THÈSE

Pour obtenir le grade de

Docteur

Délivré par le

Centre international d'études supérieures en sciences agronomiques de Montpellier

Préparée au sein de l'école doctorale **SIBAGHE**
et de l'unité de recherche **CEFE-CNRS (UMR 5175)**

Spécialité :
Ecologie, Evolution, Resources Génétiques, Paléontologie

Présentée par **Karim BARKAOUI**

Functional structure and ecohydrology of Mediterranean rangelands along a soil water availability gradient

-

Structure fonctionnelle et fonctionnement écohydrologique de parcours méditerranéens selon un gradient de disponibilité en eau

Soutenue le **12 décembre 2013** devant le jury composé de :

Mme Anne BONIS, Chargée de Recherche, CNRS	Rapporteur
M. Francisco I. PUGNAIRE, Research Professor, EEZA	Rapporteur
M. Hendrik DAVI, Chargé de Recherche, INRA	Examinateur
M. Jacques WERY, Professeur, SupAgro	Président du jury
Mme Florence VOLAIRE, Chargée de Recherche, INRA	Examinatrice
Mme Marie-Laure NAVAS, Professeure, SupAgro	Directrice de thèse

Abstract: Understanding the relationships between ecohydrology and functional structure of plant communities is a timely issue in plant ecology. From ecosystem- to species level, this works investigated the effects of a resource availability gradient on the structure and functioning of Mediterranean rangelands in Southern France. Using a water-balance modeling approach, differences in water-use at the ecosystem level were quantified across five contrasting years, accounting for the variability in both soil characteristics and vegetation functioning. We showed that plant water-use and productivity scaled with soil water storage capacity and its filling rate, suggesting a functional equilibrium between plant communities and local soil water availability. Using a trait-based approach, we then showed that the ratio of total leaf area of the community on total root area determined an 'allometric' relationship between potential evapotranspiration rate and water uptake capacity. The coordination between above- and belowground compartment of the vegetation was achieved by a suite of architectural and morphological adaptations. Modifications of species relative abundances and species turn-over explained morphological and functioning adaptations to cope with limiting resources. Finally, we aimed to unravel the role of biotic interactions in community assembly rules. At species level, a removal experiment allowed us to quantify the relative importance of water limitations and effects of plant-plant interactions on individual plant productivity of three dominant target species. The novelty of our approach was to decompose the net effect of neighbors by considering their standing biomass and their functional structure separately. We confirmed that the importance of competition increased with soil resource availability, and we showed that competition depended more on trait-trait hierarchical distances among species than on standing biomass. Competition among plants affected the axes of the functional niche of species independently, thereby leading to the convergence of leaf dry matter content towards low values when competition was important, but to greater functional divergence of plant height. Overall, this functional approach provided an integrative understanding of the role of plant diversity in the response of ecological systems to changes in water availability. Furthermore, these results on ecohydrological properties of natural communities can contribute to the design of complex agro-ecosystem better adapted to intense droughts predicted under climate change.

Résumé: Comprendre les relations entre écohydrologie et structure fonctionnelle des communautés végétales est une problématique croissante en écologie. Cette thèse a pour objectif d'analyser les effets d'un gradient de ressource édaphique sur la structure et le fonctionnement de parcours méditerranéens en intégrant les niveaux d'organisation de l'espèce à l'écosystème. Par modélisation du bilan hydrique, les différences d'utilisation de l'eau au niveau de l'écosystème ont été quantifiées pour cinq années contrastées révélatrices de la variabilité des propriétés hydrologique des sols et du fonctionnement végétal. Nous avons montré que l'utilisation de l'eau et la productivité des plantes sont proportionnelles à la réserve utile des sols et à son taux de remplissage, suggérant un équilibre fonctionnel entre les communautés végétales et la disponibilité hydrique locale. Au niveau de la communauté, nous avons ensuite montré par une approche fondée sur les traits fonctionnels des espèces que la coordination entre les compartiments racinaire et aérien de la végétation vis-à-vis de l'utilisation de l'eau dépendait d'une suite d'adaptations architecturales et morphologiques des plantes. En conséquence, le ratio entre surface foliaire et surface racinaire totales de la communauté détermine une relation 'allométrique' entre évapotranspiration potentielle et capacité de prélèvements hydriques de la végétation. Des modifications d'abondances relatives et le turn-over des espèces le long du gradient génèrent donc une variabilité de morphologie et de fonctionnement des communautés permettant leur adaptation à des ressources limitées. Afin de préciser le rôle des interactions biotiques dans les règles d'assemblage de la communauté, une expérimentation d'exclusion du voisinage a été réalisée. Elle a permis de quantifier l'importance relative de la disponibilité de l'eau et des interactions entre plantes sur la productivité individuelle de trois espèces-cibles. L'originalité de l'approche choisie a été de décomposer l'effet net du voisinage en considérant à la fois sa biomasse aérienne totale et sa structure fonctionnelle. Nous avons confirmé que l'importance de la compétition augmentait avec la disponibilité des ressources du sol et nous avons montré qu'elle dépendait plus fortement des rapports hiérarchiques entre les traits des espèces que de leur biomasse. La compétition entre plantes a affecté les axes de la niche fonctionnelle des espèces de façon indépendante conduisant à une convergence de la teneur en matière sèche des feuilles vers de plus faibles valeurs lorsque la compétition était importante, mais à une plus forte divergence fonctionnelle de la hauteur des plantes. L'approche fonctionnelle choisie dans ce travail de thèse a permis d'éclairer le rôle de la diversité végétale dans la réponse des systèmes écologiques à des ressources hydriques variables. De plus, ces résultats sur les propriétés écohydrologiques des communautés naturelles peuvent contribuer à la conception d'agro-écosytèmes complexes mieux adaptés aux sécheresses intenses prévues dans le contexte actuel de changement climatique.

Remerciements

Montpellier, le 05/03/2014

Bientôt trois mois se sont écoulés depuis la soutenance de thèse : il est désormais grand temps de déposer ce manuscrit 'pour de vrai' auprès de l'école doctorale, et cette étape commence par la rédaction des fameux remerciements. Un véritable défi après une aventure comme la thèse tant le travail présenté résulte de multiples rencontres durant trois ans. J'espère donc n'oublier personne ci-après… Le cas échéant, je vous remercie tous dès à présent.

Je souhaite évidemment remercier vivement celles et ceux qui m'ont encadré, guidé, encouragé et aidé durant ces trois années de thèse. Je commencerai donc par vous remercier Marie-Laure et Florence pour votre encadrement et votre implication dans mon travail. Malgré nos différences de point de vue sur le système d'étude, les questions à traiter et les réponses à donner, je trouve que nous avons réussi à chaque fois à faire sonner un bel accord à 3 voix lors de nos discussions, pas toujours parfait certes, voire parfois assez 'jazz' comme on pourrait dire, mais qui n'a pas été sans me déplaire, bien au contraire. Vous m'avez toute deux apporté énormément. Marie-Laure, je te remercie particulièrement de m'avoir fait confiance, tout agronome 'pur jus' que je suis (ou j'étais, je ne sais plus bien), et de m'avoir laissé cette grande liberté qui m'a permis de louvoyer au départ parmi les concepts d'écologie pour finalement mettre le cap vers des horizons nouveaux pour nous deux. Je te remercie également pour ta disponibilité, ta patience, ta pédagogie et ton regard critique tout au long de ce travail. Florence, je te remercie pour ton enthousiasme, notamment vis-à-vis de « l'écohydrologie », sortie imprévue de ce travail, car il m'a permis de passer certaines périodes de doute sans chavirer. Je te remercie également pour tes nombreux conseils, pour nos discussions, ainsi que pour ton aide répétée que je sollicitais (un peu trop) souvent 'à la dernière minute' comme avant chacun de mes oraux. J'espère que nous parviendrons tous les trois à poursuivre les travaux issus de cette thèse, tout en conciliant toujours nos différences.

Je remercie aussi les membres de mon comité de thèse, Christian Damgaard qui m'a accueilli à l'université d'Aarhus au Danemark pour discuter de modélisation bayésienne, Jean-Louis Durand qui s'est montré très enthousiaste dès le début et m'a beaucoup encouragé à poursuivre cette approche de modélisation (projet encore non abouti malheureusement…), François Mesléard pour son expertise en écologie des communautés, Philippe Choler qui m'a ouvert les yeux sur l'existence de l'écohydrologie (merci !), et Laurent Hazard qui a suivi l'évolution de ce travail lors des réunions de pilotage, mais également lors des réunions du programme ANR O2LA. De la même façon, je remercie mes rapporteurs de thèse, Francisco Pugnaire et Anne Bonnis, ainsi que les autres membres du jury, Hendrik Davi et Jacques Wery pour avoir évalué mon travail, avoir apporté leurs commentaires et suggestions, et avoir stimulé la discussion le jour de ma soutenance.

Mes remerciements vont ensuite tout droit à l'ensemble de l'équipe ECOPAR du CEFE. J'ai trouvé dans cette équipe un environnement de travail à la fois stimulant et exigeant mais aussi convivial et détendu: ç'a été précieux pour s'initier à la recherche ! Je vous remercie donc tous pour cela. Catherine, je te remercie pour ton implication dans l'ensemble des recherches de l'équipe qui ont été conduits à La Fage et sur le TE (et donc le mien aussi !), pour tes retours constructifs, et pour nos discussions sur le terrain ou au labo. Eric, je te remercie également pour ce regard que tu portais 'de loin' sur ce que je faisais et que tu partageais sans hésiter au détour d'un couloir ou lors des fameux SEKOS, ainsi que pour tes encouragements. Elena, Cyrille, un grand grand merci ! Je vous remercie particulièrement pour votre bienveillance vis-à-vis du jeune thésard que j'étais, votre écoute, vos nombreux conseils, vos coups de pouce quand il fallait débloquer un paragraphe ou relire un abstract, mais aussi pour votre complicité qui nous a valu des moments de franche rigolade !

Pour les thésards d'ECOPAR, il y a la 'dure' vie de labo au CEFE, mais également la 'dure' vie de terrain. Et à ce propos, je te remercie tout particulièrement Jean. Tu m'as accompagné maintes et maintes fois sur le Larzac, tu m'as

Remerciements



(un mouton du Larzac, *Anais Gentit 2013*)

appris à reconnaître la flore du Causse à chaque saison, tu as eu la patience de compter avec moi les innombrables brindilles des communautés de plantes que j'échantillonnais, tu as toujours été de bon conseil lorsque je doutais face à la complexité du terrain, et puis je garde un très bon souvenir des moments passés ensemble : ce travail de thèse te doit donc beaucoup, et je te suis profondément reconnaissant ! Merci pour les champignons, les jujubes, le chocolat etc. ainsi que pour tes commentaires sur mes fiches de relevés qui sont de supers souvenirs ! Bien sûr, Alain, Pascal, je vous remercie également pour l'immense travail que vous avez accompli, à La Fage, sur le TE ou au labo au CEFE, et qui a également très largement contribué à cette thèse et bien au-delà. Merci donc pour votre énergie, votre efficacité à toute épreuve, vos conseils techniques et pour les sympathiques pauses repas au grand air que vous saviez si bien agrémenter d'anecdotes ! Je remercie aussi Paul Autran, ainsi que tout le personnel de la station INRA de La Fage pour m'avoir reçu et épaulé lors des longues phases de terrain sur les parcours : merci pour l'accueil, merci pour les repas au roquefort, merci pour la chambre du pigeonnier (ma préférée), merci pour les cafés chauds l'hiver et les menthes l'eau l'été ! Enfin, je remercie ici Maria et Stéphane pour leur aide indispensable au labo et sur le terrain d'expérience à Montpellier : sans vous, certainement que je serai encore en train de broyer des feuilles, faire des micropesées, récolter de la biomasse, faire des trous dans le sol… Je n'oublie pas non plus la PACE pour son appui au labo (merci Bruno et Raphaëlle !) ainsi que l'ensemble du personnel du TE pour leur travail quotidien sur ce superbe site expérimental en plein cœur de Montpellier, et pour leurs coups de pouce salvateurs quand il s'agissait de préparer les éclats de graminées dans la chaleur des serres, d'arroser et de désherber nos expérimentations etc.

Je comparais la thèse à une aventure. Or comme dans toute aventure, il y a des compagnons de galère, sans qui rien n'est possible. J'ai eu la chance d'avoir deux supers compagnons de galère, avec qui je partageais le bureau et le Larzac aux 4 saisons (et c'était parfois une épreuve), les stress et bonheurs de la thèse, les questions philosophiques (les grandes comme les petites), les voyages à Montréal, à Londres, les fous rires: je vous remercie pour tout Marine et Ezequiel ! Vous étiez irremplaçables. On a commencé ensemble, on a (presque) fini ensemble, mais surtout on a fait tout le chemin ensemble. Donc merci pour votre complicité, pour votre humour, pour votre soutien, pour votre solidarité, bref pour toutes vos qualités qui ont fait que 'ça l'a fait grave'. Marinesita, un merci particulier pour m'avoir toléré en face de toi durant mes crackages quotidiens le soir, d'avoir su de temps à autre me rappeler des infos que j'oubliais souvent, d'être venu mettre en forme ma biblio dans les derniers rushs (et ce n'est pas un euphémisme) - remercie François-Louis également ! - et puis d'avoir su instauré autour de toi cette simplicité relationnelle qui a fait de toi un véritable moteur de convivialité ! Nos routes se croiseront à nouveau ! Ezequilito, el mero macho, salsero de Venezuela, un merci spécial à toi aussi, pour ton énergie et ta bonne humeur inaltérable : on aura bien rigolé ensemble, aïe aïe aïe, et ce, aussi bien dans les moments 'détente' autour d'une (ou plusieurs) pinte(s) de bière, que dans les périodes les plus sombres… souviens toi, la nuit au CEFE, quand on a traversé ce long tunnel de la fin de thèse à courir derrière les heures qui défilaient, à base de pizzas/cocas, pour arriver enfin à ce 'moment où la vie se coupe en deux…'. We did it ! A très vite je l'espère !

Et puis il y a eu les 'mentors' bien sûr : les thésards qui ont 'déblayé' le chemin avant nous, qui nous ont servi d'exemple et qui ont eu un 'input' considérable dans ce travail. Un grand grand merci à toi Maud ! Bien au-delà de notre 'collaboration' sur les pinpoint, tu as été une personne ressource pour de nombreuses interrogations que j'avais, aussi bien en stat, en R, qu'en botanique, en écologie, en méthodo, en rédaction, ou encore en anglais… tu m'as appris énormément de choses ! Je crois pouvoir dire maintenant que tu as été quelqu'un de très important dans mes premiers pas de thésards. Merci donc pour ta disponibilité (alors même que tu rédigeais ta thèse !) et surtout pour ta pédagogie infaillible. Marie, je te remercie également : bien que l'on n'ait pas eu la même interaction 'professionnelle' du fait de ta discipline 'obscure' (l'éco-quoi ?), cela ne nous a pas empêché d'avoir de bonnes discussions (sérieuses ou pas d'ailleurs) qui m'ont parfois permis de prendre un certain recul (sur le partage de données, l'interdisciplinarité, si, si ça aussi ça compte malgré tout !). Et puis franchement, 5 min de pause avec toi en valait bien 15 tout seul car on rigolait plutôt bien ! Alors merci pour tout ça ! Après les mentors, je n'oublie bien sûr pas la 'relève' : Iris, je te remercie pour ta motivation et ta bonne humeur naturelle car ça rebooste sur plusieurs plans et ça ouvre des perspectives ! Bon courage pour la suite !

Je souhaite également remercier les stagiaires, autres thésards et post-docs d'ECOPAR et de BIOFLUX, qui au gré de leurs allers et venues durant ces 3 années au CEFE ont apporté leur cocktail d'idées venues d'ailleurs et m'ont beaucoup aidé ponctuellement. Dans le désordre, je remercie Baptiste Testi pour son introduction au bayésien 'à la cool' et

Remerciements



(un (aigle)-vautour du Larzac, *Anais Gentit 2013*)

pour avoir partagé le bonheur des fameuses 'baptistades' ; Antoine Gardarin pour les nombreuses discussions et ses conseils ; Benjamin Borgy pour son expertise en statistiques et en R, mais également pour les nombreux cafés partagés (même si tu préférais le café d'Amérique Centrale) et pour tous ces moments où on a bien rigolé depuis les bureaux montpelliérains jusqu'à la piste de danse d'INTECOL à Londres ; Ivan Prieto pour nos discussions sur les interactions entre plantes, l'eau du sol, les modèles mixtes et pour tous les bons moments passés ensemble ; Jessie Loranger pour avoir instauré le beer time chez les temporaires (source d'échange et d'inspiration indispensable !) ; Emmanuel Defossez pour ses différents conseils lors de mes répétitions orales ou sur le reviewing d'article, et pour avoir détendu (encore plus) l'ambiance de notre cher bureau (c'est bien sûr sans compter les inextricables débats sur les VGs) ; Bérengère Leys parce que trop de détente dans le bureau, tout de même, nécessitait un peu de rangement ;) mais aussi pour ses précieux conseils en fin de parcours (comment organiser une intro de thèse ?) et les quelques gigas de musique qui m'ont aidé à tenir la dernière semaine. Je remercie également le 'bureau du fond' avec ses habitants successifs : merci à vous Mathieu, Nico, FX, Amar ! C'était bien agréable de venir faire des pauses chez vous, de discuter de vos projets sur les bestioles, de faire des contrepétries au tableau et de passer des apéros/soirées ensemble ! Mathieu, merci pour tes conseils, tes encouragements, ta solidarité tout au long de ces trois années et puis pour avoir été cette oreille rassurante quand on a besoin parfois de raconter à quelqu'un 'où on en est'. FX, merci d'avoir cadré Mathieu ;) et d'avoir (ar)rangé votre bureau pour que les pauses soient encore plus agréables. Je remercie aussi ces 'jeunes agros innocentes' que j'emmenais sur le Larzac : merci Caroline et Orianne, vous avez été d'une grande aide, et chacune d'entre vous a finalement participé à un chapitre de cette thèse ! Anaïs, je te remercie également pour tes aquarelles qui ont illustré ma soutenance et dont je mets une copie ci-contre…

Décidément, plus j'écris ces remerciements, plus je réalise qu'il y a du monde (je ne sais décidément pas synthétiser), mais surtout une belle diversité de personnalités et d'interactions ! Sans vous tous, je n'imagine pas la fadeur qu'aurait eu cette thèse : alors vraiment un grand merci pour tout ce que vous m'avez apporté !

Bien sûr, au-delà du cercle 'recherche', il y a tous les autres, ceux qui m'ont accompagné durant ces trois ans, ceux qui ont subi mes retards, mes coups de stress, mes monologues et excès d'enthousiasme, et qui ont finalement eu une importance clé dans le 'méta' projet de la thèse : je remercie ici très brièvement tous mes amis de Montpellier, la famille des Nostalguys, la famille des agros toutes générations confondues, la famille des fanfarons élargie (Coco Fanfare et Bakchich) et le CIF (Comité Interprofessionnel de la Fête), véritable soupape à pression. Je vous remercie tous pour votre confiance, pour votre présence, pour votre écoute, pour vos conseils, pour tous ces moments de détente, de sport au grand air, de musique en tout genre, de fête, et de re-fête, de rigolade, mais aussi de brainstorming, de discussions, de débats, bref. La vie quoi. La plupart d'entre vous s'en moque, mais cette thèse, je vous la doit en partie, alors merci à vous tous ! Tout de même je me dois de nommer ici au moins deux personnes : un merci immense à toi EuSBe pour tout ce que tu as fait, tes attentions répétées quand ça allait et quand ça allait moins, ainsi qu'à toi Nico car nos nombreuses discussions 'scientifico-déconnantes' à rallonge ont été en fait fructueuses à plus d'un égard (un jour, on aura notre labo !).

Enfin, je remercie affectueusement mes parents et mes soeurs de m'avoir soutenu dans ce long parcours vers la recherche qui a commencé bien avant la thèse : merci à vous, infiniement. J'ai également une pensée pour mes grand-parents qui n'auront finalement pas pu voir l'aboutissement de ce projet...

Juju, merci d'avoir partagé nos espérances, d'être là, toujours : cette thèse c'est avant tout grâce à toi.

Karim Barkaoui.

Table of contents

General introduction……..……1

A. Ecohydrology : Towards an interdisciplinary research ?

B. Contribution of the functional approach of diversity

C. Water-limited ecosystems as case study

Chapter 1 – Materials & methods…….……..……33

A. The study site: La Fage experimental station

B. Quantifying the edaphic gradient

C. Assessing the structure of vegetation

Manuscript I. *Questionning the reliability of the point-intercept method for assessing community functional structure in low productive and highly diverse Mediterranean grasslands*……...……..45

Chapter 2 – Rangeland ecohydrology…….…...…96

Manuscript II. *Which trade-off between productivity and water stress ? Evidence from a 5-years measurement series in a Mediterranean rangeland*

Chapter 3 – Functional structure of plant communities……...……..……149

Manuscript III. *Effect of the functional structure of plant communities on emergent properties of vegetation and ecosystem water flows in a Mediterranean rangeland*

Chapter 4 – Importance of plant-plant interactions…….……...209

Manuscript IV. *Competitive interactions are driven by species' trait hierarchy and not by standing biomass in a Mediteranean rangeland*

General discussion……259

A. The functional structure of plant communities

B. Towards a better understanding of ecohydrology

C. Applications in agro-ecology

References……..…….……283

Appendix……295

Manuscript V. *Designing resilient and sustainable grasslands for a drier future: adaptive strategies, functional traits and biotic interactions*

General introduction



(Mediterranean rangeland landscape at La Fage-station, *spring 2011*)

The search for general principles to elucidate the response of species communities and ecosystems to environmental changes is a longstanding theoretical issue in ecology, and has become an urgent applied avenue in the face of ongoing global changes. Since at least two decades, several attempts have been tried to reach a more predictive ecology, covering a wide range of approaches, from purely correlative statistical methods to complex mechanistic models. Nevertheless, our ability to predict the future remains a Holy Grail mostly because of the huge challenge to understand and incorporate the physiological and ecological mechanisms, as well as related uncertainties, underlying the response of organisms, communities and ecosystems.

Species distribution models (SDMs) and Landscape surface models (LSMs) are striking examples of such attempts for plant species and ecosystems. At the species level, while SDMs have been found to successfully describe species distribution worldwide (Thuiller et al. 2008), their ability to predict future distribution remains questionable since they do not explicitly account for physiological responses of the species (Morin & Thuiller 2009) and because they mostly focused on climatic conditions as forcing variable, ignoring edaphic conditions such as soil water availability (Thuiller 2013). At the ecosystem level, LSMs simulate large patterns of energy partitioning and nutrient cycling along the soil-plant-atmosphere continuum, but their representation of vegetation is oversimplified since they involve only a small number of parameter values for describing the diversity of ecosystems. Therefore, their predictive power may be strongly limited by the fact that they neglect the variations of parameters within a given ecosystem and do not account for how these variations may relate with external forcing (climate or land use) or internal pressures (biotic interactions). In this way, such modeling approach contributes to maintain a stark disconnection between the science of biological diversity and the science of the earth system.

Given the considerable importance of water availability in Mediterranean ecosystems, my general aim was to call for knowledge from several disciplines (including functional ecology, ecophysiology, hydrology, climate science and agronomy) to provide a better understanding of the interactions between the biotic component of the vegetation and soil water resources. To this end, I emphasized the importance of ecohydrology, a relatively recent discipline, as a core satellite discipline for functional ecology, in particular to make a clear linkage between water balance and ecological communities which are obviously more complex than agricultural monocultures for which water balances have been mostly developed in the past. To overcome the complexity of precisely understanding all the interactions processes within species-rich ecosystems, an approach describing organisms by their functional traits (as emerged from decades of research in

ecophysiology and functional ecology; Violle et al. 2007) appears to be a relevant and powerful tool towards the predictions of the response of co-occurring organisms (namely communities) to environmental changes.

A. Ecohydrology: towards an interdisciplinary research

The first clear definition of 'ecohydrology' was "an application driven discipline" that "aims at a better understanding of hydrological factors determining the natural development of wet ecosystems" (Wassen & Grootjans 1996) since it was first applied to wetlands. The range of application was however rapidly extended to wider environmental contexts, including all plant ecological systems (wetlands, drylands, forests, freshwater systems etc.). Over the past decade, the scope and aims of ecohydrology have been enthusiastically debated (Zalewski 200; Bonnell 2002; Kundzewicz 2002, Nuttle 2002; Bond 2003) and it was acknowledged that issues in ecohydrology should go beyond the limited objectives of water resource management and biological conservation towards the exploration and understanding of fundamental processes. The term 'ecohydrology' now refers to an interdisciplinary research between hydrology and ecology, which aims to elucidate (i) how hydrological processes influence the distribution, structure, function and dynamics of ecological systems, and (ii) how feedbacks from ecological systems affect the water cycle (Rodriguez- Iturbe 2000, Rodriguez- Iturbe et al. 2001; Nuttle 2002; Newman et al. 2006). In 2008, following the publication of seminal textbooks (*e.g,* Eagleson 2002; Rodriguez-Iturbe & Porporato 2005), the launch of *Ecohydrology* (http://onlinelibrary.wiley.com/journal/ 10.1002/ (ISSN) 1936-0592), a dedicated peer-reviewed journal, illustrates the recent emphasis of research towards the elaboration of a strong conceptual background for this emerging research field (Smetten 2008).

1. The meeting between hydrology and ecology: what is really new ?

Merging hydrological and ecological issues does not necessarily imply new ideas (Bonnell 2002), nor design a new paradigm (Hannah et al. 2004, 2007). Typically, how soil and atmospheric water (among other factors) influence the distribution and function of plant species has been a fundamental issue of plant functional ecology for several decades. Furthermore, the pivotal role that plants play in modulating many hydrologic processes has long been recognized by both ecologists and hydrologists, leading to efforts to refine and deepen understanding of water fluxes, flows and transport within these respective disciplines. However, collaboration and integration across hydrology and ecology has been historically limited.

The novelty of ecohydrology mainly relies on the reconciliation of two contrasting views of science: the Newtonian view of hydrology for which understanding broadly applicable and general mechanisms is desired, and the Darwinian view of ecology for which an understanding of the specific details of particular systems is desired (Harte, 2002). While the generality of a mechanistic understanding of processes is a common objective shared by both hydrology and ecology, research in both fields is usually faced with the variability of natural systems that hinders a comprehensive reductionist understanding. In other words, combining Newtonian principles of simplification, ideal systems, and predictive understanding with Darwinian principles of complexity, contingency, and interdependence offers a promising way towards deep improvement of our understanding of 'universal' natural processes.

Recently, ecohydrological investigations have increased the emphasis on general understanding of plant-water relations, especially as related to patterns in vegetation water use and mechanisms controlling water-balance responses to environmental change. Reflecting the synthesis between the reductionist and holistic approach, the reviews by Newman et al. (2006) and Asbjornsen et al. (2011) identified three main cross-cutting challenges for addressing this research question in ecohydrology. The first issue concerns spatial complexity and scaling which requires the identification of scaling patterns and laws to improve prediction of cross-scale interactions. While mechanisms controlling water fluxes often need to be examined at the scale of individual leaves, the effect of accumulated fluxes are better understood at the ecosystem level (Asbjornsen et al. 2011). Likewise, scaling in the timing of processes has a critical importance for evaluating the dynamics of water availability within the ecosystems and thereby integrating plant water stress across years and seasons. The second issue refers to threshold behavior, due to the non-linearity in ecohydrological relationships and, hence, threshold crossing. Shifts to alternate stable states in response to either progressive or abrupt triggers in the environment have been observed by both ecologists and hydrologists (Scheffer & Carpenter 2003; Scheffer et al. 2005), but the dynamics of the threshold behavior of ecosystem processes and the underlying causes still need to be identified. The third issue stems from complex plant-soil feedbacks and interactions, which may be better understood by long-term, place-based empirical studies to test theoretical models. Specifically, the idea that system dynamics equilibrates with forcing variables because the vegetation adapt and optimize its water-use over long-time periods has led to promising predictive framework. However, the underlying hypothesis needs to be empirically tested at the ecosystem level.

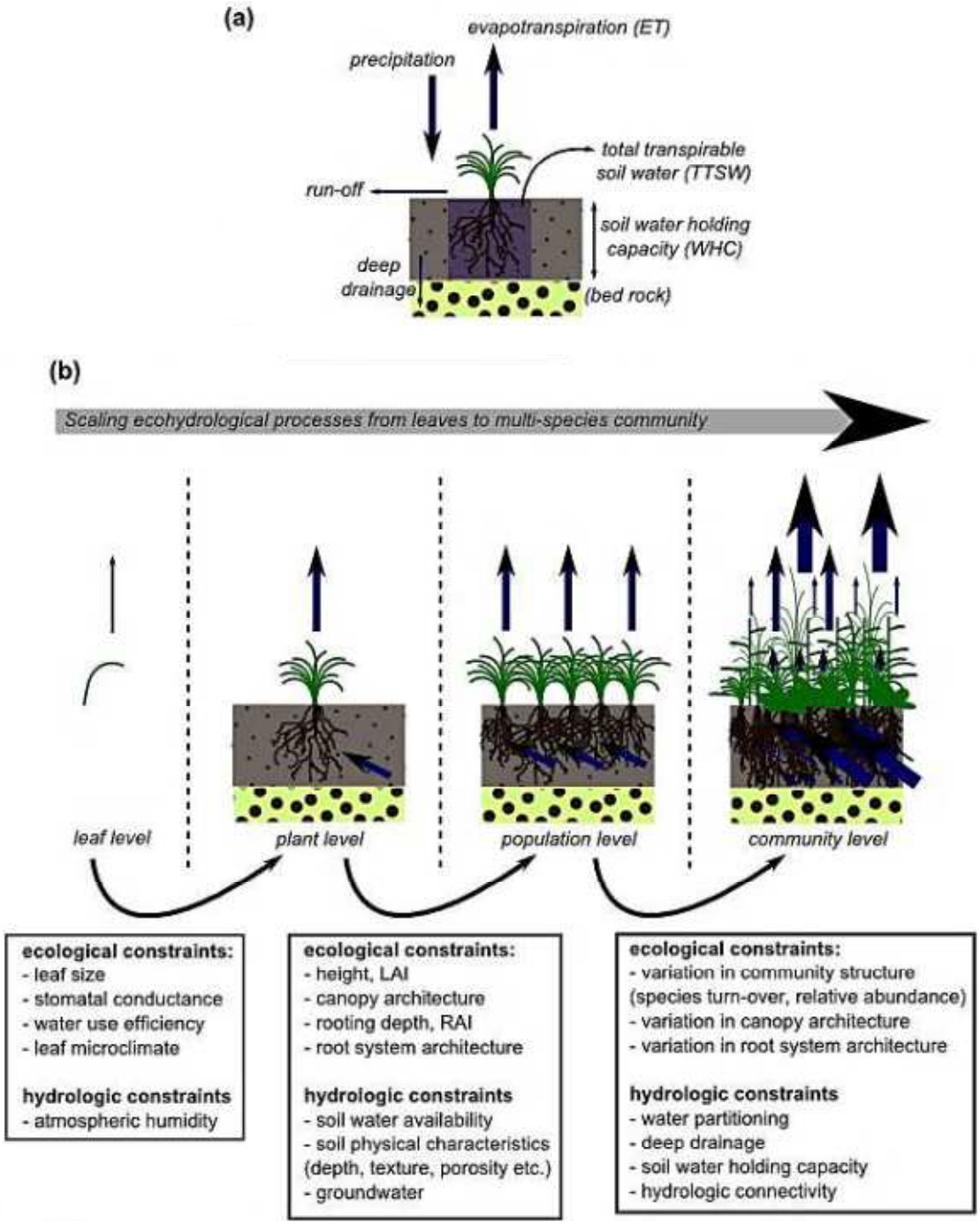


Fig. 1 (a) simplified water balance along the soil-plant-atmosphere continuum. Blue arrows represent main water fluxes involved in the water balance. Incoming flux is precipitation; outcoming fluxes are evapotranspiration (=plant transpiration + soil evaporation), run-off and deep drainage. (b) conceptual model for scaling ecohydrological processes from the leaf- to multispecies community-level, with an emphasis on evapotranspiration and water uptake fluxes. Bowes indicate the ecological and hydrologic controls on water fluxes that must be quantified and understood when transferring ecohydrological processes across scales (adapted from Asbjornsen et al. 2011).

2. Rethinking water balance as a research theme

A fundamental equation in ecohydrology is the water balance at a defined place. Based on mass conservation principle, the water balance states that the amount of water entering the soil is equal to the amount of water leaving the soil plus the change in the amount of water stored in the soil. The water balance has four main components: infiltration of precipitation into the soil, evapotranspiration, leakage of water into deeper soil layers which are not accessible to plant roots, and runoff from the ground surface (Fig. 1a). As pointed out by Asbjornsen et al. (2011), a central challenge concerning plant-water relations is the ability of scaling up from fine processes occurring at the leaf-level to stand- level (Mackay et al. 2010) where many species of different nature can coexist. Issues related to water-balance have been usually analyzed at contrasting hierarchical scales, but rarely with the objective of integration across scales. Plant-water relations have been widely studied by physiologists and ecophysiologists at the leaf-level or whole plant-level, namely with the identification of mechanisms controlling water fluxes and their integration into plant functioning models. Conversely, hydrologists have usually conceptualized water balance at the watershed scale, working with simplified representation of water cycle and general physical laws governing water flows, but with a limited description of processes related to the diversity of vegetation. How water is partitioned at intermediate scales, *i.e* within a plant community, has been therefore little explored.

The predominant water flow driving water balance is evapotranspiration (*i.e* the sum of plant transpiration and soil evaporation) which may account for a large proportion of incoming precipitation in dry areas (often > 90 % Wilcox et al. 2003). At the community-level, regulation of evapotranspiration rate with respect to available water and atmospheric demand may result from the combination of many mechanisms operating at different time scales and hierarchical levels (Fig. 1b). At the leaf- or plant level, a suite of physiological, morphological and phenological adaptations defines species strategies to drought (Maseda & Fernandez 2006; Moore & Heilman 2011; Manzoni et al. 2013; Zeppel 2013). Temporal water stress trigger a reduction of transpiration through stomata closure to prevent for internal hydraulic failure; more prolonged drought periods affect total leaf area through the senescence of leaf tissue; recurrent moderate droughts may select for species with more sclerophyllous leaves and deeper root system; permanent droughts select species with adapted phenology to avoid drought events or with specific morphology to store water, etc. At the stand-level, evapotranspiration depends also on canopy structure, plant density, soil cover etc. The lack of accurate predictions at the community level therefore arises from the difficulty of combining responses acting at different spatial and

temporal scales (Fig. 1b). In case of monocultures, agronomists have developed an effective approach for estimating evapotranspiration fluxes based on the 'crop coefficient' which is calculated as the ratio between the maximum evapotranspiration rate of a species (or genotype) and the theoretical potential evapotranspiration rate (Allen et al. 1998; Allen et al. 2005). However, whether such an approach is applicable in ecological systems characterized by a high species diversity remains questionable (Mata-Gonzales et al. 2005).

Central to the calculation of the water balance is the assessment of dynamics of soil moisture, which can be considered as an integrative factor of ecohydrological processes (Rodriguez-Iturbe & Porporato 2005), since it reflects the net effect of the different involved variables (Breshears et al. 2009), especially climate forcing and vegetation functioning. The most important shift for ecologists in moving towards an ecohydrological approach is the need to take into account more variables than only 'precipitations' (Loik et al. 2004). This will allow a more comprehensive understanding of the water balance as well as more accurate quantitative prediction of 'plant-available water' which results of the partitioning components of evapotranspiration at a site (Fig. 1a). Precipitation has extensively been used as the main predictor of plant productivity and other ecosystem properties across many systems (Knapp et al. 2002; Huxman et al. 2004; Wu et al. 2011; Ruppert et al. 2012). Yet vegetation dynamics may arguably be much more closely related to soil moisture, while soil moisture dynamics can markedly differ from patterns of precipitation alone (Vicca et al. 2012).

3. The 'ecohydrological optimality' hypothesis

The development of a quantitative theory of coupled atmosphere-soil-vegetation dynamics is therefore a fundamental issue in ecohydrology. It is essential for understanding the global distribution of biomes in relation with climatic patterns as well as the changes in vegetation structure and composition observed along microclimatic gradients (Whittaker 1967). In addition, it represents a strong potential for the improvement of fluxes models and for predicting ecosystem responses to disturbance and climate change. Accounting for the fundamental role of water-balance in many ecological processes, the 'ecohydrological optimality' hypothesis, essentially promoted by the pioneering work of Eagleson (*e.g.* Eagleson 1982ab; Eagleson & Segarra 1985; Eagleson 2002), is likely to provide the basis of such theory.

Eagleson suggested that there may be ecological pressures for evolutions in natural soil-vegetation systems which driving a synergistic modifications towards an equilibrium state in a given environment. The hypothesis was built upon three main assumptions which were used to

derive the optimal properties: (1) over short time scales (within one or a few generation), vegetation canopy density will equilibrate with climate and soil conditions to maximize soil moisture, and thereby minimize plant water stress; (2) over longer time scales (a few generation), vegetation will maximize water use efficiency through succession and selection of adapted species, thus again maximizing soil water; and (3) over much longer time scales, vegetation will modify soil development to maximize canopy density in the long-term. Following Eagleson's hypothesis, it could be assumed that the vegetation should minimize evapotranspiration in order to maximize soil moisture over time. However, this conclusion appeared unrealistic from an ecological point of view as it would effectively also result in a minimization of photosynthetic activity (Hatton et al. 1997; Kerkhoff et al. 2004). Other inconsistencies with the current knowledge of vegetation ecology have been reported. In particular, limitations of the theory have been pointed out such as the lack of a dynamic interaction between water availability and productivity (or more broadly C cycling), and the fact that physiologic differences between species at the community level are not taken into account (Rodriguez-Iturbe et al. 1999; Sankaran et al. 2004; Jenerette et al. 2012). Another unrealistic assumption underlying Eagleson's equilibrium is the constancy of selective pressures over time which ignores trends in the long-term reflecting climatic oscillations, plant-plant interactions (*e.g.* community succession) as well as inherent fluctuations in natural populations that occur even in the absence of a major disturbance. For this reason, some authors preferred to use words such as 'regime' instead of terms 'equilibrium' or 'stable state' that seems to exclude these dynamics (Scheffer et al. 2003). However, for the sake of simplicity, we will continue to use 'equilibrium' thereafter.

More recently, several studies have developed additional optimality-based hypotheses to overcome missing elements in Egleson's model. For instance, the vertical structure of the root system within an ecosystem has been incorporated for better describing water use by the vegetation (Guswa 2008; Schymanski et al. 2008). Moreover, the optimization of the trade-off between water loss and carbon uptake or between maximizing photosynthesis and minimizing organ costs have been formalized in models (Poporato et al. 2004; Schymanski et al. 2008; Caylor et al. 2009; Guswa 2010). The main assumption beyond all these studies is that natural vegetation has coevolved with its environment over a long period of time and that natural selection has resulted in ecosystem structure, function and species composition that are 'optimally' adapted to a given set of environmental conditions. Therefore, ecohydrological systems may be considered as self-organized systems, in which environmental variability will govern functional responses towards an equilibrium state (Jenerette et al. 2012). However, many ecohydrological studies have been based on typical hydrologic frameworks, using reductionist modeling approaches for which

vegetation is usually considered as a 'black box' with some mathematical properties. It is worth noticing that in most cases, these modeling approaches failed to parameterize species-rich communities. Despite the fact these studies provided valuable insights into theoretical mechanisms of coupled atmosphere-soil-vegetation dynamics, a step forward would be to open the 'black box' and to analyze the diversity of vegetation structure and functioning using both ecological concepts and empirical knowledge in ecohydrology.

B. Contribution of the functional approach of diversity

Functional ecology falls within a comparative approach which aims to synthesize the role of key biological functions across levels of organization and environments. Conversely to autoecology studies for which a precise description of the response of a particular species to the environment is desired, the comparative approach seeks to study the diversity of responses through a large number of different taxa, communities and ecosystems in order to extract broadly applicable knowledge (Garnier & Navas 2012). Concepts underlying how the diversity of functions scales up across the levels of organization in response to the environment may have critical contribution in better understanding multi-scale plant-water relationships needed in ecohydrology. These concepts are presented in the following section, starting from how the plant functional diversity has been defined and apprehended from the niche theory in ecology, and then how it may provide a 'deterministic' view of the relationships between the environment, structure of plant communities and ecosystem processes.

1. The functional approach of plant diversity

The concept of niche has a central place in ecology since it is the cornerstone of many fundamental theories of species coexistence. However, a clear definition of the niche theory has been delayed due to contrasting conception. In its first definition (Grinnell 1917), the niche referred exclusively to the habitat, identifying all the abiotic factors necessary for the existence of species. An alternative definition was proposed ten years later based on the properties of species (Elton 1927), outlining the role of species within the community as well as the importance of the biotic environment. Thirty years later, Hutchinson (1957) proposed that the niche gathers both the abiotic and the biotic factors that affect species growth and reproduction within a community. According to this view, the niche is an *n*-dimensional hyper-volume where the dimensions are environmental conditions that a species may tolerate.

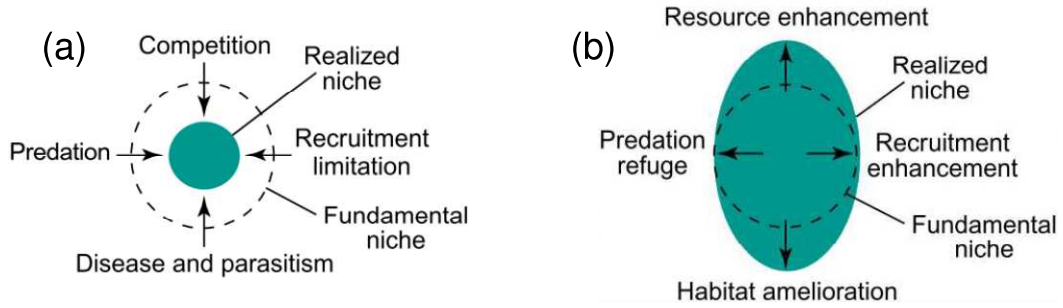


Fig. 2 Representation of the niche theory. Dashed circles delimit the fundamental niche; green circles represent the realized niche. (a) model of Hutchison (1957) where the realized niche is a sub-part of the fundamental niche reflecting several biotic constraints. (b) model of Bruno et al. (2003) which incorporates facilitation effects extending the realized niche beyond the fundamental niche (taken from Bruno et al. 2003).

Hutchinson (1957) definitely operated a conceptual shift when formalizing the niche as an attribute of species rather than of the environment. By integrating the competition exclusion principle of Gause (1937), which states that 'ecologically similar' competing species cannot coexist in the long-term, he made the distinction between the 'fundamental niche' and the 'realized niche'. The fundamental niche of a species, sometimes also referred to 'physiological niche', corresponds to the entire range of abiotic conditions under which the species can survive and reproduce, whereas the realized niche, or 'ecological niche', is the subpart of the fundamental niche that is actually occupied by the species under the influence of other species in the community (Fig. 2). Indeed, biotic interactions among plants may change the distribution of species by affecting their accessibility to resources, either negatively in case of competition (Grime 1973) or positively in case of facilitation (Bruno et al. 2003). However, how the description of species niche should be achieved was not straightforward, resulting in an important emphasis on species specificities, and thereby species identity, in community ecology.

More recently, the 'functional niche' was defined directly from the physiological functions of the species (Rosenfeld 2002). In line with the definition of Hutchinson (1957), the functional niche is an *n*-dimensional hyper-volume but the axes correspond to functional attributes of the species (growth, reproduction, regeneration, phenology, etc.) describing their resource use strategy and their tolerance to the abiotic constraints. This functional approach of the niche allows for a more objective and comparable assessment of species fitness under a given set of environmental conditions, favoring the emergence of more mechanistic and predictive theory of species coexistence (Keddy 1990; Weiher & Keddy 1995; McGill et al. 2006; Garnier & Navas 2012).

2. The use of plant functional traits: tools for comparing adaptative strategies

In functional ecology, species diversity is apprehended through the functions of species instead of using their taxonomic name. Specifically, the trait-based approach consists in using relevant functional markers (Fig. 3) to describe the variety of functions fulfilled by the species (McGill et al. 2006; Garnier & Navas 2012). A functional trait is "any morphological, physiological or phenological feature measurable at the individual level, from the cell to the whole-organism level, without reference to the environment or any other level of organization" (McGill et al. 2006; Violle et al. 2007). Among the multiple traits that can be measured on a plant, relevant traits (i) enable to apprehend clear functions of plants, (ii) are easy to measure repeatedly on a large number of individuals with standardized protocols, and (iii) can be used to rank the species consistently within contrasting environments (Garnier & Navas 2012). During the past decades,

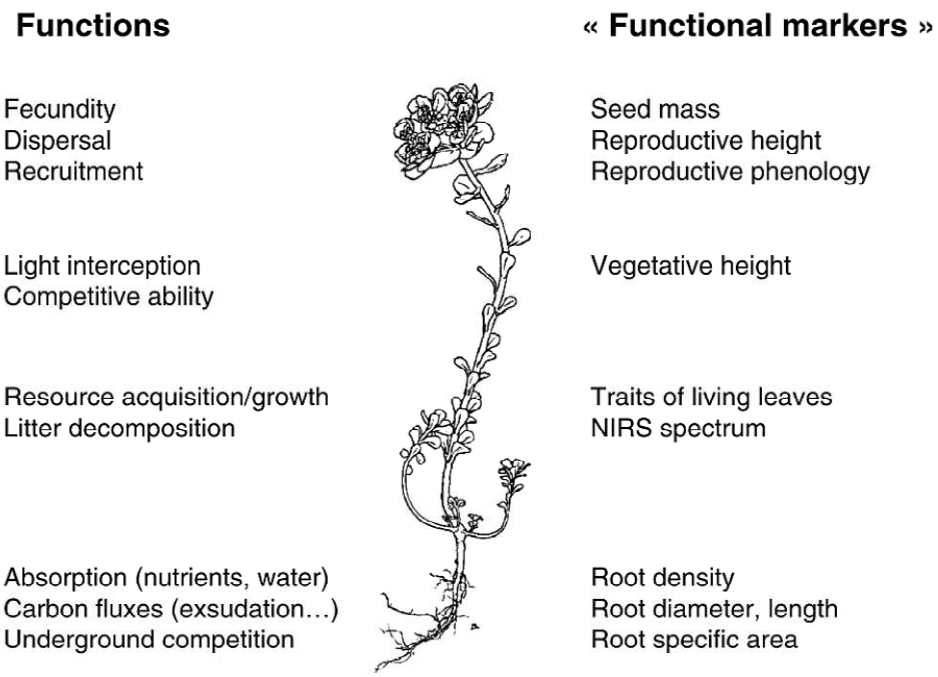


Fig. 3 Examples of functions and associated 'functional markers' (functional traits). Hand drawing of *Euphorbia Helioscopa* by Baptiste Testi (taken from Garnier & Navas 2012).

several plant functional traits have been identified in relation with sound physiological pathways (*e.g.*, Fig. 3), comprehending many fundamental plant functions such as photosynthesis, root nutrient uptake etc., and standardized measurement protocols have been proposed (Cornelissen et al. 2003).

Despite the fact they are associated with different physiological functions, plant functional traits are rarely independent of each other (Chapin et al. 1993; Grime 1997; Reich et al. 1997). On the contrary, many of them co-vary therefore defining 'trait syndromes' at the whole-plant level (Fig. 3). In most cases, such trait co-variations reveal physiological or anatomical constraints (Weiher et al. 1999). For instance, it is now well admitted that growth forms (Warming 1909) or life forms (Raunkiaer 1934) reflect the wide range of the multivariate trait co-variation among species. The 'leaf economic spectrum' (Wright et al. 2004) is another trait syndrome which has received a lot of attention across almost all biomes. It corresponds to the negative correlation between carbon assimilation rate of leaves and leaf life span, highlighting a fundamental trade-off among plants between acquisitive strategies of resources, associated with rapid plant growth and high tissue turn-over, against conservative strategies, associated with slow plant growth and low tissue turn-over. Specifically, a series of traits related to leaf physiology and morphology has been identified to be involved in the leaf economic spectrum. Among them, specific leaf area (SLA, the ratio between leaf area and biomass) and leaf dry matter content (LDMC) have been widely used to position species along the continuum between acquisition/conservation of resources (Westoby et al. 2002; Weiher et al. 1999; Wilson et al. 1999). While this trade-off was repeatedly evidenced in response to light or nutrient availability (Poorter et al. 2012), the close coupling of photosynthesis processes and transpiration also gives a sound ecological interpretation to the trade-off in response to water availability.

The identification of such recurrent co-variation patterns among a large number of species has suggested the existence of general axes defining the functional niche of species. Westoby (1998) therefore proposed to describe the functional niche of plant species using three fundamental axes (the 'L-H-S' space), each one being related to one major functional trait (or group of traits): (i) specific leaf area which is the main trait involved in the leaf economic spectrum (Wright et al. 2004), indicating species resource use strategy; (ii) maximum plant height which indicates the competitive ability of species for light, and thereby their carbon assimilation strategy (Westoby et al. 2002); and (iii) seed mass which indicates the regeneration strategy of species through the relationship between seed mass, dispersion ability and seedlings establishment and survival (Westoby 1998; Weiher et al. 1999). However,

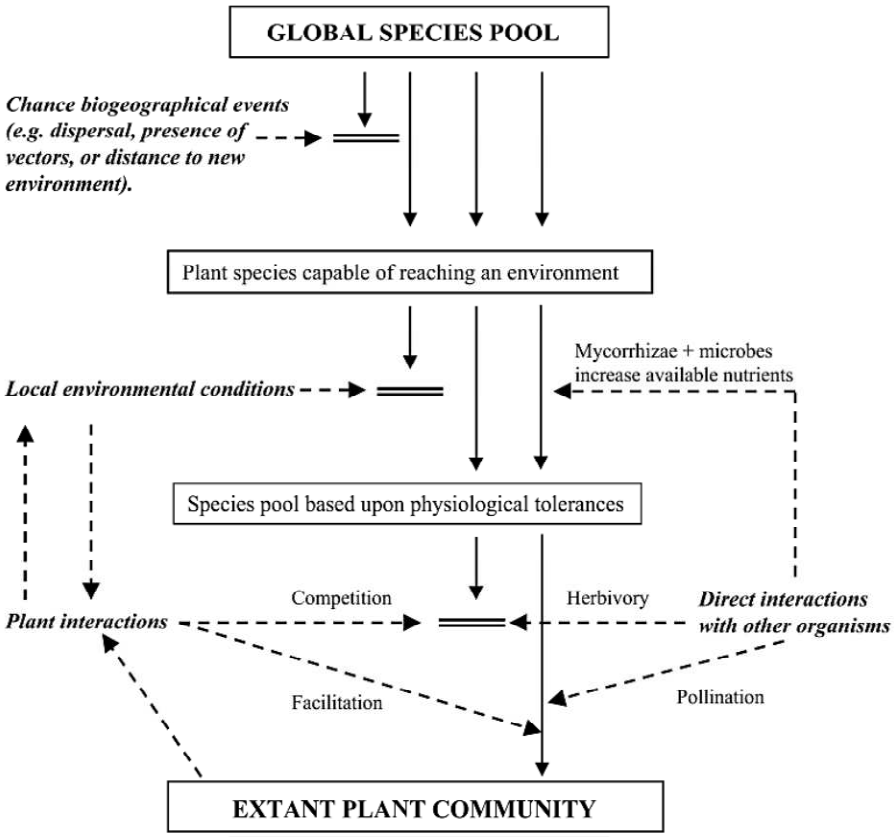


Fig. 4 The main processes (filters) that structure a plant community. The concept of successive filtering propose that a community is determined by synergistic interactions among (i) stochastic processes, (ii) the specific tolerances of species to the suite of local abiotic conditions, (iii) positive and negative direct and indirect interactions among plants, and (iv) direct interaction with other organisms. All four processes can be important in determining the extant plant community at a given site but that the relative importance of each process will vary in space and time. Solid arrows represent species movement through the filters; dashed arrows represent where the effect of each processes may influence the plant community (taken from Lortie et al. 2004).

while the L-H-S space has provided a simple representation of the functional niche of plant species, initiating important meta-analyses towards the emergence of general rules, it focused on a limited series of plant traits. For instance, it is still unclear to which axes species phenology or belowground traits are associated, despite their expected fundamental role in plant-water relations. Their integration in such representation constitutes now a challenging issue in functional ecology.

3. Trait-based response-and-effect framework: inferring processes through the community level

Changes in the environment can affect ecosystem processes directly through effects on abiotic controls and indirectly through effects on the physiology, morphology of organisms, structure of populations and composition of communities. The scaling of physiological processes from organisms to ecosystems therefore requires integrating the characteristics of each organism across levels of organization accounting for demographic processes. Therefore, it appears that the community level plays a pivotal role for scaling processes from the organisms to ecosystems. Based on the functional niche of species, functional traits were suggested to be relevant tools for analyzing the response of plants to the abiotic and biotic environment, and in turn their effect on varying ecosystem processes (Lavorel & Garnier 2002; Suding et al. 2008).

There is growing evidence that community assembly operates as a series of successive filters (Fig. 4) selecting species according to their functional traits from a regional pool into a local habitat (Keddy 1992; Belyea & Lancaster 1999; Lortie et al. 2004a). Each level of filtering modifies non-randomly the distribution of trait values within the community (Bernard-Verdier et al. 2012) depending on their selective pressure at the site and at the scale of study (Bell 2005). For instance, environmental filtering excludes species with unfit trait values (Fig. 4), and therefore strong abiotic constraints are expected to restrict the range of trait values and to lead to convergent local trait distribution (Weiher et al. 1998; Cornwell et al. 2006; Grime 2006). This is typically the case for traits related to resource-use strategy along gradients in resource availability (Cornwell & Ackerly 2009; Pérez-Ramos et al. 2012; Bernard-Verdier et al. 2012). Conversely, in line with the 'limiting similarity' hypothesis (MacArthur & Levins 1967), competition is usually expected to create a divergent distribution of trait values reflecting the niche differentiation among locally co-existing species (Wilson 2007; Schamp et al. 2008; Wilson & Stubbs 2011). However, there are conflicting views regarding the existence of competition-driven trait divergence in plant communities (Grime 2006; Navas & Violle 2009). Some authors argue that a high level of competition itself represent a strong filter, thereby leading to trait converge through mechanisms of niche equalizing (Chesson 2000; Grime 2006). To some extent, it is possible that

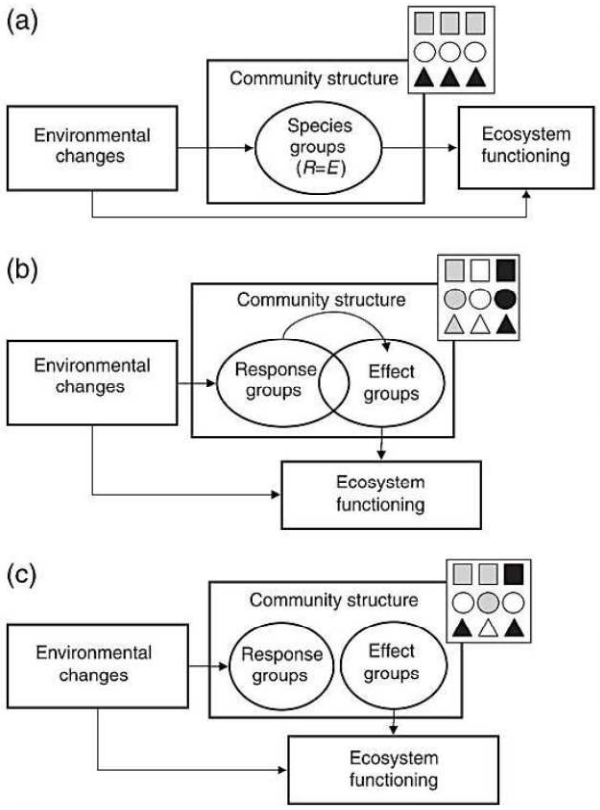


Fig. 5 The trait-based response-and-effect framework for scaling through the community level distinguishes between traits that respond to environmental change (R, response traits) and traits that impact ecosystem processes (E, effect traits). These two groups of traits may (a) completely overlap reinforcing the effects of environmental change through community dynamics. Specifically, this is what the expected situation for water related processes. In other cases the two groups of traits may (b) be more or less correlated to each other, leading to different level of resistance and resilience to environmental change, or (c) effect traits may be random with respect to response traits (taken from Suding et al. 2008).

both trait divergence and convergence co-occur under strong competition with a varying relative importance depending on the considered traits: traits related to the use of a limiting resource or to asymmetric competition may show convergent patterns, while other traits may more likely show divergent patterns (Weiher & Keddy 1995; Grime 2006; Bernard-Verdier et al. 2012).

More than the number of species, it is now well recognized that the functional structure of the communities, *i.e* the mean and the variance of the distribution of trait values, drives the ecosystem functioning (Eviner & Chapin 2003; Diaz et al. 2007). For instance, the 'mass ratio' hypothesis (Grime 1998; Smith & Knapp 2003) suggests that dominant species, determining the biomass-weighted mean trait value of the community, should have a predominant effect on ecosystem functioning, mainly by influencing instantaneous rates of processes. Another hypothesis suggests on the contrary that functional divergence, *i.e* the variance of trait values within the community reflecting the levels of niche complementarity among species, should influence ecosystem functioning through more complete use of resources (Petchey & Gaston 2006). While the mass ratio hypothesis have found strong supports for key biogeochemical processes such as primary productivity (Vile et al. 2006; Gross et al. 2008; Mokany et al. 2008; Klumpp & Soussana 2009) or litter decomposition (Garnier et al. 2004; Cortez et al. 2007; Quested et al. 2007; Fortunel et al. 2009), far less empirical evidence have been provided to support the effect of niche complementarity (see Klumpp & Soussana 2009; Schumacher et Roscher 2009). Beyond analytical reasons (Schumacher & Roscher 2009; Garnier & Navas 2012), the non-detection of effect related to functional divergence may be a question of time-scale of observation. Although the mean trait value of the community has a maximum effect on processes over short-time periods (*e.g* a growing season), the variance may have stronger effect over long-time periods in relation with the expected greater robustness of more diverse systems (Fridley et al. 2001; Hooper et al. 2005; Loreau & Mazancourt 2013).

The relationship between the response of species to the environment and their effect on ecosystem processes appears crucial for assessing how the whole system equilibrates in the long term (Diaz et al. 2013). Evidence indicates that the response of ecosystem processes to environmental changes is rarely linear: a small change in abiotic factors can lead to rapid and large changes in structure and function (Ackerly & Bazzaz 1995; Groffman et al. 2006). As it was pointed out by Suding et al. (2008), the nature of the relationship between response and effect traits may undergo this non-linearity. If response and effect traits are highly correlated to each other (Fig. 5), strong non-linearity in functioning alteration may be expected. Conversely, independent response and effect traits should confer to the system a high resistance to any

environmental change. Our ability to predict such non-linear behavior (from highly stable to highly sensitive) of the ecosystems facing varying abiotic fluctuations therefore depends on our understanding of the community assembly processes, of the effect of the resulting trait distribution on ecosystem functioning, but largely also on internal response-and-effect trait-trait linkages (Fig. 5). Since water is a key resource for plant growth, response and effect traits should be at least highly correlated to each other as it was repeatedly shown for the elements involved in biogeochemistry cycles: a reduction in water availability should directly affects plant productivity. However, how the diversity of physiological adaptation to droughts, or on the contrary emergent properties of vegetation may provide different levels of resistance to drought remains to be fully explored.

D. Water-limited ecosystems as case study

1. Mediterranean grasslands: an example of water-limited ecosystems

Broadly defined, water-limited ecosystems (WLES) are characterized by potential rates of evapotranspiration (PET) greatly exceeding precipitation (P), such that the ratio between PET:P, reflecting climatic aridity, ranges from about 0.03 to 0.75. Furthermore, these systems are usually also characterized by extreme temporal variability resulting in extended periods with no or little precipitation (Guswa et al. 2005) during which the mean rate of hydrologic cycling and biological functioning is low. However, when water becomes available after a precipitation event, large pulse of activity are triggered and resulting rates of activity can often exceed those from more humid environment (Noy-Meir 1973; Lee et al. 2004; Jenerette 2008). Such pulsed dynamics, which has been formalized into the 'pulse-reserve' paradigm (Noy-Meir 1973; Reynolds 2004), leads to a strong non-linear sensitivity to environmental variability, making WLES particularly threatened by the ongoing climate change.

WLES comprehend both arid and semi-arid lands, which cover 35-45 % of the terrestrial surface (Asner et al. 2003; Reynolds et al. 2007). However, areas where WLES-like processes are prominent for some periods of the year are likely to extend much further. Namely, because Mediterranean areas display a strong seasonality in precipitation pattern, with precipitation events markedly out of phase of the growing season of vegetation, the ecosystem functioning is also strictly controlled by water availability, which more specifically acts as an environmental constraint (low amount of water available) and also as a perturbation (recurrent severe summer droughts) in these regions. The interplay between low water availability and strong seasonality in

drought events has been argued to explain the specificities of Mediterranean plant ecosystems which are characterized by a high species-diversity and structural complexity (Blondel & Aronson 1999). Beyond the societal interest of Mediterranean grasslands (preservation of biodiversity of plants but also of birds, insects, reptiles etc., pastoral sheep breeding, water catchment etc.), these grassland ecosystems therefore present key characteristics to be good models for ecohydrology research: (i) an hydrologic regime alternating possibly both source/sink-driven processes following seasonal dynamics in water availability, (ii) a wide range of coexisting plant strategies allowing a comparative approach at different levels of ecological organization (species-communities-ecosystems), and (iii) strong ecological backgrounds for this type of plants (herbaceous species, dwarf shrubs) due to historical development of functional ecology on grasslands.

2. The use of edaphic gradients

Natural plant communities that are distributed along environmental gradients represent powerful case studies to detect the influence of abiotic factors on the structure and function of vegetation. For instance, it is assumed that the spatial variability in environmental conditions can provide accurate information on the processes underlying community assembly over time and on the drivers of ecosystem functioning. Since the concept of 'gradient' was formalized by Whittaker (1967), the study of contrasting environmental gradients has been the cornerstone of many experimental and observational approaches in comparative ecology. However, because the variations in underlying abiotic factors were in most cases poorly quantified (Austin 1980; Austin & Gaywood 1994), the use of gradients has generated considerable confusion in the identification of relevant mechanisms.

Among the different types of environmental gradients (*e.g.* Austin 1980; Austin & Gaywood 1994; Garnier & Navas 2012), the edaphic gradients are classified as 'resource' gradients since the varying factors usually include the soil resources that plants consumed for their growth and reproduction. In natural habitats, edaphic gradients are complex because they integrate the co-variation of several abiotic factors that may affect plant metabolism. Typically, the availability of water and nutrients (nitrogen, phosphorus) has been shown to co-vary, particularly in Mediterranean grasslands (Delgado-Baquerizo et al. 2013), due to the tight coupling between water and nutrient cycles. Facing the difficulty of disentangling the interlaced variations in resources, the biomass of vegetation was proposed as an alternative and integrative way of quantifying the range of constraints exerted along gradients. Resource gradients were

therefore generally associated to 'productivity' gradients (Grime 1977; Tilman 1982), and any cause of a decrease in productivity was assumed to reflect an environmental stress, leading to the emergence of a series of hypotheses about community assembly processes in relation with 'stress gradients' (Bertness & Callaway 1994; Maestre et al. 2009). Recognizing that the concept of 'stress' may not be well defined, even confusing when referring to physiological definition of stress (see the debate between Körner 2004, Lortie et al. 2004b, Marrs 2004, Weiher 2004), the conceptualization stress gradients permitted to address a series of theoretical issues in community ecology (Lortie et al. 2004b).

However, in case of a dynamic resource such as water, the distinction between longstanding water limitations due to environmental characteristics (*e.g.*, shallow soils) and periodic drought-induced stress (*e.g.*, interannual fluctuations) has now become critical for a better understanding of water-related processes between communities. The quantification of water availability gradients continues to remain problematic since strong plant-soil water interaction and feedbacks may be expected. Independent measurements of soil water availability are therefore precisely needed to identify the nature and important of such interactions and feedbacks at the community level (Vicca et al. 2012).

3. General issue

From the perspective of the emergence of ecohydrology, functional ecology should have a determinant role to play in the identification of general rules linking ecosystem functioning and water balance. The main objective of this PhD work is to determine the relative importance of abiotic and biotic factors in both structuring plant communities and ecosystem functioning. To this end, we aim to provide some general insights into how vegetation adapt and govern water flows along a gradient of soil water availability in a Mediterranean rangeland of south of France. Following the response-and-effect framework (Lavorel & Garnier 2002; Suding et al. 2008), we used an approach based simultaneously on both the modeling of soil water dynamics and on plant functional traits to identify which ecological strategies are selected at the community level when soil water decreases, and in turn how the functional structure of plant communities determines emergent properties of vegetation and thereby water-use rates of ecosystems. The following dissertation is divided into five chapters, scaling down from the ecosystem- to the species-level through the community-level. Each chapter includes a manuscript which is intended to be submitted to an international peer-reviewed journal.

In chapter 1, we present the study site and how we determined the edaphic gradient that structured the whole work. General aspects of the experimental design and field measurements are described. The chapter also includes a study (**manuscript I, accepted in *Folia Geobotanica***), in which we evaluated the reliability of the point-intercept method as a sampling method for describing the functional structure in such species-rich Mediterranean rangelands.

In chapter 2, we present a study based on a 5-years measurement series in which we explored whether the 'ecohydrological optimality' hypothesis may be supported based on a 'productivity-water stress' trade-off among contrasting plant communities (**manuscript II, in prep.**). To this end, we followed both a synchronic (the edaphic gradient) and a diachronic approach (five contrasting years) to take into account large variations in soil water availability and in climatic evaporative demand. We also compared situations in spring and summer.

In chapter 3, we present an analysis of the functional structure of contrasting plant communities distributed along the edaphic gradient (**manuscript III, in prep.**). Using a trait-based approach, we identified which axes of species functional niche are under selection when soil water availability varies, including both above- and belowground components of vegetation. We then tested the relationship between community structure and emergent properties of vegetation with the purpose of predicting ecosystem evapotranspiration rates.

In chapter 4, we present a removal experiment in which we disentangled the respective role of plant-plant interaction and environmental constraints in plant performance along the edaphic gradient (**manuscript IV, in prep.**). Three co-occurring grass species were used as target species. We then specified mechanisms underlying plant-plant interaction in relation with the functional structure of surrounding plant communities.

In chapter 5, we present a general synthesis and discussion of the whole work. We propose research perspectives for pursuing the analysis of the data set, initiating future experiments at the site or projects. We end the dissertation with exploratory ideas on how the combination of species adaptative strategies may be used in an agricultural context to manage the water balance components, and thereby stability in biomass production under drought (**manuscript V, accepted in *European Journal of Agronomy***), or more generally, how the trait-based approach can provide cues for designing multi-specific agricultural systems under varying environmental contexts.

