Ecosystem functioning in streams:
Disentangling the roles of biodiversity, stoichiometry, and anthropogenic drivers

André Frainer

To Björn Malmqvist

"If all mankind were to disappear, the world would regenerate back to the rich state of equilibrium that existed ten thousand years ago. If insects were to vanish, the environment would collapse into chaos."

E. O. Wilson

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Author contribution

Paper I

AF, WX, MM, and MOG conceived the study. AF and MM collected the data. AF analysed the data and wrote the first draft with substantial contribution from MOG.

Paper II

AF conceived the study with contribution from MOG and BGM. AF and JJ conducted the experiment, and AF, AB, and JJ collected the data. AF analysed the data and wrote the first drafts of the paper, with substantial contribution from BGM and MOG. All authors contributed with text to the paper.

Paper III

BM and BGM conceived the study with contribution from AF. AF collected and analysed the data. AF wrote the first draft of the paper. AF and BGM contributed with data interpretation and text writing.

Paper IV

AF and BGM conceived the study. AF collected and analysed the data. AF wrote the first draft, and AF and BGM contributed with data interpretation and text writing.

Paper V

BGM conceived the study. AF and BGM collected the data. AF analysed and wrote the first draft. All authors contributed with text to the manuscript.

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Abstract

What will happen to ecosystems if species continue to go extinct at the high rates seen today? Although ecosystems are often threatened by a myriad of physical or chemical stressors, recent evidence has suggested that the loss of species may have impacts on the functions and services of ecosystems that equal or exceed other major environmental disturbances. The underlying causes that link species diversity to ecosystem functioning include species niche complementarity, facilitative interactions, or selection effects, which cause process rates to be enhanced in more diverse communities. Interference competition, antagonistic interactions, or negative selection effects may otherwise reduce the efficiency or resource processing in diverse communities. While several of these mechanisms have been investigated in controlled experiments, there is an urgent need to understand how species diversity affects ecosystem functioning in nature, where variability of both biotic and abiotic factors is usually high. Species functional traits provide an important conceptual link between the effects of disturbances on community composition and diversity, and their ultimate outcomes for ecosystem functioning. Within this framework, I investigated relationships between the decomposition of leaf litter, a fundamental ecosystem process in stream ecosystems, and the composition and diversity of functional traits within the detritivore feeding guild. These include traits related to species habitat and resource preferences, phenology, and size. I focused on disentangling the biotic and abiotic drivers, including functional diversity, regulating ecosystem functioning in streams in a series of field experiments that captured real-world environmental gradients. Leaf decomposition rates were assessed using litter-bags of 0.5 and 10 mm opening size which allow the quantification of microbial and invertebrate + microbial contributions, respectively, to litter decomposition. I also used PVC chambers where leaf litter and a fixed number of invertebrate detritivores were enclosed in the field for a set time-period. The chemical characterisation of stream detritivores and leaf litter, by means of their nitrogen, phosphorus, and carbon concentration, was used to investigate how stoichiometric imbalance between detritivores and leaf litter may affect consumer growth and resource consumption. I found that the diversity and composition of functional traits within the stream detritivore feeding guild sometimes had effects on ecosystem functioning as strong as those of other major biotic factors (e.g. detritivore density and biomass), and abiotic factors (e.g. habitat complexity and agricultural stressors). However, the occurrence of diversity-functioning relationships was patchy in space and time, highlighting ongoing challenges in predicting the role of diversity *a priori*. The stoichiometric imbalance between consumers and resource was also identified as an important driver of functioning, affecting consumer growth rates, but not leaf decomposition rates. Overall, these results shed light on the understanding of species functional diversity effect on ecosystems, and indicate that the shifts in the functional diversity and composition of consumer guilds can have important outcomes for the functioning of stream ecosystems.

Key-words: detrital food web, functional diversity, stoichiometry, nitrogen and phosphorus concentrations, recalcitrant carbon, spatial and temporal species distribution, pools and riffles, isotopes, leaf decomposition rates, land use, restoration, habitat complexity.

Preamble

The healthy functioning of ecosystems is fundamental to life on Earth and human civilization. From the cycling of nutrients to biomass production and plant pollination, ecosystems support us with the most fundamental biological and biochemical processes (Millennium Ecosystem Assessment 2005). Among all these, the decomposition of terrestrially-derived organic matter in running water habitats is particularly notable for its importance in carbon and nutrient cycles at local and landscape scales (Webster & Benfield 1986). The carbon and nutrients released from the leaf litter by micro-organisms and detritivores fuel the detrital foodweb (Graça 2001), which is the dominant resource pathway in most forested streams (Petersen & Cummins 1974; Webster & Benfield 1986), across the globe (Boyero *et al.* 2011).

Although fundamental to human water security and well being (Vörösmarty *et al.* 2010), streams are threatened worldwide by a multitude of human disturbances (Vitousek *et al.* 1997). Stressors associated with the excessive release of nutrients from urban sewers or farmlands (Hagen, Webster & Benfield 2011), alterations to riparian vegetation (Hladyz *et al.* 2011), and reduced habitat complexity following hydromorphological modification (Nilsson *et al.* 2005; Palmer, Menninger & Bernhardt 2010) compromise the environmental integrity of running waters. Historically, assessment of these impacts has focussed on the composition and diversity of biological assemblages (Karr 1991), but it is increasingly recognised that direct measures of ecosystem processes can be used as indices of functional integrity. Due to its central role in stream ecosystems, leaf litter decomposition has been proposed (Gessner & Chauvet 2002) and used (Pascoal, Cássio & Marvanová 2005; McKie, Petrin & Malmqvist 2006) as a reliable indicator of ecosystem integrity across a broad range of disturbances (Lepori, Palm & Malmqvist 2005a; Pascoal *et al.* 2005; McKie & Malmqvist 2009). However, the limited understanding of how different biotic and abiotic drivers regulate ecosystem processes such as litter decomposition often hinders environmental status classifications based on functional metrics, particularly when complex interactions between abiotic factors and the composition and diversity of biota drive non-monotonic relationships between environmental change and functional responses (Woodward *et al.* 2012).

The potential for abiotic stressors related to human disturbances to affect ecosystem functioning is widely appreciated (Ferreira, Gulis & Graça 2006; Woodward *et al.* 2012), but compelling evidence has recently suggested that ecosystem functioning is also altered, in some cases strongly, by species loss (Balvanera *et al.* 2006; Cardinale *et al.* 2006). What is more, the effects of species loss may even surpass those of other biotic and abiotic disturbances on ecosystem functioning in some cases (Hooper *et al.* 2012; Eisenhauer *et al.* 2013). Most evidence underlying these claims is derived from studies of plant production in terrestrial ecosystems, but it has been suggested that species loss also affects the processes involved with leaf decomposition in streams (Gessner *et al.* 2010; Lecerf & Kominoski 2010).

To date, most studies on species diversity effects on ecosystem functioning have utilised taxonomic measures of diversity. This approach, although valid, imposes limitations to the understanding of the mechanisms behind that relationship (Mouillot *et al.* 2013). In particular, there is an urgent need to understand how the role of species in ecosystem functioning relates to their functional traits, to facilitate a more predictive approach to forecasting the effects of changes in both species composition and diversity. In this thesis I aimed to assess the spatio-temporal variability of how species diversity influences functioning in stream ecosystems. I was particularly interested in disentangling the role of functional diversity from other biotic and abiotic factors potentially affecting ecosystem functioning.

Introduction

Ecosystem functioning and functional diversity

Ecosystem functioning encompasses the processes and properties of a given ecosystem. Properties are the components that allow for the stability of an ecosystem, such as resistance to invasion, resilience to perturbation, and persistence through time (Loreau, Naeem & Inchausti 2002). Ecosystem processes regulate transformation of energy and nutrients in space and time, and include the biomass production of primary producers, the biomass accumulation of consumers (secondary production), rates of nutrient cycling, and rates of resource consumption and biomass depletion, including the decomposition of biological detritus. Variability in these processes can typically be explained by abiotic (e.g. nutrients, temperature) and biotic (e.g. species composition, biomass) environmental controls. Additionally, the role of species diversity in affecting these functions has recently been suggested to be at least as important as these other factors (Hooper *et al.* 2012; Tilman, Reich & Isbell 2012; Eisenhauer *et al.* 2013).

If two similar systems are composed of equal organismal density and biomass, a higher diversity of primary producers can positively affect biomass production (Spehn *et al.* 2005) and variation in consumer diversity may positively alter consumption rates (Srivastava *et al.* 2009). Positive effects of diversity on ecosystem functioning can be attributed to one or more underlying classes of mechanisms. First, trait complementarity (Loreau & Hector 2001), e.g. when species share a similar diet but make use of the resource in different ways, is hypothesised to positively affect ecosystem functioning (Polley, Wilsey & Derner 2003; Jousset *et al.* 2011). Another type of complementarity takes the form of facilitative interactions (Cardinale, Palmer & Collins 2002), where the presence of one species facilitates and enhances the effects of a second species (Tiunov & Scheu 2005). Finally, selection effects (Loreau & Hector 2001) are in play when observed diversity effects are largely explained by the dominance of particular species that drive increased processing rates in multispecies assemblages (Polley *et al.* 2003). In addition to positive effects, negative diversity effects may also occur when interference competition or antagonistic interactions

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increase in intensity with increasing richness, reducing the efficiency of resource processing, biomass production or similar processes (Hargrave, Hambright & Weider 2011; Jousset *et al.* 2011), or in cases where the dominant species in multispecies assemblages is an inefficient consumer of resources or producer of biomass (a *negative selection effect*).

