Direct and indirect effects of nitrogen on ecosystem functioning

Inaugural dissertation
of the Faculty of Science
University of Bern

presented by

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from France

Supervisor of the doctoral thesis:
Prof. Dr. Eric Allan

Institute of Plant Sciences, University of Bern

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The Dean
Prof. Dr. Zoltan Balogh

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21st of January 2020

Promotion Committee

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Wageningen University and Research

Chair

Prof. Dr. Matthias Erb

Institute of Plant Sciences

University of Bern

‘How is it you can all talk so nicely?’ Alice said […]. ‘I’ve been in many gardens before, but none of the flowers could talk.’
[…]
‘In most gardens,’ the Tiger-lily said, ‘they make the beds too soft – so that the flowers are always asleep.’
This sounded a very good reason, and Alice was quite pleased to know it. ‘I never thought of that before!’ she said.

Through the looking glass - Lewis Carroll

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Chapter 1

General introduction

Nitrogen enrichment is one of the major ongoing global changes (Galloway et al. 2008; Vitousek et al. 1997). Due to the scale at which this change is occurring, it has been described as "the greatest single experiment in global geo-engineering that humans have ever made" (Sutton 2011). Nitrogen (N) represents 78% of earth atmosphere but in its gaseous di-nitrogen (N_2) form is unavailable for plants. Until the beginning of the 20th century, N in the plant-available form (NO3-, NH4+) was a rare resource that constrained food production. The Haber-Bosch process development at the beginning of 1900 and its wide application after the first world war changed the picture, by fixing atmospheric N_2 to produce plant-available ammonia (NH_3). From this moment on, food production, N inputs and human population kept increasing. In addition to direct N inputs, combustion processes (from industry and transport) further increased N enrichment by releasing gaseous N into the atmosphere, which comes back to the soil through deposition. For instance in Switzerland, where we conducted the experiment described in this thesis, atmospheric N deposition rate in 2010 could go up to ten times the deposition rate of low human-impacted ecosystems (Figure1, www.bafu.ch). The worldwide increase of N in ecosystems affects ecosystem functioning through multiple direct and indirect mechanisms.

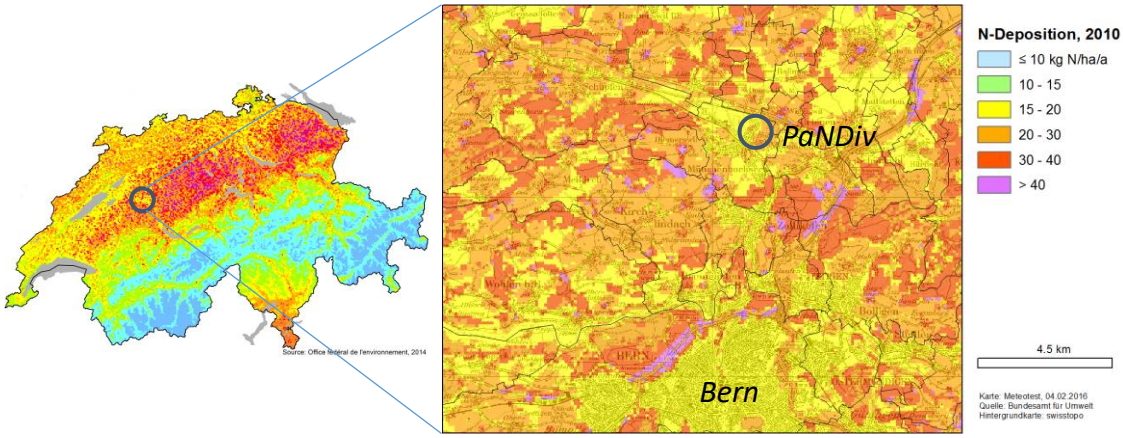


Figure 1: Atmospheric nitrogen deposition in kg ha^{-1} y^{-1} in Switzerland and near the city of Bern, where we conducted the experiment described in this thesis (the PaNDiv experiment). The deposition rate in the area is between 15 and 20 kg N ha^{-1} y^{-1}. Source: federal office for the environment (www.bafu.ch).

Direct effects

We define nitrogen enrichment direct effects as those affecting ecosystem functioning by changing soil abiotic conditions, and indirect effects as those affecting functioning through changes in the plant community, in the interactions with higher trophic levels such as plant pathogens, and in the soil fauna. These effects can be considered separately, although we have to keep in mind that they are closely linked and enhance each other through feedback processes. Nitrogen enrichment changes ecosystem functioning directly by altering biogeochemical conditions. By stimulating primary production, N can increase carbon (C) uptake and storage in soils (Vitousek et al. 1997). However, the potential for ecosystems to retain N is limited and leads to a decrease in N use efficiency, and ultimately to N leaching (Vitousek et al. 1997; Bobbink et al. 2010). Nitrogen changes ecosystems stoichiometry, increasing N:P ratios in plants and decreasing C:N ratios in plants and soil (Sardans et al. 2012). Micronutrients such as calcium and magnesium are more susceptible to be lost by leaching (Vitousek et al. 1997; Aber et al. 1995) and a decrease in soil pH under N enrichment can induce further nutrient imbalance (Tian and Niu 2015; Sardans et al. 2012). A stimulated biomass production under N enrichment also leads to higher biomass inputs in the soil, which together with higher cycling rates increases decomposition rates of plant material (Laliberté and Tylianakis 2012). However, the decomposition rate depends on N enrichment quantity and duration (Knorr et al. 2005), and a reduced C use efficiency (Sardans et al. 2012) or an inhibition of lignin degrading enzymes (Carreiro et al. 2000) can lead to an overall reduction of decomposition rates on the long term. These effects combined lead to an overall increase of nutrient cycling in the ecosystem which is also associated with nutrient losses, a decrease in nutrient use efficiency, and ultimately affects carbon storage (Laliberté and Tylianakis 2012; Bobbink et al. 2010). These changes affect ecosystem functioning, in parallel to changes occurring due to the effect of N enrichment on the biotic community.

Indirect effects

Within the plant community, N decreases species diversity (Suding et al. 2005; Stevens et al. 2004) and shifts community functional composition (Lavorel and Grigulis 2012; de Vries et al. 2012), and these two mechanisms will in turn affect ecosystem functioning. The biodiversity-ecosystem functioning relationship has been extensively studied in experimental and real-world ecosystems (van der Plas 2019). The use of functional traits to determine species and community composition effects on functioning, also known as trait-based ecology (Lavorel and Garnier 2002; Lavorel and Grigulis 2012), was studied separately from diversity effects until recent attempts to combine the two

approaches (Allan et al. 2015). Because species diversity loss and change in composition are closely linked under N enrichment, it is important to test their individual and combined effects on ecosystem functioning.

Diversity

Nitrogen enrichment reduces plant diversity by a variety of mechanisms. Due to higher biomass production, competition for light increases and the less competitive species are excluded (Hautier et al. 2009) and/or seedlings cannot gain access to light (Suding et al. 2005). Diversity can also decrease due to higher competition for soil resources (Rajaniemi et al. 2003), or following soil acidification (Crawley et al. 2005). N enrichment favours species adapted to resource-rich environments, which can decrease species richness and functional diversity (Stevens et al. 2004; Clark and Tilman 2008; Reich 2009). This decrease in diversity due to N enrichment has large effects on ecosystem functioning. The study of biodiversity-ecosystem functioning (BEF) relationships began more than twenty years ago with a shift in the way biodiversity was understood; not as an outcome of environmental conditions and ecosystem functions, but as a driver of ecosystem functions (van der Plas 2019). This led to a series of experimental setups with manipulated diversity levels (Tilman et al. 1996; Roscher et al. 2004) testing the effect of random diversity loss on functioning. Plant species diversity increases functions such as biomass production (Tilman et al. 2001; Hector et al. 1999; Cardinale et al. 2011) and litter decomposition (Cardinale et al. 2012) and can modify pathogen loads (Rottstock et al. 2014; Power and Mitchell 2004). The effects of diversity on function can be grouped under two main mechanisms: selection and complementarity effects (Loreau et al. 2001; Tilman et al. 1997). The selection effect relates to the probability of a community including species with a particular trait combination that drives the effect on function, and this probability increases with increasing diversity. The complementarity effect relies on niche differentiation between species (Tilman et al. 1997), and relates to complementarity in resource use leading to higher resource use efficiency with increasing diversity, hence higher functioning. Studies testing the direct evidence for resource complementarity are scarce and inconclusive (Barry et al. 2019; Oram et al. 2018; Bachmann et al. 2015; Kahmen et al. 2006). However, the quantification of these effects has been made possible by Loreau and Hector's (2001) additive partitioning, which provided a mathematical framework to partition diversity effects on function between selection and complementarity effects. In BEF experiments, biodiversity effects on productivity are mainly due to positive complementarity and negative selection effects (Cardinale et al. 2007). The first generation of BEF experiments tested diversity mechanisms, with reduced environmental variation. The next generation tested BEF relationships in different contexts (Loreau

2000), in combination with global change drivers such as N enrichment (e.g. Isbell et al. 2013; Reich and Hobbie 2013), drought (e.g. Vogel et al. 2013), or CO2 increase (e.g. Craven et al. 2016).

Further studies investigated how diversity influenced the ability of ecosystems to provide multiple functions (multifunctionality) (Hector and Bagchi 2007; Gamfeldt et al. 2008; Manning et al. 2018). The effect of diversity has been suggested to be even more important for the provision of multifunctionality than for individual functions (Hector and Bagchi 2007), and this is because different assemblages of species contribute to different functions, so a higher number of species are needed to maintain multiple functions at a high level. While there is no clear consensus on what functions to include or how to calculate multifunctionality, the idea is to assess high cycling rates, such as productivity and transfer to higher trophic levels, but also low nutrient losses, like C storage or nutrient use efficiency. It is now well established that diversity increases multifunctionality (Lefcheck et al. 2015). However, the assessment of multifunctionality depending on environmental conditions or community characteristics has been poorly studied (Giling et al. 2019), and we know little about how diversity-multifunctionality relationships change depending on the context in which they are measured. This context-dependency is due to interactions between diversity and other ecosystem properties. For instance, N enrichment reduces niche dimensions (Harpole et al. 2007), which reduces the complementarity and coexistence potential between species. Therefore, adding N could dampen the positive effect of diversity on functioning. If complementarity for different resources differs between functional groups, community functional composition could also modulate the effect of diversity. Due to the correlation of diversity and functional composition under N enrichment, this interaction has never been tested (Allan et al. 2015). Pathogen presence could similarly influence diversity and its effects on ecosystem functioning. Pathogens could help maintain diversity by decreasing competition between species (Chesson 2000; Mordecai 2011), due to higher infection of the dominant species. Due to these complex effects between diversity and environmental conditions, further studies have been established in order to test if the diversity-ecosystem functioning relationships are also present in the real world, where these effects occur in interaction.

At the end of the 2000's, research in BEF increased using non-experimental setups (van der Plas 2019) in order to assess whether BEF relationship hold in real-world systems, i.e. under a non-random loss of species, allowing community invasion after diversity loss, and including possible feedbacks from functions to biodiversity (see Grace et al. 2016 for instance). Consequently, these studies also took into account the shift in species composition associated with diversity loss under intensive land use (like in Fischer et al. 2010). The recent reviews and meta-analysis comparing experimental and real-world BEF research tend to show that diversity is a major driver of ecosystem functions in real-world setups as well, together with functional composition shifts (van der Plas 2019; Jochum et al. 2019). As

N enrichment affects not only the number of plant species but also the community functional composition, the biodiversity-ecosystem functioning relationships might differ depending on the functional traits of plant communities.

Functional composition

The field of trait-based ecology is linked with the idea that species influence on ecosystem functioning is determined by the characteristics of the dominant species in a community (mass ratio hypothesis, Grime 1998). This led to several protocols regarding which functional traits were characterising community composition, and how to measure them (Cornelissen et al. 2003; Garnier et al. 2001b). From the attempts to characterise the different growth strategies of plant species, a few major axes have been identified. Among them is the leaf economics spectrum (LES) (Wright et al. 2004). The LES distinguishes a gradient of growth strategies. On one side, "slow growing" species, with a conservative resource strategy, are adapted to low nutrient environments and produce dense, small, low N content leaves with a lot of fibres. These leaves have a relatively low photosynthetic rate and a long lifespan. On the other side of the LES, "fast" species with a nutrient acquisitive strategy, produce larger, high N and low fibre content leaves, and are more adapted to nutrient-rich environments. The photosynthetic rate is higher for fast species leaves than for slow growing ones, but their lifespan is shorter. The distinction between the two sides of the LES is based on this trade-off between high photosynthetic rate and leaf longevity (Wright et al. 2004). The LES has been identified as one of the main axes of resource-use strategies in plants worldwide (Díaz et al. 2015; Reich 2014).

The LES can be described using easily measured plant traits as proxies of growth strategies, like leaf N content, specific leaf area (SLA) and leaf dry matter content (LDMC). Fast growing communities tend to have a high SLA, high leaf N, and low LDMC, whereas slow growing species tend to have a low SLA, low leaf N, and high LDMC. On a large scale, these traits are correlated (Díaz et al. 2015). This correlation can be less clear at a smaller scale (Messier et al. 2017), with species having a larger range of possible trait combinations. The effect on ecosystem functioning can also be driven more strongly by one trait rather than another, depending on the function considered (Ibanez et al. 2013; Cornwell et al. 2008; Smart et al. 2017). It is therefore interesting to consider multiple traits in a community, also because functioning can also be indicated by other traits than the typical LES ones. For instance, litter decomposition has been found to be determined by plant micronutrient content like calcium and magnesium (Makkonen et al. 2012; García-Palacios et al. 2016a).

Since the identification and description of the LES, the use of functional traits to predict ecosystem functioning became more straightforward. Conservative strategies relate to low nutrient cycling rates (low biomass production, slow decomposition), whereas acquisitive strategies relate to fast cycling (high biomass production and decomposition rate) (Lavorel and Garnier 2002; Cornwell et al. 2008; de Vries et al. 2012; García-Palacios et al. 2016a; Lavorel and Grigulis 2012). This distinction between fast and slow communities also influences pathogen load, with fast growing species being more infected than slow ones because of a trade-off in the energy invested in growth versus defence (Blumenthal et al. 2009; Heckman et al. 2019). Pathogen could then modify the effect of functional composition on biomass production by attacking preferentially fast strategies than slow ones.

Nitrogen enrichment affects functional composition by shifting the plant communities composition from slow growing to fast growing strategies, which further increases nutrient cycling and ecosystem functioning in addition to N direct effects (Lavorel and Garnier 2002). It also reduces functional diversity, because it favours the growth of fast growing strategies only. The functional shift also affects soil community composition and their effect on functions (de Vries et al. 2012). Functional composition shifts following N enrichment occur through a change in species identity, but also a change in species relative abundance and a change in individual functional traits (Lepš et al. 2011), due to the increase in leaf N content and SLA under N enrichment (Siefert and Ritchie 2016). It is not well known how much the change in community composition might be due to interspecific or intraspecific variation in functional traits, and how intraspecific variation is linked to ecosystem functioning (Albert et al. 2011a; Roscher et al. 2018). The shifts in composition could then be independent of identity or the number of species if the shifts are mainly due to variation in abundance or in individual traits. Therefore, the changes in functional composition and in species diversity are linked but their relative importance in affecting ecosystem functioning is still poorly known, as their effects cannot be easily measured separately in natural communities.

Further indirect effects

Nitrogen enrichment can affect functioning through an effect on higher trophic levels, for instance by increasing plant pathogen infection. Plant pathogens have been poorly studied in natural communities, and the evidence of direct increase in pathogen infection under N enrichment comes from agricultural systems (Dordas 2008). However, pathogens can have as large an effect on functioning as herbivore insects (Allan et al. 2010). In addition, pathogen infection could have further effect on functioning depending on diversity and functional composition. The pathogen infection rate

is expected to increase in low diversity communities, because of denser host presence (host-concentration hypothesis, Mitchell et al. 2002; Rottstock et al. 2014). However, diverse communities could also have higher pathogen infection due to spillover effects (Power and Mitchell 2004). Communities comprised mostly of fast species are expected to be more infected than communities comprised mostly of slow species due to the trade-off between the energy invested in growth and in defence (Blumenthal et al. 2009; Heckman et al. 2019). Therefore, pathogens could modify the effect of diversity and composition on biomass by preferentially attacking the biomass of fast species (Jefferies and Maron 1997) or by reducing biomass production more in low diversity communities (Maron et al. 2011; Schnitzer et al. 2011), or in highly diverse communities (Seabloom et al. 2017).

Nitrogen enrichment affects soil communities. It shifts the relative abundance of microorganisms by decreasing the fungal/bacterial ratio, which tends to increase nutrient cycling rates (Bardgett and McAlister 1999; Bardgett and Wardle 2012; de Vries et al. 2006). Higher N concentrations might also decrease the abundance of microbial communities, especially under long-term enrichment (Treseder 2008; Liu and Greaver 2010). These effects have direct consequences for ecosystem functions such as decomposition (Milcu and Manning 2011), and soil respiration and C storage (Liu and Greaver 2010). N enrichment can also change plant functional traits like SLA or LDMC, which together with an increased N content and a reduced C:N ratio further affects functioning. As N enrichment changes both plant material composition, and soil biotic and abiotic conditions through a shift in plant community, diversity loss, and direct effects, a particularly interesting function to describe is litter decomposition, as it is affected by all the aforementioned mechanisms.

Disentangling direct and indirect effects

A few studies tried to estimate the relative importance of diversity loss and N enrichment and other global change drivers (Tilman et al. 2012; Hooper et al. 2012), but these studies took into account the total effect of N on functioning, and did not separate the indirect effects of N from the direct ones. Attempts to separate N direct effects from diversity loss (Isbell et al. 2013) and/or from community composition (Allan et al. 2015) have come from observational studies (but see Manning et al. 2006). These studies struggled to separate diversity effects from changes in composition due to the correlation of the two factors. They did however show that indirect effects of N can be as large as direct effects on multiple functions. These findings underline the need to consider these effects separately in an experimental setup, which could also test for potential interactions between them. This has never been possible before.

The direct and indirect effects of N enrichment on functioning can be summarised by the example of biomass production (Figure 2). Nitrogen directly increases biomass production due to higher resource availability (Laliberté and Tylianakis 2012), species diversity increases biomass production through higher complementarity or selection effects (Tilman et al. 1997), and fast community composition is linked to higher productivity strategies (Lavorel and Grigulis 2012). N further increases biomass production by shifting composition towards faster growing communities (Reich 2014), and decreases diversity due to multiple possible mechanisms (Reich 2009). Therefore, N has both positive and negative effects on biomass production. N increases the presence of fungal pathogens by increasing leaf palatability (Dordas 2008). These effects could also interact. For instance N could reduce the diversity effect by decreasing potential niches between species (Harpole and Tilman 2007), and functional composition could increase or decrease diversity effects if the complementarity between species differs between fast and slow strategies (Harpole and Tilman 2007; Harpole et al. 2016). In addition, there could be further interactions between composition and pathogens (Blumenthal et al. 2009; Jefferies and Maron 1997), or pathogens and diversity (Rottstock et al. 2014; Maron et al. 2011; Schnitzer et al. 2011). All of these mechanisms have been studied in isolation, but the strength and the relative importance of these mechanisms is not known. This led to the setup of the PaNDiv experiment, in order to disentangle direct and indirect effects of nitrogen on ecosystem functions.

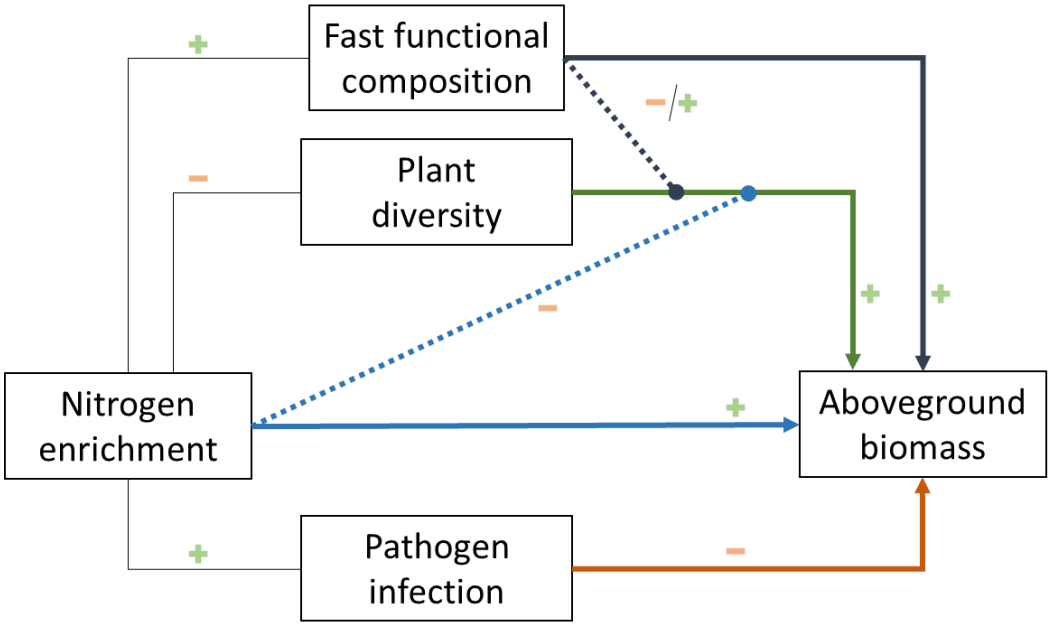


Figure 2: Theoretical framework of the PaNDiv experiment showing the direct and indirect effects of nitrogen enrichment on above ground biomass production. N directly increases biomass. It further increases biomass by shifting community composition towards fast growing species, and decreases biomass by reducing species diversity. These effects could potentially all interact. For instance, nitrogen and composition also affect diversity, which can have additional effects on the biomass production.

The PaNDiv Experiment

The PaNDiv experiment is located in Münchenbuchsee near the city of Bern (Switzerland, 47°03'N, 7°46'E, 564 m a.s.l., see Figure 1). It has a mean annual temperature of 9.2 ± 0.61°C and mean annual precipitation rate of 1051.78 ± 168.42 mm y^{-1} (mean over the last 30 years, data from the Federal Office of Meteorology and Climatology MeteoSwiss). The soil is characterized as "0.7 to 1 m deep brown soil" according to the Geoportal of the Canton Bern (http://www.geo.apps.be.ch). We measured nitrogen (N), carbon concentrations (C) and pH at the start of the experiment and found concentrations of 3.1-3.7% C and 0.34-0.40% N and a mean pH of 7.4. The field site was extensively managed (no fertilization) for at least 10 years, and was used for fodder production and grazing. The grassland was an Arrhenaterion, a moderately nutrient rich grassland type, typical of the Swiss lowlands. Due to dryness and most likely additional sowing from the farmer, there are also some characteristic plant species of a Mesobromion community. Occasional sheep grazing has led to some characteristics of a Polygono-Trisetion (classification based on Delarze 2015).

The species sown on the field were selected from a pool of 20 species commonly found in both extensively and intensively managed Central European grasslands. We divided our 20 species into 10 fast and 10 slow species according to Specific Leaf Area (SLA) and leaf N content (Table 1), which are traits related to plant growth strategy. The fast growing or acquisitive strategy is characterised by large, N rich leaves and high SLA. The slow, conservative strategy plants have smaller and denser leaves, long lasting, with a low N content and a low SLA (Wright et al. 2004). The fast growing pool therefore corresponds to species found in N enriched sites, whereas the slow growing pool comprises species found in less productive sites. We excluded legumes from the selection as few legume species will grow well at high N levels and including legumes in the slow growing pool would have caused an additional, large difference between the species pools.