Chapter 1

Materials & methods



(aerial photography of the 'INRA-La Fage' experimental station)

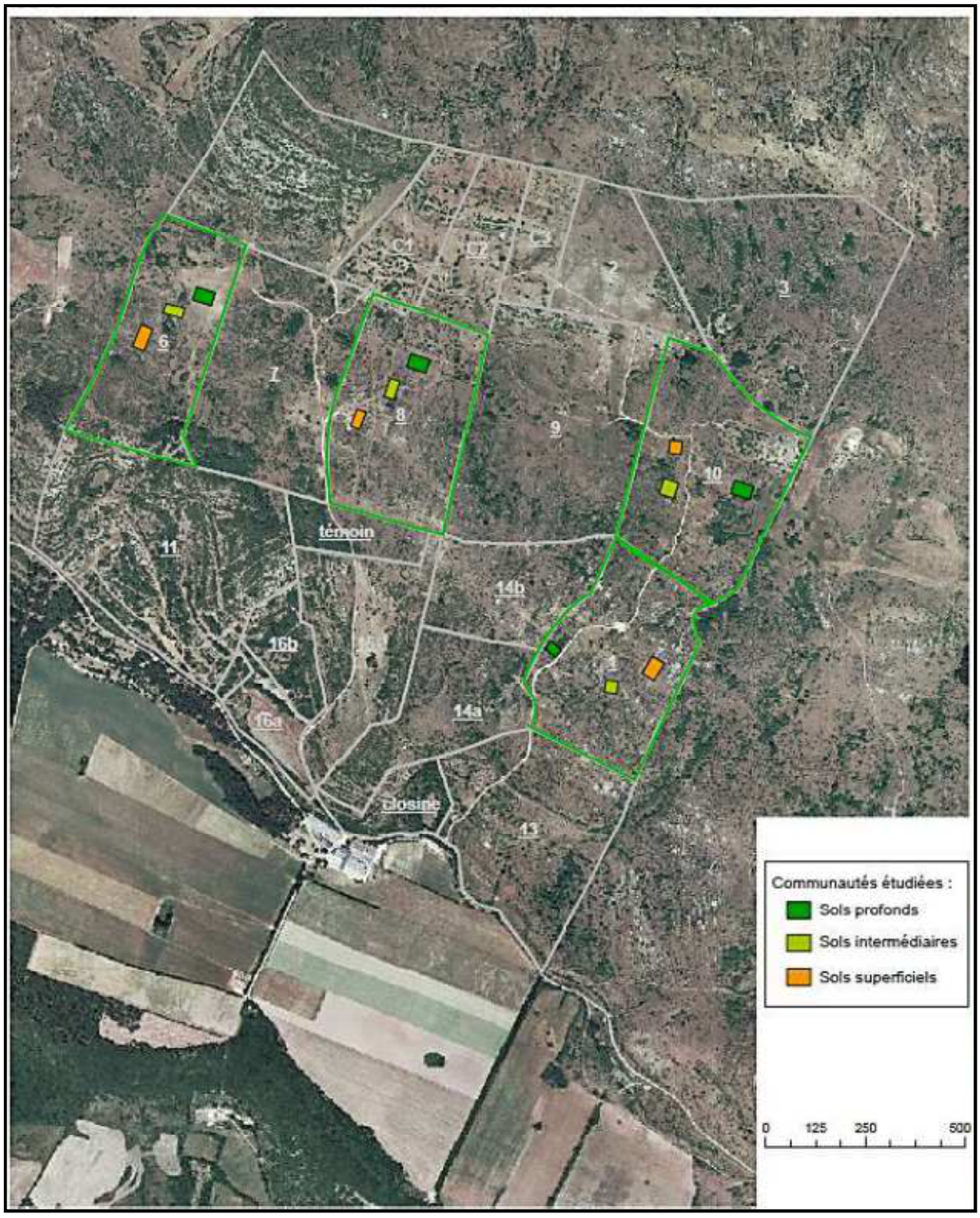


Fig.1 Aerial photography of the 'INRA-La Fage' experimental station (43°55´N, 3°05´E) showing the twelve selected plots. The edaphic gradient is represented by three colors, ranging from deep clay soils (green) to shallow sandy soils (orange) (taken from Maud Bernard-Verdier 2012).

A. The study site: La Fage experimental station

The study was carried out on dry calcareous rangelands of southern France, located on a limestone plateau (Larzac Causse, Aveyron) at the INRA experimental station La Fage (43°55´N, 3°05´E, 790 m a.s.l.), 100 km northwest of Montpellier (Fig. 1). Climate on the plateau is sub-humid with a strong Mediterranean influence. Cool and wet winters alternate with warm and dry summers. The long-term mean annual precipitation range from 680 to 1790 mm, occurring mainly during early spring and autumn (Fig. 2, data from 1973–2013). Mean monthly temperatures vary from 1°C in January to 19°C in August (Fig. 2). The growing season can last approximately eight months (from March to November), principally limited by low temperatures and snow cover during winter. However, plant growth can be interrupted by intense drought during summer, resulting some years in two distinct growing seasons (spring and autumn).

The plateau is a typical calcareous karstic system, resulting from the dissolution of the bed rock. Soils consist in dolomitic rendzinas and limestone soils distributed as a mosaic of different depths (mean soil depth ranges between 10 cm and 120 cm) and texture (sandy soils to clay soils). Overall, soils are characterized by very low water retention capacity (about 50 mm) and high leakage rate. Consequently, despite relatively high water supply provided by rainfall, water is regularly the most limiting factor for plant growth at the site. Together with mineral limitations, these constraining edaphic conditions are directly responsible for the low vegetation productivity of the system (mean biomass at the peak of vegetation is about 100 g m^{-2}).

At the landscape level, vegetation is dominated by perennial herbaceous species (*Bromus erectus*, *Festuca christiani-bernardii*, *Carex humilis)*, along with loosely scattered shrubs (*Buxus sempervirens*, *Juniperus communis*) and trees (*Quercus pubescens*). At a finer scale (approximately few meters), plant communities are highly variable in terms of composition and structure, including many different growth forms in varying proportions, such as grasses, woody dwarf shrubs, forbs and rosettes. The combination of several climatic influences allows the coexistence of typical Mediterranean plant species together with temperate and alpine species. The plant species richness is therefore particularly high, characterized by considerable endemism rate, making the whole system as a French biodiversity 'hotspot' (INPN 2012).

Historically, rangelands on the Larzac plateau have been maintained open by extensive sheep grazing for almost one thousand years. However, the recent decline of livestock farming in the region has initiated a profound change in land use which progressively leads to shrub encroachment and landscape closing. As part of a long-term experimental breeding research, the

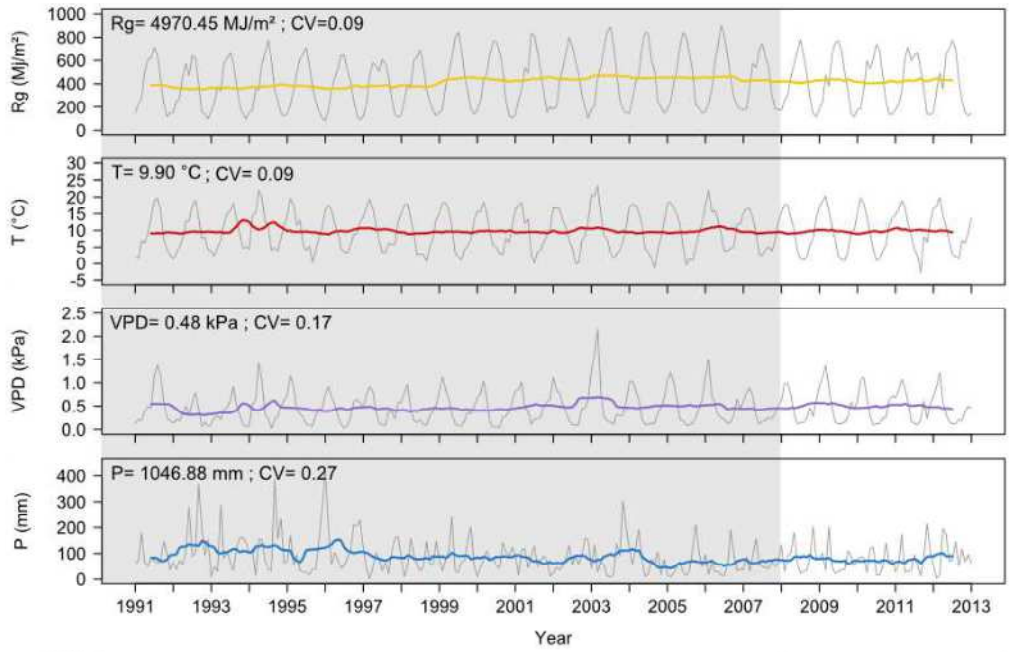


Fig. 2 Long-term mean and variation in global irradiance (Rg; MJ m^{-2}), temperature (T; °C), vapor deficit pressure (VPD; kPa) and precipitation (P; mm). Grey lines represent interpolation from daily measurements; colored lines represent the moving average over 5 years.

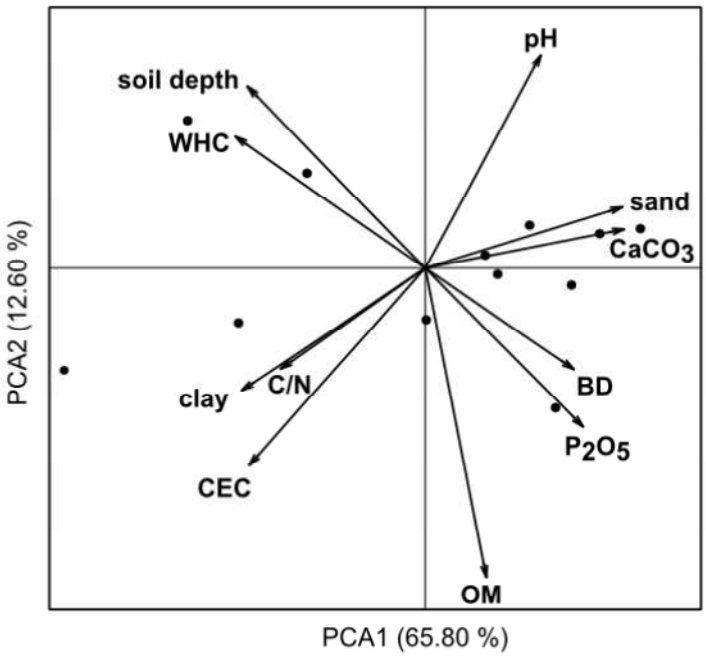


Fig. 3 Principal component analysis (PCA) on soil parameters. Data points represent the 12 plots of grassland along the soil gradient. Eight soil parameters were used: mean soil depth (cm); clay and sand content (g g^{-1}); organic carbon content (OM, g g^{-1}); soil bulk density (BD; g cm^{-3}); soil water holding capacity (WHC, mm); $CaCO_3$ and P_2O_5 content (g g^{-1}). Axis 1 of the PCA explained 65.80 % of the variation.

300 ha of vegetation at the station have been homogeneously grazed by a sheep herd (Romane breed) under a controlled grazing regime since 1972. The sheep herd is raised outdoor year-round for meat production (see Molénat et al. 2005 for further details).

B. Quantifying the edaphic gradient

The study was restricted to dolomitic rendzinas, representing an area of 160 ha at the station. Soil characteristics were highly variable due to different levels of degradation of the calcareous bed rock, favoring the accumulation of colluvium towards small topographic depressions (called 'dolines'). Twelve plots (6 × 9 m) up to 1500 m apart were selected to span the widest possible range of soil types (Fig. 1).

1. Soil physico-chemical properties

Soil physico-chemical properties were assessed in each plot to quantitatively characterize the edaphic gradient (Perez-Ramos et al. 2012; Bernard-Verdier et al. 2012). Mean soil depth as well as eight soil characteristics were measured using three randomly distributed soil cores (5 cm wide) following standard procedures (Afnor 1994): texture, water holding capacity (-0.015 MPa), calcium carbonate content, pH (in water), organic matter content, C:N ratio, cation exchange capacity (CEC) and available phosphorus (using the Olsen method).

2. Soil water reserve

Additionally, soil water content (SWC) was measured bi-weekly since spring 2008 using capacitance moisture probes (*DIVINER 2000*, Sentek Pty Ltd, Stepney, Australia), which provided a complete profile of soil humidity at three permanent locations in each plot. The probes were previously calibrated according to soil texture to account for the differences in soil water retention (Geesing et al. 2004; Groves & Rose 2004). In each plot, the total transpirable soil water (TTSW), representing the potential amount of water that plants can extract for transpiration (Ritchies 1981; Sinclair & Ludlow 1986), was derived from the course of SWC.

3. Multivariate analysis of edaphic conditions

Soil variables were analyzed using a principal component analysis (PCA). We found that most of the soil characteristics were correlated to the first axis of PCA which explained 65.80 % of the total variation (Fig. 3). In particular, the first axis of PCA was associated to soil depth, water

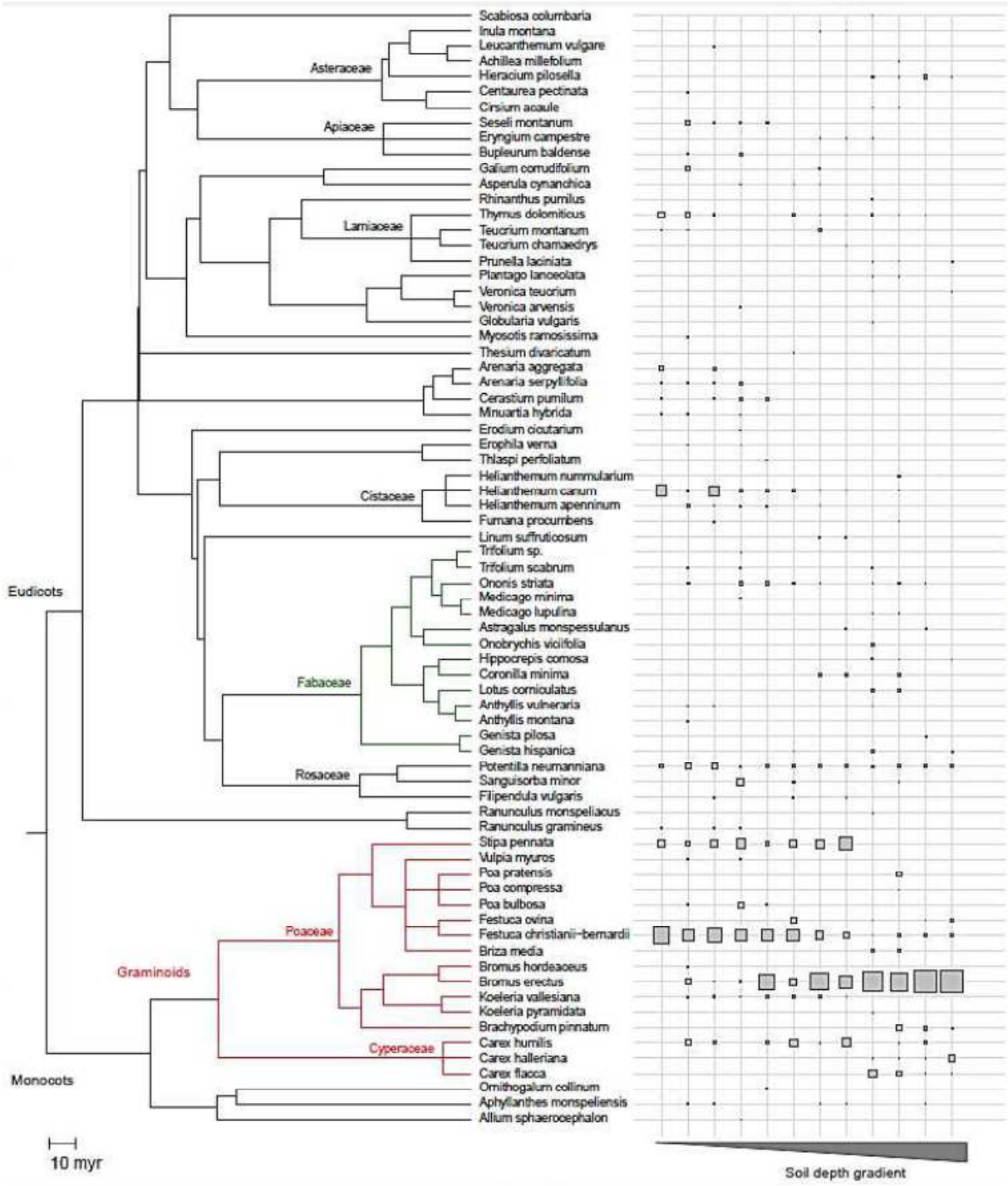


Fig. 4 Phylogenetic relationships and relative abundances for the 73 most abundant species along the soil depth gradient. The names of major families and clades are indicated on the phylogeny. Unit of branch length is indicated in million years. On the right panel, species relative abundances (2009) are represented vertically of the 12 plots along the soil gradient. The size of each square is proportional to the relative abundance of the species in a given community -maximum value corresponds to 71 % of cover – (taken from Bernard-Verdier et al. 2013).

holding capacity, sand content and calcium carbonate content. The second axis was associated to organic matter content, but explained less variation (12.60 %). The twelve selected plots were evenly distributed along the first axis highlighting the existence of a gradient in edaphic conditions along this axis, ranging from shallow (< 20 cm) sandy (> 80 % sand content) soils with low water holding capacity (< 30 mm), to deeper (> 1 m) clay (> 30 % clay content) soils with greater soil water holding capacity (>200 mm). Thereafter, we considered soil depth as a proxy variable of this first PCA axis in order to facilitate the interpretation.

4. Plant productivity along the edaphic gradients

In order to evaluate the effect of the edaphic conditions on ecosystem productivity, aboveground biomass (AGB) was collected from 2-3 quadrats (0.25 m^2) located within each plot along the soil gradient during five years (2008-2012). Quadrats were each year replaced in a different location within the plot to avoid reharvesting the same quadrat. AGB was harvested by clipping all vascular plant biomass to ground level. Harvests occurred both at the of peak vegetation before summer drying (end of May-June) and during summer (end of July-August). Mosses, lichens were sorted out as well as dead plant tissues. Samples were then oven-dried at 60°C for 48 h, weighted and averaged at the plot level (see Manuscript II). Live AGB at peak of vegetation was considered as a good proxy of spring ANPP given that winter frost repeatedly causes a complete senescence of plant tissue (Scurlock et al. 2002).

C. Assessing the structure of vegetation

The vegetation was characterized in each plot during the two successive years of study (2011 and 2012). Only the main characteristics of vegetation are presented hereafter in order to draw a general picture of the study. More specific measurements and calculations may be found in dedicated sections in the following chapters.

1. Species composition

In May 2011 and 2012, the abundance of each species was estimated using the point-intercept method (Levy & Madden 1933) using a sample frame containing a 4 x 4 cm grid pattern (1044.98 points/m^2). We recorded a total of 77 species along the soil gradient (Fig. 4). Once calibrated to plant growth form, the method provides a good estimate of green aboveground biomass of the species (Jonasson e1988; Manuscript I), although it tends to underestimate rare species. As part

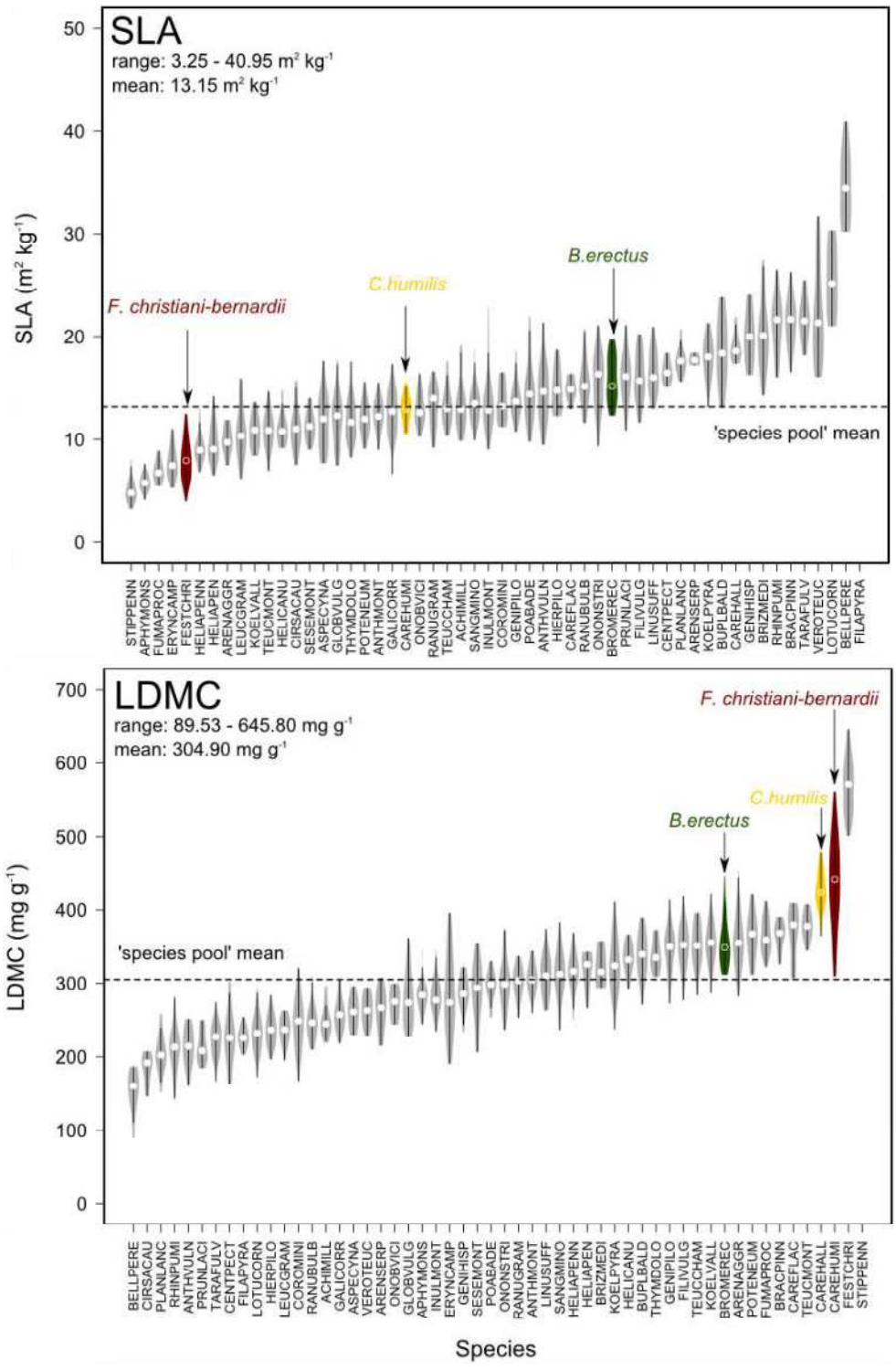


Fig. 5: Extent of inter- and intraspecific variability of specific leaf area (SLA) and leaf dry matter (LDMC) across the 53 species for which data trait are available. Violin plot represent (i) the density of the data estimated by kernel method (in grey) and (ii) the median value (white points). Data are presented by species ordered by their mean SLA value. Horizontal dashed lines indicate the unweighted mean trait value across all the 53 species. Colored species correspond to the most dominant species along the gradient.

of our methodological investigation, we evaluated the reliability of the method, which was generally used in more productive and homogeneous vegetation (see Manucript I thereafter).

2. Trait data

Aboveground traits

Eight plant functional traits related to plant stature, leaf morphology and chemicals were selected to characterize the recorded species at the study site. These traits were associated to the 'leaf' and 'height' axes of the 'Leaf-Height-Seed' space (Westoby 1998) describing the strategy of resource-use and the competitive ability of species (Fig. 5). The regenerative phase ('seed' axis) was not characterized in this study. Data traits were available for 53 most abundant species amongst the 77 species recorded (*i.e* representing > 80 % of total plant biomass; Pakeman & Quested 2009). During the study, we measured the leaf trait values of 27 species distributed across the plots. Data traits for the 26 remaining species were taken from a previous work conducted at the study site (Bernard-Verdier et al. 2012). In May 2011, we sampled at least 12 individuals per species. Dominant species were sampled in each single plot along the edaphic gradient, while species with more restricted tolerance to edaphic conditions were sampled only in some plots where they occurred. For each individual plant sample, leaf length (LL; cm), leaf area (LA; cm^2), leaf dry matter content (LDMC; mg g^{-1}), specific leaf area (SLA, m^2 kg^{-1}), leaf carbon content (LCC; % of leaf dry mass) and leaf nitrogen content (LNC; % of leaf dry mass) were measured following standard protocols (Cornelissen et al. 2003). Leaf thickness (LT; µm) was taken from Bernard-Verdier et al. (2012) or calculated using corresponding SLA and LDMC values following the method of Vile et al. (2005). Mean reproductive height (Hrep; cm) was taken from both Fayolle (2008) and Bernard-Verdier et al. (2012).

Belowground traits

Five root traits were also measured to characterize the belowground compartment of vegetation. However, because separating the root system of coexisting species in a natural community is hardly feasible, root traits were directly measured at the community level from three soil cores per plot (5 cm wide, 10-100 cm length depending on soil depth) in May 2011 as part as the ongoing PhD work of Marine Birouste. Cores were divided into 10 cm thick layers from the soil surface down to the mother rock. In the laboratory, root material was carefully washed in clear water, and a representative sub-sample of fresh roots was scanned at 400 dpi following Perez-Ramos et al. (2012). Specific root length (SRL; m g^{-1}), specific root area (SRA; m^2 kg^{-1}), mean diameter (Rdiam; mm) and root dry matter content (RDMC; mg g^{-1}) were measured from the

digital images using Winrhizo software for image analysis (Winrhizo ver. 2003b, Regent Instruments Inc., Quebec, Canada). Maximum rooting depth (RD; cm) was estimated based on the root biomass distribution along the whole soil profile. Following Gale and Grigal (1987), an asymptotic function ($Y = 1 - \beta^z$, where Y is the cumulative fraction of roots between soil surface and depth z, and β is an empirical fitting parameter that determines the root distribution with depth) was adjusted to the biomass data ($0.62 < r^2 < 0.89$, $p<0.001$) in order to determine the soil depth that contains 95 % of total dry root biomass (see Manuscript III).

Chapter 1

Reliability of the point-intercept method



(the point-intercept grid frame that was used for sampling the vegetation, *spring 2011*)

MANUSCRIPT I

Questioning the reliability of the point-intercept method for assessing community functional structure in low productive and highly diverse Mediterranean grasslands.

-

Evaluation de la méthode des points de contact pour estimer la structure fonctionnelle des communautés de plantes dans des prairies méditerranéennes peu productives et très diversifiées.

Karim Barkaoui, Maud Bernard-Verdier and Marie-Laure Navas.

Abstract

Quantifying species relative abundances in plant communities remains a key issue for the assessment of community functional structure. This is particularly challenging when non-destructive estimates are required over time. We tested whether the point-intercept method (PIM), originally developed for low-diverse communities, is relevant to assess the aboveground biomass and functional structure of highly diverse, low-productive Mediterranean grasslands. We sampled 18 communities with the PIM along a gradient of soil depth and texture, twice over the growing season. After each sampling period, we harvested the aboveground biomass in order to measure species biomass and to assess species richness and community functional structure with plant height, leaf area and leaf dry matter content (LDMC). We investigated the relationship between point-intercept measurements and aboveground biomass at three hierarchical levels (species, growth-form and community) to find generalizable calibration equations for estimating community biomass, and tested for sensitivity of estimates to community structure. We then compared the community weighted mean (CWM) and variance (CWV) of LDMC, calculated with and without calibration. Differences in species growth strategy and phenology strongly impacted biomass estimates at both species and community levels. But these differences were successfully accounted for by growth-form specific calibrations, which provided accurate estimates without any influence of community structure. Lack of calibration may have dramatic consequences on functional structure assessment, by inducing errors in estimates of CWV up to 80%, depending on growth form proportions. This work contributes to a better understanding of the possible methodological biases induced during sampling with the PIM, when quantifying species relative abundances for functional structure assessment in complex communities.

Résumé

La quantification de l'abondance relative des espèces végétales au sein des communautés de plantes continue d'être un problème central pour décrire la structure fonctionnelle des communautés. Cela est encore plus difficile lorsque des estimations non destructives sont nécessaires au cours du temps. Dans cette étude, nous avons testé dans quelle mesure la méthode des points de contact (PIM), développée à l'origine pour étudier des communautés végétales peu diversifiées, permettait d'estimer correctement la biomasse aérienne et la structure fonctionnelle des communautés de plantes de prairies méditerranéennes très diversifiées et peu productive. Nous avons échantillonné 18 communautés différentes le long d'un gradient de profondeur et de texture de sol en utilisant la méthode PIM, répétant la procédure à deux moments distincts lors de la période de croissance végétative. Après chaque période d'échantillonnage, nous avons récolté la biomasse aérienne et nous avons quantifié la richesse spécifique des communautés ainsi que la biomasse de chaque espèce présente. La structure fonctionnelle des communautés a été estimée pour trois traits différents : la hauteur des plantes, la surface foliaire et la teneur en matière sèche des feuilles (LDMC). Nous avons ensuite analysé la relation entre le nombre de contact obtenus avec la méthode PIM et les valeurs de biomasse réelles à trois niveaux d'organisation différents (espèce, forme de croissance et communauté entière). Cela nous a permis de chercher des équations de calibration générique de la méthode pour estimer la biomasse de façon non destructive à ces différents niveaux, puis nous avons testé la sensibilité des calibrations obtenues vis-à-vis de la structure des communautés. Enfin, afin d'évaluer l'importance de la calibration, nous avons comparé la moyenne (CWM) et la variance (CWV) agrégées du LDMC à l'échelle de la communauté, calculées avec les valeurs de biomasse réelles des espèces, puis en utilisation des estimations de leur abondance avec ou sans calibration. Nous avons trouvé que la diversité des stratégies de croissance et de phénologie des espèces avait un impact significatif sur les estimations non-destructives de la biomasse à l'échelle de la communauté entière. Notre calibration saisonnière de la méthode PIM, prenant en compte les différences de forme de croissance, a permis de corriger correctement les estimations de biomasse des communautés, sans être biaisée par la structure des communautés. En conclusion, nous avons montré que l'absence de calibration peut avoir des conséquences considérables lors de l'estimation de la structure fonctionnelle, et induire une erreur allant jusqu'à 80 % pour le CWV selon les proportions des différentes formes de croissance dans les communautés. Cette étude contribue donc à une meilleure compréhension des biais méthodologiques que la méthode PIM peut induire lors de l'estimation des abondances relatives des espèces pour décrire la structure fonctionnelle des communautés végétales complexes.

Introduction

Assessing the functional structure of plant communities has become a major goal in many ecological studies in order to unravel the effect of environmental variation on ecosystem processes (Lavorel & Garnier 2002; Garnier et al. 2004; Grime 2006; Diaz et al. 2007). Community functional structure can be defined as the distribution of trait values within a community (Diaz & Cabido 2001; Garnier & Navas 2012), which is a result of both species trait values and species relative abundances. It can be described by two widely used components: the '*community weighted mean*' (CWM, Garnier et al. 2004) and the '*community weighted variance*' (CWV, Sonnier et al. 2010) of traits. Yet, a critical point is to accurately quantify both species relative abundances and trait values at the same time. As pointed out by Lavorel et al. (2008), standard protocols already exist for the accurate measurement of species traits (Garnier et al. 2001a; Cornelissen et al. 2003), but a methodological consensus for species abundance estimation has not been reached. The quantification of species aboveground biomass is generally recognized to be the most relevant proxy of species abundance (Mason et al. 2003), but there is still no agreement on which method to be used to estimate it (Catchpole & Wheeler 1992; Harmoney et al. 1997; Ganguli et al. 2000; Whitbeck & Grace 2006; Byrne et al. 2011; Redjadj et al. 2012). Although destructive harvest and sorting of the biomass constitutes the most commonly used and thorough method, it may not be as relevant when fine-scale monitoring repeated over time is intended.

Many different non-destructive methods have been proposed so far in order to obtain a fast although accurate assessment of plant biomass. Most of them, namely those used for agricultural purposes, are based on a visual estimation of vegetation cover in combination or not with an estimation of the average plant height (Catchpole & Wheeler 1992; Harmoney et al. 1997; Vermeire et al. 2002; Martin et al. 2005). For example, the use of the plate meter method was recently shown to provide good estimates of plant aboveground biomass within natural and cultivated ecosystems (Axmanová et al. 2012; Hakl et al. 2012). Nevertheless, while these techniques performed well when the total aboveground biomass is targeted, they are not advisable for the assessment of the biomass of each of the constitutive species individually within the community, as it is required for the assessment of the community functional structure. An alternative and non-destructive method is the point-intercept method (*PIM* hereafter), which allows one to capture the spatial and temporal variability of individual species aboveground biomass. Basically, the PIM records the number of contacts between living parts of plants and a pin passed through the vegetation (*point-intercepts* hereafter) at a large number of positions. The point-intercept frequency, sometimes also called 'compactness' (Damgaard 2009) or 'repeated

cover' (Wilson 2010), expressed as the total number of hits per species divided by the total number of points recorded (Goodall 1952), is proportional to plant cover and estimates species total leaf area and aboveground biomass, at least when species are considered separately (Jonasson 1988; Franck & McNaughton 1990; Goenenvel 1997; Hobbie et al. 1999). The PIM was originally developed to analyze the floristic composition and the vegetation cover of relatively high-productive pastures, in which a few dominant species modulated ecosystem functioning (Cockayne 1926; Levy & Madden 1933; Goodall 1952; Daget & Poissonnet 1969). Thereafter, plant ecologists have extensively used the PIM to monitor the dynamics of target species, either within artificial herbaceous communities with low and controlled species richness, or within relatively homogeneous and productive natural grasslands that are strongly dominated by a few species, such as subarctic tundras or temperate bogs (Shaver et al. 2001; Bret-Harte et al. 2004; Lorentzen et al 2008; Hudson & Henry 2010; McLaren & Turkington 2010; Yurkonis et al. 2012). These characteristics of communities explain why the calibration of the PIM was often not necessary or limited to a small number of species to guarantee good estimates of species relative abundances. Moreover, a number of studies have led to interesting statistical recommendations in terms of sampling design and calibration efficiency (Jonasson 1988; Brathen & Hagberg 2004; Clark et al. 2008), making the PIM possibly one of the most accurate non-destructive sampling method within these kind of communities (Vittoz and Guisan 2007; Godinez-Alvarez et al. 2009).

However, despite these advantages, the relevance of the PIM in low productive and highly diverse grasslands remains to be established. A literature survey conducted on 91 peer-reviewed papers published since 1980 (Table S1) showed that the PIM was mainly used for sampling moderate to high productive communities with low to intermediate levels of species richness. Furthermore, we noticed that the importance of calibration for estimating plant productivity and community functional structure has been poorly tested within species-rich communities, especially when several plant growth forms may coexist. Since the PIM measures the tridimensional space occupancy of species, differences in plant biomass allocation (*e.g.* leaf:stem ratios) and plant architecture may be expected to influence measurements and thus different growth forms may not be represented equally by the PIM. Consequently, an absence of calibration may strongly limit the reliability of the PIM, particularly when comparing communities with contrasting leaf:stem ratios together. Likewise, since the PIM is often carried out only once per year at the same phenological stage (*e.g.* at the peak of vegetation), the effect of seasonal variations in plant architecture on the calibration has never been assessed and may differ according to plant growth form. Therefore, we could hypothesize that the risk of over- or under-

estimation of species biomass may increase when species with diverse growth-form strategies coexist within the community. Ultimately, the effect of local variations in community characteristics such as total aboveground biomass, species composition and functional structure has never been explicitly tested on the accuracy of estimates, even though it is likely to create some methodological bias when comparing highly contrasting plant communities along environmental gradients. For instance, low levels of aboveground biomass may induce supplementary biases because, under a certain threshold, discontinuous vegetation patterns may increase non-linearly the probability for a pin of hitting bare ground and miss plant patches. Sampling accuracy may also decrease in the case of dense and vertically multi-stratified communities because of higher challenge for the observer to record the plant hits and to identify the right species.

The aim of this study was to test the reliability of the PIM within species-rich, growth-form diverse and low productive Mediterranean communities for assessing seasonal changes in functional structure. We propose that seasonal calibrations of the PIM for dominant species and growth-forms may considerably enhance the accuracy of estimates of the species relative abundances over a range of communities differing in biomass and functional structure. We addressed four questions: (i) Do the well-demonstrated relationships between total leaf area/aboveground biomass and point-intercepts still hold at the species- and growth-form levels within complex communities and despite the seasonal variations in plant architecture? (ii) At the community level, does the PIM correctly predict the total plant aboveground biomass over the growing season by combining growth-forms relationships? (iii) How sensitive is the PIM to the changes in species and trait distribution along an environmental gradient? (iv) What is the impact of growth-form calibration on estimates of community functional structure?

Materials and methods

Study site

The study was carried out on species-rich rangelands in Southern France, located on a limestone plateau (Larzac Causse) at the INRA La Fage experimental station (43°55´N, 3°05´E, 790 m above sea level). Climate on the plateau is sub-humid with a strong Mediterranean influence with cool and wet winters and warm and very dry summers. Mean annual precipitations range from 680 to 1790 mm occurring mainly during spring and autumn. Mean monthly temperatures vary from 1°C in January to 19°C in August (data from 1973–2006). The main growing season lasts from March to the end of June.

At the landscape level, the vegetation is dominated by perennial herbaceous species such as *Bromus erectus* and *Festuca christiani-bernardii* (see Table S2), along with loosely scattered shrubs such as *Buxus sempervirens*, or *Juniperus communis*. Spatial heterogeneity in soil properties (mainly soil depth and texture), creates a high variability at small spatial scale in terms of vegetation aboveground biomass, species composition and functional structure (Bernard-Verdier et al. 2012; Perez-Ramos et al. 2012). Plant communities located on shallow sandy soils (0-30 cm soil depth)are co-dominated by different perennial graminoïds (*e.g. Festuca christiani-bernardii*, *Stipa pennata* or *Carex humilis*) and include dwarf shrubs (*e.g. Thymus dolomiticus, Helianthemum canum*, *Fumana procumbens* or *Arenaria aggregata*) whereas communities located on deeper clay soils (60-120 cm soil depth) are largely dominated by *Bromus erectus*, coexisting with various sub-dominant grasses, forbs and rosettes (*e.g. Carex flacca, Poa badensis, Lotus corniculatus*, *Potentilla neumanniana* or *Hieracium pilosella*).

Point-intercept sampling

18 different plant communities were selected along a gradient of soil depth and texture over a 300 ha area, in order to maximize the range of standing biomass and community structure (species composition and relative abundance). Species composition continuously changed along this gradient, as represented by Sørensen similarity indices varying from 0.15 to 0.7 (Bernard-Verdier et al. 2012), with a large shift in the relative abundance of growth forms. Large shrubs such as *Buxus sempervirens* or *Juniperus communis* were avoided during the selection.

Each plant community was sampled using a circular frame of 25 cm diameter (490 cm^2) containing a 4x4 cm grid pattern: 29 equally-spaced sample points (592 pins/m^2) were finally kept after removing the points which were closer than 4 cm from the border of the frame to avoid edge effects. The distance between two adjacent points (4 cm) was defined according to our appreciation of the patchiness of the vegetation in order to guarantee a satisfying sampling intensity, particularly within communities with the lowest plant cover. Comparison with other studies using the PIM revealed that such a point density was close to that usually found in the literature and more than 4-fold higher to that defined for sampling similar dry and Mediterranean grasslands (Table S1). At each sample point within the frame, a metal rod (3 mm diameter) was inserted vertically through the vegetation and all individual hits of living photosynthetic tissues (including all green leaves and stems) were recorded for each species from the top of the canopy down to the soil surface. For example, if one species hit the rod three times at a given point, then three hits were recorded for this species. Species tridimensional space occupancy was then estimated from the point-intercept frequency calculated as the total number of hits with the species divided by the total number of points recorded. Since the point-intercept frequency of the

species combines estimates of both the species relative cover and plant vertical density (Jonasson 1988; Damgaard 2009), it was assumed to be an appropriate variable for the PIM calibration. For each community, data collection was performed twice in spring 2011 at the beginning of the growing season (March) and at the peak of vegetation (May), providing two independent data sets of 18 sampled frames each. In the following, because our aim was to test the sampling accuracy of the PIM within the frames rather than their representativeness of the surrounding communities, each individual frame was roughly assumed as representing a single community at a time.

Across the 18 communities, we recorded a total of 45 species belonging to 15 botanical families (Table S2). Considering the small area sampled with the frame, the species density ranges from low (3 species/frame) to relatively high levels (13 species/frame), at least compared to that usually found in other studies using the PIM (Table S1). We classified the species according to growth-forms (*sensu* Cornelissen et al. 2003): (i) "dwarf shrub", which includes all woody species up to 0.8 m tall; (ii) "tussock", which includes all graminoïd species and is characterized by abundant leaves sprouting from basal meristems and forming prominent tufts; (iii) "rosette", which includes all rosette or prostrate growth-form species with short leaves (<0.5 m) deployed close to the ground; and (iv) "erect leafy" which includes erected forbs with leaves located in the middle and/or top parts of the plants. Nine of the most abundant species (Table S2) provided sufficient data points over the 36 quadrats to be analyzed individually in order to obtain species-level calibrations of the PIM to estimate both total leaf biomass and leaf area (see below). In the same manner, growth-form level calibrations were obtained only for the three most dominant growth-forms (dwarf shrub, rosette and tussock) which included 40 species; erect leafy species were insufficiently represented in the data set for that growth-form to be included in analyses.

After the point-intercept sampling was completed, live aboveground biomass of each quadrat was clipped to ground level. In the laboratory, mosses and lichens were excluded from all samples as well as dead tissues. The live biomass of the nine abundant species was sorted first. For each sample of these nine species, a representative sub-sample was selected, in which leaves and stems were carefully separated, and the total leaf area was measured on fresh leaves with an area meter (model MK2, Delta-T Devices, Cambridge, UK). The bulk remaining biomass was sorted according to plant species without separating leaves from stems. All samples were then oven-dried for two days at 60°C and weighed with an accuracy of ± 0.0001g.

Data analysis

A four-step data analysis was performed at three hierarchical levels (species, growth-form and community levels), following the four questions from the introduction. All statistical analyses

were conducted in R 2.14.0 (R Development Core Team 2011).

In a first step, we used simple allometric relationships to estimate the plant leaf:stem ratio and the leaf area of each of the nine abundant species as follows:

$$Pm_{i,j}(t) = Lm_{i,j}(t) + Sm_{i,j}(t) \quad \text{eqn. 1}$$

$$Lm_{i,j}(t) = \alpha_i(t) \times Sm_{i,j}(t) + \beta_i(t) \quad \text{eqn. 2}$$

$$La_{i,j}(t) = SLA_i \times Lm_{i,j}(t) \quad \text{eqn. 3}$$

where $Pm_{i,j}(t)$, $Lm_{i,j}(t)$ and $Sm_{i,j}(t)$ are respectively the whole plant aboveground dry mass, the leaf dry mass and the stem dry mass of a given species *i* measured on a sub-sample *j* at the period *t*; $La_{i,j}(t)$ is the corresponding leaf area; $\alpha_i(t)$ and $\beta_i(t)$ are species-specific allometric parameters. Eqn.1 describes the decomposition of the whole plant aboveground biomass into leaf and stem biomass. Eqn. 2 describes more specifically the species-specific biomass allocation between plant stems and leaves which could change throughout the growing season. eqn. 3 describes the species-specific conversion of leaf biomass into leaf area with the SLA_i as specific leaf area, the ratio of leaf area on mass. Contrary to the other parameters, we assumed that SLA_i did not vary throughout the growing season (Garnier et al. 2001b). When combined together, eqn.2 and eqn.3 lead to a general relationship between whole-plant dry mass $Pm_{i,.}(t)$ and total leaf area $La_{i,.}(t)$:

$$La_{i,.}(t) = SLA_i \times \frac{Lm_{i,j}(t) \times Pm_{i,.}(t)}{Pm_{i,j}(t)} \quad \text{eqn. 4}$$

Allometric relationships were assessed by 'standardized major axis' estimation (R package *smatr*, Warton et al. 2012). This method is particularly well adapted for allometry when the purpose is to describe how two size variables are related (Warton et al. 2006). All estimations (eqn.2 and eqn.3 for 9 species x 2 periods) were highly significant (p<0.0001). Explained variances (R^2) ranged from 0.72 to 0.99 for eqn.2 and from 0.92 to 0.99 for eqn.3.

In a second step, we successively quantified the relationships between frequencies of point-intercepts and aboveground biomass (and/or leaf area), first at the species level, and then at the growth-form level. At the species level, a linear model of calibration ($Y_i = a_i X_i + b_i + \varepsilon_i$) was selected for species biomass and leaf area against a multiplicative model ($Y_i = a_i X_i^{b_i} \varepsilon_i$) because of higher explained variances (results not shown). Differences in calibration between the

Table 1 Community features of the 18 sampled communities. Mean, minimum and maximum aboveground biomass (g/m²), species richness (No. of species/quadrat), proportions of each three major growth-forms (% by quadrat), and CWM and CWV calculated with reproductive height (cm), leaf area (cm²) and LDMC (mg/g) are reported respectively for the beginning of the growing season (March 2011) and at the peak of vegetation (May 2011). Seasonal differences were tested with a Student *t*-test. Levels of significance are noted as following: *ns* non-significant, * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

Community feature	March			May			Seasonal differences
	mean	min	max	mean	min	max	Student *t*-test
Biomass (g/m²)	61.77	37.49	114.5	107.9	34.22	205.2	-4.45***
Species richness (/490 cm²)	7.33	3.00	13.00	8.05	4.00	13.00	-0.87*ns*
% of dwarf shrub species	38.93	5.61	84.57	43.39	1.03	78.02	-0.50*ns*
% of rosette species	12.30	0.37	27.76	2.60	0.32	8.16	3.51**
% of tussock species	52.22	15.06	83.75	54.96	21.98	97.04	-0.35*ns*
CWM_Hrep (cm)	22.50	8.35	42.98	20.14	8.39	46.14	0.63*ns*
CWV_Hrep (cm)	269.4	9.15	594.3	196.7	38.27	581.8	1.24*ns*
CWM_LA (cm²)	1.50	0.26	3.31	1.24	0.30	2.40	1.04*ns*
CWV_LA (cm²)	1.35	0.05	8.78	0.98	0.04	2.33	0.69*ns*
CWM_LDMC (mg/g)	363.6	340.9	397.1	379.5	354.3	446.3	-2.21*
CWV_LDMC (mg/g)	2194	519.2	8487	2992	699.3	7787	-0.31*ns*

nine abundant species were evaluated at each period by pair-wise slope tests using a likelihood ratio statistic (Warton et al. 2006). In the same manner, within-species differences in calibration due to seasonal changes in biomass allocation were tested by comparing the slopes obtained from March and May harvests. A second analysis using the same procedures was performed at the growth-form level which provided calibration equations fitted for each of the three dominant growth-forms (dwarf shrub, rosette and tussock). To assess the reliability of growth-form calibrations, we also predicted individual species biomass separately using the corresponding growth-form equation, and tested the congruence with observed values within each community using a linear regression. In a complementary manner, a Spearman correlation test was performed to test if growth-form calibrated prediction conserves species ranking within communities. In a third step, we combined the three growth-form fitted equations in a single equation to predict the total aboveground biomass of the communities:

$$Y_j(t) = \sum_{i=1}^{3}\left[a_i(t).X_{i,j}(t) + b_i(t) + \varepsilon_i(t)\right] \qquad \text{eqn. 5}$$

where $Y_j(t)$ is the total aboveground biomass of a given community *j* at the period *t*; $X_{i,j}(t)$ is the corresponding point-intercepts of growth form; $a_i(t), b_i(t)$ are the fitted model parameters; and $\varepsilon_i(t)$ is the residual biomass error. Because we lacked a different dataset to validate the calibration, a bootstrap re-sampling procedure with 10 000 iterations was performed in order to evaluate the predictive power of eqn. 5 at the community level. At each iteration, the 18 communities were randomly divided into two independent subsets of nine different communities each. The first subset was used to re-estimate the calibration parameters for each of the three growth-forms separately and thus creates an alternative eqn. 5. This re-combined eqn. 5 was then used to predict total community biomasses for the second subset of communities, and these predictions were tested against observed biomass values. After 10 000 iterations, the procedure provided an empirical distribution of explained variance (R^2), slope and intercept of the model of calibration, which accounted for possible sampling effects and heterogeneity in the dataset. Relative mean absolute error (RMAE) was also calculated for each iteration as following:

$$RMAE = \sum_{i=1}^{9}\frac{|\varepsilon_i|}{Y_i} \qquad \text{eqn. 6}$$

where $|\varepsilon_i|$ is the absolute residual error value of predicted biomass for the community *i*; and Y_i is the corresponding observed amount of biomass.

We hypothesized that community features may have a strong incidence on the PIM accuracy during sampling. Thus, we tested for the existence of any measurement bias or threshold effect with a residual analysis. Three variables were used to describe the structure of each community. The total aboveground biomass was measured as explained above. The species richness was determined by counting all the species by quadrat on harvested biomass. The functional structure was assessed through the distribution of growth-forms proportion and of three plant traits, species reproductive height (Hrepro), leaf area (LA) and leaf dry matter content (LDMC), which are related to the vertical stratification of the communities and the distribution of plant photosynthetic tissues. Trait data was collected for the 24 to 30 most abundant species (*i.e.* representing in each case over than 80% of biomass) from previous studies conducted at the same site (Fayolle 2008; Bernard-Verdier et al. 2012). For each trait, the community weighted mean (CWM) and variance (CWV) were calculated in each community as follows:

$$CWM_j = \sum_{i=1}^{n} p_{i,j} \times trait_i \qquad \text{eqn. 7}$$

$$CWV_j = \sum_{i=1}^{n} p_{i,j} \times (trait_i)^2 - CWM_j \qquad \text{eqn. 8}$$

where n is the total number of species in community j; $p_{i,j}$ is the relative abundance of species i in the community; and $trait_i$ is the trait value of species i. Indices were weighted using species relative abundance values measured with the biomass harvest method.

In a fourth and final step, we evaluated how the choice between different methods of abundance measurement may impact our assessment of community functional structure. We calculated the CWM and CWV (see eqn. 6 and eqn. 7) for a single plant trait, LDMC, using three estimates of species relative abundances: (i) non-calibrated PIM (raw point-intercepts values); (ii) growth-form calibrated PIM (predicted biomass values); (iii) harvested biomass measurements (reference values). LDMC was chosen as an example trait because it is one of the traits showing the highest response to soil depth variations at the community level in our study site, both in terms of mean and variance (Bernard-Verdier et al. 2012). Additionally it is one of the most stable leaf traits over the growing season (Garnier et al. 2001b), allowing direct comparisons between March and May samples. RMAE was calculated in each case by analogy with Eqn. 6 as a synthetic variable indicating the global accuracy of estimates. Variations of RMAE was tested against variations of species richness, growth-forms proportions, CWM and CWV for reproductive height and leaf area, in order to detect any over- or under-estimation bias with varying

Table 2 Species-level calibration of the point-intercept method against a) total plant biomass and b) total leaf area. Mean, minimum and maximum aboveground biomass (g/m²) and leaf area (m²/m²), as well as the fitted regression parameters are represented for the nine most abundant species sampled at the beginning of the growing season (March 2011) and at the peak of vegetation (May 2011). Species are grouped (lower-case letters) according to slope parameters using a likelihood ratio test. "*R²*": coefficient of determination, "int.": intercept of the regression line, "slope": slope of the regression line, "*LLR*": likelihood ratio statistics for pair-wise common slope tests. Missing values are represented by a dash (-). Levels of significance are noted as following: *ns* non-significant, * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

Species	March							May							Seasonal differences in slopes
	mean	min	max	int.	slope	R^2	Species slope comparison	mean	min	Max	int.	slope	R^2	Species slope comparison	*LLR*
a) Biomass (g/m²)															
B.erectus	17.61	3.98	31.84	0.79	20.47	0.91***	a	31.71	0.51	80.47	0.03	20.46	0.99***	a	<0.01ns
C.humilis	3.91	0.41	9.23	0.21	21.15	0.96***	a	8.00	0.48	19.97	0.06	20.51	0.96***	a	0.10ns
F.christiani-bernardii	13.02	0.31	37.07	-0.94	22.22	0.87***	a	28.19	6.80	107.20	-0.68	24.58	0.98***	a	0.98ns
K.vallesiana	4.50	0.62	9.80	0.31	22.30	0.93**	a	6.05	0.55	14.64	0.38	23.90	0.97***	a	0.19ns
S.pennata	-	-	-	-	-	-	-	13.39	0.41	30.70	-0.14	24.75	0.99***	a	-
H.canum	17.02	0.29	38.12	-0.70	105.4	0.95***	b	26.59	4.15	48.23	2.31	43.75	0.91***	b	15.5***
P.neumanniana	10.60	0.59	28.70	-0.47	78.88	0.98***	b	16.32	0.34	97.83	-3.33	44.06	0.96***	b	30.7***
T.dolomiticus	5.17	0.77	11.38	-0.41	108.3	0.95**	b	12.24	0.24	21.27	1.10	42.63	0.96***	b	10.8**
H.pilosella	4.68	1.26	12.16	0.23	43.05	0.99***	c	-	-	-	-	-	-	-	-
b) Leaf area (m²/m²)															
B.erectus	0.175	0.039	0.318	0.001	0.205	0.91***	a	0.336	0.006	0.851	0.001	0.216	0.99***	a	0.28ns
C.humilis	0.035	0.004	0.083	0.002	0.191	0.96**	a	0.076	0.005	0.190	0.006	0.195	0.96***	a	0.04ns
F.christiani-bernardii	0.068	0.002	0.193	-0.005	0.116	0.87***	b	0.142	0.035	0.538	-0.003	0.123	0.98***	b	0.41ns
H.canum	0.049	0.003	0.106	0.000	0.245	0.93***	a	0.120	0.021	0.215	0.013	0.192	0.91***	a	2.80ns
K.vallesiana	0.030	0.004	0.66	0.002	0.175	0.93**	a	0.043	0.005	0.104	0.003	0.197	0.97***	a	0.56ns
S.pennata	-	-	-	-	-	-		0.044	0.002	0.101	0.000	0.081	0.92**	b	-
P.neumanniana	0.044	0.003	0.119	-0.001	0.326	0.98***	a	0.094	0.004	0.551	-0.016	0.247	0.96***	a	13.2***
T.dolomiticus	0.018	0.003	0.040	0.000	0.371	0.95**	a	0.061	0.002	0.115	0.006	0.228	0.96***	a	5.89*
H.pilosella	0.057	0.016	0.145	0.004	0.509	0.85**	c	-	-	-	-	-	-	-	-

community functional structure.

Results

The sampled communities were low productive communities with relatively high variable species density. Total aboveground biomass ranged from 34.22 g/m² to 205.21 g/m² over the whole dataset (Table 1), and was significantly higher in May than in March (Student *t*-test; t=-4.45, p<0.0001), reflecting plant growth over the season. Species richness ranged from 3 to 13 species per quadrat without any significant change between March and May. Communities had contrasted growth-form proportions: while some communities were strongly dominated by either considerably from one community to another, covering a wide range of CWM and CWV values for reproductive height, leaf area and LDMC (see Table 1 for mean, minimum and maximum values). However, as for species composition, community functional structure did not vary significantly throughout the growing season, apart from the significant decrease in the proportion of rosette species which reflected the senescence of early spring species such as *Hornungia petraea* or *Erophila verna*. The CWM LDMC also slightly increased between March and May, supporting our assumption that LDMC was one key response trait within these communities.

Species-level analysis

Linear regressions between aboveground biomass and point-intercepts were significant (p<0.01) for each of the nine abundant species with R^2 ranging from 0.87 to 0.99 (Table 2). As expected, the regression slopes at one date generally differed significantly among species (slope difference test; *LLR*>5, p<0.01). However, species with similar growth-form shared common slopes: dwarf shrub species (*Helianthemum canum, Potentilla neumanniana* and *Thymus dolomiticus*) had similar higher slopes than tussock species. The forb *Hieracium pilosella* had a distinct slope from all other species (slope difference test; *LLR*>12.8, p<0.001) probably because it was the only one of the nine abundants that had a rosette growth-form. Slope differences among species were higher in May than in March (Table 2). Between March and May, slopes for the three dwarf shrub species decreased significantly (slope difference test; *LLR*>10, p<0.001), whereas slopes did not change for tussock species (Table 2).

Linear regressions between total leaf area and point-intercepts gave similar results than previously (Table 2), but with less differences in slopes between species and between seasons than for regressions against biomass. We did not find species slopes to be grouped according to growth forms. Only, two species (*Festuca christiani-bernardii* and *Stipa pennata*) showed slopes which differed significantly from the others (slope difference test ; *LLR*>10, p<0.01), possibly because of their tightly rolled-up needle-like leaves, whose area may either have been incorrectly measured

Table 3 Growth form-level calibration of the point-intercept method against plant biomass. Mean, minimum and maximum aboveground biomass (g/m²), as well as the fitted regression parameters, are represented for the three major growth sampled at the beginning of the growing season (March 2011) and at the peak of vegetation (May 2011). R^2: coefficient of determination, "int.": intercept of the regression line, "slope": slope of the regression line, *LLR*: likelihood ratio statistics of pair-wise common slope tests between both periods. Levels of significance are noted as following: *ns* non-significant, * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

Growth form	March						May						Seasonal differences in slopes
	mean	min	max	int.	slope	R^2	mean	min	max	int.	slope	R^2	*LLR*
dwarf shrub	25.71	3.24	75.36	-5.00	108.47	0.94***	41.88	1.08	97.83	-0.52	42.34	0.96***	49.03***
rosette	7.71	0.19	23.43	-1.35	52.68	0.95***	2.88	0.43	8.59	-0.11	37.32	0.90***	4.64*
tussock	30.64	7.81	48.33	3.08	18.03	0.88***	62.97	13.38	146.54	1.05	22.59	0.97***	6.64*

with the area meter and/or differently captured by the PIM. Considering the relatively low differences among species slopes in both March and May, a common slope ($y=0.187x - 0.002$) was fitted for all nine species regardless of the season ($R^2=0.87$, $p<0.0001$). Relative mean absolute error was 8.99% and did not vary significantly over the growing season (Student *t*-test; $t=0.67$, $p=0.51$).

Growth-form-level analysis

After pooling all 40 species samples by growth-form, regressions between aboveground biomass and point-intercepts were highly significant ($p<0.0001$) for each growth-form, with R^2 ranging from 0.88 to 0.97 (Fig. 1, Table 3). Regression slopes differed significantly according to growth-forms ($LLR>15$, $p<0.001$): they were the steepest for dwarf shrub species, the smallest for tussock species and intermediate for rosette species (Table 3). Biomass allocation significantly changed for all growth-forms over the season, with a significant decrease in the regression slope that was larger for dwarf shrub species than for the other growth-forms (Fig. 1b, Table 3). In addition, the three growth-form equations provided accurate predictions for the biomass of each of the 40 species when they were considered individually within communities, both in March and in May ($R^2=0.95$, $p<0.0001$), and respected their relative ranking at the quadrat level (Spearman correlation test; $rho=0.97$, $p<0.0001$).

Community-level analysis

Assembling the three growth-form calibrated regressions into a single equation (Eqn. 4) provided accurate predictions of the total plant biomass both in March and in May (Fig. 2). Repeated regressions following the bootstrap procedure were all highly significant (mean $R^2=0.88$, $p<0.0001$, Fig.3a) with parameters close to the 1:1 line. The relative mean absolute error (RMAE) of the biomass prediction was satisfactory (mean RMAE=12.32 %, Fig.3b). No significant trend was found between fitted values of aboveground biomass and calibrated prediction residuals ($R^2<0.09$, $p>0.23$), suggesting that the calibration was not biased for low or large values of biomass. Furthermore, the structure of the communities successively described by species richness, growth-forms proportion and functional structure (CWM and CWV for reproductive height, leaf area and LDMC) did not have any significant effect on the accuracy of the prediction ($R^2<0.13$, $p>0.14$).

Both calibrated and non-calibrated (raw point-intercepts) PIM estimates of relative abundance provided accurate quantifications of functional structure for LDMC when compared to the reference values of CWM and CWV obtained with harvested biomass (Fig 4a,b). However, non-calibrated estimates lead to systematic over-estimation of CWM values. Growth-form calibrations

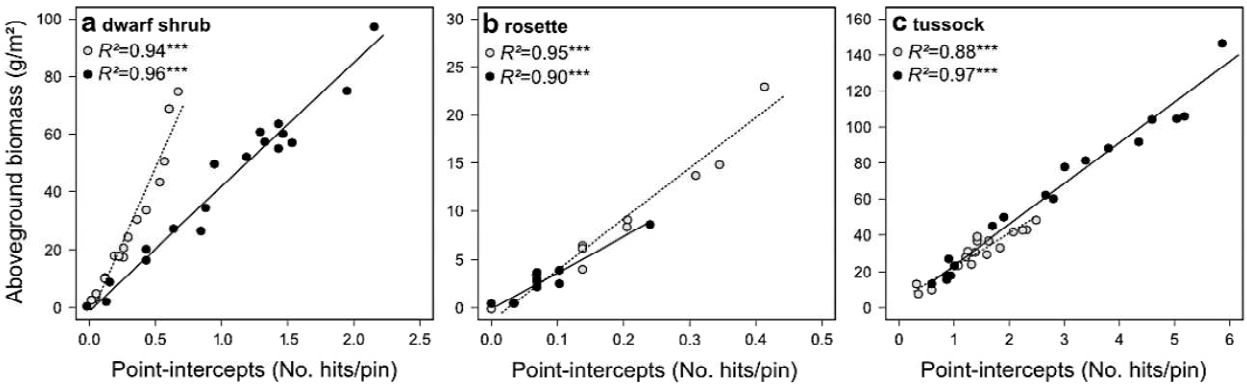


Fig. 1 Relationship between aboveground plant biomass and the mean number of hits per pin for the three main growth forms (*sensu* Cornelissen et al., 2003). Data points represent individual quadrats. Grey points and black points correspond respectively to samples collected at the beginning of the growing season (March) and at the peak of vegetation (May). Dotted and solid lines were calculated by linear regressions (OLS method). Corresponding statistics and parameters are given in Table 3.

reduced significantly the RMAE of CWM (Student paired *t*-test; t=4.11, p=0.0002) and CWV (Student paired *t*-test; t=4.23, p<0.0001). Nevertheless, CWM had always a low RMAE (<4.16 %) and appeared less sensitive to the calibration than CWV estimates, for which the RMAE decreased from 21.6% to 8.05% thanks to calibration. Community structure had a significant effect on the error terms of the CWM and CWV values calculated with non-calibrated estimates of species relative abundances, showing increasing biases with increasing biomass proportion of dwarf shrub species (Fig. 4c,d). Similar increasing biases were also found with decreasing CWM values calculated for reproductive height (R^2=0.30, p<0.0001 for CWM, and R^2=0.11, p<0.05 for CWV) and leaf area (R^2=0.29, p<0.0001 for CWM). This effect was not recorded for CWM and CWV values calculated with calibrated estimates of species relative abundances, for which relative errors were more or less randomly distributed (Fig 4cd).

Discussion

In this study, we investigated whether differences in species growth-form and phenology, and in community structure influence the accuracy of the point-intercept method (PIM) for estimating species relative abundances in low productive and highly diverse Mediterranean grasslands. Our experiment was challenged by the fact that: (i) the studied communities did not range with the communities usually sampled with the PIM (see Table S1), and (ii) comparisons with calibration equations from other studies remained limited insofar as authors used very different grid arrangements - from 20 pins/m² to 6400 pins/m² - inducing different levels of spatial autocorrelation in the data (Clark et al. 2008; Robroek et al. 2010; Stampfli & Fuhrer 2010; Jarchow & Liebman 2012). We show that calibrations of the PIM at the growth-form level allows for a more accurate prediction of aboveground biomass, both for individual species and for communities. These results allow us to evaluate the effect of calibration in quantifying species relative abundances for estimating CWM and CWV of traits in such complex Mediterranean grasslands.

Calibration of the PIM

Our results confirm that the PIM can accurately capture the aboveground biomass of individual plant species, but only if the differences in growth-form and phenological stage are accounted for by an adequate calibration method (Table 2). Calibration slopes were found to be different among growth-forms, with a steepest slope for the dwarf shrub species (Table 3). Because of their woody stems, narrow leaves and procumbent habit, each pin-point hit on a dwarf shrub

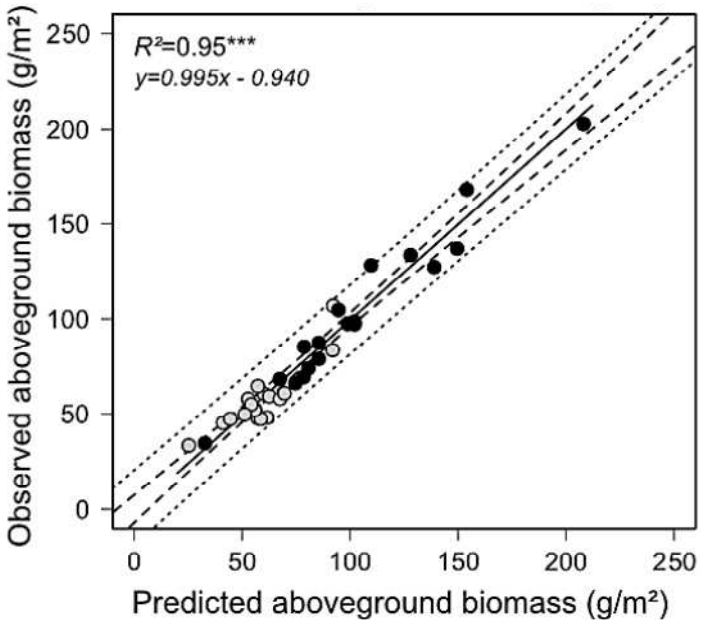


Fig. 2 Observed versus PIM-predicted values of total aboveground biomass (g/m²) at the community level. Data points represent individual quadrats. Grey and black points correspond respectively to samples collected at the beginning of the growing season (March) and at the peak of vegetation (May). Bold solid line was determined by linear regressions (OLS method). Dashed and dotted lines define respectively the confidence interval at 95% and the prediction interval.

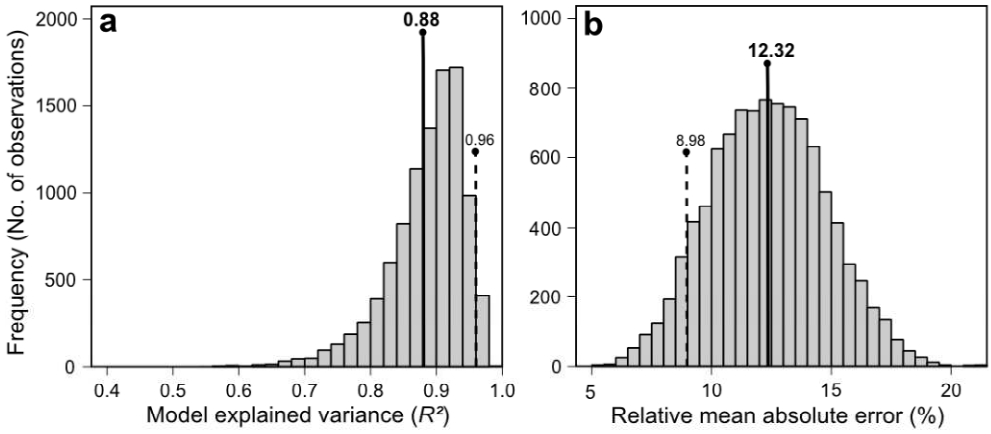


Fig. 3 Empirical bootstrap distributions after 10000 iterations: (a) of the total explained variance, and (b) of the relative mean absolute error (%) of aboveground biomass prediction at the community level. Bold solid lines indicate the empirical mean values of the distributions. Dashed lines indicate the observed values with the entire dataset.

plant captured a higher biomass (when adding both stems and leaves) than for rosette and tussock plants. Inversely, tussock species had the flattest slopes because of their erected habit and high vertical leaf density. Moreover, we show that phenological stages may affect calibration parameters, although to a different extent depending on growth-forms (Table 2 and 3, Fig 1).
From the end of the winter until the peak of vegetation, the leaf:stem ratio of all species increased (Appendix 3). Dwarf shrub species presented the largest decrease in slope across the season because they had relatively few leaves comparatively to the large amount of woody stems at the beginning of the growing season. By contrast, rosette and tussock species had a relatively stable leaf:stem ratio and the slight significant change in slopes found throughout the growing season may be considered as negligible.

