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École doctorale SIReNa
Sciences et Ingénierie des Ressources Naturelles

Stoichiometric traits in ecological analyses:
A tool to understand community responses to global changes

A dissertation submitted by

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Verachte nur Vernunft und Wissenschaft,
des Menschen allerhöchste Kraft.
Lass nur in Blend- und Zauberwerten
Dich vom Lügengeist bestärken,
so hab ich dich schon unbedingt.
…..

Reason and Knowledge only thou despise,
The highest strength in man that lies!
Let but the Lying Spirit bind thee
With magic works and shows that blind thee,
And I shall have thee fast and sure! —

(Mephisto, in Goethes Faust)

Abstract

Worldwide, changing nutrient concentrations pose main stressors in many ecosystems, which is likely to continue in the future. Consumer communities are often not directly affected but changes in resource quantity and quality have led to shifts in taxonomic as well as functional composition. For example, under phosphorous-rich conditions, some studies showed that small and fast-growing taxa were favoured and also some shifts towards P-rich taxa were reported.

Ecological stoichiometry theory (=EST) offers a framework to investigate the equilibrium and fluxes of elements (i.e. nutrients) between organisms one another and the environment. Stoichiometric traits, that is the elemental composition of an organism's body tissue, has been successfully linked to resource quality or functional traits, especially growth rate and feeding groups. However, so far, most studies did not go beyond taxon or population level and explicit links towards nutrient availabilities are rare.

It was thus the aim of my PhD to investigate whether – and to what extent – the integration of stoichiometric traits into community analyses can improve our understanding of a community response towards global stressors, i.e. changes in nutrient concentration and their stoichiometry. Using stream macroinvertebrates as model organisms, I tested assumptions from EST on community level relying to three databases comprising (i) stoichiometric traits (which I constructed based on field samplings campaigns and a literature research), (ii) functional traits and (iii) community datasets (abundances & environmental parameters).

I found significant links between stoichiometric traits and (other) functional traits, as well as between water nutrient concentration and community stoichiometry. These results could complementarily be explained by EST and phylogeny. Phylogeny was found to largely control a taxon's body stoichiometry leading to partly distinct responses among major taxonomic groups. Also confounding factors affecting community stoichiometry via shifts among those groups seemed to have weaken the global trends over a spatial nutrient gradient. On a temporal scale however the community stoichiometry and functional response followed our expectations based on EST: Under decreasing phosphorous concentrations, the community shifted towards P-poor taxa which decreased community %P. Further, high phosphorous conditions favoured traits related to fast development (e.g. small body sizes, short life duration) and detritus feeding; whereas low phosphorus conditions favoured slow-developing and predatory taxa. Stoichiometric traits were largely involved in this link, underlining their potential to explain ecological responses towards nutrient changes.

It remains to be investigated how such a changed consumer nutrient pool affect nutrient cycling towards higher/lower trophic levels or adjacent terrestrial ecosystems. It might also be worth to study its application towards – and interaction with – other global stressors, notably global warming, which already was shown to affect stoichiometry and/or traits related to body stoichiometry (i.e. growth, development). The extension of the stoichiometric trait database could further help broadening the application of this approach and testing our findings also for other systems/regions.

Abstract (french)

Dans de nombreux écosystèmes à travers le monde, les changements de concentrations et d'abondance relative des nutriments constituent un facteur de stress majeur. Les communautés de consommateurs ne sont souvent pas directement affectées par ces changements, mais plutôt indirectement par les modifications induites dans la quantité et la qualité des ressources de base. Par exemple, dans des conditions de forte disponibilité en phosphore, les taxons de petite taille et à croissance rapide sont favorisés tous comme ceux étant naturellement riches en phosphore.

La théorie de la Stœchiométrie Ecologique (EST, Ecological Stoichiometry Theory) offre un cadre conceptuel pour étudier l'équilibre et les flux d'éléments (c.a.d. de nutriments) entre les organismes et l'environnement. Les traits stœchiométriques, ici considérés sous l'angle de la composition élémentaire des tissus corporels d'un organisme, ont pu être liés avec succès à la qualité des ressources ou à des traits fonctionnels des taxons, notamment le taux de croissance et le groupe trophique des organismes. Néanmoins, jusqu'à présent, la plupart des études n'ont pas dépassé le niveau du taxon ou de la population et les liens explicites entre la structure des communautés de consommateurs et la disponibilité en nutriments restent rares.

L'objectif de mon doctorat était d'étudier si - et dans quelle mesure - l'intégration de traits stœchiométriques dans les analyses de communautés pouvait améliorer notre compréhension de leur réponse aux changements de concentrations en nutriments. En prenant pour communautés modèles les macroinvertébrés des cours d'eau, j'ai testé les hypothèses de l'EST en m'appuyant sur trois bases de données comprenant (i) des traits stœchiométriques (base de données construite dans le cadre de cette thèse), (ii) des traits fonctionnels et (iii) la structure des communautés de macroinvertébrés en France (abondances et paramètres environnementaux).

J'ai ainsi pu mettre en évidence des liens entre les traits stœchiométriques et les autres traits fonctionnels des taxons, ainsi qu'entre la concentration des nutriments de l'eau et la stœchiométrie des communautés. Ces résultats pourraient être expliqués de manière complémentaire par l'EST et la phylogénie. La phylogénie contrôle largement la stœchiométrie des taxons, entraînant des réponses distinctes parmi les principaux groupes taxonomiques. De même, les variations d'abondance relative des différents taxons selon la disponibilité des nutriments tendent à réduire l'importance des traits stœchiométriques comme facteur explicatif. Cependant, en considérant un seul cours d'eau subissant une réduction progressive des concentrations en phosphore, la stœchiométrie de la communauté et sa réponse fonctionnelle ont suivi nos attentes basées sur l'EST: Une réduction du phosphore sélectionne des taxons pauvres en P et réduit le %P de la communauté. En outre, des conditions de phosphore élevées ont favorisé les traits liés au développement rapide (par ex. petites tailles de corps, courte durée de vie) et à la détritivorie, tandis que des conditions de phosphore faibles ont favorisé les taxons à développement lent et à dominance prédateurs. Les traits stœchiométriques étaient largement impliqués dans ce lien, soulignant leur potentiel pour expliquer les réponses écologiques aux changements de concentrations en nutriments.

Il reste maintenant à étudier comment les changements de stocks de nutriments constitués par les consommateurs affecte *in fine* le cycle des nutriments, et le transfert de ces nutriments vers les niveaux trophiques supérieurs et les écosystèmes adjacents. Il pourrait également être intéressant d'étudier l'applicabilité de ces approches à d'autres facteurs de stress globaux, notamment le réchauffement climatique. L'extension de la base de données de traits stœchiométriques pourrait ainsi contribuer à élargir l'application de cette approche et à tester nos résultats sur d'autres types d'écosystèmes.

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Chapter I:
Introduction

Considering, what's everything made from

So far, we know 103 elements that build the world. A small subset of these is sufficient to build all life forms that we know, from microbes to plants and animals. They can be divided into macroelements (C, H, O, N, P) and trace elements, which appear at lower quantities but still are involved in essential metabolic functions (e.g. Fe, Mg, Ca, K). These elements cycle through the abiotic and biotic environment: They are consumed and built into new structures (=growth) or used to fuel biochemical reactions, re-packed and released back into the environment. They travel from one organisms to another, or are object of competition. Elements thereby rarely occur freely in the environment and are generally available as ions or molecules often bound in complex organic packages. In ecology, elements are often referred to as "nutrients" pointing out their essential importance to build and sustain life and growth. It is therefore not only interesting but rather essential to consider this very basic level of building blocks when studying more complex levels of biological organization.

Nutrients – a global stressor

Human activities have altered global nutrient cycles by drastically increasing carbon (C), nitrogen (N) and phosphorous (P) availabilities compared to natural fluxes (Smil, 2000; Fowler *et al.*, 2013; Galloway *et al.*, 2014; Battye *et al.*, 2017; Yuan *et al.*, 2018; Friedlingstein *et al.*, 2020). These nutrient inputs however are not balanced. A faster accumulation of phosphorus than of nitrogen in freshwaters (Yan *et al.*, 2016) or a lower deposition of nitrogen than of phosphorous (Isles *et al.*, 2018) confronts organisms not only with changes in the quantity of elements but also in their ratios (Elser *et al.*, 2009; Peñuelas *et al.*, 2012, 2013; Yan *et al.*, 2016; Isles *et al.*, 2018; Yuan *et al.*, 2018). To counteract the negative effects of eutrophication on ecosystems, management programs have been established and successfully reduced the levels of phosphorous in freshwaters (Jeppesen *et al.*, 2005; Floury *et al.*, 2013; Latli *et al.*, 2017). In terms of nitrogen however many lake systems remained at high concentrations, leading – again – to changing N:P conditions (Jeppesen *et al.*, 2005).

Nutrients are directly affecting autotrophic organisms in marine, freshwater and terrestrial systems (Elser *et al.*, 2007). Increased nitrogen and phosphorous concentrations generally increase the quantity of phytoplankton and periphyton (Hessen, 2002; Hillebrand, 2002; Qin *et al.*, 2007) as well as the quality of primary producers' biomass for herbivorous consumers. They are variable in their elemental composition and mirror the nutrient conditions of the environment, i.e. containing higher amounts of phosphorous as a response to phosphorous enrichment (Hessen, 2002; Cross *et al.*, 2003; Fink *et al.*, 2006; Liess & Hillebrand, 2006; Ventura *et al.*, 2008). Also the quality of detritus, the basal resource in "brown" food webs (compared to the "green" food webs relaying on autotrophic production), responds positively towards nutrient enrichment (Cross *et al.*, 2007; Danger *et al.*, 2013; Demi *et al.*, 2018). Colonizing microbes are able to store excess nutrients, which increases the nutritional value for detritivores (Danger *et al.*, 2016; Gulis *et al.*, 2017).

Heterotrophic organisms are less subjected to direct nutrient effects, although toxic effects of nitrate can occur under rather high concentrations in invertebrates (Camargo *et al.*, 2005). The indirect effect however can be substantial: by feeding on basal resources, primary consumers can be faced with a reduced quality of their food sources when environmental nutrient conditions change (see above). Compared to autotrophs their elemental composition is rather stable (Sterner & Elser, 2002; Fink *et al.*, 2006; Persson *et al.*, 2010; Danger *et al.*, 2013) and they rely on a specific amount of nutrients. Low-nutrient diet reduces growth (Sterner & Schulz, 1998; Frost & Elser, 2002; Danger *et al.*, 2013; Bracken, 2017) and development (Malzahn *et al.*, 2010) of herbivores and detritivores and in most cases, nutrient increases in resources thus are beneficial for consumers. However, also excess nutrients are problematic, so that a deviation from the optimal resource quality in either direction can lead to reductions of the overall fitness (Boersma & Elser, 2006; Elser *et al.*, 2016). Heterotrophs thus use regulating mechanisms to meet their nutritional needs even under non-optimal resource conditions. The strategies include changes in diet or feeding rate (Fink *et al.*, 2006; Hood *et al.*, 2014; Jochum *et al.*, 2017a; Zhang *et al.*, 2018), adapting nutrient excretion (Balseiro & Albariño, 2006) or assimilation efficiency (Anderson *et al.*, 2005; Hessen *et al.*(2013) and references therein). This also buffers nutritional imbalances for higher trophic levels which therefore often do not reach secondary consumers (Malzahn *et al.*, 2010), but in some cases can decrease their performance (Malzahn *et al.*, 2007; Boersma *et al.*, 2008). However, those regulations are energy-consuming (Anderson *et al.*, 2005) so that in general taxa with a minimal mismatch are favoured under certain nutrient conditions. We thus expect to find more P-rich taxa under high phosphorous conditions (Singer & Battin, 2007; Prater *et al.*, 2015; Teurlincx *et al.*, 2017). Overall, the responses to nutrient enrichment are taxon specific (Friberg *et al.*, 2010; Demi *et al.*, 2019) but in the end affect consumer assemblages as a whole.

From community ecology to ecosystem processes

Community ecology studies the organization of and the interaction between different taxa that share a common space and how that is influenced by abiotic factors. It aims at understanding – and ideally predicting – how a community responds to stressors.

Moderate nutrient enrichment increases overall taxa richness, abundances (Blumenshine *et al.*, 1997; Biggs *et al.*, 2000; Cross *et al.*, 2007; Ortiz & Puig, 2007; Wimp *et al.*, 2010) and productivity (Spivak *et al.*, 2009) of consumer assemblages. Higher concentrations however cause negative effects (King & Richardson, 2007; Ortiz & Puig, 2007) and can lead to homogenization of communities (Singer & Battin, 2007; Zhang *et al.*, 2019, Donohue et al. 2009). Conducting a meta-analysis, Nessel *et al.* (2021) found that globally and across ecosystems, invertebrate abundances were decreasing and were more affected than overall biomass or taxonomic richness. However, they also pointed out the absence of a universal response of taxa, and in turn communities. Generally, invertebrate communities under nutrient pollution shift towards oligochaetes and snails, while abundances of sensitive taxa (e.g. Ephemeroptera,

Plecoptera, Trichoptera) decrease or these species vanish completely from these sites (McCormick *et al.*, 2004; Ortiz & Puig, 2007; Friberg *et al.*, 2010; Zhang *et al.*, 2019).

Due to their position between autotrophic producers and higher trophic levels, consumer communities are not only concerned by but also themselves affecting energy and nutrient cycling: By transforming consumed nutrients (*via* basal resources) *via* growth and reproduction into new biomass, i.e. secondary production, they are providing resources for secondary consumers and higher trophic levels. Generally, nutrient enrichment increases biomass, abundances and/or activity of all trophic levels and travel up from autotrophic or heterotrophic basal resources to secondary consumers or higher trophic levels (Ardón *et al.*, 2021). But this does not represent uniform responses and positive effects might not reach the level of macroinvertebrate predators, for example due to prey shifting towards taxa outside the handling spectra (Spivak *et al.*, 2009). Taxa shifts under high phosphorous conditions can further cause food chains to become shorter (Singer & Battin, 2007) and less susceptible to collapse (Canning & Death, 2021).

A top-down contribution to nutrient cycling is the release of nutrients *via* excretion. This can stimulate growth or quality of primary resources (Evans-White & Lamberti, 2005; Liess & Hillebrand, 2006; Knoll *et al.*, 2009) and also depends on resource quality (Elser & Urabe, 1999). Detritivore consumers additionally contribute to the decomposition of plant material, which also can increase nutrient concentrations (Crowl *et al.*, 2001).

Ecosystem ecology investigates the interaction between biotic and abiotic systems on a large spatial scale. Studies have generally used overall community indices such as diversity and/or taxa richness. Especially biodiversity has often been linked to ecosystem functions or services, generally suggesting a generally positive correlation (reviews/meta-analyses by (Balvanera *et al.*, 2006; Cardinale *et al.*, 2011; Brockerhoff *et al.*, 2017) although it depends on the function and the spatial scale (Plas, 2019; Gonzalez *et al.*, 2020). This however lacks the consideration of taxa identity (or taxonomic composition). Although the overall taxonomic richness or diversity of a community might not be affected by nutrient enrichment, there can still be a shift in taxonomic composition (Nessel *et al.*2021; McCormick *et al.*, 2004; Hillebrand *et al.*, 2018). The contribution to ecosystem functions however depends on taxa identity and can be influenced by biotic interactions or habitat conditions (Crowl *et al.*, 2001; O'Connor & Crowe, 2005; Ieno *et al.*, 2006; Bulling *et al.*, 2008; Slade *et al.*, 2017; Emery *et al.*, 2021). Using community weighted measures improved trait relationships towards climate stressors in plants (Griffin-Nolan *et al.*, 2017). The authors and several others thus pointed out the importance of integrating the community level and species interactions when studying ecosystem processes (Suding *et al.*, 2008; Griffin-Nolan *et al.*, 2017; Bannar-Martin *et al.*, 2018; Slade *et al.*, 2019).

The functional trait approach

When questions address the functioning of a community, it is described by functional traits rather than taxonomic units where each organism is described by its morphological (body size, leaf morphology), physiological (growth rate, respiration rate, nutrient uptake rate), phenological (life duration, voltinism) or behavioral (feeding habit, locomotion) characteristics. Often combined to a multi-metric trait profile comprising several functional traits, they are linked to the organism's fitness and thereby describe its interactions with the biotic and abiotic environment (Violle *et al.*, 2007). Species are selected based on their trait profile, i.e. tolerances and preferences for certain habitat characteristics (e.g. for a substrate, temperature, food source).

This allows a functional perspective of species distributions over several levels of biological organization (McGill *et al.*, 2006; Cadotte *et al.*, 2011). Ratios among trait groups, for example feeding groups, can give information such as whether the system is autotrophic or heterotrophic-based without considering taxonomic identity (Cummins, 2018). Life history traits such as development or growth rate are linked to taxa and population growth and therewith to productivity (Huryn & Wallace, 2000). It also enables to differentiate human-impacted from un-impacted sites (Dolédec & Statzner, 2008) or to identify different stressors (Lange *et al.*, 2014) and can help explaining taxonomic shifts (Bonada *et al.*, 2007).

Compared to taxonomic composition, the trait-based composition of communities is less affected by biogeography and observed patterns remain within and between regions, allowing analyses on large spatial scales (Charvet *et al.*, 2000; Statzner *et al.*, 2001; Archaimbault *et al.*, 2005; Schriever & Lytle, 2016; Brown *et al.*, 2018). Trait profiles however can vary broadly also between taxonomically close groups (Usseglio-Polatera *et al.*, 2000; Pilière *et al.*, 2016). Taxonomic and trait-based approaches therefore can lead to divergent results (Su *et al.*, 2015; Crabot *et al.*, 2020, 2021, see also Cadotte *et al.*, 2011), suggesting that both approaches should be considered complementary and not used blindly as a proxy for each other. Meta analyses (Gagic *et al.*, 2015; Plas, 2019) and experiments (Tilman *et al.*, 1997; Hulot *et al.*, 2000) also identified functional diversity as a better predictor for ecosystem functions than taxonomic based indices, especially when multiple ecosystem responses are addressed simultaneously (Bagousse-Pinguet *et al.*, 2019).

Large trait databases are available for a range of different groups of organisms (Tachet *et al.*, 2010; Schmidt-Kloiber & Hering, 2015; Sarremejane *et al.*, 2020). This data has often been combined with long-term or large scale data from monitoring programs to study the effect of climate change or nutrient concentration changes on community structure and functions (Bonada *et al.*, 2007; Floury *et al.*, 2013, 2018). Many of the observed results are coherent, but often lack the understanding of the actual underlying mechanisms.

Ecological stoichiometry

Like functional traits improve our understanding of taxonomic responses, stoichiometric traits have the potential to explain the underlying mechanisms both in functional (and taxonomic) changes. Stoichiometry generally describes the proportion or the ratio at which constituents occur, for example the percentage of phosphorus or C:P ratio in a molecule. In ecology stoichiometric traits refer to the elemental composition of an organism's body tissue (i.e. body stoichiometry) and can be seen as one kind of functional (biological) traits. They are considered to be taxon-specific and to represent an organism's nutritional demand. There are differences between taxonomic groups (Fagan *et al.*, 2002; Frost *et al.*, 2003; Evans-White *et al.*, 2005; González *et al.*, 2018; Andrieux *et al.*, 2020) but heterotrophs generally maintain a stable elemental composition (discussed above) although in some taxa it changes during ontogeny (Frost & Elser, 2002; Villar-Argaiz *et al.*, 2002; Back *et al.*, 2008).

The framework of ecological stoichiometry theory (EST) considers stoichiometry in an ecological context. It investigates the equilibrium and fluxes of elements between an organism and its environment, and their interaction with each other (Sterner & Elser, 2002). Although some works extended the approach to other biomolecules or elements (Karimi & Folt, 2006; Xing *et al.*, 2015; Grosse *et al.*, 2017; Jochum *et al.*, 2017c), most focus on the main elements carbon, nitrogen and phosphorous. It has studied organisms from microbes and phytoplankton to vertebrates, was applied to marine, freshwater and terrestrial systems and has scaled from single organisms to whole ecosystems (Nessel *et al.*, 2021).

Stoichiometric traits already helped explaining the expression of functional traits by an organism's nutrient demand. The most known example is the Growth Rate Hypothesis (=GRH, Elser *et al.*, 1996): Ribosomal RNA (=rRNA) provides the main pool of cellular phosphorus in invertebrates, but organisms differ in their phosphorous content. GRH states that alterations in C:P and N:P ratios result from varying demands for rRNA under different growth rates. Ribosomal RNA plays a major role in protein synthesis, which is essential for growth. High growth rates thus require a higher amount of rRNA, meaning that fast-growing organisms will show low body C:P or N:P ratios. Vice versa, slow-growing organisms that are characterized by a lower demand for rRNA, will have lower phosphorous content (corresponding to high C:P, high N:P). This link has been demonstrated for a range of taxa from unicellular plankton to invertebrates (Main *et al.*, 1997; Elser *et al.*, 2003a; Acharya *et al.*, 2004; Hillebrand *et al.*, 2013).
Although assuming such a link between nutritional demand and functional traits seems intuitive, most trait-based studies integrating stoichiometric traits focus on life history (Cross *et al.*, 2005b, 2006; Singer & Battin, 2007) and feeding-related traits (Fagan *et al.*, 2002; Evans-White *et al.*, 2005; Cross *et al.*, 2007; Evans-White *et al.*, 2009; González *et al.*, 2011a) and the majority of commonly used traits remains unstudied.

Stoichiometric traits already explained species invasions (González *et al.*, 2010) or species distribution along a pollution gradient (Dang *et al.*, 2009). Differences in stoichiometric traits and stoichiometric

imbalances, explained the distinct responses of different feeding groups along a nutrient gradient (Evans-White *et al.,* 2009). Multi-year nutrient enrichment experiments and mesocosm studies have demonstrated that the effect of nutrient enrichment on basal resources and consumer communities (as described above) followed predictions of EST (Cross *et al.,* 2006; Singer & Battin, 2007; Teurlincx *et al.*, 2017; Demi *et al.*, 2019). This underlines the potential of stoichiometric traits to explain community responses towards nutrient stressors. However, most studies remained focused on species level or studied the response of individual taxa within assemblages towards stressors. Studies addressing whole communities are rare, but reported interesting results suggesting that whole community stoichiometry might respond to nutrient enrichment (Singer & Battin, 2007; Prater *et al.*, 2015; Teurlincx *et al.*, 2017). Scaling up to whole communities however is important for the understanding of ecosystem dynamics (Cross *et al.*, 2005a; Moe *et al.*, 2005), as already discussed above.

Since the birth of EST, many authors have continuously pointed out the potential of EST for improving the understanding and enabling the prediction of ecological responses towards global stressors (van de Waal *et al.*, 2010; Hessen *et al.*, 2013; Cherif *et al.*, 2017; Meunier *et al.*, 2017; Welti *et al.*, 2017; Sardans *et al.*, 2021). Indeed, with the major global stressors nowadays concerning altered nutrient pools and -cycles, the ultimate link of stoichiometric traits to nutrients on one hand and to functional traits on the other hand, seems a promising tool to causally understand the consequences.

Objective and structure of the PhD

My PhD aimed at investigating, whether – and to what extent – the integration of stoichiometric traits into community analyses can improve our understanding of a community response towards global stressors, taking as an example the human-induced changes in nutrient concentrations.

The underlying assumption based on ecology stoichiometry theory and trait-based approaches seems quite intuitive: In an environment that is rich in nutrients, e.g. phosphorous, we expect more taxa with a higher demand for this nutrient and that are rich in phosphorous themselves, than in a phosphorus-depleted environment. Heterotrophs are thereby not directly affected by the nutrient concentrations but the stoichiometric flexibility of (basal) resources confronts them with changes in resource quality. As a result, other taxa with a better suited nutritional demand will be favoured, thus leading to a shift in community composition. This should also affect the overall "community stoichiometry", that is the nutrient pool provided by the community, towards values of higher phosphorous content (i.e. low C:P, N:P). Stoichiometric differences not only between taxonomic groups but also between taxa expressing different functional traits (e.g. growth rate, feeding group) suggest that such a shift should also concern the communities' functional composition. Further investigating the general link between stoichiometric and other functional traits and to extend it to community level thus might enable to better understand the functional (and taxonomic) community response towards nutrient enrichment.

To answer this question I mainly relied on large databases, comprising information of three axes:

1. Stoichiometric traits
2. (Other) functional traits and
3. Community composition datasets comprising information about macroinvertebrate abundances all over France as well as detailed site information (e.g. water chemistry, hydrogeological parameters)

Each of the following chapters will combine these databases to address a specific question about the use of stoichiometric traits, from individual level (Chap. I & II) to community level (Chap. III & IV)(Fig. 1).

I relied on freshwater macroinvertebrates as study organisms, a taxonomically and ecologically diverse group comprising crustaceans, leeches, insects, snails and mussels. They constitute the lower trophic levels in streams and rivers and are thus – due to their role as consumers – important when studying the ecology of these systems. Their use in biomonitoring programs made large-scale community abundance data available and likewise detailed trait information is available. This enables investigating general ecological questions via large-scale data sets and further would allow testing and using results of my thesis in other systems where data is available. Last but not least, many colleagues in our lab have worked and work with freshwater macroinvertebrates, so that I could benefit from their great knowledge and experience.

First, I will give a general overview about stoichiometric traits. I am going to present the stoichiometric trait database and how it has been constructed based on field samplings and a literature research (Chap. 2a). Based on the data obtained from these field campaigns I will analyse general patterns in macroinvertebrate stoichiometry (Chap. 2b), e.g. comparing taxonomic or functional trait groups. Further, the question of homeostasis is addressed by investigating the effect of taxonomy, body mass and environmental factors on body stoichiometry (Chap. 2c). Homeostasis – which here refers to the ability of maintaining a stable elemental composition – of heterotrophs is a base assumption in ecological stoichiometry theory. To which extent this concept applies however is still under debate (Halvorson & Small, 2016) as the literature has reported varying results (e.g. Cross *et al.*, 2003; Evans-White *et al.*, 2005; Persson *et al.*, 2010; Small & Pringle, 2010; Back & King, 2013; Danger *et al.*, 2013).

In the next chapter, I link stoichiometric to other functional traits. Since body stoichiometry represents an organism's nutrient demand and different behaviours or physiological characteristics should cause and/or require different body stoichiometry (e.g. due to different allocation in N- or P-rich cell structures; differences in resource quality), I expected general stoichiometric differences between taxa expressing distinct traits. As already discussed, several works have demonstrated a link between life history traits (i.e. growth rate) or feeding groups and body stoichiometry. It thus seemed interesting to

investigate for which functional traits such a link to body stoichiometry exists, as it could help understanding the mechanisms in the observed functional response of a community towards nutrient changes. By applying multivariate analyses, I thus investigated the general link between a taxon's body stoichiometry and its biological and ecological traits and tested assumptions based on ecological stoichiometry theory.

The fourth chapter is dedicated to investigate community stoichiometry, the nutrient pool provided by a community. Is it related to water phosphorous and nitrogen concentrations? Is there a link to community functional trait profiles? Can we extend the Growth Rate Hypothesis to development rate and does it apply on community level? Assuming that changes in resource quality along a nutrient gradient should select for different taxa based on their nutrient requirements will shift the taxonomic composition of a community, I expect this to also affect community stoichiometry. It should follow the nutrient concentrations in the water, i.e. increase under high phosphorous conditions due to a higher proportion of phosphorous-rich taxa (Chap. IVa).
Due to the link between stoichiometric and functional traits (Chap. III), a shift in community stoichiometry should come along with a shift in its functional composition. I will therefore repeat the analyses from Chapter III on community level (Chap. IVb).
For the last question, I will analyse the link between developmental rate – as defined using three traits related to organism development – and body stoichiometry on taxa as well as community level. I will then use field abundance data to investigate an expected link towards a water nutrient gradient (Chap. IVc).

The last chapter addresses similar questions as the previous one, but focussing on environmental changes over time. I will test my hypotheses regarding community stoichiometry and the shift in stoichiometric and other functional traits using data from a 34-year period of the Loire river (France). It thus can be seen as a case study for global changes (Chap. V).

I further addressed questions of possible co-factors on community stoichiometry or the use of abundance vs. biomass-weighted community data. Small notes/summaries on these aspects throughout the chapters will thus complement the thesis, while detailed information in the form of drafts can be found in the Annex.

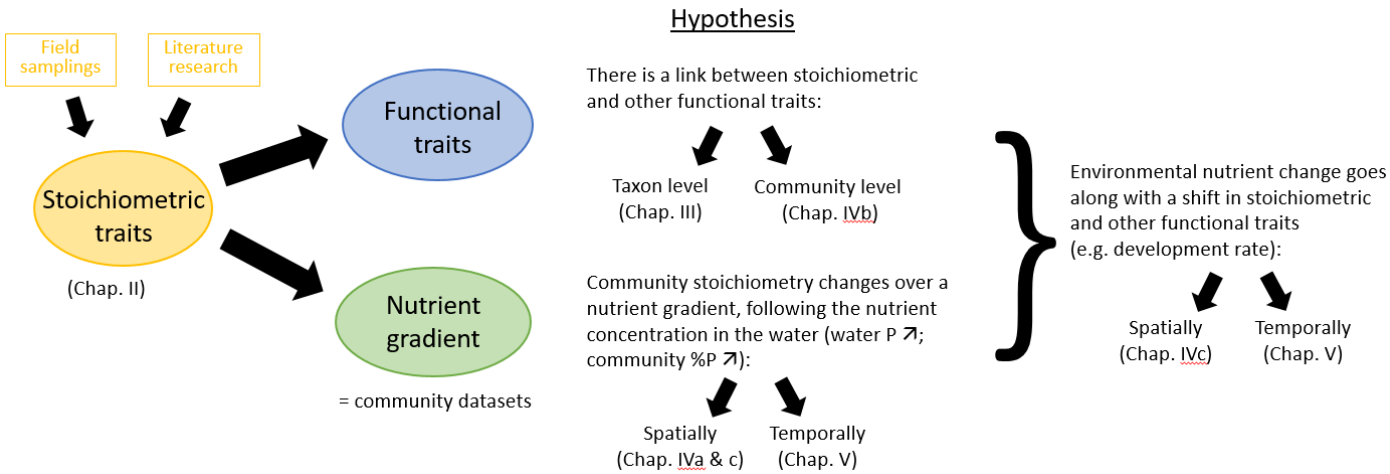

Fig. 1 : General structure of my PhD work, showing how the different databases (stoichiometric traits, functional traits) and community data sets have been combined to investigate different questions of macroinvertebrate stoichiometry on different levels of biological organization.

Chapter II:
Macroinvertebrate stoichiometry

A database of West European headwater macroinvertebrate stoichiometric traits

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Abstract

Motivation: Ecological stoichiometry investigates the equilibrium and the fluxes of chemical elements and energy between organisms and their environment. Several studies pointed out the value of integrating stoichiometric traits for a better understanding of the mechanisms behind community and ecosystem processes. Especially in the light of globally changing nutrient levels in freshwater systems, the link of stoichiometric traits to nutrient cycling and trophic interactions can improve our investigations of a species or community response to these changes.

Benthic macroinvertebrate assemblages are widely used for biomonitoring in freshwaters, providing a large number of data sets gathering information about their taxonomic diversity at many different spatial and temporal scales. Likewise, an increasing number of large databases gives ready access to information on a variety of macroinvertebrate biological traits. The addition of stoichiometric traits to this pool of available information could thus expand the application of trait approaches and facilitate the study of the links between nutrient cycling and ecosystem functioning.

Main types of variables contained: The database contains information on elemental content of 188 taxa, in terms of the major nutrients %C, %N and %P and the corresponding molar ratios C:N, C:P and N:P. We provide calculated taxon mean values as well as the underlying raw data on individual level that was obtained from field sampling, supplemented by a literature research (n = 1555, 174 taxa).

Major taxa and level of measurements: The database contains information on 188 macroinvertebrate taxa, available at species (n = 33), genus (96), tribe (9), subfamily (6), family (42), order (1) or class (1) levels.

Software format: .csv file

1. Introduction

Biological and ecological traits are used to describe communities in a more functional way than with taxonomy alone, allowing to more directly investigate – and possibly understand – ecosystem functioning (Menezes et al. 2010, Cadotte et al. 2011, Verberk et al. 2013). If referring to the "functional trait" definition of McGill et al. (2006), *i.e.* "*a well-defined, measurable property of organisms, usually measured at the individual level and used comparatively across species, that strongly influences*

organismal performance", stoichiometric traits describing the elemental composition of an organism can be seen as biological traits, allowing a direct link towards nutrient pools and cycling. The framework of ecological stoichiometry aims at investigating the interaction between the organisms and elements in their environment (Sterner & Elser, 2002). Body stoichiometry has been linked to biological traits, especially growth or body size, for a wide range of taxa (Elser *et al.*, 1996, 2003b; Naddafi *et al.*, 2012; González *et al.*, 2018). Applied to species level, body stoichiometry can help to explain species invasion (González *et al.*, 2010), pollution tolerance (Dang *et al.*, 2009) or host-parasite interactions (Sanders & Taylor, 2018). On a larger scale, community composition over a nutrient gradient has been found to follow assumptions based on ecological stoichiometry theory (Sterner & Elser 2002): species were selected based on their nutrient requirements, represented by their elemental composition (Beck et al. 2021, Singer and Battin 2007, Evans-White and Lamberti 2009). Although up to date there is a lack of large-scale studies, several researchers have pointed out that the integration of stoichiometric traits could improve our understanding of nutrient cycling (Meunier *et al.*, 2017; Welti *et al.*, 2017; Van de Waal *et al.*, 2018). Especially in the light of changes in nutrient levels in aquatic systems and changes in the elemental ratios (Falkowski *et al.*, 2000; Peñuelas *et al.*, 2013) studying stoichiometric constraints and traits could enable us to understand and predict how populations, communities and ecosystems would locally respond to such changes (*e.g.*, see Sentis et al. 2019).

Macroinvertebrates are widely used in biomonitoring programs of freshwaters. A large amount of information on their distribution, from local to global scales and under reference conditions as well as under a wide range of stressors, is thus readily available. Further, large databases containing biological and ecological trait information are easily accessible (Vieira *et al.*, 2006; Tachet *et al.*, 2010; Schmidt-Kloiber & Hering, 2015; Kefford *et al.*, 2020; Sarremejane *et al.*, 2020; Twardochleb *et al.*, 2021). The integration of stoichiometric trait information would allow – on its own or in combination with other traits – a functional analysis directly linked to nutrient pools and to test hypotheses based on ecological stoichiometry theories.

Here, we provide a database containing stoichiometric information (mass elemental contents and the corresponding ratios) of the major nutrients: carbon (C), nitrogen (N) and phosphorus (P) of macroinvertebrates (that can be found in North-Western European headwaters). We describe its compilation based on our own measurements following field sampling campaigns, further completed by data from literature. We also provide the underlying raw data at individual level which (partly) includes biomass information. In addition, we make a general comparison of stoichiometric traits among macroinvertebrate main taxonomic groups and discuss these results in the context of available literature.

2. Material & Methods

Data acquisition and compilation

The data originate mainly from field samplings we conducted with the aim of constructing a database of stoichiometric information for headwater macroinvertebrates (~64% of data presented in our database, n = 998). The sampling campaigns took place from May 2019 to January 2020 in small streams located in North-Eastern France and the Vosges mountains. We additionally integrated data from previous field campaigns done by our lab in the same region and similar stream types (~27%, n = 424) and data from the literature (~9%, n = 133). The literature research was not exhaustive and not filtered by sampling region, but tried to gather information on the most common macroinvertebrates in Western Europe. As a result, the stoichiometric information was gathered mainly from studies conducted within Western Europe, but sometimes completed by information from studies in the US.
For some taxa the provided values have been exclusively based on literature, but those values have only extended the number of taxa within groups (mainly family) we had already found in the field. Additionally, we graphically verified that literature values (and therewith also those from different regions) did not substantially increase the range of values associated to a given taxon.

The information was gathered at the finest taxonomic level of identification feasible (mainly genus), or taken as provided by the literature. Data were included when at least information for one stoichiometric variable (%C, %N, %P, C:N, C:P, N:P) was available for one individual or taxon. Mass ratios were transformed to molar ratios if necessary.

Details on the sampling sites and the laboratory nutrient analyses are provided in the supplementary material (S1+S2).

Data curation

The molar ratio values (C:N, C:P, N:P) were calculated for each individual from the corresponding elemental stoichiometric values. When measurements were done on a pool of individuals, they were treated like individuals in the following calculations.

To construct the stoichiometric database, the mean of elemental contents and ratios was calculated for each taxon and several coarser taxonomic levels, up to the family level (genus, sub-family, family). For a given taxonomic level (*e.g.* family) and a given stoichiometric variable, we averaged the information provided by all the individuals belonging to this taxonomic group, independently of their identification level. For example, if for a given family and a given stoichiometric variable, values were available for five individuals of a first genus and three individuals of a second genus, these eight values were averaged to provide the value of this family for this stoichiometric variable. If information was available for one genus (four individuals) and two species (three and four individuals), the family value was calculated as the mean of these eleven individuals. The geometric mean was used for all the stoichiometric variables, as suggested by Isles (2020).

Data availability and data use guidelines

The data are freely available, but should be referenced by appropriately citing this paper. They are available in Dryad (doi.org/10.5061/dryad.g4f4qrfr5).

3. Description of the data

The database comprises stoichiometric information on 33 species, 96 genera ("*Silo-Lithax*" thereby being a combination of two genera), 9 tribes ("Stenophylacini-Chaetopterygini" being a combination of two tribes), 6 subfamilies, 42 families, one order and one class.

The identification level of the underlying raw data was mainly the genus level (66.4% of raw data), species level (14.8%) or family level (12.7%); only Oligochaeta were not identified beyond class level; Hydracarina (Arachnida) beyond order level. The number of individuals used for calculating the database values ranged between one for some taxa and 137 for Gammaridae.

The provided data on individual level ("raw data") include biomass information (dry weight in mg) when this information was available.

Insecta are the dominant group, regarding both the overall number of taxa as well as the number of taxa at each taxonomic level.

Of note, data from Mollusca (stoichiometry, biomass) represent measurements on complete organism, *i.e.*, including the soft tissues as well as the shell, for both our measurements and data extracted from the literature.

General patterns in stoichiometry among macroinvertebrate classes and comparison with other datasets

The eight larger macroinvertebrate taxonomic groups differed significantly in their stoichiometry (Kruskal-Wallis tests; $p < 0.05$ for all the elements and ratios; Fig. 2a.1). The observed patterns in our database values are thereby in line with what has already been reported in the literature. Oligochaeta (only considered at class level) and Hydracarina (only represented by one order identified as such) as well as the comparison between "Bivalvia" and "Turbellaria" were not included into pair-wise comparisons because the corresponding sample sizes were too small ($n \leq 3$).

Bivalvia and Gastropoda (Mollusca) differed significantly from the other macroinvertebrate groups. They had lower carbon, nitrogen and phosphorous mass contents compared to taxa belonging to the other groups. The reason is most likely that data from Mollusca in the database we used were obtained from measurements on complete individuals, including the shell that primarily consists of calcium carbonate ($CaCO_3$) and makes a large proportion of individuals' body mass. Even though it is generally considered non-living material, it is part of the organism and requires the corresponding elements (but also proteins like conchiolin) for its construction. Most studies in the literature only analysed the soft tissues of Mollusca, making it impossible to compare their values with those obtained in our study.

Nevertheless, previous works have also demonstrated that Mollusca could be separated from Malacostraca (Crustacea) and/ or Insecta by their stoichiometry, although it was the inverse pattern: Mollusca had higher carbon and nitrogen mass contents and lower C: nutrient ratios compared to the other taxonomic groups (Evans-White et al. 2005, Liess and Hillebrand 2005). When measuring complete individuals, Liess & Hillebrand (2005) also found higher ratios for Gastropoda and Bivalvia (Bivalvia: only for C:N), similarly to the values in our study. Although both groups of Mollusca were more similar to one another than to other groups in most stoichiometric variables (Fig. 2a.1), they differed significantly in %P and C:P ratio, indicating a lower phosphorous content for Bivalvia.

The lower %C, %N and %P as well as C:P and N:P observed by Evans-White et al. (2005) for Malacostraca when compared to Insecta are in line with our observation. As potential reasons for the higher phosphorus content, the authors discussed general phylogenetic differences and/ or calcium that can form complexes with phosphorus in crustacean carapaces (Vrede *et al.*, 1999). Contrasting observations, such as a more similar (*i.e*. not significantly different) phosphorus content in our analyses, might be due to different taxa considered in the studies, as both taxonomic groups, especially the taxon-rich class of Insecta in our study, have shown large variation in values.

Our results regarding Hirudinea, which exhibited higher %C and %N and slightly lower %P than Malacostraca, are in accordance with the results of Frost et al. (2003). Nevertheless, carbon and nitrogen contents and C:N ratio of Hirudinea were closer to those of Insecta compared to other taxonomic groups. Their phosphorus content (as well as C:P ratio) ranged between those of Insecta and Gastropoda; their N:P ratio between those of Bivalvia and Gastropoda.

Turbellaria also had carbon and nitrogen contents close to those of Insecta, but their phosphorus content was the highest among all the taxonomic groups. This is probably due to their predaceous feeding habits, as studies have reported higher nitrogen and/or phosphorus content in predators compared to other feeding groups (Cross *et al.*, 2003; Evans-White *et al.*, 2005). C:P and N:P ratios were among the lowest and close to the values of Malacostraca and Oligochaeta.

Our values for Oligochaeta are close to those reported by Singer et al. (2007). Their mass contents (%C, %N, %P) are of intermediate values whereas C:P and N:P ratios are at the lower end of values compared to the main taxonomic groups. Their stoichiometric traits are similar to those of Malacostraca in all measured metrics (only C:N is closer to that of Insecta). Although we could not include them into the statistical analysis, their stoichiometry (especially %C, %N and N:P ratio) seems to differ from that of phylogenetically close groups (*e.g.* Hirudinea). This difference is probably partially caused by different feeding behaviors, as studies reported differences in nitrogen and phosphorous content between macroinvertebrate predators and primary consumers (Cross et al. 2003, Evans-White et al. 2005, Ventura et al. 2008).

For some of the groups only a low number of taxa has been included in our database (n = 3 for Bivalvia and Turbellaria) and the trends discussed above should therefore not blindly be generalized to these groups. However for Bivalvia the values were well in line with what is reported by the literature (up to date and to our knowledge there is no literature available for Turbellaria) and/or were in line with what one could expect due to their feeding habit. For Oligochaeta we cannot tell how many taxa contributed to the presented mean value since this group is often considered as such due to its difficult identification at a finer level.

Stoichiometric differences can also be found on finer taxonomic levels, for example among orders or families (results not shown), and have also been reported by other authors (Morse *et al.*, 2012; Mehler *et al.*, 2013; González *et al.*, 2018). Besides these studies, Andrieux et al. (2020) pointed out the importance of taxonomy in determining heterotrophs' stoichiometry.

Our database provides stoichiometric information for 188 taxa at different taxonomic levels of identification to be used by researchers who aim at integrating stoichiometric traits in works addressing individual or community level. The underlying raw data are also provided for full transparency and for people interested in using data in another way than taxa-wise mean values.

The taxa included in this database represent most of the more abundant taxa in macroinvertebrate communities of West European headwater streams. The data provided must be seen as the mean values observed for a given taxon, without considering other factors that might influence its body stoichiometry. Despite some studies reported intra-taxon differences in body stoichiometry with life stage (Elser *et al.*, 2006; Back & King, 2013), sex (Back *et al.*, 2008) or body size (Evans-White *et al.*, 2005; Liess & Hillebrand, 2005; Naddafi *et al.*, 2012) for some taxa, we think that the information we present here provides a pertinent idea of (i) the elemental composition of taxa over the aquatic instars of their life cycle and (ii) inter-taxa differences.

In the future, the number of taxa could be increased to allow better and higher resolution coverage of taxonomic groups (*e.g.* Oligochaeta) or other stream types. Small-sized taxa might indeed still be underrepresented in the current dataset, as it is hard to identify them alive and/or to obtain sufficient biomass for elemental analyses.

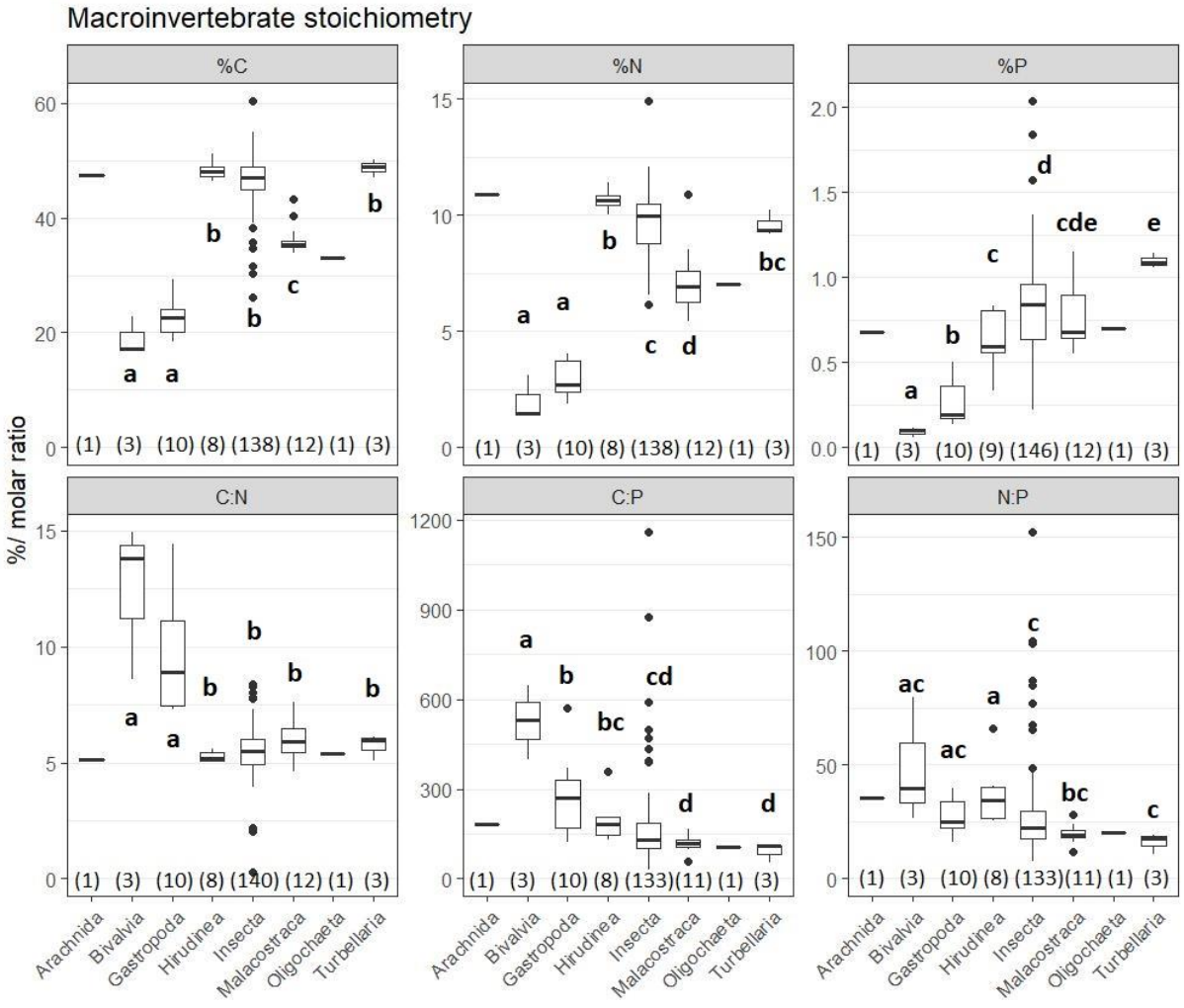


Fig. 2a.1: Stoichiometry of the main macroinvertebrate taxonomic groups. Mass contents of C, N and P are shown as % (upper panel), C:N, C:P and N:P as molar ratios (lower panel). The horizontal line represents the median, the boxes range from the 25th to the 75th percentiles and the whiskers extend to the last datapoints within 1.5 times the inter-quartile range with black dots representing outliers. N indicates the number of taxa within each taxonomic group. Different letters indicate significant differences using pairwise Wilcoxon tests with controlling the false discovery rate (adjustment of p-values for multiple testing using Benjamini-Hochberg method) following a significant Kruskal-Wallis test. Significance threshold has been set to $\alpha = 0.05$. Please note that pairwise test did not include the groups Arachnida and Oligochaeta and the comparison between Bivalvia and Turbellaria.

Supplementary Material

S 2a,1. Sampling sites

Macroinvertebrates were collected in headwater streams in North-Eastern France and the Vosges mountains. All the streams belong to the same size class (TP = very small) and the same hydroecoregion (HER10 = Côtes Calcaires Est), expressing similar characteristics regarding geology, relief and climate (Wasson *et al.*, 2002; Chandesris *et al.*, 2006). The sampling campaigns took place between May 2019 to January 2020.

S 2a.2. Laboratory measurements

After collections, the organisms were let in clear water for several hours in order to clear gut content before freezing (-20°C) until the elemental analysis.

The frozen invertebrate individuals were freeze-dried and grinded. When enough material was available (~3 mg) the elemental analyses for phosphorus and carbon/ nitrogen were done on the same individual. Otherwise, several individuals of the same taxon were pooled before grinding to obtain a sufficient large sample. When there was enough material but individuals were too small to be grinded, complete (*i.e.* un-grinded) organisms were analysed.

Carbon and nitrogen were measured using an elemental combustion analyser (model Carlo Erba NA 1500NC).

Organic phosphorus was measured by oxidizing the samples with sodium persulfate under alkaline conditions. The samples (~1 mg) were suspended in 4 mL Milli-Q water and then 1 mL sodium hydroxide (NaOH 1M) and 2.38 mL sodium persulfate ($Na_2S_2O_8$ 125 g L^{-1}) were added to the sample suspension. The solutions were adjusted to 10 mL with Milli-Q water before put in heat chamber (120°C for two hours) to accelerate the process of phosphorus mineralization. Samples were cooled, before adding 2 mL of reagent solution (H_2SO_4: 15% v/v; $K(SbO)C_4H_4O_6$, 0.5 H_2O: 2.8 g L^{-1}; $(NH_4)_6Mo_7O_{24}$, 4 H_2O: 40 g L^{-1}) and 0.5 mL of ascorbic acid (20 g L^{-1}) to each sample vial. After 30 minutes, 2 mL of each solution were used to measure absorbance at 880 nm by spectrophotometry (Analytik Jena-Specord 205 spectrophotometer). The phosphorus concentration was then calculated by comparing absorbances of samples to those of standard solutions.

General patterns in macroinvertebrate stoichiometric traits

1. Introduction

Stoichiometric traits are generally assumed to be taxon-specific, meaning that intra-taxon variability – for example between life stages (Villar-Argaiz *et al.*, 2002, 2020; Elser *et al.*, 2003a; Back *et al.*, 2008) or sexes (Back & King, 2013) in some taxa, but see Chapter IIb, – is smaller than the variability between taxa. Indeed, several studies showed homeostatic body stoichiometry for a range of taxa (Sterner & Elser, 2002; Cross *et al.*, 2003; Persson *et al.*, 2010), while there were differences between taxonomic groups. Taxonomy, *via* phylogeny, generally seems to play a major role in determining organisms stoichiometric traits (Fagan *et al.*, 2002; Morse *et al.*, 2012; Mehler *et al.*, 2013; Andrieux *et al.*, 2020) although there are variable results about the importance of lower vs. high taxonomic groups (González *et al.*, 2011b, 2018).

As with taxonomy, there also seems to be a link towards functional traits. Taxa belonging to different functional feeding groups can differ in their body stoichiometry (Fagan *et al.*, 2002; Evans-White *et al.*, 2005; González *et al.*, 2011b, 2018) or are differently affected by nutrient enrichment (Demi *et al.*, 2019). Another well-known example is the Growth Rate Hypothesis (=GRH, Elser *et al.* (1996)), which confirmed a positive correlation of body phosphorous content and growth rate for a range of taxa (Main *et al.*, 1997; Elser *et al.*, 2003a; Acharya *et al.*, 2004).

Stoichiometric differences between macroinvertebrate larger taxonomic groups have already been investigated and discussed in the previous sub-chapter describing the construction of the stoichiometric trait database (MS 1). It relied on taxon mean values, weighing each taxon equally. However, the underlying raw data set allows exploring patterns in macroinvertebrate stoichiometry in more detail, *i.e*. on lower taxonomic levels and including the group of Oligochaeta. This chapter can thus be seen as an extension of the previous one. It remains rather descriptive and should provide a general idea about stoichiometric traits that will be used throughout the manuscript. I will investigate stoichiometric differences between larger taxonomic groups (classes, orders, families) as well as between functional feeding groups and discuss the findings in the context of results already reported in the literature.

2. Methods

The data

The data used in this analysis is the underlying raw data of the construction of a stoichiometric trait database of freshwater macroinvertebrates (MS 1).

The largest part (64%) originated from field samplings in small headwater streams in North-Eastern France and the Vosges mountains. Other data originated from previous field samplings in the same kind of streams done by our lab (27%) or the literature (9%). Details on the composition and laboratory measurements are described in MS 1 (S1 & S2). Taxa were identified to the finest level possible (mainly genus). The data covers 174 taxa belonging to seven larger groups (Arachnida, Bivalvia, Gastropoda,

Hirudinea, Insecta, Malacostraca, Oligochaeta, Turbellaria), with sample sizes ranging between 1 and 94 (*Baetis*). Due to the low sample size of Hydracarina (n=3), this group and therewith the class or Arachnida will not be part of the statistical analyses. Nevertheless it will appear in some figures to allow a comparison with the other taxonomic groups.

Statistical analyses

Stoichiometric differences between macroinvertebrate taxonomic groups

To test for differences between macroinvertebrate lower taxonomic groups (generally class, *i.e*. Bivalvia, Gastropoda, Hirudinea, Insecta, Malacostraca, Oligochaeta, Turbellaria), their means were compared by applying Kruskal-Wallis tests for each stoichiometric variable (%C, %N, %P, molar C:N, C:P, N:P). In cases of significance, a Dunn-Test was conducted to further investigate these differences while adjusting p-values for multiple testing after Bonferroni's method.

Within-group differences on order-level were investigated for groups for which data of more than two orders was available. Kruskal-Wallis tests, in case of significance followed by Dunn-tests, were applied separately for each stoichiometric variable as described above. Likewise, within-order differences between families were assessed for all orders with at least two families.

To further compare the stoichiometric traits of macroinvertebrate groups, I calculated stoichiometric niches and their properties as proposed by González *et al.* (2017). The stoichiometric niche is seen as the space occupied in a three-dimensional space constrained by carbon, nitrogen and phosphorous content, which can be displayed as the convex hull defined by the mass content of those elements. We calculated the relative niche volumes for each group compared to a niche occupied by all individuals (ignoring their taxonomy) as well as their overlap and nestedness (in %). All calculations were done using the function CHVintersect (Villéger *et al.*, 2013) as in González *et al.* (2017). Niche size can be interpreted as stoichiometric variability within the corresponding group; nestedness and overlap allows comparing the stoichiometric niche of different groups. A high overlap and nestedness means that the groups are similar regarding their stoichiometric traits. Nestedness refers to the degree to which one niche is nested into the other, *i.e*. a subset. Due to the nature of this method only taxa for which information of all three stoichiometric variables was available, were used.

Stoichiometric differences between functional feeding groups

Functional feeding groups were defined based on trait information from (Tachet *et al.*, 2010). This database contains fuzzy-coded trait information, indicating for a taxon its affinity for each category of a trait. The values range from 0 (= no affinity) to 5 (high affinity) and were transformed into relative frequencies for each trait. Taxa were classified based on their affinities of the trait "food resource": Taxa with an affinity >0.5 for feeding on living plant material (living microphytes, living macrophyte) were considered "herbivore". Taxa with an affinity >0.5 for animal- based food (living microinvertbrate, living macroinvertebrate, vertebrate) were classified "predator". The group of "detritivores" was defined

as having an affinity >0.5 for feeding on detritus, microorganisms, dead plant or dead animal. When a taxon had no affinity >0.5 for any of these food types it was considered "omnivore".
For each group, the mean was calculated for each stoichiometric variable and compared *via* separate Krauskal-Wallis Tests for each stoichiometric variable. In cases of significance, a Dunn-Test was conducted to further identify differences.

Since data of some taxonomic groups and/ or stoichiometric variables did not follow normality or fulfil the assumption of homogenous variances, non-parametric tests were applied in all comparisons to keep a homogenous approach.

All analyses were conducted using R (R Development Core Team, 2019).

3. Results

Mean values provided in the following correspond to geometric mean (and geometric standard deviation) as suggested by Isles (2020) for the use of ratio data. This does not concern the statistical testing, since rank-based tests were used to compare stoichiometry of taxonomic and feeding groups.

Differences in stoichiometry between macroinvertebrate groups

Macroinvertebrate classes differed in their elemental composition, both regarding element mass content and the corresponding molar ratios (KW with $p<0.05$, Fig 2b.1).
Bivalvia and Gastropoda had the lowest C contents ($18.10^{\times}/_{\div}1.28$ and $23.06^{\times}/_{\div}1.18$, respectively), which was significant lower than that of Hirudinea, Insecta and Turbellaria. %C of Hirudinea, Insecta and Turbellaria were close together ($46.75^{\times}/_{\div}1.11$ – $49.71^{\times}/_{\div}1.05$) and significantly higher than that of Malacostraca ($35.27^{\times}/_{\div}1.11$) and Oligochaeta ($33.02^{\times}/_{\div}1.50$) who had intermediate values. Also Hirudinea and Turbellaria differed significantly in %C, with the latter having the highest value of all groups.
N content showed a similar pattern and %N was significantly lower in Bivalvia ($1.62^{\times}/_{\div}1.69$) and Gastropoda ($3.39^{\times}/_{\div}1.40$) compared to the other classes except for the difference Bivalvia-Malacostraca. The intermediate values of Malacostraca ($6.72^{\times}/_{\div}1.27$) and Oligochaeta ($7.04^{\times}/_{\div}1.45$) differed additionally significantly to those from Hirudinea ($10.80^{\times}/_{\div}1.10$), Insecta ($9.50^{\times}/_{\div}1.19\%$) and Turbellaria ($9.86^{\times}/_{\div}1.09$). %N of Hirudinea was significantly higher than that of Insecta and the highest among groups.
The phosphorous content of Bivalvia and Gastropoda ($0.09^{\times}/_{\div}2.11$ and $0.30^{\times}/_{\div}0.17$) was significantly lower than in all other groups. Turbellaria had the highest %P of $1.10^{\times}/_{\div}0.23$, which differed significantly from all other groups. Additionally, Insecta ($0.87^{\times}/_{\div}1.49$) contained significantly more phosphorous than Hirudinea ($0.70^{\times}/_{\div}1.49$) and Malacostraca ($0.67^{\times}/_{\div}1.48$).
C:N ratio was significantly higher in Bivalvia ($13.06^{\times}/_{\div}1.38$) and Gastropoda ($7.94^{\times}/_{\div}1.22$) than in all other classes. Hirudinea had the lowest values ($5.08^{\times}/_{\div}1.08$) which additionally differed significantly to

values from Insecta (5.32×/÷1.44) and Malacostraca (6.02×/÷1.26), whose values also differed significantly.

Bivalvia had the highest C:P value (467.95×/÷2.02) which differed significantly to that of all groups except for Gastropoda (199.16×/÷1.66). The values of Gastropoda and Hirudinea (152.76×/÷1.66) were significantly higher than that of Insecta, Malacostraca, Oligochaeta and Turbellaria with values between 70.07×/÷1.71 in Turbellaria and 122.39×/÷1.73 in Malacostraca. Turbellaria had significantly lower values than Insecta (102.90×/÷1.98).

Bivalvia had the highest N:P ratio among the groups (35.79×/÷2.35), although this difference was only significant compared to values of Malacostraca (20.24×/÷1.76), Oligochaeta (20.00×/÷1.85) and Turbellaria (12.90×/÷1.59). N:P of Hirudinea (30.09×/÷1.68) was significantly higher than that of all other groups except for Bivalvia. Turbellaria had the lowest N:P ratio, differing significantly to that of Gastropoda (25.02.18×/÷1.59), Hirudinea and Insecta (18.99×/÷1.76).

Stoichiometry of Arachnida was similar to that of Hirudinea in all stoichiometric variables, that is a high content of carbon, nitrogen and N:P ratio, intermediate values of phosphorous and C:P ratio and low values of C:N compared to the other groups.

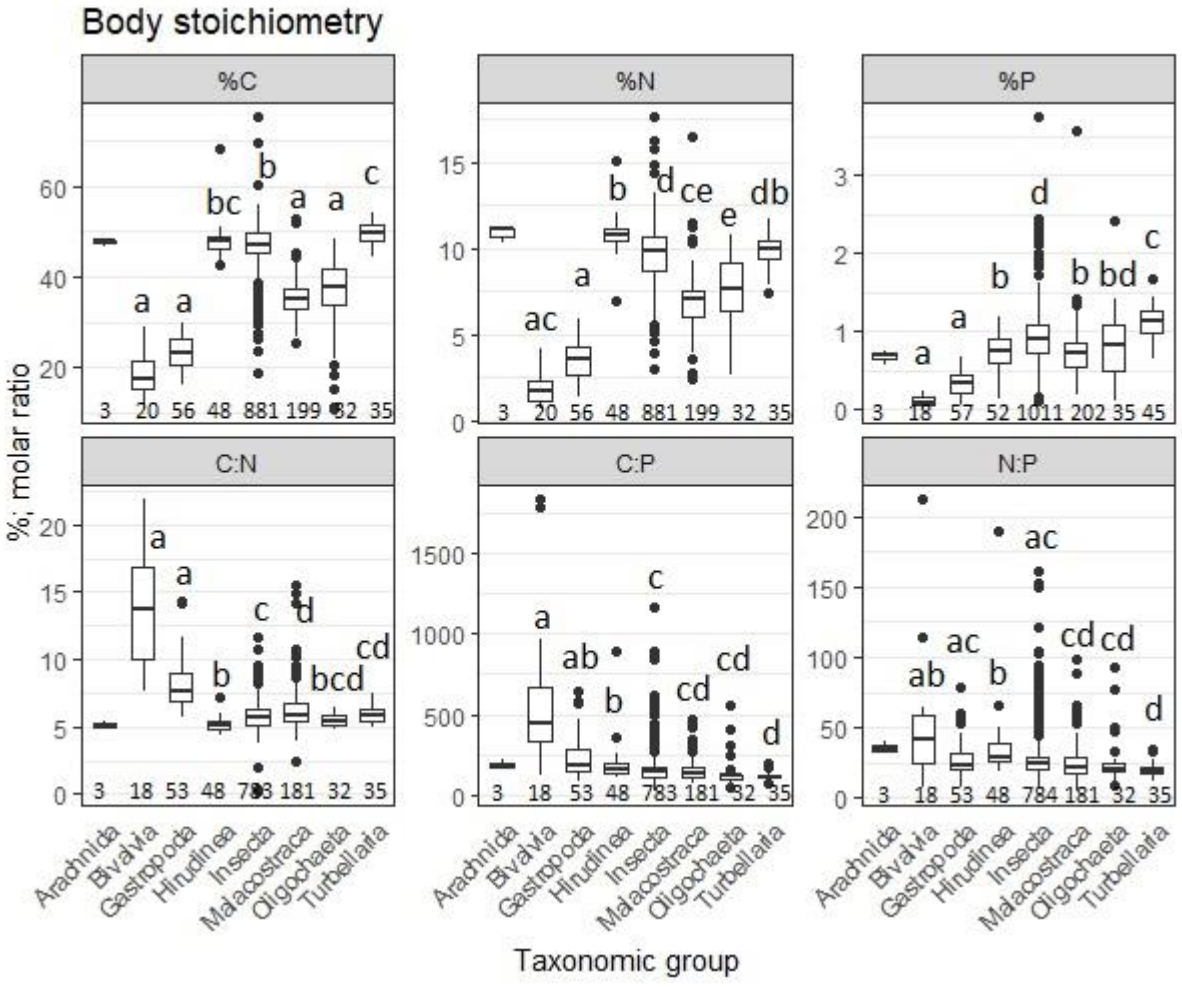


Fig. 2b.1: Stoichiometry of macroinvertebrate classes. Mass contents of C, N and P are shown as %, C:N, C:P and N:P as molar ratio. The horizontal line represents the median, the boxes range from the 25th to the 75th percentiles and the whiskers extend to the last data points within 1.5 times the inter-quartile range. Outliers are displayed as black dots. Lettering indicates significant differences in pairwise comparisons after a significant Kruskal-Wallis test (α=0.05 with Bonferroni adjustment for multiple testing). Data for Arachnida are represented for comparison only; they were not part of the pairwise testing due to the low sample size. The numbers at the bottom of each panel indicate the sample size for each stoichiometric variable x class combination.

Stoichiometric niches of the larger taxonomic groups differed in size but were generally separated. Insecta clearly occupied the largest niche, which was ~4.5x larger than the second largest niche of Malacostraca (Fig. 2b.2, Tab. 2b.1). The niches of Bivalvia and Gastropoda were the smallest.

The niche of Malacostraca showed the largest overlap with that of other groups, as they shared 16.36% and 17.18% of their niche with Insecta and Oligochaeta, respectively.

The niche of Bivalvia had an overlap of 13.12% to that of Gastropoda and was otherwise separated to the other groups. Hirudinea shared smaller parts of their niche with Turbellaria (6.39%) and Malacostraca (5.08%); as did Insecta and Oligochaeta (5.92%). Other niches were totally separated from one another or shared <5%.

The stoichiometric niches of Hirudinea and Turbellaria were almost completely positioned within that of Insecta (nestedness of 0.94 and 0.98, respectively), that of Oligochaeta to a smaller but still substantial degree (0.68).

The niches of Bivalvia, Gastropoda and Oligochaeta were mainly driven by variation in carbon and nitrogen content, leading to elongated niches shapes (S1). The more sphere-shaped niches of Hirudinea and Malacostraca resulted from a more equal variation in all three mass element contents. The upright shape of Insecta and Turbellaria niches suggest a rather strong variation in phosphorous content.

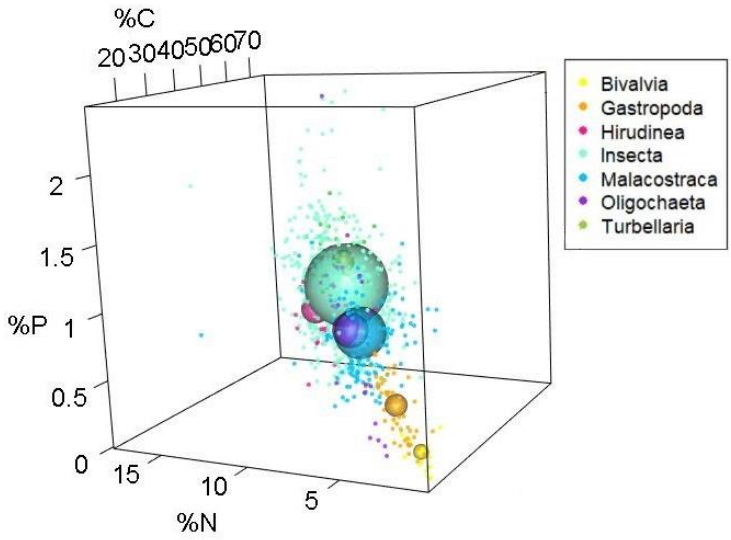


Fig. 2b.2: Relative sizes of stoichiometric niches of taxonomic groups. For each group, the niche volume is represented by a sphere centered around the average contents of %C, %N and %P of all individuals within the corresponding group (scattered dots of the same colour).

Tab. 2b.1: Stoichiometric niches of larger macroinvertebrate groups. For each group the relative volume of its niche compared to an overall niche is given; as well as the overlap (%) and nestedness (%, in italics) with niches of the other groups. Please note that all niche parameters refer to the convex hulls (S1), while Fig. 2b.2 simply represents the volume as a sphere. "n" indicates the sample size of each group.

		Overlap/ Nestedness							
Group	Rel. Vol.	Biv	Gas	Hir	Ins	Mal	Oli	Turb	n
Bivalvia	0.31		*0.21*	-	-	-	-	-	18
Gastropoda	1.03	13.12		-	*0.11*	*0.05*	*0.01*	-	53
Hirudinea	3.04	-	-		*0.94*	*0.12*	*0.01*	*0.04*	48
Insecta	68.72	-	0.30	4.39		*0.49*	*0.68*	*0.98*	782
Malacostraca	14.93	-	0.71	5.08	16.36		*0.26*	*0.20*	180
Oligochaeta	4.84	-	0.28	1.81	5.92	17.18		*0.02*	32
Turbellaria	1.38	-	-	6.39	2.02	3.39	1.62		35

Intra-group differences between finer taxonomic levels

The two orders of Hirudinea differed significantly in phosphorous-related stoichiometry: Pharyngobdelliformes had significantly higher %P and C:P ratio than Rhynchobdelliformes; while the later had a higher N:P ratio (Kruskal-Wallis test with $p<0.05$ for %P, C:P and N:P, Fig. 2b.3). The order of Gnathobdelliformes was only represented by one individual and therefore not part of the analysis.

Decapoda had the highest carbon content within Malacostraca, which was significantly higher than that of Isopoda (KW with $p<0.05$ and following post hoc with $p<0.05$ for Decapoda/ Isopoda). %N was significantly lower and C:N significantly higher in Amphipoda than in both other orders. Decapoda had the significantly highest %P and lowest C:P among the orders. There was no difference among groups in N:P ratio.

Within highest %C, which differet dignificantly to that from Diptera, Ephemeroptera, Odonata and Trichoptera (Fig. 2b.3). Diptera had the lowest carbon content which additionally was significantly different to that from Ephemeroptera, Plecoptera and Trichoptera. The carbon contents of Hemiptera and Megaloptera did not differ from that of any other group.

%N was highest in Odonata and differed significantly to that from Coleoptera, Diptera, Ephemeroptera and Trichoptera. Trichoptera had the lowest nitrogen content, which additionally differed significantly to that from Ephemeroptera, Hemiptera, Odonata and Plecoptera. Megalopteran %N did not differ to that from any other group.

Megaloptera had the highest %P, although it was only significantly different to that of Coleoptera and Odonata, who had the lowest phosphorous contents. %P of both groups was additionally significantly lower than that of Diptera, Ephemeroptera, Plecoptera and Trichoptera.

C:N ratio was lowest in Trichoptera, differing significantly to all other groups except Coleoptera and Megaloptera. Odonata had the lowest C:N ratios which was additionally significantly lower than that of Coleoptera, Diptera and Plecoptera.

C:P ratios in Coleoptera were the highest among the groups and differed significantly to that of Diptera, Ephemeroptera, Megaloptera, Plecoptera and Trichoptera. Diptera had the lowest C:P ratios, which differed significantly to that of Hemiptera, Odonata and Trichoptera, which all had rather intermediate values.

Coleoptera also had the highest N:P ratios. It differed significantly to that of Diptera, Ephemeroptera, Plecoptera and Trichoptera. N:P was lowest in Diptera, which was significantly different to that of Hemiptera, Odonata, Plecoptera and Trichoptera. Hymenoptera were not integrated into the statistical comparisons due to the low sample size (n=1).

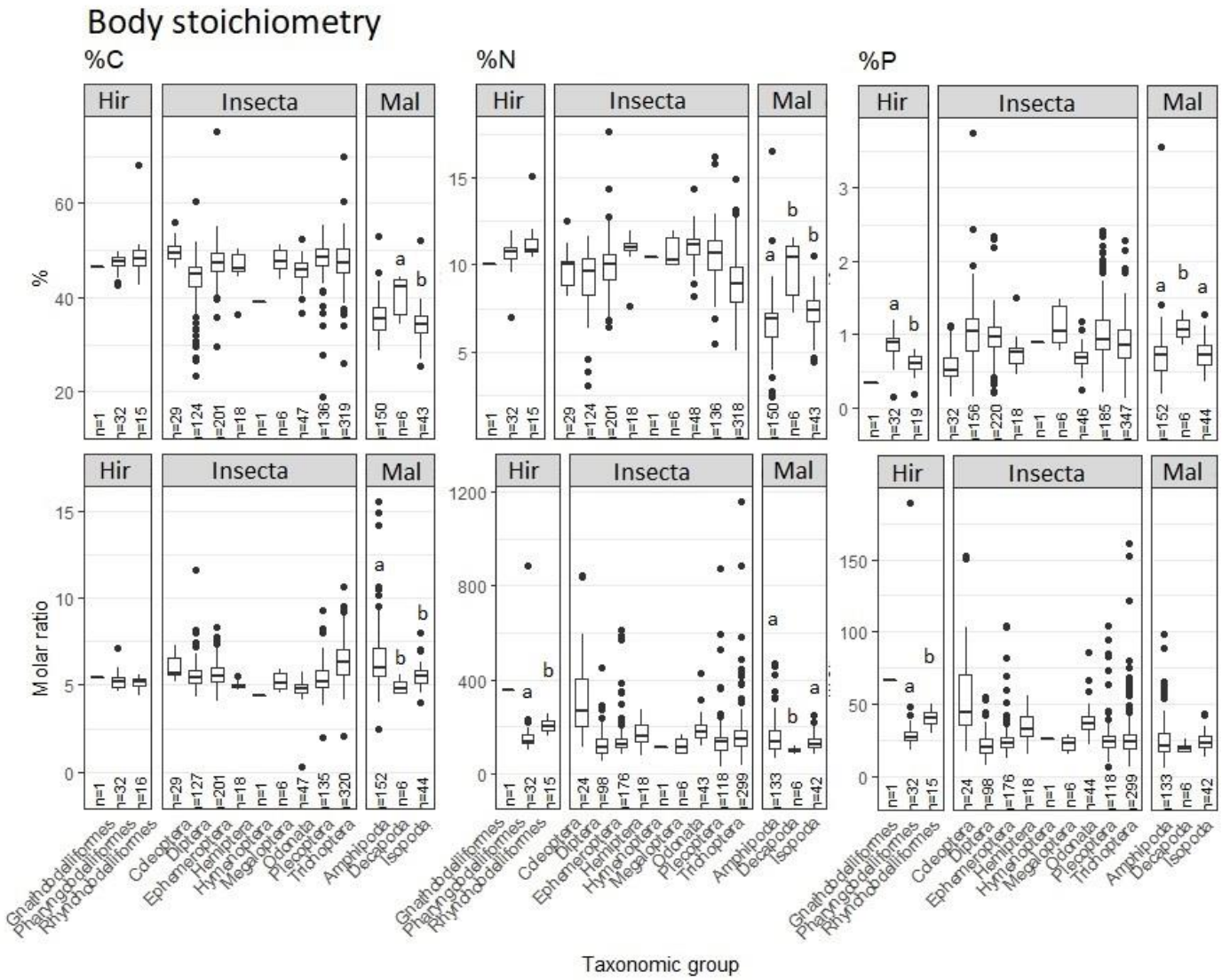


Fig. 2b.3: Stoichiometry among macroinvertebrate orders. Upper panels show elemental mass contents of C, N and P (%), lower panels the corresponding rations C:N, C:P and N:P. The horizontal line represents the median of each order, the boxes range from the 25th to the 75th percentiles and the whiskers extend to the last data points within 1.5 times the inter-quartile range. Outliers are displayed as black dots. Data for Gnathobdelliformes and Hymenoptera are represented for comparison only and were not part of the statistical analysis due to their low sample size (n<3). "n" indicates the sample size.

Within the insect orders of Coleoptera and Hemiptera, there were no differences in stoichoimetry between families. Contrastingly, families within Diptera and Trichoptera showed differences in all six stoichiometric variables, and Plecoptera in all variables except for %C. Within Ephemeroptera there were differences on family level in %C, %N and C:N ratio; families within Odonata showed differences in %C and %N.

Within the crustacean order Amphipoda there were no differences between the two families. The two families within Tricladida (class of Turbellaria) differed in %N and N:P ratio.

When comparing the stoichiometry within Bivalvia, there were significant differences in %N, C:N and N:P between the three families. In Gastropoda, the six families varied significantly in all six stoichiometric variables.

Differences between functional feeding groups

Taxa belonging to different feeding groups differed significantly in their stoichiometry (Fig 2b.4). Omnivores had a significantly lower carbon content ($37.88^{\times}/_{\div}1.33$) than the other groups (Kruskal-Wallis test with $p<0.05$ and following Dunn test with $p<0.05$ for omnivore/ detritivore, omnivore/ herbivore & omnivore/ predator). In addition, the intermediate carbon content of detritivores ($41.27^{\times}/_{\div}1.28$) differed significantly to all other groups. Herbivore and predators had the highest carbon contents of ($45.56^{\times}/_{\div}1.21$) and ($47.45^{\times}/_{\div}1.09$) respectively.

Nitrogen content of predators ($10.46^{\times}/_{\div}1.12$) was significantly higher, that of omnivores ($6.52^{\times}/_{\div}1.60$) significantly lower than that of the other groups. Detritivores and herbivores had very similar nitrogen contents with values of $8.66\ ^{\times}/_{\div}\ 1.32$ and $8.97\ ^{\times}/_{\div}\ 1.35$, respectively.