The relationship between diversity and ecosystem functioning has mostly been explored through the use of taxonomic measures of diversity. Although species taxonomic units can often be easily characterized, the use of species functional characteristics is more appropriate for developing a more mechanistic understanding of diversity–functioning relationships (Mason *et al.* 2005). Functional diversity refers to the distribution of a set of traits that directly influence habitat use and organismal performance. Such traits provide a more direct predictor of functioning than species identities, particularly given that taxonomic and functional diversity are not always linearly correlated (Laliberté & Legendre 2010).

A wide array of functional diversity measures have been developed, ranging from measurements of functional dissimilarity (Walker, Kinzig & Langridge 1999), to functional richness (Petchey & Gaston 2002) and evenness (Mason *et al.* 2005), among others (Schleuter *et al.* 2010). Indices which characterise variability in trait distribution and trait identity may offer the best prospects for understanding the interplay between biodiversity, species identity and functioning (Mouillot *et al.* 2013). Since a set of functional traits may be shared by several different species, measures of functional distribution have to weight those traits by the abundance of all species sharing it (Fig. 1). This is successfully done by Rao's quadratic entropy (Botta-Dukát 2005) and by the 'functional dispersion' index (Laliberté & Legendre 2010), as both indices calculate functional dispersion by controlling the degree of ecological dissimilarity among the traits. Information on the distribution of functional traits can be complemented by an assessment of the identity of dominant traits. According to the mass-ratio hypothesis (Grime 1998), the most abundant traits within a community are the ones driving its ecosystem functioning more strongly, and this can be assessed using the Community-Weighted Mean Trait value (CWM; Fig. 1) (Grime 1998).

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Other biotic drivers of ecosystem functioning

Apart from biodiversity, several other biotic variables can also have strong influences on ecosystem functioning, and influence diversity-functioning relationships. These include community density and biomass. Antagonistic interactions may increase at high densities, due to negative density-dependent competition for resources (McKie *et al.* 2008; Klemmer *et al.* 2012), which reduces per capita resource use efficiency (Amundsen, Knudsen & Klemetsen 2007). Alternatively, higher densities may also favour resource processing if increased species encounter rates favour greater facilitative interactions (Jonsson & Malmqvist 2003). Community biomass reflects the metabolic capacity of the system (Brown *et al.* 2004), which is related to the amount of energy needed for a given set of organisms to sustain itself. Consumer biomass is expected to positively affect consumption, but this also depends on the size of the individuals present. Smaller animals should consume more than larger animals relative to their biomass, due to the higher mass-specific metabolic rates of smaller organisms (Brown *et al.* 2004). However, the tight linkage between consumer biomass and consumption of their main target resource can be weakened by phenological shifts in consumer diets towards alternative resources (Frainer, McKie & Malmqvist 2013).

Finally, ecosystem processes related to resource consumption can be regulated by the degree of stoichiometric imbalance between consumers and their resources. Detritus-based food webs are characterised by a very high degree of chemical imbalance between consumers and their resource (Frost *et al.* 2006; Hladyz *et al.* 2009), as the typical detritivore diet consists of plant litter that is strongly deficient in nitrogen (N) and phosphorus (P), and rich in recalcitrant carbon (C), such as lignin. As consumers normally require N, P, and C in specific rations (C:N, C:P, N:P), they must change feeding preferences or feeding rates to compensate for the limiting nutrients available. As a consequence, the stoichiometric imbalance between consumers and their resource may affect both consumer growth and consumption rates, two important drivers of ecosystem functioning (Frost *et al.* 2002).

Biodiversity, ecosystem functioning, and anthropogenic disturbances in streams

Streams cover only 0.8% of the Earth's surface, but deliver several fundamental ecosystem services to mankind (Vörösmarty *et al.* 2010) which are threatened by the multiple environmental pressures impacting these ecosystems (Malmqvist & Rundle 2002; Dudgeon *et al.* 2005). Among the major threats to streams, habitat simplification associated with hydromorphological modification (Muotka *et al.* 2002; Palmer *et al.* 2010), and alterations of water quality due to urban or agricultural land uses (Paul & Meyer 2001; Hladyz *et al.* 2010) are perhaps the most pervasive, threatening not only freshwater biodiversity and ecosystem functioning at local scales, but also landscape-scale carbon and nutrient cycles (Hering *et al.* 2013). For example, impaired function of stream catchments can result in increased delivery of nutrients and carbon to the oceans (Alexander, Smith & Schwarz 2000; Bianchi 2011), causing eutrophication and even hypoxia in coastal areas (Alexander *et al.* 2000).

Carbon and nutrient cycling in streams are largely controlled by aquatic invertebrate consumers (Covich, Palmer & Crowl 1999; Vanni 2002) that feed on organic particulate matter (gatherers and filterers), graze on periphython (grazers), and/or consume dead organic matter (detritivores). Among these aquatic consumers, detritivores have a key role in the decomposition of leaf detritus (Graça 2001), which forms the basis of the detrital food web. As several detritivore species have potential to feed on alternative food sources (e.g., algae, seston, or even other invertebrates) (Wissinger *et al.* 2004; Layer, Hildrew & Woodward 2013), the relationships between the different detritivore species and ecosystem functioning are highly dependent on the functional traits present within the community (Statzner, Doledec & Hugueny 2004). The presence of different traits in turn depends on habitat and other environmental characteristics, and human disturbances.

My research focussed on the biotic and abiotic drivers, including diversity, influencing leaf decomposition in streams, with algal productivity additionally covered in one study. All my research was conducted in the field, exploiting existing environmental gradients, including gradients in functional diversity. This approach contrasts with most previous diversity-functioning research, which has relied on manipulative

approaches, from small-scale microcosm manipulations to larger scale field experiments (Wardle & Zackrisson 2005), and statistical/mathematical simulations (Morin *et al.* 2011). While powerful, these approaches inevitably miss a great deal of the variability in both environmental conditions and community structure that characterizes natural ecosystems. It is therefore necessary to directly test predictions from these studies in the field, to clarify the importance of species diversity for ecosystem functioning versus other abiotic variables, and under distinct ecological regimes (McKie *et al.* 2009; Cardinale 2011).
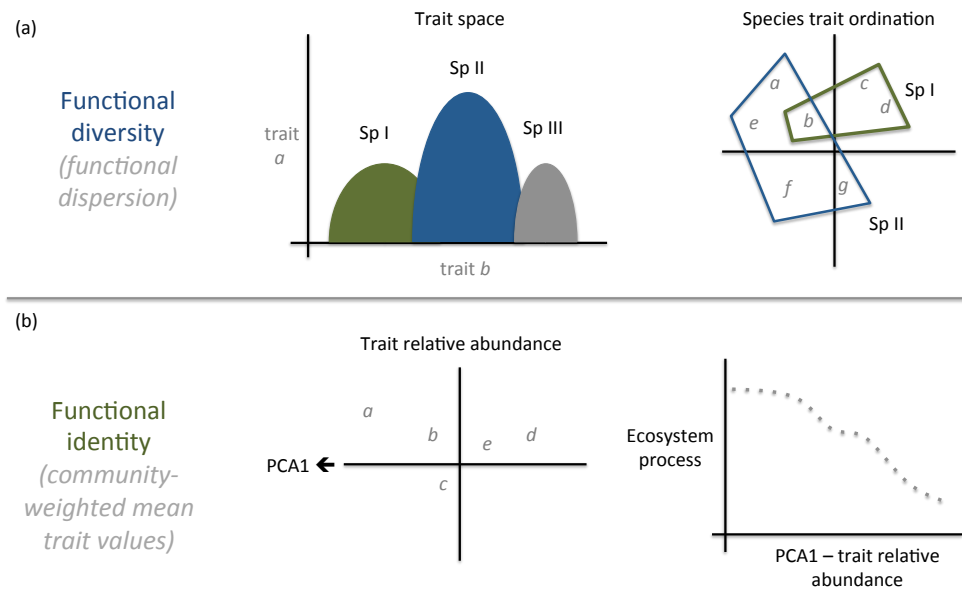

Figure 1. Hypothetical functional traits (a, b, c, d, *etc*) of detritivore species (I, II, and III) within an assemblage of consumers. (a) Niche complementarity may be related to functional dissimilarity, meaning that the most functionally dissimilar species are the ones more likely to complement each other's function. In this thesis, functional dissimilarity was calculated as Functional Dispersion (FD), which further takes into account the relative abundance of each species to calculate how dispersed the functional trait are. (b) Functional identity was assessed as the Community-Weighted Mean trait value (CWM), which indicates the identity of the trait (or set of traits) with highest relative abundance in a sample. The relationship of (a) and/or (b) to ecosystem functioning indicates the main mechanisms by which functional diversity affects ecosystems.