By distinguishing growth-forms and phenological stages, we accounted for differences in biomass allocation among plants. This is apparent in the calibration equations, which integrate two major plant functional traits related to plant growth strategies: (i) the leaf:stem ratio, which expresses the specific biomass allocation in photosynthetic tissues, and (ii) the SLA, which expresses the conversion of leaf area into leaf biomass. Both traits are important components of species relative growth rate (Poorter et al. 1995, Poorter et al. 2012) which is a major descriptor of species resource use strategy (Poorter & Garnier 2007). On the one hand, within a given growth-form (*i.e.* species with similar leaf:stem ratio), species with more conservative strategies (*i.e* low SLA) generally have steeper calibration slopes than species with more exploitative strategies (*i.e* high SLA) as they usually have lower leaf area for a higher corresponding plant biomass (Appendix 3). On the other hand, species with lower leaf:stem ratios may have always steeper calibration slopes, as discussed above for dwarf shrub species in our study. Such influence of species resource strategy on PIM calibration, documented here for nine abundant species, was already emphasized by Frank and McNaughton (1990) and Shaver et al. (2001) making differences in PIM parameters ecologically meaningful.

Our results may also help to extend the conditions of use of the PIM. First, because non-calibrated estimates may lead to systematic underestimation of plant biomass for species with low leaf:stem ratios, we recommend to perform two different sets of calibration equations, one at the beginning of the growing season and one at the peak of vegetation, in order to take into account the allometric changes occurring over the season. Second, our results show that the PIM can be used in communities others than uneven communities dominated by a few species. We show here that calibrations obtained at the growth-form appear to be accurate enough to assess individual species biomass and may therefore be particularly relevant in the context of species-rich communities where all the species-specific calibration parameters cannot be established.

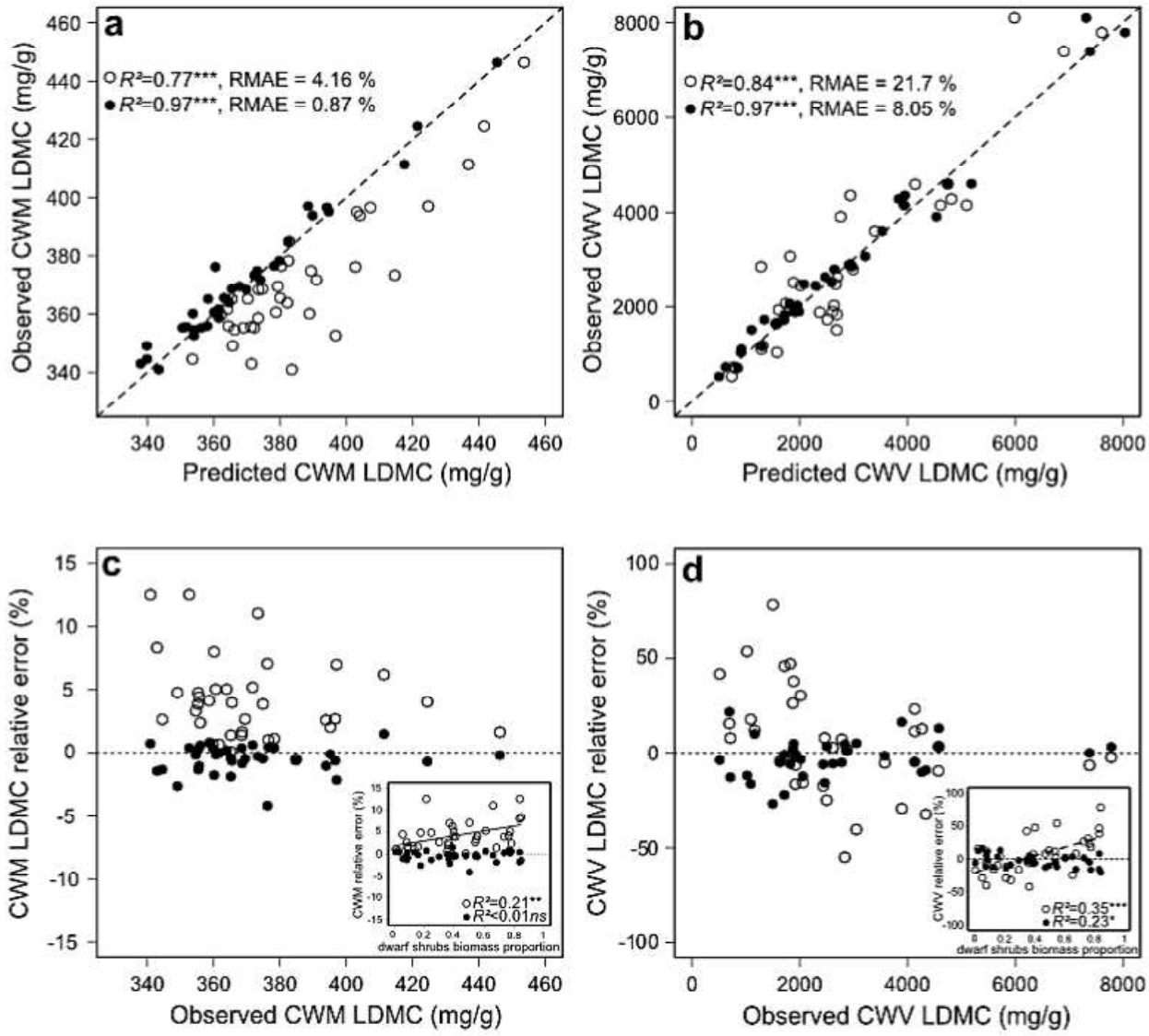


Fig. 4 Observed versus PIM-predicted values of (a) 'community weighted mean' (CWM), (b) 'community weighted variance' (CWV), (c) CWM relative error and (d) CWV relative error calculated for leaf dry matter content (LDMC). Data points represent individual quadrats, pooling samples from March and May samples. Open and black circles correspond to the PIM-estimated values of CWM and CWV using point-intercepts or biomass calibration respectively. On plots (a) and (b), dashed lines represent the 1:1 lines. On plots (c) and (d), horizontal dotted lines represent the y=0 lines. The inserts shows the relationship between relative error and dwarf shrub species biomass proportion within the community. Note the differences in scale between graphs (a) and (b) and (c) and (d).

Whether this is a generalizable result, which might provide generic equations for the PIM across different types of grassland and environment, requires further studies. Lastly, our results suggest that this calibration approach is especially relevant for assessing processes related to ecosystem productivity. Indeed, we showed that accurate estimates of species leaf area can be obtained more directly than estimates of plant biomass with low differences among species (Table 2), as already documented by Groeneveld (1997) and Shaver et al. (2001). This suggests that processes related to plant leaf area, such as leaf transpiration, could be studied using a single calibration, whatever the species abundance distribution of the communities.

Validity of the PIM for sampling complex plant communities

Contrary to our hypothesis, the accuracy of calibrated estimates did not vary with the floristic composition, functional structure nor biomass production of the community. We show that the PIM may detect even slight variations in individual species biomass observed from one community to another and provide good estimates of total aboveground biomass. The calibration approach based on the additivity of simple linear relationships of the three main growth-forms appeared to be robust enough to accurately capture the diversity of species and plant architectures of complex Mediterranean grasslands. However, it should be noted that Redjadj et al. (2012) recently pointed out an increasing heterosedasticity with increasing biomass as they studied the effect of a broader range of aboveground biomass values on the performance of the PIM. Considering this, we would suggest that the accuracy of the PIM is maintained until a certain level of vegetation density has been reached. Based on a simple graphical analysis of data points and regressed curves of other calibration studies (Jonasson 1988; Shaver et al. 2001; Clark et al. 2008), this threshold seems to be consistent and to vary between 200g/m² and 300g/m². It may correspond to a visual saturation of the experimenter during sampling when leaf density is high. Because this increasing heteroscedasticity shows a trend of overestimation for high biomass values (> 300g/m²), some authors used non-linear regression models for calibration (Olofsson 2001; Clark et al. 2008; Suter et al. 2010). But this was not needed here because the aboveground biomass of communities was below this threshold.

Importantly, the PIM provides not only a good estimation of community biomass, but also a reliable and objective representation of the distribution of species abundances within complex communities. It is often considered sufficient to quantify only a few very abundant species in order to assess relevant functional structures in response to environmental factors. Thus, methodological studies often recommend to use visual estimations (BOTANAL-method or other ocular methods) to monitor species composition and relative abundances, because they

allow to save time during field sampling and usually conserve abundance rankings of the most abundant species (Lavorel et al. 2008; Godinez-Alvarez et al. 2009; Redjadj et al. 2012). However, one characteristic of species-rich perennial communities may be their high spatial variability in species evenness resulting from different co-occurring ecological processes (dispersal stochasticity, response to environmental micro-heterogeneity, response to plant-plant interactions). Consequently, such communities are often not homogeneously structured by a few species, but by many different locally dominant species of contrasting growth forms. In such communities, it has been argued that the value of visual methods is thus strongly limited due to a poor performance in quantifying the variations in evenness among local communities (Vittoz & Guisan 2007; Godinez-Alvarez et al. 2009). In the same manner, modern digital methods based on picture analyses have a great potential for quantifying community cover and aboveground biomass over large areas (Cagney et al. 2011; Shen et al. 2008), but they are very poorly efficient for the species identification and thus for the monitoring of biomass proportions within species-rich communities. In comparison with these methods, the PIM provides an advisable trade-off between sampling intensity, the accuracy and the objectivity needed for fine-scale monitoring of community abundance structure.

Effect of the PIM sampling on community functional structure assessment

Our results reveal how an absence of calibration of the PIM may lead to significant errors in the estimation of community functional structure, both for community weighted mean (CWM) and variance (CWV) of traits (Fig. 4). This results from a biased estimation of the proportion of different growth forms, with tussock species being over-represented compared to dwarf shrub species. Consequently, in the case of a trait such as LDMC which tended to be lower for dwarf shrubs in our dataset (Appendix 4), CWM and CWV values calculated without calibration were systematically over-estimated in communities with a high proportion of dwarf shrubs (Fig. 4c,d). More generally, the same bias will be found for any trait which differs among growth forms, such as leaf:stem ratio, reproductive height and leaf area. However, several recent studies have used uncalibrated PIM measurements to estimate community functional structure (e.g. Bernard-Verdier at al. 2012; Frenette-Dussault et al. 2012; Spasojevic et al. 2012). We suggest such studies to take into account such possible biases, in particular when comparing contrasted communities with varying growth-form proportions.

Moreover, by quantifying this error in estimation, we were able to show that CWM and CWV of traits are not similarly sensitive to calibration, CWV being much biased than CWM. This may essentially reflect differences in mathematical properties between mean and variance, and

confirms the results reported by Lepš et al. (2006) and Lavorel et al. (2008) who also found a lower sensitivity of CWM compared to estimates of functional diversity, although they used a different index (FD; Mason et al. 2005; Petchey & Gaston 2006). Although errors on the CWM did reach up to 15% in some communities with a high dwarf shrub proportion (Fig. 4c), they remained on average relatively low (less than 5%) and systematically in the same direction. Thus, we suggest that CWM of traits may be accurately and rapidly assessed even with non-calibrated estimates of species abundances. By contrast, calibration of the PIM appears crucial when analyzing CWV patterns, given that methodological artifacts may in some cases inflate estimation errors up to 80% without the calibration (Fig 4d). In addition, errors in CWV estimation shifted with community structure, suggesting possibly a greater impact of calibration errors on the interpretation of functional divergence.

Conclusion

We conclude that the scope of application of the PIM may be extended to diverse and low-productive grasslands while still retaining high accuracy. Despite being a relatively time-consuming method, the PIM provides an advisable approach to study community functional structure, and could benefit a wide range of ecological studies, such as ecosystem productivity monitoring, comparative community dynamic studies or even ecological modeling. Although PIM calibration may not be essential when analyzing processes related to leaf area, such as transpiration or space occupancy, it does matter for predicting aboveground biomass because it takes into account differences in species growth strategies and biomass allocation. In the same manner, the choice of calibration should be carefully considered when assessing CWM and CWV of traits, and more particularly CWV because of its high sensitivity to calibration. An interesting challenge for the PIM approach would then be to create standardized sampling protocols, which could enable the comparison of different plant community studies and their compilation into databases. Further methodological studies are still needed, in particular concerning the choice of point-grid arrangement and its relationship to vegetation patchiness, and a possibly promising approach would be to combine field studies with simulation analyses.

Acknowledgments

This work was funded by the ANR program O_2LA (09-STRA-09). We thank the experimental station 'INRA-La Fage' for access to the facilities, and Jean Richarte and Caroline Demange for

their valuable supports during field work and laboratory sorting. We also thank Philippe Choler and Florence Volaire for helpful discussions and comments on early version of the manuscript.

References

Axmanová I, Tichý L, Fajmonová Z, Hájková P, Hettenbergerová E, Li C-F, Merunková K, Nejezchlebová M, Otýpková Z, Vymazalová M, Zelený D (2012) Estimation of herbaceous biomass from species composition and cover (R Ejrnaes, Ed.). *Appl Veg Sci* 15:580–589

Bernard-verdier M, Navas M-L, Vellend M, Violle C, Fayolle A, Garnier E (2012) Community assembly along a soil depth gradient : contrasting patterns of plant trait convergence and divergence in a Mediterranean rangeland. *J Ecol* 100:1422–1433

Bret-Harte MS, Garcia EA, Sacre VM, Whorley JR, Wagner JL, Lippert SC, Chapin FS (2004) Plant and soil responses to neighbour removal and fertilization in Alaskan tussock tundra. *J Ecol* 92:635–647

Bråthen KA, Hagberg O (2004) More efficient estimation of plant biomass. *J Veg Sci* 15:653–660

Byrne KM, Lauenroth WK, Adler PB, Byrne CM (2011) Estimating Aboveground Net Primary Production in Grasslands: A Comparison of Nondestructive Methods. *Rangeland Ecol Manage* 64:498–505

Cagney J, Cox SE, Booth DT (2011) Comparison of point intercept and image analysis for monitoring rangeland transects. *Rangeland Ecol Manage* 64:309–315

Catchpole WR, Wheeler CJ (1992) Estimating plant biomass: A review of techniques. *Austral Ecol* 17:121–131

Clark PE, Hardegree SP, Moffet CA, Pierson FB (2008) Point sampling to stratify biomass variability in sagebrush steppe vegetation. *Rangeland Ecol Manage* 61:614–622

Cockayne L (1926) Aims and methods in the study of vegetation. In Tansley AG, Chipp TF (eds) *Tussock grassland investigation in New Zealand*, pp 349-361

Cornelissen JHC, Lavorel S, Garnier E, Díaz S, Buchmann N, Gurvich DE, Reich PB, Steege H Ter, Morgan HD, Heijden MG Van Der, Pausas JG, Poorter H (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Aus J Bot* 51:335

Daget P, Poissonet J (1969) Analyse phytologique des prairies : applications agronomiques. In *CNRS-CEPE Doc 48*, Montpellier Fr

Damgaard C, Riis-Nielsen T, Schmidt IK (2009) Estimating plant competition coefficients and predicting community dynamics from non-destructive pin-point data: a case study with Calluna vulgaris and Deschampsia flexuosa. *Plant Ecol* 201:687–697

Díaz S, Cabido M (2001) Vive la différence: plant functional diversity matters to ecosystem processes. *Trends Ecol Evol* 16:646–655

Frank D, McNaughton S (1990) Aboveground biomass estimation with the canopy intercept method: a plant growth form caveat. *Oikos* 57:57–60

Frenette-Dussault C, Shipley B, Léger J-F, Meziane D, Hingrat Y (2012) Functional structure of an arid steppe plant community reveals similarities with Grime's C-S-R theory. *J Veg Sci* 23:208–222

Ganguli AC, Vermeire LT, Mitchell RB, Wallace MC (2000) Comparison of four nondestructive techniques for estimating standing crop in shortgrass plains. *Agron J* 92:1211

Garnier E, Navas M-L (2012) A trait-based approach to comparative functional plant ecology: concepts, methods and applications for agroecology. A review. *Agron Sustain Dev* 32:365–399

Garnier E, Shipley B, Roumet C, Laurent G (2001a) A standardized protocol for the determination of specific leaf area and leaf dry matter content. *Func Ecol* 15:688–695

Garnier E, Laurent G, Bellmann A, Debain S, Berthelier P, Ducout B, Roumet C, Navas M-L (2001b) Consistency of species ranking based on functional leaf traits. *New Phytol* 152:69–83

Garnier E, Cortez J, Billès G, Navas M-L, Roumet C, Debussche M, Laurent G, Blanchard A, Aubry D, Bellman A, Neill C, Toussaint J-P (2004) Plant functional markers capture ecosystem properties. *Ecology* 85:2630–2637

Godínez-Alvarez H, Herrick JE, Mattocks M, Toledo D, Van Zee J (2009) Comparison of three vegetation monitoring methods: Their relative utility for ecological assessment and monitoring. *Ecol Indic* 9:1001–1008

Goodall DW (1952) Some considerations in the use of point quadrats for the analysis of vegetation. *Aust J Sci Res Ser* B5:1–41

Grime JP (2006) Trait convergence and trait divergence in herbaceous plant communities: Mechanisms and consequences. *J Veg Sci* 17:255

Groeneveld D (1997) Vertical point quadrat sampling and an extinction factor to calculate leaf area index. *J Arid Environ* 36:475–485

Hakl J, Hrevušová Z, Hejcman M, Fuksa P (2012) The use of a rising plate meter to evaluate lucerne (Medicago sativa L.) height as an important agronomic trait enabling yield estimation. *Grass Forage Sci* 67:589–596

Harmoney KR, Moore KJ, George JR, Brummer EC, Russell JR (1997) Determination of Pasture Biomass Using Four Indirect Methods. *Agron J* 89:665

Hobbie SE, Shevtsova A, III FSC (1999) Plant responses to species removal and experimental warming in Alaskan tussock tundra. *Oikos* 84:417–434

Hudson JMG, Henry GHR (2010) High Arctic plant community resists 15 years of experimental warming. *J Ecol* 98:1035–1041

Jarchow ME, Liebman M (2012) Nutrient enrichment reduces complementarity and increases priority effects in prairies managed for bioenergy. *Biomass Bioenerg* 36:381–389

Jonasson S (1988) Evaluation of the point intercept method for the estimation of plant biomass. *Oikos* 52:101–106

Lavorel S, Garnier E (2002) Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Func Ecol* 16:545–556

Lavorel S, Grigulis K, McIntyre S, Williams NSG, Garden D, Dorrough J, Berman S, Quétier F, Thébault A, Bonis A (2008) Assessing functional diversity in the field – methodology matters! *Func Ecol* 22:134–147

Lepš J, De Bello F, Lavorel S, Berman S (2006) Quantifying and interpreting functional diversity of natural communities : practical considerations matter. *Preslia* 78:481–501

Levy EB, Madden EA (1933) The point method of pasture analysis. *N Z J Agr Res* 46:267–279

Lorentzen S, Roscher C, Schumacher J, Schulze E-D, Schmid B (2008) Species richness and identity affect the use of aboveground space in experimental grasslands. *Perspect Plant Ecol* 10:73–87

Martin RC, Astatkie T, Cooper JM, Fredeen a. H (2005) A comparison of methods used to determine biomass on naturalized swards. *J Agron Crop Sci* 191:152–160

Mason NWH, MacGillivray K, Steel JB, Wilson JB (2003) An index of functional diversity. *J Veg Sci* 14:571–578

Mason NWH, Mouillot D, Lee WG, Wilson JB (2005) Functional richness, functional evenness and functional divergence: the primary components of functional diversity. *Oikos* 111:112–118

McLaren JR, Turkington R (2010) Ecosystem properties determined by plant functional group identity. *J Ecol* 98:459–469

Olofsson J (2001) Influence of herbivory and abiotic factors on the distribution of tall forbs along a productivity gradient: a transplantation experiment. *Oikos* 94:351–357

Petchey OL, Gaston KJ (2006) Functional diversity: back to basics and looking forward. *Ecol Lett* 9:741–58

Poorter H, Vijver CADM, Boot RGA, Lambers H (1995) Growth and carbon economy of a fast-growing and a slow-growing grass species as dependent on nitrate supply. *Plant and Soil* 171:217–227

Poorter H, Garnier E (2007) Ecological significance of inherent variation in relative growth rate and its components. In Pugnaire FI & Valladares F (eds) *Functional plant ecology (2nd ed.).* CRC Press, Boca Raton, Florida, pp 67-100

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L (2012) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytol* 193:30–50

Pérez-Ramos IM, Roumet C, Cruz P, Blanchard A, Autran P, Garnier E (2012) Evidence for a " plant community economics spectrum " driven by nutrient and water limitations in a Mediterranean rangeland of southern France. *J Ecol* 100:1315–1327

R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.r-project.org/.

Redjadj C, Duparc A, Lavorel S, Grigulis K, Bonenfant C, Maillard D, Saïd S, Loison A (2012) Estimating herbaceous plant biomass in mountain grasslands: a comparative study using three different methods. *Alpine Bot* 122:57–63

Robroek BJM, Smart RP, Holden J (2010) Sensitivity of blanket peat vegetation and hydrochemistry to local disturbances. *Sci Total Environ* 408:5028–34

Shaver GR, Bret-harte MS, Jones MH, Johnstone J, Laundre J, Chapin FS (2001) Species composition interacts with fertilizer to control long-term change in tundra productivity. *Ecology* 82:3163–3181

Shen M, Tang Y, Klein J, Zhang P, Gu S, Shimono A, Chen J (2008) Estimation of aboveground biomass using in situ hyperspectral measurements in five major grassland ecosystems on the Tibetan Plateau. *J Plant Ecol* 1:247–257

Sonnier G, Shipley B, Navas M-L (2010) Quantifying relationships between traits and explicitly measured gradients of stress and disturbance in early successional plant communities. *J Veg Sci* 21:1014–1024

Spasojevic MJ, Suding KN (2012) Inferring community assembly mechanisms from functional diversity patterns: the importance of multiple assembly processes. *J Ecol* 100:652–661

Stampfli A, Fuhrer J (2010) Spatial heterogeneity confounded ozone-exposure experiment in semi-natural grassland. *Oecologia* 162:515–22

Suter M, Ramseier D, Connolly J, Edwards PJ (2010) Species identity and negative density dependence lead to convergence in designed plant mixtures of twelve species. *Basic Appl Ecol* 11:627–637

Vermeire LT, Ganguli AC, Gillen RL (2002) A robust model for estimating standing crop across vegetation types. Rangeland Ecol Manage 55:494–497

Vittoz P, Guisan A (2007) How reliable is the monitoring of permanent vegetation plots ? A test with multiple observers. *J Veg Sci* 18:413–422

Warton DI, Wright IJ, Falster DS, Westoby M (2006) Bivariate line-fitting methods for allometry. *Biol Rev* 81:259–91

Warton DI, Duursma R a., Falster DS, Taskinen S (2012) smatr 3- an R package for estimation and inference about allometric lines. *Methods Ecol Evol* 3:257–259

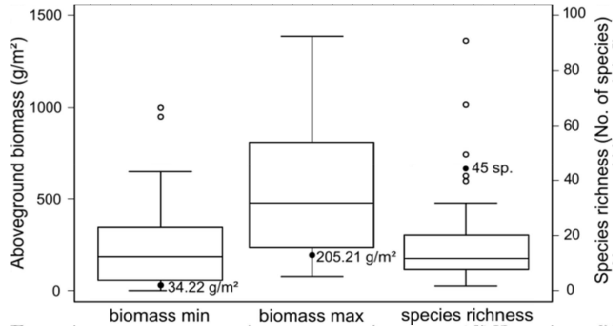
Whitbeck M, Grace JB (2006) Evaluation of non-destructive methods for estimating biomass in marshes of the upper Texas, USA coast. *Wetlands* 26:278–282

Yurkonis KA, Wilsey BJ, Moloney KA (2012) Initial species pattern affects invasion resistance in experimental grassland plots. *J Veg Sci* 23:4–12

Supporting information

Table S1 Results from a literature survey conducted on 91 peer-reviewed papers[1] on terrestrial vegetation containing either the terms 'pin-point', 'point-intercept method' or 'point-quadrat' from 1980 to 2012 (source: Web of Science, 01/11/2012). For each selected study, we classified the vegetation into 5 categories according to study location and vegetation description (dry and Mediterranean grassland, alpine grassland, temperate grassland, subarctic and temperate bog, subarctic and arctic tundra). Corresponding mean species richness, range of aboveground biomass and point density were calculated (see values in the following table). In addition, we quantified the number of studies where the PIM was calibrated for at least one species. Minimum and maximum aboveground biomass and species richness of the different studied communities were represented by 25%-quartiles (see the following boxplot). This study was positioned in the first quartile in terms of aboveground biomass and in the fourth quartile in terms of species richness (black bullets).

Vegetation type	% of total studies	Species richness (No. of species)	Range of aboveground biomass (g/m²)	Mean point density (No. points/m²)	% of studies with a calibration (for at least one species)
Dry and Mediterranean grassland	8.64 %	18.75	130.5 - 434.3	125.04	71.43 %
Alpine grassland	8.64 %	34.4	182.5 - 455	3574.44	14.28 %
Temperate grassland	28.40 %	17.25	313.19 - 1032.3	1093.02	34.78 %
Subarctic and temperate bog	11.11 %	8.88	333 - 679.3	9157.77	44.44 %
Subarctic and arctic tundra	43.21 %	15.52	199.9 - 475.2	679.61	34.29 %
Total mean	**-**	**17.32**	**240.7 - 629.8**	**792.61**	**37.03 %**



[1]List of references :

Abella SR, Covington WW (2004) Monitoring an Arizona Ponderosa Pine Restoration: Sampling Efficiency and Multivariate Analysis of Understory Vegetation. *Restor Ecol* 12:359–367

Abrahamson IL, Nelson CR, Affleck DLR (2011) Assessing the performance of sampling designs for measuring the abundance of understory plants. *Ecol Appl* 21:452–64

Arévalo JR, De Nascimento L, Fernández-Lugo S, Mata J, Bermejo L (2011) Grazing effects on species composition in different vegetation types (La Palma, Canary Islands). *Acta Oecol* 37:230–238

Aunapuu M, Dahlgren J, Oksanen T, Grellmann D, Oksanen L, Olofsson J, Rammul U, Schneider M, Johansen B, Hygen HO (2008) Spatial patterns and dynamic responses of arctic food webs corroborate the exploitation ecosystems hypothesis (EEH). *Am Nat* 171:249–62

Bassin S, Volk M, Suter M, Buchmann N, Fuhrer J (2007) Nitrogen deposition but not ozone affects productivity and community composition of subalpine grassland after 3 yr of treatment. *New Phytol* 175:523–34

Bokhorst SF, Bjerke JW, Tømmervik H, Callaghan T V., Phoenix GK (2009) Winter warming events damage sub-Arctic vegetation: consistent evidence from an experimental manipulation and a natural event. *J Ecol* 97:1408–1415

Bokhorst SF, Tømmervik H, Callaghan TV., Phoenix GK, Bjerke JW (2012) Vegetation recovery following extreme winter warming events in the sub-Arctic estimated using NDVI from remote sensing and handheld passive proximal sensors. *Environ Exp Bot* 81:18–25

Booth DT, Cox SE, Meikle TW, Fitzgerald C (2006) The accuracy of ground-cover measurements. *Rangeland Ecol Manage* 59:179–188

Borges PAV, Brown VK (2001) Phytophagous insects and web-building spiders in relation to pasture vegetation complexity. *Ecography* 24:68–82

Bragazza L, Buttler A, Habermacher J, Brancaleoni L, Gerdol R, Fritze H, Hanajík P, Laiho R, Johnson D (2012) High nitrogen deposition alters the decomposition of bog plant litter and reduces carbon accumulation. *Global Change Biol* 18:1163–1172

Brancaleoni L, Gualmini M, Tomaselli M, Gerdol R (2007) Responses of subalpine dwarf-shrub heath to irrigation and fertilization. *J Veg Sci* 18:337

Breeuwer A, Robroek BJM, Limpens J, Heijmans MMPD, Schouten MGC, Berendse F (2009) Decreased summer water table depth affects peatland vegetation. *Basic Appl Ecol* 10:330–339

Bruce LB, Panciera MT, Gavlak RG, Tilman BA, Cadle JM (1995) Observation: botanical and other characteristics in arctic salt-affected coastal Areas. *Rangeland Ecol Manage* 48:206-210

Bråthen KA, Oksanen J (2001) Reindeer reduce biomass of preferred plant species. *J Veg Sci* 12:473–480

Bråthen KA, Ims RA, Yoccoz NG, Fauchald P, Tveraa T, Hausner VH (2007) Induced Shift in Ecosystem Productivity? Extensive Scale Effects of Abundant Large Herbivores. *Ecosystems* 10:773–789

Buttler A (1992) Permanent plot research in wet meadows and cutting experiment. *Vegetatio* 103:113–124

Chiarucci A, Maccherini S (2007) Long-term effects of climate and phosphorus fertilisation on serpentine vegetation. *Plant and Soil* 293:133–144

Cornelissen JHC, Callaghan T V., Alatalo JM, Michelsen A, Graglia E, Hartley a. E, Hik DS, Hobbie SE, Press MC, Robinson CH, Henry GHR, Shaver GR, Phoenix GK, Gwynn Jones D, Jonasson S, Chapin FS, Molau U, Neill C, Lee JA, Melillo JM, Sveinbjornsson B, Aerts R (2001) Global change and arctic ecosystems: is lichen decline a function of increases in vascular plant biomass? *J Ecol* 89:984–994

Dahlgren J, Oksanen L, Oksanen T, Olofsson J, Hambäck PA, Lindgren Å (2009) Plant defences to no avail ? Responses of plants of varying edibility to food web manipulations in a low arctic scrubland. *Evol Ecol Res* 11:1189–1203

Damgaard C, Strandberg B, Mathiassen SK, Kudsk P (2011) The combined effect of nitrogen and glyphosate on the competitive growth, survival and establishment of Festuca ovina and Agrostis capillaris. *Agri, Ecosyst & Environ* 142:374–381

Dietz H, Steinlein T (1996) Determination of plant species cover by means of image analysis. *J Veg Sci* 7:131–136

Eek L, Zobel K (1997) Effects of additional illumination and fertilization on seasonal changes in fine-scale grassland community structure. *J Veg Sci* 8:225–234

Ekberg A, Arneth A, Holst T (2011) Isoprene emission from Sphagnum species occupying different growth positions above the water table. *Boreal Environ Res* 16:47–59

Emilsson T (2008) Vegetation development on extensive vegetated green roofs: Influence of substrate composition, establishment method and species mix. *Ecol Eng* 33:265–277

Faubert P, Tiiva P, Michelsen A, Rinnan Å, Ro-Poulsen H, Rinnan R (2012) The shift in plant species composition in a subarctic mountain birch forest floor due to climate change would modify the biogenic volatile organic compound emission profile. *Plant and Soil* 352:199–215

Fehmi JS, Stevens JM (2009) A plate meter inadequately estimated herbage mass in a semi-arid grassland. *Grass Forage Sci* 64:322–327

Filella I (2004) Reflectance assessment of seasonal and annual changes in biomass and CO2 uptake of a Mediterranean shrubland submitted to experimental warming and drought. *Remote Sens Environ* 90:308–318

Focardi S, Tinelli A (2005) Herbivory in a Mediterranean forest: browsing impact and plant compensation. *Acta Oecol* 28:239–247

Ganguli AC, Vermeire LT, Mitchell RB, Wallace MC (2000) Comparison of four nondestructive techniques for estimating standing crop in shortgrass plains. *Agron J* 92:1211

Grellmann D (2002) Plant responses to fertilization and exclusion of grazers on an arctic tundra heath. *Oikos* 98:190–204

Heijmans MMPD, Berendse F, Arp WJ, Masselink AK, Klees H, De Visser W, Van Breemen N (2001) Effects of elevated carbon dioxide and increased nitrogen deposition on bog vegetation in the Netherlands. *J Ecol* 89:268–279

Helm DJ, Mead BR (2004) Reproducibility of vegetation cover estimates in south-central Alaska forests. *J Veg Sci* 15:33–40

Hoosbeek MR, Breemen N van, Berendse F, Grosvernier P, Vasander H, Wallén B, Hoosbeek MR (2001) Limited effect of increased atmospheric CO2 concentration on ombrotrophic bog vegetation. *New Phytol* 150:459–463

James MB, Joe F, Mark J. S, Jonathan S, Sarah J. C, Steve J. G, Richard T (2001) A plant trait analysis of responses to grazing in a long-term experiment. *J Appl Ecol* 38:253–267

Jarchow ME, Liebman M (2012) Nutrient enrichment reduces complementarity and increases priority effects in prairies managed for bioenergy. *Biomass Bioenerg* 36:381–389

Keuper F, Dorrepaal E, Van Bodegom PM, Aerts R, Van Logtestijn RSP, Callaghan TV., Cornelissen JHC (2011) A Race for Space? How Sphagnum fuscum stabilizes vegetation composition during long-term climate manipulations. *Global Change Biol* 17:2162–2171

Killengreen ST, Ims RA, Yoccoz NG, Bråthen KA, Henden J-A, Schott T (2007) Structural characteristics of a low Arctic tundra ecosystem and the retreat of the Arctic fox. *Biol Conserv* 135:459–472

Kohler F, Gillet F, Gobat J-M, Buttler A (2004) Seasonal vegetation changes in mountain pastures due to simulated effects of cattle grazing. *J Veg Sci* 15:143–150

Kongstad J, Schmidt IK, Riis-Nielsen T, Arndal MF, Mikkelsen TN, Beier C (2011) High Resilience in Heathland Plants to Changes in Temperature, Drought, and CO2 in Combination: Results from the CLIMAITE Experiment. *Ecosystems* 15:269–283

Korb JE, Covington WW, Fulé PZ (2003) Sampling techniques influence understory plant rajectories after restoration : an example from Ponderosa Pine restoration. *Restor Ecol* 11:504–515

Laliberte A, Rango A, Herrick JE, Fredrickson E, Burkett L (2007) An object-based image analysis approach for determining fractional cover of senescent and green vegetation with digital plot photography. *J Arid Environ* 69:1–14

Lang SI, Cornelissen JHC, Shaver GR, Ahrens M, Callaghan TV., Molau U, Ter Braak CJF, Hölzer A, Aerts R (2012) Arctic warming on two continents has consistent negative effects on lichen diversity and mixed effects on bryophyte diversity. *Global Change Biol* 18:1096–1107

Larsen KS, Andresen LC, Beier C, Jonasson S, Albert KR, Ambus P, Arndal MF, Carter MS, Christensen S, Holmstrup M, Ibrom A, Kongstad J, Van der Linden L, Maraldo K, Michelsen A, Mikkelsen TN, Pilegaard K, Priemé A, Ro-Poulsen H, Schmidt IK, Selsted MB, Stevnbak K (2011) Reduced N cycling in response to elevated CO2, warming, and drought in a Danish heathland: Synthesizing results of the CLIMAITE project after two years of treatments. *Global Change Biol* 17:1884–1899

Lenssen JPM, Van de Steeg HM, Kroon H (2004) Does disturbance favour weak competitors? Mechanisms of changing plant abundance after flooding. *J Veg Sci* 15:305–314

Limpens J, Berendse F, Klees H (2004) How Phosphorus Availability Affects the Impact of Nitrogen Deposition on Sphagnum and Vascular Plants in Bogs. *Ecosystems* 7:793–804

Lonati M, Gorlier A, Ascoli D, Marzano R, Lombardi G (2009) Response of the alien species Panicum acuminatum to disturbance in an Italian lowland heathland. *Bot Helv* 119:105–111

Montès N, Maestre FT, Ballini C, Baldy V, Gauquelin T (2008) On the relative importance of the effects of selection and complementarity as drivers of diversity productivity relationships in Mediterranean shrublands. *Oikos* 117:1345–1350

Marissink M, Hansson M (2002) Floristic composition of a Swedish semi-natural grassland during six years of elevated atmospheric CO2. *J Veg Sci* 13:733–742

Mason NWH, Wilson JB (2006) Mechanisms of species coexistence in a lawn community: mutual corroboration between two independent assembly rules. *Community Ecol* 7:109–116

Michelsen A, Graglia E, Schmidt IK, Jonasson S, Sleep D, Quarmby C (1999) Differential responses of grass and a dwarf shrub to long-term changes in soil microbial biomass C, N and P following factorial addition of NPK fertilizer, fungicide and labile carbon to a heath. *New Phytol* 143:523–538

Milla R, Cornelissen JHC, Logtestijn RSP van, Toet S, Aerts R (2006) Vascular plant responses to elevated CO2 in a temperate lowland Sphagnum peatland. *Plant Ecology* 182:13–24

Mitchley J, Willems JH (1995) Vertical canopy structure of Dutch chalk grasslands in relation to their management. *Vegetatio* 117:17–27

Murphy MT, Moore TR (2010) Linking root production to aboveground plant characteristics and water table in a temperate bog. *Plant and Soil* 336:219–231

Mänd P, Hallik L, Peñuelas J, Nilson T, Duce P, Emmett BA, Beier C, Estiarte M, Garadnai J, Kalapos T (2010) Responses of the reflectance indices PRI and NDVI to experimental warming and drought in European shrublands along a north–south climatic gradient. *Remote Sens Environ* 114:626–636

Obinson CHR, Ookey PAW, Ee JAL, Allaghan TVC (1998) Plant community responses to simulated environmental change at high artic polar semi-desert. *Ecology* 79:856–866

Olofsson J (2001) Influence of herbivory and abiotic factors on the distribution of tall forbs along a productivity gradient: a transplantation experiment. *Oikos* 94:351–357

Olofsson J (2006) Short- and long-term effects of changes in reindeer grazing pressure on tundra heath vegetation. *J Ecol* 94:431–440

Olofsson J, Hulme PE, Oksanen L, Suominen O (2004) Importance of large and small mammalian herbivores for the plant community structure in the forest tundra ecotone. *Oikos* 2:324–334

Plassmann K, Edwards-Jones G, Jones MLM (2009) The effects of low levels of nitrogen deposition and grazing on dune grassland. *Sci Total Environ* 407:1391–404

Prakash C, Vinod B (2002) Patterns of plant species distribution in the Trans-Himalayan region of Ladakh, India. *J Veg Sci* 13:751–754

Press MC, Potter JA, Burke MJW, Callaghan TV, Lee JA (1998) Responses of a subarctic dwarf shrub heath community to simulated environmental change. *J Ecol* 86:315–327

Ravolainen VT, Yoccoz NG, Bråthen KA, Ims RA, Iversen M, González VT (2010) Additive partitioning of diversity reveals no scale-dependent impacts of large ungulates on the structure of tundra plant communities. *Ecosystems* 13:157–170

Ravolainen VT, Bråthen KA, Ims RA, Yoccoz NG, Henden J-A, Killengreen ST (2011) Rapid, landscape scale responses in riparian tundra vegetation to exclusion of small and large mammalian herbivores. *Basic Appl Ecol* 12:643–653

Renard D, Birk JJ, Glaser B, Iriarte J, Grisard G, Karl J, McKey D (2011) Origin of mound-field landscapes: a multi-proxy approach combining contemporary vegetation, carbon stable isotopes and phytoliths. *Plant and Soil* 351:337–353

Robin M, Chapuis J-L, Lebouvier M (2011) Remote sensing of vegetation cover change in islands of the Kerguelen archipelago. *Polar Biol* 34:1689–1700

Robroek BJM, Smart RP, Holden J (2010) Sensitivity of blanket peat vegetation and hydrochemistry to local disturbances. *Sci Total Environ* 408:5028–34

Roxburgh SH, Wilson JB (2000) Stability and coexistence in a lawn community: experimental assessment of the stability of the actual community. *Oikos* 88:409–423

Rtzel C, Paul W, Krner C (1997) Non-destructive assessment of the effects of elevated CO2 on plant community structure in a calcareous grassland. *Acta Oecol* 18:231–239

Santin-Janin H, Garel M, Chapuis J-L, Pontier D (2009) Assessing the performance of NDVI as a proxy for plant biomass using non-linear models: a case study on the Kerguelen archipelago. *Polar Biol* 32:861–871

Saïd S, Pellerin M, Guillon N, Débias F, Fritz H (2005) Assessment of forage availability in ecological studies. *Eur J Wildlife Res* 51:242–247

Schaepman-Strub G, Limpens J, Menken M, Bartholomeus HM, Schaepman ME (2009) Towards spatial assessment of carbon sequestration in peatlands: spectroscopy based estimation of fractional cover of three plant functional types. *Biogeosciences* 6:275–284

Selsted MB, Linden L, Ibrom A, Michelsen A, Larsen KS, Pedersen JK, Mikkelsen TN, Pilegaard K, Beier C, Ambus P (2012) Soil respiration is stimulated by elevated CO2 and reduced by summer drought: three years of measurements in a multifactor ecosystem manipulation experiment in a temperate heathland (CLIMAITE). *Global Change Biol* 18:1216–1230

Skalova H, Krahulec F, During HJ, Hadincova V, Pechackova S, Herben T (1999) Grassland canopy composition and spatial heterogeneity in the light quality. *Plant Ecol* 143:129–139

Sorensen PL, Michelsen A (2011) Long-term warming and litter addition affects nitrogen fixation in a subarctic heath. *Global Change Biol* 17:528–537

Stampfli A (1991) Accurate determination of vegetational change in meadows by successive point quadrat analysis. *Vegetatio* 96:185–194

Stampfli A, Fuhrer J (2010) Spatial heterogeneity confounded ozone-exposure experiment in semi-natural grassland. *Oecologia* 162:515–22

Stampfli A, Zeiter M (2004) Plant regeneration directs changes in grassland composition after extreme drought : a 13-year study in southern Switzerland. *J Ecol* 92:568–576

Strandberg B, Axelsen JA, Pedersen MB, Jensen J, Attrill MJ (2006) Effect of a copper gradient on plant community structure. *Environ Toxicol Chem* 25:743–53

Strengbom J, Nordin A, Nasholm T, Ericson L (2002) Parasitic fungus mediates change in nitrogen-exposed boreal forest vegetation. *J Ecol* 90:61–67

Strengbom J, Olofsson J, Witzell J, Dahlgren J (2003) Effects of repeated damage and fertilization on palatability of Vaccinium myrtillus to grey sided voles, Clethrionomys rufocanus. *Oikos* 103:133–141

Suter M, Ramseier D, Connolly J, Edwards PJ (2010) Species identity and negative density dependence lead to convergence in designed plant mixtures of twelve species. *Basic Appl Ecol* 11:627–637

Tiiva P, Faubert P, Michelsen A, Holopainen T, Holopainen JK, Rinnan R (2008) Climatic warming increases isoprene emission from a subarctic heath. *New Phytol* 180:853–63

Toledo P D, Herrick JE, Abbott LB (2010) A comparison of cover pole with standard vegetation monitoring methods. *J Wildlife Manage* 74:600–604

Torp M, Olofsson J, Witzell J, Baxter R (2009) Snow-induced changes in dwarf birch chemistry increase moth larval growth rate and level of herbivory. *Polar Biol* 33:693–702

Verdoodt A, Mureithi SM, Ye L, Van Ranst E (2009) Chronosequence analysis of two enclosure management strategies in degraded rangeland of semi-arid Kenya. *Agri, Ecosyst & Environ* 129:332–339

Vermeire LT, Ganguli AC, Gillen RL (2002) A robust model for estimating standing crop across vegetation types. *Rangeland Ecol Manage* 55:494–497

Vittoz P, Bayfield N, Brooker R, Elston DA, Duff EI, Theurillat J-P, Guisan A (2010) Reproducibility of species lists, visual cover estimates and frequency methods for recording high-mountain vegetation. *J Veg Sci* 21:1035–1047

Warwick KR, Taylor G, Blum H (1998) Biomass and compositional changes occur in chalk grassland turves exposed to elevated CO2 for two sasons in FACE. *Global Change Biol* 4:375–385

Whitbeck M, Grace JB (2006) Evaluation of non-destructive methods for estimating biomass in marshes of the upper Texas, USA coast. *Wetlands* 26:278–282

Wookey PA, Robinson CH, Parsons AN, Welker JM, Press MC, Callaghan T V., Lee JA (1995) Environmental constraints on the growth, photosynthesis and reproductive development of *Dryas octopetala* at a high Arctic polar semi-desert, Svalbard. *Oecologia* 102:478–489

Yurkonis KA, Wilsey BJ, Moloney KA (2012) Initial species pattern affects invasion resistance in experimental grassland plots. *J Veg Sci* 23:4–12

Supporting information

Table S2 The 45 sampled species with botanical family and corresponding growth form (*sensu* Cornelissen et al., 2003) and their abundance within the whole data set expressed respectively as their point-intercept frequencies estimates at the beginning of the growing season (March 2011) and at the peak of vegetation (May 2011; separated by a slash). The nine most abundant species are in bold. **Plant nomenclature:** Muséum national d'Histoire naturelle [Ed]. 2003-2013. *Inventaire national du Patrimoine naturel, site Web :* http://inpn.mnhn.fr.

Species	Botanical family	Growth form	Intercept frequency
Achillea millefolium	Asteraceae	rosette	0.012/0.000
Anthyllis vulneraria	Fabaceae	rosette	0.032/0.000
Aphyllanthes monspeliensis	Asparagaceae	tussock	0.006/0.101
Arenaria aggregata	Caryophyllaceae	dwarf shrub	0.040/0.000
Arenaria serpyllifolia	Caryophyllaceae	erect leafy	0.000/0.012
Brachypodium pinnatum	Poaceae	tussock	0.000/0.000
Bromus erectus	**Poaceae**	**tussock**	**0.713/1.548**
Bupleurum baldense	Apiaceae	erect leafy	0.000/0.017
Carex flacca	Cyperaceae	tussock	0.000/0.046
Carex halleriana	Cyperaceae	tussock	0.000/0.052
Carex humilis	**Cyperaceae**	**tussock**	**0.089/0.359**
Centaurea pectinata	Asteraceae	rosette	0.000/0.006
Cerastium pumilum	Caryophyllaceae	erect leafy	0.011/0.022
Coronilla minima	Fabaceae	dwarf shrub	0.006/0.017
Erophila verna	Brassicaceae	rosette	0.006/0.000
Festuca christiani-bernardii	**Poaceae**	**tussock**	**0.686/1.174**
Filago pyramidata	Asteraceae	rosette	0.000/0.006
Filipendula vulgaris	Rosaceae	rosette	0.046/0.029
Fumana procumbens	Cistaceae	dwarf shrub	0.023/0.000
Galium corrudifolium	Rubiaceae	dwarf shrub	0.000/0.023
Genista hispanica	Fabaceae	dwarf shrub	0.000/0.017
Globularia bisnagarica	Plantaginaceae	rosette	0.006/0.000
Helianthemum apenninum	Cistaceae	dwarf shrub	0.006/0.063
Helianthemum canum	**Cistaceae**	**dwarf shrub**	**0.190/0.542**
Hieracium pilosella	**Asteraceae**	**rosette**	**0.074/0.046**
Hornungia petrea	Brassicaceae	rosette	0.006/0.000
Koeleria vallesiana	**Poaceae**	**tussock**	**0.138/0.203**

(continued)

Leucanthemum vulgare	Asteraceae	rosette	0.006/0.000
Liliaceae spp.	Liliaceae	tussock	0.006/0.000
Lotus corniculatus	Fabaceae	dwarf shrub	0.006/0.006
Muscari neglectum	Asparagaceae	tussock	0.012/0.006
Ononis striata	Fabaceae	dwarf shrub	0.000/0.269
Potentilla neumanniana	**Rosaceae**	**dwarf shrub**	**0.146/0.442**
Poa badensis	Poaceae	tussock	0.000/0.063
Poa bulbosa	Poaceae	tussock	0.086/0.000
Ranunculus gramineus	Ranunculaceae	tussock	0.000/0.017
Sanguisorba minor	Rosaceae	dwarf shrub	0.000/0.023
Seseli montanum	Apiaceae	erect leafy	0.023/0.006
Stipa pennata	**Poaceae**	**tussock**	**0.000/0.236**
Taraxacum fulvum	Asteraceae	rosette	0.025/0.023
Teucrium chamaedrys	Lamiaceae	dwarf shrub	0.000/0.017
Teucrium montanum	Lamiaceae	dwarf shrub	0.017/0.040
Microthlaspi perfoliatum	Brassicaceae	erect leafy	0.006/0.006
Thymus dolomiticus	**Lamiaceae**	**dwarf shrub**	**0.029/0.218**
Veronica austriaca	Plantaginaceae	rosette	0.000/0.006

Chapter 2

Rangeland ecohydrology



(experimental plot at La Fage-station, *spring 2011*)

MANUSCRIPT II

Which trade-off between ecosystem productivity and water stress? Evidence from a 5-years measurement series in a Mediterranean rangeland.

-

Quel compromis entre productivité et stress hydrique à l'échelle de l'écosystème ? Apports et précisions après 5 ans de mesure dans des parcours méditerranéens

Karim Barkaoui, Marie-Laure Navas, Alain Blanchard, Pablo Cruz, Eric Garnier, Catherine Roumet, Florence Volaire

Abstract

Attempts to formulate general principles coupling plant water consumption and ecosystem processes have emphasized the existence of equilibrium conditions between water demand and supply, suggesting that ecohydrological optimization mechanisms underlie the structure of natural vegetation. However, such equilibrium assumptions usually focused on hydrologic processes while oversimplifying the representation of ecological processes. In this study, we aimed to clarify the 'ecohydrological optimality' framework by focusing on ecosystem functioning in relation to soil hydrology. Building on the hypothesis that vegetation of water-limited environments is constrained in its ability to maximize water use by the need of simultaneously minimize water stress, we tested whether biomass productivity and water-use efficiency (WUE) control the water balance of the ecosystem, and thereby soil water deficit, within contrasting plant communities from a Mediterranean rangeland. We used both a synchronic (12 plant communities) and diachronic (5-years measurement series) approach to evaluate the importance of both soil properties and climate on local water availability, water stress and ecosystem processes. Successive field measurements of aboveground biomass combined with a modeling approach of soil water dynamics enabled us to analyze the patterns of productivity, WUE and water stress for each plant community across seasons. We showed that biomass production in spring was always proportional to soil water availability while WUE did not differ among communities. In summer, pulses of growth occurred when rainfall water was sufficient during the season. We also found that water stress was similar for all plant communities across the 5 years of study whatever the soil water storage capacity. These results suggest that water stress was strongly buffered by vegetation productivity, but not by WUE, in accordance with local soil water availability over time, supporting the existence of an invariant 'productivity-water stress' trade-off in this Mediterranean rangeland. Applying the allometric theory to ecosystems, we therefore propose that the 'ecohydrological optimality' may be better understood as a dynamic allometric relationship between the adjusted aboveground biomass of vegetation and corresponding evapotranspiration flux. Such an integrated understanding of water dynamics within soil-vegetation systems in relation to climate is essential to predict both short- and long-term responses of ecosystems to water shortage, especially in sensitive areas where water availability is already scarce such as in the Mediterranean.

Résumé

La recherche de principes généraux reliant l'utilisation de l'eau par les plantes et les fonctions écosystémiques a suggéré que des mécanismes d'optimisation écohydrogique sous-jacents à la structuration de la végétation devaient conduire à des conditions d'équilibre entre la demande et l'approvisionnement d'eau. Cependant, ces hypothèses d'équilibre fonctionnel ont été essentiellement construites sur des processus hydrologiques avec une représentation souvent simpliste des processus écologiques. Dans cette étude, nous avons cherché à clarifier le cadre conceptuel de 'l'optimalité écohydrologique' en nous intéressant plus spécifiquement au fonctionnement des écosystèmes en relation avec le fonctionnement hydrologique des sols. Partant de l'idée que la capacité de la végétation des environnements secs à maximiser son utilisation de l'eau est fortement limitée par la nécessité de minimiser les stress hydriques, nous avons testé dans quelles mesures la productivité et l'efficacité d'utilisation de l'eau (WUE) contrôlait le bilan hydrique des écosystèmes, et donc le déficit hydrique des sols, au sein de différentes communautés de plantes dans des parcours méditerranéens. Nous avons eu à la fois une approche synchronique (12 communautés) et diachronique (5 ans de suivi) pour évaluer l'importance des propriétés physiques des sols et du climat dans la disponibilité en eau locale, le stress hydrique et les fonctions écosystémiques. Des mesures séquentielles de la biomasse aérienne, combinées à une démarche de modélisation de la dynamique de l'eau du sol, nous ont permis d'analyser les patrons de productivité, de WUE et de stress hydrique pour chaque communautés de plantes. Au printemps, nous avons montré que la production de biomasse était toujours proportionnelle à la disponibilité en eau du sol, sans modification de WUE d'une communauté à l'autre. En été, des pics de croissance ont eu lieu lorsque l'eau des pluies a été suffisante au cours de la saison. Par ailleurs, nous avons trouvé que le stress hydrique subi était similaire pour toutes les communautés au cours des 5 ans de l'étude, quel que soit la capacité de rétention du sol. Ces résultats suggèrent que le stress hydrique a été fortement tamponné par la productivité de la végétation mais non pas par sa WUE, en réponse à la disponibilité en eau locale au cours du temps. Cela soutient l'hypothèse de l'existence d'un compromis stable entre productivité et stress hydrique dans ces parcours méditerranéens. En appliquant la théorie allométrique aux écosystèmes, nous proposons donc que 'l'optimalité écohydrologique' peut être interprétée comme une relation allométrique dynamique entre la biomasse aérienne ajustée de la végétation et les flux d'évapotranspiration. Une compréhension intégrée de la dynamique de l'eau dans les systèmes plante-sol en relation avec le climat est essentielle pour prédire la réponse des écosystèmes à la réduction de la disponibilité en eau prévue par le changement climatique, particulièrement dans les régions sensibles où l'eau est déjà limitante comme en Méditerranée.

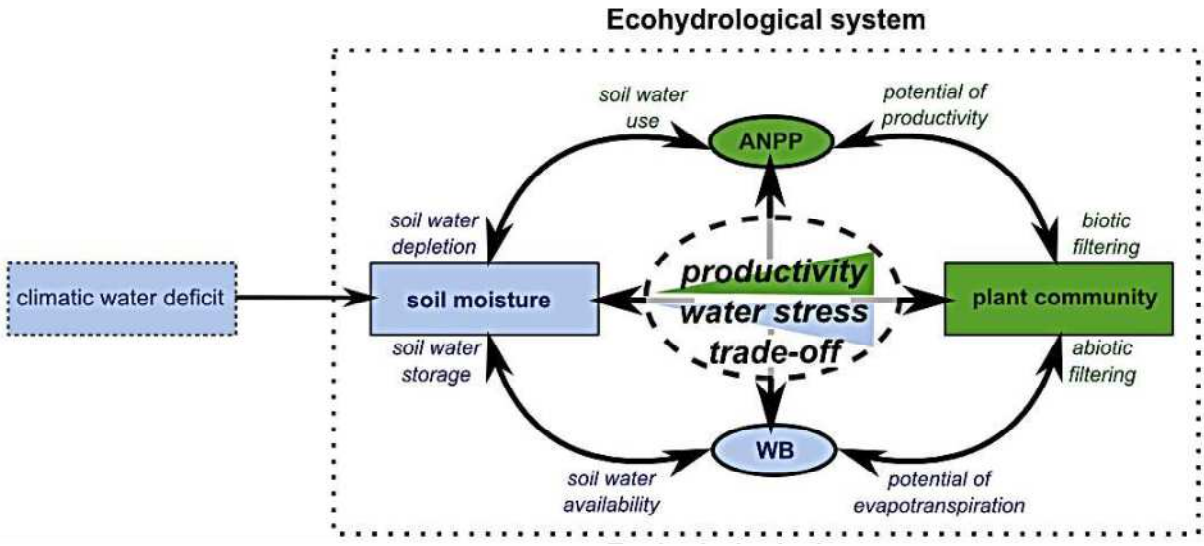


Fig. 1 Conceptual representation of the 'ecohydrological optimality' hypothesis (Egleason et al. 1982ab; Hatton et al. 1997; Egleason 2002; Kerkhoff et al. 2004) highlighting interactive relationships between hydrological and ecological processes, as well as the 'productivity-stress' trade-off. Boxes represent state (or forcing) variables, while grey ellipses represent processes.

Introduction

Despite a long history of scientific studies linking water cycle and ecosystem functioning, a comprehensive understanding of the interdependencies and interactions between hydrologic and ecological processes is still needed to provide integrated insights into ecosystem dynamics (Newman et al. 2006; Asbjornsen et al. 2011; Jenerette et al. 2012). In the context of deep alteration of hydrologic regime predicted by IPCC scenarios (IPCC 2007; Zhang et al. 2007), a central challenge for ecohydrology is the identification of relevant underlying mechanisms that govern vegetation structure and functioning in both space and time with respect to water dynamics (Solé et al. 2007).

Part of the challenge concerns the determinism of the relationships between plant aboveground net primary productivity (ANPP) and ecosystem water balance (WB) which could be conceptualized as a double-feedback relationship (Fig. 1). It is now well documented that ANPP, a key integrative property of ecosystem functioning, is mainly driven by water availability since it generally increases across ecosystems with increasing mean annual precipitation (Le Houérou et al. 1988; Sala et al. 1988; Knapp & Smith 2001; Huxman et al. 2004; Wu et al. 2011; Ruppert et al. 2012). In water-limited environments, the 'pulse-reserve paradigm' predicts that a 'pulse' of biomass production is stimulated as soon a precipitation event exceeds a certain 'biologically active' amount of water (Noy-Meir 1973; Schwinning et al. 2004; Jenerette et al. 2008). Over time, temporal water stress due to delayed water supply results almost always in an ANPP reduction (Fay et al. 2003; Miranda et al. 2009). Conversely, several studies outlined the reciprocal effect of vegetation activity on soil water content (Breshears & Barnes 1999; Nippert & Knapp 2007; Salve et al. 2011; Liancourt et al. 2012) showing that more productive species cause larger soil water depletion. In the absence of significant disturbance, such a tight coupling between plant water consumption for growth and soil water availability suggests that natural soil-vegetation systems may have co-evolved to achieve an optimal WB that maximizes plant water use while minimizing the overall occurrence of water stress.

Theoretical investigations of soil-plant-climate dynamics (Feddes et al. 1988; Rodriguez-Iturbe et al. 1999; Laio et al. 2001; Poporato et al. 2001; Schymanski et al. 2009) have suggested a series of general principles that may underlie the co-organization between vegetation properties and hydrological constraints in water-limited environments. Based on the seminal work of Eagleson (Eagleson 1982a, b; Eagleson & Segarra 1985) and accounting for subsequent re-evaluations (Salvucci & Entekhabi 1994; Hatton et al. 1997; Kerkhoff et al. 2004), an 'ecohydrological optimality' hypothesis is defined around a central trade-off between water-use (plant growth) and drought stress (plant survival) affecting both biomass production and water

flows partitioning at the ecosystem level (Rodriguez-Iturbe et al. 1999; MacKay 2001; Kerkhoff et al. 2004; Caylor et al. 2009). The hypothesis predicts that ecosystem ANPP, under demographic, functional and evolutionary processes, converges to a rate which balances the rate of water supply in the environment through the regulation of maximal evapotranspiration (ET_m) and water use efficiency (WUE). In some respects, these predictions are consistent with the allometric theory (Enquist & Niklas 2001) extended at the ecosystem level (Enquist et al. 2003; Savage 2004; Kerkhoff et al. 2006; Enquist et al. 2007) which predicts that ecosystem processes scale with the total plant biomass in whole plant communities. Over short-time periods, vegetation canopy density or biomass should therefore equilibrate with local climatic conditions and soil hydraulic characteristics (Rodriguez-Iturbe et al. 1999; Price et al. 2012), dimensioning an optimal ET_m to minimize water stress. Over successional time, species turn-over driven by successive droughts at a site should generate an optimal vegetation structure with the most adapted species strategies to maximize plant transpiration at the expense of soil evaporation, as well as to maximize CO2 uptake rates in relation with water-use (Schymanski et al. 2007).

Using a modeling approach, several studies have proposed mechanistic refinements of the optimality hypothesis, integrating both carbon and water economies of photosynthesis processes from the leaf - (Cowen and Farqhar 1977) and the canopy level (Schymanski et al. 2008) towards the 'productivity-water stress' trade-off at the ecosystem level (Schymanski et al. 2009). Yet, comparative field-oriented studies are still lacking among contrasting plant communities and environments to provide empirical evidence for this renewed 'ecohydrological optimality hypothesis'. For instance, despite the well-known importance of soil characteristics in controlling and mediating the hydrological processes, only few experiments have explicitly taken into account soil variability to analyze the responses of ecosystem processes (Vicca et al. 2012; Beier et al. 2012). In particular, soil properties such as soil depth and texture have a key role in WB since they determine the water holding capacity and thereby the dynamics of water availability as well as the ability of the ecosystem to buffer water fluctuations under a certain climate (Noy-Meir 1973; Fernandez-Illescas et al. 2001; Weng & Luo 2008). In addition, the characterization of vegetation properties is poorly documented in most studies related to WB modeling although plant community composition and structure can strongly influence water partitioning into either infiltration, evapotranspiration or run-off through their effect on ecosystem functioning (Huxman et al. 2005; Scott et al. 2009; Urgeghe et al. 2010). Refining the assessment of ecosystem water-related responses and testing the general relevance of the 'ecohydrological optimality' hypothesis should therefore rest on comparative studies providing an accurate description of

environmental gradients and accounting for both soil and vegetation properties variability (Vicca et al. 2012; Austin 2013).

In this study, we aim to explore whether the 'ecohydrological optimality hypothesis' holds across a range of contrasting plant communities distributed along a gradient of soil water availability. We hypothesized that adjustments in ANPP and WUE equilibrate the water balance in relation with soil water availability towards an invariant 'productivity-water stress' trade-off at the ecosystem level. We use a comparative approach based on a five years data set from a species-rich Mediterranean rangeland with varying edaphic conditions and a strong seasonality in precipitation to address three questions: are patterns of ANPP, WUE and soil WB associated with (i) maximized productivity and (ii) minimized water stress despite decreasing water availability? And finally, (iii) does the scaling relationship between ecosystem evapotranspiration and ANPP support the 'productivity-water stress' trade-off, both annually and seasonally?

Materials and methods

Study site and climatic conditions

The study was carried out on dry calcareous rangelands of southern France, located on a limestone plateau (Larzac Causse) at the INRA La Fage experimental station (43°55´N, 3°05´E, 790 m a.s.l.), 100 km northwest of Montpellier. At landscape level, vegetation is dominated by perennial herbaceous species (*Bromus erectus*, *Festuca christiani-bernardii*, *Carex humilis)*, along with loosely scattered shrubs (*Buxus sempervirens*, *Juniperus communis)*. At local scale (approximately few meters), plant communities are highly variable in composition and structure, including many different growth forms in different proportions, such as grasses, woody dwarf shrubs, forbs and rosettes.

Climate on the plateau is sub-humid with a strong Mediterranean influence. Cool and wet winters alternate with warm and dry summers. The long-term mean annual precipitation range from 680 to 1790 mm, occurring mainly during early spring and autumn. Mean monthly temperatures vary from 1°C in January to 19°C in August (data from 1973–2013). Precipitation, temperatures, solar radiation, air humidity and wind speed were recorded at a daily time-step by a meteorological station located at our study site. Growing degree days (GDD) were calculated as the difference between mean daily temperatures and base temperature (2.5 °C). If the mean daily temperature was lower than the base temperature, then GDD=0. Potential evapotranspiration (PET) was calculated using standard Penman-Monteith equation (Allen et al. 1998; Allen et al. 2005), and climatic deficit (P-ETP) was calculated as the difference between precipitation and PET on a given period.

Environmental gradient and study design

Soils are dolomitic rendzinas distributed as a mosaic of different depths and texture. Twelve plots (6 x 9 m) up to 1500 m apart were selected to span a gradient of the widest possible range of soil types: from the shallow and dry soils of dolomitic sand to deeper and moister clay soils. Soil characteristics were assessed in each plot to quantitatively characterize the gradient (for further details see also Perez-Ramos et al. 2012; Bernard-Verdier et al. 2012). Mean soil depth (ranging from 20 to 96 cm) was determined in each plot using 11-13 randomly distributed soil cores (5 cm wide). Soil physico-chemical properties were assessed for three of these cores, which were divided into 10 cm thick layers from the soil surface down to the bed rock in order to account for the variability along the whole soil profile. In particular, soil texture and organic matter content, which are known to strongly influence soil water retention properties (Cosby et al. 1984), were measured in each soil layers with standard procedures (Afnor 1994).

Soil water content (SWC) was measured bi-weekly since spring 2008 using capacitance moisture probes (*DIVINER 2000*, Sentek Pty Ltd, Stepney, Australia), which provided a complete profile of soil humidity at three permanent locations in each plot. The probes were previously calibrated according to soil texture to account for the differences in soil water retention of each soil layer (Geesing et al. 2004; Groves & Rose 2004).

Modeling soil water dynamics (water balance model)

Temporal dynamics of soil water was interpolated from SWC measurements with a bucket type modeling approach by applying a volume-balance equation over the entire root zone of vegetation (Rodriguez-Iturbe et al. 1999; Laio et al. 2001):

$$n \cdot Z_r \cdot \frac{ds}{dt} = \varphi[s(t),t] - \chi[s(t),t] \qquad \text{eqn 1}$$

where n is the soil porosity, Z_r is the average rooting depth of vegetation, $s(t)$ is the relative soil water content over the root zone ($0 \leq s(t) \leq 1$), $\varphi[s(t),t]$ is the incoming water flow and $\chi[s(t),t]$ is the outcoming water flow. Both n and Z_r are time-invariant parameters, and it is assumed that n depends only on soil texture while Z_r depends only on vegetation properties. Their product, $n \cdot Z_r$, gives the volume in the soil that may be filled with water over the root system, *i.e* the soil water holding capacity, and $s(t)$ represents its relative saturation over time. Three physical states of soil moisture are remarkable; (i) 'soil saturation' ($s(t) \to 1$): soil is completely saturated of water and flows of free water occur through the soil column; (ii) 'field capacity' ($s(t) = s_{fc}$): after water excess has drained away, the system reaches an equilibrium state,

and the amount of water hold by soil at this state is maximal; (iii) 'hygroscopic point' ($s(t) \to 0$): soil has completely dried out and only a minor amount of water remains. Dynamics from one state to another as well as the quantity of water involved in corresponding water flows are highly dependent on soil texture. For instance, coarse texture soils (*e.g* sandy soils) have lower porosity, and thus lower water holding capacity than fine textured soils (*e.g* clay soils), but they have greater soil conductivity favoring more rapid soil water dynamics.

The infiltration rate of rainfall $\varphi[s(t),t]$ represents the part of rainfall that effectively supplies the soil reserve, accounting for water losses before infiltration:

$$\varphi[s(t),t] = R[t] - I[t] - Q[(s(t),t)] \qquad \text{eqn 2}$$

where $R[t]$ is rainfall; $I[t]$ and $Q[(s(t),t)]$ are the amount of rainfall water lost by canopy interception and runoff respectively. Contrary to original modeling approach by Rodriguez-Iturbe et al. (1999), rainfall was not described by a statistical law, but observed precipitation data were used. Canopy interception has been usually incorporated into such water-balance model by fixing a threshold of minimum rainfall height below which no water reaches the ground, but for the sake of simplicity, we neglected these processes here, *i.e* $I[t] = 0$, because vegetation at our study site was essentially constituted by small plants (mean plant height in spring 2012 was 8.21±2.70 cm) with relatively low values of LAI (mean LAI in spring 2012 was 1.21±0.38 m m^{-2}). Runoff $Q[s(t),t]$ was modeled by a Dunnian saturation mechanism, *i.e* runoff was assumed to take place only when rainfall exceeded soil water holding capacity at a certain time.