Omnivores had a significantly lower phosphorus content ($0.62^{\times}/_{\div}1.91$) than the others groups, whose values only ranged between ($0.82^{\times}/_{\div}1.58$) in herbivores and ($0.87^{\times}/_{\div}1.55$) in predators.

C:N ratio of all groups differed significantly among each other. Omnivores had the highest values ($6.77^{\times}/_{\div}1.29$); predators the lowest ($5.07^{\times}/_{\div}1.47$).

C:P was highest in omnivores ($148.74^{\times}/_{\div}1.53$) which differed significantly to that of detritivores ($132.45^{\times}/_{\div}1.52$) and predators ($141.00^{\times}/_{\div}1.56$). C:P of detritivores additionally differed to that of herbivores ($148.74^{\times}/_{\div}1.53$) and was the lowest among the groups.

Predators had the highest N:P among the groups ($26.54^{\times}/_{\div}1.58$) which differed significantly to that of detritivores ($23.52^{\times}/_{\div}1.48$) and omnivores ($23.54^{\times}/_{\div}1.64$), but not to herbivores ($24.83^{\times}/_{\div}1.49$).

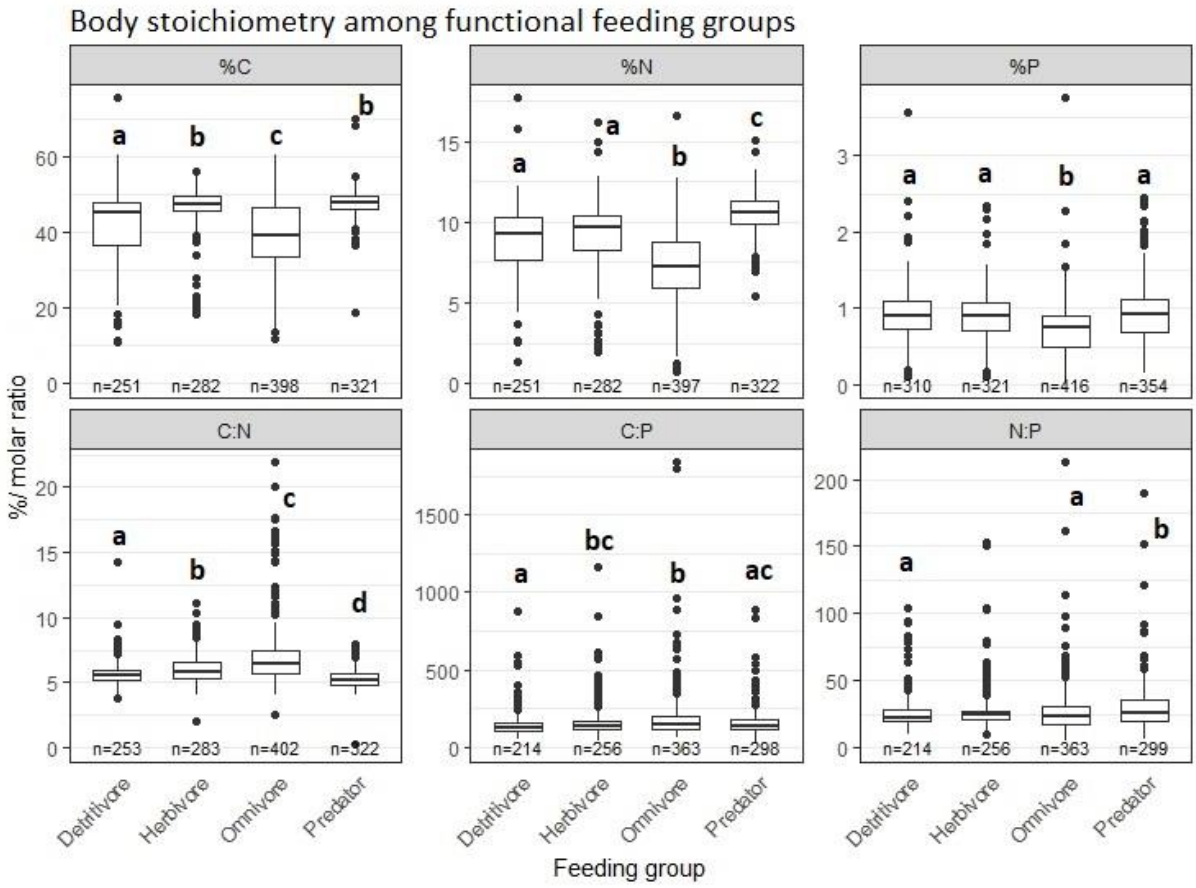


Fig. 2b.4: Stoichiometry among taxa belonging to different feeding groups. Mass contents of C, N and P are presented as percent, the corresponding ratios (C:N, C:P and N:P) as molar ratios. Lettering marks significant differences. The horizontal line represents the median of each order, the boxes range from the 25th to the 75th percentiles and the whiskers extend to the last data points within 1.5 times the inter-quartile range. Outliers are displayed as black dots. "n" indicates the sample size.

The stoichiometric niches of functional feeding groups were of similar sizes and partly overlapping. The largest niche was that of omnivores; the smallest that of herbivores (Tab. 2b.2, Fig. 2b.5).

The largest overlap was between the niches of detritivores and predators (48.53%), and detritivores also shared >40% of their niche with herbivores and omnivores. Herbivores and omnivore shared 41.28% of their niches. The lowest overlaps (25%) was between niches of predators and herbivore and predators and omnivores. The nestedness among niches was generally low. Only the niches of detritivores and herbivores were partly nested into that of omnivores, but only to a minor extend (values of 0.1 and 0.15, respectively).

The niches of detritivores, herbivores and omnivores were similar in their elongated shape shape as well as in their orientation in the three-dimensional space (S2). It was mainly driven by variation in carbon and nitrogen content. Also predators showed an elongated niche, but compared to that of the others groups it was mainly driven by a variation in phosphorous content.

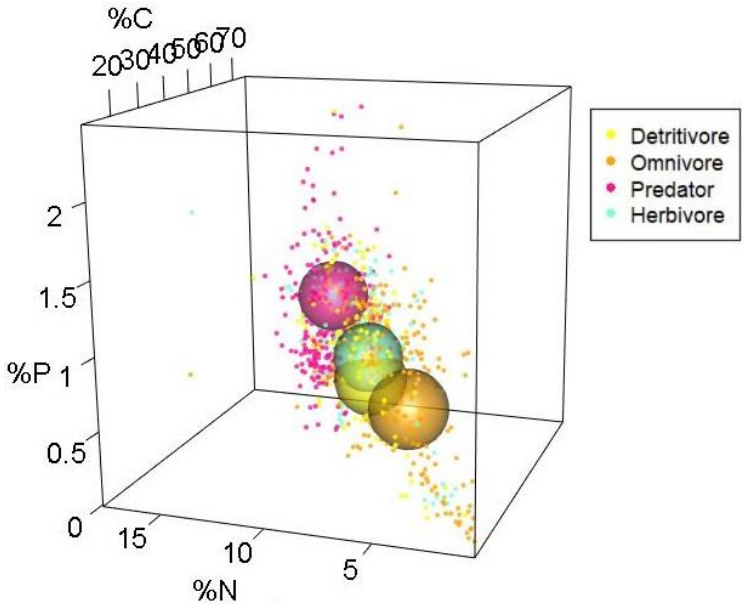


Fig. 2b.5: Relative sizes of stoichiometric niches of functional feeding groups. For each group, the niche volume is represented by a sphere centered around the average contents of %C, %N and %P of all individuals within the corresponding group (scattered dots of the same colour).

Tab. 2b.2: Stoichiometric niches of functional feeding groups. For each group the relative volume of its niche compared to an overall niche is given; as well as the overlap (%) and nestedness (%, in italics) with niches of the other groups. Please note that all niche parameters refer to the convex hulls (2b.S2), while Fig. 2b.5 simply represents the volume as a sphere. "n" indicates the sample size of each group.

		Overlap/ Nestedness				
Group	Rel. Vol.	Detritivore	Herbivore	Omnivore	Predator	n
Detritivore	30.14		*0.04*	*0.1*	*0.01*	213
Herbivore	26.75	44.17		*0.15*	*0.02*	255
Omnivore	39.41	41.53	41.28		*0.06*	362
Predator	29.32	48.53	25.75	25.26		299

4. Discussion

There were general differences in body stoichiometry between taxonomic groups – on larger and lower levels – as well as between functional feeding groups.

Differences in stoichiometry between larger macroinvertebrate groups

The differences between larger taxonomic groups (generally class level) were in line with results when comparing taxa mean values (MS 1) and the literature as discussed in that chapter. Mollusca, *i.e.* Bivalvia and Gastropoda had a significantly lower carbon nitrogen and phosphorous content while their C:N ratios were the highest among groups. Also C:P of Bivalvia was clearly higher than that of the other groups; that of Gastropoda was also higher but the difference was not significant. Arachnida, Hirudinea,

Insecta and Turbellaria had (partly) similar and the highest carbon and nitrogen contents, while Turbellaria had clearly the highest phosphorous content. Malacostraca and Oligochaeta had intermediate mass element contents and ratios.

These differences were also visible from the multi-elemental stoichiometric niches. This approach of González *et al.* (2017) is another way of representing group stoichiometry using all three mass contents at a time (rather than comparing stoichiometry separately). It thereby comprises the results obtained from comparing carbon, nitrogen and phosphorous content separately, but also allows estimating the molar ratios. *Via* the niche shapes, it gets visible whether the within-group variation is larger for a certain element, as for example %P in Insecta. It further confirmed the similarity of certain groups, such as Bivalvia-Gastropoda or Malacostraca-Oligochaeta *via* largely overlapping niches; but also the general distinction of taxonomic groups indicating different nutrient requirements. The absolute niche sizes were driven by the underlying sample sizes – Insecta were clearly dominating the datasets and also had the largest niche – but the result could well represent a macroinvertebrate community under un-disturbed conditions, which are also often dominated by insects. Although we did not aim at obtaining natural representative abundances during our field samplings and neither verified this for this analysis, some community dominating taxa (in terms of abundances) were included with more individuals than rarer ones. Also González *et al.* (2017) proposed to base sample sizes on natural abundances when applying this approach.

Differences in stoichiometry between finer macroinvertebrate groups

Also on order-level within larger taxonomic groups and family-level within orders, taxonomic groups differed significantly in their stoichiometric traits. Some orders were more similar to those of other larger taxonomic groups than to other orders within their own class, *e.g.* the nitrogen and phosphorous content of the crustacean Decapoda that was closer to some insect orders than to Amphipoda or Isopoda.

For insects we observed that part of the differences among orders were in line with ecological stoichiometry theory. Coleoptera had a high carbon content and C:N ratio, and a low phosphorous content (low %P, high C:P, N:P). They often exhibit small body sizes and undergo a rather long life cycle, suggesting slow growth and development rates.

Diptera, Ephemeroptera and Megaloptera, comprising taxa that tend to have a fast development and/ or growth rate, had high phosphorous contents (high %P, low C:P, N:P). This is well in line with Growth Rate Hypothesis, stating that body growth requires high amounts of rRNA to fuel protein synthesis. Since rRNA-molecules are phosphorous rich and can constitute the main pool of this element in invertebrates (Elser *et al.*, 2003a), fast growing taxa should contain higher amounts of phosphorous than slow-growing ones (Elser *et al.*, 2003a; Acharya *et al.*, 2004). Fast development, *i.e.* transformation towards maturity, often goes along with fast-growth rates, and indeed the link towards phosphorous content seems also to hold for development rate (MS 4). Also Odonata and Hemiptera contained only

low amounts of phosphorous, which according to GRH fits with their rather slow development. Odonata are rather long-lived and generally only undergo a single reproduction event; many Hemiptera taxa undergo only one reproductive event generally within one year, which can also be seen as rather slow compared to clear r-strategists. Taxa of both orders also contain high amounts of nitrogen. At least Odonata are almost exclusively predators with a high quality – *i.e*. nitrogen rich – diet. Both as larvae and as adult they move actively and therefore probably also require a high amount of N-rich muscle tissue, especially after emergence to allow flight.

Although the described differences were not significant towards all other groups, those groups positioned at the two extreme ends of the stoichiometric range showed the highest numbers of significant differences. There are not many studies that explicitly compared stoichiometry among macroinvertebrate or insect orders. The results suggested by Morse *et al.*(2012) are in line with some of our observations *i.e.* the highest carbon content and lowest phosphorous content (high C:P) in Coleoptera. For Diptera their results suggest an intermediate phosphorous content (C:P, N:P ratio) which was not what we found. However, their study aimed at answering another research question and most likely comprised other/a lower number of taxa. Generally, within-order differences in stoichiometry between families (Mehler *et al.*, 2013) as also found in this analysis and (partly) significant variation on up to species level (González *et al.*, 2018) makes it difficult to compare the results of different studies.

Differences in stoichiometry between functional feeding groups

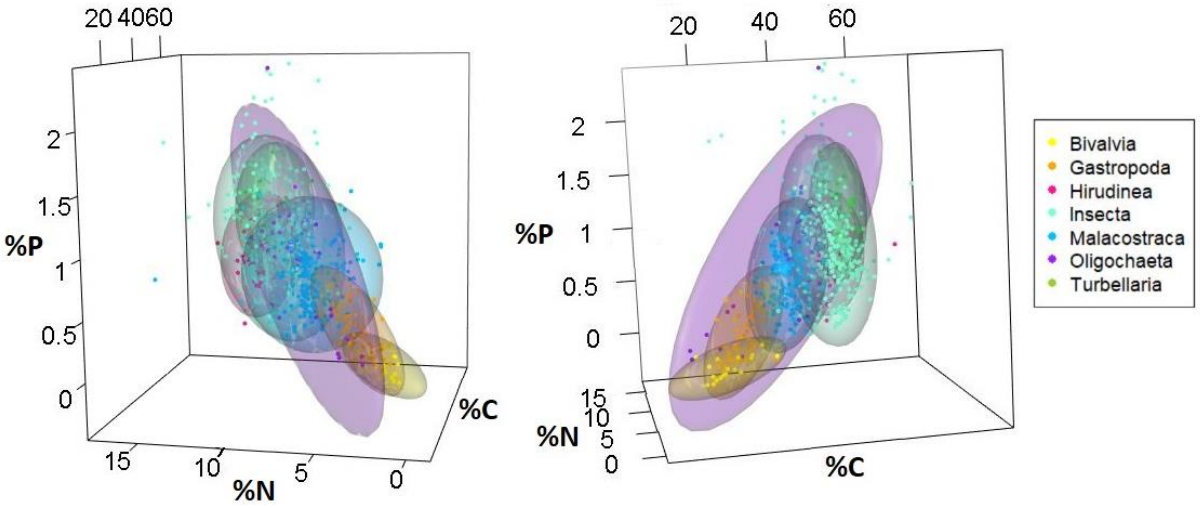
While the differences between taxonomic groups are most likely a direct effect of phylogeny, with groups that are departed already long ago also being more distinct in their stoichiometry than those that parted more recently (Fagan *et al.*, 2002), differences between trait groups probably are also partly caused by physiological/biological restrictions.

Predators had a significant higher nitrogen content and lower C:N than taxa with other feeding habits, which is in line with previous works (Fagan *et al.*, 2002; Evans-White *et al.*, 2005; Ventura *et al.*, 2008; González *et al.*, 2011b, 2018). By feeding on other (macro-) invertebrates, their resources are of a higher nutritional quality (esp. regarding nitrogen) compared to that of taxa feeding on primary resources or detritus (Evans-White *et al.*, 2005). Another reason might also be a higher investment in N-rich muscle tissue, that allows foraging the often mobile prey. The high nitrogen content most likely also caused the significantly higher N:P ratio as also suggested by Evans-White *et al.*(2005), since there was no difference in phosphorous content towards 2/3 other groups. Cross *et al.*(2003) and González *et al.* (2011) also reported a higher phosphorous content of predators, a finding not confirmed by our data and other studies (Evans-White *et al.*, 2005; González *et al.*, 2018). Predators showed the lowest intra-group variation in nitrogen and carbon content, which probably underlines the stricter stoichiometry of their food compared to algae or detritus with elemental compositions reflecting the nutrient concentrations in the environment (Cross *et al.*, 2003; Danger *et al.*, 2016; Farrell *et al.*, 2018). The predators stoichiometric niche also was the most separated one.

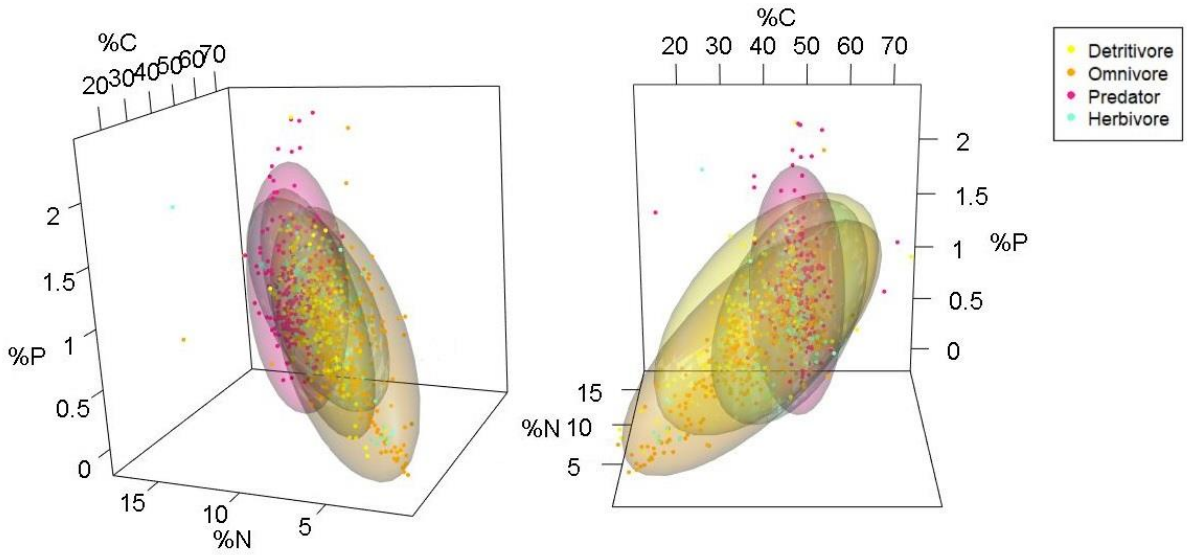
Detritivores were rather intermediate in all six stoichiometric variables, but nevertheless varying significantly from other groups. Although they are feeding on both, rather nutrient-poor plant- and nutrient-rich animal-based resources, omnivores had the lowest carbon, nitrogen and phosphorous content among the feeding groups; rather than intermediate values as expected. This was probably (partly) driven by phylogeny, since most Bivalvia and Gastropoda (and Malacostraca) were classified as omnivores and have lower mass element contents than the other taxonomic groups. However, the general pattern described for overall feeding groups remained when considering only Insecta. Evans-White *et al.* (2005) already pointed out the difficulty to disentangle effects caused by the feeding behaviour or phylogeny and González *et al.*(2018) suggested that taxonomy is more important in explaining variation in body stoichiometry than functional feeding group. Also the use of different functional feeding groups (*i.e.* defining groups based on feeding habit vs. the resource consumed) can lead to difficulties when comparing studies.

The knowledge of such general stoichiometric differences between taxonomic or trait groups is important and can help when working within ecological stoichiometry: It could allow a first estimation of the composition of a community or a rough idea of its trophic structure, for example under change nutrient concentrations. Knowing up to which taxonomic level there can be substantial variation in body stoichiometry should be considered when deciding about the taxonomic resolution when using stoichiometric data.

Supplementary Material



S 2b.1: Stoichiometric niches of taxonomic groups spanned by the axes of %C, %N and %P. For each group, the convex hull containing 95% of data points (scattered dots) is shown.



S 2b.2: Stoichiometric niches of functional feeding groups spanned by the axes of %C, %N and %P. For each group, the convex hull containing 95% of data points (scattered dots) is shown.

Homeostasis of macroinvertebrate stoichiometry: The effect of taxonomy, body mass, water N:P and season

1. Introduction

Homeostasis describes the ability of a system to maintain a stable internal state, regardless of external changing conditions. Stoichiometric homeostasis is a key assumption in ecological stoichiometry, a discipline that investigates the equilibrium and the flux of elements between organisms and their environment (Sterner & Elser, 2002). Here, it refers to a stable elemental composition of an organism. While autotrophic organisms are generally considered non-homeostatic and to directly display the nutrient conditions of the environment in their stoichiometry (Cross *et al.*, 2003; Prater *et al.*, 2015; Demi *et al.*, 2019), heterotrophs compositions are stricter constrained (Sterner & Elser, 2002; Cross *et al.*, 2003; Persson *et al.*, 2010). They developed regulating mechanisms to deal with imbalances between food sources and their nutritional demand for certain elements. Such imbalances can limit growth and reproduction in consumers if their nutritional demand is not met (Stelzer & Lamberti, 2002; Frost *et al.*, 2005; Fink *et al.*, 2006). Behavioural adaptations include increasing feeding rates to ingest enough nutrients also from low quality resources (Fink *et al.*, 2006; Ott *et al.*, 2012; Jochum *et al.*, 2017b) or a change in diet to a more suitable food source, closer to nutritional demand (Arsuffi & Suberkropp, 1985; Hood *et al.*, 2014). On the cellular level, assimilation efficiency can improve nutrient extraction (Rasmussen & Anderson, 2005; Cox & Murray, 2006).

Besides many studies confirming homeostasis in macroinvertebrates under high-nutrient conditions or artificially enriched sites (Evans-White *et al.*, 2009; Persson *et al.*, 2010; Danger *et al.*, 2013), others found some taxa deviating from strict homeostasis under such scenarios (Cross *et al.*, 2003; Small & Pringle, 2010). Such divergent results indicate that the assumption of strict homeostasis does not apply to all taxa and that there are taxon-specific responses to changes in resource quality. In some taxa stoichiometry varied over ontogeny (Frost & Elser, 2002; Villar-Argaiz *et al.*, 2002; Back *et al.*, 2008; Back & King, 2013) or between sexes (Markow *et al.*, 2001; Back & King, 2013). For some aquatic macroinvertebrate taxa seasonal changes were reported (Liess & Hillebrand, 2005) most likely resulting from seasonal fluctuations in resource quality. Due to such variations, Halvorson & Small, (2016) pointed out that field data might not be suitable to determine homeostasis of a species as such intra-specific differences are almost impossible to consider.

A deviation from homeostasis in heterotrophs can have ecological consequences by altering consumer-resource interactions and the energy and nutrient transfer to higher trophic levels (Malzahn *et al.*, 2007). Another problem arises from the use of stoichiometric data in studies. Simply assuming homeostasis and applying taxon mean values *(e.g.* from a database) could lead to biased results and thus wrong conclusions when effects due to the community structure regarding for example size, sex or life stage are ignored. It is therefore the aim of this chapter to analyse if and how macroinvertebrate taxa deviate from assumed homeostasis, and to investigate the role of environmental parameters as well as taxonomy.

Identifying the possible drivers of varying stoichiometry can improve the design of future studies and allow a better interpretation of results when working with stoichiometric data.

I used raw data obtained to build a database on stoichiometric information (elemental mass contents of C, N, P as well as their molar ratios C:N, C:P and N:P) of stream macroinvertebrates, comprising 174 taxa belonging to seven larger taxonomic groups. The data originate from a range of field sampling campaigns, supplemented by literature review and allows sub-setting to answer the following questions: Q1) How much do taxonomic levels contribute to variation in stoichiometry Q2) Is stoichiometry linked to biomass? And Q3) Does the assumption of homeostasis remains true under varying environmental parameters (water N:P, season)? The questions each will be addressed both considering all taxa together as well as individually for the most abundant taxa in the data set. A last analysis investigates stoichiometric differences between sexes (Q4). All questions will be assessed separately for the six stoichiometric variables.

I hypothesize that for Q1) Stoichiometry should vary more broadly between coarser taxonomic levels (*e.g.* class) due to general larger differences in physiology, morphology or ecology between taxa. There should be less variation between finer taxonomic levels (*e.g.* family).

Biomass should be correlated to stoichiometry (Q2). It is ultimately linked to body size and growth rate, which is known to be linked to phosphorous stoichiometry (Growth rate Hypothesis, Elser *et al.* (1996)). Due to a faster growth rate, smaller organisms should have a higher phosphorous content (lower C:P, N:P) than larger individuals. Carbon content should increase with body size due to larger proportion of carbon-rich structure molecules.

Under varying environmental parameters, we expect a stable stoichiometry of taxa when water N:P varies, due to regulating mechanisms (Q3a). Some taxa might deviate from homeostasis over the season as their demand for nutrients changes with life stage (Q3b). Females could vary from males due to different phosphorous allocation in reproductive tissue (Q4).

2. Material and methods

2.1. Field sampling

The stoichiometric data originated from a series of field samplings campaigns, supplemented by a literature research. Details on the compilation of data, the sampling procedure in the field as well as laboratory measurements are described in (Chap. IIa).

Two of the field sampling campaigns were arranged to obtain data under the scenario of 1) a gradient in water N:P caused by phosphorous pollution and 2) seasonal changes.

For the first, ten streams in North-Eastern France were selected based on their nutrient concentrations regarding nitrogen and phosphorous. The streams display a (relatively) stable concentration of nitrogen and varying levels of phosphorous to cover a gradient of water N:P ratios (Tab. 2c.1). The samplings took place during five consecutive days in May 2019.

One of the selected sites with intermediate water N:P ("Marche") was further visited during a one year period to answer question regarding seasonality of stoichiometric traits (2). In total samples were collected in May, September and December 2019. During some field campaigns, the sex of some individuals has been identified.

Tab. 2c.1: Nitrogen and phosphorous concentrations at the ten samplings sites. Concentrations of compounds given in mg/ L, the N:P ratio is based on phosphate and nitrate.

Site	Date	PO_4^{3-}	NO_3^{2-}	NO_2^-	NH_4^+	N:P	Long(E)	Lat(N)
Aisne	14/05/2019	0.08	43	0.05	0.02	573.33	5.14585	48.95549
Alzette	15/05/2019	0.56	12.6	0.46	1.35	22.54	5.9657911	49.4855656
Dorlon	13/05/2019	0.06	19.2	0.03	0.05	314.75	5.5199742	49.4897471
Marche	15/05/2019	0.04	10	0.04	0.03	243.90	5.3181483	49.6142335
Moulaine	13/05/2019	0.02	12.4	0.00	0.01	620.00	5.8150051	49.510411
Girondelle	14/05/2019	0.03	7.2	0.07	0.01	266.67	4.3985174	49.8489991
Récourt	14/05/2019	0.02	19.5	0.00	0.00	975.00	5.3469	48.99317
Tucquegnieux	16/05/2019	0.03	22.4	0.19	0.10	746.67	5.88167	49.29985
Natagne	17/05/2019	0.30	16.2	0.07	0.63	54.00	6.1453301	48.8430455
Volmerange	16/05/2019	0.04	9.38	0.02	0.01	234.50	6.0783548	49.4545167

2.2. Statistical analysis

The research questions were then addressed on this whole database (Q1) or a corresponding subset based on the field campaigns described in 2.1 (Q2, Q3, Q4).

Statistical models were applied to answer the questions generally, for the whole range of taxa or larger taxonomic group, as well as for individual taxa (mainly species or genus level). Throughout the analysis, models and tests for the stoichiometric molar ratios were applied on logit-transformed inverse ratios, *i.e.* logit(N:C) instead of C:N. This helped achieving normally distributed model residuals and avoided a bias resulting from intrinsic properties of ratio data (Isles 2020).

To investigate the effect of taxonomy on macroinvertebrate stoichiometry, linear mixed models were constructed, separately for each stoichiometric variable, which was set as dependent variable. Taxonomy was added as nested random factor of the taxonomic groups (class/ order/ family). The nature of the model implies that only taxa whose classification includes these taxonomic groups were considered. A variance component analysis was conducted using the R function varcomp for each model to quantify the amount of variance explained by each level of the random factor, *i.e.* each taxonomic level. Marginal and conditional r^2 values were calculated using the R function r.squaredGLMM from the MuMIn package to quantify the amount of variance explained by random factor levels. Linear models were built using the lmer function from lme4 package (Bates *et al.*, 2015).

The effect of biomass on stoichiometry was analysed using simple linear models with log-transformed biomass as independent variable and either %C, %N, %P, C:N, C:P or N:P as dependent variable.

Models for the ratios were applied on logit transformed data of the inverse ratio (*e.g.* logit(N:C)) to achieve normally distributed model residuals and to avoid bias resulting from intrinsic properties of ratio data (Isles 2020). The models were applied separately for each major taxonomic group (*i.e.* Bivalvia, Gastropoda, Hirudinea, Insecta, Malacostraca, Oligochaeta, Turbellaria). Taxa for which biomass information of a sufficiently large sample size was available ($n > 10$ for each stoichiometric variable) were analysed separately. Simple linear regressions as described above were applied for each stoichiometric variable and taxon.

Sets of linear mixed models were constructed to investigate 1) the effect of water N:P and 2) season on each stoichiometry variable. Elemental mass content or molar ratio was set as dependent variable. Water N:P or season was added as fixed factor (numerical in case of water N:P, categorical in case of season) and taxonomic group as described above as nested random factor of the levels class, order and family. Biomass was added to the model as additional fixed factor, when it improved the model as indicated by model comparison using anova function. Water N:P and biomass values were log-transformed before model application. For each analysis, the models were applied on the corresponding subsets of dataset (see 2.1.).

Additionally, taxa for which a sufficient large sample size was available (taxa appearing in ≥ 2 streams resp. $\geq$ seasons with $n > 2$ for each stoichiometric variable) were analysed separately. Simple linear models with the stoichiometric variable as dependent variable and water N:P as continuous independent variable were applied. For taxa where the stoichiometric variable had previously been found to be significantly correlated to biomass, log-transformend biomass was added as co-variable (without assuming an interaction between the two) to the model.

For the individual analysis of seasonal variation, a set of ANOVAs were conducted to compare each stoichiometric variable between seasons. Biomass was added as covariate assuming to interact with season, when the stoichiometric variable was previously found to be significantly correlated to biomass.

In taxa for which information on the sex was available, the mean values of females and males were compared by applying for each taxon an ANCOVA on each of the six stoichiometric variables. Log-transformed biomass thereby was set as co-variable, assuming an interaction with sex. In case the interaction term was not significant, it was excluded from the model.

3. Results

3.1. Effect of taxonomy

The taxonomic level that explained the largest part of variance in macroinvertebrate stoichiometry differed between mass element contents and their molar ratios (Tab. 2c.2).

In %C and %N, “class” clearly accounted for the largest fraction of variation (55.82% and 29.37%). Family level accounted for the second largest fraction of variation in %C and %N and the lowest fraction of variation among the taxonomic groups investigated was on order level. Class level had no effect on

%P and C:N. The largest parts of variation (26.31% and 22.45% respectively) were attributed to family level; order level also here accounted for the smallest part of variation.

In C:P and N:P ratio, order level explained most of the variation. Class level was least important in explaining C:P ratio, while for N:P ratio it was family level.

Generally, %C and %N were better explained by taxonomy than %P and the molar ratios, where ~60% of the variation remained unexplained by taxonomy.

Tab. 2c.2: Results of variance component analysis of linear mixed to investigate the effect of taxonomy on macroinvertebrate stoichiometry. For each stoichiometric variable (%C, %N, %P, C:N, C:P, N:P), the values indicate the residuals of each level of random factor, as well as the residuals. r^2 gives the conditional r^2 and n the sample size (total n as well as the size of random factor levels (family/ order/ class)).

	%C	%N	%P	C:N	C:P	N:P
Class	55.30	28.98	0	0	1.81	12.01
Order	6.513	12.92	13.78	12.26	25.18	21.08
Family	11.05	18.14	26.31	22.45	14.87	7.88
Resid	27.13	39.96	59.91	65.29	58.14	59.03
r^2	0.73	0.60	0.40	0.35	0.42	0.41
n	1158(69/16/4)	1158(69/16/4)	1305(73/16/4)	1157(69/16/4)	1040(67/16/4)	1041(67/16/4)

3.2. Effect of biomass

In some macroinvertebrate groups, stoichiometry was significantly correlated to biomass, showing group-specific directions of the relationship (Fig. 2c.1, 2c.S1).

In Gastropoda, nitrogen and phosphorus content increased with increasing biomass. Results for the transformed stoichiometric ratios (logit(N:C), logit(P:C), logit(P:N)) also showed a positive relationship with biomass, which corresponds to a negative correlation of the untransformed stoichiometric ratios.

In Hirudinea, there was a significant positive correlation between %P and biomass.

Insecta %C and C:N were positively correlated to biomass (negative model slope of logit(N:C)) while in nitrogen and phosphorous content the correlation was negative.

%P was negatively correlated to biomass in Malacostraca.

In Oligochaeta, the mass contents of C, N, and P decreased with increasing biomass ($p<0.05$, $r^2=0.4939$, 0.4037 and 0.2129 for %C, %N and %P). C:N ratio also decreased with biomass as indicated by a positive slope of transformed values by the model.

Transformed N:P ratio significantly increased with increasing biomass, suggesting a negative relationship of the untransformed stoichiometric variable.

In Bivalvia, none of the stoichiometric variables was significantly related to biomass.

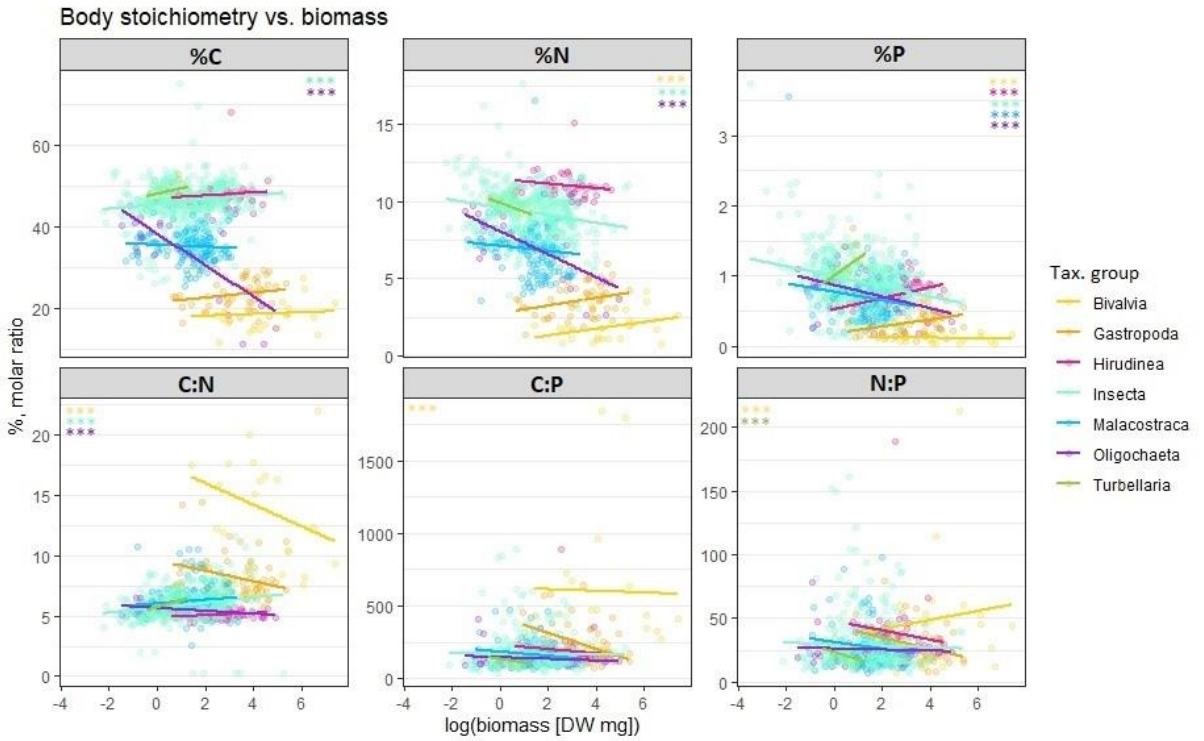


Fig. 2c.1: Correlation between body stoichiometry (%C, %N, %P, C:N, C:P, N:P) and biomass (log transformed dry weight in mg) for largest taxonomic groups. Models were applied separately for each group and stoichiometric variable. Colored stars in upper right or left corner indicate significant models. Please note that untransformed values of stoichiometric ratios are presented for easier comparison with the literature, while models were applied on logit-transformed inverse ratios (*i.e.* logit(N:C) instead of C:N). Model parameters can be found in the supplementary (2c.S1).

Of the 19 taxa investigated separately, 14 showed a significant correlation between biomass and one to all stoichiometric variables (Fig 2c.2, 2c.S2). %C, %N and C:N were the most concerned variables and resulted in significant correlations in five to eight taxa, explaining on average 31.56-39.69% of its variation. %P, C:P or %N only showed a response to biomass in two or three taxa where it explained on average 25.15-28.86% of stoichiometric variation.

In most insect taxa where the relationship of %C and/ or %N to biomass was significant (*Baetis*, *Ephemera*, *Ephemerella*, *Hydropsyche*, *Limnephilinae*, *Rhyacophila*, *Sericostoma*, Simuliidae, Stenophylacini-Chaetopterygini) it was positive for %C and negative for %N. This probably lead to an increase in C:N when biomass increases. This positive relationship with biomass was also found in *Ecdyonurus* and fits with the result found on of class level described above. %P decreased with biomass in *Baetis*, but increased in *Sericostoma*. In *Rhycophila* and *Sericostoma* N:P was negatively correlated to biomass, as it was also the case for C:P of the latter.

In the crustacean *G. pulex* %C and C:P ratio decreased with increasing biomass. In *G. roeseli* %N was negatively correlated to biomass. C:N increased with biomass in this crustacean, while it decreased in *Asellus.* For %C and %N this result is in line with the trend indicated for the class of Malacostraca.

In the hirudinean *Glossiphonia*, both C:P and N:P ratios were positively correlated to biomass, which is opposite to the trend found on class level for Hirudinea.

Atherix, *Dugesia*, *Erpobdella*, *G. fossarum* and *Radix* did not show any stoichiometric dependence on biomass.

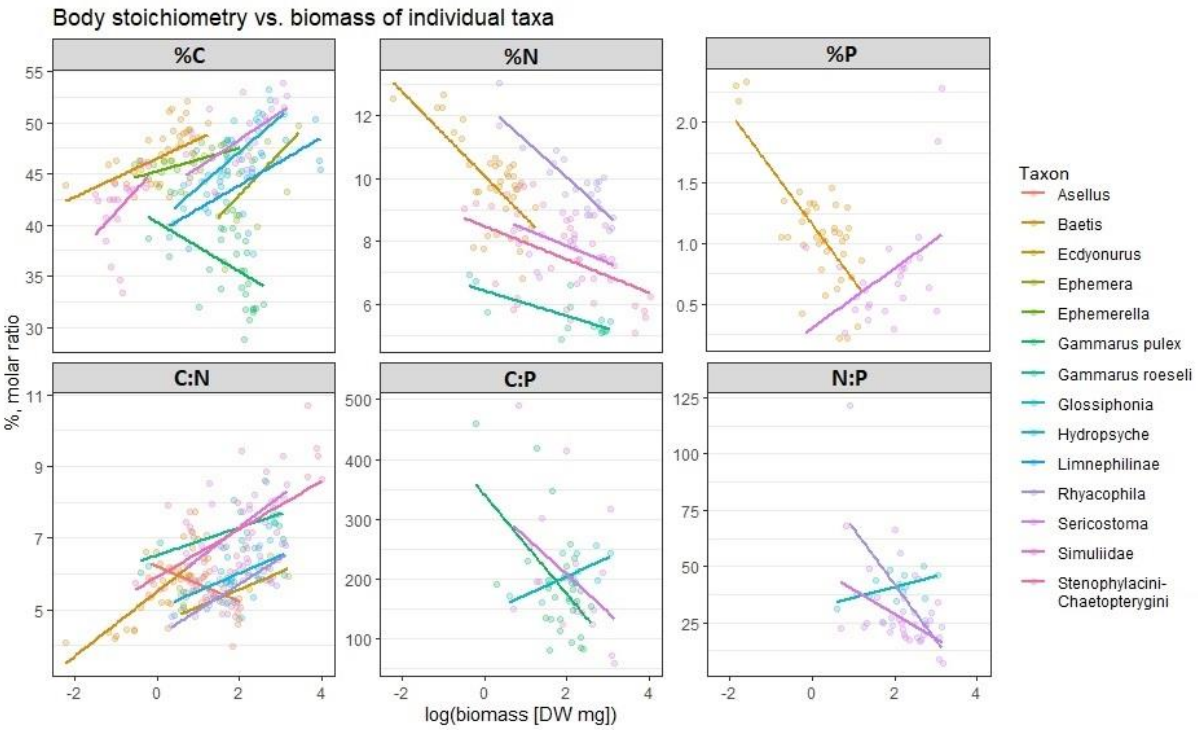


Fig. 2c.2: Correlation between body stoichiometry (%C, %N, %P, C:N, C:P, N:P) and biomass (log transformed dry weight in mg) of individual taxa. Shown are only taxa for which the model of the corresponding stoichiometric variable was significant. Please note that untransformed values of stoichiometric ratios are presented for easier comparison with the literature, while models were applied on logit-transformed inverse ratios (*i.e.* logit(N:C) instead of C:N). Model parameters can be found in the supplementary (2c.S2).

3.3.Effect of water N:P

Overall, water N:P did not have a large effect on any stoichiometric variable. The correlation was only significant for %C which increased with increasing water N:P (Tab. 2c.3).

%N tended to increase with water N:P, while %P tended to decrease. For C:N the models indicated a negative correlation to water N:P, while for C:P and N:P the relationship tended to be positive indicated by opposite slopes of transformed variable values in the model.

In cases were biomass was part of the model (%C, C:P), it had either a positive significant (%C) or a negative (C:P) effect on stoichiometry.

All models revealed a larger effect of taxonomy than of water N:P on stoichiometry, with this effect being larger on %C, %N and C:N (conditional r^2 of 0.72-0.84) than on %P, C:P and N:P (conditional r^2 0.35- 0.47).

Tab. 2c.3: Results of linear mixed models applied to investigate the effect of water N:P and biomass on body stoichiometry, with taxonomy considered as random factor. The values for fixed effects indicate their effect, the values for random factors their contribution to the overall variance. Water N:P values have been log-transformed before model application. "n" gives the total number of individuals used, as well as the number of groups for each random factor level. Bold characters indicate significance of fixed effects (based on CI)

	Fixed effect				Random factor				
Stoichio	WaterN:P	Biomass	r^2m	r^2c	Family	Order	Class	Resid	n (family/order/class)
%C	**0.6189**	**1.1131**	0.0102	0.7254	26.57	8.14	48.73	16.56	346 (37/14/4)
%N	0.1853	NA	0.0061	0.7913	33.87	11,64	33,5	20.99	346 (37/14/4)
%P	-0.0181	NA	0.0026	0.4652	34.99	11.39	0	53.62	351 (37/14/4)
C:N	0.0112	**-0.0272**	0.0446	0.8418	50.44	21.82	0	27.75	346 (37/14/4)
C:P	-0.0141	NA	0.0008	0.3943	22.57	16.81	0	60.62	335 (36/14/4)
N:P	-0.0215	NA	0.0013	0.3514	9.87	25.18	0	64.94	335 (36/14/4)

Thirteen taxa (identified on species, genus, tribe or family level, belonging to the classes/ groups of Gastropoda, Insecta and Malacostraca) were found in more than two streams. Stoichiometry of six taxa (*Baetis, Ephemerella, G. fossarum, G. pulex, O. albicorne, Radix*) was not affected but seven taxa showed a significant correlation between water N:P and at least one stoichiometric variable (Fig. 2c.3). The content of C and N and their ratio (C:N) was most often concerned and only in *Rhyacophila* and *N. punctatolineatus* phosphorous stoichiometry (%P, C:P or N:P) was affected.

Generally, the effect of water N:P on stoichoimetry differed between the individual taxa.

While %C increased with water N:P in *N. punctatolineatus* and *Simuliidae* (non sig in *Ephemerella)*, it decreased in *Ephemera* (non sig *Hydropsyche)*. Similarily, %N also increased in *N. punctatolineatus* and decreased in *Ephemera* and the crustacean *Asellus* along this gradient.

The correlation between C:N and water N:P was negative for *Asellus*, *N. punctatolineatus* and *Stenophylacini-Chaetopterygini*. in *Rhyacophila* and *Stenophylacini-Chaetopterygini* and negative in *Ephemerella* and *Asellus*.

The phosphorous content decreased with increasing water N:P in *N. punctatolineatus* and *Rhyacophila*. C:P and N:P ratio also increased along this gradient in *N. punctatolineatus* as indicated by negative model slopes of the transformed stoichiometric variables. The same trend was indicated in *G. pulex*, but only the effect of biomass was significant in this model.

The variance explained by water N:P varied between 9.42 (%N *Asellus*) and 89.03 (N:P in *N. punctatolineatus*). In *N. punctatolineatus* all stoichiometric variables were effected by water N :P. Six taxa additionally showed an effect of biomass on stoichiometry (C:N in *Asellus* and *Stenophylacini-Chaetopterygini*, %C in *Ephemerella*, *Hydropsyche* and Simuliidae, C:P *G. pulex*), showing the same patterns as indicated in previous analysis focusing only on biomass (see 3.2.).

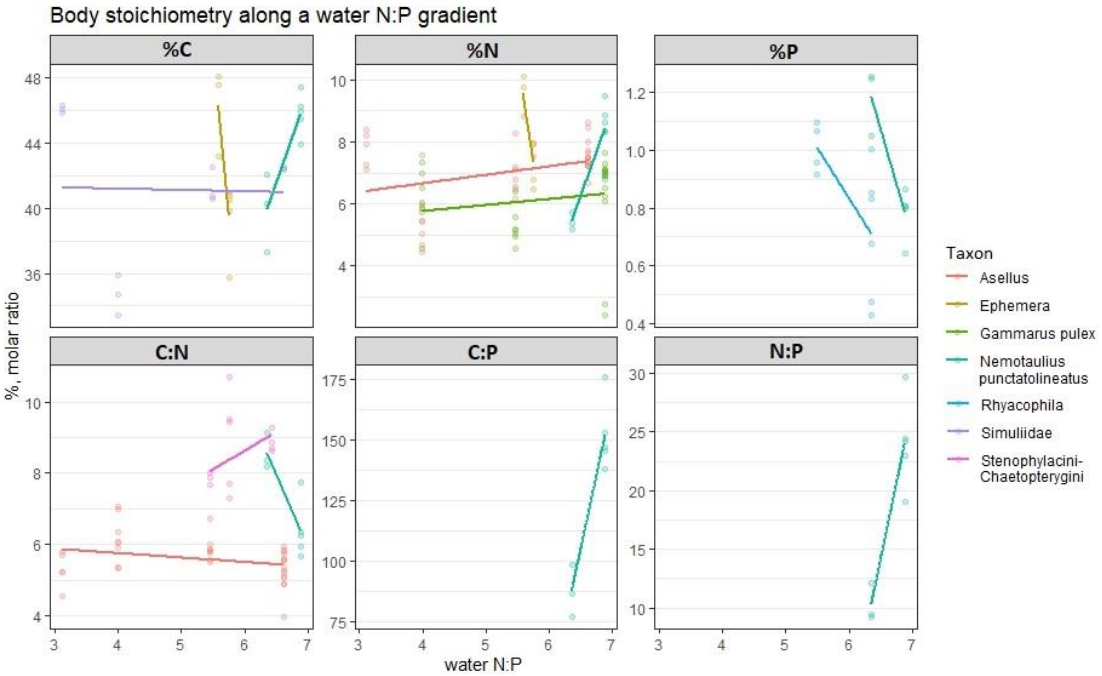


Fig. 2c.3: Correlation between body stoichiometry (%C, %N, %P, C:N, C:P, N:P) and water N:P (log-transformed) of individual taxa. Shown are only taxa for which the model of the corresponding stoichiometric variable was significant. Please note that untransformed values of stoichiometric ratios are presented for easier comparison with the literature, while models were applied on logit transformation of the inverse ratio (*i.e.* logit(N:C) instead of C:N). Model parameters for all taxa can be found in the supplementary (2c.S3).

3.4. Effect of season

Season had a significant effect on all stoichiometric variables except for %P when all individuals were pooled (Tab. 2c.4). The trend observed varied between stoichiometric variables and was not linear, *i.e.* increasing or decreasing from May over September to December. Carbon content was significantly higher in December than in May, and also biomass had a positive effect on %C.

%N was highest in September, and macroinvertebrates had significant higher values both in September and December compared to May.

C:N was significantly lower both in September and December compared to May, while biomass had a negative effect.

C:P increased from May to September and December, but only the difference between May and December was significant.

N:P likewise increased over the season, but taxa had higher N:P both in September and December compared to May.

The effect of season however was not very strong. A large part of the variation in stoichiometry was explained by taxonomic groups as indicated by clearly larger conditional r^2 values of the overall model (on average 0.49 ranging between 0.25 in %P and 0.86 in %C) compared to the marginal r^2 only referring to the fixed effects season and/ or biomass (on average 0.07 ranging between 0.02 in %P and 0.21 in C:N).

Individuals of ten taxa (species, genus, subfamily or class level, belonging to six major taxonomic groups (Bivalvia, Hirudinea, Insecta, Malacostraca, Oligochaeta, Turbellaria)) were found in more than one season, of which eight showed an effect of season on at least one to all stoichiometric variables (Tab. 2c.5). %C, %N and C:N ratio were the most concerned ratios.

In *Baetis* and *G. fossarum*, all stoichiometric variables were affected by season and biomass additionally had an effect on %N, %P and C:N.

In *Ecdyonurus* and *Sericostoma* there were seasonal differences in %C, %N and C:N, which in *Sericostoma* were also affected by biomass. Limnephilinae and Pisidium showed differences in carbon and nitrogen content and the carbon content of the first was additionally affected by biomass. In Oligochaeta, season had an effect on %C and C:N ratio.

In *Glossiphonia* and *Hydropsyche* there was no significant difference between seasons in any stoichiometry variable.

Tab. 2c.4: Results of linear mixed models applied to investigate the effect of season and biomass on stoichiometry, with taxonomy considered as random factor. The values for fixed effects indicate their effect, the values for random factors their contribution to the overall variance. N gives the total number of individuals used, as well as the number of groups for each random factor level. Bold characters indicate significance of fixed effects (based on CI)

	Fixed effect						Random factor				
Stoichio	Mai	Sept	Dec	Biomass	r^2m	r^2c	Family	Order	Class	Resid	n
%C	42.3173	-0.3034	**2.0779**	**1.1946**	0.0674	0.8579	23.31	10.50	50.94	15.24	233(25/10/4)
%N	8.5892	**1.2004**	**1.0901**	NA	0.0629	0.6500	20.31	9.61	32.73	37.34	233(25/10/4)
%P	0.7991	0.0283	-0.0753	NA	0.0163	0.2572	2.02	22.47	0	75.51	247(29/10/4)
C:N	-1.3888	**0.2022**	**0.1189**	**-0.0445**	0.2079	0.4890	13.7	11.71	10.08	64.51	233(25/10/4)
C:P	-4.1083	-0.0905	**-0.2405**	NA	0.0322	0.3448	0	32.3	0	67.70	179(24/10/4)
N:P	-2.3606	**-0.2718**	**-0.3563**	NA	0.0491	0.3608	0	32.78	0	67.22	179(24/10/4)

Tab 2c.5. Results of ANOVAs to compare stoichiometry of the most abundant macroinvertebrate taxa between seasons. Assignment to the columns indicates for which stoichiometric variable there was a significant difference between seasons and/ or a significant effect of the co-variable biomass. Underlining indicates models integrating biomass. N_{season} indicates the number of seasons at which the taxon was found.

Taxon	Season	Biomass	No effect	n_{season}	η^2(season)
Baetis	%C, %N, %P, C:N, C:P, N:P	%N, %P, C:N		2	
Dugesia	%N		%C, %P, C:N, C:P, N:P	3	
Ecdyonurus	%C, %N, C:N		%P, C:P, N:P	2	
G. fossarum	%C, %N, %P, C:N, C:P, N:P			3	
Glossiphonia			%C, %N, %P, C:N, C:P, N:P	2	
Hydropsyche			%C, %N, %P, C:N, C:P, N:P	3	
Limnephilinae	%C, %N	%C	%P, C:N, C:P, N:P	2	
Oligochaeta	%C, C:N	%C, %N, %P	C:P, N:P	2	
Pisidium	%C, %N		%P, C:N, C:P, N:P	2	
Sericostoma	%C, %N, C:N	%C, %N, C:N; N:P	%P, C:P	3	

3.5. Effect of sex

Of the five crustacean taxa tested (*Asellus*, *Dikerogammerus*, *G. fossarum*, *G. pulex*, *G. roeseli*) only *Asellus* and *G. roeseli* showed a significant difference between male and females in one stoichiometric variable: In *Asellus*, C:N was significantly higher in females than in males (ANCOVA with $p<0.05$)(fig. 2c.4, 2c.S4), and in *G. roesli* females had significantly higher %C. In neither of the models, there was an interaction between sex and log-transformed biomass.

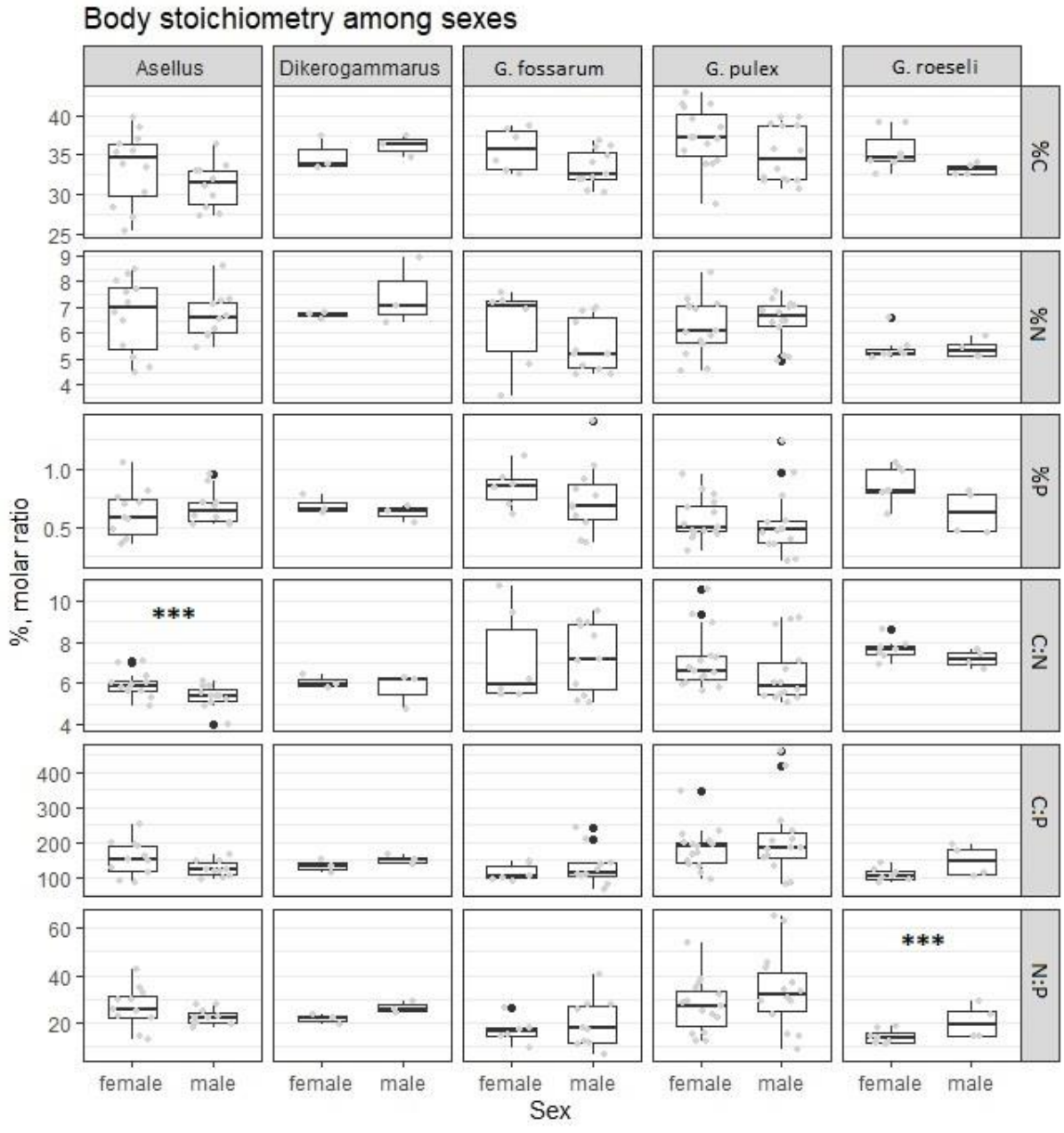


Fig. 2c.4: Differences in body stoichiometry (%C, %N, %P, C:N, C:P, N:P) between female and male crustacean taxa. Asterisks indicate significant ANCOVA models with log(biomass) as co-variable ($\alpha = 0.05$). Please note that untransformed values of stoichiometric ratios are presented for easier comparison with the literature, while models were applied on logit transformation of the inverse ratio (*i.e.* logit(N:C) instead of C:N).

4. Discussion

The results showed that taxonomy explained the largest part of variation in macroinvertebrate stoichiometry, while environmental factors such as season or water N:P only played a minor role and only affected stoichiometric traits of few taxa.

The effect of taxonomy on stoichiometric traits

This result is not surprising, as the dependence of stoichiometric traits on taxonomic groups has already been demonstrated for aquatic and terrestrial macroinvertebrates (Mehler *et al.*, 2013; González *et al.*, 2018; Andrieux *et al.*, 2020). The influence of taxonomic levels varied between elements and ratios and the effect of taxonomy was larger on mass contents (esp. %C and %N) than on elemental ratios. Throughout the study the largest part of variance was attributed to the highest level included (*i.e*. class or order) for %C and %N, while lower taxonomic groups played a larger role in explaining variation in %P and the molar ratios (C:N, C:P, N:P), indicating that there is a substantial variation in %P content on family level. It is likely that general differences between larger taxonomic groups, *e.g.* in body size (see below) constrain carbon and nitrogen stoichoimetry, while the phosphorous content is stronger influenced by other functional traits which can also vary substantially between taxonomically close taxa. This is in accordance with other studies, showing that phosphorous content is more variable between and within macroinvertebrate groups than nitrogen or carbon (Morse *et al.*, 2012; Mehler *et al.*, 2013). Gonzalez *et* al. (2018) found high variation of %P, C:P and N:P (41 – 63%) even within species. This might explain why we found rather low variation of these stoichiometric variables explained by taxonomy (residual of 59 – 61%) and that our models, which did not include species level, only reached rather low r^2 values. There seem to be other influences besides taxonomy on especially these stoichiometric variables that cause this intra-specific variation.

Besides the rather simple approach of using the Linnean taxonomic groups to investigate the effect of taxonomy on stoichiometry, one could relate to phylogeny that uses molecular signals to describe the relatedness of taxa. For example, Fagan *et al.* (2002) and Woods *et al.* (2004) showed that ancient lineages of herbivorous insects contained more nitrogen and phosphorous than more recent lineages. However, González *et al.* (2018) found no or only a weak of such phylogenetic signal, independent of the phylogenetic method used.

The effect of biomass on stoichiometric traits

Although biomass is directly linked to body size and therewith to metabolic, thus scaling with physiological rates and processes from production to life history characteristics (Kleiber, 1932; Peters, 1983; Hall *et al.*, 2007), it did not contribute much to explaining macroinvertebrate stoichiometry. Carbon and nitrogen as well as their ratio thereby were the most concerned variables when analyzing individual taxa, although on class level it was only significant for Insecta and Oligochaeta. In Insecta, the carbon content increased while the nitrogen content decreased with increasing body mass, leading

to a positive correlation of C:N ratio to body mass. This pattern was also expressed by the other groups, although insignificant. In Oligochaeta all three variables decreased in larger individuals; Gastropoda showed a positive relationship of carbon and nitrogen with biomass and a negative relationship of C:N. One reason for the general positive linkage of carbon with biomass is most likely an increasing proportion of structural material in larger, *i.e*. heavier, organisms. The exoskeletons of insects and crustaceans are based on carbon-rich Chitin molecules (Schimmelmann, 2011). Also the investment into energy storage when approaching energy-consuming metamorphosis could be a reason for a positive relationship between carbon content and biomass in aquatic insects (Veldboom & Haro, 2011). Decreasing nitrogen content with increasing biomass observed in the same study, was attributed by the authors to the simply lower relative amount of nitrogen when carbon content is increased. González *et al.* (2018) explained a positive correlation between nitrogen content and biomass in a multi-taxa model with an allocation to N-rich flight muscles during later development stages in certain invertebrates (Stjernholm & Karlsson, 2008).

Phosphorous stoichiometry was the most affected on class-level, but on taxon level only two taxa showed a significant relationship. Supporting findings from González *et al.* (2018), the response of phosphorous was more heterogenous than for the other elements, both on class and taxon level. Within insects, the relationship was negative in *Baetis* and positive in *Sericostoma*. The negative correlations of C:P and N:P ratios of *Rhyacophila* and *Serocostoma* were also contradictory to the overall negative relationship for insects.

One reason for decreasing phosphorous content is probably, as for nitrogen content, simply a lower relative amount of %P due to increasing carbon content.

Another reason that has often been suggested by the literature are decreasing growth rates in larger organisms. This, according to Growth rate Hypothesis (GRH, Sterner & Elser, 2002) leads to a lower demand for phosphorous-rich rRNA molecules to fuel protein synthesis, *i.e*. growth. Small (= young) taxa might express faster growth rates than larger (=older) ones as they still have to reach maturity, which would lead to a negative relationship between phosphorous content and biomass. This is supported by a negative correlation of RNA content and body size (not investigating biomass) as reported for larvae of Chironomidae (Small *et al.*, 2011). Some aquatic insects (Back & King, 2013) and early-stages of mayfly larvae (Frost & Elser, 2002) and Drosophila (Elser *et al.*, 2006) also express decreasing phosphorous content during the development, although it increased in later stages may fly larvae approaching maturity (Back *et al.*, 2008). Carbon and nitrogen content remained stable or were affected to a lesser degree in those studies. Except for *Baetis*, none of the mayflies in our study showed an effect of biomass on its phosphorous stoichiometry; and in *Baetis* also the carbon and nitrogen content changed with biomass. Opposing this theory, the change in the phosphorous content of *Brachycentrus occidentalis* (Trichoptera) did not follow GRH (Veldboom & Haro, 2011), but rather was in line with phosphorous allocation into reproduction as observed for mayflies (Back et al. 2008, see above). The

phosphorous content in this study was not correlated to biomass, but the taxa for which we found an increasing %P (and/ or decreasing C:P, N:P) with biomass belonged to the order of Trichoptera.
Negative scaling between both phosphorous and/or nitrogen content and biomass was also reported for terrestrial insects and arachnids (Fagan *et al.*, 2002; Woods *et al.*, 2004; Bertram *et al.*, 2008; González *et al.*, 2011, 2018) although on species level it was only significant for 50% of arthropods (González *et al.*, 2011a). Villar-Argaiz *et al.* (2002) attributed ontogenetic variation in body stoichiometry of copepods to different requirements – especially for phosphorus – during different developmental stages.

The effect of biomass mainly concerned carbon and nitrogen stoichoimetry and was taxon-specific. Some taxa (*Atherix*, *Dugesia*, *Erpobdella*, *G. fossarum* and *Radix*) did not show any effect of biomass, while in others (*Baetis*, *Sericostoma*) all mass content as well as some or all ratios were correlated to biomass. Other studies also only found responses only in some taxa (Evans-White *et al.*, 2005; Liess & Hillebrand, 2005; Mehler *et al.*, 2013) or some feeding groups (Cross *et al.*, 2003), but their patterns are in accordance with our results, at least for insects.

The effect of environmental factors on stoichiometric traits
Both environmental parameters investigated – water N:P and season – had, if any, only a low effect on macroinvertebrate stoichiometry and our models rather again confirmed the importance of taxonomy. As we expected, most macroinvertebrate taxa maintained a stable elemental composition even under changing environmental conditions.

Macroinvertebrate stoichiometry was partially affected by water N:P, indicating homeostasis. Our overall models indicated that a large part in stoichiometry is explained by taxonomy rather than water N:P (and biomass) alone; and stoichiometry of most taxa was unaffected by water N:P when analysed individually. Only in the trichopterans *Rhyacophila* and *N. punctolineatus* the phosphorous content decreased with increasing water N:P, which followed the pattern we expected in case of a deviation from homeostasis. This is in line with other studies reporting that even over a large gradient of resource quality, macroinvertebrates remained a stable elemental composition (Fink *et al.*, 2006; Evans-White *et al.*, 2009; Persson *et al.*, 2010; Danger *et al.*, 2013). Other studies reported a deviation from homeostasis over a nutrient gradient (Cross *et al.*, 2003; Small *et al.*, 2011). In addition to the general difficulty of excluding confounding factors when studying homeostasis based on field data (see Halvorson & Small (2016)), the range of the nutrient gradient might contribute to such varying observations. Our gradient in terms of water phosphorous concentration was within the range of cited studies, but regarding water N:P it tended to be larger.
Although the gradient in water N:P in was caused by increasing concentrations of phosphorous, carbon and nitrogen stoichiometry (%C, %N, C:N) of macroinvertebrates were affected in a higher number of taxa (three resp. four) and additionally showed no consistent pattern.

Other studies already observed a larger effect of nutrient enrichment (water nitrogen, phosphorous) on nitrogen stoichiometry, while phosphorous content remained unaffected (Morse *et al.*, 2012; Feijoó *et al.*, 2014; Prater *et al.*, 2015). However, Fink *et* al. (2006) found that the highest variability occurred in phosphorous content, both in macroinvertebrates as well in seston.

Generally, macroinvertebrate stoichiometry is less affected than that of basal resources (Bowman *et al.*, 2005; Feijoó *et al.*, 2014; Prater *et al.*, 2015). Studies observing deviations from homeostasis in macroinvertebrates (including phosphorous content) report a high variability in food resources following different nutrient concentrations in the water (DeMott, 2003; Bowman *et al.*, 2005; Small & Pringle, 2010). Water nutrient concentrations at our sampling sites were rather stable two months before sampling, so the basal resources should reflect differences in water nutrient concentrations over the long term.

Also season explained only a low part of stoichiometric variability. Divergent patterns when analyzing individual taxa underlines also here the taxon-specificity of stoichiometric responses.

Both the overall model (C:N) and the individual taxa responses (C, N) suggest that the effect of season is largest on carbon and nitrogen stoichiometry. This suggests an indirect effect due to changes in body size over the year as reasons for this stoichiometry change, but in most taxa there was no interaction between season and biomass.

Only in two taxa (*Baetis*, *G. fossarum*) the phosphorous stoichiometry was affected by season and decreased from May (September) to December. So even if the elemental composition changes with development *e.g via* increased investment in reproductive tissue as observed for some insects (Back *et al.*, 2008), this did generally not affect macroinvertebrate phosphorous stoichiometry in our study. Mehler *et al.* (2013) already showed that season helped explaining the variation in nitrogen content and C:N in some taxa, but not phosphorous stoichiometry. Liess & Hillebrand (2005) reported a higher importance of taxonomy and reported taxon-specific responses towards season. The general trend of highest C:N and lowest C:P and N:P in autumn observed by these authors could not be confirmed for taxa analysed in our study as generally, there was no clear trend within class or order in our study. The overall model indicated the lowest C:N value in September, as well as a (slightly) higher C:P and N:P in autumn and/ or winter

For example, for poly-voltin taxa that produce several generations per year, it might be possible with our sampling design that we caught individuals of similar sizes, missing different live stages. So that we did not cover any (possible) seasonal variation within this taxon. Conducting monthly field samplings might prevent this bias.

Confounding factors might cause or mask an effect of season: Basal resource quality, *e.g.* seston or periphyton could change over the year (Fink *et al.*, 2006; Veldboom & Haro, 2011) due to changes in nutrient availabilities or different light regimes. Seasonally altered periphyton quality however did not affect lake macroinvertebrate stoichiometry (Fink *et al.*, 2006).

Poly-voltinism of some taxa could mask an effect of season, when specimen of similar sizes but successive generations dominate the samples.

The effect of sex on stoichiometric traits

There were no significant differences in stoichiometry between male and females in most stoichiometric variables and taxa. Only in *G. roesli* and *Asellus* we observed significantly higher %P or higher C:N in females compared to males. While Back & King (2013) reported higher phosphorous content in females for some aquatic insects, to our knowledge there are no studies investigating crustaceans. However stoichiometric differences among tissues and especially a lower phosphorous content (and higher C:P) in eggs than juveniles as found for *Daphnia* and *Astacus* (Sterner & Schulz, 1998; Faerovig & Hessen, 2003), suggest a general difference between male and females, especially in mature individuals.

A larger analysis on the effect of sex on body stoichiometry in other macroinvertebrate classes would be interesting. In field campaigns, collected organisms are often not separated by sex due to the difficulties in identifying morphological characteristics in living/ intact organisms. Our results suggest that, at least for the analysed crustacean species, there is no worry regarding such bulk samplings.

Consequences for the use of stoichiometric databases

Considering that taxonomy played a major role in determining macroinvertebrate stoichiometry and that some variation (%P, C:N, C:P, N:P) mainly occurs on lower taxonomic levels, it is advised to use the highest taxonomic resolution possible in stoichiometric studies, both for data acquisition and for database use.

Phosphorous stoichiometry was unaffected in most taxa, suggesting that mean value databases probably can be used without worry. Most deviations from strict homeostasis was observed for carbon and nitrogen stoichiometry and is probably mainly driven by differences in body size. This problem might become negligible, when investigating very large datasets with the aim of detecting general patterns on a large-scale (*e.g.* MS 3). When the question is quantitatively precise and/or has a seasonal component (*i.e.* is likely to include individuals at different life stages and therewith of different body sizes), the use of mean taxon values might pose a problem. Several studies point out, that ontogeny should be taken into account when investigating macroinvertebrate growth and/ or nutrient release (Halvorson et al. 2019). Generally, databases fed by field sampling data probably comprise a range of body size classes of a taxon, which is subjected to the same bias – evidently depending on the sampling method – of underrepresenting small-bodied individuals as in community abundance data sets.

Conclusion

Our results support the findings of previous studies that taxonomy (*via* phylogeny) plays the major role in determining body stoichiometry. Deviations from homeostasis occurred mainly for carbon and nitrogen stoichoimetry (%C, %N, C:N) while most taxa showed a stable elemental composition

regarding phosphorous. Biomass affected stoichiometry in some taxa, suggesting an effect of changing nutrient allocation during the growth and/or development, for example in structural or reproductive tissue.

Only few taxa responded towards changing nutrient concentrations in the environment and most taxa were able to keep a stable elemental composition. Likewise, phosphorous stoichiometry remained unaffected by season in all but two/ most taxa. C:N ratio, carbon and nitrogen content were more concerned, although there was no linear seasonal trend.

Only in two taxa females and males differed in one stoichiometric variable (%P or N:P).

Deviations from strict homeostasis generally was taxon-specific, both regarding the presence of an effect as well as its direction or strength. Overall, these results suggest that most taxa keep a stable body stoichiometry under changing environmental conditions.

Supplementary material

2c.S1: Results of linear regressions applied to investigate the effect of biomass on macroinvertebrate stoichiometry for each larger taxonomic group. Provided are the intercept, the slope, adjusted r^2 and sample size (n). Bold lettering indicate significance of a model (α =0.05). Please note that models of stoichiometric ratios were applied on logit transformation of the inverse ratio (*i.e.* logit(N:C) instead of C:N) so that a positive slope in the results can has to be interpreted as a negative relationship of the untransformed stoichiometric variable, and vice versa.

Class	Stoichio	Intercept	Slope	r^2_{adj}	n
Bivalvia	%C	17.6804	0.2107	-0.0502	20
	%N	0.857	0.2185	0.0751	20
	%P	0.1376	-0.0054	-0.0446	18
	C:N	-2.7148	0.0881	0.1082	20
	C:P	-4.9348	-0.0825	-0.0239	18
	N:P	-2.0165	-0.1778	0.056	18
Gastropoda	%C	21.3195	0.6048	0.0192	56
	%N	**2.7399**	**0.2468**	0.0523	55
	%P	**0.1743**	**0.0499**	0.1099	57
	C:N	**-1.9369**	**0.0539**	0.059	56
	C:P	**-5.0988**	**0.2182**	0.1796	53
	N:P	**-2.881**	**0.1594**	0.085	53
Hirudinea	%C	46.8797	0.3659	-0.0155	41
	%N	11.4094	-0.1456	0.0021	43
	%P	**0.5202**	**0.0786**	0.1198	47
	C:N	-1.1332	-0.0273	0.0628	44
	C:P	-4.4062	0.0794	0.0161	43
	N:P	-2.9538	0.1061	0.0392	43
Insecta	**%C**	**45.4449**	**0.5436**	0.0206	445
	%N	**9.5731**	**-0.2456**	0.0385	405
	%P	**0.9901**	**-0.0722**	0.0682	503
	C:N	**-1.3224**	**-0.0529**	0.0847	488
	C:P	-3.9922	-0.0267	0.0021	406
	N:P	-2.2991	-0.0055	-0.0023	406
Malacostraca	%C	35.514	-0.2365	-0.002	155
	%N	7.0929	-0.1667	0.0048	142
	%P	**0.7694**	**-0.061**	0.0276	159
	C:N	-1.3967	-0.0276	0.0053	162
	C:P	-4.1293	0.0683	0.0156	142
	N:P	-2.4015	0.0823	0.0115	142
Oligochaeta	**%C**	**38.2641**	**-3.843**	0.464	23
	%N	**8.0427**	**-0.7426**	0.3938	23
	%P	**0.8693**	**-0.0834**	0.1632	25
	C:N	**-1.3254**	**0.0262**	0.1566	24
	C:P	-3.8651	0.0144	-0.0425	23
	N:P	-2.2109	-0.0083	-0.0463	23
Turbellaria	%C	48.0492	1.2811	0.0301	17
	%N	9.8716	-0.5736	0.0714	16

%P	1.0027	0.244	0.1426	18
C:N	-1.3518	-0.1126	0.1408	17
C:P	-3.8786	0.2043	0.0852	17
N:P	**-2.2087**	**0.3283**	0.2004	17

2c.S2: Results of linear regressions applied separately on most abundant taxa to investigate the effect of biomass on stoichiometry. For each stoichiometric variable (%C, %N, %P, C:N, C:P, N:P), the slope, adjusted r^2 and sample size (n) are indicated. Please note that models of stoichiometric ratios were applied on logit transformation of the inverse ratio (*i.e.* logit(N:C) instead of C:N) so that a positive slope in the results can be/ has to be interpreted as a negative relationship of the untransformed stoichiometric variable, and vice versa. Models for which $p < 0.05$ are printed in bold.

