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Amendement de composts dans un écosystème méditerranéen après incendie: effets sur le sol, les micro-organismes et la végétation.

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Chapitre I - Introduction

De l'utilisation du compost post incendie dans les écosystèmes méditerranéens

La région méditerranéenne : une région particulièrement sensible aux incendies

Le climat méditerranéen est caractérisé par l'existence d'une période sèche plus ou moins longue se superposant à la saison chaude (Bagnouls et Gaussen, 1957), La sécheresse estivale ainsi que la présence d'une végétation souvent dominée par des espèces pyrophytiques facilement inflammables conduisent au développement d'incendies forestiers parfois attisés par les vents violents tels la tramontane en Catalogne et en Italie et le mistral qui descend la vallée du Rhône (Scarascia-Mugnozza *et al*., 2000; De Luis *et al*., 2001). De plus, l'abandon des terres agricoles depuis les années 1970 ainsi que l'augmentation de la population et des activités humaines ont entraîné une forte augmentation du nombre et de la taille des feux dans toutes les régions méditerranéennes (Pausas, 2004).

En France durant l'année 2010, 10.300 ha ont été touchés par des feux de forêt dont 6.180 ha en région méditerranéenne soit environ 65% (Direction de la Sécurité Civile, 2010). Sur les dix dernières années, seuls 9% des feux de forêts sont d'origine naturelle. La part la plus importante des incendies (52%) est due à des accidents (27% par des particuliers et 15% lors de travaux d'entretien agricole ou forestier) ou est d'origine criminelle (39%; Direction de la Sécurité Civile, 2011). Depuis 1987, le Conservatoire de la forêt méditerranéenne a mis en place des actions de prévention ainsi que des plans de protection des forêts contre les incendies (article L.321-6 du code forestier) dont celui du 2 février 1995 qui soumet tous les risques naturels à une procédure unique : les plans de prévention des risques naturels (PPR). Ces PPR définissent des interdictions ou des conditions de construction par les collectivités publiques et les particuliers dans les zones à risque (article L.562 du code de l'environnement). De plus, la loi n°2001-602 du 9 juillet 2001 ajoute la prévention des incendies dans le code forestier grâce à des dispositions permettant la prévention des feux et limitant leur propagation. Depuis la mise en place de ces mesures, le nombre de départs d'incendie ainsi que la surface impliquée ont diminué significativement, à l'exception de l'année 2003 qui a présenté des conditions météorologiques fortement défavorables. Ainsi, en 2010 sur les départements méditerranéens, la surface impactée représentait 40% de la moyenne des dix derniers étés et le nombre de feux 65% (Direction de la Sécurité Civile, 2011).

Effet des incendies sur les écosystèmes méditerranéens

Les feux de forêts sont considérés comme la perturbation principale sur les écosystèmes méditerranéens (Whelan, 1995), notamment sur la rive nord de la Méditerranée, la rive sud étant encore l'objet de dégradations anthropiques importantes. Ces effets, affectant divers compartiments de l'écosystème que ce soit la végétation, le sol ou les organismes, sont décalés dans le temps. Dans un premier temps, les incendies induisent une destruction partielle ou totale du couvert végétal et de la couche superficielle de matière organique du sol (Hernández *et al.*, 1997; Guerrero *et al.*, 2001). Il en résulte notamment une baisse de la protection du sol conduisant à une stabilité moindre et, par conséquent, une vulnérabilité accrue au risque d'érosion (Kutiel et Inbar, 1993; Hart *et al.*, 2005). L'impact immédiat des incendies sur les paramètres biologiques, chimiques et physiques du sol est fonction de la fréquence, la durée et l'intensité : plus ils sont fréquents et de forte intensité, plus les effets sont importants (Boerner, 1982). Cependant, il est établi que, juste après le passage du feu, une augmentation de la disponibilité des éléments minéraux est observée pour les végétaux principalement par le dépôt de cendres et par la libération de minéraux du sol (Kutiel et Inbar, 1993; Dumontet *et al.*, 1996). Mais, durant les incendies, des pertes de ces éléments sont aussi constatées, causées par leur oxydation et, dans une moindre mesure, leur volatilisation (Fisher et

Binkley, 2000). Outre ces pertes faibles et conditionnées par les propriétés de l'incendie, des pertes plus importante sont causées par l'érosion ultérieure qu'elle soit éolienne ou hydrique par ruissellement lors des pluies (DeBano et Conrad, 1978; Boener, 1982). En effet, la combinaison de vents violents et de forts orages fréquents durant la période estivale en région méditerranéenne ainsi que des reliefs souvent accidentés augmentent le risque d'érosion lié aux feux de forêt (De Luis *et al.*, 2001). De fait, plus ces événements climatiques se produisent rapidement après l'incendie et plus leur effet est prononcé (Thomas *et al.*, 1999; Gonzalez-Pérez *et al.*, 2004). En ce qui concerne le stock d'éléments nutritifs d'autres paramètres entrent en compte comme l'historique du site en matière d'incendies ou la gestion *via* l'enlèvement des troncs brûlés (Johnson *et al.*, 2005).

Outre la perte de nutriments, les feux de forêt peuvent aussi induire une augmentation de l'hydrophobicité du sol. Ceci peut être dû à l'assèchement de la couche superficielle du sol (DeBano, 2000) ou à la production de composés hydrophobes suite à la dégradation de la matière organique (Varela *et al.*, 2005; DeBano, 2000). La synthèse de ces composés est liée aux caractéristiques du site et de l'incendie. D'une part, les espèces végétales présentes déterminent le type de composants libérés lors de leur dégradation (Doerr *et al.*, 1998; Mataix-Solera et Doerr, 2004). D'autre part, comme l'ont montré Doerr *et al.* (2004) ou Varela *et al.* (2005), la durée d'exposition du sol au feu mais aussi son intensité peuvent faire varier l'hydrophobicité résultante.

En plus de ces modifications physiques et chimiques, les incendies de forêt perturbent les propriétés biologiques du sol qui sont plus sensibles à la hausse de température (DeBano *et al.*, 1998). Celle-ci entraîne la lyse des cellules et la dégradation des capacités de reproduction des microorganismes (Covington et DeBano, 1990). Ainsi, suite aux incendies, on observe une diminution de la biomasse microbienne (Dumontet *et al.*, 1996; Prieto-Fernandez *et al.* 1998; Smith *et al.*, 2008), de la diversité des microorganismes (Díaz-Fierros *et al.*, 1990; Vásquez *et al.*, 1993; Smith *et al.*, 2008) ainsi que des activités enzymatiques (Hernández *et al.*, 1997). La communauté fongique, plus sensible, est plus affectée que les bactéries (Dunn et DeBano, 1977; Bååth *et al.*, 1995). Ces effets sont principalement observés dans la couche superficielle du sol où les microorganismes sont plus abondants (Neary *et al.*, 1999).

La végétation méditerranéenne

La végétation méditerranéenne est caractérisée par la dominance d'espèces sclérophylles dont les feuilles sont lignifiées, pour limiter les pertes en eau, et sempervirentes, permettant de réaliser la photosynthèse hors de la période estivale (Rundel, 1988; Aerts, 1995; Archibold, 1995). De plus, elles favorisent le développement de tissus pérennes et peu concentrés en nutriments (tiges et racines) ce qui, associé à la persistance de leurs feuilles, limite la restitution des éléments nutritifs au sol (Rundel, 1988; Aerts, 1995). Ceci entraîne la persistance et l'extension des communautés végétales les plus oligotrophes, principalement suite à des incendies répétés qui causent des effets cumulés à long terme négatifs sur la fertilité du sol (Eugenio et al., 2006). La recolonisation végétale devient de plus en plus difficile lorsque la fréquence des incendies augmente ce qui entraîne le développement d'un couvert végétal morcelé et l'accélération de la dégradation du sol mais induit le développement de populations d'herbacées riches en espèces (Keeley et Keeley, 1981). Les espèces herbacées, telles les poacées comme *Brachypodium retusum* peuvent être, par exemple, favorisés dans les premiers stades de recolonisation, ce qui explique d'ailleurs les pratiques d'incendie volontaire qui conduisent à l'apparition d'une végétation appétante pour les troupeaux.

En Provence calcaire, les sols les plus souvent incendiés et dégradés sont colonisés par des garrigues à *Quercus coccifera* L. (Trabaud, 1987; Barbero, 1990). Suite aux perturbations, les communautés végétales se ré-établissent selon deux stratégies reproductives différentes : soit par rejet de souche (Baeza *et al.*, 2003) mais aussi par la germination de la banque de graines (Rundel, 1988; Lloret et Vilà, 1997; Calvo *et al.*, 2002). Elles sont toutes deux dépendantes des incendies (Le Houerou, 1973). Le rejet de souche a lieu rapidement après l'incendie par développement de nouvelles tiges après que les parties aériennes des plantes ont brûlé. Quant à la germination de banque de graines, elle est permise grâce à la levée de dormance ainsi que la destruction de composés phénoliques inhibiteurs de la germination du sol par la chaleur (Christensen et Muller, 1975; Keeley et Keeley, 1989). Durant les premières étapes, la recolonisation post-incendie est souvent contrôlée par les espèces rejetant de souches qui perdent ensuite leur dominance en faveur de celles germant à partir de graines (Bellingham and Sparrow, 2000; Sparrow et Bellingham, 2001).

Le compost

Production et gestion des déchets en France.

En France, la production de déchets est de plus en plus importante (868 Mt en 2006). La part d'ordure ménagère a ainsi doublée en quarante ans même si on observe une stabilisation de la production annuelle à 354 kg par habitant depuis 2002 (ADEME, 2009). Est ainsi posé de manière récurrente la question de leur gestion.

En 2007, le traitement des déchets se répartissait en France pour 33,8% par recyclage (valorisation et gestion biologique), 30,6% par incinération dont 29,2% avec valorisation énergétique, et 35,6% par stockage (ADEME, 2009). La politique actuelle, que ce soit à l'échelle française ou européenne, vise aux orientations suivantes (Ministère de l'Ecologie, de l'Energie, du Développement Durable et de la Mer, 2009) :

- diminution de la production d'ordures ménagères par habitants de 7% pour les 5 prochaines années;
- augmentation du recyclage des déchets ménagers à 35% en 2012 et 45% en 2015;
- réduction de 15% des déchets incinérés et stockés.

Les déchets organiques peuvent être valorisés par le compostage. En 2006, 5,2 Mt de déchets ménagers (13.7%) ont été traitées par cette filière et ont permis la production de 1,8 Mt de compost (ITOM 2006) issus de déchets verts, boues de station d'épuration ou ordures ménagères résiduelles. Depuis 2002, la part de traitement par compostage est passé de 9% à 13,7% en 2006 (soit plus de 50% d'augmentation; ITOM 2002, 2006). Au cours du compostage, la dégradation des matériaux fermentescibles et la synthèse de composés humifiés conduisent à la formation d'un produit organique stabilisé et hygiénisé par l'action de microorganismes (Leclerc, 2001). La part de déchets traitée par cette voie pourrait représenter environ 50% des ordures ménagères résiduelles alors que seulement 13,7% le sont actuellement (Ministère EEDDM 2009).

La gestion des déchets urbains par cette voie permet d'obtenir quatre principaux types de compost :

- les composts de déchets verts obtenus à partir des résidus d'élagage, de tonte, et d'entretien des jardins publics ou privés;
- les composts de biodéchets obtenus à partir des résidus fermentescibles des ordures ménagères collectés sélectivement ou à partir des déchets de Marché d'Intérêts Nationaux (M.I.N). En pratique, le compostage de biodéchets requiert l'incorporation de déchets verts structurants en début de procédé;
- les composts d'ordures ménagères résiduelles obtenus à partir de la fraction résiduelle des ordures ménagères après collecte sélective des emballages propres et secs;
- les composts de boues de station d'épuration obtenus à partir des boues de station d'épuration mélangées à des co-substrats servant de structurants.

Sur les plates-formes industrielles, diverses techniques de tri et de broyage sont réalisées pour améliorer la qualité des composts. La phase active de dégradation peut être accélérée par l'utilisation de systèmes d'aération forcée. Les composts obtenus sont alors mis sur le marché en tant que produit s'ils répondent aux critères des normes NFU 44-095 (AFNOR, 2002) pour les composts de boues et NFU 44-051 (AFNOR, 2006) pour les autres composts. Ces normes fixent notamment des valeurs limites sur les teneurs en azote, éléments traces métalliques (ETM), composé traces organiques (CTO) et germes pathogènes, assurant la qualité sanitaire et environnementale des produits.

Il existe un label européen de qualité (Ecolabel) qui possède des seuils plus sévères.

Utilisations des composts.

Actuellement, la principale utilisation des composts est faite en agriculture (Tableau 1) pour leur valeur amendante.

Tableau 1. Utilisation des composts en France en (ADEME, 2002).

Grandes cultures	69%
Cultures	13,5%
Paysage -	7,9%
Formulateurs	3,1%
Réaménagement	6,4%
Particuliers	0,1%

Il existe peu de travaux sur l'utilisation de composts en milieu naturel (Albaladejo *et al.*, 1994; Borken *et al.*, 2002; Caravaca *et al.*, 2003). Parmi ceux-ci, seuls quelques-uns concernent les sols brûlés et montrent un effet bénéfique sur la restauration des sols et la régénération de la végétation suite à ces apports (Guerrero *et al.*, 2000; Caravaca *et al.*, 2003; Martinez *et al.*, 2003b; Larchevêque *et al.*, 2006a; Walter *et al.*, 2006; Kowaljow and Mazzarino., 2007; Hemmat *et al.*, 2010; Turrión *et al.*, 2012). Les composts riches en matière organique humifiée peuvent être utilisés comme source de nutriments à libération lente (Barker, 1997). L'amendement de compost améliore les propriétés physiques, chimiques et biologiques du sol notamment en augmentant les nutriments disponibles dans le compartiment organique du sol (Bodet et Carioli, 2001; Chenu, 2002; Larchevêque *et al.*, 2006a; Annabi *et al.*, 2007). L'apport de compost permet aussi une augmentation de la rétention en eau due à deux facteurs : une augmentation de la porosité (Giusquiani *et al.*, 1995) et une capacité de rétention plus grande des composts par rapport au sol (Villar *et al.*, 1998; Serra-Wittling, 1995). Cette hausse ainsi que celle en nutriments permettent un meilleur développement des populations microbiennes et des racines des plantes (Díaz *et al.*, 1994; Borken *et al.*, 2002; Kowaljow et Mazzarino, 2007).

Une augmentation de la stabilité des agrégats est souvent observée (Guerrero *et al.*, 2000; Caravaca *et al.*, 2003; Roldán *et al.*, 2006). Tout d'abord elle peut être liée à la synthèse de substances humiques à fort pouvoir agrégatif (Gerzabek *et al.*, 1995; Albiach *et al.*, 2001). Elle peut aussi provenir d'une diminution de la mouillabilité des agrégats induite par des lipides, ce qui ralentit la vitesse de pénétration de l'eau et réduit le risque d'éclatement pendant l'humidification (Guidi *et al.*, 1983; Paré *et al.*, 1999; Chenu *et al.*, 2000). Les microorganismes jouent un rôle important dans l'agrégation du sol (Oades, 1993; Annabi, 2007; Villar *et al.*, 1998) soit de façon mécanique principalement grâce aux hyphes (Metzger *et al.* 1987; Kinsbursky *et al.*, 1989; Degens *et al.*, 1996) mais aussi chimique via les sécrétions extracellulaires des champignons et des bactéries (Robert et Chenu, 1992; Abiven *et al.*, 2007).

L'apport de compost améliore aussi la nutrition et la croissance des plantes (Villar *et al.*, 1998; Guerrero *et al.*, 2000, 2001; Caravaca *et al.*, 2003; Larchevêque *et al.*, 2005). En favorisant le développement des végétaux, l'amendement peut aussi en affecter la richesse et la diversité (Madejón *et al.*, 2006; Walter *et al.*, 2006; Walter et Calvo, 2009). L'augmentation de la teneur en nutriments peut ainsi entraîner l'exclusion des espèces les moins compétitives dans ces nouvelles conditions environnementales et diminuer la richesse (Martinez *et al.*, 2003b; Moreno-Peñaranda *et al.*, 2004). Les interactions entre plantes peuvent donc être modifiées et, par conséquent, la composition de la communauté végétale (Tilman, 1984).

Néanmoins, l'utilisation de composts urbains pour la régénération de sites brûlés peut amener à se poser des questions vis-à-vis de certains paramètres. Tout d'abord il est nécessaire de suivre l'évolution des teneurs en phosphore et en azote du sol, car ils pourraient entraîner par percolation ou ruissellement l'eutrophisation du milieu terrestre et aquatique. Il existe aussi un risque de pollution par les métaux lourds (Cd, Cr, Pb, Zn) (Toribio et Romanyà, 2005; Larchevêque *et al.*, 2006b). Ces questions sont d'autant plus importantes dans le cas d'épandage sur sol brûlé car l'apport est ponctuel et les quantités supérieures (jusqu'à 160 t de matière fraiche par hectare) à celles en milieu agricole (< 20 t MS/ha; Navas *et al.*, 1999; ADEME, 2006).

L'apport de compost pourrait donc être une aide à la régénération d'un écosystème après un incendie tant du point de vue des propriétés physiques et microbiologiques du sol que de la biomasse et de la diversité végétale. Mais ces effets bénéfiques pourraient être contre-balancés par des effets nefastes tels l'apport d'éléments trace ou une eutrophisation du milieu.

Afin de répondre à ces questions, deux campagnes d'études ont été menées. Dans un premier temps, en laboratoire, les effets de différents types de compost et de la modalité d'apport sur un sol prélevé dans un écosystème incendié ont été observés au niveau de la minéralisation du carbone et de l'azote, du développement de la biomasse microbienne ainsi que de la limitation du risque d'érosion. Dans un deuxième temps, une étude a été réalisée *in situ,* avec des prélèvements réguliers, afin de suivre l'évolution de la végatation ainsi que des propriétés chimiques et microbiologiques du sol d'un écosystème incendié au cours du temps suite à l'apport d'un compost de boue de station d'épuration.

Le site de prélèvement de la première étude et le site d'étude de la seconde sont caractérisés par un climat méditerranéen avec des étés chauds et sec et des hivers tempérés et humides.

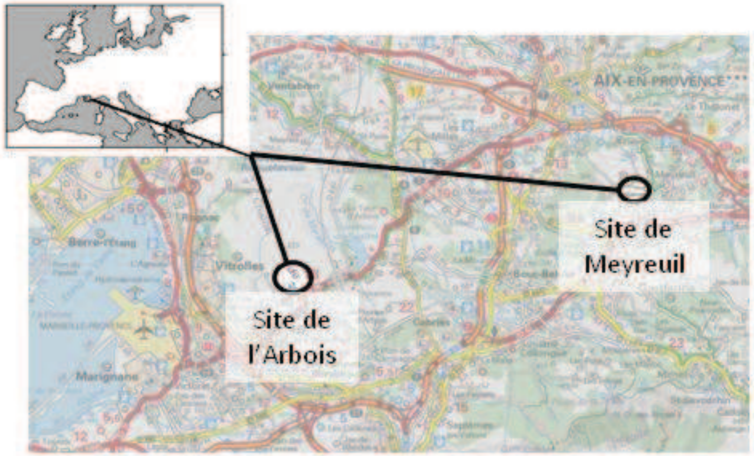

Figure 1. Localisation géographique des sites d'étude.

Photographie 1. Site d'étude de Meyreuil.

Photographie 2. Site d'étude de l'Arbois le 1er avril 2008

Photographie 3. Site d'étude de l'Arbois le 25 mai 2010

Approche expérimentale.

Site de Meyreuil

Ce site se trouve dans un écosystème de *Pinus halpensis* (Mill.) sur un substrat calcaire (Provence, Sud-est de la France; 43°28'47''N - 5°27'55''; altitude : 245 m; Fig.1; Photographie 1) et a été incendié en août 2005.

Trois types de composts provenant de plateformes industrielles ont été utilisés pour cette étude : un compost de déchets verts, un compost de boues de station d'épuration et un compost d'ordures ménagères résiduelles. Les trois composts ont été tamisés à 10 mm puis conservés à 4°C avant les incubations.

Les effets de l'apport des composts sur le sol ont été évalués lors d'incubations en conditions contrôlées de laboratoire (28°C, obscurité) sur des mélanges sol-compost. Au cours de ces incubations, deux modalités d'apport des composts (en surface ou enfouis) ont été testées et plusieurs paramètres ont été mesurés :

- la minéralisation du carbone et de l'azote dans les mélanges;
- les biomasses microbiennes totale et fongique;
- les caractéristiques physico-chimiques des mélanges.

Echantillonnage

Lors de cette étude, le sol a été prélevé en février 2006 (six mois après incendie) sur une profondeur de 20 cm comprenant la couche de cendres. Il a ensuite été tamisé à 4 mm puis conservé à 4°C avant les incubations.

Analyses en laboratoire

Mise en place des incubations pour le suivi de la minéralisation du carbone et de l'azote

Pour le suivi de la minéralisation du carbone, les incubations ont été réalisées dans des bocaux de deux litres fermant hermétiquement et entreposés à 28°C à l'obscurité. Chaque bocal contenait 150 g de sol à la capacité au champ et 10 g de compost (matière brute) ce qui correspondait à un amendement de 27 $t.ha^{-1}$. Les composts ont été soit apportés en mulch (à la surface du sol) soit mélangés avec le sol. Cinq réplicats ont été réalisés par traitement ainsi que cinq témoins sans compost.

La matière organique du sol, l'azote total, le carbone organique, la concentration en P_2O_5 et K_2O, le rapport C/N ainsi que la minéralisation du carbone et de l'azote et les activités biologiques ont été périodiquement mesurées durant 77 jours.

Dosage du carbone minéralisé

La mesure de la minéralisation du carbone est basée sur la quantité de CO_2 produite pendant l'incubation. Un pilulier contenant de la soude 1N est placé dans les bocaux. Les bocaux ont été périodiquement ouverts afin de changer les piluliers et de renouveler l'atmosphère. Le $C\text{-}CO_2$ piégé par la soude a été mesuré par colorimétrie à l’aide d’un analyseur à flux continu (Skalar, Pays-Bas). Il est libéré par acidification de la solution sous l'action de l’acide sulfurique à 100 $ml.L^{-1}$. Il est alors repris dans une solution colorée de phénolphtaléine de pH = 8,6 à 50°C. Ce nouveau piégeage entraîne une acidification de la solution et une décoloration proportionnelle à l’apport en CO_2. Celle-ci est mesurée à 550 nm après étalonnage de l’appareil (INRA, Grignon).

Dosage de l'azote minéral

Dans un premier temps, l'azote minéral a été extrait du sol par une agitation de 25 g d'échantillon dans 100 ml de KCl 1N. Le surnageant a ensuite été filtré au moyen de filtres en vibre de verre (Whatman, GF/C) puis congelé jusqu'à l'analyse. Celle-ci a été réalisée par dosage de deux formes de l'azote : $N\text{-}NH_4^+$ par la méthode de Berthelot et $N\text{-}NO_3+NO_2$ par celle de Griess et Ilossay. Les concentrations ont alors été déterminées par colorimétrie à 540 nm pour $N\text{-}(NO_3+NO_2)$ et 660 nm pour $N\text{-}NH_4^+$ après étalonnage de l'appareil (SKALAR, Pays-Bas) à l'INRA de Grignon.

Ruissellement et percolation

Les effets du compost sur le ruissellement et la percolation ont été étudiés en utilisant un simulateur de pluie. Pour cela, les mêmes sols et les mêmes composts que ceux précédemment décrits pour les incubations ont été utilisés selon les mêmes traitements. Ces préparations ont été placées dans des bacs percés de 31,5 cm de long, 24,6 cm de large et 6 cm de haut. Au fond de ces bacs, un géotextile a été placé afin d'éviter les pertes de matière à travers les trous. Ensuite, une couche de sable d'un centimètre (996 g) à été répandue sur le fond puis 2500 g de sol et 996 g de compost soit en mulch soit mélangé au sol. Trois réplicats pour chaque modalité ont été faits ainsi que trois témoins avec seulement du sol. Les bacs ont ensuite été placés sous le simulateur de pluie avec une pente de 10%. Le ruissellement a été mesuré en pesant l'eau accumulée dans un récipient placé à l'exutoire des bacs et la percolation grâce à celle contenue dans un récipient situé en dessous. La rétention des sols a été estimée par la différence entre la masse des bacs après et avant la pluie. Cette dernière a été simulée pour obetnir une intensité équivalente à 45 $mm.h^{-1}$ pour une durée de 40 min. Les concentrations en P_2O_5, $N\text{-}NO_3$, $N\text{-}NH_4^+$, SO_4^{2-}, Ca^{2+}, K^+, Na^+ et Cl^- des eaux collectées ont été déterminées.

Biomasse fongique

Cette mesure est basée sur le dosage de l'ergostérol (Gessner *et al.*, 1991; Gessner and Schmitt, 1996; Cortet *et al.*, 2003), un stérol de membrane présent dans la plupart des champignons. Il n'existe pas chez les plantes vasculaires, il est donc un bon indicateur de la colonisation fongique. L'ergostérol possède une double liaison rare ou absente chez les plantes vasculaires, qui crée une voie unique d'absorption dans l'ultra-violet, avec un maximum à 282 nm, longueur d'ondes à laquelle les stérols dépourvus de cette liaison ont une absorption négligeable. Cette caractéristique est utilisée pour quantifier l'ergostérol fongique après une séparation par HPLC (Gessner et Schmitt, 1996).

Les échantillons prélevés ont, dans un premier temps, été tamisés à 2mm puis congelés avant d'être lyophilisés puis conservés au sec dans un dessiccateur. L'ergostérol a été extrait par saponification alcaline à l'aide d'hydroxyde de potassium (8 $g.L^{-1}$) dans du méthanol (MeOH) sur 0,15 g de chaque échantillon. Cet extrait a été purifié en phase d'extraction solide (« Solid Phase Extraction ») à l'aide de cartouches Oasis HLB 3cc Waters® conditionnées par 1 ml de MeOH puis 1 ml de solution de conditionnement (MeOH KOH 8 $g.L^{-1}$ + HCl 0,65 M; 5/1 (V/V)). La fraction piégée a été éluée avec quatre fois 350µl d'isopropanol. L'éluât a été analysé en chromatographie liquide haute performance (HPLC) et comparé à une gamme réalisée avec de l'ergostérol standard commercial (pureté > 98%, Fluka) (Gessner et Schmitt, 1996).

Site de l'Arbois

Ce site se trouve sur le plateau de l'Arbois (Provence, Sud-est de la France; 43°27'16.28"N – 5°17'57.21"E, alt. 216 m; Fig.1; Photographies 2 et 3). Il a été incendié pour la dernière fois le 4 septembre 2007 et le feu s'est étendu sur une surface d'environ 82 ha de végétation. Selon la classification WRB (2006), il s'agit d'un sol calcaire de type Rendzic Leptosol.

Durant la seconde étude, les précipitations et températures annuelles moyennes étaient de 740 mm et 13,6°C.

Dans la zone d'étude, la majeure partie de la biomasse aérienne des plantes a été détruite à l'exception de quelques bosquets de *Quercus ilex L.* dont il restait quelques tiges calcinées mortes. D'après la végétation adjacente non-incendiée, la communauté végétale initiale était dominée par les espèces arbustives *Cistus albidus* L., *Cistus salviaefolius* L., *Quercus coccifera* L. et *Ulex parviflorus* Pourr*.,* l'herbacée *Brachypodium retusum (Pers.) P. Beauv.* Quelques massifs isolés de *Quercus ilex L.* étaient aussi présent sur le site. Ces cinq espèces dominantes peuvent être séparées en deux groupes selon leur type biologique : une herbacée rhizomateuse pérenne (*B. retusum*), et quatre espèces ligneuses (*C. albidus*, *C. salviaefolius*, *Q. coccifera* et *U. parviflorus*). *Q. coccifera* est un arbuste à feuilles persistantes sclérophylles, *C. albidus* et *C. salviaefolius* des arbustes malcophylles semi-décidues et *U. parviflorus* une légumineuse avec des tiges épineuses photosynthétiques. La stratégie de régénération de ces espèces est dépendante du feu : soit elles rejettent de souche (*Q. coccifera* et *B. retusum*), soit elles germent à partir de la banque de graines (*C. albidus*, *C. salviaefolius* et *U. parviflorus*).

Le compost utilisé lors de cette étude a été produit par une entreprise locale (Biotechna, Ensuès-La-Redonne, France). Il est composé de boues de station d'épuration mélangées avec des écorces de pin et des déchets verts (1/3 de volume chacun). L'ensemble a été composté durant trente jours à 75°C afin de neutraliser les microorganismes pathogènes et de décomposer les substances phytotoxiques. Il est ensuite tamisé à 40 mm afin de retirer les morceaux d'écorces de grande taille et entreposé en andains. Ces derniers sont régulièrement brassés lors des six mois suivants afin de favoriser l'humification de la matière organique. Le produit final répond aux normes françaises (NF U 44-095, 2002) pour les ETM, les CTO et les germes pathogènes. Suite à ce traitement, aucune graine viable ne persistait.

Huit parcelles de 300 m² chacune (15 m x 20 m), organisées en deux colonnes de quatre parcelles séparées par une zone tampon de deux mètres ont été choisies et matérialisés dans la zone incendiée. Le plan expérimental a été réalisé par le tirage aléatoire de quatre parcelles qui ont été amendées avec le compost, les quatre autres correspondant aux parcelles témoin. L'épandage du compost a été fait le 1er avril 2008 soit sept mois après l'incendie mais avant la reprise de végétation. Dans chacune des parcelles, 1,5 t de compost frais a été dispersé manuellement à la surface du sol, ce qui correspond à une dose d'environ 50 $t.ha^{-1}$ et environ 1 cm d'épaisseur. de matière fraîche.

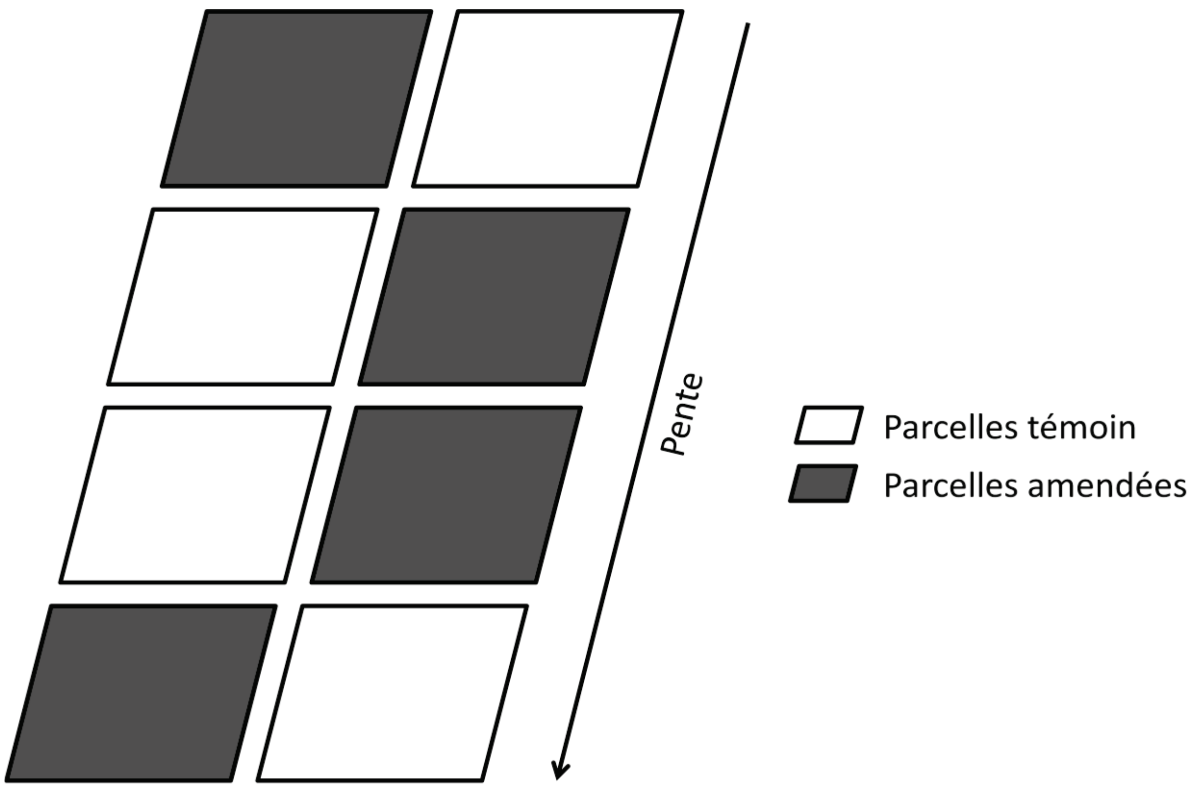

Figure 2. Plan expérimental.