In order to separate direct and indirect effects of N enrichment, we established a factorial cross of four treatments representing the direct (N enrichment) and three indirect effects (diversity loss, functional composition change and altered multitrophic interactions). Fertilised plots received N in the form of urea twice a year in April and late June (beginning of the growing season and following the first cut, see below), for a total amount of 100 kg N ha^{-1} y^{-1} which corresponds to intensive farming management (Blüthgen et al. 2012). To manipulate diversity, we established plots with 1, 4, 8 or all 20 species. To manipulate the functional composition we established plots with only fast growing, only slow growing or a mix of fast and slow growing species. This allowed us to realise a large gradient in community weighted mean trait values, similar to those occurring in naturally assembled community

along a land use intensity gradient (Breitschwerdt et al. 2018). In order to manipulate multitrophic interactions we removed fungal pathogens using fungicide. The plots were sprayed with fungicide ("Score Profi", 24.8 % Difenoconazol 250 $g.L^{-1}$) 4 times during the growing season (beginning of April and June, late July and September) and the same amount of water was applied to the untreated plots.

Table 1: The PaNDiv experiment species and corresponding growth strategy.

Grasses	**Leaf economics spectrum**	**Herbs**	**Leaf economics spectrum**
Poa trivialis *Lolium perenne* *Holcus lanatus* *Dactylis glomerata*	Fast	*Crepis biennis* *Taraxacum officinale* *Anthriscus sylvestris* *Heracleum sphondylium* *Galium album* *Rumex acetosa*	Fast
Helictotrichon pubescens *Festuca rubra* *Bromus erectus* *Anthoxanthum odoratum*	Slow	*Achillea millefolium* *Centaurea jacea* *Daucus carota* *Salvia pratensis* *Prunella grandiflora* *Plantago media*	Slow

Functional composition and diversity were completely crossed at the 4 and 8 species levels (monocultures and 20 species plots could only contain one functional composition). We sowed all plants in monoculture and we established 4 replicates of the 20 species together. At the four and eight species levels we randomly selected species compositions: we selected 10 species compositions for each combination of richness (4 and 8), times functional composition (fast, slow, mixed). This meant we had a total of 20 monocultures, 30 four species compositions, 30 eight species compositions and one 20 species composition (replicated four times, see Table 2). We constrained the random selection to ensure that all plots contained both grasses and herbs. The 84 compositions received the four combinations of N and fungicide treatments, giving a total of 336 plots in the experiment. The whole field was divided into four blocks. Each block contained all 84 compositions but the particular N x fungicide that a treatment received was randomly allocated per block.

Table 2: The numbers of species compositions and plots for different treatment combinations

Species richness	Functional composition (number of species compositions)	N x fungicide
Monocultures	10 fast	40 plots
	10 slow	40 plots
4 species	10 fast	40 plots
	10 slow	40 plots
	10 mixed	40 plots
8 species	10 fast	40 plots
	10 slow	40 plots
	10 mixed	40 plots
20 species	4 mixed	16 plots

All species within a plot were sown at equal density in October 2015, with proportions corrected by species specific germination rates, to obtain a total density of 1000 seedlings per m^2. The seeds were sourced from commercial suppliers (UFA Samen, Switzerland, and Rieger-Hofmann, Germany). Some species (*H. sphondylium, A. sylvestris, D. carota, S. pratensis, P. grandiflora, P. media*) were resown in summer 2016 because of their extremely low establishment rate, because they were mixed with other seeds to begin with (*H. pubescens, B. erectus*) or because their seedlings froze in autumn or spring (*H. lanatus, D. glomerata, A. odoratum*). In order to maintain the diversity levels the plots were weeded three times a year in April, July and September. This regime was highly successful and most plots contained very low weed covers (Figure 3). The whole experiment was mown twice a year in June and August which corresponds to intermediate to extensive grassland management.

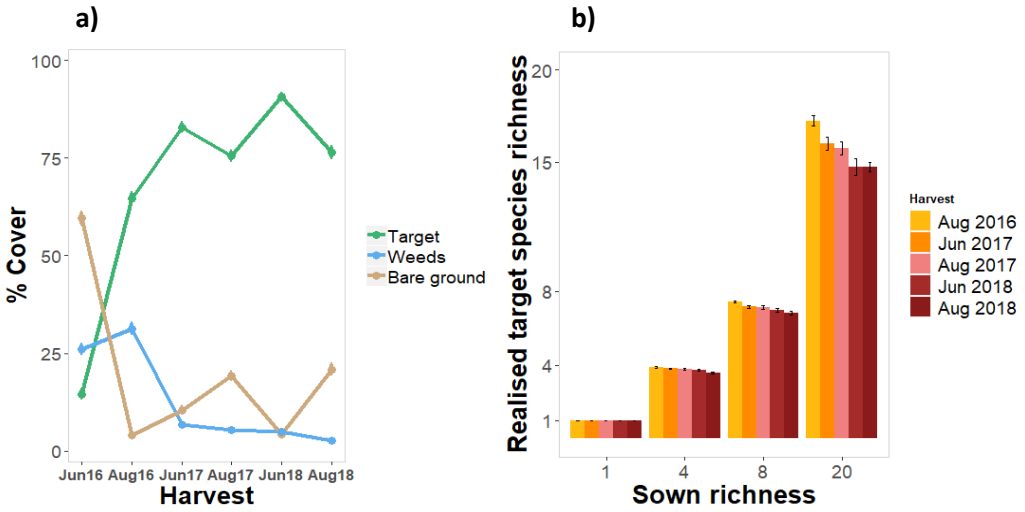


Figure 3: Establishment of the plots. a) Changes in average target (sown species), weeds (all species not sown into a plot) and bare ground cover over time. b) Realised diversity over time.

This thesis

This thesis aims to understand the mechanisms behind changes induced by N enrichment, species richness, functional composition shift and pathogen removal, in order to estimate the relative importance of these four factors in driving ecosystem functioning and at testing how they interact. We measured different ecosystem functions on the communities of the PaNDiv experiment and investigated how the manipulated factors influenced the capacity of ecosystems to supply multiple functions at a high level. We then focused on litter decomposition because it integrates direct and indirect effects of N on both above and belowground characteristics. We finally took apart the functional composition shift to understand how much of its effect on functioning was due to interspecific and to intraspecific changes under N enrichment.

In Chapter 2, we tested the direct and indirect effects of N on the ability of ecosystems to supply multiple functions. It is now well established that multifunctionality increases with diversity. However, the effect of other drivers, their relative importance and the potential interactions between them has been poorly studied. In particular, species diversity is difficult to separate from changes in the community composition, and their interaction has never been tested. The PaNDiv experiment provided an ideal framework to answer these questions. We measured a set of ten functions above and belowground on 216 plots and looked at the effect of species richness, functional diversity and composition, N enrichment, fungicide spraying and their interaction on individual functions and multifunctionality across multiple thresholds.

In Chapter 3, we focused on one particular function, litter decomposition. N enrichment can affect functioning through changes in the plant species community but also through the soil community. By looking at litter decomposition, we were able to quantify the overall effect of changes in the soil on litter decomposition and therefore explore these further indirect effects. We measured decomposition rates in 168 plots using litter bags with different mesh sizes and litter material. We measured leaf traits and nutrient and fibre content, and tested the relative importance of different aspects of functional composition, together with N enrichment and species richness on litter and soil mediated changes in decomposition rate.

In Chapter 4, we investigated how important intraspecific changes are in driving indirect effects on functioning. Functional composition shift can occur due to a change in species identity, a change in species relative abundance, or due to intraspecific variation. Intraspecific variation is often considered less important than relative abundance shifts, but only a few studies partitioned the different components of community composition to test their relative influence on functions. We measured

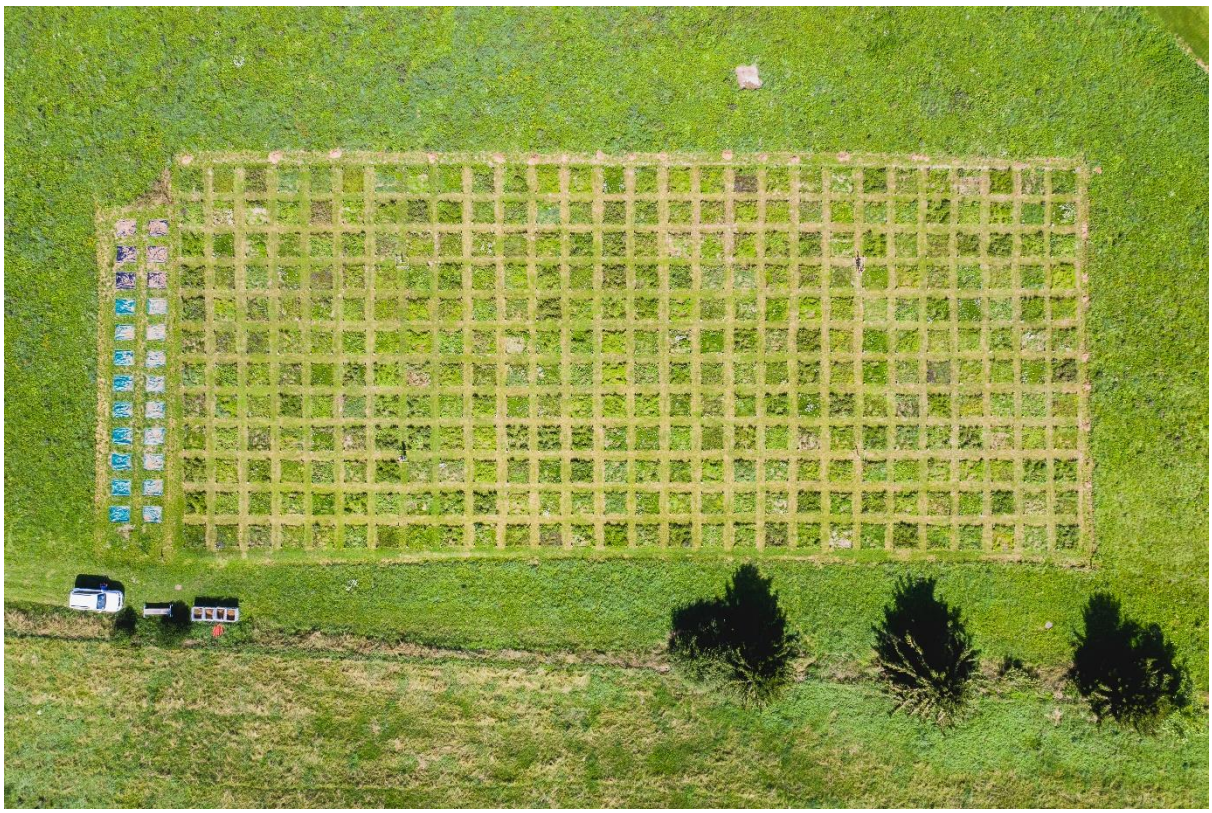
leaf traits in approximately 3500 individuals and tested community composition changes due to intraspecific or abundance shifts depending on N enrichment, fungicide spraying, species richness and sown community composition, and how their relative effect translated in above and belowground biomass production.

Finally, in Chapter 5, I summarise the most important findings of this thesis and draw general conclusions.

Chapter 2

Context-dependency in biodiversity-multifunctionality relationships is driven by nitrogen availability and plant functional composition

Noémie A. Pichon, Seraina L. Cappelli, Tosca Mannall, Thu Zar Nwe, Santiago Soliveres and Eric Allan



Aerial view of the PaNDiv Experiment, 2019
Credit: Hugo Vincent

Summary

The ability of an ecosystem to provide multiple functions simultaneously (multifunctionality) typically increases with biodiversity. There is, however, substantial variation in the strength and direction of biodiversity effects on multifunctionality, suggesting context-dependency. This context dependency is largely unexplored due to the lack of experimental studies manipulating diversity in different contexts. To understand how different factors modulate the effect of diversity on multifunctionality, we conducted a large grassland experiment with 216 communities, crossing a manipulation of plant species richness (1-20 species) with manipulations of resource levels (nitrogen enrichment), plant functional composition (gradient in sown specific leaf area [SLA] to manipulate abundances of fast vs. slow growing species), plant functional diversity (species differences in SLA) and enemy abundance (fungal pathogen removal). We measured ten functions above and belowground related to productivity, nutrient cycling and transfer of energy between trophic levels, and calculated multifunctionality at different thresholds. Plant species richness and functional diversity both had positive effects on multifunctionality, but their effects were context dependent. Multifunctionality increased with species richness, only in high SLA, fast growing communities, perhaps because fast growing species supplied more different functions than slow growing ones. Functional diversity increased multifunctionality but this effect was removed by nitrogen addition. The drivers of context dependency in diversity-multifunctionality relationships were generally different to those for individual functions, suggesting that different mechanisms and different types of complementarity are important for understanding diversity-multifunctionality relationships. The high degree of context dependency in, and multiple drivers of, diversity-multifunctionality relationships highlights the potentially complex effects of global change on multifunctionality and the need for multivariate approaches to understand these effects.

Introduction

The ability of an ecosystem to provide many functions simultaneously (multifunctionality) generally increases with diversity (Hector and Bagchi 2007; Gamfeldt et al. 2008; Manning et al. 2018; Lefcheck et al. 2015; Meyer et al. 2018; van der Plas 2019). Although positive biodiversity-ecosystem multifunctionality relationships are common, there is substantial variation in the direction and strength of this relationship among studies (Soliveres et al. 2016b; van der Plas 2019; Balvanera et al. 2006). The strength of biodiversity effects on individual functions have been shown to depend on environmental conditions, with relationships often stronger in less favourable environments (Ratcliffe et al. 2017), and on the presence of other trophic groups (Maron et al. 2011; Douglass et al. 2008). Few studies have addressed the drivers of context-dependency in biodiversity-multifunctionality relationships and these are either observational (Allan et al. 2015; van der Plas 2019) or focus on one of the multiple potential drivers (Giling et al. 2019 but see Alsterberg et al. 2014; Liu et al. 2017). It is therefore not clear how important different environmental or biotic drivers are in determining context-dependency in biodiversity-multifunctionality relationships and whether the drivers of context dependency differ from those affecting individual functions. We will focus on three key drivers: resource levels, pathogen pressure and community composition.

Two of the major mechanisms behind positive effects of species diversity on ecosystem functioning are likely to be complementary resource use amongst species in diverse communities and increased enemy attack in low diversity communities (Barry et al. 2018; Tilman et al. 2012). If species vary in their resource uptake, then they should use resources more efficiently together (Tilman 1982), and increasing resource levels would disrupt this complementarity (Ratcliffe et al. 2017). We can therefore expect a decrease in the effect of diversity on ecosystem functioning with nitrogen (N) enrichment. However, experimental studies have shown neutral (Hooper et al. 2012; Wacker et al. 2009) and positive effects of increased resource levels on the diversity-functioning relationship (Weigelt et al. 2009; Craven et al. 2016; Eisenhauer et al. 2018). These contrasting effects of resource addition on diversity-functioning relationships could arise because of variation in other environmental or biotic factors, such as the presence of enemies or plant functional composition. For example, soil pathogens can drive biodiversity-productivity relationships by reducing biomass production in monocultures (Maron et al. 2011; Schnitzer et al. 2011). However, Seabloom et al. (2017) showed that foliar fungal pathogens can dampen biodiversity-productivity relationships by removing more biomass at high diversity, meaning there is little consensus on the role of plant enemies in affecting diversity-

functioning relationships. Further, the role of pathogens and nitrogen in individually and jointly modifying biodiversity-multifunctionality relationships is completely unknown.

In addition to resources and enemies, the composition of the species pool could also modify biodiversity-multifunctionality relationships. Species with different resource use strategies might vary in their degree of resource use complementarity. The leaf economics spectrum (Wright et al. 2004; Díaz et al. 2015) distinguishes slow growing, conservative species from fast growing, acquisitive ones and is indicated by several traits, such as specific leaf area (SLA). Slow growing species, with low SLA, are more competitive in low nutrient environments (Reich 2014; Tilman and Wedin 1991) and might therefore be expected to differ more in their competitive abilities for different nutrients. Faster growing species, with high SLA, are abundant in high resource environments, where asymmetric light competition is prevalent (Hautier et al. 2009), and complementarity between fast growing species might therefore be lower. However, fast growing species are also likely to be more sensitive to both resource addition and enemies, due to a growth defence trade-off (Endara and Coley 2011), and enemies might therefore drive stronger diversity-functioning relationships in fast dominated species pools. Functional diversity, in turn, can also affect multifunctionality (van der Plas 2019; Gross et al. 2017), both positively, if species with different resource use strategies (fast vs slow) have complementary interactions (Handa et al. 2014; Cadotte et al. 2011), or negatively, in harsh environments where adaptation to local conditions is important (Le Bagousse-Pinguet et al. 2019). Overall changes in functional composition and diversity have large effects on multifunctionality (Allan et al. 2015; Ratcliffe et al. 2017) and on individual functions (Díaz et al. 2007; Lavorel et al. 2011; Le Bagousse-Pinguet et al. 2019). However, changes in diversity, and in functional traits linked to resource-use strategy, are typically correlated in observational studies and have never been manipulated independently in experiments. No studies have, therefore, tested for an interaction between changes in functional traits and diversity in affecting multifunctionality.

To understand how different factors modulate the effect of diversity on multifunctionality, we manipulated plant species richness, N enrichment, fungal pathogen exclusion and plant functional composition (as a gradient of specific leaf area [SLA]) in a full-factorial experiment with 216 plots. We measured ten functions on each plot, related to biotic interactions and biogeochemical cycling: above and belowground biomass, herbivory damage and foliar fungal pathogen infection, plant nitrogen and phosphorus uptake (plant/soil nitrogen and phosphorus ratios), two enzymatic activities related to carbon (β-glucosidase) and phosphorus (phosphatase) cycling, soil respiration and soil carbon storage. Each plot was characterised by its species richness, community weighted mean SLA and by its functional diversity (mean pairwise distance of SLA). We calculated equal weight ecosystem function multifunctionality (Manning et al. 2018) at multiple thresholds (from 50% to 80%). We then

constructed models including each multifunctionality measure, with threshold as a fixed continuous term which could interact with the treatment variables, for more details see methods. The combined model allowed us to determine which treatments and interactions had consistent effects across thresholds. Changes in the identity (Allan et al. 2015) and number of functions (Meyer et al. 2018) included in the multifunctionality index can also alter biodiversity-multifunctionality relationships. We therefore tested how the effects of our treatments changed when we varied the number and identity of functions used to calculate multifunctionality. We hypothesised that: (1) species richness and functional diversity both have an effect on multifunctionality (2) resource level and fungal presence will alter the diversity effect on multifunctionality, and (3) the number and identity of functions considered will affect these results.

Results

Diversity effects on multifunctionality were highly context-dependent in our experiment. Nitrogen enrichment and community functional composition both had an effect on multifunctionality, in interaction with functional diversity and species richness respectively. These interactions were significant across all tested thresholds, while multifunctionality decreased consistently with an increasing threshold.

Richness-multifunctionality relationships depend on community functional composition

The effect of species richness on multifunctionality was modified by plant functional composition (community weighted mean SLA). Species richness (SR) decreased multifunctionality slightly in species pools with low mean SLA but increased multifunctionality at high SLA (Figure 1a and b and Table S1). Species richness therefore had a positive effect on multifunctionality in fast growing communities, but a neutral to negative effect in the slowest growing ones. This effect was significant across thresholds. Analysing each threshold individually suggested that the SLA x richness interaction was most important at intermediate thresholds, rather than at high ones (Table S2 and Table S3). We also analysed each function individually and found that the SLA x SR interaction was significant for only two functions, phosphatase and N ratio, but the interaction was negative for phosphatase and present

only in N plots for N ratio (Figure 2), meaning that these functions did not drive the overall SLA x SR effect on multifunctionality. SLA itself had contrasting effects on individual functions, with a positive effect on aboveground measured functions (pathogen presence, herbivory, N and P ratios), and a negative effect belowground (belowground biomass, phosphatase activity, soil respiration).

High resource levels dampen effects of functional diversity on multifunctionality

Functional diversity, calculated as the mean pairwise distance between species in their SLA, increased multifunctionality in control plots (Figure 1a and c). However, nitrogen enrichment dampened this positive effect of functional diversity by increasing multifunctionality most in the low functional diversity plots. Nitrogen enrichment increased several individual functions (above and belowground biomass, β-glucosidase, P ratio) but the interaction with functional diversity was not as clear as for multifunctionality and was significant only for soil respiration (Figure 2). Interestingly, N also interacted with species richness to affect N ratio, belowground biomass and C storage (Figure 2), but this interaction depended on SLA and fungicide and did not affect multifunctionality (Table S2 and Table S3).

Fungicide reduced overall fungal damage and herbivory in species rich communities, but increased total soil respiration in the high SLA communities and belowground biomass in the species rich fertilised communities. Fungicide was also involved in some other complex interactions for other C storage and phosphatase. These contrasting effects translated into a non-significant effect on multifunctionality (Figure 2).

Effects of abundance changes on MPD

The sown functional diversity of the plots (MPD, equally weighted per species) increased multifunctionality. This indicates that communities with species that differ in growth strategies maintain more functions at a high level than those with species that are similar in their growth strategies. However, species have shifted their relative abundances since the start of the experiment and to test the effect of these abundance changes on functional diversity, we calculated MPDw, weighted by species abundance. As weighted and unweighted MPD were highly correlated overall, we fitted a second model in which we replaced MPD by MPDw. Abundance weighted functional diversity

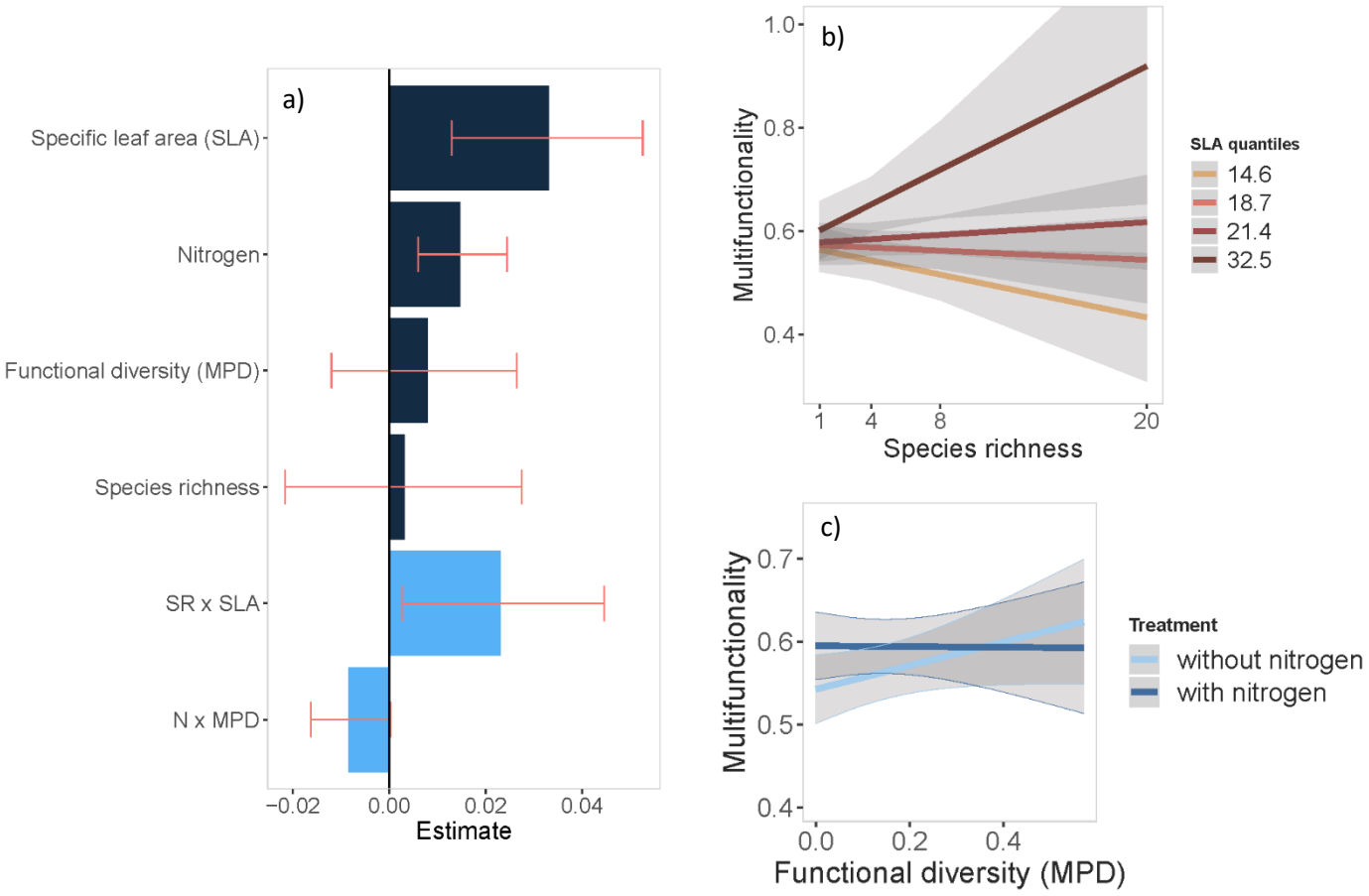


Figure 1: Effect (+/- confidence intervals) of each variable and their interaction on multifunctionality across thresholds: a) scaled centred estimates, b) interaction between species richness and specific leaf area, and c) interaction between functional diversity and nitrogen enrichment. Multifunctionality was calculated for 216 plots across 7 thresholds. Details of the model output can be found in the Table S1.

had an opposite effect and decreased multifunctionality (Figure 3). These contradictory effects indicate that the communities with the highest multifunctionality were those sown with functionally distinct species (higher unweighted MPD), but in which functional redundancy increased (lower abundance weighted MPDw). Such high multifunctionality communities are therefore dominated by functionally similar species but contain functionally distinct rare species.