Taxon	Stoichio	Slope	Intercept	r^2_{adj}	n
Asellus	%C	-1.5878	35.6625	0.0263	35
	%N	0.2259	6.7853	-0.0165	34
	%P	-0.0868	0.7876	0.057	36
	C:N	**0.0982**	**-1.4556**	0.1795	37
	C:P	0.0554	-4.0061	-0.0159	35
	N:P	-0.0168	-2.2744	-0.0292	35
Atherix	%C	2.0331	41.4493	0.0933	12
	%N	-0.5295	10.5845	0.0638	12
	%P	-0.0054	1.06	-0.1	12
	C:N	-0.1225	-1.0837	0.1628	12
	C:P	0.0329	-3.911	-0.0988	12
	N:P	0.1332	-2.4489	-0.0856	12
Baetis	**%C**	**1.8839**	**46.505**	0.2157	38
	%N	**-1.3398**	**10.0798**	0.4571	37
	%P	**-0.462**	**1.1711**	0.5153	39
	C:N	**-0.2189**	**-1.2918**	0.5729	40
	C:P	-0.4788	-3.7402	0.0878	28
	N:P	-0.4303	-2.0164	0.0452	28
Dugesia	%C	1.4044	48.1031	-0.0076	12
	%N	-0.5695	9.7412	0.0316	12
	%P	0.1369	1.0912	-0.0128	13
	C:N	-0.1173	-1.3681	0.1093	12
	C:P	0.0542	-3.7532	-0.0855	12
	N:P	0.1701	-2.0579	-0.0047	12
Ecdyonurus	%C	-1.5157	51.7101	-0.0778	12
	%N	-1.2787	12.8317	0.1581	12
	%P	0.0211	0.8073	-0.0869	12
	C:N	**-0.1104**	**-1.0973**	0.5778	12
	C:P	0.0868	-4.2078	-0.0329	12
	N:P	0.1844	-2.7752	0.1443	12
Ephemera	**%C**	**4.2108**	**34.5015**	0.3015	14
	%N	0.9496	7.063	0.0342	14
	%P	0.1035	0.6489	0.0325	14
	C:N	0.0235	-1.3963	-0.0756	14

	C:P	0.0217	-3.9571	-0.0808	14
	N:P	-0.0001	-2.246	-0.0833	14
Ephemerella	**%C**	**1.1432**	**45.2251**	0.3046	22
	%N	0.529	8.7711	0.0784	23
	%P	0.0392	0.9318	-0.0192	26
	C:N	0.0437	-1.4324	0.0064	22
	C:P	-0.0528	-3.8115	-0.0211	22
	N:P	-0.0965	-2.0594	0.0148	22
Erpobdella	%C	0.2315	46.3309	-0.0306	24
	%N	-0.1192	11.3312	-0.0108	24
	%P	0.0612	0.6482	0.0076	24
	C:N	-0.02	-1.1295	0.0104	24
	C:P	0.1167	-4.4251	0.0052	24
	N:P	0.1376	-2.9702	0.0154	24
G. fossarum	%C	-0.2855	35.0549	-0.0075	57
	%N	-0.1192	7.0444	-0.0139	56
	%P	-0.0356	0.6697	0.001	56
	C:N	-0.0162	-1.3999	-0.0153	58
	C:P	-0.0005	-4.0944	-0.0222	47
	N:P	-0.0187	-2.3348	-0.0216	47
G. pulex	**%C**	**-2.3968**	**40.2358**	0.1149	31
	%N	-0.2262	6.7008	-0.0153	30
	%P	0.1131	0.3716	0.0482	31
	C:N	0.0371	-1.6201	-0.0254	31
	C:P	**0.3464**	**-4.8186**	0.2014	31
	N:P	0.3348	-2.9609	0.0907	31
G. roeseli	%C	-0.4007	35.5098	-0.0182	15
	%N	**-0.4011**	**6.4092**	0.5094	14
	%P	-0.0625	0.9498	0.0623	15
	C:N	**-0.067**	**-1.5173**	0.4653	15
	C:P	-0.062	-3.6238	0.0112	15
	N:P	-0.0044	-1.7751	-0.0766	15
Glossiphonia	%C	4.3073	40.3304	0.1248	13
	%N	0.6253	10.139	0.0281	13
	%P	-0.0012	0.584	-0.0714	16
	C:N	-0.0398	-1.1105	-0.021	14
	C:P	**-0.1567**	**-4.0342**	0.3413	13
	N:P	**-0.1329**	**-2.5814**	0.2569	13
Hydropsyche	**%C**	**3.46**	**40.2495**	0.3667	34
	%N	-0.1052	9.4502	-0.0269	34
	%P	-0.0297	0.9157	-0.0282	33
	C:N	**-0.1046**	**-1.2051**	0.2104	34
	C:P	0.0238	-4.1028	-0.0337	31
	N:P	0.0904	-2.5189	-0.026	31
Limnephilinae	**%C**	**2.3271**	**39.2357**	0.5226	18
	%N	0.2839	7.4059	0.0111	17

	%P	0.0389	0.6963	-0.0427	18
	C:N	-0.021	-1.4709	-0.0286	18
	C:P	0.0415	-4.2089	-0.0534	18
	N:P	0.0595	-2.4429	-0.0485	18
Radix	%C	0.2031	23.5992	-0.0372	28
	%N	0.077	3.6844	-0.0364	28
	%P	0.0323	0.2717	-0.0107	29
	C:N	0.0098	-1.7023	-0.0372	28
	C:P	0.1276	-4.6882	-0.0059	28
	N:P	0.1279	-2.7542	-0.0136	28
Rhyacophila	%C	0.7321	46.9109	0.027	23
	%N	**-1.1843**	**12.3911**	0.6415	23
	%P	-0.0445	0.8953	-0.0093	24
	C:N	**-0.1653**	**-1.0166**	0.657	23
	C:P	0.3248	-4.9631	0.1339	20
	N:P	**0.4892**	**-3.6602**	0.3132	20
Sericostoma	**%C**	**2.6641**	**42.994**	0.379	25
	%N	**-0.5348**	**8.9048**	0.2062	24
	%P	**0.2443**	**0.2983**	0.169	26
	C:N	**-0.1457**	**-1.3523**	0.3633	25
	C:P	**0.3499**	**-4.9493**	0.2122	21
	N:P	**0.4908**	**-3.2924**	0.2958	21
Simuliidae	**%C**	**4.4**	**45.6642**	0.1718	18
	%N	**1.4587**	**10.537**	0.1481	20
	%P	-0.2189	1.0187	0.1221	22
	C:N	**0.0719**	**-1.1996**	0.0002	20
	C:P	-0.286	-3.7791	0.0192	15
	N:P	-0.4084	-2.2057	0.0767	15
Stenophylacini-Chaetopterygini	%C	0.7273	42.9051	0.0877	28
	%N	-0.5303	8.4754	0.1735	28
	%P	-0.0316	1.0658	-0.0206	28
	C:N	-0.1063	-1.4119	0.3239	28
	C:P	0.0075	-3.8591	-0.043	25
	N:P	0.0947	-2.0896	0.0081	25

2c.S3: Results of linear regressions applied separately on most abundant taxa to investigate the effect of water N:P (and biomass) on stoichiometry. For each stoichiometric variable (%C, %N, %P, C:N, C:P, N:P), the intercept, slopes for water N:P (and biomass if it was integrated in the model), adjusted r^2 and sample size (n) is indicated. Factors for which $p < 0.05$ are printed in bold.

Taxon	Stoichio	Intercept	WaterNP	Biomass	adj.r2	n
Asellus	%C	28.6600	0.9268	NA	0.0856	34
	%N	5.5631	**0.2740**	NA	0.0942	33
	%P	0.4862	0.0349	NA	0.0565	32
	C:N	-1.6428	**0.0310**	**0.1122**	0.2624	34
	C:P	-4.0717	0.0288	NA	-0.0059	33

	N:P	-2.3727	0.0190	NA	-0.0224	33
Baetis	%C	53.5892	-1.4067	3.9705	0.0582	13
	%N	15.1584	-1.1577	0.1774	0.0818	13
	%P	1.1140	0.0247	-0.3078	0.0927	13
	C:N	-0.7132	-0.1323	-0.0805	0.4010	13
	C:P	-2.6894	-0.1817	NA	0.0012	13
	N:P	-1.7208	-0.0323	NA	-0.0884	13
Ephemera	**%C**	319.2043	**-48.1216**	-1.5138	0.6251	8
	%N	84.4707	**-13.4104**	NA	0.7348	8
	%P	2.8239	-0.3371	NA	-0.1490	8
	C:N	3.2527	-0.8233	NA	0.3159	8
	C:P	-6.5719	0.4786	NA	-0.1487	8
	N:P	-9.4323	1.2880	NA	-0.0569	8
Ephemerella	**%C**	38.8355	0.9366	**1.9643**	0.5278	17
	%N	8.2900	0.1491	NA	-0.0608	18
	%P	0.6514	0.0428	NA	-0.0430	17
	C:N	-1.6221	0.0371	NA	-0.0433	17
	C:P	-4.4726	0.0922	NA	0.0003	17
	N:P	-2.6196	0.0681	NA	-0.0393	17
G. fossarum	%C	35.7051	-0.1024	NA	-0.0711	16
	%N	4.4176	0.1854	NA	-0.0692	16
	%P	1.2440	-0.0916	NA	-0.0576	16
	C:N	-1.3276	-0.0691	NA	-0.0653	16
	C:P	-2.6659	-0.2265	NA	-0.0508	15
	N:P	-1.4927	-0.0765	NA	-0.0757	15
G. pulex	%C	35.9298	0.6812	-2.0992	0.0948	31
	%N	4.3476	**0.3840**	-0.1203	0.1804	31
	%P	0.7684	-0.0322	NA	-0.0078	30
	C:N	-1.6549	0.0088	NA	-0.0334	31
	C:P	-4.1399	-0.1075	**0.3005**	0.2284	31
	N:P	-1.8643	-0.0724	NA	-0.0185	31
Hydropsyche	**%C**	47.6519	-1.6561	**4.3112**	0.6118	11
	%N	14.3405	-0.9909	NA	0.0480	10
	%P	-1.0693	0.3248	NA	0.0046	11
	C:N	-0.6738	-0.1034	-0.1071	0.4150	11
	C:P	-7.9185	0.6452	NA	0.0222	10
	N:P	-7.0757	0.8042	NA	0.0488	10
N. punctatolineatus	**%C**	-30.6703	**11.1104**	NA	0.7446	8
	%N	-30.4934	**5.6575**	NA	0.7885	8
	%P	5.9912	**-0.7566**	NA	0.8225	8
	C:N	-6.0749	**0.6659**	NA	0.6732	8
	C:P	3.2950	**-1.0679**	NA	0.8827	8
	N:P	10.6759	**-1.8821**	NA	0.8903	8
O. albicorne	%C	10.3029	6.0837	NA	0.3594	6
	%N	4.6805	0.5918	NA	0.1678	6
	%P	1.6936	-0.1395	NA	0.1678	6

	C:N	-1.0863	-0.0750	NA	-0.0031	6
	C:P	-2.1151	-0.3126	NA	0.2869	6
	N:P	-0.5264	-0.2732	NA	0.3760	6
Radix	%C	22.9371	0.3674	NA	-0.0317	18
	%N	3.4874	0.1115	NA	-0.0275	18
	%P	0.4253	0.0022	NA	-0.0620	18
	C:N	-1.7112	0.0117	NA	-0.0461	18
	C:P	-3.8721	-0.0402	NA	-0.0396	18
	N:P	-1.8723	-0.0541	NA	-0.0274	18
Rhyacophila	%C	41.0725	1.3901	NA	0.1971	10
	%N	14.9948	-0.6589	-0.6929	0.2496	10
	%P	2.9255	**-0.3488**	NA	0.3606	10
	C:N	-0.5276	-0.1241	-0.0772	0.3602	10
	C:P	-0.9695	-0.5227	NA	0.2893	9
	N:P	-1.7103	-0.5388	0.9576	0.4972	9
Simuliidae	**%C**	39.9107	**3.0067**	**14.8268**	0.4200	11
	%N	10.4341	-0.3453	NA	0.1588	12
	%P	1.7284	-0.0938	NA	0.1416	12
	C:N	-1.0807	**-0.0466**	NA	0.8269	12
	C:P	-3.0531	-0.0845	NA	0.0900	12
	N:P	-1.5372	-0.0518	NA	-0.0365	12
Stenophylacini-Chaetopterygini	%C	27.5379	2.7351	NA	0.0775	13
	%N	5.3221	0.1913	-0.1992	-0.0436	13
	%P	0.5463	0.0697	NA	-0.0753	13
	C:N	-1.8593	0.0458	**-0.0976**	0.4177	13
	C:P	-3.9923	0.0287	NA	-0.0891	13
	N:P	-2.7738	0.1839	NA	-0.0307	13

2c.S4: Mean body stoichiometry of male and female crustacean taxa. Significant differences ($p < 0.05$) between male and females are written in bold. "n" indicates sample size. Mean and standard deviation are calculated as geometrically, so please remember that the values are described by mean ×/÷ sd.

		%C		%N		%P		C:N		C:P		N:P	
Taxon	Sex	mean(sd)	n	mean(sd)	n	mean(sd)	n	mean(sd)	n	mean(sd)	n	mean(sd)	n
Asellus	♀	33.07(1.16)	11	6.53(1.26)	12	0.59(1.42)	12	**5.91(1.11)**	12	147.04(1.41)	11	25.26(1.43)	11
	♂	31.04(1.09)	10	6.65(1.14)	10	0.66(1.24)	10	**5.29(1.12)**	11	121.81(1.2)	10	22.35(1.16)	10
Dikero-	♀	34.89(1.06)	3	6.71(1.02)	3	0.68(1.12)	3	6.07(1.06)	3	131.93(1.169)	3	21.73(1.11)	3
gammarus	♂	36.11(1.04)	3	7.39(1.19)	3	0.62(1.12)	3	5.7(1.17)	3	150.08(1.09)	3	26.35(1.09)	3
G. fossarum	♀	35.53(1.08)	6	6.01(1.35)	6	0.83(1.24)	6	6.9(1.35)	6	110.4(1.23)	6	16.01(1.4)	6
	♂	33.3(1.07)	11	5.46(1.2)	11	0.69(1.49)	11	7.12(1.28)	11	122.91(1.48)	10	17.67(1.71)	10
G. pulex	♀	37.07(1.11)	15	6.2(1.19)	15	0.55(1.36)	15	6.98(1.2)	15	174.46(1.38)	15	25.01(1.54)	15
	♂	34.86(1.1)	14	6.41(1.15)	14	0.48(1.63)	14	6.34(1.24)	14	188.04(1.63)	14	29.65(1.75)	14
G. roeseli	♀	35.47(1.07)	7	5.42(1.09)	7	0.86(1.2)	7	7.67(1.07)	7	106.82(1.2)	7	**13.99(1.24)**	7
	♂	33.16(1.02)	4	5.39(1.07)	4	0.61(1.37)	4	7.18(1.06)	4	141.45(1.37)	4	**19.71(1.41)**	4

Chapter III:

Macroinvertebrate stoichiometry and functional traits

Towards understanding the functional role of invertebrates: lessons from linking bio/ecological to stoichiometric traits in stream macroinvertebrates

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Abstract

Aim: Investigating the general link between stoichiometric and other functional traits and testing predictions from trait-based and ecological stoichiometry theory. Ecologists rely on various biological and habitat-related traits when it comes to understanding the functioning of ecological systems and its responses to global changes. Increasing nutrient levels are a global stressor in many aquatic systems. They can affect taxa with contrasted functional traits in various ways, for example favoring small, fast-growing species under high phosphorous conditions. Considering stoichiometric traits, describing the elemental composition of organism body tissues, can help to understand the mechanisms behind such functional shifts, as some works have already demonstrated a link between body (phosphorus) stoichiometry and (mainly) life history traits (*e.g.* the Growth Rate Hypothesis).

Major taxa studied: Freshwater macroinvertebrates

Methods: We applied a series of multivariate analyses (co-inertia analyses) on six stoichiometric traits (%C, %N, %P, C:N, C:P, N:P) and 23 of their biological and habitat-related traits.

Results: We found significant co-structures between both types of traits, when analyzing single traits as well as all the traits together. Patterns found within traits related to organism development or nutrient cycling were in line with our assumptions based on concepts developed within the ecological stoichiometry framework: *e.g.* traits describing predators were associated with high %N, traits suggesting a fast development (small maximum body size, high molting frequency) with high %P. Associations between habitat-related traits and body stoichiometry could be explained by the longitudinal stream gradient: taxa preferring headwater habitats (*i.e.* high altitude, coarse substrate material, cold temperature) were rich in %N and %P.

Main conclusions: Demonstrating the link between stoichiometric and functional traits underlines the potential of integrating stoichiometric traits into the ecological analysis of ecosystems to improve our understanding of taxonomic and functional responses of communities – and then ecosystems – to changing nutrient conditions worldwide.

1. Introduction

Ecologists describe communities using two different ways: taxonomic units as descriptors of assemblages or traits intended to be proxies of functions. While the first approach gives a better picture of phylogenetic signals, the latter one can provide a functional view relating species distributions to environmental factors up to the ecosystem scale (McGill *et al.*, 2006; Cadotte *et al.*, 2011). Functional traits encompass morphological, physiological or phenological characteristics of an organism. They impact the organism fitness and influence its interactions with the environment and other organisms (Violle *et al.*, 2007). Since taxa of distinct taxonomic lineages may express more similar traits than closer related ones (Usseglio-Polatera *et al.*, 2000; Pilière *et al.*, 2016), taxonomic and functional diversity can provide distinct – sometimes divergent – but complementary information (Crabot *et al.*, 2020, 2021).

Especially when it comes to studying ecosystem functioning or ecosystem responses to (global) stressors, investigating changes in both taxonomic and trait composition becomes more interesting rather than taxonomic changes alone. Since species have to find a trade-off between their ecological demands and tolerances – closely related to key life history traits – and the abiotic/biotic features of habitats, community trait composition is closely depending on environmental parameters (Göthe *et al.*, 2017). Further, the trait structure of assemblages is far less constrained by biogeography than their taxonomic structure because trait-based functions persist within and among regions even when species pools are changing (Charvet *et al.*, 2000; Statzner *et al.*, 2001; Archaimbault *et al.*, 2005). This enables addressing also large spatial scales. Worldwide, freshwater environments have been subjected to changing nutrient levels. On one hand, increasing nitrogen and phosphorous concentrations due to anthropogenic activities can act as major stressors of biotic communities in these systems (Falkowski *et al.*, 2000; Peñuelas *et al.*, 2013; Yan *et al.*, 2016). On the other hand, management measures can successfully decrease phosphorous load but often increase water N:P ratio due to only marginal changes in nitrogen (Jeppesen *et al.*, 2005; Floury *et al.*, 2013; Latli *et al.*, 2017). Nutrients act indirectly on macroinvertebrate communities *via* shifts in resource quantity and quality and changes in habitats (*e.g.* driving macrophyte development; Rasmussen & Anderson, 2005). Although these effects are secondary compared to physical habitat conditions (Richards *et al.*, 1993; Genito *et al.*, 2002; Friberg *et al.*, 2009; Wagenhoff *et al.*, 2012; Juvigny-Khenafou *et al.*, 2021), effects of nutrient changes may directly be reflected by several structural properties of macroinvertebrate communities: slight to moderate inputs of nitrogen and/or phosphorous increase taxa richness, diversity and biomass (Rader & Richardson, 1994; Azevêdo *et al.*, 2015; Wang *et al.*, 2021). These positive effects of nutrient enrichment turn into negative ones when optimal conditions are exceeded (King & Richardson, 2007), as described by the Intermediate Disturbance Hypothesis (Connell, 1978). Thus, large nutrient inputs can decrease taxon richness (Ortiz & Puig, 2007; Yuan, 2010; Wagenhoff *et al.*, 2012; Piggott *et al.*, 2015), taxonomic (Evans-White & Lamberti, 2009; Friberg *et al.*, 2009; Zhang *et al.*, 2019) as well as functional diversity (Zhang *et al.*,

2019). Taxonomic groups thereby differ in their responses (King & Richardson, 2007; Juvigny-Khenafou *et al.*, 2021) and the occurrence of sensitive taxa especially depends on nutrient concentrations (Ortiz & Puig, 2007; Wagenhoff *et al.*, 2012).

Functional trait groups are affected by nutrient enrichment to a similar degree than individual taxa (Lecerf *et al.*, 2006; Wagenhoff *et al.*, 2012) with certain traits being favoured under high nutrient conditions. Manipulative and observational studies have reported increasing relative abundances of small, fast-growing, short-lived, fast-reproducing taxa under high nutrient conditions (Ortiz & Puig, 2007; Singer & Battin, 2007; Statzner & Bêche, 2010; Prater *et al.*, 2015). Additionally, taxa differing in life history traits or feeding types seem to be differently affected by nutrient enrichment (Cross *et al.*, 2005b, 2006; Townsend *et al.*, 2008; Evans-White & Lamberti, 2009). For example, while short-living chironomids increased growth rates and production under high nutrient conditioned, a longer-lived species remained unaffected.

Including stoichiometric traits as a third set of metrics, besides traditional functional traits and taxonomic groups, might help to understand the mechanism behind the previous observations. The framework of ecological stoichiometry investigates the link between environmental nutrient concentrations and the nutrient content in organisms' body tissues in a given ecological context (Sterner & Elser, 2002). Assuming that the latter partly reflects the organisms' demand for the corresponding nutrient, it can help to explain species distribution over a pollution gradient (Dang *et al.*, 2009) or species dominances in an ecosystem (Schade *et al.*, 2005). One well-known example for a link between stoichiometric and functional traits is the Growth Rate Hypothesis (= GRH; Elser *et al.*, 1996), which explains the increasing body P-content with increasing growth rate by the increased demand for P-rich rRNA necessary to achieve fast growth. *Vice versa*, slow growing taxa have a lower P-demand, and thus a lower P-content (Elser *et al.*, 2003b; Acharya *et al.*, 2004). For other traits such as feeding type (Fagan *et al.*, 2002; González *et al.*, 2017), body size (Liess & Hillebrand, 2005; Meunier *et al.*, 2016) or development rate (Beck *et al.*, 2021), differences in stoichiometry between taxa expressing different trait adaptations have also been identified. Linking such growth-related assumptions to nutrient concentrations in the environment, Singer & Battin (2007) and Prater *et al.* (2015) have shown that the shift towards small, fast-growing taxa under high nutrient conditions goes along with increasing abundances of P-rich taxa. This indicates the role that stoichiometry could play in understanding the response to nutrient enrichment in community composition and functions.

To our knowledge, there is so far no study that has explicitly linked stoichiometric traits to functional traits and analysed their relationships. We therefore aim to investigate the general link between functional and stoichiometric traits, by applying a multivariate approach. We focus on freshwater benthic macroinvertebrates, a group widely used for biomonitoring and for which detailed information

on their traits is available. Besides biological traits referring to characteristics of the organism itself, we also included habitat-related (or ecological) traits that describe habitat preferences of taxa.

We hypothesize that most functional traits should be related to body stoichiometry (Tab. 3.1). Functional traits that are directly linked to nutrient cycling (feeding type, preferred food resources) or organism growth rate and life history (maximum size, voltinism, life duration, number of reproductive events or number of molting events per individual) should exhibit the strongest associations with stoichiometric traits. The habitat-related traits should be indirectly related to organism body stoichiometry. Substrate preference for example is linked to resource preference and therewith also to feeding mode. The association observed for substrate preference should thus be in line with those found in the nutrient-linked traits. We additionally expect variation in the pattern of stoichiometric/habitat-related traits relationships along the longitudinal gradient of rivers, as categories describing habitat-related traits should be used differently along the river continuum (*e.g.* temperature, flow velocity, substrate). Due to the linkages between some functional traits, we expect the stoichiometric responses of such traits to be linked to one another (*e.g*. feeding type and food resource, substrate and locomotion, feeding type and locomotion or substrate attachment). Precise hypotheses on the expected associations between stoichiometric traits and biological or habitat-related traits are provided in Table 3.1.

For some functional traits we do not list clear *a priori* hypotheses on associations to stoichiometric traits due to complex relationships between trait expressions, for example the favouring of a trait under either high or low nutrient levels depending on its combination with other traits. For other traits, we suspect an important role of phylogeny due to general differences among major invertebrate lineages (*e.g.* aquatic life stage, dispersal, resistance form, respiration). Nevertheless, we decided to include both groups of traits in the analysis as they are part of the organisms' trait profile and such an analysis can reveal some interesting relationships with stoichiometric traits.

Tab. 3.1: Expected associations between functional and stoichiometric traits (a detailed description of the traits and all trait categories are provided in tab. 3.2).

Functional trait	Hypotheses
Biological	
Development	
Maximum body size	Small maximum body sizes → fast growth rates and thus high body %P and low C:P & N:P (GRH, Elser *et al.*, 1996; Main *et al.*, 1997; Carrillo *et al.*, 2001; Meunier *et al.*, 2016)
Life duration, Molting events, Voltinism	Trait categories indicating fast development (short life duration, several generations per year, high frequency (*i.e*. also number) of molting events) → high body %P, low C:P & N:P due to fast growth (*cf.* "max. body size" above, Beck *et al.*, 2021) and increased P demand during molts (Villar-Argaiz *et al.*, 2020)
Feeding	
Feeding habits, Food resource	Linked to nutritional quality of the resource: Nutrient-rich (in)vertebrates, consumed by swallower/chewers → high body %N & %P, low C:P & N:P; Living plant-based resources (macroalgae, microphytes) of intermediate quality, consumed by filter-feeders or scrapers → intermediate values of body %N & %P Nutrient-poor resources (detritus, dead plants or animals), consumed by deposit-feeders & shredders → low body %N & %P, high C:P % N:P (Cross *et al.*, 2003; Evans-White *et al.*, 2005; Lauridsen *et al.*, 2012)
Others	
Locomotion	Linked to energy and muscle investment: Swimmer → high body %N due to investment in muscle tissue (Stjernholm & Karlsson, 2008; González *et al.*, 2018) to perform swimming; Attached → low %N because no high muscle demand for locomotion
Habitat-related	
Altitude, Longitudinal distribution	Linked to substrate and resource preferences: Alpine & upstream river sections with dominating leaf litter/detritus and boulders/flags (following RCC; Vannote *et al.*, 1980) → high body C:P & N:P; Lowland & metapotamon/estuary with high quantities of mud/sand and macroalgae/microphytes (following RCC) & larger anthropogenic nutrient inputs (Paisley *et al.*, 2011; Aguilera *et al.*, 2012) → high body %N & %P due to nutrient-richer resources (*cf*. "Feeding habits")
Substrate	Linked to nutritional quality of the substrate (*cf.* "Feeding habits"): Detritus/ litter → low body %N & %P, high C:P % N:P due to low nutritional quality; Macro-/microphytes → higher body %N & %P due to higher nutritional quality
Temperature	Linked to metabolism: Higher energy demand (due to C loss from increased respiration) under warmer temperatures and preferences for carbon-rich resources (Hamburger & Dall, 1990; Boersma *et al.*, 2016) → low body C:N, C:P ratios Probably counteracting with: faster growth/development rates under warmer temperature (Levinton, 1983; Hoegh-Guldberg & Pearse, 1995; Nilsson-Örtman *et al.*, 2012) → high %P (Janssens *et al.*, 2015; cf. "development-related traits")
Trophic status	Eutrophic environments → high body %N & %P due to increased food quality of basal resources under high nutrient conditions (Cross *et al.*, 2003; Danger *et al.*, 2016; Demi *et al.*, 2019)
Velocity	Linked to mobility (*cf.* "locomotion"): Fast → high body %N due to more active mobility which requires N-rich muscle tissue; Slow/null → low body %N due to lower proportion of muscles

2. Material & methods

2.1. Data sets

Stoichiometric trait information was obtained from a database comprising 259 macroinvertebrate taxa and the elemental mass content of %C, %N, %P as well as the corresponding molar ratios (C:N, C:P, N:P) (Beck *et al.*, in press (MS 1)). For this study, only taxa at family level or lower have been considered for the taxonomic groups Bivalvia, Gastropoda, Hirudinea, Insecta, Malacostraca and Turbellaria. Oligochaeta have been considered as such.

Functional trait information was obtained from a database comprising information about >400 macroinvertebrate taxa and their biological and habitat-related traits (updated version of Tachet *et al.*, 2010). The traits "number of reproductive cycles per individual" and "number of molting events" were taken from Beck *et al.* (2021) and coded for this study, respectively. The trait profiles have been described *via* fuzzy coding: each trait is represented by several trait categories and for each category the affinity of a taxon is given by a score ranging from 0 (= no affinity) to 5 (= high affinity) (Chevenet *et al.*, 1994). The trait profiles of taxa thereby are taking into account intra-taxon variation and describe the variety of attributes potentially exhibited by a given taxon (*i.e.* by the successive aquatic instars during development). When only a very low proportion of rare taxa used a trait category, it was merged with the more biologically – or ecologically – similar category of the same trait, by summing their respective relative frequency values. As an example, the two extreme categories at both ends of the gradient of "maximum body size" (*i.e.* "< 0.25 cm" and "> 8 cm") were merged with their nearest categories (*i.e.* "≥ 0.25-0.50 cm" and "≥ 4-8 cm", respectively), into the new categories "≤ 0.5cm" and "> 4cm". All these modifications of trait categories are provided in the supplementary material (S 3.1).

For the analysis, the relative frequencies of trait categories have been calculated for each trait and each taxon, based on these affinity scores. In case of missing trait information due to differences in taxonomic resolution between the two databases, the trait information of the nearest higher taxonomic level (up to family level) was attributed when available. In total, we analysed 13 biological and 10 habitat-related traits (Tab. 3.2).

Tab. 3.2: Functional traits and their corresponding trait categories.

Type	Functional trait	Categories
Biological		
Development	Life duration	≤ 1 year, >1 year
	Maximum body size	≤0.5cm, >0.5-1cm, >1-2cm, >2-4cm, >4cm
	Molting events	0, 1, 2-4, 5-9, 10-14, 15-19, >20 (= number of molting events)
	Reproductive cycles	1, 2, >2-6, >6 (= number of reproductive cycles per individual)
	Voltinism	<1, 1, >1 (= number of generations per year)
Feeding	Feeding habits	Deposit-feeder, filter-feeder, piercer, parasite, scraper, shredder, swallowers/chewers
	Food resources	dead animal (≥1mm), dead plant (≥1mm), detritus (<1mm), living macrophytes, living microphytes, living microinvertebrates, (in)vertebrates
Others	Aquatic life stages	egg, larva, nymph, adult
	Dispersal	aquatic-active, aquatic-passive, aerial-active, aerial-passive
	Locomotion	attached, burrower, crawler, interstitial, swimmer
	Reproduction mode	ovoviviparity, isolated eggs (free), isolated eggs (cemented), clutches (cemented/fixed), clutches (free), clutches (in vegetation), clutches (terrestrial), asexual reproduction
	Resistance forms	eggs/statoblasts, housing/cocoons, diapause/dormancy, none
	Respiration	tegument, gill, plastron/spiracle
Habitat-related	Altitude	lowlands, piedmont, alpine
	Longitudinal distribution	crenon, epirithron, metarithron, hyporithron, epipotamon, metapotamon, estuary, outside river
	Low pH tolerance	≤4, >4-4.5, >4.5-5, >5-5.5, >5.5-6, >6
	Salinity tolerance	brackish water, freshwater
	Saprobity	xenosaprobic, oligosaprobic, β-mesosaprobic, α-mesosaprobic, polysaprobic
	Substrate	detritus/litter, flags/boulders/cobbles/pebbles, gravel, macrophytes, microphytes, mud, sand, silt, twigs/roots
	Temperature	eurythermic, psychrophilic, thermophilic
	Transversal distribution	banks/connected side-arms, lakes, marshes/peat bogs, ponds/pools/disconnected side-arms, river channel, temporary waters
	Trophic status	eutrophic, mesotrophic, oligotrophic
	Velocity	null, slow, medium, fast

2.2. Data analysis

The co-structure between the functional trait profiles of taxa and their stoichiometric profiles was investigated *via* co-inertia analysis (Dolédec & Chessel, 1994). This multivariate approach identifies the common structure in two tables, both describing the same set of individuals (taxa in this study). The co-inertia analysis thereby follows a simple ordination analysis applied to each set of variables (stoichiometric and bio-/ecological traits) separately. Since both tables are analysed simultaneously as a symmetric canonical ordination technique, neither of them is treated as a table of explanatory or response variables. This is important since it is not possible to make statements about the direction of

causation, *i.e*. whether a certain stoichiometric profile causes the expression of certain bio-/ecological traits, or whether these other traits cause the observed body stoichiometry. A RV-coefficient is calculated as the sum of eigenvalues of the co-inertia analysis divided by the square root of the product of the squared total inertia from the individual analyses. It varies within the [0-1] range, with a high RV-value indicating a high degree of co-structure.

Co-inertia analyses (CoI) were conducted (1) separately for each functional trait or (2) considering the "complete trait profile" taking into account simultaneously all the biological and habitat-related traits. The procedure was the same in both approaches. Prior to co-inertia analysis, a FCA (= Fuzzy Correspondence Analysis, Chevenet et al. 1994) was applied to the functional trait profile array. Stoichiometric data were analysed by normalized PCA. The stoichiometric variables were scaled before the analysis to avoid a bias due to large variances when variables are measured in different units. To correct for the characteristics of ratio data that can lead to biased results, the stoichiometric ratios (C:N, C:P, N:P) were log-transformed before manipulation as advised by Isles (2020). Mass element stoichiometry was used without prior transformation. A co-inertia analysis was applied on the results of each couple of unconstrained ordinations. The significance of RV-values, *i.e.* of the co-structures, was tested *via* permutation tests (9999 permutations). Since the stoichiometric trait information was used in all the separate analyses as well as in the analysis of the complete trait profiles, the p-values were corrected for multiple testing with the Bonferroni method.

Due to requirements of the statistical methods applied, only taxa with complete information (both bio-/ecological and stoichiometric traits) were used in the analyses, leading to slightly varying taxa lists used in the different analyses (n = 160-224 for the CoI analyses of single functional traits, n = 131 for the CoI analysis of the complete set of functional traits).

All analyses were performed using R software (R Development Core Team, 2019, version 3.5.2) and t he package ade4 (Dray & Dufour, 2007).

3. Results

Single traits

Of the 23 traits, 17 showed significant co-structure between functional and stoichiometric traits, with RV-coefficients ranging between 0.25 in the trait describing salinity tolerance and 0.07 in the temperature preference (Tab. 3.3). The largest part of co-structure was projected on the first axis (66.15 – 98.83% for traits with at least three trait categories *i.e.* allowing for at least two axes); the second axis explaining between 1.17 and 32.26% of the co-structure.

Tab. 3.3: Results of the co-inertia analyses between individual functional traits and stoichiometric profiles (%C, %N, %P, C:N, C:P, N:P) of macroinvertebrates. The RV-coefficient indicates the level of co-structure between the two sets of traits, with bold lettering indicating a significance at $\alpha = 0.05$ (p-values were adjusted for multiple testing after Bonferroni correction). n_{mod} indicates the number of trait-modalities, n_{taxa} indicates the number of taxa included in the corresponding analysis. Additionally, the proportion of projected inertia on the first two axes is given. Traits marked with an exposed "x" correspond to traits for which we had no clear *a priori* hypotheses or expected a strong effect of phylogeny and whose detailed results can be found in the supplementary material.

					Projected inertia (%)	
Type	Functional trait	RV	n_{mod}	n_{taxa}	Axis 1	Axis 2
Biological						
Development	Life duration	**0.0816**	2	224	100.00	NA
	Maximum body size	**0.0968**	5	160	88.98	10.70
	Molting events	**0.1606**	7	223	90.52	6.82
	Reproductive cycles [x]	0.0583	4	182	95.53	4.08
	Voltinism	**0.0694**	3	224	93.55	6.45
Feeding	Feeding habits	**0.1416**	7	224	70.81	26.66
	Food resources	**0.1508**	7	224	79.82	19.20
Others	Aquatic life stages [x]	**0.2504**	4	224	94.75	5.20
	Dispersal [x]	**0.1600**	4	224	85.20	14.45
	Locomotion	0.0198	5	224	66.90	29.89
	Reproduction mode [x]	**0.0980**	8	224	78.79	13.95
	Resistance forms [x]	0.0495	4	220	66.95	32.04
	Respiration [x]	0.0324	3	224	91.94	8.06
Habitat	Altitude	**0.0804**	3	224	98.83	1.17
	Longitudinal distribution	**0.1104**	8	224	66.15	32.26
	Low pH tolerance [x]	0.0234	6	220	84.50	14.85
	Salinity tolerance [x]	**0.2508**	2	224	100.00	NA
	Saprobity [x]	**0.0747**	5	219	96.57	2.73
	Substrate	**0.0745**	9	224	86.42	8.05
	Temperature	**0.0715**	3	221	85.93	14.07
	Transversal distribution [x]	**0.0941**	6	224	78.74	19.58
	Trophic status	0.0200	3	223	96.86	3.14
	Velocity	**0.1024**	4	224	96.53	3.33

Development-related traits

Life duration

Having a life duration of ≤ 1 year was associated to high %P and C:N, all being negatively related to the first axis (not shown). A life duration of > 1 year was positively related to the first axis as were the other stoichiometric traits, with N:P and C:P showing the most positive coordinates.

Maximum body size

%P was negatively associated with the first axis and positively related to small and intermediate maximum sizes (0.5-1cm, 1-2cm)(Fig. 3.1). C:P and N:P were positively related to the first axis, such were taxa of the two extreme categories with largest (> 4cm) and smallest (< 0.5cm) maximum size. %C and C:N were strongly and positively associated with the second axis, and thus negatively associated with taxa with intermediate (2-4cm) and the largest maximum sizes (> 4cm) (F2 < 0).

Molting events

The first axis clearly separated %C, %N and %P from the molar ratios. The trait categories of no molting and one molting event were strongly and positively associated with C:P. The trait-categories 2-4 and 10-14 molting events were co-varying with %N, while taxa potentially passing 15-19 or >20 molting events were positively related to %P and %C. C:N and the trait modality of 5-9 molting events both were negatively related to the second axis.

Voltinism

The first axis mainly separated % N and N:P (F1 > 0) from C:N ratio (F1 < 0), as well as semivoltin taxa (<1 generation per year) from those with fast development (>1 generation per year). Taxa potentially expressing the intermediate trait category of 1 generation per year (*i.e.* monovoltin) were negatively related to the second axis, as well as those with high C:P ratio and %P.

Feeding-traits

Feeding habits

Filter-feeders exhibited the strongest response and were – together with scrapers – positively related to the first axis (76.60% of total inertia), both being positioned between high C:N and high C:P. Swallowers/chewers were varying in the opposite direction, as were %C and %N (F1 < 0). Deposit-feeders and shredders were positively varying along the second axis (21.55%), together with %P. Taxa with high C:P and N:P were positioned opposite of %P (F2 < 0), and thus co-varied with piercers or parasites.

Food resources

The first axis (78.51% of total inertia) mainly separated %C and %N (F1 < 0) from C:N (F1 > 0), the second axis %P (F2 < 0) from C:P and N:P ratio (F2 > 0), still projecting 20.05% of the total inertia. C:N was associated to taxa feeding on dead animal, while %C and %N were strongly positively co-varying with taxa using living macroinvertebrates as resources. Taxa feeding on living microinvertebrates were strongly related to the second axis (F2 > 0), opposing %P. The trait categories “living macrophytes”, “dead plant” and “detritus” were slightly varying in this direction, thus positioned between taxa with high C:N or high %P.

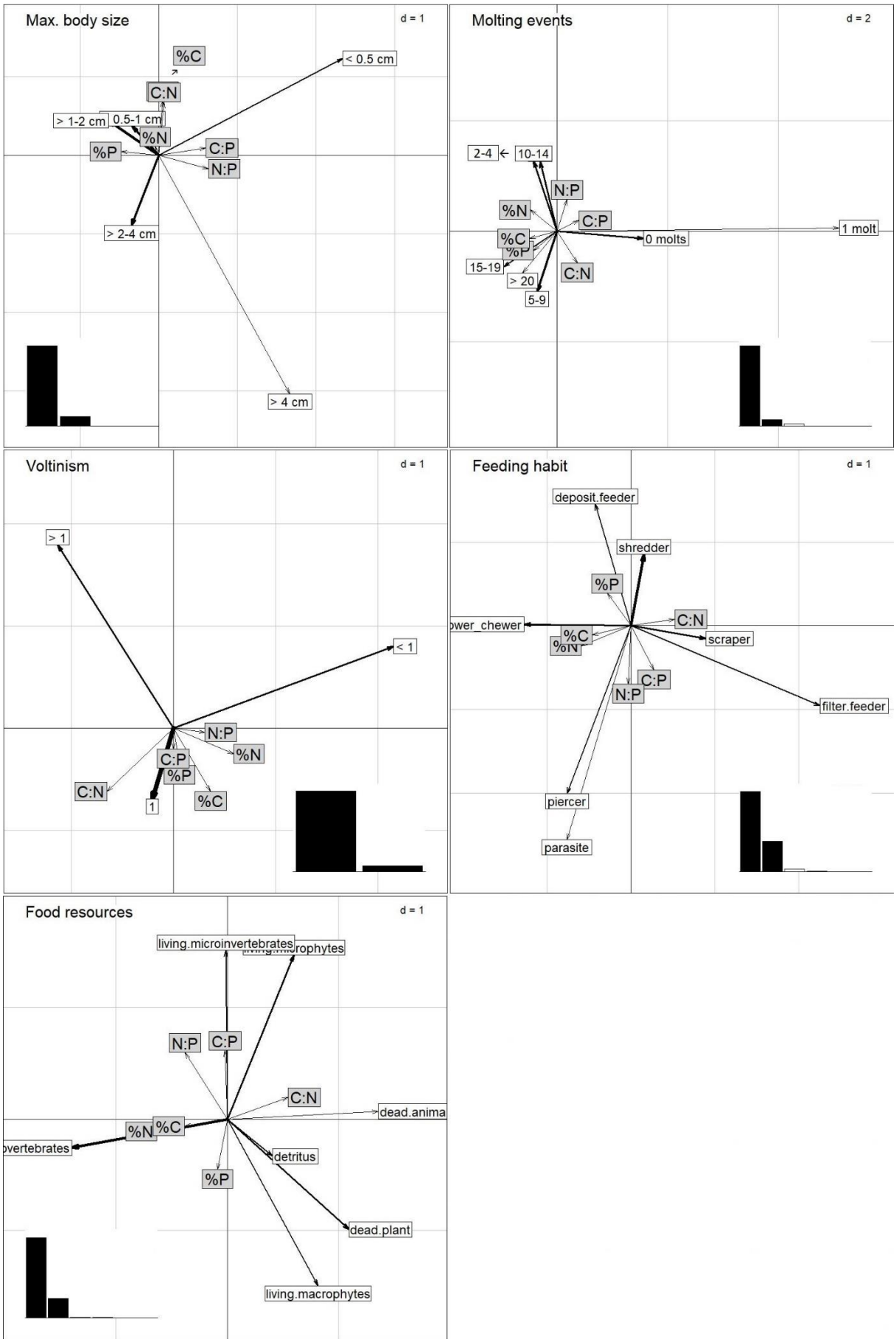

Max. body size
d = 1
%C
< 0.5 cm
C:N
> 1-2 cm
0.5-1 cm
%N
%P
C:P
N:P
> 2-4 cm
> 4 cm
Molting events
d = 2
2-4
10-14
N:P
%N
C:P
%C
%P
0 molts
1 molt
15-19
> 20
C:N
5-9
Voltinism
d = 1
> 1
< 1
N:P
%N
C:P
%P
C:N
1
%C
Feeding habit
d = 1
deposit.feeder
shredder
%P
C:N
%C
scraper
C:P
N:P
filter.feeder
piercer
parasite
Food resources
d = 1
living.microinvertebrates
N:P
C:P
C:N
dead.anima
%N
%C
detritus
%P
dead.plant
living.macrophytes

Fig. 3.1: Results from co-inertia analyses on single biological traits. The functional trait categories and stoichiometric traits are located on the first co-inertia plane. The small histogram illustrates the eigenvalues of the first (two to five) co-inertia axes and indicates the proportion of co-variation that is projected on these axes. The thickness of arrows pointing to functional traits corresponds to trait categories most used by macroinvertebrates. For the traits "life duration" described by only two categories no graphical representation is provided as the whole inertia was projected on a single axis.

Habitat-related traits

Altitude

More than 98% of the co-structure was explained by the first co-inertia axis, separating the stoichiometric elemental mass contents (mainly %P; F1 < 0) from molar ratios (mainly N:P and C:P; F1 > 0)(Fig. 3.2). The affinity for alpine environments was associated to high %N and %P, that for piedmont habitats to high %C. At the opposite side of the first axis, C:P and N:P were co-varying with the preference for lowland habitats.

Longitudinal distribution

%P varied together with the headwater habitats from springs (crenon) to metarithron along the first axis (F1 < 0; 68.30% of total inertia). The second axis still explained a large part of co-inertia (30.87%) and mainly separated %C and %N (F2 < 0) from C:N (F2 > 0). The latter was related to the lower sections of streams (epipotamon and metapotamon) and estuarine habitats. Taxa with high C:P and N:P were associated with water bodies disconnected from the river (*e.g.* ponds, peat bogs, temporary waters). The epi- and hypo-rithral habitats were related to taxa exhibiting high %P and %C.

Substrate

The first axis separated the stoichiometric mass elements (F1 < 0) from molar ratios (F1 > 0) while the second axis separated the molar ratios C:N from C:P and N:P. Both C:P and N:P were associated with a preference for habitats "mud" and "macrophytes" (F2 < 0), opposing taxa with high C:N and a preference for "twigs/roots" (F2 > 0). %C and %N were positively related to "sand", while %P varied in the same direction as "detritus/litter".

Temperature

With negative coordinates along the first axis, eurythermic taxa were associated to C:P and N:P (F2 > 0) and thermophilic taxa to C:N (F2 < 0). Psychrophilic taxa and those rich in %C, %N and %P were positively associated along the first axis, but the trait categories were not strongly co-varying.

Velocity

Taxa preferring standing waters (category "null") were also taxa exhibiting high C:P ratio. Taxa with high %C and %N preferred medium current velocity range. Rheophilic taxa (category "fast") were related to both high %C and high %P, all being positively related to the first axis.

Figures related to biological traits without significant relationship with stoichiometric traits (Tab. 3.3) or for which no *a priori* hypotheses have been made can be found in the supplementary material (S 3.2).

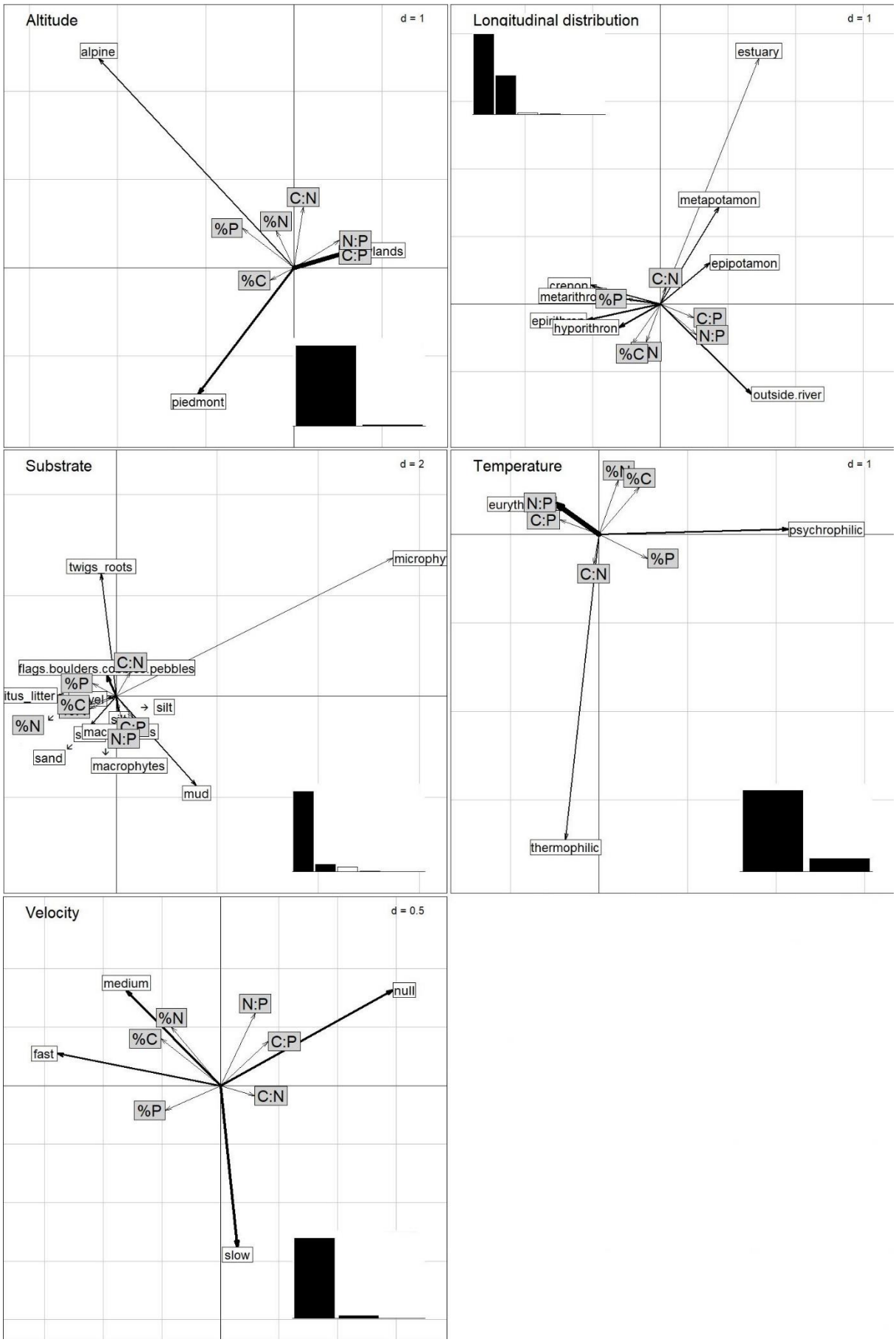

Altitude
d = 1
alpine
C:N
%P
%N
N:P
C:P
lands
%C
piedmont
Longitudinal distribution
d = 1
estuary
metapotamon
epipotamon
C:N
%P
C:P
N:P
%C
N
outside.river
hyporithron
Substrate
d = 2
twigs_roots
microphy
C:N
%P
%C
%N
silt
N:P
sand
macrophytes
mud
Temperature
d = 1
%C
euryth
N:P
C:P
psychrophilic
%P
C:N
thermophilic
Velocity
d = 0.5
medium
N:P
null
%N
%C
C:P
fast
C:N
%P
slow

Fig. 3.2: Results from co-inertia analyses on single habitat-related traits. The functional trait categories and stoichiometric traits are located on the first co-inertia plane. The small histogram illustrates the eigenvalues of the first (two to five) co-inertia axes and indicates the proportion of co-variation that is projected on these axes. The thickness of arrows pointing to functional traits corresponds to trait categories most used by macroinvertebrates. For the trait describing the salinity tolerance described by only two categories, no graphical representation is provided as the whole inertia was projected on a single axis.

Complete trait profile

The complete functional trait profiles were significantly related to the stoichiometric trait profiles (RV = 0.27). This relationship was stronger than those observed for single traits, but the associations among functional and between functional and stoichiometric traits were - in general - similar to those in single trait analyses described above (S 3.3). The largest part of the co-structure was explained by the first axis (71.95%), but still 24.58% fell onto the second axis (Tab. 3.4). As in (most) single trait analyses, variation in stoichiometric profiles was better captured by co-inertia axes than that of functional traits, whose fit was still > 75%.

Tab. 3.4: Results of the co-inertia analysis between macroinvertebrate complete functional and stoichiometric trait profiles. The RV-coefficient indicates the level of co-structure between the two sets of traits and was significant at $\alpha = 0.05$. For the first three axes, projected inertia (%) and the ratio between inertia and co-inertia indicate the fitting level of the analysis. n_{traits} and n_{taxa} indicate the number of functional traits and taxa included in the analysis.

						Projected inertia	Ratio co-inertia/inertia	
	RV	p	n_{traits}	n_{taxa}	Axis	(%)	Fun. traits	Stoichio. traits
Complete trait profile	0.2744	0.0017	23	131	Ax 1	71.95	0.7614	0.9787
					Ax 2	24.58	0.7731	0.9862
					Ax 3	2.65		

4. Discussion

We found significant co-structures between functional and stoichiometric traits in macroinvertebrates, both when considering organism's overall trait profiles as well as when analyzing traits individually. Many of these patterns followed our assumptions based on concepts within the ecological stoichiometry framework.

Development-related traits

We expected that traits corresponding to fast growth or development would be associated to high body phosphorous content. For some trait categories we were able to show this link, but we also found phylogenetic signals. The highest numbers of molting events were positively related to a high phosphorous (and carbon) content. Villar-Argaiz *et al.* (2020) found increasing %P over ontogeny in several hemimetabolous insects and suggested a high phosphorous demand during molts as one explanation. A high number of molting events might also reflect a high growth rate which, according to GRH, requires high amounts of phosphorous and leads to a high body %P. This is supported as Ephemeroptera is the order with the highest affinities for these trait modalities, an insect order with a

high number of species living less than one year and thus growing and developing fast. Additionally, phosphorous content was positively related to small and intermediate maximum body sizes (0.5-1cm, 1-2cm) and negatively related to the categories of largest maximum sizes (2-4 cm, > 4cm), supporting GRH that small taxa tend to be short-lived and fast-growing. However, the category of the smallest maximum body size (< 0.5cm) was associated with low phosphorous content, and was mainly driven by Coleoptera which have one of the lowest phosphorous content among insect orders (Beck *et al.*, in press (MS 1); Frost *et al.*, 2003; Liess & Hillebrand, 2005). In a field study, Villar-Argaiz *et al.* (2020) did not find a correlation between %P and the individual maximum biomass they found for a given taxon. These different results might simply be due to differences in study design (field study with actual measurements vs. a more theoretical approach in our case) and the number of taxa included.
Some trait categories and also their link towards body stoichiometry are strongly coupled to phylogeny. Growing without molting (*i.e*. direct development) was associated with a high C:N ratio, both are characteristics of Bivalvia and Gastropoda which generally have a significantly lower nitrogen (and phosphorous) content than other macroinvertebrate classes (Beck *et al.*, in press (MS 1); Evans-White *et al.*, 2005). Furthermore, molluscs have a complete aquatic life cycle (*i.e.* aquatic "adults") and are either ovoviviparous (Bivalvia) or produce fixed cemented clutches during reproduction (Gastropoda). These biological characteristics have most likely caused the association of these trait groups with high C:nutrient ratios, even though organisms from other taxonomic groups can also exhibit these traits.

Semivoltinism, when analyzing multi-trait profiles, was associated to long life durations and a large number of reproductive events (>6), suggesting, that this trait category was mainly expressed by slow-developing taxa. They were richer in nitrogen and carbon than multivoltine taxa. This was generally also observed in the single trait analysis where stoichiometric traits were not well separated, probably because 89% of the taxa can express one generation per year but still differ for their growth rate (slower or faster development).

Feeding-related traits

Our observations regarding the traits that are linked to food utilization – feeding habits and preferred food resources – are in line with other studies reporting higher nitrogen content of predators compared to herbivores and detritivores (Fagan *et al.*, 2002; Cross *et al.*, 2003; Evans-White *et al.*, 2005). Although our study does not use these categories, we found that swallowers/chewers and taxa preferentially feeding on living macroinvertebrates and vertebrates, to which the common concept of "predator" applies, were linked to high nitrogen and carbon contents. The reason is probably the consumption of animal resources, which are more nutrient-rich (%N, %P) than primary resources and detritus (Cross *et al.*, 2003), as well as higher proportion of N-rich muscle tissues that are necessary to forage preys that are actively moving themselves and/or often exhibit a patchy distribution. Many of these predators are

crawlers, which – although the association was not significant in our study – tended to be related to high nitrogen (and phosphorous) content.

Interestingly, feeding on living macroinvertebrates was not clearly associated with being rich in phosphorous, even if we had assumed a higher content of phosphorous in this food resource. While Cross *et al.* (2003) reported this finding, other authors have not confirmed this observation (Evans-White *et al.*, 2005), similarly to this study. Although several taxa exclusively feed on macroinvertebrates (*e.g.* almost all analysed taxa of Hirudinea and Turbellaria, but also Odonata), other taxa, such as Coleoptera and Plecoptera, exhibit a more opportunistic diet. Their food spectrum is wider and contains animal preys among other food resources, which can explain the deviation from our expectation.

Scraping the biofilm on surfaces and filter-feeding was associated with low concentrations of nitrogen (high C:N, opposed to %N and %C) and is probably influenced by taxonomy, as it is the primary feeding mode of Gastropoda and Bivalves, which are generally poor in nitrogen and phosphorous as stated above. Food items that tend to be consumed by nutrient-poor taxa (high C:P, C:N) included living microphytes, microinvertebrates and dead animal tissues, that all fall in the potential resource spectrum of these feeding habits. Mehler *et al.* (2013) reported significantly lower C:N and higher nitrogen content in scrapers compared to gatherers and/or filter-feeders, but their study only included insects which probably explains this contrasting finding. Evans-White *et al.* (2005) found no difference in stoichiometry among those feeding groups and our analyses also suggest that they express close stoichiometric traits. Generally, detritus is considered a nutrient-poor resource compared to other plant or animal-based food resources and detritivores exhibited also themselves low body nutrient concentrations, especially shredders (Cross *et al.*, 2003; Lauridsen *et al.*, 2014).

Apart from studies showing similar or opposite differences in stoichiometry between feeding groups, a range of studies did not find any differences (Villar-Argaiz *et al.*, 2020). A limited number of taxa per group or in total as well as difficulties or differences in allocating taxa (mostly) to one single feeding category, thus masking the flexibility to feed on a range of different resources (Blanchette *et al.*, 2014; Zhang *et al.*, 2018; Figueroa *et al.*, 2019), could have contributed to these contrasting observations. Also, focusing on larger individuals and therewith later life stages of certain taxa in field studies – unconsciously or to facilitate identification/sample processing – can have caused a bias by underestimating the intra-taxon variability (Villar-Argaiz *et al.*, 2020).

Habitat-related traits

For habitat-related traits, the patterns we found could at least partly be explained by the River Continuum Concept (= RCC, Vannote *et al.*, 1980; Craig, 2002) – originally linking invertebrate community composition in terms of functional feeding groups to patterns of geo-morphological and organic matter properties from river source to mouth – as we hypothesized. This link thereby is indirect *via* biological

traits, mainly those describing mobility and feeding habits. High phosphorous taxa were associated with taxa preferring freshwaters and living in the crenel and metarithral parts of streams, at high altitudes (> 2000 m) and on detritus/leaf litter and coarse mineral substrates. These are all river characteristics that would, according to the RCC, occur in headwaters. The affinities for large rivers (metapotamon), brackish habitats (estuary), as well as microphytes, mud and fine mineral substrates – all attributes of downstream river areas – were associated with high C:nutrient ratios (*i.e.* low nutrient contents %P and %N). The salinity tolerance could also be explained on cellular level and a lower demand of freshwater organisms for ATP (and N-rich proteins) to fuel osmoregulation *via* the active sodium pump (Lucu & Towle, 2003).

Thermophilic taxa co-varied with high C:N ratios, while psychrophilic were richer in phosphorous and carbon. This corresponds to the extended RCC (Craig, 2002), suggesting that temperatures are lower in mountain parts of a stream and increase in downstream direction, and is in line with the observations for other traits described just above. While *a priori* we had two opposing hypotheses for the link between body stoichiometry and temperature preference based on metabolic theory – either high nutrient content (%P) due to increased growth rates at higher temperatures (Janssens *et al.*, 2015) or a higher energy demand that results in high C:P and C:N ratios – our results suggest that the latter drove the overall result.

We expected a positive association between %N and %P stoichiometry and eutrophic habitats, due to better nutritional quality – *i.e*. a higher nutrient content – of basal resources at higher water nutrient concentration (Ventura *et al.*, 2008; Danger *et al.*, 2016; Demi *et al.*, 2019). Nitrogen and phosphorus levels are expected to increase downstream, where nutrient flow from upstream parts and anthropogenic inputs from agriculture are high (Paisley *et al.*, 2011; Aguilera *et al.*, 2012). Our analysis however found no significant link, but indicates (also in the multi-trait approach) an association between P- and N-rich taxa and oligotrophic habitats, suggesting that nutrient rich taxa are favoured in nutrient-poor rather than nutrient-rich habitats.

Based on the prevailing habitat characteristics, the RCC predicts a change in community composition in terms of feeding modes along the stream. Headwaters are expected to be dominated by collectors and shredders. Towards the lower reaches of the stream, the RCC predicts a continuous increase in the proportion of collectors as a result of increasing availability in fine particulate organic matter. In contrast, the proportion of shredders is expected to decrease along the longitudinal gradient, following the gradual reduction of allochthonous inputs of coarse organic matter as riparian forest cover declines. Grazers should reach their highest proportion in intermediate reaches representing optimal conditions (low water depth, low turbidity and reduced shadow effect of woody cover) for biofilm growth. Considering our results regarding both biological and habitat-related traits our findings support this concept, based on stoichiometric trait profiles of taxa.

It is to note that we investigated co-structures between the two sets of traits, *i.e.* the common distribution of biological/habitat-related and stoichiometric traits in macroinvertebrate taxa. Consequently, we can therefore not make statements about causation.

Some functional traits clearly display taxonomic groups in their trait modalities as already discussed above: the aquatic life stage (only some insects exhibit aquatic pupae or emerging imagines), dispersal or the number of molting events (mainly an opposition between direct development vs. indirect development, *i.e. via* successive molting events). Likewise, there are significant differences in stoichiometry among macroinvertebrates belonging to different higher taxonomic groups (Beck *et al.*, in press (MS 1); Frost *et al.*, 2003; Evans-White *et al.*, 2005). Other studies have also reported stoichiometric differences between orders or families (Lauridsen *et al.*, 2012; Mehler *et al.*, 2013) or have pointed out the importance of phylogeny in explaining macroinvertebrate stoichiometry (Morse *et al.*, 2012). Thus, trait syndromes related to phylogeny alone could explain parts of the observed associations between functional and stoichiometric traits.

However, assumptions from ecological stoichiometry can help explaining such observed patterns. For example, active, aerial dispersal – only expressed by insects – was linked to high contents of nitrogen which most likely indicates investment in N-rich muscle tissue needed for flight. On the other hand, aquatic, passive dispersal, which should require less muscles than active movement, was associated to high C:N (and C:P) ratio and all these traits are – among others and with exceptions – largely prominent in bivalves and gastropoda. This clearly demonstrates the difficulty of defining which trait is the "cause" or the "response", keeping in mind the important role of phylogeny for both types of traits.

Understanding the general link between functional and stoichiometric traits might help to directly understand changes, not only in the taxonomic composition, but also in the functional composition of macroinvertebrate assemblages. Macroinvertebrates play an important role at ecosystem scale (Wallace & Webster, 1996; Covich *et al.*, 1999) due to their contribution to stream secondary production (Poepperl, 2000; Stagliano & Whiles, 2002), organic matter decomposition (Halvorson *et al.*, 2015), or nutrient release *via* excretion (Knoll *et al.*, 2009). Considering that shifts in community stoichiometric composition should follow changes in water nutrient concentration after nutrient enrichment, such changes should go along with shifts in functional trait composition, leading to changes in nutrient cycling, for example *via* shorter food chains due to larger body sizes and increased growth rates (Singer & Battin, 2007). Field studies along a gradient of nutrient enrichment or the analysis of detailed datasets could help to verify that the relationships we found stand practical application in functional ecology. We think that such links exist also in other ecosystems and for other groups of organisms and could be used to improve analyses in those fields.

Differences and difficulties to compare our results to previous studies also originate in the high number of functional traits and trait modalities our study has considered. Considering potential, fuzzy coded traits - rather than allocating only one single category to a taxon for a given trait (*i.e.* a completely disjunctive coding system) – has the advantage to integrate the full intra-taxon variability that can occur in nature. Macroinvertebrates can be ecologically flexible and, for example, change their diet according to resource availability of habitats (Tomanova *et al.*, 2006; Wellard Kelly *et al.*, 2013; Zhang *et al.*, 2018). Moreover, their life history characteristics such as the annual number of generations (voltinism) or the number of successive reproductive cycles for a given individual are not always constant and can be influenced by external factors such as temperature (Corbet *et al.*, 2006; Winder *et al.*, 2009; Altermatt, 2010; Lehmkuhl, 2011). This further complexifies the interpretation of results, but on the other hand captures the nature more accurately.

Insects were by far the largest group of taxa represented in this study, but in general, they make up the largest proportion of a macroinvertebrate community, at least in springs and wadeable streams. Thus, our study list of taxa is fairly representative of the natural composition of macroinvertebrate assemblages in such systems.

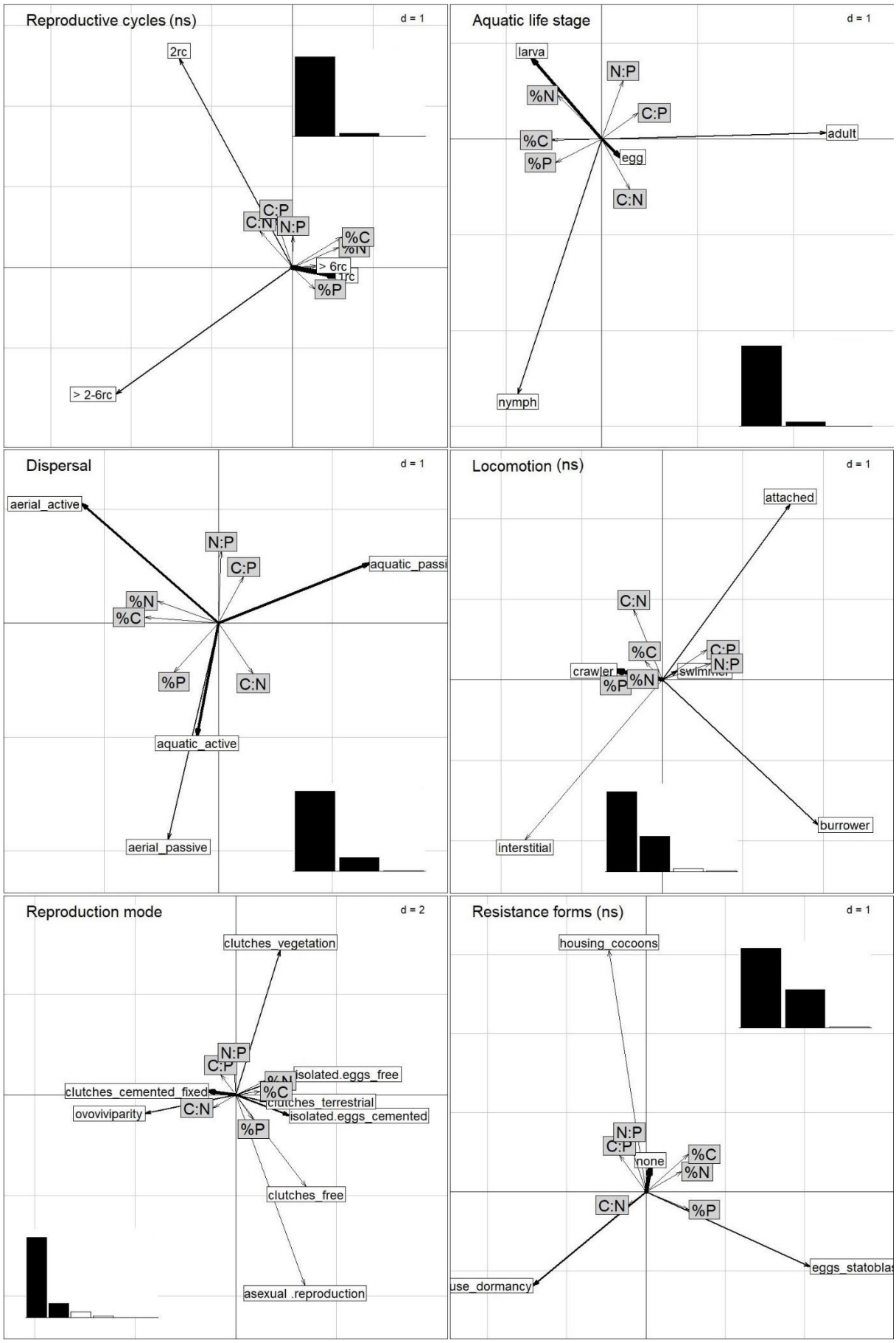
Conclusion

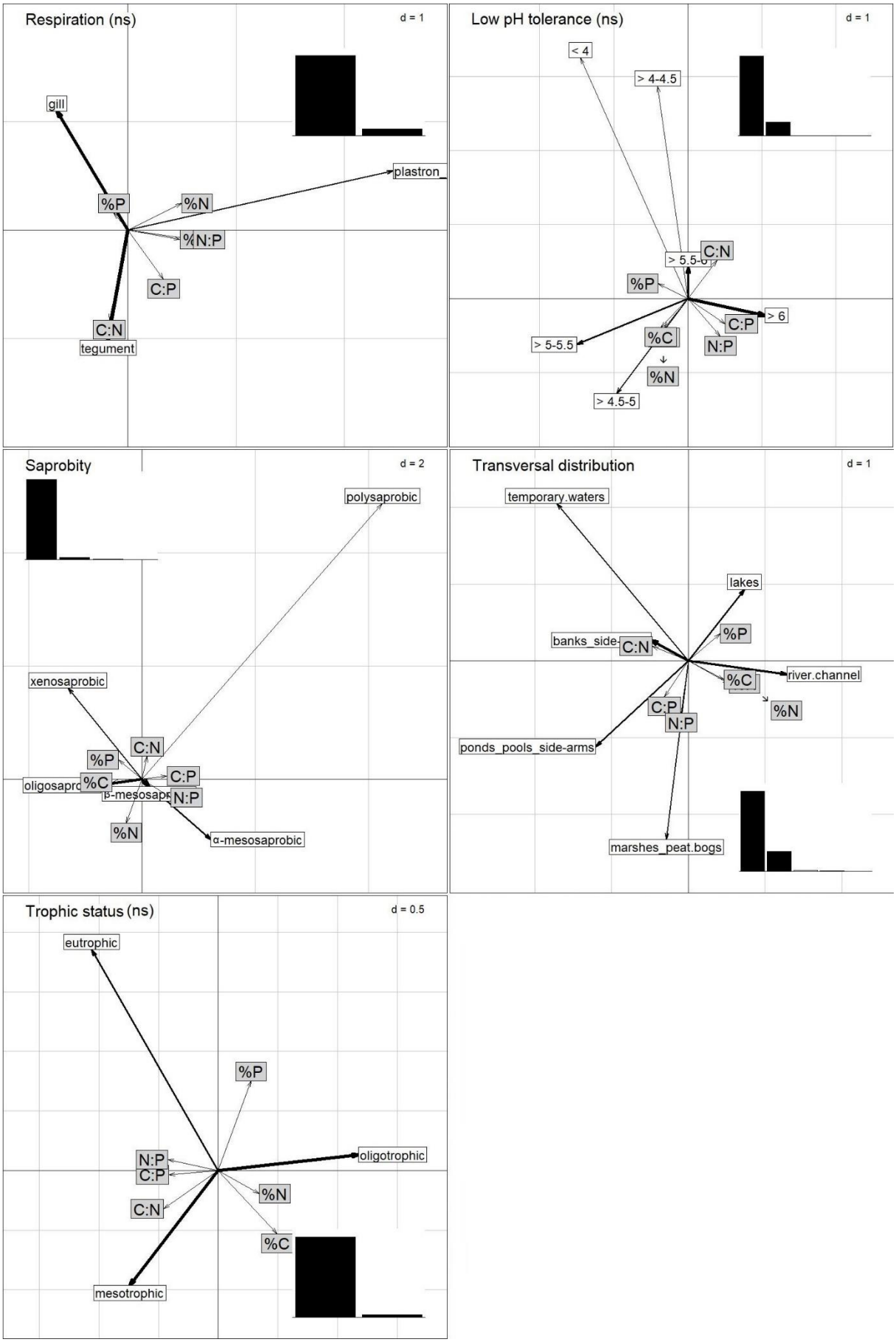
We have shown that there is a significant co-occurrence between stoichiometric and biological as well as habitat-related traits in benthic macroinvertebrates, both when analyzing single traits or overall trait profiles. The associations for traits related to organism development, nutrient cycling (*via* feeding) or locomotion thereby are in line with assumptions based on concepts and observations within ecological stoichiometry. For habitat-related traits, the link towards stoichiometric traits probably was indirect *via* biological traits, additionally displaying the longitudinal stream gradient. Some functional traits have shown a strong effect of phylogeny, which can be explained by group-specific trait syndromes but partly also by ecological stoichiometry theory. Demonstrating the link between stoichiometric and functional traits underlines the potential of integrating stoichiometry into ecological studies to better understand and predict the taxonomic and functional responses of macroinvertebrate communities to nutrient concentration changes.

Supplementary material

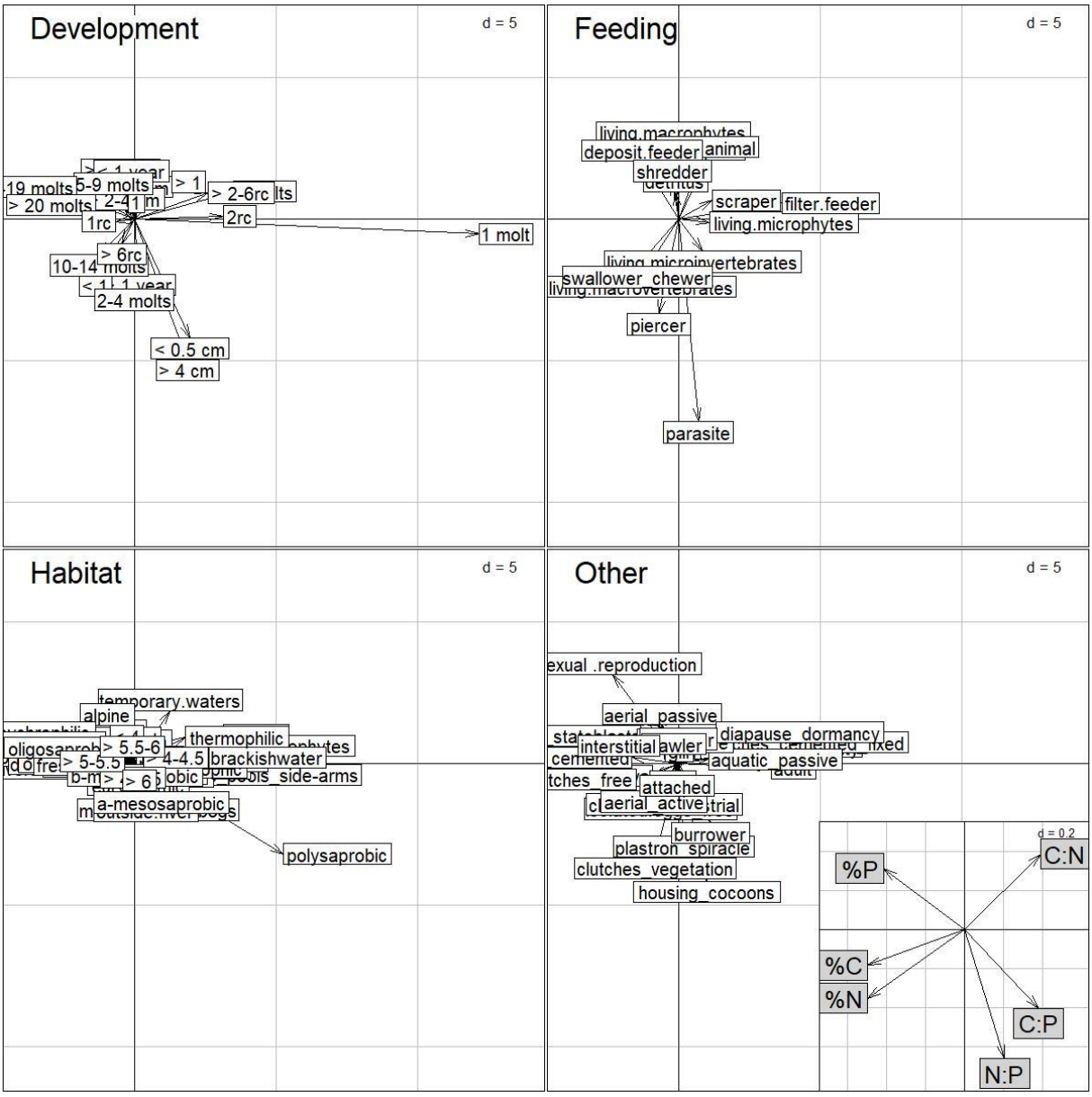
S 3.1.: Modifications of trait-categories compared to the original database from Tachet et al. (2010).

Functional Trait	Modification
Maximum body size	"<0.25 cm" + "0.25-0.5 cm" → "<0.5 cm"
	"4-8cm" + ">8 cm" → ">4 cm"
Food resources	"living macroinvertebrates" + "vertebrates" → "(in)vertebrates"
	"detritus" + "microorganisms" → "detritus"
Locomotion	"housing" + "cocoons" → "housing/cocoons"
	"plastron" + "spiracle" → "plastron/spiracle"
Resistance forms	"surface swimmer" + "full water swimmer" →"swimmer"
Respiration	"temporarily attached" + "permanently attached" → "attached"
Transversal distribution	"groundwater" has been removed and relative frequencies calculated without considering this trait category


Reproductive cycles (ns)
d = 1
2rc
C:P
C:N
N:P
%C
%N
> 6rc
Trc
%P
> 2-6rc
Aquatic life stage
d = 1
larva
N:P
%N
C:P
adult
%C
%P
egg
C:N
nymph
Dispersal
d = 1
aerial_active
N:P
C:P
aquatic_passi
%N
%C
%P
C:N
aquatic_active
aerial_passive
Locomotion (ns)
d = 1
attached
C:N
%C
C:P
N:P
crawler
%P
%N
swimmer
burrower
interstitial
Reproduction mode
d = 2
clutches_vegetation
N:P
C:P
isolated.eggs_free
%N
%C
clutches_cemented_fixed
clutches_terrestrial
ovoviviparity
C:N
isolated.eggs_cemented
%P
clutches_free
asexual .reproduction
Resistance forms (ns)
d = 1
housing_cocoons
N:P
C:P
%C
none
%N
C:N
%P
use_dormancy
eggs_statobla


Respiration (ns)
d = 1
gill
plastron
%P
%N
%N:P
C:P
C:N
tegument
Low pH tolerance (ns)
d = 1
< 4
> 4-4.5
C:N
%P
> 6
C:P
%C
N:P
> 5-5.5
%N
> 4.5-5
Saprobity
d = 2
polysaprobic
xenosaprobic
C:N
%P
C:P
%C
N:P
%N
α-mesosaprobic
Transversal distribution
d = 1
temporary.waters
lakes
%P
C:N
%C
river.channel
C:P
N:P
%N
ponds_pools_side-arms
marshes_peat.bogs
Trophic status (ns)
d = 0.5
eutrophic
%P
N:P
C:P
oligotrophic
%N
C:N
%C
mesotrophic

S 3.2: Results from co-inertia analyses on single biological and ecological traits for which we had no *a priori* hypothesis and/or for which the results were not significant. The functional trait categories and stoichiometric traits are located on the first co-inertia plane. The small histogram illustrates the eigenvalues of the first (two to five) co-inertia axes and indicates the proportion of co-variation that is projected on these axes. The thickness of arrows pointing to functional traits corresponds to trait categories most used by macroinvertebrates.