Outcoming water flow $\chi[s(t),t]$ is theoretically determined by water losses from the soil due to plant transpiration, passive soil evaporation and leakage. Although plant transpiration and soil evaporation are governed by different mechanisms, they were merged together and evapotranspiration was modeled as the unique water flow towards the atmosphere:

$$\chi[s(t),t] = ET[s(t)] + L[(s(t)] \qquad \text{eqn. 3}$$

where $ET[s(t)]$ and $L[(s(t)]$ are the amounts of water lost by evapotranspiration and leakage respectively. Leakage losses $L[(s(t)]$ towards deeper soil layers were neglected here because the entire soil profile was extensively and entirely explored by roots. We therefore assumed that water infiltration into the underneath bed rock should represent only a minor fraction compared to evapotranspiration. Following Laio et al. (2001), evapotranspiration $ET[s(t)]$ has been described by a four-step function that depends on actual value of $s(t)$ (see rationale in supporting information, Fig. S2):

$$ET[s(t),t)] = \begin{cases} 0 & s(t) < s_h \\ \frac{s(t)-s_h}{s_w-s_h} \cdot E_w & s_h \le s(t) \le s_w \\ E_w + \frac{s(t)-s_w}{s^*-s_w} \cdot (ET_m - E_w) & s_w \le s(t) \le s^* \\ ET_m & s(t) > s^* \end{cases} \quad \text{eqn. 4}$$

where s_h is hygroscopic point, s_w is wilting point, s^* is stress threshold below which evapotranspiration rates are reduced, ET_m is maximum rate of evapotranspiration when soil water is above the stress threshold, and E_w is soil evaporation when soil water is at the wilting point. Field capacity s_{fc} (-0.03 MPa) and hygroscopic point s_h (-3.1 MPa) were estimated using empirical equations from Saxton & Rawls (2006) with texture data for each soil layer. While wilting point s_w has been broadly estimated at a standard soil water potential in modeling approaches (-1.5 Mpa), it actually results from the interaction between soil physical characteristics and vegetation properties. Given the wide variability among soil types and plant communities in this study, we preferred to consider s_w as a model parameter to be fitted for each community and derive the 'total transpirable soil water' (TTSW) which represents the *potential* (maximal) amount of water that plants of a given community can extract for transpiration (Ritchie 1981; Sinclair & Ludlow 1986) as:

$$TTSW = s_{fc} - s_w \quad \text{eqn. 5}$$

The stress threshold s^* was then fixed at 30 % of TTSW (Vicca et al. 2012). Similarly, maximum rate of evapotranspiration ET_m was assumed to vary depending on local vegetation properties. For instance, we applied the 'crop coefficient method' (Wight 1990; Allen et al. 1998; Allen et al. 2005) considering each plant community as a whole:

$$ET_m = k \cdot PET \quad \text{eqn. 6}$$

where PET is potential evapotranspiration; and k is model parameter to be fitted for each plant community. Soil evaporation at wilting point E_w was fixed at 0.1 mm day^{-1} independently of soil characteristics (Laio et al. 2001).

The water-balance model was adjusted to the repeated measurements of SWC across the five years of study for each community separately. We used pseudo-random search

optimization (Price, 1977) to estimate the two parameters (s_w, k) using ordinary least square method, and calculate the *TTSW* of each community. We then performed a bootstrap re-sampling procedure

with 1000 iterations to evaluate the predictive power of the model. At each iteration, predictions were tested against observed values, and after 1000 iterations, the procedure provided an empirical distribution of model efficiency (R^2), slope and intercept of the relationship between predicted and observed values accounting for the heterogeneity in dataset. Relative mean absolute error (RMAE) was also calculated for each iteration.

Assessing plant water stress at the community level

A way of assessing plant response to water stress at the community level is to quantify the reduction of evapotranspiration rate over time. The difference between maximal and actual evapotranspiration rate may indicate how much energy plants have to develop to extract water and therefore how far they are from their optimal functioning. With this purpose, we calculated the 'dynamic water stress' (Porporato et al. 2001; Bartholomeus et al. 2010) which is an integrative index including the reduction of evapotranspiration during drought in combination with mean intensity, mean duration and mean frequency of drought events over a given period, usually over the growing season. We calculated this index for each plot across the five years of study as follows:

$$\bar{\theta}[s(t)] = \begin{cases} \left(\dfrac{\overline{\Delta ET_{s^*}} \cdot \overline{D}}{r \cdot D_{seas}} \right)^{\left(1/\sqrt{\overline{F}}\right)} & \overline{\Delta ET_{s^*}} \cdot \overline{D} < r \cdot Dseas \\ 1 & otherwise \end{cases} \qquad \text{eqn. 7}$$

where $\bar{\theta}[s(t)]$ is dynamic stress index (dimensionless); $\overline{\Delta ET_{s^*}}$ is mean reduction of evapotranspiration during drought period compared to ET_m (mm day^{-1}); $\overline{D}$ is mean duration of drought events (days); D_{seas} is length of the growing season (days); $\overline{F}$ is mean frequency of drought events over the growing season; and r is a parameter describing vegetation resistance to drought (mm day^{-1}). Length of growing season D_{seas} was considered to be restricted by temperature constraints and was calculated for each year as the number of days for which GDD>0. Plant resistance parameter r was unknown and was therefore fixed to 0.5 for all type of vegetation (Laio et al. 2001). The other variables $\overline{\Delta ET_{s^*}}$, $\overline{D}$ and $\overline{F}$ were derived from

modeled soil water dynamics. In particular, a drought event was defined as the time course of soil water content below the stress threshold $s*$ (30 % of TTSW, see Fig. S3).

The index gives a good description of how the vegetation locally 'perceives' water stress in a given environment. However, to unravel the role of both soil conditions and vegetation properties, we followed an approach initiated by Dyer (2009) and Bartholomeus et al. (2010), and computed the reduction of evapotranspiration $\overline{\Delta ET_{s*}}$ of a reference vegetation instead of the actual vegetation using simulations of soil water dynamics from the WB model. The resulting 'reference stress index' reflects the hydrological behavior of soil independently of the actual vegetation, and is therefore better representative of the energy required for plants to extract water. As reference vegetation, we used a virtual standardized grassland as proposed by the Food and Agriculture Organization (Allen et al. 1998; Allen et al. 2005). The same reference vegetation was used for the calculation of potential evapotranspiration PET in the WB model, *i.e* $ET_m = PET \quad (k = 1)$ for this vegetation. The effect of an 'adaptation' of the actual vegetation on water stress was calculated the relative difference with the reference stress index:

$$RD_{\bar{\theta}} = \frac{\left|\bar{\theta}_{actual} - \bar{\theta}_{reference}\right|}{\bar{\theta}_{reference}} \qquad \text{eqn. 8}$$

Under a Mediterranean climate, the occurrence of drought events is not uniformly distributed during the growing season since there is a strong seasonality with more intense and more frequent drought events during summer. Therefore, in addition to the dynamic water stress which gives an integrated overview of the selective pressure exerted by water stress at a site across several successive years, we accounted for the seasonality in drought events by calculating the reduction of evapotranspiration rates in spring and summer separately. Both seasons were compared using one-way ANOVA, and the differences along the soil gradient were assessed for each season and each year using linear regressions between actual ET and ET_m.

Seasonal ANPP and WUE of vegetation along the gradient

Aboveground biomass (AGB) was collected from 2-3 quadrats (0.25 m^2) located within each plot along the soil gradient during the five years of study. Quadrats were each year replaced in a different location within the plot to avoid reharvesting the same quadrat. AGB was harvested by clipping all vascular plant biomass to ground level. Harvests occurred both at peak vegetation before summer drying (end of May-June) and during summer (end of July-August). Mosses, lichens were sorted out as well as dead plant tissues. Samples were then oven-dried at 60°C for

Table 1 Model selection for the relationship between ANPP and soil depth ('depth'), cumulated growing degree day ('GDD'), and cumulated climatic deficit ('P-PET'). Selection was performed separately in spring and summer. Best models showing the lowest AIC (ΔAIC=0) and the highest AIC weight are indicated in bold; models with ΔAIC<2 are indicated in italic. Marginal and conditional R^2 (R^2_m and R^2_c, respectively) are given for each model.

		spring				summer			
model	df	ΔAIC	AIC weight	R^2_m	R^2_c	ΔAIC	AIC weight	R^2_m	R^2_c
(intercept)	3	40.37	<0.0001	-	0.60	59.46	<0.0001	-	0.48
~'depth'	4	2.06	0.1344	0.19	0.81	57.74	<0.0001	0.03	0.51
~'GDD'	4	38.41	<0.0001	0.27	0.62	60.65	<0.0001	0.06	0.55
~'P-PET'	4	40.61	<0.0001	0.14	0.64	55.98	<0.0001	0.29	0.48
~'depth'+'GDD'	5	**0**	**0.3586**	**0.46**	**0.81**	58.93	<0.0001	0.08	0.87
~'depth'+'P-PET'	5	2.20	0.1978	0.31	0.83	54.25	<0.0001	0.32	0.51
~'GDD'+'P-PET'	5	39.74	<0.0001	0.27	0.66	56.93	<0.0001	0.29	0.52
~'depth'x'GDD'	6	*1.72*	*0.1520*	*0.46*	*0.81*	39.43	<0.0001	0.21	0.71
~'depth'x'P-PET'	6	4.19	0.0440	0.31	0.82	4.42	0.0989	0.59	0.81
~'GDD'x'P-PET'	6	41.08	<0.0001	0.21	0.75	50.44	<0.0001	0.44	0.46
~'depth'+'GDD'+'P-PET'	6	*1.33*	*0.1844*	*0.43*	*0.84*	55.20	<0.0001	0.32	0.55
~'depth'x'GDD'x'P-PET'	10	7.85	0.0071	0.33	0.87	**0**	**0.9011**	**0.74**	**0.79**

48 h, weighted and averaged at the plot level. Live AGB at peak of vegetation was considered as a good proxy of spring ANPP given that winter frost repeatedly causes a complete senescence of plant tissue (Scurlock et al. 2002). Summer ANPP was estimated by the difference between live AGB from spring and summer harvests. WUE was estimated by dividing ANPP values by the corresponding cumulated ET values derived from soil water dynamics over the same time period. In spring, cumulative GDD were computed from the beginning of the growing season, when GDD was above zero for several consecutive days at the end of winter.

We used linear mixed models (Bolker et al. 2009) to test the effect of soil depth on ANPP and WUE accounting for seasonal climatic conditions of each year. Data from spring and summer were analyzed in the same way but separately. We first started with intercept random models (*e.g variable~1|random*) with year ('*year*') as random variable to decompose the inter-annual variability. More complex models were then built (*e.g variable~fixed+1|random*), including successively soil depth ('*depth*'), cumulated degree day ('*GDD*'), cumulated climatic deficit ('*P-PET*') as well as their interaction terms as fixed effects. Model selection was performed using the Akaike Information Criteria (AIC) by considering that lower AIC value indicated better fit to data. Models with ΔAIC<2 were considered equivalent to each other, and in this case, the most parsimonious model (*e.g* with smaller *df*) was preferred. In addition, we calculated AIC weights giving the likelihood of each model to be the best model in comparison to all the competing models tested. Using this approach, significance of random effect was tested by comparison with simple linear regression without random effect. Furthermore, significance of fixed effects in the best model was tested using Wald χ^2 test procedure. However, while AIC values provide an estimate of relative goodness of fit of several alternative models, they are limited in quantifying variance explained by these models (Orelien & Edwards 2008). Therefore, following the proposal of Nakagawa & Schielzeth (2013), we calculated the marginal R^2 (R^2_m) which describes the variance explained by the fixed factors alone, and the conditional R^2 (R^2_c) which describes the variance explained by both fixed and random factors. Error standard deviations of both fixed and random effects were estimated by maximizing the restricted log-likelihood (REML) using *lme4* package in *R*.

Testing the relationship between ANPP and ET across seasons

For the spring periods, the relationship between actual ET and ANPP at the ecosystem level was tested using 'standardized major axis' regressions (SMA, *smatr* package in *R*, Warton et al. 2012) after data were log-transformed. SMA regression minimizes residual variation in both variables (Warton et al. 2006) and is therefore more appropriate than ordinary least square regression for

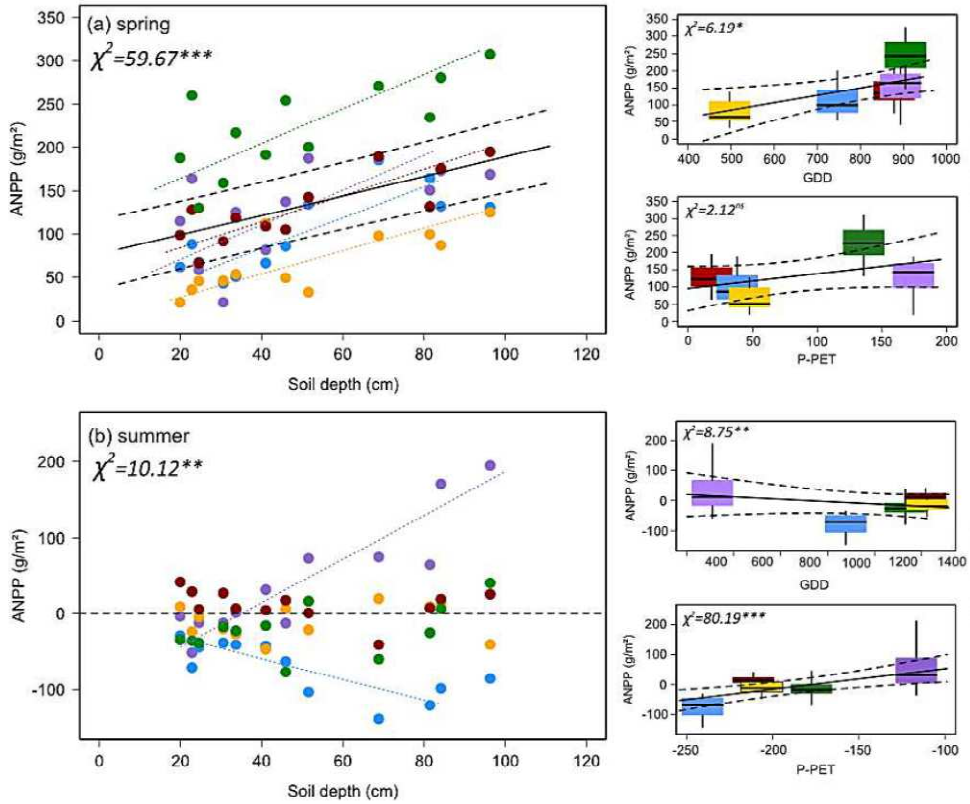


Fig. 2 Seasonal patterns of ANPP along the soil gradient resulting from (a) spring harvest of biomass, and (b) summer harvest of biomass. Data points represent mean ANPP values for each plot. Colors correspond to the five years of study (purple: 2008; blue: 2009; yellow:2010; red: 2011; green: 2012). Colored dotted lines were calculated by OLS-regression for year independently while black solid lines are the fixed effects as calculated from linear mixed model including year as random effect (see model selection in Table 1). Black dashed lines represent the confidence intervals at 95 %. Boxplot inserts show the effect of seasonal climatic conditions (GDD and P-ETP) on ANPP.

describing functional relationships among variables which are not clearly independent. Assuming that the values of ET rate and ANPP were maximum at the peak of vegetation in spring because of no water stress, we used spring values of ET and ANPP to define potential values for the successive summer periods (ET_m and $ANPP_m$ thereafter) as if there was no additional water limitation. Reductions in ET rates during summer were then quantified for each year by calculating the relative differences with corresponding ET_m values; *i.e* $(ET - ET_m)/ET_m$. Similarly, reductions in ANPP were quantified for each year by calculating the relative differences with corresponding $ANPP_m$ values from spring, *i.e* $(ANPP - ANPP_m)/ANPP_m$. A positive (negative) relative difference means that summer ET (or ANPP) was greater (lower) than the potential value of rates defined in spring; zero values indicates that ET rates and ANPP were similar in summer than in spring. For ANPP, differences in cumulated GDD between spring and summer were accounted for by normalizing values of ANPP by the corresponding cumulated GDD over the considered period.

Results

Seasonal ANPP patterns

Linear mixed models provided general better fit to data than simple linear model (Table 1), indicating the relevance of including 'year' as random effect. In spring, the best model explaining variations in ANPP included 'soil depth' and 'GDD' as fixed effects (R^2_m=0.46, R^2_c=0.81, Table 1). Soil depth had a strong positive effect on ANPP (χ^2=59.67, p<0.0001), and slopes of the relationship between ANPP and soil depth were found to be very similar from one year to another (Fig. 2a) when years were analyzed separately (LLR=2.741, df=4, p=0.60). The relationship between ANPP and cumulated GDD was also significant, influencing positively intercept values (χ^2=6.19, p=0.01, Fig. 2a), but the relationship with cumulated P-PET was not (χ^2=2.12, p=0.15, Fig. 2). In summer, variations in ANPP were best explained by a full model including 'soil depth', 'GDD', 'P-PET' and their interaction terms as fixed effects (R^2_m=0.72, R^2_c=0.79, Table 1). Similarly to spring, soil depth had a significant positive although weaker effect on ANPP (χ^2=10.12, p<0.01), and cumulated GDD influenced mean values of ANPP (χ^2=8.75, p<0.01). By contrast however, cumulated P-PET had a strong negative effect on ANPP (χ^2=80.19, p<0.0001) and effect of cumulated GDD shifted from positive to negative (Fig. 2b). Moreover, the interaction term between soil depth and P-PET was found to be the strongest in summer (χ^2=41.63, p<0.0001) and to negatively influence mean values of ANPP (Fig. 2b). Consequently, slopes of the relationship between ANPP and soil depth differed significantly

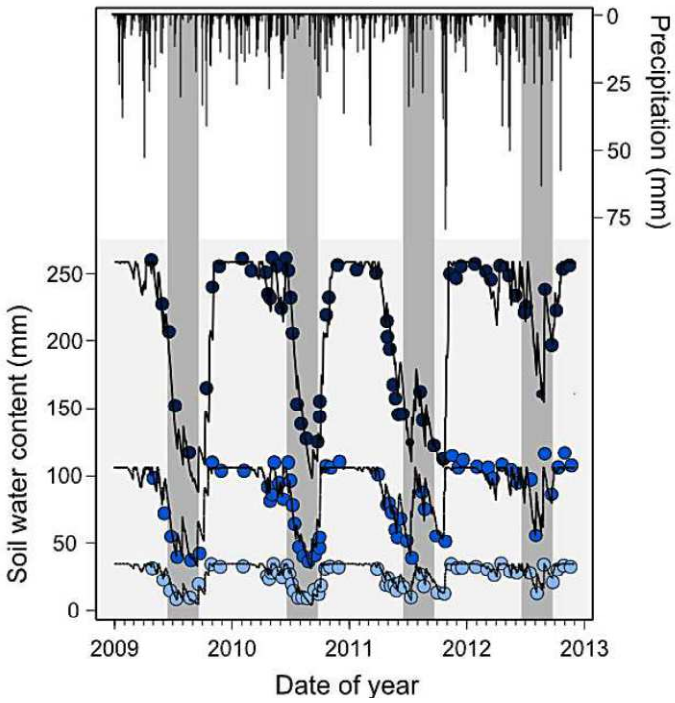


Fig. 3 Examples of soil water dynamics over the five years of study (2008-2013) within three contrasting communities along the soil gradient. Data points represent measurements of SWC in the field using permanent Diviner probes. Probes were previously calibrated according to soil texture. Black solid, dashed and dotted lines correspond to predictions from the adjusted water-balance model. Black vertical bars represent precipitation data provided by the local meteorological station at a daily-time step. Grey areas highlight summer periods (21st of June – 21st of September).

among years (LLR=20.60, df=4, p=0.0004). For instance, the slope was positive in summer 2008 for which cumulated P-PET was the lowest, whereas it was negative in summer 2009 for which cumulated P-PET was the highest. Years 2010, 2011 and 2012 were intermediate, with no significant slope. Overall, WUE did not vary significantly along the soil gradient neither in spring (χ^2=2.800, p=0.094) but it slightly did in summer (χ^2=8.677, p=0.003); especially a significant increase in WUE with soil depth (r^2=0.80, p=0.0001) was found in summer 2008 when years were analyzed separately. Moreover, variations in intercept among years were significantly explained by climatic deficit: greater P-PET resulted in lower WUE in summer (χ^2=13.08, p=0.0003).

Soil water dynamics and water stress at the community level

Once calibrated, the water balance model provided accurate predictions of soil water dynamics across the five years of study for each plant community (Fig. 3). Repeated regressions following the bootstrap procedure between predicted and observed values of soil water content (SWC) were all highly significant (mean r^2=0.99, p<0.0001, see Fig. S4) and did not differ significantly from the 1:1 line (see Fig. S5). The relative mean absolute error (RMAE) of predictions was very low (mean RMAE=0.092 %, Fig S3, S4 in supporting information), indicating that the calibration was not biased for low or large values of SWC. Parameter estimation of the model revealed that the total transpirable soil water (TTSW) varied significantly among communities (r^2=0.94, p>0.0001, Fig. 4): sites with deeper soils had higher TTSW. Similarly, maximum rates of evapotranspiration (ET_m) increased significantly with increasing soil depth (r^2=0.82, p<0.0001, Fig. 4).

The dynamic water stress $\bar{\theta}$ increased significantly towards more water-limited soils both for reference (r^2=0.87, p<0.0001, Fig. 5) and actual vegetation (r^2=0.22, p<0.01, Fig. 5), although values were always lower for the actual vegetation across the five years of study (Student t-test; t=-13.91, p<0.0001). The slope was however steeper for the reference vegetation than for actual vegetation along the soil gradient (Fig. 5). Consequently, the relative difference with reference significantly increased toward shallower soils (r^2=0.30, p=0.002, Fig.). Moreover, while less than 15 % of sites were below $\bar{\theta} = 0.5$ for reference vegetation, all sites were below this threshold for the actual vegetation.

Reduction of evapotranspiration rates differed significantly among seasons (F=596.99, p<0.0001) and years (F=36.82, p<0.0001), with a significant interaction effect (F=32.36, p<0.0001). While actual ET did not significantly differ from ET_m during spring (Fig. 6), it was systematically lower during summer drought (Fig. 6). Furthermore, relationships between actual

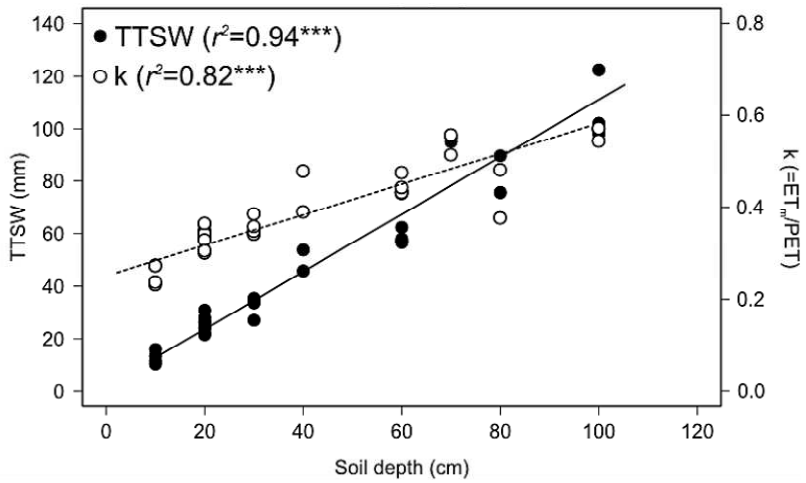


Fig. 4 Parameter values of the adjusted water-balance model along the soil gradient. Data points represent individual Diviner probes located in the selected plots. Black filled points correspond to TTSW values; open white points correspond to k values (ET_m/PET). Lines were calculated by OLS-regression.

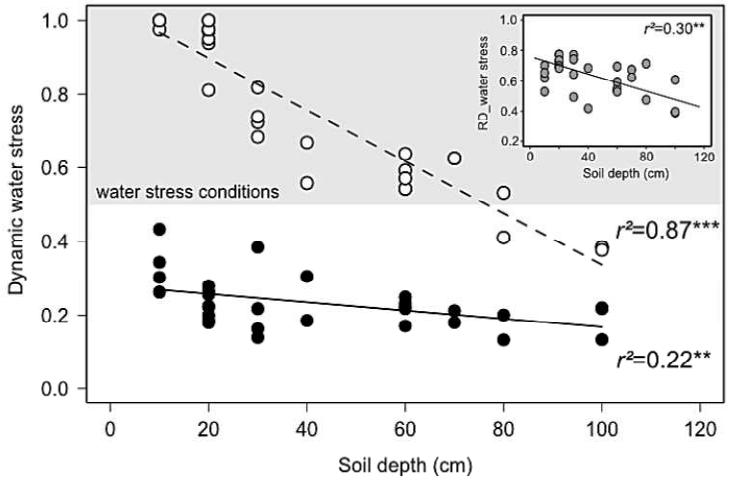


Fig. 5 Changes in dynamic water stress $\overline{\theta}$ along the soil gradient. Data points represent individual Diviner probes. Open white points correspond to the reference vegetation as defined by the FAO (Allen et al. 1998; Allen et al. 2005); black filled points correspond to actual vegetation. Lines were calculated by OLS-regression. The insert shows the relative difference (RD) with reference values along the soil gradient.

ET and ET_m did not differ from the 1:1 line during spring (Fig. 6, Table 2), but slopes were significantly larger than one during summer 2009, 2010 and 2012 (Fig. 6, Table 2), indicating that deviation from the 1:1 line tended to increase towards more water-limited soils.

Scaling relationships between ET and ANPP

In spring, ET varied allometrically with ANPP (r^2=0.48, p<0.0001, Fig. 7) with a general exponent equal to 0.47 (95 % CI: 0.39-0.57) and only slight differences in exponents were found among the five years of study (range: 0.32-0.81). In summer, the relative changes in both ET and ANPP with respect to corresponding spring values (ET_m and $ANPP_m$) were found to be significantly related (r^2=0.33, p>0.0001, Fig. 7).

Discussion

It is urgent to identify general principles underlying the functioning of ecohydrological systems (Fig. 1) in order improve the predictions of the impacts of climate change. The 'ecohydrological optimality' hypothesis is a suitable framework to analyze the equilibrium state that may be achieved between water balance and vegetation through a plant 'productivity-water stress' trade-off at the ecosystem level. Yet, despite several theoretical and modeling investigations, it is still unclear whether the predictions of the optimality hypothesis provide a basis for a general ecohydrological theory and thus it should be tested across a large range of plant communities and environments. In this comparative study, we used both a synchronic (gradient of soil water availability) and a diachronic approach (five years of data) to compare patterns of aboveground net primary productivity, water-use efficiency and water balance under different levels of water limitations (edaphic and climatic). We show that variations in ANPP, more than in WUE, controlled the level of water stress which was maintained at a similar level within all plant communities despite decreasing soil water availability. Moreover, the allometric relationship between ET and total plant biomass in spring, as well as the proportional reductions of both ANPP and ET in summer, suggest a strong scaling in processes related to water-balance at the ecosystem level.

Variations in ANPP along the soil gradient between years and seasons

Our results clearly support the relevance of a seasonal ANPP dynamics framework based on the coupling of both temperature and precipitation regimes (Knapp et al. 2006; Muldavin et al. 2008; Chollet et al. 2013). We highlight that seasonal climatic deficit, *i.e* the difference between precipitation and PET, may be a fundamental driver of ANPP dynamics in combination with

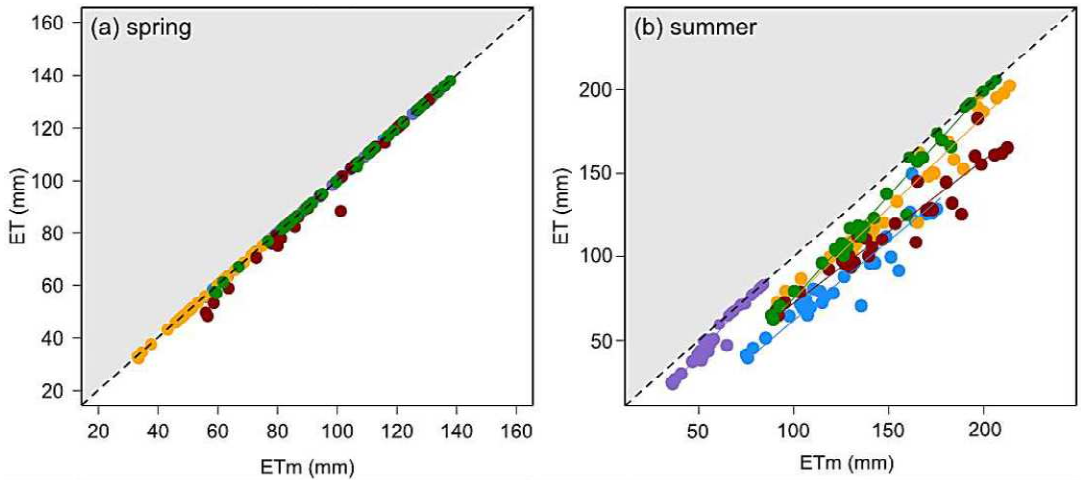


Fig. 5 Comparison between actual evapotranspiration ET and corresponding ET_m along the soil gradient with (a) spring values of ET and (b) summer values of ET. Data points represent individual Diviner probes. Colors correspond to the five years of study (purple: 2008; blue: 2009; yellow:2010; red: 2011; green: 2012). Black dashed lines represent the 1:1 line ($ET=ET_m$) and grey areas delimit the unrealistic situation where $ET>ET_m$. Lines were calculated by linear regression (SMA method) for each year independently. Relative deviation from the 1:1 line was tested using slope tests (Table 2).

Table 2 Reduction in rate of evapotranspiration during spring and summer. Mean and range of relative difference, as well as slope and R^2 of relationships between actual ET and corresponding ET_m are represented across the five years of study. Italic values in brackets are the confidence interval at 95 % of regressed slopes. Levels of significance are noted as follows: ^{ns} non-significant, * $p<0.05$, *** $p<0.0001$.

	spring			summer		
year	slope	R^2	*r* slope.test	slope	R^2	*r* slope.test
2008	1.00 *(1.01-0.99)*	1.00***	0.065^{ns}	1.31 *(1.22-1.41)*	0.96***	0.819***
2009	1.00 *(1.00-1.01)*	1.00***	0.386^{ns}	0.95 *(0.83-1.09)*	0.95***	0.881***
2010	1.00 *(1.00-1.01)*	1.00***	0.405^{ns}	1.09 *(1.01-1.19)*	0.95***	0.457*
2011	1.08 *(1.04-1.13)*	0.99***	0.581***	0.86 *(0.77-0.96)*	0.91***	0.380^{ns}
2012	1.01 *(1.00-1.02)*	1.00***	0.467*	1.24 *(1.17-1.31)*	0.98***	0.831***

cumulated GDD. The classical 'pulse-reserve paradigm' (Noy-Meir 1973) in which water is assumed to be the dominant limiting factor over the entire year has proven to be useful for the description of vegetation structure and dynamics in most arid and semi-arid lands, but there may be seasonal climatic patterns where it may not apply straightforward (Muldavin et al. 2008). This is especially the case in Mediterranean areas because of the strong seasonality of precipitation and temperature. In spring, precipitation was found to systematically exceed PET (see Table S1 and Fig. S1 in supporting information) reflecting typical temperate climate, and therefore the inter-annual variability of ANPP was essentially controlled by temperature (Fig. 2). By contrast, the relative importance of temperature and climatic deficit reversed during summer. Specifically, hydrologic conditions during summer were similar to that in semi-arid regions. Negative values of climatic deficit, indicating that precipitation was insufficient to meet the evaporative demand, primarily determined ANPP values (Fig. 2). Moreover, the amount of summer precipitation as well as climatic deficits, which varied considerably among years generated a large range of response for summer ANPP (from negative to positive), reflecting typical pulse-reserve phenomena. At our study site, we therefore suggest that spring plant growth benefited from the soil water stored in winter and responded to GDD rather than pulse precipitation, whereas the infrequent precipitation events occurring during summer reduced the strong climatic deficit and favored opportunistic pulses of biomass production.

The comparison of contrasting communities from one unique site with the same climate but distributed along an edaphic gradient allowed us to provide empirical insights into the role of soil characteristics on ANPP patterns. The revision of the 'pulse-reserve paradigm' (Reynolds et al. 2004; Ogle & Reynolds 2004) emphasizes that plant productivity under water-limited conditions may not be a direct response to rainfall -or climatic deficit-, but rather to soil water availability at local scale (Le Houérou et al. 1988; Knapp et al. 2006; Swemmer et al. 2007). We also show that greater soil water holding capacity, resulting from both greater soil depth and texture, may support greater ANPP in spring accounting for inter-annual variations in seasonal climatic conditions (Fig. 2). Moreover, we show that soil water holding capacity may also determine the extent of ANPP pulse or decline during summer. Deeper clay soils permitted greater ANPP pulse in wet summer 2008 (Fig. 2), reflecting that a greater amount of water stored following precipitation events may advantage plant growth. This difference in storage effect due to soil characteristics should be even more visible as storm events are getting larger. On the other hand, the ANPP decline was more important on deeper clay soils during severe drought in summer 2009 (Fig. 2), certainly because these soils exerted stronger suction forces as they dried out due to their finer texture (Cosby et al. 1984).

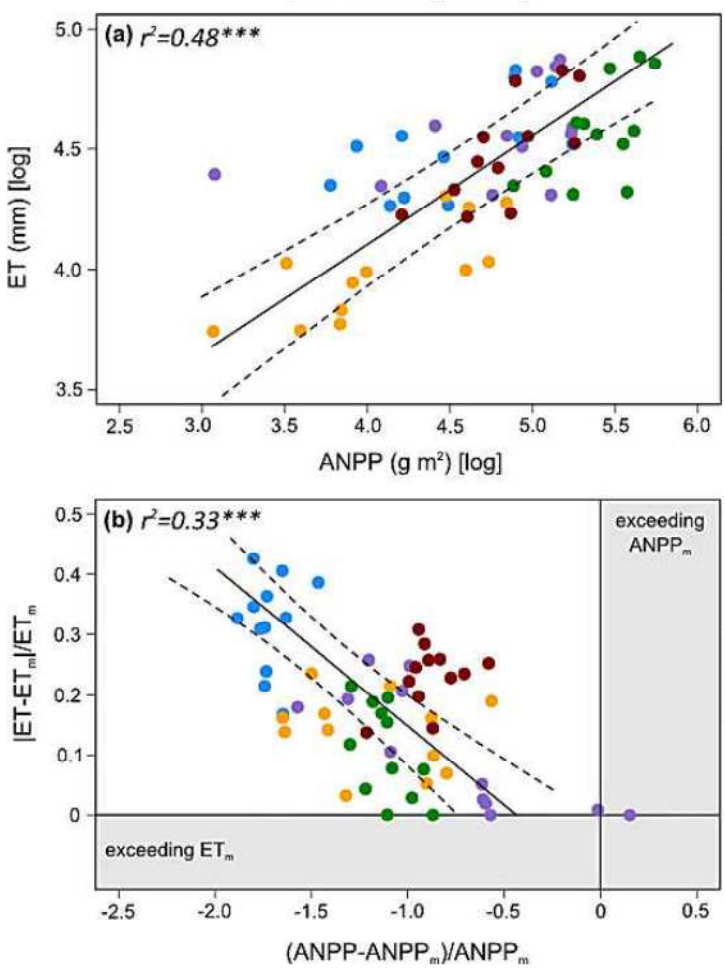


Fig. 7 Scaling relationships (a) between ET and ANPP at peak of vegetation in spring; and (b) between relative changes of ET and ANPP after summer drought. Data points represent mean values for each plot. Colors correspond to the five years of study (purple: 2008; blue: 2009; yellow:2010; red: 2011; green: 2012). Black solid lines were calculated by SMA-regression; black dashed lines are the corresponding confidence interval at 95 %. Grey areas delimit the situation where ET> ET_m (unrealistic), and ANPP>$ANPP_m$.

Overall, these ANPP patterns are compatible with the 'ecohydrological optimality' hypothesis which predicts that ecosystem productivity should be maximized. In spring, we show that temperature was limiting growth more than soil water availability, indicating that water use was maximum under these climatic conditions whatever the soil water holding capacity. In summer, ANPP was clearly limited by drought across years, but the pulse-induced growth in summer 2008 also suggests a maximization of productivity as soon as enough water is available in the environment. However, while the optimality hypothesis additionally predicts that WUE should be higher in water-limited environments or during drier periods, our results showed on the contrary that it did not have a significant role in the equilibrium between vegetation and soil water availability, highlighting the dominant role effects of size rather than efficiency.

Variations in water stress along the soil gradient between years and seasons

Our results highlight significant 'adaptative' modulation of vegetation functioning in response to decreasing soil water availability. Namely, plant communities greatly differed in their value of both TTSW and ET_m (Fig. 4) influencing soil water dynamics over time (Fig. 4). We show that TTSW was strictly proportional to soil water holding capacity, confirming our assumption that the whole soil profile was entirely explored by roots regardless to soil depth, and that the amount of water extractable by plants was primarily limited by soil volume and retention properties. Interestingly, ET_m was also proportional to soil water holding capacity, suggesting that vegetation may exert a strong control on water loss rates in relation with soil water availability. Because TTSW and ET_m linearly co-varied along the gradient, soil water dynamics was found similar among communities, although water flows were more important when soil water holding capacity was higher.

By modeling the soil water dynamics for each plant community separately using the adjusted values of TTSW and ET_m, we were able to evaluate the combined effect of both soil characteristics and vegetation properties on water stress. Our results provide strong evidence that an overall 'functional equilibrium' was achieved between the actual vegetation and local hydrologic conditions, supporting the 'ecohydrological optimality' hypothesis. More than half of the situations exhibited high values (>0.5) of the reference dynamic water stress index (Fig. 5) when calculated for the reference vegetation, indicating that the combination of climate and soil characteristics at our study site creates harsh conditions for plant growth (Porporato et al. 2001). However, despite these abiotic variations, we show that the dynamic water stress remained more or less constant for the actual 'adapted' vegetation. This suggests that 'adaptative' changes in vegetation properties, influencing both TTSW and ET_m, may mitigate the dynamic water stress

towards a certain common value across all plant communities.

As expected, the strong seasonality in climatic conditions resulted in a marked seasonality of water stress, which mainly occurred during summer and also tended to converge towards a common value under severe drought across the five years of study. Indeed, deeper soils had a noticeable buffering effect in comparison with shallow soils (slopes >1, Fig. 6), reflecting a delay in timing of water stress due to greater water storage effect. These results therefore suggest that a minimization of water stress during summer is a major structuring determinism of plant communities within this Mediterranean rangeland.

Scaling relationships between ET and ANPP

Our results clearly support the existence of an 'ecosystem allometry' (Enquist et al. 2003; Kerkhoff et al. 2006) for water exchange, showing that the ecohydrological optimality may be largely underlined by general allometric constraints. At the 'optimum' stage, *i.e* during spring where no significant water stress occurred, we show that actual ET may vary allometrically with standing biomass, as it was previously found for nutrients and productivity (Kerkhoff et al. 2006; Enquist et al. 2007). This allometric relationship for water, although reported here for the first time at the ecosystem level, is not completely surprising since the allometric theory has been initially developed based on hydraulic laws (West et al 1997, 1999; Mencuccini 2003; West & Brown 2005). Nevertheless, it clarifies some expectations concerning the future of vegetation properties facing water shortage. Namely, a general rule seems to predict that decreasing soil water availability should reduce the density of plants within communities, reverse the size distribution towards smaller plants with reduced leaf exchange surface and favor less productive species (Enquist et al. 1998).

A scaling relationship may also exist between the reductions of both ET and ANPP during summer, highlighting the fundamental interdependency of water flows and plant ecosystem processes. We therefore suggest that regulation of ANPP may be the dominant mechanism for the vegetation to control ET reduction in relation with soil water availability, and thereby to limit the soil water depletion. Limiting plant growth reduces the physiological needs of water by lowering metabolic rates towards a more conservative behavior. Furthermore, we show that ANPP does not simply regress but can also switch to negative values during prolonged dry periods, indicating that the senescence of aboveground tissue at the community level is a way of reducing water losses in the absence of significant precipitation. It is known that tissue senescence is involved in drought resistance (Munne Bosch & Alegre 2004). Moreover, the scaling relationship between the reductions of both ET and ANPP in summer was mostly driven

by strong inter annual variations rather than variations along the soil gradient. This suggests that such regulation in ANPP is not context-dependent (soil, vegetation) but possibly represents a general mechanism at the ecosystem level promoting long-term equilibrium WB. While the allometric theory requires plants to be at their optimum stage, we extend here the predictions at a longer time scale, accounting for changes in processes rates due to resource shortage. We therefore argue that the 'ecohydrological optimality' hypothesis can be conceptualized as a dynamic scaling relationship between ET and ANPP over the entire life cycle. However, other factors may reduce ANPP during summer in addition to the only determinism of water conservation, such as phenology effect (most of perennial species have already flowered at this period), reserve accumulation priority, or seasonal turn-over of less-efficient species.

What does water stress really means for a plant community?

Finally, this study raises this issue of the assessment of water stress at the community level. While the concept of 'stress' has been precisely delimited at the plant level with the identification of a suite of physiological responses that can be directly measured on the individuals, the definition and quantification of 'stress' at higher level of organization is not straightforward and has aroused intense debate (Körner 2003, 2004; Lortie et al. 2004; Marrs 2004; Weiher 2004). When soil water availability is too low to meet the demand for transpiration, a plant suffers from water stress. Several physiological symptoms such as stomata closure, reduction of cell division, etc. have been found to be relevant indicators of plant water stress (Ludlow & Muchow 1989; Larcher 2003; Schulze 1986) and appropriate methods have been developed to assess water stress in the field such as those based on plant water potential or soil water deficit (Myers 1988; Rambal et al. 2003; Vicca et al. 2012). However, while plant responses to water stress are species-specific (Larcher 2003), natural plant communities are constituted by a set of individual plants belonging to different species with contrasting resource-use strategies. Therefore the response to water shortage may not be similar nor synchronous for all individuals within the community complicating the evaluation of water status of the whole.

According to Grime (1989, 2001), 'stress' occurs when plant biomass production is lower than expected regardless to underlying cause (resource limitation, heat damage or disturbance regime). This definition has been extensively used to describe and analyze the relationships between species and their environment at multiple levels and scales (Brooker 2006; Michalet et al. 2006). However, when trying to scale up the notion of 'stress' at the community or ecosystem level, Grime's definition doesn't provide clues to identify underlying mechanisms since the 'optimal reference under no stress' is not always clear to apprehend. For instance, while a

reference may be intuitively conceptualized and quantified at the plant level, namely by suppressing the factor that limits growth in the environment, the definition of such reference for a community is questionable since the suppression of limiting factors may also, in a different time-scale, alter community composition by favoring more competitive species, and thereby change the identity of the community (Grime 2006). We therefore propose to define community water stress as the deviation of any ecosystem process rates from a maximum value that is reached during the most favorable period over the life cycle in a given environment. To avoid misleading comparisons between sites under restricted rainfalls or under a range of soil water availabilities, we advocate to monitor soil water dynamics as well as the balance between precipitations and evapotranspiration during the analyzed periods since they will distinctly impact the levels and dynamics of stress.

Conclusion

This study provides empirical supports for the 'ecohydrological optimality' hypothesis driven by a plant 'productivity-water stress' trade-off. Differences in vegetation properties strongly buffer the effects of abiotic constraints of the environment towards the minimization of summer water stress under a Mediterranean climate. For instance, the important degree of 'adaptation' (and self-organization) in sites with low water availability were characterized by very low ANPP generating limited ET_m that contributed to preventing further severe soil water deficits. Yet, the mechanisms underlying these main differences in vegetation properties with decreasing soil water availability remain to be understood and should be related to species functional strategies within the different communities as well as to demographic processes. In the current context of climate change, it would be relevant to identify the thresholds of water deficits (intensity, duration and frequency) and maximum temperatures that may trigger a shift in plant community structure (species turn-over and species relative abundances). Therefore, to ensure the sustainability of an 'ecohydrological optimality', more studies should combine experimental and modeling approaches manipulating resources and climatic variables.

Acknowledgements

This work was funded by the ANR program O_2LA (09-STRA-09). We thank the experimental station 'INRA-La Fage' for access to the facilities, as well as Jean Richarte and Alain Blanchard for their valuable supports during field work.

References

Allen R, Pereira L, Raes D, Smith M (1998) Crop evapotranspiration-Guidelines for computing crop water requirements-FAO Irrigation and drainage paper 56. FAO, Rome

Allen R, Tasumi M, Morse A, Trezza R (2005) A Landsat-based energy balance and evapotranspiration model in Western US water rights regulation and planning. Irrig Drain Syst 19:251–268

Asbjornsen H, Goldsmith GR, Alvarado-Barrientos MS, Rebel K, Van Osch FP, Rietkerk M, Chen J, Gotsch S, Tobon C, Geissert DR, Gomez-Tagle A, Vache K, Dawson TE (2011) Ecohydrological advances and applications in plant-water relations research: a review. J Plant Ecol 4:3–22

Bartholomeus RP, Witte J-PM, Runhaar J (2011) Drought stress and vegetation characteristics on sites with different slopes and orientations. Ecohydrol 5:808–818

Beier C, Beierkuhnlein C, Wohlgemuth T, Penuelas J, Emmett B, Körner C, Boeck H, Christensen JH, Leuzinger S, Janssens IA, Hansen K (2012) Precipitation manipulation experiments - challenges and recommendations for the future. Ecol Lett 15:899–911

Bernard-Verdier M, Navas M-L, Vellend M, Violle C, Fayolle A, Garnier E (2012) Community assembly along a soil depth gradient: contrasting patterns of plant trait convergence and divergence in a Mediterranean rangeland. J Ecol 100:1422–1433

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White J-SS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evol 24:127–35

Brooker RW (2006) Plant-plant interactions and environmental change. New Phytol 171:271–84

Caylor KK, Scanlon TM, Rodriguez-Iturbe I (2009) Ecohydrological optimization of pattern and processes in water-limited ecosystems: A trade-off-based hypothesis. Water Resour Res 45:407

Dyer JM (2009) Assessing topographic patterns in moisture use and stress using a water balance approach. Landsc Ecol 24:391–403

Eagleson PS (1982a) Ecological Optimality in Water-Limited Natural Soil-Vegetation Systems 1. Theory and Hypothesis. Water Resour Res 18:325–340

Eagleson PS (1982b) Ecological Optimality in Water-Limited Natural Soil-Vegetation Systems 2. Tests and Applications. Water Resour Res 18:341–354

Eagleson PS, Segarra RI (1985) Water-Limited Equilibrium of Savanna Vegetation Systems. Water Resour Res 21:1483–1493

Enquist BJ, Brown JH (1998) Allometric scaling of plant energetics and population density. Nature 395:163–165

Enquist BJ, Niklas KJ (2002) Global allocation rules for patterns of biomass partitioning in seed plants. Science 295(5559):1517–1520

Enquist BJ, Economo EP, Huxman TE, Allen AP, Ignace DD, Gillooly JF (2003) Scaling metabolism from organisms to ecosystems. Nature 423:639–42

Enquist BJ, Kerkhoff AJ, Huxman TE, Economo EP (2007) Adaptive differences in plant physiology and ecosystem paradoxes: insights from metabolic scaling theory. Glob Chang Biol 13:591–609

Fay PA, Carlisle JD, Knapp AK, Blair JM, Collins SL (2003) Productivity responses to altered rainfall patterns in a C4-dominated grassland. Oecologia 137:245–51

Fernandez-Illescas CP, Porporato A, Laio F, Rodriguez-Iturbe I (2001) The ecohydrological role of soil texture in a water-limited ecosystem. Water Resour Res 37:2863

Geesing D, Bachmaier M, Schmidhalter U (2004) Field calibration of a capacitance soil water probe in heterogeneous fields. Aust J Soil Res 42:289–299

Grime JP (1989) The stress debate : symptom of impending synthesis ? Biol J Linn Soc 37:3–17

Grime JP (2001) Plant strategies, vegetation processes, and ecosystem properties. Wiley, Chichester

Grime JP (2006) Trait convergence and trait divergence in herbaceous plant communities: Mechanisms and consequences. J Veg Sci 17:255

Groves SJ, Rose SC (2004) Calibration equations for Diviner 2000 capacitance measurements of volumetric soil water content of six soils. Soil Use Manag 20:96–97

Hatton TJ, Salvucci GD, Wu HI (1997) Eagleson' s optimality theory of an ecohydrological equilibrium : quo vadis ? Funct Ecol 11:665–674

Jenerette GD, Scott RL, Huxman TE (2008) Whole ecosystem metabolic pulses following precipitation events. Funct Ecol 22:924–930

Jenerette GD, Barron-gafford GA, Guswa AJ, Mcdonnell JJ, Villegas JC (2012) Organization of complexity in water limited ecohydrology. Ecohydrol 5:184–199

Kerkhoff AJ, Enquist BJ (2006) Ecosystem allometry: the scaling of nutrient stocks and primary productivity across plant communities. Ecol Lett 9:419–27

Kerkhoff AJ, Martens SN, Milne BT (2004) An ecological evaluation of Eagleson's optimality. Funct Ecol 18:404–413

Knapp AK, Smith MD (2001) Variation among biomes in temporal dynamics of aboveground primary production. Science 291(5503):481–4

Knapp AK, Burns CE, Fynn RWS, Kirkman KP, Morris CD, Smith MD (2006) Convergence and contingency in production-precipitation relationships in North American and South African C4 grasslands. Oecologia 149:456–64

Laio F, Porporato A, Ridolfi L, Rodriguez-Iturbe I (2001) Plants in water-controlled ecosystems : active role in hydrologic processes and response to water stress II: Probabilistic soil moisture dynamics. Adv Water Resour 24:707–723

Liancourt P, Spence LA, Boldgiv A, Lkhagva A, Helliker BR, CAsper BB, Petraitis PS (2012) Vulnerability of the northern Mongolian steppe to climate change : insights from flower production and phenology. Ecology 93:815–824

Lortie CJ, Brooker RW, Kikvidze Z, Callaway RM (2004) The value of stress and limitation in an imperfect world: A reply to Körner. J Veg Sci 15:577

Mackay DS (2001) Evaluation of hydrologic equilibrium in a mountainous watershed: incorporating forest canopy spatial adjustment to soil biogeochemical processes. Adv Water Resour 24:1211–1227

Marrs RH (2004) Why we should conserve "limiting factors", at least sometimes! J Veg Sci 15:573

Mencuccini M (2003) The ecological significance of long-distance water transport : short-term regulation , long-term acclimation and the hydraulic costs of stature across plant life forms. Plant, Cell Environ 26:163–182

Michalet R, Brooker RW, Cavieres LA, Kikvidze Z, Lortie CJ, Pugnaire FI, Valiente-Banuet A, Callaway RM (2006) Do biotic interactions shape both sides of the humped-back model of species richness in plant communities? Ecol Lett 9:767–73

Mokany K, Raison RJ, Prokushkin AS (2006) Critical analysis of root : shoot ratios in terrestrial biomes. Glob Chang Biol 12:84–96

Muldavin EH, Moore DI, Collins SL, Wetherill KR, Lightfoot DC (2008) Aboveground net primary production dynamics in a northern Chihuahuan Desert ecosystem. Oecologia 155:123–32

Nakagawa S, Schielzeth H (2013) A general and simple method for obtaining R^2 from generalized linear mixed-effects models (RB O'Hara, Ed.). Methods Ecol Evol 4:133–142

Newman BD, Wilcox BP, Archer SR, Breshears DD, Dahm CN, Duffy CJ, McDowell NG, Phillips FM, Scanlon BR, Vivoni ER (2006) Ecohydrology of water-limited environments: A scientific vision. Water Resour Res 42: W06302

Ogle K, Reynolds JF (2004) Plant responses to precipitation in desert ecosystems: integrating functional types, pulses, thresholds, and delays. Oecologia 141:282–94

Orelien JG, Edwards LJ (2008) Fixed-effect variable selection in linear mixed models using R2 statistics. Comput Stat Data Anal 52:1896–1907

Porporato A, Laio F, Ridolfi L, Rodriguez-Iturbe I (2001) Plants in water-controlled ecosystems : active role in hydrologic processes and response to water stress III: Vegetation water stress. Adv Water Resour 24:725–744

Rambal S, Ourcival JM, Joffre R, Mouillot D, Nouvellon Y, Reichstein M, Rocheteau A (2003) Drought controls over conductance and assimilation of a Mediterranean evergreen ecosystem : scaling from leaf to canopy. Glob Chang Biol 9:1813–1824

Reynolds JF, Kemp PR, Ogle K, Fernández RJ (2004) Modifying the "pulse-reserve" paradigm for deserts of North America: precipitation pulses, soil water, and plant responses. Oecologia 141:194–210

Ritchie JT (1981) Soil water availability. Plant Soil 58:327–338

Rodriguez-Iturbe I, Porporato A, Ridolfi L, Isham V, Cox DR (1999) Probabilistic modelling of water balance at a point : the role of climate, soil and vegetation. Proc R Soc Sci Math Phys Eng Sci 455:3789–3805

Ruppert JC, Holm A, Miehe S, Muldavin EH, Snyman HA, Wesche K, Linstädter A (2012) Meta-analysis of ANPP and rain-use efficiency confirms indicative value for degradation and supports non-linear response along precipitation gradients in drylands. J Veg Sci 23:1035–1050

Salve R, Sudderth EA, St. Clair SB, Torn MS (2011) Effect of grassland vegetation type on the responses of hydrological processes to seasonal precipitation patterns. J Hydrol 410:51–61

Salvucci GD, Entekhabi D (1994) Comparison of the Eagleson balance model with numerical simulations. Water Resour Res 30:2751–2757

Savage VM (2004) Improved approximations to scaling relationships for species, populations, and ecosystems across latitudinal and elevational gradients. J Theor Biol 227:525–34

Saxton KE, Rawls WJ (2006) Soil water characteristic estimates by texture and organic matter for hydrologic solutions. Soil Sci Soc Am J 70:1569–1578

Schwinning S, Sala OE, Loik ME, Ehleringer JR (2004) Thresholds, memory, and seasonality: understanding pulse dynamics in arid/semi-arid ecosystems. Oecologia 141:191–3

Schymanski SJ, Roderick ML, Sivapalan M, Hutley LB, Beringer J (2007) A test of the optimality approach to modelling canopy properties and CO2 uptake by natural vegetation. Plant Cell Environ 30:1586–98

Schymanski SJ, Roderick ML, Sivapalan M, Hutley LB, Beringer J (2008) A canopy-scale test of the optimal water-use hypothesis. Plant Cell Environ 31:97–111

Schymanski SJ, Sivapalan M, Roderick ML, Hutley LB, Beringer J (2009) An optimality-based model of the dynamic feedbacks between natural vegetation and the water balance. Water Resour Res 45:412

Scurlock JMO, Johnson K, Olson RJ (2002) Estimating net primary productivity from grassland biomass dynamics measurements. Glob Chang Biol 8:736–753

Sinclair T, Ludlow M (1986) Influence of Soil Water Supply on the Plant Water Balance of Four Tropical Grain Legumes. Aust J Plant Physiol 13:329

Solé R (2007) Scaling laws in the drier. Nature 449:151–153

Swemmer AM, Knapp AK, Snyman HA (2007) Intra-seasonal precipitation patterns and above-ground productivity in three perennial grasslands. J Ecol 95:780–788

Urgeghe AM, Breshears DD, Martens SN, Beeson PC (2010) Redistribution of runoff among vegetation patch types: on ecohydrological optimality of herbaceous capture of run-on. Rangel Ecol Manag 63:497–504

Vicca S, Gilgen AK, Serrano MC, Dreesen FE, Dukes JS, Estiarte M, Gray SB, Guidolotti G, Hoeppner SS, Leakey ADB, Ogaya R, Ort DR, Ostrogovic MZ, Rambal S, Sardans J, Schmitt M, Siebers M, van der Linden L, van Straaten O, Granier A (2012) Urgent need for a common metric to make precipitation manipulation experiments comparable. New Phytol 195:518–522

Warton DI, Wright IJ, Falster DS, Westoby M (2006) Bivariate line-fitting methods for allometry. Biol Rev 81:259–91

Warton DI, Duursma R a., Falster DS, Taskinen S (2012) smatr 3- an R package for estimation and inference about allometric lines. Methods Ecol Evol 3:257–259

Weiher E (2004) Why should we constrain stress and limitation ? Why conceptual terms deserve broad definitions. J Veg Sci 15:569–571

Weng E, Luo Y (2008) Soil hydrological properties regulate grassland ecosystem responses to multifactor global change: A modeling analysis. J Geophys Res 113:1–16

West GB, Brown JH (2005) The origin of allometric scaling laws in biology from genomes to ecosystems: towards a quantitative unifying theory of biological structure and organization. J Exp Biol 208:1575–92

West GB, Brown JH, Enquist BJ (1997) A general model for the origin of allometric scaling laws in biology. Science 276(5309):122–126

West GB, Brown JH, Enquist BJ (1999) A general model for the structure and allometry of plant vascular systems. Nature 400:664–667

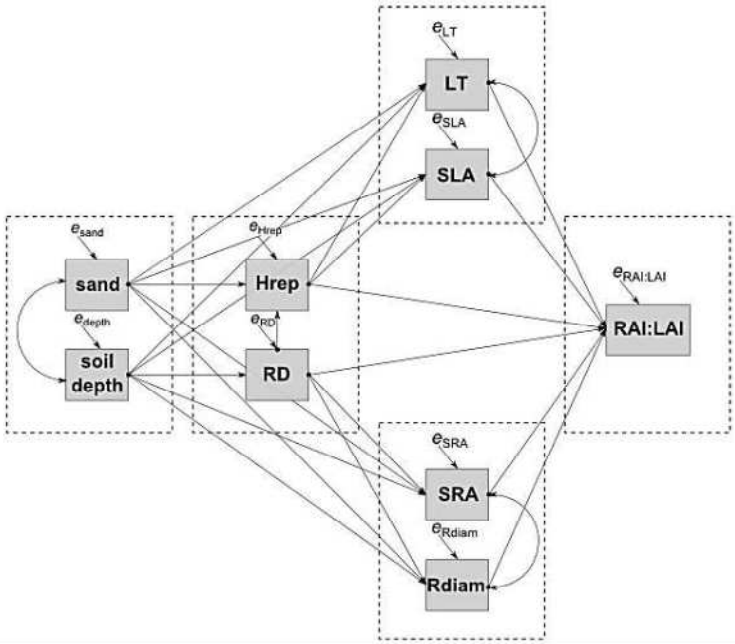
Wight JR, Hanson CL (1990) Crop coefficients for rangeland. J Range Manag 43:482–485

Wu Z, Dijkstra P, Koch GW, Peñuelas J, Hungate BA (2011) Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. Glob Chang Biol 17:927–942

Zhang X, Zwiers FW, Hegerl GC, Lambert FH, Gillett NP, Solomon S, Stott PA, Nozawa T (2007) Detection of human influence on twentieth-century precipitation trends. Nature 448:461–465

Supporting information

Fig S1 Hypothesized model specified with nine observed variables (grey boxes). Soil was characterized with soil depth and sand content; plant size was characterized by CWM of reproductive height (Hrep) and rooting depth (RD); leaf morphology was characterized by CWM of leaf thickness (LT) and specific leaf area (SLA); root morphology was characterized by root diameter (Rdiam) and specific root area (SRA); biomass partitioning was characterized by the ratio between the root area index and the leaf area index (RAI:LAI). Causal relationships are represented by one-headed arrows; free correlations are represented by double-headed arrows. Residual errors variables (e_x) represent effects of unexplained causes.



Description of the hypothetical model

Based on results from PCA and RDA, nine variables were selected to specify the hypothetical model (Fig. 1b). The soil was specified by two physical characteristics (soil depth and sand content) both defining the volume of soil available to plants and its texture. The vegetation was specified by four plant functional traits: two size-related traits (Hrep and RD), two morphological leaf traits (SLA and LT) and their two analogous root traits (SRA and Rdiam). Output of the model was the ratio between the root area index and the leaf area index (RAI:LAI ratio). A first set of paths describes how plant communities respond to soil characteristics (Fig. 2b). Together with climate and disturbance, soil is a major environmental factor affecting plant growth strategy and performance (Ordoñez et al. 2009). Community plant size was hypothesized to respond

directly to soil characteristics since climate and disturbance were held constant. For example, plant height was expected to be negatively affected by unfavourable soil conditions at community scale, especially in response to low water availability (Cingolani et al. 2007; Sonnier et al. 2010; Bernard-Verdier et al. 2012; Frenette-Dussault et al. 2012). Plant rooting depth was also expected to respond significantly to soil characteristics, although the direction of response is not clear in case of varying soil water availability, because it strongly depends on climate. Under a Mediterranean climate with seasonal drought periods, rooting depth may be potentially greater in cases of coarse structured soils with lower water holding capacity because of deeper water infiltration (Schenk & Jackson 2005). This may enable a more complete exploitation of water stored in the soil during stress periods. On the contrary, roots may rather preferentially develop in top soil layers for a more opportunistic use of rain water as it is the case in environments with prolonged periods of drought and deserts (Schenk & Jackson 2002).

In addition to plant size, leaf and root morphology have a pivotal role in resource-use strategy of plants and were therefore also expected to respond directly to soil characteristics (Fig. 2b). For instance, leaf traits involved in the global leaf economic spectrum (Wright et al. 2004) such as SLA were shown to shift at the community level from values associated with high metabolic rates and high resource turn-over (*e.g.* high SLA value) under favorable soil conditions towards values associated with lower metabolic rates (*e.g.* lower SLA value) favoring resource retention under water-limited conditions (Bernard-Verdier et al. 2012; Frenette-Dussault et al. 2012). Similarly, root morphological traits such as SRA were shown to drive resource uptake from soil (Jackson et al. 1996), although far less empirical evidence are available at the community level. On this basis, root traits were also hypothesized to respond directly to soil characteristics, but in the opposite direction compared to leaf traits. In order to enhance water uptake capacity at drier sites, root traits were expected to shift from low values to higher values at the community level with deceasing water availability. Moreover, because roots have an important role in plant mechanics, namely for plant anchoring in soil, root traits were also expected to respond directly to physical constraints limiting root foraging such as soil porosity. For example, root diameter was shown to decrease in response to decreasing soil porosity (Schymanski et al. 2009). Because soil texture strongly influences soil porosity, fine texture having greater soil porosity, root diameter was therefore hypothesized to decrease when sand content increases.

A second set of paths describes the allometric and functional linkages among plant traits. Most importantly, an allometric relationship was expected between average rooting depth and plant height at the community level (Fig. 2b). According the allometric theory, root biomass is predicted to scale isometrically with stem biomass. From a mechanical point of view, it seems

therefore also reasonable to expect that below- and aboveground plant dimensions should be somehow interrelated. For example plant height should be limited if rooting depth is limited by a physical barrier in soil. Furthermore, allometric theory predicts that leaf biomass should scale with the ¾ power of stem, suggesting possible constraints of plant size on the range of leaf morphology (Enquist & Nikals 2002; Niklas & Enquist 2002). While leaf morphology and plant height have been considered as two independent axes of variation in plant strategy (Westoby 1998; Westoby et al. 2002), recent studies supported significant linkages between leaf morphological traits and height in response to environmental drivers at both species and community level. Therefore we hypothesized a relationship between plant height and leaf morphology at the community level, greater heights allowing stronger SLA (Fig. S1). A relationship was also expected between root morphological traits and rooting depth at community level for analogous reasons.

Finally, a third set of paths describes the effect of community structure on emergent properties of vegetation such as RAI:LAI ratio which should strongly influence ecosystem water flows. Such relationships between community weighted mean traits and ecosystem functioning were already shown for processes such as biomass production and litter decomposition (*e.g.*, Garnier et al. 2004). Here, average plant size and organ morphology at the community level were hypothesized to determine the coordination between both total root and leaf areas of the vegetation.

References

Bernard-Verdier M, Navas M-L, Vellend M, Violle C, Fayolle A, Garnier E (2012) Community assembly along a soil depth gradient : contrasting patterns of plant trait convergence and divergence in a Mediterranean rangeland. J Ecol 100:1422–1433

Cingolani AM, Cabido M, Gurvich DE, Renison D, Díaz S (2007) Filtering processes in the assembly of plant communities: Are species presence and abundance driven by the same traits ? J Veg Sci 18:911–920

Enquist BJ, Niklas KJ (2002) Global allocation rules for patterns of biomass partitioning in seed plants. Science 295(5503):1517–20

Frenette-Dussault C, Shipley B, Léger J-F, Meziane D, Hingrat Y (2012) Functional structure of an arid steppe plant community reveals similarities with Grime's C-S-R theory (P Adler, Ed.). J Veg Sci 23:208–222

Garnier E, Cortez J, Billès G, Navas M-L, Roumet C, Debussche M, Laurent G, Blanchard A, Aubry D, Bellman A, Neill C, Toussaint J-P (2004) Plant functional markers capture ecosystem properties. Ecology 85:2630–2637

Jackson RB, Canadell J, Ehleringer JR, Mooney HA, Sala OE, Schulze ED (1996) A global analysis of root distributions for terrestrial biomes. Oecologia 108:389–411

Niklas KJ, Enquist BJ (2002) On the vegetative biomass partitioning of seed plant leaves, stems, and roots. Am Nat 159:482–497

Ordoñez JC, van Bodegom PM, Witte J-PM, Wright IJ, Reich PB, Aerts R (2009) A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. Glob Ecol Biogeogr 18:137–149

Schenk HJ, Jackson RB (2005) Mapping the global distribution of deep roots in relation to climate and soil characteristics. Geoderma 126:129–140

Schymanski SJ, Sivapalan M, Roderick ML, Hutley LB, Beringer J (2009) An optimality-based model of the dynamic feedbacks between natural vegetation and the water balance. Water Resour Res 45:412

Sonnier G, Shipley B, Navas M-L (2010) Quantifying relationships between traits and explicitly measured gradients of stress and disturbance in early successional plant communities. J Veg Sci 21:1014–1024

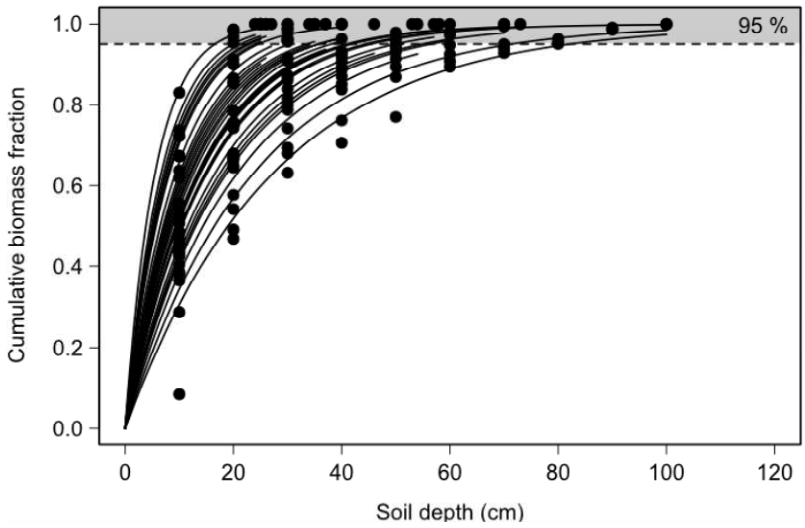
Westoby M (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. Plant Soil 199:213–227

Wright IJ, Westoby M (2002) Leaves at low versus high rainfall: coordination of structure, lifespan and physiology. New Phytol 155:403–416

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee WG, Lusk C, Midgley JJ, Navas M-L, Niinemets U, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R (2004) The worldwide leaf economics spectrum. Nature 428:821–827

Supporting information

Fig. S2 Cumulative root distribution (cumulative proportion) as a function of soil depth. Data points represent individual 10 cm-soil layer for each community. Lines correspond to the fitted model of Gale & Grigal (1987). Grey area indicates when the 95 %.