Effect of number and identity of functions

In order to test how changes in the number and identity of functions altered the multifunctionality index, we calculated multifunctionality with all possible combinations of functions and for four thresholds (0.5, 0.6, 0.7 and 0.8). For each of 4052 multifunctionality measures we extracted the effect of each treatment and interaction and then calculated the mean effect and confidence interval each effect and number of functions. The number of functions included did not significantly change the mean effect of our variables on multifunctionality, except for the interaction between SLA and SR, which increased the more functions were considered, although only at the 50% threshold. The variation around the mean got larger with a decreasing number of functions, going from positive to negative depending on the functions included (Figure S1 and Figure S2). However, this variation was driven more by variation in the identity rather than the number of functions included (Figure S2).

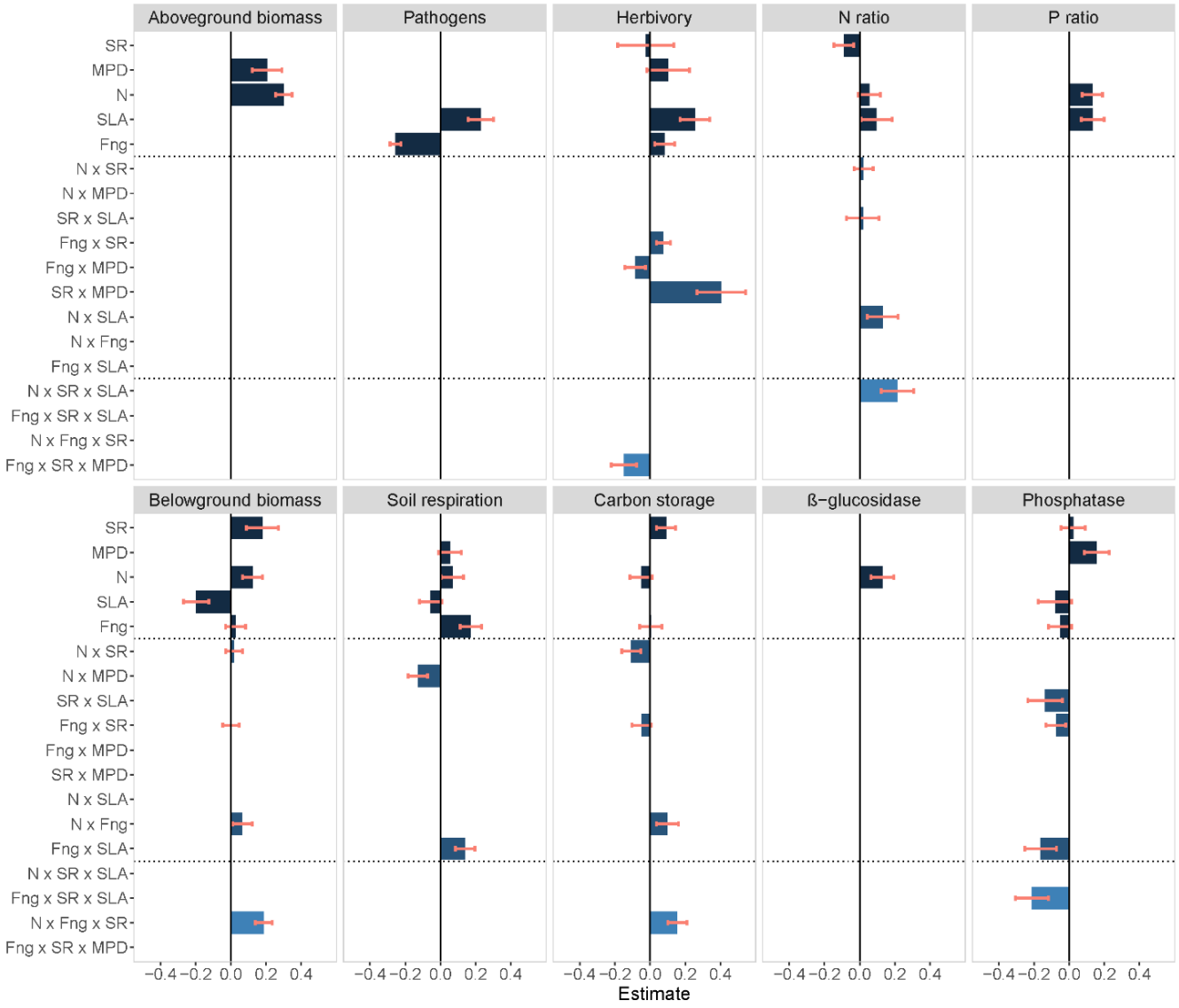


Figure 2: Mean effect (+/- standard errors) of each variable and their interaction on functions after model simplification. SR: plant species richness, MPD: mean pairwise distance in SLA equally weighted, N: nitrogen enrichment, SLA: abundance weighted community specific leaf area, Fng: fungicide treatment. Each function was measured on 216 plots. See Table S4 for function transformation detail.

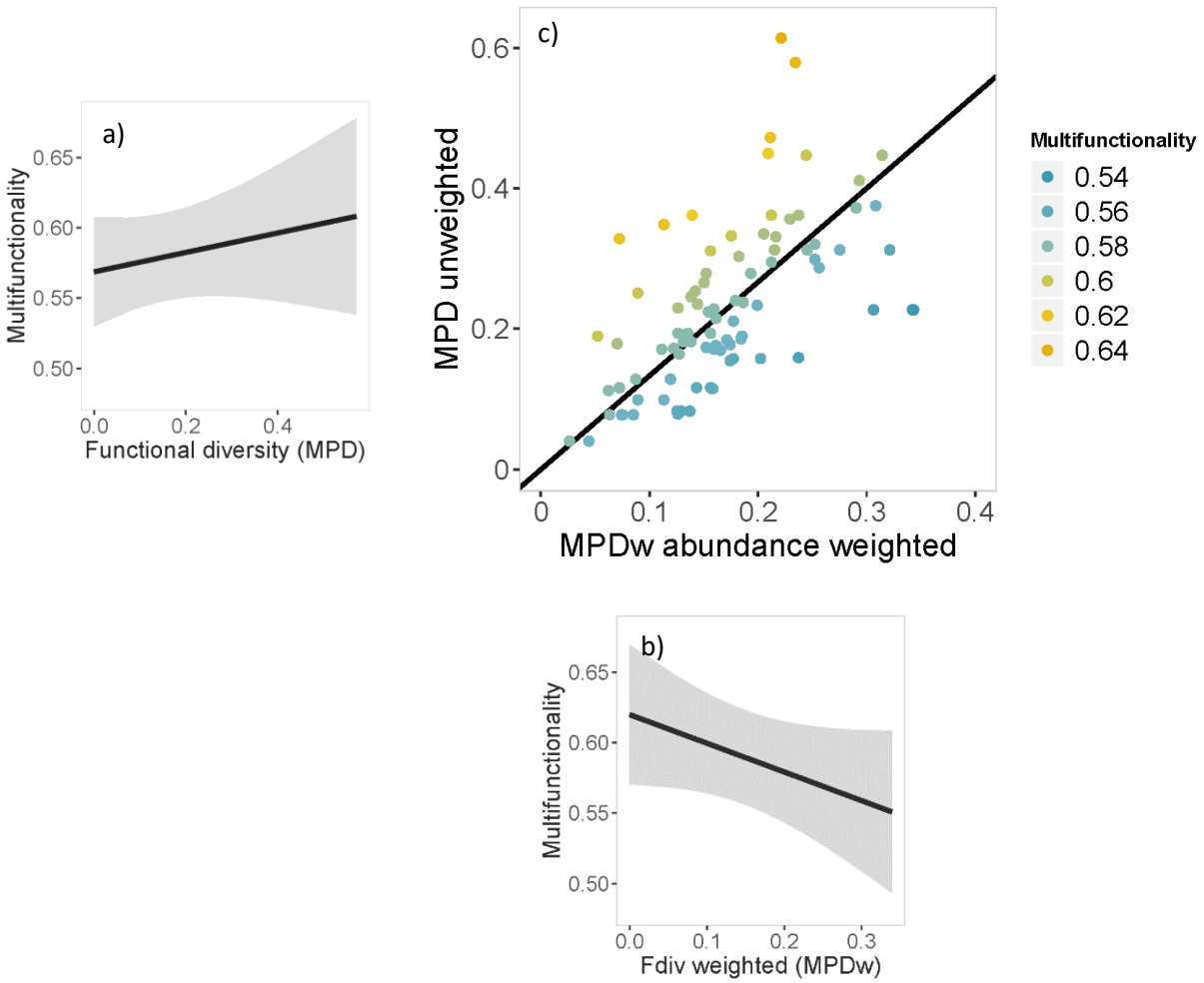


Figure 3: Effect of weighting functional diversity (MPD) by species relative abundance (MPDw). Effect (+/- confidence intervals) from individual models: (a) MPD increases multifunctionality, (b) MPDw decreases multifunctionality. The high multifunctionality plots are those with both a relatively high sown MPD and a relatively low MPDw: data points are coloured according to the model predicted multifunctionality, at a 0.65 threshold (c), monocultures are not shown as they are all coded as 0. See Table **S5** for details on the model output.

Discussion

Our results show that there is substantial context dependency in biodiversity-multifunctionality relationships and that this is explained mainly by the functional composition of the species pool and by resource levels. We found that functional composition was the strongest moderator of the biodiversity-multifunctionality relationship. Previous diversity experiments have generally isolated diversity effects from compositional variation, and when experimental (Hector et al. 2011) or observational (e.g. Allan et al. 2015) studies have considered compositional and diversity effects they have compared their relative importance but have never tested whether they might interact. In general, biodiversity experiments have focused on diversity as a driver of functioning (e.g. Cardinale et al. 2012), while many observational studies have considered mean trait values and mass ratio effects (e.g. Garnier et al. 2004; Lavorel and Grigulis 2012). By experimentally crossing functional composition and diversity treatments, we were able to show that composition and diversity are not independent drivers of multifunctionality but rather enhance each other's effects. Our results support recent observational evidence (Le Bagousse-Pinguet et al. 2019) that multiple biodiversity dimensions (including species richness, functional diversity and functional redundancy) need to be considered together and emphasise that a better understanding of multifunctionality requires approaches that can disentangle the influence of multiple, interacting drivers.

Community composition modulates richness effects

Species richness increased multifunctionality, but only in fast growing plant communities. Our results, therefore, suggest a higher complementarity between fast growing species than between slow ones. This shows a novel mechanism modulating biodiversity-functioning relationships. Previously, some studies have considered how the functional diversity of the species pool impacts richness-functioning relationships (Ratcliffe et al. 2017; Wagg et al. 2017) but our results show that the functional composition of the species pool may be an even larger context driver. The finding that high diversity of fast but not slow species promotes multifunctionality, is, however, surprising as complementarity would be expected to be higher in resource limited environments due to greater variety of nutrient niches (Harpole and Tilman 2007; Harpole et al. 2016). Therefore niche partitioning might be expected to be greater between slow than fast species. In line with this, we do find that complementarity effects on biomass are lower in communities with high SLA species (Cappelli et al. unpublished). A positive

SLA x richness effect could instead be explained if individual fast species supply different functions whereas slower species are more redundant in the functions they supply. In particular, fast growing species supplied several aboveground functions at high levels, while belowground functions tended to decrease with high community mean SLA (Table S6). This SLA effect is consistent with the negative response of soil functions to fast growing tall species (Fry et al. 2018). Diverse, acquisitive communities would therefore supply multiple different functions whereas diverse, conservative ones would all supply the same set of functions. This idea is supported by the fact that a similar diversity x composition interaction was only seen for one function (N ratio, and then only on N addition plots), instead species richness increased belowground biomass and carbon storage regardless of the community SLA, and increased phosphatase activity most in plots dominated by slow growing species. Diversity effects did therefore increase several individual functions, but not multifunctionality, within slow species pools. In addition, the species richness x SLA interaction was mostly significant at intermediate thresholds (<0.55) but not at higher ones (Table S2 and Table S3). This pattern is similar to the jack-of-all trades effect on multifunctionality (van der Plas et al. 2016), and suggests that a diversity of fast growing species supplies several functions but only at intermediate levels. While species richness and composition have usually been seen as alternative or opposing drivers of functioning (i.e. mass ratio vs. diversity effects), we show here that they are dependent and enhance each other's effects.

Resource level, but not fungal presence, modulates functional diversity effects on ecosystem functioning

In addition to species richness, functional diversity was an important driver of multifunctionality but its positive effect was dampened by N enrichment. N enrichment increased functioning in low diversity communities, but not in high diversity ones. However, high diversity, unfertilised communities still provided slightly higher multifunctionality than low diversity, fertilised communities, suggesting management for diversity could still deliver multiple benefits relative to intensive systems. Previous studies have found mixed effects of increased resource supply on diversity-functioning relationships. One study in the Jena Experiment showed that the strength of the diversity-productivity relationship increased under nutrient enrichment (Weigelt et al. 2009). However, the Jena Experiment added multiple nutrients, which increased total resource levels, whereas we added nitrogen alone which may have led to unbalanced nutrient ratios and therefore a reduction in complementarity (Cardinale et al. 2009). In a recent meta-analysis Craven et al. (2016), found that the effect of diversity on productivity

was independent of nutrient levels, although complementarity was reduced in fertilised communities due to higher productivity in monocultures. Our study also found that the effect of diversity on aboveground productivity was independent of fertilisation (Figure 2), and the interaction between N and either species richness or functional diversity was not consistent across individual functions. However, we do find a clear effect of N enrichment on the diversity-multifunctionality relationship. This effect could be due to trade-offs between functions increased under N enrichment and those increased in high diversity communities. Therefore, adding N in high MPD plots would not further increase multifunctionality. This result is partially supported by a recent study by Eisenhauer et al. (2018), who found a consistent positive effect of diversity on multifunctionality under N enrichment, but showed that the diversity effect was weaker at higher thresholds under N enrichment. Our findings underline the importance of considering multiple functions simultaneously and further demonstrate that the drivers of context dependency in diversity-multifunctionality relationships differ from those for individual functions.

Foliar fungal pathogen removal had no significant effect on multifunctionality or on diversity-multifunctionality relationships. It reduced the diversity effect for two individual functions: herbivory and phosphatase activity. This indicates that although it modulated the diversity effect for individual functions, pathogen presence was less important than N enrichment in modulating diversity-multifunctionality relationships. Previous studies have shown that specialist pathogens can drive diversity-productivity relationships by reducing biomass particularly at low diversity (Maron et al. 2011; Schnitzer et al. 2011). However, in our experiment foliar pathogen impact was not reduced in high diversity communities, perhaps due to a shift towards generalist pathogens or more spillovers in diverse mixtures, counteracting resource concentration effects (Power and Mitchell 2004). We also find evidence that the fungicide application may alter pathogen community composition, perhaps shifting the pathogen community towards more generalist fungi (Cappelli et al. 2019). The absence of an effect of foliar fungal exclusion on biodiversity-multifunctionality relationships suggests resources are more important context drivers than enemies but changes in other enemy groups or in foliar fungal species composition need to be investigated further.

Functionally rare species and redundant communities are key to maintain high levels of ecosystem multifunctionality

Multifunctionality was maximised at high levels of sown functional diversity. However, this effect was not related to a more even distribution of traits in the community but rather to the presence of species

with distinct traits (Figure 3), as multifunctionality increased with sown functional diversity (non-abundance weighted MPD) but decreased with high abundance weighted MPD. Multifunctionality was therefore highest when communities were dominated by functionally similar species but included some distinct rare species. The positive effects of non-abundance weighted functional diversity emphasize the importance of species identity effects on multifunctionality (McLaren and Turkington 2010). These findings are also in accordance with a body of literature on the importance of locally rare species for the provision of multiple ecosystem functions (Soliveres et al. 2016a; Lyons et al. 2005; Mouillot et al. 2013). The negative effects of functional evenness on multifunctionality indicate that mass ratio effects were simultaneously important for multifunctionality. Evenness has been shown to reduce multifunctionality in biocrust species rich systems (Maestre et al. 2012), and a recent study showed the importance of functional redundancy for multifunctionality in dryland ecosystems (Le Bagousse-Pinguet et al. 2019). In our experiment, an increase in functional evenness could reduce the levels of certain functions because more even communities contained a higher proportion of low functioning species, for instance a higher proportion of low SLA species would reduce functions related to fast biogeochemical cycling (herbivory, pathogen infection, N and P ratio, see Figure 2). On the other hand, the presence of low SLA species led to an increase in belowground biomass (Figure 2) and several other belowground functions (Table S6), perhaps indicating increased microbial activity and diversity (Delgado-Baquerizo et al. 2018). Addition of a few rarer slow growing species could therefore have increased levels of certain soil functions and boosted multifunctionality in fast dominated communities. In the PaNDiv experiment, the highly functioning communities contained a large proportion of species linked to acquisitive strategies, as well as a few functionally distinct species supplying a complementary set of functions, further highlighting the complex interplay between diversity and mass ratio effects in driving multifunctionality.

Conclusions

Our results shed light on the context dependence of diversity effects on multifunctionality. The most important driver of context dependency was the functional composition of the species pool, a novel context driver not considered in previous biodiversity-functioning experiments. Our findings show that shifts in functional composition due to global change will not just alter ecosystem functioning themselves but will also alter the strength of diversity-functioning relationships. The functional

impacts of diversity loss will therefore depend on the initial functional composition of the ecosystem. This could result in complex interactions between global change drivers, for instance, if some drivers alter functional composition and others subsequently change diversity. We also found that the drivers of context dependency in multifunctionality were different from the drivers of context dependency in diversity effects on individual functions. This highlights the different mechanisms driving effects of species diversity on individual functions vs. on multifunctionality. Managing for multifunctionality may therefore require different diversity dimension to be maximised and our results suggest that several need to be considered. In our study and for the considered functions, the optimal community for multifunctionality was one with several different fast growing species at high abundance and some functionally distinct slow species at low abundance. Overall, these findings underline the need to consider multiple diversity dimensions and interactions between them and abiotic conditions to optimise multifunctionality in the context of multiple simultaneous global changes.

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Material and methods

The PaNDiv Experiment

We set up a field experiment in October 2015 in Münchenbuchsee (Switzerland) with factorial manipulations of plant species richness, plant functional composition, N enrichment and foliar fungal pathogens. The area was cleared from vegetation and ploughed before the establishment of experimental plots. The whole experiment consisted of 216 plots of 2x2m separated from one another by a 1m path and arranged in four blocks. Taking into account the germination success of each species, we sowed the different communities to obtain a density of 1000 seedlings per m^2, equally divided between the species. The species pool consisted of 20 common herbs and grasses (see list of species Table S7). We divided the species into 10 fast and 10 slow growing strategies according to their leaf N content and their specific leaf area (SLA), which are indicators of the leaf economics spectrum (Wright et al. 2004). We excluded legumes from the species pool as they are expected to decline with N enrichment and including them in the slow growing pool only would add an additional difference between the pools.

All 20 monocultures were established, crossed with N and fungicide application (80 plots), together with 60 four species, 60 eight species and 16 twenty species plots. Plots with four and eight species could contain only fast or only slow growing species, or a mix of strategies. This allowed us to obtain a large gradient in functional composition and functional diversity, independent of species richness. Species compositions for each plot were randomly selected from their respective species pool, with the constraint that all plots (except monocultures) had to contain both grasses and herbs. Each particular species composition received the four combinations of N and fungicide. Fertilised plots received N in the form of urea twice a year in April and late June, for an annual addition of 100 kg N $ha^{-1}y^{-1}$, which corresponds to intermediately intensive grassland management (Blüthgen et al. 2012). In order to manipulate multitrophic interactions we removed foliar fungal pathogens using fungicide. The plots were sprayed with fungicide ("Score Profi", 24.8 % Difenoconazol 250 $g.L^{-1}$ and "Ortiva", 32.8% Chlorothalonil 400 $g.L^{-1}$ 6.56% Azoxystrobin 80 $g.L^{-1}$) four times during the growing season (beginning of April and June, late July and September) and the same amount of water was applied to the untreated plots. The experiment was weeded three times a year to maintain the diversity levels. All plots were mown twice a year, in mid-June and mid-August, which corresponds to intermediate to extensive grassland management.

Further details about the site characteristics and experimental setup of the PaNDiv Experiment can be found in Pichon et al. (2019), the third chapter of this thesis.

Basic measurements on the plant community

Plant traits

We measured specific leaf area (SLA = surface/dry weight, in $m^2 g^{-1}$) in June 2018 and in August 2017 and 18, taking one leaf from each of five individuals in all the monocultures. We rehydrated the leaves and measured leaf surface, and dry weight according to the protocol of Garnier et al. 2001b. The SLA per species is the average of the trait values across the five samples.

Cover

We visually estimated bare ground percentage and plant cover of each species (target species and all weeds together) before mowing. We calculated community weighted mean (CWM) SLA per plot using the cover estimates: CWM = $\sum p_i * x_i$; with p_i the relative abundance of the species i and x_i the trait value of i in each monoculture of the same treatment (nitrogen and fungicide added or not).

Functional diversity (MPD)

As a measure of functional diversity, we calculated a community mean pairwise distance, accounting for the variation in SLA between the present species (de Bello et al. 2016). The SLA values used were the control monoculture traits, and all monocultures had an MPD value of 0 by definition. We used only SLA to calculate MPD as our experimental design involved manipulating the mean and variation in SLA within communities (species were defined as fast and slow based on SLA).

To assess the effect of species relative abundance on MPD, we calculated an abundance weighted index, MPDw. The two indices were highly correlated, partly due to coding the monocultures as 0. We therefore analysed the effect of MPD and MPDw in two separate models (Table S5).

Set of functions

The functions described in this study were measured between June 2017 and autumn 2018. We excluded June 2016 aboveground measurements as the experiment had just started to establish and

the weeds and bare ground cover were still large (see Pichon et al. 2019). For each function measured more than once, we used the mean value per plot over the whole period (see Table S4 and Figure S3).

Aboveground biomass

We collected aboveground biomass twice a year, each year before the mowing. The samples were taken in two quadrats of 20x50 cm in the centre of each plot by clipping plant material above 5cm. We weighed the biomass after 2 days of drying at 65°C. The plot biomass production was then corrected by removing the percentage cover of weeds from the total weight. To control if the estimation was accurate, we sorted the biomass per species in June 2017 for all plots of one block. The correlation coefficient was 0.997 between the weight of sorted biomass without the weeds and the estimated target biomass, corrected by the weed percentage cover (R^2 = 0.994, data not shown). The mean weed percentage was around five percent (Pichon et al. 2019).

Herbivory

Herbivory was assessed in May and August 2018. Five individuals of each target species were randomly selected from each plot and five leaves per individual were assessed for damage. Leaves were selected from the middle tier of each individual, and juvenile and senescent leaves were excluded. Damage was characterised by the type of herbivory; chewing, sucking, leaf mining and rasping damage based on the methods of Loranger et al. 2014. In this study, we calculated the community weighted mean total damage using the sum of damage types per leaf, per species, weighted by the cover of the particular species.