S 3.3: Results from co-inertia analysis on multiple-trait profiles comprising all biological and ecological traits, showing the location of the functional trait categories on the first co-inertia plane. Note that all traits were part of one analysis and simple faceted according to the four trait groups as also discussed separately throughout the manuscript (development, feeding, habitat, other) for easier readability. The small figure illustrates the position of stoichiometric traits on the first co-inertia plane.

Chapter IV:

Macroinvertebrate community stoichiometry

Large scale effects of stream water nutrient concentrations on macroinvertebrate community stoichiometry

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Abstract

Resource quality is one factor determining community composition. Due to plasticity of basal resource elemental composition which tend to mirror nutrient conditions in the environment, the global stressor of nutrient alternations reach consumer communities: An increased nutrient content in primary and detrital resources under nutrient enriched conditions will favour taxa with a higher demand for this nutrient. The nutrient demand of a taxon is correlated to the elemental composition of its body tissue, i.e. its stoichiometric traits (e.g. phosphorous content). Such above described species shifts towards nutrient rich taxa therefore seem to also affect the overall community stoichiometry. This is in line with ecological stoichiometry theory (=EST), a framework that investigates the equilibrium and the fluxes of elements in the biotic and abiotic world. However, such studies addressing community level beyond planktonic systems are rare and limited to single streams or experimental setups.

Relying on a stoichiometric trait database and >1300 abundance datasets from the French national monitoring programme, we investigated the effect of water phosphorous and nitrogen load on macroinvertebrate community stoichiometry. For a better understanding of the observed patterns, we additionally separately analysed main taxonomic groups and functional feeding groups.

Community stoichiometry was significantly affected by water phosphorous concentration and the effect was strongest at low levels of nitrogen. While we could not confirm our hypothesis of increasing community %P (and decreasing C:P, N:P) along a gradient of water phosphorous concentrations for the overall community, for some taxonomic groups (e.g. Insecta, Malacostraca) it clearly followed this pattern. General differences in the elemental composition among main macroinvertebrate groups and a shift among these larger taxonomic groups over the nutrient gradient probably hindered the expected pattern in community stoichiometry, but could also explain this deviation. Our results clearly show that assumptions from EST also hold on community level. The consequences of a change in community stoichiometry remain to be investigated. It surely means an altered nutrient pool that the community is providing to higher trophic levels, and as suggested by a link between body stoichiometry and functional traits it could further indicate a change in the community functional composition (e.g. as our results indicate for functional feeding groups).

1. Introduction

Community ecology studies the structure and functioning of communities, that is the presence/absence or abundance of taxa in a certain space and their interactions among each other and with the environment. Generally, taxa are favoured based on their preferences (*e.g.* for food resources or substrates) and tolerances (*e.g*. towards pollution), thereby favouring those for which the mismatch between current environmental conditions and individual requirements is lowest. Imbalances – for example between resource quality and organism nutrient demand – can occur under changing nutrient conditions in the environment: while heterotrophs are homeostatic and able to keep a constant nutrient level, the nutrient content of basal resources changes accordingly and represents nutrient availabilities in the environment (Fink *et al.*, 2006). Nutrient enrichment for example alters their elemental composition towards lower C: nutrient ratios, thus increasing its quality for herbivore consumers (Cross *et al.*, 2003; Ventura *et al.*, 2008). Similarly, detritus quality is increased at higher nutrient concentrations *via* increased microbial activity leading to changed stoichiometry(Gulis & Suberkropp, 2003; Cross *et al.*, 2007; Danger *et al.*, 2016; Demi *et al.*, 2018). For herbivore consumers, feeding on resources that largely deviate from the individual nutrient demand in any direction reduces the overall fitness (Boersma & Elser, 2006; Elser *et al.*, 2016; Zhou & Declerck, 2019). Therefore, taxa whose nutrient demand is closer to the altered nutrient conditions will be favoured and nutrient concentrations in the environment indirectly affect community structure. Nutrient-sensitive taxa that are adapted to low nutrient conditions will disappear even at moderate nutrient increases, shifting the community towards more tolerant taxa (Ortiz & Puig, 2007; Friberg *et al.*, 2010).

For some taxa, the response follows assumptions from ecological stoichiometry theory (= EST): assuming that body stoichiometry, that is the elemental composition, indicates organism's nutrient demand (Sterner & Elser, 2002), abundances of phosphorous-rich taxa increased, following increased phosphorous levels in the environment (Singer & Battin, 2007; Prater *et al.*, 2015; Teurlincx *et al.*, 2017). This shift also decreased the overall community C: P stoichiometry (Singer & Battin, 2007; Teurlincx *et al.*, 2017), which can be seen as a descriptor of the elemental composition of the nutrient pool provided by the community. Community stoichiometry, which followed the nutrient gradient, is thus directly linked to nutrient cycling and could provide information about the trophic structure of a system.

Ecological stoichiometry investigates the link and the interaction between elements in an organism and its environment to understand processes from individual to ecosystem level (Sterner & Elser, 2002). On taxon level it has already explained growth rate for a range of organisms (Elser *et al.*, 2003a; Acharya *et al.*, 2004) and has helped explaining diverse ecological questions such as host-parasite interactions (Sanders & Taylor, 2018), species invasions (González *et al.*, 2010) or size-spectra of consumers (Mulder & Elser, 2009). Stoichiometric differences between taxonomic groups (Fagan *et al.*, 2002; Evans-White *et al.*, 2005; Teurlincx *et al.*, 2017, MS 1) as well as a general link between stoichiometric and functional traits (MS 2), for example feeding habits described by functional feeding groups (Fagan

et al., 2002; Cross *et al.*, 2003; Evans-White *et al.*, 2005) can be used to address questions also in terms of (ecosystem) functioning.

The integration of body stoichiometry into ecological studies thus promises to be a great tool in understanding the mechanisms underlying community or ecosystem responses towards global stressors (van de Waal *et al.*, 2010; Hessen *et al.*, 2013; Leroux *et al.*, 2017; Meunier *et al.*, 2017). With changing nutrient concentrations being a main stressor in worldwide ecosystems – either increasing due to anthropogenic activities (Falkowski *et al.*, 2000; Peñuelas *et al.*, 2013) or decreasing when counteracting management measures have been successful (Jeppesen *et al.*, 2005; Floury *et al.*, 2013; Latli *et al.*, 2017)(the latter ones only single stream examples) – it seems indeed useful to benefit from the ultimate link towards nutrients that this approach offers.

Up to date, not many works have addressed stoichiometric questions considering consumer communities as a whole, and no study yet has explicitly investigated community stoichiometry on a large scale. In this study, we aimed at analyzing the effect of water phosphorous and nitrogen concentration on community stoichiometry. We considered coarser taxonomic groups (generally class) and feeding groups to investigate further details of these effects.

We hypothesize that changing concentrations of water nutrients will alter community stoichiometry. More specifically, we assume that community phosphorus stoichiometry will be higher at sites where water phosphorous concentration is high, while community C:P and N:P stoichiometry decreases along this gradient. This should be due to shifts in taxonomic composition of the communities along such nutrient gradient, as taxa are selected based on their individual nutrient demand, represented by body stoichoimetry. We expect to find this pattern for the stoichiometry of the complete community, as well as within each major macroinvertebrate group. The change in overall community stoichiometry should thereby mainly be caused by a change in abundances among larger taxonomic groups, accompanied by a within-group shift towards P-rich taxa. The latter change will alter within-group stoichiometry. The reason is a general difference in stoichiometry between (macroinvertebrate) classes and families (Fagan *et al.*, 2002; Evans-White *et al.*, 2005, MS 1).

As overarching mechanism for the taxa selection, we assume altered quality and/or availability of basal resources under different nutrient scenarios. Due to this variability, we expect that the community stoichiometry of primary consumers (detritivores, herbivores and omnivores) will be stronger affected than that of secondary consumers (predators), whose resources are stoichiometrically more stable. The community stoichiometry within each feeding group should mainly follow the same pattern as described above for the complete community.

We tested our hypotheses using information from a database containing stoichiometric information about >200 macroinvertebrate taxa and a dataset including >1300 site sampling events from a French national biomonitoring program, comprising macroinvertebrate abundance information and water chemistry. Macroinvertebrates are widely used in freshwater monitoring programs, thus providing

extensive information on abundances and traits enabling such analyses. Although our main hypotheses refer to water phosphorous concentration, we also integrate the level of water nitrogen into our analyses since not only absolute concentrations of both elements are changing but also their ratio (Peñuelas *et al.*, 2013; Yan *et al.*, 2016).

2. Material & Methods

The data

Under the WFD, benthic macroinvertebrate assemblages found in French streams are routinely monitored using standardized procedures (AFNOR 2009 for the field samplings; AFNOR 2010 for the sorting and identification steps). It allowed us to consolidate a large and standardized faunal dataset comprising the taxonomic composition and abundance of macroinvertebrates assemblages found in wadeable streams all over France from 2004 to 2017, thus covering various regions and stream sizes. The general identification level was the genus level, whereas the family level was used for Diptera, Hirudinea and Turbellaria; Oligochaeta and Nematoda were identified as such.

The faunal dataset was complemented with a second dataset describing 17 pressure categories, including ten pressure categories linked to water quality (Mondy *et al.*, 2012). Each pressure category was described using a set of five pressure levels, from worst to best: "bad", "poor", "moderate", "good" and "high". In order to avoid additional effects in heavily polluted sites we first selected site sampling events (SSE; station x sampling date) which exhibited pressure levels either "high", "good" or "moderate" for the following pressure categories linked to water quality: organic and mineral micro-pollutants, pesticides, suspended particulate matter, HAP (=polycyclic aromatic hydrocarbon), and nitrogen compounds (excluding nitrates). We then filtered by stream size, only keeping SEE from very small, small and intermediate streams (*i.e.* exhibiting a Strahler rank comprised between 1 and 4 (5 for sites located in the basin Loire-Bretagne)). Finally, we only kept SSE for which we had stoichiometric information available for at least 95% of the individuals. Based on these criteria, 1308 SSE were included in this study, with sampling years ranging between 2005 and 2017 and including information about the abundances of 146 different taxa.

Last, we characterized the pressure level for phosphorus and nitrogen for our reduced dataset (S 4a.1). Phosphorus level was used quantitatively and corresponds to the concentration of phosphates (PO_4^{3-}; mg/L). Nitrogen level refers to nitrate (NO_3^{2-}; mg/L) concentration and was considered qualitatively as categorical variable with three categories: low (<10 mg/L), intermediate (10-25 mg/L) and high (>25 mg/L). It has to be noted that the quantitative values of phosphorus concentration contain a certain amount of imprecise values due to detection limits. In these cases, the true values might be lower than the reported value. Due to a lack of possibility to identify such values with certainty and to overcome the problem, we decided against excluding suspicious datasets. Most of these sites should be positioned at the real low end of the phosphorus concentration gradient, so that the overall trend we aim to find in

the data should largely be unbiased. Also the nitrate values suffer from this problem, but due to the use of categories this does not pose a problem as all probably concerned SSE will fall into the same category (*i.e.* "low", <10 mg/L).

Data analysis

Community stoichiometry

Stoichiometric information on each taxon observed in our reduced dataset was obtained from a database comprising information of elemental mass content of major nutrients (%C, %N, %P) and the corresponding molar ratios (C:N, C:P, N:P) for > 250 taxa at different taxonomic levels (data paper). For each SSE, the community stoichiometry was calculated as the abundance-weighted mean stoichiometry of the community: the relative abundance of each taxon was multiplied by its corresponding stoichiometry value (%C, %N, %P, C:N, C:P, N:P) before summing up the values of all taxa in the community. The relative abundance thereby was based only on taxa for which the stoichiometry information was available, but covered ≥ 95% of the community in terms of individuals, *i.e*., the number of taxa used can slightly vary between stoichiometric variables, but since we did not plan to compare stoichiometric variables among each other we decided for best data coverage. Values of the stoichiometric ratios (C:N, C:P, N:P) were log-transformed before the calculation to avoid biased results due to inert properties of ratio data as suggested by Isles (2020). Mass contents of the elements (%C, %N, %P) were directly used from the database.

Group-wise (macroinvertebrate lower taxonomic groups) and consumer-wise community ratios were calculated as described above, but by weighting the mean by the relative frequency of a taxon within its taxonomic or feeding group, rather than the complete community. An updated (by the authors) version of the macroinvertebrate trait database from (Tachet *et al.,* 2010) was used to assign each taxon to a feeding group, based on resource preference. This trait database provides fuzzy-coded trait information and contains the affinity (ranging from 0 = no affinity to 3 = high affinity) for each trait modality of a trait. For a given taxon, affinities for each resource preference were first expressed as relative frequencies. We defined our feeding groups as follows, based on calculated relative frequencies of the affinities: (i) taxa with an affinity >0.5 for the resources "living microinvertebrates" and "living macroinvertebrates" were considered predators; (ii) taxa with an affinity >0.5 for plant-based resources ("living microphytes", "living macrophytes") were considered herbivores; (iii) taxa with an affinity >0.5 for detritus-based resources ("dead plant", "dead animal", "detritus") were considered detritivores; and (iv) taxa with no affinity >0.5 for any of these resource groups were considered omnivores.

Proportion of taxonomic and feeding groups

The proportion of lower taxonomic groups and feeding groups based on abundance was calculated as the relative frequency of each group in the overall community of the SSE. For that, the sum of all individuals of a group was divided by the total number of individuals found at a site. In the case of

feeding groups, individuals taxa for which this trait information was not available were included in the total number of individuals found at a site so that the obtained values represent the proportion of the complete community.

Statistical analysis

Multiple linear models were separately applied for each stoichiometric variable to analyse the effect of water nutrient concentrations on community stoichiometry. Community stoichiometry was set as the dependent variable, whereas water P and level of N were integrated as quantitative and categorical independent variables, respectively. An interaction term between the two independent variables was integrated and its contributing effect verified by comparison to a model without interaction term *via* the anova() function. In case the integration of the interaction term did not improve the model, the simple model was preferred. Model assumptions, that is normality of residuals and homoscedasticity, were checked graphically by inspecting QQ-plots.

The same models were applied to group-wise community ratios, setting one model per stoichiometric variable and taxonomic or feeding group. Due to the limited number of taxa in some taxonomic groups, only those groups that made the largest part of most communities and for which stoichiometric information was available for a sufficient number of taxa were considered for this part of the analysis (*i.e.* Gastropoda, Hirudinea, Insecta, Malacostraca). For the groups Gastropoda and Hirudinea, the assumptions of normal distributed residuals was not met. Since this is most likely due to the limited number of taxa which led to numerous identical values for community stoichiometry when only few taxa were present in a community, we still consider the results in this study, under the corresponding reservation.

To analyse feeding group-wise community stoichiometry, individual models for each group were applied as described above.

The effect of water phosphorus and nitrogen levels on the proportions of lower taxonomic groups and feeding groups in the macroinvertebrate community was investigated by applying the same multiple linear models, setting the proportion of the corresponding group as dependent variable.

All analyses were conducted in R (version 3.5.3; R Core Team, 2020).

3. Results

Please note that since our main hypotheses concerned phosphorous, we limit the detailed description to phosphorous-related stoichiometry and provide figures and results of the others (%C, %N, C:N) for anyone interested in the supplementary material.

Effect of water nutrient level on community stoichiometry

In all community stoichiometry values there was a significant effect of water P concentration and an interaction with the level of N (*i.e.* waterP:levelN), with the effect of water P being largest at low N-

levels (Fig.4a.1, S 4a.2). Overall, the models explained between 3.17 (N:P) and 19.02% (%P) of the variation in phosphorous-related community stoichiometry.

Community %P stoichiometry values significantly decreased with increasing water P, with the strength of the effect decreasing from low to intermediate/high N levels. In contrast, the ratio community stoichiometry values (C:P, N:P) significantly increased with increasing water P concentration at low and intermediate water N levels. Also here the effect was larger at low compared to intermediate levels of water N in both ratios. At high N levels however, the community ratios decreased but with a very low slope.

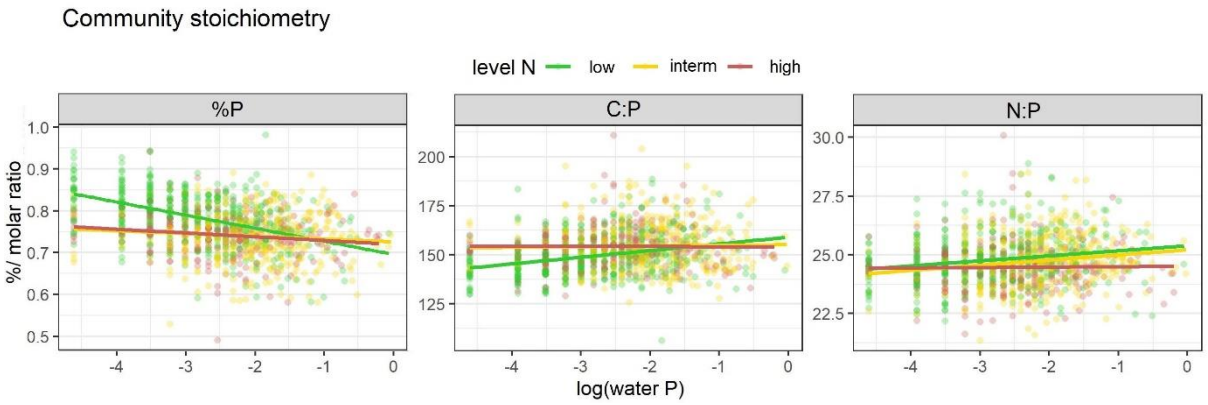


Fig. 4a.1: Community stoichiometry (%P, C:P, N:P) along a gradient of water P concentration (log scale). Regression lines allow different slopes and intercept for low, intermediate and high levels of water N, indicated by colors. Scattered points represent raw data (calculated community stoichiometry for the different SSE) with n=678/ 599/ 341 SSE, respectively for low/ intermediate/ high N levels. Values of the molar ratios have been exponentiated (exp(x)) in order to facilitate to facilitate the comparison with other studies that mainly report untransformed ratio values.

Community stoichoimetry within taxonomic groups

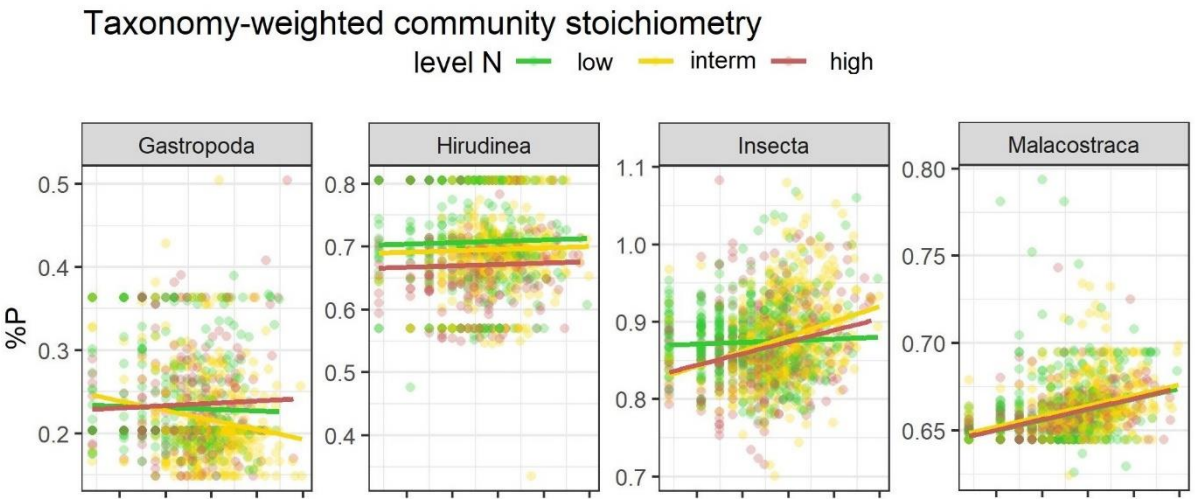
In all major macroinvertebrate taxonomic groups, community stoichiometry was significantly correlated to water P concentration and/or nitrogen level.

Within Gastropoda, all community stoichiometry values were influenced by water phosphorous concentration with the effect depending on water N level and being strongest at intermediate N concentrations (Fig. 4a.2, S 4a.4). Community %P decreased with increasing water P concentration at intermediate N levels, and tended to slightly decrease under low N levels. Community C:P and N:P were positively correlated to water P concentration at intermediate N, but tended to decrease under high N conditions. At low N levels, C:P ratios slightly increased while N:P ratios decreased along the water P gradient (both not significant).

Community stoichiometry of Hirudinea was not affected by water P concentration. However, community %P was significantly lower under intermediate and high levels of N compared to low N conditions. Community C:P and N:P ratios showed the opposite pattern and values increased with N load, However, only the difference between low and high levels was significant.

Insect community %P was positively correlated to water P concentration on all three levels of water N, but this effect was only significant under intermediate and high N conditions. Community ratios decreased with increasing water P concentration and this effect increased with increasing levels of N (significantly for both intermediate & bad N levels for C:P and only for intermediate N levels for N:P).

In Malacostraca, all stoichiometric variables were significantly affected by water P concentration but with an interaction between water nutrients only in community N:P. In community N:P the effect was positive and increased with increasing concentration of N. Community %P was positively correlated to water P concentration and significantly higher at intermediate N levels compared to low N levels. The correlation of community C:P to water P concentration was negative and values at intermediate and high N sites were significantly lower compared to low N sites.



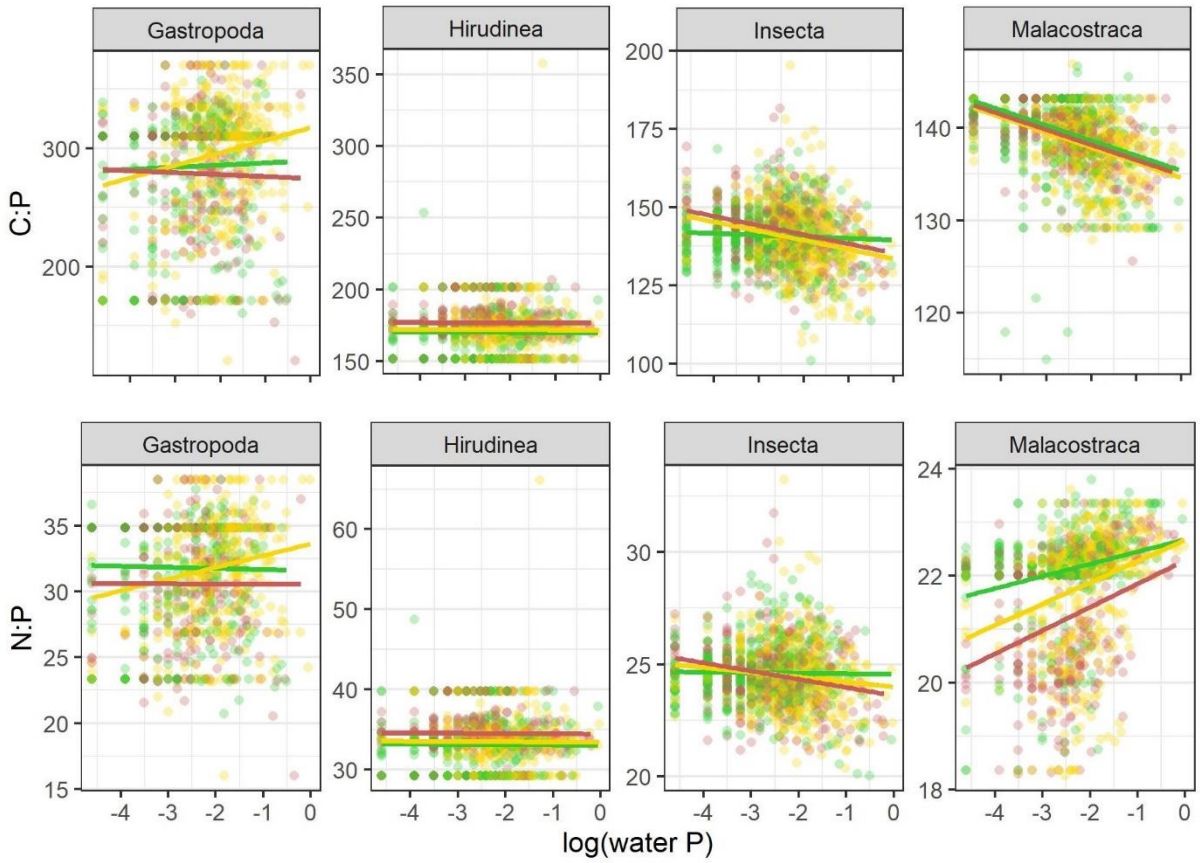


Fig. 4a.2: Taxonomy-weighted community stoichiometry (%P, molar C:P, molar N:P) along water P concentration (log scale). Depending on the suggestion by model comparison, regression lines allow different slopes and intercepts for different levels of water N (low, intermediate, high, indicated by colors) or assume the same slope for each level of water N. Scattered data points represent raw data (calculated community stoichiometry at the different sites). Values of the molar ratios have been exponentiated (exp(x)) in order to facilitate to facilitate the comparison with other studies that mainly report untransformed ratio values.

Proportions of major taxonomic macroinvertebrate groups

Except for Turbellaria, the proportion of the taxonomic group in the macroinvertebrate community depended on water phosphorous concentration and/or the level of nitrogen. While for Insecta, Malacostraca and Oligochaeta the effect of water P concentration on their proportion in the community depended on the level of water N, there was no such interaction in Bivalvia, Gastropoda and Hirudinea (Fig. 4a.3, S 4a.6). Overall, the models explained 1.53 % (Bivalvia) – 11.71% (Insecta) in the variation of taxonomic proportion.

Bivalvia and Gastropoda tended to slightly – but insignificantly – increase in proportion with increasing water P concentration. At intermediate water N levels their proportions were significantly higher compared to low N sites. The proportion of Hirudinea increased significantly with water P concentration, but was unaffected by the level of N. The effect of water P concentration on the proportion of Insecta was largest when N levels were low. Insect proportion then decreased significantly with increasing water

P concentration, while it increased significantly at intermediate and at high N levels. Malacostraca proportion showed the opposite pattern: When N levels were low, the significant correlation between Malacostraca proportion and water P concentration was positive, while it was negative when N levels were intermediate or high. The effect of water P then also was larger compared to low-N sites. The proportion of Oligochaeta significantly increased with increasing water P concentration at low and high levels of water N, with the effect being largest at high N levels. However, at intermediate N levels this increase was not significant.

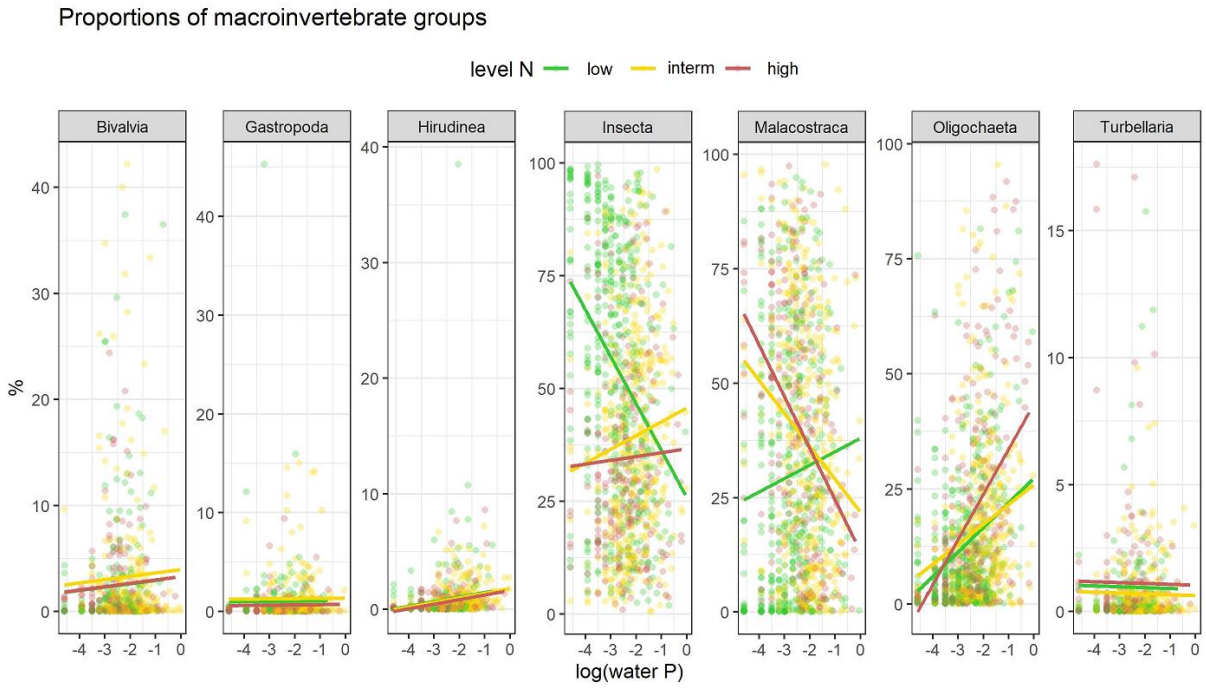


Fig. 4a.3: Proportion based on abundance of taxonomic groups in the macroinvertebrate community along water P concentration (log scale). Depending on the suggestion by model comparison, regression lines allow different slopes and intercepts for different levels/ status of water N (low, intermediate, high, indicated by colors) or assume the same slope for each level of water N. Scattered data points represent raw data. Note that for better readability of the effects the scaling of y-axis differs between groups.

Community stoichiometry among functional feeding groups

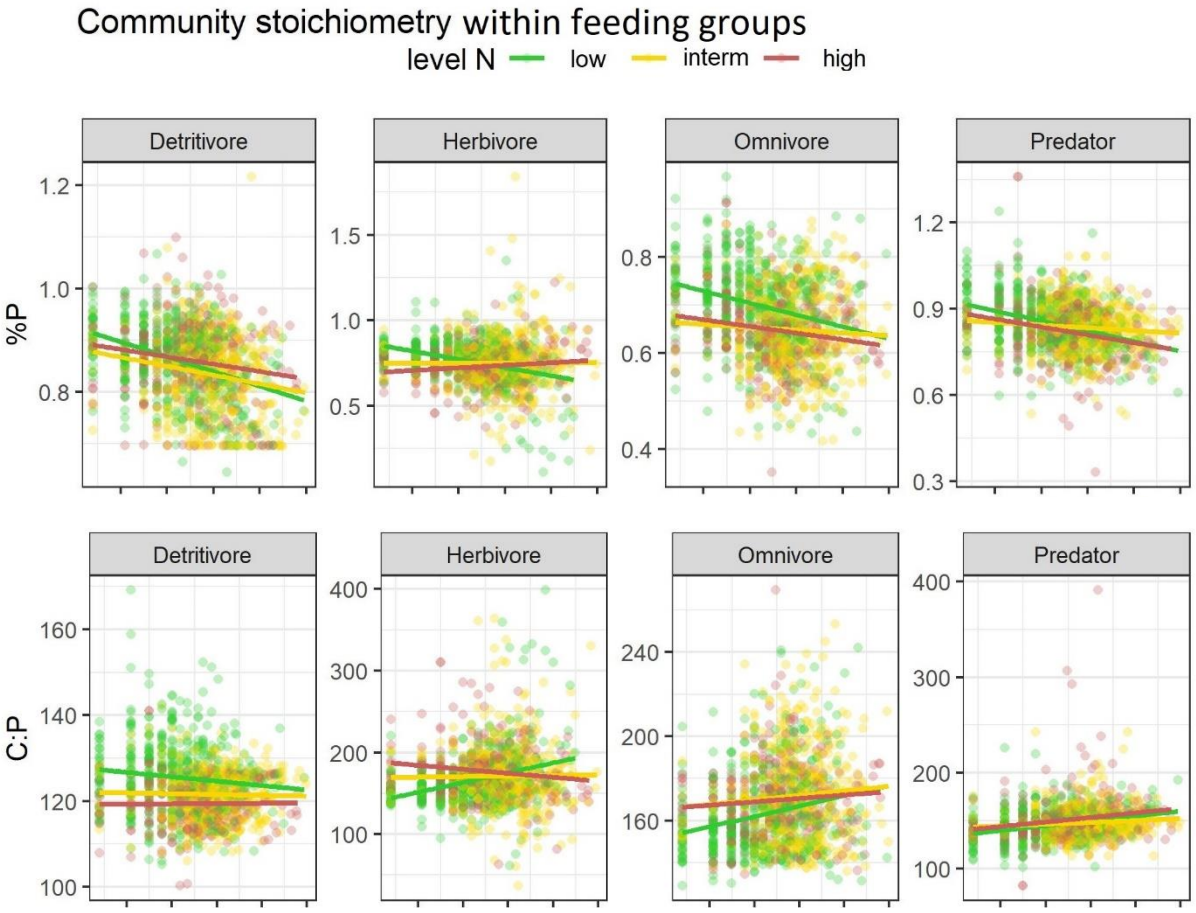
Within detritivores, all stoichiometric variables significantly decreased with increasing water P concentration at low levels of N (Fig. 4a.4, S 4a.7). In community %P increasing levels of N decreased this effect, but it remained significant. Community C:P values insignificantly decreased under intermediate N levels and to increase when N levels were high. N:P ratios were positively correlated to water P concentration under intermediate and high N loads.

In herbivores, increasing levels of water N opposed the effect of water P concentration on community stoichiometry values compared to low levels of N. %P was negatively correlated to water P

concentration under low levels of N, while this correlation was positive under intermediate and high N load. Community C:P and N:P ratio both significantly increased with increasing water P under low N scenarios, but significantly decreased under higher levels of N).

In the omnivore community the effect of %P and C:P stoichiometry depended on the level of N. Community %P significantly decreased and community C:P increased with increasing water P concentration. These effects were reduced under higher levels of N effect). Community N:P was significantly and positively correlated to water P concentration. There was no interaction with water N level, but increasing N load decreased community N:P.

Predator community %P significantly decreased with increasing water P concentration while C:P and N:P ratios significantly increased. For all three community stoichiometry values this effect was reduced under intermediate levels of water N.



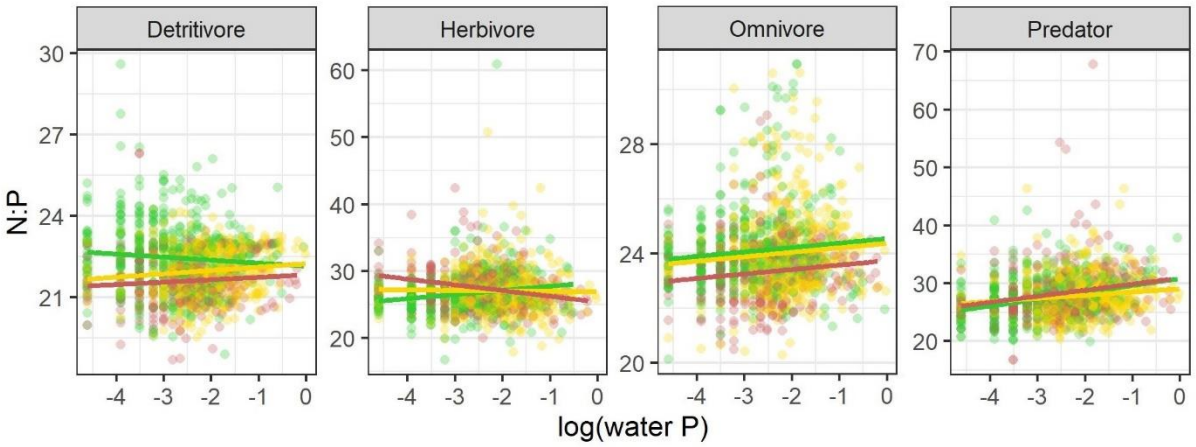


Fig. 4a.4: Community stoichiometry (%P, molar C:P, molar N:P) within functional feeding groups along water P concentration (log scale). Depending on the suggestion by model comparison, regression lines allow different slopes and intercepts for different levels/ status of water N (low, intermediate, high, indicated by colors) or assume the same slope for each level of water N. Scattered data points represent raw data (calculated community stoichiometry at the different sites). Values of the molar ratios have been exponentiated (exp(x)) in order to facilitate to facilitate the comparison with other studies that mainly report untransformed ratio values.

Proportions of feeding groups

For all feeding groups, there was a significant interacting effect of water P concentration and the level of N on their proportion in terms of abundance in the community (Fig. 4a.5, S 4a.9).

The proportion of detritivores increased significantly with increasing water P concentration at all the levels of N, with the effect of phosphorus being largest at high levels of N. In contrast, herbivore proportion within the community significantly decreased over this gradient under all the water N scenarios. Here, the effect of water P concentration was strongest at low N levels. Omnivore proportion significantly decreased with increasing water P concentration under high and intermediate N levels, but significantly increased at low N levels. The effect of water P concentration and its interaction with water N level was lower on predator proportion compared to that of the other groups, but still significant for low and intermediate levels of N. The relationship was negative at low and high levels of N; but positive at intermediate N levels.

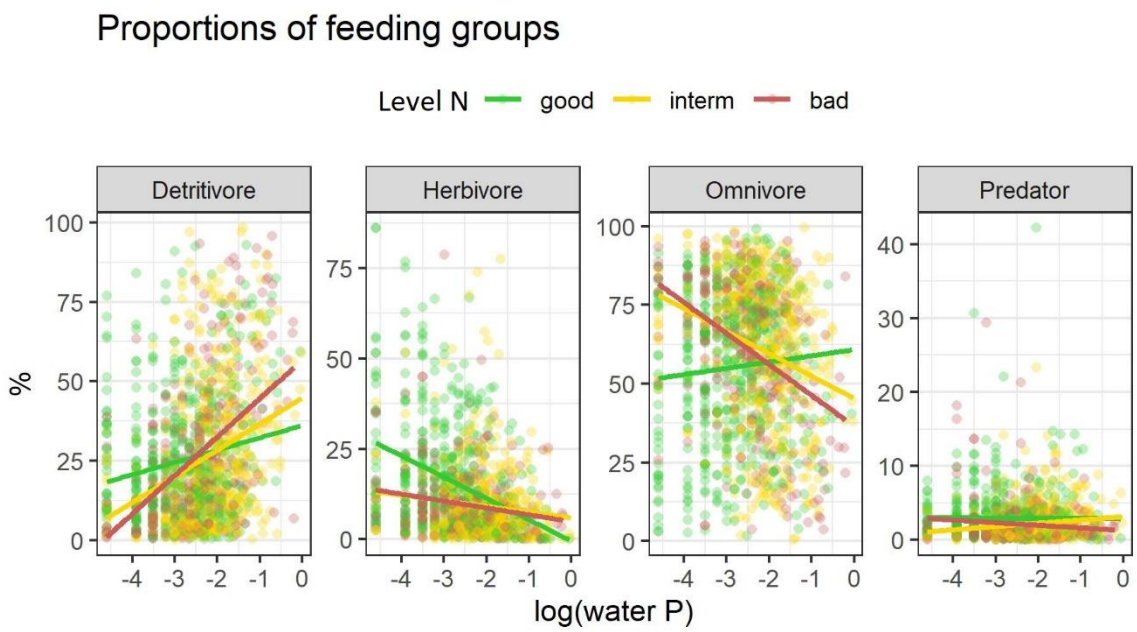


Fig. 4a.5: Proportion based on abundance of feeding groups in the macroinvertebrate community along water P concentration (log scale). Depending on the suggestion by model comparison, regression lines allow different slopes and intercepts for different levels of water N (low, intermediate, high, indicated by colors) or assume the same slope for each level of water N. Scattered data points represent raw data. Note that for better readability of the effects the scaling of y-axis differs between groups.

4. Discussion

We showed that community stoichiometry of most taxonomic and functional groups was significantly affected by water P concentration and that this effect depended on the level of N. Changes in taxonomic composition seem to be the main driver of this pattern, suggesting that species selection along a nutrient gradient also alters the nutrient pool provided by a consumer community.

Interestingly, community stoichiometry values did not follow the P concentration in the water which was in contrast to our first hypothesis that communities at high P sites should show higher %P and lower C:P and N:P than at low P sites. This is surprising, as Singer & Battin, (2007), also studying macroinvertebrates, found increasing community %P with increasing water P concentration and similarly to Prater *et al.* (2015) reported shifts towards P-rich taxa. Community stoichiometry is most likely mainly driven by community composition and originates from the proportion of taxa that are rich and/or poor for a given nutrient. Teurlincx *et al.* (2017) demonstrated that a shift in plankton community C:P was caused by a change in community composition following nutrient enrichment, rather than

stoichiometric plasticity of the individual taxa. Although there are stoichiometric variations among families or genera (McManamay *et al.*, 2011; Morse *et al.*, 2012; Mehler *et al.*, 2013), those variations should be larger among low classes or orders (Fagan *et al.*, 2002; González *et al.*, 2011; but see also González *et al.*, 2018) due to general differences in body structure or metabolism between more distant lineages resulting in more contrasted nutrient requirements. In our study, the decrease in community %P with increasing water P went along with a sharp decrease of insect abundances at low N sites – a generally P-rich class with many species being sensitive to high nutrient levels (Friberg *et al.*, 2010). Malacostraca and Oligochaeta, which together with Insecta were the most abundant taxonomic groups, are less rich in P and increased in proportion with increasing water P at low N level. This probably caused the overall decline in community %P stoichoimetry. The proportion of Oligochaeta increased independently of N level (and especially at high N sites), which is not surprising as taxa of this group tend to dominate under high nutrient conditions (McCormick *et al.*, 2004; Ortiz & Puig, 2007). The opposite pattern in Malacostraca and Insecta under higher levels of N – a moderate increase of Insecta and a decrease of Malacostraca within the community – could explain the lower effect on community %P under these conditions.

Zooming in on class level, we found that community stoichiometry was also not stable within taxonomic groups, indicating that the mechanism of shifting taxonomic composition causing overall community stoichiometry shifts, also happens within lower taxonomic groups. The groups however varied in their response. For Insecta and Malacostraca we could confirm our hypothesis of increasing community %P (and decreasing C:P) with increasing water P concentration, independently of N level. Within these groups there seemed to be a shift towards P-rich taxa under high P concentrations, for example increase in P-rich Diptera in Insecta while proportions of P-poor Coleoptera decreased (data not shown).

This effect of taxonomy on community stoichiometry seemed to over-shade that of feeding behavior. Community stoichiometry values of feeding groups were less distinct from one another compared to taxonomic groups. They generally were affected in the same way by water nutrient concentrations and showed the same pattern as we found for the whole community. This is contradictory to our hypothesis, that community stoichiometry within feeding groups will follow nutrient concentrations in the water and, as a result, quality of their resources to reduce stoichiometric imbalances. It underlines the major effect of taxonomy: Feeding groups are taxonomically diverse and several studies already demonstrated that taxonomy better explains organism stoichiometry than its feeding group (Allgeier *et al.*, 2020; Andrieux *et al.*, 2020). Considering only taxon richness rather than abundances Evans-White *et al.*(2009) found mean C:P stoichiometry values decreasing under high water P conditions within some (shredders collector-gatherers) – but not all – feeding groups.

Although the stoichiometric difference between feeding groups was lower than between taxonomic groups, their abundances were significantly affected by water P and N concentrations, thus indicating probably a minor effect on overall community stoichiometry. Likewise, it suggests a shift in functional

composition, with detritivores being favoured under high P conditions, while herbivore abundances decrease, which tend to follow expectations based on their stoichiometric traits (González *et al.*, 2011a; McManamay *et al.*, 2011). Compared to primary consumers, predators were only slightly affected by water nutrient conditions, which is in line with observations regarding taxon richness (Evans-White *et al.*, 2009). Although they can have a relatively high body P content (Cross *et al.*, 2003; González *et al.*, 2011a), their prey are stoichiometrically more stable than resources of primary consumers and prey identity has probably a more important influence on abundances. Some studies nevertheless reported increases in predators (Wimp *et al.*, 2010; Murphy *et al.*, 2012; Demi *et al.*, 2019), probably as a consequence of increased prey availability.

Outlook – community stoichiometry and ecosystem function

Nutrient cycling

Besides serving as resource for higher trophic levels, consumers also control autotroph production *via* the release of nutrients (Covich *et al.*, 1999; McManamay *et al.*, 2011; Metcalfe *et al.*, 2014). The individual contribution depends on the elemental ratio of the excreted material, which was negatively related to the body stoichiometry in some taxa (Elser & Urabe, 1999; Vanni *et al.*, 2002; McManamay *et al.*, 2011) although it seems to only play a minor role compared to taxonomy or feeding group. Nevertheless, it remains interesting to investigate whether the indicated link found for individual taxa also holds on community level, thus allowing the use of community stoichiometry to estimate nutrient release.

Our study focused on community stoichiometry as relative values and did not quantify available nutrients in terms of biomass. Ecological theory and some studies suggest a shift towards small-bodied taxa under high nutrient conditions (Singer & Battin, 2007; Demi *et al.*, 2019), which could decrease the overall mass of phosphorous despite these taxa having higher body %P.

A first estimation based on the data used in this study shows that although community %P decreased, the mass of phosphorous that is available in the community was not affected by neither water nutrient..

One specificity of freshwater systems is the biomass loss due to emergence of semi-aquatic insects (Raitif *et al.*, 2018). After passing successive aquatic larval stages, the organism transforms into a terrestrial adult and leaves the stream. From the ecosystem perspective, this provides an energy and nutrient transfer between the aquatic and terrestrial systems. Such material flow can be substantial (Martin-Creuzburg *et al.*, 2017; Bartrons *et al.*, 2018; Raitif *et al.*, 2018). For our data, the amount of phosphorous that should be lost from the system with emergence showed a strong decline when water phosphorous load increases (S 4a.10), indicating that a larger part of phosphorous will remain in the system for a longer time.

Accurately quantifying nutrient budgets and answering these questions could help understanding and estimating effects of nutrient enrichment on nutrient cycling beyond community scale.

Functional community composition

Due to (i) the link between body stoichiometry and biological traits, and (ii) taxa selection by water nutrient concentrations, changes in community stoichiometry likely come along with changes in the functioning of a system. One could assume a higher proportion of traits linked to high P body stoichiometry (*e.g.* fast growth rate, fast development, predatory feeding behavior) when the community phosphorous stoichiometry (%P) is high. In a previous study we already showed that communities under high water N:P conditions tend to have a higher community N:P stoichiometry and had higher abundances of slow-developing taxa (Beck *et al.*, 2021). Although they did not calculate community stoichiometry, other studies have reported decreases in taxon body size and life duration (Cross *et al.*, 2003; Ortiz & Puig, 2007). This suggests a faster cycling of nutrients through this part of the assemblage compared to a system dominated by long-living taxa. Building up on these results, investigating the link between community stoichiometry and biological trait-based groups could help to better understand and/or predict how changes in nutrient condition will alter community functioning.

It is to note that our study, by using stoichiometric trait information from a database, assumes macroinvertebrate homeostasis in a way that intra-taxon variation is smaller than inter-taxon variation. Even though deviations from strict homeostasis with ontogeny (Back & King, 2013), body size (Naddafi *et al.*, 2012; González *et al.*, 2018) or stoichiometric differences between sexes (Back & King, 2013) have been reported for some taxa, the large number of data used in this analysis makes us confident that this assumption still leads to meaningful results.

Compared to most works cited in this study that either conducted mesocosm experiments (Teurlincx *et al.*, 2017) or stream manipulations (Cross *et al.*, 2003; Demi *et al.*, 2019) to investigate the effect of

Infobox 1: And nothing else matters?

Nutrient concentrations are not the only factor in structuring communities. Habitat characteristics tend to be more important, and some communities might also show seasonal taxonomic shifts due to changes in environmental conditions (e.g. temperature, resources) or life cycle characteristics. This in turn is likely to affect community stoichiometry.

I therefore investigated the effects of season, stream size and hydroeco-region (HER) on community stoichiometry, relying to the data from the national database used in MS 3.

Generally, the integration of all three factors increased the variance explained by models describing the effect of water nutrient concentration on community stoichiometry, which clearly suggest a general importance. Stream size clearly was the most important parameter for all stoichiometric variables, except for %P where season was more important. Also in %C and other P-related variables season was more important than HER; whereas for %N and C:N this pattern was inverse. However, the general response of community stoichiometry towards water phosphorous and nitrogen condition did not vary among seasons or stream sizes, with few exceptions (e.g. intermediate streams where community ratios decreased at intermediate or high levels of N) and was in line with the overall result (MS 3). The responses among HERs were more variable, but nevertheless the general patterns (decreasing community mass contents & increasing community ratios with water phosphorous concentration) were found within most HERs.

Differences in community stoichiometry, i.e. higher values in %C, %N and %P in winter or lower values in very large streams corresponded to differences in community composition in terms of larger taxonomic groups (i.e. larger proportions of N-/ P-rich Insecta in winter; low proportions in very large streams), suggesting this parameter as the main driver of community stoichiometry. However almost all the streams sampled in winter had low levels of water phosphorous, thus providing conditions under which insects are more likely to dominate over pollutant resistant taxa groups. Although most of the observed patterns remained – partly insignificant and less clear – also within one nutrient level or within insects, this bias in the data sets prevented to clearly disentangle how the additional factors influence community stoichiometry, despite the large number of datasets.

Generally, community stoichiometry values were not well separated in terms of any of these factors and sampling events were rather grouped on a PCA plane. When grouped by water phosphorous or nitrogen status there was at least a slight trend from low over intermediate to high levels, but there was still no clear separation.

Details of this comparison and an extended discussion can be found in in Appendix a.

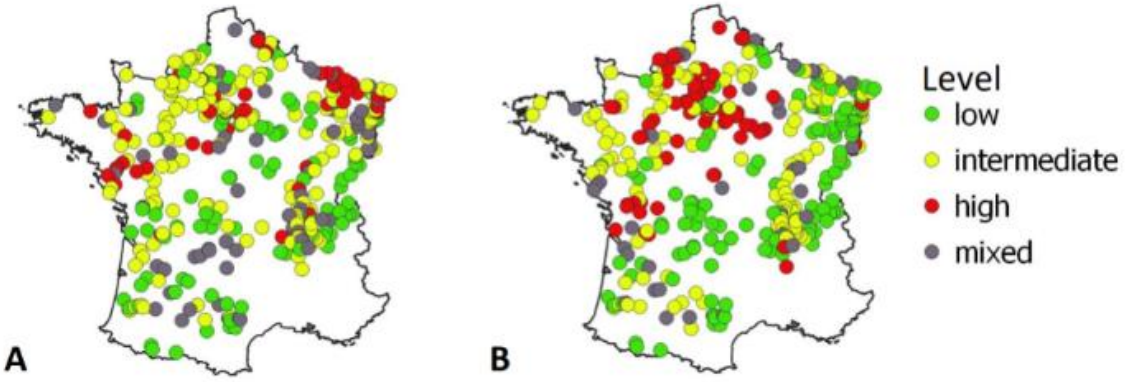
nutrient enrichment on consumer communities, we used data from large-scale field sampling of sites varying in nutrient levels. This strategy allows analysing a large number of sampling events, but it also

means a larger risk of non-controlled confounding factors. Although we reduced this risk by selecting sites of similar sizes and excluding polluted streams, additional morphological or hydro-geographical stream characteristics might have reduced the stoichiometric trends in our analysis. Despite this noise we have found a significant relationship in all stoichiometric variables, suggesting that there is a strong general link between environmental nutrient levels and community stoichiometry.

Conclusion

We showed that a shift in taxonomic composition that followed nutrient enrichment leads to a change in community stoichiometry. Even though this change did not perfectly follow our assumptions, it demonstrates that assumptions from ecological stoichiometry theory also hold on community level. Such changes mean an altered nutrient pool for higher trophic levels and were accompanied by shifts regarding feeding groups. Further consequences that come along with such changes in community stoichiometry (*e.g.* functional trait composition) or that follow from this altered nutrient pool (*e.g.* regarding nutrient cycling) remain to be more deeply investigated.

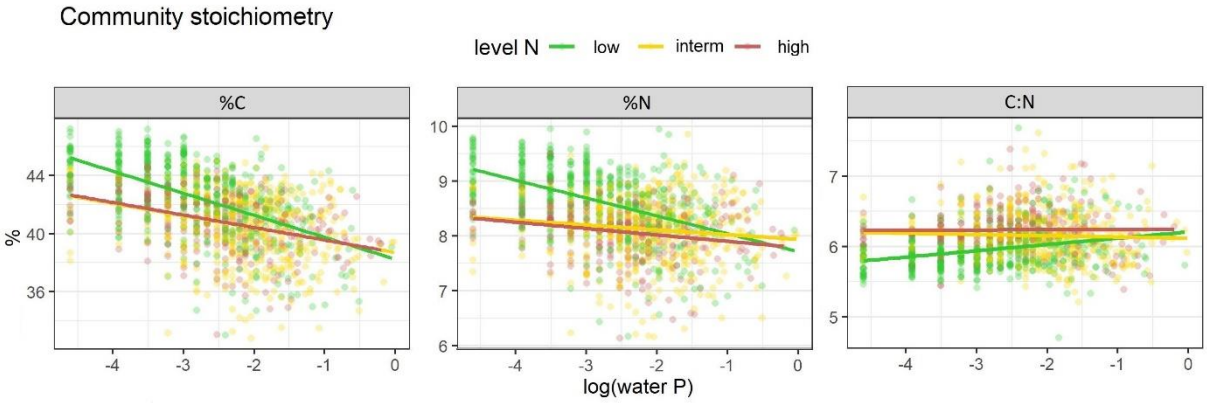
Supplementary material



S 4a.1: Positions of sampling sites (n=504) and their level regarding phosphate (A) and nitrogen (B) ("low", "intermediate", "high") indicated by different colors. Grey dots ("mixed") have been attributed when the nutrient level at a site changed between sampling events.

S 4a.2: Results of linear regressions for community stoichiometry. All models include an interaction term between water P concentration and level of N as suggested by model selection. The intercept of "low N" serves as reference to which the given values for "interm N" and "high N" indicate the change; for the slope the true values under the different N levels are given. Asterisks indicate significance of slopes/intercept with: * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$.

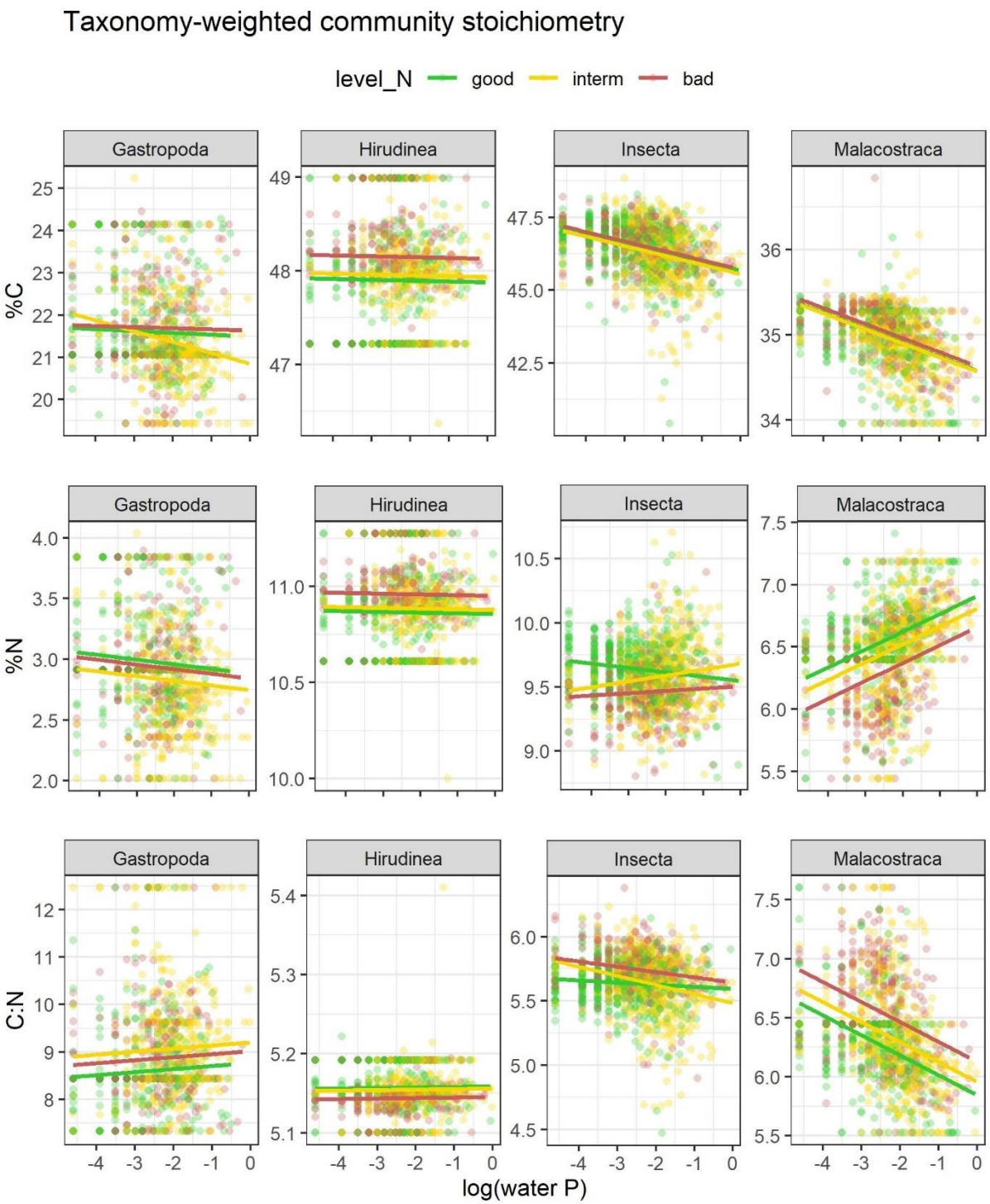
	Slope			Intercept			
Stoichio	Low N	Interm. N	High N	Low N	Interm. N	High N	r^2.adj
%C	-1.5127***	-0.8420***	-0.8605***	38.2451	0.4681	0.4632	0.2992
%N	-0.3249***	-0.0878***	-0.1163***	7.7211	0.2196*	0.0690	0.2552
%P	-0.0303***	-0.0061***	-0.0082***	0.6997	0.0329 *	0.0255	0.1902
C:N	0.0146***	-0.0032***	0.0002***	1.8246	-0.0158	0.0050	0.1415
C:P	0.0215***	0.0021***	-0.0012***	5.0653	-0.0288*	-0.0343*	0.0659
N:P	0.0081***	0.0066	-0.0006***	3.2315	-0.0111	-0.0381***	0.0317



S 4a.3: Community stoichiometry (%C, %N, C:N) along water P concentration (log scale). Regression lines allow different slopes and intercept for low, intermediate and high levels of water N, indicated by colors. Scattered points represent raw data (calculated community stoichiometry for the different SSE) with n=678/ 599/ 341 SSE, respectively for low/ intermediate/ high N levels. C:N values have been exponentiated (exp(x)) in order to facilitate to facilitate the comparison with other studies that mainly report untransformed ratio values.

S 4a.4: Results of linear regression for group-weighted community stoichiometry. The choice of whether to include an interaction between water nutrients was made based on model comparison *via* anova() function and models including such interaction term are indicated by "+". In models without interaction, the slope indicated for "low N" refers to the general slope of the model and consequently there are no slopes for intermediate and high levels of N available. The intercept of "low N" serves as reference to which the given values for "interm N" and "high N" indicate the change; for the slope the true values under the different N levels are given. Asterisks indicate significance of slopes/intercept with: * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$.

		Slope			Intercept				
Class	St.	Low N	Interm. N	High N	Low N	Interm. N	High N	r^2.adj	PXN
Gas	%C	-0.0441	-0.2568*	-0.0269	21.4878	-0.6395**	0.1423	0.0227	+
	%N	-0.0380**			2.8826	-0.1361***	-0.0399	0.0336	
	%P	-0.0019	-0.0115*	0.0028	0.2249	-0.0317**	0.0167	0.0218	+
	C:N	0.0079*			2.1674	0.0460***	0.0267**	0.0420	
	C:P	0.0069	0.0416*	-0.0089	5.6507	0.1073*	-0.0650	0.0211	+
	N:P	-0.0027	0.0297***	-0.0014	3.4431	0.0679*	-0.0379	0.0197	+
Hir	%C	-0.0091			47.8749	0.0563	0.2509***	0.0300	
	%N	-0.0037			10.8559	0.0207	0.0947***	0.0299	
	%P	0.0034			0.7138	-0.0141*	-0.0373***	0.0322	
	C:N	0.0001			1.6406	-0.0005	-0.0026***	0.0285	
	C:P	-0.0004			5.1307	0.0109	0.0397***	0.0248	
	N:P	-0.0006			3.4924	0.0116	0.0432***	0.0257	
Ins	%C	-0.3216***			45.6881	-0.1295*	0.0302	0.1278	
	%N	-0.0342***	0.0461***	0.0182***	9.5457	0.1368**	-0.0404	0.0851	+
	%P	0.0022	0.0193***	0.0150**	0.8799	0.0402***	0.0244*	0.0535	+
	C:N	-0.0031	-0.0128***	-0.0072	1.7204	-0.0197**	0.0093	0.0768	+
	C:P	-0.0045	-0.0217***	-0.0207**	4.9341	-0.0428***	-0.0270	0.0508	+
	N:P	-0.0016	-0.0091	-0.0151**	3.1972	-0.0219*	-0.0376**	0.0185	+
Crust	%C	-0.1713***			34.5757	-0.0085	0.0537**	0.2036	
	%N	0.1435***			6.9100	-0.1012***	-0.2522***	0.1864	
	%P	0.0060***			0.6740	0.0021*	0.0004	0.1242	
	C:N	-0.0269***			1.7669	0.0170***	0.0431***	0.1910	
	C:P	-0.0119***			4.9076	-0.0052***	-0.0031	0.1723	
	N:P	0.0104***	0.0185*	0.0205**	3.1212	-0.0003	-0.0178	0.1565	+



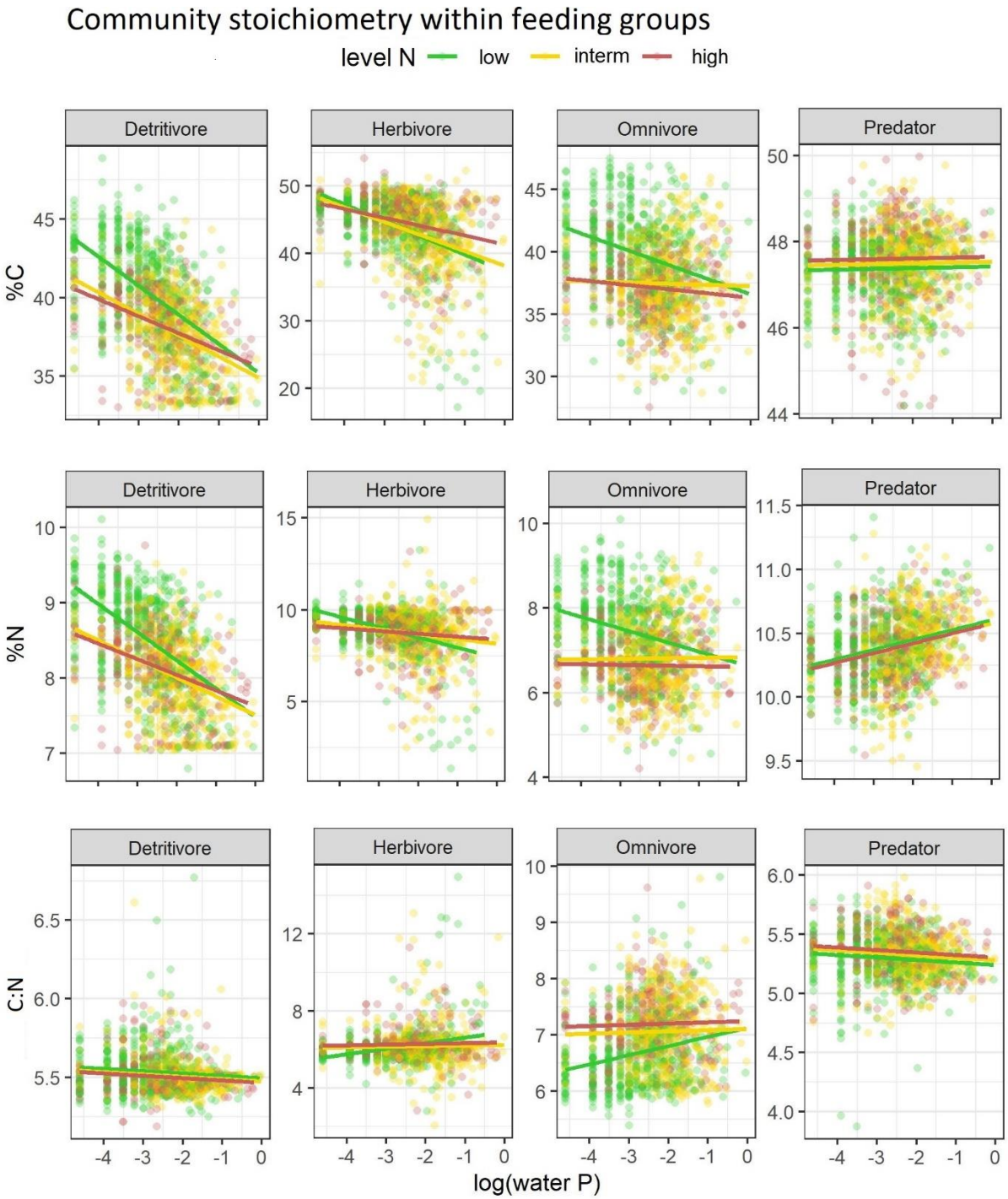
S 4a.5: Taxonomy-weighted community stoichiometry (%C, %N, C:N) along water P concentration (log scale). Depending on the suggestion by model comparison, regression lines allow different slopes and intercepts for different levels of water N (low, intermediate, high, indicated by colors) or assume the same slope for each level of water N. Scattered data points represent raw data (calculated community stoichiometry at the different sites). C:N values have been exponentiated (exp(x)) in order to facilitate to facilitate the comparison with other studies that mainly report untransformed ratio values.

S 4a.6: Results of linear regression on the proportion of taxonomic groups within the macroinverbrate community under the effect of water phosphorous concentration and nitrogen level. The choice of whether to include an interaction between water nutrients was made based on model comparison *via* anova() function and models including such interaction term are indicated by "+". In models without interaction, the slope indicated for "low N" refers to the general slope of the model and consequently there are no slopes for intermediate and high levels of N available. The intercept of "low N" serves as reference to which the given values for "interm N" and "high N" indicate the change; for the slope the true values under the different N levels are given. Asterisks indicate significance of slopes/intercept with: * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$. "(ns)" indicates models with an overall p value >0.05.

Taxonomic	Slope			Intercept				
group	Low N	Interm. N	High N	Low N	Interm. N	High N	r^2.adj	PXN
Bivalvia	0.0081			0.0235	0.0149**	0.0055	0.0153	
Gastropoda	0.0021			0.0118	0.0047*	-0.0034	0.0180	
Hirudinea	0.0044***			0.0193	-0.0006	-0.0025	0.0367	
Insecta	-0.1038***	0.0305***	0.0085***	0.2588	0.1981***	0.1074	0.1171	+
Malacostraca	0.0293*	-0.0719***	-0.1128***	0.3799	-0.1614**	-0.2479***	0.0380	+
Oligochaeta	0.0533***	0.0432	0.0983**	0.2733	-0.0136	0.1622***	0.1117	+
Turbellaria (ns)	-0.0002			0.0092	-0.0024	-0.0012	0.0008	

S 4a.7: Results of linear regression for community stoichiometry under a gradient if water P concentration and different levels of water N within functional feeding groups. The choice of whether to include an interaction between water nutrients was made based on model comparison *via* anova() function and models including such interaction term are indicated by "+". In models without interaction, the slope indicated for "low N" refers to the general slope of the model and consequently there are no slopes for intermediate and high levels of N available. The intercept of "low N" serves as reference to which the given values for "interm N" and "high N" indicate the change; for the slope the true values under the different N levels are given. Asterisks indicate significance of slopes/intercept with: * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$.

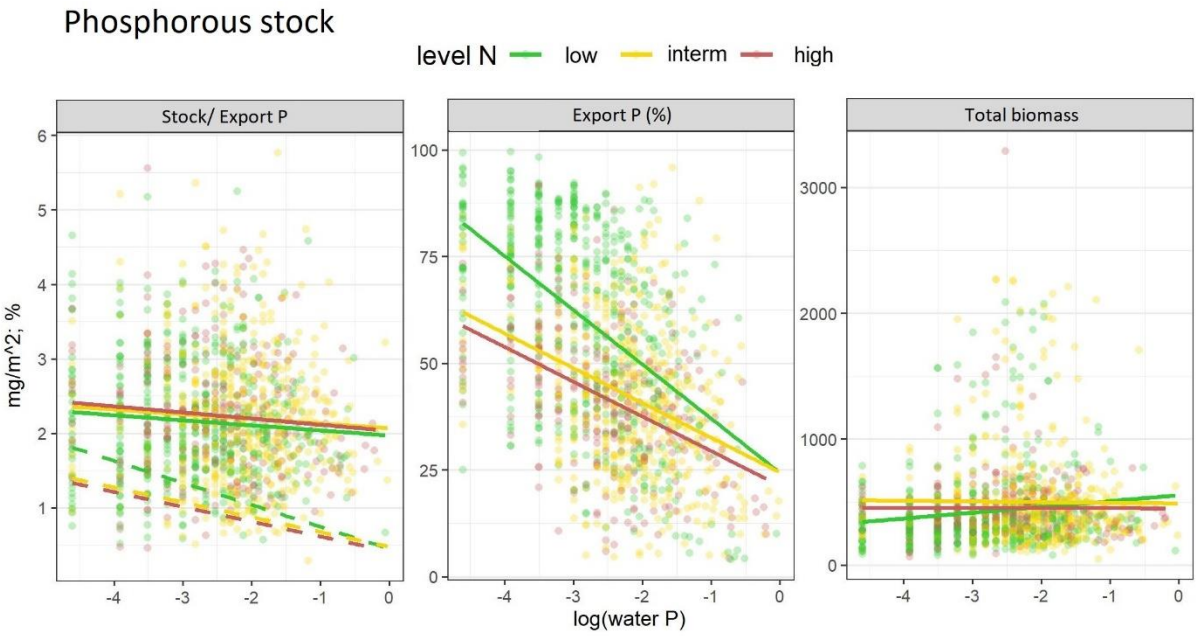
		Slope			Intercept				
	St.	Low N	Interm. N	High N	Low N	Interm. N	High N	r^2.adj	PXN
Detritivore	%C	-1.8490***	-1.3574**	-1.0802***	35.2058	-0.3043	0.3711	0.3456	+
	%N	0.0253***	0.1926***	0.8085***	-0.3747	0.1445	0.1267	0.3058	+
	%P	-0.0286***	-0.0172*	-0.0142*	0.7832	0.0159	0.0428**	0.0960	+
	C:N	-0.0026***			1.7035	-0.0035*	-0.0051**	0.0232	
	C:P	-0.0077*	-0.0013	0.0008	4.8083	-0.0118	-0.0251*	0.1145	+
	N:P	-0.0046*	0.0058**	0.0044*	3.0970	0.0039	-0.0146	0.0933	+
Herbivore	%C	-2.4873***	-2.1683	-1.3066***	37.3685	0.8328	4.0249	0.1579	+
	%N	-0.5587***	-0.2587***	-0.1568***	7.3907	0.7648***	0.9902***	0.0823	+
	%P	-0.0482***	0.0006***	0.0150***	0.6289	0.1224***	0.1382***	0.0504	+
	C:N	0.0370***	-0.0002***	0.0031**	1.9012	-0.0962***	-0.0667*	0.0252	+
	C:P	0.0599***	-0.0035***	-0.0287***	5.2606	-0.1523***	-0.1735***	0.0442	+
	N:P	0.0213***	-0.0039***	-0.0306***	3.3360	-0.0529***	-0.1033***	0.0364	+
Omnivore	%C	-1.1524***	-0.0957***	-0.3325**	36.6002	0.6423	-0.2776	0.1583	+
	%N	-0.2732***	0.0129***	-0.0165***	6.6999	0.1355	-0.0943	0.1641	+
	%P	-0.0247***	-0.0059**	-0.0138	0.6303	0.0057	-0.0161	0.1126	+
	C:N	0.0236***	0.0022***	0.0028	1.9597	-0.0053	0.0167	0.1317	+
	C:P	0.0282*	0.0114*	0.0096	5.1662	-0.0039	-0.0135	0.0604	+
	N:P	0.0067***			3.1987	-0.0077*	-0.0336***	0.0501	
Predator	%C	0.0184			47.4213	0.1110*	0.2249***	0.0094	
	%N	0.0772***			10.6028	-0.0259	-0.0264	0.0643	
	%P	-0.0353***	-0.0094***	-0.0276	0.7524	0.0624***	0.0021	0.0835	+
	C:N	-0.0037**			1.6561	0.0075**	0.0118***	0.0151	
	C:P	0.0360***	0.0127**	0.0308	5.0791	-0.0597**	0.0046	0.0554	+
	N:P	0.0437***	0.0200**	0.0366	3.4295	-0.0664**	-0.0071	0.0639	+



S 4a.8: Community stoichiometry (%C, %N, molar C:N) within functional feeding groups along water P concentration (log scale). Depending on the suggestion by model comparison, regression lines allow different slopes and intercepts for different levels/ status of water N (low, intermediate, high, indicated by colors) or assume the same slope for each level of water N. Scattered data points represent raw data (calculated community stoichiometry at the different sites). C:N values have been exponentiated (exp(x)) in order to facilitate to facilitate the comparison with other studies that mainly report untransformed ratio values.

S 4a.9: Results of linear regression on the proportion of feeding groups within the macroinvertebrate community under the effect of water P concentration and nitrogen level. All models include an interaction term between water P concentration and level of N as suggested by model selection. The intercept of "low N" serves as reference to which the given values for "interm N" and "high N" indicate the change; for the slope the true values under the different N levels are given. Asterisks indicate significance of slopes/intercept with: * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$.

Feeding	Slope			Intercept			
group	Low N	Interm. N	High N	Low N	Interm. N	High N	r^2.adj
Detritivore	0.0387***	0.0824**	0.1212***	0.3611	0.4485*	0.5673***	0.1047
Herbivore	-0.0590***	-0.0157***	-0.0190***	-0.0049	0.0594**	0.0487*	0.1405
Omnivore	0.0198	-0.0715***	-0.0983***	0.6081	0.4526***	0.3649***	0.0677
Predator	-0.0003	0.0044*	-0.0035	0.0285	0.0314	0.0127*	0.0191



S 4a.10: Phosphorous stock (mg/m^2), export (% of stored P), and total biomass (mg/m^2) groups along water P concentration (log scale). Solid lines in the first panel represent stored phosphorous, dashed lines represent the exported phosphorous. Scattered data points represent raw data (calculated community stoichiometry at the different sites).

Linking bio/ecological to stoichiometric traits – at community level

1. Introduction

Understanding how environmental parameters impact a community, in terms of both taxonomic and functional composition is one aim of community ecology as this will help to understand its response towards future stressors. Worldwide, one main stressor in freshwater ecosystems are changing – mainly increasing but also decreasing – nutrient concentrations, notably nitrogen (N) and phosphorous (P). This often also alters the ratio of these nutrients (Peñuelas *et al.*, 2012, 2013; Yuan *et al.*, 2018). In stream environments, nutrient enrichment changed macroinvertebrate taxonomic as well as functional composition: Communities shifted towards more tolerant taxa, *e.g.* oligochaets, snails or Chironomidae (McCormick *et al.*, 2004; Ortiz & Puig, 2007; Friberg *et al.*, 2010) and abundances of small and fast-growing and –developing taxa increased (Singer & Battin, 2007; Prater *et al.*, 2015).

Ecological stoichiometry provides a framework that investigates the interaction between nutrients in an organism and the environment, aiming to understand ecological processes and interactions (Sterner & Elser, 2002). Taxa are selected (among other factors) based on their nutrient requirement and will be present where the resource quality is close to their requirements. Assuming that body stoichiometry – approximated here by the elemental composition of an organism – represents the (taxon-specific) demand for an element, abundances of phosphorous-rich taxa (high body %P, low C:P and/or N:P) thus will be higher under high phosphorous conditions, while nutrient depleted environments will favour taxa that require less nutrients (Singer & Battin, 2007; Teurlincx *et al.*, 2017; see also MS 5). These shifts affected also the overall community stoichiometry and followed that of water phosphorous concentration.