Cette étude a porté sur deux composantes essentielles de l'écosystème. D'une part, l'évolution de la végétation a été suivie à travers l'estimation de sa biomasse et de sa biodiversité ainsi que par le suivi de plusieurs éléments minéraux du feuillage. D'autre part, le compartiment sol a été suivi au niveau de ces caractéristiques physico-chimiques ainsi que de sa biodiversité et de ses activités microbiologiques.

Echantillonnage et mesures in situ

Pour cette étude, des échantillons de sol ont été collectés avant l'amendement (fin mars 2008), puis six semaines après (mi-mai) et ensuite tous les trois mois de juillet 2008 à avril 2010. Seul l'horizon minéral du sol (horizon A) a été prélevé au moyen d'une tarière sur une profondeur maximale de 20 cm sans compost ni litière végétale. Des sous-échantillons ont été immédiatement réfrigérés après la collecte puis tamisés à 2 mm et conservés à 4°C ou congelés (pour les mesures d'azote minéral et d'ergostérol).

La biomasse aérienne végétale a été mesurée en juin 2008, mars et juin 2009 ainsi qu'en mars et juillet 2010 pour les cinq espèces dominantes. Elle a été estimée par la méthode des points de contact (Jonasson, 1983) qui est basée sur la relation entre la biomasse de la plante et le nombre de points de contact avec une tige métallique (5 mm de diamètre) passée verticalement dans la végétation jusqu'au sol. Pour chaque parcelle, trois transects de 2 m chacun ont été matérialisés et les points de contacts ont été notés tous les 10 cm. Dans une étude précédente, il a été établi que la biomasse aérienne de toutes les espèces étudiées suit une régression linéaire positive avec le nombre total de contacts (Larchevêque *et al.*, 2010) ou avec la hauteur du plus haut point de contact pour *U. parviflorus* (Montès *et al.*, 2008). Les régressions utilisées pour estimer les biomasses aériennes sur les parcelles expérimentales sont présentées dans le tableau 2.

Tableau 2. Régressions linéaires entre la biomasse aérienne des plantes (y, g.m^{-2}) et le nombre total de points de contact ou la hauteur du plus haut point de contact (x).

Espèces	Equation de régression	R²
B. retusum	y = 0.146 x	0.96
C. albidus	y = 1.092 x	0.89
C. salviaefolius	y = 0.560 x	0.97
Q. coccifera	y = 1.186 x	0.98
*U. parviflorus**	y = 0.472 x	0.91

** pour cette espèce, x est la hauteur du plus haut point de contact.*

A partir de ces données obtenues avec la méthode des points de contact, la fréquence centésimale, qui est une estimation du recouvrement végétal, peut être calculée :

$$FC = (P/n) \times 100$$

avec P, la somme du nombre de points de contact où l'espèce est présente sur le transect et n, le nombre total de points de contact.

La contribution spécifique est la proportion de la fréquence centésimale de chaque espèce et est calculée selon la formule suivante :

$$CS = (FC / \Sigma FC) \times 100$$

Deux ans après l'amendement (mai 2010), un relevé de diversité des espèces végétales vasculaires a été réalisé. Pour cela, vingt placettes (1m x 1m) ont été délimitées. Sur chacune des placettes, chaque espèce présente est notée en fonction de son recouvrement grâce à l'échelle d'abondance-dominance de Braun-Blanquet (1932) :

- 1 = très peu d'individus (<1%)
- 2 = recouvrement < 5%
- 3 = recouvrement de 5 à 25%
- 4 = recouvrement de 25 à 75%
- 5 = recouvrement de 75 à 100%

Ces résultats ont permis de calculer la richesse spécifique, l'indice de Shannon qui permet d'estimer la biodiversité des parcelles ainsi que celui de Jaccard qui permet de comparer la similarité entre les parcelles.

L'indice de Shannon est calculé selon la formule :

$$H' = - \Sigma\, p_i * \ln p_i$$

avec p_i, la proportion de recouvrement de l'espèce i en utilisant le centre des classes décrites précédemment (respectivement 0,5, 2,5, 15, 37,5, 62,5 et 87,5).

L'indice de Jaccard est calculé selon l'équation :

$$I_{A\text{-}B} = \frac{\text{Nombre d'espèces communes à A et B}}{\text{Nombre total des espèces de A et B}}$$

avec A et B, deux placettes différentes.

L'échantillonnage des végétaux pour les analyses de nutriments et d'éléments traces a été réalisé dans les stades précoces de la régénération en juillet 2009 et juillet 2010. Quatre échantillons par traitement (parcelles témoin et amendées) et par espèces ont été analysés. Chaque échantillon correspondait à un regroupement de trois à vingt individus selon leur taille. Seules les feuilles furent analysées pour *C. albidus*, *C. salviaefolius* et *Q. coccifera* alors que tout l'organe photosynthétique l'a été pour *B. retusum* and *U. parviflorus*

Analyses en laboratoire.

Analyses de nutriments et d'éléments trace

Les mesures de concentration des feuilles en azote, phosphore, cations (Ca, Mg, K, Na), éléments traces (B, Fe, Mn, Cu, Zn) ont été réalisées par le Laboratoire Centre Atlantique sur des échantillons séchés (Tableau 3) puis broyés (Foss Tecator Sample Mill 1093 Cyclotec, Foss Tecator, Nanterre, France). Les éléments trace ont été dosés uniquement en juillet 2010.

La concentration du sol en P, Mg, K ainsi qu'en métaux lourds (Cu, Zn, Cr, Ni, Cd, Hg) a été mesurée par le Laboratoire d'Analyses des Sols (Inra d'Arras; Tableau 4) sur les échantillons tamisés à 2 mm. Les métaux lourds n'ont été analysés qu'en mars 2008 et avril 2010.

Tableau 4. Méthodes et normes des analyses de sol.

	Méthode	Norme
Phosphore	méthode Olsen	NF ISO 11263
Magnésium	échangeable à l'acétate d'ammonium (AAF)	NF X 31-130
Potassium	échangeable à l'acétate d'ammonium (EAF)	NF X 31-108
Sodium	échangeable à l'acétate d'ammonium (EAF)	NF X 31-108
Cuivre	ICP-AES	NF EN ISO 11885
Zinc	ICP-AES	NF EN ISO 11885
Chrome	ICP-AES	NF EN ISO 11885
Nickel	ICP-AES	NF EN ISO 11885
Cadmium	ICP-MS	Méthode INRA
Mercure	méthode INRA	Méthode INRA

Teneur en eau du sol

La teneur en eau du sol (%H) a été estimée par méthode gravimétrique. Les échantillons de sol ont été pesés avant (M_h) et après (M_s) avoir été séchés 24h dans une étuve à 105°C. La teneur en eau du sol a été calculée comme étant la différence entre ces deux masses divisée par la masse de sol sec.

$$\%H = (M_h\text{-}M_s) / M_s \times 100$$

Densité apparente

La densité apparente a été déterminée sur le sol collecté au moyen de tubes de Siegrist dont le volume est connu. Le sol prélevé est pesé après avoir été séché à l'étuve à l'étuve (105°C). Cette masse (g) est divisée par le volume du tube de Siegrist (cm^{-3}) pour obtenir la densité apparente ($g.cm^{-3}$).

Carbone et azote total

Tout d'abord, les échantillons de sol secs ont été broyés au moyen d'un broyeur à bille (MM400, Retsch GmbH, Haan, Germany).Trente milligrammes ont ensuite été analysés au moyen d'un analyseur NA 1500 CN (Fisons instrument - Thermoelectron) à l'Inra de Grignon selon la méthode de Dumas.

Dosage de l'azote minéral

Ces mesures ont été réalisées comme décrit dans l'expérience précédente (page 23) au moyen d'une extraction sur 25 g d'échantillon et 100 ml de KCl 1N.

Teneur en carbonates

La teneur en carbonates dans le sol a été mesurée avec un calcimètre de Bernard avec une solution de HCl ½. Une gamme étalon préalablement réalisée au moyen de quantité connues de $CaCO_3$ a permis de calculer la teneur en carbonate des échantillons de sol prélevés.

La teneur en carbone organique des sols est ensuite calculée comme la différence entre la teneur en carbone total et celle en carbone calcaire.

Paramètres microbiologiques

Respiration basale et biomasse microbienne

L'état physiologique des communautés microbiennes du sol a été estimé par la méthode de respiration basale (Anderson et Domsch, 1978).

Une masse de sol frais (équivalente à 10 g de sol sec) de chaque échantillon a été placée dans deux flacons (250 ml). Le premier a été laissé à la teneur en eau observée *in situ*. La teneur en eau du second échantillon a été normalisée à 20% pour observer l'effet de ce paramètre sur les micro-organismes. Les flacons ont été laissés une nuit à température ambiante. Ensuite, leur atmosphère a été remplacée par injection d'un air contrôle durant 3 min puis ils ont été clos hermétiquement. Après incubation (4 h à température ambiante), 1 ml de l'air du flacon a été prélevé au moyen d'une seringue puis injecté dans un chromatographe à phase gazeuse (Chrompack CHROM3 - CP9001) équipé d'un détecteur TCD et d'une colonne (Porapack) dans laquelle circule un flux d'hélium à 60 $ml.h^{-1}$. Les valeurs obtenues ont été ajustées à 22°C. La quantité de CO_2 produite par les microorganismes est calculée comme étant la différence entre la concentration mesurée dans l'atmosphère du flacon et celle de l'air contrôle injecté.

La biomasse microbienne a été estimée selon la méthode de respiration induite par ajout de glucose (Anderson et Domsch, 1978). Les échantillons utilisés pour cette mesure étaient les mêmes que ceux précédemment utilisés pour la respiration basale. Un mélange de talc et de glucose (1.000 $\mu gC.g^{-1}$ de sol) a été ajouté et incorporé dans le sol puis l'ensemble est incubé pendant 90 min. Comme pour la respiration basale, l'atmosphère interne des flacons a été remplacée par un air contrôlé et les flacons ont ensuite été fermés hermétiquement. Une nouvelle période d'incubation a lieu à température ambiante durant 60 min. Comme décrite pour la respiration basale, la mesure de concentration en CO_2 dans les flacons a été réalisée par chromatographie gazeuse. La valeur de la biomasse microbienne est ensuite obtenue par l'équation de Beare *et al.* (1990) à partir du taux de respiration induite.

Le quotient métabolique (qCO_2) est calculé pour chaque échantillon comme étant le rapport entre la respiration basale et la biomasse microbienne. Ceci permet d'estimer l'efficacité métabolique de la communauté microbienne.

Pour la mesure de la biomasse microbienne lors des incubations, la méthode de fumigation-extraction (Wu *et al.*, 2002) a été utilisée. Le dosage du carbone organique total (COT) a été effectué avec un analyseur de carbone Shimadzu TOC5050A à l'Inra de Grignon soit sur des échantillons ayant subi une fumigation (16h) dans des vapeurs de chloroforme ou non. La différence entre ces deux COT permet de d'obtenir l'extractile microbien (E_c). La biomasse microbienne est ensuite calculée selon la formule :

$$\text{Biomasse} = 2{,}22 * E_c$$

Biomasse fongique

Cette mesure est basée sur le dosage de l'ergostérol (Gessner *et al.*, 1991; Gessner and Schmitt, 1996; Cortet *et al.*, 2003), un stérol de membrane présent dans la plupart des champignons. Il n'existe pas chez les plantes vasculaires, il est donc un bon indicateur de la colonisation fongique. L'ergostérol possède une double liaison rare ou absente chez les plantes vasculaires, qui crée une voie unique d'absorption dans l'ultra-violet, avec un maximum à 282 nm, longueur d'ondes à laquelle les stérols dépourvus de cette liaison ont une absorption négligeable. Cette caractéristique est utilisée pour quantifier l'ergostérol fongique après une séparation par HPLC (Gessner et Schmitt, 1996).

Les échantillons prélevés ont, dans un premier temps, été tamisés à 2mm puis congelés avant d'être lyophilisés puis conservés au sec dans un dessiccateur. L'ergostérol a été extrait par saponification alcaline à l'aide d'hydroxyde de potassium ($8\ g.L^{-1}$) dans du méthanol (MeOH) sur 0,15 g de chaque échantillon. Cet extrait a été purifié en phase d'extraction solide (« Solid Phase Extraction ») à l'aide de cartouches Oasis HLB 3cc Waters® conditionnées par 1 ml de MeOH puis 1 ml de solution de conditionnement (MeOH KOH 8 $g.L^{-1}$ + HCl 0,65 M; 5/1 (V/V)). La fraction piégée a été éluée avec quatre fois 350µl d'isopropanol. L'éluât a été analysé en chromatographie liquide haute performance (HPLC) et comparé à une gamme réalisée avec de l'ergostérol standard commercial (pureté > 98%, Fluka) (Gessner et Schmitt, 1996).

Profils cataboliques des communautés microbiennes cultivables

La structure et la diversité des fonctions cataboliques pour les communautés microbiennes cultivables ont été déterminées par la méthode Biolog® Ecoplates (BIOLOG Inc., Hayward, CA). Cette méthode consiste à mesurer la croissance des micro-organismes et le métabolisme résultant de l'utilisation d'une grande variété de substrats carbonés. Les microplaques BIOLOG® ECO (Figure 3) sont subdivisées en trois zones identiques comprenant chacune trente et une sources de carbone et un puit contrôle sans substrats. L'oxydation des substrats engendre la réduction proportionnelle du bleu de nitro-tetrazolium incolore en formazan pourpre. De véritables empreintes métaboliques, sortes de cartes d'identité des fonctions cataboliques des communautés, peuvent alors être établies et comparées. Les sources de carbone peuvent être regroupées en six classes chimiques : les acides carboxyliques, les amides et amines, les polymères, les carbohydrates, les acides aminés et les composés divers.

Les profils cataboliques ont été mesurés selon le protocole modifié de Garland et Mills (1991). Dix grammes (équivalent sec) de sol frais ont été mis en suspension dans 100 ml d'une solution stérile de pyrophosphate de sodium à 0,1% (pH 7) puis agités pendant 60 min. La solution a été laissée à décanter 30 min puis le surnageant a été dilué 100 fois dans une solution saline stérile (0.85% NaCl). Les puits ont été inoculés avec 125 µl de cette nouvelle solution et les microplaques Biolog ont été incubées à 25°C pendant cinq à dix jours. Le développement de densité optique de chaque puits a été enregistré deux fois par jour à une longueur d'onde de 595 nm (spectrophotomètre Elisa 960 Metertech®) jusqu'à la fin du développement de la densité otique moyenne des trente et un puits. Pour chaque échantillon et chaque temps de mesure, l'absorbance du puits contrôle a été soustraite de celle des autres puits de la microplaque pour éliminer l'absorbance de la suspension de sol (particules et bactéries).

A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine
B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine
C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine
D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine
E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine
F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid
G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine
H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine

Figure 3. Substrats carbonés disponibles dans une plaque BIOLOG® ECO.

Le temps d'incubation pour lequel la densité optique moyenne de l'ensemble des substrats atteignait une valeur de 0,5 ($AWCD_{0,5}$ ou Average Well Colour Development) a été calculé selon la méthode de Garland et Mills (1991) pour chaque échantillon. Ce temps a été utilisé pour déterminer par extrapolation la densité optique de chaque substrat. Un tableau contenant, pour chaque échantillon une valeur d'absorbance standardisée pour les trente et un substrats, est analysé par des tests statistiques multivariés ou des calculs d'indice de diversité.

Activités enzymatiques

Mesure de l'activité des hydrolases du diacétate de fluorescéine (FDA)

L'activité FDA a été mesurée selon la méthode modifiée de Green et al. (2006). Six millilitres de tampon phosphate de potassium à 50 mM (pH 7) plus 50 µl de solution FDA (2 mg/ml d'acétone) ont été ajoutés à 1 g de sol frais et incubés à température ambiante pendant une heure. La réaction a été arrêtée en ajoutant 2 ml d'acétone et le mélange est immédiatement centrifugé (3 min, 10.000*g,* 4°C). La fluorescéine libérée à partir de la FDA a été mesurée dans le surnageant à 490 nm. Pour chaque échantillon, un témoin est réalisé dans des conditions identiques en ajoutant seulement le tampon phosphate de potassium au sol mais pas la solution FDA.

L'activité FDA est exprimée en µmole de fluorescéine libérée par minute (U) et par gramme de sol sec ($U.g^{-1}$).

Mesure de l'activité des phosphomonoestérases alcalines (Pmb)

L'activité Pmb a été mesurée selon la méthode de Tabatabai et Bremner (1969). Le milieu réactionnel est constitué d'1 g de sol frais, de 4 ml de tampon NaOH-glycine (0,1M, pH 9) et d'1 ml de p-nitrophenyl phosphate (pNPP, 5 mM). Après une incubation de une heure à 30°C, la réaction a été arrêtée par l'ajout d'1 ml de $CaCl_2$ (0,5M) et 4 ml de NaOH (0,5M). Après une centrifugation de 3 min à 10.000*g* (4°C), la quantité de p-nitrophenol (pNP) libérée à partir du p-NPP a été mesurée sur le surnageant à 412 nm. Pour chaque échantillon, un témoin est réalisé dans des conditions identiques en remplaçant la solution pNPP par 5 ml de tampon acétate seul.

L'activité Pmb est exprimée en µmole de p-NP libéré par minute (U) et par gramme de sol sec ($U.g^{-1}$).

Mesure de l'activité des uréases (Ur)

L'activité Ur a été évaluée selon la méthode adaptée de Tabatabai et Bremmer (1972). Dans un tube à essai, 1 g de sol frais a été pesé puis mélangé avec 6 ml de tampon acétate de sodium (50 mM, pH 6) contenant 20 mM d'urée. Un témoin sans urée est réalisé pour chaque échantillon. Le milieu réactionnel a été incubé pendant deux heures à 37°C puis centrifugé (3min, 4°C, 10.000*g*). La teneur en azote sous la forme d'ammonium ($N\text{-}NH_4^+$) libéré par les uréases a été quantifiée par la méthode de (Mulvaney, 1996). Le surnageant (1,5 ml) est transféré dans un tube à hémolyse auquel sont ajouté 0,5 ml d'EDTA, 2ml de salicylate Na-nitroprusside et 1 ml d'HOCl puis le mélange est incubé 30 min à 30°C. L'intensité de la coloration vert émeraude qui se forme après ajout de salicylate est mesurée au spectrophotomètre à la longueur d'onde de 667 nm.

L'activité Ur est exprimée en µmole de $N\text{-}NH_4^+$ libéré par minute (U) et par gramme de sol sec (U.g-1).

Mesure de l'activité des phénols oxydases (PO)

L'activité PO de type tyrosinases (EC 1.14.18.1) a été mesurée en utilisant le protocole de Saiya-Cork et al. (2002). Un gramme de sol frais est placé dans un tube à essai auquel a été ajouté 6 ml d'une solution de L-Dopa (3,4 dihydroxyphénylalanine, εM= 620 $L.mol^{-1}.cm^{-1}$)) à 25 mM dans un tampon acétate (pH 6,5, 50 mM). Des témoins ont été réalisés pour chaque échantillon en ajoutant 6 ml du tampon acétate ne contenant pas de L-Dopa. Après vingt minutes d'incubation à l'obscurité et à température ambiante, les tubes ont été centrifugés pendant trois minutes à 10.000*g* à 4°C. La densité optique du surnageant est mesuré à 590 nm.

L'activité PO est exprimée en $U.g^{-1}$ de sol sec (1U = 1µmole de $Dopachrome.min^{-1}$).

Chapitre II - Etude des effets de l'apport de composts en laboratoire.

Utilisation de composts urbains pour la régénération d'un sol méditerranéen incendié : approche en laboratoire.

Use of urban composts for the regeneration of a burnt Mediterranean soil: a laboratory approach

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Abstract

In Mediterranean region, forest fires are a major problem leading to the desertification of the environment. Use of composts is considered as a solution for soil and vegetation rehabilitation. In this study, we determined under laboratory conditions the effects of three urban composts and their mode of application (laid on the soil surface or mixed into the soil) on soil restoration after fire: a municipal waste compost (MWC), a compost of sewage sludge mixed with green waste (SSC) and a green waste compost (GWC). Carbon (C) and nitrogen (N) mineralisation, total microbial biomass, fungal biomass and soil characteristics were measured during 77-day incubations in microcosms. The impact of composts input on hydrological behaviour related to erodibility was estimated by measuring runoff, retention and percolation (i.e. infiltration) of water using a rainfall simulator under laboratory conditions. Input of composts increased organic matter and soil nutrient content, and enhanced C and N mineralisation and total microbial biomass throughout the incubations, whereas it increased sporadically fungal biomass. For all these parameters, the MWC induced the highest improvement while GWC input had no significant effect compared to the control. Composts mixed with soil weakly limited runoff and infiltration whereas composts laid at the soil surface significantly reduced runoff and increased percolation and retention, particularly with the MWC.

Keywords: Forest fire; municipal wastes compost; sewage sludge; green wastes compost; mineralisation; total microbial biomass; fungal biomass; soil characteristics; runoff; infiltration.

Introduction

The Mediterranean climate is characterized by a long dry summer and strong winds favouring recurrent forest fires (Bagnouls & Gaussen, 1957; Scarascia-Mugnozza *et al.*, 2000; De Luis *et al.*, 2001). Fires induce major alterations to the ecosystem. Their frequency, duration and intensity are important factors determining the impact on biological, chemical and physical properties of the ecosystem: the more fires are recurrent and serious, the more their marked impact (Boerner, 1982).

Fire can produce a partial or total destruction of the vegetal cover and the soil organic horizons (Guerrero *et al.*, 2001). Subsequently, burned soils are prone to erosion and could decline in stability (Kutiel & Inbar, 1993; Hart *et al.*, 2005). For example, part of the nutrients are oxidised and volatilised by fire (Grogan *et al.*, 2000; Hart *et al.*, 2005) and can easily be lost by wind erosion and runoff (DeBano and Conrad, 1978; Boerner, 1982; De Luis *et al.*, 2001) aggravated by an increase of soil hydrophobicity (DeBano, 2000). Thus, Mediterranean soils are often deficient in organic matter (OM) (Archibold, 1995). Another biological change due to fire is the activity and structure of the microbial community, with a shift to communities in which heat-resistant microorganisms dominate (Vasquez *et al.*, 1993; Hart *et al.*, 2005).

The use of composts as an amendment for soil restoration and forest regeneration in frequently burnt or degraded Mediterranean ecosystems is increasing (Navas *et al.*, 1999; Martinez *et al.*, 2003; Román *et al.*, 2003; Curtis & Claassen, 2009; Kowaljow and Mazzarino, 2007). The spreading of biosolids stabilized by composting, can improve the low fertility of soils and constitutes an alternative to landfill disposal. Moreover, this stabilization decreases risks of heavy metal leaching (Garcia *et al*., 1990; Planquart *et al*., 1999).

Compost amendment improves physical, chemical and biological properties of soils, in particular by increasing available nutrients mainly in the organic soil fractions (Larchevêque *et al.*, 2005a). This induces an increase of soil microbial biomass (Borken *et al.*, 2002) and positively affects plant cover by an improvment of plant nutrition (Villar *et al.*, 1998; Guerrero *et al.*, 2000, 2001; Caravaca *et al.*, 2003; Larchevêque *et al.*, 2005b, 2006, Larchevêque *et al.*, in press). The increase of microbial activity can induce a better aggregate stability (Guerrero *et al.*, 2001;

Caravaca *et al.*, 2003), and, combined with the development of plant biomass, reduces the risk of erosion (Guerrero *et al.* 2000).

The objective of this study was to i) compare the effects of different types of composts and the mode of application on some chemical and microbial properties under laboratory conditions in a Mediterranean burned soil, and ii) determine how these amendments modify the soil's hydrologic response using rainfall simulations.

Materials & Methods

Soil and composts characteristics

The soil was collected in a burnt *Pinus halepensis* (Mill.) ecosystem on calcareous substrate under Mediterranean climate in south-eastern France (43°28'47''N-5°27'55''E, 245 m altitude). The sampling was realised on 30 sampling points in the burnt area over 20cm depth (including surface ashes) in February 2006, 6 months after the fire. The fire was intense (high calorific power) but rapidly progressed because of a strong wind. Fire affected crown and soil surface. Consumption of litter layer could be observed, organo-mineral layer had been eroded or was missing, but there was no visible alteration of the surface of the mineral soil. Burned trees were still standing but no vegetation had begun to grow. The soil was homogenized by sieving (<4 mm) for the experiments. Three urban composts sieved at 10mm were studied: green waste compost (GWC), sewage sludge (1/5 volume) mixed with green waste compost (SSC) and municipal solid waste compost (only organic wastes, MWC). Levels of heavy metals in composts studied were below the minimum current standards (NFU 44-095 AFNOR, 2002; NFU 44-051 AFNOR, 2006). Soil and compost were stored at 4°C before incubations. According to the self-heating test depending on the maximum temperature reached (T_{max}) (FCQAO, 1994), GWC was stabilized whereas SSC and MWC were unstabilized (Table 1).

Soil and compost initial characteristics are presented in Table 1.

Soil is a Haplic Cambisol (Calcaric) (FAO, 1998).

Table 1: Initial microbiological, chemical and physical characteristics of composts and soil. Values of the DEWAR indice.

		Soil	MWC	SSC	GWC	Methods
pH		8.1	7.8	6.6	8.0	NF EN 12176
Water content	%	27.09±0.86	21.94±4.83	22.03±5.00	26.52±0.88	
Conductivity	mS/m	15	3.02	0.85	1.38	Extr. Water 1/5 (V/V) & Conductivity NF EN 13039
OM	g/kg	84.4	712	454	357	NF EN 12879
Total N	g/kg	2.4	15.1	22.1	14.2	Attack Kjeldahl + colorimetry
C/N		17	23	10	12	Organic C / Total N
$N\text{-}NH_4^+$	mg/kg	0.06	3419.0	3407.7	147.9	Extraction KCl M & dosage, Berthelot
$N\text{-}(NO_3 + NO_2)$	mg/kg		3.48	0.80	12.67	Extraction KCl M & dosage, Griess and Ilossay's
Total phosphorus	g/kg	0.04	0.32	0.08	0.34	ISO 11-263-1 adapted
K_2O	g/kg	3.4	8	7.91	12.6	NF EN 13346, Dosage ICF AES NF EN ISO 11885 extraction with *aqua regia*
Total microbial biomass	mgC/kg	269	4788	2481	2324	Vance *et al.* , 1987
Fungal biomass	mg/kg	4.61	12.17	0	4.64	Gessner et Schmitt, 1996
Tmax	°C	-	71	58.5	22	FCQAO, 1994
Dewar indice		-	I	II	V	

Units are related to dry matter of soil.

Laboratory incubations

Soil-compost mixtures were incubated in 2L jars hermetically closed at 28±1°C, in the dark. Composts were mulched or mixed with soil at field capacity. The rate of compost used was 10 g of compost in each jar corresponding to 27 $Tm.ha^{-1}$ of fresh matter. The amount of soil in each jar was 150g and corresponded to 20cm depth of soil sampling. Soil OM, total N, organic C, total phosphorus, K2O contents, soil C/N ratio, C and N mineralisation and biological activities were measured periodically during 77 days. Measurements were made only on the soil fraction for the mulched composts.

Carbon mineralisation

C mineralisation during incubation was based on the amount of CO_2 produced during the incubation time. A flask with 25ml of NaOH 1N and another with 10ml of water were introduced into each 2L jar. The jars were opened to change the flasks and to renew the atmosphere on days 2, 4, 7, 10, 14, 21, 28, 35, 49, 63 and 77. Five replicates for the 2 modes of application were made for the 3 composts. 5 controls of soil without compost were also done. C-CO_2 trapped in NaOH was analysed by colorimetry at 550nm with a continuous flux analyser (SKALAR, Netherlands) after acidification with H_2SO_4 solution (100 mg/L), and addition of phenolphthalein (pH = 8.6, 50°C).

Nitrogen mineralisation

The measurements of N mineralisation were destructive and were carried out on days 0, 14, 28, 49 and 77. At each date, 5 controls of soil without compost and 5 replicates for each mode of application and for the 3 composts were performed. For mulch application, the compost layer was removed before the sampling. Mineral N was extracted with KCl 1N (1/4, soil mass/KCl volume) by agitation during 1 hour and decantation. Mineral N was measured on the filtered supernatant (Whatman, GF/C) by colorimetric methods (Berthelot's method for N-NH_4^+ at 660nm and Griess and Ilossay's method for N-(NO_3+NO_2) at 540nm; SKALAR, Netherlands).

Total microbial and fungal biomass

The measurements of total microbial and fungal biomass were destructive and were carried out on days 14, 28, 49 and 77 for 5 controls of soil without compost, 5 replicates for each mode of application and for the three composts. As for nitrogen mineralisation, only the soil below compost was analysed for mulch application. Total microbial biomass was measured on 24g of the soil-compost mixture or the soil under mulch by fumigation-extraction (Vance *et al*., 1987). Microbial extractable C was estimated from the difference in C released between fumigated and unfumigated samples (Wu *et al.*, 1990). Fungal biomass in soil was determined from ergosterol concentrations using solid-phase extraction and high-performance liquid chromatography (HPLC; Gessner & Schmitt, 1996).

Rainfall simulation: runoff and infiltration (retention and percolation)

Compost effects on runoff and percolation were studied in laboratory by using a rainfall simulator, on another set of soil samples. Amended soils were placed in pierced bottom containers of 31.5cm in length, 24.6cm width and 6cm height. From the bottom to the top of the container, we placed respectively: a geotextile to avoid matter loss through holes, 996g of sand (depth = 1cm), 2500g of homogenized soil, and 167g of compost homogenously distributed on soil surface for mulch treatment or mixed with soil. We performed 3 controls of soil without compost and 3 replicates for each mode of application and for the 3 composts. Containers with soil and composts were placed under the rainfall simulator with a 10 degree slope. The rainfall simulator, similar to the one used by Foster *et al.* (1979), was equipped with oscillating nozzles allowing a rainfall equivalent to 45mm.h^{-1} during 40 min, at a pressure of 0.9 bar. Runoff and percolation were measured by weighting the water respectively from runoff and from percolation after rainfall. Retention was estimated by the difference between the weight of the soil before and after the rainfall. We also determined dissolved organic carbon (COD) (NF EN 1484), suspended solids (SS) (NF T 90-105-2), total phosphorus (NF EN ISO 11885 before mineralization with *aqua regia*), chloride and N-NO_3^- (NF EN ISO 10304-2), N-NH_4^+ (NF T 90-015-1), SO_4^{2-} (NF EN ISO 10304-2), Ca^{2+}, K^+ and Na^+ (NF EN ISO 11885 before mineralization with *aqua regia*) contents of the water collected from runoff and percolation.

Statistical analyses

The effect of compost type, mode of application and incubation duration were analysed by one-way ANOVA combined with Tukey tests (Zar, 1984). Previously, normality and homocedasticity were verified by Shapiro-Wilks and Bartlett tests respectively (Zar, 1984). We used Kruskal-Wallis analyses and post-hoc Student-Newman-Keuls when these conditions were not met (for C and N mineralisation) Significance was defined by $p<0.05$. The software MINITAB® (Minitab Inc., 2000) was used.

Results

Soil-compost characterization after 49 days of incubation

After 49 days of incubation, all treatments increased OM, total N and K content (Table 2; one-way ANOVA, $p<0.05$). Only SSC input induced an increase of P content and MWC of NH_4^+ content (Table 2; one-way ANOVA, $p<0.001$). Total phosphorus, OM, total N, organic C and total potassium content were greater for mixed compost than for mulched compost (one-way ANOVA, $p<0.05$).

Table 2: Chemical characteristics of soil after 49 days of incubation

		Control	Incubation with MWC		Incubation with GWC		Incubation with SSC	
			Mulched	Mixed	Mulched	Mixed	Mulched	Mixed
pH		7.88 ± 0.04^{bc}	7.86 ± 0.05^{bc}	7.74 ± 0.05^{d}	7.90 ± 0.00^{ab}	7.98 ± 0.04^{a}	7.84 ± 0.05^{bc}	7.80 ± 0.00^{cd}
Conductivity	mS/cm	1.28 ± 0.10^{c}	1.31 ± 0.08^{bc}	1.44 ± 0.02^{ab}	1.23 ± 0.00^{c}	1.29 ± 0.08^{c}	1.49 ± 0.06^{a}	1.59 ± 0.02^{a}
Total phosphorus	g/kg	0.03 ± 0.00^{cd}	0.02 ± 0.00^{d}	0.03 ± 0.00^{cd}	0.03 ± 0.00^{cd}	0.04 ± 0.00^{b}	0.03 ± 0.00^{c}	0.12 ± 0.01^{a}
OM	g/kg	66.48 ± 3.19^{c}	70.02 ± 3.37^{bc}	80.72 ± 6.39^{a}	68.46 ± 3.91^{bc}	74.86 ± 4.63^{abc}	69.56 ± 6.66^{bc}	78.52 ± 7.16^{ab}
C/N		14.40 ± 0.89^{a}	13.60 ± 0.55^{a}	14.20 ± 1.10^{a}	13.60 ± 0.89^{a}	13.40 ± 1.34^{a}	14.00 ± 1.22^{a}	10.67 ± 0.58^{b}
$N\text{-}NH_4^+$	mg/kg	11.34 ± 2.58^{bc}	22.40 ± 5.18^{a}	14.20 ± 1.92^{b}	7.44 ± 1.35^{c}	8.20 ± 1.85^{c}	7.18 ± 1.76^{c}	14.00 ± 1.87^{b}
K_2O	g/kg	0.31 ± 0.01^{f}	0.38 ± 0.00^{e}	0.47 ± 0.01^{c}	0.43 ± 0.01^{d}	0.61 ± 0.02^{a}	0.39 ± 0.03^{e}	0.54 ± 0.01^{b}

Mean ± SD, N=5. Units are related to dry matter of soil. Results of the comparison are given by an exponent letter: values that do not differ at the 0.05 level are noted with the same letter (one-way ANOVA and Tukey test, a > b > c > d > e > f).