Pathogens

Fungal infection was measured per species per plot once in October 2017 and twice in 2018, in July and October. Ten randomly chosen individuals in the central square meter of the plots were screened for signs of infection and the percentage of infected individuals was recorded. If there were less than 10 individuals in the central square meter, individuals in the rest of the plot were screened. If there were fewer individuals, the percentage of infected individuals was calculated based on all individuals present. The species level infection and the percentage cover data were used to calculate a community weighted mean of fungal infection per plot and season.

Plant/Soil N and P ratio

We included two ecosystem functions representing the potential N and P flux within a community. The goal was to compare the resource use efficiency independently of biomass output or nutrient stock. We calculated plant/soil N and P ratios using total biomass and soil available N and P content. The biomass collected in August 2017 was homogenised per plot and we ground a minimum of 5g per plot with a cyclone mill to obtain a fine powder. We then used near infrared reflectance spectroscopy

(NIRS) analyses to estimate biomass N and P content using calibrations by Kleinebecker et al. (2011). Soil samples were taken in autumn 2017 using two homogenised cores per plot. Soil NH_4^+ and PO_4^{3-} were extracted with $CaCl_2$ and analysed using a Continuous Flow Optical-Absorption Spectrometer (CF-OAP; model Skalar Scan+; Skalar Analytical, Breda, The Netherlands). As we only measured ammonium but not nitrate, the N ratio function represents only part of the community nitrogen use efficiency and might omit information if the different N fractions are uncorrelated.

Enzymatic activity

The activity of two enzymes related to carbon and phosphorus cycling was measured in autumn 2017 and in April, May, July and August 2018. We took two soil samples per plot to 20 cm depth. The fresh samples were homogenised then sieved through 2mm meshes. In each sample, we measured the concentration of nytrophenyl released by the β-glucosidase and phosphatase enzymatic activities, following the protocol of Tabatabai (1982). For the analyses presented here, we calculated the mean value of each enzymatic activity for all the periods sampled.

Belowground biomass

Root biomass was measured once in autumn 2017. We took two cores per plot to 20 cm depth (440 cm^3 of soil). We homogenised the two samples and used a subset of 40g fresh soil in which we sorted out the roots. The samples were washed in 200 µm sieves and the roots sorted out with tweezers. We dried the roots at 65°C for 48 hours. We estimated soil bulk density by weighing 40g of soil from the same plot before and after drying for 24 hours at 105°C. Bulk density did not differ between the treatments (data not shown). The final belowground biomass per plot is the weight of roots per g of dry soil.

Respiration

We measured overall soil respiration five times during 2018. We placed one PVC ring per plot at least a week before taking the measurements. The vegetation was clipped before starting the measures without disturbing the soil. We took two measures per plot and repeated the measures in case of large difference between the respiration rates. We took the measurements using a soil CO_2 flux chamber (6400-09) attached to a LI-6400XT (Li-Cor Inc.). Soil respiration varies during the day and between days. To minimise this variation, we took the measurements starting at 10am and finishing the latest at 2pm, as this time span is expected to provide rates representative of the mean daily respiration (Mielnick and Dugas 2000; Castillo-Monroy et al. 2011). We measured one block per day (54 plots), avoided too humid days and kept the measurement days as close to each other as possible (max 6 days for one sampling session). The final soil respiration measure is the mean measure per plot.

Carbon storage

We measured soil carbon concentration on soil samples of 440 cm^3, taken to 25 cm depth in autumn 2017. We took two samples per plot, homogenised them and removed stones and living material (roots, fauna). We weighed the samples before and after drying at 65°C for 48 hours. To calculate carbon storage, we compared the 2017 samples to soil samples taken in autumn 2015 before the start of the experiment. In 2015, we took 5 soil samples per plot from a subset of 89 plots distributed across the field. As the field had just been ploughed and homogenised there was relatively little variation between adjacent plots and we estimate soil C concentrations for all plots using kriging (package geoR, estimating sill and range of the data variogram using the variofit function). Analyses of soil total C were conducted in a CNS Analyser at the Institute of Geography of the University of Bern. The carbon storage per plot is the difference between the percentage carbon in autumn 2017 and in autumn 2015.

Analyses

Multifunctionality

We calculated multifunctionality from our 10 ecosystem functions using a multiple threshold approach (Byrnes et al. 2014). We therefore calculated "ecosystem function multifunctionality" (Manning et al. 2018) using a set of distinct functions, representing key above and belowground carbon and nutrient fluxes. We first standardised each function to a common scale (see Table S4 and Figure S4) and then calculated multifunctionality using thresholds from 0.5 to 0.8 with an interval of 0.05. The thresholds were defined relative to the mean of the five highest values of each function. Multifunctionality was the proportion of functions reaching this threshold per plot, calculated using the multidiv function (available at https://github.com/eric-allan/multidiversity).

We first analysed multifunctionality at each threshold separately, using linear mixed models, testing the effects of N enrichment, fungicide (Fng), species richness (SR), SLA, MPD and their interaction on multifunctionality. Analysing realised instead of sown species richness gave similar results (Table S8). Our data met the model assumptions of homogeneity of variance and normal distribution of residuals. All explanatory variables were scaled and centred to a mean of 0 and standard deviation of 1, so that their effect sizes are comparable. We included block and combination (specific set of species per richness and functional composition level) as random terms. We did not test for the SLA x MPD interactions, as the two factors are not fully orthogonal in the experimental design (MPD is inevitably maximal at intermediate SLA).

The effects of different factors can vary according to the threshold used to calculate multifunctionality. Previous approaches have tested how diversity effects vary depending on the threshold (Byrnes et al. 2014) but visualising how multiple factors vary with multifunctionality thresholds is more challenging. We therefore used a new approach to test which treatment effects were consistent across thresholds and which depended on the threshold level. To do this, we constructed models with all multifunctionality measures (calculated using different thresholds) combined. This meant that each plot had seven measures of multifunctionality (at the 50, 55, 60, 65, 70, 75 and 80% thresholds). We analysed all the multifunctionality values with linear mixed effects models, including threshold as a fixed continuous term which could interact with the treatment variables, and including plot as a random effect to correct for the fact that we had several measures of multifunctionality per plot. We also included random slopes for the effect of threshold on multifunctionality in each plot, which corrects for additional autocorrelation between multifunctionality values measured in the same plot. This is a similar approach to that used in Soliveres et al. (2016b) but extended to multifunctionality calculated at different thresholds. Using this method, we could estimate which effects were significant across all thresholds, and which ones changed continuously as the threshold was increased. We then estimated the overall effect of each treatment and interaction across the thresholds and calculated a confidence interval around the effect. Confidence intervals of lmer were predicted using Ben Bolker's script (available at https://bbolker.github.io/mixedmodels-misc/glmmFAQ.html#predictions-andor-confidence-or-prediction-intervals-on-predictions).

In addition, all functions were analysed separately using linear mixed effect models.

Monocultures are not widespread in natural communities and all get an arbitrary MPD of 0 causing some correlation between MPD and species richness. To ensure that the effect of diversity is not driven only by the difference between monocultures and polycultures, we added one analysis accounting for this distinction with a discrete variable (monoculture/not monoculture). Results using this approach were qualitatively similar to those presented in the main text (Table S9).

All analyses were conducted in R using the package lme4 (Bates et al. 2015; R Core Team 2018). We simplified full models by dropping terms that did not significantly improve the overall model fit using likelihood-ratios.

Number of functions

In addition to the threshold chosen, multifunctionality values depend on the number and identity of functions included, which makes generalisation across studies challenging (Bradford et al. 2014; Giling et al. 2019; Meyer et al. 2018). In order to test the sensitivity of our results to the number and identity of functions, we calculated multifunctionality with all possible combinations of between two and ten functions (1013 combinations) for four thresholds from 0.5 to 0.8 (4052 multifunctionality measures). We extracted the effects of our factors and interactions from a linear mixed effect model for each multifunctionality measure. In order to correct for the uncertainty of each estimate, we weighted each estimate by the inverse of the standard error. We then calculated an overall mean and confidence interval for multifunctionality per threshold depending on the number of functions included.

Supporting information

Table S1: Effect on multifunctionality of species richness (SR), functional diversity (MPD), community weighted mean specific leaf area (SLA), fungicide (Fng), nitrogen enrichment (N), multifunctionality threshold and their interaction. Output of the linear mixed effects model after model simplification (scaled variables).

Initial model in R:

Threshold * (N + SLA + Fng) ^3 * (SR + MPD)
+ (1|Block) + (1|Combination) + (Threshold|Plot_Nr)

Factor	Estimate	Std.Error	Significance
(Intercept)	0.579	0.016	
Threshold	-0.184	0.004	<0.001
N	0.015	0.005	
SR	0.003	0.011	
SLA	0.033	0.010	
MPD	0.008	0.009	
N x MPD	-0.008	0.004	0.049
SR x SLA	0.023	0.010	0.016

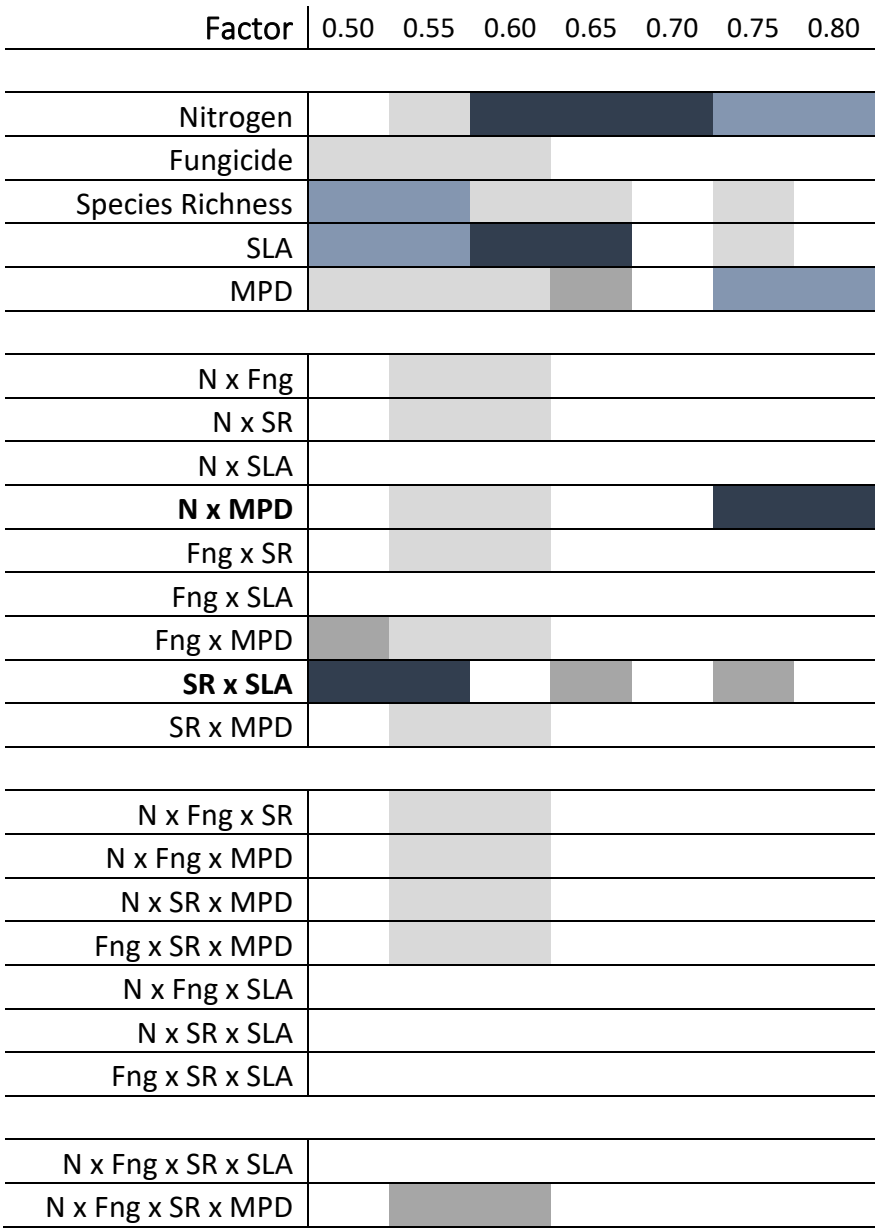
Table S2: Effect on multifunctionality per threshold of species richness (SR), functional diversity (MPD), community weighted mean specific leaf area (SLA), fungicide (Fng), nitrogen enrichment (N) and their interaction. Output of the linear mixed effects model after model simplification (scaled variables).

Initial model:

(N + Fng + SR)^3 * (MPD + SLA) + (1|Block) + (1|Comb)

	Factor	Estimate	Std.Error	Significance
0.5				
	(Intercept)	0.814	0.012	
	SR	0.010	0.010	
	SLA	0.041	0.011	
	SR x SLA	0.032	0.011	0.004
0.55				
	(Intercept)	0.769	0.016	
	SR	0.006	0.010	
	SLA	0.043	0.011	
	SR x SLA	0.031	0.011	0.004
0.6				
	(Intercept)	0.700	0.018	
N		0.023	0.006	<0.001
SLA		0.022	0.009	0.013
0.65				
	(Intercept)	0.606	0.023	
	N	0.020	0.008	0.009
	SLA	0.020	0.010	0.036
0.7				
	(Intercept)	0.498	0.023	
	N	0.030	0.008	<0.001
0.75				
	(Intercept)	0.377	0.022	
	N	0.024	0.009	
	MPD	0.009	0.009	
	N x MPD	-0.021	0.008	0.008
	0.8			
	(Intercept)	0.275	0.021	
	N	0.011	0.007	
	MPD	0.001	0.008	
	N x MPD	-0.021	0.007	0.002

Table S3: Visual summary of the Table S2. Dark blue: significant, light blue: part of a significant interaction. Grey: marginally significant, light grey: part of a marginally significant interaction.



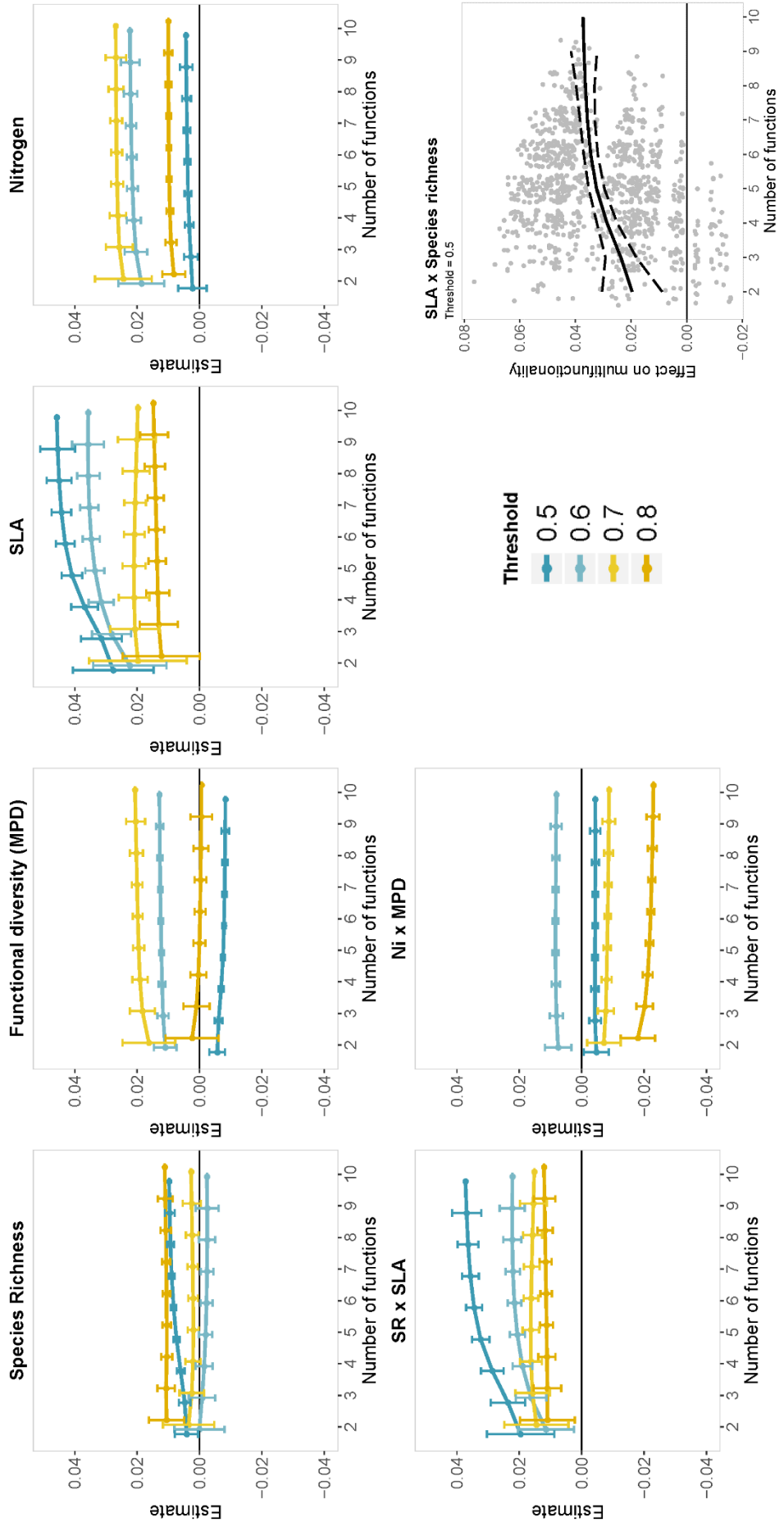


Figure S1: Estimate (+/- confidence interval) of manipulated variables and interactions effect on multifunctionality, depending on the threshold and the number of functions. Mean effect (+/- CI) of the species richness x SLA interaction on multifunctionality at a 0.5 threshold depending on the number of functions. In grey are shown the transformed estimates for each combination of function.

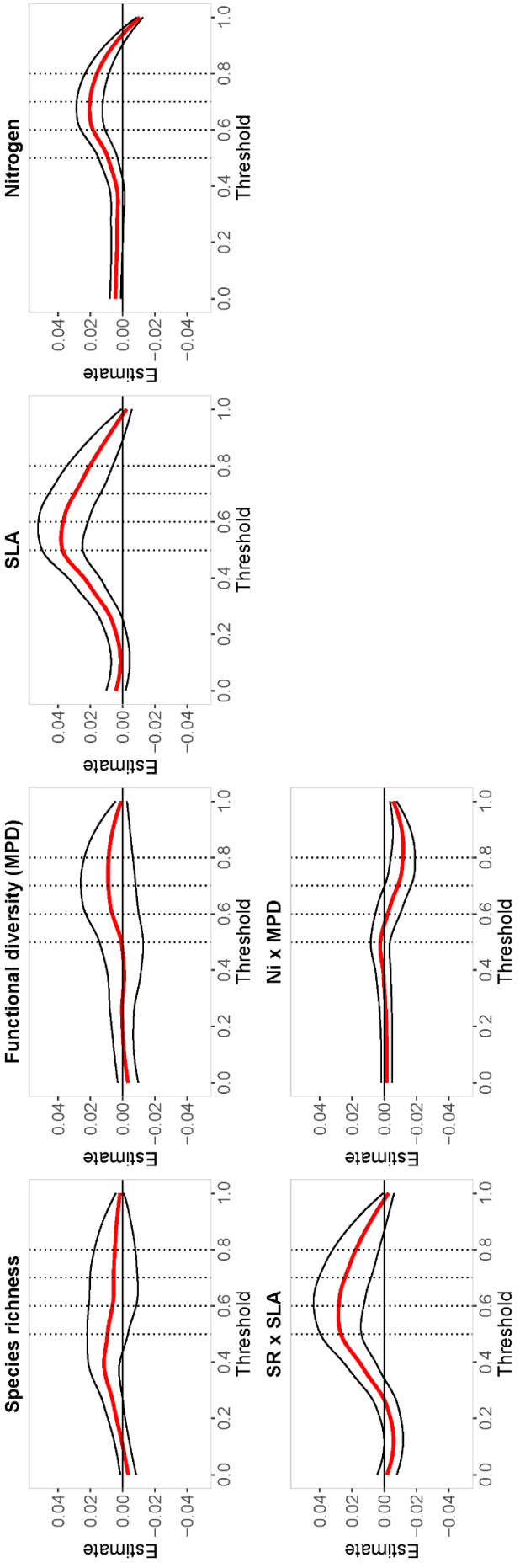


Figure S2: Estimate (+/- confidence interval) of manipulated variables and interactions effect on multifunctionality, depending on the multifunctionality threshold.

Table S4: Description of the functions included in our multifunctionality measure. The PaNDiv technicians Hugo Vincent, Mervi Laitinen and Marlise Zimmermann helped with data collection for all measurements.

Variable / Measurement	Number of measurements	Time of measurement	Contributors	Related function	Transformation
Aboveground biomass	4	June & Aug 17, June & Aug 18	NP, SC	Aboveground primary production	Sqrt
Pathogens	3	Oct 17, July & Oct 18	SC	Transfer of energy to higher trophic levels	-
Herbivory	2	June & Aug 18	TM	Transfer of energy to higher trophic levels	Sqrt
N ratio	1	Aug 17, Autumn 17	NP, TZN, Münster	N cycling, community N uptake efficiency	Log
P ratio	1	Aug 17, Autumn 17	NP, TZN, Münster	P cycling, community P uptake efficiency	Log
Belowground biomass	1	Autumn 17	NP	Belowground primary production	Log
Respiration	5	Mai to Sept 2018	NP	C cycling, microbial activity	Log
Carbon storage	1	Autumn 2015 and 2017	NP	C cycling	Sqrt +1
β -glucosidase	5	Autumn 17, Apr, Mai, July & Aug 18	TZN	C cycling	Sqrt
Phosphatase	5	Autumn 17, Apr, Mai, July & Aug 18	TZN	P cycling	Log
Plant % cover	4	June & Aug 17, June & Aug 18	SC, EA		
Plant traits	3	Aug 17, June & Aug 18	NP, SC		

Table S5: Effect on multifunctionality of species richness (SR), abundance weighted functional diversity (MPDw), community weighted mean specific leaf area (SLA), fungicide (Fng), nitrogen enrichment (N), multifunctionality threshold and their interaction. Output of the linear mixed effects model after model simplification (scaled variables).

Initial model in R:

Threshold * (N + SLA + Fng + SR + MPDw) ^2 – Threshold : SLA : MPDw – SLA : MPDw
+ (1|Block) + (1|Combination) + (Threshold|Plot_Nr)

Factor	Estimate	Std. Error	Significance
(Intercept)	0.594	0.017	
Threshold	-0.184	0.004	<0.001
N	0.015	0.005	
SR	0.016	0.012	
SLA	0.041	0.010	
MPDw	-0.018	0.011	
N x MPDw	-0.011	0.004	0.01
SR x SLA	0.030	0.010	0.001
SR x MPDw	-0.026	0.011	0.014

Multifunctionality increased with unweighted MPD (presence/absence), decreased with abundance weighted MPDw. The difference between the two (here “MPD redundancy”) indicates how much the community decreased in MPD with a shift in abundance. The main effect of “MPD redundancy” was never significant for individual functions (data not shown).

Initial model in R:
m ~ Threshold * (N + Fng + SR + SLA + MPD)^2 - Threshold : SLA : MPD - SLA : MPD
+ MPD_redundancy
+ (1|Block) + (1|Comb) + (Threshold|Plot_Nr)

Fixed effects:	Estimate	Std. Error	Pvalue
(Intercept)	0.578	0.016	<0.001
Threshold	-0.184	0.004	
N	0.016	0.005	
SR	0.008	0.012	
SLA	0.033	0.010	
MPD	0.002	0.010	
MPD redundancy	0.025	0.014	0.048
N x MPD	-0.008	0.004	0.053
SR x SLA	0.023	0.010	0.019

Table S6: Effect on above/belowground multifunctionality of species richness (SR), functional diversity (MPD), community weighted mean specific leaf area (SLA), fungicide (Fng), nitrogen enrichment (N), multifunctionality threshold and their interaction. Output of the linear mixed effects model after model simplification (scaled variables).