Functional traits have already been integrated into the ecological stoichiometry framework, demonstrating a link between body stoichiometry and functional feeding groups (Fagan *et al.*, 2002; Evans-White *et al.*, 2005; González *et al.*, 2011a) or life history traits (Main *et al.*, 1997; Elser *et al.*, 2003; Acharya *et al.*, 2004; see also MS 4). Likewise, EST could explain distinct responses of functional feeding groups towards nutrient enrichment (Evans-White *et al.*, 2009) or the increasing abundances of small, fast-growing taxa mentioned above. In a previous chapter (MS 2) we investigated the link between a range of potential biological and habitat-related traits and body stoichiometry in stream macroinvertebrates, showing that indeed there is a correlation for a range of individual traits as well as for overall trait profiles. So far, there is no study analyzing this link on community level. The aim of this chapter is therefore to investigate whether the link between stoichiometric and other functional traits as demonstrated on taxon level (MS 2) also holds on community level. This can improve our understanding of how nutrients in the environment might affect the community functional structure.

Assuming that taxa will be selected based on their requirement (*e.g.* for nutrients, substrate) and that different functional traits lead to different nutrient demands of an organism– expressed as body

stoichiometry –, communities differing in the overall stoichiometry should also vary in their functional composition. We thus hypothesize that community trait profiles are linked to community stoichiometry. More precisely, the co-variation should follow that found on taxon level (MS 2), for example communities rich in phosphorous (high community %P, low community C:P % and/or N:P) should have a high affinity for small-bodied, frequently reproducing and molting taxa. They should also favour deposit-feeders and/or taxa preferentially feeding on detritus and dead plant material.

2. Material & methods

The analysis was based on the same abundance data sets as in MS 3 where details on the data sets and selection filters (*e.g.* stream size) can be found. The selected data sets comprises 1158 sampling events from small and medium sized streams distributed all over France over a 14-year period.

Community stoichiometry values for data set were taken from MS 3. They have been calculated as abundance weighted mean stoichiometric values (%C, %N, %P, molar C:N, C:P, N:P). Details on the calculation are provided in that chapter (MS 3).

Community trait profiles were calculated for each sampling event as community-weighted mean affinities. The trait information was obtained from (Tachet *et al.*, 2010) and modified as described in MS 2, where also information on the categories of each trait can be found (Tab. 3.2, p. 84). The data provided is fuzzy coded, meaning that each trait consists of several trait categories (or modalities) for which the affinity of each taxon for this category is indicated by a number ranging between 0 (=no affinity) and 5 (= high affinity). This data was first transformed to obtain relative frequencies for each trait for each taxon. For each trait, the mean affinity of a community for each trait category was then calculated: The relative abundance of each taxon was multiplied by the relative frequency for each trait category, before summing up the values for all taxa of the community. Then, relative frequencies for each trait category within a trait were calculated based on the obtained values. Abundances were log(n+1) transformed prior to the analysis.

Co-inertia analyses were then applied to investigate the co-structure between community stoichiometry and community trait affinities, both on single traits or overall trait profiles comprising all 23 biological and habitat-related traits. Details on the method and the application can be found in MS 2. Compared to the analysis on individual level, this analysis treated sampling events *i.e.* community values as “taxon”. Further, stoichiometric ratio values were not log-transformed prior to the analysis, because community stoichiometry values have already been calculated on log-transformed values to avoid bias due to ratio data (Isles, 2020).

Please note that the comparison towards the analysis on taxon level is only qualitative without applying statistical methods to quantify the differences between both approaches.

3. Results

Co-variation between community functional trait and stoichiometric trait profiles

There was a significant co-variation between community functional trait affinities and community stoichiometry, both for all individual traits as well as the overall trait profile. RV values ranged between 0.09 for "respiration" and 0.62 for "aquatic life stage; that of the overall trait profile was 0.52.

In all analyses – either using single traits or overall trait profiles – the RV values were clearly higher when analysed on community level, indicating a stronger co-variation than on taxon level. Only for the trait "food resources", it was higher in the latter. Additionally, the co-variation was significant for all traits on community level, while on taxon level that was the case for only 16/23 traits.

As in the analyses on taxon level, clearly the largest part of variation was along the first axis also when analysed on community level. This proportion compared to what was explained by the second axis here was even larger for most traits and in 18 of the 21 traits with more than two trait categories >95 of the variation was along the first axis (vs. 9/21 on taxon level).

The following descriptions of single trait analyses will focus on the same traits discussed in detail in MS 2 (Fig. 4b.1 + 4b.2) and due to its clearly dominating importance will be limited to positions along the first axis only. Figures of all other traits are provided in the supplementary (S 4b.1).

Associations between stoichiometric and functional community traits found in the multi-trait analysis were – for most trait categories – in line with findings from the single trait analyses (S 4b.2).

Tab. 1: Results of the co-inertia analyses between functional traits and stoichiometric profiles (%C, %N, %P, C:N, C:P, N:P) of macroinvertebrate communities, either as single or multiple-trait profiles. The RV-coefficient indicates the level of co-structure between the two sets of traits, additionally the proportion of projected inertia on the first two axes is given. Bold writing indicates significant p-values after adjustment for multiple testing after Bonferroni. n_{mod} indicates the number of trait-modalities, the number of sites was 1158 for each analysis.

				Projected inertia (%)	
Type	Functional trait	RV	n	Axis 1	Axis 2
Bological					
Development	Life duration	**0.3063**	2	100	NA
	Maximum body size	**0.24**	5	86.9565	8.6548
	Molting events	**0.4859**	7	98.2364	1.5012
	Reproductive events	**0.3173**	4	98.2322	1.507
	Voltinism	**0.1857**	3	99.8439	0.1561
Feeding	Feeding habits	**0.215**	7	87.4243	7.2231
	Food	**0.1213**	7	77.1319	15.4054
Other	Aquatic life stages	**0.6164**	4	99.0087	0.9756
	Dispersal	**0.4441**	4	98.9453	0.871

	Locomotion	**0.243**	5	95.0631	4.7158
	Reproduction mode	**0.5233**	8	97.8872	1.5771
	Resistance forms	**0.1772**	4	97.7006	2.0105
	Respiration	**0.0954**	4	90.8282	9.1077
Habitat	Altitude	**0.3966**	3	99.8965	0.1035
	Longitudinal distribution	**0.4452**	8	98.6328	0.9251
	Low pH sensitivity	**0.112**	6	92.7464	5.3876
	Salinity Tolerance	**0.5324**	2	100	NA
	Saprobity	**0.3868**	5	98.5573	0.986
	Substrate	**0.4178**	9	95.9469	3.4331
	Temperature	**0.1673**	3	97.1827	2.8173
	Trophic status	**0.2711**	3	99.6012	0.3988
	Transversal distribution	**0.3547**	6	99.0722	0.7049
	Velocity	**0.5035**	4	99.573	0.3815
Multi- trait profile	(all the above)	**0.521**	-	94.64	0.94

Developmental traits

Life duration

Communities with high %C, %N and %P were associated to a high affinity for a life duration of less than one year while longer life spans were associated to high community molar ratios. Regarding P-related stoichiometry, this was also observed on taxon level. C:N ratio however was associated to short life spans and %C and %N were (weakly) associated to taxa living longer than one year.

Maximal body size

On both taxon- and community level, small maximal body sizes (“0.5-2cm”; “1-2cm”) were positively associated to high %P and high %N. While on taxon level, also intermediate sizes (“2-4cm”) were co-varying with the small body sizes, on community level they were associated to the two extreme categories (“<0.5cm”, “>4cm”) and co-varying with high C:N, C:P and N:P.

Molting events

Undergoing the highest numbers or an intermediate number of molting events (“>20”, “15-19”, “5-9”) was associated to high %P, %N and %C. Also showing 2-4 molts was co-varying along the first axis, but with a lower strength. Direct development without molting or undergoing one or 10-14 molts was associated to high community C:N, C:P and N:P ratios. This is generally in line with the results from taxon level, although in that analysis only no and one molt were co-varying with high community ratios.

Voltinism

Affinities for producing less than one or one generation per year were associated to high community %C, %N, %P and N:P ratios. Poly-voltinism and high C:P and C:N ratios were positioned at the opposite

side of the first axis. These associations were also found on taxon level, with the exception mono-voltinism was co-varying with poly-voltinism rather than slow generation times.

Feeding traits

Feeding habits

High community affinities for "scraping" were "swallowing/chewing" strongly; "shredding" and "deposit-feeding" only weakly negatively associated to the first axis, as were high community mass elements. "Piercing", "filter-feeding" and parasitic feeding were co-varying with high community molar ratios. On taxon level, "scraping" and "shredding" were associated to high C:N and C:P ratios, while parasitic feeding was co-varying with "piercing".

Food resources

Although the first axis clearly accounted for the largest part of variation (77.13%), the second axis still captured 15.41%. Feeding on macro- and microphytes and dead plants was co-varying with high community %C, %N and %P along the first axis, with the second axis separating %C, %N and "microphytes" from "%P", "macrophytes" and "dead plant". The negative association of detritus towards the first axis was only very weak, but it was strongly negatively associated towards the second axis, co-varying with "dead plant". Feeding on animal prey and high community ratios had positive coordinates along the first axis. Considering associations to the second axis, feeding on living macrovertebrates and microinvertebrates was co-varying with high community C:P and N:P ratios. High C:N ratios were associated to feeding on "dead animal". On taxon level, taxa feeding on macroinvertebrates were strongly co-varying with high %C and %N contents. Relying on microinvertebrates or microphytes was associated to high C:P and C:N ratios. As on community level, feeding on dead animal was clearly associated to high body C:N, and taxa relying on detritus, dead plant or microphytes were positioned between high %P and high C:N ratios.

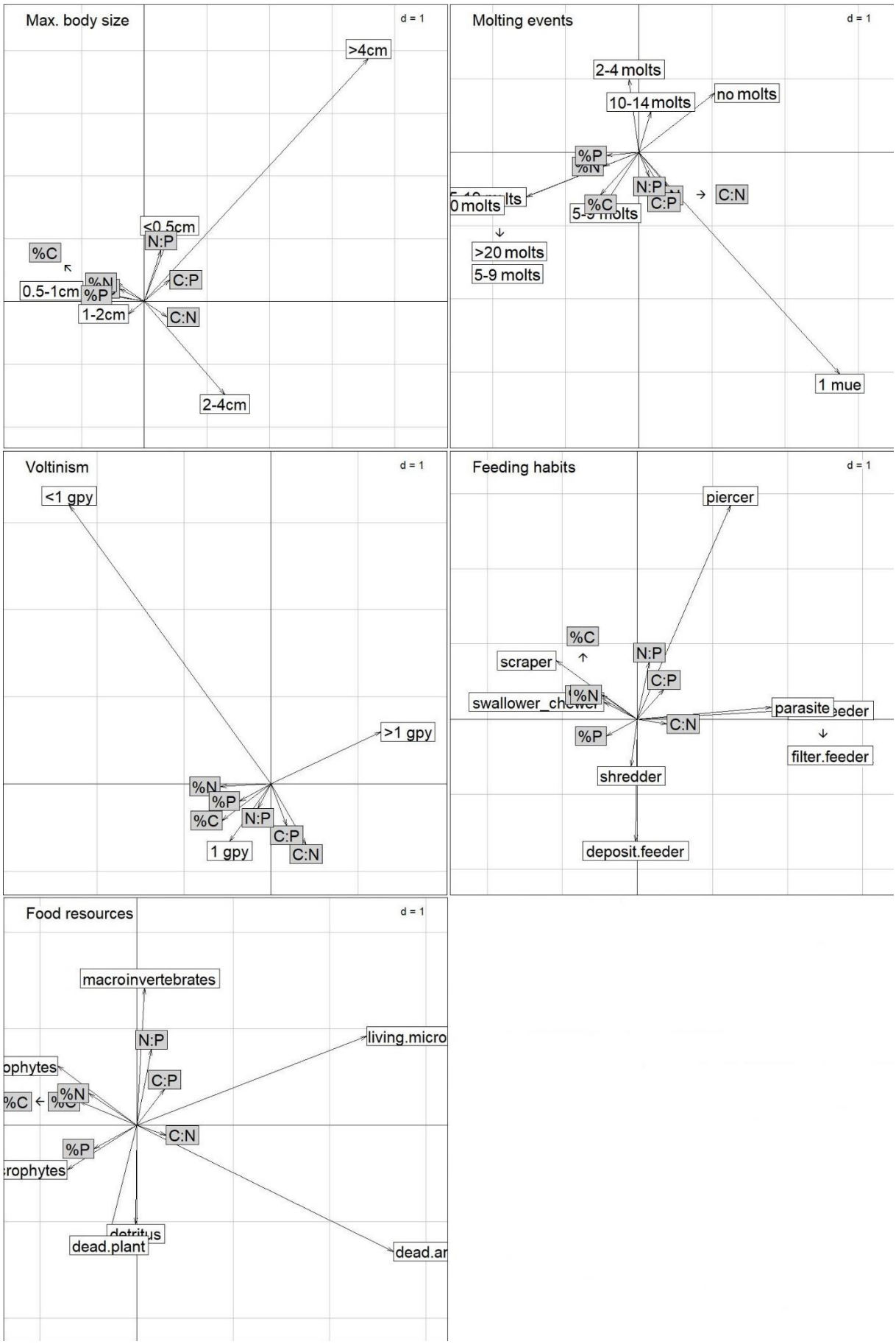

Max. body size
d = 1
>4cm
<0.5cm
N:P
%C
C:P
0.5-1cm
%N
%P
1-2cm
C:N
2-4cm
Molting events
d = 1
2-4 molts
10-14 molts
no molts
%P
%N
N:P
%C
C:P
C:N
0 molts
5-9 molts
>20 molts
5-9 molts
1 mue
Voltinism
d = 1
<1 gpy
>1 gpy
%N
%P
%C
N:P
C:P
1 gpy
C:N
Feeding habits
d = 1
piercer
%C
N:P
scraper
C:P
%N
parasite
C:N
%P
filter.feeder
shredder
deposit.feeder
Food resources
d = 1
macroinvertebrates
N:P
living.micro
C:P
%N
C:N
%P
dead.plant
dead.ar

Fig. 4b.1: Results from single trait co-inertia analyses investigating the co-structure between community stoichiometric and biological traits. The functional trait categories and stoichiometric traits are located on the first co-inertia plane. For the trait "life duration" described by only two categories no graphical representation is provided as the whole inertia was projected on a single axis.

Habitat-related traits

Altitude

As on taxon level, the first axis separated alpine and piedmont habitats from lowlands. The first were co-varying with high community nutrient contents, while the latter was associated to high community ratios.

Longitudinal distribution

Preferences for upper stream parts ("crenon"," epirithron", "metathritron", "hyporithron") were co-varying with high community %C, %N and %P. Communities with high affinities for epipotamon, metapotamon, estuary or outside river habitats were associated to high community C:N, C:P and N:P ratios. This was also found on taxon level.

Substrate

The first axis separated preferences for fine-grained substrates ("sand", "silt", "mud"), micro- and microphytes and detritus/litter from coarser materials ("gravel", "flags/boulders/pepples") and twigs/roots. The first were associated to high community molar ratios; the latter to high community %C, %N and %P. This was similar to the links found on taxon level, regarding fine-and coarser-grained material and microphytes, but communities with a high preference for detritus/litter were associated to high %C, %N and %P rather than high molar ratios. The association of detritus on community level however was only weak.

Temperature

Termophily and eurythermy were associated to high C:N, C:P and N:P ratios, both on taxon- and community level. Low temperature preference was co-varying with high body and community mass elements.

Velocity

Both on community- and taxon level, high affinities for medium and fast water velocities were associated to high %C, %N and %P, while standing ("null") or slow flowing waters were associated to high molar ratios

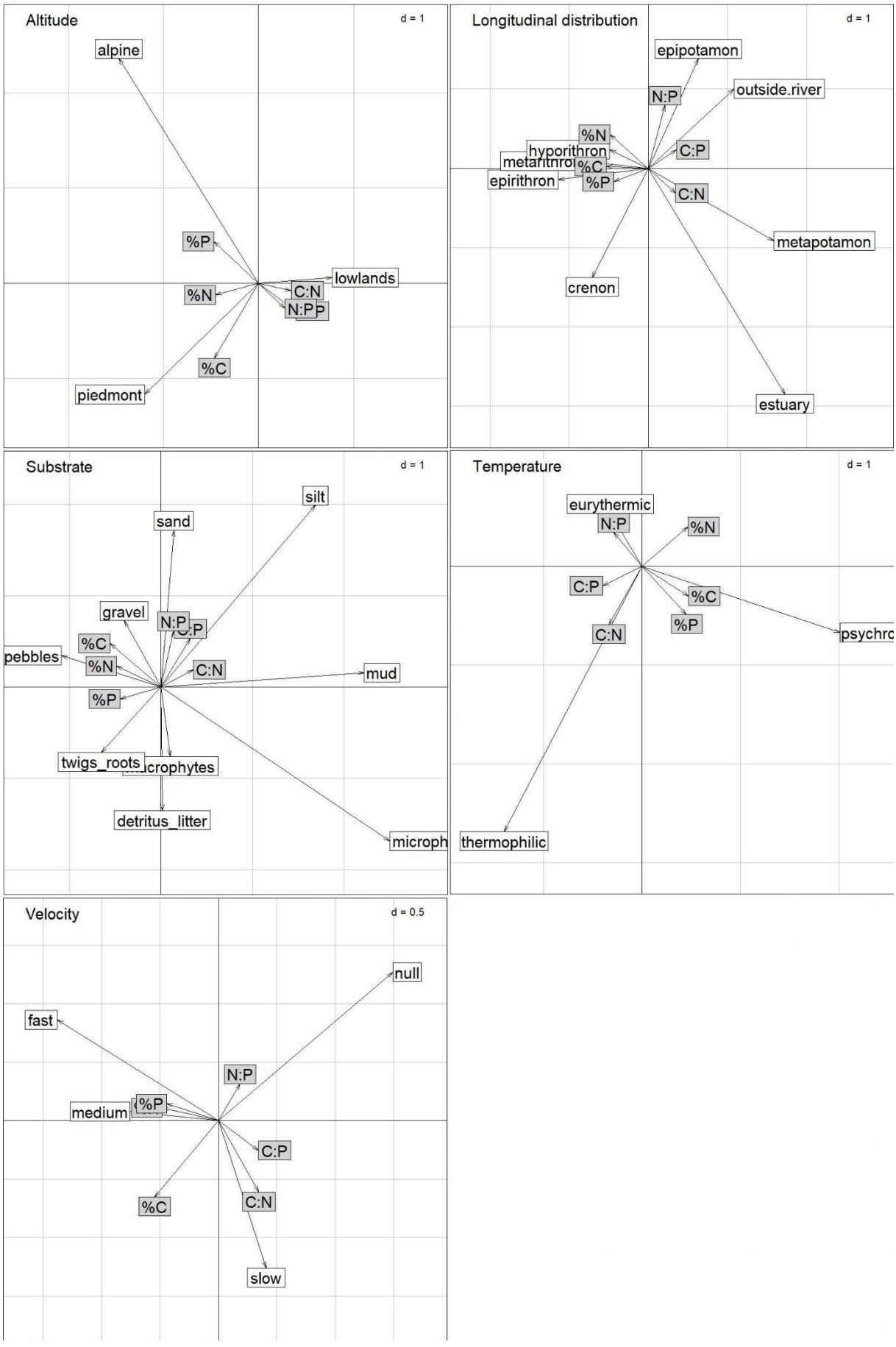

Altitude
d = 1
alpine
%P
lowlands
%N
C:N
N:P
%C
piedmont
Longitudinal distribution
d = 1
epipotamon
N:P
outside.river
%N
hyporithron
C:P
%C
epirithron
%P
C:N
metapotamon
crenon
estuary
Substrate
d = 1
silt
sand
gravel
N:P
C:P
%C
pebbles
%N
C:N
mud
%P
twigs_roots
detritus_litter
microph
Temperature
d = 1
eurythermic
N:P
%N
C:P
%C
%P
C:N
psychro
thermophilic
Velocity
d = 0.5
null
fast
N:P
%P
medium
C:P
%C
C:N
slow

Fig. 4b.2: Results from single trait co-inertia analyses investigating the co-structure between community stoichiometric and habitat-related traits. The functional trait categories and stoichiometric traits are located on the first co-inertia plane. For the trait "life duration" described by only two categories no graphical representation is provided as the whole inertia was projected on a single axis.

4. Discussion

We showed that there is a strong link between community stoichiometry and traits, both for individual traits as well as for overall trait profiles or trait-groups. This association was stronger than on taxon level (MS 2) but the general co-structures found between both types of traits were similar and are in line with EST.

For most traits the analysis of single trait and multiple-trait profiles confirmed the hypotheses we made prior to our previous analysis based on concepts in ecological stoichiometry, that have already been demonstrated on taxon level (MS 2).

Communities that are rich in phosphorous and nitrogen show a high affinity for small, short-lived taxa with high number of molting & reproduction events. All of these trait categories often correspond to taxa with fast body growth and/ or development rates. These correlations were generally also found on taxon level and are well in line with assumptions based on Growth Rate Hypothesis (=GRH, Elser *et al.*, 1996): Taxa with high growth rates require high amounts of phosphorous for mRNA synthesis which is required for protein synthesis and therewith growth. This high phosphorous demand leads to high body %P. While this link has been shown for several taxa (Elser *et al.*, 2003a; Acharya *et al.*, 2004), results on community level beyond planktonic assemblage are rare. However, it has already been demonstrated that nutrient enrichment increased community %P and went along with increasing abundances of fast-growing taxa (Singer & Battin, 2007; Prater *et al.*, 2015) and also fast-developing taxa were more abundant in phosphorous-rich streams (Beck *et al.*, 2021 (MS 4)). Also our results suggest a change in the size and/or age structure of the community which is likely to affect the secondary production and the nutrient flow through trophic levels (Huryn & Wallace, 2000).

Initially, we also assumed multi-voltinism to be an indicator of fast development and therewith to be associated to high phosphorous contents. However, as on taxon level, it was linked to communities with high C:N and C:P ratios. Generally the stoichiometric traits were not well separated by the analysis, suggesting that – as on taxon level – the analysis was mainly driven by mono-voltinism. Since this can be expressed by 89% of taxa, also community affinities were higher than for a semi- or multi-voltinism.

High community %N and %P were associated with high affinities for feeding by scraping (and chewing/swallowing) and feeding on micro- and macrophytes, although on taxon level these functional trait categories were associated to low nutrient taxa. Low nutrient communities had high affinities for filter-feeding and piercing/parasitic feeding habit and high preferences for animal resources (both living and dead). Surprisingly, predatory feeding was not clearly associated to nutrient-rich communities as we expected due to a generally higher nutrient content (esp. nitrogen) of predators (Fagan *et al.*, 2002;

Evans-White *et al.*, 2005; González *et al.*, 2011a) and results from the taxon level analysis. Although they showed a high affinity for the corresponding feeding habit (swallowing/chewing), feeding on macroinvertebrates was linked to nutrient poor communities. One reason is probably that also other feeding modes allow consuming macroinvertebrates (*i.e*. piercers, parasites), which were linked to low nutrient content communities. Additionally, predators tend to only account for a small proportion of the communities (MS 3) so that although they are nutrient rich they likely do not drive the overall community stoichiometry. On taxon level, feeding-related traits seemed (partly) driven by taxonomy, notably Mollusca. Their common feeding habits scraping and filter-feeding and the corresponding resources (dead animal, microphytes and –invertebrates) were associated to taxa with low body nutrient contents. Bivalves and gastropods generally have higher C:N and C:P ratios than other macroinvertebrate groups (MS 1, Chap. 2b). However they often only account for a minor part of the macroinvertebrate community in terms of abundances (see *e.g*. MS 3 (for these datasets), MS 5), which probably suppressed a large dominance compared to the analysis on taxon level. Nevertheless, also within insects, scraping and feeding on microphytes and dead plant was associated to taxa and communities with high C:nutrient ratios (results not shown).

High nitrogen contents – both on taxon and community level – were linked to aerial active dispersal. As discussed in MS 2 this could either be due to the rather high nitrogen content of insects compared to other macroinvertebrate groups or/and a large investment into N-rich muscle tissue of flight organs. Considering the community, we could thus assume a higher proportion of emerging insects in nutrient-rich communities, which affect nutrient-cycling in the aquatic (*via* energy/nutrient loss) and the surrounding terrestrial system (*via* energy/nutrient input) (Martin-Creuzburg *et al.*, 2017; Bartrons *et al.*, 2018).

For habitat-related traits, results between community and taxon level were generally more similar than for biological traits. Also here, the co-structures between stoichiometric and functional traits can be well explained by concepts from River Continuum Concept (=RRC, Vannote *et al.*, 1980), describing biological and physical geomorphic characteristics of a stream from its source to its mouth. Headwater streams have a high amount of allochthonous material input (leaf litter) and provide (large) boulders and gravel as substrate. Temperatures are low due to their location at high altitudes. All these characteristics were associated to communities rich in %N and %P, indicating a selection for taxa with high body nutrient contents. Moving further downstream, fine-grained substrates (sand, mud) become dominating and temperatures and nutrient load (due to increased agriculture activities and an inflow from upper stream parts) increase, while communities become poorer in nutrients (higher C:N, C:P and/ or N:P ratios). With these habitats the proportion of bivalves and gastropods in the community increases, which both have significant lower body C:N and C:P stoichiometry than other taxonomic macroinvertebrate groups. On the contrary, insects generally have a high %P content and often dominate headwater

streams. The link between stoichiometric and functional traits thus well represents and helps explaining the taxonomic distribution we expect to find along the longitudinal river continuum.
Contradictory to our initial hypothesis – but in line with results on taxon level – communities with a high affinity for eutrophic habitats were low rather than rich in nitrogen and phosphorous. This supports our finding that community stoichiometry (%N, %P) decreased along a gradient of water phosphorous concentration, *i.e*. that increased resource quality did not favour nutrient-rich taxa when considering the whole community. Considering only the insects in the communitites however supported our initial hypothesis and high P communitites had high affinitites for eutrophic habitats (data now shown).

Infobox 2: Does size matter after all?

Even though taxa might dominate a community in terms of numbers, when they are only small, their overall impact on functioning and nutrient cycling might be reduced low. Integrating biomass in community analyses could therefore lead to different conclusions.

Redoing parts of my analyses with biomass-weighted abundances, I found that the general pattern (e.g. correlation to an environmental variable) was the same in most indices analysed, but for others went in the opposite direction.
Even if the direction of response was the same, biomass-weighting often lead to steeper slopes, which one would interpret as a larger effect of the environmental variable. The absolute values differed quite substantially, especially when addressing the whole community, clearly demonstrating the large impact of heavy taxa – in our case shell-bearing bivalves. Diversity values were lower when integrating biomass, whereas for the proportions of taxonomic groups this was group-specific (values of heavy taxa such as Bivalves, Gastropoda and Crustacea were increased when weighted by biomass; while those of light Insecta decreased). Also, community stoichiometry was clearly driven by the low nutrient contents of Bivalvia when abundances were weighted by biomass. When analyzing only the insect community, the difference between simple abundance and biomass-weighted values was smaller, due to a narrower biomass range of this group.

Also multivariate analyses detected stronger links between functional traits and environmental variables when biomass was considered. This was the case in both types of analyses, that either used taxa abundances directly (RLQ-analysis) or only indirectly *via* calculated community mean values (co-inertia).

This shows, that calculations or analyses done using one kind of data should not simply be translated to the other. The observation that some variables showed varying responses depending on the underlying community used (complete vs. insect community) underlines this complexity. The choice therefore should be made in advance, based on the ecological question addressed.

Details of this comparison and an extended discussion can be found in Appendix b.

It is obvious, that phylogeny had an effect also on the other functional traits *via* general stoichiometric differences between taxonomic groups and taxon-specific trait syndromes. This effect however was larger on taxon level and community-wise calculated trait affinities seemed to partly dampen this effect, at least for biological traits (scraper not clearly related to high C: N). Compared to the study on individual taxa and potential traits, this analysis was based on abundance data and therewith gives information on the realized potential traits. However, community-weighted mean values do not necessarily represent the optimal strategy in a community as there could be quite different trait expressions. Muscarella & Uriarte (2016) showed that most taxa with traits close to community mean trait appeared at higher abundances in the corresponding plant community, but that many taxa also deviated in several traits from the mean value. Nevertheless, this approach is often used in plant ecology but has also been applied on potential traits, *i.e.* trait affinities, of macroinvertebrate communities (Gayraud *et al.*, 2003; Statzner *et al.*, 2004; Tillin *et al.*, 2006; Floury *et al.*, 2017; Aspin *et al.*, 2019).

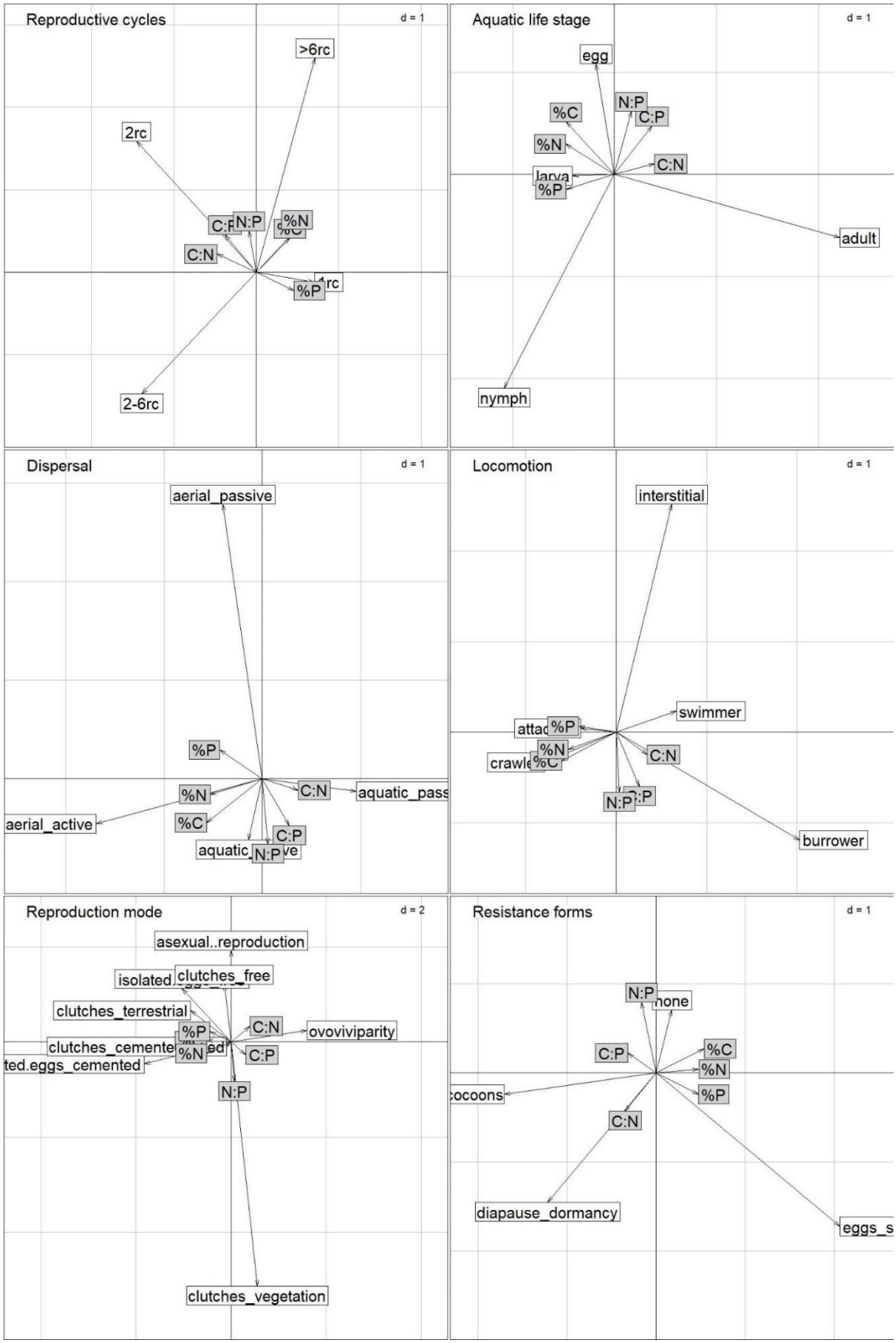
The use of community weighted mean values also lead to a closer to normal distribution of trait affinity values and a lower number of zeros in the dataset compared to taxon trait profiles, which could explain the higher RV values on community level.

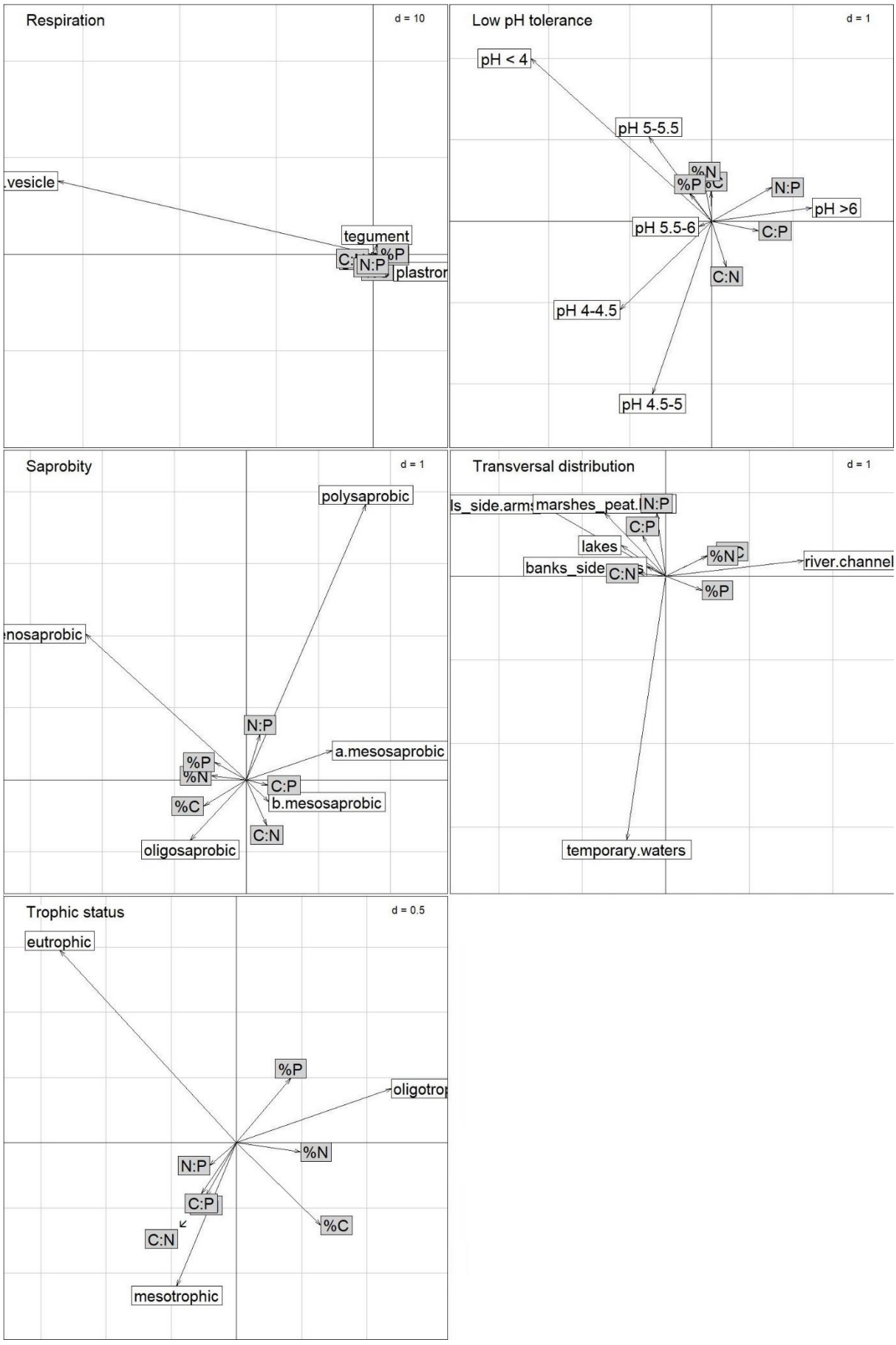
Community stoichiometry can change over a nutrient gradient or as a response to nutrient enrichment (Singer & Battin, 2007; Prater *et al.*, 2015, Beck *et al.* 2021; also MS 3, MS 5). As our results suggest, this is likely to go along with a change in the functional composition of the community, following assumptions from ecological stoichiometry theory. Many studies already showed shifts in functional community composition as a response to changing nutrient conditions, but did not consider stoichiometric traits (Floury *et al.*, 2017) or only relied it to single taxa (Cross *et al.*, 2006). Our demonstration of the link between community stoichiometry and community trait profiles suggest that it would be promising to combine all three axes – stoichiometric traits, (other) functional traits, environmental nutrient concentrations – into one analysis to understand (taxonomic and functional) community shifts as a response to nutrient stressors.

Conclusion

Our results showed that there is a significant link between community stoichoimetry and community trait profiles. This link is, in large parts, in line with findings on taxon level and assumptions from ecological stoichiometry theory. A phosphorous and nitrogen-rich community also has a high affinity for traits describing fast growth and/or development (*e.g.* small body sizes, high number of molting and reproduction events). It should also have a high number of scrapers, swallowers/chewers and taxa feeding on plant-material. The link towards habitat preferences well represented stream characteristics along the longitudinal gradient and the expected shift among larger taxonomic groups. Overall, these links suggest that stoichiometric traits, when applied on community level, could help understanding functional (and taxonomic) community responses towards changing nutrient concentrations.

Supplementary material




Respiration
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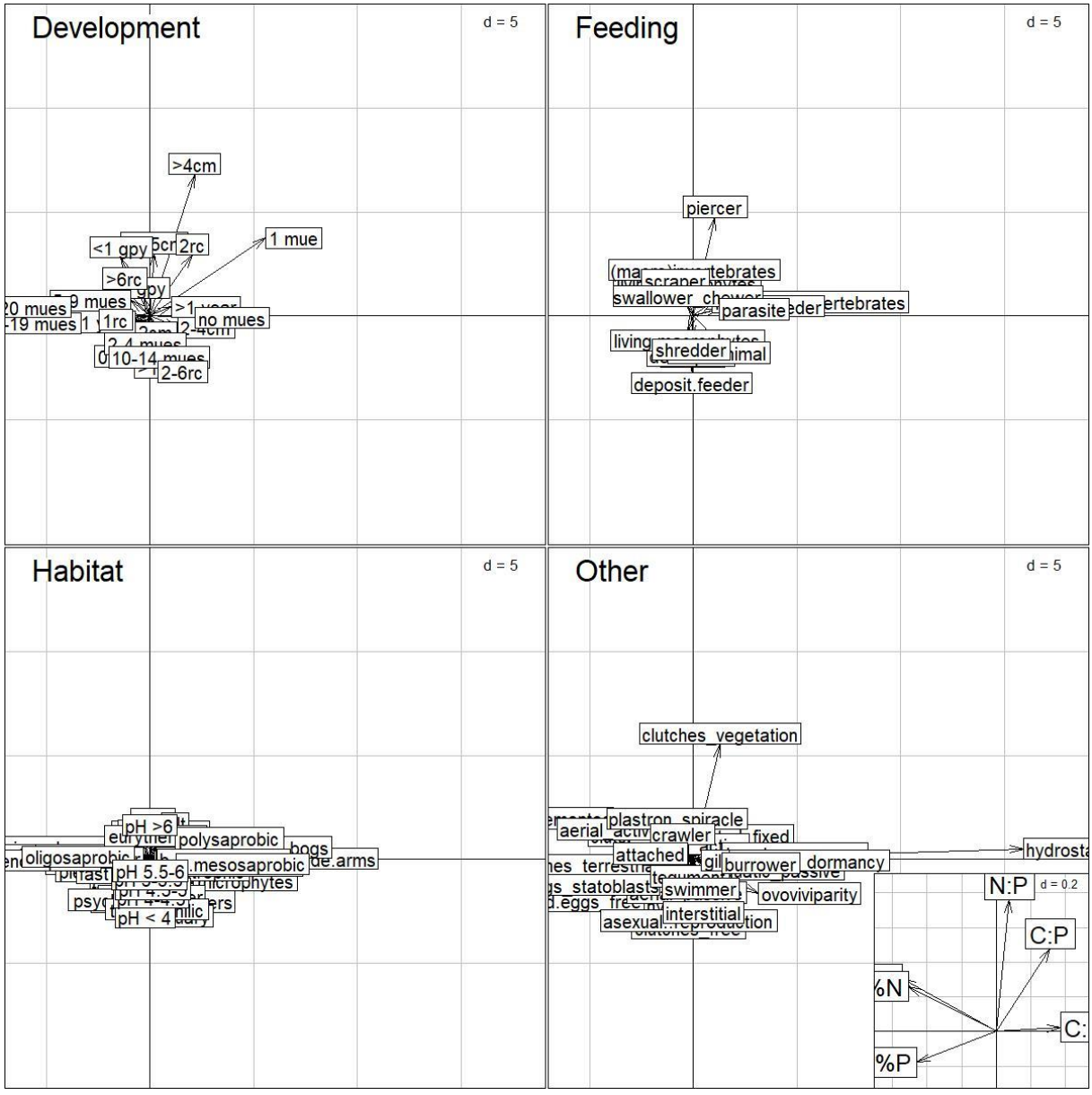
tegument
%P
C:
N:P
plastror
Low pH tolerance
d = 1
pH < 4
pH 5-5.5
%N
%P
N:P
pH >6
pH 5.5-6
C:P
C:N
pH 4-4.5
pH 4.5-5
Saprobity
d = 1
polysaprobic
nosaprobic
N:P
a.mesosaprobic
%P
%N
C:P
b.mesosaprobic
%C
C:N
oligosaprobic
Transversal distribution
d = 1
ls_side.arm
marshes_peat.
N:P
C:P
lakes
%N
river.channel
banks_side
C:N
%P
temporary.waters
Trophic status
d = 0.5
eutrophic
%P
oligotro
%N
N:P
C:P
%C
C:N
mesotrophic

S 4b.1: Results from single trait co-inertia analyses investigating the co-structure between community stoichiometric and functional traits. The functional trait categories and stoichiometric traits are located on the first co-inertia plane.



S 4b.2: Results from co-inertia analyses on multi-trait profiles. The functional trait categories and stoichiometric traits (small panel in the lower right corner) are located on the first co-inertia plane. Please note that all traits were part of one analysis and that the plot has simply been faceted by trait groups for easier readability and comparison.

Extending the Growth Rate Hypothesis to species development: Can stoichiometric traits help to explain the composition of macroinvertebrate communities?

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Abstract

1. Ecological stoichiometry seeks to understand the ecological consequences of elemental imbalances between consumers and their resources. Therein, the well-accepted Growth Rate Hypothesis (GRH) states that organisms exhibiting rapid growth have higher phosphorus (P) demand – and thus lower C:P and N:P ratios – than slow growing ones, due to a higher allocation to P-rich rRNA. However, GRH has rarely been extended to other biological traits than growth, especially at the community level.
2. In this study, we investigated whether macroinvertebrate stoichiometric traits (*e.g.* C:P and N:P ratios) can be linked to their development traits, and whether these stoichiometric traits are related to macroinvertebrate community assemblage under different nutrient conditions.
3. We allocated more than 400 European taxa to different groups, defined using available information about three development-related traits: "life span", "voltinism", and "number of reproductive cycles per individual". We sampled 18 invertebrate taxa in six streams exhibiting different levels of nutrient concentration and measured their stoichiometric traits. Further, we quantified invertebrate taxon abundances in these streams during an annual survey. Based on these data, we tested whether community composition regarding the developmental groups differs, depending on nutrient concentration.
4. We found significant differences in the proportions of the developmental groups along a gradient of water N:P, in relation to their stoichiometric traits. Taxa with low C:P and N:P ratios were generally associated with faster development groups, and these taxa tended to occur at higher proportions in streams exhibiting low dissolved N:P ratios. In contrast, communities from P-poor, high dissolved N:P streams, were dominated by slowly developing taxa with high N:P ratios.
5. Our results highlight that extending the GRH to species development rate might give some insights about the mechanisms by which nutrient concentrations in ecosystems influence consumers' community composition.

1. Introduction

In the past decades, human activities, among which agriculture, dramatically increased the nutrient load of streams and terrestrial ecosystems compared to natural conditions (Smil, 2000). The large anthropogenic inputs of phosphorus (P) and nitrogen (N) have largely altered nutrient cycles and ecosystem functions (Falkowski 2000, Elser et al. 2009), and nutrient releasing processes such as fertilization and sewage discharge are going to continue in the future. However, the input of both elements is imbalanced, altering also the proportions of available nutrients (Peñuelas *et al.*, 2013; Yan *et al.*, 2016).

Basal resources such as plants, algae or leaf litter are variable in their elemental composition and directly display nutrient availability in the environment (Cross et al. 2003, Ventura et al. 2008, Farrell et al. 2018), thereby exhibiting different stoichiometric quality as resources for consumers. Changes in resource quantity, *e.g*. following nutrient enrichment, often go along with changes in resource quality, although the strength of their respective effects differ from one context to another (Marcarelli *et al.*, 2011; Sauvadet *et al.*, 2016; Jochum *et al.*, 2017c). This can strongly impact consumer' life history traits (Elser et al. 2001, Danger et al. 2013), alter consumer biomass (Greenwood *et al.*, 2007; Spivak *et al.*, 2009) and lead to major changes in community structure over various trophic levels (Singer & Battin, 2007; Ott *et al.*, 2012). Therefore it is not only worth investigating community and ecosystem responses to increasing nutrient levels, but also the effects towards their changing ratio.

Ecological stoichiometry specifically investigates the balance of elements (mainly carbon, nitrogen, phosphorus) in an ecological context, permitting to relate the natural availability of several nutrients to nutrient cycling and trophic interactions (Sterner & Elser, 2002; González *et al.*, 2010). This allows (i) understanding how organisms and their environment interact *via* their elemental composition and (ii) answering ecological questions related to species dominance in ecosystems (Schade *et al.*, 2005), patterns of species invasions (González *et al.*, 2010) or species distribution along pollution gradients (Dang *et al.*, 2009). Within the ecological stoichiometry framework, the Growth Rate Hypothesis (GRH; Elser et al. 1996) links stoichiometry to species life history traits. This concept states that organism requirements for phosphorus result, in a large part and in particular for invertebrates, from varying demands for ribosomal RNA (rRNA) (Elser et al. 1996, Elser et al. 2003). Ribosomal RNA plays a major role in protein synthesis, which is essential for growth, *i.e*. the increase in size or mass of an organism. High growth rates involve large quantities of synthesized proteins thus requiring a higher content of P-rich rRNA. This means that fast-growing organisms will exhibit lower body C:P or N:P ratios than slow-growing ones. This linkage between growth and stoichiometry has been examined and confirmed for various organisms, ranging from microbes to crustaceans (Elser et al. 2003, Acharya et al. 2004).

The question arises whether and how this relationship affects assemblages of different taxa. Despite the recurrence of laboratory tests carried out on individual species, there is to date limited knowledge about the applicability of stoichiometric traits as predictors for consumer community composition (Moe *et al.*, 2005; Van de Waal *et al.*, 2018). Yet, the use of stoichiometric traits could largely improve our understanding of complex ecosystem dynamics (Leal *et al.*, 2017; Meunier *et al.*, 2017). Among the few studies dealing with stoichiometric traits and community structures, Evans-White et al. (2009) showed that higher nutrient loads influenced stream macroinvertebrate diversity. Species richness in some functional groups declined, following GRH. More recently, Demi et al. (2019) demonstrated that experimental instream manipulations of dissolved N:P ratios led to large changes in invertebrate community structure. Predator and shredder biomass increased under high-phosphorus conditions, with small, short-lived taxa tending to be more positively affected than larger taxa. However, they did not show any relationship between consumer body stoichiometry and species replacements. This link towards body stoichiometry was demonstrated by Singer and Battin (2007), who found a community shifting towards P-rich and fast-growing taxa after nutrient enrichment, and changes in community structure due to different responses of functional feeding groups.

In this context, to go further in the understanding of the ecological consequences of stoichiometric relationships within consumer communities and with the environment, we investigated whether the link between organism stoichiometry and growth rate as – proposed by GRH – can be generalized to other functional traits, *e.g*. related to species development. Functional traits can describe physiological, morphological, behavioural or ecological features of organisms such as life history, body form, mobility or ecological tolerances (Usseglio-Polatera et al. 2000, Vieira et al. 2006). Using species biological traits rather than taxonomy alone to describe communities would provide a more functional view on the investigated ecosystem, and could help to more deeply understand the role of stoichiometric traits in community structuration. In contrast to growth rate, which concerns body size at individual-level, development can be seen as its transformation towards maturity, affecting the growth and structure of a whole population.

In this study, we aimed at investigating the relationships between species biological traits – including stoichiometric traits – and nutrient concentrations in ecosystems, focusing on aquatic macroinvertebrate assemblages. This group of organisms is widely used in water quality biomonitoring.Large scale routine surveys have provided huge datasets gathering information on their distribution, community composition, and some of their traits (Tachet *et al.*, 2010; Schmidt-Kloiber & Hering, 2015; Sarremejane *et al.*, 2020). Based on the assumption that macroinvertebrate development follows predictions of GRH originally addressing growth rate, we specifically addressed the questions whether (i) the stoichiometry (N:P, C:P, C:N) of macroinvertebrates can be linked to their development-related traits and (ii) the community composition regarding development characteristics can be related to changes in

environmental nutrient concentrations. As development is closely linked to (body) growth in macroinvertebrates, the underlying mechanism is probably similar to that stated in GRH. Organisms with a fast development might also express high growth rates to quickly reach a body size that allows reproduction (*i.e*. body size at maturity), as shown for example for mayfly larvae (Back & King, 2013). We thus hypothesize that, due to different demands for P-rich rRNA synthesis, macroinvertebrates with trait characteristics of fast development would exhibit low N:P and/or C:P ratios, whereas macroinvertebrates with trait characteristics of slow development would exhibit high N:P and/or C:P ratios. Therefore, macroinvertebrates with traits related to fast development would be favoured in low N:P environments while high N:P conditions would select for slow developing taxa. This would lead to differences in community stoichiometry and composition in different nutrient availability conditions. We tested these hypotheses based on functional groups specifically defined in this study, using information on three development-related characteristics of macroinvertebrates – "life span", "voltinism", and "number of reproductive cycles per individual" – and organisms sampled in six streams exhibiting contrasting levels of dissolved N:P concentrations. We thereby aim to consider the community as a whole, an approach that distinguishes our study from previous studies in this field which have generally looked at individual taxa when comparing communities.

2. Material and Methods

2.1. Stoichiometric data

2.1.1. Sampling

Six streams were sampled in the Vosges mountains (North-Eastern France). They display similar natural environmental conditions, such as belonging to the same stream category (TP4 = very small streams from the hydroecoregion "Vosges" (Wasson *et al.*, 2002; Chandesris *et al.*, 2006)), presenting comparable land cover of catchment area dominated by forest as well as similar resource types (leaf litter, perilithon, FPOM, bryophytes, algae; with only algae and leaf litter appearing in minor quantities from one (low1) and two sites (medium2, high2), respectively) (Tab. 4c.1, Supp. Fig. 4c.S1). The study sites were selected to represent three site types, well discriminated based on water physico-chemical parameters (*e.g*. nutrients, oxygen, pH, cations; see Supp. Fig. S2). In particular, these three site types cover a gradient of dissolved nutrient level with either high, medium or low water N:P. We also verified that these three site types differed by their basal resource elemental quality, these resources matching well the availability of dissolved nutrients (Tab. 4c.2, Supp. Fig. 4c.S3).

The sampling of the most abundant macroinvertebrates for stoichiometric analyses was conducted in March 2011. The different types of substrates (defined in the French standard AFNOR XP T90-333 (2009), *e.g.* boulders/rocks, pebbles/cobbles, gravel, sand, organic litter, bryophytes, (other) submerged macrophytes, etc.) present on a given site were sampled in proportion to their individual benthic coverages. Individuals were gathered until a sufficient biomass of each identified taxon was reached for stoichiometric trait analyses (minimum of ~1mg dry mass per taxon and per site).The organisms were

collected with a Surber net (1/20 m^2, 500µm mesh size) and directly identified to genus level or the highest level possible under a microscope (Olympus SZ40) using identification keys of Tachet et al. (2010). In the field, larger organisms (≈1cm and above) were dissected to remove the gut in order to avoid a possible impact of gut content on stoichiometry measurement. All organisms were stored in a freezer at -20°C until further analysis.

Tab.4c.1: Chemical, physical and geographical information of the study sites. For chemical variables, the mean over the whole sampling period is given in mg/ L with values in parentheses indicating standard variation. (13 visits between 03/2009 and 04/2010). Forest cover was evaluated within a riparian corridor of 50m wide and 1km long upstream from each site using forest cover data from the BD Forêt version 2 (IGN) and the hydrogaphic referential from the BD Topage (IGN, OFB & Sandre). Temperatures refer to the mean water temperature measured in the two coldest (January, February) and warmest (July, August) months using temperature loggers.

Site	lat N	long E	Width (m)	Forest cover (%)	Temp min (°C)	Temp max (°C)	NO_3^-	PO_4^-	N:P	O_2
low1	48°40'13"	7°08'34"	2.75	41.15	5.02 (± 1.24)	16.15 (±1.18)	1.61 (± 0.40)	0.13 (± 0.04)	12.38 (±0.39)	9.21 (±1.43)
low2	48°30'15"	6°52'40"	2.50	72.60	4.71 (± 1.15)	16.63 (±1.14)	1.21 (± 0.34)	0.19 (± 0.06)	6.37 (±0.42)	6.99 (±1.90)
medium 1	48°30'33"	7°05'14"	6.30	75.56	3.89 (± 0.85)	14.19 (±1.31)	0.81 (± 0.14)	0.02 (± 0.01)	40.50 (±0.53)	10.46 (±1.04)
medium 2	48°29'27"	7°03'12"	3.35	100	5.03 (± 0.67)	11.56 (±0.63)	0.70 (± 0.03)	0.02 (± 0.00)	35.00 (±0.00)	10.63 (±0.68)
high1	48°26'59"	7°03'41"	2.60	100	2.71 (± 0.96)	13.75 (±1.35)	0.81 (± 0.15)	< 0.01	> 81.00	10.77 (±1.23)
high2	48°24'48"	6°55'23"	6.60	100	5.10 (± 0.87)	12.94 (±1.07)	0.48 (± 0.05)	0.01 (± 0.00)	48.00 (±0.14	11.19 (±0.84)

Tab. 4c.2: Stoichiometry (N:P, C:P. C:N) of resources (bryophytes, FPOM, litter, perilithon) at the three different site types with either low, medium or high water N:P (n indicates sample size). Values correspond to the mean of the molar ratios, values in parentheses indicate the range of raw data. For each resource, letters indicate significant differences in stoichiometry between site types (Kruskal-Wallis Anova followed by Dunn's rank-sum test with Bonferroni correction with $\alpha = 0.05$). The resources were sampled during the sampling of macroinvertebrates for stoichiometric analysis.

Resource	Water N:P (n)	N:P	C:P	C:N
Bryophytes	low (3)	11.83 (10.38 - 12.62)	139.59 (121.77 - 149.66) [a]	11.80 (11.73 - 11.86) [a]
	medium (6)	14.78 (12.32 - 17.48)	297.84 (236.30 - 364.07) [b]	20.06 (17.81 - 21.74) [a]
	high (6)	25.21 (7.07 - 45.60)	930.90 (177.43 - 1774.24) [ab]	32.58 (23.63 - 39.11) [b]
FPOM	low (6)	7.30 (2.01 - 12.16)	91.48 (30.33 - 143.76) [a]	13.47 (11.73 - 15.20) [a]
	medium (6)	13.35 (10.99 - 15.81)	299.81 (275.93 - 345.77) [b]	22.77 (19.75 - 26.71) [b]
	high (6)	12.77 (7.30 - 18.51)	318.81 (201.16 - 464.48) [b]	25.35 (23.67 - 27.54) [b]
Litter	low (3)	12.95 (12.66 - 13.11) [a]	337.48 (302.25 - 368.08) [a]	26.07 (23.10 - 28.08) [a]
	medium (6)	26.04 (22.02 - 32.46) [ab]	1016.31 (816.98 - 1244.65) [ab]	39.12 (33.78 - 48.10) [ab]
	high (6)	57.58 (26.63 - 91.68) [b]	2578.93 (1199.17 - 4303.98) [b]	44.74 (40.20 - 47.43) [b]
Perilithon	low (3)	7.77 (7.27 - 8.26) [a]	55.31 (52.55 - 58.16) [a]	7.12 (7.04 - 7.23) [a]
	medium (6)	16.26 (14.91 - 17.99) [b]	190.19 (141.18 - 241.55) [ab]	11.8 (8.83 - 14.63) [ab]
	high (6)	24.90 (13.44 - 37.94) [ab]	352.47 (178.72 - 544.84) [b]	13.90 (13.29 - 14.81) [b]

2.1.2. Elemental analysis

Invertebrate individuals were oven-dried (48h at 65°C) and grinded. Carbon and nitrogen were measured from samples (~1 mg) using an elemental combustion analyser (model Carlo Erba NA 1500NC).

Organic phosphorus was measured by oxidizing the samples with sodium persulfate under alkaline conditions. The samples (~1 mg) were suspended in 4ml Milli-Q water and then 1ml sodium hydroxide (NaOH 1M) and 2.38 ml sodium persulfate ($Na_2S_2O_8$ 125 g L^{-1}) were added to the sample suspension. Each solution was adjusted to 10 ml with Milli-Q water before put in heat chamber at 120°C for two hours to accelerate the process of phosphorus mineralization. After cooling, 2 ml of reagent solution (H_2SO_4: 15% v/v; $K(SbO)C_4H_4O_6$, 0.5 H_2O: 2.8 g L^{-1}; $(NH_4)_6Mo_7O_{24}$, 4 H_2O: 40 g L^{-1}) and 0.5 ml of ascorbic acid (20 g L^{-1}) were added to each sample vial. After 30 minutes, 2 ml of each solution were used to measure absorbance at 880 nm by spectrophotometry (Analytik Jena-Specord 205 spectrophotometer). The phosphorus concentration was calculated by comparing absorbances of samples to those of standard solutions.

2.2. Faunal community survey

The sites at which macroinvertebrates were collected for stoichiometric measurements (see 2.1.1.), were additionally visited every four weeks from March 2009 to April 2010 (thirteen campaigns) to quantify abundances. At each site, coarse gravel, as the common dominant habitat at the six streams, was sampled three times per site and per campaign. Samples were taken with a Surber net (1/20 m^2, 500 µm mesh

size) and directly fixed with 3% formaldehyde.

Invertebrates were identified at the genus level [except for Oligochaeta, some Diptera (mainly family level), trichopteran Limnephilidae, coleopteran Dytiscidae and Hydrophilidae (sub-family level)] and counted.

During each sampling campaign a water sample (1 L) was taken to analyse water chemistry. The sample bottle was stored at 5°C until analysis within the next few (< 6) days. Water was filtered (Whatmann GF/C in case of N measurement; filter paper of pore size 0.25µm in case of P measurement) before nitrate concentration was determined *via* ionic chromatography and phosphate was measured using molecular absorption spectrophotometry.

2.3. Data analysis

2.3.1. Groups based on developmental traits

We used biological traits as descriptors of specific aspects of the biology and life history of species. Macroinvertebrate trait profiles were described using a fuzzy coding approach, where each trait is described by several trait categories and the affinity of taxa for each category is coded based on numerical scores from 0 (= "no affinity for the corresponding category") to 3 (= "high affinity") (Chevenet *et al.*, 1994).

For characterizing macroinvertebrate development, the biological traits "life span" (with the trait categories: "one year or less", "more than one year"), "voltinism" ("less than one generation per year", "one generation per year", "more than one generation per year") and "number of reproductive cycles per individual" ("one", "two", "three to six", "more than six") were used to gather macroinvertebrate taxa in development trait-based homogeneous groups. A Fuzzy Correspondence Analysis (= FCA, Chevenet et al. 1994) was applied to the profiles of 447 taxa for which information for these traits was available (mainly from Juget and Lafont 1994, Richoux 1994, Tachet et al. 1994, 2010, Usseglio-Polatera 1994, and Usseglio-Polatera and Tachet 1994; further information in Supp. Tab. 4c.S1). A hierarchical cluster analysis (applying the minimum variance criterion (Ward, 1963) based on a matrix of Euclidean distances) was then performed using the first three axes of the FCA (explaining 70% of the total trait variability among taxa) to aggregate taxa in groups with similar suites of traits. The final number of groups was determined according to the shape of the dendrogram, by selecting a partitioning level (*i.e.* a given distance value) corresponding to the best compromise between (i) the number of groups and (ii) the homogeneity of group size (*i.e.* maximizing evenness of taxa among groups). The definition of these groups of taxa was supported by their trait profiles (see Fig. 4c.1 in the results section).

2.3.2. Stoichiometric traits

After measuring the molar concentrations of carbon (C), nitrogen (N) and phosphorus (P), the organisms' nutrient molar ratios C:N, C:P and N:P were calculated. After assigning the organisms to

their corresponding trait-based group (see 2.3.1.) the mean nutrient ratio of each group was calculated. Mean group nutrient ratios were statistically compared for identifying between-groups differences.

Community ratios were calculated as the mean of stoichiometric values of all the individuals found at a site type, independently of their trait-based group. We tested for an effect of water N:P on community stoichiometry by comparing ratios of all the invertebrates sampled at the different site types. This analysis was performed considering either both inter-taxa and intra-taxon ratio variations (raw elemental ratios) or only the intra-taxon ratio variation. To analyse intra-taxon variation only, we standardized the elemental ratios using a z-transformation: $(obs_{i,s} - mu_s) / sd_s$ where $obs_{i,s}$ is the observed elemental ratio for taxon "i" at site "s" whereas mu_s and sd_s, represent the mean and the standard deviation of the elemental ratio for taxon "i" among all the sites, respectively. Note that since the stoichiometric measurements only covered the most abundant taxa at the sites without further quantification, the calculated community ratios represent only this subset of the whole community. Nevertheless, since collected organisms were among the dominant ones in terms of biomass, our analyses should be quite representative of the whole community.

A non-parametric approach (*i.e.* Kruskal-Wallis tests followed by Dunn's rank-sum tests with Bonferroni correction) was applied for comparing stoichiometry between the trait-based groups as well as community stoichiometry between site types, as graphical examination of residual plots and tests (Shapiro test, Levene test) revealed non-normality of data and/ or heterogeneity of variances.

2.3.3. Community composition

Community composition regarding developmental groups was assessed in two ways: 1) based on taxon richness and 2) based on taxon abundance, both using data from the one-year community survey performed between March 2009 and April 2010. The approaches are complementary, since the first approach permits to test the impact of water N:P on the proportion of the taxa that belong to each developmental group, while the second one permits to evaluate the impact of water N:P on the proportion of macroinvertebrate individuals that belong to the different groups.

The number of taxa within each trait-based group during each sampling event was assessed, separately for the different site types. Likewise, the number of individuals within each trait-based group was calculated for each sampling event and site type. To test whether taxon richness and abundance of the developmental groups differ between site types, Friedman tests were applied for each developmental group, followed by paired Wilcoxon rank tests in case of significance. The use of blocks in the design of Friedman test allows controlling for additional variation among the seasons.

All the statistical analyses were done using R (R Development Core Team, 2019)

3. Results

3.1. Trait-based groups of macroinvertebrates

Using information about three biological traits related to species development: "life span", "voltinism"

and “number of reproductive cycles per individual”, from a database covering 447 European taxa, it was possible to define seven groups of taxa with contrasted trait profiles (Fig. 4c.1, Supp. Fig. 4c.S4). The clustering resulted in six groups, one of which was further subdivided into two sub-groups (I_3 and F), to achieve a more balanced number of taxa in each group. This additional splitting followed that of the clustering process and was supported by differences in trait profiles. The final classification explained 73.47% of the trait variation among taxa (between-group Monte Carlo permutation test with n = 9999 permutations; $p = 10^{-4}$) and the number of taxa within each group varied between 40 and 142 (Tab. 4c.3). The groups vary in their affinity for the different trait categories with taxa of groups S and F clearly expressing either slow or fast development (Fig. 4c.1). Most of the taxa of group S, *e.g*. including the genus *Cordulegaster* (Odonata), tend to live longer than one year, although some of them have also some affinity for a shorter life span. Further, these taxa are characterized by having just one reproductive cycle per individual and producing less than one generation per year. In contrast, taxa from group F live less than one year and produce one or more than one generation per year. This group includes among others the insect genera *Baetis* (Ephemeroptera) and *Hydropsyche* (Trichoptera). Regarding the number of potential reproductive cycles per individual, they show a similar profile as group S. Profiles of the five other groups expressed characteristics of both modes and thus show intermediate development rate. Labelling among these groups was based on decreasing voltinism (but this could have been based on one of the other traits). Taxa in group I_1, such as the crustaceans *Asellus* and *Gammarus* and group I_2 (*e.g.* Hydrozoa and Oligochaeta) tend to live longer than one year while producing more than one generation per year. Individuals tend to go through two to six (group I_1) or more than six (group I_2) reproductive cycles. Group I_3 contains taxa with short life duration (less than one year) and only one reproductive cycle per individual. Representatives of this group are *Brachyptera*, *Protonemura* (Plecoptera) and *Plectrocnemia* (Trichoptera). The majority of taxa in groups I_4 and I_5 tend to live longer than one year by producing mainly one generation per year. While almost all taxa in group I_5 (*e.g*. *Rhyacophila* and *Sericostoma* (Trichoptera)) are supposed to express only one reproductive cycle per individual, taxa in group I_4 (*e.g*. *Limnius* (Coleoptera) and Erpobdellidae (Hirudinea)) are able to have two reproductive cycles during their life.

Tab. 4c.3: Composition of the trait-based groups regarding the number of taxa (genus level or highest level of identification) and their taxonomic homogeneity (Shannon diversity index H’). H’ for each group was calculated, using the relative number of taxa per taxonomic group (order for insects, class for other branches).

Group	Number of taxa	Taxonomic homogeneity
F	52	2.3442
I_1	53	1.9766
I_2	40	2.2670
I_3	142	2.8560

I_4	49	1.6256
I_5	64	2.9331
S	47	2.1037

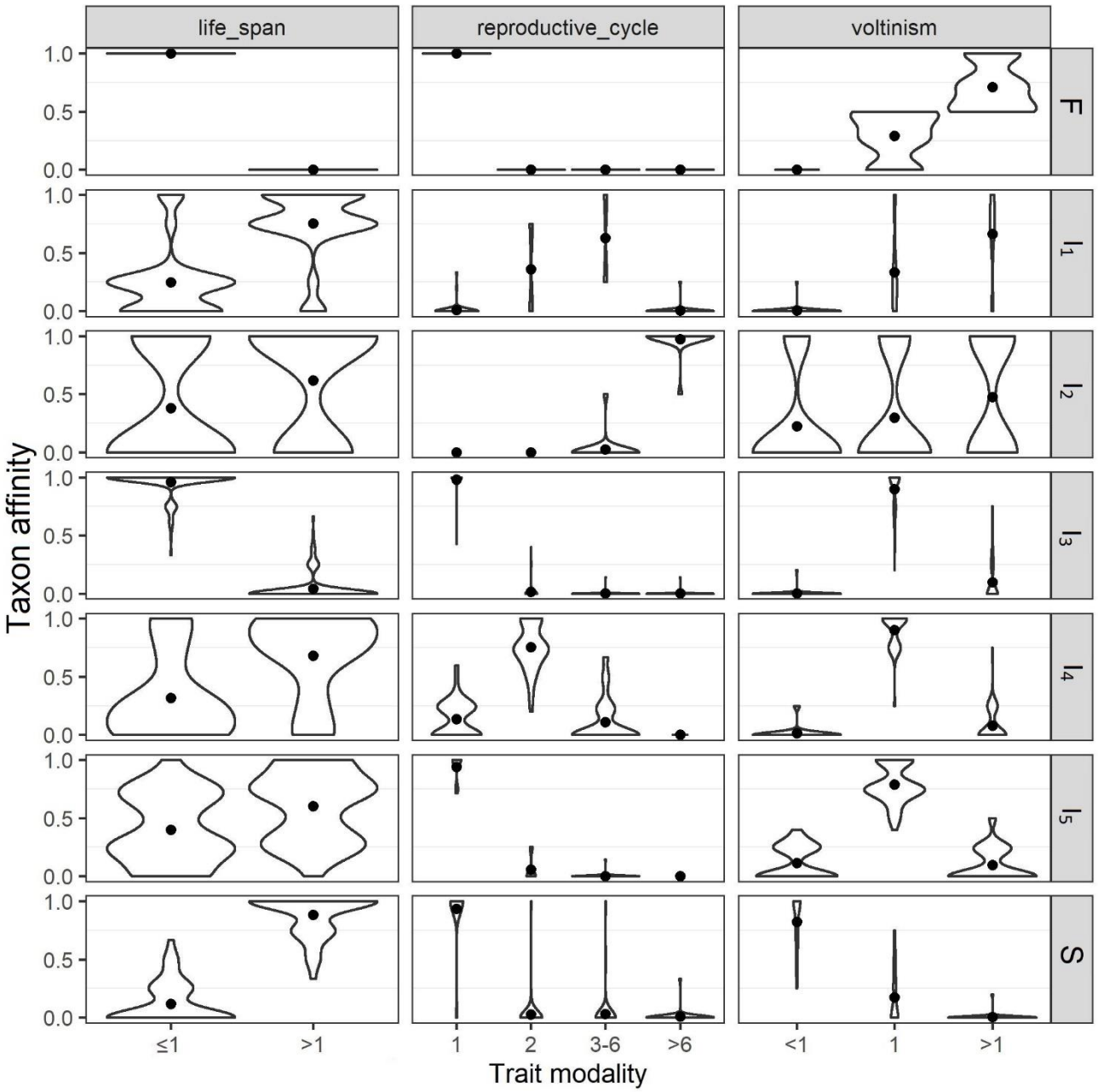


Fig. 4c.1: Trait profiles of the seven groups with their affinity for each category of the traits “life span”, “voltinism” and “number of reproductive cycles per individual”. Violin plots represent the symmetric density of the trait affinity distribution. Black dots represent the median affinity of the group for each trait category.

3.2. Macroinvertebrate elemental ratios among groups

The taxa collected for stoichiometric analyses belonged to groups I_3 (6 taxa), I_5 (4), S (4), I_1 (2) and F (2). Taxa belonging to groups I_2 and I_4 were not found during this sampling event (Supp. Tab. 4c.S2). There was a trend of increasing N:P ratio from group I_1 to groups F, I_3, I_5 and S, with group I_1 having a significantly lower N:P ratio value than groups I_3, I_5 and S (Fig. 4c.2, Kruskal-Wallis test with $p < 0.05$ followed by Dunn's rank-sum test with $p < 0.05$ for groups I_1/ I_3, I_1/ I_5, I_1/ S). The C:P ratio tended also to increase in this order, with group I_1 having the lowest ratio value which was significantly different from those of groups F, I_5 and S. The C:N ratio was highest in group F and it differed significantly from those of groups I_3 and S. The lowest C:N ratio found in group I_3 differed significantly from those of groups I_5 and F.

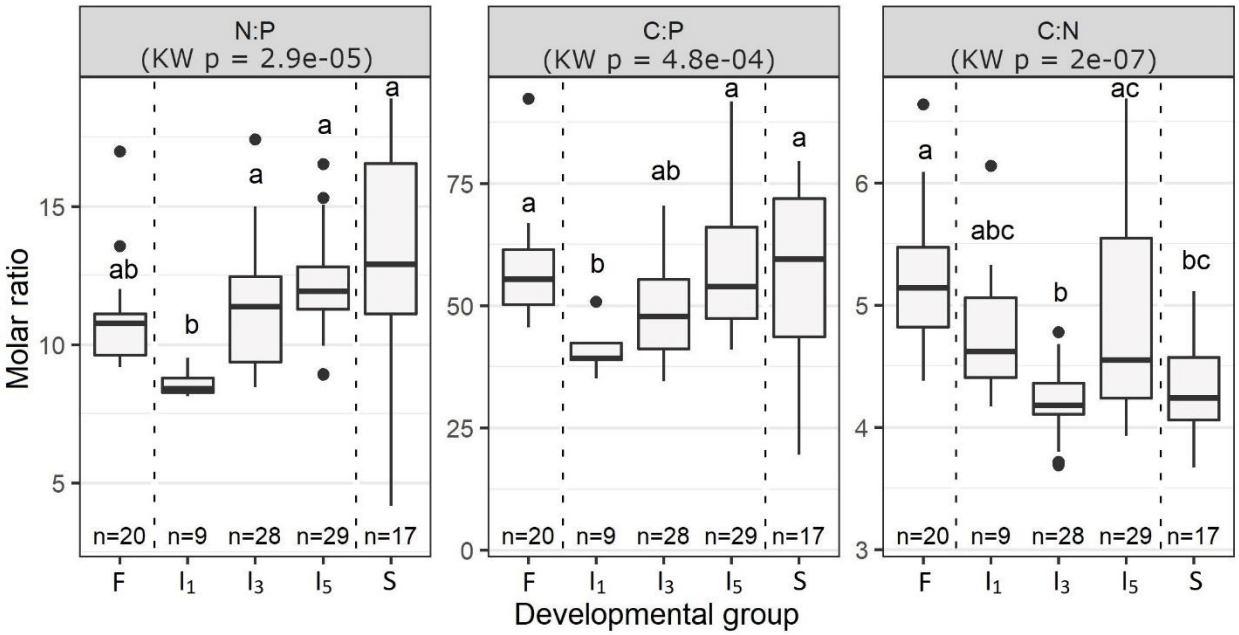


Fig. 4c.2: Differences in macroinvertebrate stoichiometry (N:P, C:P, C:N) among developmental groups. For each ratio and each group, the thick horizontal line represents the median, the boxes range from the 25th to the 75th percentiles and the whiskers extend to 1.5 times the inter-quartile range with black dots representing outliers. Different letters indicate significant differences using Dunn's rank-sum test with Bonferroni correction following a significant Kruskal-Wallis test with significance threshold set to $\alpha = 0.05$. Groups are ordered along the x-axis according to decreasing development rate, n indicates the number of individuals analysed within each developmental group.

3.3. Macroinvertebrate stoichiometry along gradient of water N:P

Macroinvertebrate stoichiometry at the community level changed along a gradient of water N:P. Community N:P ratio increased with increasing water N:P. When considering inter-taxa differences, *i.e.* changes in community composition, macroinvertebrate N:P ratios at high water N:P sites were significantly higher than at medium and low water N:P sites (Fig. 4c.3 top panel, $p < 0.05$ in Kruskal-Wallis test with following Dunn's rank-sum test with $p < 0.05$ for high/ medium and high/ low N:P ratio). Within each taxon the elemental ratios did not differ between site types, but tended to decrease

with lower water N:P (Fig. 4c.3 bottom panel, Kruskal-Wallis test with $p > 0.05$ for each ratio).
The C:N ratios of communities showed the opposite, but not significant, trend and tended to decrease with increasing water N:P. Community C:P ratio did not differ significantly between the three water N:P conditions. For both C:P and C:N ratios the differences were not significant, but the trend was the same in scenarios including intra-taxon and/ or inter-taxa variation.