Objectives

In this thesis, I aimed at assessing and quantifying the drivers of ecosystem functioning, with a particular emphasis on the role of biodiversity – characterised as functional diversity – relative to other important drivers. I also aimed at describing the mechanisms behind *how* species diversity affects ecosystem functioning, and *when* and *where* these species diversity effects occur in both natural and anthropogenically altered ecosystems.

To achieve these goals, I addressed the following core questions:

- What is the relative importance of functional diversity for ecosystem functioning (**papers III** and **IV**)? In particular, does it affect decomposition rates (**papers I**, **III**, **IV**, and **V**), leaf litter nutrient dynamics (**paper I**), and fungal biomass accumulation (**paper I**) to a similar, lesser or greater extent than other abiotic and biotic drivers?

- Does the relationship between detritivore functional diversity and leaf litter decomposition differ across seasons (**papers III** and **IV**) and habitats (**papers III**, **IV**, and **V**)?

- Which detritivore traits are more relevant for leaf litter decomposition rates (**paper IV** and **V**)?

- How do anthropogenic disturbances affect stream ecosystem functioning (**papers IV** and **V**)?

- Can the nutrient imbalance between leaf litter and detritivores explain variation in consumer growth and resource consumption (**paper II**)?

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Methods

Study sites

This thesis consists entirely of field-based research, with around 50 different stream reaches sampled to compile the data analysed. Field sites for **papers I** and **II** were located in the German Black Forest and in the French Pyrenees, respectively, while field sites for **papers III**, **IV**, and **V** were located in northern Sweden. Streams were characterized according to their water nutrient concentrations (**papers I**, **II**, and **IV and V**), water flow velocity and discharge (**papers III**, **IV**, and **V**), and substrate type (**all papers**). I also measured water temperature (all papers), and pH and dissolved oxygen (**paper I**, **III**, and **IV**). In **paper IV** I further estimated the extent of riparian vegetation surrounding the stream reach.

The province of Västerbotten in northern Sweden was particularly scrutinized for streams that could help answering the different questions addressed here. Streams were chosen based largely on their surrounding land use type: natural standing forest or agricultural landscapes. In **paper III**, six well-forested streams with minimal human influence were chosen. Within each of these six streams, two adjacent habitats – pool and riffle – were assessed. In **paper IV**, five forested and five agricultural streams with variable levels of disturbance were assessed. In **paper V** restoration effects on ecosystem functioning were tested using 20 stream reaches that presented different levels of habitat complexity: channelized, channelized reaches restored with addition of boulders to the stream channel, and reaches restored with the addition of large boulders, tree trunks, and gravel beds.

Ecosystem functioning

In **papers I**, **III**, **IV**, and **V**, I used leaf litter enclosed in plastic meshes (the 'litter-bags'; Fig. 2) to test whether invertebrates and microbes influenced organic matter decomposition. The litter-bags were constructed with one of two different mesh sizes: 10 (plastic mesh) and 0.5 mm (nylon mesh), hereafter referred to as coarse- and fine-mesh bags, respectively. The two sizes allowed for the assessment of the microbial (fine-mesh) and the microbial + invertebrates (coarse-mesh) mediated components of

decomposition. Leaf-litter was collected from the ground just after leaf abscission, and was air-dried for one or two weeks. In each litter-bag I enclosed 4 ± 0.1g of leaf litter. In **paper II** I used enclosures constructed from PVC pipes (27 cm long, 7 cm diameter), and covered with 0.33 mm mesh-size on both openings. These pipes were used to retain invertebrates and/or leaf litter according to the experimental design of **paper II**. In all experiments I aimed at retrieving the leaf litter at 50% decomposition stage, which allows the comparison of drivers of leaf decomposition without the possibly confounding effects related to decomposition stage.

After retrieval the leaf litter was sorted to species when applicable (**paper I**) and cleaned under tap water (**all papers**). In **papers III** and **IV**, leaf litter was further incinerated at 550°C for 3h to analyse the ash-free dry-mass, and thereby remove the confounding influences of fine sediments adhered to the leaves. Leaf litter decomposition rates were calculated using the negative exponential equation (Petersen & Cummins 1974): $k = \ln(W_t/W_o)/t$, where t = exposure period of the experiment (in days or in degree-days), W_t = leaf litter dry mass at t, and W_o = leaf litter dry mass at time 0. In **paper II** and **III** I calculated the contribution of invertebrates alone by adding the lost fraction of biomass due to microbes alone to the remaining mass of the microbe + invertebrate decomposition, thereby modifying W_t.

I measured algal growth in the streams to investigate how agriculture (**paper IV**) and habitat complexity (**paper V**) might be related to algae production. I used ceramic tiles (16 x 16 cm) tied to a plastic mesh at the stream bottom to allow algal growth. Using a field probe (the *Benthotorch*), the fluorescence of chlorophyll a at 8 points on each tile was quantified, which was then converted to estimates of diatom, green algae, and cyanobacteria biomass. These measurements were conducted 30 days after the tiles were first placed in the stream.

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Figure 2. The litter-bag field method. (a) Weighed four grams of air-dried leaf litter waits in tin foil before being sprayed up with tap water and inserted into the litter-bags. Green meshes have 10 mm openings, and the grey meshes have 0.5 mm openings. (b) Litter-bags deployed in a stream pool. The litter-bags are attached with plastic cable-binders to metal chains, which are anchored at the stream bottom using long metal bars.

I also measured fine particulate organic matter (FPOM) transport and deposition in **paper V**. To measure FPOM transport I took 1L water samples from the top water layer at three different locations in each stream reach. FPOM deposition was estimated with the use of 20 x 20 cm astroturfs tied on a plastic mesh at the stream bottom, which were left in the stream for three days. Both transport and deposition samples were filtered over two sieves of 0.063 and 1 mm mesh size. Deposition samples were later burned for 3h at 550°C to estimate ash-free dry-mass.

Fungal biomass was analysed in **papers I**, **III**, and **IV** to evaluate the responses of fungal decomposers. From coarse (**paper I**) or fine-mesh bags (**papers III** and **IV**) five leaf discs of 11 mm diameter were cut from five randomly chosen leaves immediately after the litter-bags were retrieved from the field and brought to the laboratory. Fungal biomass was quantified as ergosterol biomass – a sterol present only in fungi cell membranes – following Gessner (2005a) (**paper I**) and Dahlman et al. (2002) (**papers III** and **IV**).

In **papers I** and **II,** leaf litter nutrients (N and P) and lignin concentration were analysed following Ebina *et al.* (1983) and Gessner (2005b), respectively. I analysed the difference in nutrient concentration from before and after each experiment to

determine if litter mixture (**papers I** and **II**) or the chemical characteristics of the consumers (**paper II**) were related to changes in nutrient concentration in the leaf. In both papers, initial leaf litter nutrient concentrations were analysed after immersing the air-dried leaf litter in water for 24h.

I measured invertebrate growth rates in **paper II** with the aid of image analysis. Invertebrates from each replicate were photographed both immediately prior to and after the experiment, before being preserved and later oven-dried at 60°C. Through differences in length, and based on allometric equations calculated on another set of invertebrates which were photographed and later oven-dried and weighed, I was able to determine the biomass gain of each set of invertebrates.

Measures of diversity

There are currently several possible measures of functional diversity available, which range from measurements of the ecological distance between two species (Walker *et al.* 1999), to the analyses of trait distribution in a multivariate trait space (Botta-Dukát 2005; Laliberté & Legendre 2010). In **paper I,** I tested for litter mixture effects by measuring the functional distance between pairs of leaf species. These distance measurements were based on three important chemical variables describing leaf litter, nitrogen (N), phosphorus (P), and lignin (Melillo, Aber & Muratore 1982; Taylor, Parkinson & Parsons 1989). Euclidean distances (Walker *et al.* 1999) and the dendrogram branch length (Petchey & Gaston 2002) separating every two species were used as measures of functional diversity.

In **papers III**, **IV**, and **V** I assessed the relative contribution of functional traits to a community of consumers while accounting for the dissimilarity among those traits (the 'functional dispersion'). To characterise species, I used traits that described variation in habitat preferences, as well as phenologic characteristics and feeding potential. These traits were mostly assessed from the Freshwater Ecology Database (Schmidt-Kloiber & Hering 2011). Functional dispersion was calculated based on a distance matrix between the species, taking into account their functional traits (Fig. 1). A centroid was calculated based on this distance matrix and was weighed by species

relative abundances (Laliberté & Legendre 2010). In **papers IV** and **V** I further investigated the contribution of the most abundant set of traits in each community using the community-weighted mean trait values (CWM), related to the mass-ratio hypothesis (Grime 1998; Ricotta & Moretti 2011).