Chapter 3

Functional structure of plant communities



(a plant community at the 'INRA-La Fage' experimental station, *spring 2012*)

MANUSCRIPT III

Influence of the functional structure of plant communities on emergent properties of vegetation and associated ecosystem water flows in a Mediterranean rangeland

-

Influence de la structure fonctionnelle des communautés de plantes sur les propriétés émergentes de la végétation et les flux d'eau associés à l'échelle de l'écosystème dans des parcours méditerranéens

Karim Barkaoui, Marine Birouste, Catherine Roumet, Marie-Laure Navas

Abstract

Aboveground and belowground vegetation properties have a fundamental role in ecosystem functioning because they strongly influence both biogeochemical and hydrological cycles. Yet, predicting how variations in abiotic conditions may impact such properties which in turn will affect the rate of ecosystem processes requires more integrated understanding of underlying mechanisms. In particular, the role of the functional structure of plant communities in driving water-related processes was scarcely analyzed. Here, we investigated how plant traits at the community-level may mediate the effect of varying soil water availability on ecosystem water flows in a species-rich Mediterranean rangeland. We assessed multivariate trait co-variation among 36 plant communities distributed along a soil water availability gradient. Community weighted means (CWMs) were calculated for a suite of 15 above- and belowground traits related to plant stature, plant allometry, organ morphology and chemistry which reflects the integrated nature of plant form and function for resource economy. We then used structural equation modeling to test for response-and-effect relationships between key environmental variables, CWMs and vegetation properties defined by the ratio between total leaf area (LAI) and total root area (RAI). Finally, we evaluated the ecological importance of both LAI and RAI for predicting two major components of the ecosystem water balance: the potential evapotranspiration rate (ET_m) and the total transpirable soil water (TTSW). Results showed that above- and belowground traits may not respond in a coordinated fashion to water shortage. While plant height and leaf morphology showed a strong response pattern at the community-level along the soil gradient, only the rooting depth and not root morphology was affected by the variation in soil properties. The variations in RAI:LAI ratio, which were strongly correlated to the variations in soil water availability, were essentially determined by the average plant height followed by specific leaf area and leaf thickness within the community. Besides, RAI and LAI were shown to be key controlling factors of local ecosystem water balance in this rangeland, regardless to species identity and diversity. Under climate change, we therefore provide strong empirical evidence that changes in local soil water availability may directly affect a limited set of size-related traits having a pivotal role which in turn will induce important modulations of ecosystem water flows through changes in exchange surfaces of vegetation.

Résumé

Les propriétés aériennes et souterraines de la végétation ont un rôle fondamental dans le fonctionnement des écosystèmes car elles influencent fortement les cycles biogéochimiques et hydrologiques. Cependant une compréhension plus intégrée des mécanismes sous-jacents est aujourd'hui nécessaire afin de prédire comment des variations des conditions abiotiques modifient ces propriétés, qui à leur tour vont moduler les processus écologiques. En particulier, le rôle que joue la structure fonctionnelle des communautés de plantes dans le contrôle des processus liés à l'eau est encore très peu connu. Dans cette étude, nous nous sommes intéressés à la structure des communautés dans des parcours méditerranéens, et nous avons exploré comment les traits fonctionnels des plantes pouvaient déterminer les flux d'eau de l'écosystème en réponse à différents niveaux de disponibilité en eau du sol. Nous avons examiné les co-variations entre traits au sein de 36 communautés établies le long d'un gradient édaphique. Nous avons calculé les moyennes des valeurs de traits à l'échelle de la communauté (CWMs) pour 15 traits aériens et souterrains liés à la stature des plantes et à leur trajectoire allométrique, ainsi qu'à la morphologie et la chimie des organes végétatifs. Ces traits ont été choisis car ils reflètent l'intégration entre forme de croissance et fonction des plantes vis-à-vis de l'utilisation des ressources. Nous avons utilisé la modélisation en équations structurales pour tester des relations de type 'réponse-effet' entre les variables de l'environnement, les CWMs et les propriétés de la végétation, définies ici par le rapport entre la surface foliaire totale (LAI) et la surface racinaire totale (RAI). Puis, nous avons évalué l'importance écologique du LAI et du RAI dans le contrôle de l'évapotranspiration potentielle (ET_m) et la détermination de la quantité d'eau transpirable du sol (TTSW) qui sont deux composantes majeures du bilan hydrique de l'écosystème. Nos résultats montrent que les traits aériens et souterrains ne répondent pas de façon similaire à la réduction de la disponibilité de l'eau. Alors que la hauteur des plantes et la morphologie des feuilles répondent fortement au gradient édaphique, seule la profondeur racinaire, et non pas la morphologie des racines, a été affecté. Les modifications du rapport RAI:LAI, fortement corrélées à la disponibilité en eau du sol, ont été déterminées essentiellement par la hauteur des plantes, puis par la surface foliaire spécifique et l'épaisseur des feuilles au sein des communautés. Par ailleurs, nous avons montré que le LAI et le RAI sont deux facteurs clés dans le contrôle du bilan hydrique au sein des parcours étudiés, quel que soit l'identité et la diversité des espèces présentes. Dans un contexte de changement climatique, notre étude met donc en évidence que des changements dans la disponibilité en eau affectent un nombre limité de traits 'pivots', liés à la taille des plantes et à la morphologie des feuilles, qui peuvent induire des modulations considérables des flux d'eau à l'échelle de l'écosystème via des modifications des surfaces d'échange de la végétation.

Introduction

In the context of climate change characterized by more intense fluctuations of water supply among years and seasons (IPCC 2007; Zhang et al. 2007), the study of macroscopic factors governing water exchange through the soil plant-atmosphere-continuum (SPAC) is necessary to understand how ecosystem ecohydrology might be affected. From this perspective, applying the trait-based response-effect framework (Lavorel & Garnier 2002; Suding et al. 2008) across plant communities allows a combined analysis of vegetation responses and subsequent effects on ecosystem properties, accounting for species turn-over, changes in species relative abundances and plant intraspecific variability. The approach, now widely used in biodiversity-ecosystem functioning studies, provided growing evidence that, being determined by environmental conditions, the functional structure of plant communities was in turn a good predictor of many ecosystem processes such as primary productivity (Garnier et al. 2004; Mokany et al. 2008) or biogeochemical processes (Garnier et al. 2004; Fortunel et al. 2009; de Deyn et al. 2008; Klumpp & Soussana 2009). However, far fewer studies have been using the approach in case of water-related processes, and such an integrated vision between community response and ecosystem hydrologic functioning continues to be missing in functional ecology.

While the aboveground trait-based response of plant communities has been well described along gradients in soil water availability (Ackerly 2004; Bernard-Verdier et al. 2012; Frenette-Dussault et al. 2012), relevant traits having an effect on the ecosystem water balance still need to be identified and tested. Because of the tight coupling between transpiration and CO2 uptake (Reich et al. 1999; Westoby et al. 2002), aboveground traits related to plant growth strategy such as the specific leaf area were expected to impact water flows toward the atmosphere (Schwinning & Ehleringer 2001; Larcher 2003). However, except some physiological studies at the species level (Mitchell et al. 2008; Nardini et al. 2012; Bartlett et al. 2012), an ecological test of such expectation at the community level has not been proposed yet. Moreover, plant stature seems to have a conflictual role: plant height was argued in some cases to enhance water flows toward the atmosphere because taller plants have greater resource turn-over rates, but in other cases, it was shown to promote soil water retention by reducing incident radiation on the ground and thereby soil evaporation (Liancourt et al. 2005; Gross et al. 2008). Otherwise, the belowground compartment of the vegetation is particularly missing in the ecological literature to draw a complete picture of ecosystem ecohydrology. To date, only a few works have been studying the relationships between soil water and root traits in species-rich communities along environmental gradients. For instance, community root length and specific root area were both shown to be negatively correlated to soil moisture in alpine and Australian grasslands (Eviner &

Chapin 2003; Gross et al. 2008; Mokany et al. 2008) and they were formally predicted to drive soil water uptake (Garnier & Navas 2012). In the same way, average rooting depth was shown to determine the depth of plant water uptake and thereby the extent of soil water depletion (Schwinning & Ehleringer 2001; Eviner & Chapin 2003). Despite these promising findings, a reason why the trait-based approach was not incorporated into water balance studies and modeling is that absolute rates of processes, and thereby quantitative values of fluxes, are still poorly predicted from trait values solely.

Another classical approach to assess the ecohydrology of plant ecosystems is the so-called 'big leaf' approach which simplifies the vegetation to one cumulative leaf and root. Compared to the trait-based approach, the 'big leaf' approach is entirely based on the absolute amount of biomass allocated to exchange organs of plants and does not distinguish the different species within the community. The flow of water through the SPAC is therefore conceptualized, in analogy with Ohm's law, as proportional to the difference of water potentials between soil, root, leaf and the atmosphere accounting for successive diffusive resistance (Maseda & Fernandez 2006). Neglecting the internal hydraulic resistance of plants, *i.e* considering that roots and leaves always have equal water potentials, the flow of water through the SPAC is entirely driven by diffusive resistances at exchange surfaces of plants with the environment. For instance, increasing the amount of leaves may lead to decreasing diffusive resistance between vegetation and the atmosphere enabling higher water loss rates through transpiration while increasing the amount of roots favors greater capacity of water uptake. The approach allowed for a more mechanistic view of hydrological functioning of simple and homogeneous plant community types and it has been widely adopted in water-balance modeling whether driven by leaf evaporative demand (Schymanski et al. 2009) or root water uptake capacity (Guswa 2010). For more complex communities, including many different plant species of different sizes and growth forms, the reliability of the approach may however be questioned because the diversity of adaptative strategies is not explicitly accounted for.

Whilst the trait-based approach and the 'big leaf' approach may be implicitly presented exclusive to each other, they should more likely be thought as complementary when incorporating plant allometric strategy. Allometric theory predicts a series of scaling relationships that reflect how plants should efficiently partition biomass with the constraint of maximizing photosynthetic and resource harvesting capacity while minimizing hydrodynamic resistance and transport times (Enquist & Niklas 2002; Niklas & Enquist 2002). Such relationships were shown to hold at the species level for many different plant species in contrasting environments, and they were more recently also suggested to hold at the community level (Cheng & Niklas 2007;

Yang et al. 2010). According to this alternative view, dimensions of exchange surfaces of vegetation may thus depend on both plant traits related to organ morphology defining how biomass is converted into area, and on plant allometric trajectory defining how much biomass is invested to the different organ types accounting for plant size and ontogeny (Müller et al. 2000; Weiner et al. 2004). Furthermore, allometric relationships highlight the 'coordination' between above- and belowground components of vegetation which may balance the water uptake capacity of roots with the potential (maximal) evaporative demand of leaves according to local water availability. For instance, species tend to allocate more biomass toward belowground development in water-limited environments (Chapin et al; 1993; Schenk & Jackson 2002). Since then, root:shoot ratio appears to be a key emergent property of vegetation to study for understanding the ecohydrology of ecosystems. More precisely, the ratio between water absorbing and transpiring surfaces, *i.e* between the root area index (RAI) and the leaf area index (LAI), should even better indicate plant water use strategy under contrasting water supplies.

In this study, we aim to explore how the functional structure of species-rich Mediterranean rangelands may determine emergent properties of vegetation such as the RAI:LAI ratio, which in turn may drive ecosystem water flows in response to varying soil water availability along an edaphic gradient. We hypothesized that shifts in plant trait values at the community level may mediate the coordination between aboveground and belowground components of vegetation towards a 'functional equilibrium' between water demand and supply, accounting for plant allometric constraints and trait-trait scaling relationships. We address three questions: (i) how do traits related to plant size, allometry and morphology co-vary across communities in response to varying soil characteristics? (ii) how does the multivariate trait-based response of plant communities translate into RAI:LAI ratio of vegetation? (iii) following the "big leaf" approach, how important is RAI:LAI ratio of vegetation in driving ecosystem water flows?

Materials and Methods

Study site

The study was carried out on dry calcareous rangelands of southern France, located on a limestone plateau (Larzac Causse) at the INRA experimental station La Fage (43°55´N, 3°05´E, 790 m a.s.l.), 100 km northwest of Montpellier. Climate on the plateau is sub-humid with a strong Mediterranean influence. Cool and wet winters alternate with warm and dry summers. Mean annual precipitations range from 680 to 1790 mm occurring mainly during spring and autumn. Mean monthly temperatures vary from 1°C in January to 19°C in August (data from 1973–2013).

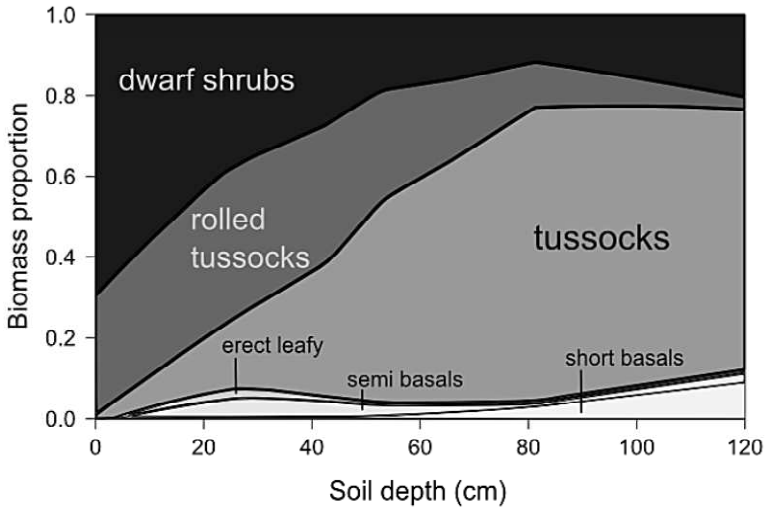


Fig. 1 Biomass proportion of each plant growth form within communities along the soil gradient. Trends were extrapolated from the 36 vegetation surveys distributed along the gradient using a spline method. Growth forms are those mentioned in the text.

The main growing season lasts from March to the end of June. At the landscape level, vegetation is dominated by perennial herbaceous species (*Bromus erectus*, *Festuca christiani-bernardii*, *Carex humilis)*, along with loosely scattered shrubs (*Buxus sempervirens*, *Juniperus communis).* For the past 35 years, the rangeland at the station has been homogeneously grazed by a sheep herd year-round under a controlled grazing regime.

Environmental gradient and study design

Soils consist of dolomitic rendzinas arranged as a mosaic of different depths and texture. Twelve plots (6 x 9 m) up to 1500 m apart were selected to span the widest possible range of soil types: from the shallow and dry soils of dolomitic sand to deeper and moister clay soils. Soil characteristics were assessed in each plot to quantitatively characterize the gradient (Table 2; for further details see also Perez-Ramos et al. 2012; Bernard-Verdier et al. 2012). Mean soil depth (ranging from 10 to 100 cm) was determined in each plot using 11-13 randomly distributed soil cores (5 cm wide). Soil physico-chemical properties were assessed for three of these cores, which were divided into 10 cm thick layers from the soil surface down to the bed rock in order to account for the variability along the whole soil profile. Eight soil characteristics were measured following standard procedures (Afnor 1994): texture, water holding capacity (-0.015 MPa), calcium carbonate content, pH (in water), organic matter content, C:N ratio, cation exchange capacity (CEC) and available phosphorus (using the Olsen method).

Soil water content (SWC) was measured bi-weekly since spring 2008 using capacitance moisture probes (*DIVINER 2000*, Sentek Pty Ltd, Stepney, Australia), which provided a complete profile of soil humidity at three permanent locations in each plot. The probes were previously calibrated according to soil texture to account for the differences in soil water retention (Geesing et al. 2004; Groves & Rose 2004). To interpolate the SWC measurements at a daily time step, we have fitted the single-layer bucket model initially proposed by Laio et al. (2001) that describes soil water dynamics by considering the soil as a reservoir to be intermittently filled by rainfall events and emptied by surface runoff, deep drainage and evapotranspiration processes.

Vegetation surveys

In May 2011, the abundance of each species was estimated using the point-intercept method (Levy & Madden 1933) within a total of 36 circular quadrats (three quadrats/plot) of 16 cm diameter (200.96 cm^2) containing a 4 x 4 cm grid pattern (1044.98 points/m^2). We recorded a total of 46 species belonging to 14 botanical families (Table S1) and classified them according to

Table 1 Studied traits and ecosystem properties with corresponding abbreviation and unit. Mean and range values (community weighted mean for traits) as well as loadings of each variable on the two first axes of PCA (Fig. 2a) are reported. Ecosystem properties were used as illustrative variables in the PCA.

Vegetation variables	Abbreviation	Unit	Mean	Range	PCA1	PCA2
a) Plant stature traits						
Reproductive height	Hrep	cm	29.18	10.39-48.10	-0,957	-0,084
Rooting depth	RD	cm	28.43	10.00-63.17	-0,621	0,210
b) Plant allocation traits						
Leaf mass fraction	LMF	$g\ g^{-1}$	0.133	0.046-0.322	-0,486	-0,682
Root mass fraction	RMF	$g\ g^{-1}$	0.825	0.571-0.942	0,486	0,676
c) Leaf traits						
Leaf length	LL	cm	9.533	2.606-18.90	-0,689	-0,278
Leaf area	LA	cm^2	1.540	0.430-3.069	-0,837	-0,050
Leaf thickness	LT	µm	234.9	178.8-302.2	0,852	-0,075
Specific leaf area	SLA	$m^2\ kg^{-1}$	12.68	7.733-18.28	-0,926	0,025
Leaf dry matter content	LDMC	$mg\ g^{-1}$	374.6	308.3-460.4	0,753	-0,115
Leaf nitrogen content	LNC	$g\ g^{-1}$	8.786	6.851-10.01	-0,937	-0,058
Leaf carbon content	LCC	$g\ g^{-1}$	22.96	20.03-26.65	0,704	0,040
d) Root traits						
Root diameter	Rdiam	mm	0.348	0.276-0.441	-0,643	0,467
Specific root length	SRL	$m\ g^{-1}$	44.40	19.99-82.48	0,359	-0,829
Specific root area	SRA	$m^2\ kg^{-1}$	39.07	23.17-60.94	0,162	-0,875
Root dry matter content	RDMC	$mg\ g^{-1}$	388.7	315.5-444.0	0,017	0,411
e) Illustrative variables						
Total community biomass	TCB	$g\ m^{-2}$	598.0	222.6-1388	0.105	0,493
Aboveground biomass	AGB	$g\ m^{-2}$	92.32	38.62-170.0	-0,621	-0,141
Belowground biomass	BGB	$g\ m^{-2}$	505.7	143.0-1303	0,189	0,529
Leaf area index	LAI	$m^2\ m^{-2}$	0.672	0.249-1.229	-0,706	-0,190
Root area index	RAI	$m^2\ m^{-2}$	18.93	5.005-46.36	0,240	0,146

growth-forms (*sensu* Cornelissen et al. 2003): (i) 'short basal' (15.22 % of the species) includes all rosette or prostrate growth-form species with short leaves (<0.5 m) deployed very close to the ground; (ii) 'semi basal' (19.57 % of the species) includes several forbs characterized by significant leaf area deployed both close to the ground and higher on plant; (iii) 'erect leafy' (13.04 % of the species) includes erected forbs with leaves located in the middle and/or top parts of the plants; (iv) 'tussock' (19.57 % of the species) includes almost all graminoïd species characterized by abundant and flat leaves sprouting from basal meristems and forming prominent tufts; (v) 'rolled tussock' (6.52 % of the species) has similar characteristics than tussock but includes graminoïd species that have rolled-up leaves, dividing by up to half their exposed leaf blade area; (vi) 'dwarf shrub' (23.91 % of the species) includes all woody species up to 0.8 m tall.

The proportion of contrasting growth forms within communities varied significantly along the soil gradient (Fig. 1): communities located on deep clay soils were largely dominated by tussocks (*Bromus erectus*, *Carex hallerianna*) whereas communities located on shallow sandy soils were co-dominated by rolled tussocks (*Festuca christiani-bernardii, Stipa pennata*) and dwarf shrubs (*Helianthemum canum, Thymus dolomiticus*). Communities located on intermediate soil conditions were composed by a more or less even mixture of tussocks (Bromus erectus, Carex humilis), rolled tussocks (*Festuca christiani-bernardii, Koeleria valesianna, Stipa pennata*) and dwarf shrubs (*Helianthemum canum, Potentilla neumanniana, Coronilla minima*). Short basals, semi basals and erect leafy only contributed to a minor proportion of community biomass without any particular trend along the soil gradient (Fig. 1).

Trait measurements and functional structure of plant communities

We selected 15 plant functional traits to characterize the vegetation (Table 1). As far as it was possible, above- and belowground components of the vegetation were characterized with analogous traits, related to whole plant stature and allocation, leaf and root morphology and plant resource-use strategy. Leaf trait were available for the 36 most abundant species (*i.e* representing > 80 % of total biomass, Pakeman & Quested 2009) from a previous study conducted in 2009 (Bernard-Verdier et al. 2012) and completed in late spring 2011 with the same protocol on at least 12 individuals distributed across the plots. For each individual sample, leaf length (LL; cm), leaf thickness (LT; µm), leaf area (LA; cm^2), leaf dry matter content (LDMC; mg g^{-1}), specific leaf area (SLA, m^2 kg^{-1}), leaf carbon content (LCC; % leaf dry mass) and leaf nitrogen content (LNC; % leaf dry mass) were measured following standard protocols (Cornelissen et al. 2003; for leaf thickness see Vile et al. 2005). Mean reproductive height (Hrep; cm) was taken from both

Fayolle (2008) and Bernard-Verdier et al. (2012). Community-weighted mean for each aboveground trait ($CWM_trait_{above,i}$) was calculated for each circular quadrat following Garnier et al. (2004):

$$CWM_trait_{above,i} = \sum_{j=1}^{n} p_{i,j} \times trait_j \quad \text{eqn. 1}$$

where n is the total number of species in the quadrat i, $p_{i,j}$ is the relative abundance of species j in quadrat i, and $trait_j$ is the mean trait value of species j.

Because separating the rooting system of coexisting species in a natural community is hardly feasible, root traits were directly measured at the community level. Soil cores (5 cm wide, 10-100 cm length depending on soil depth) were collected from the center of each of the 36 circular quadrats in spring 2011. Cores were divided into 10 cm thick layers from the soil surface down to the mother rock. In the laboratory, root material was carefully washed in water, and a representative sub-sample of fresh roots was scanned at 400 dpi following Perez-Ramos et al. (2012). Root length, area and mean diameter were measured from the digital images using Winrhizo software for image analysis (Winrhizo ver. 2003b, Regent Instruments Inc., Quebec, Canada). The whole root material was weighted fresh, then oven dried at 60 °C for 48 h and re-weighted. For each core, means of root dry matter content (RDMC; mg g^{-1}), root diameter (Rdiam; mm), specific root length (SRL; m g^{-1}) and specific root area (SRA; m^2 kg^{-1}) were calculated as the means of measured trait values in each 10 cm soil layer weighted by the relative root biomass over the whole soil profile:

$$CWM_trait_{below,i} = \frac{1}{n} \times \sum_{j=1}^{n} CWM_trait_{below,i,j} \times \left(\frac{root_biomass_{i,j}}{total_root_biomass_i} \right) \quad \text{eqn. 2}$$

where n is the number of 10 cm soil layers at community i, $CWM_trait_{below,i,j}$ is the value of trait measured in the j-th soil layer of community i.

Finally, community mean rooting depth (RD; cm) was estimated based on the root biomass distribution along the whole soil profile. Following Gale and Grigal (1987), an asymptotic function ($Y = 1 - \beta^z$, where Y is the cumulative fraction of roots between soil surface and depth z, and β is an empirical fitting parameter that determines the root distribution with depth) was adjusted to the biomass data ($0.62 < r^2 < 0.89$, $p<0.001$) in order to determine the soil depth that contains 95 % of total dry root biomass (see Fig. S3). For communities with very

shallow soil (soil depth < 20 cm, *i.e* 5 cases over 36), rooting was assumed to be equal to soil depth.

Ecosystem properties

Above- and belowground primary productivity were respectively estimated by community above- and below ground biomass at the peak of vegetation (Table 1). Aboveground biomass (AGB; g m^{-2}) was assessed non-destructively for each circular quadrat in late spring 2011 before the summer drought began. The number of contacts recorded by species from the point intercept method was converted into aboveground biomass using appropriate calibration equations (Barkaoui et al. 2013). Community leaf biomass was assessed in a similar way using data from Barkaoui et al. (2013). Belowground biomass (BGB; g m^{-2}) was assessed at the same date for each circular quadrat from the soil cores (as previously described). In addition, plant leaf mass fraction (LMF; g g^{-1}) and root mass fraction (RMF; g g^{-1}) were calculated as the inverse ratio of total community biomass (TCB; g m^{-2}) on community leaf biomass and belowground biomass respectively.

Above- and belowground exchange surfaces were respectively estimated at the peak of vegetation by the leaf area index and its belowground analogous, the root area index, defined as the ratio of the total leaf (or root) area on the corresponding ground area. The leaf area index (LAI; m^2 m^{-2}) was assessed for each circular quadrat in a similar way than aboveground biomass: the number of contacts recorded by species from the point intercept method was converted into aboveground leaf area using an appropriate calibration equation (Barkaoui et al. 2013). The root area index (RAI; m^2 m^{-2}) was calculated for each circular quadrat using SRA and root biomass values of all 10 cm layers constituting the whole soil profile.

Differences in water-use at the community level were assessed along the soil gradient by the maximal evapotranspiration (ET_m; mm) and the total transpirable soil water (TTSW; mm), which were both directly derived from the adjusted water-balance model (as previously described) for each plot.

Explaining multivariate co-variation of traits among communities.

A principal component analysis (PCA; ter Braak 1987) was performed with the CWMs of traits for each circular quadrat, followed by a redundancy analysis (RDA; ter Braak 1987) based on environmental data in order to characterize the variations of multivariate trait syndrome at the community level and how soil characteristics may underlie the observed pattern. Gross and net effects of each soil characteristic were tested in turn following the methodology of

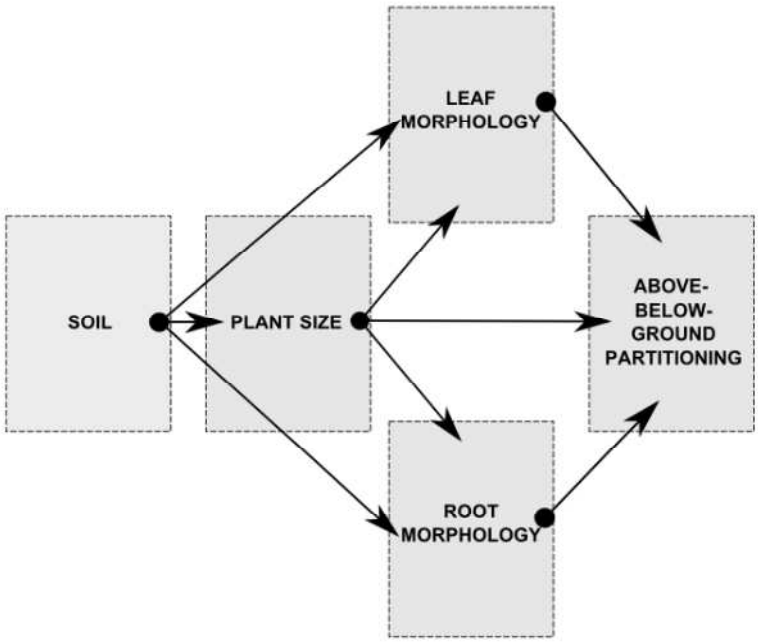


Fig. 2 Hypothesized meta-model of cascading relationships between soil properties, vegetation characteristics (average plant size, leaf morphology, root morphology) and the above- vs. belowground biomass partitioning at community level.

Fried et al. (2008). Separate RDAs with each soil characteristic as single explanatory variable were performed to quantify gross effects. Similarly, net effects were quantified using partial RDAs with single explanatory variable but the effect of the other nine variables was accounted for by including them as covariates in the analysis. Ratio of resulting eigenvalue over the total inertia of the PCA was used as a measure of the proportion of variation explained by each soil characteristic. A Monte-Carlo test with 999 permutations was then performed to ensure the significance of the results. Finally, bivariate regressions were performed between both biomass-based or area based root:shoot ratios (BGB:AGB or RAI:LAI) and the first axis of PCA explaining most of the variation in traits averages. Root:shoot ratios were previously log-transformed to meet normality assumption. Moreover, allometric relationships between AGB and BGB, as well as between RAI and LAI were tested after log-transformation using 'standardized major axis' (SMA) regressions (Warton 2006; Warton et al. 2012).

Explaining the differences in root:shoot ratio by causal relationships.

Causal relationships between the environment, CWMs of traits and root-to-shoot ratios were tested using a structural equation modeling approach (SEM; Shipley 2002; Grace 2010). Based on our initial hypotheses, an *a priori* meta-model was set up linking soil, average plant size in the community, average morphology of leaves and roots and above- vs. belowground partitioning together (Fig. 2). The initial causal structure posits that 1) at the community level, average plant dimension should respond to soil characteristics as well as leaf and root morphology; 2) morphology of leaves and roots should be moreover partly determined by plant dimension due to internal allometric constraints; 3) altogether, both mean plant dimension and organ morphology determine root:shoot ratio at community level (for further description of underlying rationale, see Fig. S2 and associated description). Soil characteristics were considered as exogenous variables with no incoming arrows whereas variables relative to vegetation were endogenous variables, assumed to be influenced by the exogenous variables. The meta-model was then specified with relevant variables selected from the PCA and RDA (Fig. 2), whose number was deliberately limited in order to prevent from model over-fitting.

The expected covariance structure generated by the hypothetical model was compared to the covariance matrix from the empirical data. The overall model fit was tested using a combination of different statistical metrics (Grace 2006), namely the χ^2 value and its associated *p*-value, the *comparative fit index* (CFI) and the *root mean square error of approximation* (RMSEA). The $\chi 2$ test determines whether the fit between model and data was adequate. CFI and RMSEA both

Table 2 Studied soil characteristics with corresponding abbreviation and unit. Mean and range values are reported for each factor, as well as their gross and net effect on multivariate trait pattern derived from successive RDAs. Significance of effects was tested using a Monte-Carlo test with 999 permutations.

Soil characteristic	Abbreviation	Unit	Mean	Range	Gross effect	Net effect	Monte-Carlo test
Cation exchange capacity	CEC	cmol+ kg^{-1}	12.92	6.936-21.65	0.279	0.010	$p<0.001$
Sand content	Sand	g g^{-1}	0.593	0.055-0.909	0.274	0.033	$p<0.001$
C:N ratio	C:N	-	10.49	8.751-13.00	0.262	0.027	$p=0.003$
Soil depth	Soil depth	cm	41.92	10.00-100.0	0.223	0.009	$p<0.001$
Water holding capacity	WHC	mm	43.44	8.200-127.0	0.221	0.015	p<0.001
Soil pH	pH	-	7.804	6.990-8.137	0.195	0.007	$p=0.038$
Calcium carbonate content	$CaCO_3$	g g^{-1}	62.43	0.161-95.30	0.126	0.021	$p<0.001$
Organic matter content	OM	g g^{-1}	0.051	0.029-0.085	0.114	0.020	$p=0.057$
Clay content	Clay	g g^{-1}	0.171	0.049-0.519	0.072	0.011	$p<0.001$
Available phophorus	P_2O_5	g g^{-1}	0.010	0.006-0.016	0.064	0.044	$p=0.006$
All factors			-	-	0.597	0.597	$p<0.001$

take sample size into account and assess the closeness of fit. Good models generally have non-significant $\chi 2$ associated with high p-value, $\chi 2/df < 2$, CFI > 0.95 and RMSEA < 0.05.

Path analysis was used to quantify both direct and indirect relationships between the selected variables. Relationships between variables may be either causal or correlation. Standardized coefficients specify the strength of the relationship and measure the degree to which one variable affects another given the overall structure of the model. The direct effect of one variable on another was directly measured by the value of standardized path coefficient, while its indirect effect was measured by the product of all intermediate standardized path coefficients. Summing both direct and indirect effects together allowed to compare total effects of variables on RAI:LAI ratio. Parameters were all tested for significance using z statistics after a 999 bootstrap procedure.

Predicting TTSW and ET_m using a "big root" and "big leaf" approach.

Total transpirable soil water (TTSW) was estimated using geometrical properties of the whole root system by defining the amount of water contained in the volume of soil that is under the influence of root activity (Casper et al. 2003, Hinsinger et al. 2009). Following the model of Gardner (1960), each single root was considered as a cylinder of uniform radius r_0 and an effective length L having uniform water-absorbing capacities. Water depletion was hypothesized to occur from uptake in a cylindrical soil volume of radius r_1 surrounding the root along the entire root length L. Thus, assuming an uniform distribution of roots in each soil layer, the volume of soil that is under the influence of roots can be calculated by summing all the soil cylindrical volumes surrounding each single root. In order to account for differences in water retention properties depending on soil texture, this volume was multiplied by the volumetric soil water holding capacity. Doing so, we predicted TTSW as follows:

$$TTSW_i = \sum_{j}^{RD_i} 0.5 \times RAI_{i,j} \times r_1 \times \frac{WHC_j}{h_j} \qquad \text{eqn. 3}$$

where $TTSW_i$ is the total transpirable soil water of community i, RD_i is maximal rooting depth of community i defining the number of soil layers with effective roots, $RAI_{i,j}$ is root area index of community i in each soil layer, r_1 is radius of the cylindrical zone of influence around each root as described above, WHC is water holding capacity for each soil layer containing roots, and h_j is the layer depth (10 cm in most cases, see above). Value of r_1 depends on diffusion constraints in the soil and was fixed here to 3 cm following the empirical results of Doussan et al. (2003).

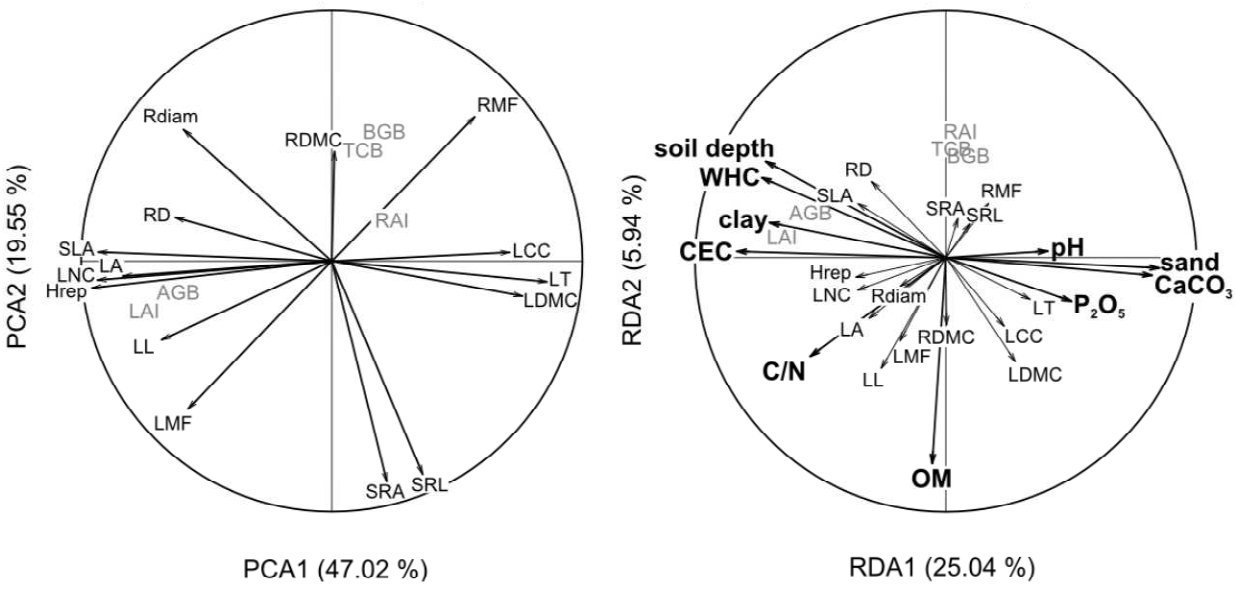


Fig. 3 (a) Principal component analysis (PCA) of the CWM traits matrix (36 relevés x 15 traits). The first two axes were significant after a test of dimensionality (p<0.0001) and explained 66.57 % of the total variation. Total community biomass (TCB), aboveground biomass (AGB), belowground biomass (BGB) leaf area index (LAI) and root area index (RAI) were added as illustrative variables (in grey). Correlations with axes and loadings are in Table 1. (b) Redundancy analysis (RDA) with the CWM traits matrix (36 relevés x 15 traits) constrained by 10 soil characteristics: soil depth, clay content (clay), sand content (sand), water holding capacity (WHC), carbonate calcium content ($CaCO_3$), pH, organic matter content (OM), C/N ratio, cation exchange capacity (CEC) and phosphorus content (P_2O_5). These soil characteristics significantly explained 59.57 % of the total PCA inertia after a Monte-Carlo test based on 9999 permutations (p<0.0001). The first two axes cumulated 80.25 % of the constrained inertia. Correlations with axes and loadings are in Table 2.

WHC was calculated using an empirical equation from Saxton & Rawls (2006) with texture data for each soil layer.

Maximal evapotranspiration (ET_m) was derived from the energy-balance of the community using the equation of Penman-Monteith (Allen et al. 1998; Allen et al. 2005), as follows:

$$\lambda \times ET_{m_i} = \frac{(\Delta \times \varepsilon_i \times Rg) + (\rho_a \times Cp \times \frac{VPD}{ra_i})}{\Delta + \gamma \times (1 + \frac{rc_i}{ra_i})} \quad \text{eqn. 4}$$

where ET_{m_i} is the maximal evapotranspiration of community *i*, λ is latent heat of vaporization, Δ is slope of saturation vapour pressure-temperature relationship, ε_i is efficiency of light interception of community *i*, Rg is total solar radiation, ρ_a is density of air, Cp is specific heat of air, VPD is saturation vapour pressure deficit, ra_i is aerodynamic resistance of community *i*, rc_i is bulk stand resistance of community *i*, and γ is psychrometric constant. Among all the variables involved in eqn. 4, many are related to climate and physical properties of the air (λ, Δ, Rg, ρ_a, Cp, VPD and γ). These variables have been considered as constant along the soil gradient, and their values have been provided at daily time step by the local meteorological station. However, the efficiency of light interception (ε_i), aerodynamic resistance (ra_i) and bulk stand resistance (rc_i) depend on vegetation characteristics, such as height and LAI, and were therefore specifically calculated for each community using corresponding LAI and height data.

A direct pairwise comparison between predicted and empirical values of the parameters (ET_m and TTSW) was not possible because the Diviner probes and the circular quadrats were not rigorously positioned at the same place within each 12 selected plots. The important spatial variability in soil characteristics and vegetation structure could have led to misleading results. Thus, congruence between predicted and empirical values were evaluated using a slope (and intercept) comparison test of their respective relationship along the soil gradient using soil depth as explicative variable in regressions. Slope differences were tested using a likelihood ratio statistic (LLR), while intercept shifts were tested using a Wald statistic (Warton 2006; Warton et al. 2012).

Table 3 Standardized total, direct and indirect effects of trait and soil variables on RAI:LAI ratio at community level. Direct effects are standardized partial regression coefficients. Total effects were calculated by adding direct and indirect effects. All effects were significant at $p<0.05$.

Variable	Total effect	Direct effect	Indirect effect
Hrep	-0.94	-1.23	0.29
LT	-0.89	-0.89	-
Sand	-0.78	-	-0.78
RD	0.55	0.55	-
SLA	-0.54	-0.54	-
Soil depth	0.48	-	0.48
SRA	0.22	0.22	
Rdiam	-	-	-

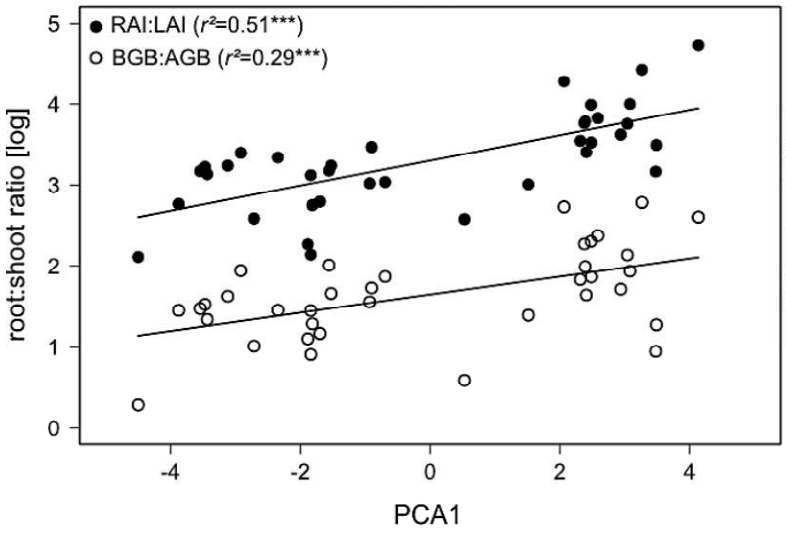


Fig. 4 Relationship between root:shoot ratios and the first axis of PCA explaining 48.07 % of the total co-variation in traits. Root:shoot ratios were calculated with community above- and belowground biomass (BGB:ABG; white open points), and with community leaf area index and root area index (RAI:LAI; black filled points). Data points represent individual circular quadrats. Lines were calculated by linear regressions (OLS method).

Results

Multivariate trait co-variation among communities along the soil gradient

A large percentage (66.57 %) of functional variation in CWM_traits was explained by the first two axes of PCA (Fig. 3a). Size-related traits (Hrep and RD), leaf morphological traits (LL, LA, LT, SLA and LDMC) and chemical traits (LCC and LNC) were related together with the first PCA axis explaining 47.02 % of the variation. Root morphological traits (SRL, SRA and RDMC) were associated with the second PCA axis explaining 19.55 % of the variation. Root diameter and allometric traits (RMF and LMF) took an intermediate position between the two set of traits. The supplementary variables LAI and AGB were strongly and positively associated with the first PCA axis, BGB and TCB were rather associated to the second axis, whereas RAI was only weakly associated with the first axis (Fig. 3a, Table 1). BGB:AGB and RAI:LAI ratios were both significantly related to the first axis of trait PCA (Fig. 4), although noticeably better for the latter (r^2=0.51, p<0.0001) than the former (r^2=0.39, p<0.0001). Allometric relationships were not significant between BGB and AGB (r^2=0.05, p=0.19), nor between RAI and LAI (r^2=0.04, p=0.24).

Soil characteristics explained 39.74 % of the total multivariate CWM_traits co-variation in the RDA: soil depth, texture and water holding capacity as well as soil CEC and $CaCO_3$ content were all significantly explicative (p<0.001, Table 2) and were associated together with the first axis explaining 25.04 % of the total variation (Fig. 3b). Soil organic matter content, C:N ratio and P-Olsen content were less explicative (Table 3) and were most related to the second axis explaining 5.94 % of the variation (Fig. 3b). Soil pH did not have an important role in structuring multivariate trait patterns. LAI and AGB were both positively associated with the first axis whereas RAI, BGB and TCB were poorly explained by soil characteristics.

Cause-effect relationships between environment and traits explaining RAI:LAI ratio

The specified model was consistent with the data (χ^2=13.29, $\chi 2/df$=1.33, p=0.208, *CFI*=0.99, *RMSEA*=0.096). However, several paths were not significant at p<0.05 such as the path from rooting depth to height, or from soil depth and SLA (Fig. 5). These non-significant paths were removed from the initial model except the path from 'sand' to 'Rdiam' which was kept despite marginal significance (p=0.086). The modified model provided a better fit to the data (χ^2=23.55, χ^2/df=1.07, p=0.371, *CFI*=0.995, *RMSEA*=0.044) than the initial model and explained 80.70 % of the variation in RAI:LAI ratio (Fig. 5).

Size related traits (RD and Hrep) responded directly to soil characteristics. RD responded positively to soil depth while Hrep responded negatively to sand content (Fig 5, Table 3), two soil

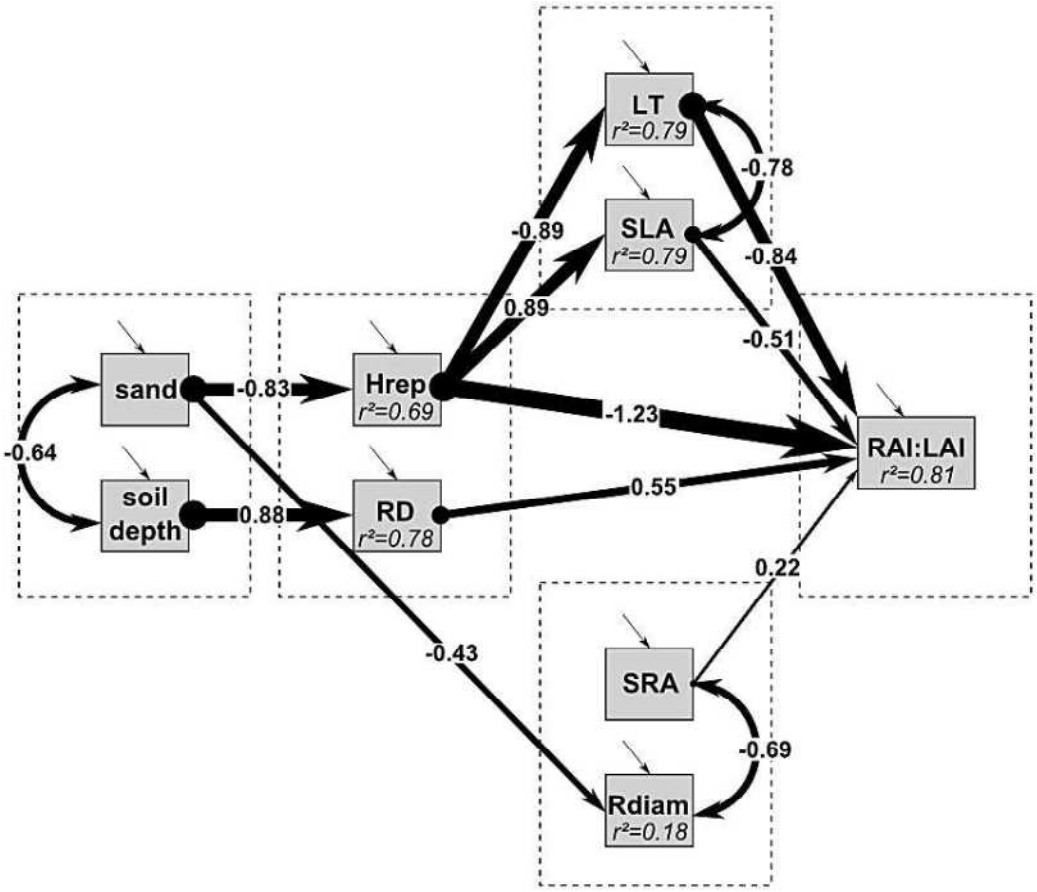


Fig. 5 Final structural model derived from initial model in Fig. 4. Only significant pathways ($p<0.05$) are represented by arrows. Single headed arrows represent direct effects; double-headed arrows represent correlations. Path coefficients on single-headed arrows between variables are standardized partial regression coefficients of direct effects. For total and indirect effects, see Table 3. Arrows widths are proportional to the standardized path coefficient. Variances explained by the model (R^2) are given under the variable names.

characteristics that were negatively correlated to each other: communities are composed by smaller plants with lower average rooting depth at sites with shallow sandy soils. There were no significant relationships between average rooting depth and root morphological traits. Although root morphological traits were negatively inter-correlated (r=-0.69), Rdiam only had a significant negative response to sand content. Conversely, leaf morphological traits were also negatively inter-correlated (r=-0.78) but they did not respond directly to soil characteristics. They were however both related to plant height, SLA positively and LT negatively (Fig. 5, Table 3). Finally, as expected, size related traits together with leaf and root morphological traits had all direct effects on RAI:LAI ratio, although no allometric was found between average rooting depth and plant height at the community level.

Combining both direct and indirect paths, Hrep and LT had the strongest total effect on RAI:LAI ratio (respectively -0.94 and -0.84, Table 3). Rooting depth contributed to a smaller proportion (0.55) whereas SRA had only a minor effect (0.22). Sand content was however an important factor (-0.78) influencing RAI:LAI ratio through indirect paths only, revealing the importance of indirect effects in such cascading response-effect model. Indirect effects of soil depth were intermediate (0.48).

Ecological importance of RAI:LAI ratio for water-use at community level.

Parameter estimation of the water-balance model revealed that the total transpirable soil water (TTSW) varied significantly among communities along the soil gradient (r^2=0.94, p<0.0001, Fig. 6a). Sites with deeper soil water holding capacity had greater TTSW. Similarly, potential rates of water evapotranspiration (ET_m) also increased significantly with increasing water availability (r^2=0.82, p<0.0001, Fig. 6b).

Predictions of both TTSW and ET_m respectively based RAI and LAI were highly congruent to the observed trends along the soil gradient. RAI provided a good prediction of TTSW along the soil gradient (r^2=0.45, p<0.0001, Fig. 6a), although weak but significant differences in slope (LLR=5.361, p=0.02) and intercept ($Wald$=8.529, p=0.003) were found. Similarly, accounting for LAI variation allowed to accurately predict the variations in ET_m along the soil gradient (r^2=0.41, p<0.0001, Fig. 6b) with a weak but significant difference in slope was noticed (LLR=5.212, p=0.02). Predictions however significantly under-estimated ET_m values ($Wald$=96.03, p<0.0001, Fig. 6b).

Highly significant and positive relationships were found between TTSW and ET_m (Fig. 6c), both with fitted values from the water balance model (r^2=0.83, p<0.0001) and with predicted values (r^2=0.39, p<0.0001). Slopes did not differ significantly between the two types of values

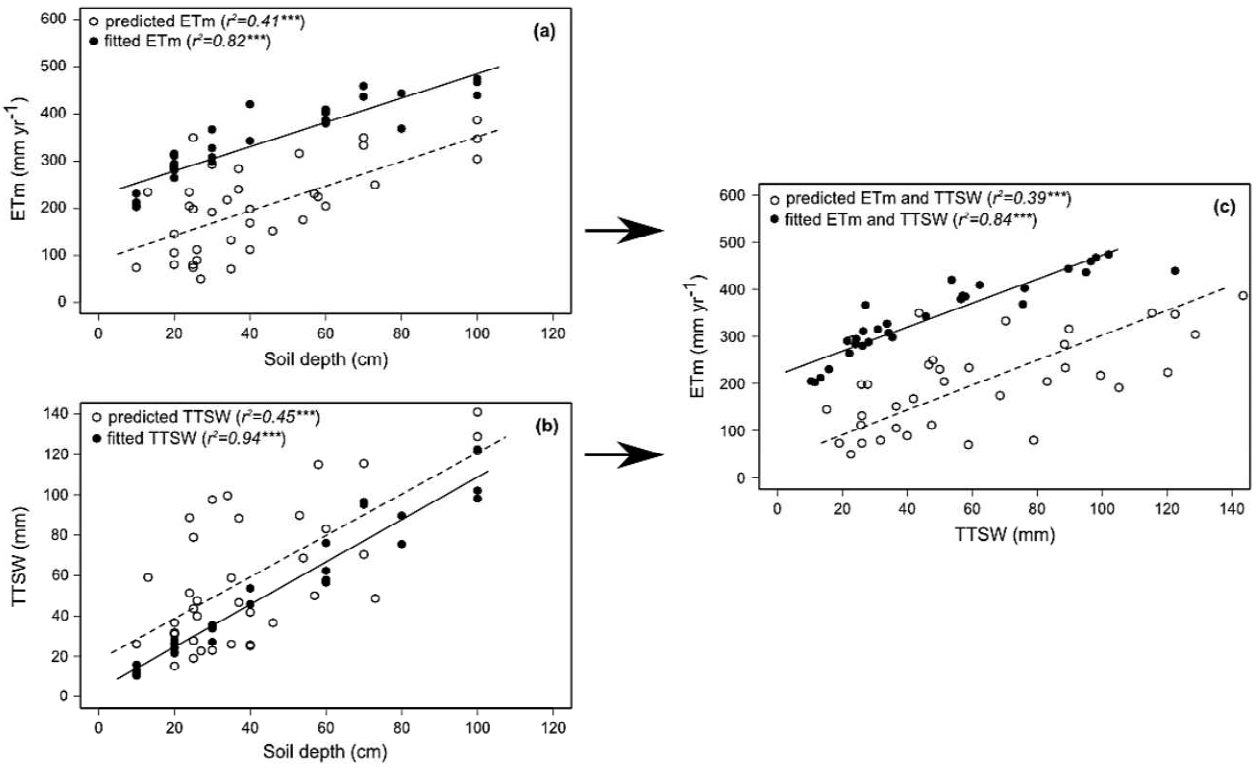


Fig. 6 Relationship between (a) TTSW and soil depth, (b) ET_m and soil depth, and (c) ET_m and TTSW. Empirical values of TTSW and ET_m were estimated from the adjustment of the water balance model to soil water content data (black filled points). Predicted values resulted from calculations using theoretical equations and vegetation data (open white points). Thus, data points represent individual diviner probes and circular quadrats respectively. Lines were calculated by linear regressions (OLS method), solid lines corresponding to empirical values of TTSW and ET_m (black filled points) and dashed lines to predicted values (open white points).

(*LLR*=0.061, *p*=0.81), suggesting that the slight differences in slope found previously were not decisive. The intercept was however lower with predicted values of TTSW and ET_m (*Wald*=130.7, *p*<0.0001), arising from cumulated differences in intercept found previously.

Discussion

Aboveground and belowground vegetation properties such as leaf and root areas have a fundamental role in ecosystem functioning because they strongly influence both biogeochemical and hydrological cycles. Yet, how variations in abiotic conditions may impact such properties which in turn will affect the rate of ecosystem processes requires more integrated understanding of underlying mechanisms. In this study, we investigated how plant traits at the community level may mediate the effect of varying soil water availability on ecosystem water flows in species-rich Mediterranean rangelands. We provide evidence that both leaf and root exchange surfaces may be determinant drivers of local water balance in such ecosystems regardless to species identity and diversity. Moreover, we show that multivariate response of community weighted mean traits related to plant stature and organ morphology may principally drive the coordinated dimensioning of above and belowground exchange surfaces.

Functional structure of plant communities in response to soil water availability

Our results confirm that the fundamental trade-off between rapid acquisition and conservation of resources (Wright et al. 2004) which was extensively displayed in relation with varying soil fertility over the past decade (Ordoñez et al. 2009, 2010), may also be a primary structuring response along water availability gradients (Ackerly & Cornwell 2007; Frenette-Dussault et al. 2012). In line with previous work on the study site (Bernard-Verdier et al. 2012; Perez-Ramos et al. 2012), the first PCA axis (Fig. 3a) revealed a continuum between two opposing syndromes of CWM_traits related to plant stature and resource economy, ranging from "stress tolerant" communities (*e.g.,* low SLA, low LNC, high LDMC) dominated by short plants, towards more "competitive" communities (*e.g.,* high SLA, high LNC, low LDMC) with taller plants. Furthermore, AGB and LAI were strongly correlated to this PCA axis which is consistent with the idea that "stress-tolerant" communities should be less productive than more "competitive" communities.

By including a suite of root traits in the analysis we complete previous vision of plant ecological strategy schemes and specify how aboveground and belowground traits may be coordinated at the community level. To date, root traits only begin to be considered in comparative ecology approach (Hummel et al. 2007; Freschet et al; 2010; Fortunel et al. 2012), and we present here one of the first field studies which provide patterns of co-variation of

belowground traits at the community level (see also Perez-Ramos et al. 2012). Interestingly, our results show that aboveground and belowground plant traits may not necessarily behave similarly at the community level when abiotic conditions vary. At the species level, while it has been repeatedly suggested that leaf, stem and root traits should be coordinated towards an integrated 'plant economics spectrum' (Freschet et al. 2010), empirical tests across a broad range of species tended to reveal conflicting correlations, particularly in case of morphological traits (Withington et al. 2006; Hobbie et al. 2010; Kembel & Cahill 2011). In this study, only rooting depth and root diameter were found to be consistent together with aboveground traits along the first axis of PCA. In contrast, SRA, SRL and RDMC which have been proposed to reflect belowground resource economy strategy, by analogy with SLA and LDMC, determined here an independent second axis of variation explaining a far less proportion of the total variation in traits at the community level (Fig. 3a). Otherwise, whereas the optimal partitioning theory predicts greater allocation to root tissue when soil resources are limiting (Shipley & Meziane 2002), allometric traits (LMF and RMF) appeared to be facultative in multivariate co-variation of traits among communities and were associated to the third axis of trait PCA (data not shown).

This apparent non- (or weak) coordination of above- and belowground traits at the community level may however hide an adaptative decoupling of both root and leaf strategies. In broad outline, acquisitive strategies (*e.g.,* strong SLA and SRA) may have been selected for both leaves and roots at the less resource-limited end of the gradient, favoring resource turn-over, rapid plant growth and greater competitive ability. At the opposite end of the gradient, strong water shortage in soil may have selected for species with more conservative leaf strategy but in combination with an acquisitive strategy for roots intensifying soil foraging to maximize water exploitation. Thus, the correlation between leaf and root traits may inverse along a gradient of soil resource, obscuring overall expected patterns of above- and belowground coordination.

Furthermore, previous work on the study site showed that more diversified strategies may coexist in more constrained environments (Bernard-Verdier et al. 2012), involving certainly different suites of traits (Chapin et al. 1993), with different scaling relationships among traits according to both abiotic factors and biotic interaction (Wright et al. 2005; Liu et al. 2010; de Bello et al. 2012). In particular, what is optimal for one species may not be for another. While one species may allocate increased biomass primarily to roots in response to decreasing water availability, another would preferentially reduce plant height and SLA. Additionally, the fact that several abiotic constraints are usually crossed to each other along complex edaphic gradients may even intensify the functional divergence in resource-poor environments. Typically, because roots are fundamentally involved in both resource economy and plant anchoring, variations in soil

characteristics may have additional constraints on root traits compared to leaf traits, explaining their decoupling at the community level. Considering that root morphological traits vary with depth (Vanguelova et al 2005; Makita et al. 2011), these results may differ if root traits are measured across the entire soil profile or only on the first soil layers as it is usually done. Thus, even locally the coordination between above- and belowground components of vegetation may not be warranted to result in clear correlations among CWM_traits at the community level because traits are under multiple selective pressure forces.

Our results clearly support the hypothesis that species are sorted on the basis of their trait values by abiotic conditions, endorsing previous evidence of a strong trait-based habitat filtering during community assembly (Cornwell et al. 2007; Bernard-Verdier et al. 2012; Maire et al. 2012; Spasojevic & Suding 2012). Soil characteristics were confirmed to be determinant drivers of the selection of species traits related to plant size and resource economy. Moreover, we suggest that entire trait syndrome rather than individual traits may be the target of species selection within the community because they should better describe how species cope with local abiotic conditions in an integrated fashion. We would therefore recommend to be careful when interpreting community response to water shortage on the basis of single traits only because reliable and easy to measure traits specifically related to drought tolerance or resistance have not been identified yet.

Balancing effect of vegetation properties on ecosystem water-use

Theoretically, biomass partitioning between root and shoot can be predicted using plant allometric relationships (Enquist & Niklas 2002; Niklas 2004), but we show that such predictions may not be reliable at the community level when environmental conditions vary greatly. The allometric theory suggests that AGB and BGB should scale isometrically across individual plants regardless to environmental conditions (Enquist & Niklas 2002). It was supported by several empirical studies at the species level including a broad range of vascular plant species (Niklas & Enquist 2001), and was more recently suggested to be also valid at the community level across diverse ecosystems (Cheng & Niklas 2007; Yang et al. 2010). However, the allometric theory predicts that the scaling exponent of the power function between BGB and AGB should be common to all species and be close to 1, but it allows the *y*-intercept to be variable and species-specific. This indicates that absolute value of AGB could vary substantially with respect to BGB between plant growth forms and community types (Cheng & Niklas 2007; Yang et al. 2010). Thus, detecting general scaling relationships at the community level may be limited due to important noise caused by different *y*-intercepts in data points. This may be a reason why AGB

and BGB of communities with contrasting species composition and plant growth forms do not obviously fall into line with each other as it is the case along our study gradient as well as for other European grasslands at a more global scale (Mokany et al. 2006).

By contrast, we show that variations in vegetation properties may result in a stable scaling relationship between resource demand and supply at the ecosystem level. Both leaf and root areas were confirmed to be key drivers of ecosystem water-use at our study site, despite high variability in community structure and biomass. Using the Penman Monteith equation (Allen et al. 1998; Allen et al. 2005), LAI of vegetation enabled to predict accurately the potential annual outgoing water flows by evapotranspiration (ET_m) under certain climatic conditions. Likewise, accounting for the soil water retention properties, RAI of vegetation indicated the effective amount of soil water that were extractable by roots (TTSW) thanks to simple geometrical approximation of the root system (Gardner 1960). These results clearly validate the reliability of the "big leaf"-"big roots" approach for species-rich communities, and suggest that the primary response of vegetation to long-term changes in water availability in the environment may be the coordinated adjustment of exchange surfaces, namely RAI:LAI ratio. All other things being equal, reducing potential transpiration rates through lower LAI will allow for more parsimonious use of the amount of water extractable by roots, whereas intensifying soil exploration through higher RAI per unit of soil will increase the amount of water available for transpiration. One important implication of such coordination between RAI and LAI under Mediterranean climate may be the maintenance of potential rate of water-use during longer periods, delaying the negative effect of water stress of summer drought forcing stomata closure.

By analogy with plant allometry, which controls individual plant size based on internal hydraulic constraints, we therefore support the existence of an 'ecosystem allometry' (Kerkhoff et al. 2006) which controls the potential rate of ecosystem processes with respect to constraining resource limitation in the environment. Based on the initial allometric theory, the quantitative framework initiated by Kerkhoff et al. (2006) suggests that ecosystem functioning could be accurately predicted by integrating physiological function that occurs at the plant level across a collection of individual plants using only their size distribution within the community (*i.e* regardless to species identity). Predictions hold true for processes such as plant primary productivity and nutrient content across diverse communities (Kerkhoff et al. 2006). However, the authors did not explicitly quantify the rate of resource supply in the environment, assuming that plant biomass was a good proxy of the functional equilibrium between vegetation and abiotic conditions (Enquist & Niklas 2002). Here, we provide additional insights into how vegetation

dimension and morphology mediate the interplay between capture of resource and resource use along a gradient of water availability.

From community trait response to vegetation properties

By using structural equation modeling (SEM), we were able to get further in the analysis and test causal relationships between the environment, plant traits and vegetation properties in an integrated response-effect framework (Lavorel & Garnier 2002; Suding et al. 2008). We show that effects of soil characteristics on vegetation biomass partitioning may be principally mediated by a tight set of traits related to plant size (average plant height and rooting depth) and organ morphology (SLA, LT, SRA and Rdiam). Surprisingly however, rooting depth and root morphology only had a minor effect compared to plant height and leaf morphology (Fig. 6, Table 3), suggesting greater responsiveness of plant aboveground component to variations in water availability compared to belowground component. This may provide an explanation why the variations in RAI:LAI ratio were essentially due to the variations of LAI along the first PCA axis despite larger absolute values of RAI. Furthermore, among aboveground traits, we show that average plant height was predominant in determining biomass partitioning of vegetation, leaf morphological traits having lower explicative power.

To that extent, our results support theoretical eco-evolutionnary schemes which depict viable plant strategies as an optimization between plant hydraulics and light acquisition based on plant size (Falster & Westoby 2003). Physiologically, the same conditions that are favorable for plant growth (high water and light availability) are those that are conducive to greater height and LAI. However, driving mechanisms such as water limitation at dry sites and competition for light at wetter sites may more likely underlie the observed pattern linking height and vegetation LAI. When soil water is not limiting growth, plants are essentially in competition for light (Falster & Westoby 2003). Because taller plants may have greater access to light than shorter understory plants, competition for light is strongly asymmetric and there is greater benefit for increasing height (Falster & Westoby 2003), particularly in high LAI-sites where light is largely preempted by the vegetation. Being advantaged, taller plants have greater opportunity to better develop themselves, keep on producing higher LAI for preempting light and thus ensure their dominance within the community but also to fulfill their maintenance costs and construction requirements. Therefore, the race for light within the community may lead to increasing overall LAI through the selection of species with taller plant strategy. Indeed, increasing SLA (Fig. 6, Table 3) was also often interpreted as part of the adaptative strategy for light competition by increasing the efficiency of light interception (Schieving & Poorter 1999). Conversely, when soil water is a

limiting resource tall plant strategy may not be the successful strategy as illustrated by global patterns of plant height (Moles et al. 2009), namely because increasing height should concomitantly increase construction and maintenance costs that may not be supported under low water availability (Enquist 2003; Mencuccini 2003). As expected here (Fig. 1), average plant height significantly decreased toward shallower and sandy soils which have lower water holding capacity (Fig. 4, Table 1). Belowground however, the picture is not straightforward because root competition for soil resources is symmetric (Cahill 2003). Typically, soil water cannot be preempted in an analogous way than for light: while vertically deep-rooted plants have greater access to deep soil water reserve, horizontally shallower-rooted plants better intercept rainfall water (Viola et al. 2008). Therefore, the effect of belowground competition on community structure and biomass was rather shown to be unimportant (Cahill 2003; Lamb & Cahill 2008).

Our results reveal that cascading trait-trait relationships may have important implication when studying how changes in the environment may impact vegetation properties, namely because abiotic factors could have both direct effect and several indirect effects through many co-varying traits. Previous works at the community level either provided growing evidence of trait-based response to abiotic factors, identifying key processes of community assembly, or highlighted governing trait-based effect of plant community on major ecosystem properties. However, both aspects were more often studied independently to each other, and therefore only direct relationships were investigated. The link between response traits, which are targets of species selection at the community level, and effect traits which control ecosystem functioning was more scarcely accounted for across habitats (but see Garnier et al. 2007; Klumpp & Soussana 2009, Minden & Kleyer 2011; Lienin & Kleyer 2012; Garcia-Palacios et al. 2013). Though, depending on environmental drivers, response and effect trait groupings may be more or less correlated to each other (Lavorel & Garnier 2002; Suding et al. 2008). For example, community response to disturbance involves traits related to fecundity, regeneration and dispersal (Grime 2006; Douma et al. 2012) while ecosystem processes such as productivity or biogeochemical cycling depend more importantly on traits related to resource economy. Consequently, ecosystem functioning could be more or less sensitive to changes in environmental conditions, depending on functional linkages among response and effect traits at the community level: more independent sets of traits would possibly allow greater buffering effect. Here however, the fact that a single set of traits, namely size-related traits (Fig. 6), both responded to soil characteristics and in turn strongly determined RAI:LAI ratio suggests that even slight variations in edaphic conditions may directly impact vegetation properties.

Conclusion

Considering that plant traits were shown to have a pivotal role between soil characteristics and RAI:LAI ratio at the community level, we provide evidence that the functional structure of plant communities may be key driver of ecosystem water flows in this Mediterranean rangeland. Decreasing soil water availability may select for plants with more adapted growth form and trait values, namely with reduced height and more sclerophyllous leaves, resulting in higher RAI:LAI ratio. In turn, modifying root:shoot ratios at the community level may allow a 'functional equilibrium' between water loss by leaf transpiring surfaces and water extraction by root surfaces. By analogy with the phenotypic plasticity commonly observed for species along resource gradients, we suggest therefore that changes in species relative abundances and species turn-over occurring at the community level drive a morphological variability of communities explaining functioning modulations in the face of limiting resources. Further research should better incorporate plant demography into such trait-based approach in order to predict the effect of the absolute amount of biomass on ecosystem water flows.

Acknowledgements

This work was funded by the ANR program O_2LA (09-STRA-09). We thank the experimental station 'INRA-La Fage' for access to the facilities, as well as Jean Richarte and Alain Blanchard for their valuable support during field work and laboratory sorting. We also thank Maud Bernard Verdier and Florence Volaire for helpful discussions and comments on early version of the manuscript.

References

Ackerly DD (2004) Functional strategies of chaparral shrubs in relation to seasonal water deficit and disturbance. Ecological Monographs, 74, 25–44.

Barkaoui K, Bernard-Verdier M, Navas M-L (2013) Questioning the Reliability of the Point Intercept Method for Assessing Community Functional Structure in Low-Productive and Highly Diverse Mediterranean Grasslands. Folia Geobotanica, 48, 393–414.

Bartlett MK, Scoffoni C, Sack L (2012) The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. Ecology Letters, 15, 393–405.

De Bello F, Janeček Š, Lepš J, Doležal J, Macková J, Lanta V, Klimešová J (2012) Different plant trait scaling in dry versus wet Central European meadows. Journal of Vegetation Science, 23, 709–720.