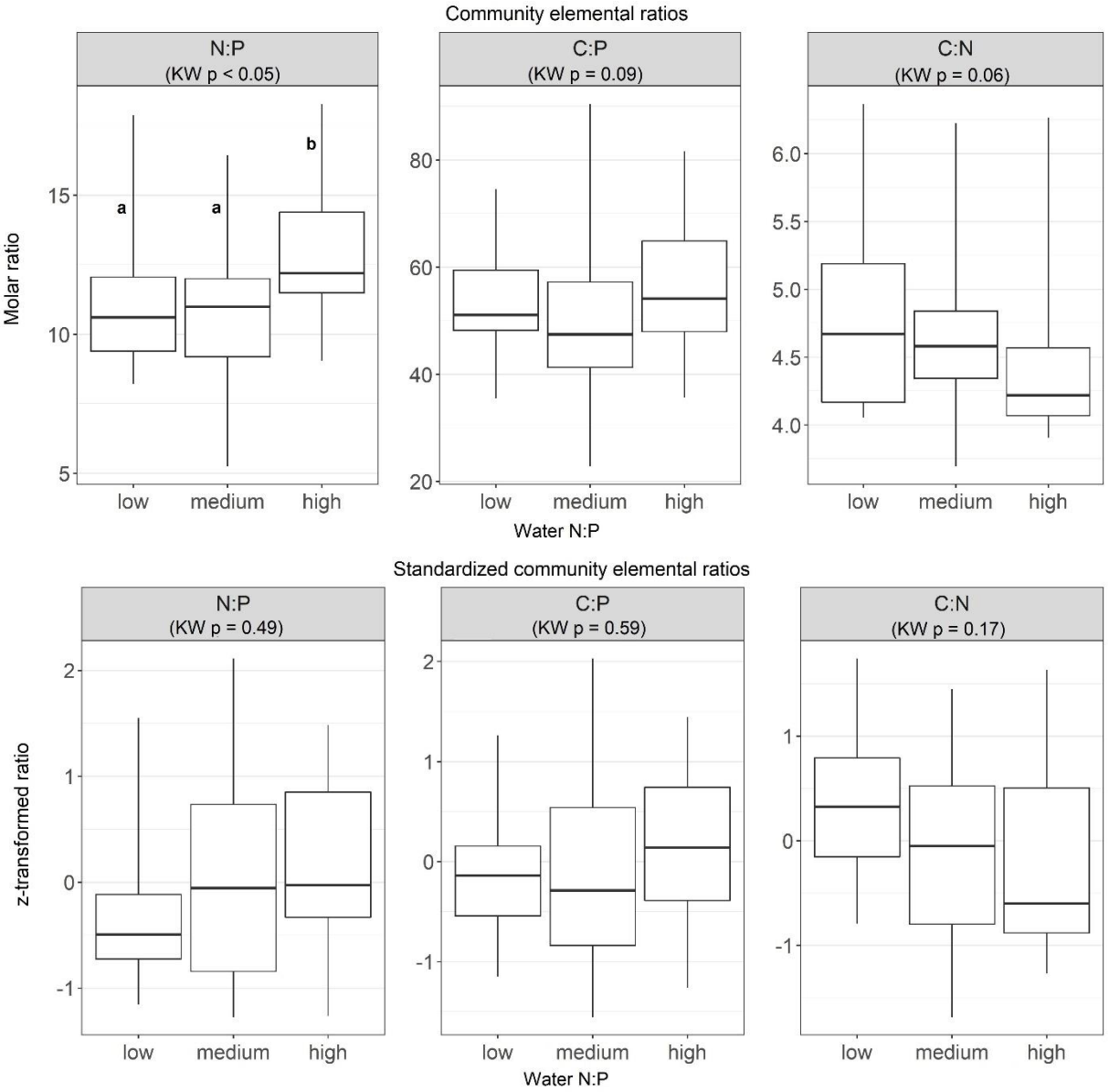


Fig. 4c.3: Changes in macroinvertebrate stoichiometry among groups of sites with increasing N:P ratios. Top panel graphs consider intra- and interspecific variation, while bottom panel shows only intra-specific variation. Inter-specific variation was suppressed by standardization of elemental ratios using z-transformation. For each macroinvertebrate ratio (N:P, C:P, C:N) and each group of sites (low, medium, high water N:P), the thick horizontal line represents the median, the boxes range from the 25th to the 75th percentiles and the whiskers cover 95% of the values. Different letters indicate significant differences ($\alpha = 0.05$) based on the multiple comparison Dunn's rank-sum test with Bonferroni correction following a Kruskal-Wallis rank-sum test. Sample size was n=23/51/29 for low/ medium/ high water N:P sites.

3.4. Community composition based on trait-based groups

During the annual faunal survey, taxa from all seven trait-based groups were found. The composition of macroinvertebrate communities differed between sites with different water N:P conditions in terms of these developmental groups.

Taxa richness of all seven groups differed significantly between sites with different water N:P ratios (Fig. 4c.4, individual Friedman test with $p<0.05$ for each group). In all groups, taxa richness was the highest at sites with medium water N:P and on average 1.7 – 14.3 taxa were found in each group during the sampling events over the year. This difference was significant compared to either both other site types (groups I_3, I_5 & F) or only low (group S) or high water N:P sites (groups I_1, I_2 & I_3) (Wilcoxon rank tests with $p<0.05$ for pair-wise comparisons between medium/low and medium/high sites for groups I_3, I_5 & F, medium/low for group S and medium/high for groups I_1, I_2 & I_3). While in groups F, I_1, I_2 and I_4 taxa richness was significantly higher at low water N:P sites than at high water N:P sites, the number of taxa in groups I_3 and S showed the inverse pattern between these site types (Wilcoxon rank tests with $p<0.05$ for high/ low). Taxa richness of group I_5 was also higher in high water N:P sites than in low ones, but this difference was not significant.

Also the abundances of developmental groups differed between site types, except for group I_4 (Fig. 4c.5, Friedman test with $p<0.05$). The abundance of individuals decreased in groups F, I_1 and I_2 with increasing water N:P and differed significantly between all site types for groups F and I_2 (Wilcoxon rank tests with $p<0.05$ for medium/low, medium/high and high/low sites for groups F & I_2, medium/high and high/low for group I_1). For example in group F on average 1465.3 individuals were found at low water N:P sites compared to 247.8 individuals at high water N:P sites, abundances in group I_1 decreased from 371.8 at low water N:P sites to only two individuals at high water N:P sites. Abundances of group S increased along this gradient. At low water N:P sites only one individual of this group was found during the whole sampling period, which was significantly less compared to medium and high water N:P sites (30.5 and 60.8, respectively). Groups I_3, I_4 and I_5 had the highest abundances at sites with medium water N:P (956.8, 272.1 and 211.1, respectively). In groups I_3 and I_5 the number of individuals was significantly higher at high water N:P sites compared to low water N:P sites (which also differed significantly), while in group I_4 it was slightly higher at low water N:P sites, although this difference was not significant. A list of all the taxa found during the annual sampling campaigns and their corresponding development group can be found in the supplementary information (Supp. Tab. 4c.S3).

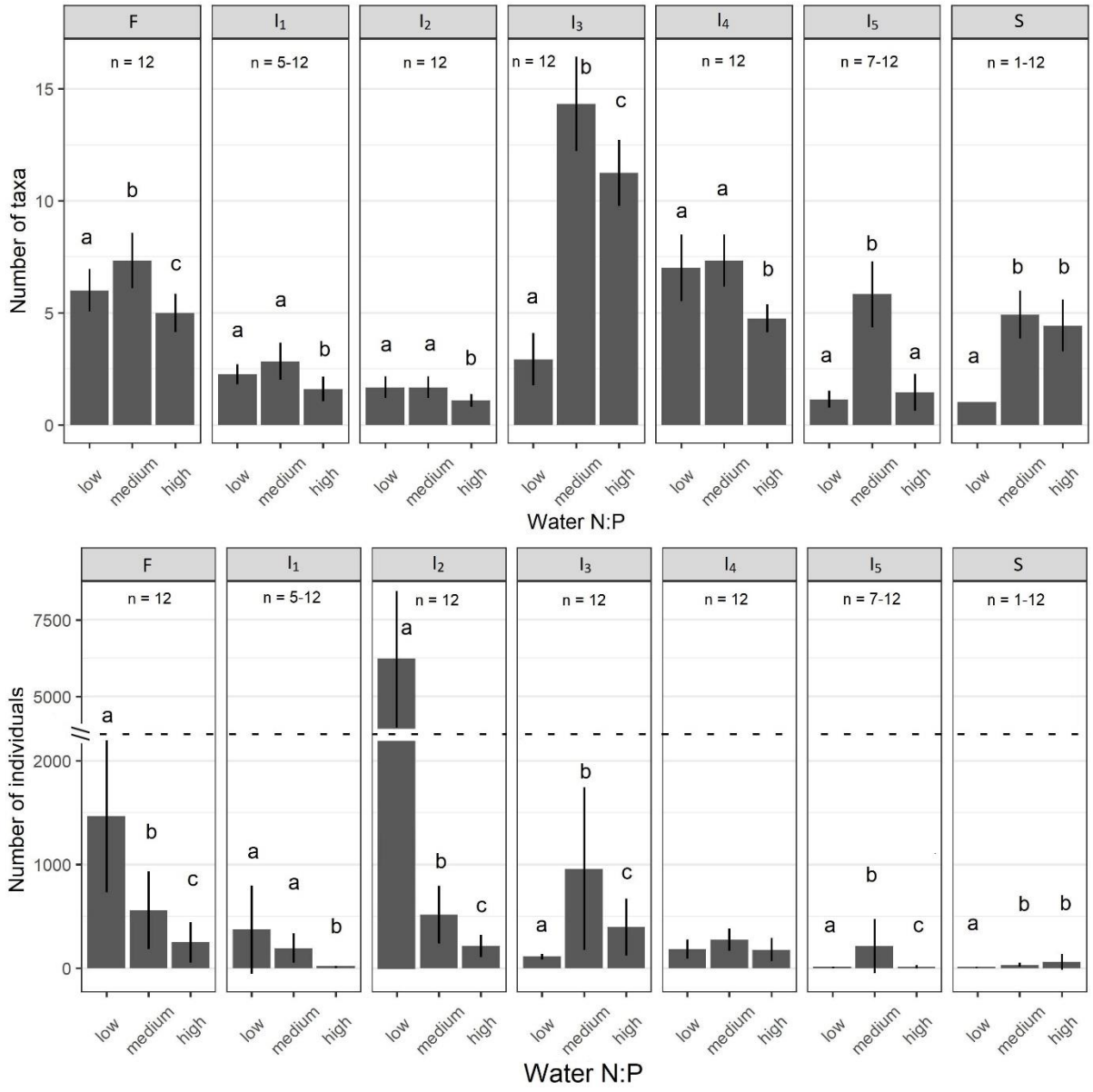


Fig. 4c.4: Community composition regarding taxonomic richness (upper panel) and abundances (lower panel) of the developmental groups among sites with different water N:P ratio levels (low, medium and high). For each group, bar height shows mean number of taxa or mean number of individuals per 0.15 m^2 of each group over the different sampling events during the one-year survey with error bars indicating the variation between sampling events as standard deviation and n indicating the range of the numbers of sampling events for each group.

4. Discussion

In this study, we were able to show that macroinvertebrate groups with contrasted developmental traits differ in their stoichiometry and their relative frequency in communities under different stream nutrient regimes, both in terms of number of individuals and taxa. These results suggest the potential interest of incorporating stoichiometric traits into trait-based approaches of community ecology.

4.1 Links between organism development rates and body stoichiometry

For two trait-based groups, the trend of the relationship between developmental traits and stoichiometry was in accordance with our first hypothesis, *i.e.* taxa with fast development exhibit lower N:P and C:P ratios than slow development taxa. Taxa from both groups S and F mostly have only one reproductive cycle per individual, but taxa from group F displayed characteristics of fast development such as a short life span with more than one generation per year, whereas taxa from group S exhibited a slow development, indicated by traits such as a longer life span with less than one generation per year. We showed that differences in C:N, C:P and N:P stoichiometry exist between these two groups, although only significant for C:N, (Fig. 4c.2), at least for species and populations collected at our sites. Having a high content of phosphorus (low N:P and C:P) thus not only allows fast growth as predicted by GRH (Elser et al. 1996), but also seems to favour a fast development of species. The opposite trend in C:N compared to C:P ratio underlines different requirements and suggests a trade-off between nitrogen and phosphorus acquisition: Taxa with a high demand for phosphorus (often related to low body C:P) probably require less nitrogen and thus express higher body C:N ratios. In contrast, slow-developing taxa with a lower demand for phosphorus are richer in nitrogen, probably displaying higher requirements for N-rich molecules such as proteins (Elser et al. 1996). This could be the case due to a greater investment in somatic development, for example in muscle tissue for predators such as the long-lived larvae of *Cordulegaster*. Considering that all the taxa in group S that were analysed for stoichiometry may be viewed as predators, this might have also led to the significantly lower C:N ratio compared to group F containing herbivorous and omnivorous species. Predators generally show higher N-content than primary consumers (Fagan et al. 2002, Evans-White et al. 2005).

Using the three selected traits, it was not possible to define a clear gradient of potential development rate for the other groups. Groups I_1, I_3 and I_5 had intermediate trait profiles, exhibiting a variable combination of characteristics of both fast and slow development, according to their position between the two extreme groups. This corresponds to smaller (non-significant) differences in stoichiometry between those groups, compared to the two extreme groups. N:P ratio of groups I_3 and I_5 was located between the values of fast- and slow-developing groups, suggesting a link to their developmental traits. A further analysis on an extended dataset, including more taxa, could help to clarify this link.

Group I_1 was the only group containing crustaceans and only consisted of the crustaceans *Gammarus* sp. and *Asellus* sp. in our stoichiometric dataset. In addition, when using data sets of the annual survey, crustaceans clearly dominated the individuals belonging to group I_1. This may explain its significantly higher phosphorus content compared to the three other groups (groups I_3, I_5, S), for which only insects were sampled and analysed. This is in accordance with the findings of Evans-White et al. (2005), who found that crustaceans had on average lower C:P and N:P stoichiometry and that such phylogenetic differences might explain large differences with other groups mainly composed of insects and mollusks. The authors assigned this phylogenetic difference to a generally higher content of rRNA of crustaceans

and/ or complexes of phosphorus and calcium found in their carapace as suggested by Vrede et al. (1999). In contrast, insects generally have higher N:P ratios, due to their high protein contents but also their chitinous cuticle (Elser et al. 1996). Furthermore, crustaceans generally exhibit clear differences in life history to most of the insects considered in this study. They are fully aquatic and mature by growing and moulting while juveniles of most of amphibiotic insects emerge into terrestrial adults after metamorphosis with major morphological changes. This may lead to varying demands for phosphorus in new morphological structures in amphibiotic taxa even during their aquatic instars, compared to fully aquatic taxa. Mayflies for example were found to vary in stoichiometry with life stage with later development instars of females (nearing emergence) allocating higher amounts of P into reproductive tissues (Back et al. 2008). Other aquatic and terrestrial insects have shown decreasing P content during ontogeny (Back and King 2013, Elser et al. 2006), which is probably due to different life history characteristics of adults. Also crustaceans may face a decreasing ontogenic demand for phosphorus during their development (Main et al. 1997, Villar-Argaiz et al. 2002), which might simply be the result of decreasing growth rate at older age. Therefore, although similar patterns occur during development, general differences in the speed or degree in which the stoichiometry changes, might exist between organisms with fully aquatic or amphibiotic life cycle. In our stoichiometric analysis, no fully aquatic insect was present, but it might be important to consider this difference in future studies.

4.2 Response of community stoichiometry along a gradient of stream N:P ratio

We expected that stoichiometry on community level follows the gradient in water N:P and basal resource N:P. Changing nutrient concentrations in the environment has generally been associated with changes in basal resource quality, which in turn can affect consumer levels (Cross et al. 2003, Demi et al. 2018, Demi et al. 2019). In our study, analyses of bryophytes, FPOM, leaf litter and perilithon confirmed that changes in the elemental composition of these basal resources were clearly associated with water N:P gradient. These changes are generally mediated by the non-homeostatic nature of primary producers and decomposers. For example, leaf litter, the main basal resource in detritus-based systems, varies broadly in stoichiometry and reflects nutrient availability in ecosystems (Cross *et al.*, 2003; Farrell *et al.*, 2018; Demi *et al.*, 2019). Nutrient enrichment (N, P) increases the activity of and colonization by microbial decomposers, thereby increasing leaf litter phosphorus content and its quality as resource (Cross et al. 2003, Danger et al. 2016). Some microbial decomposers themselves are not homeostatic and can store excess phosphorus in their biomass which lowers their body N:P and C:P ratios and those of the whole leaf litter detritus (Danger *et al.*, 2013; Gulis *et al.*, 2017). Due to the high number of studies confirming the link between water nutrient concentrations, resource stoichiometry and primary as well as secondary consumers as cited above, we assume that this could be the mechanism by which community composition has been affected. Besides resource quality, also its quantity may change under different nutrient concentrations, which might especially affect long-lived taxa (Cross et al. 2005).

Generally, there are two ways in which a community might adapt to changing nutrient concentrations: (i) the taxa adapt their elemental composition to the nutrient content of their resources (Small & Pringle, 2010) or (ii) the composition of benthic community changes, supporting taxa whose elemental demands best fit the resources provided (Singer & Battin, 2007). Both of these strategies will lead to changes in community stoichiometry. Our findings indicate that the elemental composition of the overall community tends to change, reflecting the differences in dissolved N:P concentrations in the water. Although the differences in nutrient concentrations between low, medium and high water N:P sites did not significantly impact the macroinvertebrate community C:P ratios, we observed a significant increase in community N:P and a marginal but insignificant decrease in community C:N along this N:P gradient. The opposite patterns in community C:P (and N:P) versus C:N ratio indicate that a trade-off between nitrogen and phosphorus stoichiometry as discussed above (4.1.) can also be observed on community level. The loss of these effects when focusing on intraspecific differences (see Fig. 4c.3) suggests that the observed community changes in stoichiometry along the water N:P gradient likely results for a large part from taxonomic changes and not from stoichiometric plasticity of similar taxa. Sites with a high N:P ratio seem thus to select for taxa with a high N:P demand and vice versa. In agreement with our results, similar changes in community stoichiometry and composition over a nutrient gradient or after nutrient enrichment have already been observed in previous studies (Singer and Battin 2007, Prater et al. 2015). In both studies, the community C:P decreased when water phosphorus load increased, due to a shift towards P-rich taxa. The preponderance of taxonomic shifts over taxon stoichiometry plasticity for explaining changes in community stoichiometry, is further confirmed in this study, insofar as seven out of nine taxa that were found at more than one site type, showed no difference in stoichiometric ratios (C:N, C:P, N:P) when nutrient load in the stream varied (see Supp. Fig. 4c.S5). This observation is a major assumption in GRH (Elser et al. 1996) and in accordance with several results in the literature stating general homeostasis for aquatic macroinvertebrates when nutrient content changes in the environment (Evans-White et al. 2005, Persson et al. 2010). However other studies found deviation from strict homeostasis under different nutrient regimes (Cross et al. 2003, Small and Pringle 2010), which underlines the potential confounding effect of factors such as sex or size/age on the variability of C:N:P ratios and the difficulty to actually confirm homeostasis using field data (Halvorson & Small, 2016). We are aware that it is therefore questionable to apply the concept of strict homeostasis without accounting for these additional factors, but we think that here it can serve as an indication that macroinvertebrates keep a stable stoichiometry under changing nutrient concentration.

4.3 Community trait composition shifts with stream water N:P ratio

Changes in community stoichiometry along a gradient of water N:P ratio were linked to a selection of species according to certain biological traits, as the communities differed regarding macroinvertebrate groups with distinct developmental traits. With increasing water N:P level, the abundances of fast-developing taxa (group F) decreased, while that of slow-developing taxa (group S) increased. Taxon

richness followed a similar pattern of response for part of the water N:P gradient. This is in agreement with our second hypothesis, predicting differences in developmental trait composition in stream communities. Singer and Battin (2007) and Prater et al. (2015) have already shown a shift in traits towards fast-growing and small-bodied species under high phosphorus conditions. These observations on species growth and body size, following assumptions based on GRH, seem to be applicable to development and could be extended to the whole community as well. Taxa with fast growth and/or development seem to be favoured at P-rich sites. Their high phosphorus requirements cannot be met at phosphorus depleted sites. At these sites, taxa with lower phosphorus demand are favoured, leading to the dominance of taxa with slow growth and/or development in communities. This matches with the expected and observed differences in stoichiometry between the trait-based groups (discussed above). The groups to which no clear development speed could be assigned, also partly showed a pattern of increasing (group I_3) or decreasing (group I_1 & I_2) abundances with increasing water N:P, but the relationship between this pattern and developmental traits needs to be further investigated.
The ability of some taxa to regulate growth rate and to maintain it also under varying resource quality (Fuller et al. 2015), might further hinder the detection of a clear response to nutrient concentration changes in some groups.

Communities at low water N:P sites were clearly dominated by taxa belonging to group I_2. This was due to the large number of Oligochaeta, whose dominance at this site type might be explained by low body N:P ratios as reported by Singer et al. (2007).

4.4 Perspectives

This study, combining stoichiometric traits with other biological traits to investigate patterns of changes in community C:N:P ratios, suggests that there is a great potential of using and extending this approach for investigating numerous questions. Specifically, it would be interesting to:

i) *Integrate other biological traits.* There is further evidence that nutrient load is linked to functional traits, as taxa differing in life span or feeding type respond differently to nutrient changes in the environment (Cross et al. 2005, Cross et al. 2006). In other studies, only some functional groups were affected by nutrient enrichment (Dang *et al.*, 2009; Evans-White *et al.*, 2009). The integration of feeding and/or food type seems to be particularly promising, as basal resources vary in stoichiometry with nutrient load in the stream (Farrell *et al.*, 2018), leading to a larger effect of nutrient enrichment on primary consumers than predators (Demi et al. 2019). Additionally, stoichiometric differences between feeding groups have already been reported (Cross et al. 2003, Evans-White et al. 2005) and also in this study we found a signal in C:N stoichiometry probably related to trophic level.

ii) *Increase the number of taxa and environmental situations*. The scope of our analysis has been restricted by the number of taxa that we were able to collect and analyse for stoichiometry. Especially very small taxa are often difficult to identify and to collect in sufficient quantities. Therefore, they have

often not been considered or only integrated as pooled groups at lower taxonomic level, although they can make large proportions of a community. Improving the stoichiometric data set by increasing the number of taxa as well as their sample size would allow a finer analysis and might strengthen the first observations. Using the dataset of a one-year survey on community composition has already supported our approach of using stoichiometry and developmental traits to distinguish macroinvertebrate communities along a nutrient gradient. Testing community hypotheses on a larger number of situations might be useful (*e.g.* increasing the number and the environmental diversity of stream types). Likewise, increasing the number of study sites could help to reduce the confounding effect of other non-nutrient water parameters (*e.g.* pH, O_2), that are necessary to account for when relying to field data rather than controlled experimental set-ups. In general, the approachmight be extended to other ecosystem types and animal communities, as similar trait databases are available for example for zooplankton (Brun *et al.*, 2016) or for terrestrial invertebrates (Pey *et al.*, 2014).

iii) *Define the consequences of changes in the stoichiometric structure of animal communities on ecosystem functioning.* In this study, changes in community stoichiometry went along with changes in community composition and community traits. Reported ecosystem responses following such changes on community level after nutrient enrichment involve changes in nutrient flux and cycling (Cross et al. 2007). More precisely, food chains become smaller (Singer and Battin 2007) and consumer biomass increases due to a higher amount of faster growing species (Prater *et al.*, 2015). The production of macroinvertebrate primary consumers increases as the result of increased resource quality. This effect only reaches predators, *i.e*. secondary consumers, in some studies (Cross et al. 2006, Demi et al. 2018) and seems to depend on prey identity (Spivak *et al.*, 2009). Davis et al. (2010) observed a shift towards larger prey organisms, which turned the positive effects of increased consumer production into negative effects for secondary consumers, as preys became morphologically not suited for consumption. This underlines the complexity in tracing such bottom-up effects along trophic levels and the importance of integrating traits in such analyses. Body stoichiometry is also linked to nutrient recycling. Besides taxonomic differences, P-rich taxa tend to excrete material that is less rich in P. The same negative relationship holds for N:P ratio (Vanni *et al.*, 2002). Consequently, the stoichiometric traits of a consumer affect the elemental composition of the basal resources *via* its nutrient release (Evans-White and Lamberti 2006, Knoll et al. 2009). Although the strength of consumer-driven nutrient recycling seems to depend on nutrient availability, changes in community stoichiometry will alter the concentration of the nutrients released into the environment.

Conclusion

We have shown that the community stoichiometry changed over a nutrient gradient of water N:P, following the nutrient concentrations. This went along with a change in community composition regarding developmental groups, which, for some trait-based groups, clearly followed our assumptions based on GRH. By extending the GRH to developmental traits, we have demonstrated the potential of

using stoichiometric traits to address changes in community composition. Integrating other functional traits to stoichiometric traits and linking them to alterations in nutrient loads could help to better understand the consequences of nutrient changes on community structure, and, in turn, on ecosystem functioning.

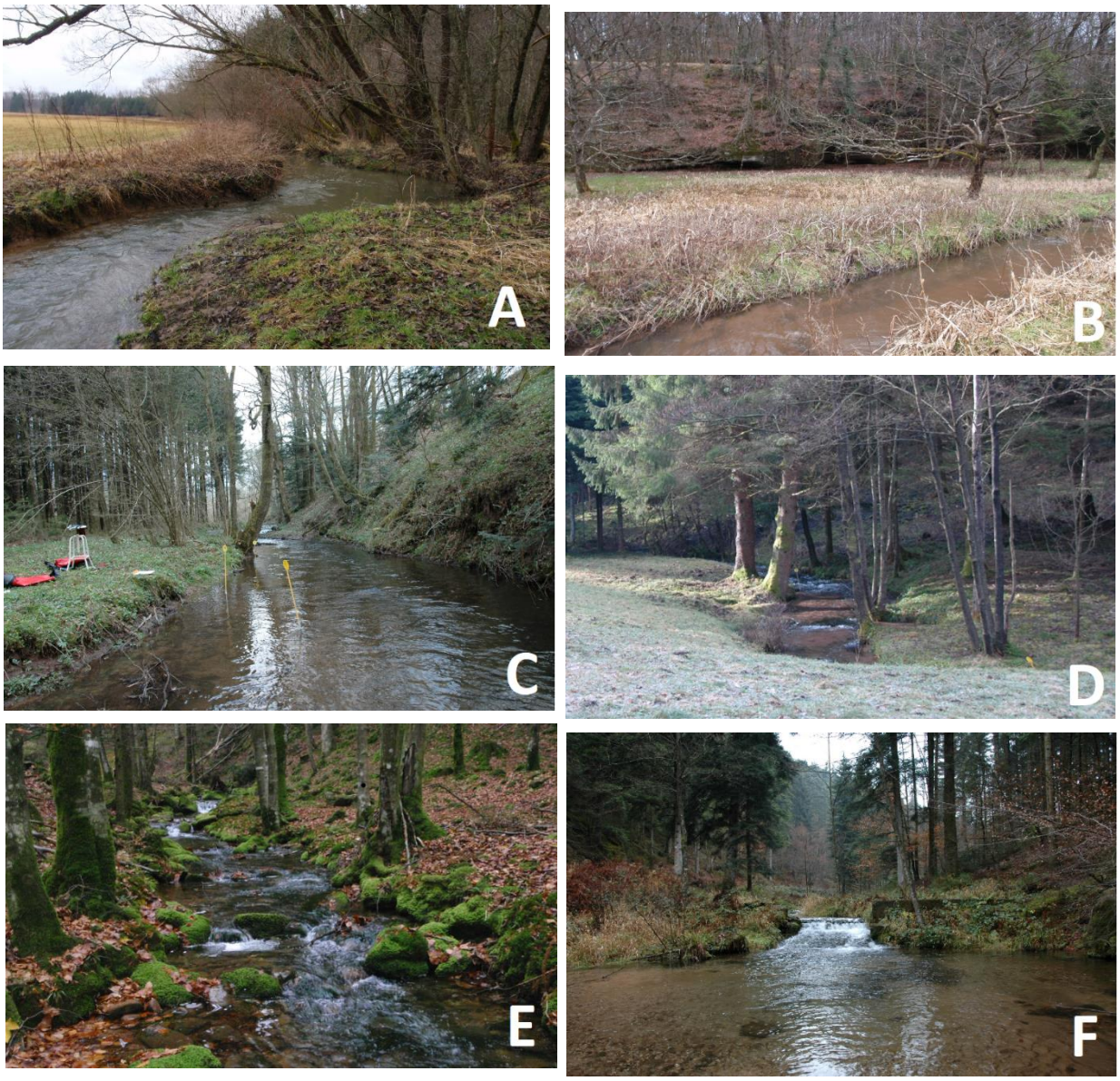


Fig. 4c.S1: Photographs of the sampling sites representing either low (A & B), medium (C & D) or high (E & F) water N:P. Photos were taken by Philippe Wagner.

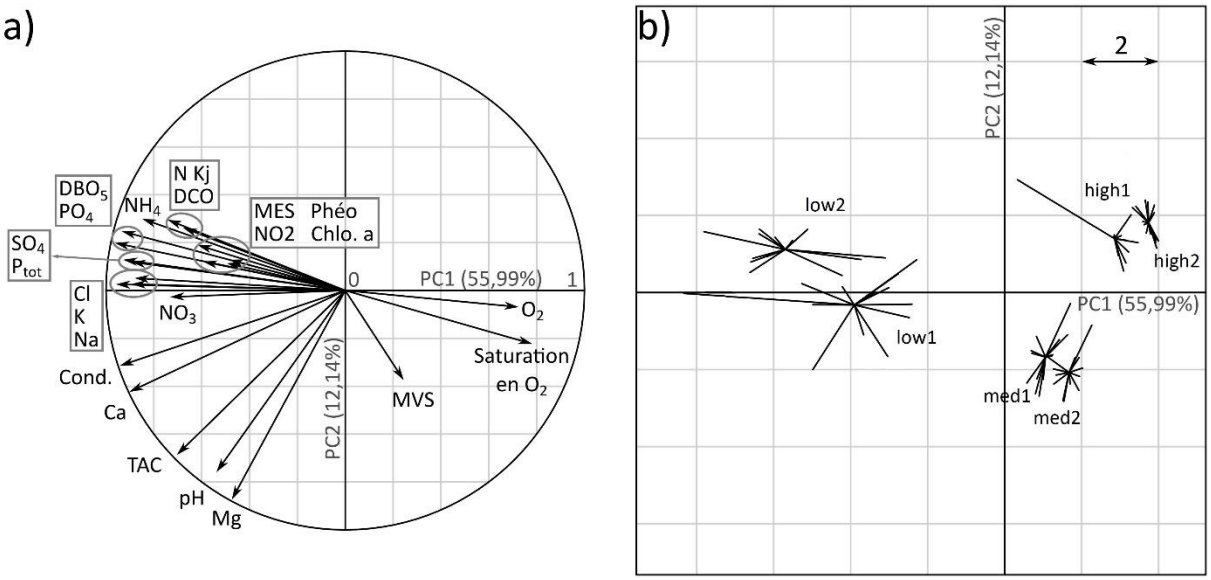
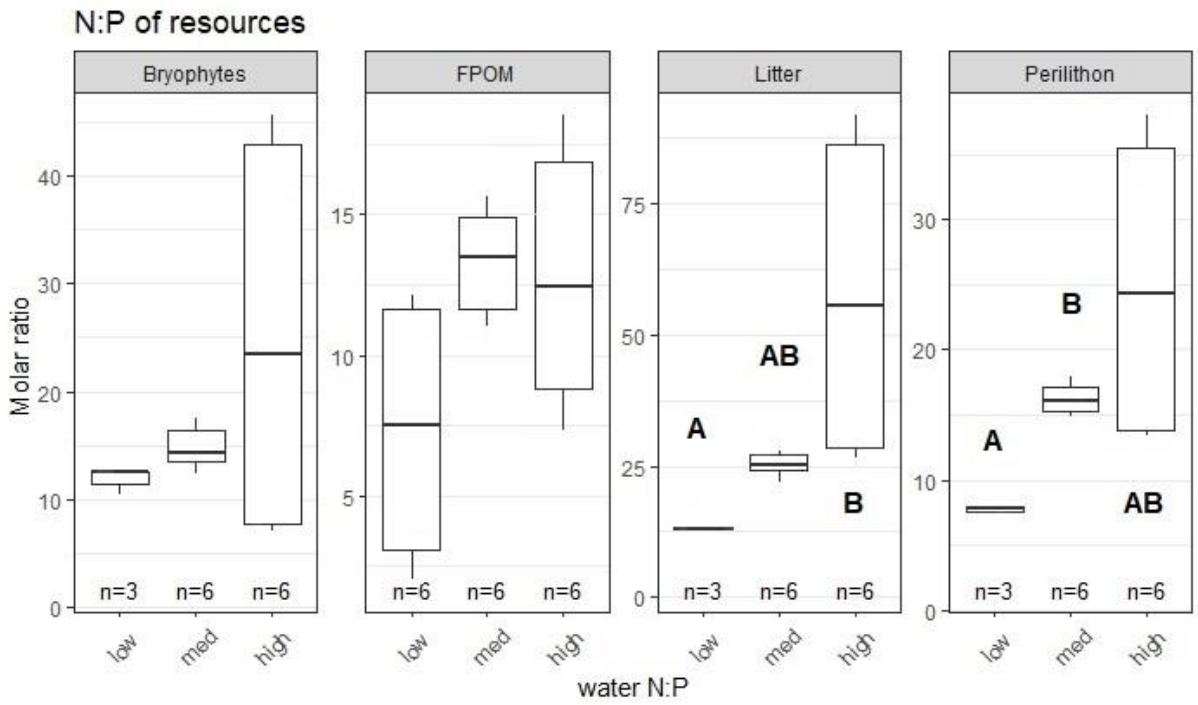


Fig. 4c.S2: Results of a principal component analysis (PCA) applied to the array gathering information on 23 water chemistry-related parameters for the six sampling sites surveyed over fourteen months (twelve campaigns). a) Correlation circle providing the correlations of the 23 parameters with the two first principal components; b) Mean locations of sites in the first factorial plane. Each site is located at the average of the locations of the corresponding twelve campaigns (each campaign being related to the main location of the site by a row). The percentage of the total variance explained by each PCA axis is provided.



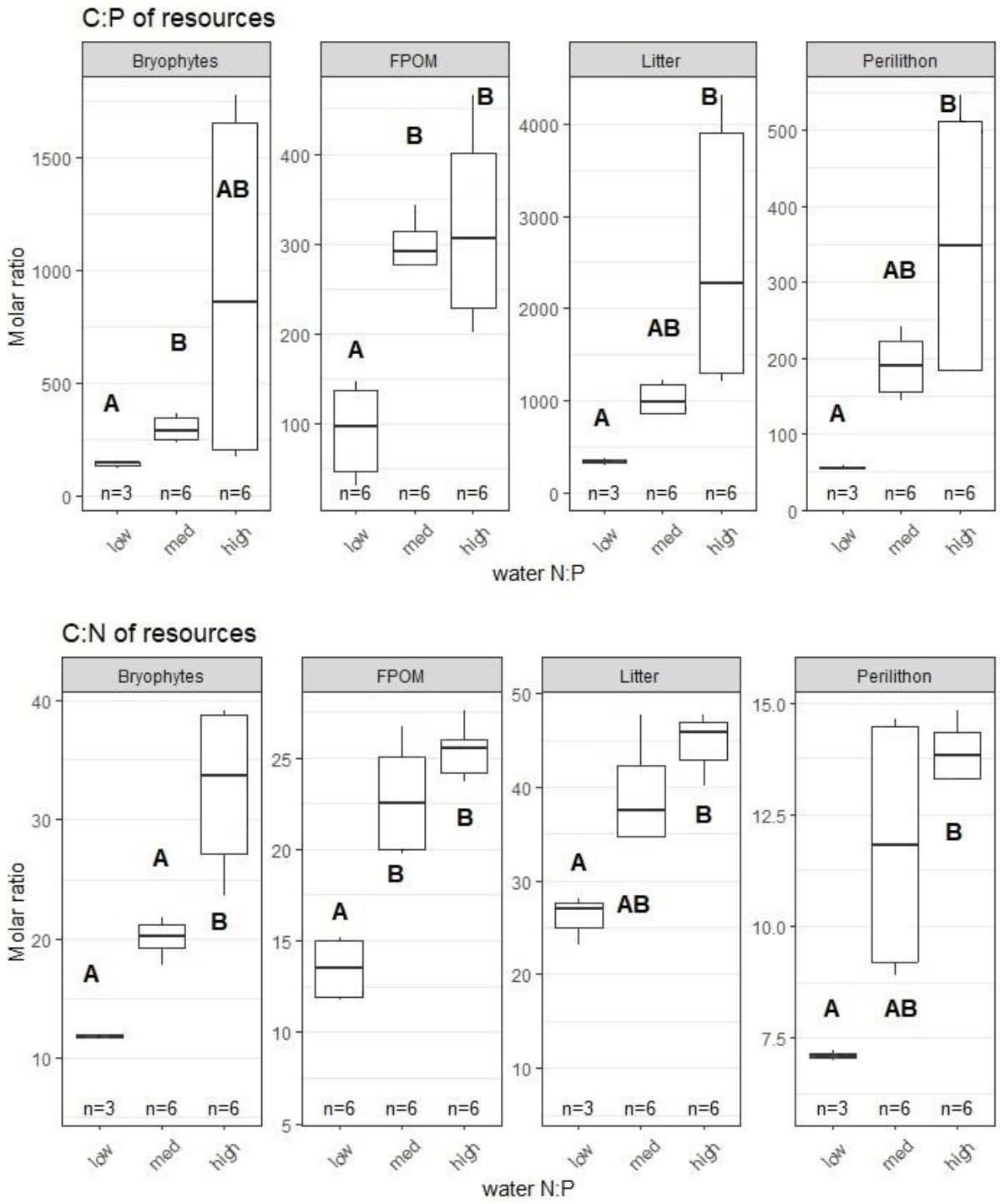


Fig. 4c.S3: Stoichiometric ratios (N:P, C:P, C:N) of resources (bryophytes, FPOM, leaf litter, perilithon) at the three levels of water N:P (high/ medium/ low). The thick horizontal line represents the median, the boxes range from the 25th to the 75th percentiles and the whiskers cover extend to 1.5 times the inter-quartile range. Letters indicate significant differences ($\alpha < 0.05$) using Dunn's rank-sum test with Bonferroni correction following a Kruskal-Wallis test. Sample size was n=6 for high and medium water N:P streams and n=3 for low water N:P streams.

Tab. 4c.S1: Trait profiles of invertebrate taxa for the three developmental traits ("life duration", "voltinism", "number of reproductive cycles per individual") used to define the developmental groups. For each trait category, the values indicate the affinities of taxa for the corresponding modality as relative frequencies. Trait information was gathered based mainly on Tachet et al. (2010), Juget & Lafont (1994), Richoux P. (1994), Tachet et al. (1994), Usseglio-Polatera (1994) and Usseglio-Polatera & Tachet (1994), and an unpublished trait database constructed during the project "Biologische Merkmale von Fusswirbellosen als Basis einer überregionalen Bewertung ökologischer Funktionsfähigkeit" (BFG/University Lyon1; 2001-2003).

-> This supplementary material can be found with the online article (https://doi-org.bases-doc.univ-lorraine.fr/10.1111/oik.08090)

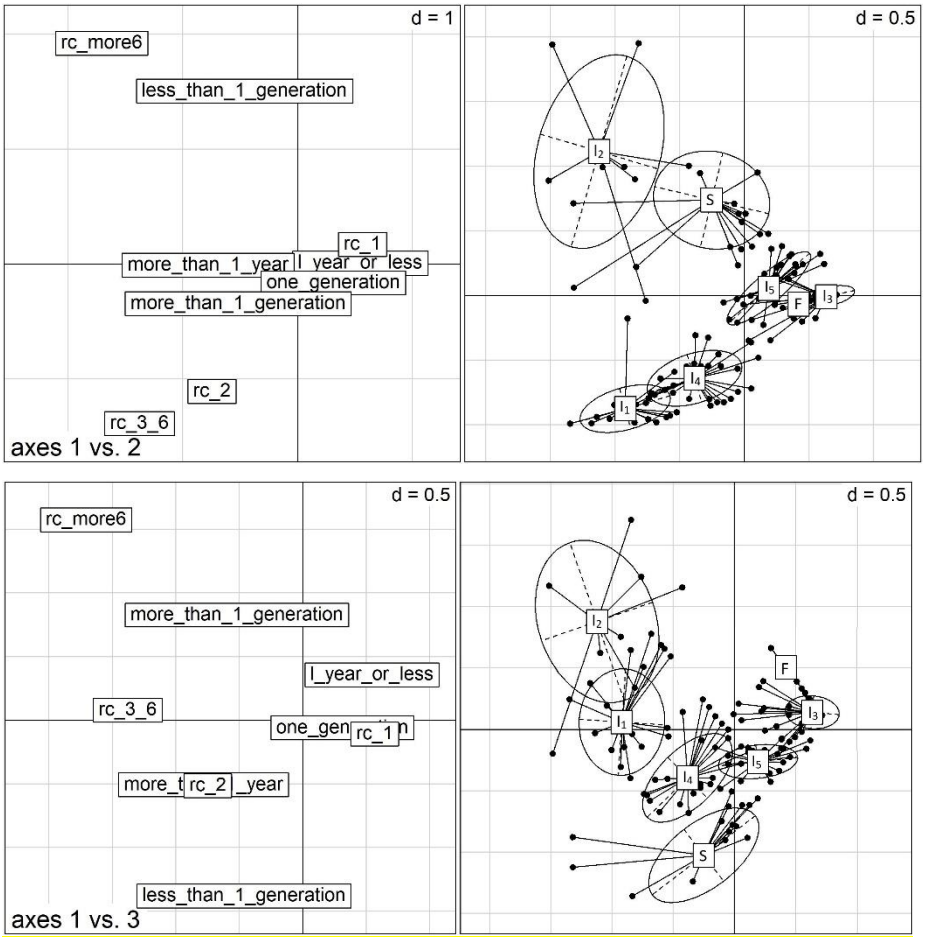


Fig. 4c.S4: Homogeneous groups of macroinvertebrate taxa with respect to their trait characteristics. The groups were defined using a Fuzzy Correspondence Analysis (FCA) performed on the affinity profiles of 447 European taxa (dots) regarding life span ("one year or less", "more than one year"), voltinism ("less than one generation per year", "one generation per year", "more than one generation per year") and the number of reproductive cycles per individual ("one", "two", "three to six", "more than six"). The following hierarchical clustering (Ward algorithm) using the Euclidean distance between taxa on the first three axes of FCA (explaining 69.87% of the total trait variance). The groups explain 73.47% of the total trait variance (Monte Carlo between group permutation test: n = 9999; $p = 10^{-4}$). For each plane defined by the axes used in the clustering analysis, the left panel shows the distribution of trait categories) and the right panel shows how groups are distributed on those planes.

Tab. 4c.S2: Macroinvertebrate stoichiometry across sites with different water N:P levels and corresponding group.

group	taxa	site type	n	C:N	C:P	N:P
I_1	*Asellus*	Low	3	4.25 (± 0.14)	36.67 (± 2.12)	8.63 (± 0.65)
I_1	*Gammarus*	Low	3	5.51 (± 0.56)	47.99 (± 4.84)	8.72 (± 0.69)
I_1	*Gammarus*	Medium	3	4.60 (± 0.13)	39.44 (± 0.55)	8.58 (± 0.27)
F	*Baetis*	Low	6	4.99 (± 0.20)	51.55 (± 3.08)	10.34 (± 0.66)
F	*Baetis*	Medium	6	4.88 (± 0.38)	56.99 (± 17.69)	11.61 (± 2.87)
F	*Hydropsyche*	Low	3	6.06 (± 0.60)	58.78 (± 2.52)	9.75 (± 0.57)
F	*Hydropsyche*	Medium	5	5.34 (± 0.46)	61.95 (± 3.66)	11.66 (± 1.16)
I_3	*Brachyptera*	High	2	4.20 (± 0.02)	49.47 (± 2.14)	11.79 (± 0.57)
I_3	*Brachyptera*	Medium	3	4.42 (± 0.07)	38.93 (± 1.77)	8.82 (± 0.51)
I_3	*Ephemerella*	Medium	6	4.18 (± 0.48)	41.36 (± 1.44)	9.98 (± 0.93)
I_3	*Nepa*	Low	6	4.12 (± 0.06)	56.56 (± 8.93)	13.75 (± 2.34)
I_3	*Plectrocnemia*	High	3	3.98 (± 0.14)	39.54 (± 7.34)	9.910 (± 1.51)
I_3	*Protonemura*	High	2	4.50 (± 0.40)	50.73 (± 6.65)	11.24 (± 0.49)
I_3	*Protonemura*	Medium	3	4.28 (± 0.11)	51.04 (± 4.88)	11.90 (± 0.85)
I_3	*Velia*	High	3	4.23 (± 0.08)	62.09 (± 2.40)	14.69 (± 0.28)
I_5	*Leuctra*	High	6	4.11 (± 0.13)	49.18 (± 2.94)	11.97 (± 0.51)
I_5	*Leuctra*	Medium	3	4.55 (± 0.04)	54.91 (± 2.53)	12.07 (± 0.65)
I_5	*Rhithrogena*	Medium	5	4.44 (± 0.26)	48.05 (± 6.05)	10.83 (± 1.34)
I_5	*Rhyacophila*	High	3	4.45 (± 0.54)	53.81 (± 10.45)	12.12 (± 2.09)
I_5	*Rhyacophila*	Medium	3	4.42 (± 0.19)	46.60 (± 6.78)	10.59 (± 2.02)
I_5	*Sericostoma*	High	3	6.14 (± 0.52)	80.73 (± 2.19)	13.19 (± 0.76)
I_5	*Sericostoma*	Medium	6	5.86 (± 0.51)	77.64 (± 11.02)	13.40 (± 2.59)
S	*Cordulegaster*	High	4	4.33 (± 0.26)	75.17 (± 2.94)	17.43 (± 1.31)
S	*Cordulegaster*	Low	2	4.22 (± 0.13)	73.92 (± 7.98)	17.48 (± 1.32)
S	*Cordulegaster*	Medium	1	4.06 (± NA)	65.36 (± NA)	16.10 (± NA)
S	*Isoperla*	Medium	3	3.73 (± 0.05)	38.93 (± 6.79)	10.44 (± 1.89)
S	*Perlodes*	Medium	4	4.51 (± 0.24)	35.32 (± 18.52)	7.99 (± 4.47)
S	*Sialis*	High	3	4.73 (± 0.58)	57.80 (± 8.07)	12.22 (± 0.65)

Tab. 4c.S3: Developmental groups of macroinvertebrate taxa found during the annual sampling campaign.

Group	Taxon	Group	Taxon	Group	Taxon
F	Anthomyidae	I3	*Brachycentrus*	I4	*Myxas*
	Baetis		*Brachyptera*		*Orectochilus*
	Caenis		Chaetopterygini		*Oulimnius*
	Ceratopogonidae		Dixidae		*Pisidium*
	Chironomidae		Drusinae		Planariidae
	Holocentropus		*Empididae*		Planorbidae
	Hydropsyche		*Epeorus*		*Radix*
	Hydroptila		*Ephemerella*		*Riolus*
	Lepidoptera		*Glossosoma*		*Sphaerium*
	Mesoveliidae		*Goera*	I5	Athericidae
	Notidobia		*Habroleptoides*		Calopterygidae
	Psychodidae		*Habrophlebia*		Curculionidae
	Psychomyia		*Lepidostoma*		*Ecdyonurus*
	Simuliidae		Leuctridae		*Elodes*
	Tinodes		Limoniidae		*Heptagenia*
I1	Asellidae		*Lithax*		*Hydraena*
	Brychius		*Mystacides*		Hydrophilinae
	Bythinella		Nematoda		*Micrasema*
	Colymbetinae		*Plectrocnemia*		*Perlodes*
	Corophiidae		*Polycentropus*		Rhagionidae
	Galba		*Protonemura*		*Rhithrogena*
	Gammarus		Tabanidae		*Sericostoma*
	Hydroporinae		Tipulidae		*Siphonoperla*
	Lithoglyphus		*Torleya*		*Synagapetus*
	Potamopyrgus		Valvatidae	S	*Ancylus*
I2	Hydrozoa	I4	Dugesiidae		*Chloroperla*
	Oligochaeta		Erpobdellidae		*Cordulegaster*
I3	*Adicella*		*Esolus*		*Diura*
	Agapetus		Glossiphoniidae		*Ephemera*
	Amphinemura		Hydracarina		*Isoperla*
	Ancylidae		*Lathelmis*		*Nemoura*
	Beraea		*Limnius*		*Odontocerum*
					Rhyacophila

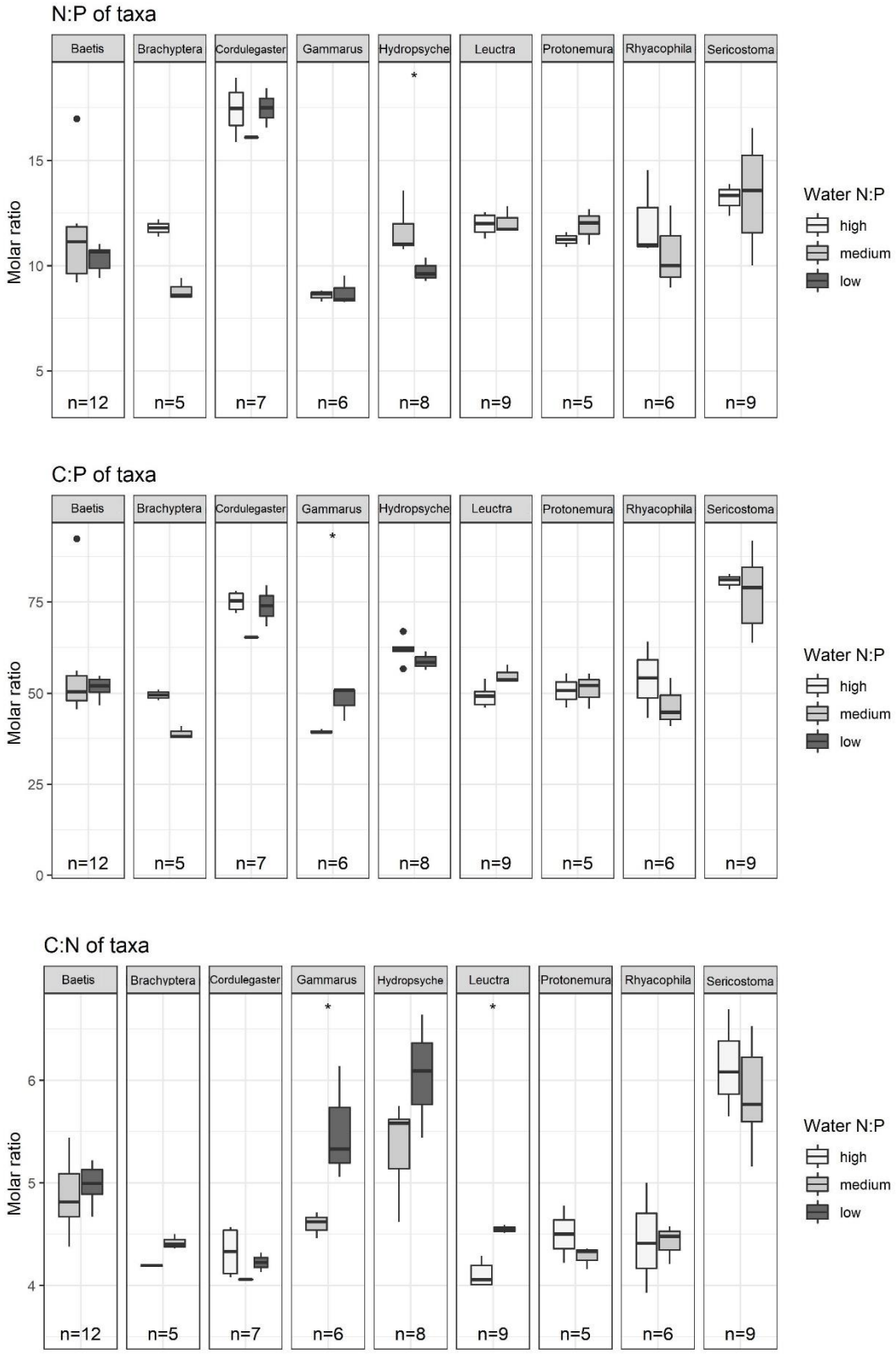


Fig. 4c.S5: Stoichiometric ratios (N:P. C:P. C:N) at different levels of water N:P of macroinvertebrate genera that were found at least at two different site types (nine out of 18 taxa). The thick horizontal line represents the median, the boxes range from the 25th to the 75th percentiles and the whiskers extend to 1.5 times the inter-quartile range. Outliers are displayed as black dots. Asteriks indicate significant differences ($\alpha < 0.05$) using Dunn's rank-sum test with Bonferroni correction following a Kruskal-Wallis test. "n" indicates the number of individuals of a given genus.

Chapter V:

Community stoichiometry under global change

34-year of phosphorous decline: Responses in community stoichiometry and functional trait composition

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Abstract

Nutrient changes, both in terms of quantity and ratio are and will continue to be main stressors in worldwide ecosystems. Phosphorous and nitrogen enrichment for example shifted consumer communities towards small-bodied, fast-growing taxa and taxa rich in phosphorous, supporting the well-known link between body (phosphorous) stoichiometry and growth rate. As it has recently been shown, also other traits, *e.g*. related to development or feeding are linked to body stoichiometry. Ecological stoichiometry theory (=EST) and the integration of stoichiometric traits, *i.e.* body stoichiometry, seem thus a promising tool to help understanding the mechanisms behind such taxonomic and functional community responses. However, analyses on community level and/or on a temporal nutrient gradient so far are missing.

Using a dataset of the French Loire river spanning a 34-year period we applied multivariate analysis and investigated long-term trends in water nutrient concentration and community stoichiometry. We expected that – due to species shifts – community stoichiometry would follow the nutrient concentration in the water and that changes in the functional trait structure should follow assumptions from EST. Over the study period, water phosphorous concentration significantly decreased while water N:P increased. The macroinvertebrate community shifted towards P-poor taxa and we observed significant trends in overall community stoichiometry that were in line with our expectations and indicates a change in the nutrient pool provided by these consumers. Further, high phosphorous conditions favoured traits often associated to a fast development (small maximum body sizes, short life duration, high number of molting events) and detritus feeding. Recent conditions, *i.e*. high N:P ratios, favoured slow-developing and predatory taxa. Stoichiometric traits were largely contributing to the link between functional traits and environmental parameters, which further underlines the potential of integrating stoichiometric traits when investigating ecological responses towards nutrient changes.

1. Introduction

Human activities such as fertilization or fossil fuel burning, have dramatically increased nitrogen and phosphorous concentrations in freshwater systems (Peñuelas *et al.*, 2013; Battye *et al.*, 2017). On the other hand, successful management programs have led to declines in phosphorous concentrations (Jeppesen *et al.*, 2005). Nitrogen levels that often remained unaffected by these measures and therewith high as well as a faster accumulation of phosphorus rather than nitrogen in freshwaters (Yan *et al.*, 2016), increased also the N:P ratio in the water (Jeppesen *et al.*, 2005; Peñuelas *et al.*, 2013; Yan *et al.*, 2016; Isles *et al.*, 2018). Changing nutrient conditions – either in absolute values and/or in their ratio – therefore are among the main stressors in worldwide ecosystems.

Apart from toxic effects at rather high increases (Camargo *et al.*, 2005), the effect on consumer communities is only indirect, mainly *via* changes in resource quality and quantity (Evans & Sanderson, 2018; Nessel *et al.*, 2021). Globally, total invertebrate abundances and biomass increased following nutrient enrichment (Nessel *et al.*, 2021). Biodiversity decreased (Singer & Battin, 2007; Zhang *et al.*, 2019), but a species shift not always affects diversity or taxa richness (McCormick *et al.*, 2004; Nessel *et al.*, 2021). The communities thereby tend to shift towards pollution-tolerant taxa, increasing abundances of oligochaets and snails over insects (McCormick *et al.*, 2004; Ortiz & Puig, 2007; Friberg *et al.*, 2010). Although the effects of water hydrology and/or physical habitat characteristics are often more important than nutrients, nutrient load can influence the community responses towards these stressors (Wagenhoff *et al.*, 2012; Piggott *et al.*, 2015; Juvigny-Khenafou *et al.*, 2021) or disturbances (Gafner & Robinson, 2007).

Trait-based approaches aim at investigating how such stressors affect the functional structure of a community. Feeding groups for example respond differently to nutrient-enrichment, which favoured scrapers and collector-gatherers over shredders and filter/suspension feeders (McCormick *et al.*, 2004; Demi *et al.*, 2019); but responses of predators vary among studies (McCormick *et al.*, 2004; Wimp *et al.*, 2010; Demi *et al.*, 2019). Life history traits shifted towards small and fast-growing/-developing taxa (Singer & Battin, 2007; Demi *et al.*, 2019).

Stoichiometric traits are one kind of biological traits, describing the elemental composition, *i.e.* the nutrient content or ratio, of an organism's body tissue. It is assumed to represent the organism's nutrient demand depending on its physiology and biology and is considered to be taxon-specific. Regulating mechanisms in heterotrophs allow for (general) homeostasis and – although there is intra-taxon variation *e.g.* over ontogeny (Back & King, 2013) – most taxa keep a constant body stoichiometry even under changing nutrient conditions (Evans-White *et al.*, 2005; Persson *et al.*, 2010; Danger *et al.*, 2013). Therefore, they are a key tool in ecological stoichiometry to investigate the equilibrium and fluxes of elements between the environment and the organisms and how they interact (Sterner & Elser, 2002).

Studies have demonstrated a link to functional traits (MS 2), especially growth rate (Elser *et al.*, 2003a; Acharya *et al.*, 2004). Taxa differing in development-related traits (Beck *et al.*, 2021) or feeding group differ also in their stoichiometric traits (Fagan *et al.*, 2002; Cross *et al.*, 2003; González *et al.*, 2011a). This direct link towards both, nutrients and functional traits, makes the integration of stoichiometric traits into trait-based community analyses interesting to better understand the mechanisms behind taxonomic and/or trait shifts described above.

The few studies that investigated the link between environmental nutrient conditions and body stoichiometry or stoichiometric trait composition at the community scale suggest that community stoichiometry follows the nutrient gradient in the water by favouring phosphorous-rich taxa under high phosphorous conditions (Singer & Battin, 2007; Prater *et al.*, 2015; Teurlincx *et al.*, 2017). These studies investigated artificially enriched streams or compared streams with distinct nutrient regimes. Both approaches allowed for interesting results, but also have the limitations of being restricted to a relatively short period of time or facing other, non-controlled geophysical differences among study sites. Additionally, most studies remained largely focused on individual taxa within communities. The mono-site characteristic of a time series excludes such additional variations and, due to the longer time period covered, increases the chance to detect true long-term trends rather than short-term effects.

Based on a long-term data set, this study aims at investigating whether community stoichiometry follows changes in water nutrient concentration over time. We expect that community %P will decrease over time (likely going along with increasing community C:P and N:P ratios), thus following the water phosphorous concentration. This should be caused by a shift of the community towards P-poor taxa that require lower quantities of phosphorous. Since body stoichiometry has already been related to other functional traits (MS 2), we will further analyse if and how these expected changes in stoichiometric traits within the community are also associated to changes in other biological traits. For example, we expect that trait attributes linked to fast growth and development (such as small body size, short life duration, high number of molting events) would be associated to high body %P, both being positively correlated to water phosphorous concentration. To test these hypotheses, we combined stoichiometric and biological trait information available from databases with abundance data collected over a 34-year period at Dampierre-en-Burly, on the upper section of the Middle Loire River (France). Continuous detailed information on macroinvertebrate abundances and water parameters are available and already revealed a significant decrease in phosphorous concentration over this period (Floury *et al.*, 2013). This site also showed effects of climate change, that are a significant increase in temperature (+0.9°C over the 30-year period) and a decline in water discharge (c. -82 $m^3 s^{-1}$, from 387 to 305 $m^3 s^{-1}$ over the same period). These stressors have caused a shift in taxonomic and functional composition of the macroinvertebrate community (Floury *et al.*, 2013, 2017), thereby making it attractive to add the stoichiometric view in order to help explaining the observed patterns of changes.

2. Material & methods

Datasets

Community and environmental data

The study site is located in the upper section of the Middle Loire River in France. Hydro-climatic parameters (temperature, discharge) have been monitored at "Dampierre-en-Burly" (47.4°N; 2.1° E) continuously over the 1980-2013 study period, due to a regulatory survey at the *Electricité de France* power plant, positioned downstream of the study site. Water chemistry information (NO_3, PO_4) was obtained from the monitoring program of the *Loire-Bretagne Basin Water Authority* at a site located 40km downstream ("Jargeau", 47.5° N; 2.1° E). These data provide the best homogeneous data coverage available over the study period regarding these parameters and are representative for Dampierre-en-Burly (Floury *et al.*, 2013). The nutrient concentrations were monthly recorded.

Invertebrate communities were sampled at Dampierre-en-Burly four times per year (except for 2008 with only three sampling events), providing in total 135 benthic invertebrate community samples over the study period between 1980 and 2013. Most sampling events were conducted during summer (June, July, August, September) but the sampling period ranged from April to November.

The invertebrate sampling followed the IQBG protocol ("Indice de Qualité Biologique Globale"; Verneaux *et al.*, 1976) for 1980-1994 and the normalized IGBN field protocol ("Indice Biologique Global Normalisé"; French Norm NF T90-350, 2004) between 1995 and 2013. The taxa Bryozoa, Hydrozoa, Hydrachnidia, Nematoda, Oligochaeta and Porifera have been excluded to avoid identification or quantification bias. Taxonomic resolution of the other groups was harmonized to family level. For the period 1980-2008 both data sets have already been used in previous studies (Floury *et al.*, 2013, 2017).

Stoichiometric and biological trait information

Stoichiometric traits were available from a database (MS 1) gathering stoichiometric information as mass contents of carbon (%C), nitrogen (%N) and phosphorous (%P) as well as the molar ratios C:N, C:P and N:P. It provides taxon mean values, based mainly on field sampling data but also including values from the literature. In case the stoichiometric information was not available for a taxon, the values from a lower taxonomic level (*i.e.* genus) were used ($n \leq 3$ taxa). The data finally covered on average 85% of the communities in terms of abundances.

Eleven biological traits were taken from Tachet *et al.* (2010), supplemented by two traits used already in other works (molting events(Ref), number of reproductive events). The information is fuzzy coded (Chevenet *et al.*, 1994): a trait is resolved in different categories (or modalities), for which each taxon's affinity is described by affinity scores. These scores range between 0 (= no affinity) and 5 (= high affinity) and have been transformed into relative frequencies within each trait before further analysis. By using this fuzzy coding approach, we rely on potential traits that capture intra-taxon variation. The

description of some trait categories was slightly modified, *i.e.* by merging ecologically/functionally similar categories to avoid categories only occupied by a very low number of taxa (sup. S 5.1). The traits and the final categories used in this study can be found in Tab. 5.1.

Data analysis

Trends in community stoichiometry and water parameters

Community stoichiometry was calculated as abundance-weighted mean of individual taxon values. Macroinvertebrate abundances (n) were log(n+1) transformed, before multiplying the relative frequency of each taxon (its log-transformed abundance divided by overall cumulated log-transformed abundances) with its stoichiometric value (%C, %N, %P, C:N, C:P, N:P). The values of all the taxa in a faunal sample were then summed up to obtain the community stoichiometric composition for each sampling event. Thereby, the calculations are based only on those taxa, for which the corresponding stoichiometric information was available. To avoid a bias when calculating with ratio data, taxon body C:N, C:P and N:P ratios were log-transformed prior to calculations. For each year, the mean of each community stoichiometry variable was calculated (n = 4, except for 2008 where n = 3).

Water N:P ratio was calculated from NO_3 and PO_4 concentrations on the raw data set, that is for each measurement with both variables available. For the analysis only water parameter information (concentrations of NO_3, PO_4, water N:P, temperature) that was assumed relevant for the macroinvertebrate community, was considered. Therefore, available information on the 126-day period before each sampling day was averaged for each community sampling date. Using this same dataset, such ~4-month period has been defined the optimal proxy for the response time of macroinvertebrate communities to these water parameters in this site (Floury *et al.*, 2013). As for community stoichiometry, the yearly mean of each water parameter was calculated.

To detect possible trends, Mann-Kendall trend tests were applied to each community stoichiometric variable and water parameter. To overcome the problem of autocorrelation in time series data a modified version of the test that removes autocorrelation by Spearman rank correlations (Hamed & Rao, 1998) was used.

Association between water parameters and stoichiometric & biological traits

A RLQ analysis was applied to investigate the co-structure between functional traits (stoichiometric and biological) and water parameters (PO_4, NO_3, water N:P, temperature). This kind of analysis was designed to link an environment table (site x environmental parameter, 'R') *via* an abundance table (site x taxa, 'L') to a trait table (traits x taxa, 'Q'). The dimension of sites in R & L (*i.e.* rows) and of taxa in L & Q (*i.e.* columns) thereby must be the same.

In a first step, each of the tables was analysed separately, to capture the total variation, (inertia), within each of them. Yearly mean abundances were calculated from the abundance tables, so that "year" in our analysis could be treated as "site" from the original design. A Correspondence Analysis (CA) was then

applied on this log(n+1) transformed mean abundance data ('L'). The column and row weights obtained by this analysis were used in individual analyses of the R and Q table: The environment ('R') table was subjected to a PCA, weighing the sites by the row weights (corresponding to the sites) obtained by the CA. Likewise, the trait table ('Q') was subjected to a PCA using the CA column weights to weigh the taxa. Water chemistry variables (NO_3, PO_4, water N:P) and stoichiometric ratios (C:N, C:P, N:P) were log-transformed prior to the analysis.

In a second step the rlq() function was applied using the three output objects from the individual analyses described above to perform a double inertia analysis ('R', 'Q') linked by a contingency table ('L') . The total inertia indicates the link between functional traits and water parameters as mediated by abundances. Its significance was estimated by a permutation test (9999 permutations). We decided for the two-step procedure suggested by Dray & Legendre (2008) for a better control of type I error.

Differences in body stoichiometry between groups of taxa

Over the study period, Floury *et al.* (2013) identified seven groups of macroinvertebrate families that show distinct temporal distributions. They used a cluster analysis on the taxon scores on the first factorial plane obtained from a Correspondence Analysis (CA) applied on taxa abundances over the period from 1979-2008. We gathered these groups according to their response curve: Group A contains taxa that were present in the first years of the study and showed decreasing abundances (groups 1 and 2 in Floury et al. 2013), taxa of group B were present over the whole period showing unimodal or saturating response curves over time (originally groups 3, 4 and 5) and group C gathers taxa only appearing during the last decade (original groups 6 and 7).

We calculated the mean stoichiometric traits (%C, %N, %P, C:N, C:P, N:P) for each group and applied Kruskal-Wallis tests on each stoichiometric variable to test for differences between groups. Pairwise comparisons were applied when the result was significant (Dunn test, with Bonferroni correction for multiple testing). Non-parametric tests were used because of unequal sample sizes and deviations from homogeneity and normality assumptions as judged from graphical inspection.

All the analyses were performed using R (R Development Core Team, 2019, version 3.5.2) and the package ade4 (Dray & Dufour, 2007).

Tab. 5.1: Biological traits and their corresponding trait categories.

Functional trait	Categories
Aquatic life stages	egg, larva, nymph, adult
Dispersal	aquatic-active, aquatic-passive, aerial-active, aerial-passive
Feeding habits	deposit feeder, filter-feeder, piercer, parasite, scraper, shredder, swallower/chewer
Food resources	dead animal (≥1mm), dead plant (≥1mm), detritus (<1mm), living macrophytes, living microphytes, living microinvertebrates, living macroinvertebrates
Life duration	≤ 1 year, >1 year
Locomotion	attached, burrower, crawler, interstitial, swimmer
Maximum body size	≤0.5cm, >0.5-1cm, >1-2cm, >2-4cm, >4cm
Molting events	0, 1, 2-4, 5-9, 10-14, 15-19, >20 (= number of molting events)
Resistance forms	eggs/statoblasts, housing/cocoons, diapause/dormancy, none
Respiration	tegument, gill, plastron/spiracle
Reproductive cycles	1, 2, >2-6, >6 (= number of reproductive cycles per individual)
Reproduction mode	ovoviviparity, isolated eggs, clutches, clutches.vegter (vegetation/terrestrial), asexual reproduction
Voltinism	<1, 1, >1

3. Results

Trends in community stoichiometry and water parameters

All community stoichiometry variables showed a significant trend over the period 1980-2013. This trend was negative for community %C, %N and %P and positive in community molar ratios (C:N, C:P, N:P) (Fig. 5.1, Tab. 5.2). From the first decade (1980-1989) to the last decade of the study period (2004-2013), mean yearly community %C decreased from 43.05 to 39.53. Likewise, community %N and %P decreased from 8.75 to 7.82 and 0.79 to 0.65, respectively. Community C:N increased from 5.87 during the first decade to 6.42 during the last one. Community C:P and N:P changed from 146.94 to 184.93 and 24.53 to 28.50.

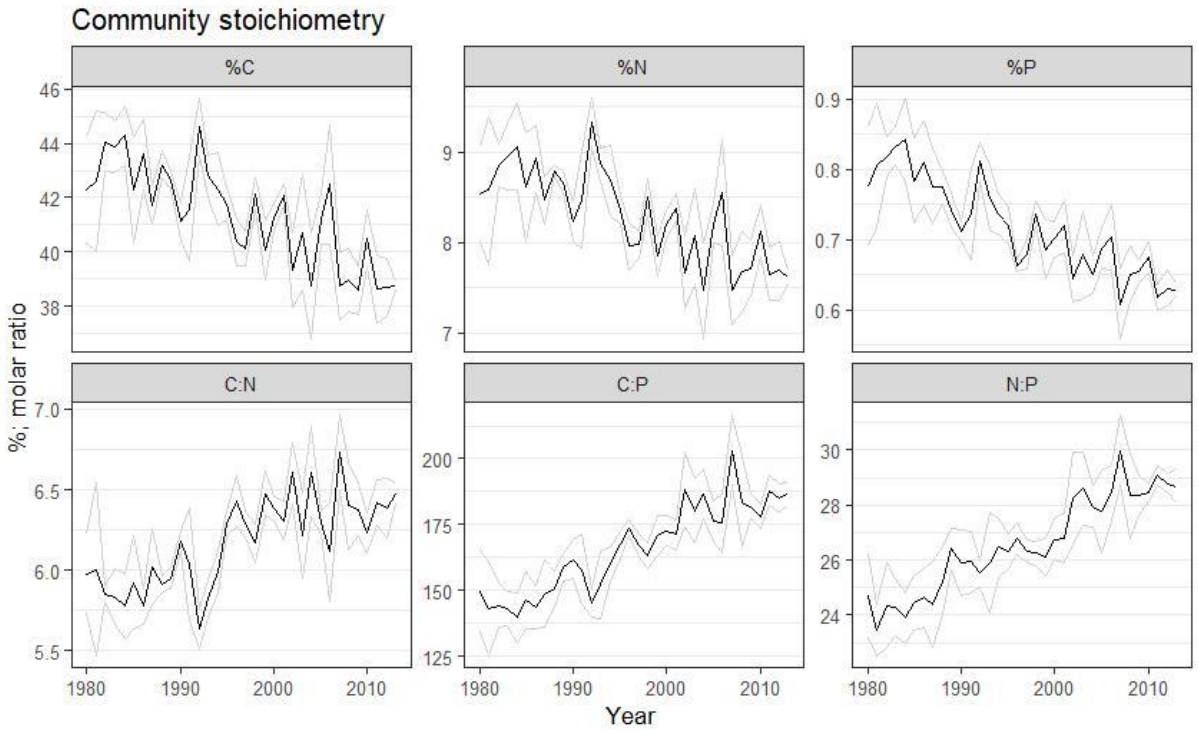


Fig. 5.1: Community stoichiometry (%C, %N, %P and molar C:N, C:P, N:P) over the study period 1980-2013. The black lines represent yearly mean values; light gray lines the mean +/- the standard deviations. The number of sampling events per year was n = 4 for all the years except 2008 with n = 3. Please note that ratio values (C:N, C:P, N:P) have been exponentiated in the figure to facilitate interpretation and comparison with other studies, while all analyses have been conducted using log-transformed values.

Water phosphorous concentration decreased significantly over the 34-year period, even though it slightly increased again during the years from 2003 to 2010 (Fig. 5.2, Tab. 5.2). Compared to the first decade (0.21mg/ L), the mean concentration during the last decade declined by 61.90% to 0.08 mg/ L. Water nitrogen concentration showed fluctuations between the years, but exhibited no overall trend. Between the first and the last decade, mean concentrations increased from 6.11 to 6.60 mg/ L (+8.02%). Water N:P ratio showed a significant increase and values increased more than fivefold from 31.52 calculated over the first decade to 174.67 over the last decade. In 2003 (when water P was the lowest), water N:P drastically increased from 98.73 to a maximum of 377.09 in 2004 and then decreased to values around 50 in 2009.

Water temperature showed a significant and positive trend, with temperatures increasing from 14.90°C during the first decade to 18.98°C during the last decade, corresponding to an increase by 27.38%.

Tab. 5.2: Results of Mann-Kendall trend tests applied on water parameters and community stoichiometry. Bold lettering indicates significance of Kendall Tau at $\alpha = 0.05$. Additionally, mean values (and standard deviation) for the first (1980-1989) and last (2004-2013) decade are provided.

	Parameter	Trend	Tau
Water	NO_3	-	0.11
	PO_4	↘	**-0.58**
	N:P	↗	**0.57**
	Temperature	↗	**0.57**
Community stoichiometry	%C	↘	**-0.60**
	%N	↘	**-0.56**
	%P	↘	**-0.70**
	C:N	↗	**0.56**
	C:P	↗	**0.76**
	N:P	↗	**0.79**

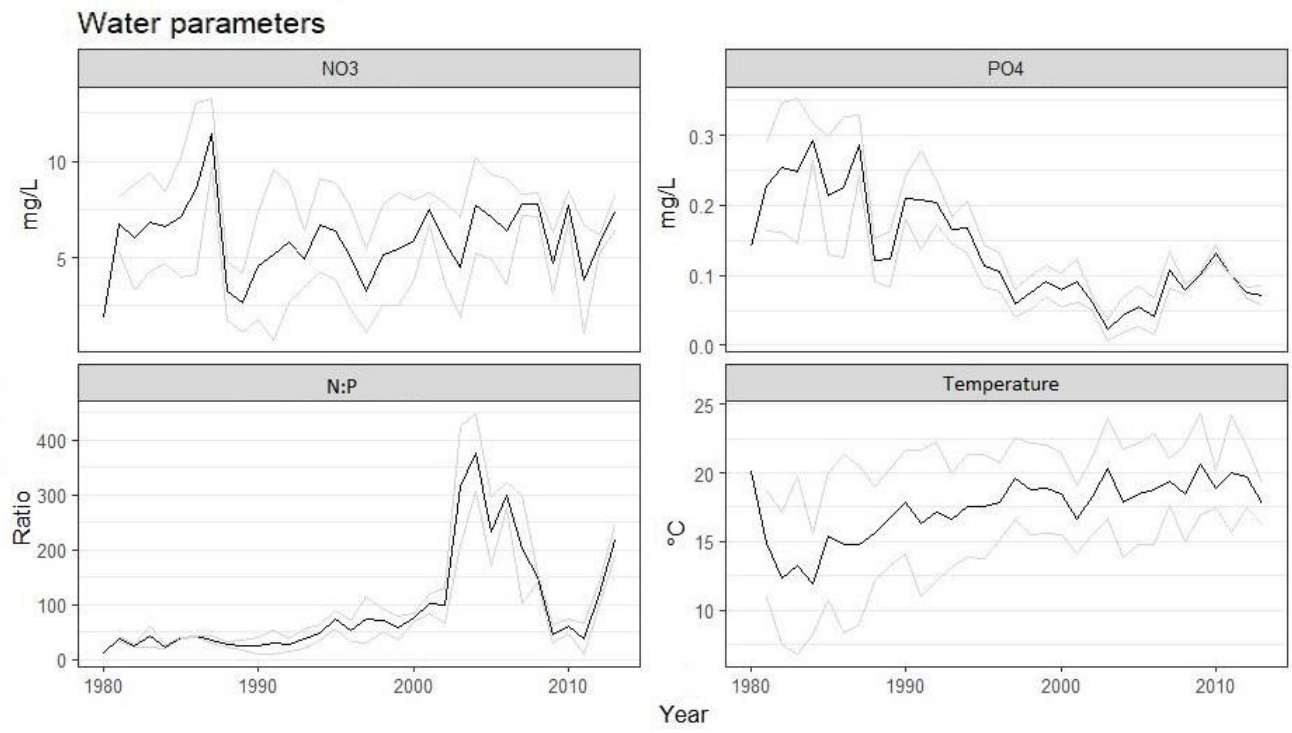


Fig. 5.2: Water quality parameters (concentrations of NO_3 & PO_4 in mg/ L and their ratio) and temperature (°C) over the study period 1980-2013. The black lines represent yearly mean values; light gray lines the mean +/- the standard deviation. The number of sampling events per year was n = 4 for all the years except 2008 with n = 3. The calculations are based on mean values over the 126-day period before each faunal sampling date.

Links between water parameters and stoichiometric & biological traits

There was a significant link between water parameters and stoichiometric and biological traits ($p < 0.05$, Tab. 5.2). Clearly the largest part of variation was captured by the first RLQ axis (94.81%). which separated water phosphorous concentration from water N:P and temperature, thus well representing the changes in these water parameters found in the annual mean values described above and representing the change in time. Water nitrogen concentration was slightly negatively correlated to the first axis, but showed a strong correlation to the second axis, which explained only 4.27% of variance. The following description of the results will focus on trait category involvement in the definition of the first RLQ axis only.

Body %C, %N and %P were all negatively varying along the first axis (Fig. 5.3c), positively related to water phosphorous concentration (Fig. 5.3a). Body molar ratios (C:N, C:P, N:P) were varying in the opposite direction, positively linked to water N:P and temperature. All six stoichiometric variables were among the highest involved trait categories in the link between invertebrate traits and environmental parameters.

The proportion of large-sized invertebrates (> 4 cm) increased along the first RLQ axis and linked to high water N:P ratio values, while the proportion of small-sized taxa (> 0.5-1 cm) decreased and was related to high water phosphorous concentrations (Fig. 5.3d). Other size classes exhibited only a slight association with the first RLQ axis but were ordered according to their size between these extremes (except for the category of smallest maximum sizes).