Initial model in R:

Threshold * (N + SLA + Fng + SR + MPDw) ^2 - Threshold:SLA:MPDw - SLA:MPDw
+ (1|Block) + (1|Combination) + (Threshold|Plot_Nr)

Aboveground functions: aboveground biomass, pathogens presence, herbivory, N and P ratios.

Factor	Estimate	Std.Error	Pvalue
(Intercept)	0.449	0.012	
Threshold	-0.128	0.005	
N	0.017	0.007	0.01
SR	-0.005	0.013	
SLA	0.068	0.013	
SR x SLA	0.046	0.013	
Threshold x SR	-0.008	0.004	
Threshold x SLA	-0.010	0.006	
Threshold x SR x SLA	-0.014	0.007	0.035

Belowground functions: belowground biomass, soil respiration, C storage, β-glucosidase, and phosphatase.

Factor	Estimate	Std.Error	Pvalue
(Intercept)	0.707	0.020	
Threshold	-0.241	0.006	
N	0.013	0.007	
Fng	0.002	0.005	
SR	0.017	0.009	
SLA	-0.007	0.007	
MPD	0.009	0.009	
N x MPD	-0.018	0.007	
Fng x MPD	-0.010	0.005	0.049
Threshold x N	0.008	0.006	
Threshold x SR	0.013	0.005	0.019
Threshold x SLA	-0.010	0.005	0.049
Threshold x MPD	-0.003	0.006	
Threshold x N x MPD	-0.011	0.005	0.025

Table S7: PaNDiv Experiment list of species and corresponding growth strategy.

Grasses	**Leaf economics spectrum**	**Herbs**	**Leaf economics spectrum**
Poa trivialis *Lolium perenne* *Holcus lanatus* *Dactylis glomerata*	Fast	*Crepis biennis* *Taraxacum officinale* *Anthriscus sylvestris* *Heracleum sphondylium* *Galium album* *Rumex acetosa*	Fast
Helictotrichon pubescens *Festuca rubra* *Bromus erectus* *Anthoxanthum odoratum*	Slow	*Achillea millefolium* *Centaurea jacea* *Daucus carota* *Salvia pratensis* *Prunella grandiflora* *Plantago media*	Slow

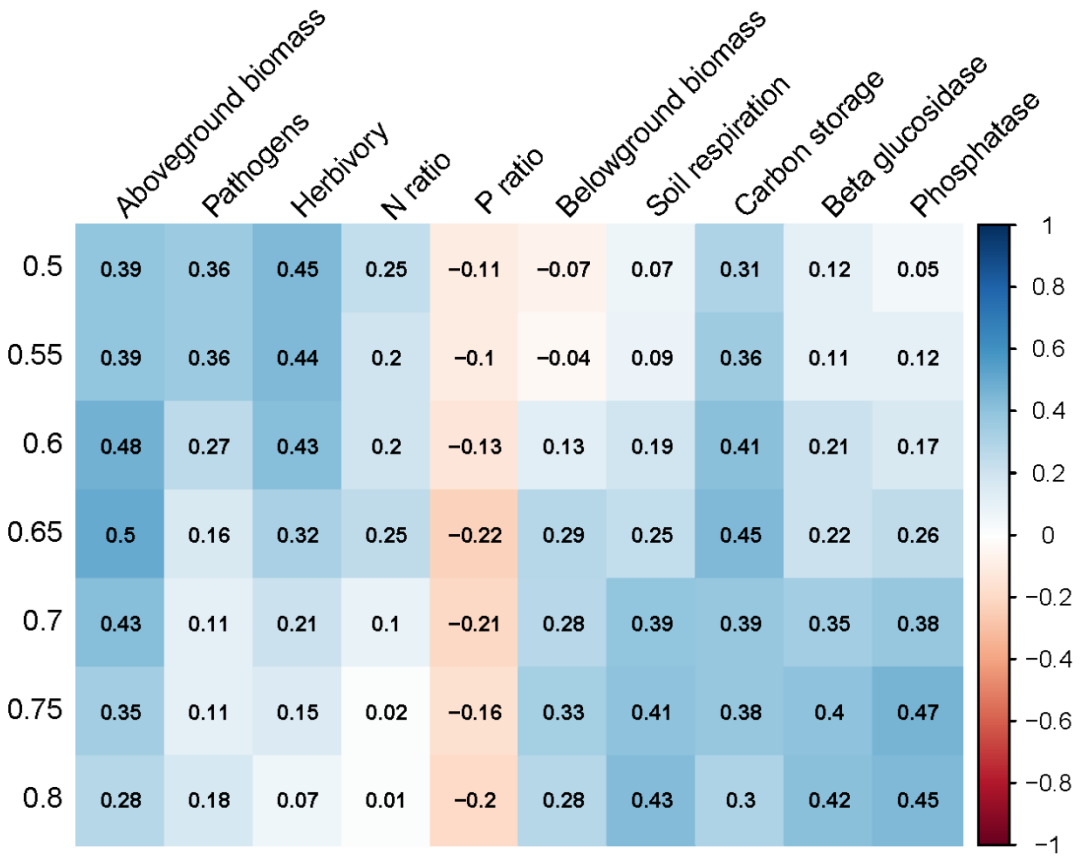


Figure S3: Pearson's correlations between the functions after normalisation (see Table SX for the transformation detail) and multifunctionality at each threshold. Each function was measured on 216 plots.

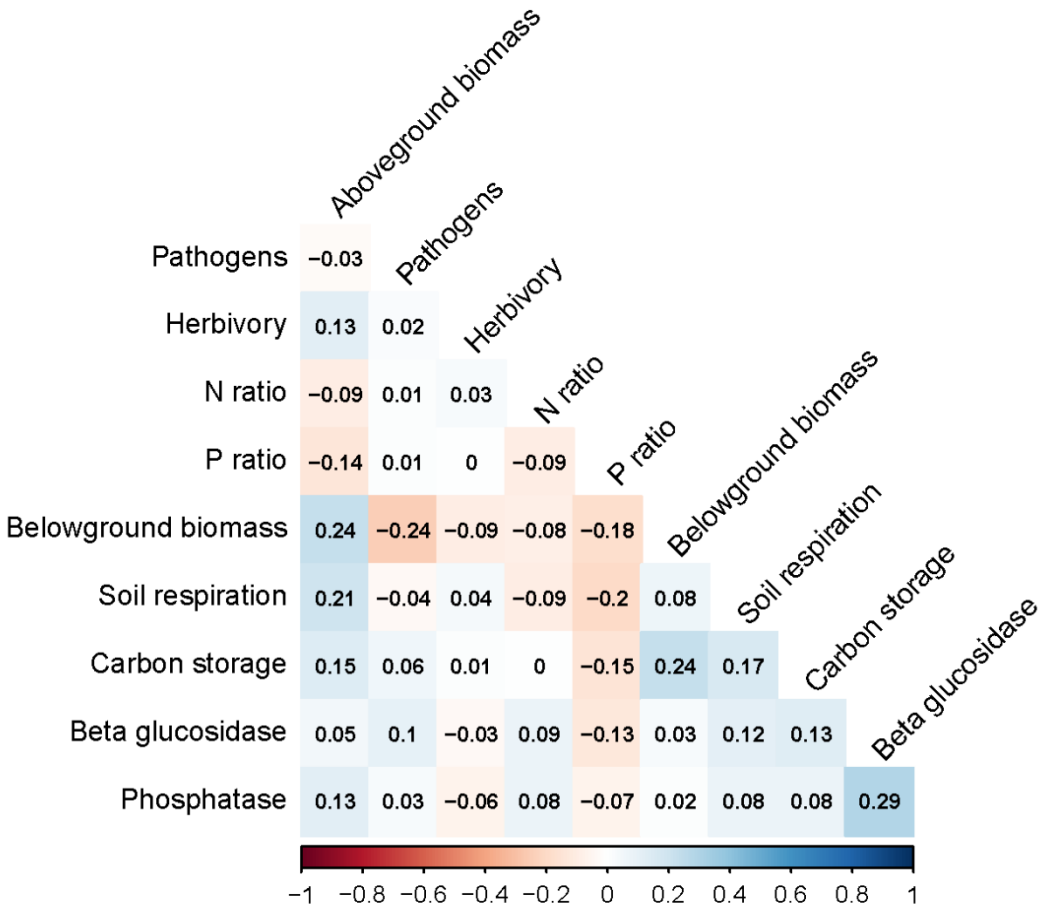


Figure S4: Pearson's correlations between the functions measured on 216 plots after log or square root transformation to fit a normal distribution (see Table SX for the transformation detail).

Table S8: Effect on multifunctionality of realised species richness (RDiv), functional diversity (MPD), community weighted mean specific leaf area (SLA), fungicide (Fng), nitrogen enrichment (N), multifunctionality threshold and their interaction. Output of the linear mixed effects model after model simplification (scaled variables). Realised species richness includes per plot all species that were recorded at least once between 2017 and 2018.

Initial model:
Threshold * (N + SLA + Fng + RDiv + MPD) ^2
- Threshold : SLA : MPD - SLA : MPD
+ (1|Block) + (1|Comb) + (Threshold |Plot_Nr)

Factor	Estimate	Std.Error	Significance
(Intercept)	0.579	0.016	
Threshold	-0.184	0.004	<0.001
N	0.015	0.005	
RDiv	-0.001	0.013	
SLA	0.031	0.009	
MPD	0.010	0.009	
RDiv x SLA	0.027	0.011	0.009
N x MPD	-0.008	0.004	0.053

Table S9: Lmer output correcting for the fact that monocultures are all coded as 0 MPD. The term "mixture" is a categorical variable distinguishing monoculture from mixture (0/1). The mixture term drops out from the final model.

Initial model:
Threshold * (N + SLA + Fng + SR + MPD) ^2 - Threshold : SLA : MPD - SLA : MPD
+ mixture : SLA + mixture : N
+ (1|Block) + (1|Comb) + (Threshold |Plot_Nr)

Factor	Estimate	Std.Error	Significance
(Intercept)	0.579	0.016	
Threshold	-0.184	0.004	<0.001
N	0.015	0.005	
SR	0.003	0.011	
SLA	0.033	0.010	
MPD	0.008	0.009	
N x MPD	-0.008	0.004	0.049
SR x SLA	0.023	0.010	0.016

Chapter 3

Decomposition disentangled: a test of the multiple mechanisms by which nitrogen enrichment alters litter decomposition

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Credit: Hugo Vincent

Summary

Nitrogen (N) enrichment has direct effects on ecosystem functioning by altering soil abiotic conditions and indirect effects by reducing plant diversity and shifting plant functional composition from dominance by slow to fast growing species. Litter decomposition is a key ecosystem function and is affected by N enrichment either by a change in litter quality (the recalcitrance of the plant material) or through a change in soil quality (the abiotic and biotic components of the soil that affect decomposition). How the direct and indirect effects of N alter soil and litter quality remains poorly known. We designed a large grassland field experiment manipulating N enrichment, plant species richness and functional composition in a full factorial design. We used three complementary litter bag experiments and a novel structural equation modelling (SEM) approach to quantify the effects of the treatments and various measures of functional composition and diversity on litter and soil quality and total decomposition. Our results revealed multiple drivers of litter quality and showed that nutrient contents (N and calcium) were about twice as important as structural components (leaf dry matter content, fibres) in determining litter quality. Overall the experimental results suggest that N enrichment increases litter decomposition mostly indirectly through a shift in functional composition toward faster growing plant species producing higher quality litter. N enrichment also altered soil quality by directly and indirectly affecting vegetation cover. Our novel SEM approach showed that we could partition overall decomposition rate into the contributions of litter and soil quality. Our approach provides a mechanistic tool to test the drivers of litter decomposition across different ecosystems. Our results show that litter quality is determined by several nutrient and structure traits and highlight the importance of considering shifts in plant species composition when assessing the effects of N enrichment on decomposition.

Introduction

Soil nitrogen enrichment is one of the major global changes ecosystems are currently facing (Galloway et al. 2008). Nitrogen (N) enrichment alters ecosystem functioning directly and through several indirect mechanisms. It directly alters functions related to nutrient stocks and fluxes by changing soil abiotic conditions, stoichiometry and pH (Sardans et al. 2012; Laliberté and Tylianakis 2012). In addition, N enrichment indirectly affects ecosystem functioning by altering biotic community properties such as plant diversity and composition. N enrichment typically reduces the number of plant species able to coexist (Suding et al. 2005) and this loss of diversity could affect ecosystem functioning as much as N enrichment per se (Hooper et al. 2012; Tilman et al. 2012). However, plant community change following N enrichment, also involves compositional turnover and in particular a shift towards faster growing plant species (Isbell et al. 2013; Lavorel and Grigulis 2012; de Vries et al. 2012). Such changes in functional composition are indicated by an increase in mean values of leaf economics spectrum traits, such as specific leaf area and leaf N content (Wright et al. 2004), and this shift is a key driver of ecosystem functioning (Lavorel and Grigulis 2012). However, we still have little mechanistic insight into the relative importance of these direct (abiotic) and indirect (plant richness and composition) effects of N enrichment on ecosystem functioning. Observational studies have separated direct effects of N from indirect effects mediated through species richness (Isbell et al. 2013) and/or functional composition (Allan et al. 2015). However, observational studies struggle to separate effects of correlated drivers, such as diversity loss and compositional turnover. Experimental approaches are therefore needed to separate these effects and to fully understand and predict the mechanisms by which N enrichment affects ecosystem functioning.

The decomposition of plant litter is a key ecosystem function that influences rates of soil biogeochemical cycling and which is strongly affected by N deposition (Finn et al. 2015; Knorr et al. 2005; Hobbie et al. 2012). Depending on the ecosystem and the enrichment level and duration, N can have either positive or negative effects on decomposition (Bardgett and Wardle 2012; Knorr et al. 2005; Hobbie et al. 2012; Riggs et al. 2015). Plant litter decomposition is determined by multiple mechanisms: it depends principally on the physical and chemical properties of the litter and on soil biotic and abiotic conditions (Cebrian 1999; Handa et al. 2014; Cornwell et al. 2008; Garnier et al. 2004). To distinguish these drivers of litter decomposition, we will refer to “litter quality”, and to “soil quality” respectively.

N enrichment is likely to directly and indirectly alter litter quality. Litter quality is largely determined by its chemical properties (nutrient contents and the presence of defence compounds) and by physical

factors such as leaf dry matter and fibre contents (Garnier et al. 2004; Cornwell et al. 2008). Soil N enrichment will directly increase the nutrient content of leaves: in addition to an increase in N, changes in macronutrients like Ca and Mg may also influence litter decomposability (García-Palacios et al. 2016a) but are less well studied. Indirect effects of N are also likely to be important: a shift to fast growing plant communities further enhances litter quality because fast growing plants have generally higher leaf N and lower fibre contents. N enrichment is therefore likely to increase decomposability by increasing nutrient contents and by changing leaf physical structure, however, the relative importance of N induced changes in nutrients vs. structure is not well known. Other indirect effects of N may reduce decomposition, in particular a loss of species or functional diversity could reduce decomposability (Handa et al. 2014). We therefore lack a comprehensive picture of how N enrichment alters different aspects of litter quality.

The direct and indirect effects of N enrichment could also influence decomposition through changes in soil quality, i.e. by altering soil properties and faunal and microbial composition (Treseder 2008; Milcu et al. 2013; Bardgett and Wardle 2012; de Vries et al. 2006). These effects may have contrasting signs (e.g., fast growing species have more decomposable litter, but N enrichment can reduce the efficiency of soil microbes in decomposing it) and follow multiple pathways. To understand the impacts of N enrichment on decomposition we therefore need experimental and analytical approaches that can quantify the net effects of N enrichment on ecosystem functioning and the mechanisms behind these effects.

In this study, we tested the effects of N enrichment on litter decomposition and disentangled its direct effects on soil and litter quality from its indirect effects mediated by plant species richness and functional composition. We created experimental plant communities to realise a full factorial cross of plant functional composition, plant species richness and N enrichment. Plant functional composition was manipulated by creating a gradient in community mean specific leaf area and leaf N, as these traits are key indicators of the resource economics spectrum. Three complementary litter bag experiments were used to test direct and indirect effects of N enrichment on litter quality, on soil quality and on both combined. We also looked at the effect of macro and mesofauna on decomposition by using different mesh sized litter bags. This framework enabled us to test the following questions:

What is the relative importance of direct effects of N enrichment on decomposition relative to indirect effects mediated through changes in the plant community (species richness and functional composition)?

Which aspects of litter quality are most important in determining decomposition?

Can we partition the overall decomposition rate into the contributions of soil and litter quality?

How important are meso and macro fauna in determining decomposition and how does their relative importance change with N enrichment?

Material and methods

The PaNDiv Experiment

The PaNDiv Experiment is located in Münchenbuchsee near the city of Bern (Switzerland, 47°03'N, 7°46'E, 564 m.a.s.l.). It has a mean annual temperature of 9.2°C and mean annual precipitation of 1051 mm y^{-1} (Federal Office of Meteorology and Climatology MeteoSwiss). The soil is characterized as 0.7 to 1m deep cambisol (http://www.geo.apps.be.ch). We measured total soil N and carbon (C) concentrations and pH at the start of the experiment and found concentrations of 3.4 (sd 0.3) %C, 0.37 (sd 0.03) %N and a mean pH of 7.4. The site had been unfertilised for at least 10 years before the start of the experiment. The vegetation was cleared and the area ploughed before the experimental plots were established.

The species sown were selected from a pool of 20 species commonly found in both extensively and intensively managed Central European grasslands. We divided our 20 species into 10 fast and 10 slow growing species according to their specific leaf area (SLA) and leaf N content, which are related to resource use strategy (see Figures S1 and S2) (Wright et al. 2004). We excluded legumes from the species pool as few legume species will grow well at high N levels and including legumes only in the slow growing pool would have caused a large difference between the species pools.

In order to separate direct and indirect effects of N enrichment, we established a factorial cross of treatments representing the direct (N enrichment) and indirect effects (plant diversity loss and change in functional composition) on 2x2m plots. Fertilised plots received N in the form of urea twice a year in April and late June, for an annual addition of 100 kg N $ha^{-1}y^{-1}$, corresponding to intermediately intensive grassland management (Blüthgen et al. 2012). To manipulate diversity, we established plots with 1, 4, 8 or all 20 species. To manipulate functional composition and diversity we established plots with only fast growing, only slow growing or a mix of fast and slow growing species. This created a large gradient in mean trait values, see below. We sowed all plants in monoculture and we established four replicates of the 20 species together. At the four and eight species levels we randomly selected

10 species compositions for each combination of richness (4 and 8), times functional composition (fast, slow, mixed), so effects of changes in mean traits are independent of particular species. All polycultures contained both grasses and herbs. The 84 different species compositions were grown once in control conditions and once with N enrichment. In addition to the N treatment, we also applied a fungicide treatment and a fungicide x N treatment, resulting in 336 plots in total. However, for logistical reasons the litter bag experiment was only conducted on the 168 control (no fungicide) plots (see Table S1). The whole field was divided into four blocks. Each block contained all 84 compositions but the particular N x fungicide treatment was randomly allocated per block. A regularly mown 1m path sown with a grass seed mixture (*Lolium perenne* and *Poa pratensis*, UFA-Regeneration Highspeed) separated the plots.

All species within a plot were sown at equal density in October 2015, with proportions corrected by species specific germination rates, to obtain a total density close to 1000 seedlings m^{-2}. The seeds were obtained from commercial suppliers (UFA Samen, Switzerland, and Rieger-Hofmann, Germany). In order to maintain the diversity levels, the plots were weeded three times a year in April, July and September, keeping weed cover always below 5% (Figure S3). The whole experiment was mown twice a year in mid-June and mid-August which corresponds to intermediate to extensive management.

Measuring decomposition of litter bags

We conducted three complementary litter bag experiments simultaneously to test the mechanisms by which our treatments affected decomposition. To test the effect of our treatments on litter quality, we filled the first set of bags (common garden bags) with biomass collected from each plot and let them decompose in a common garden, established in the grassland surrounding the experimental plots. The soil conditions in the common garden are therefore very similar to those in the plots. The second set of bags tested effects on soil quality (standard bags). We filled these bags with rapeseed straw (*Brassica napus*) as a standard material and placed them on every plot. No Brassicaceae are present in the experiment and this litter should therefore be equally foreign for all plots. We filled the third set of bags, called plot bags, with aboveground dry biomass from each plot and let them decompose on their own plot (i.e. the plot from which the biomass was collected) to test the combined effect of soil and litter quality on decomposition. With this design it is not possible to test for interactions between soil and litter quality, as this would require crossing soil and litter quality, i.e. decomposing a range of litters on each plot, which would not have been feasible. However, by combining data from these three experiments in a structural equation model (see below), we can

disentangle the relative importance of soil and litter quality in driving overall decomposition and test if the additive effects of soil and litter quality can adequately explain overall variation in decomposition.

We sewed the litterbags using nylon fabric with a mesh size of 5 mm above and 0.2 mm for the fabric in contact with the soil, to avoid loss of material during transport and manipulation (Bradford et al. 2002). To investigate the effects of different sized groups of detritivores on overall decomposition, we sewed two additional plot bags with smaller top mesh size: 2 mm to exclude the macrofauna, and 0.2 mm to exclude meso and macrofauna (Milcu and Manning 2011; Bardgett 2005). By comparing decomposition rates in the bags with different mesh sizes we could estimate the effect of different aspects of the soil community on the overall decomposition rate.

The plant biomass used to fill the common garden and plot bags was collected on the field before the mowing in June 2017 (with some very unproductive plots sampled again in August in order to have enough material). The biomass was dried at 65°C for 48h, chopped, homogenized and split into equal parts (Biomass splitter, RT 6.5–RT 7; Retsch, Haan, Germany). We filled each bag with a maximum of 20g dry material and weighed the litterbags again after closing. Because some experimental communities produced only a small amount of biomass, we could not include 20g in all bags and the initial biomass varied from 5 to 20g. The bags decomposed on top of the soil for 2.5 months between September and December 2017. We then collected the bags, cleaned them of debris and soil, dried them and weighed them again. We measured decomposition rate as the percentage biomass lost between September and December, to correct for differences in initial weight. Initial bag weight was included as a covariate in our models but it never affected percentage mass loss (see Table S2).

Plant traits used to calculate functional composition

Our experimental designed created large gradients in mean leaf economic traits. Although plots were designed to differ in SLA, we also created a large gradient in mean LDMC, which was only partially correlated with SLA (-0.33, Figure S4). We calculated community weighted means for Specific Leaf Area (SLA) and Leaf Dry Matter Content (LDMC) and the range in these (SLA 10-30m^{-2} kg^{-1} and LDMC 150-400mg g^{-1}; Fig. S4) was comparable to that seen for the traits along typical central European land use intensity gradients (SLA 13-32m^{-2} kg^{-1} and LDMC 220-420mg g^{-1}; Breitschwerdt et al. 2018). We measured SLA and LDMC in the control (unfertilised) monocultures sampling one leaf from five individuals per species, and measuring the fresh weight and leaf area with a leaf area meter (LI-3000C,

LI-COR Biosciences) following the protocol of Garnier et al. (2001b). We dried the samples at 65°C for two days and measured their dry weight. To measure the abundances of the plant species, we visually estimated the percentage cover of our target and weed species on every plot before the biomass was cut. We calculated a community weighted mean (CWM) trait measure for each plot by multiplying each species' relative abundance (cover) by the mean trait value of the species in monoculture.

Litter quality

Two key aspects of litter quality are nutrient and fibre contents. We measured the concentration of several nutrients and fibre fractions in the plant biomass samples used to fill the litter bags from June and August 2017 using Near Infrared Reflectance Spectrometry (NIRS). A minimum of 5 g of biomass per plot (pooled sample, including all species present and their relative abundance) was ground with a cyclone mill to obtain a fine powder. The infrared spectrum of the powder was used to estimate the nutrient and fibre contents (see Kleinebecker et al. (2011)). We estimated several nutrients and fibre concentration but could not use all of them in the analyses as some were highly correlated (e.g. and magnesium (Mg) and calcium (Ca), see Figures S4 and S5). We decided to select widely used variables that did not correlate strongly and which account for structural components and nutritional quality of litter: biomass N, fibres (sum of acid detergent fibres: cellulose, lignin and silica) and Ca content (García-Palacios et al. 2016a; Smith and Bradford 2003; Cornwell et al. 2008).