Bernard-Verdier M, Navas M-L, Vellend M, Violle C, Fayolle A, Garnier E (2012) Community assembly along a soil depth gradient: contrasting patterns of plant trait convergence and divergence in a Mediterranean rangeland. Journal of Ecology, 100, 1422–1433.

Casper BB, Schenk HJ, Jackson RB (2003) Defining a plant's belowground zone of influence. Ecology, 84, 2313–2321.

Chapin III FS, Autumn K, Pugnaire F (1993) Evolution of suites of traits in response to environmental stress. American Naturalist, 142, S78–S92.

Cheng D-L, Niklas KJ (2007) Above- and below-ground biomass relationships across 1534 forested communities. Annals of botany, 99, 95–102.

Cornelissen JHC, Lavorel S, Garnier E et al. (2003) A handbook of protocols for standardized and easy measurement of plant functional traits worldwide. Australian Journal of Botany, 51, 335.

Cornwell WK, Schwilk LDW, Ackerly DD (2006) A trait-based test for habitat filtering: convex hull volume. Ecology, 87, 1465–71.

De Deyn GB, Cornelissen JHC, Bardgett RD (2008) Plant functional traits and soil carbon sequestration in contrasting biomes. Ecology Letters, 11, 516–31.

Douma JC, Shipley B, Witte JM, Aerts R, Bodegom PM Van (2012) Disturbance and resource availability act differently on the same suite of plant traits: revisiting assembly hypotheses. Ecology, 93, 825–835.

Enquist BJ, Niklas KJ (2002) Global allocation rules for patterns of biomass partitioning in seed plants. Science, 295, 1517–20.

Enquist BJ, Economo EP, Huxman TE, Allen AP, Ignace DD, Gillooly JF (2003) Scaling metabolism from organisms to ecosystems. Nature, 423, 639–42.

Eviner VT, Chapin III FS (2003) Functional matrix: a conceptual framework for predicting multiple plant effects on ecosystem processes. Annual Review of Ecology, Evolution, and Systematics, 34, 455–485.

Falster DS, Westoby M (2003) Plant height and evolutionary games. Trends in Ecology & Evolution, 18, 337–343.

Fortunel C, Fine PVA, Baraloto C (2012) Leaf, stem and root tissue strategies across 758 Neotropical tree species. Functional Ecology, 26, 1153–1161.

Frenette-Dussault C, Shipley B, Léger J-F, Meziane D, Hingrat Y (2012) Functional structure of an arid steppe plant community reveals similarities with Grime's C-S-R theory. Journal of Vegetation Science, 23, 208–222.

Freschet GT, Cornelissen JHC, van Logtestijn RSP, Aerts R (2010) Evidence of the "plant economics spectrum" in a subarctic flora. Journal of Ecology, 98, 362–373.

Garnier E, Navas M-L (2012) A trait-based approach to comparative functional plant ecology: concepts, methods and applications for agroecology. A review. Agronomy for Sustainable Development, 32, 365–399.

Garnier E, Cortez J, Billès G et al. (2004) Plant functional markers capture ecosystem properties. Ecology, 85, 2630–2637.

Garnier E, Lavorel S, Ansquer P et al. (2007) Assessing the effects of land-use change on plant traits, communities and ecosystem functioning in grasslands: a standardized methodology and lessons from an application to 11 European sites. Annals of botany, 99, 967–85.

Geesing D, Bachmaier M, Schmidhalter U (2004) Field calibration of a capacitance soil water probe in heterogeneous fields. Australian Journal of Soil Research, 42, 289–299.

Grace JB, Anderson, Michael T, Olff H, Scheiner SM (2010) On the specification of structural equation models for ecological systems. Ecological Monographs, 80, 67–87.

Grime JP (2006) Trait convergence and trait divergence in herbaceous plant communities: Mechanisms and consequences. Journal of Vegetation Science, 17, 255.

Gross N, Robson TM, Lavorel S, Albert CH, Le Bagousse-Pinguet Y, Guillemin R (2008) Plant response traits mediate the effects of subalpine grasslands on soil moisture. New Phytologist, 180, 652–62.

Groves SJ, Rose SC (2004) Calibration equations for Diviner 2000 capacitance measurements of volumetric soil water content of six soils. Soil Use and Management, 20, 96–97.

Guswa AJ (2010) Effect of plant uptake strategy on the water−optimal root depth. Water Resources Research, 46, 601.

Hinsinger P, Bengough AG, Vetterlein D, Young IM (2009) Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant and Soil, 321, 117–152.

Hummel I, Vile D, Violle C et al. (2007) Relating root structure and anatomy to whole-plant functioning in 14 herbaceous Mediterranean species. New Phytologist, 173, 313–21.

Kerkhoff AJ, Enquist BJ (2006) Ecosystem allometry: the scaling of nutrient stocks and primary productivity across plant communities. Ecology Letters, 9, 419–27.

Klumpp K, Soussana J-F (2009) Using functional traits to predict grassland ecosystem change: a mathematical test of the response-and-effect trait approach. Global Change Biology, 15, 2921–2934.

Laio F, Porporato A, Ridolfi L, Rodriguez-Iturbe I (2001) Plants in water-controlled ecosystems: active role in hydrologic processes and response to water stress II: Probabilistic soil moisture dynamics. Advances in Water Resources, 24, 707–723.

Lavorel S, Garnier E (2002) Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. Functional Ecology, 16, 545–556.

Levy EB, Madden EA (1933) The point method of pasture analysis. New Zealand Journal of Agriculture Research, 46, 267–279.

Liancourt P, Callaway RM, Michalet R (2005) Stress tolerance and competitive-response ability determine the outcome of biotic interactions. Ecology, 86, 1611–1618.

Lienin P, Kleyer M (2012) Plant trait responses to the environment and effects on ecosystem properties. Basic and Applied Ecology, 13, 301–311.

Liu G, Freschet GT, Pan X, Cornelissen JHC, Li Y, Dong M (2010) Coordinated variation in leaf and root traits across multiple spatial scales in Chinese semi-arid and arid ecosystems. New Phytologist, 188, 543–53.

Maire V, Gross N, Börger L et al. (2012) Habitat filtering and niche differentiation jointly explain species relative abundance within grassland communities along fertility and disturbance gradients. New Phytologist, 196, 497–509.

Maseda PH, Fernández RJ (2006) Stay wet or else: three ways in which plants can adjust hydraulically to their environment. Journal of experimental botany, 57, 3963–77.

Mencuccini M (2003) The ecological significance of long-distance water transport: short-term regulation, long-term acclimation and the hydraulic costs of stature across plant life forms. Plant, Cell and Environment, 26, 163–182.

Minden V, Kleyer M (2011) Testing the effect-response framework: key response and effect traits determining above-ground biomass of salt marshes. Journal of Vegetation Science, 22, 387–401.

Mitchell PJ, Veneklaas EJ, Lambers H, Burgess SSO (2008) Using multiple trait associations to define hydraulic functional types in plant communities of south-western Australia. Oecologia, 158, 385–97.

Mokany K, Ash J, Roxburgh S (2008) Functional identity is more important than diversity in influencing ecosystem processes in a temperate native grassland. Journal of Ecology, 96, 884–893.

Müller I, Schmid B, Weiner J (2000) The effect of nutrient availability on biomass allocation patterns in 27 species of herbaceous plants. Perspectives in Plant Ecology, Evolution and Systematics, 3, 115–127.

Nardini A, Pedà G, La Rocca N (2012) Trade-offs between leaf hydraulic capacity and drought vulnerability: morpho-anatomical bases, carbon costs and ecological consequences. New Phytologist, 196, 788–98.

Niklas KJ (2004) Plant allometry: is there a grand unifying theory? Biological reviews of the Cambridge Philosophical Society, 79, 871–89.

Niklas KJ, Enquist BJ (2002) On the vegetative biomass partitioning of seed plant leaves, stems, and roots. American Naturalist, 159, 482–97.

Ordoñez JC, van Bodegom PM, Witte J-PM, Wright IJ, Reich PB, Aerts R (2009) A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. Global Ecology and Biogeography, 18, 137–149.

Reich PB, Ellsworth DS, Walters MB, Vose JM, Gresham C, Volin JC, Bowman WD (1999) Generality of leaf trait relationships: a test across six biomes. Ecology, 80, 1955–1969.

Saxton KE, Rawls WJ (2006) Soil water characteristic estimates by texture and organic matter for hydrologic solutions. Soil Science Society of America Journal, 70, 1569–1578.

Schenk HJ, Jackson RB (2002) Rooting depths, lateral root spreads and below-ground/above-ground allometries of plants in water-limited ecosystems. Journal of Ecology, 90, 480–494.

Schieving F, Poorter H (1999) Carbon gain in a multispecies canopy: the role of specific leaf area and photosynthetic nitrogen-use efficiency in the tragedy of the commons. New Phytologist, 143, 201–211.

Schymanski SJ, Sivapalan M, Roderick ML, Hutley LB, Beringer J (2009) An optimality-based model of the dynamic feedbacks between natural vegetation and the water balance. Water Resources Research, 45, 412.

Shipley B, Meziane D (2002) The balanced-growth hypothesis and the allometry of leaf and root biomass allocation. Functional Ecology, 16, 326–331.

Spasojevic MJ, Suding KN (2012) Inferring community assembly mechanisms from functional diversity patterns: the importance of multiple assembly processes. Journal of Ecology, 100, 652–661.

Suding KN, Lavorel S, Chapin FS et al. (2008) Scaling environmental change through the community-level: a trait-based response-and-effect framework for plants. Global Change Biology, 14, 1125–1140.

Vile D, Garnier E, Shipley B et al. (2005) Specific leaf area and dry matter content estimate thickness in laminar leaves. Annals of botany, 96, 1129–36.

Viola F, Daly E, Vico G, Cannarozzo M, Porporato A (2008) Transient soil-moisture dynamics and climate change in Mediterranean ecosystems. Water Resources Research, 44, W11412.

Warton DI, Wright IJ, Falster DS, Westoby M (2006) Bivariate line-fitting methods for allometry. Biological Reviews, 81, 259–91.

Warton DI, Duursma RA., Falster DS, Taskinen S (2012) smatr 3- an R package for estimation and inference about allometric lines. Methods in Ecology and Evolution, 3, 257–259.

Weiner J (2004) Allocation, plasticity and allometry in plants. Perspectives in Plant Ecology, Evolution and Systematics, 6, 207–215.

Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ (2002) Plant ecological strategies: some leading dimensions of variation between species. Annual Review of Ecology and Systematics, 33, 125–159.

Wright IJ, Reich PB, Westoby M et al. (2004) The worldwide leaf economics spectrum. Nature, 428, 821–7.

Wright IJ, Reich PB, Cornelissen JHC et al. (2005) Modulation of leaf economic traits and trait relationships by climate. Global Ecology and Biogeography, 14, 411–421.

Yang Y, Fang J, Ma W, Guo D, Mohammat A (2010) Large-scale pattern of biomass partitioning across China's grasslands. Global Ecology and Biogeography, 19, 268–277.

Zhang X, Zwiers FW, Hegerl GC et al. (2007) Detection of human influence on twentieth-century precipitation trends. Nature, 448, 461–5.

Supporting information

Fig. S1 Photography of three contrasting communities that were sampled along the edaphic gradient at the 'INRA-La Fage' experimental station (spring 2012).

(a) Plant community in a doline with deep-clay soil (>100 cm)



(b) Plant community at intermediate position with intermediate soil depth (40-80 cm)

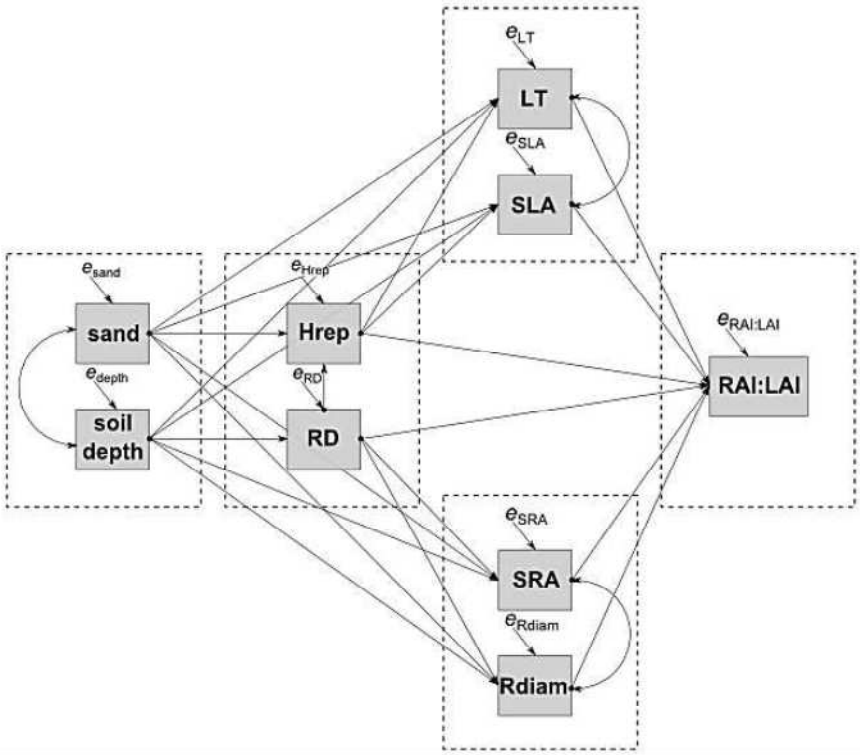


(c) Plant community at uphill position with shallow-sandy soil (<20 cm)



Supporting information

Fig S2 Hypothesized model specified with nine observed variables (grey boxes). Soil was characterized with soil depth and sand content; plant size was characterized by CWM of reproductive height (Hrep) and rooting depth (RD); leaf morphology was characterized by CWM of leaf thickness (LT) and specific leaf area (SLA); root morphology was characterized by root diameter (Rdiam) and specific root area (SRA); biomass partitioning was characterized by the ratio between the root area index and the leaf area index (RAI:LAI). Causal relationships are represented by one-headed arrows; free correlations are represented by double-headed arrows. Residual errors variables (e_x) represent effects of unexplained causes.



Description of the hypothetical model

Based on results from PCA and RDA, nine variables were selected to specify the hypothetical model (Fig. S2). The soil was specified by two physical characteristics (soil depth and sand content) both defining the volume of soil available to plants and its texture. The vegetation was specified by four plant functional traits: two size-related traits (Hrep and RD), two morphological leaf traits (SLA and LT) and their two analogous root traits (SRA and Rdiam). Output of the model was the ratio between the root area index and the leaf area index (RAI:LAI ratio). A first set of paths describes how plant communities respond to soil characteristics (Fig. S2). Together

with climate and disturbance, soil is a major environmental factor affecting plant growth strategy and performance (Ordoñez et al. 2009). Community plant size was hypothesized to respond directly to soil characteristics since climate and disturbance were held constant. For example, plant height was expected to be negatively affected by unfavourable soil conditions at community scale, especially in response to low water availability (Cingolani et al. 2007; Sonnier et al. 2010; Bernard-Verdier et al. 2012; Frenette-Dussault et al. 2012). Plant rooting depth was also expected to respond significantly to soil characteristics, although the direction of response is not clear in case of varying soil water availability, because it strongly depends on climate. Under a Mediterranean climate with seasonal drought periods, rooting depth may be potentially greater in cases of coarse structured soils with lower water holding capacity because of deeper water infiltration (Schenk & Jackson 2005). This may enable a more complete exploitation of water stored in the soil during stress periods. On the contrary, roots may rather preferentially develop in top soil layers for a more opportunistic use of rain water as it is the case in environments with prolonged periods of drought and deserts (Schenk & Jackson 2002).

In addition to plant size, leaf and root morphology have a pivotal role in resource-use strategy of plants and were therefore also expected to respond directly to soil characteristics (Fig. S2). For instance, leaf traits involved in the global leaf economic spectrum (Wright et al. 2004) such as SLA were shown to shift at the community level from values associated with high metabolic rates and high resource turn-over (*e.g.,* high SLA value) under favorable soil conditions towards values associated with lower metabolic rates (*e.g.,* lower SLA value) favoring resource retention under water-limited conditions (Bernard-Verdier et al. 2012; Frenette-Dussault et al. 2012). Similarly, root morphological traits such as SRA were shown to drive resource uptake from soil (Jackson et al. 1996), although far less empirical evidence are available at the community level. On this basis, root traits were also hypothesized to respond directly to soil characteristics, but in the opposite direction compared to leaf traits. In order to enhance water uptake capacity at drier sites, root traits were expected to shift from low values to higher values at the community level with deceasing water availability. Moreover, because roots have an important role in plant mechanics, namely for plant anchoring in soil, root traits were also expected to respond directly to physical constraints limiting root foraging such as soil porosity. For example, root diameter was shown to decrease in response to decreasing soil porosity (Schymanski et al. 2009). Because soil texture strongly influences soil porosity, fine texture having greater soil porosity, root diameter was therefore hypothesized to decrease when sand content increases.

A second set of paths describes the allometric and functional linkages among plant traits. Most importantly, an allometric relationship was expected between average rooting depth and

plant height at the community level (Fig. S2). According the allometric theory, root biomass is predicted to scale isometrically with stem biomass. From a mechanical point of view, it seems therefore also reasonable to expect that below- and aboveground plant dimensions should be somehow interrelated. For example plant height should be limited if rooting depth is limited by a physical barrier in soil. Furthermore, allometric theory predicts that leaf biomass should scale with the ¾ power of stem, suggesting possible constraints of plant size on the range of leaf morphology (Enquist & Nikals 2002; Niklas & Enquist 2002). While leaf morphology and plant height have been considered as two independent axes of variation in plant strategy (Westoby 1998; Westoby et al. 2002), recent studies supported significant linkages between leaf morphological traits and height in response to environmental drivers at both species and community level. Therefore we hypothesized a relationship between plant height and leaf morphology at the community level, greater heights allowing stronger SLA (Fig. S2). A relationship was also expected between root morphological traits and rooting depth at community level for analogous reasons.

Finally, a third set of paths describes the effect of community structure on emergent properties of vegetation such as RAI:LAI ratio which should strongly influence ecosystem water flows. Such relationships between community weighted mean traits and ecosystem functioning were already shown for processes such as biomass production and litter decomposition (*e.g.*, Garnier et al. 2004). Here, average plant size and organ morphology at the community level were hypothesized to determine the coordination between both total root and leaf areas of the vegetation.

References

Bernard-Verdier M, Navas M-L, Vellend M, Violle C, Fayolle A, Garnier E (2012) Community assembly along a soil depth gradient : contrasting patterns of plant trait convergence and divergence in a Mediterranean rangeland. J Ecol 100:1422–1433

Cingolani AM, Cabido M, Gurvich DE, Renison D, Díaz S (2007) Filtering processes in the assembly of plant communities: Are species presence and abundance driven by the same traits ? J Veg Sci 18:911–920

Enquist BJ, Niklas KJ (2002) Global allocation rules for patterns of biomass partitioning in seed plants. Science 295(5559):1517–1520

Frenette-Dussault C, Shipley B, Léger J-F, Meziane D, Hingrat Y (2012) Functional structure of an arid steppe plant community reveals similarities with Grime's C-S-R theory (P Adler, Ed.). J Veg Sci 23:208–222

Garnier E, Cortez J, Billès G, Navas M-L, Roumet C, Debussche M, Laurent G, Blanchard A, Aubry D, Bellman A, Neill C, Toussaint J-P (2004) Plant functional markers capture ecosystem properties. Ecology 85:2630–2637

Jackson RB, Canadell J, Ehleringer JR, Mooney HA, Sala OE, Schulze ED (1996) A global analysis of root distributions for terrestrial biomes. Oecologia 108:389–411

Niklas KJ, Enquist BJ (2002) On the vegetative biomass partitioning of seed plant leaves, stems, and roots. Am Nat 159:482–97

Ordoñez JC, van Bodegom PM, Witte J-PM, Wright IJ, Reich PB, Aerts R (2009) A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. Glob Ecol Biogeogr 18:137–149

Schenk HJ, Jackson RB (2005) Mapping the global distribution of deep roots in relation to climate and soil characteristics. Geoderma 126:129–140

Schymanski SJ, Sivapalan M, Roderick ML, Hutley LB, Beringer J (2009) An optimality-based model of the dynamic feedbacks between natural vegetation and the water balance. Water Resour Res 45:412

Sonnier G, Shipley B, Navas M-L (2010) Quantifying relationships between traits and explicitly measured gradients of stress and disturbance in early successional plant communities. J Veg Sci 21:1014–1024

Westoby M (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. Plant Soil 199:213–227

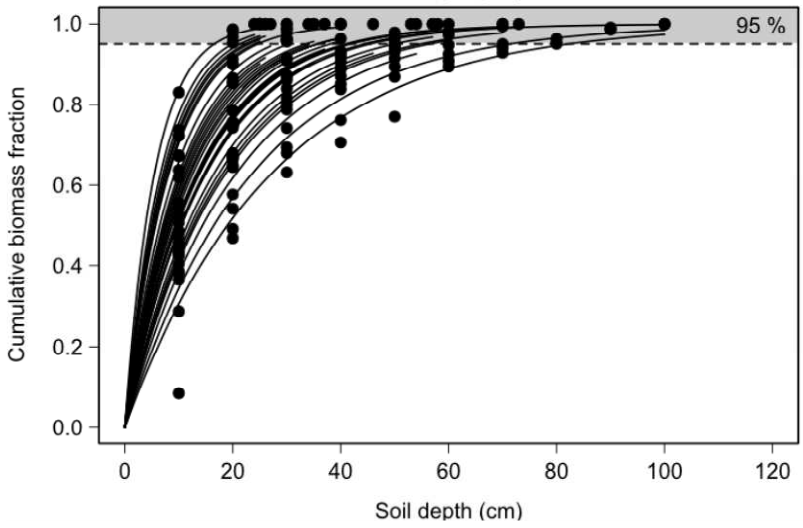
Wright IJ, Westoby M (2002) Leaves at low versus high rainfall: coordination of structure, lifespan and physiology. New Phytol 155:403–416

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont

BB, Lee T, Lee WG, Lusk C, Midgley JJ, Navas M-L, Niinemets U, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R (2004) The worldwide leaf economics spectrum. Nature 428:821–827

Supporting information

Fig. S3 Cumulative root distribution (cumulative proportion) as a function of soil depth. Data points represent individual 10 cm-soil layer for each community. Lines correspond to the fitted model of Gale & Grigal (1987). Grey area indicates when the 95 %.



Chapter 4

Importance of plant-plant interactions



(an isolated individual of *Carex humilis* in a removal experiment at La Fage-station, *spring 2012*)

MANUSCRIPT IV

Competitive interactions are driven by species' trait hierarchy and not by standing biomass in a Mediterranean rangeland.

-

Les interactions de compétition entre plantes sont régies par la hiérarchie des traits des espèces et non pas par les rapports de biomasse dans des parcours méditerranéens.

Karim Barkaoui, Marie-Laure Navas

Abstract

The assembly of plants into communities is a dynamic equilibrium that reflects continuous interactions between multiple processes including both habitat and biotic filtering. Yet, understanding how these processes work within communities under contrasting environmental conditions remains a longstanding issue in ecology for identifying reliable assembly rules. In particular, the importance of biotic processes in sorting species traits at the community level needs to be clarified since plant-plant interactions can lead to trait overdispersion in case of species niche differentiation ('limiting similarity') or to trait underdispersion in case of competitive exclusion ('competitive hierarchy'). In this study, we aimed to specify the mechanisms of biotic filtering by evaluating the relative effect of both species niche difference and competitive hierarchies in determining the importance of competition across a range of varying edaphic conditions. We carried out a removal experiment using established individuals of three co-occurring species of contrasting strategies within 60 plant communities from a Mediterranean rangeland to quantify how plant performance was affected by the presence of neighbors. Accounting for local abiotic limitations, the novelty of our approach was to decompose the impacts of neighbors into separated effects related (i) to aboveground biomass, and (ii) to the functional structure of the surrounding community. Three major traits, plant height, specific leaf area (SLA) and leaf dry matter content (LDMC), representing two distinct axes of species strategy related to resource economy were used to calculate absolute vs. relative trait distances (*i.e*, species similarity vs. competitive hierarchy). Our results confirmed that the importance of plant competition increases with increasing soil resource availability regardless to the identity of the target species. We found that hierarchical trait distances calculated with both plant height and LDMC were more important drivers of changes in plant performance within communities than species niche difference or aboveground biomass. However, while hierarchies in LDMC were predominant all along the gradient, both niche differences and hierarchies presumably acted for plant height depending on local abiotic conditions. Overall, these results suggest that competitive processes associated with light and soil resources preemption prevails in this Mediterranean rangeland, explaining more precisely how biotic interactions may determine the niche structure of communities. Although to a different extent depending on the considered trait, this is consistent with the action of equalizing mechanisms of species coexistence over short time periods. Moreover, we showed that independent axes of plant strategy may be involved in plant competition along such complex gradient, reflecting multiple effects of competition processes on species trait sorting that must accounted for when inferring community assembly from trait patterns.

Résumé

L'assemblage des communautés résulte des interactions continues entre différents processus comme la sélection par l'environnement physique ou biologique. Comprendre comment ces processus agissent au sein des communautés constitue une question fondamentale en écologie pour identifier des règles d'assemblage pertinentes. En particulier, l'importance de la compétition dans la sélection des traits des espèces doit être clarifiée dans la mesure où elle peut conduire à une sur-dispersion des valeurs de traits en cas de différentiation de niche ('limite à la similarité') ou à une sous-dispersion des valeurs de traits en cas d'exclusion compétitive ('hiérarchie compétitive'). Dans cette étude, nous avons cherché à préciser les mécanismes associés au filtre biotique en évaluant les effets relatifs de la dissimilarité de niche et des hiérarchies compétitives dans l'importance de la compétition. Nous avons conduit une expérimentation de suppression du voisinage pour quantifier *in natura* les modifications de performance de trois espèces cibles ayant des stratégies différentes en réponse à la compétition au sein de 60 communautés distribuées le long d'un gradient édaphique dans des parcours méditerranéens. L'originalité de notre approche a été de décomposé l'impact du voisinage en considérant séparément sa biomasse aérienne et sa structure fonctionnelle, tout en prenant en compte les limitations abiotiques locales. Nous avons calculé des distances absolues vs. relatives des valeurs de traits entre les espèces cibles et leur voisinage (*i.e.*, dissimilarité des espèces vs. hiérarchie compétitive) pour trois traits fonctionnels : la hauteur de la plante, la surface spécifique foliaire (SLA) et la teneur en matière sèche des feuilles (LDMC). Ces derniers ont été choisis car ils représentent des axes distincts de la stratégie d'utilisation des ressources des plantes. Nos résultats confirment que l'importance de la compétition augmente avec la disponibilité des ressources du sol, quel que soit l'identité des espèces cibles. Nous avons trouvé que les distances hiérarchiques des traits expliquaient mieux la modification de performance des plantes que la dissimilarité des espèces et la biomasse du voisinage. Cependant, bien que la hiérarchie des LDMC ait eu un rôle prédominant tout au long du gradient, il est probable que la dissimilarité et la hiérarchie des hauteurs aient eu un effet conjoint selon les conditions abiotiques. Ces résultats suggèrent que les processus de compétition dominent dans ces parcours via la préemption de la lumière et des ressources du sol. Ils contribuent à mieux expliquer du rôle des interactions biotiques dans la structuration des communautés et sont cohérents avec les actions des mécanismes d'homogénéisation de la coexistence des espèces au court-terme. Enfin, nous avons montré que des axes indépendants de la stratégie des plantes pouvaient être impliqués le long de gradient complexe, ce qui reflète la multiplicité des effets des interactions biotiques dans la sélection des traits des espèces dont il faut tenir compte lorsque l'on infère l'assemblage des communautés par les patrons de traits.

Introduction

Understanding processes that underlie community assembly remains a major issue in ecology (Diamond 1975) in a context of environmental variations. The assembly of species into local communities has been conceptualized as a series of filters (Belea & Lancaster 1999; Lortie et al. 2004) selecting species from a regional pool according to their ability to colonize the habitat (stochasticity), to cope with the local abiotic conditions (environmental filtering), and to survive despite multiple interactions with the established individuals in the community (biotic filtering). However, the relative effect of these filters on species sorting varies with environmental severity, in relation with changes in nature and strength of biotic interactions (Grime 2006). Although a large body of studies tried to characterize changes in competition of facilitation with environmental gradients (Grime 1973; Newman 1973; Tilman 1988; Keddy 1989; Bertness & Callaway 1994), the net effect of plant-plant interactions on species fitness remains to be established with regards to environmental conditions in a community.

Following Welden & Slauson (1986), the analysis of biotic interactions should therefore distinguish between *intensity* and *importance* of interactions. Intensity is defined by the decrease (or the increase) in plant performance (*e.g.* growth, metabolism rate, fecundity *etc.*) caused by the presence of neighbors while importance is the change in plant performance from an interaction relative to all other factors in the environment (*e.g.,* resource shortage, disturbance *etc.*). These two components of plant-plant interactions are not necessarily correlated to each other along environmental gradients since they reflect distinct niche-based mechanisms with intensity and importance relating more to realized and fundamental niches respectively (Brooker et al. 2005; Brooker & Kikvidze 2008; Kunstler et al. 2010; Gross et al. 2010). Patterns of intensity along environmental gradients have been repeatedly identified: competition has been shown to be the least intense in resource-poor environments and the greatest in resource-rich environments (Grime 1973, 1977), whereas the reverse was true for facilitation according to the 'stress gradient hypothesis' (Bertness & Callaway 1994). However, it is still unclear how the importance of interactions varies along environmental gradients (Lamb & Cahill 2008; Freckelton et al. 2009; Mitchell et al. 2009; Kunstler et al. 2010).

Instead of considering gradients in abiotic factors only, another way of understanding the variations in plant-plant interaction is to test intensity and importance across a range of productivity and community structure. While several experimental studies have been carried out on a limited set of environmental conditions, typically opposing a 'low' versus a 'high' environmental stress levels, often with a poor description of the underlying variations in abiotic

factors (Brooker et al. 2008; Maestre et al. 2009), the majority of ecosystems contain multiple interlaced environmental gradients. The advantage of better considering the properties of the established vegetation is to integrate these multifaceted environmental constraints on the outcome of biotic interactions. Productivity, usually estimated with measurement of the aboveground biomass in grassland ecosystems (Scurlock et al. 2002), has been proposed as a major driver of plant-plant interaction reflecting the availability of resources (Grime 1977; Tilman 1982). Moreover, it directly refers to the density dependency of interaction processes. However, interaction intensity either increased with increasing productivity (Kadmon 1995; Sammul et al. 2000; Zhang et al. 2008) or did not change significantly (Wilson & Tilman 1993; Cahill et al. 1999; Gaucherand et al. 2006; Kunstler et al. 2010), questioning the reliability of productivity itself as a sufficient predictor of plant-plant interactions.

Surprisingly, the role of community composition and structure in driving the interactions has been little explored along environmental gradients (but see Elmendorf & Moore 2007; Kunstler et al. 2012; Navas & Fayolle 2012). Theoretical considerations on plant coexistence have linked species niche and plant competition through two fundamental hypotheses. The 'limiting similarity hypothesis' (MacArthur & Levins 1967) predicts that ecologically similar species should compete more intensely for resources than dissimilar species, and therefore should less likely coexist locally, leading to greater niche differentiation among species within a community. In contrast, the 'competitive ability hierarchy hypothesis' (Keddy 1989) predicts that species with greater competitive ability should pre-empt resources and thereby suppress species with inferior competitive ability, suggesting the possibility of 'enhancing similarity' processes among species towards similar competitive abilities at the community level. If certainly both niche difference and competitive hierarchy may together determine the outcome of plant-plant interactions (Chesson 2000; Adler et al. 2007; Mayfield & Levine 2010), their relative impact on community structuring should depend on the considered time scale (Herben & Goldberg *in press*). For instance, competitive hierarchy should prevail over short-time periods, reflecting on-going importance of competition, while niche differentiation should be initiated over long-time periods, reflecting past importance of competition. Chesson (2000) formalized the idea by distinguishing niche equalizing processes due to competitive hierarchy which act along with niche stabilizing processes through niche partitioning. Consequently, intensity and importance of competition may be more or less correlated depending on the degree of achievement of niche differentiation within the community. Furthermore, the interplay between competitive hierarchy and niche differentiation may also depend on the resources involved and therefore on the position of the community on the environmental gradient.

Assuming that species similarity/dissimilarity may be assessed on the basis of functional traits reflecting their resource acquisition strategy, the description of the functional structure of the community, based on the distribution of trait values within the community (Diaz et al. 2007), should help to unravel the effect of niche difference and competitive hierarchy on intensity and importance of plant-plant interactions at the community level (Kunstler et al. 2012; Navas & Fayolle 2012). Although plant height is a proxy of species ability to compete for light because it may capture several other architectural traits related to light interception (Westoby 1998; Falster & Westoby 2003; Violle et al. 2009), other traits have been identified as good descriptors of competitive ability. Typically, specific leaf area or leaf dry matter content which have been both shown to accurately indicate the strategy of acquisition of nutrients and water (Wright et al. 2004; Westoby et al. 2002; Hodgson et al. 2011) may be associated with response to competition(Violle et al. 2009). Thus, distinguishing niche difference from competitive hierarchy may be achieved following the approach of Mayfield & Levine (2010) and Kunstler et al. (2012) by calculating the absolute and the hierarchical distances between species trait values respectively.

In this study, we aim to evaluate the relative importance of niche differentiation and competitive-ability hierarchy in determining the outcome of plant-plant interaction across a range of varying edaphic conditions level in a Mediterranean rangeland. We carry out a removal experiment using established individuals of three co-occurring species with contrasting strategies and abundance along the gradient to quantify how plant performance is affected by community aboveground biomass and community structure accounting for local abiotic limitations. We hypothesized that trait distances between the target species and their neighbors would better explain the variations in intensity and importance of interactions than community aboveground biomass. Furthermore, extrapolating the values of importance obtained for these three dominant species to the whole community, we hypothesized that greater importance of competition would in turn generate higher functional variability in plant height and leaf traits at the community level. We address the following questions: (i) do intensity and importance of interactions co-vary with aboveground biomass along the soil gradient? (ii) once the effect of biomass has been accounted for, which of the absolute or the hierarchical trait distances best explain the remaining variations in intensity and importance? (iii) If trait distances significantly explain intensity and importance patterns along the soil gradient, is there a link between variability in trait values and importance of competition at the community level?

Materials and methods

Study site

The study was carried out on dry calcareous rangelands of southern France, located on a limestone plateau (Larzac Causse) at the INRA experimental station La Fage (43°55´N, 3°05´E, 790 m a.s.l.), 100 km northwest of Montpellier. Climate on the plateau is sub-humid with a strong Mediterranean influence. Cool and wet winters alternate with warm and dry summers. Mean annual precipitations range from 680 to 1790 mm occurring mainly during spring and autumn. Mean monthly temperatures vary from 1°C in January to 19°C in August (data from 1973–2013). The main growing season lasts from March to the end of June. At the landscape level, vegetation is dominated by perennial herbaceous species, along with loosely scattered shrubs and soils consist of dolomitic rendzinas arranged as a mosaic of different depths and texture. For the past 35 years, the rangeland at the station has been homogeneously grazed by a sheep herd year-round under a controlled grazing regime.

Twelve plots (6 x 9 m) up to 1500 m apart were selected to span the widest possible range of soil types: from the shallow and dry soils of dolomitic sand to deeper and moister clay soils. Soil physico-chemical properties were assessed in each plot to quantitatively characterize the gradient (Perez-Ramos et al. 2012; Bernard-Verdier et al. 2012). Mean soil depth as well as eight soil characteristics were measured using three randomly distributed soil cores (5 cm wide) following standard procedures (Afnor 1994): texture, water holding capacity (-0.015 MPa), calcium carbonate content, pH (in water), organic matter content, C:N ratio, cation exchange capacity (CEC) and available phosphorus (using the Olsen method). Additionally, soil water content (SWC) was measured bi-weekly since spring 2008 using capacitance moisture probes (*DIVINER 2000*, Sentek Pty Ltd, Stepney, Australia), which provided a complete profile of soil humidity at three permanent locations in each plot. The probes were previously calibrated according to soil texture to account for the differences in soil water retention (Geesing et al. 2004; Groves & Rose 2004). In each plot, the total transpirable soil water (TTSW), representing the potential amount of water that plants can extract for transpiration (Ritchies 1981; Sinclair & Ludlow 1986), was derived from the course of SWC. Environmental variables were then analyzed using a principal component analysis. We found that the first axis of PCA explained 65.80 % of the total variation at the plot level, and that soil depth was well correlated to this axis (Fig. S2). We therefore used soil depth as a proxy of the edaphic conditions.

Target species and experimental design

In 2012, a neighborhood removal experiment was carried out to assess the effect of varying edaphic conditions and plant-plant interaction on species performance. Based on previous vegetation surveys (Perez-Ramos et al. 2012; Bernard-Verdier et al. 2012), three co-dominant perennial grass species differing in their abundance pattern (% of biomass) along the soil gradient (Table 1, Fig S1) were chosen as target species: *Bromus erectus* (dominant at the less limited end of the gradient), *Carex humilis* (constant abundance all along the gradient), *Festuca christiani-bernardii* (dominant at the most severe end of the gradient). In each plot, when the target species was present, three individual established plants per species were randomly selected and the neighboring plant biomass was carefully removed by hand within a 0.30 × 0.30 m square around, taking care to cut roots around the edge of the area as well. These plants were kept isolated by periodic inspections and re-weeding throughout the study. In addition, five undisturbed quadrats of the same size (0.30 × 0.30 m) containing the three target species (as far as it was possible) were distributed within the plot. Soil depth was measured at the end of the experiment by sliding a metal probe into the soil at three places within each quadrat.

Aboveground biomass and the functional structure of plant communities

Vegetation was successively sampled at the end of winter (March) and at the peak of vegetation (end of June). Vascular plants were identified to species within each undisturbed quadrat and their abundance was estimated using the point-intercept method (Levy & Madden 1933) with a 4 cm x 4 cm grid pattern (1044.98 points/m²). For each quadrat, the functional structure of communities was assessed with functional traits related to whole plant stature and leaf morphology. Leaf trait were available for the 53 most abundant species (*i.e.,* representing at least > 80 % of total biomass in each quadrat) from a previous study conducted in 2009 (Bernard-Verdier et al. 2012) and completed in late spring 2011 with the same protocol on at least 12 individuals distributed across the plots. For each individual sample, leaf length (LL; cm), leaf area (LA; cm^2), leaf dry matter content (LDMC; mg g^{-1}) and specific leaf area (SLA, m^2 kg^{-1}) were measured following standard protocols (Cornelissen et al. 2003). Mean reproductive height (Hrep; cm) was taken from both Fayolle (2008) and Bernard-Verdier et al. (2012).

The functional structure of each plant community was described using two complementary metrics at the peak of vegetation. The community weighted mean (CWM, Garnier et al. 2004; Diaz et al. 2007) of traits was calculated as follows:

$$CWM_k = \sum_{i=1}^{S} p_{i,k} \times trait_i \qquad \text{eqn. 1}$$

Table 1 Mean trait values, vegetative height (June 2012) and aboveground biomass (June 2012) of the three target species across communities. Values in brackets are minimum and maximum values in data set.

species	SLA (m^2 kg^{-1})	LDMC (mg g^{-1})	Hrep (cm)	Hveg (cm)	AGB (g m^2)
Bromus erectus	18.28	308.4	48.10	12.5 (5.70-18.1)	66.09 (2.09-146)
Carex humilis	12.55	427.1	8.87	8.46 (14.0-4.00)	21.00 (3.88-47.1)
Festuca christiani-bernardii	8.00	438.0	7.88	7.79 (3.70-15.6)	48.15 (1.36-225)

where S is the number of species in community k; $p_{i,k}$ is the relative abundance (*e.g.,* biomass proportion) within the community; $trait_i$ is the value of trait of species i. CWM quantifies the average trait value expressed by the vegetation. In addition, the functional divergence (FD) of trait was assessed using the Rao index (Leps et al. 2006; Ricotta & Moretti 2011):

$$FD_k = \sum_{i=1}^{S}\sum_{j=1}^{S} p_{i,k} \times p_{j,k} \times d_{i,j,k} \qquad \text{eqn. 2}$$

where S is the number of species in community k; $p_{i,k}$ and $p_{j,k}$ are the relative abundances of species i and j within the community ; $d_{i,j,k} = 1 - O_{i,j,k}$ is the dissimilarity between species i and j, with $O_{i,j,k}$ representing the overlap between the probability density function of trait values of species i and j within the community k. FD quantifies the heterogeneity of trait values within the vegetation.

Trait distances between the target species and surrounding communities

Plant traits (LL, LA, LDMC, SLA and Hveg) were measured per species within each undisturbed quadrat and for each individual plant. These traits were selected because they are related to species resource-use strategy and therefore we assumed that they may be somehow involved in competitive interactions among plants. The niche distance between the target species and the neighboring vegetation were determined using each of these traits alternately. Within the undisturbed quadrats, we quantified the absolute trait distance (ATD) and the hierarchical trait distance (HTD) following Kunstler et al. (2012):

$$ATD_{i,k} = \frac{\left| trait_{i,k} - CWM_k \right|}{\max(trait) - \min(trait)} \qquad \text{eqn. 3}$$

$$HTD_{i,k} = \frac{trait_{i,k} - CWM_k}{\max(trait) - \min(trait)} \qquad \text{eqn. 4}$$

where i is the target species; $trait_i$ is the trait value of target species within the community k; CWM_k is the corresponding community weighted mean value of trait; $\max(trait) - \min(trait)$ is the range of trait values over the whole data set (including all the species recorded).

Intensity and importance of plant-plant interaction

After one year, the performance of the three target species was estimated based on their biomass production over the growing season. We calculated the specific aboveground net primary productivity (SANPP; Garnier et al. 2004) for each quadrat, including those with the isolated plants:

$$SANPP_{i,k} = \frac{AGB_{i,k,t_1} - AGB_{i,k,t_0}}{AGB_{i,k,t_0}} \times \frac{1}{GDD_{t_0 t_1}} \quad \text{eqn. 5}$$

where i is the target species; AGB_{i,t_0} and AGB_{i,t_1} are respectively the aboveground biomass at the beginning of growing season (March) and at the peak of vegetation (end of June) of species i in community k; $GDD_{t_0 t_1}$ is the cumulated growing degree day between the two dates, with 2.5°C as base temperature. Aboveground biomass was estimated using species-specific calibration of the point-intercept method (Barkaoui et al. 2013).

The effect of edaphic conditions on species SANPP was tested for each species separately with linear regressions against soil depth using data from the isolated plants. To account for differences in soil depth among quadrats, the regression coefficients were used to calculate theoretical values of SANPP within the undisturbed quadrats as if only soil depth had an effect. The effect of plant-plant interactions was then estimated by quantifying the deviation from this expectation. For each quadrat, we calculated both the intensity and importance of interactions, using the 'relative interaction index' (*RII*, Armas et al. 2004) and the 'interaction importance index' (I_{imp}, Seifan et al. 2010) respectively:

$$RII_{i,k} = \frac{SANPP_{i,k,+N} - SANPP_{i,k,-N}}{SANPP_{i,k,+N} + SANPP_{i,k,-N}} \quad \text{eqn. 6}$$

$$I_{imp_{i,k}} = \frac{SANPP_{i,k,+N} - SANPP_{i,k,-N}}{\left|SANPP_{i,k,+N} - SANPP_{i,k,-N}\right| + \left|SANPP_{i,k,-N} - Max\left(SANPP_{i,k,\pm N}\right)\right|} \quad \text{eqn. 7}$$

where i is the target species; $SANPP_{i,k,+N}$ is the species performance with neighbors in community k; $SANPP_{i,k,-N}$ is the corresponding expected performance without neighbors; $Max\left(SANPP_{i,k,\pm N}\right)$ is the maximum performance of species i, with or without neighbors, recorded along the soil gradient. Both *RII* and I_{imp} indexes are continuous, symmetric around zero and range from -1 to +1. Values are negative when competition prevails, positive when

Table 2 Model selection for the relationship between I_{imp}, absolute trait distance (ATD) and hierarchical trait distance HTD using plant height (Hveg), specific leaf area (SLA) and leaf dry matter content (LDMC). Selection was performed separately in spring and summer. Best models showing the lowest AIC (ΔAIC=0) and the highest AIC weight are indicated in bold; models with ΔAIC<2 are indicated in italic. Marginal and conditional R^2 (R^2_m and R^2_c, respectively) are given for each model.

		ATD				HTD			
model	df	ΔAIC	AIC weight	R^2_m	R^2_c	ΔAIC	AIC weight	R^2_m	R^2_c
(intercept)	3	45.25	<0.0001	0.00	0.08	46.96	<0.0001	0.00	0.08
~'Hveg'	4	42.15	<0.0001	0.02	0.12	38.28	<0.0001	0.06	0.17
~'SLA'	4	22.68	<0.0001	0.03	0.20	20.64	<0.0001	0.10	0.40
~'LDMC'	4	7.179	0.0265	0.12	0.33	6.440	0.0225	0.14	0.37
~'Hveg'+'SLA'	5	21.94	<0.0001	0.06	0.25	15.94	0.0002	0.14	0.44
~'Hveg'+'LDMC'	5	8.183	0.0159	0.13	0.33	**0.000**	**0.6818**	**0.19**	**0.43**
~'Hveg'×'SLA'	6	13.91	0.0010	0.16	0.46	15.61	0.0003	0.16	0.46
~'Hveg'×'LDMC'	6	**0.000**	**0.9565**	**0.19**	**0.43**	*1.710*	*0.2904*	*0.19*	*0.43*

facilitation prevails, and are close to zero when the interactions are neutral or unimportant. Thereafter, only the analysis performed on the importance of interactions and corresponding results are presented because *RII* and I_{imp} were strongly correlated to each other along the soil gradient (*r-pearson*=0.93, $p<0.0001$, Fig. S3).

Statistical analyses

We used linear mixed models (Bolker et al. 2009) to test the effect of neighbors on the importance of interactions. The advantage of using such models here is to test the generality of the relationship across different taxa with contrasting strategy (Goldberg et al. 1999). We first started with intercept random models (*e.g variable~1|random*) with the target species ('*species*') as random variable to decompose the variability between the three target species. More complex models were then built (*e.g variable~fixed+1|random*) including successively community aboveground biomass ('AGB'), absolute trait distances ('ATD_trait') and hierarchical trait distances ('HTD_trait'). To identify the effect of trait distances, we first tested the effect of AGB on I_{imp}, and we used the residuals into the next models. To avoid further collinearity, we tested the correlation of ATD and HTD for the different traits and we only included the combinations having non-significant or low correlations (*r-pearson* < 0.5). Model selection was performed using the Akaike Information Criteria (AIC) by considering that lower AIC value indicated better fit to data. Models with $\Delta AIC<2$ were considered equivalent to each other, and in this case the most parsimonious model (*e.g.,* with smaller *df*) was preferred. In addition, we calculated AIC weights giving the likelihood of each model to be the best model in comparison to all the competing models tested. Using this approach, significance of random effect was tested by comparison with simple linear regression without random effect. Furthermore, significance of fixed effects in the best model was tested using Wald χ^2 test procedure (Fox 2008). However, while AIC values provide an estimate of relative goodness of fit of several alternative models, they are limited in quantifying variance explained by these models (Orelien & Edwards 2008). Therefore, following the proposal of Nakagawa & Schielzeth (2013), we calculated the marginal R^2 (R^2_m) which describes the variance explained by the fixed factors alone, and the conditional R^2 (R^2_c) which describes the variance explained by both fixed and random factors. Error standard deviations of both fixed and random effects were estimated by maximizing the restricted log-likelihood (REML) using *lme4* package in *R* (Bates et al. 2011).

Finally, the relationships between the importance of interactions and the components of the functional structure (CWM and FD) of the communities were tested using simple linear regressions for the traits involved previously. In cases where two or three of the target species

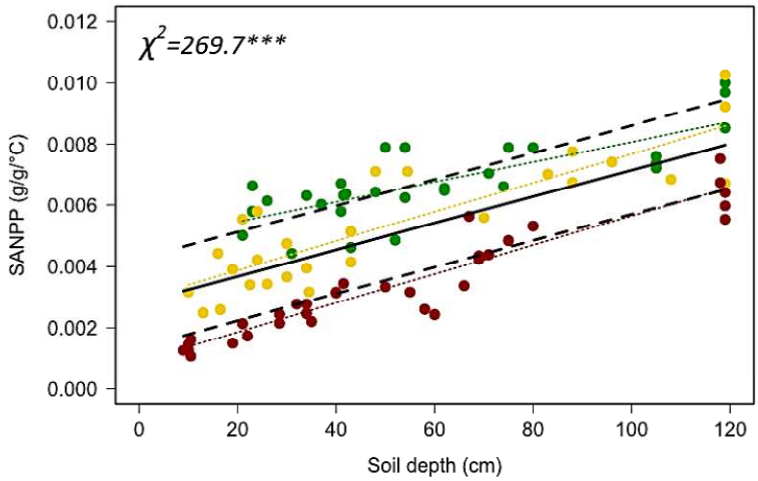


Fig. 1 Specific aboveground net primary productivity (SANPP) of the three target species along the soil gradient. Data points represent SANPP of the species within the communities. Colors indicate species identity (green: *Bromus erectus*; yellow: *Carex humilis*; red: *Festuca christiani-bernardii*). Black solid line is the mean effect of soil depth on SANPP from a linear mixed model with species as random variable. Dashed black lines are 95%-confidence interval. The colored dotted lines were calculated by linear regression for each species separately.

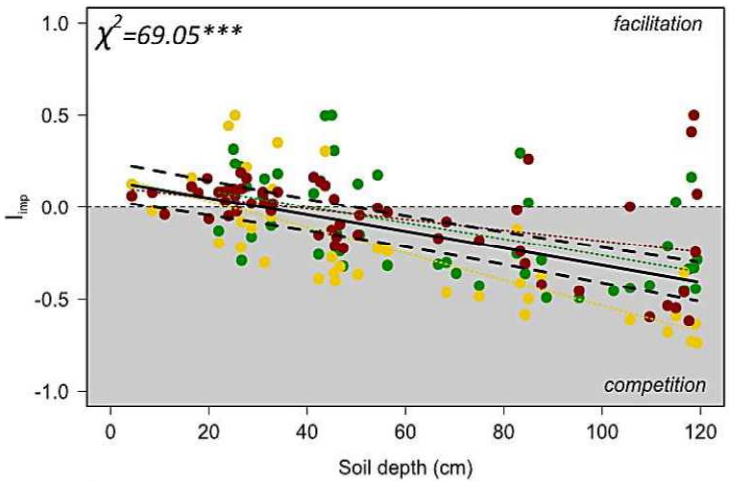


Fig. 2 Relationship between the importance of interaction (I_{imp}) and soil depth across the communities. Data points represent the values for the species in each community. Colors indicate species identity (green: *Bromus erectus*; yellow: *Carex humilis*; red: *Festuca christiani-bernardii*). Black solid line is the mean effect of soil depth on I_{imp} from a simple linear mixed model with species identity as random variable. Dashed black lines are 95%-confidence interval. The colored dotted lines were calculated by linear regression for each species separately.

were present in the same community, the different values of I_{imp} were averaged before the analysis, assuming that the average represented the importance of interaction for the whole community.

Results

Change in plant-plant interactions along the soil gradient

Edaphic conditions had a strong effect on growth performance. SANPP of the isolated plants increased significantly with increasing soil depth (Fig. 1) without difference in slope among the three target species, although intercepts differed significantly. As expected, *Bromus erectus* had the highest SANPP while *Festuca christiani-bernardii* had the lowest SANPP. The presence of neighboring plants significantly affected species SANPP along the soil gradient. Plant competition similarly affected the three target species, and the importance of competition significantly increased towards deeper clay soils (χ^2=69.05, p<0.0001, Fig. 2). Conversely, plant-plant interactions were neutral or even shifted to facilitation on shallow sandy soils.

Effect of aboveground biomass and trait distances on the importance of plant-plant interactions

The aboveground biomass (AGB) of the communities significantly increased with increasing soil depth (Fig. 3). However, the variations in AGB only explained a small part of the variations in the importance of interactions (r^2=0.06, p=0.003). The explicative power of AGB was even lower when the three species were analyzed separately, and was significant only for *Carex humilis* (r^2=0.15, p =0.014, Fig. 4). After removing the general effect of AGB, the best model explaining the residual variation in I_{imp} included the hierarchical trait distances for both plant height ('HTD_Hveg') and leaf dry matter content ('HTD_LDMC') as fixed effects (R^2_m=0.19, R^2_c=0.43, Table 2). Models with absolute trait distances were generally less explicative than model with hierarchical distances (quite similar R^2_m and R^2_c but with higher *df*), but the best model with absolute trait distances also included plant height and LDMC as fixed effects (Table 2). These two trait distances were not correlated to each other along the soil gradient (*r-pearson*=-0.098, p=0.257). HTD_LDMC had a strong general negative effect on I_{imp} (χ^2=25.39, p<0.0001), although the slopes slightly but significantly differed when the species were analyzed separately (Fig. 5b, *LLR*=9.291, p=0.01). In the same manner, HTD_Hveg had a general positive effect on I_{imp} although to a lower extent (χ^2=8.734, p=0.003) with small significant differences among slopes when the species were analyzed separately (Fig. 5a, *LLR*=10.67, p=0.005).

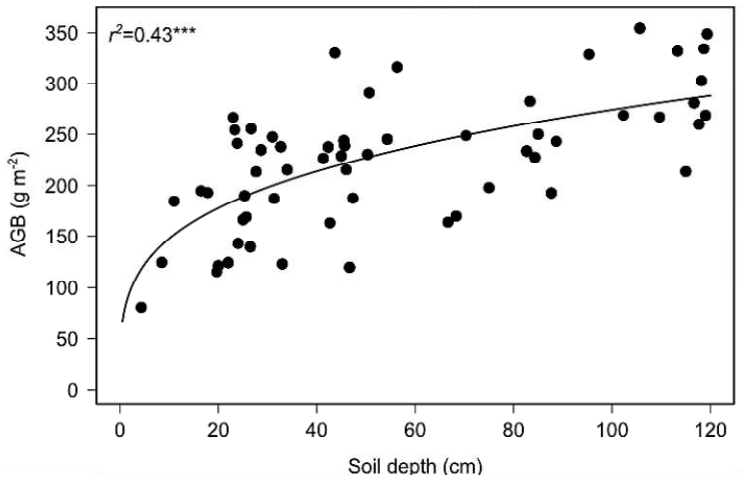


Fig. 3 Aboveground biomass (AGB) of the communities along the soil gradient. Data points represent individual quadrats. The line was calculated by nonlinear regression.

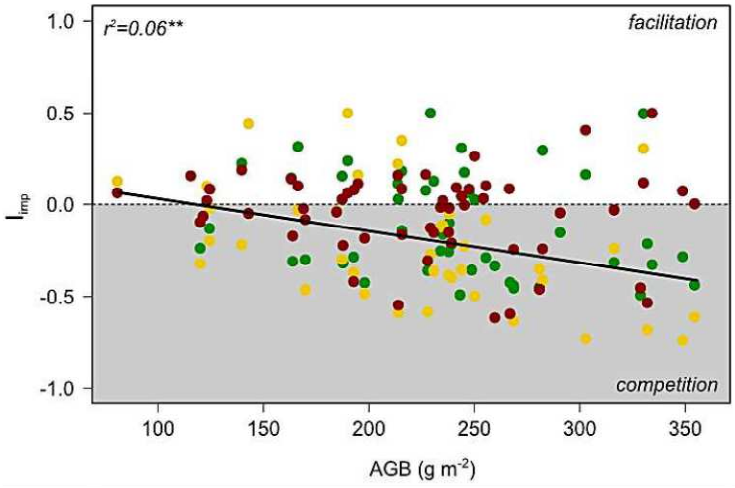


Fig. 4 Relationship between the importance of interaction (I_{imp}) and aboveground biomass (AGB) across the communities. Data points represent the values for the species in each community. Colors indicate species identity (green: *Bromus erectus*; yellow: *Carex humilis*; red: *Festuca christiani-bernardii*). Black solid line is the mean effect of AGB on I_{imp} provided by the linear mixed model with species identity as random variable. Dashed black lines are 95%-confidence interval. The colored dotted lines were calculated by linear regression for each species separately.

Effect of plant-plant interactions on the functional structure of the communities

In most cases, significant relationships were found between I_{imp} and the components of the functional structure across the communities. For plant height, the relationships were negative for both components of the functional structure, although I_{imp} better explained the variations in CWM_Hrep (r^2=0.45, p<0.0001, Fig. 6a) than in FD_Hrep (r^2=0.27, p<0.0001, Fig. 6b). Conversely, the relationship was positive for CWM_LDMC (r^2=0.45, p<0.0001, Fig. 6c) whereas FD_LDMC did not vary linearly with I_{imp} (r^2=0.04, p=0.133), but tended to follow a humped-back trend (Fig. 6d).

Discussion

In this study, we aim to evaluate the relative effect of niche difference and competitive hierarchy in determining the outcome of plant competition. We carried out a removal experiment along a complex edaphic gradient which was mainly associated with contrasting soil depth and texture (Fig. S1). Compared to other gradients in the literature, our study gradient range within the least productive ones (50 g m^{-2} < AGB < 350 g m^{-2}). As such, *Bromus erectus* was considered here as a 'competitive fast growing' species while this species is usually found at severe end of environmental gradients within temperate grasslands (Liancourt et al. 2005, 2009; Gross et al. 2010). However, we found comparable patterns to that found within more productive grasslands: plant-plant interactions progressively shifted from facilitative to competition along the gradient. The novelty of our approach was to test explicitly the effect of the functional identity of neighboring plants on plant-plant interaction at the community level. Hierarchical trait distances calculated with plant height and leaf dry matter content (LDMC) did better explain the variation of competition importance than did standing biomass. Moreover, the three target species responded similarly to trait distances despite their ecological differences, highlighting a possible general mechanism of plant competition in this Mediterranean rangeland.

Methodological consideration for the study of plant-plant interactions in the field

The study of the relationship between importance of competition and the functional structure of the community revealed key methodological aspect to be considered. In line with previous studies (Grace 1991; Damgaard & Fayolle 2009; Navas & Fayolle 2012), we followed an approach based on the performance of species at the community level. Instead of assessing competition with classical phytometer transplantations, we chose to work with established

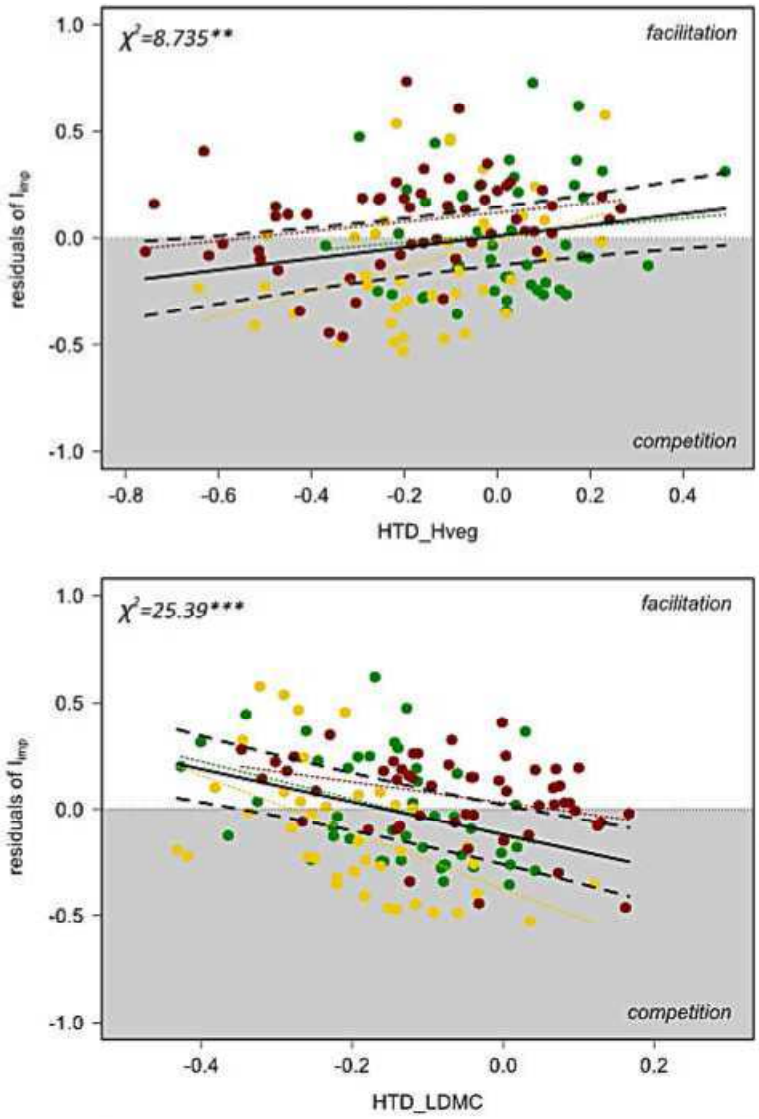


Fig. 5 Relationship between the residuals of importance of interaction (I_{imp}) once the general biomass effect has been accounted for, and the hierarchical trait distance across the communities of (a) plant height (HTD_Hveg), and (b) leaf dry matter content (HTD_LDMC). Data points represent the values for the species in each community. Colors indicate species identity (green: *Bromus erectus*; yellow: *Carex humilis*; red: *Festuca christiani-bernardii*). Black solid line is the mean effect of HTD on I_{imp} provided by the linear mixed model with species identity as random variable (see model selection in Table 2). Dashed black lines are 95%-confidence interval. The colored dotted lines were calculated by linear regression for each species separately.

populations of species. Despite considerable advantages of phytometers in the experimental approach, namely through the normalization of response along gradients, the use of external individuals imposes strong limitations for unraveling the community-level processes that drive changes in interactions along gradients, such as those related to population dynamics (Freckleton et al. 2009). Moreover, the identity of the species used as phytometer may cause important bias in studying processes related to species similarity or competitive hierarchy if the species does not occur 'naturally' in the targeted communities. Because a community is usually considered as resulting from the long-term dynamic equilibrium between the constitutive species and abiotic factors, the assessment of competition using external phytometers may not be representative of what actually happens within the community since it will be strongly dependent on the size, strategy and ontogenic stage of the phytometer. Finally, the study of established populations avoids heavy disturbance due to any transplantation.

Effect of the environmental gradient on species performance and plant-plant interaction

Our results revealed that abiotic conditions have a predominant role in determining species performance at our study site. In the absence of neighbors, the three target species similarly benefited from an increase in soil resource availability despite their contrasting resource-use strategies and their difference in relative abundance along the soil gradient (Fig. S2). This indicates that none of these three species were growing at their environmental optimum, highlighting that multiple constraints generally co-limit plant growth along edaphic gradient (Körner 2003) such as soil water and nutrients. We show here that even for *Festuca christiani-bernardii*, one the most stress-tolerant species at the study site, resource availability at the most favorable end of the gradient were below the optimal requirement.

To a certain extent, plant-plant interactions significantly modulated species performance along the soil gradient in a way that is predicted by the 'stress gradient hypothesis' (Bertness & Callaway 1994). We show that both intensity and importance of competition increased towards deeper soils which represent more favorable growth conditions, while facilitation was more important on water-limited shallow sandy soils. Competition and facilitation generally co-occur in the habitat, but the interplay between the two is often argued to switch when the positive effect of neighbors on the abiotic conditions becomes higher than resource depletion due their activity (Bertness & Callaway 1994; Choler et al. 2001; Callaway et al. 2002). In our case, the net effect of facilitation may be explained by a reduction of soil water evaporation due to plant cover, ameliorating soil water availability which was by far the most limiting factor for growth at the most severe end of the gradient. However, the absolute effect of facilitation was generally low,

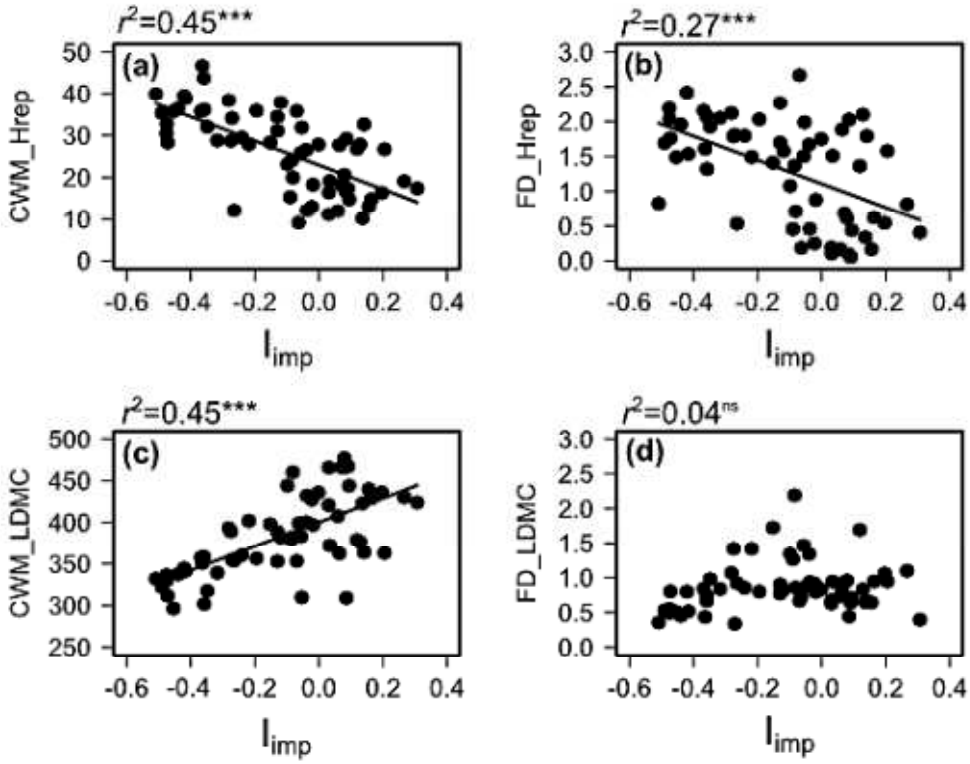


Fig. 6 Relationship between importance of interaction (I_{imp}) and different components of the functional structure of the communities for plant height and LDMC: (a) CWM_Hrep; (b) FD_Hrep; (c) CWM_LDMC; (d) FD_LDMC. Data points represent individual quadrats. The average of I_{imp} of the three target species was used for each community. Lines were calculated using linear regressions.

and competition was likely a more determinant biotic process for driving community assembly at our study site.

Despite they are controlled by distinct underlying mechanisms (Welden & Slauson 1986; Brooker 2005), the two components of competition, intensity and importance, were found to be strongly correlated to each to other for the three target species, confirming the assumption that intensity can in some instance be a strong indicator of importance (Grace 1991). While intensity refers to the realized niche of a species delimited by the interactions with the neighbors under given environmental conditions, importance rather expresses how a species deviates from the fundamental niche optima (*sensu* Hutchison 1957) due to interactions with neighbors. Grime's theories relate specifically to the importance of competition as a mechanism that structure plant communities (Grime 1977). It has been suggested that systems where shoot competition prevails generally show a positive relationship between intensity and importance (*e.g.*, Grime 2001; Keddy 2001). In contrast, it has been shown that root competition only has little effect on community structure (Lamb & Cahill 2008; Lamb et al. 2009), and therefore that the intensity of root competition, even if high, does not inform on the importance of competition during community assembly (Mitchell et al. 2009). The authors argued that the difference between root and shoot competition mainly relies in the fact that, unlike shoot competition, the intensity of root competition is symmetric (Cahill & Casper 2000) and may not be related to plant root biomass (Cahill 1999, 2002; Lamb et al. 2007), nor species diversity (Cahill 2003). Despite important changes in soil water and nutrients, we therefore conclude that biotic processes influencing community assembly can be depicted as shoot competition in this Mediterranean rangeland. Understanding if root competition could even though have had an indirect effect through root-shoot interdependency at the community level clearly requires more comprehensive studies.