Other development-related trait categories highly involved in the link between environmental variables and biological traits and related to high water phosphorous concentrations (F1<0) included a short life duration ("≤ 1 year"), polyvoltinism (">1 generation/year"), one single reproductive cycle per individual ("1rc") and a high number of molting events ("15-19", "> 20"). They were associated with aquatic larvae, an aerial, passive dispersal, eggs and statoblasts as resistance stages and deposit (*i.e*. fine detritus) feeding (Fig. 5.3e+f).

At the opposite side (F1>0), long-lived (>1 year), monovoltin (1 generation/year) organisms with several reproductive events per individual (mainly two), a direct development (0 moult) were related to both high water temperature and high values of water N:P ratios. Such organisms exhibited also aquatic adults, living macroinvertebrates (and vertebrates; mainly fish eggs and juveniles) and an aquatic, active dissemination mode (Fig. 5.3a)

Positioned opposing this were taxa with a high affinity to live ">1 year", producing one generation per year, the highest number of reproductive cycles per individual (">6 rc") and showing no molting events. For both the number of molting events and the number of reproductive cycles per individual intermediate categories were positioned between the extremes mentioned above. A high affinity for feeding on "detritus" and the feeding mode of "deposit feeding" had the most negative coordinates on the first axis

and therewith were associated to high water phosphorous concentrations. Feeding on "(in)vertebrates" as well as the feeding modes "piercer" and "swallower/ chewer" were positively associated to the first axis.

Being aquatic during larvae and pupa (="nymph") life stages had negative coordinates on the first axis, opposing the life stages "adult" and "egg".
Reproduction *via* "isolated eggs" and "asexual reproduction" were negatively associated towards the first axis, while coordinates of both forms of "clutches" and ovoviviparity were positive, although less strong.
The first axis separated aerial dispersal (both active and passive), which had negative coordinates on the first axis from both forms of aquatic dispersal. The associations were stronger for aerial passive and aquatic active dispersal.
A resistance stage of "statoblasts" had strong negative coordinates for the first axis, while those of other resistance forms was slightly positive.
Taxa with "gills" were negatively positioned on the first axis, opposing those with aerial and skin respiration systems.
The coordinates on the first axis were positive for "burrowers" and negative for all other types of locomotion. Burrowing and living in the substrate interstices ("interstitial") thereby showed the strongest associations.

Tab. 5.3: Results of RLQ analysis linking water chemistry (NO_3, PO_4, water N:P) and temperature to species stoichiometric and biological traits *via* their abundances over a 34-year period. Total inertia indicates the global link between environment and functional traits, bold lettering indicates significance at $\alpha = 0.05$ (permutations with n= 9999 with applying two separate tests to correct for type I error (Dray & Legendre 2008)). Inertia/co-inertia ratios of environment and functional trait table as well as the correlation of species abundances indicates the fit between prior analysis of each table compared to RLQ analysis for the first two axes. Additionally the projected inertia along the first three RLQ axes is indicated.

Total		Inertia/ Co-inertia		Correlation	
inertia	Axis	Env	Traits	species	Proj. inertia
1.015	1	0.99	0.86	0.52	94.81
	2	0.98	0.69	0.33	4.27
	3				0.89

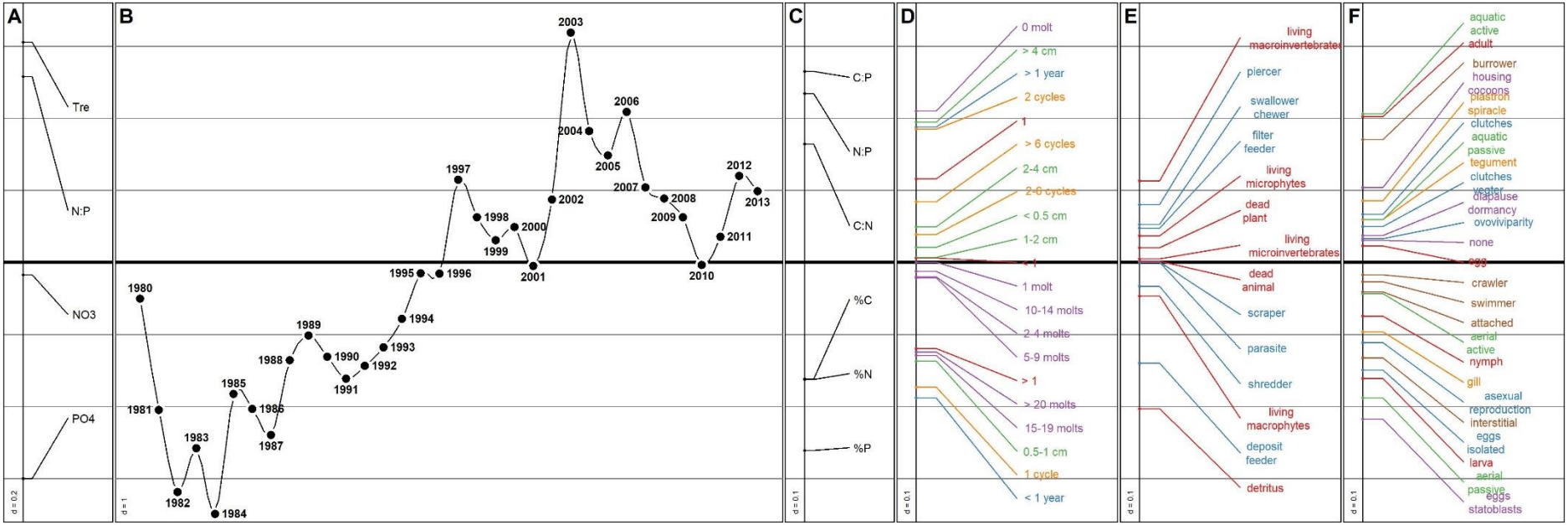


Fig. 5.3 : Canonical weights of A) environmental parameters (water PO_4, NO_3, N:P, temperature) and C-F) biological trait categories along the first RLQ axis. Biological trait categories were all equally part of the analysis and are simply presented group-wise (stoichiometric (C), develpoment (D), feeding (E), other (F)) for better readability. Panel B shows chronologically the position of years along the first axis.

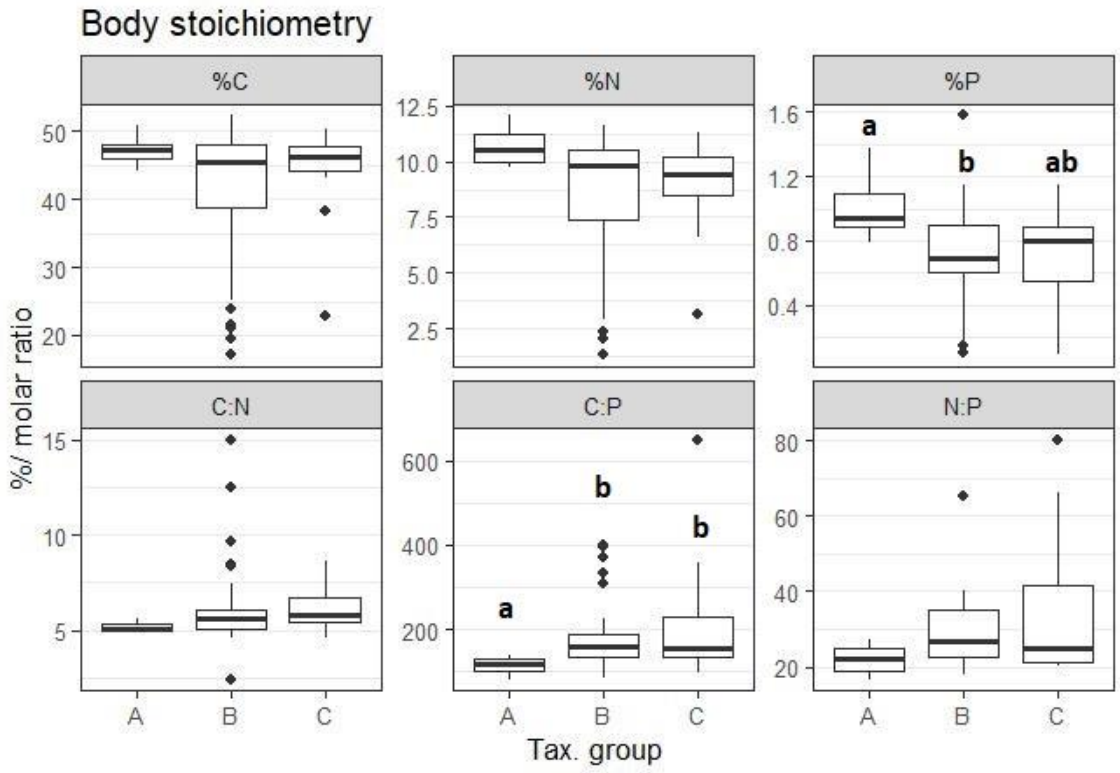


Fig. 5.4: Body stoichiometry (%C, %N, %P, C:N, C:P, N:P) of macroinvertebrate families belonging to groups with distinct abundance patterns over the study period as defined by Floury *et al.* (2013). The horizontal line represents the median, the boxes range from the 25th to the 75th percentiles and the whiskers extend to the last values within 1.5 times the inter-quartile range with black dots representing outliers. Different letters indicate significant differences using pairwise Dunn-tests following a significant Wilcoxon test (adjustment of p-values for multiple testing using Bonferroni method). Sample size was n = 6/ 36/ 13 for groups A/ B/ C.

Taxa groups only differed significantly in %P and C:P stoichiometry (Fig. 5.4). Taxa belonging to group A had the highest %P among groups, although this difference was only significant compared to group B (Dunn-test with $p<0.05$). Groups B and C did not differ.

C:P ratio was significantly lower in taxa of group A than in both other groups, which did not differ ($p>0.05$). A non-significant trend for highest values of %C and %N in group A was also observed, while values in groups B and C hardly differed. Body C:N and N:P ratios increased from group A over group B to group C, but neither groups differed significantly.

4. Discussion

Phosphorous-related community stoichiometry increased (%P) or decreased (C:P, N:P) significantly over the 34-year study period, thus following the decline in water phosphorous concentration (and increase in water N:P ratio) and confirming our hypothesis. Also the other community stoichiometric traits showed significant trends. Generally, stoichiometric traits were the trait-based metrics most involved in the link between environmental parameters and biological traits of benthic macroinvertebrate assemblages in the study site over the study period. Additionally, the shift in water

quality and temperature was significantly linked to community biological and stoichiometric trait profiles, following assumptions based on ecological stoichoimetry.

Trends in community stoichiometry

Community mass contents (%C, %N, %P) significantly increased over the 34-year study period, while community molar ratios decreased. For phosphorous-related stoichiometry this confirms our hypothesis and findings of previous studies that community stoichiometry would follow the decrease in phosphorous concentration (Singer & Battin, 2007; Prater *et al.*, 2015; Teurlincx *et al.*, 2017). The likely reason for this is a shift among (major) taxonomic groups, that generally differ in their body stoichiometry (Evans-White *et al.*, 2005; Mehler *et al.*, 2013, MS 1): the increase in community C:N and C:P went along with increasing proportions of Bivalvia and Gastropoda (S 5.2), two groups that have generally lower C:N and C:P ratios than the other groups (MS 1). Insecta, which are relatively rich in phosphorous, decreased over the study period, in parallel with overall community %P and water phosphorous concentration.

Families that tended to decrease or even vanished during the first years of the study period (Floury *et al.*, 2013) had significantly higher %P and lower C:P than those occurring only during the last years or that were present during the whole period. The RLQ analysis further confirmed this selection for phosphorous-rich taxa under high phosphorous conditions.

In an ecological context, community stoichiometry can be seen as a descriptor of the nutrient pool provided by the community. Serving as food for higher trophic levels, releasing nutrients into the water *via* excretion (Elser & Urabe, 1999; Vanni *et al.*, 2002 (vertebrates); Liess & Hillebrand, 2006) or providing an energy and nutrient flow from the aquatic to the terrestrial system *via* emergence of insects (Martin-Creuzburg *et al.*, 2017; Raitif *et al.*, 2018); macroinvertebrates are important contributors to nutrient cycling in many freshwaters. Any shift in this nutrient pool thus can cause effects within and beyond community level, for example by providing lower quality prey or reduced nutrient release (Elser & Urabe, 1999; Vanni *et al.*, 2002; McManamay *et al.*, 2011; but see also Vanni & McIntyre, 2016) due to low C:P, C:N taxa.

Links between water parameters and stoichiometric & biological traits

All six stoichiometric traits showed a strong association to the first axis and overall seemed to be more affected by environmental parameters than the other biological traits.

The correlations we found in other functional traits are in line with our hypotheses based on EST and/or with what has already been reported. Taxa with high body %P and expressing traits indicating a fast growth and/or development and often attributed to r-strategists (small body size, short life duration, high number of generations and molting events) were positively related to high water phosphorus concentrations and therewith had higher abundances early in the study period. In recent years, the community shifted towards larger, longer-lived taxa with direct development (no molt) and several

reproductive events per individual. The recent environmental conditions, notably the reduction in phosphorous load, also selected for taxa with high N:P (and C:N, C:P) ratio. This is in line with the Growth Rate Hypothesis (= GRH, Elser *et al.*, 1996), assuming a high demand for phosphorous and thus higher phosphorous content in the body tissues of fast growing taxa (Elser *et al.*, 2003a; Acharya *et al.*, 2004). This link also seems to hold for development rate (Beck *et al.*, 2021). Other studies found also a shift towards small-bodied, fast-developing taxa (Singer & Battin, 2007; Demi *et al.*, 2019), and decreasing live duration (Floury *et al.*, 2013), under high nutrient conditions.

Such an overall reduction of body size is also generally expected as a community response towards climate change, *i.e*. increasing temperatures, either at the population or community levels (Daufresne *et al.*, 2009; O'Gorman *et al.*, 2012; Latli *et al.*, 2017). Likewise, growth rate and development of taxa should be increased (Hannesdóttir *et al.*, 2013). Our results and those of Floury *et al.* (2013) suggest that water quality (esp. phosphorous concentration) was a more important driver on these traits than hydro-climatic parameters (temperature, discharge). In other multiple-stressor experiments the effect of nutrients on taxa or trait groups was also only minor compared to physical changes of the habitat (sediment deposition, flow velocity) or temperature, but affected body sizes and community size-structure (Piggott *et al.*, 2015; Juvigny-Khenafou *et al.*, 2021). Other works suggest also an effect of increased predation risk (due to exogenous species whose settlement has been facilitated by new environmental conditions) rather than a direct effect of temperature or water quality on traits related to life history (Latli *et al.*, 2017).

With decreasing water phosphorous concentration, feeding habits shifted from deposit-feeders, shredders and feeding on detritus towards predatory feeding strategies (swallowing/chewing, piercing, (in)vertebrates as resource)

The increase of the later could probably be due to the increasing proportion of Bivalvia over the study period. A general link between filter-feeding and high C:P and C:N ratios has already been found (MS 2), and was also (partly) attributed to an effect of phylogeny. The same study also reported a positive association between deposit-feeders and shredders towards high body phosphorous content. Decrease in deposit-feeding has already been observed under the same environmental scenarios (Floury *et al.*, 2013; Latli *et al.*, 2017), but these works reported either no effect on predators or a decrease and therewith the opposite to our finding. The decrease however was mainly influenced by phytoplankton concentration, a parameter that was not considered in our study. Generally, responses of predators towards nutrient enrichment have been divergent (Hulot *et al.*, 2000; McCormick *et al.*, 2004; Wimp *et al.*, 2010; Murphy *et al.*, 2012; Demi *et al.*, 2019) and are most likely not only driven by prey quality but also quantity. Predatory feeding behaviour has already been found to be linked to body nitrogen content (Fagan *et al.*, 2002; Cross *et al.*, 2003, MS 2), but not to phosphorous. Although in our study the link was among the strongest among the feeding-related traits, feeding traits overall contributed only little to the link between traits and environmental parameters.

The strong association of phosphorus concentration along the first axis and its position opposite to water N:P and temperature clearly represents the trends in environmental parameters and suggests these as the main drivers of functional trait composition over nitrogen concentration which was fluctuating among years but did not show a continuous trend.

Like community stoichiometry, part of the observed trait patterns were clearly driven by trait syndromes, *i.e.* groups of traits that originate from phylogenetic constraints, and the shift among larger taxonomic groups, especially the increase of Mollusca. However, most of these observations can also be explained by ecological stoichiometry theory. For example, the decreasing affinity for aerial dispersal could either be due to a generally rather high nitrogen content of Insecta (data paper), the only group expressing this trait, or due to a higher investment into nitrogen-rich muscle tissue necessary for flight. This makes it impossible to disentangle causes and effects among stoichiometric traits, other biological traits and taxonomy. In case of community stoichiometry however, the observed decrease in community phosphorous content remained when only analyzing the insect community and therewith reducing the effect of taxonomy (results not shown).

Outlook

The main advantage of stoichiometric traits is their ultimate link to elements that allows understanding shifts in taxonomical and functional community structure (as demonstrated in this work), but could also help answering nutrient-related questions beyond community level. More specifically, integrating biomass information would allow quantifying nutrient stocks or exports. A fist estimation using this dataset suggests that although community %P decreased, the overall amount of phosphorous storage increased (in line with a general increase in overall biomass, S3). Although the estimated phosphorous export by emerging insects increased, the element seemed to remain in the system for a longer period of time as suggested by the sharp decline in relative phosphorous export. This indicates that the change in community (trait) structure, also affects the community nutrient pool structure, and it would be interesting to investigate whether there is a general response towards changing nutrient conditions and/or towards community stoichiometric trait structure. Additionally, investigating whether the link stoichiometric/biological traits – environment nutrient levels holds for other systems (*e.g.* soil, lakes) or over a spatial rather than temporal nutrient gradient could underline the potential of stoichiometric traits in ecological works.

Conclusion

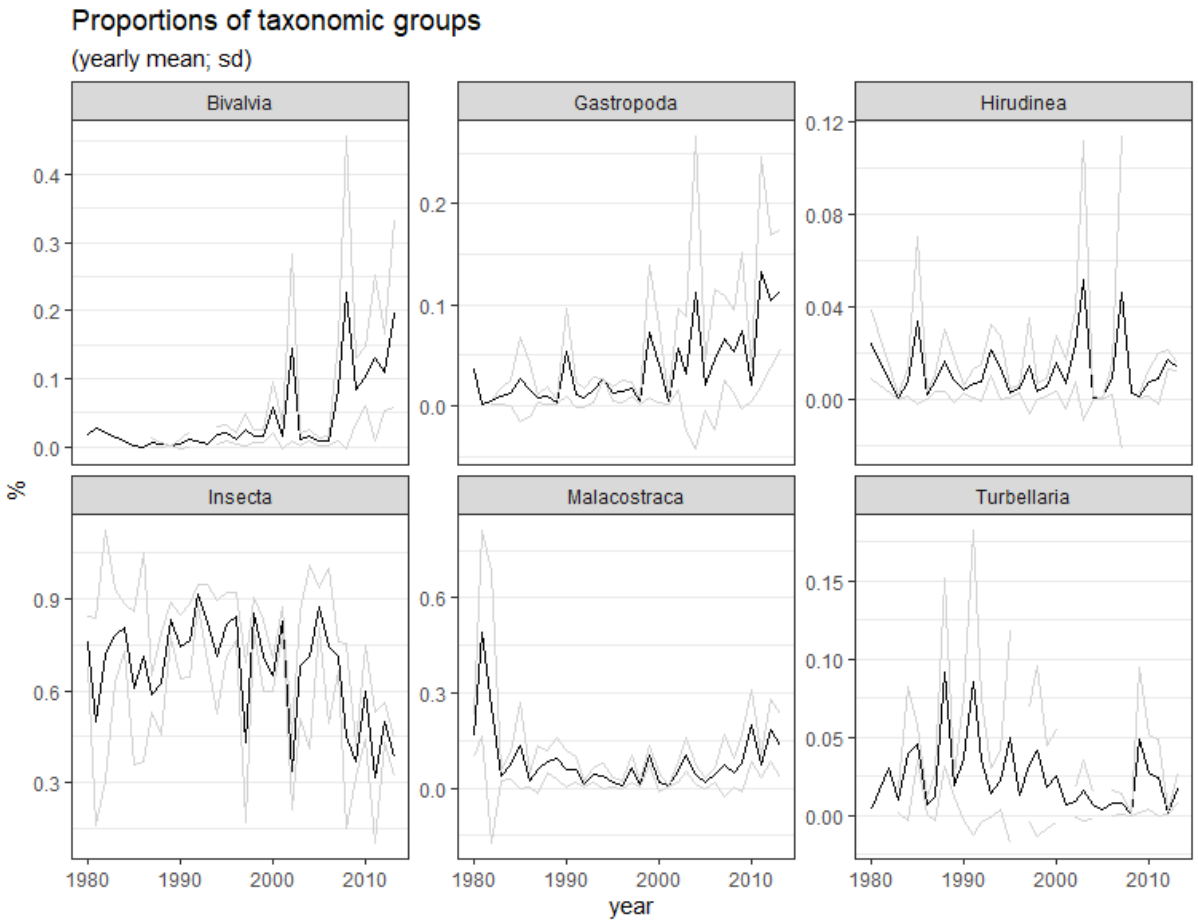
We demonstrated that over a 34-year period community (phosphorous) stoichoimetry followed the decrease in water phosphorous concentration, which thus directly affected the nutrient pool provided by the community *via* a shift towards P-poor taxa. Shifts regarding functional traits were also in line with assumptions based on ecological stoichiometry theory. Recent communities consisted of larger-bodied taxa with traits indicating slow growth/development and predatory and filter-feeding feeding habits

rather than deposit-feeding or shredding. Our results have underlined the potential of integrating stoichiometric traits into (trait-based) analyses, to better understand the mechanisms behind functional community responses towards nutrient-related stressors. Integrating variables such as biomass could further help quantifying nutrient stocks and fluxes under global stressors.

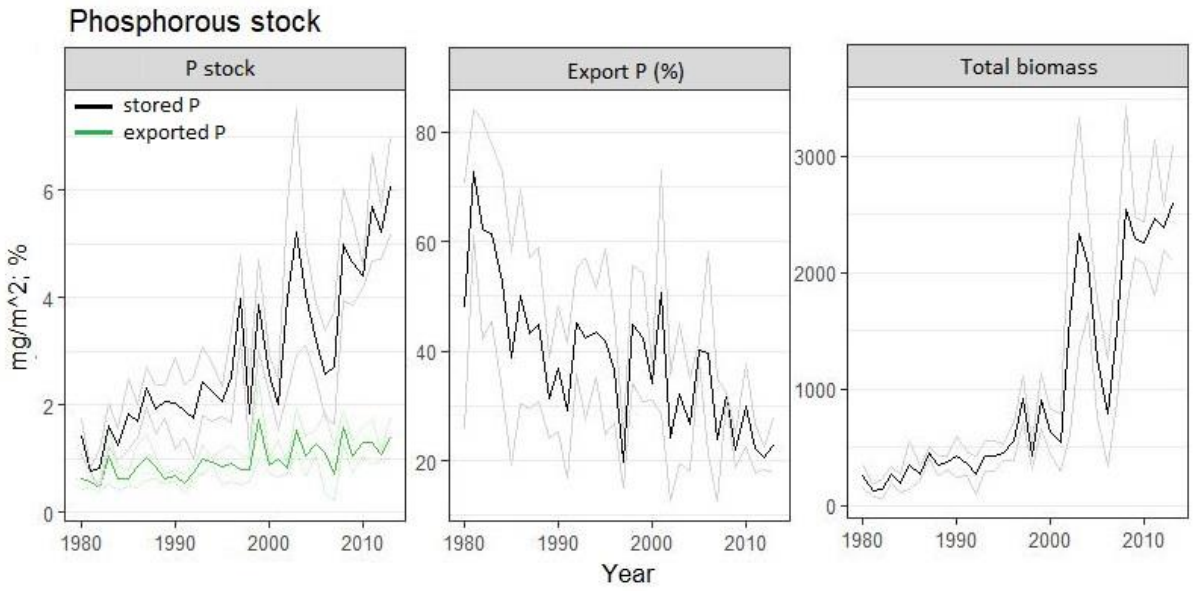
Supplementary Material

S 5.1.: Modifications of trait-categories compared to the original database from Tachet et al. (2010).

Functional Trait	Modification
Maximum body size	"<0.25 cm" + "0.25-0.5 cm" → "<0.5 cm"
	"4-8cm" + ">8 cm" → ">4 cm"
Food resources	"living macroinvertebrates" + "invertebrates" → "(macro)invertebrates"
	"detritus" + "microorganisms" → "detritus"
Locomotion	"housing" + "cocoons" → "housing/cocoons"
	"plastron" + "spiracle" → "plastron/spiracle"
Reproduction mode	"isolated eggs free" + "isolated eggs cemented → "isolated eggs"
	"clutches cemented" + "clutches isolated" → "clutches"
	"clutches vegetation" + "clutches terrestrial" → "clutches.vegter"
Resistance forms	"surface swimmer" + "full water swimmer" +"flier" →"swimmer"
Respiration	"temporarily attached" + "permanently attached" → "attached"



S 5.2.: Proportions of macroinvertebrate groups in the community over the study period 1980-2013. The black lines represent yearly mean values. light gray lines the mean +/- the standard deviations (the number of sampling events per year was n = 4 for all the years except 2008 with n = 3).



S 5.3: Phosphorous stock (stored P, exported P (in green), proportion of exported P) and total biomass over the study period 1980-2013. The dark lines represent yearly mean values, light lines the mean +/- the standard deviations (the number of sampling events per year was n = 4 for all the years except 2008 with n = 3). Biomass and masses of phosphorous are given in mg/ m^2, the proportion of exported phosphorous in % of stored P.

Chapter VI:
General discussion

General discussion

Most results obtained during my thesis work demonstrate that – overall – assumptions based on Ecological Stoichiometry Theory (=EST) also apply on functional traits beyond those related to growth or feeding and that it is indeed a promising approach when applied on community level.

Stoichiometric and other functional traits

Many studies have been dedicated to investigate the correlation between growth rate and body stoichiometry, have compared different functional feeding groups or have relied to these trait groups when explaining taxa shifts as a response to nutrient enrichment. I demonstrated a link between taxon's body stoichoimetry, more or less related to nutrient demand (see infobox 3 p. 181), and a range of other biological and habitat-related functional traits. These links generally followed assumptions based on EST, *e.*g. suggesting a link *via* resource quality, the investment in muscle tissue or the allocation in biomolecules (*e.g.* ATP, proteins, rRNA). My results for example confirmed a higher nitrogen content of predators, as already reported by other works (*e.g.* Fagan *et al.*, 2002; Cross *et al.*, 2003; González *et al.*, 2011), but also indicated a link to other feeding modes and resource preferences. For example, detritivorous feeding habits (*e.g.* deposit-feeding, shredders) tend to have a high phosphorous content; filter-feeders and scrapers high C:N ratios. Further they confirmed the stoichiometric link to (maximum) body size *via* growth rate as suggested by Growth Rate Hypothesis (Elser *et al.*, 1996), and demonstrated this link also for molting frequency and development rate (MS 4) both linked in the literature to body growth rate. An analysis on community level applying the same methods, confirmed most of these associations, suggesting that the link between stoichiometric and (other) functional traits, *via* species selection, reaches community level.

That these links towards stoichiometric traits are in line among each other (*e.g.* feeding habits being associated to the same stoichiometric traits as their corresponding potential food resources) or (partly) represent certain taxonomic groups or species distribution along the longitudinal stream gradient is on one hand conclusive as it is supporting our finding. On the other hand, it underlines the complexity of ecological systems, as it is difficult to make any statement regarding causation. From an ecological point of view, this question is probably less important for the use of stoichiometric traits to estimate community responses, since organisms exist "as such" and it is of minor importance if a scraping mollusc is present because being a mollusc, or because being a scraper. For community ecologists interested in the drivers of community structure, it might be interesting to try further disentangling these interactions.

However, the finding of a stoichiometric link – both on taxon as well as on community level – clearly underlines its potential for a better (and causal) understanding of taxa and function shifts under nutrient changes. Two analyses confirmed this applicability in practice, one on a nutrient gradient on a smaller spatial scale but relying on personal field data (MS 4), one over a long-term temporal nutrient gradient (MS 5). Although a large part of functional shifts could probably be explained by a shift among larger taxonomic groups alone, the integration of stoichiometric traits helped explaining the probable underlying mechanisms, for example the favouring of phosphorous-rich taxa and/or phosphorous-demanding traits under high phosphorous levels. Stoichiometric traits were also found to be among the trait categories that were most involved in the link between environmental variables and functional traits. Most observations remained when focussing on one larger taxonomic group only (i.e. insects, data not shown), suggesting that this species sorting is not only due to phylogenetic constraints (among coarser taxonomic groups), but also happens on a finer scaler.

Infobox 3: You are what you eat and that's what you need?

Throughout my work, I may have equated stoichiometric traits (e.g. phosphorous content) with a taxon's demand for this nutrient. This is a rather simplifying depiction, as other factors such as growth efficiency contribute to the latter. The calculation of such threshold elemental ratios (=TER) as proposed by Frost *et al.* (2006) however is based on and seems to be correlated to body stoichiometry. While nutrient use efficiencies and therewith the correlation between a stoichiometric trait and its TER might be similar among phylogenetically close taxa (i.e. within insects), groups that are more distinct might differ in these coefficients. The approach of stoichiometric traits is lacking this physiology aspect, but therefore is more direct and easy to obtain. It considers the organism as it is at the moment of study, including – as in our studies – the shells of Mollusca (likewise bones of vertebrates). Even though they are generally considered dead tissue, the organism had/has to assimilate the required nutrients, which should be taken into account.

Stoichiometric traits on community level

Few studies so far had suggested that taxonomic shifts following nutrient enrichment (i.e. a favouring of phosphorous-rich taxa under high phosphorous conditions) affect also the stoichiometry on community level, which should follow the nutrient concentration in the water (Singer & Battin, 2007; Prater *et al.*, 2015; Teurlincx *et al.*, 2017). My results confirm such a link, although the direction in which community stoichiometry is affected was less clear. While for certain coarser taxonomic groups (Insecta, Crustacea, Hirudinea, partly Gastropoda) we could confirm our hypothesis, considering the complete community in some analyses lead to opposite findings. This was the case when relying on large-scale data from a monitoring program (MS 3) as well as when investigating data from a field campaign conducted by colleagues and me (i.e. visiting ten small streams in eastern France representing a water N:P gradient driven mainly by water PO_4^{3-}, results not shown). In the first case, it is likely that

general differences between the sites (*e.g.* habitat structure, hydro-eco-region) over-shaded the effect of nutrients and/or caused different community responses. Habitat properties often have major influences on community composition compared to nutrient concentrations alone (Richards *et al.*, 1993; Yuan, 2010; Juvigny-Khenafou *et al.*, 2021), which nevertheless contributed to models. In the second case, such confounding factors were further reduced as we personally visited and knew the sites. Attempts to disentangle the additional environmental factors that probably had an influence on community stoichiometry (season, stream size, hydroecoregion, Appendix a) suggested that especially stream size might be important. However, despite the large number of abundance data sets and the detailed site information, it was not possible to satisfactorily disentangle these confounding factors due to unbalanced data availabilities. To a larger extend the trends in overall community stoichiometry seemed to be driven by shifts among coarser taxonomic groups: the decreasing phosphorous community content under increased nutrient conditions went along with increased proportions of phosphorous-poor Mollusca (and Oligochaeta with rather high %P) while that of Insecta (high %P, low C:P) decreased. Such taxonomic shifts are generally observed under (nutrient) polluted conditions (McCormick *et al.*, 2004; Ortiz & Puig, 2007; Zhang *et al.*, 2019) and also confounding factors (as those mentioned above) influenced the community composition regarding lower taxonomic groups.

In contrast, studying a temporal rather than spatial nutrient gradient (MS 5) almost perfectly supported our hypothesis also for the whole community: the decrease in water phosphorous concentration shifted the community towards P-poorer taxa, which decreased community %P. Additionally, the integration of stoichiometric traits proved to be suited to explain shifts in taxonomic and functional community composition, which were in line with findings regarding the link between stoichiometric and functional traits. The global examination of further single sites relying on the French national monitoring data did not provide clear results, neither in support nor in rejection of our hypothesis. Although quite a high number of sites was included, most time series only covered less than ten years, with numerous missing points, which impeded the detection of trends. It should be worth to repeat this analysis in some years when the available data has grown to investigate the temporal correlation of environmental parameters and community stoichiometry on a large (spatial) scale.

Recommendation for the use of stoichiometric traits on community scale

There are three additional aspects that should be kept in mind: The first concerns the way community data is used. Community studies often rely on taxa abundances, but might use biomass-weighing for example when addressing questions of functioning or nutrient fluxes. Both approaches partly resulted in opposing responses, especially when heavy taxa such as Mollusca were considered (Appendix b, Infobox 2 p. 202). Results from one approach should therefore not blindly be used to estimate the other or used to quantify nutrient pools.

Second, the community composition regarding coarser taxonomic groups (*e.g.* class) clearly influenced community stoichiometry and its link to functional traits or an environmental nutrient gradient (already

discussed above). For example, while – on taxon as well as community level – deposit-feeders were associated to high %P both addressing all macroinvertebrates as well as only insects, filter-feeding was either associated to high C:P (i.e. low %P, for all taxonomic groups) or co-varying with deposit-feeding and high %P (insects only). Likewise, while our assumptions regarding an affinity for phosphorous-rich taxa and communities for eutrophic habitats was not supported for the overall taxa, insects followed this assumption. That is not necessarily a problem, as also most natural communities will comprise taxa of these stoichiometric distinct groups and the results of such analyses is meaningful when addressing whole communities. However, they should not be used to make predictions for insect or crustacean only assemblages. Also the translation from taxon to community level can be surprisingly different when it is not the general stoichiometric differences between coarser taxonomic groups that are driving the result. While our hypothesis of a large number of molting events in phosphorous-rich taxa worked perfectly within insect taxa, on community level these trait categories were associated to high C:P and N:P ratios and high P communities had a high affinity for rather few molting events ("2-4 molts"). Focussing on one taxonomic group leaves only phylogenetically closer taxa with more similar trait profiles, which probably caused this discrepancy.

The last aspect concerns the homeostasis of taxa. My analysis has shown that most taxa used in the analyses showed no significant intra-taxon variability in their body stoichiometry regarding environmental parameters (water nutrient concentration, season) or sex (tested for crustaceans). However, some taxon's carbon and nitrogen content changed with biomass, which is not captured by the database. This is a general shortcoming of mean value usage and in the next paragraph I will point out how intra-taxon variability could be taken into account in the future.

Outlook

The extension to other global stressors – My work focused on nutrient changes as global stressors, but nowadays many systems are confronted with multiple stressors and notably increasing temperatures due to climate change are ubiquitous (Parry *et al.*, 2008). Ecologists therefore are trying to understand how multiple stressors are interacting and acting on communities. Compared to nutrients, temperature is (also) acting directly on an organism by affecting its metabolism and increasing physiological rates such as respiration (Hamburger & Dall, 1990), growth (Sutcliffe *et al.*, 1981; Pöckl, 1992; Janssens *et al.*, 2015) or development (Pöckl, 1992; Gillooly *et al.*, 2002) under warmer conditions. This is also observed as inter-taxa variability with species from colder regions having slower development rates than those from/adapted to warmer regions (Lonsdale & Levinton, 1985; Hoegh-Guldberg & Pearse, 1995). Respiration is directly linked to energy demand and therewith the carbon budget and also growth, reproduction and feeding are linked to body stoichiometry as demonstrated and discussed throughout my work. With both of these attributes – body size and stoichiometry – controlling ecological interactions from taxon to ecosystem level, several studies already proposed a combination of

stoichiometric and metabolic theory (Allen & Gillooly, 2009; Hillebrand *et al.*, 2009; Ott *et al.*, 2014) to understand constraints acting on different levels of organization up to ecosystem.

Under warming, communities shifted towards smaller and/or short-lived taxa from phyto- and zooplankton (Strecker *et al.*, 2004; Rasconi *et al.*, 2015) to macroinvertebrate communities (Daufresne *et al.*, 2009; O'Gorman *et al.*, 2012; Floury *et al.*, 2017; Latli *et al.*, 2017). From the stoichiometric point of view, this would go along with a shift towards phosphorous-rich taxa and thus increase the overall phosphorous community content. Another response could arise due to decreased resource quality, i.e. increased C: nutrient ratios of phytoplankton and seston under elevated temperatures, both as individual response as well as *via* species shift on community level (Domis *et al.*, 2014; Yvon-Durocher *et al.*, 2017). Van de Waal *et al.* (2010) proposed thus a shift towards consumers with lower nutrient demand, which – according to EST and results of my thesis (MS 5) – would come along with a shift towards slower developing and probably less active taxa. Generally, studies referring to ecological stoichiometry theory and/or integrating stoichiometric traits when investigating effects of global warming beyond phytoplankton systems are missing. In my analysis of the Loire river community, the response of development- and growth-related traits seemed to have been driven by declining phosphorous concentration rather than increasing temperature. However, there seems to be an interaction between temperature and nutrient load on ecological processes: Over the long-term, phosphorous reduction seemed to mitigate negative impacts of warming on macroinvertebrate community taxonomic or functional composition (Daufresne *et al.*, 2009; Floury *et al.*, 2017) and to reduce the risk of harmful algae blooms (Minaudo *et al.*, 2021). Likewise, increased nutrient concentrations amplified the effects of warming on seston stoichiometry (Domis *et al.*, 2014) and warming increased quality reduction under nutrient depletion (Verbeek *et al.*, 2018). It seems thus interesting to investigate how these stressors also interact with stoichiometric traits in assemblages of more complex organisms.

Warming can also alter stoichiometric traits as observed in *Daphnia* and Odonata where a reduced energy storage under elevated temperatures lead to lower C:N ratios (Janssens *et al.*, 2015; Zhang *et al.*, 2016). In the damselfly (but not *Daphnia*), also C:P decreased as growth rate increased, as expected due to EST. Generally, the correlation between phosphorous demand and temperature is probably U-shaped as shown for *Daphnia*: A temperature increase at moderate temperatures increases consumer's growth and the demand for phosphorous, but when temperatures continue to increase beyond a threshold, the demand for carbon – due to increased metabolism – dominates (Ruiz *et al.*, 2020). Although as an individual response this might not directly concern community responses in terms of functioning, it could translate the stoichiometric mismatch between primary producer–herbivore to higher trophic levels (prey–predator). Obviously, this renders ecological analyses more complex, but considering the growing importance of this stressor the stoichiometric response of individual taxa to warming should be investigated to ensure an adequate application of stoichiometric traits. Also the investigation of a link

between stoichiometric traits and thermal tolerance and/or thermal optimal temperature would be interesting.

Going up to the ecosystem scale – My thesis answered questions from taxon to community level, but did not reach the ecosystem scale. As mentioned throughout several chapters, (macroinvertebrate) consumers are positioned between primary resources and higher trophic levels and thus mediate both bottom-up as well as top-down control. Many of these controls depend on body stoichiometry, for example the nutrients provided to the next higher trophic level or, at least partly, the nutrients released by excretion (Vanni & McIntyre, 2016). Since community stoichiometry describes the nutrient pool provided by a community it would be interesting to study if it can also be a proxy for estimating the effects on primary producers or vertebrate predators. Studies showed that effects of nutrient enrichment probably do not reach secondary consumers (Malzahn *et al.*, 2010, but see Boersma *et al.*, 2008) since they buffer the mismatch due to their general homeostasis. This however investigated simplified trophic chains and therefore did not consider stoichiometric imbalances due to species shifts.
First rough estimations of quantitative nutrient pools and fluxes were promising as there were clear trends in nutrient (phosphorous) stock and the export from the stream *via* emerging insects (supplementary material of MS 3 & 5). It suggests that the shift in taxonomic and stoichiometric composition of a community is reaching beyond the studied stream communities, *e.g.* by a reduced phosphorous export into the surrounding terrestrial ecosystem under high phosphorous conditions. However, more accurate biomass data and more precise information about the actual proportion of insects surviving until emergence is necessary to verify and build up on these findings.

Extension to other environmental systems – My analyses studied stream macroinvertebrates. Translating these findings onto other systems (and probably organism groups) therefore is interesting, for example different freshwater systems (lakes), marine or terrestrial environments. Similarly to freshwaters, most works in these systems have largely been limited to taxon (or population) level or studied planktonic or microbe communities, but there is no evident reason why analyses on community level should not reveal promising results as my thesis.

Future of the stoichio database – Without question, it would be great to expand the stoichiometric trait database by missing macroinvertebrate taxa, thereby maybe including also dominating taxa from other systems. This would increase the data coverage and allow an application on community data beyond Western-European headwater streams on which we focused during its construction. During my analyses of community stoichiometry, I aimed to cover the majority of taxa in terms of abundances (95%), but a slight reduction of the data coverage, allowing analysing a larger number of community datasets, resulted in the same overall pattern.
I additionally propose to increase the underlying raw data of already represented taxa, especially those with only scarce sample sizes, as this would render values more precise and minimize biases due to

intra-taxon variation. This could time and resource friendly be achieved by using individuals used by colleagues and/or collected on the side during field campaigns serving another aim.

To minimize the uncertainty of intra-specific variation, i.e. deviation from stoichiometric homeostasis, one could firstly add size classes or life stage categories for taxon likely undergoing significant changes in body stoichoimetry during their lifetime (*e.g. Baetis*, *Sericostoma*). Such high resolution in a database however requires the same resolution in the data to which it will be applied, *e.g*. abundance data, which is often not available. A second option is the fuzzy-coding of stoichiometric traits: Each stoichiometric trait (*e.g*. %P) will be described by several categories corresponding to different levels of nutrient content (e.g. very poor, poor, intermediate, rich, very rich), providing for each the relative frequency a taxon might occupy this category. Like the previous one, this approach would require a larger number of underlying raw data, ideally covering the whole range of body sizes and/or life stages in a representative way. This option would be well suited to be applied on abundance data that is likely to contain intra-taxon variation in terms of body size and/ or life stage, which has not been considered during sampling. Such fuzzy-coding approach is already widely used in other functional trait databases (Tachet *et al.*, 2010; Degen & Faulwetter, 2019; Sarremejane *et al.*, 2020) and would allow an easier combination of stoichiometric and other functional traits.

As shortly discussed, in the infobox above, TERs are another, probably more precise approach of nutritional requirements. Differences in the correlation slopes between stoichiometric traits and assimilation efficiencies could help to understand the different responses that we observed among coarser taxonomic groups throughout the analyses. The acquisition of such data however is much more laborious than that of elemental composition measurements and is therefore probably not feasible for a large number of taxa// the large number of taxa covered by our database.

Conclusion

My PhD aimed at extending the stoichiometric approach and assumptions from EST to community level, as this would enable to come closer to understanding how nutrient effects *via* taxa responses might reach larger scales. I found that throughout my work phylogeny played a large role in controlling a taxon's body stoichiometry and therefore also drove community stoichiometry along a nutrient gradient and its link towards other functional traits. This was in most cases not contradictory to our assumptions based on EST, so that taxonomy and EST complementary explained the observed patterns. Confounding factors that, *via* effects on the taxonomic community composition, probably also affect community stoichiometry need to be further investigated and disentangled from the pure nutrient effect. Likewise, distinct responses among taxonomic groups or in comparison with that of the overall community can occur. On a temporal scale however the community stoichiometry and functional response towards changing nutrient concentrations followed our expectations based on EST and stoichiometric traits proved to be well suited to explain the community response towards this stressor. It remains to be investigated how such a changed consumer nutrient pool affects nutrient cycling towards higher/lower

trophic levels or adjacent terrestrial ecosystems, for which this approach clearly has the potential. It might also be worth to study its application towards – and interaction with – other global stressors, notably global warming, which already was shown to affect stoichiometry and/or traits related to body stoichiometry (i.e. growth, development). The provision of a stoichiometric trait database together with the large availability of abundance and trait data for the group of macroinvertebrates makes it easy to adapt this approach to study other systems and/or regions to verify or extend these results.

Appendix

Effects of season, stream size and hydroeco-region on community stoichiometry

1. Introduction

Nutrient concentrations can indirectly – *via* resource quality and quantity – affect the composition of a community as discussed in more detail in several chapters of this thesis. However, physical habitat characteristics such as substrate composition, particle size or channel morphology are often the main factor in determining macroinvertebrate community composition (Richards *et al.*, 1993; Yuan, 2010; Juvigny-Khenafou *et al.*, 2021). Taxa richness and density tend to be larger on loose and larger-grained substrate rather than sand (Erman & Erman, 1984; Flecker & David Allan, 1984) and also taxa diversity and the dominating taxa depends on grain size (Duan *et al.*, 2008). Grain size determines the suitability of a substrate as living space (*i.e.* in interstices or borrowed within) and the provisioning of food resources (*e.g.* by algal colonization (Allan & Castillo, 2007) or retention of coarse particulate organic matter (Larrañaga *et al.*, 2003). Besides the substrate itself, heterogeneous (meso-) habitats composed of different substrates and thus providing several niches for invertebrates lead to a high taxa richness and diversity (Beisel *et al.*, 2000) and also increases functional diversity (Milesi *et al.*, 2016). Although such local physical (and also chemical) factors are more important in determining communities (Sandin, 2003), there are general differences in macroinvertebrate community structure between larger (eco-) regions that differ also in climate, soil type or geology (Sandin & Johnson, 2000; Dovciak & Perry, 2002).

In my previous analysis of community stoichoimetry, I focused on the effect of water nutrient condition (phosphorous, nitrogen) due to the direct link to organism body stoichiometry. However, the datasets originated from sites all over France with samplings being conducted throughout the year and therefore covering a range of different hydroeco-regions (HER), stream sizes and seasons. It is the aim of this chapter to explore the effect of these environmental factors on community stoichiometry and its response towards nutrient levels. I expect significant differences in community stoichiometry between different seasons, stream sizes and HERs, *via* an effect on community composition. For example, the proportions of Bivalvia and Gastropoda tend to be higher in large streams, which due to the low body nutrient content of Mollusca, should decrease community stoichiometric values; season should mainly have an impact *via* life cycle characteristics of taxa. Due to the high number of HERs, I do not have clear hypotheses and therefore only expect a general difference due to differences related to climate or geology that should favour different taxa.

I further hypothesize, that the general response of community stoichiometry towards water phosphorous concentration and nitrogen level should not change with season, stream size or HER. The community composition – and therewith community stoichiometry – should follow the same pattern over the nutrient gradient in all categories, but differences in the communities and taxa selection might lead to different strengths of the response.

2. Material & methods

The macroinvertebrate abundance datasets used in this chapter originate from the French National database and were selected based on the same criteria as in MS 3. Only the filter of stream sizes was not applied to include a larger number of categories. This resulted in 1620 sampling site events, *i.e*. data sets. Community stoichiometry values were then calculated as described in the same chapter.

The additional factors considered in this analysis and their categories were:

- HER (= Hydroeco-Region; a classification of streams based on geology, relief and climate and indicated by a number (1-22) (Wasson *et al.*, 2002; Chandesris *et al.*, 2006, Fig. 1, S1). The present dataset comprises the following HERs: 1, 3-15, 16-22.
- Season: spring (March, April, Mai), summer (June, July, August), fall (September, October, November), winter (December, January, February)
- Stream size: very small (VS, Strahler rank 1 & 2 (in the basin Loire-Bretagne: rank 1-3), small (S; 3 (4)), small/medium (SM), medium (M; 4 (5)), medium/large (ML), large (L; 5 (6)), very large (VL; 6-8 (7 & 8).

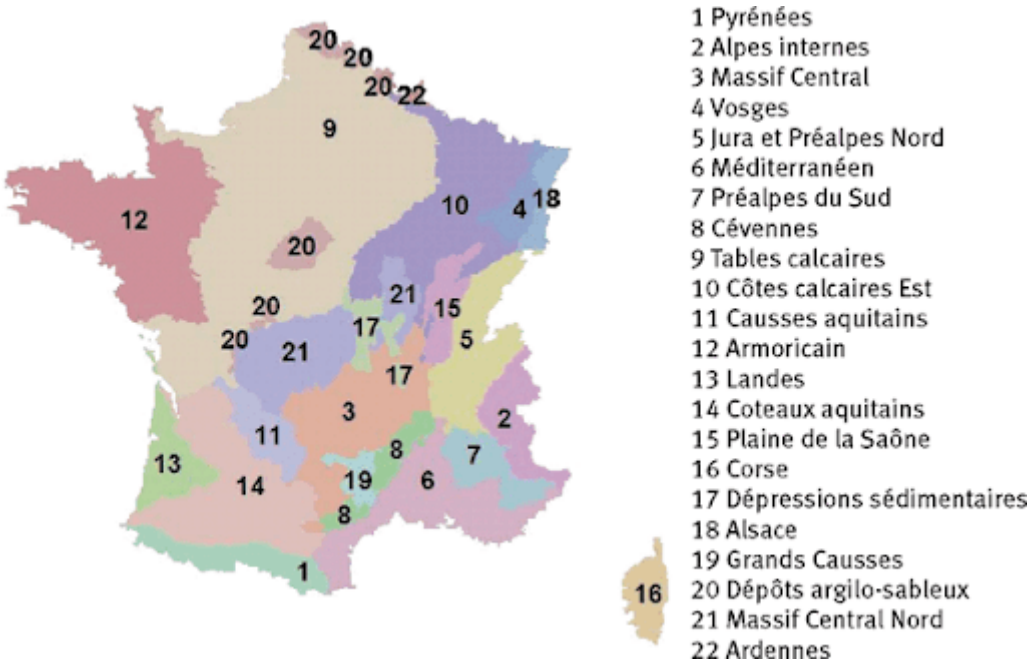


Fig. Aa.1: Carte of the 22 hydro-eco-regions in France, defined by characteristics in climate, geology and relief. Details on these characteristics can be found in the supplementary Tab. Aa.S1. ©image: Gauroy et al. 2012

Differences in community stoichiometry between additional factors

To compare the community stoichiometry values between different stream sizes, seasons and HERS, ANOVAS were applied separately for each stoichiometric variable and each of the three additional factors. For the comparison between HERs, those with a sample size <10 were excluded (HER 6, 7 and 19). In cases of significance, a Tukey HSD test was applied to investigate pairwise comparisons, correcting for multiple testing with the Bonferroni method. Model assumptions, *i.e.* homogeneity of variance and normality of residuals were checked graphically.

The effect of additional factors on the effect of water parameters on community stoichiometry

A set of linear mixed models was applied to investigate the effect of the three additional factors on the effect of water nutrient levels on community stoichiometry, which has already been investigated in MS 3. Log-transformed community phosphorous concentration (quantitative) and nitrogen level (categorical, with the categories "low", "intermediate", "high") were set as fixed factors. Stream size, season and HER were added as random factor. Individual models were applied for each stoichiometric variable (%C, %N, %P, C:N, C:P, N:P), in case of the stoichiometric ratios on log-transformed values following the suggestion of Isles (2020).

The contribution of the three random factors to the overall variance was estimated by variance partitioning using the varcomp() function.

All analyses were conducted using R (R Development Core Team, 2019).

3. Results

Differences in community stoichiometry between additional factors

All six community stoichiometry variables differed significantly between seasons, stream sizes and HERs (Fig. Aa.2 & 3).

Community mass elements (%C, %N, %P) were significantly higher in winter compared to the other seasons (Fig. Aa.2). Community %P additionally was only slightly but significantly higher in spring than in summer. Community ratios (C:N, C:P, N:P) were significantly lower in winter compared to the other seasons. C:P and N:P ratios additionally were significantly higher in summer than in spring. Generally, community stoichiometry did not vary much between spring, summer and fall and all three seasons showed a broad range of values, compared to winter, which also had a clearly lower sample size.

Very large streams ("VL") had significantly lower community mass element contents and significantly higher community stoichiometric ratios than all other size classes.
Carbon and nitrogen contents were significantly higher in medium ("M") and medium/large ("ML") streams than in all other size classes, with no difference between the two. Carbon content differed

additionally between very small (“VS”), small (“P”) and small/medium (“SM”) streams whereas there was no difference in nitrogen content between these size classes. Phosphorous content was significantly higher in communities from medium/large streams compared to all other stream sizes. The phosphorous content in communities from very small and small/ medium streams additionally was significantly lower than in medium sized streams. Medium/large streams had the lowest values, with the difference being significant compared to small and very small streams.
Community C:P ratios were lowest in medium/large streams, with this difference being significant towards all size classes except for medium sized streams.
Community N:P was significantly higher in small/medium and large streams than in very small, small and medium/large streams with no significant differences within these two groups of size classes.

All six community stoichiometry variables also varied among HERs. However, due to the large number of HERs and their basis on different climatic and geomorphology characteristics, this finding is interesting enough and pairwise differences will not be further investigated.

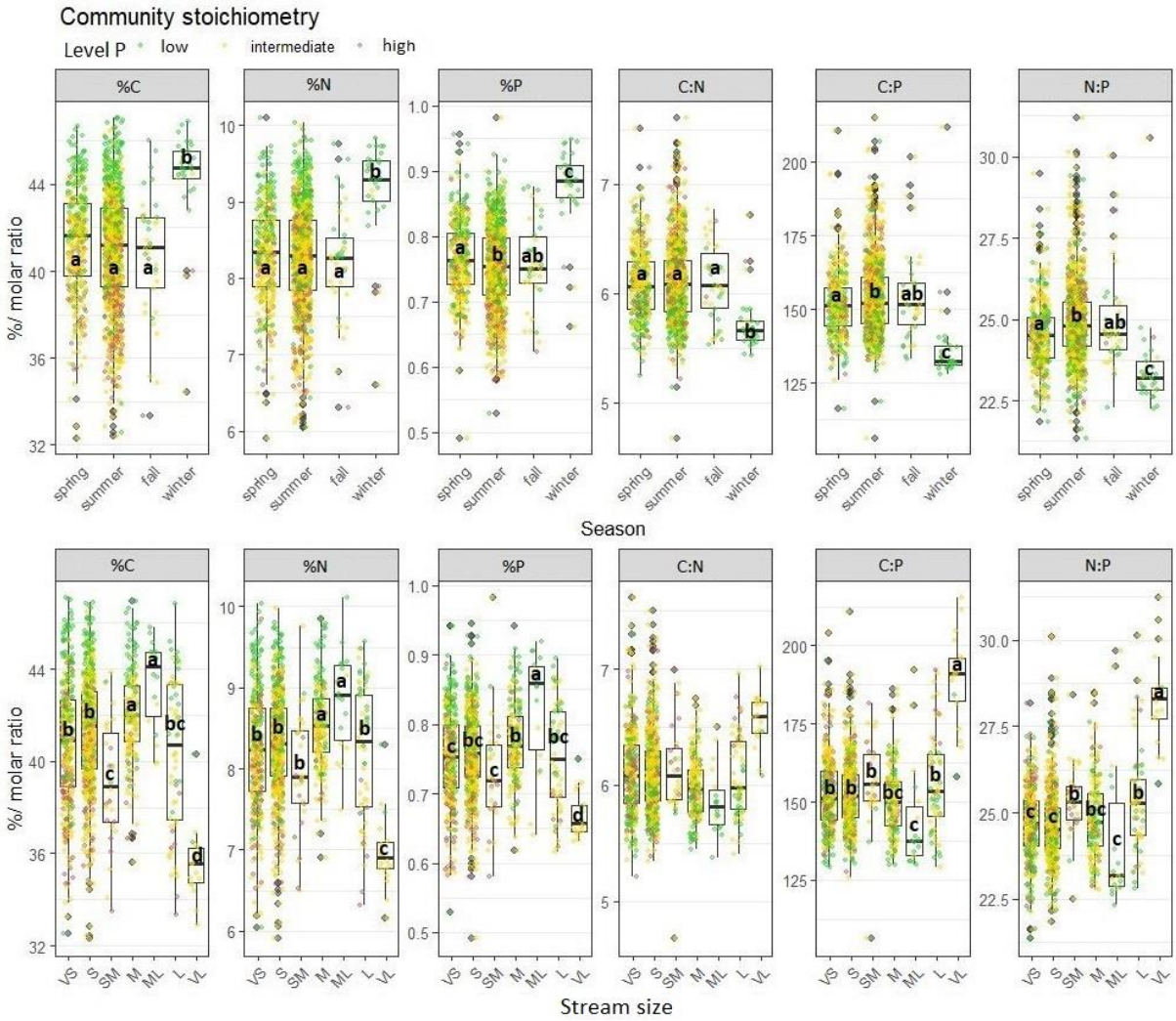


Fig. Aa.2: Community mass contents (%C, %N, %P) and molar ratios (C:N, C:P, N:P) among different seasons (upper panels) and different stream sizes (lower panels). The horizontal line represents the median, the boxes range from the 25th to the 75th percentiles and the whiskers extend to the last values within 1.5 times the inter-quartile range with black dots representing outliers. The scattered dots represent underlying data points with the color indicating the level of phosphorous in the water (low, intermediate, high). Different letters indicate significant differences using Tukey HSD test following a significant ANOVA (adjustment of p-values for multiple testing using Bonferroni method). Sample size was n=354/ 983/ 38/ 29 for spring/ summer/ fall/ winter and n=345/ 577/ 30/ 77/ 18/ 62/ 18/ 177 for stream sizes VS/ S/ SM/ M/ ML/ L/ VL.

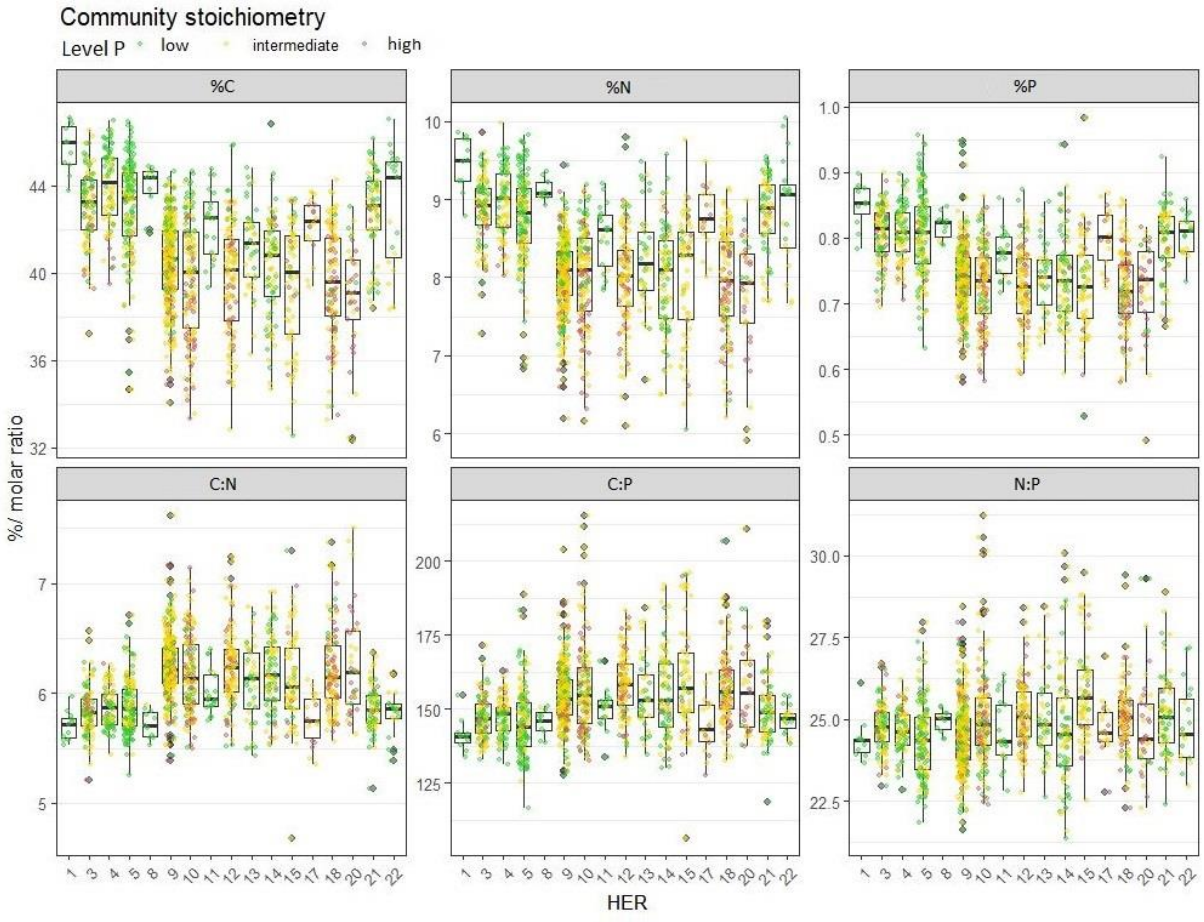


Fig. Aa.3: Community mass contents (%C, %N, %P) and molar ratios (C:N, C:P, N:P) among different hydro-eco-regions (HER). The horizontal line represents the median, the boxes range from the 25th to the 75th percentiles and the whiskers extend to the last values within 1.5 times the inter-quartile range with black dots representing outliers. The scattered dots represent underlying data points with the color indicating the level of phosphorous in the water (low, intermediate, high). Sample size was n=11/ 101/ 80/ 147/ 11/ 344/ 138/ 20/ 109/ 40/ 83/ 66/ 18/ 111/ 37/ 69/ 19 for the different HERs.

The effect of additional factors on the effect of water parameters on community stoichiometry

The integration of random factors clearly increased the variance explained by the model, from 0.41 - 8.73% to 46.7 - 66.09% (Tab. Aa.1).

In all stoichiometric variables except for community N:P ratio there was an interaction between water phosphorous concentration and nitrogen level. The effects of fixed effects on community stoichiometry were generally the same as found with simple linear models on a slightly smaller data set (excluding

large streams of the categories “ML”, “L”, “VL”) in MS 3. Community mass elements (%C, %N, %P) decreased with increasing water phosphorous concentration, while community ratio values increased. In all stoichiometric variables, this effect was largest at low levels of water N; the difference between intermediate and high levels of N was only small.

Stream size explained the largest part of variance in all stoichiometric variables; only in community %P most variance was explained by season. Here, the difference between the three factors also clearly was lower than for the other factors. In %N and C:N community ratios, HER was more important than season, while for %C, C:P and N:P ratios more variance was explained by season.

Tab. Aa.1: Model parameters of linear mixed models to investigate the effect of additional factors (HER, season, stream size) on the effect of water nutrient levels on community stoichiometry. Marginal (r^2m) and conditional (r^2c) r^2 values indicate model fit regarding fixed effects only and including random factors. Variance partitioning was applied to quantify the proportion of residual variance explained by each of the additional factors. Sample size was n = 1223 with 16/ 7/ 4 categories within HER/ Season/ stream size.

	Slope			Intercept				
Stoichio	Low N	Interm N	High N	Low N	Interm N	High N	r^2m	r^2c
%C	**-1.1512**	**-0.7924**	**-0.7684**	38.0379	0.3821	0.5748	0.0862	0.6616
%N	**-0.2437**	**-0.1009**	**-0.1071**	7.7193	0.1750	0.1699	0.0574	0.6449
%P	**-0.0194**	**-0.0070**	**-0.0071**	0.7288	0.0157	0.0190	0.0428	0.5771
C:N	**0.0086**	**-0.0024**	0.0007	1.8163	-0.0129	-0.0079	0.0207	0.4711
C:P	**0.0125**	-0.0001	**-0.0022**	5.0616	-0.0096	-0.0259	0.0101	0.6507
N:P	0.0047	NA	NA	3.2332	-0.0001	-0.0034	0.0044	0.5992

	Variance			
Stoichio	HER	Season	Stream size	Resid
%C	1.8860	11.8026	46.1107	40.2007
%N	18.5631	5.0495	38.7174	37.6699
%P	14.8782	23.4629	17.4792	44.1798
C:N	16.6743	5.1543	24.1587	54.0127
C:P	5.3435	9.9489	49.4206	35.2870
N:P	1.8279	12.0940	45.8172	40.2609

4. Discussion

All three additional factors significantly affect community stoichiometry and there were significant differences between seasons, stream sizes and HERs as well as a large increase in the variance explained by models including these factors.

Generally, the differences between these groups can be explained by differences in the proportion of larger taxonomic groups, notably Mollusca and Insecta. For example, during winter the proportion of Bivalvia and Gastropoda – both groups with significantly lower %C, %N and %P and higher C: nutrient ratios than the other groups (Evans-White & Lamberti, 2005, MS 1) – was lower compared to the other

seasons. Additionally, the proportion of P-rich Insecta was clearly higher during this season (Aa.S2). However, it is not clear whether this is really an effect of season since the sites sampled during winter had almost exclusively low levels of phosphorous, therewith providing conditions under which the proportion of Insects tends to be larger than under high phosphorous conditions (*e.g.* shown in MS 3). Nevertheless, the trend remained when considering only site sampling events of low or intermediate levels of phosphorous or when only considering the insect community (although the difference was smaller under the latter conditions)(not shown).

Similarly, the lower community mass contents and higher stoichiometric ratios in very large streams ("VL") can be explained by a low proportion of Insects and higher proportions of Bivalves and Gastropoda. Particle size tend to decrease in downstream direction (*e.g.* River Continuum Concept (Vannote *et al.*, 1980; Knighton, 1982), leading to a dominance of fine grained substrates (sand, mud) in large streams, the preferred substrate of certain Molluscans. Also (macro-) algae abundances increases along the longitudinal gradient which can serve as substrate and food resource for Gastropoda.

Due to this strong link of stream size to the longitudinal stream gradient and therewith important habitat characteristics such as food resources, substrate or stream velocity, it is probably most driving community composition in terms of larger taxonomic groups among the factors investigated. As a result, it is also explaining the largest part of variance in most stoichiometric variables as suggested by our models.

The importance of season in explaining community %P was probably caused by the clearly higher proportion of P-rich Insecta in winter, which is at least partly an artefact due to datasets mainly from good water quality streams.

Hydroeco-region was only of minor importance for %C, C:P and N:P stoichiometry, but accounted for a substantial part of variance in %N and C:N (and %P although here it was also the least important among the factors). This is probably due to differences in the intensity of agriculture among regions, which often result in large nutrient inputs (especially nitrogen). Regions that tended to have high water nitrogen levels (12="Armoricain", 9="Tables calcaires", also the largest regions in size) are those with high intensities of agriculture and livestock farming in France. Surprisingly, community %N in both regions was rather low. This is probably due to a lower proportion of (rather) N-rich Insecta and higher abundances of Bivalvia and pollution tolerant Oligochaeta under these high levels of nitrogen (and phosphorous).

However, due to the unbalanced data availability it was not possible to accurately disentangle the effects of possible additional effects of community stoichiometry. Also on the first plane (F1 x F2) of a PCA analysis applied on the community stoichiometry values, the sampling site events were largely overlapping and not well separated by season, stream size or hydroeco-region (not shown) Nevertheless, these results confirm the finding that the taxonomic composition among major taxonomic groups is a

main driver of community stoichiometry (MS 3). The composition in terms of larger macroinvertebrate groups is probably driven by a combination of water nutrient level and environmental factors. Although the trends discussed above remained when looking separately at different levels of phosphorous, the differences between categories seemed smaller and became insignificant for some of the groups (results not shown). Likewise, this was the case when considering only the insect community thereby minimizing the effect of stoichiometric variation among taxa.

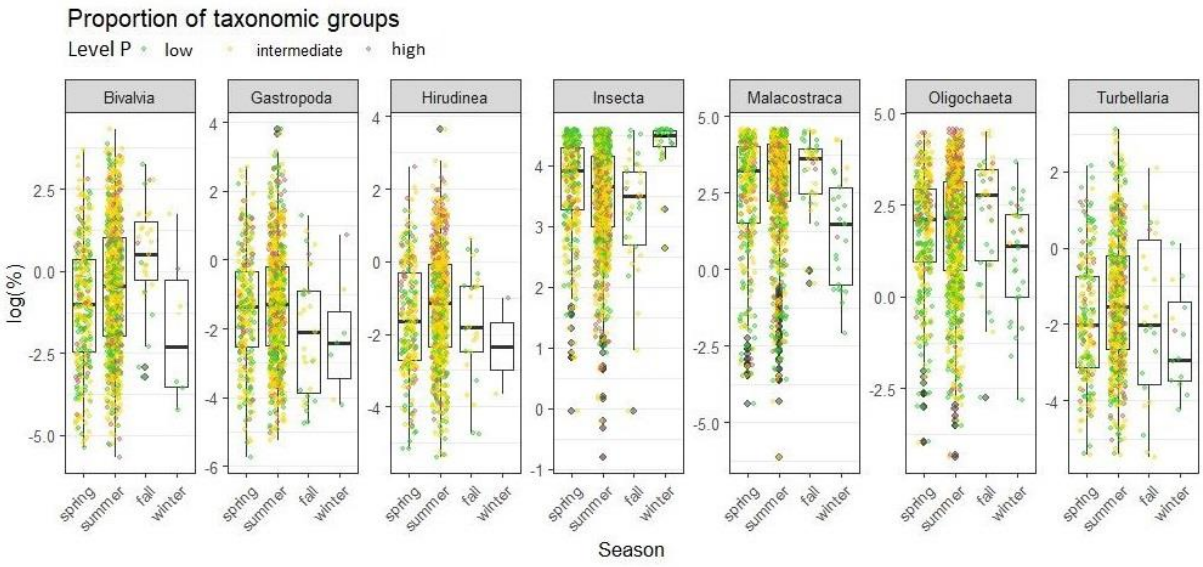
The observation that community stoichiometry tended to respond in the same way towards nutrient changes in different seasons and stream sizes (and most HERs), suggests that indeed community composition and therewith community stoichiometry changes in a similar way despite general differences among communities.

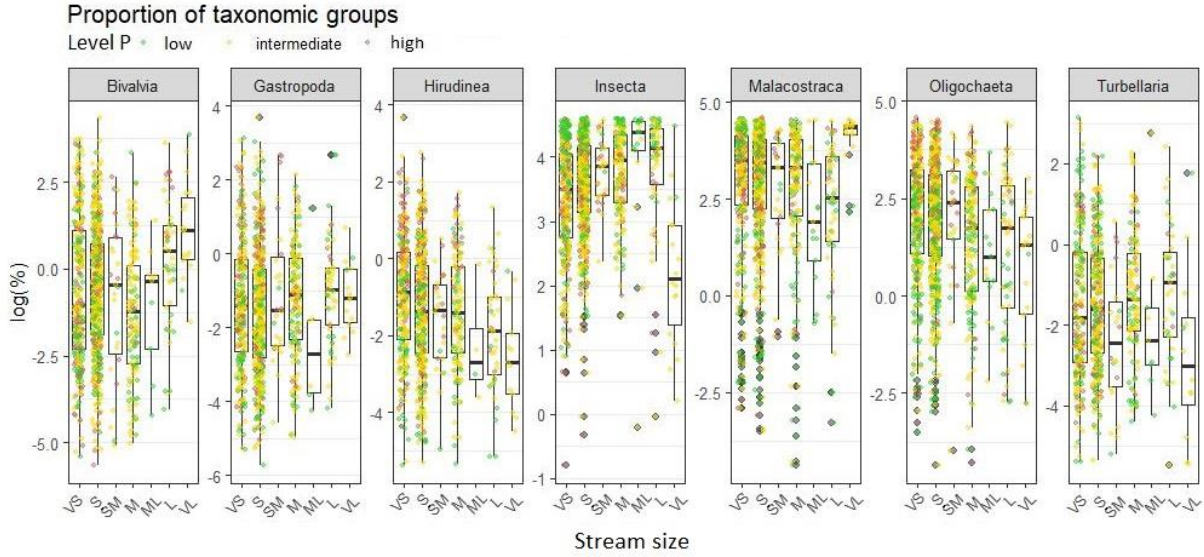
Although these results confirmed the general effect of water phosphorous concentration on community stoichiometry (MS 3), it is necessary to understand the role of confounding factors such as season, stream size and hydroeco-region, that surely have an effect on community composition and therewith community stoichiometry. Only this would allow to make predictions based on stoichiometric traits one day.

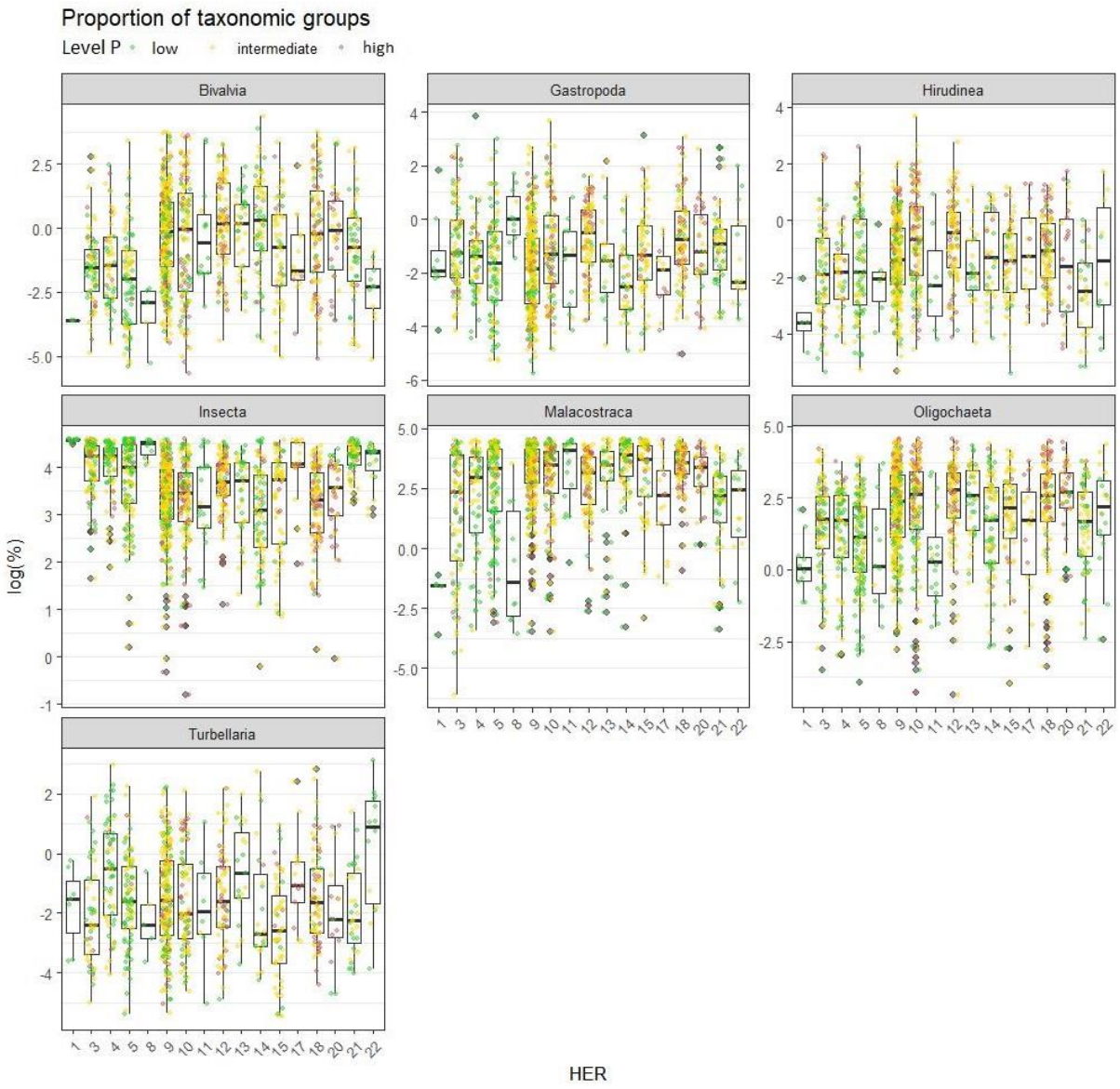
Supplementary Material

Aa.S1: Characteristics in climate, geology and relief in French Hydroeco-Regions (HER1).