Carbon mineralisation

Input of compost induced an increase of C mineralisation in comparison with the control except for the GWC (Figure 1; Kruskal-Wallis, $p<0.001$). C mineralisation of amended soil was the highest with MWC, the lowest with the GWC and intermediate with SSC (Figure 1a and 1b; Tukey test $p<0.05$).

Differences between mulched and mixed compost modes were significant but depended on the type of compost (Kruskal-Wallis, $p<0.05$). C mineralisation was higher with mixed mode compared to mulched mode at the beginning of the incubation for MWC and SSC(Kruskal-Wallis, $p<0.05$), but was lower with the mixed mode for GWC from 4 to 77 d of incubation (Kruskal-Wallis, $p=0.009$).

Nitrogen mineralisation

All compost types induced an increase of NH_4^+ content immediately after input (Figure 2a & 2b; Kruskal-Wallis, $p<0.001$), with the highest ammonium content observed for MWC and SSC (Figure 2a & 2b). Then ammonium strongly decreased to a value close to zero and remained constant until the end of incubation whatever the compost (Figure 2a & 2b; Kruskal-Wallis, $p>0.05$).

Except for MWC during the first 14 days and GWC throughout the incubation time, we observed an increase of N-(NO_3^-+NO_2^-) for amended soil compared to control (soil) with maximum values reached at the end of the incubation for SSC (Figure 2c & 2d; Kruskal-Wallis, $p<0.001$; Student-Newman-Keuls, $p<0.05$).

No effect of the mode of application was observed on N mineralisation (NH_4^+ and N-(NO_3^-+NO_2^-); Kruskal-Wallis, $p>0.05$).

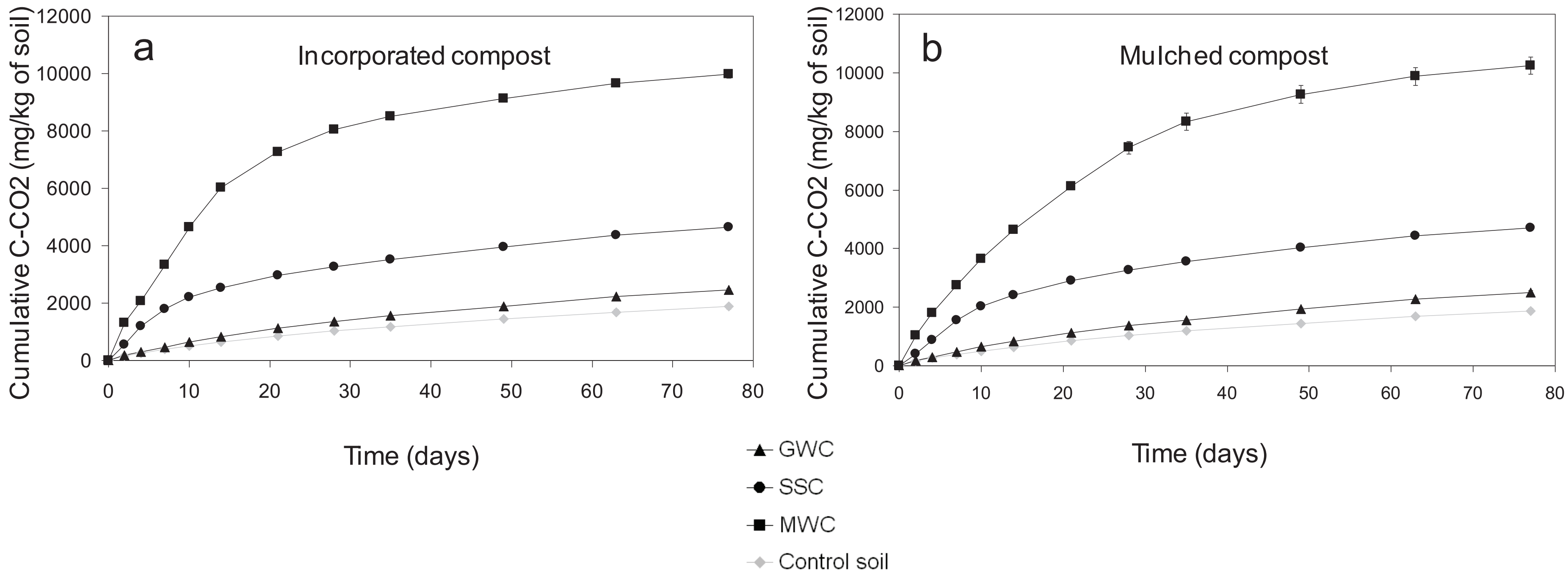

Figure 1: Carbon mineralised during soil-compost incubation (Mean ± SD, N=5)

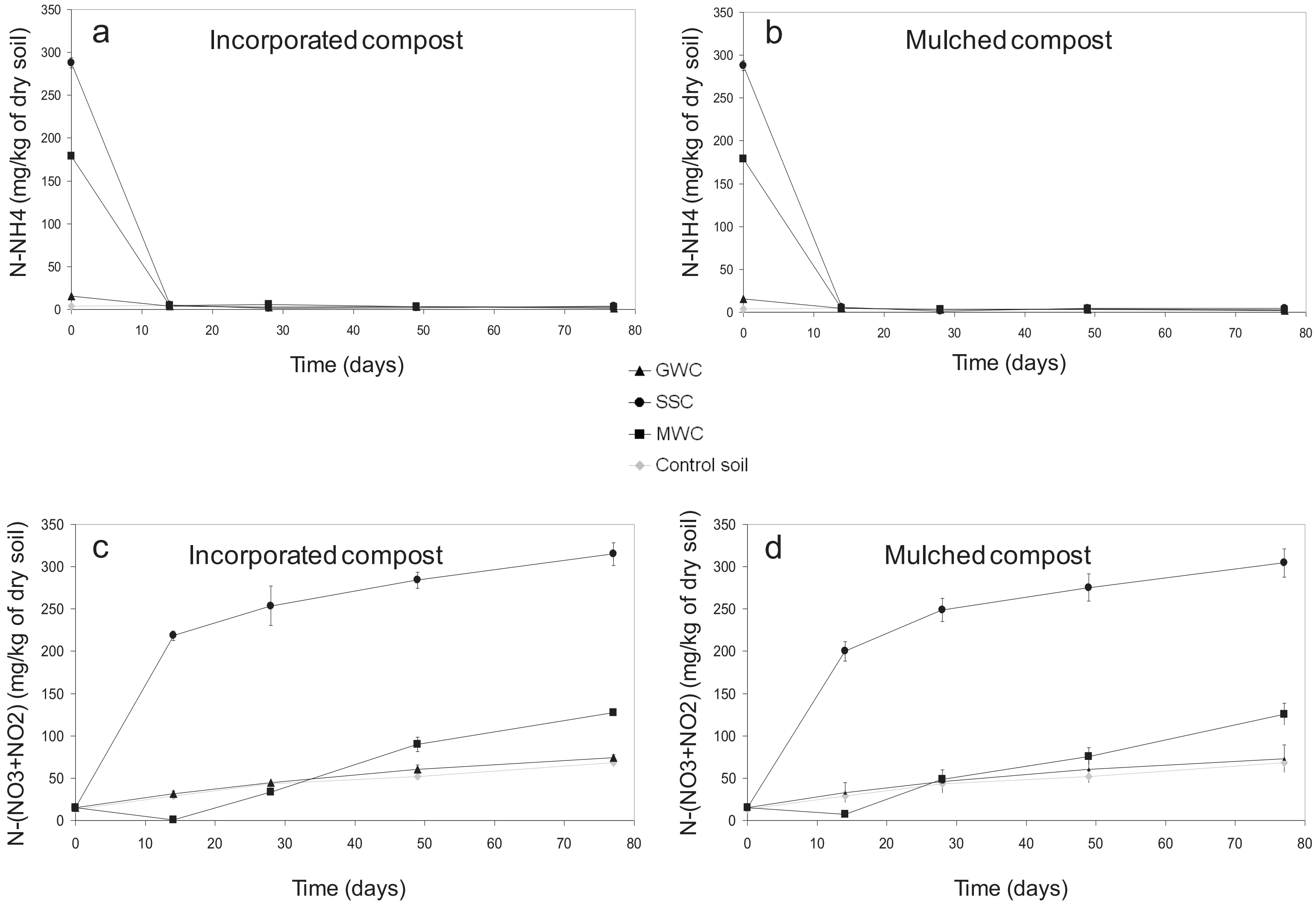

Figure 2: Nitrogen mineralised during soil-compost incubation (Mean ± SD, N=5)

Total microbial biomass

Amendment increased total microbial biomass especially for MWC (Figures 3a & 3b; Kruskal-Wallis, $p<0.001$). For this compost, the microbial biomass was lower with the mulched mode than with the mixed mode between days 0 and 49 and higher from the 49th until the 77th day (Figure 3a & 3b; Kruskal-Wallis, $p<0.05$).

Fungal biomass

Fungal biomass increased just after composts were applied except for SSC (Figure 4a & 4b; Kruskal-Wallis, $p<0.001$). There were no significant differences between mulched and mixed modes except for MWC from the 14th to the 49th day.

Composts effects on runoff and infiltration

Only two ways of distribution were observed for control treatment: runoff and retention. Mixed composts weakly decreased runoff and increased retention except for mixed GWC and no percolation was observed with this mode (Figure 5; one-way ANOVA, $p<0.001$). Mulched composts induced an important decrease of runoff, and an increase of retention, and some water from percolation may have been observed. Effects were the most marked for MWC and the less for GWC (Figure 5; one-way ANOVA, $p<0.001$).

In water from runoff, input of GWC induced an increase of dissolved organic carbon (DOC) (Table 3; one-way ANOVA, $p=0.005$). MWC and SSC reduced exported NH_4^+ and NO_3^- (Table 3; one-way ANOVA, $p<0.05$). Mulched application decreased exportation of SO_4^{2-} and chloride (Table 3; one-way ANOVA, $p<0.05$).

In water from percolation, MWC induced the most important exportation of NH_4^+, SO_4^2, Ca^{2+}, K^+, Na^+, and, chloride (Table 3; one-way ANOVA, $p<0.001$). With SSC, this was observed for NH_4^+, total P, K^+ and chloride (Table 3; one-way ANOVA, $p<0.001$) and only for NO_3^- with GWC (Table 3; one-way ANOVA, $p<0.001$).

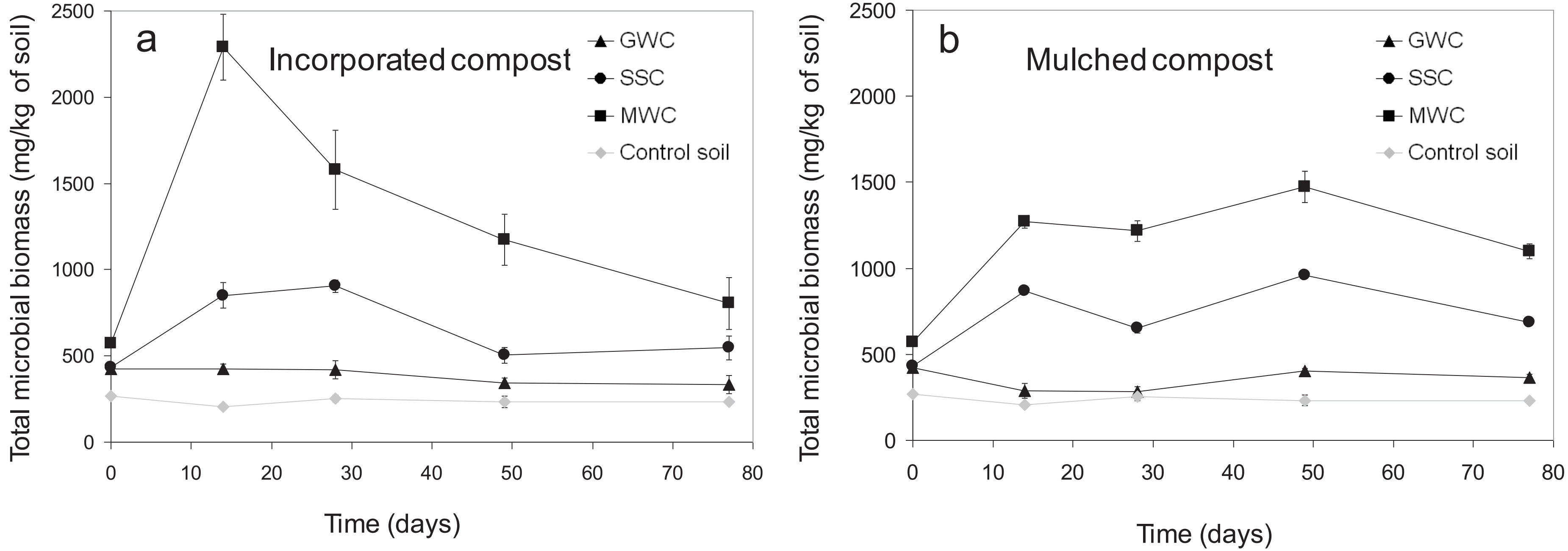

Figure 3: Dynamics of total microbial biomass during soil-compost incubation (Mean ± SD, N=5)

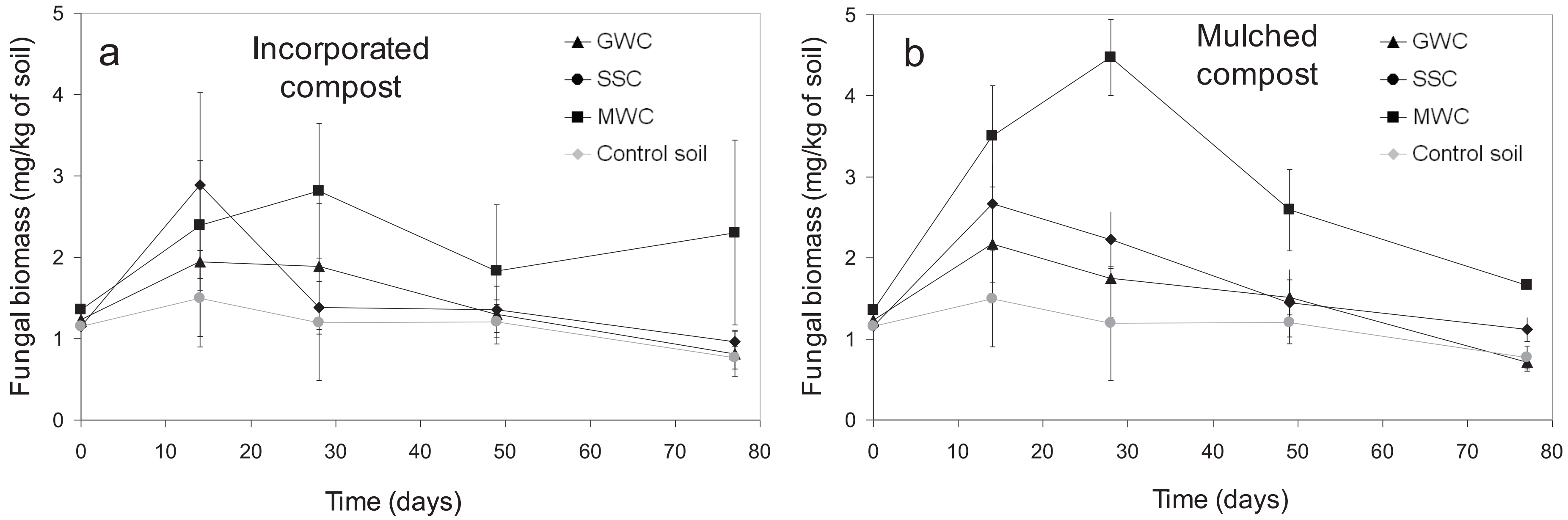

Figure 4: Dynamics of fungal biomass during soil-compost incubation (Mean ± SD, N=5

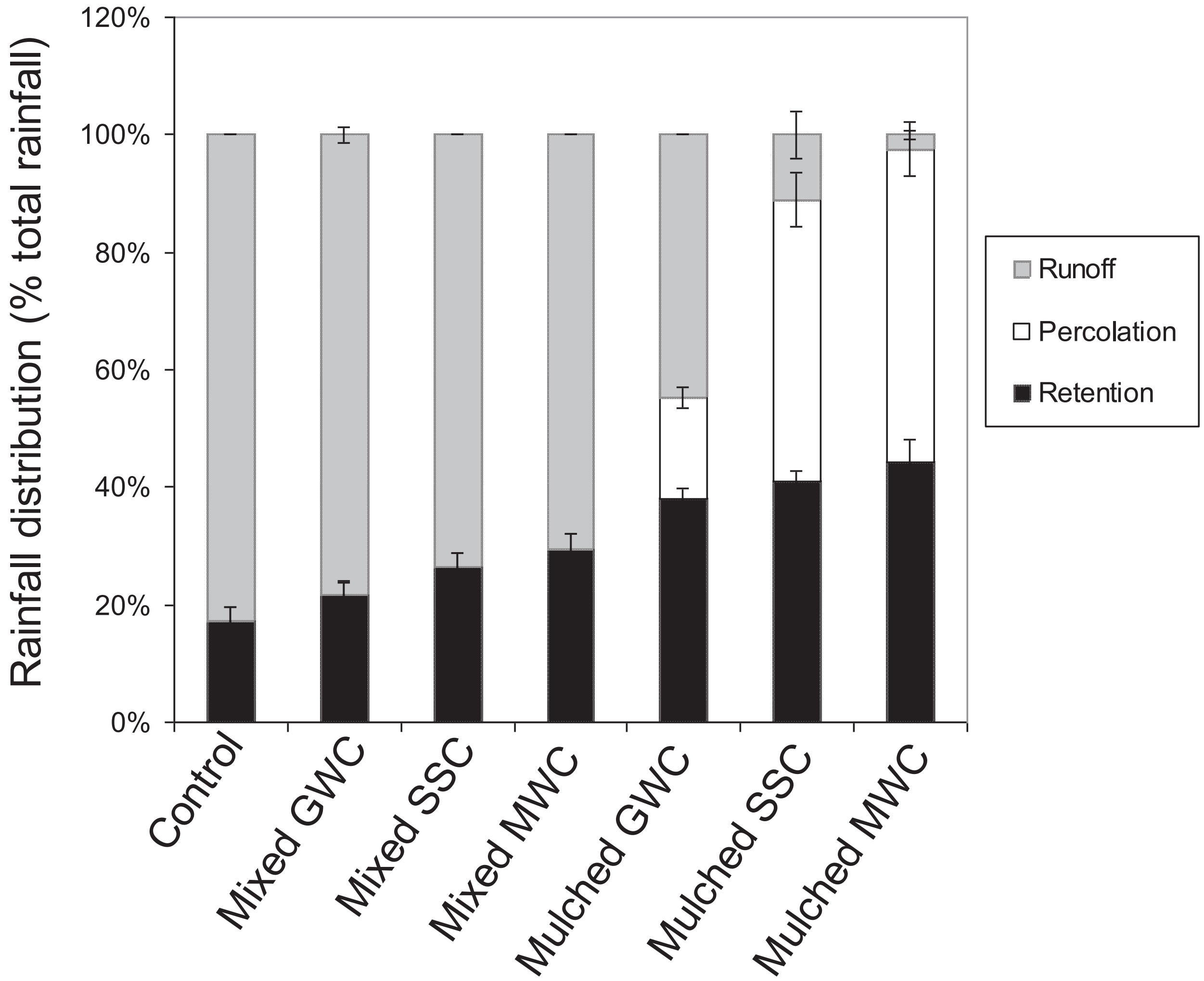

Figure 5: Water rainfall distribution (Mean ± SD, N=3)

Table 3: Chemical analysis of runoff and percolation water collected after the rainfall simulation

		Runoff							Percolation		
			MWC		SSC		GWC		MWC	SSC	GWC
		Control	Mixed	Mulched	Mixed	Mulched	Mixed	Mulched	Mulched	Mulched	Mulched
SS	g	9.33 ± 3.83^{a}	6.62 ± 1.69^{ab}	0.00^{c}	7.27 ± 2.25^{ab}	0.00^{c}	8.17 ± 2.04^{a}	0.97 ± 0.88^{bc}	0.18 ± 0.04^{A}	0.41 ± 024^{A}	-
DOC	mg C	23.9 ± 4.8^{b}	27.3 ± 5.2^{b}	0.00^{c}	24.1 ± 5.2^{b}	0.00^{c}	30.0 ± 12.5^{b}	53.6 ± 22.91^{a}	-	0.00^{A}	0.00^{A}
$N\text{-}NH_4^+$	mg	11.8 ± 2.8^{a}	2.5 ± 0.7^{bc}	0.00^{c}	7.4 ± 2.8^{ab}	0.00^{c}	10.8 ± 2.5^{a}	8.5 ± 2.9^{ab}	71.9 ± 21.5^{A}	67.7 ± 9.5^{A}	1.2 ± 0^{B}
$N\text{-}N0_3^-$	mg	2.03 ± 0.65^{a}	0.87 ± 0.09^{bc}	0.00^{c}	0.93 ± 0.06^{abc}	0.00^{c}	1.37 ± 0.66^{ab}	0.58 ± 0.11^{bc}	0.62 ± 0.03^{B}	0.57 ± 0.06^{B}	5.72 ± 0.85^{A}
Total P	mg	2.5 ± 1.0^{ab}	2.8 ± 0.8^{ab}	0.4 ± 0.5^{b}	4.4 ± 2.1^{a}	1.3 ± 1.2^{ab}	2.6 ± 0.5^{ab}	4.2 ± 0.8^{a}	3.2 ± 0.3^{B}	6.6 ± 1.3^{A}	0.81 ± 0.15^{C}
SO_4^{2-}	mg	59.0 ± 13.8^{a}	43.4 ± 4.6^{ab}	0.00^{c}	44.2 ± 1.9^{ab}	0.00^{c}	49.3 ± 4.5^{ab}	29.2 ± 5.5^{b}	226.7 ± 16.7^{A}	208.5 ± 16.6^{A}	12.9 ± 11.3^{B}
Ca^{2+}	mg	558 ± 190^{a}	415 ± 113^{a}	26.5 ± 31.5^{b}	483 ± 73^{a}	17.8 ± 16.2^{b}	533 ± 73^{a}	73.4 ± 12.0^{b}	631 ± 145^{A}	186.4 ± 25.4^{B}	68.2 ± 3.5^{B}
K^+	mg	38.0 ± 11.1^{a}	30.7 ± 4.6^{a}	15.6 ± 20.92^{a}	33.0 ± 5.3^{a}	5.4 ± 5.3^{a}	167.7 ± 218.4^{a}	94.1 ± 32.0^{a}	136.0 ± 28.4^{A}	71.1 ± 4.1^{B}	18.3 ± 1.0^{C}
Na^+	mg	3.7 ± 0.9^{a}	8.6 ± 1.7^{a}	21.7 ± 29.85^{a}	3.5 ± 0.7^{a}	1.1 ± 1.2^{a}	3.5 ± 0.3^{a}	4.1 ± 1.4^{a}	284.1 ± 26.8^{A}	39.0 ± 3.4^{B}	4.5 ± 0.3^{B}
Chloride	mg	45.7 ± 3.8^{a}	37.8 ± 2.8^{ab}	0.00^{c}	38.7 ± 2.9^{ab}	0.00^{c}	41.8 ± 5.0^{ab}	30.1 ± 0.1^{b}	485.5 ± 39.0^{A}	204.2 ± 16.1^{B}	32.1 ± 27.8^{C}

Mean ± SD, N=3. Units are related to dry matter of soil. Results of the comparison are given by an exponent letter: values that do not differ at the 0.05 level are noted with the same letter (one-way ANOVA and Tukey test, a > b > c to compare runoff water for the control and the 3 composts and their 2 application modes; A > B > C to compare percolation water for the three composts).

Discussion

Effect of compost characteristics on chemical and microbiological soil properties

As it has been previously observed (Larchevêque *et al.*, 2005a, Larchevêque *et al.*, 2006), compost input led to an increase of soil nutrients and then could enhance vegetation recolonization after fire (Navas *et al.*, 1999; Martinez *et al.*, 2003; Larchevêque *et al.*, 2005a). However, according to the different initial characteristics of composts, soil nutrient and biological responses to amendment differed: MWC induced the greatest effect, and GWC the lowest. The stability level of the composts explains those results. MWC was not stabilized and therefore rich in easily degradable OM which induced a strong mineralisation of C. In contrast, GWC presented a high stability level, and then induced low mineralisation. The dynamics of C mineralisation also varied over time whatever the type of compost, with the highest rate of mineralisation at the beginning of incubation, when labile OM is still available (Bernal *et al.,* 1997).

The three composts induced different dynamics in levels of N-NH_4^+ and N-(NO_2 + NO_3), due to initial compost C/N ratio and OM matter stability (Barbarika *et al.*, 1985; Trinsoutrot *et al.*, 2000; Parnaudeau *et al.*, 2004).

Input of compost initially increased fungal and microbial biomass (Perucci, 1992; Albiach *et al.*, 2001; Debosz *et al.*, 2002) corresponding to the decomposition of easily biodegradable OM (Bernal *et al.,* 1997). Maximum value of microbial biomass observed for MWC occurred at the same time as the lowest value of N-(NO_2 + NO_3) showing N immobilization. This compost showed the highest values of microbial biomass and C mineralization related to its labile OM content (Annabi *et al.,* 2007). In contrast, microbial biomass remained stable and low for GWC, as the labile OM of this mature compost has already been degraded during composting.

Effect of the application mode on chemical and microbiological soil properties

Composts increased soil nutrient content whatever the mode of application, indicating a migration of nutrients from the mulched composts to the soil (Larchevêque *et al.*, 2006). However, composts mixed with soil induced a greater C mineralisation and microbial biomass increase compared to mulched composts during the first stage of incubation. OM of mixed compost was rapidly degraded through a greater surface contact between soil and compost, enhancing biological activity (Schomberg *et al.*, 1994; Coppens *et al.*, 2007). Differences of C mineralisation and microbial biomass between the two modes of application were no longer observed during the second stage of incubation, because of the decrease of microbial biomass due to less available feeding substrate for microorganims during the second stage.

In contrast, the development of the fungal biomass was affected by the mode of application but it was greater with mulch mode than buried in the case of MWC. We hypothesize that this development may be explained by a fungal community specific to compost favoured by the greater availability of the substrate in the case of mulch mode.

Effect of composts on infiltration and runoff and their potential implications for soil erosion risk

Unlike the findings of Tejada and Gonzalez (2006), no significant effects of mixed composts on water infiltration and runoff were observed, probably because the rainfall intensity was different between the studies: they applied a 140mm.h^{-1} rainfall while ours was 45mm.h^{-1}. According to Albaladejo *et al.* (1994) and Agassi *et al.* (2004), mulching is a method to limit runoff and, thus, erosion. Our study under controlled conditions confirmed this assertion, as mulched composts (particularly MWC) reduced runoff by increasing water retention and percolation, and thus infiltration in the soil (Giusquiani *et al.*, 1995). The compost formed a protective layer on the surface and absorbed the kinetic energy of rain responsible for soil erosion

(Agassi *et al.*, 1985). This effect was more marked with MWC, probably due to its more fibrous texture than others that allowed better absorption of rainfall. Moreover, mulched composts allowed water percolation and the high decrease of runoff also led to a decrease of exported nutrients in surface water.

When composts were mixed into the soil, no significant effects were observed on exportation of elements.

Owing to these results, use of compost type and mode of application could affect in a different way water quality. Mulched mode compared to mixed mode lead to less surface water export.

Conclusion

Under laboratory conditions, input of compost on burned soil induced an increase in organic matter and nutrient soil content. Thus it increased the microbial biomass and its activity, depending on stability: the more the compost is unstable the more the effect is marked. The application mode had an effect on the physical protection of soil: mulched composts allow the absorption of kinetic energy of rainfall and thereby can limit losses by erosion and favour water infiltration.

Although compost effects could differ considerably under field conditions, the results obtained could help for generalizations through some implications for practice.

The use of composts for restoring burnt ecosystems shows promise as a method to speed up Mediterranean soil regeneration. Due to the high initial nutrient content, compost amendment after fire can accelerate vegetation recolonization. Unstable composts are the most efficient for rapidly enhancing soil biological activity. Their mulch application seems suitable to decrease nutrient exportation in surface water, reducing the risk of eutrophication. Moreover, as this mode of application increased water retention in the soil, it may increase water availability to plants.

Acknowledgements

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Chapitre III - Apport de compost en garrigue.

Effets de l'amendement d'un compost de boues de station d'épuration sur la régénération d'une végétation méditerranéenne après incendie.

Effect of sewage sludge compost amendment on Mediterranean vegetation regeneration after fire

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Abstract

In Mediterranean region, forest fires are a major problem towards the desertification of the environment. Use of composts is considered as a solution for soil and vegetation regeneration. In this study, we determined the effects of sewage sludge compost on vegetation regeneration after fire. In April 2008, the compost was amended on soil surface. Plant biomass and cover were estimated in March and June between 2008 and 2010 and biodiversity in May 2010. The first months after amendment, input of compost increased grass species *Brachypodium retusum* biomass. One year after amendment, we observed an important decrease of legume species *Ulex parviflorus* biomass. Although no significant effect was observed on other studied plants, plant biomass was more heterogeneous on amended plots. Moreover, compost input induced no difference in plots biodiversity but it was more homogeneous on amended plots.

Keywords: Forest fire; sewage sludge compost; plant cover; plant biomass; biodiversity.

Introduction

Forest fires are considered as the main perturabtion of Mediterranean ecosystems (Whelan, 1995). The Mediterranean-type shrublands are highly resilient to wildfires (Keeley, 1986; Trabaud, 1994) as a consequence of the ability of plant species to rapidly recover from fire by means of a wide diversity of regeneration strategies from resprouting to fire-prone seed germination (Trabaud and Lepart, 1980; Lloret and Vilà, 1997). However, the abandonment of agricultural lands since the 1970s and the increase in human population and activities have resulted in a dramatic increase in the number and size of fires in all Mediterranean regions (Pausas, 2004). Fire recurrence is also favored by the Mediterranean climate characterized by long dry summer and strong winds (Bagnouls & Gaussen, 1957; Scarascia-Mugnozza *et al.*, 2000; De Luis *et al.*, 2001). Fires induce important modifications on vegetation and soil. Fire frequency, duration and intensity are the main factors determining impacts on ecosystem: the more fires are recurrent and intense, the more their effects pronounced (Boerner, 1982). Firstly, fires induced partial or total destruction of plant cover and superficial organic layers (Hernández *et al.*, 1997; Guerrero *et al.*, 2001). Then, soil stability decrease due to the loss of organic layer and the increase of erosion risk (Kutiel and Inbar, 1993; Hart *et al.*, 2005). Immediately after fire, nutrient availability increases due to ashes and release of soil minerals (Kutiel and Inbar, 1993; Dumontet *et al.*, 1996). However, part of the nutrients is oxidized and volatilized during fires (Fisher and Binkley, 2000). Nutrient losses are more important after fire because of wind erosion, runoff (DeBano and Conrad, 1978; Boerner, 1982; Gimeno-Garcia *et al.*, 2000) and leaching into groundwater (Mohamed *et al.*, 2007). When these climatic events occur in a short delay after the fire, their effects are much more pronounced (Thomas *et al.*, 1999; Gonzalez-Pérez *et al.*, 2004). Adding to physical and chemical modifications, forest fires disturb soil biological properties (DeBano *et al.*, 1998), mainly in the superficial soil layer (Neary *et al.*, 1999). Post-fire Mediterranean ecosystems are dominated by evergreen sclerophyllous shrubs (Rundel, 1988; Aerts, 1995; Archibold, 1995) which are stress-tolerant species adapted to infertile habitats. The development of persistent leaves with low nutrient contents and slow decomposition rates induce a low restitution of nutrients to soil (Rundel, 1988; Aerts, 1995). This in turn leads to the persistence and the extension of the most oligotrophic plant communities, especially when repeated fires cause long-term cumulative negative effects on soil fertility (Eugenio *et al.*, 2006). Despite the high resilience of Mediterranean shrublands, vegetation recovery after fire becomes more and more difficult as fire frequency increases, resulting in patchy vegetation cover and accelerating soil degradation but induces the development of herbaceous plant populations rich in species (Keeley and Keeley, 1981). In calcareous lower Provence (southeastern France), the most frequently burnt and degraded soils are colonized by *Quercus coccifera* L. - shrublands (Trabaud, 1987; Barbero, 1990). After a disturbance, these communities can re-establish by two different reproductive strategies: either by resprouting or seeding (Lloret and Vilà, 1997). Both of these strategies are fire-promoted: resprouting occurs rapidly by recruitment of new shoots after aboveground organs were burnt; dormant seeds in the soil can germinate thanks to suppression of dormancy and destruction of germination soil phenolic inhibitors by heat (Christensen and Muller 1975; Keeley and

Keeley 1989). In the initial phases, post-fire recolonization is often controlled by sprouting species that early lose dominance in favor of obligate seeders (Bellingham and Sparrow, 2000; Sparrow and Bellingham, 2001).