In addition to our measures of functional composition (CWMs) and mean values of litter quality, we calculated a measure of litter functional diversity. We used the abundance weighted mean pairwise distance metric (de Bello et al. 2016). This measure quantified the distance between all species in a plot in their SLA, LDMC, biomass N, fibre and Ca values. In order to derive species specific values for biomass N, Ca and fibres, we used the values from the control monocultures as the species trait values, as for SLA and LDMC. To test if diversity in specific traits was more important for decomposition, we additionally calculated diversity for each plant trait (see Table S3).

Analyses

We first used linear mixed effect models to test the effect of our treatments (and all interactions between them) on litter decomposition (percentage mass loss), for each type of bag individually and

for all bags combined. We ran two combined models: one with plot litter, standard litter and common garden litter combined and one with the three mesh sizes combined. In the combined model we fitted interactions between bag type and the experimental treatments to test if changes in N, functional composition and diversity (fixed effects) had different effects on litter or soil quality. Note that main effects of bag type could be due to differences between litters or differences between locations (plots, common garden), however, we focus here on main effects of the treatments (i.e. effects that are consistent for soil and litter quality) and interactions between treatments and bag type, which indicate cases where the treatments have different effects on litter vs. soil quality. Although we used categorical measures of functional composition to design the experiment, we intended to create a gradient in CWM traits. We therefore also fitted models where we replaced our three level functional composition variable by a continuous measure of community weighted mean SLA and LDMC, and functional diversity. We ran the models in R (package lme4, Bates et al. 2015; R Core Team 2018) and simplified full models by dropping terms that did not significantly improve the overall model fit, using likelihood-ratio tests. All models included block and species composition (84 levels) as random terms. Species composition distinguished the randomly assembled sets of species and was included to correct for the fact that replicated species composition are pseudoreplicates for testing the species richness effects. The combined model with all the bags also included plot as a random term (168 levels), which summarises unexplained variation in both soils and litters between the plots. We added fixed covariates for the month of biomass harvest (June or August) and the initial weight of biomass put in each bag. We did not transform the data since the errors were normally distributed and the variance homogenous.

In a second step, we quantified the mechanisms by which our treatments affected decomposition using Structural Equation Modelling (SEM) (Grace 2006). We included our three decomposition experiments (and the different mesh size treatments, see below) in the same model. By doing this we were able to test the effect of our treatments on litter or soil mediated decomposition and the relative importance of litter and soil mediated decomposition for driving the final decomposition rate measured per plot. We used the mass loss in the "plot" litter bags (i.e. litter decomposing on its own plot) as a measure of the total plot decomposition rate. We then used the mass loss in the common garden litter bags as a measure of the litter mediated effects, as these bags decompose on the same soil and only variation in litter quality will determine variation in mass loss between the bags. We used mass loss from the standard litter bags as our measure of soil mediated decomposition, as the litter is always the same and only variation in soil quality between plots will determine variation in decomposition. We fitted paths from common garden and standard litter mass loss to plot litter mass loss. The size of these two standardised path coefficients indicates the relative contribution of litter

and soil quality to overall decomposition rates. In the SEM, plot litter mass loss is only affected by the mass loss measured in common garden and standard litter bags, to determine if we can explain all of the variation in overall decomposition rate based on our two measures of litter and soil quality.

We then tried to identify the traits and community properties that determined litter and soil quality. We included our manipulated variables, N enrichment and plant species richness, as well as continuous measures of plant functional composition and litter quality, SLA, LDMC, biomass N, fibres and Ca. These measures could affect functional diversity and microclimate. The microclimate measure we used in the analyses is the total plant cover on each plot. It correlates with biomass production and accounts for humidity and temperature variation among plots (Figure S6). To account for an effect of the soil fauna on decomposition, we included the log response ratio of the big mesh to the small mesh bag decomposition rate (see Figure S7). This variable "soil fauna effect" measures the relative effect of macro and mesofauna exclusion on decomposition and tests whether our treatments alter their effect.

We fitted SEMs using the lavaan package (Rosseel 2012). This meant we could not include random effects, which could bias paths from species richness to other variables (not corrected for species composition). However, we also fitted models using piecewiseSEM (Lefcheck 2016), in which we could include composition as a random effect, and this did not change the significance of any paths (see Table S4). The theoretical model and all detailed hypotheses are described in the Supplementary Information (Figure S7).

Results

Individual effects of N enrichment and plant community characteristics on litter and soil quality

Decomposition rates differed significantly among bag types. Litter decomposed faster in the common garden than on the experimental plots, and standard litter decomposed most slowly. Decomposition rates increased with mesh size (Fig.1a and Table S2).

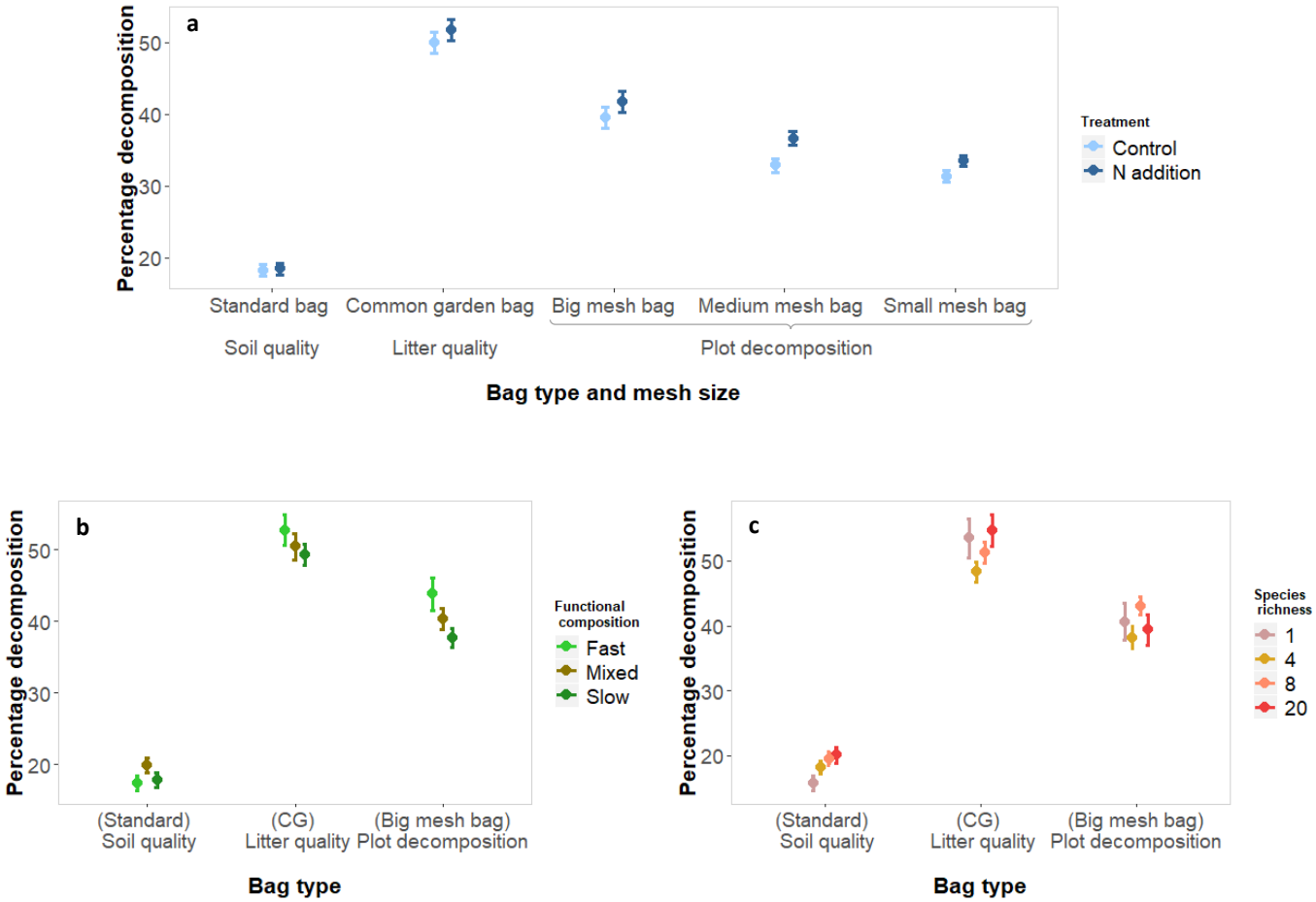

Figure 1. Effect of nitrogen addition (**a**), functional composition (**b**) and species richness (**c**) on litter decomposition depending on the litter bag type (standard, common garden and plot decomposition) and the mesh size (big, medium and small). Mean and standard error of the raw values (168 plots per bag).

N enrichment consistently increased the litter decomposition rate in all bags (significant main effect of N but no interaction between N and mesh size, Table S2 and Figure 1a). The effect was absent for standard litter bags when analysed alone but was significant when different bag types (common garden, standard, and plot big mesh size bags) were analysed together. There was no interaction between N and mesh size, meaning that N enrichment did not change the relative effect of large fauna, compared to small fauna, on decomposition.

Plant functional composition, expressed as a categorical variable (fast, mixed or slow growing species, Fig.1b), had a significant effect on the decomposition of common garden and plot litter. Litter from fast growing communities decomposed more rapidly than litter from mixed and slow communities. This effect was larger than the effect of fertilisation, as the difference between decomposition in fast and slow communities was twice that between fertilised and unfertilised plots (Table S2). We observed the same effects of continuous measures of functional composition, with a non-significant effect of SLA but a negative significant effect of LDMC on decomposition (Figure 2). Comparing the bags with different mesh sizes, LDMC had a larger negative effect on decomposition in the big mesh litter bags than in the smaller mesh sizes, suggesting a larger effect of LDMC on the activity of the macrofauna than on the activity of the meso or microfauna (Fig 2b).

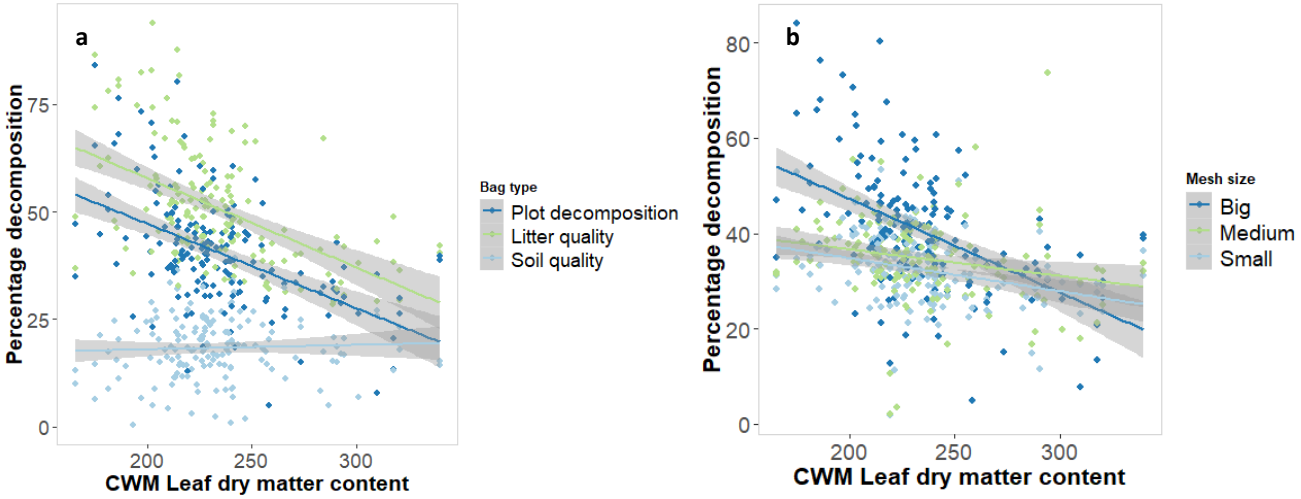

Figure 2. Effect of community weighted mean leaf dry matter content (mg g^{-1}) on decomposition depending on the bag type (**a**) and on the mesh size (**b**). Mean and standard error of the raw values (168 plots per bag).

Plant species richness had a positive effect on the decomposition of standard litter bags, when analysed separately (Fig.1c and Table S2). The effect of functional diversity depended on the bag type, with a negative effect in plot and common garden bags and a positive effect on standard bags (Table S2). These results indicate that species richness and functional diversity of communities increased soil quality, whereas the functional composition of the community increased litter quality.

Relative importance of different aspects of litter and soil quality in driving overall decomposition

We used structural equation models to test the relative importance of our different treatments in affecting soil and litter quality and the overall decomposition rate. Litter and soil quality both had a positive effect on total plot decomposition, but litter quality had a larger effect (path coefficient of 0.93, Table S5) than soil quality (path coefficient of 0.19; see Figures 3 and 4a and b). The overall fit of the model was good ($\chi^2 = 17$, $p = 0.4$) showing that we were able to explain all of the effects of the experimental treatments and traits on overall decomposition with our measures of soil

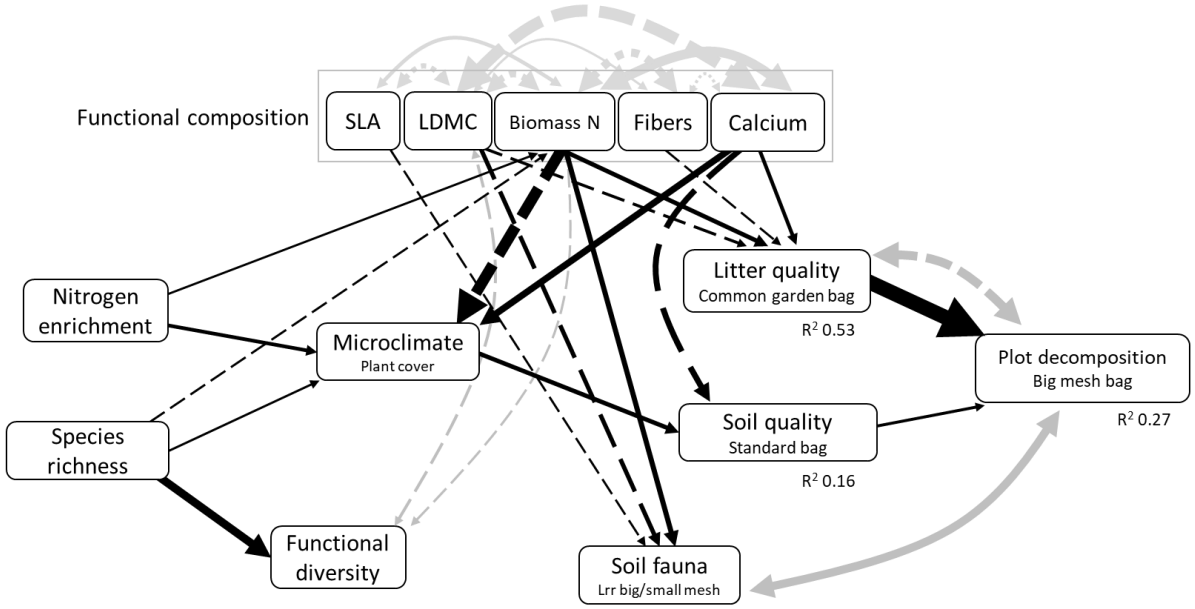

Figure 3. Final results of the structural equation model, showing effects of nitrogen enrichment, plant species richness and plant functional composition on decomposition. Dashed arrows show negative, full arrows positive path coefficients. The arrow size is proportional to the path coefficient. Double-headed grey arrows show covariances. Details of the output in Table S5. Model fit: Pvalue 0.423; Chisq 17.477; Df 17; RMSEA 0.013.

and litter quality. Although soil macro and mesofauna increased decomposition overall, they did not contribute to variation in decomposition between plots, as there was no link between the log response ratio between decomposition in big and small mesh-sized bags and the overall decomposition rates. Litter quality was mainly influenced by plant functional composition. Litter from communities with a high biomass N content, low LDMC and low fibre content, corresponding to our fast growing communities, decomposed faster than litter from slow growing communities (Fig. 4e-h). Interestingly, high Ca concentrations in the biomass also increased litter quality (path coefficient of 0.26). In addition, N enrichment and plant species richness had opposite (positive and negative, respectively) indirect effects on litter quality because they had opposing effects on the N content of the biomass.

Plant species richness increased soil quality, as observed in the mixed models (see Fig. 1b). However, this effect was indirect and mediated by a change in microclimatic conditions: increased plant cover in diverse communities presumably increased soil moisture which increased the decomposition rate. N enrichment also increased soil quality indirectly, through a change in the microclimatic conditions.

Plant functional composition also altered soil quality through changes in microclimatic conditions. Communities with high Ca contents had higher plant cover and therefore higher soil quality. Ca-rich communities were dominated by herbs and had higher cover because herbs established better than grasses at the start of the experiment, probably due to higher drought resistance. Surprisingly, however, biomass N was negatively related to plant cover. Ca also had a direct negative effect on soil quality. Ca therefore had opposing effects on total decomposition through its effects on litter quality (positive) and effects on soil quality (negative), with a total positive effect of 0.20. Biomass N increased, and LDMC and SLA decreased, the effect of the macrofauna on decomposition, i.e. the relative differences in decomposition rate in big compared to small bags (coeff. 0.40; -0.21 and -016 respectively). However, the change in the effect of the soil fauna did not influence soil quality (i.e. there was no path between soil fauna and soil quality).

Plant functional diversity had no significant effect on decomposition, despite the increase in soil quality in mixed communities, found in the linear models (Fig. 1b). According to the SEM, this effect seems to be mediated by community-weighted traits rather than functional diversity. Functional diversity increased with species richness, which is due to the coding of monocultures as zero diversity.

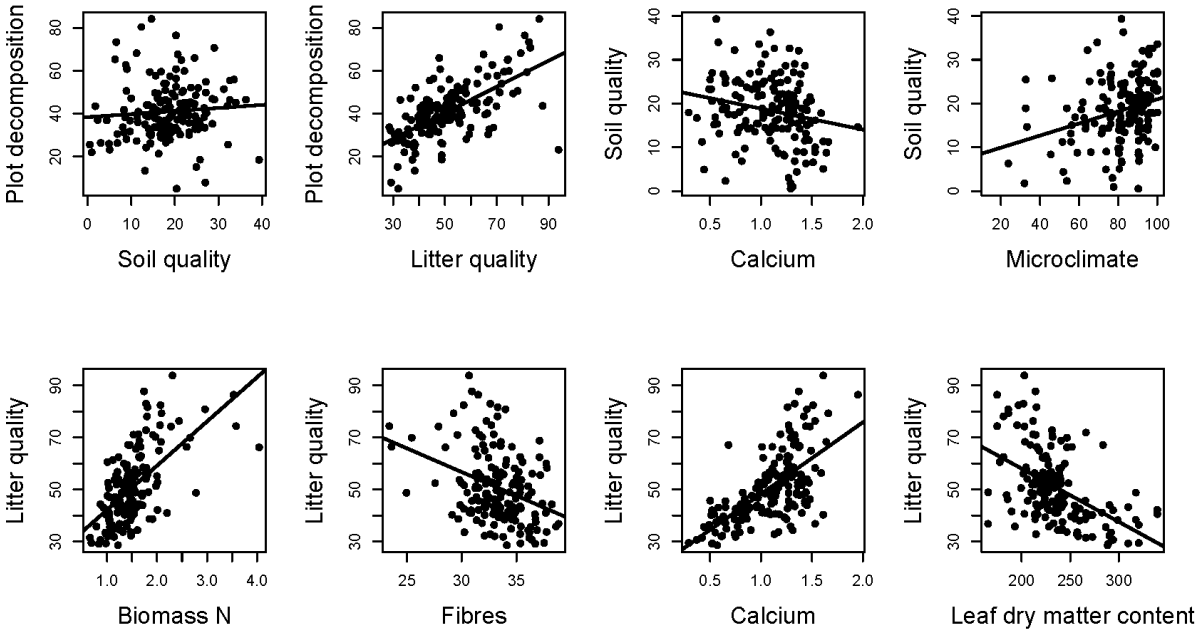

Figure 4. Partial plots visualizing SEM outputs from the Figure 3 of variables effects on overall decomposition (**a-b**), on soil quality (**c-d**) and on litter quality (**e-h**). X-axis units are back-transformed values, y-axis are back-transformed residuals of the target explanatory variable on the remaining explanatory variables. Plot decomposition, litter quality soil quality: % mass loss. Calcium, biomass N, fibres: % of total biomass. LDMC: mg g^{-1}.

Discussion

Here we disentangled the key drivers of litter decomposition in an experiment manipulating the direct (increase in soil N) and indirect (diversity and functional composition change) effects of N enrichment, which allowed us to compare the effects of different aspects of litter quality on decomposition. We also used a new approach to combine data from three types of litter bag in a structural equation model and partition the overall variation in decomposition into that due to changes in litter vs. soil quality. Our results show that the key determinant of litter quality was the functional composition of the plant community. Experimental manipulation of functional composition had a larger effect on decomposition than nitrogen fertilisation or manipulations of species and functional diversity. In addition to the litter quality effects, changes in soil quality were also important in affecting decomposition and were largely driven by microclimate effects in our experiment. Therefore, N

enrichment increases decomposition, mostly through indirect effects arising from a shift in functional composition towards faster growing plant species producing easily decomposable litter.

Functional composition is the main driver of litter quality

Litter quality was determined by leaf economics spectrum traits and we found that nutrient related traits were more important than leaf physical traits. Plant communities with biomass rich in N and Ca, with low fibre content and low LDMC produced the most degradable material but increases in nutrient contents were about twice as important as changes in structural components (combined path coefficients of 0.61 for N and Ca and -0.36 for LDMC and fibres). Overall, this result agrees with a large body of literature showing that fast growing plants have more decomposable litter (Cornwell et al. 2008; Reich 2014; Freschet et al. 2012). However, although nutrient and physical traits are both indicators of the resource economics spectrum, at some scales they are not strongly correlated (Anderegg et al. 2018). Our results suggest environmental change will have the largest effects on litter decomposition when it shifts functional composition towards plants with high tissue N concentrations. Effects of N have been shown in many studies (Garnier et al. 2004; Cornwell et al. 2008) and as pointed out in Mládková et al. (2018), Ca and Mg content (which were highly correlated in our case) also indicate a better digestibility and a higher decomposability of the litter (García-Palacios et al. 2016a). Ca and Mg are thought to have the largest effect on invertebrate decomposers as they are key components of invertebrate diets (National Research Council 2005). However, in our experiment high Ca content did not increase the effect of macrofauna on decomposition, suggesting that Ca may be important for microbes too. Litter structural components including both fibre content and LDMC were also important in determining decomposition. Most previous studies have focussed on LDMC but our results suggest that biomass fibre content added complementary information on structure, as some species had a low LDMC but still produced fibrous stems (see Figure S9). It is therefore important to measure stem in addition to leaf traits, as the two sets of traits may not correlate strongly. Our results show that several different nutrient and structure traits affect decomposition, which suggests that we need to disentangle the role of different resource economics traits in determining litter quality.

Litter diversity, calculated from the diversity of functional trait values within each community, did not have any effect on litter quality. In addition, none of the individual measures of diversity per plant trait had any effects on decomposition (see Table S3). Functional diversity might be of importance only in communities containing legumes, where a transfer of nutrients from the N-rich legume litter to more recalcitrant litter can increase decomposition (Handa et al. 2014). Our experimental design

may therefore have underestimated diversity effects. Our results do however agree with other studies which showed that functional composition is usually a good predictor of litter decomposition rate and that functional diversity is of secondary importance (see Finerty et al. 2016 and Bílá et al. 2014).