ID	HER1	Climate	Geology	Relief
1	PYRÉNÉES	Moist mountainous	Granite/ metamorphic rocks	High mountains
2	ALPES INTERNES	Moist mountainous, cold	Granite/ metamorphic rocks	High mountains
3	MASSIF-CENTRAL SUD	Moist mountainous	Granite/ metamorphic rocks	Mountains
4	VOSGES	Moist mountainous	Granite/ metamorphic rocks	Mountains
5	JURA-PRÉALPES DU NORD	Moist mountainous	Limestone/ sedimentary rocks	Mountains
6	MÉDITERRANÉEN	Mediterranean	Heterogenous	Low relief
7	PRÉALPES DU SUD	South-mediterranean	Limestone/ sedimentary rocks	Mountains
8	CÉVENNES	Mediterranean	Granite/ metamorphic rocks	Mountains
9	TABLES CALCAIRES	Temperate oceanic	Limestone/ sedimentary rocks	Plains
10	CÔTES CALCAIRES EST	Temperate oceanic	Limestone/ sedimentary rocks	Low relief
11	CAUSSES AQUITAINS	Meridional oceanic	Limestone/ sedimentary rocks	Low relief
12	ARMORICAIN	Temperate oceanic	Granite/ metamorphic rocks	Plains
13	LANDES	Meridional oceanic	Detrital?	Plains
14	CÔTEAUX AQUITAINS	Meridional oceanic	Detrital?	Low relief
15	PLAINE SAÔNE	Meridional oceanic	Detrital?	Plains
16	CORSE	Mediterranean	Granite/ metamorphic rocks	Mountains
17	DÉPRESSIONS SÉDIMENTAIRES	Meridional oceanic	Detrital?	Plains
18	ALSACE	Continental oceanic	Detrital?	Plains
19	GRANDS CAUSSES	South-mediterranean	Limestone/ sedimentary rocks	Mountains
20	DÉPOTS ARGILO SABLEUX	Temperate oceanic	Detrital?	Plains
21	MASSIF-CENTRAL NORD	Temperate oceanic	Granite/ metamorphic rocks	Mountains
22	ARDENNES	Temperate oceanic	Granite/ metamorphic rocks	Low relief


Proportion of taxonomic groups
Level P low intermediate high
Bivalvia
Gastropoda
Hirudinea
Insecta
Malacostraca
Oligochaeta
Turbellaria
log(%)
spring summer fall winter
Season


Proportion of taxonomic groups
Level P low intermediate high
Bivalvia
Gastropoda
Hirudinea
Insecta
Malacostraca
Oligochaeta
Turbellaria
log(%)
VS S SM M ML L VL
Stream size



Aa.S2: Proportions of larger taxonomic groups among different seasons (upper panels) and different stream sizes (lower panels). The horizontal line represents the median, the boxes range from the 25th to the 75th percentiles and the whiskers extend to the last values within 1.5 times the inter-quartile range with black dots representing outliers. The scattered dots represent underlying data points with the color indicating the level of phosphorous in the water (low, intermediate, high). Different letters indicate significant differences using Tukey HSD test following a significant ANOVA (adjustment of p-values for multiple testing using Bonferroni method). Sample size was n=354/ 983/ 38/ 29 for spring/summer/fall/winter, n=345/ 577/ 30/ 77/ 18/ 62/ 18/ 177 for stream sizes VS/ S/ SM/ M/ ML/ L/ VL and n=11/ 101/ 80/ 147/ 11/ 344/ 138/ 20/ 109/ 40/ 83/ 66/ 18/ 111/ 37/ 69/ 19 for the different HERs (1/ 3/ 4/ 5/ 8/ 9/ 10/ 11/ 12/ 13/ 14/ 15/ 17/ 18/ 20/ 21/ 22).

Comparison of using abundance vs. biomass-weighted data in stoichiometry and community analyses

1. Introduction

Community ecology seeks to understand community structure and functioning and to understand how it is affected by environmental stressors. The basis is therefore to describe and measure communities. Often this is done using taxa abundances, that is the number of individuals of each taxa in a sample unit, i.e. a community. The use of this measure is indeed intuitive: Each individual contributes to the whole community and the relative abundance can give information of whether a taxon can be considered dominant or rare. Above all, no extra information is required and data can simply be obtained from counting.

However, taxa occurring at the same abundances might not cause the same impact: A small species with a low body weight might only account for a small proportion of the global biomass and contribute little to the overall functioning. Likewise, a rare taxon with a high body weight might cause a larger impact than a small taxon than occur at high numbers.

When it comes to going beyond a single community or investigating energy transfer to higher trophic levels, nutrient cycling or its contribution to ecosystem functioning, abundances are generally weighted by biomass. The interest is not only on the simple number of individuals or taxa, but on how much they contribute to the overall functioning in terms of energy or nutrient. Since metabolism scales with body mass (Kleiber, 1932; Brown *et al.*, 2004) it provides a more direct link to the overall function than simple abundances.

It has been shown that taxa abundances generally are negatively correlated to its body mass (Blackburn et al. 1993), suggesting that calculations and analyses integrating biomass might lead to different results, and in the following cause different interpretations. Some studies already demonstrated that the use of abundance or biomass-weighted abundance can produce quite different results (Saint-Germain *et al.*, 2007).

During my PhD, I was also confronted with the question of whether to base my calculations on simple abundances or whether biomass weighting would better fit my research question. To see whether a decision for the other option would have changed my results, I repeated part of the analyses using biomass-weighted abundances and compared both results. The aim of this analysis is to compare the two approaches and to see if results made from one vary from those based on the other.

2. Material & methods

Stoichiometric information (%C, %N, %P, molar C:N, C:P and N:P) was obtained from the stoichiometric database described in detail in MS 1, It was mainly fed from field samplings but also includes literature values. Biomass information was taken from the underlying raw data of this database.

Taxon mean values were calculated as the geometric mean of all individuals of a taxon and all individuals of finer taxonomic groups for which dry mass information was available (vgl. “Data compilation” MS 1).
Biological and habitat-related traits were originally obtained from Tachet et al. (2010) and used with slightly modified categories as described in MS 2.

The same abundance data sets as used in other parts of my manuscript (MS 3) were used for this comparison. They were taken from the French National monitoring program and details on their selection and applied filters can be found in the corresponding chapters.
For each of these data sets (n=1140) a range of variables (community stoichiometry, diversity indices Shannon H’ and eveness, proportion of larger taxonomic groups, proportion of trait-groups) was calculated in two ways, using simple taxa abundances or biomass-weighted abundances.The analyses and calculations use relative abundances. For biomass-weighted calculations, the relative frequency of a taxon was multiplied by its biomass before continuing the calculation as described in the following. To achieve comparable results, in both approaches – abundance and biomass-based – only taxa for which biomass information was available were considered. In terms of abundances, still a minimum of 95% (mean=97.44, 95.05-10%) of the communities was represented. Additionally, all analyses and calculations were done for the complete community, i.e. considering all taxa, as well as for the insect community only. Here, the relative frequency refers to the total number of insect individuals.

Community indices
As a measure of diversity, the indices Shannon diversity (H’) and evenness were calculated. For Shannon diversity, the relative abundance of each taxon (p_i) was multiplied with its natural logarithm ($\ln(p_i)$), before summing the values of all taxa in the community. The result was multiplied by -1.

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

Eveness was obtained from Shannon diversity by dividing H’ by the natural logarithm of the number of taxa present in the community. Values range between 0 and 1 with 1 indicating complete evenness.

The proportions of lower taxonomic groups (Bivalvia, Gastropoda, Hirudinea, Insecta, Malacostraca, Oligochaeta, Turbellaria) and functional feeding groups (detritivore, herbivore, omnivore, predator) as well as community stoichoimetry (%C, %N, %P, molar ratios C:N, C:P and N:P) were calculated as described in MS 3. Multiple linear models were applied on all calculated indices to compare the effect of water phosphorous and nitrogen level on abundance or biomass-weighted indices. Details on model application are described in the same chapter.

Multivariate community analyses

Co-inertia analyses were applied to investigate the co-variation between community stoichiometry and community functional trait profiles. Community stoichiometry values were used from calculations described above. Community trait profiles were calculated as (simple or biomass-weighted) abundance weighted mean affinity of a community for a trait category.
Co-inertia analyses were applied separately for each of the functional traits as well as for larger groups of traits: Feeding ("food", "feeding mode"), development ("life duration", "maximal body size", "molting events", "number of reproductive events per individual", "voltinism"), all biological traits, all ecological traits and the overall trait profiles including all 23 traits. Details on the calculation of community trait affinities as well as on the application of co-inertia analysis can be found in Chapter 4b.

RLQ analysis uses taxa abundances to link functional trait information to environmental variables. Taxa abundances and environmental information were used from a mono-site long-term data set of the Loire river. Details about the data set, the method and application of RLQ analysis are described in MS 5. The general idea of this analysis is that the three data tables (taxa abundances, taxa traits, environmental variables) that describe the same sites resp. taxa are first analysed separately by suited ordination methods (e.g, CA, PCA) before feeding the output into the rlq() function to quantify co-structure. The total inertia is a measure of the link between the functional traits and environmental variables as mediated by taxa abundances and can be tested for significance. Taxa abundances have been weighted by biomass by multiplying log(n+1)-transformed abundances with each taxon's biomass prior to Correspondence Analysis (CA).

The comparisons between simple abundance and biomass weighted analyses in the following was qualitative and descriptive. It is based on model values but without further statistical analysis.

To note: The stoichiometric trait information used in this chapter refers to an outdated version of the cited trait database. However, differences to the final database values are small and only concern few taxa.

3. Results

Community diversity indices

Community diversity (Shannon diversity H') and evenness both were higher when calculated from abundances rather than biomass-weighted abundances (Fig. Ab.1, Ab.S1). The correlation of both indices towards water nutrient concentration was the same for both ways of calculation but the effect was stronger when values were based on biomass: Both indices decreased with increasing water phosphorous concentration and this effect was stronger at low nitrogen levels.

When only insects in the community were considered, the observed patterns were the same: Both indices had higher values when based on abundances and decreased with increasing water phosphorous concentration with the strongest slopes under low nitrogen conditions. Generally, the difference between values based on biomass or simple abundance was smaller than when the whole community was considered.

Models explained larger parts of variation when applied on abundance weighted values. Also, for both slopes the difference in slopes between abundance and biomass weighted values was larger under low nitrogen conditions compared to intermediate or high nitrogen levels.

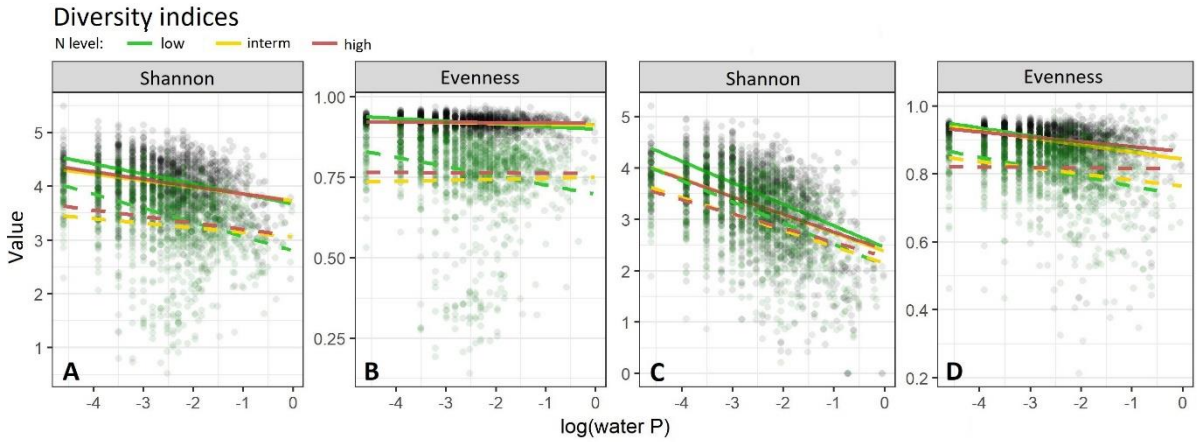


Fig. Ab.1: Shannon Index (H') and evenness of macroinvertebrate communities along a gradient of water P concentration (log transformed) calculated based on abundance (solid lines, black scatter) or biomass (dashed lines, green scatter). Regression lines represent models allowing an interaction between water P concentration and level of water N (low, intermediate, high). Left panels (A+B) show results calculated for the whole community; right panels (C+D) consider only insects. Sample size for all calculations was n=1140.

Community stoichiometry along water nutrient gradient

Abundance-based community mass elements values (%C, %N, %P) were significantly higher than biomass-weighted values, while those community ratio values (C:N, C:P, N:P) were significantly lower (Fig. Ab.2, Ab.S2). Nevertheless, the effects of water phosphorous concentration and water nitrogen level on community stoichiometry followed the same patterns between abundance- and biomass weighted values: Mass elements community values decreased with increasing water phosphorous concentration and this decrease was strongest under low nitrogen conditions. Community ratio values increased along this gradient, but also here the effect of water phosphorous concentration was strongest at low nitrogen levels.

Also when only insects of the community were considered, community %C, %N and %P had higher values when calculations were based on simple abundance rather than biomass, while it was the opposite in community ratios where biomass-weighted values were higher. However, this difference was lower compared to values of the complete community.

While for %C and C:N the correlation of community stoichiometry to water phosphorous concentration

was in the same direction, community %N, %P, C:P and N:P showed opposite relationships to water phosphorous concentration when based on abundance or biomass. For the ratios the biomass-based values followed the same trend as observed for the complete community – that is an increase with increasing water phosphorous concentration. In %P it were the biomass-weighted values that had a negative correlation with water phosphorous concentration, while values calculated from abundances showed a positive correlation. For community %N, only under low nitrogen conditions abundance-based values showed the same pattern as observed for the complete community. Under intermediate and high nitrogen levels as well as when calculations were based on biomass, the values were positively correlated to water phosphorous concentration.

The model slopes indicated a stronger effect on community stoichiometry when values were weighted by biomass instead of abundances at low nitrogen levels. At intermediate N levels the slopes were higher in abundance-based values (except for community %P), such as for the community ratios (C:N, C:P, N:P) at high nitrogen sites. Biomass-weighted slopes were only higher for community %C, %N and %P under high nitrogen conditions.
For all the community stoichiometry values, the model explained more variation when applied on abundance-weighted data.

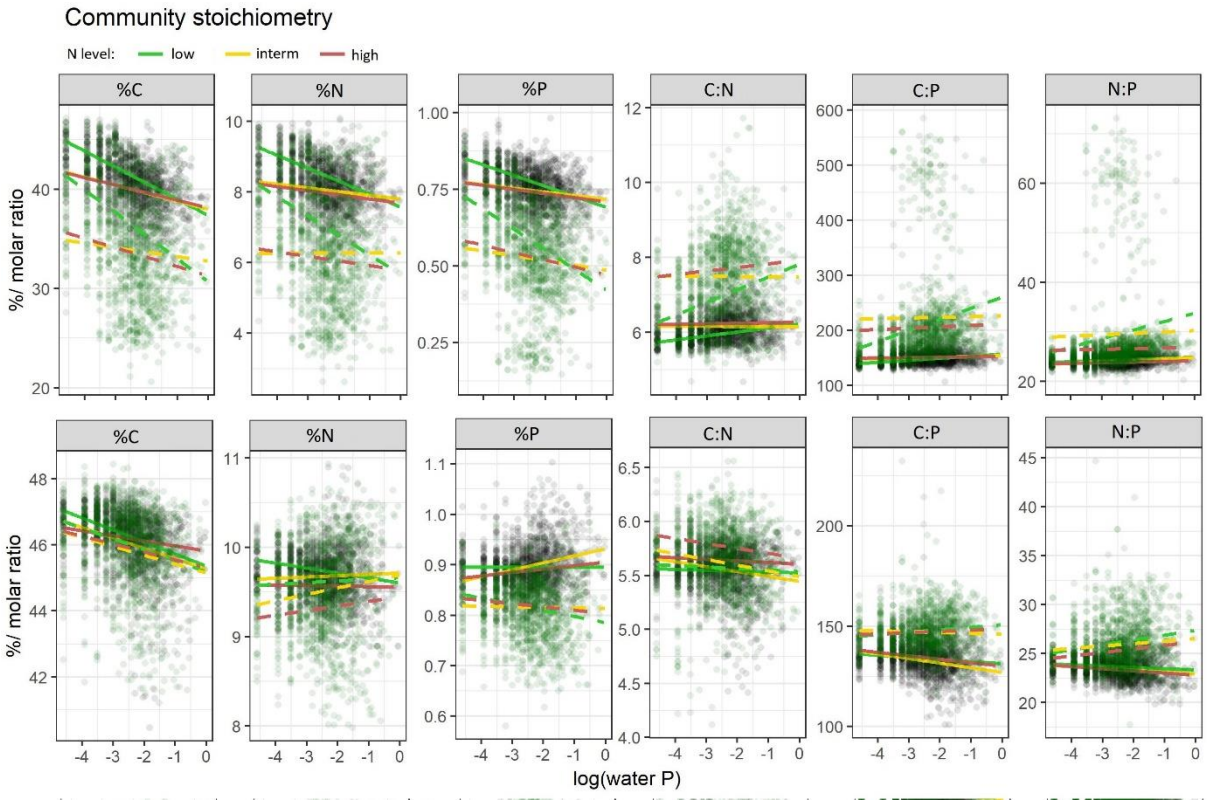


Fig. Ab.2: Community stoichiometry (%C, %N, %P, C:N, C:P, N:P) of macroinvertebrate communities along a gradient of water P concentration calculated based on abundance (solid lines, black scatter) or biomass (dashed lines, green scatter).

Regression lines represent models allowing an interaction between water P concentration (log transformed) and level of water N (low, intermediate, high). Upper panels show results calculated for the whole community; lower panels consider only insects.

Proportion of macroinvertebrate taxonomic groups

Bivalvia, Gastropoda, Hirudinea and Malacostraca accounted for higher proportions of the community when the calculations were based on biomass-weighted abundances (Fig. Ab.3, Ab.S3). Proportions of Insecta, Oligochaeta and Turbellaria were higher when simple abundances were used. This difference was smallest in Oligochaeta and Turbellaria.

Generally, the correlation to water phosphorous concentration and its dependence on water nitrogen level showed the same pattern, independently which way of calculation was used. When in some taxa the slope went in the opposite direction, it was only a very low slope (*e.g.* proportion of insects under high nitrogen levels).

Biomass-weighted abundances lead to a better model fit (higher r^2) in Bivalvia, Gastropoda, Hirudinea, Insecta and Malacostraca, while for Oligochaeta and Turbellaria a higher variation in the value was explained when simple abundances were used in the calculation.

Under all water nitrogen conditions, the model slopes of Bivalvia, Gastropoda, Hirudinea and Insecta were higher for biomass-weighted values, while for Oligochaeta and Turbellaria this was the case for abundance-based values. For Gastropoda and Turbellaria this difference however was only marginal (<0.01). For proportions of Malacostraca, the effect of water phosphorous concentration was larger using abundance-based values under low nitrogen levels, while these values leaded to lower slopes compared to biomass-weighted values under intermediate and high nitrogen levels.

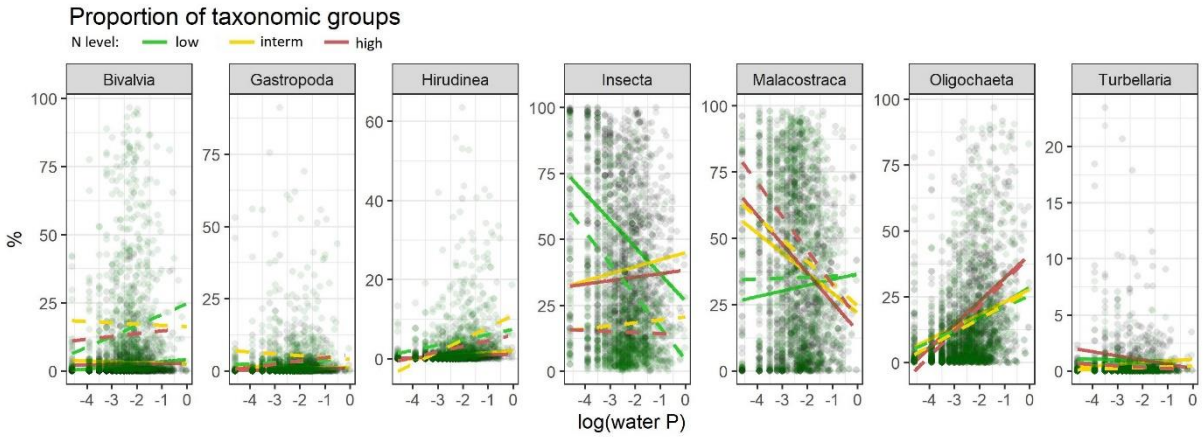


Fig. Ab.3: Proportion of macroinvertebrate classes in the communities along a gradient of water P concentration calculated based on abundance (solid lines, black scatter) or biomass (dashed lines, green scatter). Regression lines represent models allowing an interaction between water P concentration (log transformed) and level of water N (low, intermediate, high).

Proportion of functional feeding groups

For herbivores and predators, the proportions based on simple abundances were higher than when weighted by biomass but generally showed the same correlation to water phosphorous concentration

and nitrogen level (Fig. Ab.4, Ab.S4). Only the difference between the different levels of water nitrogen (esp. “low” N levels) was larger when data was biomass-weighted.

The trend of increasing detritivore proportions with increasing water phosphorous concentration was present in both ways of calculation. But while the slope was rather low when based on abundance, it was steep for biomass-weighted values (at intermediate and high levels of nitrogen) leading to higher detritivore proportions in terms of biomass at high concentrations of water phosphorous. Abundance based calculations resulted in higher proportions under low levels of phosphorous.

In omnivores, proportions were higher in terms of biomass than in terms of simple abundances. The difference in slopes was the largest among all feeding groups and the proportions of omnivores drastically declines with increasing water phosphorous level under intermediate and high water nitrogen conditions. Using simple abundances, this decline was only marginal.

Considering only the insect community, the proportion of herbivores according to abundances was very close to that based on biomass, although value of the latter one tend to be slightly lower than when biomass was not considered. Additionally, the effect of water phosphorous concentration on abundance-based proportions depended on the level of water N, while there was no such interaction when abundances were weighted by biomass.

The proportions of predators were clearly higher when calculated based on biomass rather than simple abundances, but both showed the same response to water nutrient concentrations (*i.e.* the effect of water phosphorous concentration was weakest at low N levels).

Abundance and biomass-weighted detritivore proportions were overlapping over the gradient of water phosphorous concentration. While there was no effect of water N level on the response to water phosphorous concentration in biomass-weighted proportions, the response of abundance based values decreased with decreasing level of water N.

Omnivore proportions weighted by biomass were stronger affected by water phosphorous concentrations than values based on simple abundances. Therefore, both response curves were overlapping with omnivore proportions at low levels of water P concentration being larger when based on abundances only, while biomass-weighted values were larger at high concentrations.

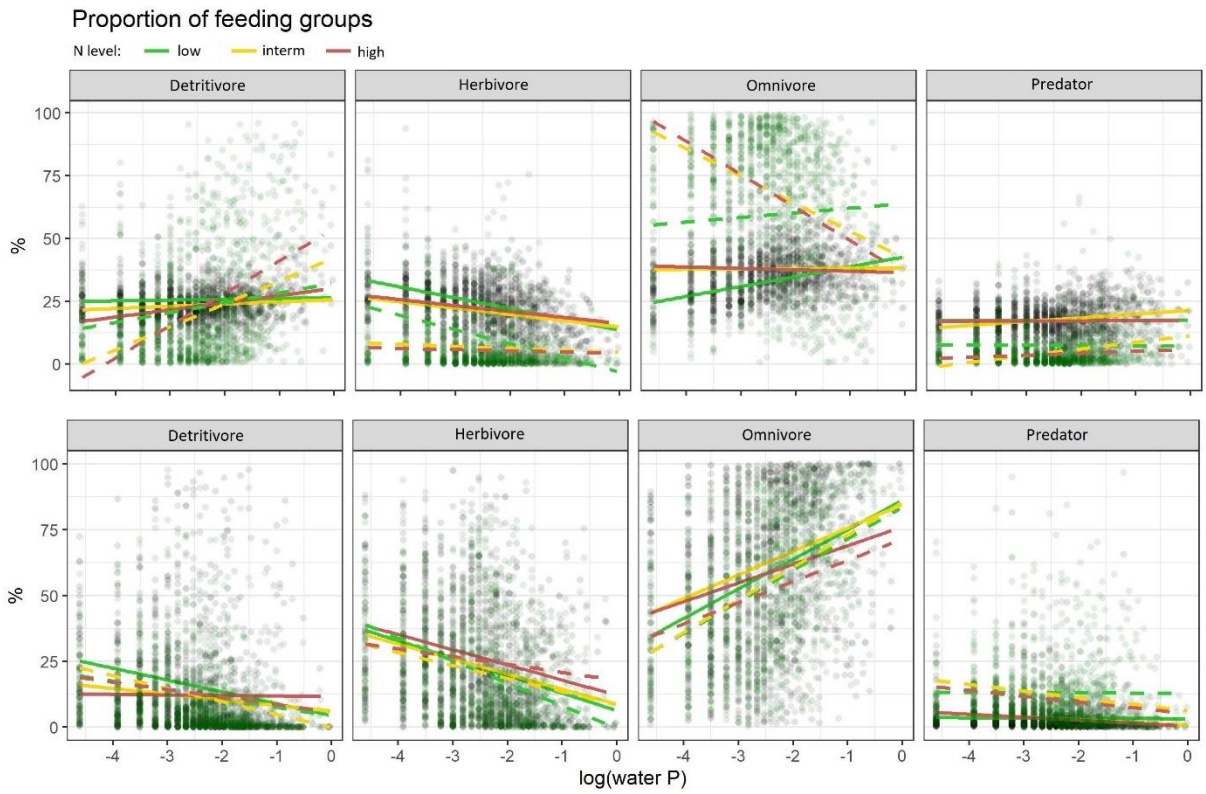


Fig. Ab.4: Proportion of functional feeding groups in macroinvertebrate communities along a gradient of water P concentration calculated based on abundance (solid lines, black scatter) or biomass (dashed lines, green scatter). egression lines represent models allowing an interaction between water P concentration (log transformed) and level of water N (low, intermediate, high). Upper panels show results calculated for the whole community; lower panels consider only insects.

The link between community stoichiometric and functional trait

Using co-inertia analysis, the use of biomass-weighted community traits resulted in a stronger co-variation between community stoichiometry and functional traits in 19/ 23 individual traits and in all groups of traits (Tab. Ab.1). Only for the traits "aquatic life stage", "longitudinal distribution", "number of molting events" and "resistance form" the use of abundance based values results in higher values. This difference was quite substantial and values differed by up to >0.5 (0.52 and 0.56 in traits related to feeding). The group of feeding traits was also the most concerned of all trait groups analysed. Only in the traits "aquatic life stage" and "longitudinal distribution" the difference between abundance and biomass-based values was only marginal (0.1).

When only insects were considered the difference between both approaches was lower, but still 19/ 23 traits had a stronger co-variation when biomass-weighted values were used to calculate the community traits. Exceptions were "aquatic life stage", "life duration", "voltinism" and "water velocity" with the differences in "aquatic life stage" being only marginal. The difference when analyzing groups of traits was larger than it was in the analyses of the complete community.

Tab. Ab.1: Results of Co-inertia analyses to investigate the co-variation between community stoichiometric and functional trait profiles. For each individual trait or group of traits, RV-values are provided either based on abundance or biomass-weighted abundances community traits; for either the complete community or only considering insects. RV-values is a measure of the strength of the link between both tables and varies between 0 (=no correlation) and 1 (=perfect co-variation). (ns) indicates insignificant RV values. The number of communities in each analysis was n=1140.

		Complete community		Insect community	
	Funct. trait	Abundance	Bm-weighted	Abundance	Bm-weighted
Individual traits					
Biological	Aquatic life stages	0.6234	0.61	0.2896	0.2734
	Dispersal	0.4507	0.6581	0.2487	0.3409
	Feeding habit	0.218	0.7785	0.1602	0.3918
	Food	0.1272	0.6445	0.1999	0.5678
	Life duration	0.3142	0.5548	0.2659	0.2489
	Locomotion	0.2725	0.4365	0.0891	0.1968
	Maximal body size	0.2288	0.495	0.1637	0.4593
	Moulting events	0.3839	0.2896	0.1652	0.2857
	Reproductive cycles	0.3254	0.5223	0.1088	0.1806
	Reproduction mode	0.532	0.6426	0.1611	0.305
	Resistance forms	0.1817	0.1089	0.0604	0.3458
	Respiration	0.0997	0.2229	0.0923 (ns)	0.2493
	Voltinism	0.2023	0.4251	0.1602	0.1003
Habitat-related	Altitude	0.4545	0.6377	0.1448	0.1778
	Longitudinal distribution	0.3827	0.3766	0.1044	0.3046
	Low pH sesitivity	0.4991	0.7054	0.1417	0.4855
	Salinity	0.1521	0.4602	0.1261	0.2376
	Substrate	0.5233	0.7168	0.1119	0.2357
	Saprobity	0.0889	0.458	0.1287	0.1646
	Temperature	0.3878	0.5755	0.194	0.2136
	Transversal distribution	0.4482	0.6164	0.1558	0.4228
	Trophic status	0.5434	0.7589	0.1677	0.2382
	Water velocity	0.2749	0.5519	0.1778	0.0584
Trait groups	All traits	0.5441	0.7382	0.2612	0.5691
	Biolog. traits	0.5292	0.7561	0.2692	0.5823
	Ecol. traits	0.4626	0.6349	0.1887	0.3658
	Development	0.5177	0.7016	0.2407	0.5254
	Feeding	0.1992	0.7411	0.1940	0.5057

The link between functional traits and environmental variables, according to RLQ-analysis, was clearly stronger (*i.e.* a higher total inertia) when abundances were weighted by biomass, independent of the community analysed (0.84 vs. 7.04 for the complete community; 0.93 vs. 2.34 for the insect community for which both values were not significant) (Tab. Ab.2). Additionally, the proportion of variance explained by the first axis was even larger under biomass-weighting. The co-structure for the insect community was not significant in both approaches.

The association between functional traits and environmental parameters or among functional trait categories was for some traits similar in both approaches (*e.g.* stoichiometric traits, life duration, voltinism, feeding habit, food resource)(Ab.S5). In others however, it varied quite substantial: multiple reproduction cycles per individual were co-varying with high body C:N, C:P and N:P ratios and associated to high levels of water N:P when simple abundances were used. Integrating biomass, an intermediate ("2-6") number of reproduction cycles was associated to these stoichiometric traits/ environmental conditions, opposing taxa with one, two or more than six reproduction events. While aerial dispersal (both active & passive) was separated from aquatic dispersal modes (active & passive) using simple abundances, aquatic active dispersal was co-varying with aerial dispersal modes when abundances were weighted by biomass. Using simple abundances swimmers and burrowers had opposite coordinates along the first axis, while they were co-varying and associated to high water N:P when weighted by biomass.

When considering only the insect community the effect of biomass-weighting also depended on the trait. Associations between trait categories and environment parameters were similar for life duration, feeding type, food resource and number of reproduction cycles per individual. Only some trait categories were positioned differently, for example producing one generation per year was co-varying with the category "<1 generation/year" and associated to high water N:P when using simple abundances, while it was positioned opposite and co-varying with ">1 generations/ year" when abundances were weighted by biomass. The first axis clearly separated high number of molting events (15-20, >20) from few/ intermediate number of molting events (1, 2-4, 10-14), but the category 5-9 molting events was associated to either of them, depending on the approach used. This was similar for maximum body sizes, where the category of smallest sizes (<0.5cm) was co-varying with water phosphorous concentration and small/ intermediate body sizes when including biomass; but with water N:P and large body sizes when only considering simple abundances.

Tab. Ab.2: Results of RLQ-analyses to investigate the link between community stoichiometric and functional trait profile *via* abundance tables. Values of total inertia and projected inertia on the first and second axis are provided, either based on abundance or biomass-weighted abundances; for either the complete community or only considering insects. The total inertia indicates the global link between stoichiometric and functional trait profiles and was tested for significance by permutation tests (Monte-Carlo permutation teste, rep=9999, for models 2 and 4 to best control for type I error (Dray & Legendre 2008)). The sample size in each analysis was n=1140.

			Proj.ineratia	
	Weighting	Total inertia	ax1	ax2
Complete community	abd	0.8411	93.77	5.36
	bm	7.044	99.04	0.84
Insect community	abd	0.9309 (ns)	86.56	11.21
	bm	2.341 (ns)	93.60	3.79

4. Discussion

The results of this comparison revealed large differences between the use of simple or biomass-weighted abundances in community analyses. This concerned both absolute values as well as the strengths of responses and links towards environmental parameters.

Compared to the use of simple abundance data the integration of biomass put more weight on large taxa, while reducing the impact of smaller ones. In our case, molluscs, especially bivalves clearly drove these results. Since for both, bivalves and gastropods, the shell was included in dry mass values – as it is part of the organism such as the carapace of crustaceans or insects – their biomass values exceeded those of the other taxonomic (Ab.S6).

While the difference in community diversity indices (Shannon H', eveness) can simply be explained by the high influence of some heavy taxa – reducing the diversity and eveness values when biomass is included in the calculation – other indices showed a clear influence of molluscs, notably bivalves, and their characteristics.

Based on biomass, bivalves made up to 30% of the communities, while in terms of abundances they accounted for only around max. 5%. This extreme difference became also visible in community stoichiometry values: molluscs have lower mass element contents (%C, %N, %P) than the other taxonomic groups (and especially insects which tend to account for the largest proportion of communities in terms of abundances) and higher C:N ratios (MS 1). Due to their large proportion in the community in terms of biomass, biomass-weighting lead to decreased community mass contents and increases in community C:N (and other molar ratios). Nevertheless, the indices still followed the same pattern along a gradient of water phosphorous and nitrogen levels in most cases, indicating that the same general trend of a community can be found with both methods when the absolute value is of minor importance.

Heavy taxa also largely influenced the proportion of feeding groups: Both for the complete community as well as the insect community, biomass-weighted values showed a stronger response towards water phosphorous concentration, although the trends were going into the same direction.

Also when only considering insects and thereby excluding shell-bearing taxa and therewith extreme body weights, abundance and biomass-weighted values differed in all diversity and community stoichiometry variables. The pattern of absolute values was the same as for the complete community. Overall, the difference in values between biomass and abundance-based values was smaller than for the complete community indicating that there were less extreme differences in biomass between insect taxa. Interestingly, the correlation of some community stoichiometry variables (%N, %P, C:P, N:P) to water phosphorous concentration went in opposite directions when based on biomass-weighted rather than simple abundances. Since this has not been observed for the complete community, it indicates that

biomass-weighting can result in the same pattern as the use of simple abundances, but can also suggest opposite responses.

Also in multivariate analyses the integration of biomass suggested a stronger link between functional traits and environmental variables than when unweighted abundances were used. This was the case in both RLQ and co-inertia analysis, and therewith independently of using taxa abundances directly (RLQ) or only indirectly *via* community weighted mean values (co-inertia analysis). Also here, this difference was lower – but still present – when only the insect community was considered. For most traits the general association of trait groups among each other and towards environmental stressors was similar. For some traits, biomass-weighing changed the interpretation for one or few categories, partly resulting in opposing directions of association. This might be different when a larger part of variation is captured by the second and/or third axis and more than coordinates along the first axis have to be considered when interpreting the results.

General advantages and disadvantages of biomass data

The use of biomass-weighting requires the additional information of weight of the organisms. Depending on the system studied and the further processing of samples, it might be an (almost) impossible task to obtain this information of the corresponding community. Database values or the use of body size/biomass relationship formulas (Poepperl, 1998; Benke *et al.*, 1999; Baumgärtner & Rothhaupt, 2003) could be an alternative, but are less accurate. Intra-taxon variation in biomass that occurs due to different life stages are most likely not considered, or only integrated as more or less rough approximation when size-classes of each taxon are recorded separately. The use of body size/biomass relationship instead still leaves either the problem of time-consuming acquisition of body size data of all collected individuals, or the fall back on size information taken from a database that comes along with the same restrictions as for biomass itself.

Following their comparison made on arthropod communities, Saint-Germain *et al.* (2007) suggested to – if both approaches are ecologically useful – check whether a community show a strong correlation between taxa abundances and taxa biomass. If that is the case, both methods were likely to give the same results as they demonstrated for correspondence analyses. The authors also point out, that there are ecological functions for which the body weight of a taxon or individual is not important. (*e.g.* pollination).

Conclusion

This qualitative comparison showed that there can be a notable difference between using abundances or biomass-weighted abundances in community analyses. The relative pattern (*e.g.* correlation to an environmental gradient such as water phosphorous concentration) can be the same, but can also go in the opposite direction leading to a different conclusion. Also the strength of the effect or link towards an independent variable (*i.e.* water nutrient concentration) can vary substantially.

This shows that calculations or analyses done using one kind of data should not simply be translated to the other, especially if the interest is on absolute values. The choice to base an analysis on simple abundance or to integrate biomass therefore should be made in advance, based on the (ecological) question addressed.

Supplementary Material

Ab.S1: Results of linear regressions applied to Shannon index and eveness values with water P concentration (log transformed) and water N level (low, intermediate, high) as explanatory variable. Both indices have been calculated by abundance (abd) - and biomass-weighting (bm). The choice of whether to include an interaction between water nutrients was made based on model comparison *via* anova() function and models including such interaction term are indicated by "+". In models without interaction, the slope indicated for "low N" refers to the general slope of the model and consequently there are no slopes for intermediate and high levels of N available. The intercept of "low N" serves as reference to which the given values for "interm N" and "high N" indicate the change; for the slope the true values under the different N levels are given. Asterisks indicate significance of slopes/intercept with: * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$.

			Slope			Intercept			
	Wght	Index	Low N	Interm N	High N	Low N	Interm N	High N	r_2
Complete community	abd	Shannon	-0.1536***			3.7617	-0.0933**	-0.0804*	0.1131
		Eveness	-0.0083***	-0.0021***	-0.0008***	0.8998	0.0135**	0.0187***	0.0551
	bm	Shannon	-0.2626***	-0.0847***	-0.1197**	2.8031	0.2557*	0.2760	0.0871
		Eveness	-0.0288***	0.0031***	-0.0006**	0.6977	0.0524*	0.0646*	0.0276
Insects only	abd	Shannon	-0.3792***			2.5688	-0.2401***	-0.2431***	0.2691
		Eveness	-0.0202***			0.8513	-0.0064	0.0005	0.1274
	bm	Shannon	-0.4125***	-0.3193**	-0.2765*	2.1071	0.0437	0.1726	0.2360
		Eveness	-0.0282***	-0.0182	-0.0013***	0.7363	0.0274	0.0786***	0.0428

Ab.S2: Results of linear regressions applied to community stoichiometry (%C, %N, %P, C:N, C:P. N:P) with water P concentration (log transformed) and water N level (low, intermediate, high) as explanatory variable. Community stoichiometry was either calculated abundance (abd)- or biomass-weighted (bm). The choice of whether to include an interaction between water nutrients was made based on model comparison *via* anova() function and models including such interaction term are indicated by "+". In models without interaction, the slope indicated for "low N" refers to the general slope of the model and consequently there are no slopes for intermediate and high levels of N available. The intercept of "low N" serves as reference to which the given values for "interm N" and "high N" indicate the change; for the slope the true values under the different N levels are given. Asterisks indicate significance of slopes/intercept with: * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$; (ns) indicates models with $p > 0.05$.

			Slope			Intercept			
	Wght	Stoichio	Low N	Interm N	High N	Low N	Interm N	High N	r_2
Complete community	abd	%C	-1.6195 ***	-0.8094 ***	-0.7580 ***	37.3708	0.6319	0.7813	0.3065
		%N	-0.3669 ***	-0.1049 ***	-0.1179 ***	7.5704	0.2289 *	0.1109	0.2697
		%P	-0.0348 ***	-0.0121 ***	-0.0136 ***	0.6903	0.0265 *	0.0182	0.2146
		C:N	0.0176 ***	-0.0007 ***	0.0019 ***	1.8274	-0.0137	0.0058	0.1552
		C:P	0.0244 ***	0.0083 **	0.0048 ***	5.0501	-0.0189	-0.0253	0.0786
		N:P	0.0091 ***			3.2126	-0.0083 **	-0.0195 ***	0.0571
	bm	%C	-2.3155 ***	-0.4559 ***	-0.9378 ***	30.6923	2.0676 **	0.6101	0.1520
		%N	-0.5494 ***	0.0044 ***	-0.1300 ***	5.6645	0.6131 *	0.1225	0.1319
		%P	-0.0665 ***	-0.0156 ***	-0.0241 ***	0.4192	0.0670 *	0.0519	0.1319
		C:N	0.0487 ***	-0.0018 ***	0.0114 **	2.0529	-0.0546 *	0.0071	0.1334
		C:P	0.0909 ***	0.0155 ***	0.0209 **	5.5093	-0.1228 *	-0.1600 *	0.0516
		N:P	0.0309 ***			3.4159	0.0007	-0.0772 ***	0.0308
Insects only	abd	%C	-0.3640 ***	-0.3166	-0.1527 **	45.3607	-0.0997	0.4526 *	0.1320
		%N	-0.0555 ***	0.0148 ***	-0.0058 *	9.6004	0.1096 *	-0.0483	0.0733
		%P	0.0000	0.0138	0.0068 ***	0.8946	0.0369 ***	0.0104	0.0351
		C:N	-0.0017	-0.0084	-0.0027	1.7077	-0.0141	0.0153	0.0423
		C:P	-0.0126 ***			4.8626	-0.0096 *	0.0043	0.0486
		N:P	-0.0076 ***			3.1427	-0.0108 ***	-0.0127 ***	0.0373
	bm	%C	-0.2880 ***			45.3044	-0.1993 *	-0.1163	0.0587
		%N	0.0443 **			9.7305	-0.1018 **	-0.3036 ***	0.0502
		%P	-0.0122 ***	-0.0011 *	-0.0065	0.7855	0.0278 *	0.0179	0.0120
		C:N	-0.0058 **			1.7005	0.0090 *	0.0369 ***	0.0583
		C:P (ns)	0.0032			4.9983	-0.0041	-0.0018	-0.0004
		N:P	0.0140 ***			3.2919	-0.0087	-0.0301 ***	0.0270

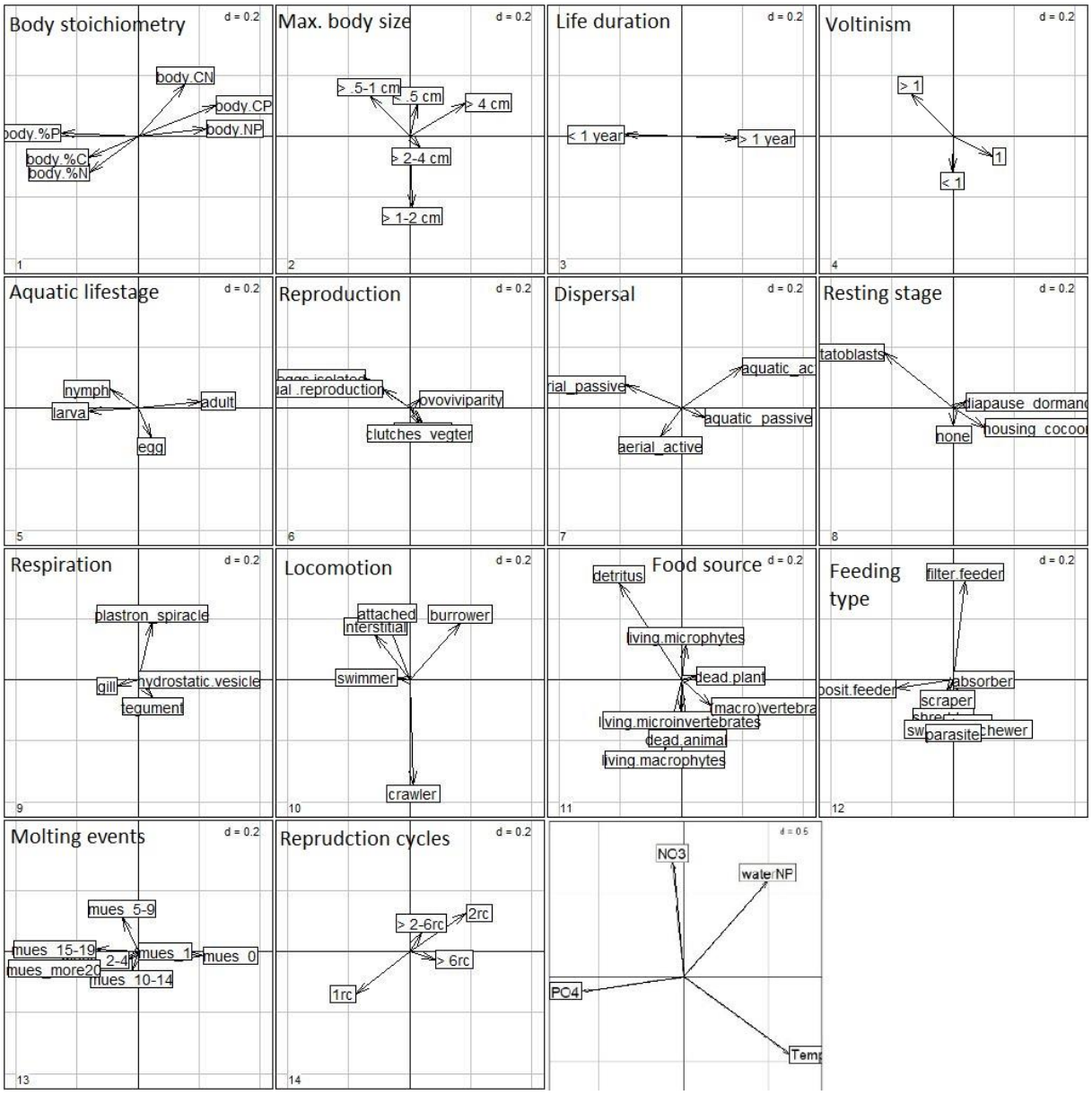
Ab.S3: Results of linear regressions applied to proportions of macroinvertebrate taxonomic groups within the community with water P concentration (log transformed) and water N level (low, intermediate, high) as explanatory variable; either calculated with abundance (abd)- or biomass-weighting (bm). The choice of whether to include an interaction between water nutrients was made based on model comparison *via* anova() function and models including such interaction term are indicated by "+". In models without interaction, the slope indicated for "low N" refers to the general slope of the model and consequently there are no slopes for intermediate and high levels of N available. The intercept of "low N" serves as reference to which the given values for "interm N" and "high N" indicate the change; for the slope the true values under the different N levels are given. Asterisks indicate significance of slopes/intercept with: * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$; (ns) indicates models with $p > 0.05$.

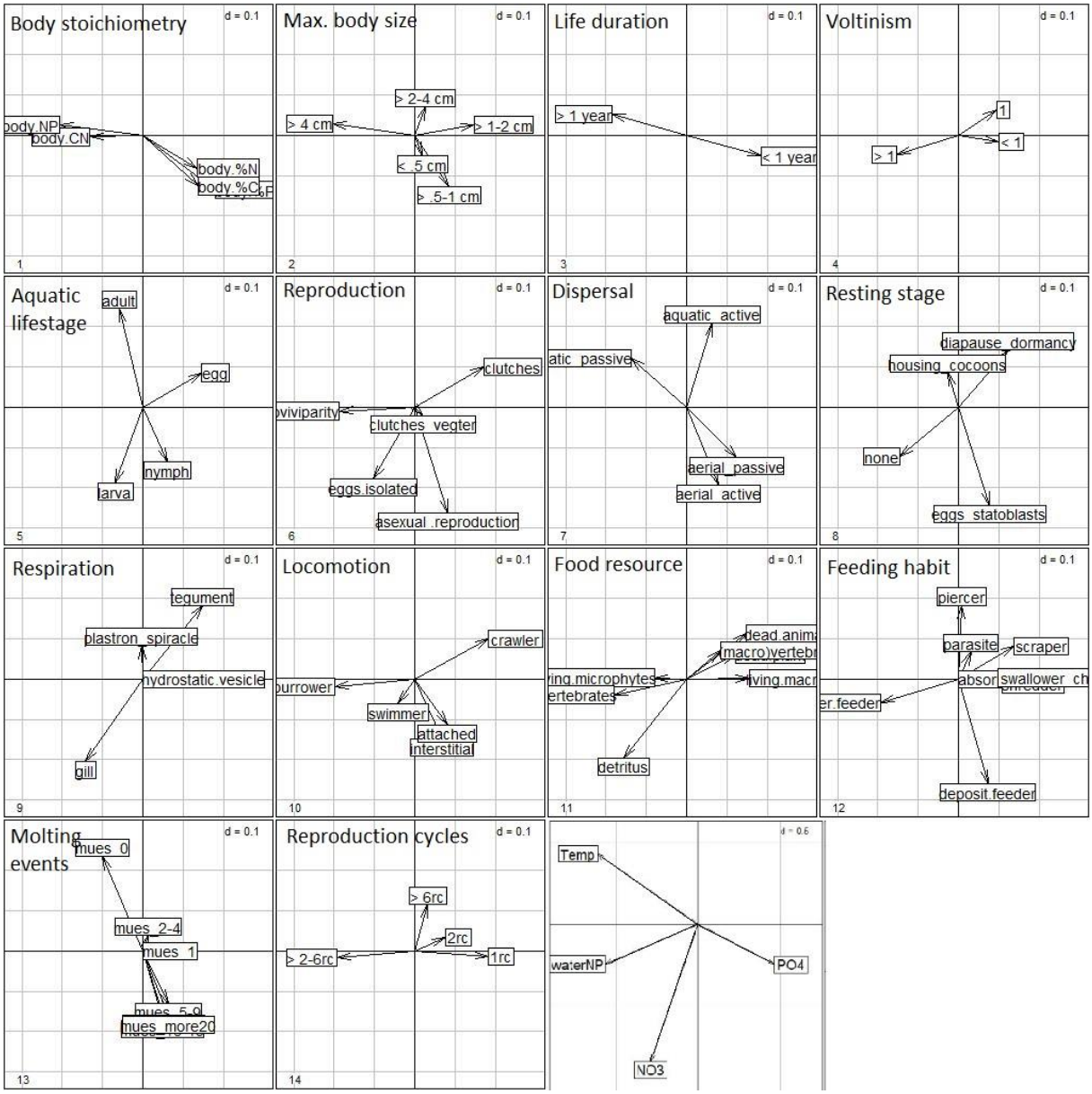
		Slope			Intercept			
Wght	Tax. group	Low N	Interm N	High N	Low N	Interm N	High N	r_2
abd	Bivalvia	0.0087*	-0.0026*	0.0014	0.0429	-0.0164	-0.0148	0.0107
	Gastropoda(ns)	0.0020	-0.0010	0.0018	0.0144	-0.0029	-0.0039	0.0111
	Hirudinea	0.0045***	0.0052	0.0023	0.0207	0.0002	-0.0084	0.0348
	Insecta	-0.1017***	0.0268***	0.0132***	0.2671	0.1833***	0.1165*	0.1435
	Malacostraca	0.0215	-0.0752***	-0.1091***	0.3673	-0.1473**	-0.2166***	0.0702
	Oligochaeta	0.0574***	0.0473	0.0886**	0.2881	-0.0095	0.1231*	0.1132
	Turbellaria(ns)	-0.0006	0.0015	-0.0037	0.0081	0.0025	-0.0057	0.0080
bm	Bivalvia	0.0399**	-0.0048*	0.0102	0.2481	-0.0852	-0.0902	0.0122
	Gastropoda(ns)	0.0041	-0.0063	0.0110	0.0417	-0.0010	0.0157	0.0157
	Hirudinea	0.0132**	0.0306**	0.0141*	0.0750	0.0351*	-0.0147	0.0709
	Insecta	-0.1205***	0.0109***	-0.0041***	0.0460	0.1600***	0.0926*	0.2416
	Malacostraca	0.0037	-0.0829***	-0.1278***	0.3614	-0.1136*	-0.1624*	0.0863
	Oligochaeta	0.0439***	0.0521	0.0947***	0.2554	0.0158	0.1487***	0.0915
	Turbellaria	-0.0011	0.0008	-0.0013	0.0024	0.0022	-0.0015	0.0095

Ab.S4: Results of linear regressions applied to proportions of functional feeding groups within the community with water P concentration (log transformed) and water N level (low, intermediate, high) as explanatory variable; either calculated weighted by abundance (abd) or biomass (bm). The choice of whether to include an interaction between water nutrients was made based on model comparison *via* anova() function and models including such interaction term are indicated by "+". In models without interaction, the slope indicated for "low N" refers to the general slope of the model and consequently there are no slopes for intermediate and high levels of N available. The intercept of "low N" serves as reference to which the given values for "interm N" and "high N" indicate the change; for the slope the true values under the different N levels are given. Asterisks indicate significance of slopes/intercept with: * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$.

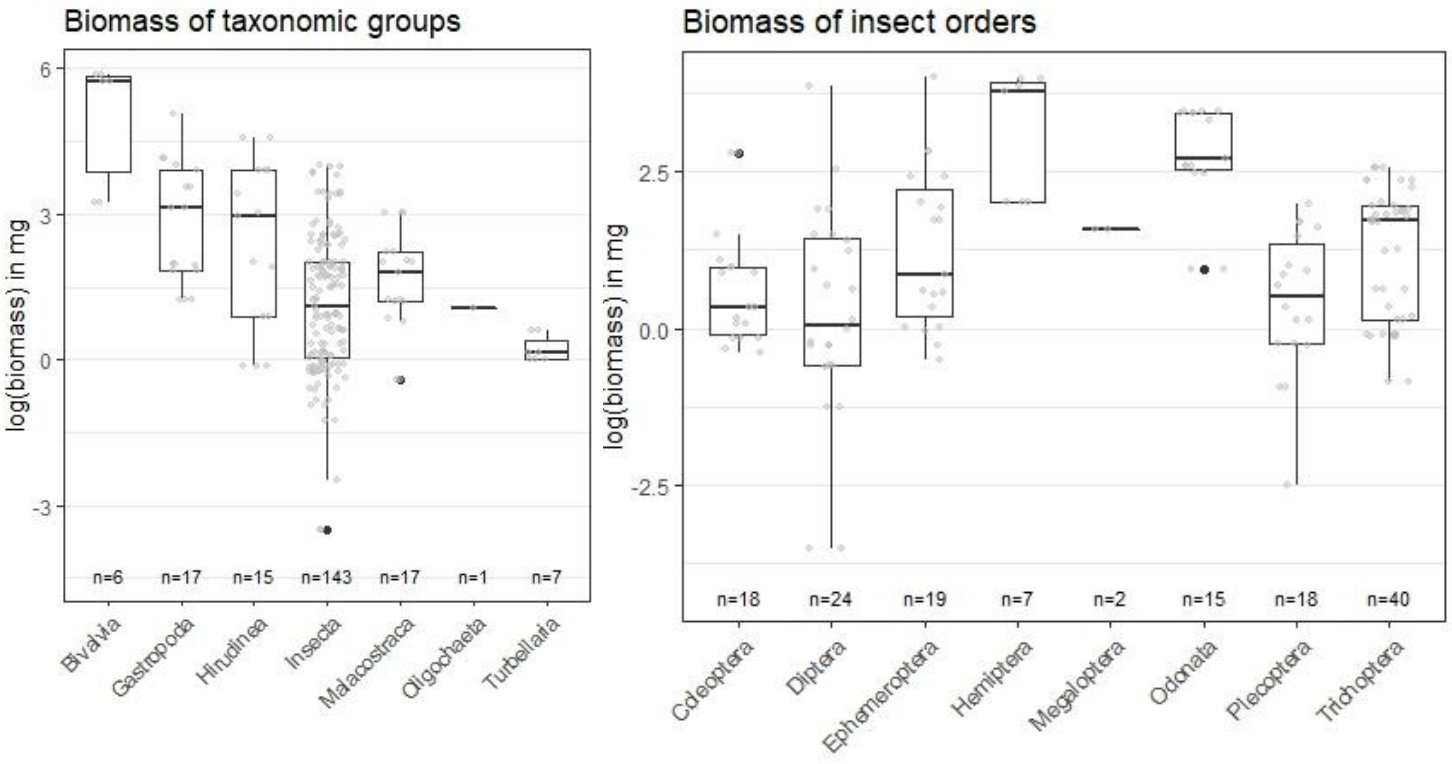
			Slope			Intercept			
	Wght	FFG	Low N	Interm N	High N	Low N	Interm N	High N	r_2
Complete community	Abd	Detritivore	0.0031	0.0088	0.0285 ***	0.2638	-0.0075	0.0376 **	0.0547
		Herbivore	-0.0429 ***	-0.0249 **	-0.0236 **	0.1348	0.0130	0.0270	0.1600
		Omnivore	0.0395 ***	0.0014 ***	-0.0056 ***	0.4276	-0.0452 ***	-0.0639 ***	0.1555
		Predator	0.0003	0.0148 **	0.0008	0.1737	0.0397 **	-0.0006	0.0128
	Bm	Detritivore	0.0391 ***	0.0916 ***	0.1289 ***	0.3223	0.1004 ***	0.2175 ***	0.1219
		Herbivore	-0.0564 ***	-0.0081 ***	-0.0048 ***	-0.0325	0.0782 ***	0.0750 **	0.1279
		Omnivore	0.0184	-0.1096 ***	-0.1319 ***	0.6391	-0.2198 ***	-0.2796 ***	0.1057
		Predator	-0.0011	0.0261 ***	0.0078	0.0711	0.0411 **	-0.0130	0.0585
Insects only	Abd	Detritivore	-0.0450 ***	-0.0215	-0.0021 **	0.0439	0.0166	0.0716	0.0438
		Herbivore	-0.0613 ***			0.0768	0.0013	0.0352 *	0.0734
		Omnivore	0.0950 ***			0.8138	0.0446 *	0.0056	0.1144
		Predator	-0.0009	-0.0106 *	-0.0112 *	0.0313	-0.0249 *	-0.0282 *	0.0155
	Bm	Detritivore	-0.0374 ***			0.0303	-0.0059	0.0047	0.0465
		Herbivore	-0.0856 ***	-0.0495 *	-0.0297 ***	-0.0052	0.0904 *	0.1837 ***	0.0883
		Omnivore	0.1118 ***			0.8133	0.0152	-0.0221	0.1551
		Predator	-0.0010	-0.0249 *	-0.0220 *	0.1277	-0.0659 **	-0.0768 **	0.0163

Ab.S5: Associations of stoichiometric and biological trait categories with the first two axes of RLQ analysis, relying either on simple abundances (upper panels) or biomass-weighted abundances (lower panels). The axes capture 93.76 and 5.35 % (simple abundances) or 99.04% and 0.84% (biomass-weighted abundances) of variation. The last panels for each approach indicates the association of environmental parameters. The whole community was considered in the analysis, results for the insect community are not shown because there was no signficant co-structure.




Body stoichiometry
d = 0.1
body.NP
body.CN
body.%N
body.%C
1
Max. body size
d = 0.1
> 2-4 cm
> 4 cm
> 1-2 cm
< .5 cm
> .5-1 cm
2
Life duration
d = 0.1
> 1 year
< 1 year
3
Voltinism
d = 0.1
1
> 1
< 1
4
Aquatic lifestage
d = 0.1
adult
egg
nymph
larva
5
Reproduction
d = 0.1
clutches
oviviparity
clutches_vegter
eggs.isolated
asexual .reproduction
6
Dispersal
d = 0.1
aquatic_active
atic_passive
aerial_passive
aerial_active
7
Resting stage
d = 0.1
diapause_dormancy
housing_cocoons
none
eggs_statoblasts
8
Respiration
d = 0.1
tegument
plastron_spiracle
hydrostatic.vesicle
gill
9
Locomotion
d = 0.1
crawler
burrower
swimmer
attached
interstitial
10
Food resource
d = 0.1
ertebrates
detritus
11
Feeding habit
d = 0.1
piercer
parasite
scraper
absor
swallower_ch
er.feeder
deposit.feeder
12
Molting events
d = 0.1
mues_0
mues_2-4
mues_1
mues_more20
13
Reproduction cycles
d = 0.1
> 6rc
2rc
> 2-6rc
1rc
14
Temp
waterNP
PO4
NO3

Ab.S6.: Log transformed dry mass (mg) among macroinvertebrate larger taxonomic groups (left panel) and orders of Insects (right panel). The horizontal line represents the median, the boxes range from the 25th to the 75th percentiles and the whiskers extend to 1.5 times the inter-quartile range with black dots representing outliers. Scattered light gray dots indicate underlying raw data points. N indicates the number of taxa within each taxonomic group.



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Scientific activities

Publications

Beck, M. Billloir, E., Felten, V., Meyer, A., Usseglio-Polaterra, P., Danger, M. (2021) A database of West European headwater macroinvertebrate stoichiometric traits *Global Ecology and biogeography, in press*

Beck, M., Mondy, C.P., Danger, M., Billoir, E. & Usseglio-Polatera, P. (2021) Extending the growth rate hypothesis to species development: Can stoichiometric traits help to explain the composition of macroinvertebrate communities? *Oikos*, **130**, 879–892

Danger, M., Arce-Funck, J., **Beck, M**., Crenier, C., Felten, V., Wang, Z. & Maunoury-Danger, F. (2021) *Stoichiometry of Plant Litter Decomposition in Stream Ecosystems*. *The Ecology of Plant Litter Decomposition in Stream Ecosystems* (ed. by C.M. Swan), L. Boyero), and C. Canhoto), pp. 23–49. Springer International Publishing, Cham

Saha, M., Barboza, F.R., Somerfield, P.J., Al-Janabi, B., **Beck, M**., Brakel, J., Ito, M., Pansch, C., Nascimento-Schulze, J.C., Jakobsson Thor, S., Weinberger, F. & Sawall, Y. (2020) Response of foundation macrophytes to near-natural simulated marine heatwaves. *Global Change Biology*, **26**, 417–430.

Conferences – oral presentations

Beck, M., Billoir E., Floury M., Usseglio-Polatera, P., Danger, M., 34 years of phosphorous decline: Responses in community stoichiometry and functional trait composition, EAB 2021, British Ecological Society, Liverpool 12-15.12.2021

Beck, M., Billoir, E., Felten, V., Meyer, A., Usseglio-Polatera, P., Danger, M., Linking functional and stoichiometric traits for connecting macroinvertebrate communities to biogeochemical cycles, SEFS12, Symposium for European Freshwater Sciences, online, 25.7.-30.7.2021

Beck, M., Mondy, C.P., Danger, M., Billoir, E. & Usseglio-Polatera, P., Extending the growth rate hypothesis to species development: Can stoichiometric traits help to explain the composition of macroinvertebrate communities?, SEFS11, Symposium for European Freshwater Sciences, Zagreb, Coratia, 30.6.-5.7.2019

Conferences – poster presentations

Beck, M., Mondy, C.P., Danger, M., Billoir, E. & Usseglio-Polatera, P. Extending the growth rate hypothesis to species development: Can stoichiometric traits help to explain the composition of macroinvertebrate communities?, Festival of Ecology, British Ecological Society, online, 14.12.-18.12.2020

Teaching activities

2020: "Biostatistics" (34h) for Master students of the programmes "Ecotoxicologie" and "Gestion des Milieux Aquatiques Restauration et Conservation"

2021: "Ecological stoichiometry" (4h) as part of ecology lectures for Master students of Ecosystem Management

Additional activities

Participitation at "Ma these en 180 secondes", regional final of Université de Lorraine (2021)

Résumé

Les activités anthropiques ont radicalement modifié les concentrations de nutriments dans les écosystèmes, ce qui constitue un facteur de stress important dans de nombreux milieux à travers le monde. Cette situation devrait se poursuivre à l'avenir et ne concerne pas seulement les changements dans les concentrations absolues, mais également le ratio dans lequel les principaux nutriments sont présents. Les communautés de consommateurs ne sont souvent pas directement affectées, mais plutôt indirectement par les modifications induites dans la quantité et la qualité des ressources de base. Par exemple, dans des conditions de forte disponibilité en phosphore, les taxons de petite taille et à croissance rapide devraient être favorisés, tout comme ceux étant naturellement riches en phosphore.

Les traits fonctionnels des taxons sont souvent utilisés en écologie lorsque les questions portent sur les communautés d'organismes et leur rôle dans le fonctionnement des écosystèmes. Au lieu d'utiliser des unités taxonomiques, chaque organisme est décrit par ses caractéristiques morphologiques, physiologiques, phénologiques et/ou comportementales. Souvent combinées dans un profil de traits multi-métriques incluant plusieurs traits fonctionnels, ces caractéristiques sont généralement liées aux aptitudes et aux fonctions de l'organisme dans son milieu et décrivent ainsi ses interactions avec l'environnement biotique et abiotique.

La Théorie de la Stœchiométrie Écologique (=EST, Ecological Stoichiometry Theory) offre un cadre conceptuel pour étudier l'équilibre et les flux d'éléments (c.a.d. de nutriments) entre les organismes et l'environnement. Les traits stœchiométriques, ici considérés sous l'angle de la composition en éléments chimiques (surtout le carbone, C, l'azote, N, et le phosphore, P) des tissus corporels d'un organisme, peuvent être considérés comme un type de traits biologiques. Ils sont considérés comme spécifiques à un taxon et comme globalement proportionnels à la demande en nutriments d'un organisme. Ces traits ont déjà pu, dans la littérature, être liés avec succès à la qualité des ressources ou à quelques traits fonctionnels des taxons, notamment le taux de croissance et le groupe trophique des organismes. Néanmoins, jusqu'à présent, la plupart des études n'ont pas dépassé le niveau du taxon ou de la population et les liens explicites entre la structure des communautés de consommateurs et la disponibilité en nutriments dans les écosystèmes restent rares.

L'objectif de mon doctorat était d'étudier si - et dans quelle mesure - l'intégration de traits stœchiométriques dans les analyses de communautés animales pouvait améliorer notre compréhension de leur réponse aux changements de concentrations en nutriments. En prenant pour communautés modèles les macroinvertébrés des cours d'eau, j'ai testé les hypothèses de l'EST en m'appuyant sur trois bases de données comprenant (i) des traits stœchiométriques (base de données construite dans le cadre de cette thèse), (ii) des traits fonctionnels et (iii) la structure des communautés de macroinvertébrés dans les rivières de France (abondances et paramètres environnementaux). Les macroinvertébrés sont un groupe taxonomiquement et écologiquement diversifié, constituant les

niveaux trophiques inférieurs des environnements aquatiques et remplissant des fonctions importantes dans le cycle de l'énergie et des nutriments. Ils sont largement utilisés pour la biosurveillance des milieux, et on dispose à ce titre de nombre d'informations sur leur abondance et leurs traits à grande échelle.

Dans un premier temps, j'ai réalisé une série d'échantillonnages de terrain et effectué une recherche bibliographique pour construire une base de données des traits stœchiométriques des macroinvertébrés. Cette base de données comprend 188 taxons de sept groupes taxonomiques principaux et des informations sur six traits stœchiométriques (%C, %N, %P, ratios molaires C:N, C:P N:P). Une analyse de ces données a montré que les plus grands groupes taxonomiques diffèrent significativement dans leur composition corporelle. Par exemple, les bivalves et les gastéropodes présentent des teneurs en N et P significativement plus faibles et des ratios molaires plus élevés que les autres groupes taxonomiques. La taxonomie joue généralement le plus grand rôle dans l'explication de la composition élémentaire des macroinvertébrés. En outre, la plupart des taxons présentaient une composition stable (homéostasie) quelle que soit leur biomasse, la saison ou les disponibilités environnementales en N et P, bien que quelques taxons aient montré une variabilité de leur composition avec ces facteurs, notamment en ce qui concerne la stœchiométrie du carbone et de l'azote (%C, %N, C:N). La teneur en phosphore (%P, C:P, N:P) n'a, quant à elle, pratiquement jamais été affectée par ces paramètres phénologiques ou environnementaux.

J'ai ensuite pu mettre en évidence des liens significatifs entre les six traits stœchiométriques investigués et 23 traits biologiques ou écologiques (liés à l'habitat) des différents taxons étudiés, tant lors de l'analyse des traits individuels que des profils de traits globaux. Les relations mises en évidence avec les traits liés au développement de l'organisme ou au cycle des nutriments étaient conformes à nos hypothèses basées sur les concepts développés dans le cadre de la stœchiométrie écologique. Par exemple, les traits décrivant les prédateurs étaient associés à un %N élevé, les traits suggérant un développement rapide (petite taille corporelle maximale, fréquence de mue élevée) à un %P élevé. Les associations entre les traits liés à l'habitat et la stœchiométrie corporelle pourraient être expliquées par le gradient longitudinal des cours d'eau : les taxons préférant les habitats les plus en amont des cours d'eau (c.a.d. une altitude élevée, un substrat grossier, une température froide) étaient riches en %N et %P. En général, les liens avec les traits stœchiométriques étaient en accord les uns avec les autres (par exemple, les habitudes alimentaires sont associées aux mêmes traits stœchiométriques que les ressources alimentaires potentielles correspondantes). Certaines relations entre traits montrent clairement un effet de la phylogénie qui, cependant, pour la plupart des traits, peuvent également être expliquées par l'EST.

La plupart de ces patrons de réponse persistent lorsque l' analyse est conduite à l'échelle de la communauté plutôt qu'à celle du taxon, et un lien significatif entre la stœchiométrie de la communauté

et les traits fonctionnels de la communauté (tous deux calculés en tant que moyenne pondérée de l'abondance) a pu être démontré.

Le lien entre les ratios stœchiométriques corporels et les traits liés au développement a ensuite été étudié plus avant, montrant que l'hypothèse du taux de croissance (=GRH) peut être étendue au taux de développement : Les taxons présentant des rapports C:P et N:P corporels faibles ont été associés à des groupes d'un développement plus rapide caractérisés par une durée de vie courte et produisant plusieurs générations par an. Le mécanisme en jeu est très probablement le même que celui décrit dans la GRH : Une teneur élevée en phosphore corporel permet un taux de croissance rapide, en fournissant suffisamment de phosphore pour construire de grandes quantités de molécules d'ARN ribosomique qui sont nécessaires pour alimenter la synthèse des protéines, c'est-à-dire la croissance. En examinant les communautés, nous avons constaté que ces taxons avaient tendance à être présents en plus grande proportion dans les cours d'eau présentant des ratios N:P dissous faibles. En revanche, les communautés des cours d'eau pauvres en phosphore et à fort ratio N:P dissous étaient dominées par des taxons à développement lent (durée de vie plus longue, moins d'une génération par an) avec des ratios N:P corporels élevés.

Un autre point important de mon travail consistait à étudier la stœchiométrie de la communauté le long d'un gradient de nutriments. La stœchiométrie de la communauté peut être considérée comme un descripteur du stock de nutriments qui est représenté par la communauté de consommateurs (ici, les macroinvertébrés) et qui a donc des conséquences sur le cycle des nutriments du système.
En utilisant un gradient spatial de nutriments basé sur plus de 1300 ensembles de données d'abondance des différents taxons de macroinvertébrés de cours d'eau provenant de toute la France, j'ai montré que la stœchiométrie de la communauté était significativement affectée par la concentration en phosphore de l'eau et que l'effet était le plus fort à de faibles niveaux d'azote. Bien que nous n'ayons pas pu confirmer notre hypothèse d'une augmentation du %P de la communauté (et d'une diminution de ratios C:P et N:P) avec l'augmentation de la concentration de phosphore dans l'eau pour l'ensemble de la communauté, pour certains groupes taxonomiques (par exemple Insecta, Malacostraca), ce modèle a clairement été vérifié. Les différences générales dans la composition élémentaire des principaux groupes de macroinvertébrés et des remplacements dans la dominance de ces grands groupes taxonomiques le long du gradient de nutriments sont probablement à l'origine de notre incapacité à démontrer les liens attendus entre la stœchiométrie de la communauté globale et la disponibilité en éléments minéraux dans le milieu.

Les facteurs confondants – saison des échantillonnages, taille du cours d'eau, hydro-écorégion – qui affectent la stœchiométrie de la communauté en jouant sur la dominance des différents groupes taxonomiques semblent avoir affaibli les tendances globales attendues sur un gradient spatial de nutriments. Cependant, dans une autre étude menée à l'échelle d'un même cours d'eau (la Loire) suivi

sur un gradient temporel de réduction de disponibilité en nutriments, la stœchiométrie de la communauté a varié dans le sens de nos hypothèses. En effet, la teneur en P de la communauté de macroinvertébrés a significativement diminué au cours des 34 ans de suivi mettant lui-même en évidence une régression du phosphore dans l'eau sur ce site. Ce changement a pu être attribué à un remplacement progressif de taxons riches en P par des taxons plus pauvres en phosphore. La réponse fonctionnelle des communautés a également suivi nos attentes basées sur les EST et nos résultats d'analyses précédentes au niveau des taxons : les conditions de phosphore élevé ont favorisé les traits liés au développement rapide (par exemple, petite taille des corps, courte durée de vie) et à l'alimentation détritique, tandis que les conditions de phosphore faible ont favorisé les taxons à développement lent et prédateurs. Les traits stœchiométriques étaient largement impliqués dans ce lien, soulignant leur potentiel pour expliquer les réponses écologiques aux changements de nutriments.

Au bilan, la plupart des résultats obtenus au cours de ma thèse démontrent que, de manière générale, les hypothèses basées sur la théorie de la stœchiométrie écologique s'appliquent également aux traits fonctionnels au-delà de ceux liés à la croissance ou à l'alimentation et qu'il s'agit d'une approche prometteuse lorsqu'elle est appliquée au niveau des communautés. Le lien entre les traits stœchiométriques et les autres traits fonctionnels ainsi que les concentrations de nutriments dans l'environnement souligne son potentiel pour une meilleure compréhension – et causale – des changements taxonomiques et fonctionnels en cas de modifications des nutriments.

A la suite de ce travail, de nombreuses perspectives s’ouvrent. L'observation que la stœchiométrie des communautés animales est affectée par les concentrations de nutriments dans l’environnement suggère que ces changements atteignent des niveaux plus élevés d'organisation biologique. Il reste à étudier comment un tel changement du stock de nutriments représentés par les consommateurs affecte le cycle des nutriments vers les niveaux trophiques supérieurs/inférieurs ou les écosystèmes terrestres adjacents. Les premières estimations grossières réalisées au cours de cette thèse sont prometteuses car il semble y avoir des tendances claires sur le rôle des nutriments dans l’environnement sur les stock et l'export de phosphore des cours d'eau, suggérant une exportation réduite de phosphore via les insectes émergents dans l'écosystème environnant dans des conditions de phosphore élevé.
Il pourrait également être intéressant d'étudier l’application de ces approches basées sur les traits stoechiométriques à – et son interaction avec – d'autres facteurs de stress mondiaux que les nutriments, notamment le réchauffement climatique dont il a déjà été montré qu'il affectait la stœchiométrie et/ou les traits liés à la stœchiométrie de l'organisme. Contrairement aux nutriments, la température agit directement sur les organismes ectothermes en affectant leur métabolisme et en augmentant leurs taux physiologiques, notamment la respiration, la croissance et le développement. La respiration est directement liée à la demande d'énergie et donc au bilan carbone, et la croissance et le développement sont également liés à la stœchiométrie de l'organisme, comme l'ont démontré mes travaux. Au niveau de la communauté, les taxons plus petits et à croissance rapide sont favorisés par le réchauffement. Il

serait donc intéressant d'étudier l'effet de la température à la fois au niveau des taxon et de la communauté et de voir comment la température interagit avec les changements des conditions nutritives.

L'extension de la base de données des traits stœchiométriques pourrait contribuer à élargir l'application de cette approche et à tester nos résultats également pour d'autres systèmes d'eau douce (lacs), mais également l'environnement marin ou terrestre. De même que pour les eaux douces, la plupart des travaux dans ces systèmes ont été limités au niveau d'un nombre réduit d'espèces (ou de leurs populations) ou se sont concentrés sur des communautés planctoniques ou microbiennes, mais il n'y a pas de raison évidente pour que les analyses au niveau des communautés ne révèlent pas des résultats prometteurs, comme ceux obtenus dans ma thèse pour les macroinvertébrés.

Conclusion générale

Ma thèse visait à étendre l'approche stœchiométrique et les hypothèses de l'EST à l'échelle des communautés animales, ce qui permettrait de mieux comprendre la façon dont les effets des nutriments sur les espèces pourraient atteindre des niveaux d'organisation plus grands. Tout au long de mon travail, j'ai constaté que la phylogénie jouait un rôle important dans le contrôle de la stœchiométrie corporelle d'un taxon et, par conséquent, déterminait également la stœchiométrie de la communauté le long d'un gradient de nutriments et son lien avec d'autres traits fonctionnels. Dans la plupart des cas, cela n'était pas en contradiction avec nos hypothèses basées sur l'EST, de sorte que la taxonomie et l'EST expliquent de manière complémentaire les schémas observés. Les facteurs de confusion qui, par le biais de leurs effets sur la composition taxonomique des communautés, affectent probablement aussi la stœchiométrie de ces communautés, doivent être étudiés plus avant et dissociés de l'effet strict des nutriments. De même, des réponses distinctes entre les groupes taxonomiques ou par rapport à celles de la communauté globale peuvent se produire. Cependant, lors d'un suivi temporel à long terme (34 ans) d'un seul cours d'eau, la stœchiométrie de la communauté et la réponse fonctionnelle aux changements de concentrations de nutriments ont suivi nos attentes basées sur l'EST et les traits stœchiométriques se sont avérés bien adaptés pour expliquer la réponse de la communauté à ce facteur de stress. Il reste à étudier comment une telle modification du stock de nutriments des consommateurs pourrait affecter le cycle des nutriments à l'échelle des écosystèmes, l'approche stœchiométrique pouvant s'avérer particulièrement pertinente pour cela. Il pourrait également être intéressant d'étudier son application à – et son interaction avec – d'autres facteurs de stress à l'échelle globale, notamment le réchauffement climatique, dont il a déjà été démontré qu'il affecte la stœchiométrie et/ou les traits liés à la stœchiométrie corporelle (c.a.d. la croissance, le développement). La mise à disposition d'une base de données sur les traits stœchiométriques ainsi que la grande disponibilité de données sur l'abondance et les traits pour le groupe des macroinvertébrés permettent d'adapter facilement cette approche pour étudier d'autres systèmes et/ou régions afin de vérifier ou d'étendre ces résultats,