The use of organic biosolids as amendments is increasing for soil restoration and vegetation regeneration in frequently burnt or degraded ecosystems (Guerrero *et al.*, 2000; Caravaca *et al.*, 2003; Martinez *et al.*, 2003; Larchevêque *et al.*, 2006a; Walter *et al.*, 2006; Kowaljow and Mazzarino., 2007; Hemmat *et al.*, 2010; Turrión *et al.*, 2012). Compost amendments improve physical, chemical and biological properties of soils, in particular by increasing available nutrients mainly in the organic soil fractions (Bodet and Carioli, 2001; Larchevêque *et al.*, 2005; Annabi *et al.*, 2007). Composted biosolids have a high water retention capacity (Giusquiani *et al.*, 1995) which induces an increase of soil water content (Villar *et al.*, 1998). These modifications positively affect plant cover through an improvement of plant nutrition and growth (Villar *et al.*, 1998; Guerrero *et al.*, 2000, 2001; Caravaca *et al.*, 2003; Larchevêque *et al.*, 2005), and contribute to reduce the risk of erosion (Guerrero *et al.* 2000). By favoring plant establishment, organic amendments may also affect plant species richness and diversity (Madejón *et al.*, 2006; Walter *et al.*, 2006; Walter and Calvo, 2009). The increase of nutrient levels due to high rates of amendment can even lead to exclusion of the less competitive species in the new environmental conditions, and decrease species richness (Martinez *et al.*, 2003; Moreno-Peñaranda *et al.*, 2004), according to the “stimulus hypothesis” (Huston, 1979). Thus, increasing nutrient availability in a low-fertility post-fire soil can modify plant-plant interactions and the resulting composition of the plant community (Tilman, 1984).

In France, there are a few studies on reclamation of burnt Mediterranean ecosystems using organic amendments, despite large burnt surfaces (about 4,360 ha in 2012 in the French Mediterranean region, Direction de la Sécurité Civile). Vegetation plays an essential role in ecosystem reconstitution after fire, by protecting soil against erosion, producing organic matter and favoring soil biological activity. Fertility of frequently burnt soils could be restored by spreading biosolids in order to enhance vegetation growth after fire and limit erosion on bare soil. In this study we examine the potential of a compost amendment to speed up vegetation recovery in a burnt Mediterranean shrubland. The objectives of this study were to monitor *in situ* the effect of a single compost input after fire on plant biomass production, plant cover

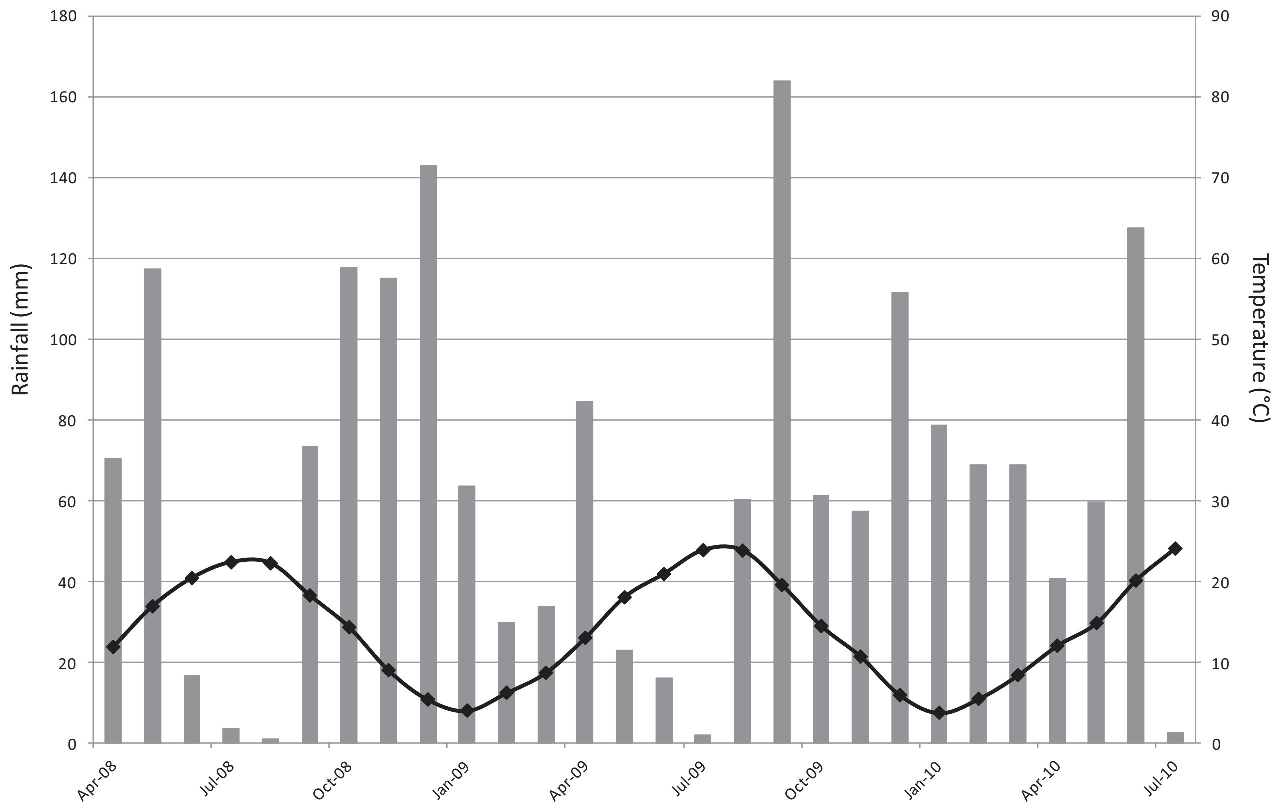

Figure 1. Monthly rainfall and mean air temperature (Météo France) during the study period.

and plant diversity for 2 years following compost addition. We also examine plant responses depending on their regeneration strategy after fire, hypothezing that obligate-seeder species would be more sensitive to compost than resprouters.

Material and methods

Study site

The experiment was carried out in a burnt site of about 82ha on the plateau of Arbois (Provence, Southeastern France; 43°27'16.28"N – 5°17'57.21"E, alt. 216m). The fire occurred on 4th September 2007 and totally burnt the above-ground vegetation. As visible in the adjacent unburnt vegetation, the initial plant community was dominated by the shrub species *Cistus albidus* L., *Cistus salviaefolius* L., *Quercus coccifera* L. and *Ulex parviflorus* Pourr., the herb *Brachypodium retusum (Pers.) P. Beauv.* Some sparse clumps of *Quercus ilex L.* were also growing in the site. The study site is characterised by a Mediterranean climate with dry, hot summers and wet and temperate winters. The mean annual precipitation and temperature are 740 mm and 13.6°C, respectively (Fig.1). The calcareous soil is a Rendzic Leptosol according to WRB (2006).

Compost characteristics and experimental design

The compost was produced by a local company (Biotechna, Ensuès-La-Redonne, France) from municipal sewage sludge mixed with pin barks and green wastes (1/3 volume each). After being composted for 30 days at 75°C to kill pathogenic microorganisms and decompose phytotoxic substances, the mixture was sieved (< 40 mm mesh) to remove the large bark pieces and stored in swathes. The swathes were mixed several times over the next 6 months to promote organic matter humification. The final compost met the French legal standards (NF U 44-095, 2002) for pathogenic microorganisms, organic trace elements, and trace metals. No viable seeds remained.

Table 1: Soil (0-20 cm: maximum depth; N = 8 to 40; mean±SD) and compost initial characteristics.

		Soil		Compost
		Control plots	Amended plots	
Organic C	$g.kg^{-1}$	41.26±9.56	35.24±9.94	312.4
Total carbonates	$g.kg^{-1}$	13.44±10.51	9.94±6.88	-
Total N	$g.kg^{-1}$	2.66±0.66	2.31±0.38	31.73
$N\text{-}NH_4^+$	$g.kg^{-1}$	0.027±0.007	0.031±0.011	2.49
$N\text{-}(NO_3^-+NO^{2-})$	$g.kg^{-1}$	0.009±0.004	0.007±0.004	0.002
P_2O_5	$g.kg^{-1}$	0.018±0.006	0.017±0.006	30.7
K_2O	$g.kg^{-1}$	0.428±0.088	0.435±0.060	8.1
MgO	$g.kg^{-1}$	0.251±0.058	0.249±0.037	7.9
Na_2O	$g.kg^{-1}$	0.024±0.003	0.022±0.004	-
CaO	$g.kg^{-1}$	-	-	99.30
CEC	$Cmol^+.kg^{-1}$	19.41±2.82	19.30±2.12	-
Total Cd	$mg.kg^{-1}$	0.26±0.06	0.22±0.02	0.81
Total Cr	$mg.kg^{-1}$	81.23±5.98	78.08±8.18	22.91
Total Cu	$mg.kg^{-1}$	21.90±2.30	19.83±1.32	181.12
Total Hg	$mg.kg^{-1}$	0.05±0.01	0.05±0.01	0.4
Total Ni	$mg.kg^{-1}$	49.50±0.94	49.88±1.75	15.06
Total Zn	$mg.kg^{-1}$	92.10±10.29	84.45±3.59	325.3
Humidity	Soil : % / Compost : %FM	14.24±2.69	13.88±2.35	28.2
Bulk density (unsieved fraction)	$t.m^{-3}$	1.08±0.16	1.06±0.16	0.31
Clay	% *	33.75±4.99	32.93±3.93	-
Silt	% *	55.40±5.16	57.30±2.87	-
Sand	% *	10.85±0.45	9.78±1.03	-

All nutrient and trace element values are expressed in kg^{-1} of soil dry matter (DM)
* : % of sieved fraction ≤ 2mm ; FM : Fresh Matter.

The experimental design was a randomized block of eight 300 m^2 plots (15 m x 20 m) delimited in a 0.5 ha flat zone of the burnt site. Four plots were amended with compost and four were the controls. About 50 $t.ha^{-1}$ of fresh compost were homogeneously spread by hand on soil surface 7 months after fire on the 1st April 2008, before plant regrowth.

Soil and compost initial characteristics are presented in Table 1.

Vegetation analysis

The five species studied can be separated into two groups based on life-form, *i.e.* a perennial rhizomatous herb (*Brachypodium retusum)*, and four woody species. Among the latter, *Quercus coccifera* is an evergreen sclerophyllous shrub, *Cistus albidus* and *Cistus salviaefolius* are semi-deciduous malacophyllous shrubs and *Ulex parviflorus* a legume with thorny photosynthetic stems. The regenerative strategy of the five species studied is fire-promoted, either as resprouters (*Q. coccifera* and *B. retusum*) or obligate-seeders (*C. albidus*, *C. salviaefolius* and *U. parviflorus*). Plant aboveground biomass was monitored in June 2008, in March and June 2009 and 2010. It was estimated indirectly by the point intercept method (Jonasson, 1983) which is based on the relationship between plant biomass and the number of contacts with a fine metal rod (5 mm diameter) passed vertically through the vegetation to the ground. In each experimental plot, we set 3 transects of 2 m long along which rod contacts with plant species were noted at 10 cm intervals. In previous studies, we have established that aboveground biomass of each species studied fitted a positive linear regression with total number of contacts (see Larchevêque *et al.* 2010 for details of field procedure and regression equations) or with the height of the highest contact for *U. parviflorus* (Montès *et al.*, 2008). We used these regressions to estimated aboveground biomass on the experimental plots (Table 2).

Table 2. Linear regression between plant aboveground biomass (y, g.m^{-2} DM) and total number of contacts or height of the highest contact (x).

Espèces	Equation de régression	R²
B. retusum	y = 0.146 x	0.96
C. albidus	y = 1.092 x	0.89
C. salviaefolius	y = 0.560 x	0.97
Q. coccifera	y = 1.186 x	0.98
*U. parviflorus**	y = 0.472 x	0.91

*for this species, x is the height of the highest contact.

Centesimal frequency estimates the plant cover and is calculated using the point intercept method data as:

CF = (P / n) x 100

with P is the sum of the number of contact points where the specie is present on the transect and, n is the total number of contact points.

Specific contribution is the proportion of each centesimal frequency species and is calculated as:

$$SC = (CF / \Sigma\ CF) \times 100$$

At the end of experimentation (May 2010), twenty sub-plots (1m x 1m) were delimited on amended and control plots (4 lines of 5 subplots across each plot). In these sub-plots, all vascular plant species were noted and each species was assigned a cover coefficient according to the abundance-dominance scale of Braun-Blanquet (1932): 1 = very low individuals cover (<1%), 2 = cover < 5%, 3 = 5 to 25% cover, 4 = 25 to 50% cover, 5 = 50% to 75% cover, 6 = 75 to 100% cover. Specific richness, diversity index and evenness were calculated using these results.

Shannon index which allows diversity measurement was calculated as:

$$H' = - \Sigma\ p_i * \ln p_i$$

with p_i, the cover proportion of the i^{th} species using the centers of the classes described before (0.5, 2.5, 15, 37.5, 62.5 and 87.5 respectively).

Jaccard index which allows to compare similarity between different plots was calculated as:

$$I_{A\text{-}B} = \frac{A \cap B}{A \cup B}$$

with A and B, the species of 2 different subplots.

Statistical analyses and calculations

Compost effects on plant cover and biomass for each date and each species were assessed using repeated measures analyses of variance (ANOVA) combined with Tukey test (Zar, 1984) or, in case of non parametric data, by Kruskal-Wallis test and Mann-Whitney test.

Plant growth was studied on the 3 growth period of the experimentation (between March and June). It was calculated as the difference between plant biomass in June and in March. Data were assessed using Mann-Whitney test.

Principal component analysis (PCA) was performed on plants biomass to determined compost effects by combining all species data.

Plant diversity was assessed by correspondent factor analysis using species presence/absence on all sub-plots. Shannon index and specific richness were assessed using Mann-Whitney test.

Significant level was considered to be 95%. The software XLSTAT 2012.4.02, Addinsoft 1995-2012) was used for statistical analysis.

Results

Plant cover

No compost effect was observed on relative cover of *C. albidus*, *C. salviaefolius,* and *Q. coccifera* (two-way ANOVA, p > 0.05) excepted the cover relative increase on amended plots for *B. retusum* (two-way ANOVA, p = 0.012) and on control plots for *U. parviflorus* (one-way ANOVA, p = 0.026). Compost input significantly increased the relative cover of *B. retusum* in March and July 2009 and July 2010 (one-way ANOVA, p = 0.027, 0.09 and 0.011 respectively; Fig.2). Inversely, relative cover of *Q. coccifera* was significantly lower on amended plots in March 2009 (Repeated measures ANOVA, p = 0.015; Fig.2) and no effect was observed at the other dates. The cover of *U. parviflorus* was higher on control plots than on amended plots in March 2009 and 2010 (one-way ANOVA, p < 0.0001 and p = 0.008 respectively; Fig.2).

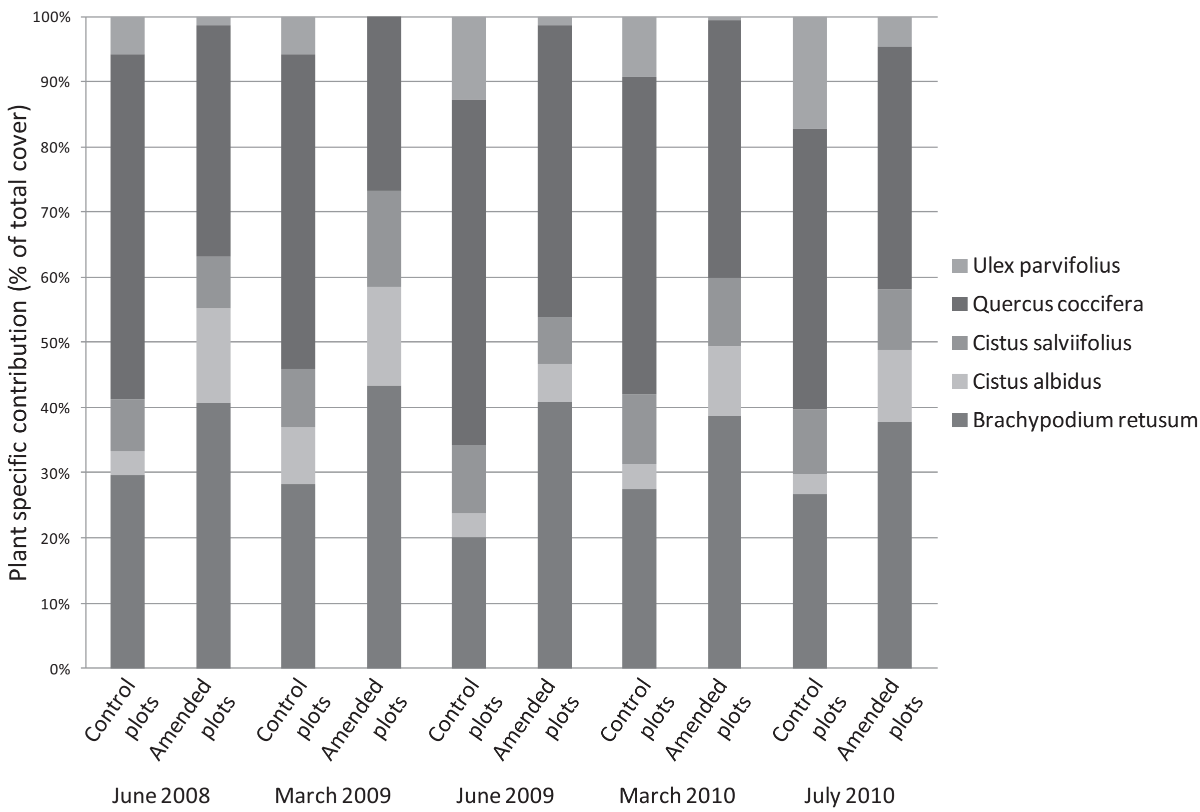

Figure 2. Evolution of each plant specific contribution at the observation dates (mean value ± SD).

Plant biomass and growth

After compost amendment, plant biomass was significantly increased for *C. albidus* and decreased for *U. parviflorus* (Kruskal-Wallis test, $p < 0.0001$) on amended plots compared to control plots. No effect was observed on *B. retusum* (Repeated measures ANOVA, $p > 0.05$), *C. albidus* and *Q. coccifera* (Kruskal-Wallis test, $p > 0.05$).

Biomass of *B. retusum* and *C. albidus* were significantly increased in June 2008 (one-way ANOVA, dose factor, $p = 0.043$ and 0.036 respectively; Fig.3) on amended plots and , then, no effect was observed (one-way ANOVA, dose factor, $p > 0.05$; Fig.3). *U. parviflorus* biomass was higher on control than on amended plots from March 2009 to July 2010 (Mann-Whitney test, $p < 0.05$; Fig.3). No significant effect of compost was observed on plant biomass for *C. salviaefolius and Q. coccifera* (Mann-Whitney test, $p > 0.05$; Fig.3). A significant date effect was detected on plant biomass for all species (Repeated measure ANOVA, $p < 0.0001$ for *B. retusum* and *Q. coccifera*; Kruskal-Wallis, $p < 0.001$ for *C. albidus*, *C. salviaefolius* and *U. parviflorus*; Fig.3). This was only due to the initial growth phase after total destruction of pre-fire aerial biomass.

Compost input had no significant effect on growth rate for *C. salviaefolius* and *Q. coccifera* (Mann-Whitney test, $p > 0.05$; Table 3) whatever the growth phase. Initial growth rate of *B. retusum* and *C. albidus* were increased by compost input between April 2008 and June 2008 (Mann-Whitney test, $p = 0.007$ and 0.036 respectively; Table 3) but no significant effect was observed during the second and the third growth seasons (Mann-Whitney test, $p > 0.05$; Table 3). *U. parviflorus* had a higher growth rate on control plots between March and June 2009 (Mann-Whitney test, $p < 0.0001$; Table 3).

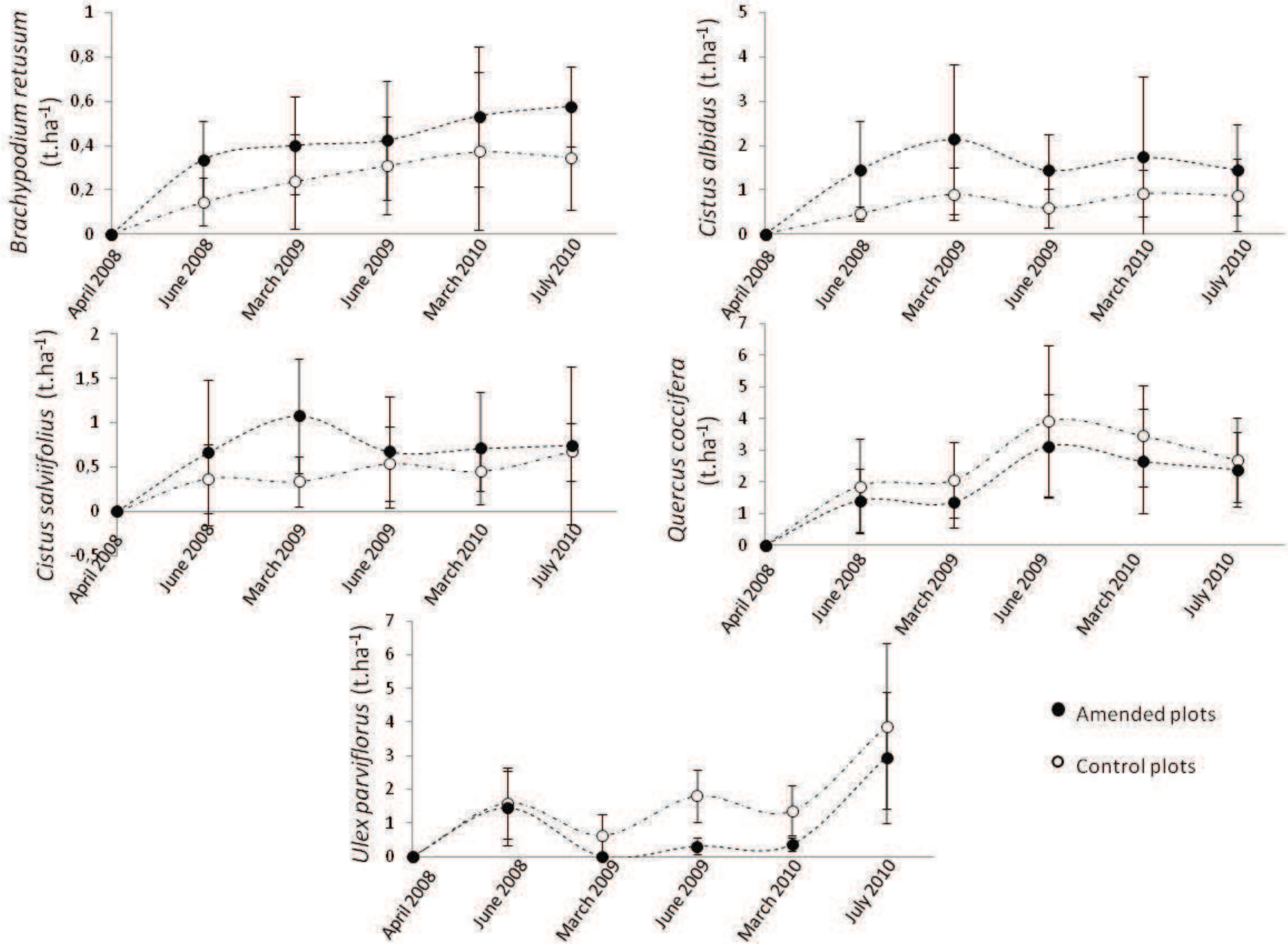

Figure 3. Evolution of plants biomass during the study period (mean value ± SD).

Table 3. Growth rate of studied species (N = 240, mean±SD). Values that differ at the 0.05 level are indicated by an exponent letter (Mann-Whitney test; a<b).

Species	April 2008 to June 2008		March 2009 to June 2009		March 2010 to June 2010	
	Control plots	Amended plots	Control plots	Amended plots	Control plots	Amended plots
B. retusum	**6.54±5.54**[a]	**13.76±10.11**[b]	1.29±5.67	0.64±3.40	-1.81±6.01	-0.24±4.22
C. albidus	**7.54±8.39**[a]	**47.56±55.74**[b]	-4.23±14.27	-21.15±33.19	4.23±9.10	-1.54±36.67
C. salvifolius	7.54±14.24	16.28±32.15	3.40±6.68	-10.59±16.73	3.59±7.15	0.80±11.13
Q. coccifera	91.87±73.16	69.27±49.04	60.67±48.18	57.72±41.21	-25.30±33.35	-8.35±40.07
U. parviflorus	45.84±56.63	17.72±39.62	**32.01±33.15**[b]	**2.50±5.69**[a]	82.59±65.98	38.19±61.50

Principal components analysis on plant biomass results showed slight differences between control and amended plots during the study period (Fig.4).

In June 2008, plant biomass was more equitably distributed between species on control plots than on amended plots according to the first axe (32.0%; Fig.4). This axe was correlated with the biomass of *C. albidus*, *C. salviaefolius* and *U. parviflorus*. The second axe explained 27.4% of the distribution and was correlated with the presence of *Q. coccifera* and *B. retusum*. The amended plots were gathered on the positive pole of the first axe which was characterized by high *C. albidus* biomass. On the negative pole of the first axe, was found an amended plot characterized by *C. salviaefolius* and *U. parviflorus*. The control plots were distributed along the second axe; some control plots were characterized by the high biomass of *Q. coccifera* whereas the other plots were characterized by *B. retusum*.

In June 2009, plant biomass on control plots was distributed along the first axe (32.6%) (Fig.4). This axe is characterized on the negative part by *C. albidus* and on the positive part by the other species. As in June 2008, the amended plots were characterized by high *C. albidus* biomass but were gathered on the negative pole of the first axe. Most of amended plots were distributed on the positive part of the second axe (25.8%) which is determined by high *B. retusum* biomass. The other species characterized the negative part of the second axe.

In July 2010, the differences were lower than in June 2008 and 2009 (Fig.4). We can observe that the correlation with the axes and plant species was different. In July 2010, *C. albidus* characterized the positive part of the second axe (26.8%) and the other species the negative part. The most of the amended plots were distributed on the positive part of this axe and the most of the control plots were on the negative part. The positive part of the first axe (37.5%) is characterized by *C. albidus*, *Q. coccifera* and *U. parviflorus* and the negative part by *B. retusum* and *C. salviaefolius*.

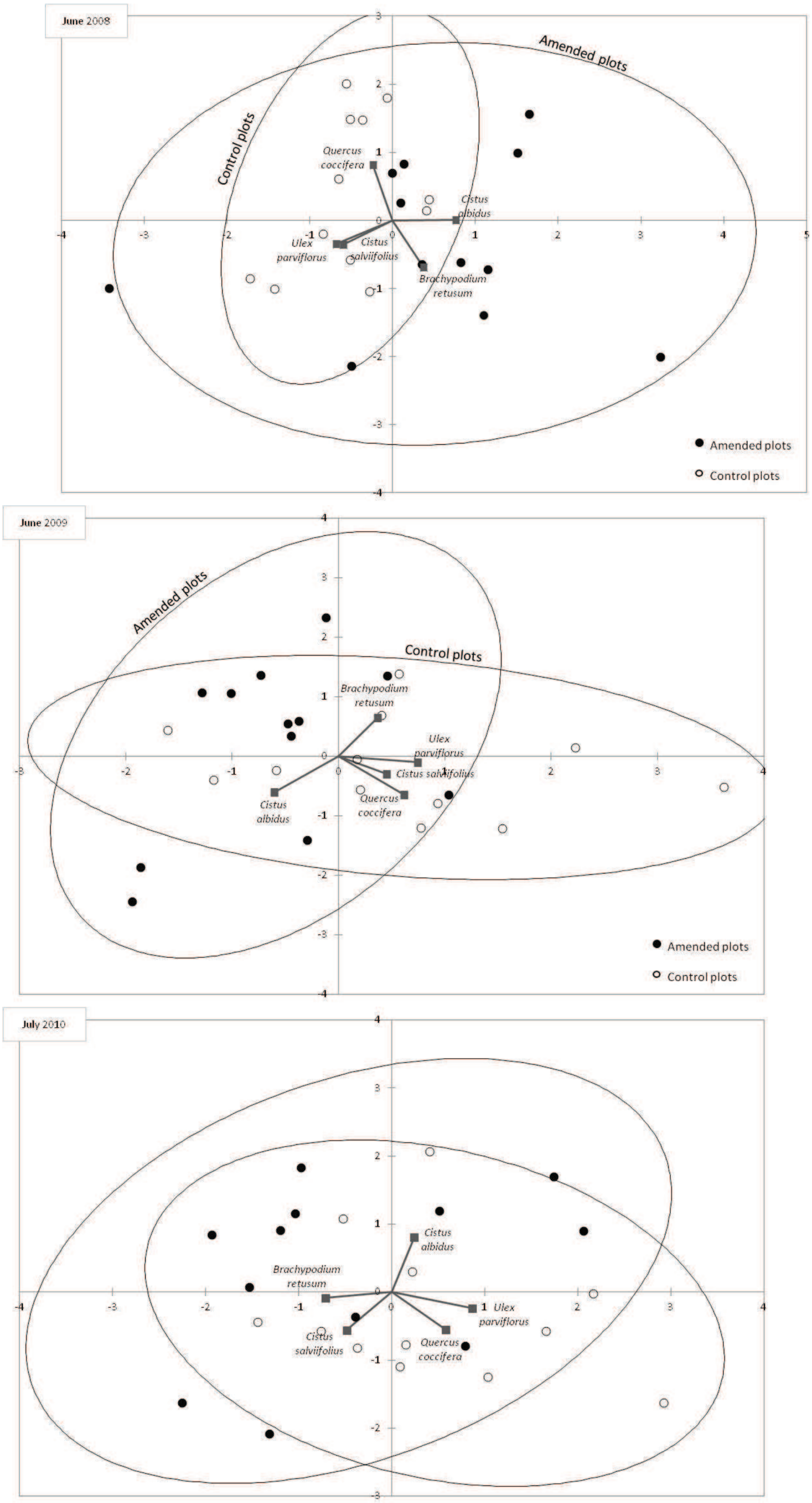

Figure 4. PCA performed on plant biomass in June 2008, June 2009 and July 2010.

Plant diversity

No effect of compost input was observed on specific richness, Shannon index and Jaccard index (Mann-Whitney test, $p < 0.05$; Table 4).

Correspondence analysis results, based on presence/absence of all species on the sub-plots, showed a more important homogeneity of species distribution on amended plots than on controls (Fig.5). Sub-plots were distributed along the first axe (10.5%).

Table 4. Species cover in July 2010 according to Braun-Blanquet indexes (N = 80; mean±SD) and Shannon index and specific richness (N = 80; mean±SD).