Changes in plant-plant interactions across communities

Our results questioned the assumption that plant competition may be primarily driven by aboveground productivity at the community level. In contrast with previous studies (Sammul et al. 2000; Corcket et al. 2003; Brooker et al. 2005; Zhang et al. 2008; Kunstler et al. 2012), we show that both the intensity and the importance of competition did not detectably change with the variations in AGB across the communities (Fig. 3). Despite productivity varied consistently with edaphic constraints, the range of AGB was relatively low (< 350 g m^{-2}) all along the gradient indicating that strong limitations still occurred even towards deeper soils. We therefore confirm that a certain threshold in productivity exist below which the 'crowding' effect does not affect plant-plant interactions (Violle et al. 2005). This is endorsed by the fact that no considerable

difference between the three target species were found, although they had contrasting status (from rare to dominant) within the communities with the highest levels of AGB (Table 1, Fig S1). Additionally, the lack of relationship between competition and productivity may be explained by the fact that contrasting growth forms coexisted within these species-rich communities. While it is generally assumed that greater AGB translate into higher plants, more important ground cover, etc. leading to greater light interception, the heterogeneity in growth-forms within the community may considerably influenced the spatial variability in biomass allocation among organs at the community level, and thereby the 'crowding' effect that is actually perceived by the species. For the same amount of biomass, tall grasses will have more negative effect on light availability than will small-leaved dwarf shrubs, or rosettes.

Instead, we argue that the description of the functional structure of plant communities was a more relevant way to assess the competitive effect at the community level than standing biomass. We show that functional traits related to both plant size and resource-use strategy were involved in the outcome of interaction, providing generic results between species. In particular plant height and LDMC, which depict two major axes of plant strategy in the LHS representation (Westoby 1998; Garnier & Navas 2012), were the most explicative of the variations in importance of competition across the communities. Because higher plants have the ability to overtop the vegetation and to pre-empt the incident light at the expense of smaller plants (Westoby et al. 2002), plant height was repeatedly associated to competition for light (Keddy et al. 2002; Turnbull et al. 2004; Falster & Westoby 2003; Liancourt et al. 2009) and more specifically to describe the competitive effect of species (Violle et al. 2009). Reflecting this typical asymmetry in competition for light, we show here that the importance of competition gradually increased when the height of the target species fell below the mean plant height of the community (Fig. 5a). The role of LDMC in plant-plant interactions was however less documented nor supported in previous studies (Navas & Moreau-Richard 2005; Liancourt et al. 2009). We show that the importance of competition was greater when the target species had higher LDMC than the surrounding community (Fig. 5b). Given that LDMC usually differentiates the species in terms of resource-use strategy (Hodgson et al. 2011), stress tolerance (Garnier & Navas 2012 and references therein) and abundance distribution along aridity or edaphic gradients (Niinemets 2001; Lavorel et al. 2007; Craine et al. 2010; Bernard-Verdier et al. 2013), this indicates that more conservative species tended to be suppressed under competition in this Mediterranean rangeland. Despite no evident relationship was found between LDMC and competitive response of species (Navas & Moreau-Richard 2005; Liancourt et al. 2009), we expected that greater LDMC values, conferring greater tolerance to low soil resources, would

have resulted on the contrary in greater tolerance to resource depletion by neighbors. Thus, even if our gradient represents overall strong constraining abiotic conditions for plant growth, there may be sufficient competitive interactions for species growing faster to be advantaged, thereby supporting more general trends that are expected on larger environmental gradients.

By distinguishing the effects of both biomass and functional structure, this study provides novel insight into underlying mechanisms of plant-plant interactions within natural plant communities. We found strong support for the hypothesis that competition between a species and its neighboring plants within a community is linked to trait hierarchical distances, at least for plant height and LDMC, rather than to functional similarity. Models based on hierarchical trait distances were more explicative and parsimonious than models based on absolute trait distances (Table 2). Only few previous studies have explicitly tested whether competition was best predicted by absolute niche difference or by the hierarchy in competitive ability (see Kunstler et al. 2012). For instance, niche difference was repeatedly found to influence interspecific competition (Uriarte et al. 2010) but studies usually did not explore if one direction in differences were more determinant than others. Conversely, the hierarchy in body size (Freckelton & Watkinson 2001) or in height (Violle et al. 2009) was also shown to drive asymmetric competition processes but in these cases, the hypothesis of similarity was not verified. Moreover, whatever the identity of target species, we show that trait distances for plant height and LDMC were not correlated to each other across the communities. This reveals that plant-plant interactions may involve multiple independent axes of plant strategy (Westoby 1998), reflecting co-occurring effect-response mechanisms along such complex environmental gradients.

Implications for the functional structure of plant communities

Our results outline multiple effects of biotic interactions on the functional structure of plant communities. We show that the importance of competition participated to shape the patterns of CWM and FD of traits involved in species competitive ability across the communities. This role of competition in trait-based community assembly was predicted to follow a humped-back model (Navas & Violle 2009), adapted from the humped-back model of species diversity (Michalet et al. 2006), but it was rarely evaluated empirically (see Kunstler et al. 2012; Navas & Fayolle 2012). Under this framework, the clustered or over-dispersed distribution of trait values within a community may result from both species niche differences and competitive ability hierarchy depending on their relative importance. Namely, communities characterized by important competition have been predicted to exhibit low functional divergence because successful competitive species generally display similar values of traits related to resource-use strategy

(Grime 2006), thus reflecting typical competitive hierarchy effect on the functional structure. It is not worth to notice that low functional divergence is also expected under very severe environmental conditions where the importance of competition is generally low, because unfit species are not able establish. By contrast, communities characterized by intermediate levels of competition should have higher functional divergence in relation with niche differentiation that occurs among the species (Weiher & Keddy 1995; Stubbs & Wilson 2004; Scheffer & Nes 2006). From this perspective, it has been suggested that plant height is a relevant trait to assess the effect of plant competition on community structure (McGill 2006; Navas & Violle 2009; Navas & Fayolle 2012). In our study, because tall species generally have an asymmetric competitive effect on light availability, smaller species tended effectively to be excluded from the most favorable sites, resulting in the increase in CWM_height (Fig. 6a). However, probably because the range of competition importance was relatively limited (Fig. 2), the competitive hierarchy exerted by tall plants was not predominant, and a considerable part of niche differentiation occurred, resulting also in the increase in FD_height towards the most favorable sites (Fig. 6b). Conversely, we show that the competitive hierarchy seemed to be predominant in case of LDMC. Because rapid acquisition of resource designed a more successful strategy under competition, CWM_LDMC decreased when competition was more important at these sites (Fig 6d), and alternative strategies, having higher LDMC values, were excluded, resulting correspondingly in low values FD_LDMC (Fig. 6d). However, the expected humped-back model of FD along the gradient of competition importance tended to be supported only for LDMC (Fig 6d) but not for plant height (Fig 6b), probably as a consequence of successive impacts of competition for light and water over the growing season.

Conclusion

In this study, we confirmed that the importance of plant competition increases with increasing soil resource availability. However, we showed that hierarchical trait distances between species and the surrounding plant community may better predict the outcome of plant competition than standing biomass itself, explaining how biotic interactions may determine the niche structure of communities, especially the functional divergence of traits. Moreover, we showed that independent axes of plant strategy may be involved in plant competition along such complex gradient, reflecting multiple effects of competition processes on species trait sorting. We highlighted that niche differentiation and competitive hierarchy should act together within plant

communities, but to a different extent depending on niche axis. For instance, while functional divergence of plant height linearly increased with the importance of competition, functional divergence of LDMC tended to follow a humped-backed pattern in this Mediterranean rangeland with a strong convergence to low values when competition was important. We therefore conclude that inferring community assembly processes from clustered or over-dispersed trait distributions only should draw particular attention to the role of traits within the community to avoid misleading interpretation of the relative importance of abiotic and biotic filters.

Acknowledgements

This work was funded by the ANR program O_2LA (09-STRA-09). We thank the experimental station 'INRA-La Fage' for access to the facilities, and Jean Richarte for his valuable support during field work.

References

Armas C, Ordiales R, Pugnaire FI (2004) Measuring plant interactions: a new comparative index. Ecology 85:2682–2686

Barkaoui K, Bernard-Verdier M, Navas M-L (2013) Questioning the Reliability of the Point Intercept Method for Assessing Community Functional Structure in Low-Productive and Highly Diverse Mediterranean Grasslands. Folia Geobot 48:393–414

Bates D, Maechler M, Bolker B (2011) lme4:Linear Mixed-Effects Models Using S4 Classes.

Beleya LR, Lancaster J (1999) Assembly rules within a contingent ecology. Oikos 86:402-416

Bernard-Verdier M, Navas M-L, Vellend M, Violle C, Fayolle A, Garnier E (2012) Community assembly along a soil depth gradient : contrasting patterns of plant trait convergence and divergence in a Mediterranean rangeland. J Ecol 100:1422–1433

Bertness MD, Callaway RM (1994) Positive interactions in communities. Trends Ecol Evol 9:191–193

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White J-SS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evol 24:127–35

Brooker RW, Kikvidze Z (2008) Importance : an overlooked concept in plant interaction research. J Ecol 96:703–708

Brooker RW, Kikvidze Z, Pugnaire FI, Callaway RM, Choler P, Lortie CJ, Michalet R (2005) The importance of importance. Oikos 109:63–70

Cahill JF (1999) Fertilization effects on interactions between above and belowground competition in an old field. Ecology 80:466–480

Cahill JF (2003) Lack of relationship between below-ground competition and allocation to roots in 10 grassland species. J Ecol 91:532–540

Cahill JF (2002) Interactions between root and shoot competition vary among species. Oikos 99:101–112

Cahill JF (1999) Fertilization effects on interactions between above- and belowground competition in an old field. Ecology 80:466–480

Chesson P (2000) Mechanisms of maintenance of species diversity. Annu Rev Ecol Syst 31:343–366

Choler P, Michalet R, Callaway RM (2001) Facilitation and Competition on Gradients in Alpine Plant Communities. Ecology 82:3295–3308

Corcket E, Liancourt P, Callaway RM & Michalet R. (2003) The relative importance of competition for two dominant grass species, as affected by environmental manipulations in the field. Ecoscience 10: 186-194.

Cornelissen JHC, Lavorel S, Garnier E, Díaz S, Buchmann N, Gurvich DE, Reich PB, Steege H Ter, Morgan HD, Heijden MG Van Der, Pausas JG, Poorter H (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Aust J Bot 51:335

Craine JM, Gelderman TM (2011) Soil moisture controls on temperature sensitivity of soil organic carbon decomposition for a mesic grassland. Soil Biol Biochem 43:455–457

Diamond JM (1975) Assembly of species communities. In Ecology and Evolution of Communities. Eds Cody, ML, Diamond, JM. Harvard University Press Cambridge, Massachusetts, pp. 342–444.

Damgaard C, Fayolle A (2010) Measuring the importance of competition: a new formulation of the problem. J Ecol 98:1–6

Díaz S, Lavorel S, de Bello F, Quétier F, Grigulis K, Robson TM (2007) Incorporating plant functional diversity effects in ecosystem service assessments. Proc Natl Acad Sci U S A 104:20684–9

Elmendorf SC, Moore KA (2007) Plant competition varies with community composition in an edaphically complex landscape. Ecology 88:2640–50

Falster DS, Westoby M (2003) Plant height and evolutionary games. Trends Ecol Evol 18:337–343

Fayolle A. (2008) Structure des communautés de plantes herbacées sur les grands causses : stratégies fonctyionelles des espèces et interactions interspécifiques. Thèse de doctorat de Montpellier SupAgro.

Freckleton RP, Watkinson AR, Rees M (2009) Measuring the importance of competition in plant communities. J Ecol 97:379–384

Fox J, Weisberg S (2011) An R Companion to Applied Regression, Second Edition, Sage

Garnier E, Navas M-L (2012) A trait-based approach to comparative functional plant ecology: concepts, methods and applications for agroecology. A review. Agron Sustain Dev 32:365–399

Garnier E, Cortez J, Billès G, Navas M-L, Roumet C, Debussche M, Laurent G, Blanchard A, Aubry D, Bellman A, Neill C, Toussaint J-P (2004) Plant functional markers capture ecosystem properties. Ecology 85:2630–2637

Gaucherand S, Liancourt P, Lavorel S (2006) Importance and intensity of competition along a fertility gradient and across species. J Veg Sci 17:455

Geesing D, Bachmaier M, Schmidhalter U (2004) Field calibration of a capacitance soil water probe in heterogeneous fields. Aust J Soil Res 42:289–299

Goldberg DE, Rajaniemi T, Gurevitch J, Stewart-Oaten A (1999) Empirical approaches to quantifying interaction intensity: competition and facilitation along productivity gradients. Ecology 80:1118–1131

Grace JB (1991) A clarification of the debate between Grime and Tilman. Funct Ecol 5:583–587

Grime JP (1973) Competitive exclusion in herbaceous vegetation. Nature 242:344–347

Grime JP (2001) Plant strategies, vegetation processes, and ecosystem properties. Wiley, Chichester

Grime JP (2006) Trait convergence and trait divergence in herbaceous plant communities: Mechanisms and consequences. J Veg Sci 17:255

Gross N, Liancourt P, Choler P, Suding KN, Lavorel S (2010) Strain and vegetation effects on local limiting resources explain the outcomes of biotic interactions. Perspect Plant Ecol Evol Syst 12:9–19

Groves SJ, Rose SC (2004) Calibration equations for Diviner 2000 capacitance measurements of volumetric soil water content of six soils. Soil Use Manag 20:96–97

Hodgson JG, Montserrat-Martí G, Charles M, Jones G, Wilson P, Shipley B, Sharafi M, Cerabolini BEL, Cornelissen JHC, Band SR, Bogard A, Castro-Díez P, Guerrero-Campo J, Palmer C, Pérez-Rontomé MC, Carter G, Hynd A, Romo-Díez A, de Torres Espuny L, Royo Pla F (2011) Is leaf dry matter content a better predictor of soil fertility than specific leaf area? Ann Bot 108:1337–45

Hutchinson GE 1957. Concluding remarks. Cold Springs Harbor Symposium in Quantitative Biology 22:415–427.

Kadmon R (1995) Plant competition along soil moisture gradients : a field with experiment the desert annual Stipa capensis. J Ecol 83:253–262

Keddy P, Nielsen K, Weiher E, Lawson R (2002) Relative competitive performance of 63 species of terrestrial herbaceous plants. J Veg Sci 13:5–16

Keddy PA (1989) Competition. Chapman and Hall, London.

Keddy PA (2001) Competition. Springer Netherlands, Dordrecht

Körner C (2003) Limitation and stress – always or never ? J Veg Sci 14:141–143

Kunstler G, Lavergne S, Courbaud B, Thuiller W, Vieilledent G, Zimmermann NE, Kattge J, Coomes D a, Grover J (2012) Competitive interactions between forest trees are driven by species' trait hierarchy, not phylogenetic or functional similarity: implications for forest community assembly. Ecol Lett 15:831–840

Lamb EG, Cahill JF (2008) When competition does not matter: grassland diversity and community composition. Am Nat 171:777–87

Lamb EG, Shore BH, Cahill JF (2007) Water and nitrogen addition differentially impact plant competition in a native rough fescue grassland. Plant Ecol 192:21–33

Lamb EG, Kembel SW, Cahill JF (2009) Shoot, but not root, competition reduces community diversity in experimental mesocosms. J Ecol 97:155–163

Lavorel S, Díaz S, Cornelissen JHC, Garnier E, Harrison SP, McIntyre S, Pausas JG, Pérez-Harguindeguy N, Roumet C, Urcelay C (2007) Plant Functional Types: Are We Getting Any Closer to the Holy Grail? In: Canadell JG, Pataki DE, Pitelka LF (eds) Terr. Ecosyst. a Chang. World. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 149–164

Lepš J, de Bello F, Lavorel S, Berman S (2006) Quantifying and interpreting functional diversity of natural communities : practical considerations matter. Preslia 78:481–501

Levy EB, Madden EA (1933) The point method of pasture analysis. New Zeal J Agric Res 46:267–279

Liancourt P, Callaway RM, Michalet R (2005) Stress tolerance and competitive-response ability determine the outcome of biotic interactions. Ecology 86:1611–1618

Liancourt P, Tielbörger K, Bangerter S, Prasse R (2009) Components of "competitive ability" in the LHS model: Implication on coexistence for twelve co-occurring Mediterranean grasses. Basic Appl Ecol 10:707–714

Lortie CJ, Brooker RW, Choler P, Kikvidze Z, Michalet R, Pugnaire FI (2004) Rethinking plant community theory. Oikos 107:433–438

MacArthur R, Levins R (1967) The limiting similarity , convergence, and divergence of coexisting species. Am Nat 101:377–385

Maestre FT, Callaway RM, Valladares F, Lortie CJ (2009) Refining the stress-gradient hypothesis for competition and facilitation in plant communities. J Ecol 97:199–205

Mayfield MM, Levine JM (2010) Opposing effects of competitive exclusion on the phylogenetic structure of communities. Ecol Lett 13:1085–93

McGill BJ, Enquist BJ, Weiher E, Westoby M (2006) Rebuilding community ecology from functional traits. Trends Ecol Evol 21:178–85

Michalet R, Brooker RW, Cavieres LA, Kikvidze Z, Lortie CJ, Pugnaire FI, Valiente-Banuet A, Callaway RM (2006) Do biotic interactions shape both sides of the humped-back model of species richness in plant communities? Ecol Lett 9:767–73

Mitchell MGE, Cahill JF, Hik DS (2009) Plant interactions are unimportant in a subarctic-alpine plant community. Ecology 90:2360–2367

Nakagawa S, Schielzeth H (2013) A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol Evol 4:133–142

Navas M-L, Fayolle A (2012) Competition depends more on the functional structure of plant community than on standing biomass. Community Ecol 13:21–29

Navas M-L, Moreau-Richard J (2005) Can traits predict the competitive response of herbaceous Mediterranean species? Acta Oecologica 27:107–114

Navas M-L, Violle C (2009) Plant traits related to competition: how do they shape the functional diversity of communities? Community Ecol 10:131–137

Newman EI (1973) Competition and Diversity in Herbaceous Vegetation. Nature 244:310–310

Niinemets Ü (2001) Global scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. Ecology 82:453–469

Orelien JG, Edwards LJ (2008) Fixed-effect variable selection in linear mixed models using R2 statistics. Comput Stat Data Anal 52:1896–1907

Ricotta C, Moretti M (2011) CWM and Rao's quadratic diversity: a unified framework for functional ecology. Oecologia 167:181–8

Ritchie JT (1981) Soil water availability. Plant Soil 58:327–338

Sammul M, Kull K, Oksanen L, Veromann P (2000) Competition intensity and its importance: results of field experiments with Anthoxanthum odoratum. Oecologia 125:18–25

Scurlock JMO, Johnson K, Olson RJ (2002) Estimating net primary productivity from grassland biomass dynamics measurements. Glob Chang Biol 8:736–753

Seifan M, Seifan T, Ariza C, Tielbörger K (2010) Facilitating an importance index. J Ecol 98:356–361

Sinclair T, Ludlow M (1986) Influence of Soil Water Supply on the Plant Water Balance of Four Tropical Grain Legumes. Aust J Plant Physiol 13:329

Stubbs WJ, Wilson JB (2004) Evidence for limiting similarity in a sand dune community. J Ecol 92:557–567

Tilman D (1982) Resource Competition and Community Structure.

Uriarte M, Swenson NG, Chazdon RL, Comita LS, John Kress W, Erickson D, Forero-Montaña J, Zimmerman JK, Thompson J (2010) Trait similarity, shared ancestry and the structure of neighbourhood interactions in a subtropical wet forest: implications for community assembly. Ecol Lett 13:1503–14

Vieilledent G, Courbaud B, Kunstler G, Dhôte J-F, Clark JS (2010) Individual variability in tree allometry determines light resource allocation in forest ecosystems: a hierarchical Bayesian approach. Oecologia 163:759–73

Violle C, Garnier E, Lecoeur J, Roumet C, Podeur C, Blanchard A, Navas M-L (2009) Competition, traits and resource depletion in plant communities. Oecologia 160:747–55

Weiher E, Keddy PA (1995) The assembly of experimental wetland plant communities. Oikos 73:323–335

Westoby M (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. Plant Soil 199:213–227

Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ (2002) Plant ecological strategies : some leading dimensions of variation between species. Annu Rev Ecol Syst 33:125–159

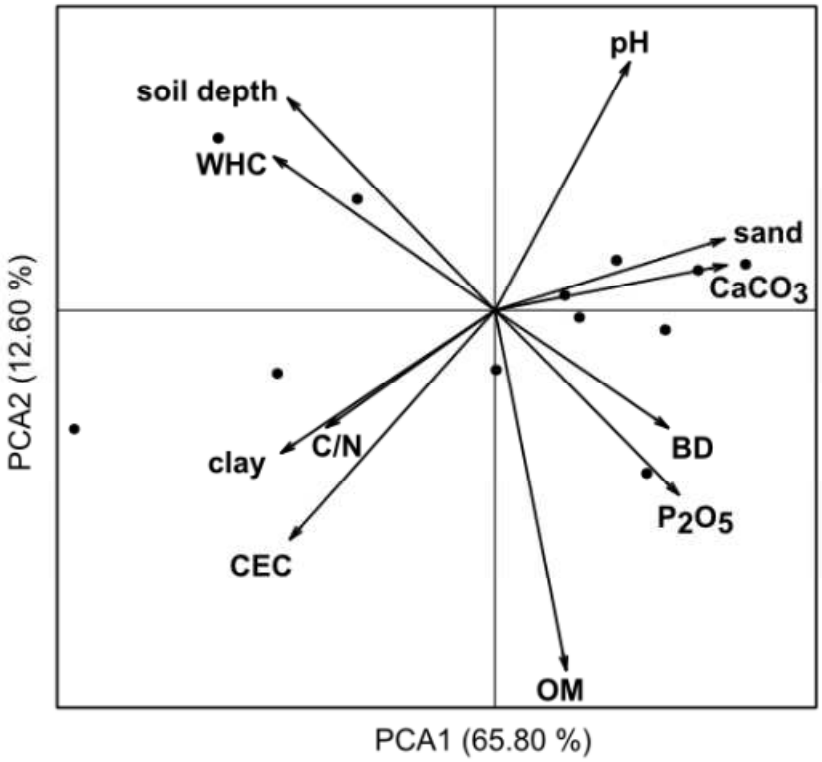
Wilson SD, Tilman D (1993) Plant Competition and Resource Availability in Response to Disturbance and Fertilization. Ecology 74:599

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee WG, Lusk C, Midgley JJ, Navas M-L, Niinemets U, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R (2004) The worldwide leaf economics spectrum. Nature 428:821–827

Zhang K, Kimball JS, Hogg EH, Zhao M, Oechel WC, Cassano JJ, Running SW (2008) Satellite-based model detection of recent climate-driven changes in northern high-latitude vegetation productivity. J Geophys Res 113:G03033

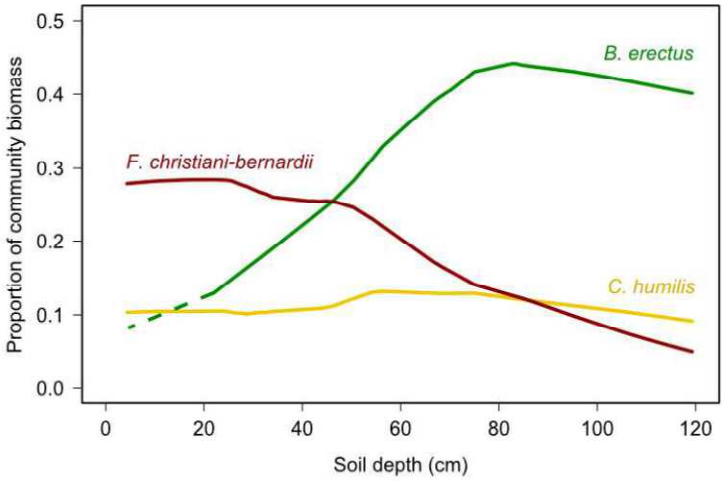
Supporting information

Fig S1 Principal Component Analysis (PCA) on soil parameters. Data points represent the 12 plots of grassland along the soil gradient. Eight soil parameters were used: mean soil depth (cm); clay and sand content (g g^{-1}); organic carbon content (OM, g g^{-1}); soil bulk density (BD; g cm^{-3}); soil water holding capacity (WHC, mm); $CaCO_3$ and P_2O_5 content (g g^{-1}). Axis 1 of the PCA explained 65.80 % of the variation.



Supporting information

Fig S2 (a) Density functions of the abundance (% of biomass) of the three target species along the soil gradient (June 2012). Colors indicate species identity (green: *Bromus erectus*; yellow: *Carex humilis*; red: *Festuca christiani-bernardii*). (b)Photography of ramets of the three target species.



Bromus erectus



Carex humilis

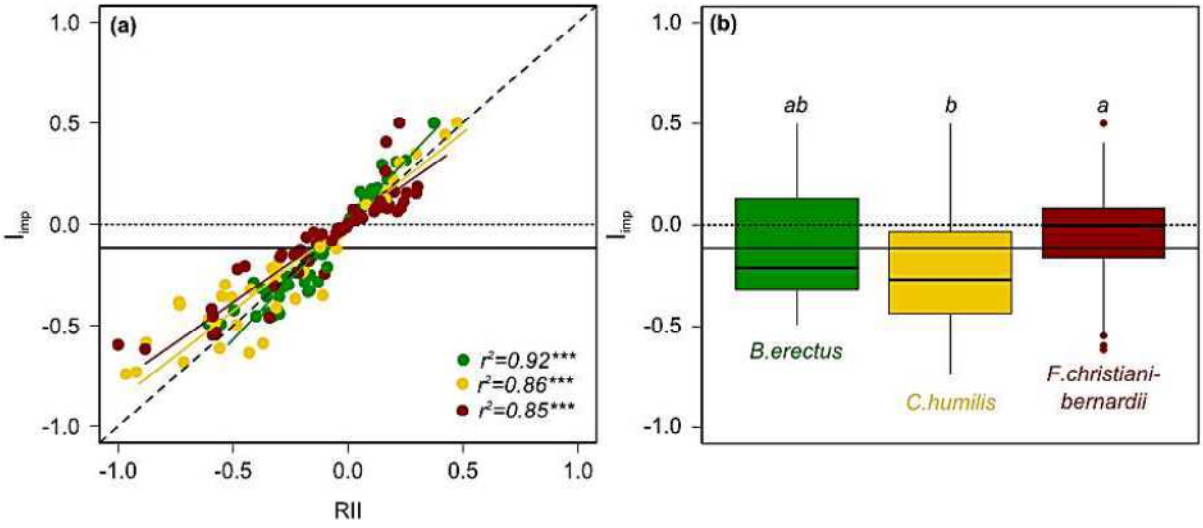


Festuca christiani-bernardii



Supporting information

Fig S3 (a) Correlation between intensity (RII) and importance (I_{imp}) of interactions across the communities. Data points represent the values for the species in each community. Colors indicate species identity (green: *Bromus erectus*; yellow: *Carex humilis*; red: *Festuca christiani-bernardii*). Lines were calculated with linear regression for each species separately. Black dashed line represents the 1:1 line. Differences between slopes are indicated in the text. (b) Boxplot of I_{imp} of the three target species. Letters indicate significant differences between species at $p<0.05$. Dotted horizontal line represents y=0 line; solid horizontal line represents the average I_{imp}.



General discussion



(Sheep herd at La Fage-station, *spring 2011*)

During this PhD work, I attempted to understand how abiotic and biotic factors co-determine the structure of species-rich Mediterranean rangelands and associated ecosystem processes. Specifically, I was interested in identifying general mechanisms underlying how vegetation adapts to contrasting soil water availability and in turn how it affects overall water flows. To do so, I used an approach based on plant functional traits related to plant stature and resource-use strategy to analyze the response of the functional structure of plant communities along an edaphic gradient, as well as its effect on vegetation emergent properties and on the water balance. The different studies of this PhD work highlight that concepts of functional ecology may provide valuable contribution for building an integrative ecohydrological framework linking the complex interactions and feedbacks between vegetation and hydrologic cycle. In the following section, I discuss the pivotal role played by the functional structure in addressing some current challenges in ecohydrology about alternate stable states and threshold behavior of ecosystems.

A. The functional structure of plant communities

1. A gradient of constraints but not of stress

Mediterranean rangelands are typical water-limited ecosystems which are under the control of recurrent severe droughts during summer. In these systems, a large part of water supply is provided by autumn and winter precipitations, and therefore is out of phase with the growing season of vegetation. Soil water holding capacity thus plays a critical role in determining the amount of water available for plant growth since it represents the capacity of the system to capitalize water before the growing season starts. Soils at our study site consist in dolomitic rendzinas of different co-varying soil depth and texture. We show that deep (120 cm) fine-textured (> 50 % clay) soils had more than 14 times greater soil water holding capacity than shallow (< 10 cm) coarse textured (> 90 % sand) soils, highlighting considerable range of edaphic constraints exerted on vegetation and making soil water availability probably the most discriminating factor in this Mediterranean rangeland (Manuscript II). As our study site was located within a small area (1500 m wide) with negligible climatic variations among plots, we had a relevant experimental design for testing the effect of these varying edaphic conditions on ecosystem ecohydrologic functioning. Furthermore, other external factors likely to affect directly plant performance (*e.g.,* grazing) were controlled and normalized for 35 years. However, even if the altitude did not vary between plots, a small effect of topography and orientation might not be completely excluded (*e.g.*, wind exposure).

Our results revealed strong plant-soil interaction and feedbacks all along the soil gradient, resulting in a functional equilibrium between the use of water by plants and soil water availability. Namely, we show that aboveground productivity and maximum evapotranspiration rates were proportional to the total soil available water (Manuscript III). In addition, dynamic water stress (Porporato et al. 2001) calculated over five years was not different between communities (Manuscript II), suggesting that water requirements and demand of the vegetation is somehow regulated with respect to water supply. The idea of such equilibrium water balance constitutes a central hypothesis in ecohydrology. Originally, the 'ecohydrological optimality' hypothesis predicted that vegetation should evolve in such a way to maximize soil moisture over time (Eagleson 1982ab, Eagleson & Segarra 1985; Eagleson 2002), namely by minimizing evapotranspiration rates, but the underlying assumptions were called for revision because they were not consistent with current knowledge of plant ecology (Hatton et al. 1997; Kerkhoff et al. 2004). Indeed, minimizing evapotranspiration would imply to stop any photosynthesis activity. Alternatively, we propose in this work that the functional equilibrium is achieved around a certain minimum level of soil water content below which plant survival may be dangerously affected (Manuscript II). In other words, the vegetation maximizes productivity while minimizing overall water stress, highlighting a fundamental productivity-water stress trade-off of plant functioning at the ecosystem level in water limited environments. This is consistent with the idea suggested at the plant level that "maximizing gas exchange (*i.e.,* carbon gain for growth) while avoiding hydraulic failure means operating on the edge of dysfunction" (Sperry 2004).

Many types of plant adaptations to drought have been reported in the literature, but essentially at the leaf- or plant level (Sperry et al. 2002; Mencuccini 2003; Maseda & Fernandez 2006; Bartlett et al. 2012; Manzoni et al. 2013). In particular, considerable knowledge has been acquired concerning the response of ecophysiological pathways to water shortage, such as cell growth, protein synthesis, stomata closure, sugar accumulation, leaf senescence, carbon allocation to root tissue etc. Anatomical characteristics of plants have also been widely studied, with a strong emphasis on organ plasticity. However, it is still unclear how these adaptations scale at the community level, where species with different strategies may coexist, and to which extend they modulate water fluxes at this level. We show here that variations in exchange surfaces were predominant in the adjustment of water fluxes at the community level. For instance, more constraining edaphic conditions (*e.g.,* shallow sandy soils) selected for greater ratio between root and leaf areas (RAI:LAI ratio), which in turn, influenced water uptake capacity and maximum evapotranspiration rates, two main drivers of ecosystem water balance (Manuscript III).

More specifically, the responsiveness of LAI was greater than that of RAI, suggesting that the control of evaporative demand constituted a more flexible lever than uptake capacity in this Mediterranean rangeland. Conversely to another expectation of the original 'ecohydrological optimality' hypothesis which predicts that water use efficiency should promote the equilibrium with soil moisture, we therefore argue that structural components of vegetation have a predominant role in plant-water relationship at the community level. However, ecophysiological mechanisms regulating water fluxes should have a more critical role in the fitness of individual plants within a community, namely to face temporal water stress (McDowell et al. 2008).

2. The pivotal role of community level: meeting of two different views of vegetation

In ecohydrology, the dominant approach is typically inherited from the reductionist view of hydrology for which downscaling of processes is preferred. The vegetation has been usually characterized by very few emergent properties such as LAI or standing biomass, which have major effects on water flows at the ecosystem level. The underlying assumption is that the vegetation behaves homogeneously, despite the diversity of organisms or species. A reason is that the parameterization of vegetation as a 'super-organism' was critical for developing a series of ecohydrological models. Typically, the 'big leaf' approach based on LAI directly arises from such representation of vegetation, and it provided accurate predictions of water-use dynamics in a range of vegetation-type. In this work, we confirmed that simple indexes such as LAI or RAI were reliable for predicting potential rates of evapotranspiration or water uptake capacity even in species-rich and structurally complex communities (Manuscript III). However, despite the apparent simplicity of using such indexes, it is still unclear how these emergent properties were determined in response to environmental variability, since integrating the diversity of species growth strategies within plant communities remains difficult. In particular, the interplay between below- or aboveground variations of exchanges areas has been rarely quantified at the community level, and therefore constituted an originality of our research.

In plant ecology, however, the vegetation has been described at several scales and levels of complexity. Specifically, the community level was argued to represent a pivotal level between natural selection of individual plants and ecosystem functioning (Lavorel & Garnier 2002; Suding et al. 2008). Despite a longstanding debate over the definition of what a 'community' is and how it should be delimited in space and time, a consensual view defines a community as an intermediate level of biological organization between the organisms (or populations of organisms) belonging to different species, and the ecosystem. For instance, community assembly

rules have been conceptualized as successive filters acting on the different components of species fitness (Beleya & Lancaster 1999; Lortie et al. 2004), leading to a complex niche structure at the community level that accounts for the abundance pattern of the different species.

In plant ecology, however, the vegetation has been described at several scales and levels of complexity. Specifically, the community level was argued to represent a pivotal level between natural selection of individual plants and ecosystem functioning (Lavorel & Garnier 2002; Suding et al. 2008). Despite a longstanding debate over the definition of what a 'community' is and how it should be delimited in space and time, a consensual view defines a community as an intermediate level of biological organization between the organisms (or populations of organisms) belonging to different species, and the ecosystem. For instance, community assembly rules have been conceptualized as successive filters acting on the different components of species fitness (Beleya & Lancaster 1999; Lortie et al. 2004), leading to a complex niche structure at the community level that accounts for the abundance pattern of the different species. There is growing evidence that the functional structure of communities reflect both abiotic and biotic pressures at site, but the cause underlying the absolute value of species abundance have not been clearly identified. In turn, the structure of the community determines how the whole assembly of organisms will interact with abiotic factors (*e.g.,* energy, resources), and thereby drive ecosystem processes. Following the response-and-effect framework, we therefore opened the vegetation 'black box' of ecohydrological systems to unravel the effects of abiotic and biotic factors on the emergent properties of vegetation.

We show that variations in RAI:LAI ratio were mainly driven by plant height and leaf traits in this Mediterranean rangeland, whereas roots traits apparently did not have an important role (Manuscript III). This result was *a priori* counterintuitive, since several studies reported on the contrary that adjustments in the root system should constitute the predominant response when the availability of soil resources (*e.g.,* water, nutrients) varied (Shipley & Meziane 2002). For instance a number of hypotheses suggest that greater root biomass, deeper root foraging, thinner roots etc. should be displayed to maximize the acquisition of the limiting resource (Jackson et al. 2000; Schenk & Jackson 2002; Casper et al. 2003; Moore & Heilman 2011). However, because our study was conducted along a complex edaphic gradient characterized by several co-variations of physico-chemical soil properties (Manuscript III), it is possible that factors other than resource availability had additional effects on the root system. Namely, water availability was limited by soil depth and texture. Although a deeper rooting depth has been repeatedly observed in previous studies under dry environments, this adaptation could not be exhibited along our gradient since the entire soil profile of all plots was extensively explored by roots down to the bed rock, which

therefore represented a physical obstacle for the expression of a deeper rooting potential. We are aware that roots can grow into cracks within the bed rock, especially in such calcareous karstic systems (Schwinning et al. 2010), but this seemed not to play a critical role for water uptake at the community level in our study, although we were not able to quantify the corresponding amount of biomass. Therefore, reducing aboveground development was the only remaining way for plants to adjust the evaporative demand to water uptake at the community level. However, the relative stability in root biomass along the gradient hide an increase in root mass density towards shallower soils, indicating that soil layers were more intensely explored by roots when the available soil volume was limited (supporting information in Manuscript III).

Plant height and leaf traits define two major axes of plant strategy (Westoby 1998). The strong variations in these traits along the edaphic gradient were therefore consistent with the idea that they indicate how plants overall cope with the environment. Based on an approach using null model, a previous studies at the site showed that patterns of traits differed significantly with what is expected by chance, indicating the co-occurrence of both trait-based habitat and biotic filtering along the gradient (Bernard-Verdier et al. 2012). Specifically, it was proposed that community assembly processes should act following two main consecutive steps. Firstly, species with unfit trait values within the regional species pool are sorted out with respect to local abiotic conditions, restricting the range of trait values within the community. Secondly, trait values may either converge or diverge depending on biotic interactions among plants. Building on this theoretical framework, we show that plant competition also had a considerable effect on plant height and LDMC values at the community level, although its relative importance varied along the gradient (Manuscript IV). For instance, the importance of competition increased towards more favorable sites, where plants have the potential to grow more, generating strong asymmetric competition for space and light. However, we observed two different niche-based mechanisms for the two trait axes: stabilizing niche processes were found for plant height, leading to greater functional divergence in plant height. Conversely, equalizing niche processes were found for LDMC leading to trait convergence. That is, when the importance of competition was high, 'tall' grasses with 'low' LDMC values dominated the community along with smaller subordinate forbs with low LDMC values, while small grasses and dwarf shrubs with high LDMC values dominated when environmental constraint were more important. Interestingly, this indicated that competition for light did not necessarily exclude small species at our site, and preserved species able to acquire limiting soil resources at the site. Therefore, because plant height and leaf traits distribution were both controlled by abiotic and biotic factors (Manuscript III), although to a varying extent along the gradient, it may provide an explanation of their greater responsiveness compared to

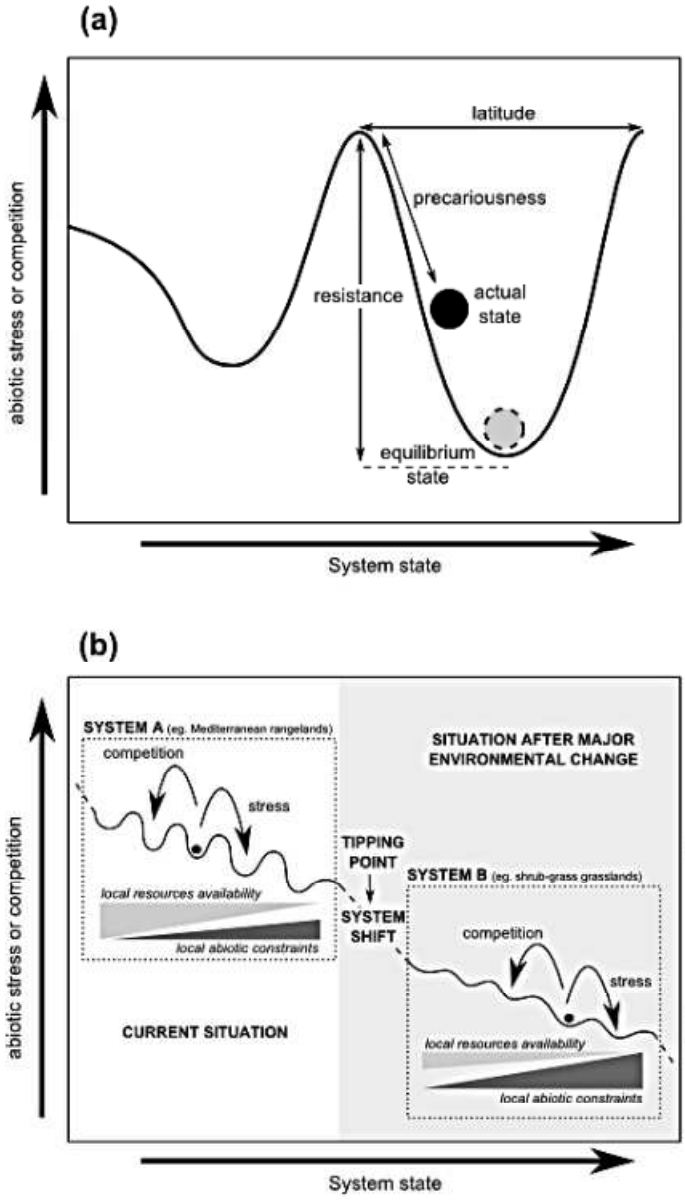


Fig. 1 Hypothesized representation of (a) Ecosystems can switch between states in a stability landscape. A stability landscape is a phase space diagram representing all possible states of an ecosystem. Ecological resilience describes the local stability of the current state of the ecosystem. The black dot represents the state of the ecosystem at a point in time and movement along the hills represents change in the ecosystem. As an ecosystem moves out of a basin, it approaches a threshold, and a regime shift occurs when the ecosystem "falls" into another basin of stability. Resilience has three aspects: latitude, resistance and precariousness (adapted from Walter et al. 2004). (b) A plant ecosystem can have several equilibrium states depending on the in the interplay between abiotic stress and competition. Each equilibrium state corresponds to a given functional structure that results from the sorting of species from the regional pool. Catastrophic shift in system structure (permanent modification of the regional species pool) may occur after a major environmental change.

belowground traits. However, although it has been suggested that belowground competition may not impact the structure of the community (Lamb & Cahill 2008), we were not able to fully assess the role of root traits since we characterize the root system at the community level only.

3. Alternative stable states: the dynamics of successive community re-assembly

In line with previous discussions (Manuscript II and Manuscript III), it is reasonable to consider that ecohydrological systems fluctuate over time around some trends or stable average. The functional structure of the communities equilibrates with the actual environmental conditions and in turn dimensions the rates of ecosystems processes that can be supported in the long-term under these conditions. We suggest that this equilibrium state can be conceptualized as a local 'basin' of stability (Fig. 1a). However because the selective pressures (abiotic stress, competition, disturbance) are not constant over time, ecosystem processes are obviously not completely stable in the sense that they do not change. Firstly, there are always slow trends, reflecting for example climatic long-term oscillations or community successions. Secondly, natural populations always fluctuate more or less, partly due to seasonal or inter-annual variations in abiotic (*e.g.,* wetter vs. drier years) and biotic conditions (*e.g.,* more or less competition in the community), but also because of intrinsic cycles or chaotic dynamics (Vandermeer 1999; Rinaldi et al. 2001; Scheffer et al. 2003). Overall, these micro-dynamics temporally increases the entropy of the ecosystem and translate into small fluctuations at both sides around the equilibrium position (Fig. 1a.). In the absence of a major disturbance, these fluctuations should not be important enough to reach a threshold towards the next minima. Occasionally, however, the equilibrium may be interrupted by an abrupt shift (tipping point) to a different state. For instance, following a major change in the environment (*e.g.,* precipitation regime or grazing pressure), the functional structure of plant communities may be significantly transformed toward an alternative stable state with direct effects on ecosystem processes. In such cases, changes are non-linear, reflecting species loss and thereby the disappearance of some trait values within the community. Depending on nature, intensity and frequency of the perturbation, the triggered shifts may be reversible (change in community structure but with species from the same 'meta-community' as observed along continuous environmental gradients) or quasi-irreversible (complete system change as observed during shrub encroachment or desertification) (Fig. 1b).

An important issue in ecohydrology is to identify tipping points that delimit these alternative stable states with respect to water-balance components (Newman et al. 2006). In water-limited environments, there are important issues in relation with predicted 'catastrophic'

shifts due to the combination of increasing drought regime and grazing pressure (Kéfi et al. 2007). From this perspective, we believe that the study of small intermediate shifts in the functional structure of plant communities in response to varying water availability should constitute a good starting point for a better understanding of the underlying mechanisms. In this work, the use of both synchronic and diachronic approaches (Manuscript II) provides some insights into how mechanisms underlying community assembly may determine the shift from one alternative stable state to another. The inter-annual or inter-seasonal variability in water availability informed on how evapotranspiration fluctuates for a given potential rate determined by vegetation properties, while the edaphic gradient gave virtual predictions about the effects of more drastic change in water availability on ecohydrologic functioning. Specifically, we argue that mechanisms related to permanent environmental constraints (*e.g.,* the edaphic gradient) does not act on similar components of functional diversity than those related to dynamic stress between years and season. The environmental constraints which exert more permanent selective pressure affect the community weighted mean values of traits (Manuscript III), while environmental fluctuations should more probably affect the functional divergence of the communities since the importance of plant competition should be more sensitive to small changes in environmental conditions.

B. Towards a better understanding of rangeland ecohydrology

1. The allometric approach of plant diversity: a biophysical view of functional equilibrium

Among the fundamental resources required for plant metabolism, water has a particular status since only a small part of the water acquired from the soil hydrates the organs; the largest part being necessarily lost during photosynthesis. The continuum between water acquisition/conservation can therefore not be conceptualized similarly to that of nutrients. Tissue turn-over has less direct impact on the water-use strategy. However, in analogy with Ohm's law, plants can be more or less resistant to water flows, thereby using more parsimoniously the amount of water available in case of water shortage. Beyond a series of physiological or anatomical adjustments, plants are able to regulate water fluxes by modifying to a certain extent their exchange surfaces through phenotypic plasticity, allocating more biomass to the organs acquiring the most limiting resource (Shipley & Meziane 2002). Regulation of water fluxes could therefore be conceptualized as the ratio between the sizes of the root system, harvesting water, and deployment of the transpiring canopy.

At the community level, we do not exclude that such plasticity in allocation patterns occurred at the plant level along the gradient, but we suggest that the adjustment between the aboveground and belowground components of vegetation resulted more likely from the turn-over of species with different allometric trajectories. This is typically illustrated by the difference in relative abundance among the different growth forms between the communities (Manuscript I and Manuscript III). More specifically, we show that the average plant height in the community had a central role in determining the RAI:LAI ratio of vegetation (Manuscript III), highlighting the fundamental role of size-related traits to equilibrate with abiotic factors. We therefore agree with Niklas (2004) who asserted that plant size, whether estimated by biomass, height or biovolume, exerts a powerful and quantifiable influence on plant form, function and life history. The size distribution of individuals may thus be the single predominant characteristic of a community (Enquist et al. 2001; Brown et al. 2004; Allen et al. 2005; Economo et al. 2005) at equilibrium.

This is in part consistent with the 'metabolic theory of ecology' (West et al. 1997, 1999) which is grounded in the assumption that the flux of energy, and thereby of water, at the whole-organism level can be predicted using biophysical principles of mass balance, hydrodynamics, biomechanics and thermodynamics. Applying the theory to vascular plants, plant size therefore represents the physical and biological constraints that limit internal transport through the vascular system, and thereby govern the biomass production and the allocation pattern. Assuming (i) that plants within a community compete for the same amount of resources, (ii) that their rate of resource use scales with the ¾ power of the plant size, and (iii) that plant grow until they are limited by resource availability, one prediction of the theory is the maximum population density that can be supported at equilibrium in an area with respect to resource supply and average plant mass in plant community (Enquist et al. 1998). Similar predictions have been proposed and tested successfully for ecosystem processes such as productivity and nutrient-use (Kerkhoff et al. 2006). Extending the empirical evidences supporting such 'ecosystem allometry', we show in this study that the potential evapotranspiration of the ecosystem allometrically scaled with respect to standing biomass at the peak of vegetation (Manuscript II). Likewise, the water uptake capacity of plants scaled with respect to the potential evapotranspiration rate (Manuscript III). Because these generalized allometric rules have a high degree of predictive power, we would suggest that the 'ecohydrological optimality' hypothesis may be better understood within the allometric conceptual framework, in particular for the identification of the functional structure of plant community at equilibrium state which can described as an 'allometrically ideal' steady-state plant community.

2. Call for future experiments on plant-plant interactions: manipulating resources

The vegetation at the experimental station is characterized by a high species richness of different growth forms including grasses, forbs, dwarf shrubs, rosettes etc. in the herbaceous layer. As shown in this work, and formally tested in previous work (Bernard-Verdier et al. 2012), these species are not randomly distributed, but reflect the spatial variability in edaphic conditions. However, due to the proximity of the different habitats (<100 m), species may possibly disperse from one community to another along the edaphic gradient. We can therefore assume that if there is a permanent change in a given habitat (*e.g.,* greater or lower resource availability, or more or less intense grazing pressure), the community will lose some species and recruit some other which are usually found in the other parts of the gradient. The amelioration in water availability may favor more competitive grasses (*e.g., Bromus erectus, Brachypodium pinnatum*) on shallow soils at the expense of more conservative species. Conversely, dwarf shrubs (*e.g., Thymus dolomiticus*, *Helianthemum canum*) and conservative grasses (*e.g., Koeleria vallesiana*, *Festuca christiani-bernardii*), species may be progressively advantaged if water availability decreases. Such rapid changes have been shown at the study site in response to increasing nutrient availability (Fayolle 2008).

Following these possible modifications in community composition, and thereby in functional structure, a question arises: how do plant communities reach alternative equilibrium states? In this work we provide evidence that the ecohydrological optimality hypothesis may hold along a gradient of soil water availability (Manuscript II), but we did not test the dynamics and resilience of the equilibrium water balance (Fig.1). While the alternative equilibrium states may be accurately predicted using an allometric approach with respect to abiotic constraints (*i.e* water availability), the transition from one alternative state to another should depend more on the importance of plant competition displayed in the biotic environment because competition processes may directly affect population dynamics and thereby have strong impact on species demography. Depending on the balance between abiotic stress and plant competition, a given species can be advantage or on the contrary be endangered and suppressed, impacting in turn the functional structure of the whole community. For example, in this study, we showed by a removal experiment that competitive exclusion may gradually lead to stronger convergence of trait values (Manucript IV). However, since the ecosystem at our study site was 'at equilibrium' (Manuscript II), identifying the effect of competition processes as transient driver at the community level may be difficult in the absence of an experimental modification in abiotic or biotic conditions. Manipulating resources (*e.g.,* water supply) will typically allow for the identification of threshold effects (how much water stress triggers a permanent modification of functional structure of the plant communities?), and testing the underlying mechanisms

(does the importance of plant competition increase by adding water?). We therefore suggest using rainout shelters and watering treatments to extend the edaphic gradient towards both extremes (dry and humid) in order to explore the potential dynamics of these ecosystems. Furthermore, since both water availability and nitrogen availability co-vary along the edaphic gradient (Chapter 1), reflecting close link between water and nutrient cycles, it would be interesting to untangle the effect of both water stress and nutrient limitation on plant performance and plant-plant interaction in order to provide further insights into community assembly rules within such Mediterranean rangeland ecosystems.

The results of the removal experiment revealed that plant height and leaf morphology (LDMC) may be both involved, but independently to each other, in competition processes along a complex edaphic gradient (Manuscript IV). This may reflect that there were a gradual interplay between different competition processes involving these two traits across communities, namely between asymmetric competition for light and competition for water. However, while plant height is admittedly associated to the competitive ability for light (Westoby et al. 2002; Falster & Westoby 2003), evidence for LDMC to indicate water competition has not been reported. Moreover, since low water availability acts also as a perturbation inducing the senescence of plant tissue, LDMC was on contrary shown to indicate resistance to stress. The balance between the two competition processes, for light and water, can be interpreted on the one hand as a limitation in photosynthesis processes (energy acquisition, carbon assimilation) in case of light competition, while being on the other hand more related to water use efficiency in case of water competition. A way to disentangle which of the two competitive processes is the most important in case of water shortage in the environment, and how it relates to response in plant height and LDMC requires assessing physiological responses of the species under different level of competition and controlled abiotic constraints. To this end, we set up an experimental design at the experimental station in Montpellier (Terrain d'expérience du CEFE), in which artificial communities were composed with native species harvested from the La Fage station. Monocultures of each species, as well as the different mixtures (2 to 3 species in mixtures) were assembled and placed under two contrasting climatic scenario thanks to rainout shelters. Standing biomass, as well as aboveground and belowground traits was measured during two consecutive years in order to assess the effect of the climatic scenario and of inter-/intra specific competition on species performance. Moreover, physiological processes related to photosynthesis and water use efficiency in response to the functional identity of neighbors are under analysis by comparing differences in carbon and oxygen isotopes from random individuals within each species mixture and monoculture.

C. Applications in agroecology

Management of production-oriented agro-ecosystems has focused on simplifying the genetic composition (the species or genotypes that surrogates the function of interest and performs the best) rather than on species diversity, namely because of management facilities and short-term higher efficiencies. However, the maintenance of high productivity over time in monocultures requires high input rates (*e.g.,* water, fertilizers, energy) and it is now recognized that such intense management may not be sustainable in the face of new worldwide challenges. In the context of increasing summer droughts as predicted by climate change models for Mediterranean areas (IPCC 2007; Zhang et al. 2007), grassland management will be challenged by: (i) maintaining current ecosystem productivity over short-term periods and under low or moderate stress, *i.e* during the main growing seasons (e.g. spring and autumn), while reducing the use of inputs, and (ii) enhancing the resistance and resilience of ecosystem functions such as productivity over long-term periods including severe stress events (*e.g.*, summer drought, change in grazing regime), resulting in pluri-annual maintenance of biomass production. To overcome these challenges as best possible, species –and variety- diversity has become a major management goal and that emerging properties of species mixtures need to be accounted for. Particularly, it is now widely expected that plant interaction processes could lead to a more complete resource use through functional complementarity and facilitation among species, and thus to parsimonious and stable plant dynamics. Moreover, increasing the temporal scale of management, we believe that species diversity would buffer environmental fluctuations and act as safeguard of ecosystem functions in case of extreme drought events. However, we outline here that a conceptual framework linking the trait values of interest and their combination with respect to the productive objective is strongly required for designing efficient multispecies stands (see Manuscript V in Appendix).

References



(Romane ewe and her lambs at La Fage-station, *spring 2011*)

Ackerly DD & Bazzaz, FA (1995) Leaf dynamics, self-shading and carbon gain in seedlings of a tropical pioneer tree. Oecologia 101(3):289-298

Afnor (1994) Qualité des sols, vol 1, Recueil des normes

Allen R, Pereira L, Raes D, Smith M (1998) Crop evapotranspiration-Guidelines for computing crop water requirements-FAO Irrigation and drainage paper 56. FAO, Rome

Allen R, Tasumi M, Morse A, Trezza R (2005) A Landsat-based energy balance and evapotranspiration model in Western US water rights regulation and planning. Irrig Drain Syst 19:251–268

Asbjornsen H, Goldsmith GR, Alvarado-Barrientos MS, Rebel K, Van Osch FP, Rietkerk M, Chen J, Gotsch S, Tobon C, Geissert DR, Gomez-Tagle A, Vache K, Dawson TE (2011) Ecohydrological advances and applications in plant-water relations research: a review. J Plant Ecol 4:3–22

Asner GP, Scurlock, J M, & A Hicke J (2003) Global synthesis of leaf area index observations: implications for ecological and remote sensing studies. Global Ecol and Biogeogr 12(3):191-205

Austin, MP (1999) A silent clash of paradigms: some inconsistencies in community ecology. Oikos 170-178

Austin M & Gaywood M (1994) Current problems of environmental gradients and species response curves in relation to continuum theory. J Veg Sci 5:473-482

Bell (2005) The co-distribution of species in relation to the neutral theory of community ecology. Ecology 86:1757-1770

Bernard-Verdier M, Navas M-L, Vellend M, Violle C, Fayolle A, Garnier E (2012) Community assembly along a soil depth gradient: contrasting patterns of plant trait convergence and divergence in a Mediterranean rangeland. J Ecol 100:1422–1433

Bertness MD, Callaway RM (1994) Positive interactions in communities. Trends Ecol Evol 9:191–193

Beleya LR, Lancaster J (1999) Assembly rules within a contingent ecology. Oikos 86:402-416

Blondel J, Aronson J (1999) Biology and wildlife of the Mediterranean region. Oxford University Press, Oxford

Bonell M (2002) Ecohydrology—a completely new idea? Hydrol Sci J 47:809–810

Breshears DD, Myers OB, Barnes FJ (2009) Horizontal heterogeneity in the frequency of plant-available water with woodland intercanopy-canopy vegetation patch type rivals that occuring vertically by soil depth. Ecohydrol 2:503–519

Brown JH, Gillooly JF, Allen AP, Savage, VM, West GB (2004) Toward a metabolic theory of ecology. Ecology 85:1771–1789

Bruno JF, Stachowicz JJ, Bertness MD (2003) Inclusion of facilitation into ecological theory. Trends Ecol Evol 18:119–125

Caylor KK, Scanlon TM, Rodriguez-Iturbe I (2009) Ecohydrological optimization of pattern and processes in water-limited ecosystems: A trade-off-based hypothesis. Water Resour Res 45:407

Chapin III FS, Autumn K, Pugnaire F (1993) Evolution of suites of traits in response to environmental stress. Am Nat 142:S78–S92

Chesson P (2000) Mechanisms of maintenance of species diversity. Annu Rev Ecol Syst 31:343–366

Cornelissen JHC, Lavorel S, Garnier E, Díaz S, Buchmann N, Gurvich DE, Reich PB, Steege H Ter, Morgan HD, Heijden MG Van Der, Pausas JG, Poorter H (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Aust J Bot 51:335

Cornwell WK, Ackerly DD (2009) Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. Ecol Monogr 79:109–126

Cornwell WK, Schwilk LDW, Ackerly DD (2006) A trait-based test for habitat filtering: convex hull volume. Ecology 87:1465–71

Delgado-Baquerizov M, Maestre FT, Gallardo A., Bowker MA, Wallenstein MD, Quero JL, ... & Ungar, E. D. (2013) Decoupling of soil nutrient cycles as a function of aridity in global drylands. Nature 502(7473):672-676

Díaz S, Lavorel S, de Bello F, Quétier F, Grigulis K, Robson TM (2007) Incorporating plant functional diversity effects in ecosystem service assessments. Proc Natl Acad Sci USA 104:20684–20689

Díaz, S, Purvis, A, Cornelissen, JH, Mace, GM, Donoghue, MJ, Ewers, RM, ... & Pearse, WD (2013) Functional traits, the phylogeny of function, and ecosystem service vulnerability. Ecol Evol 3(9):2958-2975

Eagleson P (2002) Ecohydrology: Darwinian expression of vegetation form and function. Cambridge University Press

Eagleson PS (1982a) Ecological Optimality in Water-Limited Natural Soil-Vegetation Systems 1. Theory and Hypothesis. Water Resour Res 18:325–340

Eagleson PS (1982b) Ecological Optimality in Water-Limited Natural Soil-Vegetation Systems 2. Tests and Applications. Water Resour Res 18:341–354

Eagleson PS, Segarra RI (1985) Water-Limited Equilibrium of Savanna Vegetation Systems. Water Resour Res 21:1483–1493

Economo EP, Kerkhoff, AJ, Enquist, BJ (2005) Allometric growth, life-history invariants and population energetics. Ecol Lett 8(4):353-360

Elton CS (1927) Animal ecology. Sidwick & Jackson, London (UK)

Eviner VT, Chapin III FS (2003) Functional matrix : a conceptual framework for predicting multiple plant effects on ecosystem processes. Annu Rev Ecol Evol Syst 34:455–485

Fridley JD, Carolina N, Hill C (2001) The influence of species diversity on ecosystem productivity : how, where, and why ? Oikos 93:514–526

Fortunel C, Garnier E, Joffre R, Kazakou E, Quested H, Grigulis K, ... & Zarovali M (2009) Leaf traits capture the effects of land use changes and climate on litter decomposability of grasslands across Europe. Ecology 90(3):598-611

Garnier E, Navas M-L (2012) A trait-based approach to comparative functional plant ecology: concepts, methods and applications for agroecology. A review. Agron Sustain Dev 32:365–399

Garnier E, Cortez J, Billès G, Navas M-L, Roumet C, Debussche M, Laurent G, Blanchard A, Aubry D, Bellman A, Neill C, Toussaint J-P (2004) Plant functional markers capture ecosystem properties. Ecology 85:2630–2637

Gause GF (1934) The struggle for existence. Williams & Wilkins, Baltimore, MD

Grime JP (1973) Competitive exclusion in herbaceous vegetation. Nature 242:344–347

Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am Nat 111:1169–1194

Grime JP (1997) Biodiversity and ecosystem function: the debate deepens. Science 277(5330):1260-1261

Grime JP (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. J Ecol 86:891–899

Grime JP (2006) Trait convergence and trait divergence in herbaceous plant communities: Mechanisms and consequences. J Veg Sci 17:255

Grinnell J (1917) Field tests of theories concerning distributional control. Am Nat 51:15-128

Groffman PM, Bain DJ, Band LE, Belt KT, Brush GS, Grove, JM, ... & Zipperer WC (2003) Down by the riverside: urban riparian ecology. Front Ecol Environ 1(6):315-321

Gross N, Robson TM, Lavorel S, Albert CH, Le Bagousse-Pinguet Y, Guillemin R (2008) Plant response traits mediate the effects of subalpine grasslands on soil moisture. New Phytol 180:652–62

Guswa AJ (2005) Soil-moisture limits on plant uptake: An upscaled relationship for water-limited ecosystems. Adv Water Resour 28:543–552

Guswa AJ (2008) The influence of climate on root depth: A carbon cost-benefit analysis. Water Resour Res 44:1–11

Guswa AJ (2010) Effect of plant uptake strategy on the water–optimal root depth. Water Resour Res 46:601

Hannah DM, Wood PJ, Sadler JP (2004) Ecohydrology and hydroecology: A new paradigm? Hydrol Process 18:3439–3445

Hannah DM, Sadler JP, Wood PJ (2007) Hydroecology and ecohydrology : a potential route forward ? Hydrol Process 3390:3385–3390

Harte J (2002) Toward a synthesis of the Newtonian and Darwinian worldviews. Phys Today 55:29

Hatton TJ, Salvucci GD, Wu HI (1997) Eagleson's optimality theory of an ecohydrological equilibrium : quo vadis ? Funct Ecol 11:665–674

Hooper DU, Chapin FS, Lodge DM, Ewel JJ, Loreau M, Hector A, Naeem S, Schmid B, Inchausti P, Setala H, Vendermeer AJ, Wardle DA (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecol Monogr 75:3–35

Hutchinson MF (1957) Concluding remarks. Cold Spring Harbor Symposia on Quantitative Biology 22:415-427

Jackson RB, Sperry JS, Dawson TE (2000) Root water uptake and transport: using physiological processes in global predictions. Trends Plant Sci 5:482–488

Jenerette GD, Scott RL, Huxman TE (2008) Whole ecosystem metabolic pulses following precipitation events. Funct Ecol 22:924–930

Jenerette GD, Barron-gafford GA, Guswa AJ, Mcdonnell JJ, Villegas JC (2012) Organization of complexity in water limited ecohydrology. Ecohydrol 5:184–199

Keddy (1990) Competitive hierarchies and centrifugal organization in plant communities. Pages 265-291 Perspectives on lant competition. Academic Press, Inc

Keddy PA (1992) Assembly and response rules: two goals for predictive community ecology. J Veg Sci 3:157–164

Kerkhoff AJ, Martens SN, Milne BT (2004) An ecological evaluation of Eagleson' s optimality. Funct Ecol 18:404–413

Klumpp K, Soussana J-F (2009) Using functional traits to predict grassland ecosystem change: a mathematical test of the response-and-effect trait approach. Glob Chang Biol 15:2921–2934

Knapp AK, Fay PA, Blair JM, Collins SL, Smith MD, Carlisle JD, Harper CW, Danner BT, Lett MS, McCarron JK (2002) Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. Science 298(5601):2202–2205

Körner C, Paulsen J (2004) A world-wide study of high altitude treeline temperatures. J Biogeogr 31:713–732

Kundzewicz ZW (2002) Ecohydrology—seeking consensus on interpretation of the notion. Hydrol Sci J 47:799–804

Lavorel S, Garnier E (2002) Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. Funct Ecol 16:545–556

Loik ME, Breshears DD, Lauenroth WK, Belnap J (2004) A multi-scale perspective of water pulses in dryland ecosystems: climatology and ecohydrology of the western USA. Oecologia 141:269–281

Loreau M, de Mazancourt C (2013) Biodiversity and ecosystem stability: a synthesis of underlying mechanisms. Ecol Lett 16:106–115

Lortie CJ, Brooker RW, Choler P, Kikvidze Z, Michalet R, Pugnaire FI (2004a) Rethinking plant community theory. Oikos 107:433–438

Lortie CJ, Brooker RW, Kikvidze Z, Callaway RM (2004b) The value of stress and limitation in an imperfect world: A reply to Körner. J Veg Sci 15:577

MacArthur R, Levins R (1967) The limiting similarity , convergence, and divergence of coexisting species. Am Nat 101:377–385

Maestre FT, Callaway RM, Valladares F, Lortie CJ (2009) Refining the stress-gradient hypothesis for competition and facilitation in plant communities. J Ecol 97:199–205

Manzoni S, Vico G, Porporato A, Katul G (2013) Biological constraints on water transport in the soil–plant–atmosphere system. Adv Water Resour 51:292–304

Marrs RH (2004) Why we should conserve "limiting factors", at least sometimes! J Veg Sci 15:573

Maseda PH, Fernández RJ (2006) Stay wet or else: three ways in which plants can adjust hydraulically to their environment. J Exp Bot 57:3963–77

Mata-González R, McLendon T, Martin DW (2005) The inappropriate use of crop transpiration coefficients (Kc) to estimate evapotranspiration in arid ecosystems: a review. Arid L Res Manag 19:285–295

McGill BJ, Enquist BJ, Weiher E, Westoby M (2006) Rebuilding community ecology from functional traits. Trends Ecol Evol 21:178–85

Mokany K, Ash J, Roxburgh S (2008) Functional identity is more important than diversity in influencing ecosystem processes in a temperate native grassland. J Ecol 96:884–893

Moore GW, Heilman JL (2011) Proposed principles governing how vegetation changes affect transpiration. Ecohydrol 4:351–358

Morin X & Thuiller W (2009) Comparing niche- and process-based models to reduce prediction uncertainty in species range shifts under climate change. Ecology 90(5): 1301–13

Navas M-L, Violle C (2009) Plant traits related to competition: how do they shape the functional diversity of communities? Community Ecol 10:131–137

Newman BD, Wilcox BP, Archer SR, Breshears DD, Dahm CN, Duffy CJ, McDowell NG, Phillips FM, Scanlon BR, Vivoni ER (2006) Ecohydrology of water-limited environments: A scientific vision. Water Resour Res 42: W06302

Noy-Meir I (1973) Desert ecosystems: environment and producers. Annu Rev Ecol Evol S 4:25-51.