Soil quality and soil fauna effects are indirectly mediated by biomass Ca content and microclimate

Soil quality also affected the overall decomposition rate. Soil quality was influenced by biomass Ca and microclimatic conditions. We observed no direct effect of N enrichment, plant species richness, functional diversity or soil fauna on soil quality, all their effects were mediated through changes in plant cover (microclimate; see Figure 2). N enrichment had both positive direct and negative indirect effects (through increasing the negative effect of biomass N) on plant cover. The negative indirect effect on plant cover could be explained by the dry conditions in the first year of the experiment, which allowed conservative species (with low N contents) to establish better than faster growing species (see Figure S8). As microclimate had no impact on the relative effect of macro vs. microfauna it seems likely that an increase in humidity was of equal importance for all soil decomposers. In contrast to its positive effects through microclimate, biomass Ca reduced soil quality. This means that plant communities producing more digestible litter, with a higher Ca (and/or Mg) content, were growing on a soil which was poor at decomposing standard litter. This result could indicate that inputs of Ca-rich litter stimulated soil communities that were less effective at decomposing our relatively recalcitrant standard litter. Enzymes responsible for the breakdown of resistant material have been shown to be inhibited under N enrichment (Carreiro et al. 2000). Our results may indicate that these enzymes are also inhibited by inputs of Ca-rich litter. The various direct and indirect effects of N enrichment therefore had opposing effects on soil quality: a loss of species diversity, expected under N enrichment, would reduce soil quality but this effect would be compensated for by a direct increase of plant cover under fertilisation.

The relative effect of macrofauna on decomposition increased with biomass N and decreased with LDMC. The macro and mesofauna contribution to decomposition was therefore higher, relative to the effect of microfauna, when litter contained more easily degradable material. This means that high litter quality either increased the abundance of macrofauna, such as earthworms and isopods, or their efficiency in breaking down litter. Little is known about how a change in litter quality alters the effect of different soil fauna on decomposition but we can hypothesise that macrofauna are more active

when feeding on higher quality litter because they actively forage for nutrients and make them available for microorganisms (see Smith and Bradford 2003).

Partitioning the relative importance of litter and soil quality

We used a novel SEM approach, and three litter bag experiments, to partition the importance of soil and litter quality in affecting decomposition. The SEM was able to explain all of the effects of the experimental treatments, traits and microclimate on overall decomposition through our measures of soil and litter quality, and accounted for a third of the overall variation in decomposition rate (R2 0.272, see Tables S5). This shows that our approach is able to successfully partition overall decomposition into soil and litter quality effects and this also suggests that soil and litter quality had additive effects on overall decomposition. If the treatments, traits and microclimatic conditions caused strong interactions between soil and litter quality we would expect to see unexplained direct effects of these on overall litter decomposition. By combining our different litter bag experiments in a single model, we integrate all aspects of litter and soil quality together, allowing us to test for their relative importance without the need for a complete list of all litter and soil properties that could affect decomposition. Further studies using this approach could compare the effects of soil and litter quality on decomposition across environmental gradients to determine the global importance of these factors in determining litter decomposition.

In our experiment, the overall decomposition rate was more influenced by litter quality than by soil quality (see Figure 3). This agrees with studies in multiple biomes showing that litter traits are more important than the complexity of the decomposer community (García-Palacios et al. 2013) or soil properties in determining decomposition (García-Palacios et al. 2016b). However, other studies in boreal forests have found opposing patterns (Maaroufi et al. 2017). The lower importance of soil quality indicates either that litter quality is indeed more important, or that the effects of N enrichment, diversity and functional composition take longer to fully change soil communities (Eisenhauer et al. 2011; Boeddinghaus et al. 2019). Our experiment is still relatively young and whilst we created a large gradient in litter quality by design, differences in soil conditions emerge only in response to our treatments. We might expect that the effects of the experimental treatments on soil communities will strengthen over time (Eisenhauer et al. 2012), leading to greater differences in soil quality in the future. In addition, we measured litter mass loss after 2.5 months of decomposition. While some litter bags were almost empty at the end of the experiment, the results may only capture the early stage of decomposition in some plots. It would be interesting to determine the drivers of litter decomposition

at different stages of decomposition as the relative importance of soil and litter quality, and the factors determining them, might change over time (Smith and Bradford 2003).

Conclusion

We identified several different drivers of litter quality, with biomass nutrient concentrations being about twice as important as structural composition. This suggests that multiple traits are needed to properly characterise litter quality. Soil quality was mainly affected by microclimatic conditions, driven by changes in plant cover. Our study suggests that, at least for the early stages of decomposition, N enrichment will directly increase decomposition rates by increasing litter N content and by increasing biomass which promotes a microclimate favouring high soil faunal activity. It will indirectly affect decomposition through a shift in plant functional composition towards faster growing species, which will increase litter quality, and through a loss in plant species richness, which would mainly decrease soil quality through a reduction in plant cover. The relative importance of different drivers of decomposition under N enrichment might vary between ecosystems and further studies could use our approach to quantify the relative importance of soil and litter quality in different contexts. Nevertheless, the large effect of plant functional composition, seen in both biomass nutrients and structural components, indicates that it is among the major drivers to take into consideration when assessing overall N enrichment effects on decomposition.

Acknowledgments

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Supporting information

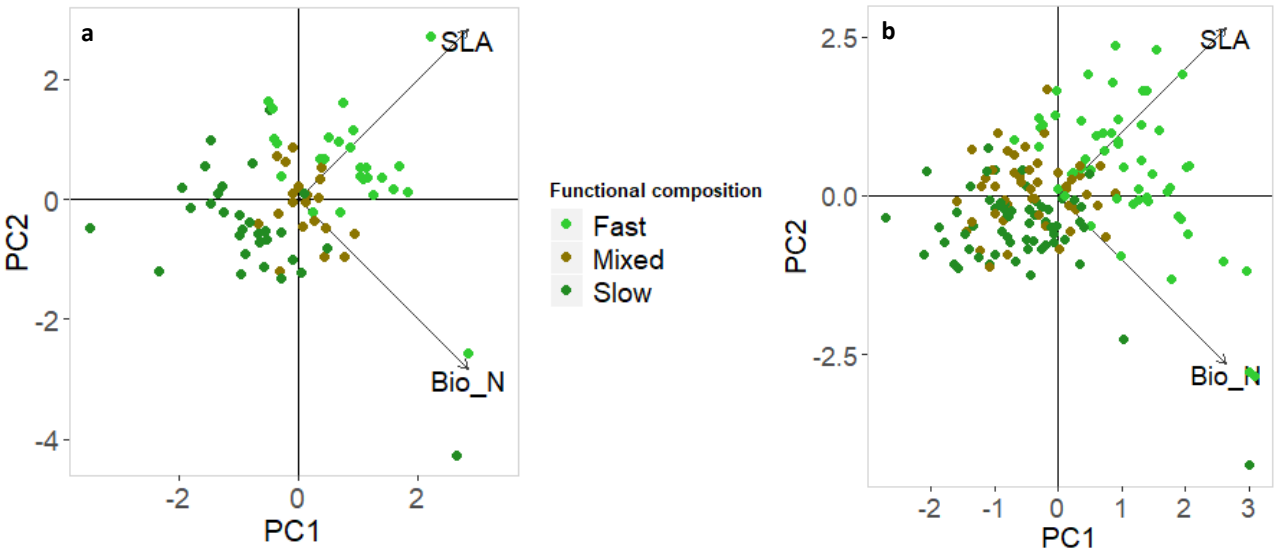


Figure S1: Community traits Specific Leaf Area (SLA) and biomass nitrogen for our 168 communities, divided by fast, mixed and slow plots. **a**) The sown community traits calculated using control monocultures SLA and biomass N (expected values). **b**) The community weighted mean traits calculated using control monocultures and species percentage cover per plot (realised values).

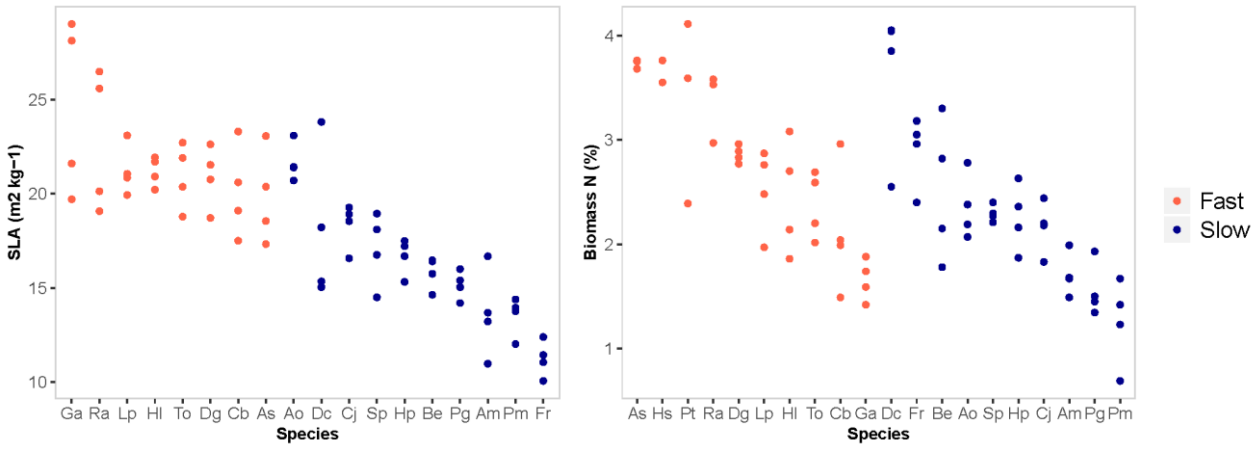


Figure S2: Specific leaf area and biomass N content measured per plot in the four monocultures. List of species:

Fast growing:
Anthriscus sylvestris (As), *Crepis biennis* (Cb), *Dactylis glomerata* (Dg), *Galium album* (Ga), *Holcus lanatus* (Hl), *Heracleum sphondylium* (Hs), *Lolium perenne* (Lp), *Poa trivialis* (Pt), *Rumex acetosa* (Ra) and *Taraxacum officinale* (To).

Slow growing:
Achillea millefolium (Am), *Anthoxanthum odoratum* (Ao), *Bromus erectus* (Be), *Centaurea jacea* (Cj), *Daucus carotta* (Dc), *Festuca rubra* (Fr), *Helictotrichon pubescens* (Hp), *Prunella grandiflora* (Pg), *Plantago media* (Pm) and *Salvia pratensis* (Sp).

Table S1: The PaNDiv Experiment manipulates species diversity, functional composition, nitrogen enrichment and foliar fungal pathogens in a full factorial design. The 4 and 8 species levels consist per treatment in 10 fast, 10 mixed and 10 slow communities. We were growing 10 fast and 10 slow monocultures and 4 replicates of the 20 species together. These 84 communities were sown in a control plot, with nitrogen, with fungicide, and with both nitrogen and fungicide. The total number of plots summed up to 336 divided in four blocks. Each block contained all 84 combination with a randomly attributed treatment.

Manipulation	**Levels**	
Species diversity (SD)	Monoculture	*20 combinations*
	4 species	*30 combinations*
	8 species	*30 combinations*
	20 species	*4 replicates*
Functional composition (FC)	Fast growing	
	Slow growing	
	Mix of both	
Nitrogen enrichment (Ni)	100 kg N ha^{-1} y^{-1}	
	no fertilisation	
Fungicide (Fz)	fungicide	
	no fungicide	

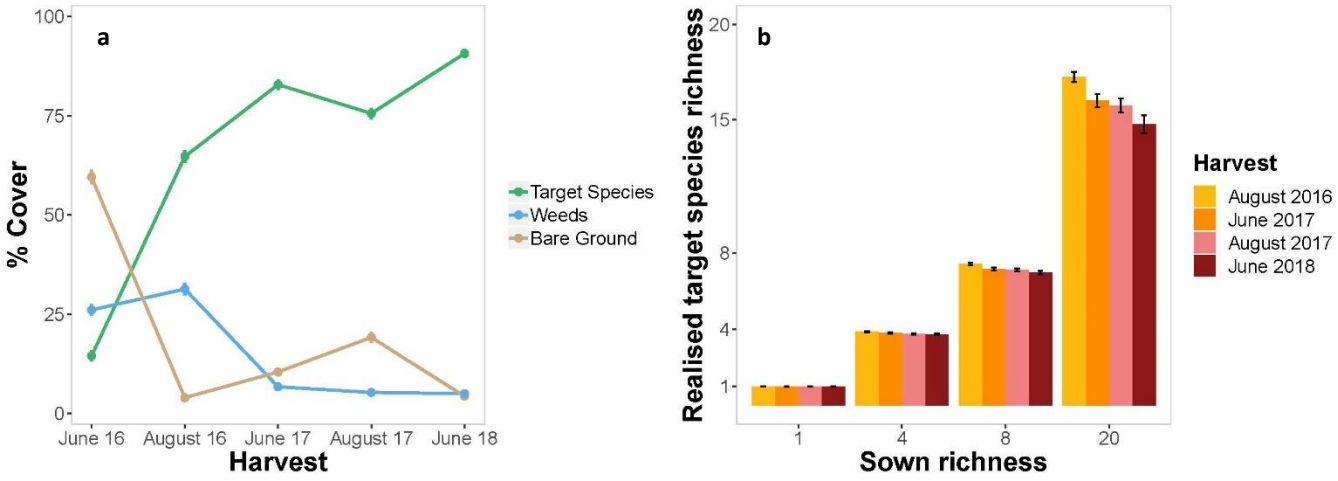


Figure S3: Field establishment across time. Percentage cover of target species, weeds and bare ground (**a**). Number of target species within each diversity level over time (**b**). Some species were resown once in spring 2016 because of poor establishment (*Heracleum sphondylium, Anthriscus sylvestris, Daucus carota, Salvia pratensis, Prunella grandiflora, Plantago media*), because they were mixed with other seeds to begin with (*Helictotrichon pubescens, Bromus erectus*) or because their seedlings froze in autumn or spring (*Holcus lanatus, Dactylis glomerata, Anthoxanthum odoratum*). No resowing was done after spring 2016.

Table S2: Linear mixed effect models standardised output. p-values were derived by dropping a term from the model and comparing models with and without the term of interest. Model simplification was done step wise and main effects that are part of significant interactions were therefore not dropped from the model, they are indicated as "marginal".

Simple model	Factor	Estimate	Std.Error	Pr(Chi)
Small Bags	(Intercept)	-0.04	0.11	
	Nitrogen	0.11	0.03	0.002
	Litter august	0.27	0.09	0.006
Medium Bags	(Intercept)	-0.06	0.07	
	Nitrogen	0.13	0.04	0.001
Big Bags	(Intercept)	0.76	0.19	
	Nitrogen	0.16	0.05	0.004
	Litter august	0.66	0.17	<0.001
Common Garden	(Intercept)	1.57	0.16	
	Nitrogen	0.12	0.05	0.02
	Litter august	0.88	0.18	<0.001
Standard Litter	(Intercept)	-1.15	0.09	
	Species richness	0.11	0.04	0.015

Combined model	Factor	Estimate	Std.Error	
Bag Type	(Intercept)	0.77	0.15	
	Nitrogen	0.11	0.03	0.002
	Mixed Plots	-0.11	0.16	
	Slow Plots	-0.26	0.15	marginal
	Litter quality	0.65	0.12	
	Soil quality	-1.78	0.12	
	Litter august	0.50	0.11	<0.001
	Mixed Plots * Litter quality	0.02	0.18	
	Slow Plots * Litter quality	0.12	0.17	0.035
	Mixed Plots * Soil quality	0.42	0.18	
	Slow Plots * Soil quality	0.47	0.17	
Mesh size	(Intercept)	0.57	0.12	
	Nitrogen	0.14	0.03	<0.001
	Medium bag	-0.40	0.05	<0.001
	Small bag	-0.54	0.05	
	Litter august	0.38	0.10	<0.001

Simple traits model	Factor	Estimate	Std.Error	
Small Bags	(Intercept)	-0.21	0.08	
	Nitrogen	0.09	0.03	0.007
	LDMC	-0.12	0.03	<0.001
Medium Bags	(Intercept)	-0.08	0.06	
	Nitrogen	0.13	0.04	0.001
	Species richness	0.01	0.06	marginal
	LDMC	-0.21	0.06	
	Species richness * LDMC	-0.15	0.07	0.033
Big Bags	(Intercept)	0.76	0.16	
	Nitrogen	0.13	0.05	0.019
	LDMC	-0.35	0.06	<0.001
	Litter august	0.65	0.18	<0.001
Common Garden	(Intercept)	1.45	0.13	
	Nitrogen	0.11	0.05	0.040
	LDMC	-0.47	0.06	<0.001
	Functional diversity	-0.16	0.07	0.017
	Litter august	0.61	0.18	0.001
	Initial litter	-0.15	0.06	0.015
Standard Litter	(Intercept)	-1.15	0.10	
	Functional diversity	0.10	0.04	0.007

Combined traits model	Factor	Estimate	Std.Error	
Bag Type	(Intercept)	0.61	0.12	
	Nitrogen	0.09	0.03	0.009
	Litter quality	0.69	0.06	marginal
	Soil quality	-1.49	0.06	

	LDMC	-0.39	0.05	
	Functional diversity	-0.06	0.06	
	Litter august	0.42	0.11	<0.001
	Litter quality * LDMC	-0.05	0.07	<0.001
	Soil quality * LDMC	0.49	0.07	
	Litter quality * Functional diversity	-0.12	0.07	<0.001
	Soil quality * Functional diversity	0.18	0.07	
Mesh size	(Intercept)	0.54	0.11	
	Nitrogen	0.13	0.03	<0.001
	Medium bag	-0.39	0.05	marginal
	Small bag	-0.54	0.05	
	LDMC	-0.38	0.05	
	Litter august	0.32	0.11	0.004
	Medium bag * LDMC	0.31	0.05	<0.001
	Small bag * LDMC	0.28	0.05	

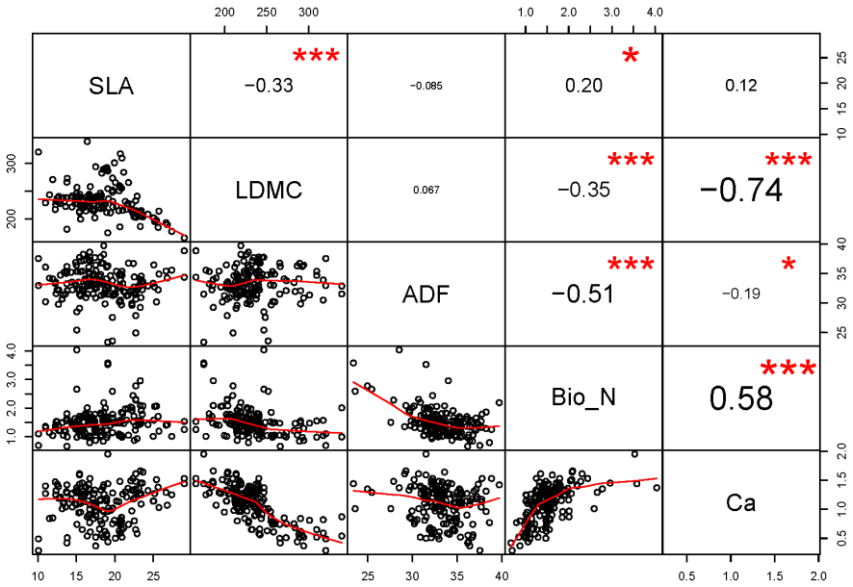


Figure S4: Spearman correlations between the community weighted mean measured traits specific leaf area and leaf dry matter content, and the NIRS (near infrared reflectance spectroscopy) analysed elements in the biomass, using the R package “PerformanceAnalytics". ADF = Acid Detergent Fibres. Calcium and LDMC do have a high correlation (-0.74) and we have to interpret results including both variables with caution, as we may underestimate their effects.

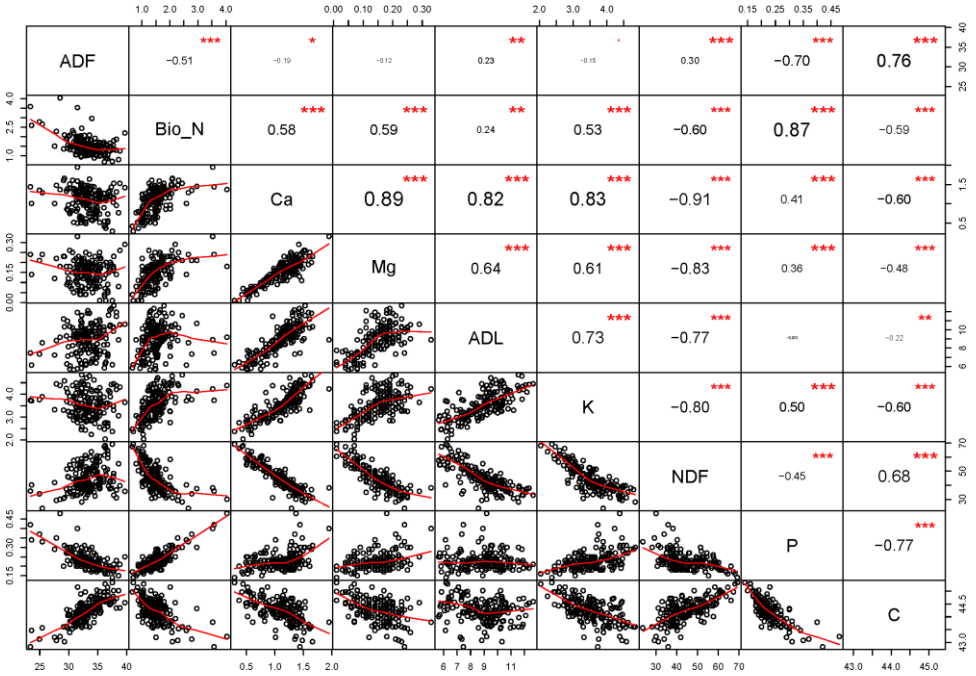


Figure S5: Spearman correlations between the NIRS analysed elements in the biomass, using the R package “PerformanceAnalytics". ADF = Acid Detergent Fibres; ADL = Acid Detergent Lignin; NDF = Neutral Detergent Fibres. We used ADF, biomass N and Ca in the structural equation model.

Table S3: Structural Equation Model with a functional diversity per trait (calcium, nitrogen and fibres). Standardised output using lavaan. We could not include functional diversity of LDMC nor of calcium as these measures were too correlated.
Model fit: Pvalue = 0.301, Chisq = 32.427, Df = 29, RMSEA = 0.027.