Other biotic variables

To estimate the role of functional diversity relative to other biotic variables related to ecosystem functioning, I also quantified the following metrics for the detritivores colonizing the litter bags: detritivore density (number of invertebrates per litter bag) (**papers III**, **IV**, and **V**), oven-dried mass (**paper III**), metabolic capacity (Brown *et al.* 2004) (**papers IV** and **V**), richness (**papers III**, **IV** and **V**), and evenness (Hurlbert 1971).

Stream leaf-eating detritivores are characterised by a high degree of dietary flexibility, with some species potentially grazing on algae (Layer *et al.* 2013) or even preying on other invertebrates (Wissinger *et al.* 2004) in addition to shredding leaves. Therefore, I analysed detritivore ^{13}C and ^{15}N isotope signal (**paper IV**) to assess the extent to which the diet of these species varied along an environmental gradient.

Data analyses

Among all the statistical approaches used in this thesis, the two most important were Mixed-Effect Model (MEM) analysis of variance, and Structural Equation Model (SEM) analysis. These two approaches are particularly useful for analyses of experiments conducted in the field, where multiple factors may be driving the response variable(s) simultaneously. MEMs were used to quantify the variation in a response variable that is attributable to variation in a set of predictors (fixed variables), taking into account the existence of random variation of error across a series of sampling units, as in a block design (random variables). MEMs were used in **papers I**, **III**, **IV**, and **V**, with the R (R Core Team 2012) package *nlme* (Pinheiro *et al.* 2012).

SEM allows the partitioning of variance among a set of predictor and response variables that may be related to each other via multiple causal pathways (Grace 2006). This method is a form of path analysis, which may be seen as a multivariate regression with multiple responses. By testing several variables that may affect each other simultaneously, it is possible to disentangle which correlations were most significant in explaining variation within a complex data set. With the use of a hypothetical-deductive framework established before model testing I could further suggest causation effects between the tested variables. SEMs were tested using the R package *lavaan* (Rosseel 2012) in papers **III** and **IV**.

Results and discussion

Potential diversity effects on ecosystem functioning cover a broad spectrum, ranging from increased biomass of primary producers (Loreau & Hector 2001; Spehn *et al.* 2005), to consumers (Duffy 2002; Polley *et al.* 2003) and decomposition of organic matter (Gessner *et al.* 2010). In this thesis I studied functional diversity at the resource and consumer levels, but whereas I found substantial evidence for consumer diversity effects, I found no evidence for resource diversity effects on litter decomposition. Consumers were mostly characterized by the aquatic invertebrate detritivores caddisflies (Insecta, Trichoptera) and stoneflies (Insecta, Plecoptera).

What is the relative importance of functional diversity for ecosystem functioning?

Several mechanisms may be hypothesised to explain positive litter diversity effects (Schindler & Gessner 2009). First, nutrient complementarity between two distinct leaf species could positively affect fungal activity, or invertebrate feeding rates, assuming that consumption of leaf litter would be otherwise reduced due to nutrient limitation in either leaf litter. Second, positive diversity effects mediated by invertebrate feeding could also be dependent on the capacity of fungi to extract nutrients from the nutrient rich species and transfer it to the nutrient poor species. Finally, physical leaching from one leaf type to another should transfer relatively abundant nutrients from the nutrient rich leaf to the nutrient poor leaf. This mechanism, if at play, could also have reversed results: leaf secondary compounds, such as phenolics, could also be physically transported to the other species negatively affecting its quality.

Despite the potential for the above mechanisms to occur, there was no evidence for resource diversity effects, as indicated by the absence of leaf litter mixture effects on either microbial or microbial + invertebrate mediated decomposition rates in **paper I**. Dynamics of N and P were also constant within each litter species, regardless of the nutrient dissimilarity with the co-occurring species (**paper I**). Also, there was no relationship between fungal biomass and litter diversity (**paper I**). These results were further supported by the calculation of levels of dissimilarity for each two-species combination using four different functional distance measurements that cover a range

of possible functional diversity measures (Walker *et al.* 1999; Petchey & Gaston 2002; Podani & Schmera 2006; Schleuter *et al.* 2010).

In contrast with litter diversity, the functional diversity of consumers was identified as an important driver of functioning, though this varied with environmental context (**papers III**, **IV**, and **V**). Notably, I found that, under some circumstances, consumer diversity can be as significant for functioning as other biotic and abiotic variables (Fig. 2), with the distribution of traits within a community (Laliberté & Legendre 2010) being of particular importance. In two experiments (**paper III** and **IV**), positive diversity effects occurred when dissimilar traits were evenly distributed, as indicated by their higher functional dispersion (Laliberté & Legendre 2010). Positive trait evenness effect is indicative of complementarity effects in which either (i) two or more species complement each other's activities in niche-space, therefore enhancing overall ecosystem functioning (Cardinale 2011), or (ii) the activities of one species facilitates another species resource consumption (Jonsson & Malmqvist 2003).

In **papers IV** and **V,** I extended the perspective on functional diversity by including another index related to functional traits: community-weighted mean trait values (Grime 1998; Ricotta & Moretti 2011). This measurement, although not a diversity measurement *per se*, characterises mean trait identities at a community level (Ricotta & Moretti 2011). In other words, it captures the set of traits that most characterises an assemblage at a given point in space and time by considering their relative abundance.

By relating both CWM and FD to a process, insights can be gained into whether functioning is strongly driven by particular traits, or whether the interplay between multiple traits is important (Fig. 1). In **paper IV** I found evidence that variation in both trait diversity and the presence of particular traits along a land-use gradient affected leaf decomposition rates, while in **paper V** only trait identity was important in explaining variation in decomposition along a habitat complexity gradient.

Although detritiore functional diversity could have both negative and positive effects on litter decomposition, and the relationship may be highly variable, it can be as important as other prominent biotic drivers in affecting ecosystem functioning. In

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paper III, where functional dispersion was positively related to functioning, detritivore functional diversity effects were as strong as density effects. In **paper IV**, only detritivore functional diversity was related to litter decomposition in autumn, whereas biomass and density affected decomposition in spring.

Does the relationship between detritivore functional diversity and leaf litter decomposition differ across seasons and habitats?

Seasonality played a large role in controlling the relationship between functional diversity and ecosystem functioning (**papers III** and **IV**). This result reflects the roles of fluctuations in abiotic environmental conditions and resource densities, as well as shifting insect phenologies. Specifically, our detritivore assemblages were dominated by insects, which develop as larvae in autumn when litter resources are extensive, and emerge as winged-adults in spring and summer when resource densities are lower (Harper 1973; Otto 1974).

In **paper III** I found that positive diversity effects occurred only during spring, and only in the habitat (pools) characterised by significant abundances of caddisflies (Fig. 3). Litter resources are scarce overall during spring, but are important for insect detritivores completing their growth and development prior to spring-summer emergence. Interestingly, positive diversity effects were observed despite the potential for intensified competition for the scarcer resource in spring. This suggests that diversity effects may actually be favoured when resource densities are lower, perhaps due to an increased concentration of potentially dissimilar traits within the restricted resource patches, and greater potential for facilitative interactions associated with higher species encounter rates.

In **paper IV**, opposite results regarding seasonality were found, as diversity effects were observed in autumn, but not in spring. Contrary to **paper III**, where only undisturbed "reference" sites were studied, the streams studied in **paper IV** represented a gradient of increasing agricultural disturbance. In autumn, functional dispersion of the detritivores decreased along this gradient, and this was negatively associated with litter decomposition rates. Detritivore assemblages in the

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agriculturally disturbed sites were dominated by caddisflies, which are notable for strong antagonistic interactions (McNeely, Finlay & Power 2007; Katano *et al.* 2007; Klemmer *et al.* 2012), which might lower overall resource consumption (McKie *et al.* 2008; Klemmer *et al.* 2012). In spring, greater functional evenness was more associated with the most disturbed areas, but functional dispersion had no relationship to ecosystem functioning.

The contrasting results across the two seasons in **papers III** and **IV** indicate that diversity effects may occur in natural communities, but they may be transient and challenging to predict *a priori* (Turnbull *et al.* 2012). Transient diversity effects may also explain the high variability in response effects observed in the literature, where null diversity effects on functioning are common (Balvanera *et al.* 2006; Cardinale *et al.* 2006). The multiple biotic and abiotic factors that contextualize natural communities must be taken into account to allow for a more mechanistic understanding of *when* and *where* diversity effects should be expected to occur.