Nuttle WK (2002) Is ecohydrology one idea or many? Hydrol Sci J 47:805–807

Pérez-Ramos IM, Roumet C, Cruz P, Blanchard A, Autran P, Garnier E (2012) Evidence for a "plant community economics spectrum" driven by nutrient and water limitations in a Mediterranean rangeland of southern France. J Ecol 100:1315–1327

Petchey OL, Gaston KJ (2006) Functional diversity: back to basics and looking forward. Ecol Lett 9:741–58

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L (2012) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytol 193:30–50

Porporato A, Daly E, Rodriguez-Iturbe I (2004) Soil water balance and ecosystem response to climate change. Am Nat 164:625–632

Quested H, Eriksson O, Fortunel C, & Garnier E (2007) Plant traits relate to whole-community litter quality and decomposition following land use change. Funct Ecol 21(6):1016-1026

Raunkiaer C 1934. The life forms of plants and statistical plant geography. The Clarendon Press, Oxford

Rinaldi S, Candaten M & Casagrandi R (2001) Evidence of peak-to-peak dynamics in ecology. Ecol Lett 4(6):610-617

Reich PB, Walters MB, & Ellsworth DS (1997) From tropics to tundra: global convergence in plant functioning. P Nat Acad Sci USA 94(25):13730-13734

Reynolds JF, Kemp PR, Ogle K, Fernández RJ (2004) Modifying the "pulse-reserve" paradigm for deserts of North America: precipitation pulses, soil water, and plant responses. Oecologia 141:194–210

Reynolds JF, Smith DMS, Lambin EF, Turner BL, Mortimore M, Batterbury SP, ... & Walker B (2007) Global desertification: building a science for dryland development. Science 316(5826):847-851

Rodriguez-Iturbe I (2000) Ecohydrology : A hydrologic perspective of climate-soil-vegetation dynamics. Water Resour Res 36:3–9

Rodriguez-Iturbe I, D'Odorico P, Porporato A, Ridolfi L (1999) On the spatial and temporal links between vegetation, climate and soil moisture. Water Resour Res 35:3709–3722

Rodriguez-Iturbe I, Porporato A, Laio F, Ridolfi L (2001) Plants in water-controlled ecosystems : active role in hydrologic processes and response to water stress I . Scope and general outline. Adv Water Resour 24:695–705

Rosenfeld JS (2002) Functional redundancy in ecology and conservation. Oikos 98:156-162

Ruppert JC, Holm A, Miehe S, Muldavin EH, Snyman HA, Wesche K, Linstädter A (2012) Meta-analysis of ANPP and rain-use efficiency confirms indicative value for degradation and supports non-linear response along precipitation gradients in drylands. J Veg Sci 23:1035–1050

Sankaran M, Ratnam J, Hanan NP (2004) Tree-grass coexistence in savannas revisited - insights from an examination of assumptions and mechanisms invoked in existing models. Ecol Lett 7:480–490

Schamp BS, Chau J, Aarssen LW (2008) Dispersion of traits related to competitive ability in an old-field plant community. J Ecol 96:204–212

Scheffer M, Carpenter SR (2003) Catastrophic regime shifts in ecosystems: linking theory to observation. Trends Ecol Evol 18:648–656

Scheffer M, Holmgren M, Brovkin V, Claussen M (2005) Synergy between small- and large-scale feedbacks of vegetation on the water cycle. Glob Chang Biol 11:1003–1012

Schumacher J & Roscher C (2009) Differential effects of functional traits on aboveground biomass in semi-natural grasslands. Oikos 118(11) 1659-1668

Schwinning S (2010) The ecohydrology of roots in rocks. Ecohydrol 3:238–245.

Schymanski SJ, Sivapalan M, Roderick ML, Beringer J, Hutley LB (2008) An optimality-based model of the coupled soil moisture and root dynamics. Hydrol Earth Syst Sci 5:51–94

Smetten KRJ (2008) Welcome address for the new "Ecohydrology" Journal. Ecohydrol 1:1–2

Smith MD, & Knapp AK (2003) Dominant species maintain ecosystem function with non-random species loss. Ecol Lett 6(6): 509-517

Sperry JS (2004) Coordinating stomatal and xylem functioning—an evolutionary perspective. New Phytol 162: 568–570

Suding KN, Lavorel S, Chapin FS, Cornelissen JHC, Díaz S, Garnier E, Goldberg DE, Hooper DU, Jackson ST, Navas M-L (2008) Scaling environmental change through the community-level: a trait-based response-and-effect framework for plants. Glob Chang Biol 14:1125–1140

Thuiller W, Albert C, Araújo MB, Berry PM, Cabeza M, Guisan A, …, Zimmermann NE (2008) Predicting global change impacts on plant species' distributions: Future challenges. Perspect Plant Ecol 9(3-4):137–152

Thuiller W (2013) Competitive effect and response of savanna tree seedlings: comparison of survival, growth and associated functional traits. J Veg Sci 24(4): 591–592

Tilman D (1982) Resource Competition and Community Structure

Vandermeer J (1989) The Ecology of Intercropping. Cambridge University Press, Cambridge

Vandermeer J & Yodzis P (1999) Basin boundary collision as a model of discontinuous change in ecosystems. Ecology 80(6):1817-1827

Vicca S, Gilgen AK, Serrano MC, Dreesen FE, Dukes JS, Estiarte M, Gray SB, Guidolotti G, Hoeppner SS, Leakey ADB, Ogaya R, Ort DR, Ostrogovic MZ, Rambal S, Sardans J, Schmitt M, Siebers M, van der Linden L, van Straaten O, Granier A (2012) Urgent need for a common metric to make precipitation manipulation experiments comparable. New Phytol 195:518–522

Vile D, Shipley B, Garnier E (2006) Ecosystem productivity can be predicted from potential relative growth rate and species abundance. Ecol Lett 9:1061–1067

Violle C, Navas M, Vile D, Kazakou E, Fortunel C (2007) Let the concept of trait be functional ! Oikos 116:882–892

Warming E (1909) Oecology of plants. Clarendon Press, Oxford

Wassen MJ, Grootjans AP (1996) Ecohydrology: an interdisciplinary approach for wetland management and restoration. Vegetatio 126:1–4

Weiher E (2004) Why should we constrain stress and limitation ? Why conceptual terms deserve broad definitions. J Veg Sci 15:569–571

Weiher E, Keddy PA (1995) The assembly of experimental wetland plant communities. Oikos 73:323–335

Weiher E, van der Werf A, Thompson K, Roderick M, Garnier E, Eriksson O (1999) Challenging Theophrastus : A common core list of plant traits for functional ecology. J Veg Sci 10:609–620

Westoby M (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. Plant Soil 199:213–227

Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ (2002) Plant ecological strategies : some leading dimensions of variation between species. Annu Rev Ecol Syst 33:125–159

Whittaker R (1967) Gradient analysis of vegetation. Biol Rev 42:207–64

Wilcox BP, Breshears DD, Allen CD (2003) Ecohydrology of a resource-conserving semiarid woodland: effects of scale and disturbance. Ecol Monogr 73:223–239

Wilson PJ, Thompson KEN, Hodgson JG (1999) Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. New Phytol 143(1):155-162.

Wilson M V. (2007) Measuring the components of competition along productivity gradients. J Ecol 95:301–308

Wilson JB, & Stubbs WJ (2012) Evidence for assembly rules: limiting similarity within a saltmarsh. J Ecol 100(1):210-221

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee WG, Lusk C, Midgley JJ, Navas M-L, Niinemets U, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R (2004) The worldwide leaf economics spectrum. Nature 428:821–827

Wu Z, Dijkstra P, Koch GW, Peñuelas J, Hungate BA (2011) Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. Glob Chang Biol 17:927–942

Zalewski M (2002) Ecohydrology — the use of ecological and hydrological processes for sustainable management of water resources. Hydrol Sci J 47:799–804

Zeppel M (2013) Convergence of tree water use and hydraulic architecture in water-limited regions: a review and synthesis. Ecohydrol 6:889–900

Appendix

Application in agroecology



(experimental garden in Montpellier, *spring 2012*)

MANUSCRIPT V

Designing resilient and sustainable grasslands for a drier future: adaptive strategies, functional traits and biotic interactions

-

Concevoir des prairies durables et résilientes pour un futur plus sec: stratégies adaptatives, traits fonctionnels des plantes et interactions biotiques

Florence Volaire, Karim Barkaoui, Mark Norton

Abstract

In many regions of the world, such as Southern Europe and most Mediterranean areas, the frequency and magnitude of droughts and heat waves are expected to increase under global warming and will challenge the sustainability of both native and sown grasslands. To analyze the adaptive strategies of species, genotypes and cultivars, we aim both (i) to understand the composition and functioning of natural grasslands and (ii) to propose ideotypes of cultivars and optimal composition for mixtures of species/genotypes under water deficit and high temperatures. This review presents a conceptual framework to analyze adaptive responses of perennial herbaceous species, starting from resistance to moderate drought with growth maintenance (dehydration avoidance and tolerance of lamina) to growth cessation and survival of plants under severe stress (dehydration avoidance and tolerance of meristems). The most discriminating functional traits vary according to these contrasting strategies because of a trade-off between resistance to moderate moisture deficit and survival of intense drought. Consequently it is crucial to measure the traits of interest in the right organs and as a function of soil water use, in order to avoid misleading interpretations of plant responses. Furthermore, collaboration between ecologists, eco-physiologists, and agronomists is required to study the combination of plant strategies in natural grasslands as only this will provide the necessary rules for species and cultivars or ecotypes assemblage. This 'agro-ecological' approach aims to identify and enhance functional complementarity and limit competition within the multi-specific or multi-genotypic material associated in mixtures since using plant biodiversity should contribute to improving grassland resistance and resilience.

Résumé

Dans un contexte de réchauffement climatique planétaire, des prévisions montrent que la fréquence et la sévérité des sécheresses ainsi que des vagues de chaleur extrême vont augmenter dans de nombreuses régions du monde comme en Europe du Sud et en Méditerranée. Parmi les nombreuses conséquences que ces nouvelles conditions climatiques vont avoir sur le fonctionnement des écosystèmes, la durabilité des prairies naturelles et semées constitue aujourd'hui une préoccupation centrale. Afin de pouvoir sélectionner au mieux les stratégies adaptatives des espèces, des génotypes et des cultivars disponibles susceptibles de constituer les prairies de demain, notre démarche générale est (i) de comprendre les déterminants de la composition et du fonctionnement des écosystèmes prairiaux naturels pour (ii) proposer des idéotypes de cultivars mieux adaptés et déterminer quelle peut être la composition optimale pour des mélanges d'espèces (ou de génotypes) soumis à de forte contraintes hydrique et de température. Cette synthèse présente un cadre conceptuel pour analyser les réponses adaptatives des espèces herbacées pérennes à la sécheresse. Elle décrit les mécanismes de résistance des plantes à des sécheresses modérées qui permettent le maintien de la croissance végétative (évitement de la déshydratation, et tolérance des limbes), puis les mécanismes de survie à des sécheresses sévères qui oblige l'arrêt de la croissance végétative (évitement de la déshydratation, et tolérance des méristèmes). Les traits fonctionnels des plantes qui permettent de caractériser de façon pertinente la gamme des stratégies adaptatives sont spécifiques à chacun de ces différents mécanismes en raison d'un compromis évolutif entre résistance à des déficits hydriques modérés et survie à des déficits hydriques intenses. En conséquence, il est primordial de mesurer les traits d'intérêt sur les bons organes végétatifs en relation avec la dynamique d'utilisation de l'eau du sol afin d'éviter de mauvaises interprétations vis-à-vis de la réponse des plantes. D'autre part, nous suggérons qu'une collaboration plus étroite entre écologues, écophysiologistes et agronomes est nécessaire pour étudier finement la combinaison des stratégies des plantes dans les prairies naturelles dans la mesure où cela devrait fournir des règles d'assemblage mobilisables pour concevoir de nouveaux mélanges d'espèces, d'écotypes ou de cultivars. Cette approche 'agroécologique' a notamment pour objectif de promouvoir la complémentarité fonctionnelle au sein des mélanges plurispécifiques pour augmenter l'efficacité globale d'utilisation de l'eau et réduire la compétition entre les plantes. L'utilisation de la biodiversité apparaît donc essentielle pour améliorer la durabilité et la résilience des prairies face à la sécheresse.

Introduction

Grasslands cover vast areas of the Earth's surface and other than producing forage provide a range of ecosystem services including carbon storage, soil protection and the preservation of biodiversity. In most rain-fed environments, the productivity and sustainability of both native and sown grasslands, depends mainly on temperature and precipitation (Boyer, 1982) and will be challenged by predicted warmer climates (I.P.C.C., 2007). In Southern Europe, a decrease in summer precipitation accompanied by increased temperatures and solar radiation would inevitably lead to more frequent and more intense droughts (Supit et al., 2010; Trnka et al., 2011). Therefore the frequency of widespread mortality events is likely to increase along with long-term pasture degradation associated with the droughts (Ciais et al., 2005). To cope with the negative effects of climate change, short-term adaptations may include changes of species or populations with greater drought tolerance (Olesen et al., 2007). However, breeding efforts in 'cool season' forage plants have taken place mainly in temperate areas and very few cultivars adapted to severe drought are currently available in Europe (Lelièvre and Volaire, 2009). It is now known that forage persistence during severe drought is governed by mechanisms different than those conferring resistance to moderate droughts (Milbau et al., 2005; Volaire et al., 2009). The plant traits conferring relevant adaptive strategies should therefore be defined according to the targeted environments. It is also advocated increasingly to maximize genetic diversity in multi-specific and multi-genotypic grasslands as a possible adaptation strategy against climate change (Kreyling et al., 2012). Therefore, this review addresses the following questions: (1) what is a drought tolerant perennial forage genotype? (2) What are the traits associated with the different adaptive strategies to drought and how are these measured reliably? And (3) how do we combine strategies (genotypes) for persistent forage mixtures under drought? Our objective is to clarify concepts and methods for the study of drought resistance of perennial forage plants since they differ from those intensively studied in major annual crops (Sinclair, 2012; Tardieu, 2012). We aim to stress the inputs of functional and community ecology applied to native grasslands in order to understand (1) the nature of trade-offs between plant strategies that should have more implications in the design of breeding programs and (2) the elaboration of a framework to rationalize the association of genotypes in forage mixtures resilient under both current and future environmental conditions.

The differences between drought resistance & drought survival

Plant growth maintenance versus plant survival: a trade-off

Drought resistance in crop plants usually defines the ability of species or varieties to grow and yield satisfactorily under periodic drought (May and Milthorpe, 1962). This definition is generally assumed without much discussion and is applied to all cultivated species, whether annual of perennial, whether producing grains or biomass and irrespective of the types of drought and environmental constraints. We believe that for perennial herbaceous species, this definition is inadequate and needs modification. Forage crops and perennial grasslands are expected to produce over many years and their sustainability is associated with yield stability and long-term resilience. Their drought resistance should be therefore analysed over the appropriate time scale and as a function of the magnitude of water deficit experienced by the plants. This drought intensity is estimated as a cumulative index of 'precipitation' minus or versus 'evapotranspiration' accumulated during the dry period (FAO, 2008; Tsakiris & Vangelis, 2005; Vicente-Serrano et al., 2012). Measurements of soil water reserve and rooting depth will also provide complementary information on water availability for plants (Vicca et al., 2012).

In the temperate and Mediterranean bioclimatic areas, we propose to make a clear distinction between drought resistance and drought survival, based in particular on recent experiments (Poirier et al., 2012). Under moderate water deficits (cumulative P-ETP lower than around -300 mm according to soil water reserve) and in temperate climates, most genotypes and cultivars of cool-season perennial forage species can be expected to grow. In this case, drought resistance complies with the general definition, i.e. the ability to maintain satisfactory aerial growth and production under a moderate water deficit. Conversely, under severe water deficits (cumulative P-ETP between -300 and -600 mm and according to soil water reserve), plants are expected 'to know when not to grow' (Bielenberg, 2011) in order to survive potential lethal conditions. In these environments, drought resistance combines the ability not to grow during the dry period albeit to survive drought and to regrow when drought is relieved. In this case 'drought survival' is a more suitable term than 'drought resistance'. This issue is exemplified by summer dormancy which confers to genotypes of some grass species the endogenous ability to cease aerial growth and senesce irrespective of the water supply in summer (Volaire and Norton, 2006). Summer dormancy has been correlated with superior survival after severe and repeated summer droughts (Norton et al., 2006, 2012), showing that the ability not to grow during the drought period is the most efficient response to maximise drought survival. This 'trade-off' between 'drought resistance' and 'drought survival' can be paralleled with plant responses under

winter and low temperatures, when winter dormant plants (no growth) are those most able to survive the winter and regrow in spring (Castonguay et al., 2006). 'Drought survival' should not therefore, only be associated with marginal cereal crops under extreme environments (Sinclair, 2011) or with desiccation tolerant species none of which are of agricultural importance (Farrant and Moore, 2011). 'Drought survival' for perennial pasture species is instead, a valuable plant adaptation during part of the plant cycle which may enhance long term persistence and productivity under increasing drought (Lelievre et al., 2011).

Importance of intra-specific variability for drought resistance/survival

For plant breeders, agronomists and eco-physiologists, the importance of intra-specific variability which is one of the major sources of genetic improvement, is an undisputable fact. In plant ecology working on native plant species, the inter-specific variability of functional traits has been recently challenged by the increasingly recognized importance of intra-specific and ecotypic variability (Albert et al., 2011; Violle et al., 2012). Adaptation of local ecotypes or cultivars to environmental conditions and to drought in particular, has been consistently shown to depend on the origin of the genotypes (Annicchiarico et al., 2011; Pecetti et al., 2011; Volaire, 1995; Volaire and Lelievre, 1997). In addition, drought resistance and drought survival were associated more with intra-specific than inter-specific variability in two major perennial grasses (Poirier et al., 2012). Consequently, the characterization and comparison of plant stress responses should be necessarily focussed at the level of the genotype or the cultivar, and not only at the 'species' level.

Adaptation strategies to drought – identification and measurement

Dehydration avoidance and dehydration tolerance

Plants respond to drought with a combination of mechanistically-linked responses and traits that comprise a particular group of behaviours during periods of water stress characterised into several different 'strategies' (Levitt, 1972; Ludlow, 1989). 'Drought escape', the ability of a plant to complete the life cycle before being subjected to serious water stress is relevant mainly for annual species which survive the dry periods as seeds (May and Milthorpe, 1962). At the other extreme, 'desiccation tolerance' is only possessed by a rare group of angiosperms termed resurrection or poikilohydric plants (Gaff, 1971), which can desiccate to air dryness for long periods, but revive rapidly upon re-watering (Scott, 2000). Perennial herbaceous plants combine the more common strategies of both 'dehydration avoidance' and 'dehydration

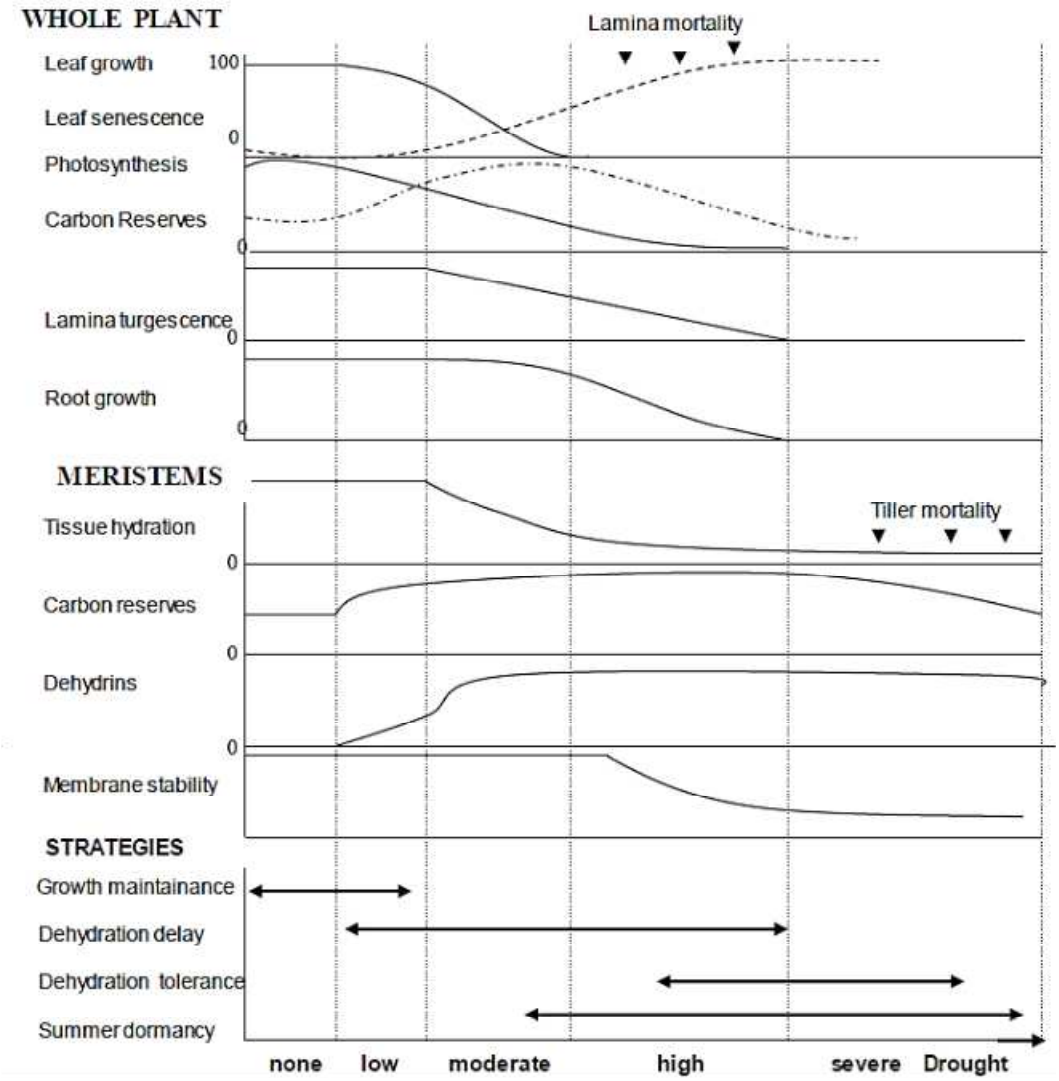


Fig. 1. Schematic responses to intensifying drought of perennial grass at the level of whole plant and aerial meristem and resulting ecophysiological strategies (from Volaire *et al.*, 2009). Scales are arbitrary.

tolerance' (Ludlow, 1989). We argue that these strategies have to be analysed in the light of the drought intensity experienced (Fig. 1).

Plant responses resulting in resistance under moderate drought through the maintenance of aerial growth have to avoid and/or tolerate leaf dehydration. Conversely, plant responses resulting in survival under severe drought are mainly associated with both dehydration avoidance and tolerance occurring in meristematic tissues. In some species and genotypes, summer dormancy is another combination of strategies which confers efficient survival of meristematic tissues through the dehydration avoidance and tolerance of these organs (Volaire and Norton, 2006). Making the distinction between the responses of mature and young tissues seems crucial to analyse properly the strategies of perennial grasses to contrasting drought intensities.

Meristematic tissues can tolerate greater dehydration than mature tissues in grasses

In both annual and perennial grasses, stress responses of growing tissues differ substantially from those of expanded mature tissues (Riazi et al., 1985), since basal meristems have been shown to survive more intense water deficits than older tissues (Barlow et al., 1980; Munns et al., 1979; West et al., 1990). Apices are protected from rapid evaporative water loss by their location enclosed within the mature leaf sheaths (Barlow et al., 1980). In addition they are strong sinks within the plants and remain so throughout severe stress resulting in carbohydrate accumulation (Schnyder and Nelson, 1989; Volaire et al., 1998a). It is indeed in this most actively growing region of the leaves that the synthesis of fructans and sucrose is the most rapid (Schnyder et al., 1988; Spollen and Nelson, 1994). Basal tissues also exhibit the greatest osmotic adjustment relative to other tissues during drought (Matsuda and Riazi, 1981; Munns et al., 1979; West et al., 1990). Therefore, basal meristems of grasses can often still regenerate when the adult blades are dead (Van Peer et al., 2004).

Drought resistance: dehydration avoidance and tolerance of lamina

Traits associated with drought resistance have been much explored and documented in annual grasses particularly in the major cultivated cereals (Ludlow and Muchow, 1990; Passioura, 1996; Passioura, 2006; Sinclair, 2011). Although cereals are grown for grain production, a number of identified traits are also relevant for those perennial grasses which are grown for forage (White & Snow, 2012). Water-use efficiency (WUE) is an integrative variable to measure the aptitude of a plant to grow and produce under moderate water deficit (White and Snow, 2012). The enhancement of biomass production under moisture limiting conditions can be achieved

primarily by dehydration avoidance through maximising soil water capture while diverting the largest part of the available soil moisture towards stomatal transpiration (Blum, 2009). A deep root system with a high density of roots at depth is a major trait to sustain higher yield in water-limited environments (Carrow, 1996; Wasson et al., 2012; White and Snow, 2012). Maintenance of leaf area, leaf relative water content and photosynthetic capacity are associated with dehydration tolerance of lamina which is based on maintenance of turgor in these tissues by osmotic adjustment (Morgan, 1988; Serraj and Sinclair, 2002).

Drought survival: dehydration avoidance and tolerance of meristems

Traits associated with drought survival have been much less explored (Volaire et al., 2009) although recent efforts to explore the physiological mechanisms of drought-induced mortality have been made in trees and show the relative importance of carbon starvation and hydraulic failure in these model plants (McDowell, 2011; Sala et al., 2010). In perennial grasses, drought survival should be measured after rehydration following the drought period, using values such as the percentage of plant survival and the recovery biomass in the subsequent seasons (Milbau et al., 2005; Poirier et al., 2012; Volaire et al., 1998b). As plant meristems (including root apices) are the key surviving organs, adaptive responses contribute to the dehydration avoidance of theses tissues, through the crucial maintenance of a minimum water supply (Karcher et al., 2008; McWilliam and Kramer, 1968) even at low water potential (Volaire and Lelievre, 2001). Dehydration avoidance is also mediated through leaf senescence and leaf shedding which play a major role in reducing total plant water losses and therefore in the survival of many species especially under drought (Gepstein, 2004; Munne Bosch and Alegre, 2004). Dehydration tolerance of meristems has been shown to be potentially very efficient in adapted genotypes since high concentrations of fructans and dehydrins contribute to osmoregulation and membrane stabilisation of these tissues (Hincha et al., 2000; Hincha et al., 2002). Indeed, high carbohydrate reserves are associated with superior plant resilience and recovery after severe drought, with a strong interaction with defoliation intensity (Boschma et al., 2003). In addition, nitrogen uptake ability was associated with greater survival and productivity stability after intense drought (Poirier et al., 2012; Zwicke et al., 2013).

Summer dormancy induces (even under irrigation) the cessation or reduction of aerial growth, various degrees of foliage senescence and a dehydration tolerance of meristems that remain viable under very severe water deficits (Volaire and Norton, 2006). It is worth underlining again that the interpretation of drought resistance versus drought survival should rest on the responses of the right organs. To interpret low leaf water potential or high foliage

senescence as responses associated with drought sensitivity and poor adaptation may be correct if drought resistance is targeted but highly misleading if drought survival is the focus. Genotypes that survive best can reach very low leaf water potentials such as -5 to -7 MPa in the remaining meristems (Norton et al., 2006; Poirier et al., 2012) even though their foliage senescence is almost complete under severe drought. The measurements of dehydration tolerance must therefore be focused on the correct organs i.e. the meristems.

Methodological aspects in the measurement of plant responses and strategies

In order to understand the balance between the different strategies and their values to plants, it is essential to measure both environmental stress and the plants' responses under experimental droughts which should be similar in intensity to the stresses that occur naturally (Bray, 1993; Jones, 2007). Moreover, it is crucial to ensure that the 'stress' treatments are truly comparable between tested genotypes. In particular, differences in water use due to differences in leaf area or root depth can strongly interfere with drought resistance/survival (Jones, 2007). It is worth stressing that the interaction with plant size complicates the interpretation of performance differences between genotypes since the depletion of soil water is a function of leaf area and total transpiration and not necessarily of any physiological or morphological acclimation (Poorter et al., 2012a). To avoid these confounding effects, the best approach should be to model the responses of each genotype as a function of its specific kinetics of soil available water and to avoid using a time scale (such as number of days) as these are mostly irrelevant when comparing contrasting genotypes under progressive drought. To disentangle the different strategies, we believe that the experimental conditions should be chosen carefully. The analysis of dehydration delay should be carried out in conditions allowing the full expression of root length and density such as long tubes (to measure root traits and water uptake in individual plants) or deep soils (to measure water uptake in dense swards). Conversely, dehydration tolerance should be tested in short pots to eliminate the effect of differences of root depth (the largest component of dehydration delay) on water availability (Pérez-Ramos et al., 2013; Volaire and Lelievre, 2001). Dehydration tolerance can be tested by measuring plant survival after rehydration once a pre-determined soil moisture is reached by all genotypes (Pérez-Ramos et al., 2013) or after successive rehydration with calculation of the soil moisture associated with 50% plant mortality (Volaire et al., 2005). The measurement of summer dormancy is based on scoring plant growth (1) under summer irrigation or (2) regrowth after a mid-summer storm (Oram, 1983), under the correct inductive day-length and temperatures and after vernalisation during the previous winter

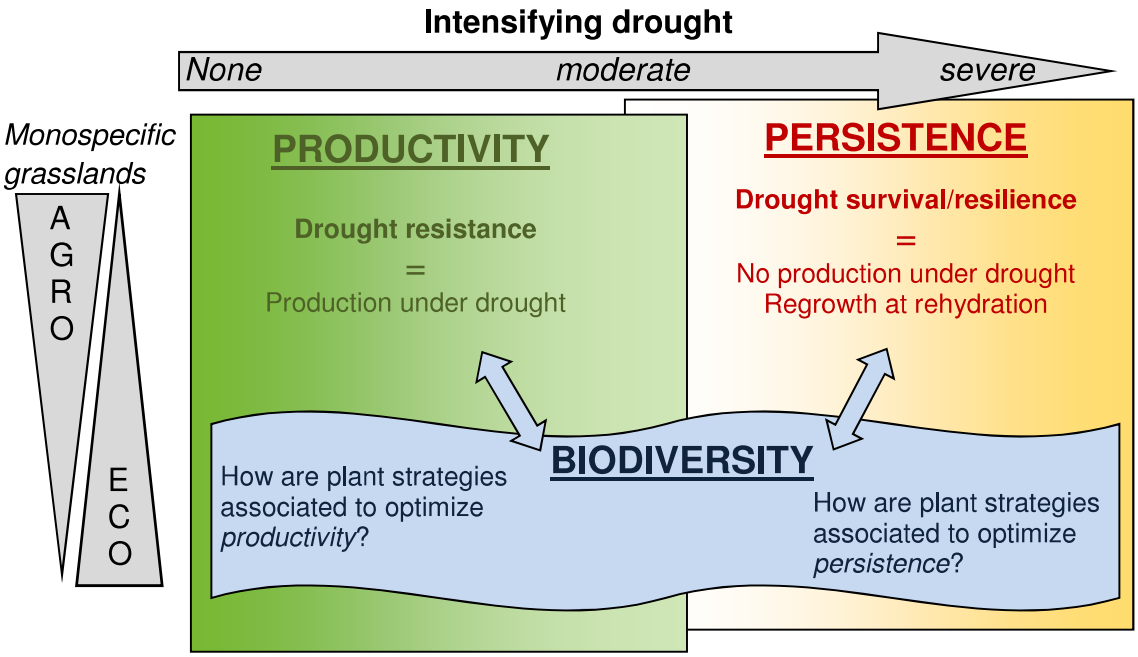


Fig. 2 The main focus of agro-ecology to design sustainable mono- to multi-specific grasslands with targeted functions (productivity, biodiversity and perenniality) under intensifying droughts.

(Norton et al., 2008; Ofir and Kerem, 1982). Using the right methodologies, a functional typology of plant material according to their adaptive strategies can be defined to predict plant responses under a range of potential drought intensities (Volaire et al., 2009).

How to combine plant strategies to develop resilient forage mixtures under drought?

The contribution of community ecology for the design of multi-species grasslands

Establishing persistent and multi-specific grasslands should become a major goal to ensure ecosystem stability in order to enhance the sustainability of agricultural production and ecosystem services. Ecosystem stability is a multi-faceted concept and it is associated in particular with both *ecosystem resistance*, which refers to the maintenance of productivity despite changes in the environment, and *ecosystem resilience*, which refers to the recovery of ecosystem functions and productivity after a temporal alteration due to changes in the environment. While grassland resistance and productivity could be reasonably expected under moderate levels of environmental stress, persistence and resilience of multi-annual productivity is a target for grasslands under severe or extreme levels of environmental stress (Fig. 2).

In natural grassland ecosystems, evolution has led to diverse plant adaptive strategies combining different functions at the community level. Following the description stage of these strategies using relevant plant traits as suggested above, agro-ecology then seeks to define how they should be associated together in order to optimize the ecosystem services provided by multi-specific grasslands. In the following section, concepts of plant community ecology underlying how species assemble in natural grasslands are assumed to provide a suitable framework to define relevant associations of forage cultivars with the most efficient biotic interactions under stressful environments.

Expected effect of biodiversity on ecosystem functioning

Biodiversity, whether at the level of genotypes, species, or communities strongly affects ecosystem functioning over time (Fridley, 2001; Hooper et al., 2005). Importantly for the conception of forage mixtures, a positive relationship between species richness and productivity is expected through plant 'overyielding', *i.e* greater plant biomass production in species mixtures compared with monocultures (Vandermeer, 1989). However empirical results obtained for sown and natural grasslands are still controversial since some studies show strong positive effects of species diversity on productivity (Hector et al.,1999; Tilman et al., 1997; Tilman et al. 2001)

whereas others reach opposite conclusions (Garnier et al., 1997; Huston, 1997). Nonetheless, a consensual view is that the increase in productivity associated with intercropping results not from the total number of species, but from the unique role of a few dominant species with adaptive value of traits and specific properties (Garnier et al., 2004; Mokany et al., 2008).

Moreover, biodiversity should also act as a safeguard of ecosystem functioning, leading to more stabilized ecosystem functions in response to environmental fluctuations when species diversity increases (Campbell et al., 2011; Grime, 1997; Ives and Carpenter, 2007). It was shown that the larger the number of species in a plant community but with a range of environmental sensitivities, the greater the probability that at least some of these species will survive changes in the environment and maintain the functions of the ecosystem (Diaz and Cabido, 2001). Especially for water-limited grasslands, primary productivity in more diverse communities is expected to be more resistant to, and recover more fully from a major drought (Grime, 1997; Tilman and Downing, 1994; van Ruijven and Berendse, 2010).

The positive role of species diversity in ecosystem functioning is driven by the following mechanisms. Firstly, species *niche differentiation* leads to different levels of *functional complementarity* among plants, reducing plant competition compared to that in a monoculture. Secondly, positive interactions or *facilitation,* occurs when one species enhances the plant performance of another because of beneficial effects on local resource availability (Callaway, 1995). Mechanisms related to the properties of dominant species and those related to species diversity may be involved simultaneously even though their relative importance is unclear (Huston, 1997). Properties of dominant species may have an overall effect of leading ecosystem functioning over short-term periods (Grime, 1998), while functional complementarity and/or facilitation may enhance the persistence of ecosystem functions over the long-term, buffering the overall environmental fluctuations (Allan et al., 2011).

Minimizing plant competition: searching for high functional complementarity among species

Interspecific competition is a key process in multi-specific communities that needs to be limited since it may alter plant performance and ecosystem functions. According to several ecological models of plant coexistence such as the *limiting similarity hypothesis* (Chesson, 2000; Macarthur and Levins, 1967), this is achieved by enhancing *functional complementarity* for resource use among plants through the association of species with different functional strategies reflecting their resource economy and plant biomass investment (Stubbs and Wilson, 2004). The higher the functional complementarity, the less the competition among plants.

Temporal and spatial partitioning of resources (Hooper, 1998) are major factors that structure the coexistence of plant species, as was recently demonstrated in Mediterranean communities (Penuelas et al., 2011). Using a trait-based approach to plant competition (Navas and Violle, 2009), functional complementarity among species can be comprehended in terms of differences between relevant plant traits related to resource acquisition/conservation and plant biomass allocation, including plant architectural or phenological traits (Weiher et al., 2011). For example, contrasting plant height and aboveground bio-volume, which reflects growth potential, as well as *specific leaf area* that reflects light acquisition (Poorter et al., 2012b), have been associated with different levels of light requirement (Gross et al., 2007; Mason et al., 2013; Violle et al., 2009). Likewise, contrasting species rooting depth potential and fine root distribution have been argued to partition belowground available resources (Verheyen et al., 2008; Yang et al., 2011). Furthermore, contrasting flowering date, a relevant marker of plant biomass allocation establishment and therefore of resource use dynamics, may segregate the periods during which resources are required by the different species (Catorci et al., 2012). Moreover, asynchrony in demographic processes within species-rich communities has been suggested as a major mechanism guaranteeing ecosystem stability through compensation effects among species over time (Hector et al., 2010).

However, many, if not most of the plant competition studies have been conducted for resources such as light or soil nutrients. Relevant traits related to soil water use have not yet been precisely identified and tested. Associating species with different rooting depth and contrasting adaptive strategies to drought (resistance, avoidance and survival) should be a high research priority given the predicted changes of future climates.

The dream of facilitation-different species helping one another

Under facilitation, plant performance may be enhanced by neighboring plants through their favorable modification of the local environment. Facilitation is distinct from functional complementarity since it involves underlying mechanisms other than species niche differentiation (Brooker et al., 2008; Bruno et al., 2003). Facilitation (Callaway, 1995) results from a favorable alteration of resource availability (e.g. soil nutrients, soil water, light), protection from wind and stressful temperatures, or a beneficial modification of biotic interactions with other species or trophic levels (e.g. herbivores, pollinisators, mycorrhizae).

A well-known facilitative mechanism among plants is the nutrient enrichment by legumes which has been a major functional mechanism addressed in grassland biodiversity-productivity experiments (Mulder et al., 2002; Spehn et al., 2002). In natural communities, evidence for

facilitation also came from high altitude areas or from deserts, where large nurse plant species often enhance the establishment of seedlings and plant growth of other species by alleviating thermal and water stresses (Butterfield et al., 2010; Schob et al., 2012).

While a combination of plant traits has been identified for the ability to form a symbiotic association with nitrogen-fixing bacteria and therefore to enhance nutrient enrichment, specificplant traits are not clear for other facilitation mechanisms such as the "nurse" effect. For the water resource, water sharing via hydraulic lift has been identified as a promising facilitative mechanism for grass-shrub mixtures (Caldwell et al., 1998; Prieto et al., 2012) but remains to be demonstrated for grasslands. Moreover, the relevancy of such positive interaction in the conception of water-limited agricultural system remains questionable since facilitation may dramatically decrease with increasing aridity (Kefi et al., 2007; Rietkerk et al., 2004).

Community assembly rules matter for persistence of grasslands!

The conception of persistent forage mixtures should be supported by the identification of *rules governing community assembly* (Grime, 2006; Keddy, 1992; Weiher et al., 2011). An important point is the strong interdependency of local environmental conditions and plant interaction processes (Brooker and Kikividze, 2008; Spasojevic and Suding, 2012). Given the major role played by abiotic factors in regulating ecosystem functioning (Ciais et al., 2005; Huston, 2012), mechanisms underlying plant interaction processes, and therefore linking species diversity to ecosystem functioning, strongly depend on the environment (Hooper et al., 2005). For instance, the outcome of plant interaction, *i.e* the net result of competition and facilitation processes, varies along environmental gradients, with facilitation becoming more important in more stressful habitats. Hence, if facilitation among species is a potential means by which plant performance may be enhanced, it should be most apparent under severe stress or in resource-poor environments (Armas et al., 2011; Callaway et al., 2002; Maestre et al., 2009). Likewise, functional complementarity should occur more commonly under moderate environmental stress or in resource-rich environments where competition is important and where resource partitioning is possible (Gross et al., 2007; Weiher and Keddy, 1995). Thus, management of plant interactions in promoting sward species coexistence across a range of environments must consider the co-variation of the effects of biotic interactions and abiotic factors on plant performance. Consequently, no ideal pattern of plant diversity can be defined.

To face an increasing frequency of droughts, the challenge of grassland agro-ecology is to design and test a range of forage mixtures based on sound community assembly rules for various types of environments where the short-term productivity and long term persistence are

targeted (Fig. 2). Further research is clearly needed, in particular in environments subjected to increasingly frequent drought and heat waves, to address the following key-questions: (1) to which extent are the assembly rules and biotic interactions identified in natural communities with low productivity, valid for the conception of multi-specific grasslands based on cultivars selected for their high productivity in monocultures and therefore with high competitiveness for resources? (2) How to ensure the long-term performance of a community by combining high functional complementarity (in space and/or time) with species or genotypes of contrasting strategies exhibiting various trade-offs between growth and stress resistance/survival (drought escape, avoidance, dormancy…)? Both experimental and modeling research is needed and should aim to define the most efficient assembly rules of plant material for various types of environments.

Conclusions

To cope with the increase of drought occurrence impacting forage production worldwide, we point out two main challenges for plant breeding and agro-ecology research.

The first challenge is to design ideotypes of forage plants to ensure long-term sustainable production, either to maintain plant growth under moderate drought as expected in temperate areas or to survive severe drought and recover actively afterwards, as expected in drier areas such as the Mediterranean regions. In this last case, expected to occur at a larger scale, the lack of adapted, commercially available cultivars contrasts starkly with the hundreds of perennial grasses and legume cultivars registered for temperate areas throughout the World (Lelièvre and Volaire, 2009). It is therefore urgently required to select new plant material, incorporating traits associated with improved long-term survival and persistence according to defined levels of drought (Blum, 1996) and then to ensure that sufficient seed of these cultivars is commercially available.

The second challenge is to develop the conception and agronomic practices for forage mixtures which ensure that species diversity would buffer environmental fluctuations and enhance resistance and resilience of grasslands. To this end, a close collaboration between agronomists and plant ecologists should promote the applications of the concepts of community ecology to the design of multi-specific grasslands adapted to present and future environments.

Acknowledgments

This work was funded by the French ANR program O2LA (09-STRA-09) and by the INRA project Climagie (ACCAF).

References

Albert, C.H., Grassein, F., Schurr, F.M., Vieilledent, G., Violle, C., 2011. When and how should intraspecific variability be considered in trait-based plant ecology? Perspect. Plant Ecol. Evol. Syst. 13, 217-225.

Allan, E., Weisser, W., Weigelt, A., Roscher, C., Fischer, M., Hillebrand, H., 2011. More diverse plant communities have higher functioning over time due to turnover in complementary dominant species. Proceedings of the National Academy of Sciences 108, 17034-17039.

Annicchiarico, P., Pecetti, L., Bouzerzour, H., Kallida, R., Khedim, A., Porqueddu, C., Simoes, N.M., Volaire, F., Lelièvre, F., 2011. Adaptation of contrasting cocksfoot plant types to agricultural environments across the Mediterranean basin. Environmental and Experimental Botany 74, 82-89.

Armas, C., Rodriguez-Echeverria, S., Pugnaire, F.I., 2011. A field test of the stress-gradient hypothesis along an aridity gradient. J. Veg. Sci. 22, 818-827.

Barlow, E.W.R., Munns, R.E., Brady, D.J., 1980. Drought responses of apical meristems, in: Turner, N.C., Kramer, P.J. (Eds.), Adaptation of Plants to Water and High Temperature Stress. John Wiley & Sons, New York, USA, pp. 191-205.

Bielenberg, D.G., 2011. Knowing when not to grow. New Phytol. 189, 3-5.

Blum, A., 1996. Crop responses to drought and the interpretation of adaptation. Plant Growth Regulation 20, 135-148.

Blum, A., 2009. Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. Field Crop. Res. 112, 119-123.

Boschma, S.P., Hill, M.J., Scott, J.M., Rapp, G.G., 2003. The response to moisture and defoliation stresses, and traits for resilience of perennial grasses on the Northern Tablelands of New South Wales, Australia. Aust. J. Agric. Res. 54, 903-916.

Boyer, J.S., 1982. Plant productivity and environment. Science 218, 443-448.

Bray, E.A., 1993. Molecular responses to water deficit. Plant Physiol. 103, 11-16.

Brooker, R.W., Kikividze, Z., 2008. Importance: an overlooked concept in plant interaction research. J. Ecol. 96, 703-708.

Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.L., Cavieres, L.A., Kunstler, G., Liancourt, P., Tielborger, K., Travis, J.M.J., Anthelme, F., Armas, C., Coll, L., Corcket, E., Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F., Quiroz, C.L., Saccone, P., Schiffers, K., Seifan, M., Touzard, B., Michalet, R., 2008. Facilitation in plant communities: the past, the present, and the future. J. Ecol. 96, 18-34.

Bruno, J.F., Stachowicz, J.J., Bertness, M.D., 2003. Inclusion of facilitation into ecological theory. Trends Ecol. Evol. 18, 119-125.

Butterfield, B.J., Betancourt, J.L., Turner, R.M., Briggs, J.M., 2010. Facilitation drives 65 years of vegetation change in the Sonoran Desert. Ecology 91, 1132-1139.

Caldwell, M.M., Dawson, T.E., Richards, J.H., 1998. Hydraulic lift: Consequences of water efflux from the roots of plants. Oecologia 113, 151-161.

Callaway, R.M., 1995. Positive interactions among plants. Bot. Rev. 61, 306-349.

Callaway, R.M., Brooker, R.W., Choler, P., Kikvidze, Z., Lortie, C.J., Michalet, R., Paolini, L., Pugnaire, F.I., Newingham, B., Aschehoug, E.T., Armas, C., Kikodze, D., Cook, B.J., 2002. Positive interactions among alpine plants increase with stress. Nature 417, 844-848.

Campbell, V., Murphy, G., Romanuk, T.N., 2011. Experimental design and the outcome and interpretation of diversity-stability relations. Oikos 120, 399-408.

Carrow, R.N., 1996. Drought avoidance characteristics of diverse tall fescue cultivars. Crop Science 36, 371-377.

Castonguay, Y., Laberge, S., Brummer, E.C., Volenec, J.J., 2006. Alfalfa winter hardiness: A research retrospective and integrated perspective, in: Sparks, D.L. (Ed.), Advances in Agronomy, Vol 90. Elsevier Academic Press Inc, San Diego, pp. 203-265.

Catorci, A., Cesaretti, S., Gatti, R., Tardella, F.M., 2012. Trait-related flowering patterns in submediterranean mountain meadows. Plant Ecol. 213, 1315-1328.

Chesson, P., 2000. Mechanisms of maintenance of species diversity. Annu. Rev. Ecol. Syst. 31, 343-+.

Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogee, J., Allard, V., Aubinet, M., Buchmann, N., Bernhofer, C., Carrara, A., Chevallier, F., De Noblet, N., Friend, A.D., Friedlingstein, P., Grunwald, T., Heinesch, B., Keronen, P., Knohl, A., Krinner, G., Loustau, D., Manca, G., Matteucci, G., Miglietta, F., Ourcival, J.M., Papale, D., Pilegaard, K., Rambal, S., Seufert, G., Soussana, J.F., Sanz, M.J., Schulze, E.D., Vesala, T., Valentini, R., 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature 437, 529-533.

Diaz, S., Cabido, M., 2001. Vive la difference: plant functional diversity matters to ecosystem processes. Trends Ecol. Evol. 16, 646-655.

FAO, 2008. Water and Cereals in drylands. Food and Agriculture Organization, Sterling, VA, London, p. 113.

Farrant, J.M., Moore, J.P., 2011. Programming desiccation-tolerance: from plants to seeds to resurrection plants. Curr. Opin. Plant Biol. 14, 340-345.

Fridley, J.D., 2001. The influence of species diversity on ecosystem productivity: how, where, and why? Oikos 93, 514-526.

Gaff, D., 1971. Desiccation tolerant flowering plants in Southern Africa. Science 174, 1033-1034.

Garnier, E., Cortez, J., Billes, G., Navas, M.L., Roumet, C., Debussche, M., Laurent, G., Blanchard, A., Aubry, D., Bellmann, A., Neill, C., Toussaint, J.P., 2004. Plant functional markers capture ecosystem properties during secondary succession. Ecology 85, 2630-2637.

Garnier, E., Navas, M.L., Austin, M.P., Lilley, J.M., Gifford, R.M., 1997. A problem for biodiversity-productivity studies: how to compare the productivity of multispecific plant mixtures to that of monocultures? Acta Oecol.-Int. J. Ecol. 18, 657-670.

Gepstein, S., 2004. Leaf senescence - not just a 'wear and tear' phenomenon. Genome Biol. 5.

Grime, J.P., 1997. Ecology - Biodiversity and ecosystem function: The debate deepens. Science 277, 1260-1261.

Grime, J.P., 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. J. Ecol. 86, 902-910.

Grime, J.P., 2006. Trait convergence and trait divergence in herbaceous plant communities: Mechanisms and consequences. J. Veg. Sci. 17, 255-260.

Gross, N., Suding, K.N., Lavorel, S., Roumet, C., 2007. Complementarity as a mechanism of coexistence between functional groups of grasses. J. Ecol. 95, 1296-1305.

Hector, A., Hautier, Y., Saner, P., Wacker, L., Bagchi, R., Joshi, J., Scherer-Lorenzen, M., Spehn, E.M., Bazeley-White, E., Weilenmann, M., Caldeira, M.C., Dimitrakopoulos, P.G., Finn, J.A., Huss-Danell, K., Jumpponen, A., Mulder, C.P.H., Palmborg, C., Pereira, J.S., Siamantziouras, A.S.D., Terry, A.C., Troumbis, A.Y., Schmid, B., Loreau, M., 2010. General stabilizing effects of plant diversity on grassland productivity through population asynchrony and overyielding. Ecology 91, 2213-2220.

Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M.C., Diemer, M., Dimitrakopoulos, P.G., Finn, J.A., Freitas, H., Giller, P.S., Good, J., Harris, R., Hogberg, P., Huss-Danell, K., Joshi, J., Jumpponen, A., Korner, C., Leadley, P.W., Loreau, M., Minns, A., Mulder, C.P.H., O'Donovan, G., Otway, S.J., Pereira, J.S., Prinz, A., Read, D.J., Scherer-Lorenzen, M., Schulze, E.D., Siamantziouras, A.S.D., Spehn, E.M., Terry, A.C., Troumbis, A.Y., Woodward, F.I., Yachi, S., Lawton, J.H., 1999. Plant diversity and productivity experiments in European grasslands. Science 286, 1123-1127.

Hincha, D.K., Hellwege, E.M., Heyer, A.G., Crowe, J.H., 2000. Plant fructans stabilize phosphatidylcholine liposomes during freeze-drying. European Journal of Biochemistry 267, 535-540.

Hincha, D.K., Zuther, E., Hellwege, E.M., Heyer, A.G., 2002. Specific effects of fructo- and gluco-oligosaccharides in the preservation of liposomes during drying. Glycobiology 12, 103-110.

Hooper, D.U., 1998. The role of complementarity and competition in ecosystem responses to variation in plant diversity. Ecology 79, 704-719.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setala, H., Symstad, A.J., Vandermeer, J., Wardle, D.A., 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecol. Monogr. 75, 3-35.

Huston, M.A., 1997. Hidden treatments in ecological experiments: Re-evaluating the ecosystem function of biodiversity. Oecologia 110, 449-460.

Huston, M.A., 2012. Precipitation, soils, NPP, and biodiversity: resurrection of Albrecht's curve. Ecol. Monogr. 82, 277-296.

I.P.C.C., 2007. International Panel of Climatic changes. Fourth assessment report (AR4), New-York.

Ives, A.R., Carpenter, S.R., 2007. Stability and diversity of ecosystems. Science 317, 58-62.

Jones, H.G., 2007. Monitoring plant and soil water status: established and novel methods revisited and their relevance to studies of drought tolerance. J. Exp. Bot. 58, 119-130.

Karcher, D.E., Richardson, M.D., Hignight, K., Rush, D., 2008. Drought tolerance of tall fescue populations selected for high root/shoot ratios and summer survival. Crop Science 48, 771-777.

Keddy, P.A., 1992. Assembly and response rules - two goals for predictive community ecology. J. Veg. Sci. 3, 157-164.

Kefi, S., Rietkerk, M., Alados, C.L., Pueyo, Y., Papanastasis, V.P., ElAich, A., de Ruiter, P.C., 2007. Spatial vegetation patterns and imminent desertification in Mediterranean arid ecosystems. Nature 449, 213-U215.

Kreyling, J., Thiel, D., Simmnacher, K., Willner, E., Jentsch, A., Beierkuhnlein, C., 2012. Geographic origin and past climatic experience influence the response to late spring frost in four common grass species in central Europe. Ecography 35, 268-275.

Lelievre, F., Seddaiu, G., Ledda, L., Porqueddu, C., Volaire, F., 2011. Water use efficiency and drought survival in Mediterranean perennial forage grasses. Field Crop. Res. 121, 333-342.

Lelièvre, F., Volaire, F., 2009. Current and potential development of perennial grasses in rainfed Mediterranean farming systems. Crop Science 49, 2371-2378.

Levitt, J.V., 1972. Responses of Plants to Environmental Stress. Academic Press, New-York.

Ludlow, M.M., 1989. Strategies of response to water stress., in: Kreeb, K.H., Richter, H., Hinckley, T.M. (Eds.), Structural and functional responses to environmental stresses: water shortage. SPB Academic Publishing BV, The Hague, pp. 269-281.

Ludlow, M.M., Muchow, R.C., 1990. A critical evaluation of traits for improving crop yields in water-limited environments. Advances-in-Agronomy. 43, 107-153.

Macarthur, R., Levins, R., 1967. Limiting similarity convergence and divergence of coexisting species. Am. Nat. 101, 377-+.

Maestre, F.T., Callaway, R.M., Valladares, F., Lortie, C.J., 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. J. Ecol. 97, 199-205.

Mason, N.W.H., Pipenbaher, N., Skornik, S., Kaligaric, M., 2013. Does complementarity in leaf phenology and inclination promote co-existence in a species-rich meadow? Evidence from functional groups. J. Veg. Sci. 24, 94-100.

Matsuda, K., Riazi, A., 1981. Stress-induced osmotic adjustment in growing regions of barley leaves. Plant Physiol. 68, 571-576.

May, L.H., Milthorpe, F.L., 1962. Drought resistance in crop plants. Field Crop Abstracts 15, 171-179

McDowell, N.G., 2011. Mechanisms Linking Drought, Hydraulics, Carbon Metabolism, and Vegetation Mortality. Plant Physiol. 155, 1051-1059.

McWilliam, J.R., Kramer, P.J., 1968. The nature of the perennial response in Mediterranean grasses. 1. Water relations and summer survival in *Phalaris*. Australian Journal Agricultural Research 19, 381-395.

Milbau, A., Scheerlinck, L., Reheul, D., De Cauwer, B., Nijs, I., 2005. Ecophysiological and morphological parameters related to survival in grass species exposed to an extreme climatic event. Physiol. Plant. 125, 500-512.

Mokany, K., Ash, J., Roxburgh, S., 2008. Functional identity is more important than diversity in influencing ecosystem processes in a temperate native grassland. J. Ecol. 96, 884-893.

Morgan, J.M., 1988. Physiological traits for drought resistance. Drought resistance in cereals CAB International, Wallingford, UK.

Mulder, C.P.H., Jumpponen, A., Hogberg, P., Huss-Danell, K., 2002. How plant diversity and legumes affect nitrogen dynamics in experimental grassland communities. Oecologia 133, 412-421.

Munne Bosch, S. , Alegre, L., 2004. Die and let live: leaf senescence contributes to plant survival under drought stress. Functional-Plant-Biology. 31, 203-216.

Munns, R., Brady, C.J., Barlow, E.W.R., 1979. Solute accumulation in the apex and leaves of wheat during water-stress. Aust. J. Plant Physiol. 6, 379-389.

Navas, M.L., Violle, C., 2009. Plant traits related to competition: how do they shape the functional diversity of communities? Community Ecol. 10, 131-137.

Norton, M.R., Lelievre, F., Fukai, S., Volaire, F., 2008. Measurement of summer dormancy in temperate perennial pasture grasses. Australian Journal Agricultural Research 59.

Norton, M.R., Lelievre, F., Volaire, F., 2006. Summer dormancy in *Dactylis glomerata* L., the influence of season of sowing and a simulated mid-summer storm on two contrasting cultivars. Aust. J. Agric. Res. 57, 1267-1277.

Norton, M.R., Lelievre, F., Volaire, F., 2012. Summer Dormancy in Phalaris aquatica L., the Influence of Season of Sowing and Summer Moisture Regime on Two Contrasting Cultivars. J. Agron. Crop Sci. 198, 1-13.

Ofir, M., Kerem, D., 1982. The effects of temperature and photoperiod on the onset of summer dormancy in *Poa bulbosa* L. Annals of Botany 50, 259-264.

Olesen, J.E., Carter, T.R., Diaz-Ambrona, C.H., Fronzek, S., Heidmann, T., Hickler, T., Holt, T., Minguez, M.I., Morales, P., Palutikof, J.P., Quemada, M., Ruiz-Ramos, M., Rubaek, G.H., Sau, F., Smith, B., Sykes, M.T., 2007. Uncertainties in projected impacts of climate change on European agriculture and terrestrial ecosystems based on scenarios from regional climate models. Climatic Change 81, 123-143.

Oram, R.N., 1983. Ecotypic differentiation for dormancy levels in oversummering buds of *Phalaris aquatica* L. Botanical Gazette 144, 544-551.

Passioura, J. B, 1996. Drought and drought tolerance. 20, 79-83.

Passioura, J., 2006. Increasing crop productivity when water is scarce - from breeding to field management. Agricultural Water Management 80, 176-196.

Pecetti, L., Annicchiarico, P., Abdelguerfi, A., Kallida, R., Mefti, M., Porqueddu, C., Simoes, N.M., Volaire, F., Lelievre, F., 2011. Response of Mediterranean Tall Fescue Cultivars to Contrasting Agricultural Environments and Implications for Selection. J. Agron. Crop Sci. 197, 12-20.

Penuelas, J., Terradas, J., Lloret, F., 2011. Solving the conundrum of plant species coexistence: water in space and time matters most. New Phytol. 189, 5-8.

Pérez-Ramos, I.M., Volaire, F., Fattet, M., Blanchard, A., Roumet, C., 2013. Tradeoffs between functional strategies for resource-use and drought-survival in Mediterranean rangeland species. Environmental and Experimental Botany 87, 126-136.

Poirier, M., Durand, J.L., Volaire, F., 2012. Persistence and production of perennial grasses under water deficits and extreme temperatures: importance of intraspecific vs. interspecific variability. Global Change Biology 18, 3632-3646.

Poorter, H., Fiorani, F., Stitt, M., Schurr, U., Finck, A., Gibon, Y., Usadel, B., Munns, R., Atkin, O.K., Tardieu, F., Pons, T.L., 2012a. The art of growing plants for experimental purposes: a practical guide for the plant biologist. Funct. Plant Biol. 39, 821-838.

Poorter, H., Niklas, K.J., Reich, P.B., Oleksyn, J., Poot, P., Mommer, L., 2012b. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytol. 193, 30-50.

Prieto, I., Armas, C., Pugnaire, F.I., 2012. Water release through plant roots: new insights into its consequences at the plant and ecosystem level. New Phytol. 193, 830-841.

Riazi, A., Matsuda, K., Arslan, A., 1985. Water-stress induced changes in concentrations of proline and other solutes in growing regions of young barley leaves. J. Exp. Bot. 36, 1716-1725.

Rietkerk, M., Dekker, S.C., de Ruiter, P.C., van de Koppel, J., 2004. Self-organized patchiness and catastrophic shifts in ecosystems. Science 305, 1926-1929.

Sala, A., Piper, F., Hoch, G., 2010. Physiological mechanisms of drought-induced tree mortality are far from being resolved. New Phytol. 186, 274-281.

Schnyder, H., Nelson, C.J., 1989. Growth rates and assimilate partitioning in the elongation zone of tall fescuue leaf blades at high and low irradiance. Plant Physiol. 90, 1201-1206.

Schnyder, H., Nelson, C.J., Spollen, W.G., 1988. Diurnal growth of tall fescue leaf blades. 2. Dry matter partitioning and carbohydrate metabolism in the elongation zone and adjacent expanded tissue. Plant Physiol. 86, 1077-1083.

Schob, C., Butterfield, B.J., Pugnaire, F.I., 2012. Foundation species influence trait-based community assembly. New Phytol. 196, 824-834.

Scott, P., 2000. Resurrection plants and the secrets of eternal leaf. Annals of Botany 85, 159-166.

Serraj, R., Sinclair, T.R., 2002. Osmolyte accumulation: can it really help increase crop yield under drought conditions? Plant Cell Environ. 25, 333-341.

Sinclair, T.R., 2011. Challenges in breeding for yield increase for drought. Trends Plant Sci. 16, 289-293.

Sinclair, T.R., 2012. Is transpiration efficiency a viable plant trait in breeding for crop improvement? Funct. Plant Biol. 39, 359-365.

Spasojevic, M.J., Suding, K.N., 2012. Inferring community assembly mechanisms from functional diversity patterns: the importance of multiple assembly processes. J. Ecol. 100, 652-661.

Spehn, E.M., Scherer-Lorenzen, M., Schmid, B., Hector, A., Caldeira, M.C., Dimitrakopoulos, P.G., Finn, J.A., Jumpponen, A., O'Donnovan, G., Pereira, J.S., Schulze, E.D., Troumbis, A.Y., Korner, C., 2002. The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. Oikos 98, 205-218.

Spollen, W.G., Nelson, C.J., 1994. Response of fructan to water deficit in growing leaves of tall fescue. Plant Physiol. 106, 329-336.

Stubbs, W.J., Wilson, J.B., 2004. Evidence for limiting similarity in a sand dune community. J. Ecol. 92, 557-567.

Supit, I., van Diepen, C.A., de Wit, A.J.W., Kabat, P., Baruth, B., Ludwig, F., 2010. Recent changes in the climatic yield potential of various crops in Europe. Agricultural Systems 103, 683-694.

Tardieu, F., 2012. Any trait or trait-related allele can confer drought tolerance: just design the right drought scenario. J. Exp. Bot. 63, 25-31.

Tilman, D., Downing, J.A., 1994. Biodiversity and Stability in Grasslands. Nature 367, 363-365.

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., Siemann, E., 1997. The influence of functional diversity and composition on ecosystem processes. Science 277, 1300-1302.

Tilman, D., Reich, P.B., Knops, J., Wedin, D., Mielke, T., Lehman, C., 2001. Diversity and productivity in a long-term grassland experiment. Science 294, 843-845.

Tilman, D., Wedin, D., Knops, J., 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 379, 718-720.

Trnka, M., Olesen, J.E., Kersebaum, K.C., Skjelvag, A.O., Eitzinger, J., Seguin, B., Peltonen-Sainio, P., Rotter, R., Iglesias, A., Orlandini, S., Dubrovsky, M., Hlavinka, P., Balek, J., Eckersten, H., Cloppet, E., Calanca, P., Gobin, A., Vucetic, V., Nejedlik, P., Kumar, S., Lalic, B., Mestre, A., Rossi, F., Kozyra, J., Alexandrov, V., Semeradova, D., Zalud, Z., 2011. Agroclimatic conditions in Europe under climate change. Global Change Biology 17, 2298-2318.

Tsakiris, G., Vangelis, H., 2005. Establishing a drought index incorporating evapotranspiration. European Water 9/10, 3-11

Van Peer, L., Nijs, I., Reheul, D., De Cauwer, B., 2004. Species richness and susceptibility to heat and drought extremes in synthesized grassland ecosystems: compositional vs physiological effects. Functional Ecology 18, 769-778.

van Ruijven, J., Berendse, F., 2010. Diversity enhances community recovery, but not resistance, after drought. J. Ecol. 98, 81-86.

Vandermeer, J., 1989. The ecology of intercropping. Cambridge University Press, Cambridge.

Verheyen, K., Bulteel, H., Palmborg, C., Olivie, B., Nijs, I., Raes, D., Muys, B., 2008. Can complementarity in water use help to explain diversity-productivity relationships in experimental grassland plots? Oecologia 156, 351-361.

Vicca, S., Gilgen, A.K., Serrano, M.C., Dreesen, F.E., Dukes, J.S., Estiarte, M., Gray, S.B., Guidolotti, G., Hoeppner, S.S., Leakey, A.D.B., Ogaya, R., Ort, D.R., Ostrogovic, M.Z., Rambal, S., Sardans, J., Schmitt, M., Siebers, M., van der Linden, L., van Straaten, O., Granier, A., 2012. Urgent need for a common metric to make precipitation manipulation experiments comparable. New Phytol. 195, 518-522.

Vicente-Serrano, S.M., Beguería, S., Lorenzo-Lacruz, J., Camarero, J.J., López-Moreno, J.I., Azorin-Molina, C., Revuelto, J., Morán-Tejeda, E., Sanchez-Lorenzo, A., 2012. Performance of Drought Indices for Ecological, Agricultural, and Hydrological Applications. Earth Interactions 16, 1-27.

Violle, C., Enquist, B.J., McGill, B.J., Jiang, L., Albert, C.H., Hulshof, C., Jung, V., Messier, J., 2012. The return of the variance: intraspecific variability in community ecology. Trends Ecol. Evol. 27, 244-252.

Violle, C., Garnier, E., Lecoeur, J., Roumet, C., Podeur, C., Blanchard, A., Navas, M.L., 2009. Competition, traits and resource depletion in plant communities. Oecologia 160, 747-755.

Volaire, F., 1995. Growth, carbohydrate reserves and drought survival strategies of contrasting *Dactylis glomerata* populations in a Mediterranean environment. J. Appl. Ecol. 32, 56-66.

Volaire, F., Lelievre, F., 1997. Production, persistence, and water-soluble carbohydrate accumulation in 21 contrasting populations of *Dactylis glomerata* L. subjected to severe drought in the south of France. Aust. J. Agric. Res. 48, 933-944.

Volaire, F., Lelievre, F., 2001. Drought survival in *Dactylis glomerata* and *Festuca arundinacea* under similar rooting conditions in tubes. Plant & Soil 229, 225-234.

Volaire, F., Norton, M., 2006. Summer dormancy in perennial temperate grasses. Annals of Botany 98, 927-933.

Volaire, F., Norton, M.R., Lelievre, F., 2009. Summer Drought Survival Strategies and Sustainability of Perennial Temperate Forage Grasses in Mediterranean Areas. Crop Science 49, 2386-2392.

Volaire, F., Norton, M.R., Norton, G.M., F., L., 2005. Seasonal patterns of growth, dehydrins and water-soluble carbohydrates in genotypes of *Dactylis glomerata* varying in summer dormancy. Annals of Botany 95, 981-990.

Volaire, F., Thomas, H., Bertagne, N., Bourgeois, E., Gautier, M.F., Lelievre, F., 1998a. Survival and recovery of perennial forage grasses under prolonged Mediterranean drought: water status, solute accumulation, abscisic acid concentration and accumulation of dehydrin transcripts in bases of immature leaves. New Phytol. 140, 451-460.

Volaire, F., Thomas, H., Lelievre, F., 1998b. Survival and recovery of perennial forage grasses under prolonged Mediterranean drought: growth, death, water relations and solute content in herbage and stubble. New Phytol. 140, 439-449.

Wasson, A.P., Richards, R.A., Chatrath, R., Misra, S.C., Prasad, S.V.S., Rebetzke, G.J., Kirkegaard, J.A., Christopher, J., Watt, M., 2012. Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. J. Exp. Bot. 63, 3485-3498.

Weiher, E., Freund, D., Bunton, T., Stefanski, A., Lee, T., Bentivenga, S., 2011. Advances, challenges and a developing synthesis of ecological community assembly theory. Philos. Trans. R. Soc. B-Biol. Sci. 366, 2403-2413.

Weiher, E., Keddy, P.A., 1995. The assembly of experimental wetland plant-communities. Oikos 73, 323-335.

West, C.P., Oosterhuis, D.M., Wullschleger, S.D., 1990. Osmotic adjustment in tissues of tall fescue in response to water deficit. Environmental and Experimental Botany 30, 149-156.

White, T.A., Snow, V.O., 2012. A modelling analysis to identify plant traits for enhanced water-use efficiency of pasture. Crop Pasture Sci. 63, 63-76.

Yang, H., Auerswald, K., Bai, Y.F., Han, X.G., 2011. Complementarity in water sources among dominant species in typical steppe ecosystems of Inner Mongolia, China. Plant Soil 340, 303-313.

Zwicke, M., Alessio, G.A., Thiery, L., Falcimagne, R., Baumont, R., Rossignol, N., Soussana, J.-F., Picon-Cochard, C., 2013. Lasting effects of climate disturbance on perennial grassland above-ground biomass production under two cutting frequencies. Global Change Biology, 19, 3435-3448.