Species	Control plots	Amended plots
Allium sp.	0.50±0.00	0.50±0.00
Anagalis arvensis	0.50±0.00	-
Aphylanthus monspeliensis	1.21±1.22	2.25±1.21
Arenaria leptoclados	0.67±0.65	0.50±0.00
Argyrolobium zanonii	0.50±0.00	0.73±0.75
Aristolochia pistolachia	-	1.00±1.12
Asterolinum stellatum	0.61±0.52	0.50±0.00
Brachypodium retusum	21.44±21.56	34.09±25.41
Catapodium rigidum	0.50±0.00	0.50±0.00
Centrenthus calatrapa	0.50±0.00	0.50±0.00
Cerastium humilum	0.50±0.00	0.50±0.00
Cistus albidus	10.07±11.43	13.26±16.03
Cistus salvaefolius	12.92±11.02	21.09±16.69
Crepis micranta	0.50±0.00	0.50±0.00
Dorycnium hirsutum	-	0.50±0.00
Dorycnium pentaphyllum	3.41±3.88	8.97±8.46
Erodium cicutarium	0.50±0.00	-
Euphorbia exigua	4.09±11.11	0.50±0.00
Filago	0.50±0.00	0.50±0.00
Gallium parisiense	0.60±0.49	0.58±0.45
Helianthenum hirtum	1.64±1.31	0.95±1.01
Hieracium murorum	0.50±0.00	0.50±0.00
Lactua perennis	0.50±0.00	-
Leuzea conifer	0.50±0.00	0.50±0.00
Linaria simplex	0.50±0.00	0.50±0.00
Muscari comosa	0.50±0.00	0.50±0.00
Ononis minutissima	-	0.50±0.00
Ornithogalum umbellatum	0.50±0.00	0.50±0.00
Phillyrea angustifolia	16.50±22.63	18.70±25.54
Quercus coccifera	49.54±27.10	50.09±22.19
Querus ilex	40.38±28.24	15.00±0.00
Rosmarinus officinalis	0.78±0.83	0.50±0.00
Rubia perigrina	0.72±0.72	0.88±0.92
Sanguisorba minor	0.50±0.00	-
Sedum sp.	0.50±0.00	0.50±0.00
Sisymbrium uncivatum	0.50±0.00	0.50±0.00
Teucrium chamaedrys	0.81±0.84	0.84±0.88
Teucrium polium	0.50±0.00	0.50±0.00
Tulipa sp.	0.50±0.00	0.50±0.00
Ulex parviforus	15.94±16.21	7.04±9.48
Valantia muralis	0.50±0.00	-
Vulpia ciliava	0.50±0.00	-
Vulpia unilateralis	0.50±0.00	-
Shannon index	1.12±0.33	1.14±0.29
Specific richness	8.05±3.06	7.60±2.12

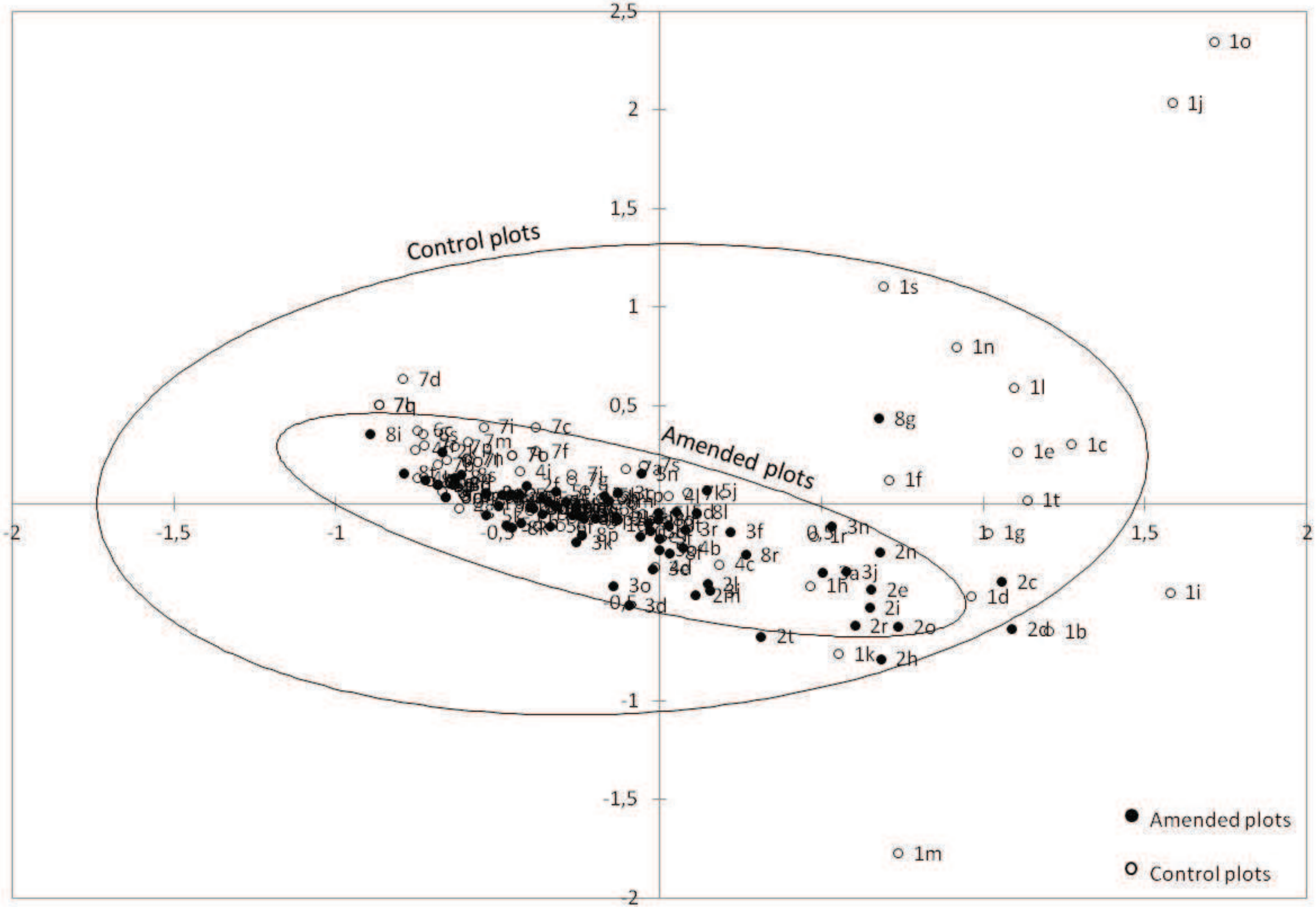

Figure 5. Correspondence analysis on species presence/absence.

Discussion

According to other studies (Navas *et al.*, 1999; Guerrero *et al.*, 2000; Caravaca *et al.*, 2003; Martínez *et al.*, 2003; Curtis and Claasen, 2009; Larchevêque *et al.*, 2010), compost amendment induce an increase of plant biomass but Walter *et al.* (2006) observed the increase of annual plants biomass and the decrease of perennial species. In our study, plants response to amendment varied with low and temporary effects. Even if statistical results were not significant, we observed that compost input tended to increase biomass of *B. retusum*, *C. albidus* and *C. salviaefolius* and to decrease biomass of *Q. coccifera* and *U. parviflorus*.

As described by Berendse (1998) and Larchevêque *et al.* (2010), we observed that the grass species *B. retusum* had the fastest response to compost amendment. Moreover, its cover was still more important on amended plots. It might be explained by the development of roots in the superficial soil layer (Caturla *et al.*, 2000) which allow an easier access to nutrients from compost.

We hypothesized that obligate-seeder species like *C. albidus* and *C. salviaefolius* would have been more sensitive to compost because their shallow roots (Keeley, 1986) could directly access to nutrients, especially the young seedlings. *B. retusum* response was similar to *Cistus* species which were also responsive to compost amendment, whereas it is a resprouter and *Cistus* species obligate-seeders. The similarity in biomass responses might be due to the fact that the three species exhibit shallow roots. Root-traits seemed to be more determinant to plant response to compost amendment (Paula and Pausas, 2011) rather than regeneration strategy.

Q. coccifera which has deeper roots (Rambal, 1984) and which is resprouter species was more independent from compost nutrients for its growth in case of mulch amendment. On the other hand, *Q. coccifera* may resprout by using nutrient reserves from lignotubers (James, 1984). These life-traits could be explained that *Q. coccifera* did not respond to amendment even when amendment was spread at the early stage of post-fire succession after complete burning of above-ground biomass. As suggested by Román *et al.* (2003), species adaptation to dry soils under the Mediterranean climate can be the reason why they did not respond to amendment.

Biomass decrease of *C. albidus* and *C. salviaefolius* during spring 2009 might be correlated with rainfalls which were lower in 2009 than in 2008. Plants might have limited their total leaf surface to decrease water loss through transpiration. This decrease might be result to mortality of seedling due to their insufficient roots system.

At the opposite of *B. retusum, U. parviflorus* biomass was lower on amended than on control plots after one year of growth. As observed by Greenle and Callaway (1996) and Raventós *et al.* (2010), the higher growth of *B. restusum* during the early stage of restoration can have a facilitation effect for seedlings of seeder species. The increased soil cover by *B. retusum* biomass could have protect *U. parviflorus* seedlings from intense irradiance and desiccation. But a stronger competition between species could have been induced by the rapid growth of *B. retusum* and impeded nutrition and growth of *U. parviflorus* seedlings. According to Rundel (1983) and Arianoutsou and Thanos (1994), N-fixing capacity is a nutritional advantage in frequently burned ecosystem where N is the more limiting element. Due to its N-fixing symbiotic activity, *U. parviflorus* is less influenced by compost input than other species. Thus it might be less competitive in ecosystems with higher nutrient level. As suggested by Hulme (1996) and Vitousek and Field (1999), grazing is more important on legumes species due to their higher protein content, especially during seedling stage. Compost input might have induced richer plant protein content and increase predation by rabbits or herbivorous insects on *U. parviflorus.* Indeed we observed during field observations that *U. parviflorus* seedlings were more frequently grazed than the other shrub species. Moreover *U. parviflorus* N content on amended plots was higher than on control plots (Cellier *et al.*, 2012, chapter III.2). This could partly explain that no *U. parviflorus* seedlings were observed on transects on amended plots. Between March and June 2009, the lower growth on amended plots might be caused by a higher concurrence between young plant of *U. parviflorus* and other species than for 1-year old plant on control plot. Between March and July 2010, its growth rate was equal on amended and control plots as during the first growth period. Thus, it seems that compost amendment had a negative effect on *U. parviflorus* growth during the first year after amended which was a contrary result to what was expected.

Factorial maps of plant biomass showed a higher heterogeneity on amended plots than on control plots in June 2008. This effect decreased in June 2009 and disappeared in July 2010. Input of compost seemed to allow a greater plant development but it was masked by the important variability of responses on the different plants and plots.

It appeared the plots discrimination was more important according to studied species than to compost input. We also observed the opposition between *C. albidus* and *C. salviaefolius*. In June 2008 and 2009, *C. albidus* was the more correlated with amended plots and *C. salviaefolius* and *U. parviflorus* with control plots. In June 2008, the main part of the plots distribution was explained by seedling species and the other part by resprouting species. Thus, in the early months after amendment, seedling species were more specific to plots treatment than resprouting species.

Analysis showed that compost input had no effect on biodiversity or specific richness but a higher homogeneity of amended plots. Control plots higher heterogeneity was caused by plants which were sub-plot specific. Amendment decreased spatial heterogeneity of biodiversity due to seed bank or local soil characteristics observed about plants biomass. Compost amendment created better and more homogenous soil conditions for seed germination whilst, in early post-fire succession, favorable soil conditions are rather scarce.

Contrary to what is frequently stated to limit compost uses in natural ecosystems (Pinamonti *et al.*, 1997; Clemente *et al.*, 2010; Farrell *et al.*, 2010), plant biodiversity of the Mediterranean ecosystem studied was not affected more than 2 years after amendment. The indigenous flora was not modified and no adventicious vegetation colonized the amended plots.

Conclusion

Compost effects were lower than expected on plant growth after fire but plant biodiversity was not modified. It mainly allowed a higher development of grass species *B. retusum* the first months after amendment. Although this amendment had no significant effect on vegetation, it induces an increase of biomass which was masked by the heterogeneity of the response. Plant responses were more affected by their life traits than by the compost input. Higher amount of compost or less mature compost might be amended to obtain higher effects but it could affect biodiversity or induce eutrophication process. However, further studies are needed to better understand these results and, especially the results of *U. parviflorus* one year after amendment.

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Chapitre III - Apport de compost en garrigue.

Effets de l'amendement d'un compost de boues de station d'épuration sur les nutriments et les éléments trace d'un écosystème méditerranéen après incendie.

Effects of sewage sludge compost amendment on nutrients and trace elements in a Mediterranean ecosystem after fire

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Abstract

Backgrounds and aims In Mediterranean frequently burnt areas, the decrease of soil fertility leads to regressive vegetation dynamics. Organic amendments could help to accelerate post-fire ecosystem resilience, by improving soil properties and stimulating plant growth. This study was conducted to assess the potential of a composted biosolid to restore an early post-fire shrubland ecosystem.

Methods About 50t.ha^{-1} of fresh co-composted sewage sludge and green wastes were surface applied seven months after fire. We monitored over a two-year period organic matter and nutrient transfers to soil, nutrient responses of dominant plant species, and ecosystem contamination by potentially toxic trace elements.

Results Over the experimental survey, compost rapidly and durably improved soil P_2O_5, MgO and K_2O content, and temporarily increased N-(NO_3^-+NO_2^-) content. Plant nutrition was improved more or less durably depending species. The most positive compost effect was on plant and soil phosphorus content. Plant nutrient storage was not improved two years after amendment, suggesting luxury consumption. No contamination by trace elements was detected in soil and plant.

Conclusions The use of compost after fire could help for rapidly restoring soil fertility and improving plant nutrition. The increase of soil nutrient pools after amendment emphazised the diversity of plant nutritional traits. Eutrophication could occur from high compost and soil P_2O_5 content.

Keywords: burnt ecosystem, phosphorus, plant nutrition, sewage sludge compost

Abbreviations:

PCA Principal components analysis

CEC Cationic exchange capacity

Introduction

Forest fires are considered as the main Mediterranean ecosystem perturbation (Whelan, 1995). The mediterranean-type shrublands are highly resilient to wildfires (Keeley, 1986; Trabaud, 1994) as a consequence of the ability of plant species to rapidly recover from fire by means of a wide diversity of regeneration strategies from resprouting to fire-prone seed germination (Trabaud and Lepart, 1980; Lloret, 1998). However, the abandonment of agricultural lands since the 1970s and the increase in human population and activities have resulted in a dramatic increase in the number and size of fires in all Mediterranean regions (Pausas, 2004). Fire recurrence is also favored by the Mediterranean climate characterized by long dry summer and strong winds (Bagnouls and Gaussen, 1957; Scarascia-Mugnozza *et al.*, 2000; De Luis *et al.,* 2001). Fire frequency, duration and intensity are the main factors determining impacts on ecosystem: the more fires are recurrent and intense, the more their effects pronounced (Boerner, 1982). Firstly, fires induced partial or total destruction of plant cover and superficial organic layers (Hernández *et al.,* 1997; Guerrero *et al.*, 2001). Then, soil stability decrease due to the loss of organic layer and the increase of erosion risk (Kutiel and Inbar, 1993; Hart *et al.*, 2005). Immediately after fire, nutrient availability increases due to ashes and release of soil minerals (Kutiel and Inbar, 1993; Dumontet *et al.*, 1996). However, part of the nutrients is oxidized and volatilized during fires (Fisher and Binkley, 2000). Nutrient losses are more important after fire because of wind erosion, runoff (DeBano and Conrad, 1978; Boerner, 1982; Gimeno-Garcia *et al.*, 2000) and leaching into groundwater (Mohamed *et al.*, 2007). When these climatic events occur in a short delay after the fire, their effects are much more pronounced (Thomas *et al.*, 1999; Gonzalez-Pérez *et al.*, 2004). Adding to physical and chemical modifications, forest fires disturb soil biological properties (DeBano *et al.*, 1998), mainly in the superficial soil layer (Neary *et al.*, 1999). Recurrent fires are known to progressively impoverish soil in organic matter and nutrients (Reich *et al.*, 2001; Eugenio *et al.,* 2006; Knicker, 2007)) and frequently burnt soils are often nitrogen and phosphorus depleted (Ferran *et al.*, 2005). These fire effects on soil worsen the summer water deficit, which is already the main constraint for Mediterranean ecosystems. Post-fire plant communities are much more water limited because cumulative losses of organic matter and erosion decrease soil water retention. This in turn limits nutrient availability and reduce plant nutrient uptake. Consequently, post-fire Mediterranean ecosystems are dominated by evergreen sclerophyllous shrubs (Rundel, 1988; Aerts, 1995; Archibold, 1995) which are stress-tolerant species adapted to infertile habitats. The development of persistent leaves with low nutrient contents and slow decomposition rates induce a low restitution of nutrients to soil (Rundel, 1988; Aerts, 1995). This leads to the persistence and the extension of the most oligotrophic plant communities. In calcareous lower Provence (southeastern France), the most frequently burnt and degraded soils are colonized by *Quercus coccifera* L. - shrublands (Trabaud, 1987; Barbero, 1990). Despite the high resilience of Mediterranean shrublands, vegetation recolonization after fire becomes more and more difficult as fire frequency increases, resulting in patchy vegetation cover and accelerating soil degradation. The use of organic biosolids as amendments is increasing for soil restoration and vegetation regeneration in frequently burnt or degraded ecosystems (Guerrero *et al.*, 2000; Caravaca *et al.*, 2003; Martinez *et al.*, 2003; Larchevêque *et al.,* 2006; Walter *et al.*, 2006; Kowaljow and Mazzarino, 2007; Hemmat *et al.*, 2010; Turrión *et al.*, 2010). In contrast to mineral fertilizers, composted biosolids are rich in humified organic matter and can be used as a slow-release source of nutrients (Barker, 1997). Compost amendments improve physical, chemical and biological properties of soils, in particular by increasing available nutrients mainly in the organic soil fractions (Bodet and Carioli, 2001; Chenu, 2002; Larchevêque *et al.*, 2006a; Annabi *et al.*, 2007). Composted biosolids have a high water retention capacity (Giusquiani *et al.*, 1995) which induces an increase of soil water content (Villar *et al.,* 1998). These modifications positively affect plant cover through an improvement of plant nutrition and growth (Villar *et al.*, 1998; Guerrero *et al.,* 2001; Caravaca *et al.*, 2003; Larchevêque *et al.*, 2005, 2006b), and contribute to reduce the risk of erosion (Guerrero *et al.,* 2000).

In France, there are few studies on restoration of burnt Mediterranean ecosystems using organic amendments, despite large burnt surfaces (about 4360 ha in 2012, in the French Mediterranean region, Direction de la Sécurité Civile). Vegetation plays an essential role in ecosystem reconstitution after fire, by protecting soil against erosion, enriching the soil with organic matter and favoring soil biological activity. Fertility of frequently burnt soils could be potentially restored by spreading biosolids in order to enhance vegetation growth after fire and limit erosion on bare soil. In this study we examine the potential of a sewage sludge and green wastes compost to speed up ecosystem restoration in a burnt Mediterranean shrubland. The aim of this study was to monitor *in situ* the effect of a single surface compost input after fire on soil fertility and plant nutrition for 2 years following compost addition. Our objectives were (1) to study organic matter and nutrient possible transfers from compost to the burnt soil, (2) to test the capacity of the dominant plant species (*Q. coccifera*, *Cistus albidus* L., *Cistus salviaefolius* L., *Ulex parviflorus* Pourr. and *Brachypodium retusum* Pers) to absorb and accumulate the exogenous nutrients in the early stages of regeneration after fire, (3) to study possible plant and soil contamination by trace elements after amendment.

Materials and methods

Study site

The experiment was carried out in a burnt site of about 82ha on the plateau of Arbois (Provence, Southeastern France; 43°27'16.28"N – 5°17'57.21"E, alt. 216m). The fire occurred the 4th September 2007 and totally burnt the above-ground vegetation. The initial plant community was dominated by the shrub species *Cistus albidus* L., *Cistus salviaefolius* L., *Quercus coccifera* L. and *Ulex parviflorus* Pourr*.,* the herb *Brachypodium retusum (Pers.) P. Beauv.,* as visible in the adjacent unburnt vegetation. Some sparse clumps of *Quercus ilex L.* were also growing in the site. The study site is characterized by a Mediterranean climate with dry, hot summers and wet and temperate winters. The mean annual precipitation and temperature are 740mm and 13.6°C, respectively (Fig.1). The calcareous soil is a Rendzic Leptosol according to WRB (2006) with silty-clayey texture. The parent bed-rock was frequently apparent, resulting in bare zones deprived of soil and vegetation.

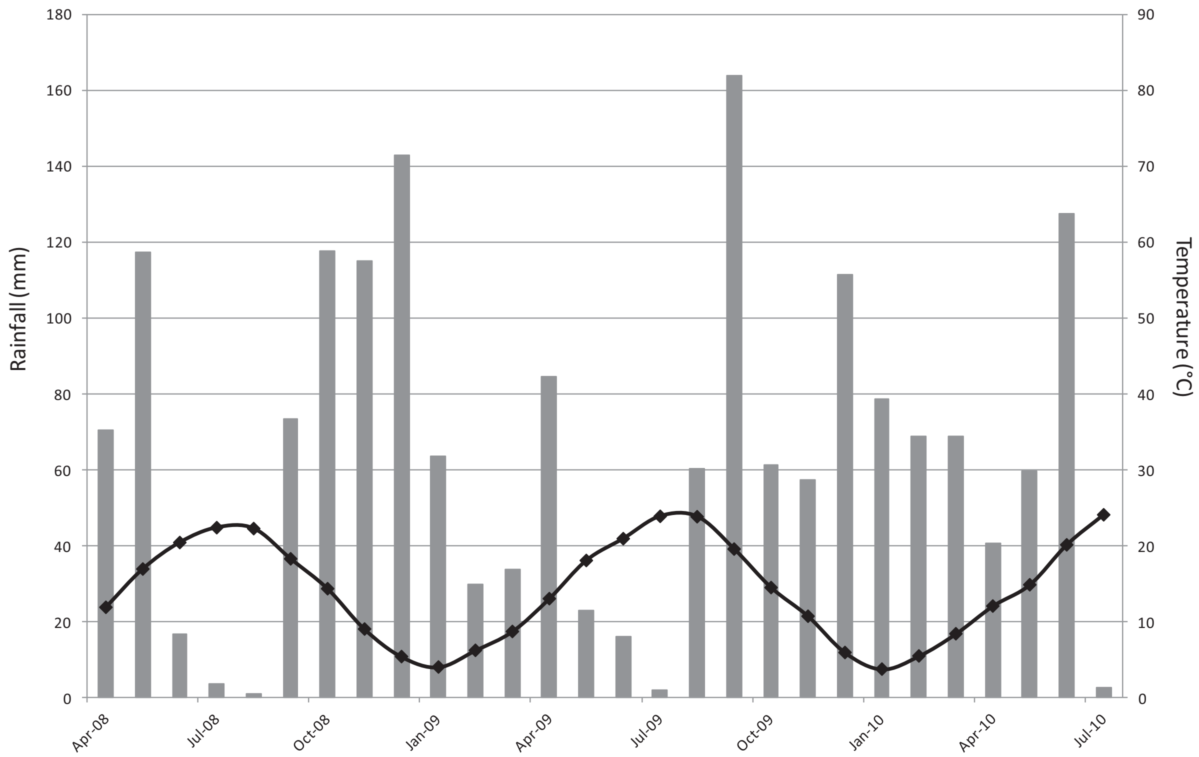

Figure 1. Monthly rainfall and mean air temperature (Météo France) during the study period. One-way ANOVA: *p<0.05, ** p<0.01, *** p<0.01

Compost characteristics and experimental design

The compost was produced by a local company (Biotechna, Ensuès-La-Redonne, France). It was made with municipal sewage sludge mixed with pin barks and green wastes (1/3 volume each). After being composted for 30 days at 75°C to kill pathogenic microorganisms and decompose phytotoxic substances, then the mixture was sieved (<40 mm mesh) to remove the large bark pieces and stored in swathes. The swathes were mixed several times over the next 6 months to promote organic matter humification. The final compost met the French legal standards (NF U 44-095, 2002) for pathogenic microorganisms, organic trace elements and trace metals, and no viable seeds remained.

The experimental design was a randomized block of eight 300 m^2 plots (15m x 20m) delimited in a 0.5 ha flat zone of the burnt site. Four plots were amended with compost and four were the controls. About $50 t.ha^{-1}$ of fresh compost were homogeneously spread by hand on soil surface 7 months after fire before plant regrowth (1^{st} April 2008).

Field procedures

The five species studied can be separated into two groups based on life-form, *i.e*. a perennial rhizomatous herb (*Brachypodium retusum)*, and four woody species. Among the latter, *Quercus coccifera* is an evergreen sclerophyllous shrub, *Cistus albidus* and *Cistus salviaefolius* are semi-deciduous malacophyllous shrubs and *Ulex parviflorus* a legume with thorny photosynthetic stems. The regenerative strategy of the five species studied is fire-promoted, either as resprouters (*Q. coccifera* and *B. retusum*) or obligate-seeders (*C. albidus*, *C. salviaefolius* and *U. parviflorus*).

Plant sampling was realized in July 2009 and July 2010. Four plant samples per compost rate (control and amended plots) and per species were analyzed (each sample corresponding to a composite of 3 to 20 individuals, depending on their size, randomly collected on each 300 m^2 plot). Only leaves were collected for *Q. coccifera,* *C. albidus* and *C. salviaefolius*, while all photosynthetic organs were collected for *B.* retusum (leaves and floral stems*)* and *U. parviflorus.*

Table 1. Soil (0-20 cm: maximum depth; N = 4 to 20; mean±SD) and compost initial characteristics

		Soil		Compost
		Control plots	Amended plots	
Organic C	$g.kg^{-1}$	41.26±9.56	35.24±9.94	312.4
Total carbonates	$g.kg^{-1}$	13.44±10.51	9.94±6.88	-
Total N	$g.kg^{-1}$	2.66±0.66	2.31±0.38	31.73
$N\text{-}NH_4^+$	$g.kg^{-1}$	0.027±0.007	0.031±0.011	2.49
$N\text{-}(NO_3^-+NO^{2-})$	$g.kg^{-1}$	0.009±0.004	0.007±0.004	0.002
P_2O_5	$g.kg^{-1}$	0.018±0.006	0.017±0.006	30.7
K_2O	$g.kg^{-1}$	0.428±0.088	0.435±0.060	8.1
MgO	$g.kg^{-1}$	0.251±0.058	0.249±0.037	7.9
Na_2O	$g.kg^{-1}$	0.024±0.003	0.022±0.004	-
CaO	$g.kg^{-1}$	-	-	99.30
CEC	$Cmol^+.kg^{-1}$	19.41±2.82	19.30±2.12	-
Total Cd	$mg.kg^{-1}$	0.26±0.06	0.22±0.02	0.81
Total Cr	$mg.kg^{-1}$	81.23±5.98	78.08±8.18	22.91
Total Cu	$mg.kg^{-1}$	21.90±2.30	19.83±1.32	181.12
Total Hg	$mg.kg^{-1}$	0.05±0.01	0.05±0.01	0.4
Total Ni	$mg.kg^{-1}$	49.50±0.94	49.88±1.75	15.06
Total Zn	$mg.kg^{-1}$	92.10±10.29	84.45±3.59	325.3
Humidity	Soil : % Compost : %FM	14.24±2.69	13.88±2.35	28.2
Bulk density (unsieved fraction)	$t.m^{-3}$	1.08±0.16	1.06±0.16	0.31
Clay	% *	33.75±4.99	32.93±3.93	-
Silt	% *	55.40±5.16	57.30±2.87	-
Sand	% *	10.85±0.45	9.78±1.03	-

All nutrient and trace element values are expressed in kg^{-1} of soil dry matter (DM)
* : % of sieved fraction ≤ 2mm ; FM : Fresh Matter.

Plant nutrient accumulation was determined by monitoring plant aboveground biomass in July 2010. It was estimated indirectly by the point intercept method (Jonasson, 1983) which is based on the relationship between plant biomass and the number of contacts with a fine metal rod (5mm diameter) passed vertically through the vegetation to the ground. In each experimental plot, we set 3 transects of 2 m long along which the number of rod contacts with plant species were noted at 10-cm intervals. We previously established in the unburnt neighboring vegetation of the study area, that aboveground biomass of each species studied fitted a positive linear regression with total number of contacts (Cellier, 2012) or height of the highest contact (for *U. parviflorus*, see Montès et al 2008 for field procedure and regression equation) along vegetation transects. Leaf and stem contacts are considered separately for *Q coccifera* and *Cistus* species. We used the regressions to estimate plant biomass on the experimental plots.

Before amendment, five soil samples per plot were collected to determine initial physico-chemical characteristics. Soil and compost initial characteristics are presented in Table 1. Soil samples were collected before amendement (at the end of March 2008) six weeks after amendment (mid-May 08) and then every 3 months from July 2008 to April 2010. Each analyzed soil sample was a composite of three sub-samples for each plot. Only organic-mineral soil (A horizon or A/S horizon) was collected with a drill (0 to 20 cm depth), after removing surface compost and plant litter. Sub-samples were immediately refrigerated after sampling and then sieved and frozen at the laboratory for determination of mineral N.

Laboratory procedures

Plant and soil analyses were performed according to French or International norms (Table 2).

Nitrogen, phosphorus, cations (Ca, Mg, K), trace element (B, Fe, Mn, Cu and Zn) concentrations were determined in plant samples. Trace elements were determined only in plant samples collected in July 2010.

Table 2 Norms used for soil analysis.

	Methods	Norms
P_2O_5	Olsen method	NF ISO 11263
MgO	ammonium acetate exchangeable (AAF)	NF X 31-108
K_2O	ammonium acetate exchangeable (EAF)	NF X 31-108
Total Cu	ICP-AES	NF EN ISO 11885
Total Zn	ICP-AES	NF EN ISO 11885
Total Cr	ICP-AES	NF EN ISO 11885
Total Ni	ICP-AES	NF EN ISO 11885
Total Cd	ICP-MS	INRA* method
Total Hg	INRA method	INRA method
CEC	Metson method	NF X 31-130
Carbonates	Bernard calcimeter	NF ISO 10693
Total C	Dry combustion	NF ISO 10694
Total N	Dry combustion	NF ISO 13878
Mineral N	KCl extraction and colorimetric measure	NF ISO 14256-2
Bulk density	Cylinder method	NF X 31-501
Texture	Sedimentation	NF ISO 11277
Soil water content	Gravimetric method	NF ISO 11465

Plant material was air-dried until constant weight, and crushed in a trace metal-free grinder (Foss Tecator Sample Mill 1093 Cyclotec, Foss Tecator, Nanterre, France). Nitrogen was determined by dry combustion and thermic conductimetry (Dumas method), phosphorus, cations and the trace elements Cu, Zn, Fe, Mn, B were measured after digestion in aqua regia using inductive-coupled plasma – atomic emission spectroscopy (ISO 11-885 norm).

Soil samples were first 2-mm mesh sieved. Sub-samples of 2-mm fraction were then air-dried for determination of physico-chemical parameters; some other sub-samples were weighted before and after oven drying 24h at 105°C for calculation of soil water content. Total carbon and total nitrogen were determined after dry combustion using an element analyser (NA 1500 CN, Fisons Instruments, Manchester, UK) after samples were ground (MM400, Retsch GmbH, Haan, Germany) and homogenized. Organic carbon concentrations were obtained by the difference between total carbon and carbonates determined by a volumetric method with a Bernard calcimeter.

Mineral N was extracted after soil lyophilization with a 1 *M* KCl solution. $N\text{-}NH_4^+$ was then analyzed by Berthelot method, $N\text{-}(NO_3^-+NO_2^-)$ by Griess & Ilossay method. Available phosphorus was determined by Olsen method. Exchangeable Mg and K were extracted by ammonium acetate. Mg was then analyzed by atomic absorption spectrophotometry, K by flame emission spectrophotometry. Total exchange capacity was measured by soil percolation with an ammonium acetate solution and analysis of ammonium ions exchanged (Metson method). Total trace metal (Cu, Zn, Cr, Ni, Cd, Hg) concentrations were measured after digestion in *aqua regia*) using inductive coupled plasma-atomic emission spectroscopy. Trace elements were determined in soil two years after amendment (April 2010).

Bulk density was determined on soil samples collected in the top 10 cm with a Siegrist steel cylinder, after drying at 105°C.

Statistical analysis and calculations

The effects of compost and sampling date on soil nutrients were assessed using two-way ANOVAs combined with Tukey test (Zar, 1984). If any interaction occurred between the two studied factors one-way ANOVAs were performed. Conditions of normality and variance homogeneity were verified by Shapiro-Wilks and Bartlett tests, respectively. In the case of non normality and non homogeneity of variances, the data were ln or square-root transformed before applying ANOVAs.

For each species, nutrient stocks were calculated from leaf nutrient concentrations and leaf biomass determined in July 2010 and weighed by species cover (for *B. retusum* and *U. parviflorus*, photosynthetic organs were considered for nutrients and biomass). We determined species cover using point-intercept data obtained along vegetation transects in July 2010. Species cover data and regression equations for biomass calculation are given in Cellier (2012). Leaf biomass was used for calculating nutrient stocks in *Q. coccifera*, *C. albidus* and *C. salviaefolius*, whereas biomass of photosynthetic organs was considered for *B. retusum* and *U. parviflorus*.