In **paper III** I also aimed at assessing the relationship between diversity and functioning in two distinct habitats: riffles and pools. Detritivore diversity effects on litter decomposition were observed in pools, whereas evidence for similar relationships in riffles was weak (Fig. 3). Detritivores potentially contribute more in terms of abundance and biomass in pools than in riffles (Cheshire, Boyero & Pearson 2005; Oliveira & Nessimian 2010), and pools accumulate leaf litter both in autumn and in spring, hosting a functionally diverse community often dominated by large-sized caddisflies, whereas riffles tend to be dominated by smaller-sized, more functionally uniform, stoneflies (Kobayashi & Kagaya 2009). By studying both riffles and pools, I was able to contrast the different dynamics associated with the two habitats. Surprisingly pool habitats are represented by a tiny fraction of all litter decomposition and diversity studies conducted so far, which are heavily biased towards riffles (Graça 2001).
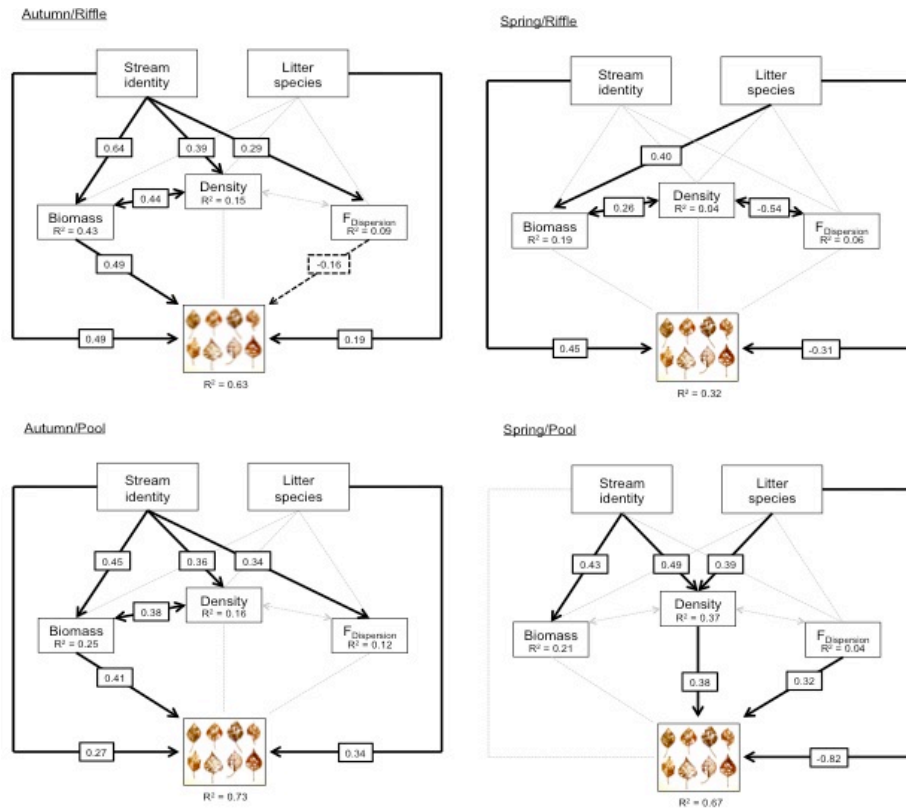
Figure 3. Structural Equation Models showing the relationship between the abiotic and biotic variables and leaf litter decomposition rates. Reproduced from **paper III** with permission from the publisher (Frainer et al. 2013).

Which detritivore traits are more relevant for leaf litter decomposition rates?

In **papers IV** and **V** I attempted to identify which functional traits were related to functioning, based on CWM values. My focus in **paper IV** was to relate variation in environmental conditions due to agricultural activities to variation in feeding traits of the detritivores. I expected scraper-detritivores, which mostly comprise stoneflies that can scrape periphyton from leaves and rocks in addition to shredding leaves, to be the dominant feeding trait in streams influenced by agricultural activities. Pristine streams were expected to have higher presence of biter-detritivores (mostly comprising

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caddisflies), which may be more dependent on leaf litter, although many have potential for predation at later stages of development (Wissinger *et al.* 2004).

However, in contrast to these expectations, scraper-detritivores were found in higher abundances at the more pristine sites, whereas biter-detirtivores were most prominent in agricultural streams. Also contrary to expectations, there was no increase in algal consumption along the gradient, as attested by the similar ^{13}C isotope signal of detritivores sampled from all sites. The effects of these two trait groups on functioning differed, with scraper-detritivores associated with higher and biting-detritivores with lower decomposition rates. As stated above, caddisflies are known for their aggressive behaviour (Katano *et al.* 2007; Klemmer *et al.* 2012), and these antagonistic interactions can reduce leaf decomposition rates (McKie *et al.* 2008; Klemmer *et al.* 2012).

In **paper V** I assessed how stream restoration, which aims at increasing habitat complexity, affected trait identity and ecosystem functioning. Restored streams normally have enhanced flow variability due to the addition of boulders and other structures to the stream channel (Gippel 1995). In this study I saw variation in the functional composition of the detritivore assemblage along the habitat complexity gradient. In particular, traits associated with preferences for high water flows and coarse sediments, and with biting as the favoured feeding mode, were related to the most complex habitats. This result is especially meaningful given the almost complete lack of evidence for positive effects of stream restoration on invertebrate community structure (Palmer *et al.* 2010; Louhi *et al.* 2011), though these previous assessments have focussed on taxonomic rather than functional characteristics.

In addition to alterations in trait identity, the most complex habitats also had the highest rates of leaf litter decomposition in coarse-mesh bags. The same result was not observed for fine-mesh bags, which indicates that alterations in the composition of detritivores explain the differences in ecosystem functioning. Again, this result contrasts to most evaluations of stream restoration to date, where leaf decomposition has shown no relationship to enhanced habitat complexity in restored streams (Lepori *et al.* 2005a; Flores *et al.* 2011), and may be a consequence of the greater range of

physical restoration conducted in this study (Gardeström *et al.* 2013), compared to other evaluations of restoration effects in the same region (Lepori *et al.* 2005b).

How do anthropogenic disturbances affect stream ecosystem functioning?

Anthropogenic disturbances may alter ecosystem functioning directly, or may affect the relationships between diversity and functioning, by altering nutrient inputs and instream hydrogeomorphology, which both can influence instream resources, assemblage diversity and composition, and species interactions. In **paper IV**, the identity (CWM) and diversity (FD) of functional traits varied along an agricultural landuse gradient in autumn, with consequences for leaf litter decomposition (see above). However, there was an absence of land-use effects on functional diversity in spring. This may be explained by the long winter during which disturbances in the stream are governed by ice dynamics (Engström *et al.* 2011), but not so much by land use characteristics. In spring, disturbances related to flood events (Lepori & Malmqvist 2009) may even surpass the effects of land use activities during the previous summer and autumn. Our results give further support to the notion that seasonality greatly affects the relationship between species diversity and ecosystem functioning (see above), even within a gradient of human-induced disturbances.

In **paper IV** I also investigated how land use could affect another important ecosystem function in streams: algal growth. Although agricultural streams, which are characterized by higher water nutrient concentrations and lower riparian cover, are expected to positively affect algal growth (Klose *et al.* 2012) our streams had negligible algal biomass in both seasons. The low water temperature and reduced sunlight in the autumn, and the extended stream ice-cover (Engström *et al.* 2011) followed by the physical abrasion caused by spring floods (Lepori & Malmqvist 2009) limit the possibility for algal-grazing detritivores to exploit disturbed areas where there is decreased vegetation cover and higher water nutrient concentrations. The lack of variation in isotopic signal of the stream detritivores along the land-use gradient gives further support to this idea.

In **paper V**, a gradient of habitat complexity, influenced by human alterations of the physical conditions of the streams (channelization) affected the identity of the traits present, which in turn was related to variation in litter decomposition rates (see above). Habitat complexity also affected other measures of ecosystem functioning in these streams. Algal growth, which was measured in the summer instead of autumn and spring (as in **paper IV**), was not related to enhanced habitat complexity at the local scale I sampled, though the overall increase in surface area of favourable habitat for algal productivity following restoration is likely to have enhanced algal productivity at the reach scale. Another measure of ecosystem functioning, fine particle deposition, was positively affected by enhanced habitat complexity. This result is in accordance to other observations of increased particle retention due to stream restoration (Muotka & Laasonen 2002; Negishi & Richardson 2003; Lepori *et al.* 2005a). Fine particle transport, however, was not related to increased habitat complexity. The transport of particles may depend on other factors happening upstream the restored reach and calls attention to the mostly small-scale restoration projects conducted worldwide, where restoration of one reach is expected to improve ecological integrity of larger areas of the catchment (Bernhardt & Palmer 2011).

Can the nutrient imbalance between leaf litter and detritivores affect consumer growth and resource consumption?

Although leaf litter diversity *per se* had no effect on decomposition rates due to chemical complementarity (**paper I**), mixtures of particular litter species may nonetheless affect detritivore consumption through the chemical mismatch between consumers and resource (Sterner & Elser 2002). In **paper II** I found that invertebrates preferred litter with nutrient ratios that were more balanced with respect to each invertebrate species' nutrient requirements. This was especially evident when mixtures of contrasting litter qualities were offered. In this situation, invertebrate detritivores increased their consumption rates of the leaf litter containing more N relative to C.

Detritivore growth rates were not affected by litter mixtures in this experiment, but were highly constrained by stoichiometric imbalances, mostly driven by N limitation.

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These results demonstrate the importance of detritivore functional characterisation to disentangle the effects that species diversity may have on ecosystems. It further highlights the importance of ecosystem stoichiometry to the processing of resource and growth of consumers in detrital food webs (Martinson *et al.* 2008; Hladyz *et al.* 2009).

Conclusions

In this thesis, I disentangled the importance of a range of biotic drivers (diversity, consumer biomass and density, and stoichiometry) from other sources of variability in functioning, with a focus on identifying the conditions in which diversity affected functioning in natural ecosystems. I found that species effects on ecosystem functioning, as summarized in Figure 4, first depend fundamentally on their functional traits. The functional composition and diversity of detritivore assemblages, in terms of functional dispersion and functional identity, can have strong effects on leaf litter decomposition, through functional complementarity and trait identity effects. The occurrence and expression of these traits vary according to the abiotic conditions, which reflect the natural variation in habitat or resource availability, or as a consequence of human disturbances. Functioning in both natural and human-altered environments can depend strongly on seasonal variations, including seasonal changes in species traits associated with phenological shifts in size, feeding or habitat preference, and behaviour. Ecosystem functioning is further affected by variation in consumer density and biomass. A key finding of my work is that the role of species functional diversity on ecosystem functioning, although highly variable under different environmental regimes, may be as important in affecting ecosystem functioning as other abiotic or biotic drivers.

My findings have major implications for understanding the role of biodiversity in the functioning of real-world ecosystems, and for evaluations of ecological integrity in stream ecosystems based on functional parameters. Since the occurrence of diversity effects may be patchy in space and time, both researchers and managers should be aware of the conditions in which a system is assessed and analysed. Although seemingly straightforward, this conclusion highlights a major obstacle for interpreting current diversity-functioning relationships, where relationships between species and ecosystem functioning have been studied in highly controlled experiments. In these experiments the biota is constrained spatially, and environmental variation in space and time is limited. The variability I have documented in the main drivers of functioning also pose problems to managers, who need to characterise human disturbances on ecosystems but fail in finding significant relationships between

disturbance and functioning. Insight into such interactions can be gained by characterising species according to their functional traits, an approach that has proven meaningful in explaining variation in ecosystem functioning in my research. Small variations in trait identity and diversity in local communities can evidently have wider consequences for the functioning of ecosystems. Future research should place functional diversity within a wider food web context, and consider consequences for the cycling of carbon and nutrients in freshwaters. The role of species diversity on the functioning of ecosystems is more than a potential insurance effect for when ecosystems loose certain species (Yachi & Loreau 1999). The services and functions provided by nature are indeed altered by variation in species functional diversity.
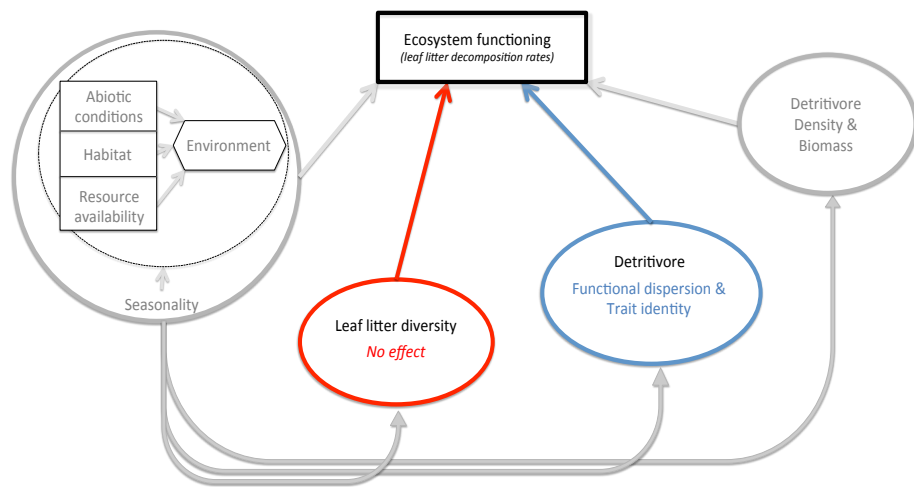

Figure 4. Summary of results of diversity effects on ecosystem functioning seen in this thesis. Functional diversity has direct effects on ecosystem functioning, while both functional diversity and ecosystem functioning may be affected by environmental factors, including seasonality. These environmental factors also affect other biotic components, such as density and biomass, which themselves play a role in affecting ecosystem functioning too.

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References

Alexander, R.B., Smith, R.A. & Schwarz, G.E. (2000) Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. *Nature*, **403**, 758–761.

Amundsen, P.-A., Knudsen, R.R. & Klemetsen, A.A. (2007) Intraspecific competition and density dependence of food consumption and growth in Arctic charr. *Journal of Animal Ecology*, **76**, 149–158.

Balvanera, P., Pfisterer, A.B., Buchmann, N., He, J.-S., Nakashizuka, T., Raffaelli, D. & Schmid, B. (2006) Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecology Letters*, **9**, 1146–1156.

Bernhardt, E.S. & Palmer, M.A. (2011) River restoration: the fuzzy logic of repairing reaches to reverse catchment scale degradation. *Ecological Applications*, **21**, 1926–1931.

Bianchi, T.S. (2011) The role of terrestrially derived organic carbon in the coastal ocean: A changing paradigm and the priming effect. *Proceedings of the National Academy of Sciences*, **108**, 19473–19481.

Botta-Dukát, Z. (2005) Rao's quadratic entropy as a measure of functional diversity based on multiple traits. *Journal of vegetation science*, **16**, 533–540.

Boyero, L., Pearson, R.G., Dudgeon, D., Ferreira, V., Graça, M.A.S., Gessner, M.O., Boulton, A.J., Chauvet, E., Yule, C.M., Albariño, R.J., Ramirez, A., Helson, J.E., Callisto, M., Arunachalam, M., Chara, J., Figueroa, R., Mathooko, J.M., Gonçalves, J.F., Jr, Moretti, M.S., Chará-Serna, A.M., Davies, J.N., Encalada, A., Lamothe, S., Buria, L.M., Castela, J., Cornejo, A., Li, A.O.Y., M'Erimba, C., Villanueva, V.D., del Carmen Zúñiga, M., Swan, C.M. & Barmuta, L.A. (2011) Global patterns of stream detritivore distribution: implications for biodiversity loss in changing climates. *Global Ecology and Biogeography*, **21**, 134–141.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. & West, G.B. (2004) Toward a metabolic theory of ecology. *Ecology*, **85**, 1771–1789.

Cardinale, B.J. (2011) Biodiversity improves water quality through niche partitioning. *Nature*, **472**, 86–89.

Cardinale, B.J., Palmer, M.A. & Collins, S.L. (2002) Species diversity enhances ecosystem functioning through interspecific facilitation. *Nature*, **415**, 426–429.

Cardinale, B.J., Srivastava, D.S., Emmett Duffy, J., Wright, J.P., Downing, A.L., Sankaran, M. & Jouseau, C. (2006) Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature*, **443**, 989–992.

Cheshire, K., Boyero, L. & Pearson, R.G. (2005) Food webs in tropical Australian streams: shredders are not scarce. *Freshwater Biology*, **50**, 748–769.

Covich, A.P., Palmer, M.A. & Crowl, T.A. (1999) The role of benthic invertebrate species in freshwater ecosystems: zoobenthic species influence energy flows and nutrient cycling. *BioScience*, **49**, 119–127.

Dahlman, L., Näsholm, T. & Palmqvist, K. (2002) Growth, nitrogen uptake, and

resource allocation in the two tripartite lichens Nephroma arcticum and Peltigera aphthosa during nitrogen stress. *New Phytologist*, **153**, 307–315.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.-I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.-H., Soto, D., Stiassny, M.L.J. & Sullivan, C.A. (2005) Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews of the Cambridge Philosophical Society*, **81**, 163.

Duffy, J.E. (2002) Biodiversity and ecosystem function: the consumer connection. *Oikos*, **99**, 201–219.

Ebina, J., Tsutsui, T. & Shirai, T. (1983) Simultaneous determination of total nitrogen and total phosphorus in water using peroxodisulfate oxidation. *Water Research*, **17**, 1721–1726.

Eisenhauer, N., Dobies, T., Cesarz, S., Hobbie, S.E., Meyer, R.J., Worm, K. & Reich, P.B. (2013) Plant diversity effects on soil food webs are stronger than those of elevated CO2 and N deposition in a long-term grassland experiment. *Proceedings of the National Academy of Sciences*, **110**, 6889–6894.

Engström, J., Jansson, R., Nilsson, C. & Weber, C. (2011) Effects of river ice on riparian vegetation. *Freshwater Biology*, **56**, 1095–1105.

Ferreira, V., Gulis, V. & Graça, M.A.S. (2006) Whole-stream nitrate addition affects litter decomposition and associated fungi but not invertebrates. *Oecologia*, **149**, 718–729.