Soil nutrient and trace element stocks ($g.m^{-2}$) were calculated as:

$$S_e = (C_e\ \rho_d\ D).10^3$$

with C_e, element (nutrient or trace element) concentration ($g.kg^{-1}$), ρ_d bulk density, and D, soil sampling depth (0.2m).

Mann-Whitney test was used to compare plant and soil trace element concentrations and stocks in control versus amended plots at the end of the study period.

Principal component analysis (PCA) was performed on plant element stocks to assess plant specific strategy of nutrient accumulation during early growth after fire.

Significant level was considered to be 95%. The software XLSTAT 2012.4.02 (Addinsoft 1995-2012) was used for statistical analysis.

Results

Plant nutrients and trace elements

Plant nutrient contents were differently affected by compost amendment (Fig.2, only significant results are shown for compost or date effects). In all species studied except *Q. coccifera*, P concentration were significantly increased in amended plots (one-way ANOVA, $p < 0.05$) at both dates, but only in July 2010 in *C. albidus.* N concentration was higher in amended plots at both dates for *U. parviflorus* (two-way ANOVA, $p = 0.001$) and only in July 2009 for *B. retusum* (one-way ANOVA, $p = 0.040$).

Cation concentrations showed both compost and date effects, depending on species and cation.

Foliar K concentration on amended plots was higher than on control in *C. salviaefolius* only in July 2009, whereas it was lower than in control at the same date in *B. retusum* (one-way ANOVA, $p= 0.025$ and $p=0.013$ respectively). This last effect was also observed in *C. albidus* at both dates, K concentration on amended plots being lower than on control (two-ways ANOVA, $p = 0.043$).

Calcium concentrations were lower in amended plots than on control in *U. parviflorus* (two-way ANOVA, $p= 0.029$) and in *Q. coccifera* only in July 2009 (one-way ANOVA,

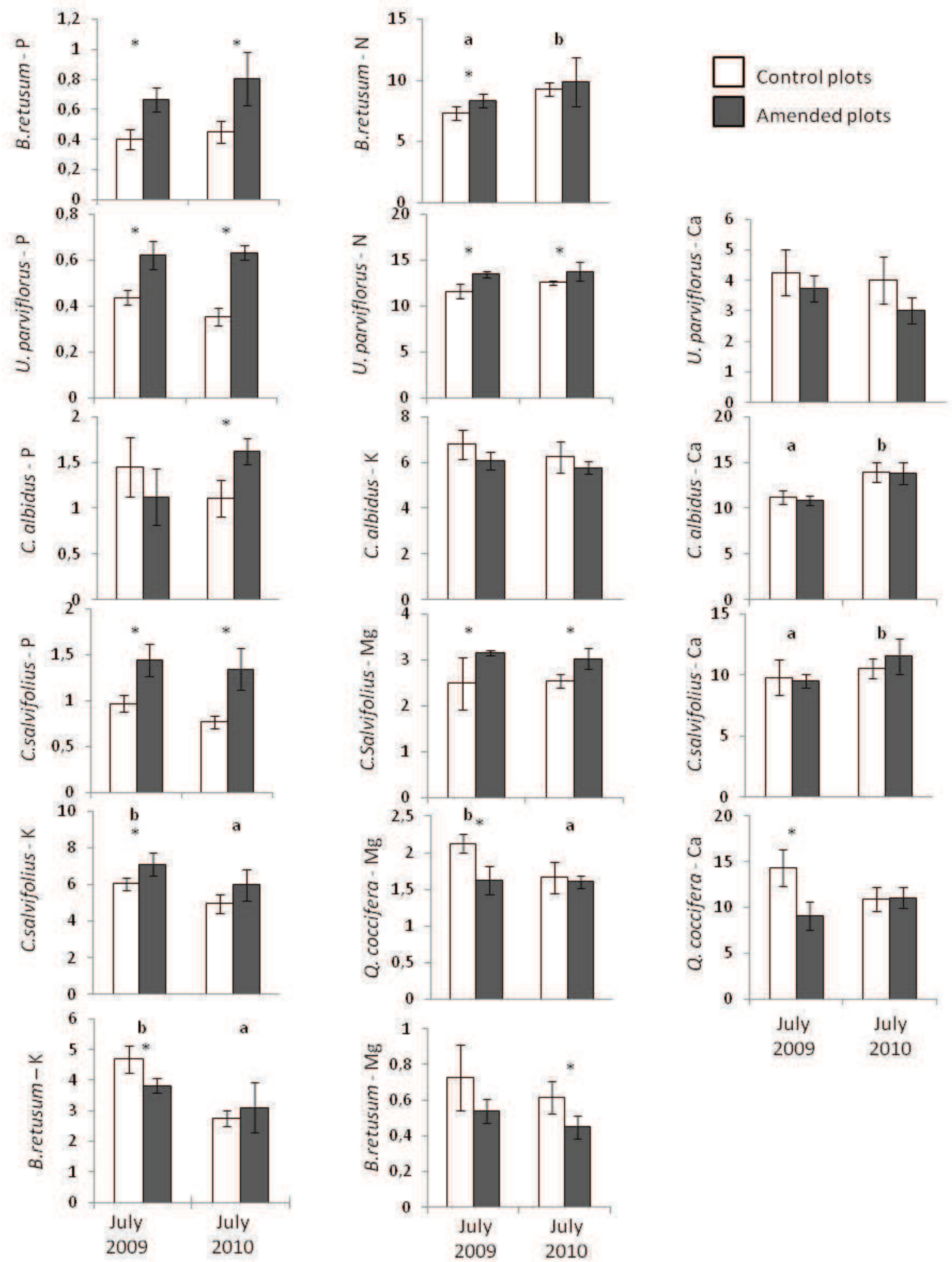

Figure 2. Nutrient concentrations in photosynthetic organs of the five species studied (mg.g^{-1} of plant dry matter; N = 4; mean±SD). Significant differences at the 0.05 level: date effect (two-way ANOVA) a<b; compost effect (one-way ANOVA): * p < 0.05

p = 0.006). For *Cistus* species, a date effect was observed, Ca concentrations in July 2009 being lower than in July 2010 (two-way ANOVA, p = 0.001 for *C. albidus*, p = 0.032 for *C. salviaefolius*).

Mg concentration in *C. salviaefolius* was higher in amended plots (two-way ANOVA, p = 0.002). Mg concentration was lower in amended plots than in control in July 2009 in *Q. coccifera* (one-way ANOVA, p = 0.005) in July 2010 in *B. retusum* (two-way ANOVA, p = 0.009*).* A date effect was also observed in *Q. coccifera* (two-way ANOVA, p = 0.011).

Compost had no effect on trace element concentrations in *Q. coccifera*, *C. albidus* and *C. salviaefolius* leaves (Table 3). Fe, Mn and Zn concentrations were significantly lower on amended plots in *B. retusum* and *U. parviflorus*, as well as Cu concentration in *U. parviflorus* (Mann-Whitney test, $p<0.05$).

Plant nutrient stocks were weakly affected by compost amendment, depending on biomass and nutrient responses (Table 4). There was no compost effect on nutrient stocks in *Q. coccifera*, *C. albidus* and *C. salviaefolius*. In *U. parviflorus*, Cu and Zn stocks were significantly lower on amended plots, twenty-eight months after amendment (Table 4). In *B. retusum*, only P stocks were significantly higher on amended plots.

Principal component analysis was performed on plant element stocks, including nutrients and only the trace elements Cu and Zn, in order to discriminate species storage strategies when increasing soil nutrients and potentially toxic trace elements. Indeed, plant concentrations and stocks in Fe, Mn and B were not or weakly modified by amendment and thus not considered for the PCA. Results of PCA showed some differences in species responses. (Fig.3). The first axis explained 88.33% of species distribution on the factorial map and was mainly correlated with the macronutrients Mg, Zn, K, Cu N and P, ordered by decreasing correlation coefficients (0.974 to 0.933). *Cistus* species and *B. retusum* gathered on the negative part of the axis, showing the lowest element stocks. *U. parviflorus* and *Q. coccifera* were globally distributed along the positive part of the first axis. Along the second axis (5.57% of variance) the species were mainly discriminated by high N stocks on the negative part and high Ca stocks on the positive part, *U. parviflorus* and *Q. coccifera* exhibiting an opposite response.

Table 3 Trace element concentrations in studied plants in July 2010 (mg.kg-1 of plant dry matter; N = 4; mean±SD). Values that differ at the 0.05 level are indicated by an exponent letter (Mann-Whitney test; a<b).

Species	Trace elements	Control plots	Amended plots
B. retusum	B	7.75±0.5	5.75±1.5
	Cu	11.5±1.3	11.5±1.3
	Zn	**37.5±2.1^{b}**	**27±2.9^{a}**
	Mn	**63.5±16.4^{b}**	**24.1±16.8^{a}**
	Fe	**2141.5±729.9^{b}**	**739±351.2^{a}**
C. albidus	B	21.75±2.22	20.5±1.29
	Cu	9.5±1.3	8.25±0.5
	Zn	49.5±8.06	45.25±1.71
	Mn	30.75±18.71	16.75±1.71
	Fe	271.5±137.5	171.25±24.7
C. salvifolius	B	45±7.12	47.25±9.6
	Cu	7.75±0.5	8.5±1.3
	Zn	38.5±3.4	41.5±6.14
	Mn	25.25±4.11	21±4.55
	Fe	208.5±37.6	160.5±25.2
Q. coccifera	B	45.5±8.1	41.5±10.7
	Cu	7±0.8	6.25±1.25
	Zn	24±3.27	23.5±3.42
	Mn	235.25±145.94	207±159.43
	Fe	410.5±211.8	190±61.14
U. parviflorus	B	17.75±2.22	21.75±4.11
	Cu	**5±0.82^{b}**	**2.25±0.5^{a}**
	Zn	**18.5±2.89^{b}**	**9.5±1^{a}**
	Mn	**17.25±1.89^{b}**	**11.25±1.71^{a}**
	Fe	**98.5±11.73^{b}**	**82.25±4.35^{a}**

Table 4 Plant nutrient and trace element stocks (mg.m^{-2} of species cover; N = 4; mean±SD) two years after amendment (July 2010). Values that differ at the 0.05 level are indicated by an exponent letter (Mann-Whitney test; a<b)

		B. retusum	*C. albidus*	*C. salvifolius*	*Q. coccifera*	*U. parviflorus*
N	Control plots	160,92±92,27	114,24±135,27	68,68±39,46	918,01±535,56	2190,36±803,83
	Amended plots	347,24±116,40	390,77±286,26	132,97±182,16	1118,37±546,55	1281,66±885,69
P	Control plots	**7,66±4,15^{a}**	11,09±14,92	4,68±2,63	49,47±26,03	61,70±24,54
	Amended plots	**27,22±3,30^{b}**	50,60±37,774	15,30±21,51	65,12±35,02	60,07±40,57
K	Control plots	47,12±24,74	56,35±71,64	30,72±18,89	468,20±292,04	959,61±430,12
	Amended plots	107,38±32,04	184,60±133,17	67,47±91,98	601,16±402,58	616,43±451,16
Mg	Control plots	10,07±4,57	17,83±23,20	15,94±9,67	123,40±74,50	168,52±66,94
	Amended plots	15,80±4,13	65,45±47,98	37,06±52,91	152,39±57,93	102,37±66,51
Ca	Control plots	58,93±35,30	122,34±155,87	67,82±46,35	787,46±436,67	681,64±201,51
	Amended plots	127,75±26,08	456,08±343,66	146,49±212,15	1110,68±585,06	273,93±180,50
B	Control plots	0,13±0,06	0,20±0,26	0,30±0,21	3,08±1,29	3,14±1,40
	Amended plots	0,19±0,02	0,63±0,46	0,64±0,96	4,16±2,73	1,93±1,11
Cu	Control plots	0,20±011	0,09±0,12	0,05±0,03	0,50±0,27	**0,87±0,33^{b}**
	Amended plots	0,40±0,09	0,26±0,19	0,09±0,13	0,64±0,39	**0,22±0,17^{a}**
Zn	Control plots	0,64±0,32	0,45±0,61	0,25±0,17	1,72±0,93	**3,12±0,89^{b}**
	Amended plots	0,94±0,19	1,44±1,07	0,49±0,67	2,34±1,33	**0,92±0,68^{a}**
Mn	Control plots	1,01±0,47	0,26±0,27	0,17±0,14	16,39±10,00	3,07±1,41
	Amended plots	0,88±0,75	0,52±0,39	0,25±0,35	24,72±24,19	1,07±0,80
Fe	Control plots	39,56±34,87	2,33±3,32	1,25±0,65	33,19±27,32	17,64±8,27
	Amended plots	28,06±16,29	4,86±3,38	1,90±2,59	19,36±12,48	7,76±5,25

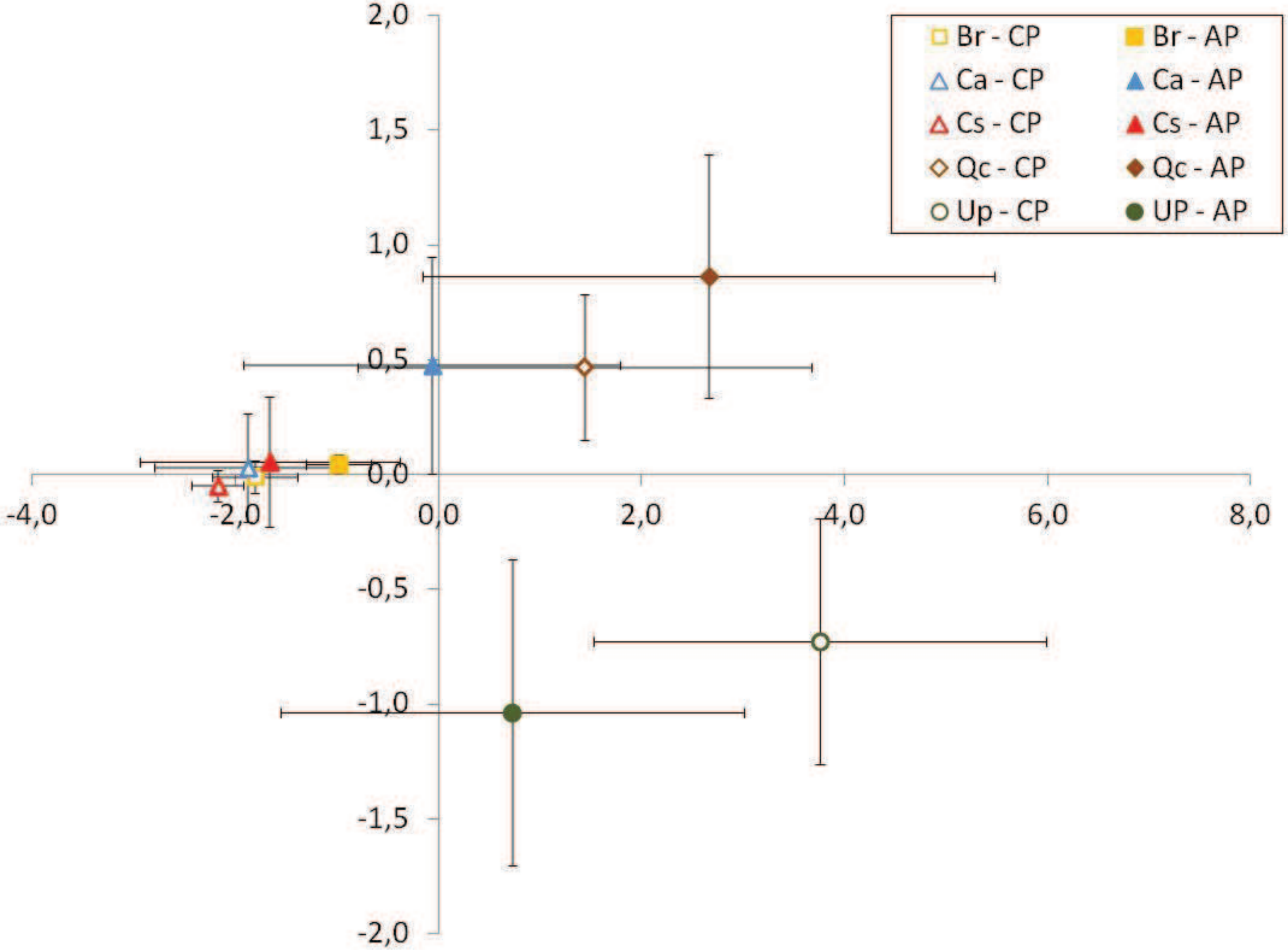

Figure 3. Factorial map of the PCA performed on plant nutrient, Cu and Zn stocks evaluated in July 2010
Br = *Brachypodium retusum*; Ca = *Cistus albidus*; Cs = *Cistus salviaefolius*; Qc = *Quercus coccifera*; Up = *Ulex parviflorus*; CP = Control plots; AP = Amended plots

Soil parameters

Compost amendment induced a significant increase in P_2O_5, N-(NO_3^-+NO_2^-), MgO and K_2O concentrations (two-way ANOVA, factor compost, Table 5, Fig.4) but no significant effect was observed on C_{org}, total and organic N, N-NH_4^+ and CEC. However, C_{org} tended to slightly increase in amended plots (Fig.4).

However, for P_2O_5, MgO, NaO, N-NH_4^+ and N-(NO_3^-+NO_2^-) contents, a significant date effect was shown (two-way ANOVA, $p < 0.001$, and Kruskal-Wallis test, $p < 0.0001$ for Na_2O; Fig.4). P_2O_5, significantly increased on amended plots from May 2008 to October 2010 (two-way ANOVA, $p < 0.05$; Fig.4). K_2O and MgO concentration showed a rapid increase after amendment and remained significantly higher in amended plots from October 2008 to April 2010 (one-way ANOVA, factor compost; Fig.4). MgO content was significantly lower in April 2010 compared to the other sampling dates (one-way ANOVA, $p < 0.05$; Fig.4). N-NH_4^+ significantly decreased after October 2008 on both amended and control plots (one-way ANOVA, $p < 0.0001$; Fig.4), and there was a peak of N-(NO_3^-+NO_2^-) content in amended only in October 08 (one-way ANOVA, $p = 0.001$; Fig.4).

Compost had no significant effect on the trace elements studied (Table 6). Soil Cr and Cd concentrations were significantly higher before amendment than two years after amendment (two-way ANOVA, factor date, $p = 0.001$ and $p = 0.016$ respectively; Tables 1 and 6). However, soil initial Cr and Ni concentrations were much higher than in compost (Table 1).

Bulk density was significantly lower in amended plots one and two years after amendment (one-way ANOVA, $p < 0.0001$; Table 5, Fig.4).

Compost amendment induced a significant increase in soil water content in May 2008, April, July and October 2009, and April 2010 (one-way ANOVA, $p < 0.0001$; Table 5, Fig.4). A seasonal effect was shown (one-way ANOVA, factor date; Table 5, Fig. 4), soil water content in July 2008 and 2009 being significantly lower than at the other dates.

Table 5. Significant results of two-way ANOVAs (factors compost and date) on soil parameters. Values that differ at the 0.05 level are indicated by an exponent letter ($a<b<c<d<e<f$); ns = non significant.

Parameter	ANOVA	Tukey test
P_2O_5	Date : F=9.096 ; p<0.0001	Apr08^{ab} May08^{a} July08^{bc} Oct08^{c} Apr09^{c} Apr10^{bc}
	Compost : F=124.486 ; p<0.0001	CP^{a} AP^{b}
	Date x Compost : F=13.583 ; p<0.0001	
MgO	Date : F=5.585 ; p=0.001	Apr08^{ab} May08^{ab} July08^{b} Oct08^{b} Apr09^{b} Apr10^{a}
	Compost : F=61.816 ; p<0.0001	CP^{a} AP^{b}
	Date x Compost : F=6.422 ; p=0.0002	
K_2O	Date : F=2.213 ; p=0.074	ns
	Compost : F=29.383 ; p<0.0001	CP^{a} AP^{b}
	Date x Compost : F=1.910 ; p=0.117	ns
$N\text{-}NH_4$	Date : F=64.892 ; p<0.0001	Apr08^{b} Oct08^{b} Apr09^{a} Apr10^{a}
	Compost : F=0.672 ; p=0.421	ns
	Date x Compost : F=0.194 ; p=0.899	ns
$N\text{-}(NO_3+NO_2)$	Date : F=46.795 ; p<0.0001	Apr08^{a} Oct08^{b} Apr09^{a} Apr10^{a}
	Compost : F=14.082 ; p=0.001	CP^{a} AP^{b}
	Date x Compost : F=22.933 ; p<0.0001	
Bulk density	Date : F=2.259 ; p=0.112	ns
	Compost : F=7.499 ; p=0.008	CP^{b} AP^{a}
	Date x Compost : F=1.338 ; p=0.270	ns
Soil water content	Date : F=125.005 ; p<0.0001	Apr08^{b} May08^{c} July08^{a} Oct08^{cd} Jan09^{de} Apr09^{de} Jul09^{a} Oct09^{b} Jan10^{f} Apr10^{e}
	Compost : F=41.016 ; p<0.0001	CP^{a} AP^{b}
	Date x Compost : F=1.838 ; p=0.023	

Table 6. Soil total trace element concentrations two years after amendment (April 2010) (mg.kg^{-1} of soil dry matter; N = 4; mean ± SD)

	Control plots	Amended plots
Total Cd	0.18±0.04	0.18±0.01
Total Cr	64.6±4.5	64.4±3.4
Total Cu	20.0±0.64	20.10±1.35
Total Hg	0.054±0.003	0.055±0.008
Total Ni	51.2±1.4	51.6±2.2
Total Zn	80.0±5.0	81.2±2.5

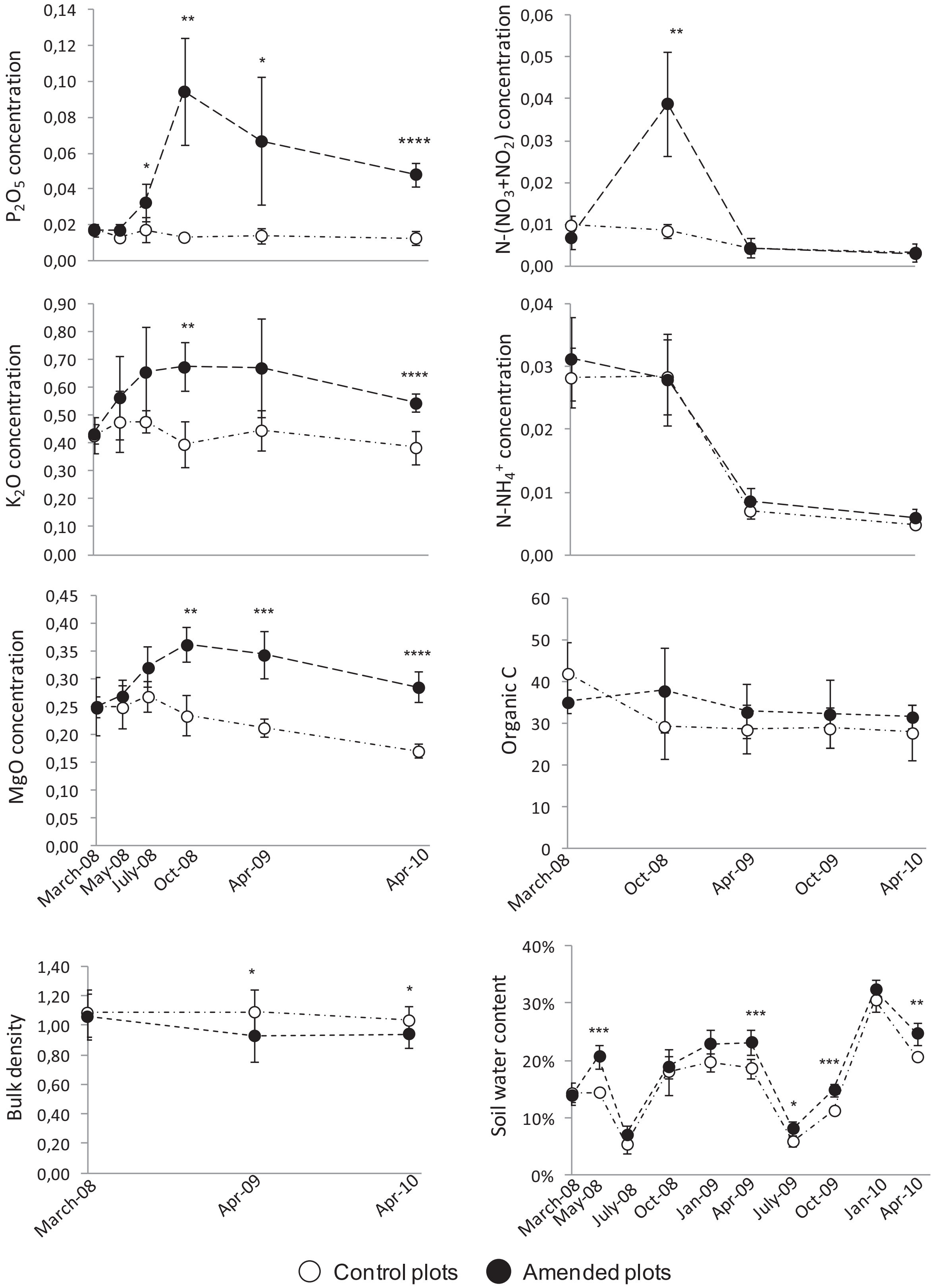

Figure 4. Soil nutrient concentrations (g.kg^{-1} of soil dry matter) bulk density (g.cm^{-3}) and soil water content (% of dry soil) (N = 4 to 12 ; mean±SD). *p<0.05, ** p<0.01, *** p<0.01, **** p<0.0001

Two years after amendment, no significant effect of compost was observed on soil nutrient and trace element stocks excepted a significant increase in P_2O_5 and MgO on amended plots (Mann-Whitney test, p = 0.029 for both nutrients; Table 7).

Table 7. Soil nutrient and trace element stocks ($g.m^{-2}$; N = 12 for nutrients, N = 4 for trace elements; mean±SD) two years after amendment (April 2010). Values that differ at the 0.05 level are indicated by an exponent letter (Mann-Whitney test; a<b).

	Control plots	Amended plots
P_2O_5	**2.58 ± 0.81^a**	**9.10 ± 1.53^b**
MgO	**35.30 ± 2.47^a**	**53.76 ± 4.22^b**
K_2O	79.87±15.81	102.09±7.96
Corg	56.78±13.99	59.44±3.79
$N\text{-}(NO_3 + NO_2)$	0.69±0.45	0.58±0.20
N-NH4	1.00±0.005	1.13±0.29
Total Cd	0.036±0.002	0.035±0.009
Total Cr	12.85±0.94	12.39±0.98
Total Cu	3.96±0.27	3.88±0.34
Total Hg	0.011±0.0003	0.011±0.002
Total Ni	10.45±1.04	9.70±0.45
Total Zn	16.19±1.22	15.38±1.73

Discussion

Effects on soil properties

Our results showed that some soil physico-chemical properties of the upper horizon were rapidly and durably improved by compost application. As observed on surface-amended burnt soils by Guerrero *et al.* (2001) with municipal solid waste compost, and Kowaljow and Mazzarino (2007) with biosolid and municipal organic waste composts, most of soil nutrient content showed a high response to compost amendment.

As sewage sludge and green waste compost contains high quantities of available P_2O_5, MgO and K_2O, amendment had a great overall effect on soil concentrations in these nutrients. Moreover, there was a rapid transfer of these nutrients from compost to soil during the first months after amendment, probably due to percolation by rainfall (Fig.1). Indeed, they all increased more or less rapidly after amendment and showed a concentration peak in autumn. In October 2008, available P_2O_5 was about five times higher in amended plots than in soil before amendment. The increase was less important for MgO and K_2O but still significant: it was about 44% and 53% of the initial soil content respectively. For the three nutrients, the increase due to compost maintained significantly in amended plots over the two-year study period, even though concentrations decreased more or less slowly after the autumn peak. Formation of inorganic calcium phosphate (Khanna and Ulrich, 1984) could explain this decrease as well as leaching or nutrient uptake by plants. Indeed, the decrease of P_2O_5, MgO and K_2O started after the onset of strong autumn rainfall (October 2008) that induced both a better root absorption and an intense leaching. Being under anionic forms, phosphorus especially can be easily leached. However, two years after amendment, P_2O_5, MgO and K_2O concentrations were still above soil levels before amendment: for instance available P_2O_5 was about three times higher than in the initial soil. This indicates an improvement of the quality of the burnt soil. Also, despite the lower soil density, P_2O_5 and MgO stocks were higher in amended plots due to the high P_2O_5 and MgO concentrations. Phosphorus is one of the most limiting nutrients in ecosystems, particularly in calcareous soils where its solubility is limited. Calcareous soils also have often low Mg and K availability for plants (Khanna and Ulrich, 1984), due to competitive effects between polyvalent cations. Plant growth could be favored by amendment during the early stages of post-fire succession. Nevertheless, the buildup of phosphorus in soil can exceed plant uptake and soil sorption capacity, and lead to groundwater eutrophication. High initial phosphorus level can be a limiting factor for the use of composted biosolids in ecosystem restoration.

There was a temporary increase in soil N-(NO_3^-+NO_2^-) with a concentration peak in October 2008, although the initial compost concentration was about four times lower than in soil. This could be due to the high compost N-NH_4^+ concentration (about 100-fold higher than in initial soil) and to the subsequent microbial nitrification of N-NH_4^+.

Indeed, amendment had no effect on soil $N\text{-}NH_4^+$ concentration despite the high compost concentration. Moreover, some other processes could explain that $N\text{-}NH_4^+$ was not transferred to soil: it could partly volatilize after amendment and immobilize in microbial biomass. (Cambardella *et al.*, 2003). This would also explain that $N\text{-}NH_4^+$ content decreased rapidly in both amended and control plots whereas initial soil $N\text{-}NH_4^+$ content was high compared to $N\text{-}(NO_3^-{+}NO_2^-)$, resulting from mineralization of plant and soil organic matter during fire (Prieto-Fernández *et al.*, 2004; Rodríguez *et al.*, 2009). As for P_2O_5, $N\text{-}(NO_3^-{+}NO_2^-)$ is very easily leached by rainfall, that can cause the rapid decrease of $N\text{-}(NO_3^-{+}NO_2^-)$ in amended plots, rather than root absorption. If excess levels of nitrates arise in soil from compost amendment, nitrate leaching can lead to pollution of groundwater.

Given the high organic C and N content in compost, we expected soil organic C, N, and CEC to be improved by amendment, even though compost effect was not supposed to be as important as when compost is incorporated into soil (Curtis and Claassen, 2007; Fernández *et al.*, 2007). The non significant compost effect observed reflected the very low incorporation rate of compost organic matter into the mineral soil. The finest organic particles of the compost could have been transferred from surface to soil with infiltrating water (Kowaljow and Mazzarino, 2007), as well as some dissolved organic compounds (Ashworth and Alloway, 2004). Two years after surface amendment with a municipal waste compost, Martinez *et al.* (2003) observed soil organic C improvement at the highest rate only (120 $Mg.ha^{-1}$, which is 2,4 times higher than our rate) and no soil total N increase. However, Kowaljow and Mazzarino (2007) obtained a significant increase in soil organic C and total N only one year after surface applying composted biosolids at the rate of 40 $Mg.ha^{-1}$. Soil texture could be involved to explain low organic matter transfer from surface compost into soil. The silty-clayey soil texture of our study site was overall less permeable to water percolation of organic particles than the sandy soils amended in the studies cited above. Moreover, some compost characteristics could prevent organic matter transfer. Compost contained particles with relatively high size because it was 40-mm mesh sieved; its density was quite low (0.31); it could exhibit some water repellency because of some hydrophobic organic compounds, for instance from pine bark or others green wastes.

High soil organic matter content is known to improve soil water holding capacity and thus increases water reserves. Soil organic matter was not significantly increased by amendment, nevertheless soil water content in the upper soil layer was overall durably improved by compost amendment. This effect was significant even sometimes in summer (July 2009) despite the rainfall deficit. So, compost spreading helped to preserve the water retained into soil during wet seasons. This was probably due to a decrease of water evaporation from the soil beneath the compost surface layer and/or to changes in soil structure and bulk density. Compost surface application on burnt bare soil prevents desiccation until the reconstitution of plant canopy plays the same protecting role on soil water reserves. Nutrient availability and root absorption are thus improved in amended plots. In turn, the long-term improvement of soil humidity after fire could facilitate the recovery of soil biological activity and plant regeneration.

A lower soil bulk density is generally observed when biosolids are incorporated into soil (Caravaca *et al.*, 2003; Curtis and Claassen, 2007; Albaladejo *et al.*, 2008). Present results showed that surface compost amendment could also decrease bulk density in amended plots. This could result from an increase of soil porosity due to root growth allowed by soil nutrient content rise (Zebarth *et al.*, 1999; Aggelides and Londra, 2000; Celik *et al.*, 2004, 2010). Most of plant species regenerating during the experimental period exhibited shallow roots growing in the upper soil layer (seedlings of obligate-seeder species or *B. retusum* superficial rhizoms and roots). Soil micro-organisms favor soil aggregate formation and stability (Annabi *et al.*, 2007) that could also increase soil porosity and thus decrease soil density. Mature composts such as used in this study, can also indirectly decrease soil density because humic substances participate to mineral particle aggregation (Piccolo and Mbagwu, 1999) and increase soil porosity.

Despite high initial levels of most of trace elements in compost, soil trace element concentrations and stocks were not increased two years after amendment. The compost used had no depreciating effect on soil quality at the rate amended (50 $Mg.ha^{-1}$). Two years after an amendment at 100 $Mg.ha^{-1}$ of the same compost, Larchevêque *et al.* (2006a) did not observed a significant increase of total trace elements in soil mineral horizon of a post-fire *Q. coccifera* shrubland. However, soil conditions in an early post-fire ecosystem are not comparable to an older one and a higher compost rate could induce negative effects on a recently burnt soil which is more sensitive to environmental constraints. Most of trace elements analyzed are known to be toxic to micro-organisms. Compost total Cu and Zn concentrations are higher than concentrations reported to have toxic effects on microbial activity in soil (Kabatas-Pendias and Pendias, 1992). Nickel is toxic at lower levels than other trace elements (Kabatas-Pendias and Pendias, 1992). The compost used was less contaminated than the control soil in Cr and Ni because our experimental site had relatively high concentrations in these elements. This contamination was assumed to have atmospheric origin, as the area receives significant atmospheric pollution from industrial activities (AIRMARAIX report 1999). We did not observe a dilution effect of the amended soil by compost (Larchevêque *et al.*, 2006a) that would induce a decrease in soil Cr and Ni concentrations. The high pH of compost could favor immobilization of trace elements and decrease their solubility (Römkens and Salomons, 1998). Trace elements might also be complexed with phosphorus or organic matter in the compost (Khabatas-Pendias and Pendias, 1992; Moreno *et al*., 1996; Planquart et al 1999). However, on the long-term, further changes in physico-chemical soil conditions and compost organic matter mineralization could induce some remobilization and solubility of trace elements, and increase their availability to plants (Planquart *et al.*, 1999).

Effects on plant nutrients and trace elements

As most of soil macronutrients rapidly and durably increased after compost amendment, positive effects on plant nutrient contents were observed during the study period (Caravaca *et al*., 2003; Walter and Calvo, 2009). Nutrient availability and root absorption were significantly improved by soil enrichment and could enhanced plant biomass production, as frequently observed (Martinez *et al*., 2003; de Andres *et al*., 2007, Curtis and Claassen, 2009, Kowaljow *et al*., 2010). The durability of compost effect on soil P_2O_5 concentration resulted in a persistant increase of P foliar concentrations in *C. salviaefolius*, *C. albidus, U. parviflorus* and *B. retusum* on amended plots. Phosphorus concentrations were still higher on amended plots more than two years after amendment. The humified organic matter of the mature compost is a slow-release source of available nutrients (Bernal *et al.*, 1998) for plant nutrition, even though P_2O_5 soil concentration tended to decrease over time. However, in terms of P stocks evaluated twenty-eight months after amendment (July 2010), compost profitable effect on plant growth was limited to *B. retusum*. This is due to the biomass increase observed on amended plots in this species, whereas biomass of the three shrub species did not increase (Cellier, 2012). Although P is a major limiting nutrient for plants and is rapidly absorbed after supply, fertilizing compost effect did not stimulate growth of *C. salviaefolius*, *C. albidus* and *U. parviflorus*, suggesting luxury consumption of high freely available P (Chapin, 1980).

Positive compost effects on plant N and cation contents were lower than for phosphorus, despite the strong and lasting compost effect on soil K and Mg content especially. Nitrogen concentration was only increased in *U. parviflorus* and *B. retusum*, and less durably in this last species. Foliar Mg concentration was durably increased in *C. salviaefolius* whereas K concentration increased only temporary (July 2009) in this species. The low species responses to N input from compost were unexpected and contrasted with results obtained in many compost amendment studies (i.e. Caravaca *et al.*, 2003; Larchevêque *et al.*, 2010; Martinez *et al.*, 2003). This could indicate that N was not limiting in the initial burnt soil. Soil mineral N content, especially $N\text{-}NH4^+$, is frequently increased immediately after fire because of the organic matter mineralization by combustion (Rodríguez *et al.*, 2009). Also the increase in soil $N\text{-}(NO_3^-{+}NO_2^-)$ concentration was maybe of too short duration to widely and durably improve plant N nutrition. In *U. parviflorus,* nitrogen absorption in excess of requirements seemed also occurred, although positive compost effect on soil N (organic or mineral) was much lower than for P. High soil Ca content may have prevented K and Mg being absorbed. Moreover, the formation of some organic complexes may have retained Mg and K in forms unavailable to plants.

In some cases, compost amendment seemed to negatively influence plant nutrition. This effect concerned the three cations whose foliar concentrations were lower on amended plots than on controls. This appeared punctually in *Q. coccifera* for Ca and Mg and in *B. retusum* for Mg and K. This response maintained over the two years in

U. parviflorus for Ca and in *C. albidus* for K. It was unlikely that this decrease was due to a dilution effect induced by a rapid growth on foliar nutrient concentrations because plant growth stops or markedly slows down during summer for most of perennial species in Mediterranean ecosystems. Root cation absorption is controlled by antagonistic relationships between soil nutrients, and root selectivity. These processes could combine with summer water depletion to impede cation availability and root absorption. In *U. parviflorus* Cu and Zn stocks were lower on amended plots than on controls. This response was due to the lower biomass on amended plots (Cellier, 2012) whilst nutrients did not increase, even though N nutrition was improved. Phosphorus stock did not differ between plots because the decrease of biomass counteracted the strong increase in P content.

A global date effect on some cation concentrations appeared in some species. These changes between July 2009 and July 2010 were due either to the decrease (K content in *C. salviaefolius* and *B. retusum*, Mg in *Q. coccifera*) or to the increase (Ca in *C. albidus* and *C. salviaefolius*) of cation content over time. Nitrogen content in *B. retusum* was also higher in July 2010 than in July 2009. These variations probably reflected the inherent soil micro-heterogeneity between plots, and the intraspecific variability in plant nutrition and metabolism, both also depending on soil nutrient and water availability and climatic conditions. In Mediterranean ecosystems especially, compost effect may be overrode by drought (Larchevêque *et al.*, 2010). Interannual variations in climatic and soil conditions could explain that compost effect on plant cation concentrations did not persist over the two years in most cases.

Compost had no or little significant short-term effect on trace element concentrations in plants. The trace elements studied are required to plant metabolism as essential oligo-elements. In spite of high compost concentrations, Cu and Zn foliar concentrations were not increase. This response was expected since no transfer of Cu and Zn occurred from compost into soil. Excess levels of Cu and Zn are toxic for plants and soil organisms but compost amendment did not induce contamination risk on the short-term. However, as mentioned for trace elements in amended soil, changes in physico-chemical soil conditions and compost organic matter mineralization could provide available toxic trace elements to plants. Trace element concentrations were in some cases lower on amended plots than on control. This occurred only in *B. retusum* and *U. parviflorus.* As an indirect compost effect, high soil nutrient concentrations in amended plots may have prevented trace elements initially present in soil to be absorbed, especially Ca, Mg and P which are the main antagonistic elements against plant absorption of several trace elements (Kabata-Pendias, 1992). The effect of high soil pH on the immobilization of most trace elements may combine with competitive ion effects to reduce trace element absorption. Planquart *et al.* (1999) showed that trace element contents in plants are more influenced by soil nature than compost application.

Besides the more or less strong immobilization of trace elements in soil, plant-specific trace element selectivity and requirement may also be involved in the response observed on amended plots. Although *B. retusum* and *U. parviflorus* are adapted to calcareous soils with high pH, they may be more sensitive to essential trace elements deficiency.

Plant nutrient strategies

This study allowed to discriminate plant nutrient strategies in early stages of post-fire regeneration of a Mediterranean shrubland, these strategies being related to differences in life traits (Hernández *et al.*, 2010; Paula and Pausas, 2011). We can separate species upon their regeneration trait: the resprouters *Q. coccifera* and *B. retusum,* the obligate-seeders *Cistus* species and *U. parviflorus*. In each group, species still exhibit different foliar and root traits. Compost positive effects seemed to depend on post-fire species regeneration strategies.

Resprouters maintain viable underground storage organs after fire and thus are less dependent of soil nutrient pools for further growth. This enables rapid regeneration after fire. However, the two species studied responded differently to nutrient input from compost. As a deeply-rooted resprouter, *Q. coccifera* was the less responsive species to nutrient input from compost. Besides, nutrients in upper soil layers were maybe less available for deep roots. Sclerophyllous evergreen species are generally considered requiring low nutrient levels because they are adapted to oligotrophic environments. Nutrient response of *B. retusum* was globally lower than that observed by Larchevêque *et al.* (2010). Grass species were observed to respond more rapidly than shrub species to an increase in nutrient supply (Berendse, 1998). Given the increased growth of *B. retusum* on amended plots, we expected that this perennial herb could rapidly accumulate nutrients after fire because of some life traits: superficial roots, faster growth rate than woody species. However, roots near the soil surface could have been altered by fire heating, explaining the low nutrient response in *B. retusum* leaf during early stages of post-fire regeneration.

Compost amendment was also thought to be profitable to nutrition of obligate-seeder shrubs because seedlings can only rely on soil nutrients after germination for immediate growth after fire. There was a direct compost effect through nutrient supply but an indirect effect could also occur because compost enhanced seedling root growth (Caravaca, 2003; Rincón *et al.*, 2006); this in turn improved nutrient uptake through a larger soil exploration and absorbing root surface. Paula and Pausas (2011) showed that seedlings of non resprouters explore more efficiently the upper soil layers than seedlings of resprouters, thanks to root structure characteristics related to soil resource acquisition. Moreover, seedling fine absorbing roots were able to colonize the soil beneath the compost layer or even directly dip into compost. But specific differences in root traits and nutrition strategy may exist between obligate-seeder species and induce specific responses. Among species studied, *C. salviaefolius* was the most responsive to compost in terms of nutrients considered, *C. albidus* the less responsive one. *U. parviflorus* exhibited an intermediate response, having unsurprisingly the highest N content globally. As a symbiotic N_2-fixing legume, *U. parviflorus* was considered to be less dependant from soil N and was not expected to exhibit a positive N response on amended plots. Symbiotic N nutrition of legume species is controlled by many environmental conditions, part of each is difficult to evaluate in field studies when ecological factors interact. A lack of soil suitable rhizobia after fire (Larson and Siemann 1997) could explain that young seedlings of *U. parviflorus* relied on compost N input, leading to the increased N concentrations on amended plots. Larchevêque *et al*. (2010) observed in an older post-fire succession that *U. parviflorus* was non responsive to N input after compost amendment. Moreover, in early post-fire stages, the temporary high level of mineral N can depress nodulation and N_2-fixation (Vitousek and Field 1999), driving N-fixers to use soil N. On the other hand, high plant P content due to high P input could stimulate N_2-fixing activity, leading to a better plant N status on amended plots. Symbiotic N_2-fixation has been shown to be P-limited in terrestrial ecosystems, especially since N-fixers have greater P requirements than non fixers (Vitousek and Field 1999).

From our results, it appeared that, more than plant regeneration strategy, specific nutritional traits related to root system and intrinsic nutrition physiology drove species responses to nutrient input from compost.The PCA performed on foliar nutrient stocks discriminated the species storage capacity mainly from N, Mg, K, Cu and Zn stocks, and regardless global compost effect. Nutrient stocks integrate both foliar nutrient concentrations and foliar biomass. Due to generally low foliar P concentrations in plants compared to the other macro-nutrients (Martin-Prével 1978), foliar P stocks appeared less important in separating species storage responses. *U. parviflorus* showed the highest nutrient stocks on control plots, the low stocks on amended plots resulting from a biomass decrease (Cellier, 2012). *Cistus* species and *B. retusum* showed the lowest foliar nutrient storage capacity due to the relatively low biomass produced after two years (Cellier, 2012), even though their foliar nutrient concentrations were globally improved on amended plots. The intermediate position of *Q. coccifera* was related to its high foliar biomass (Cellier, 2012) probably due to the production of vigourous resprouts after fire, rather than high nutrient content. Related to their nutrient stocks, each species plays a particular role in ecosystem reconstitution. *B. retusum* can rapidly protect the soil until woody species recover, and can help in the reconstitution of soil organic matter pool early after fire, especially because of its rapid biomass production. Effects of woody species on the onset of nutrient cycling are variable and more or less delayed. *Cistus* species behave as *B. retusum*. Due to their malacophyllous leaves with short lifespan, this group of species can rapidly restitute to soil nutrients accumulated in leaves through litterfall and easily decomposing litter (Fioretto *et al*., 2005; Simões *et al.*, 2009). *U. parviflorus* and *Q. coccifera* further enrich soil in nutrients either through restitution of highly accumulated nutrients or through high biomass production of long lifespan leaves, respectively.

Conclusion

During a short-term experiment in an early post-fire ecosystem, a single compost amendment induced rapid improvement of soil fertility and plant nutrition over the two-year experimental survey. The use of compost for restoring burnt ecosystems shows promise to speed up vegetation recovering, as some plant species have the capacity to accumulate nutrients during the early post-fire succession. The increase of soil nutrient pools after compost amendment emphasized the diversity of plant resource use, which was related to specific nutritional traits. It also highlights the role of dominant species in the recovery of soil fertility after fire through litter nutrient restitution.

Compost had no effect on soil and plant contamination by trace elements at the rate used (50Mg.ha^{-1}), but some changes in soil conditions over time and the progressive compost mineralization could release available trace elements to plants. On the other hand, P_2O_5 and N-NO_3^- increased more or less durably in amended soil, which induces potential negative consequences on ground water quality. Soil P_2O_5 concentration especially was still high two-years after amendment. According to our results, P was the most limiting factor for the use of this sewage sludge and green waste compost for ecosystem restoration after fire, if it is brought in excess of plant requirements. The use of moderate compost rates would prevent from eutrophication risk.

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Chapitre III - Apport de compost en garrigue.

Effets de l'amendement d'un compost de boues de station d'épuration sur les microorganisme d'un sol méditerranéen après incendie.

Effects of sewage sludge compost amendment on Mediterranean soil microbiology after fire

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Abstract

In Mediterranean region, forest fires are a major problem towards the desertification of the environment. Use of composts is considered as a solution for soil and vegetation regeneration. In this study, the effects of compost on soil microbiology after fire were determined by measuring the evolution microbial activities, biomass and diversity. In April 2008, a sewage sludge compost was amended on soil surface. Compost effects on microbial activities were low and only sporadically increased microbial biomass and diversity. However, in our study, soil microbial activities and biomass seemed to be more dependent on soil water content than soil nutrient content. Moreover, no negative effect was observed during the two years of experimentation.

Introduction

Forest fires are considered as the main Mediterranean ecosystem perturbation (Whelan, 1995). The Mediterranean-type shrublands are highly resilient to wildfires (Keeley, 1986; Trabaud, 1994) as a consequence of the ability of plant species to rapidly recover from fire by means of a wide diversity of regeneration strategies from resprouting to fire-prone seed germination (Lloret and Vilà, 1997; Trabaud and Lepart, 1980). However, the abandonment of agricultural lands since the 1970s and the increase in human population and activities have resulted in a dramatic increase in the number and size of fires in all Mediterranean regions (Pausas, 2004). Fire recurrence is also favored by the Mediterranean climate characterized by long dry summer and strong winds (Bagnouls & Gaussen, 1957; Scarascia-Mugnozza *et al.,* 2000; De Luis *et al.,* 2001). Recurrent fires, , are known to progressively impoverish soil in organic matter and nutrients through erosion and repeated burnings (Knicker, 2007; Reich *et al.,* 2001) This makes some restoration actions should be necessary to help ecosystem to recover. Fire frequency, duration and intensity are the main factors determining impacts on ecosystem: the more recurrent and intense fires are, the more pronounced their effects (Boerner, 1982). Firstly, fires induced partial or total destruction of plant cover and microbial biomass as well as organic matter in superficial organic layers (Hernández et al., 1997; Guerrero et al., 2001). The main effects on soil organic matter quality are removal of external oxygen groups, reduction in the chain length of alkyl compounds, aromatisation of sugars and lipids, conversion of amide-N into heterocyclic N compounds, macromolecular condensation of humic substances and production of black carbon (Gonzáles-Pérez *et al.,* 2004). Then, soil stability decreases due to the loss of organic matter which induces an increase in erosion risk (Kutiel and Inbar, 1993; Hart *et al.,* 2005). Immediately after fire, nutrient availability increases due to ashes and release of soil minerals (Kutiel and Inbar, 1993; Dumontet *et al.,* 1996). However, part of the nutrients is oxidized and volatilized during fires (Fisher and Binkley, 2000). Nutrient losses are more important after fire because of wind erosion, runoff (DeBano and Conrad, 1978; Boerner, 1982; Gimeno-Garcia *et al.,* 2000) and leaching into groundwater (Mohamed *et al.,* 2007). Forest fires also disturb soil biological properties (DeBano *et al.,* 1998). These effects are mainly observed in the superficial soil layer where microorganisms and soil fauna are more abundant (Neary *et al.,* 1999). Fires can induce a decrease in microbial biomass (Dumontet *et al.,* 1996; Prieto-Fernandez *et al.* 1998; Smith *et al.,* 2008) and diversity (Vásquez *et al.,* 1993; Smith *et al.,* 2008) and enzymatic activities (Hernández *et al.,* 1997). As observed by Bååth *et al.* (1995) fungi are more seriously affected than bacteria. Recovery of soil microbial community and activities depend on nutrient availability (Moore *et al.,* 1993), pH (Wardle, 1998) and soil organic matter remaining after disturbance or brought to soil (De Angelis *et al.,* 1989; Gros *et al.,* 2004, Guénon *et al.,* 2011).

Using of organic biosolids as amendments for soil restoration and vegetation regeneration is increasing in frequently burnt or degraded ecosystems (Guerrero *et al.,* 2000; Caravaca *et al.,* 2003; Martinez *et al.,* 2003; Larchevêque *et al.,* 2006a; Walter *et al.,* 2006; Kowaljow and Mazzarino., 2007; Hemmat *et al.,* 2010; Turrión *et al.,* 2012). Composted biosolids are rich in humified organic matter and can be used as a slow-release source of nutrients (Barker, 1997). Compost amendments improve physical, chemical and biological properties of soils, in particular by increasing available nutrients mainly in the organic soil fractions (Bodet and Carioli, 2001; Larchevêque *et al.,* 2006a; Annabi *et al.,* 2007). Composted biosolids have a high water retention capacity (Giusquiani *et al.,* 1995) which induces an increase of soil water content (Villar *et al.,* 1998). These modifications positively affect plant cover by

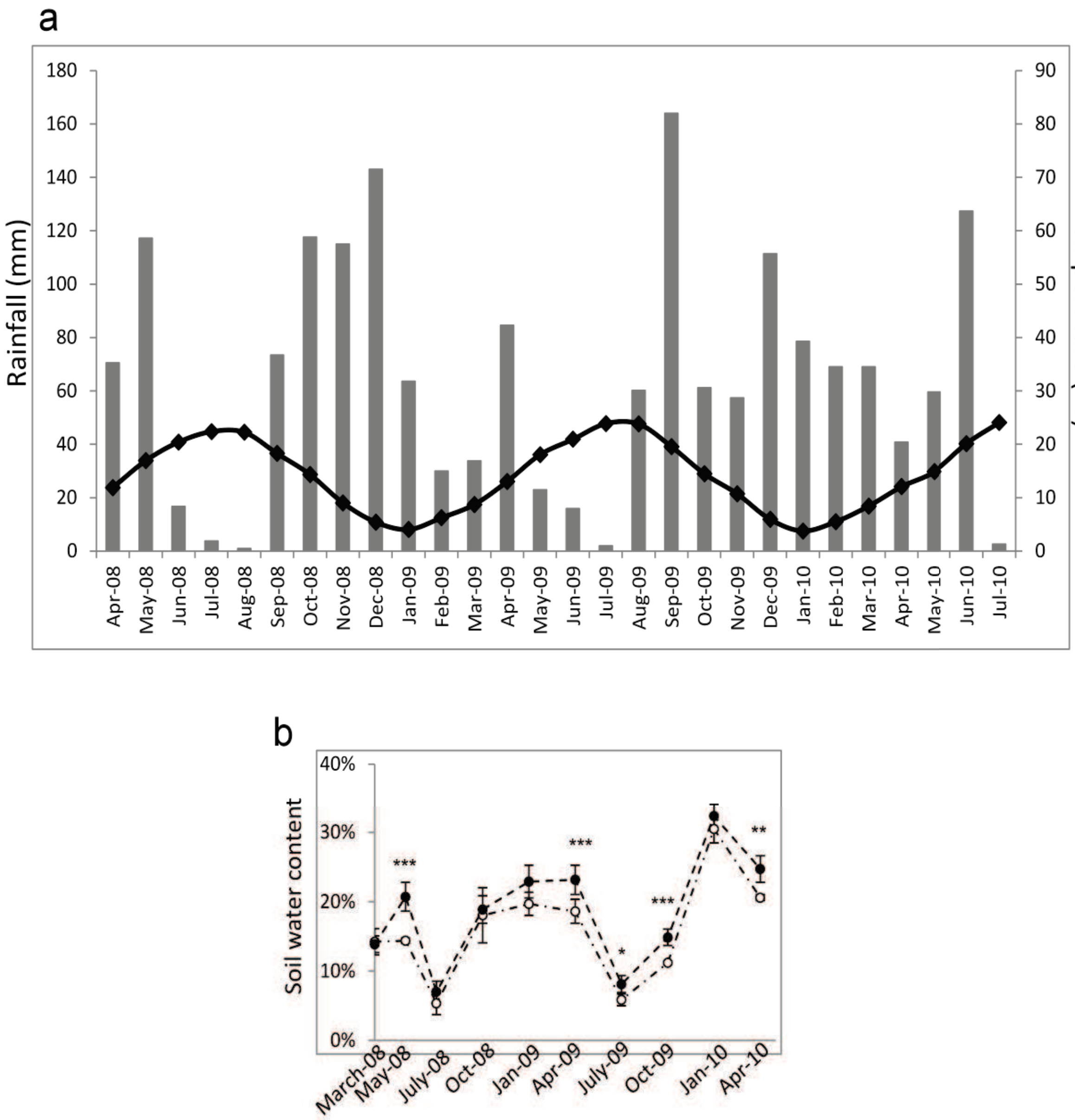

Figure 1. (a) Monthly rainfall and mean air temperature (Météo France) during the study period and (b) soil water content (% of dry soil; N = 4; mean±SD). One-way ANOVA: *p<0.05, ** p<0.01, *** p<0.01

improving plant nutrition and growth (Villar *et al.,* 1998; Guerrero *et al.,* 2001; Caravaca *et al.,* 2003; Larchevêque *et al.,* 2005b, 2006b), and contribute to reduce the risk of erosion (Guerrero et al. 2000). It was also shown to increase soil microbial biomass and activities (Borken *et al.,* 2002; Kowaljow et Mazzarino, 2007).

In this study we aimed to investigate the potential of a compost amendment to enhance microbial properties in a burned Mediterranean shrubland. We thus monitored *in situ* effects of a single compost input following fire on soil enzymatic activities, microbial basal respiration, microbial and fungal biomass and catabolic diversity during 2 years following compost addition.

Material and methods

Study site

The experiment was carried out in a burnt site of about 82ha on the plateau of Arbois (Provence, Southeastern France; 43°27'16.28"N – 5°17'57.21"E, alt. 216m). The fire occurred on 4th September 2007 and totally burnt the above-ground vegetation. As visible in the adjacent unburnt, the initial plant community was dominated by the shrub species *Cistus albidus* L., *Cistus salviaefolius* L., *Quercus coccifera* L. and *Ulex parviflorus* Pourr., the herb *Brachypodium retusum (Pers.) P. Beauv.,* vegetation. Some sparse clumps of *Quercus ilex L.* were also growing in the site. The study site is characterized by a Mediterranean climate with dry, hot summers and wet and temperate winters. The mean annual precipitation and temperature are 740 mm and 13.6°C, respectively (Fig.1). The soil is a Rendzic Leptosol according to WRB (2006).

Compost and experimental design

The compost was produced by a local company (Biotechna, Ensuès-La-Redonne, France) from municipal sewage sludge mixed with pin barks and green wastes (1/3 volume each). After being composted for 30 days at 75°C to kill pathogenic microorganisms and decompose phytotoxic substances, the mixture was sieved (< 40mm mesh) to remove the large bark pieces and stored in swathes. The swathes were mixed several times over the next 6 months to promote organic matter humification. The final compost met the French legal standards (NF U 44-095, 2002) for pathogenic microorganisms, organic trace elements, and trace metals. No viable seed remained.

The experimental design was a randomized block of eight 300 m^2 plots (15 m x 20 m) delimited in a 0.5 ha flat zone of the burnt site. Four plots were amended with compost and four were the controls. About 50 $t.ha^{-1}$ of fresh compost were homogeneously spread by hand on soil surface 7 months after fire before plant regrowth (1st April 2008). Five soil samples per plot were collected to determine physico-chemical characteristics before amendment.

Soil and compost initial characteristics are presented in Table 1.

Field procedures

Soil samples were collected before amendment (at the end of March 2008) six weeks after amendment (mid-May 08) and then every 3 months from July 2008 to April 2010. Each soil sample was a composite of three sub-samples for each plot. The 0-20 cm soil horizon, excluding compost and plant litter was collected with a drill. Sub-samples were immediately refrigerated after sampling and then sieved and frozen at the laboratory for determination of mineral N.

Table 1: Soil (0-20 cm: maximum depth; N = 8 to 40; mean±SD) and compost initial characteristics.

		Soil		Compost
		Control plots	Amended plots	
Organic C	$g.kg^{-1}$	41.26±9.56	35.24±9.94	312.4
Total carbonates	$g.kg^{-1}$	13.44±10.51	9.94±6.88	-
Total N	$g.kg^{-1}$	2.66±0.66	2.31±0.38	31.73
$N\text{-}NH_4^+$	$g.kg^{-1}$	0.027±0.007	0.031±0.011	2.49
$N\text{-}(NO_3^-+NO^{2-})$	$g.kg^{-1}$	0.009±0.004	0.007±0.004	0.002
P_2O_5	$g.kg^{-1}$	0.018±0.006	0.017±0.006	30.7
K_2O	$g.kg^{-1}$	0.428±0.088	0.435±0.060	8.1
MgO	$g.kg^{-1}$	0.251±0.058	0.249±0.037	7.9
Na_2O	$g.kg^{-1}$	0.024±0.003	0.022±0.004	-
CaO	$g.kg^{-1}$	-	-	99.30
CEC	$Cmol^+.kg^{-1}$	19.41±2.82	19.30±2.12	-
Total Cd	$mg.kg^{-1}$	0.26±0.06	0.22±0.02	0.81
Total Cr	$mg.kg^{-1}$	81.23±5.98	78.08±8.18	22.91
Total Cu	$mg.kg^{-1}$	21.90±2.30	19.83±1.32	181.12
Total Hg	$mg.kg^{-1}$	0.05±0.01	0.05±0.01	0.4
Total Ni	$mg.kg^{-1}$	49.50±0.94	49.88±1.75	15.06
Total Zn	$mg.kg^{-1}$	92.10±10.29	84.45±3.59	325.3
Humidity	Soil : % Compost : %FM	14.24±2.69	13.88±2.35	28.2
Bulk density (unsieved fraction)	$t.m^{-3}$	1.08±0.16	1.06±0.16	0.31
Clay	% *	33.75±4.99	32.93±3.93	-
Silt	% *	55.40±5.16	57.30±2.87	-
Sand	% *	10.85±0.45	9.78±1.03	-

All nutrient and trace element values are expressed in kg^{-1} of soil dry matter (DM)
* : % of sieved fraction ≤ 2mm ; FM : Fresh Matter.

Laboratory procedures

Soil characteristics

Soil samples were first 2 mm mesh sieved. Sub-samples were then air-dried for determination of physico-chemical parameters; some other sub-samples were weighted before and after oven drying 24h at 105°C for calculation of soil water content (SWC). Total carbon and total nitrogen were determined after dry combustion using an element analyzer (NA 1500 CN, Fisons Instruments, Manchester, UK) after grounding and homogenizing samples (MM400, Retsch GmbH, Haan, Germany). Organic carbon concentrations were obtained by the difference between total carbon and carbonates determined by a volumetric method with a Bernard calcimeter. Mineral N was extracted after soil lyophilization with a 1 *M* KCl solution. N-NH_4 was then analyzed by Berthelot method, N-(NO_3+NO_2) by Griess & Ilossay method. Available phosphorus was determined by Olsen method. Exchangeable Mg and K were extracted by ammonium acetate. Mg was then analyzed by atomic absorption spectrophotometry, and K by flame emission spectrophotometry.

Microbiological analysis

Basal respiration (BR) and microbial biomass (MB)

The physiological statement of soil microbial community was estimated by basal respiration method (Anderson et Domsch, 1978).

Soil samples were sieved at 2mm and stored at 4°C. A mass of fresh soil (equivalent of 10 g dry soil) of each sample was placed in 2 flasks (250 ml). The first was left at the soil water content observed during the sampling. The SWC of the second flask was normalized at 20% (of soil dry mass) to observe the effect of this parameter on microorganisms. The bottles were left one night at ambient temperature. Then, their atmosphere was substituted by the input of a control air during 3 min and they were hermetically closed. After incubation (4h at ambient temperature), 1 ml of the flask atmosphere was sampled with a syringe and injected into a gas phase chromatograph (Chrompack CHROM3 - CP9001) equipped with a TCD detector and a packed column (Porapack) in which circulates a stream of helium at 60 ml.h^{-1}. Measured values were adjusted to 22°C. The amount of C-CO_2 produced by microorganisms was calculated as the difference between the measured concentration of the atmosphere and the control air injected into the flasks.

Microbial biomass was estimated by the method of respiration induced by addition of glucose (Anderson and Domsch, 1978). The samples used for this measurement were those previously used for measuring basal respiration. A talc and glucose mixture (1000 µg C.g^{-1} soil) was added and incorporated into the soil and is incubated for 90 minutes. As for basal respiration, excess CO_2 accumulated in each glass jars were then flushed for 2 min with ambient air. The flasks were then hermetically closed and incubated at ambient temperature for 60 min. As described for basal respiration, the CO_2 concentration of the flasks was measured by gas chromatography. The value of microbial biomass is then obtained by the equation of Beare *et al.* (1990) from the respiration rate induced.

The metabolic quotient (qCO2) was calculated for each sample by the ratio between basal respiration and microbial biomass which allows estimating the metabolic efficiency of the microbial community.

Fungal biomass

Fungal biomass was estimated using soil ergosterol concentration (Gessner *et al.,* 1991; Gessner and Schmitt, 1996; Cortet *et al.,* 2003). Soil was sieved at 2 mm and lyophilized. Ergosterol was extracted by adding potassium hydroxide in methanol (8 g.L^{-1}) to 0.15g of soil (Gessner *et al.,* 1991) and purified by solid-phase extraction (Gessner and Schmitt, 1996). Quantification of ergosterol was realised by high performance liquid chromatography (HPLC; HP series 1050 chromatograph).

Catabolic profiles of the cultivable microbial communities

Structure and diversity of catabolic functions were determined for cultivable microbial communities by Biolog® Ecoplates method (BIOLOG Inc., Hayward, CA). The method consists of measuring the growth of micro-organisms and metabolism resulting from the use of a wide variety of carbon substrates. BIOLOG® ECO microplates are divided into three identical areas. Each area is divided into 32 wells with 31 carbon sources and a control without substrate. The substrate oxidation generates a proportional reduction of colorless blue of nitro tetrazolium into purple formazan. Metabolic fingerprints of catabolic functions communities can then be established and compared. Carbon sources can be grouped into six chemical classes: carboxylic acids, amides and amines, polymers, carbohydrates, amino acids and compounds.

Catabolic profiles were measured according to the modified protocol of Garland and Mills (1991). Ten grams (dry equivalent) of fresh soil were suspended in 100 ml of a sterile solution of sodium pyrophosphate 0.1% (pH 7) and then shaked for 60 min. After 20 min of decantation, the supernatant was diluted 100 times in sterile saline solution (0.85% NaCl). We purposely did not adjust the inoculum in order to obtain a uniform cell density as we considered the total microbial number as an inherent characteristic of microbial communities of each plot. The wells were inoculated with 125 µl of the new solution and Biolog microplates were incubated at 25°C for 5 to 10 days. The development of optical density of each well was recorded twice a day at a wavelength of 595 nm (spectrophotometer Elisa 960 Metertech®) until the development of the average optic density of 31 wells. For each sample and each measurement time, the absorbance of control well was subtracted from other well of the microplate to remove the absorbance of the soil suspension (particles and bacteria).