Flores, L., Larrañaga, A., Díez, J. & Elosegi, A. (2011) Experimental wood addition in streams: effects on organic matter storage and breakdown. *Freshwater Biology*, **56**, 2156–2167.

Frainer, A., McKie, B.G. & Malmqvist, B. (2013) When does diversity matter? Species functional diversity and ecosystem functioning across habitats and seasons in a field experiment. *Journal of Animal Ecology*, DOI 10.1111/1365-2656.12142.

Frost, P.C., Benstead, J.P., Cross, W.F., Hillebrand, H., Larson, J.H., Xenopoulos, M.A. & Yoshida, T. (2006) Threshold elemental ratios of carbon and phosphorus in aquatic consumers. *Ecology Letters*, **9**, 774–779.

Frost, P., Stelzer, R., Lamberti, G. & Elser, J.J. (2002) Ecological stoichiometry of trophic interactions in the benthos: understanding the role of C : N : P ratios in lentic and lotic habitats. *Journal of the North American Benthological Society*, **21**, 515–528.

Gardeström, J., Holmqvist, D., Polvi, L.E. & Nilsson, C. (2013) Demonstration restoration measures in tributaries of the Vindel River catchment. *Ecology and Society*, **18**, 8.

Gessner, M.O. (2005a) Ergosterol as a measure of fungal biomass. *Methods to Study Litter Decomposition: a practical guide* (eds M.A.S. Graça, F. Bärlocher & M.O. Gessner pp. 189–196. Springer-Verlag, Dordrecht, The Netherlands.

Gessner, M.O. (2005b) Proximate Lignin and Cellulose. *Methods to study litter*

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decomposition: a practical guide (eds M.A.S. Graça, F. Bärlocher & M.O. Gessner pp. 115–120. Springer-Verlag, Dordrecht, The Netherlands.

Gessner, M.O. & Chauvet, E. (2002) A case for using litter breakdown to assess functional stream integrity. *Ecological Applications.*

Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H. & Hättenschwiler, S. (2010) Diversity meets decomposition. *Trends in Ecology & Evolution*, **25**, 372–380.

Gippel, C.J. (1995) Environmental hydraulics of large woody debris in streams and rivers. *Journal of Environmental Engineering*, **121**, 388–395.

Grace, J.B. (2006) *Structural Equation Modeling and Natural Systems.* Cambridge Univ Press.

Graça, M.A.S. (2001) The role of invertebrates on leaf litter decomposition in streams – a review. *International Review of Hydrobiology*, **86**, 383–393.

Grime, J.P. (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *Journal of Ecology*, **86**, 902–910.

Hagen, E.M., Webster, J.R. & Benfield, E.F. (2011) Are leaf breakdown rates a useful measure of stream integrity along an agricultural landuse gradient? *Journal of the North American Benthological Society*, **25**, 330–343.

Hargrave, C.W., Hambright, K.D. & Weider, L.J. (2011) Variation in resource consumption across a gradient of increasing intra- and interspecific richness. *Ecology*, **92**, 1226–1235.

Harper, P.P. (1973) Life histories of Nemouridae and Leuctridae in Southern Ontario (Plecoptera). *Hydrobiologia*, **41**, 309–356.

Hering, D., Borja, A., Carvalho, L. & Feld, C.K. (2013) Assessment and recovery of European water bodies: key messages from the WISER project. *Hydrobiologia*, **704**, 1–9.

Hladyz, S., Åbjörnsson, K., Giller, P.S. & Woodward, G. (2011b) Impacts of an aggressive riparian invader on community structure and ecosystem functioning in stream food webs. *Journal of Applied Ecology*, **48**, 443–452.

Hladyz, S., Gessner, M.O., Giller, P.S., Pozo, J. & Woodward, G. (2009) Resource quality and stoichiometric constraints on stream ecosystem functioning. *Freshwater Biology*, **54**, 957–970.

Hladyz, S., Tiegs, S.D., Gessner, M.O., Giller, P.S., Preda, E., Risnoveanu, G., Nistorescu, M., Schindler, M. & Woodward, G. (2010) Leaf-litter breakdown in pasture and deciduous woodland streams: a comparison among three European regions. *Freshwater Biology*, **55**, 1916–1929.

Hooper, D.U., Adair, E.C., Cardinale, B.J., Byrnes, J.E.K., Hungate, B.A., Matulich, K.L., Gonzalez, A., Duffy, J.E., Gamfeldt, L. & O'Connor, M.I. (2012) A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature*, **486**, 105–108.

Hurlbert, S.H. (1971) The nonconcept of species diversity: a critique and alternative parameters. *Ecology*, **52**, 577–586.

Jonsson, M. & Malmqvist, B. (2003) Mechanisms behind positive diversity effects on ecosystem functioning: testing the facilitation and interference hypotheses. *Oecologia*, **134**, 554–559.

Jousset, A., Schmid, B., Scheu, S. & Eisenhauer, N. (2011) Genotypic richness and dissimilarity opposingly affect ecosystem functioning. *Ecology Letters*, **14**, 537–545.

Karr, J.R. (1991) Biological integrity: a long-neglected aspect of water resource management. *Ecological Applications*, **1**, 66–84.

Katano, I., Mitsuhashi, H., Isobe, Y., Sato, H. & Oishi, T. (2007) Group size of feeding stream case-bearing caddisfly grazers and resource abundance. *Basic and Applied Ecology*, **8**, 269–279.

Klemmer, A.J., Wissinger, S.A., Greig, H.S. & Ostrofsky, M.L. (2012) Nonlinear effects of consumer density on multiple ecosystem processes. *Journal of Animal Ecology*, **81**, 770–780.

Klose, K., Cooper, S.D., Leydecker, A.D. & Kreitler, J. (2012) Relationships among catchment land use and concentrations of nutrients, algae, and dissolved oxygen in a southern California river. *Freshwater Science*, **31**, 908–927.

Kobayashi, S. & Kagaya, T. (2009) Colonization of leaf patches at topographically different locations by insect shredders in a small mountain stream. *Limnology*, **10**, 143–147.

Laliberté, E. & Legendre, P. (2010) A distance-based framework for measuring functional diversity from multiple traits. *Ecology*, **91**, 299–305.

Layer, K., Hildrew, A.G. & Woodward, G. (2013) Grazing and detritivory in 20 stream food webs across a broad pH gradient. *Oecologia*, **171**, 459.

Lecerf, A. & Kominoski, J. (2010) Functional leaf traits and biodiversity effects on litter decomposition in a stream: comment. *Ecology*, **91**, 1867–1869.

Lepori, F. & Malmqvist, B. (2009) Deterministic control on community assembly peaks at intermediate levels of disturbance. *Oikos*, **118**, 471–479.

Lepori, F., Palm, D. & Malmqvist, B. (2005a) Effects of stream restoration on ecosystem functioning: detritus retentiveness and decomposition. *Journal of Applied Ecology*, **42**, 228–238.

Lepori, F., Palm, D., Brännäs, E. & Malmqvist, B. (2005b) Does restoration of structural heterogeneity in streams enhance fish and macroinvertebrate diversity? *Ecological Applications*, **15**, 2060–2071.

Loreau, M. & Hector, A. (2001) Partitioning selection and complementarity in biodiversity experiments. *Nature*, **412**, 72–76.

Loreau, M., Naeem, S. & Inchausti, P. (2002) *Biodiversity and Ecosystem Functioning: Synthesis and Perspectives*. Oxford University Press.

Louhi, P.P., Mykrä, H.H., Paavola, R.R., Huusko, A.A., Vehanen, T.T., Mäki-Petäys, A.A. & Muotka, T.T. (2011) Twenty years of stream restoration in Finland: little response by benthic macroinvertebrate communities. *Ecological Applications*, **21**, 1950–1961.

Malmqvist, B. & Rundle, S. (2002) Threats to the running water ecosystems of the world. *Environmental Conservation*, **29**, 134–153.

Martinson, H., Schneider, K., Gilbert, J., Hines, J., Hambäck, P. & Fagan, W. (2008) Detritivory: stoichiometry of a neglected trophic level. *Ecological Research*, **23**, 487–491.

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

McKie, B.G. & Malmqvist, B. (2009) Assessing ecosystem functioning in streams affected by forest management: increased leaf decomposition occurs without changes to the composition of benthic assemblages. *Freshwater Biology*, **54**, 2086–2100.

McKie, B.G., Petrin, Z. & Malmqvist, B. (2006) Mitigation or disturbance? Effects of liming on macroinvertebrate assemblage structure and leaf-litter decomposition in the humic streams of northern Sweden. *Journal of Applied Ecology*, **43**, 780–791.

McKie, B.G., Schindler, M., Gessner, M.O. & Malmqvist, B. (2009) Placing biodiversity and ecosystem functioning in context: environmental perturbations and the effects of species richness in a stream field experiment. *Oecologia*, **160**, 757–770.

McKie, B.G., Woodward, G., Hladyz, S., Nistorescu, M., Presa Abos, C., Popescu, C., Giller, P.S. & Malmqvist, B. (2008) Ecosystem functioning in stream assemblages from different regions: contrasting responses to variation in detritivore richness, evenness and density. *Journal of Animal Ecology*, **77**, 495–504.

McNeely, C., Finlay, J.C. & Power, M.E. (2007) Grazer traits, competition, and carbon sources to a headwater-stream food web. *Ecology*, **88**, 391–401.

Melillo, J.M., Aber, J.D. & Muratore, J.F. (1982) Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology*, **63**, 621–626.

Millennium Ecosystem Assessment. (2005) *Ecosystems and Human Well-Being. Synthesis*. Island Press, Washington, DC.

Morin, X., Fahse, L., Scherer-Lorenzen, M. & Bugmann, H. (2011) Tree species richness promotes productivity in temperate forests through strong complementarity between species. *Ecology Letters*, **14**, 1211–1219.

Mouillot, D., Graham, N.A.J., Villéger, S., Mason, N.W.H. & Bellwood, D.R. (2013) A functional approach reveals community responses to disturbances. *Trends in Ecology & Evolution*, **28**, 167–177.

Muotka, T. & Laasonen, P. (2002) Ecosystem recovery in restored headwater streams: the role of enhanced leaf retention. *Journal of Applied Ecology*, **39**, 145–156.

Muotka, T., Paavola, R., Haapala, A., Novikmec, M. & Laasonen, P. (2002) Long-term recovery of stream habitat structure and benthic invertebrate communities from in-stream restoration. *Biological Conservation*, **105**, 243–253.

Negishi, J.N. & Richardson, J.S. (2003) Responses of organic matter and macroinvertebrates to placements of boulder clusters in a small stream of southwestern British Columbia, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, **60**, 247–258.

Nilsson, C., Lepori, F., Malmqvist, B., Törnlund, E., Hjerdt, N., Helfield, J.M., Palm, D., Östergren, J., Jansson, R., Brännäs, E. & Lundqvist, H. (2005) Forecasting environmental responses to restoration of rivers used as log floatways: an interdisciplinary challenge. *Ecosystems*, **8**, 779–800.

Oliveira, A.L.H. de & Nessimian, J.L. (2010) Spatial distribution and functional feeding groups of aquatic insect communities in Serra da Bocaina streams, southeastern Brazil. *Acta Limnologica Brasiliensia*, **22**, 424–441.

Otto, C. (1974) Growth and energetics in a larval population of Potamophylax cingulatus (Steph.) (Trichoptera) in a south Swedish stream. *Journal of Animal Ecology*, 339–361.

Palmer, M.A., Menninger, H.L. & Bernhardt, E.S. (2010) River restoration, habitat heterogeneity and biodiversity: a failure of theory or practice? *Freshwater Biology*, **55**, 205–222.

Pascoal, C., Cássio, F. & Marvanová, L. (2005) Anthropogenic stress may affect aquatic hyphomycete diversity more than leaf decomposition in a low-order stream. *Archiv für Hydrobiologie*, **162**, 481–496.

Paul, M.J. & Meyer, J.L. (2001) Streams in the urban landscape. *Annu. Rev. Ecol. Syst.*, 207–231.

Petchey, O.L. & Gaston, K.J. (2002) Functional diversity (FD), species richness and community composition. *Ecology Letters*, **5**, 402–411.

Petersen, R.C. & Cummins, K.W. (1974) Leaf processing in a woodland stream. *Freshwater Biology*, **4**, 343–368.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & R Core Team. (2012) nlme: Linear and Nonlinear Mixed Effects Models. *R package version 31-104*.

Podani, J. & Schmera, D. (2006) On dendrogram-based measures of functional diversity. *Oikos*, **115**, 179–185.

Polley, H.W., Wilsey, B.J. & Derner, J.D. (2003) Do species evenness and plant density influence the magnitude of selection and complementarity effects in annual plant species mixtures? *Ecology Letters*, **6**, 248–256.

R Core Team. (2012) R: A language and environment for statistical computing. *www.R-project.org*.

Ricotta, C. & Moretti, M. (2011) CWM and Rao's quadratic diversity: a unified framework for functional ecology. *Oecologia*, **167**, 181–188.

Rosseel, Y. (2012) lavaan: an R package for structural equation modeling. *Journal of Statistical Software*, **48**, 1–36.

Schindler, M.H. & Gessner, M.O. (2009) Functional leaf traits and biodiversity effects on litter decomposition in a stream. *Ecology*, **90**, 1641–1649.

Schleuter, D., Daufresne, M., Massol, F. & Argillier, C. (2010) A user's guide to functional diversity indices. *Ecological Monographs*, **80**, 469–484.

Schmidt-Kloiber, A. & Hering, D. (2011) The taxa and autecology database for freshwater organisms. URL http://www.freshwaterecology.info [accessed September 2011]

Spehn, E., Hector, A., Joshi, J., Scherer-Lorenzen, M., Schmid, B., Bazeley-White, E., Beierkuhnlein, C., Caldeira, M., Diemer, M., Dimitrakopoulos, P., Finn, J., Freitas, H., Giller, P.S., Good, J., Harris, P.M., Hogberg, P., Huss-Danell, K., Jumpponen, A., Koricheva, J., Leadley, P., Loreau, M., Minns, A., Mulder, C., O'Donovan, G., Otway, S., Palmborg, C., Pereira, J., Pfisterer, A.B., Prinz, A., Read, D., Schulze, E., Siamantziouras, A., Terry, A., Troumbis, A., Woodward, F., Yachi, S. & Lawton, J.H. (2005) Ecosystem effects of biodiversity manipulations in European grasslands. *Ecological Monographs*, **75**, 37–63.

Srivastava, D.S., Cardinale, B.J., Downing, A.L., Duffy, J.E., Jouseau, C., Sankaran, M. & Wright, J.P. (2009) Diversity has stronger top-down than bottom-up effects on decomposition. *Ecology*, **90**, 1073–1083.

Statzner, B., Doledec, S. & Hugueny, B. (2004) Biological trait composition of European stream invertebrate communities: assessing the effects of various trait filter types. *Ecography*, **27**, 470–488.

Sterner, R.W. & Elser, J.J. (2002) *Ecological Stoichiometry: the Biology of Elements From Molecules to the Biosphere*. Princeton University Press, USA.

Taylor, B.R., Parkinson, D. & Parsons, W.F.J. (1989) Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. *Ecology*, **70**, 97–104.

Tilman, D., Reich, P.B. & Isbell, F. (2012) Biodiversity impacts ecosystem productivity as much as resources, disturbance, or herbivory. *Proceedings of the National Academy of Sciences*, **109**, 10394–10397.

Tiunov, A.V. & Scheu, S. (2005) Facilitative interactions rather than resource partitioning drive diversity-functioning relationships in laboratory fungal communities. *Ecology Letters*, **8**, 618–625.

Turnbull, L.A., Levine, J.M., Loreau, M. & Hector, A. (2012) Coexistence, niches and biodiversity effects on ecosystem functioning. *Ecology Letters*, **16**, 116–127.

Vanni, M.J. (2002) Nutrient cycling by animals in freshwater ecosystems. *Annual Review of Ecology and Systematics*, **33**, 341–370.

Vitousek, P.M., Mooney, H.A., Lubchenco, J. & Melillo, J.M. (1997) Human domination of Earth's ecosystems. *Science*, **277**, 494–499.

Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R. & Davies, P.M. (2010)

Global threats to human water security and river biodiversity. *Nature*, **467**, 555–561.

Walker, B., Kinzig, A. & Langridge, J. (1999) Plant attribute diversity, resilience, and ecosystem function: the nature and significance of dominant and minor species. *Ecosystems*, **2**, 95–113.

Wardle, D.A. & Zackrisson, O. (2005) Effects of species and functional group loss on island ecosystem properties. *Nature*, **435**, 806–810.

Webster, J.R. & Benfield, E.F. (1986) Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology and Systematics*, **17**, 567–594.

Wissinger, S., Steinmetz, J., Alexander, J.S. & Brown, W. (2004) Larval cannibalism, time constraints, and adult fitness in caddisflies that inhabit temporary wetlands. *Oecologia*, **138**, 39–47.

Woodward, G., Gessner, M.O., Giller, P.S., Gulis, V., Hladyz, S., Lecerf, A., Malmqvist, B., McKie, B.G., Tiegs, S.D., Cariss, H., Dobson, M., Elosegi, A., Ferreira, M.T., Graça, M.A.S., Fleituch, T., Lacoursiere, J.O., Nistorescu, M., Pozo, J., Risnoveanu, G., Schindler, M., Vadineanu, A., Vought, L.B.M. & Chauvet, E. (2012) Continental-scale effects of nutrient pollution on stream ecosystem functioning. *Science*, **336**, 1438–1440.

Yachi, S. & Loreau, M. (1999) Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proceedings of the National Academy of Sciences*, **96**, 1463–1468.

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