The incubation time for which the mean optical density of all the substrates reached a value of 0.5 (AWCD0.5 or Average Well Color Development) was calculated using the method of Garland and Mills (1991). This time was used to determine by extrapolating the optical density of each substrate. For each sample, a table with the standardized absorbance value for 31 substrates is analyzed by multivariate statistical tests or calculations of diversity index.

Enzymatic activities

Fluorescein diacetate hydrolase activity (FDA)

FDAse activity was measured according to the modified method of Green *et al.* (2006). Six ml of potassium phosphate buffer to 50 mM (pH 7) plus 50 µl of FDA solution (2mg/ml of acetone) were added to 0.5 g of fresh soil and incubated at 30°C for 1h. The reaction was stopped by adding 2 ml of acetone and the mixture was immediately centrifuged for 3 minutes at 10,000*g* (4°C). Fluorescein released from the FDA was measured in the supernatant at 490 nm. FDAse activity is expressed as µmole of fluorescein released per minute per gram of dry soil ($U.g^{-1}$ DM).

Alkaline phosphomonoesterase activity (Pmb).

Pmb activity was measured using the method of Tabatabai and Bremner (1969). The reaction medium consists of 1 g of fresh soil, 4 ml of NaOH-glycine buffer (0.1M, pH 9.0) and 1 ml p-nitrophenyl phosphate (pNPP, 5 mM). After incubation for 1h at 30°C, the reaction was stopped by adding 1 ml of CaCl2 (0.5M) and 4 ml of NaOH (0.5 M). After centrifugation for 3 min at 10,000*g*, the amount of p-nitrophenol (pNP) released from p-NPP was measured in the supernatant at 412 nm. For each sample, a control is carried out under identical conditions by replacing the p-NPP solution with 4 ml of acetate buffer. Pmb activity is expressed in µmole of p-NP released per minute per gram of dry soil ($U.g^{-1}$ DM).

Urease activity (Ur)

Ur activity was assessed by a method adapted from Tabatabai and Bremmer (1972). In a test tube, 1 g of fresh soil was weighed and then mixed with 6mL of sodium acetate buffer (50 mM, pH 6) containing 20 mM urea. The reaction medium was incubated for 90 min (37°C) and centrifuged (3 min, 4°C, 10,000*g*). The nitrogen content in the form of ammonium ($N\text{-}NH_4^+$) released by the urease was quantified by the method (Mulvaney, 1996). The intensity of the emerald green color that is formed after addition of salicylate was measured in a spectrophotometer at a wavelength of

667 nm. The Ur activity is expressed as µmole of $N\text{-}NH_4^+$ released per minute per gram of dry soil ($U.g^{-1}$ DM).

Phenol oxidase activity (PO)

PO activity type tyrosinase (EC 1.14.18.1) was measured using the protocol of Saiya-Cork *et al.* (2002). In a test tube, 1 g of fresh soil was mixed with 6ml of a solution of L-dopa (3,4-dihydroxyphenylalanine, εM = 620 $L.mol^{-1}.cm^{-1}$) to 25 mM in acetate buffer (pH 6.5, 50 mM). After 15 min of incubation in the dark at ambient temperature the tubes were centrifuged (3 min, 10,000*g*, 4°C). The optical density of the supernatant was determined by spectrophotometry at 590 nm. Controls are performed under the same conditions as the test but adding 6ml of 25 mM acetate buffer without Dopa. PO activity is expressed as µmole of Dopachrome released per minute per gram od dry soil ($U.g^{-1}$ DM).

Statistical analysis and calculations

The effects of compost and sampling date on ergosterol concentration were assessed using two-way ANOVAs combined with Tukey test (Zar, 1984). Conditions of normality and variance homogeneity were verified by Shapiro-Wilks and Bartlett tests, respectively. When normality and homogeneity conditions were not met, the data were square-root transformed before applying ANOVA.

For soil enzymatic activities, basal respiration, microbial biomass and metabolic quotient data, we used Kruskal-Wallis test for date effect and Mann-Whitney test for compost effect as well as diversity indexes.

Principal component analysis (PCA) was performed on catabolic profiles at each sampling date.

Significant level was considered to be 95%. The software XLSTAT 2012.4.02 (Addinsoft 1995-2012) was used for statistical analysis.

Results

Basal respiration (BR) and microbial biomass (MB)

No compost effect was observed on BR and qCO_2 (Mann-Whitney, $p > 0.05$; Fig.2) but MB was higher on amended plots in May 2008 and October 2009 (Mann-Whitney, $p = 0.029$; Fig.2). Basal respiration and microbial biomass were significantly lower in July 2008, July 2009 and October 2009 (Kruskal-Wallis, $p < 0.0001$; Fig.2). On the opposite, qCO_2 was significantly higher in July 2008 and 2009 (Kruskal-Wallis, $p < 0.0001$; Fig.2).

After SWC normalization at 20%, compost amendment significantly increased BR in April 2009 (Mann-Whitney, $p = 0.029$; Table 2). BR, MB and qCO_2 significantly increased in July 2009 as well as BR and qCO_2 in July 2008 (Kruskal-Wallis, $p < 0.0001$; Table 2).

Ergosterol

Compost input had no effect on soil ergosterol content and no difference was observed over the experimentation (two-way ANOVA, $p = 0.948$; Fig.2).

Enzymatic activities

Compost amendment had no effect on enzymatic activities (Kruskal-Wallis, $p > 0.05$; Fig.2) but date effects were observed (Kruskal-Wallis, $p < 0.0001$; Fig.2).

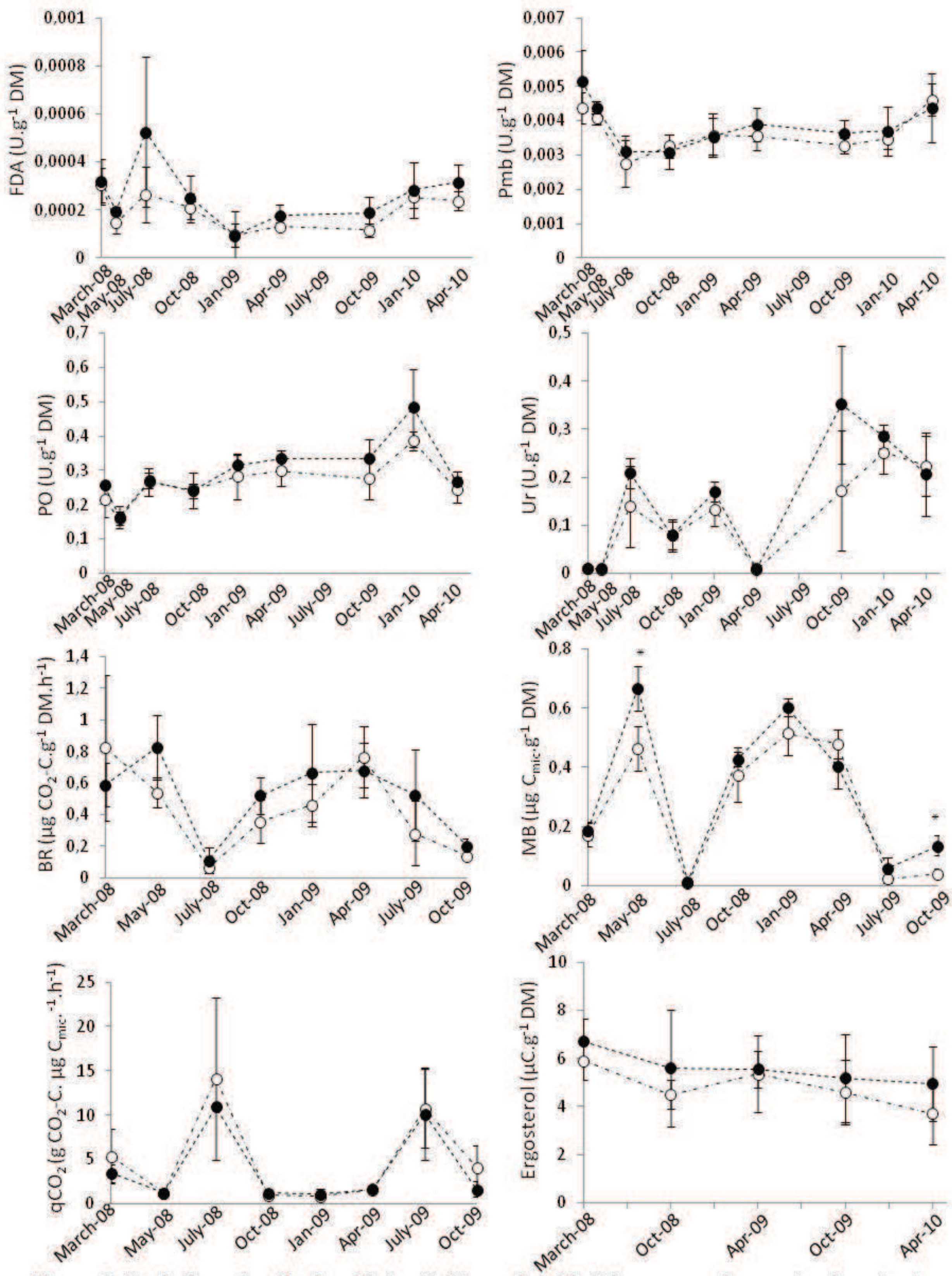

Figure 3. Evolution of soil microbial activities, microbial biomass and ergosterol content (N = 4 to 12 ; mean±SD) : Significant result of Mann-Whitney test: * ($p < 0.05$).

Catabolic profiles of the cultivable microbial communities

PCA performed on each sampling date showed no effect of compost input on catabolic activities and no seasonal effect was observed when the analysis was realized on all data (data not shown).

Diversity indexes analysis showed significant differences 18 and 24 months after amendment on amended plots (Mann-Whitney test, $p < 0.05$; Table 2). In April 2010, we observed an increase of specific richness (Mann-Whitney test, $p = 0.003$; Table 2). In October 2009 and April 2010, we observed an increase of Shannon index (Mann-Whitney test, $p = 0.037$ and 0.007 respectively; Table 2).

Soil chemical parameters

Compost amendment induced a significant increase in P_2O_5, N-(NO_3+NO_2), MgO and K_2O concentrations (two-way ANOVA, $p < 0.05$; Table 3) but no significant effect was observed on C_{org}, total and organic N and N-NH_4. However, C_{org} tended to slightly increase on amended plots.

However, for P_2O_5, MgO, NaO, N-NH_4 and N-(NO_3+NO_2) soil contents, a significant date effect was observed (two-way ANOVA, $p < 0.001$, and Kruskal-Wallis test, $p < 0.0001$ for Na_2O; Table 3). P_2O_5 significantly increased on amended plots from July 2008 to July 2010 (two-way ANOVA, $p < 0.05$; Table 3). K_2O and MgO concentration significantly increased on amended plots from October 2008 to July 2010 (one-way ANOVA, $p < 0.05$; Table 3). MgO content in April 2010 was significantly lower than the other sampling dates (one-way ANOVA, $p < 0.05$; Table 3). N-NH_4^+ significantly decreased after October 2008 on both amended and control plots (one-way ANOVA, $p < 0.0001$; Table 2), and there was a peak of N-(NO_3+NO_2) content only in October 08 (one-way ANOVA, $p = 0.001$; Table 3).

Table 2. Basal respiration (µg $C\text{-}CO_2.g^{-1}$ $DM.h^{-1}$), microbial biomass (µg $C_{mic}.g^{-1}$ $DM.h^{-1}$), metabolic quotient (g $C\text{-}CO_2$. µg $C_{mic}.^{-1}.h^{-1}$), Shannon index and specific richness (N = 4 to 12; mean±SD); values that differ at the 0.05 level are indicated by an exponent letter (Mann-Whitney test; a<b).

	Treatment	March 2008	May 2008	July 2008	October 2008	January 2009	April 2009	July 2009	October 2009	January 2010	April 2010
Basal respiration	Control plots	1.07±0.37	0.83±0.12	1.71±0.28	0.44±0.12	0.38±0.14	**0.80±0.15[a]**	3.10±0.40	0.41±0.09	-	-
	Amended plots	1.27±0.64	0.76±0.29	2.33±1.07	0.64±0.22	0.56±0.20	**1.13±0.21[b]**	3.33±0.85	0.51±0.14	-	-
Microbial biomass	Control plots	0.55±0.06	0.64±0.08	0.53±0.05	0.37±0.11	0.50±0.09	0.51±0.11	0.91±0.06	0.43±0.02	-	-
	Amended plots	0.61±0.08	0.62±0.06	0.56±0.14	0.46±0.09	0.61±0.08	0.55±0.11	0.99±0.13	0.50±0.12	-	-
qCO2	Control plots	1.92±0.61	1.32±0.32	3.30±0.32	1.35±0.76	0.74±0.20	1.70±0.42	3.42±0.58	0.94±0.19	-	-
	Amended plots	2.01±0.73	1.23±0.46	4.03±0.85	1.38±0.24	0.94±0.36	2.13±0.46	3.34±0.51	1.01±0.15	-	-
Shannon index	Control plots	2.73±0.01	2.36±0.47	2.94±0.12	2.95±0.14	3.05±0.15	2.70±0.38	-	**2.10±0.32[a]**	2.90±0.05	2.57±0.13
	Amended plots	2.67±0.04	2.67±0.35	2.71±0.43	3.01±0.03	3.00±0.06	2.76±0.40	-	**2.70±0.16[b]**	2.90±0.04	2.80±0.14
Specific richness	Control plots	17.25±0.32	15.50±3.42	21.25±2.50	21.25±2.22	22.92±2.63	18.75±3.83	-	**13.63±2.50**	20.75±0.50	**15.83±2.01**
	Amended plots	16.08±0.57	17.00±4.69	18.75±5.38	23.00±1.15	22.00±1.15	19.42±4.07	-	**18.00±2.45**	21.50±1.29	**19.67±2.09**

Table 3. Soil nutrient concentrations (N = 4 to 12; mean±SD); values that differ at the 0.05 level are indicated by an exponent letter (Mann-Whitney test; a<b).

		P_2O_5	MgO	K_2O	Na_2O	C_{org}	$N\text{-}(NO_3+NO_2)$	$N\text{-}(NH_4)$
March 2008	Control plots	0.018 ± 0.002^a	0.251 ± 0.052^a	0.428 ± 0.067^a	0.024 ± 0.0002^a	0.421 ± 0.072^a	9.788 ± 2.15^a	28.288 ± 4.817^a
	Amended plots	0.017 ± 0.003^a	0.249 ± 0.018^a	0.435 ± 0.033^a	0.022 ± 0.0025^a	0.352 ± 0.025^a	6.875 ± 2.65^a	31.307 ± 6.625^a
May 2008	Control plots	0.013 ± 0.001^a	0.250 ± 0.039^a	0.478 ± 0.109^a	0.023 ± 0.0034^a	n.d.	n.d.	n.d.
	Amended plots	0.017 ± 0.004^a	0.270 ± 0.030^a	0.566 ± 0.150^a	0.042 ± 0.0071^a	n.d.	n.d.	n.d.
July 2008	Control plots	$\mathbf{0.017\pm0.007^a}$	0.270 ± 0.027^a	0.479 ± 0.040^a	0.023 ± 0.0009^a	n.d.	n.d.	n.d.
	Amended plots	$\mathbf{0.033\pm0.010^b}$	0.322 ± 0.036^a	0.657 ± 0.162^a	0.043 ± 0.0015^a	n.d.	n.d.	n.d.
October 2008	Control plots	$\mathbf{0.013\pm0.002^a}$	$\mathbf{0.235\pm0.036^a}$	$\mathbf{0.398\pm0.081^a}$	0.029 ± 0.0047^a	0.294 ± 0.080^a	$\mathbf{8.441\pm1.64^a}$	28.431 ± 5.973^a
	Amended plots	$\mathbf{0.095\pm0.030^b}$	$\mathbf{0.363\pm0.031^b}$	$\mathbf{0.675\pm0.089^b}$	0.039 ± 0.0066^a	0.380 ± 0.101^a	$\mathbf{38.958\pm12.46^b}$	28.004 ± 7.361^a
April 2009	Control plots	$\mathbf{0.014\pm0.004^a}$	$\mathbf{0.213\pm0.016^a}$	0.448 ± 0.072^a	0.014 ± 0.013^a	0.287 ± 0.058^a	4.268 ± 1.197^a	7.060 ± 1.317^a
	Amended plots	$\mathbf{0.067\pm0.036^b}$	$\mathbf{0.344\pm0.042^b}$	0.672 ± 0.177^a	0.015 ± 0.0014^a	0.329 ± 0.065^a	4.426 ± 2.264^a	8.621 ± 2.156^a
April 2010	Control plots	$\mathbf{0.013\pm0.004^a}$	$\mathbf{0.171\pm0.012^a}$	$\mathbf{0.386\pm0.061^a}$	0.017 ± 0.0018^a	0.279 ± 0.067^a	3.286 ± 2.028^a	4.871 ± 0.625^a
	Amended plots	$\mathbf{0.048\pm0.006^b}$	$\mathbf{0.286\pm0.028^b}$	$\mathbf{0.546\pm0.034^b}$	0.016 ± 0.0030^a	0.317 ± 0.029^a	3.096 ± 1.120^a	5.996 ± 1.464^a

Compost amendment induced a significant increase SWC in May 2008, April, July and October 2009, and April 2010 (one-way ANOVA, $p < 0.0001$; Fig.1). A seasonal effect was observed (one-way ANOVA, factor date, $p < 0.0001$; Fig.1), SWC in July 2008 and 2009 being significantly lower than at the other dates.

Discussion

According to other studies (Borken *et al.,* 2002; Ros *et al.*, 2002; Kowaljow et Mazzarino, 2007), compost amendments induced an increase in microbial biomass and microbial activities. However, in the present study, even if the microbial parameters showed a global tendency to increase with compost addition, we observed only low and sporadic significant variations and only few measured parameters were higher on amended plots than on control ones.

During the first months after amendment, short-term effects were however observed. Six weeks after amendment (May 2008), we observed a significant increase in MB and a marginal increase in BR with compost addition. As shown by Schimel *et al.* (1999) and Larchevêque *et al.* (2005a), these effects might be correlated to the increase of SWC in May 2008 (Fig.1). This result is confirmed during the dry period of July 2008, with a decrease in MB and BR and an increase in qCO_2 suggesting a period of intense stress for microorganisms (Killham, 1985; Anderson, 2003). Moreover, when SWC was normalized at 20% of dry matter for all samples, MB and BR were highly increased. This supports the water stress. Adding to the potential effect of SWC in May 2008, the input of exogenous microorganisms might explain MB increase as suggested by Blagodatsky *et al.* (2000). In July 2008, FDA and Ur activities also tended to be enhanced with organic amendment. Due to the absence of other parameters increase in the same time, we could assume that this last effect could be induced by enzyme production during a previous period when microbial communities were still active (Burns, 1982; Pascual *et al.,* 2002; Burns and Wallenstein, 2010). The non-significance could be due to the high variability of these parameters among plots or to the lack of significant increase of soil nutrients with compost just after amendment.

After this first short-term period, we observed a significant increase of soil nutrients (K_2O, MgO, P_2O_5) and this effect remained until the end of the experiment. But no significant effect was observed on organic C and mineral N or on microbial activities and biomass except MB increase on amended plots in October 2009. Other studies (Sanchez-Morenedero *et al.,* 2004; Ros *et al.,* 2002; Odlare *et al.,* 2008) showed that transfer of organic C from compost to soil allowed the increase of enzymatic activities. Thus we could assume that the present transfer was too low to induce a response of microbial communities. As previously suggested, MB development is strongly linked to SWC. Thus, in October 2009 as in May 2008, the observed increase in MB might reflect better environmental conditions due to the increase of SWC on amended plots. However, even if SWC was significantly higher on amended plots also in April and July 2009, no effect was observed. In April 2009, we may assume that SWC on control plots (18.6%) was sufficient to allowed MB development and, on the opposite, too low on amended plots (8.1%) in July 2009. Moreover, in April 2009, after SWC normalization, BR and qCO_2 on amended plots significantly increased compared to samples with *in situ* soil parameters. It might result from stress due to the soil drying before the experiment because *in situ* SWC was higher than 20%.

Pmb enzymes are involved in the mineralization of organic P in soil (Magid et al., 1996). Their activities are controlled by soil properties, soil organisms interaction, leachate inputs and the presence of inhibitors or activators (Juma and Tabatabai, 1977; Tyler, 1981; Kanderler *et al.,* 1996; Chen *et al.*, 2003). Several studies showed an inhibition of Pmb activity by available P (Juma and Tabatabai, 1977; Nannipieri *et al.*, 1978; Lima *et al.*, 1996; Olander and Vitousek, 2000; Moscatelli *et al.*, 2005). According to Oshima *et al.* (1996), inorganic P could repress phosphomonoesterase synthesis by inhibiting PHO genes expression. In our study, no effect of compost amendment was observed. Thus, we might assume that Pmb activity on amended and control plots was limited by C and N availability as suggested by Allisson and Vitousek (2005).

No significant effect was observed on PO even if this activity tended to be higher on amended plots from January 2009 to April 2010 (Fig.2). Fungi are the main producers of PO enzymes (Baldrian, 2006) and a tendency to increase in soil ergosterol content was also observed on amended plots. PO is known to be involved in biodegradation of aromatic compounds such as phenols and lignin (Criquet *et al.,* 2000; Farnet *et al.,* 2004) indicating that these organic compounds have not been transferred into soil. Moreover, PO activity is regulated by substrate availability (Sinsabaugh *et al.*, 1993). But, as C and N content did not increase on amended plots, this lake of substrate might explain this result.

Contrary to other studies (Kanderler *et al.,* 1996; Wang *et al.*, 2007; Moreno *et al.*, 2009), no negative effect was observed on microbial diversity due to heavy metals input. In the present study, we observed a sporadic change of catabolic diversity with an increase only 16 months (October 2009) and 24 months (April 2010) after amendment. During a 2-years experiment by Crecchio *et al.* (2001), no effect was observed but they assumed that the duration of observation was too short. Indeed, compost effects on microbial communities depends on its nature and its amount (Pascual *et al.*, 1998; Albiach *et al.*, 2000; Garcia-Gil *et al.*, 2000; Saison *et al.*, 2006). Moreover, the delayed effect could result from the adaptation of soil microbial communities to this new nutrient resource or to the transfer of microorganisms from compost. But some changes might occur earlier without being apparent (Marschner *et al.,* 2003). This lack of apparent effect could be due to the redundancy of soil functions (Nannipieri *et al.*, 2003).

All along the experiment, we observed a seasonal effect on BR and MB (Fig.2) with the lowest values in July, the highest in January and intermediate values in April and October. As previously suggested (Schimel *et al.,* 1999; Larchevêque *et al.,* 2005a), BR and MB were more water-dependent than nutrient-dependent.

Globally, compost effects were low but it might be explained by its properties (Pérez-Piqueres *et al.,* 2006). High compost maturity could have reduced C and nutrients availability (Andrès *et al.,* 2011). Moreover, we can expect that the dose of mature compost used in this study was not adapted (*i.e.* too low) to this burned ecosystem to significantly increase C and N availability (Allisson and Vitousek, 2005).

Conclusion

Contrary to what was expected, compost effects on soil microbial biomass and activities were low. However, organic amendment induced sporadically increase of microbial biomass and microbial catabolic diversity. We observed marked seasonal effects only for basal respiration and microbial biomass suggesting that SWC was their main limiting-factor. Conversely, enzyme activities and ergosterol seemed to be nutrient-dependent. Moreover, it seemed that compost amendment increased microbial diversity 16 months after amendment. Higher amount of compost or less mature compost could be applied to improve microbial activities and biomass but it might affect microbial diversity through excessive trace elements input and induce eutrophication process. Even if no negative effect was observed, further studies are needed to understand how compost induced microbial diversity increase and to observed long term effect of this amendment on possible trace elements release and toxicity after soil parameters modifications.

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Chapitre IV. Conclusion

Dans un premier temps, l'analyse en laboratoire nous a permis d'observer l'impact de l'apport de composts urbains sur un sol méditerranéen après incendie. Nous avons tout d'abord étudié la dégradation de la matière organique et le développement de la biomasse microbienne et, ensuite, l'effet de cet apport sur le risque d'érosion. Les résultats obtenus permettent de formuler les conclusions suivantes :

- l'apport de composts urbains induit une augmentation plus ou moins importante de la teneur en matières organiques et en nutriments du sol. Plus un compost a des teneurs initiales élevées, plus l'augmentation observée est importante;
- la stabilité de la matière organique des composts conditionne sa minéralisation. Plus elle est instable et plus elle sera minéralisée rapidement ;
- la minéralisation de l'azote est conditionnée par la teneur en azote et en carbone du milieu. Ainsi plus le rapport C/N du compost est faible plus la minéralisation est importante. Inversement s'il est trop élevé, une phase d'immobilisation de l'azote par les microorganismes peut se produire;
- le développement de la biomasse microbienne totale semble principalement contrôlé par la biodégradabilité et la biodisponibilité de la matière organique et des éléments nutritifs;
- la modalité d'apport (en mulch ou incorporé) n'a que peu d'effet sur le développement de la biomasse microbienne. Si la quantité ou la disponibilité en nutriments est insuffisante en un point pour permettre leur croissance, les populations microbiennes peuvent se développer à des endroits plus favorables;
- l'apport de composts urbains permet de réduire le risque d'érosion principalement grâce à un effet de protection mécanique des composts qui

forment un mulch en surface. Selon cette modalité on observe une très forte diminution voir un arrêt du ruissellement de surface et donc de l'érosion;

- l'apport de compost en mulch pour lutter contre l'érosion peut induire une pollution par des ETM dans les eaux de ruissellement de surface;
- la réduction du ruissellement entraîne une augmentation de la rétention d'eau dans le sol et de la percolation.

Cette étude a également permis de constater que le compost le plus apte à favoriser le développement de la biomasse microbienne est le compost d'ordures ménagères résiduelles (OMR) grâce à sa forte teneur en matière organique facilement biodégradable. Mais cette propriété limite son effet à long terme vis-à-vis du maintien du taux de matière organique du sol, étant donné sa dégradation rapide. Un autre point positif de ce compost est la forte réduction du ruissellement de surface mais qui pourrait être contrebalancée par la présence d'ETM dans l'eau récupérée. Il en est de même pour le compost de boues de station d'épuration (B) mais l'effet négatif vient ici du niveau du phosphore exporté. Le compost de déchets verts (DV) a un comportement opposé aux composts OMR et B. D'une part il n'a que peu d'effet sur la minéralisation des mélanges sol/compost et sur le développement de la biomasse microbienne, d'autre part la réduction de ruissellement induite par son apport est faible mais il n'y a pas de pollution observée par les ETM et les phosphates.

Ainsi le compost le plus apte à régénérer le sol et à limiter les risques d'érosion est soit le compost OMR, soit le compost B en déterminant si les quantités d'ETM ou de phosphore ne peuvent pas être néfastes vis-à-vis de l'environnement.

Ces expériences se sont déroulées en laboratoire en plaçant les échantillons dans des conditions idéales pour le développement des microorganismes. Elles étaient axées sur l'impact au niveau des propriétés du sol. Afin d'étudier l'impact des conditions climatiques sur ces paramètres et sur la végétation (vitesse de recolonisation, discrimination de certaines espèces), une expérimentation complémentaire a été menée *in situ* avec un compost de boues de station d'épuration apporté en mulch. Pour cela, un compost de boues de station d'épuration a été épandu en mulch sur une garrigue après incendie. Cette étude a permis de faire les observations suivantes :

- augmentation rapide de la biomasse des espèces herbacées dans les premiers mois après amendement;
- la stratégie de développement racinaire des végétaux étudiés a impact plus important que leur type biologique dans l'utilisation des éléments du compost;
- la biodiversité des parcelles est plus homogène sur les parcelles amendées;
- augmentation faible des nutriments dans les plantes et variable en fonction des éléments et des espèces considérées;
- pas de baisse de diversité végétale, ni d'eutrophisation;
- augmentation rapide et durable des teneurs en nutriment. Néanmoins, aucun effet n'a été observé sur les teneurs en azote et en carbone;
- augmentation de la teneur en eau du sol grâce à la formation d'une couche protectrice par le compost limitant l'évaporation;
- pas d'augmentation de la teneur en éléments trace métalliques dans le sol et une diminution dans les plantes des parcelles amendées.
- augmentation sporadique de la biomasse microbienne. De plus, les variations de celle-ci ainsi que de la respiration basale sont dépendentes de la teneur en eau du sol plus que de l'apport de nutriments;
- pas d'effet néfaste apparent du compost sur les microorganismes et la végétation.

L'apport de compost de boues de station d'épuration a donc un effet positif sur l'écosystème considéré. Dans un premier temps, l'apport de compost en mulch semble avoir créé une couche protectrice qui limite l'assèchement de la couche superficielle du sol comme observé lors de l'étude en laboratoire. Ce paramètre semble être plus limitant au niveau de la biomasse microbienne que l'apport de nutriments par le compost mais sans toutefois permettre une hausse des activités. Outre cette augmentation de biomasse, une augmentation du développement des végétaux a été notée. Ces augmentations de biomasse permettent ensuite une stabilisation du sol et limitent l'érosion et la perte de nutriments. Cet apport a aussi induit des conditions environnementales plus homogènes et plus favorables au niveau du sol permettant un développement Du fait de la stabilité du compost, le carbone et l'azote peuvent être peu minéralisables et ne sont pas transférés au sol à la différence d'autres éléments. Ces derniers peuvent alors favoriser le développement initial des espèces herbacées rejetant de souche grâce à un réseau de racines superficielles déjà établi qui permet une meilleur captation que les espèces germant à partir de la banque de graines ou celles ayant des racines plus profondes. Néanmoins, une augmentation des nutriments contenus dans les plantes a été observée ce qui pourrait permettre un développement ultérieur accru comparé aux végétaux des parcelles témoins.

De plus, l'apport de compost n'a pas eu d'effet nocif observé sur l'écosystème étudié. D'une part, il n'a pas été observé d'accumulation d'ETM ou de composés toxiques. Du fait de la forte teneur de ces éléments dans le compost, on peut supposer qu'ils sont complexés et ne sont libérés pas dans le sol ou non assimilables par les plantes. D'autre part, une diminution de la teneur de ces éléments a été observée dans les végétaux. L'apport de compost semble donc limiter leur disponibilité du fait de leur complexation avec des éléments apportés par le compost.

Les effets observés sur l'écosystème ont été faibles. Comme noté précédemment, la maturité du compost et sa stabilité ou la quantité apportée peuvent avoir limité la libération des éléments d'intérêt et donc leur effet quant aux développements des microorganismes et de la végétation. On peut alors supposer qu'un compost moins mature ou une quantité plus importante aurait eu un effet plus significatif. Mais cela peut induire un risque de pollution plus important du fait d'une complexation moindre des éléments qui peuvent avoir un effet toxique. De plus, des teneurs plus élevées ont été mesurées pour certains éléments tel le phosphore dans les parcelles amendées. Un apport plus important de nutriments pourrait entraîner un déséquilibre dans l'écosystème avec une modification de la flore et un risque d'eutrophisation du milieu ainsi que la pollution des eaux souterraines par les excès de phosphates ou de nitrates.

Outre l'aspect lié à l'augmentation des éléments apportés par le compost, une augmentation de la quantité pourrait aussi avoir un effet négatif. La création d'une couche trop importante en surface du sol pourrait alors retarder ou empêcher la croissance des plantules par effet d'écrasement.

Il pourrait aussi être nécessaire de prendre en compte de futures modifications des conditions environnementales et des caractéristiques du sol. En effet, un changment de l'équilibre pourrait entraîner une diminution de la complexation d'éléments potentiellement toxiques pour les plantes ou les organismes du sol et donc leur libération.

L'apport de compost sur un sol méditerranéen incendié a donc un effet positif sur la recolonisation du milieu par les microorganismes et les végétaux. Il est cependant nécessaire de prendre en compte les caractéristiques du sol et du compost afin de limiter des effets nocifs dus à des apports trop excessifs d'éléments nutritifs ou d'ETM qui pourraient inhiber certains processus environnementaux essentiels à l'écosystème. Des études à plus long terme sur les différents paramètres suivi pourraient donc être nécessaire pour confirmer ou infirmer l'inocuité de ce type de compost dans le temps.

Toutefois, dans l'état actuel de l'étude, si les effets positifs ont peu de répercussions sur le développement observable de l'écosystème, aucun effet néfaste n'a été relevé. Ainsi, ce type d'apport pourrait être envisager dans la gestion des stocks de compost et permettre un développement et des débouchés pour cette filière de traitement de nos déchets.

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