Response	Predictor	Estimate	Std.error	p.value	
Plot decomposition	Litter quality	0.937	0.091	0.000	***
	Soil quality	0.182	0.043	0.000	***
Litter quality	SLA	-0.102	0.052	0.050	.
	Biomass N	0.389	0.078	0.000	***
	Fibres	-0.131	0.055	0.016	*
	Calcium	0.203	0.079	0.010	*
	LDMC	-0.262	0.074	0.000	***
	Functional div SLA	-0.030	0.063	0.635	
	Functional div bio N	-0.107	0.058	0.065	.
	Functional div fibres	0.080	0.065	0.222	
Soil quality	Soil fauna	0.052	0.078	0.508	
	Nitrogen	-0.009	0.080	0.908	
	Microclimate	0.283	0.105	0.007	**
	SLA	0.048	0.088	0.588	
	Biomass N	-0.100	0.152	0.509	
	Fibres	-0.081	0.091	0.375	
	Calcium	-0.306	0.135	0.024	*
	LDMC	-0.124	0.123	0.316	
	Species richness	0.013	0.090	0.887	
	Functional div SLA	-0.036	0.106	0.736	
	Functional div bio N	-0.009	0.096	0.926	
	Functional div fibres	0.109	0.112	0.330	
Soil fauna	Species richness	0.003	0.067	0.963	
	Nitrogen	-0.104	0.057	0.067	.
	SLA	-0.161	0.071	0.024	*
	Biomass N	0.402	0.110	0.000	***
	Fibres	0.083	0.076	0.272	
	Calcium	-0.057	0.110	0.604	
	LDMC	-0.211	0.102	0.039	*
	Functional div SLA	0.128	0.088	0.143	
	Functional div bio N	-0.114	0.080	0.156	
	Functional div fibres	0.016	0.093	0.866	
SLA	Nitrogen	0.045	0.072	0.535	
	Species richness	-0.041	0.072	0.569	
Biomass N	Nitrogen	0.140	0.071	0.048	*
	Species richness	-0.141	0.071	0.047	*
Fibres	Species richness	0.033	0.078	0.671	
	Nitrogen	0.095	0.078	0.224	
Ca	Nitrogen	-0.021	0.079	0.795	
	Species richness	0.032	0.079	0.689	

LDMC	Species richness	-0.033	0.077	0.666	
	Nitrogen	-0.137	0.077	0.077	.
Functional div SLA	Species richness	0.473	0.066	0.000	***
	Nitrogen	0.000	0.066	0.995	
Functional div bio N	Species richness	0.321	0.074	0.000	***
	Nitrogen	-0.036	0.074	0.623	
Functional div fibres	Species richness	0.509	0.066	0.000	***
	Nitrogen	-0.009	0.066	0.891	
Microclimate	Species richness	0.148	0.067	0.027	*
	Nitrogen	0.230	0.057	0.000	***
	SLA	-0.192	0.064	0.003	**
	Biomass N	-0.693	0.098	0.000	***
	Fibres	-0.091	0.068	0.179	
	Calcium	0.333	0.098	0.001	**
	LDMC	-0.207	0.091	0.022	*
	Functional div SLA	-0.062	0.079	0.433	
	Functional div bio N	0.040	0.072	0.580	
	Functional div fibres	0.091	0.084	0.282	

Covariances		Estimate	Std.error	p.value	
Functional div bio N	Functional div fibres	0.448	0.071	0.000	***
Functional div SLA	Functional div bio N	0.295	0.063	0.000	***
	Functional div fibres	0.324	0.058	0.000	***
Biomass N	Calcium	0.558	0.082	0.000	***
	Fibres	-0.485	0.079	0.000	***
	LDMC	-0.375	0.074	0.000	***
SLA	Biomass N	0.212	0.066	0.001	**
Calcium	LDMC	-0.726	0.094	0.000	***
Fibres	LDMC	0.121	0.075	0.106	
SLA	LDMC	-0.238	0.071	0.001	**
Fibres	Calcium	-0.205	0.079	0.010	*
SLA	ADF	-0.103	0.071	0.147	
	Calcium	0.129	0.073	0.074	.
Plot decomposition	Soil fauna	0.427	0.060	0.000	***
	CG	-0.316	0.061	0.000	***
LDMC	Functional div SLA	-0.137	0.038	0.000	***
Biomass N	Functional div SLA	-0.086	0.033	0.010	**

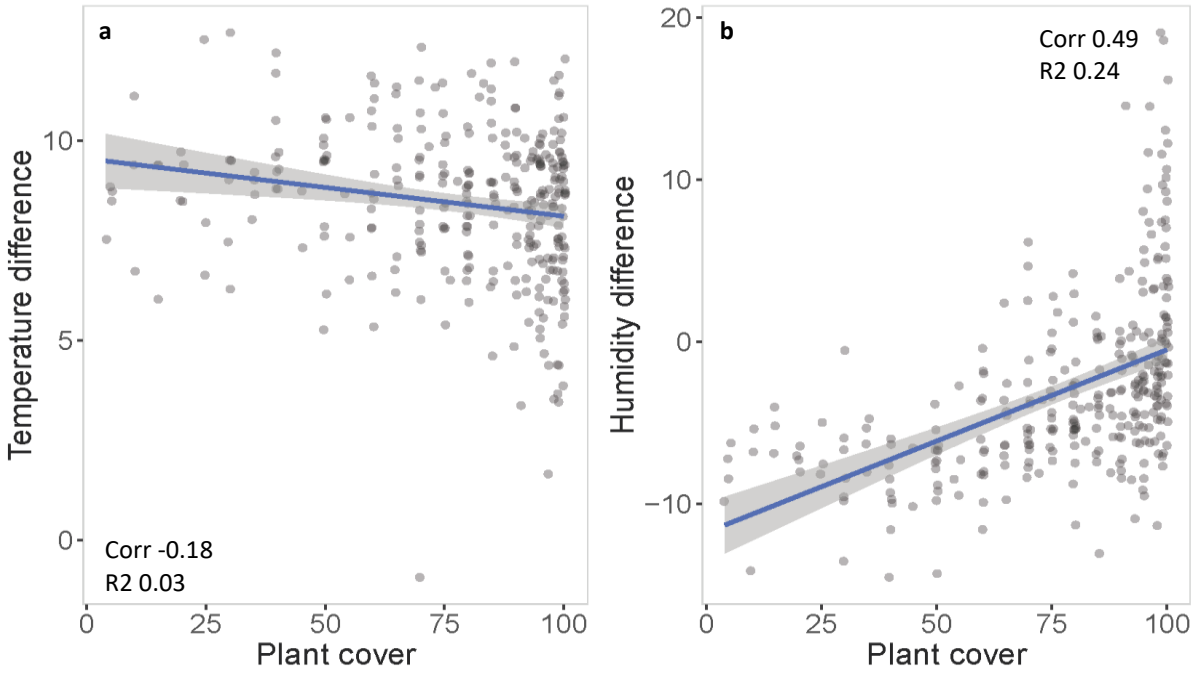


Figure S6: Effect of plant cover on microclimatic conditions: **a)** temperature and **b)** humidity. Plant cover was estimated in August 2018. The temperature and humidity were measured at 10 cm below maximum canopy and min 10 cm above ground, in July and August 2018 on each plot for minimum 2 days over a period of four weeks. Each point represents the mean deviation of the microclimatic temperature in the vegetation from the values recorded at the closest meteorological Station in Zollikhofen (ca. 4km away).

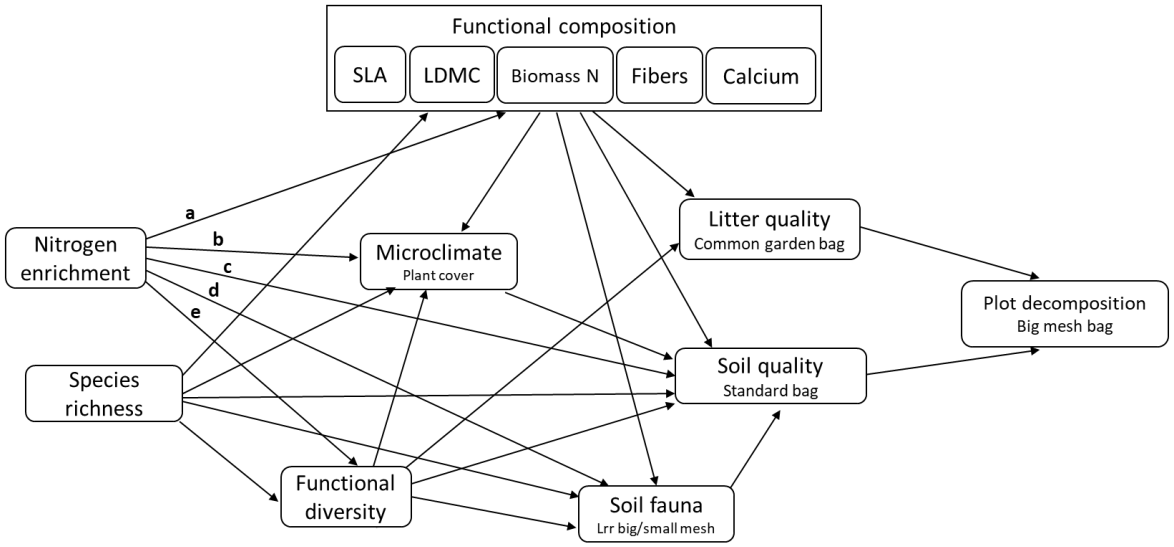


Figure S7: A priori model structure for the Structural Equation Model analysis. Hypothesis:

N enrichment shifts functional composition (**a**) and decreases functional diversity (**b**), affecting litter mediated decomposition. N enrichment affects soil quality through an increase of microclimatic conditions (**c**), shifts in soil fauna relative effect (**d**), or any other effect not taken into account here (**e**) for instance a change in available nitrogen. We expect species richness to influence the same variables. We included all these arrows because we know little about the importance of the different processes.

Functional composition and functional diversity affect litter mediated decomposition by changing litter decomposability, and soil through effects on microclimate, soil fauna or any other direct effect materialised by a direct arrow, as for N and SR.

The plot decomposition rate is affected via both litter mediated and soil mediated effect.

The final model included some correlations to increase the model fit, and because they are related to potential unmeasured variables that affected the outcome of the multiple experiments plugged in the model. We included a correlation between biomass nitrogen and functional diversity and between LDMC and functional diversity because of the way we coded monocultures (see main text). We also included a correlation between plot decomposition and soil fauna, because the fauna variable is calculated from plot decomposition. The last correlation is between litter quality and plot decomposition. In these two experiments, we used the same litter material (see main text).

Table S4: We fitted the model using piecewiseSEM (Lefcheck 2016) another package for Structural Equation Model computing several linear mixed effect models together. Unlike the lavaan package, it enabled us to include "Combination" as a random term. Each plot contains a different species combination, grown as a control or with nitrogen addition. If the random term is not included, it increases the species richness effect. Comparing lavaan and piecewiseSEM outputs informed us about the importance of this potential bias. We decided to use lavaan in the end because the differences between the packages were not major and because of lavaan's wide use. At the same time, the recent update of the piecewiseSEM package with unfixed issues made it difficult to work with. The results presented here are calculated using the earlier version 1.2.1.

When using the same initial model as for the lavaan code, the piecewiseSEM model was rejected (fit using fischer.c, p.value significant). The model fit improved when we added three residual covariances (p.value 0.117): between plot decomposition and calcium, biomass N, and fibres. However, the path coefficients did not change at all when we added these covariances.

In the piecewiseSEM output, three paths became non-significant: Litter quality ~ LDMC, Soil fauna ~ SLA and Microclimate ~ Species richness. Two other became marginally significant: biomass N ~ Species richness, Litter quality ~ Fibres. The differences are underlined in the table hereafter. The estimates of soil and litter quality on plot decomposition were lower in the piecewiseSEM output but their relative importance stays the same. On the other hand, the effect of species richness on biomass N and on Functional diversity was underestimated in lavaan. Although the model output differed slightly between the two packages, the overall conclusions of our study remains the same.

Response	Predictor	Estimate	Std.error	p.value	
Plot decomposition	Litter quality	0.652	0.061	0.000	***
	Soil quality	0.130	0.060	0.033	*
Litter quality	Biomass N	0.335	0.085	0.000	***
	Ca	0.323	0.100	0.002	**
	Fibres	**-0.120**	**0.062**	**0.060**	
	LDMC	**-0.138**	**0.101**	**0.180**	
	SLA	-0.056	0.070	0.430	
	Functional diversity	-0.012	0.068	0.863	
Soil quality	Microclimate	0.296	0.099	0.004	**
	Ca	-0.390	0.148	0.011	*
	LDMC	-0.204	0.133	0.132	
	Fibres	-0.065	0.090	0.475	
	Soil fauna	0.041	0.080	0.610	
	Species richness	0.047	0.088	0.639	
	SLA	-0.035	0.085	0.681	
	N	-0.033	0.082	0.685	
	Biomass N	-0.053	0.141	0.714	
	Functional diversity	-0.014	0.094	0.885	
Soil fauna	LDMC	-0.307	0.133	0.024	*
	Biomass N	0.277	0.123	0.027	*
	SLA	**-0.111**	**0.085**	**0.198**	
	N	-0.067	0.079	0.402	
	Ca	-0.111	0.144	0.447	

	Species richness	0.046	0.089	0.647	
	Functional diversity	-0.030	0.095	0.760	
	Fibres	0.002	0.091	0.981	
SLA	N	0.030	0.024	0.207	
	Species richness	0.039	0.148	0.791	
Biomass N	N	0.138	0.058	0.020	*
	Species richness	**-0.226**	**0.120**	**0.066**	
Fibres	Species richness	0.098	0.100	0.347	
	N	0.054	0.073	0.460	
Ca	N	-0.029	0.062	0.645	
	Species richness	0.045	0.120	0.708	
LDMC	Species richness	-0.232	0.140	0.101	
	N	-0.030	0.039	0.442	
Functional diversity	Species richness	0.837	0.117	0.000	***
	N	-0.015	0.021	0.466	
Microclimate	Biomass N	-0.710	0.098	0.000	***
	N	0.264	0.063	0.000	***
	Ca	0.447	0.115	0.000	***
	SLA	-0.120	0.068	0.083	
	Species richness	**0.148**	**0.071**	**0.106**	
	Fibres	-0.110	0.073	0.141	
	LDMC	-0.128	0.106	0.236	
	Functional diversity	0.091	0.076	0.245	

Covariances		Estimate	p.value	
Biomass N	Ca	0.631	0.000	***
	Functional diversity	0.025	0.378	
	LDMC	-0.403	1.000	
	Fibres	-0.466	1.000	
Fibres	Ca	-0.162	0.980	
LDMC	Fibres	0.098	0.109	
	Functional diversity	-0.057	0.764	
	Ca	-0.700	1.000	
SLA	Ca	0.231	0.002	**
	Biomass N	0.177	0.012	*
	Fibres	-0.059	0.772	
	LDMC	-0.636	1.000	
Plot decomposition	Soil fauna	0.643	0.000	***
	Litter quality	0.610	0.000	***
	Biomass N	0.249	0.001	***
	Ca	0.248	0.001	***
	Fibres	-0.216	0.997	

Table S5: Structural Equation Model standardised output using lavaan.

Response	Predictor	Estimate	Std.Err	P(>|z|)
Plot decomposition	Litter quality	0.933	0.092	0
	Soil quality	0.192	0.042	0
Litter quality	Functional diversity	-0.019	0.05	0.705
	SLA	-0.023	0.05	0.645
	Biomass N	0.344	0.077	0
	Fibres	-0.163	0.055	0.003
	Ca	0.262	0.084	0.002
	LDMC	-0.197	0.08	0.014
Soil quality	Soil fauna	0.045	0.083	0.59
	N enrichment	-0.033	0.079	0.674
	Microclimate	0.328	0.106	0.002
	SLA	-0.035	0.082	0.669
	Biomass N	-0.057	0.149	0.701
	Fibres	-0.066	0.089	0.453
	Ca	-0.389	0.142	0.006
	LDMC	-0.206	0.13	0.112
	Functional diversity	-0.014	0.092	0.877
	Species richness	0.047	0.085	0.578
Soil fauna	Species richness	0.038	0.059	0.513
	N enrichment	-0.101	0.052	0.054
	SLA	-0.146	0.063	0.021
	Biomass N	0.331	0.1	0.001
	Fibres	0.063	0.07	0.369
	Ca	-0.088	0.108	0.414
	LDMC	-0.269	0.101	0.008
	Functional diversity	-0.031	0.07	0.659
SLA	N enrichment	0.021	0.079	0.787
	Species richness	-0.008	0.079	0.922
Biomass N	N enrichment	0.14	0.07	0.047
	Species richness	-0.141	0.071	0.046
Fibres	N enrichment	0.052	0.077	0.5
	Species richness	0.07	0.078	0.364
Ca	N enrichment	-0.02	0.079	0.799
	Species richness	0.031	0.079	0.693
LDMC	N enrichment	-0.042	0.078	0.589
	Species richness	-0.128	0.078	0.1
Functional diversity	Species richness	0.507	0.066	0
	N enrichment	-0.022	0.066	0.745
Microclimate	Species richness	0.134	0.062	0.031
	N enrichment	0.239	0.056	0
	SLA	-0.108	0.059	0.07
	Biomass N	-0.696	0.094	0

Fibres	-0.1	0.065	0.123
Ca	0.403	0.101	0
LDMC	-0.117	0.095	0.216
Functional diversity	0.084	0.068	0.215

Covariances		Estimate	Std.Err	P(>|z|)
Biomass N	LDMC	-0.385	0.075	0
	Ca	0.548	0.082	0
	Fibres	-0.455	0.077	0
Fibres	Ca	-0.179	0.078	0.023
	LDMC	0.11	0.075	0.145
Ca	LDMC	-0.758	0.097	0
SLA	LDMC	-0.325	0.081	0
	Biomass N	0.232	0.072	0.001
	Fibres	-0.093	0.078	0.234
	Ca	0.131	0.08	0.1
Plot decomposition	Soil fauna	0.394	0.056	0
	Litter quality	-0.331	0.063	0
LDMC	Functional diversity	-0.155	0.041	0
Biomass N	Functional diversity	-0.109	0.039	0.006

R square	
Plot decomposition	0.272
Litter quality	0.531
Soil quality	0.158
Soil fauna	0.149
SLA	0.004
Biomass N	0.047
Fibres	0.01
Calcium	0.001
LDMC	0.02
Functional diversity	0.266
Microclimate	0.464

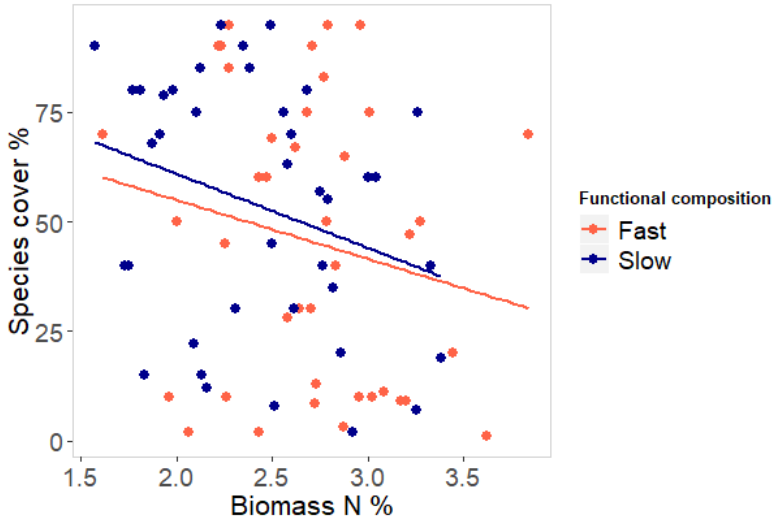


Figure S8: Correlation in August 2016 between species percentage cover and biomass N content (%) in the monocultures fast and slow growing plots. At the beginning of the experiment, species with low biomass N content established better than species with high biomass N content.

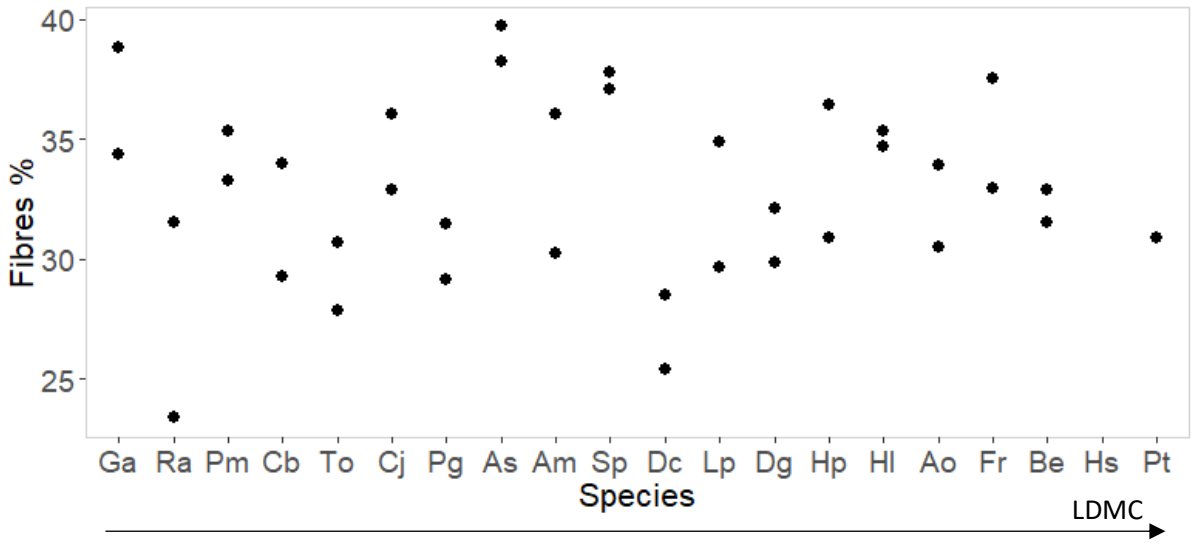
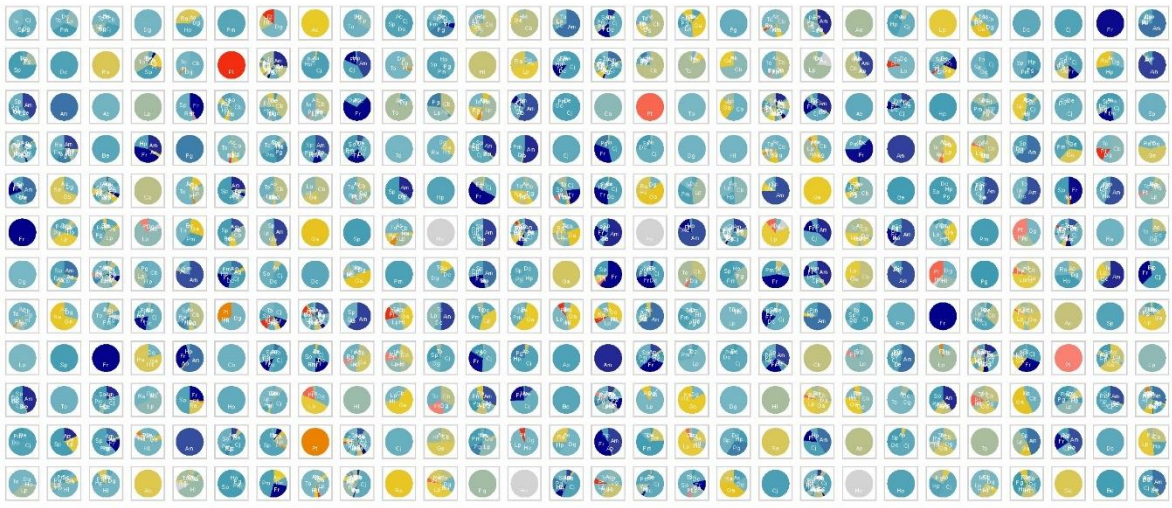


Figure S9: Percentage fibre content (acid detergent lignin) in the monoculture plots biomass. Species are ranked from left to right in increasing order of LDMC. Some species like *Galium album* or *Plantago media* present both a relatively low LDMC and a large fibre content.

Chapter 4

Response and effect of intraspecific trait changes

Noémie A. Pichon, Seraina L. Cappelli and Eric Allan



Abundance weighted specific leaf area overview of the PaNDiv experiment, from slow growing (blue) to fast growing species (red)

Summary

Plant species functional composition has a large effect on ecosystem functions, and responds to global change. In particular, nitrogen enrichment shifts functional composition and leaf economic traits from dominance by slow to fast growing plants. This change in mean functional trait values can be due to a shift in species identity, a shift in species relative abundance and a shift in intraspecific trait values. We know little about the relative importance of these three shifts in determining community responses to global change and effects on function. We quantified the relative importance of composition, abundance and intraspecific shifts for specific leaf area (SLA) and leaf dry matter content (LDMC). We collected leaf samples in a large grassland experiment, factorially manipulating functional composition, plant species richness, nitrogen enrichment and foliar fungal pathogen removal. We fitted structural equation models to test the relative importance of abundance shifts, intraspecific shift and sown trait composition in contributing to overall variation in community weighted mean traits and aboveground and belowground biomass production. We found that intraspecific shifts were as important as abundance shifts in determining overall community weighted mean traits and even had large effects relative to a wide initial gradient in trait composition. Intraspecific trait shifts tended to cause convergence towards intermediate SLA values in diverse communities, although the degree of convergence was reduced by nitrogen addition and enhanced by pathogen removal. In contrast, intraspecific shifts in LDMC were large but were not influenced by the treatments. Belowground biomass was increased by SLA and reduced by LDMC, while aboveground biomass increased in communities dominated by high SLA species. However, despite large intraspecific trait shifts, intraspecific variation in these traits had no effect on above or belowground biomass production. Our results add to a growing amount of literature showing large intraspecific trait variation and emphasise the importance of using field sampled data to determine community composition. However, they also show that intraspecific variation is not important for ecosystem functioning and therefore trait response-effect relationships may differ between vs. within species.

Introduction

The functional composition of plant communities responds to environmental change and influences ecosystem functioning (Lavorel and Grigulis 2012; de Vries et al. 2012; Allan et al. 2015). Functional composition can be measured in various ways but one of the key axes of plant functional variation is the leaf economics spectrum (Wright et al. 2004; Díaz et al. 2015). The leaf economics spectrum distinguishes slow growing species with low leaf nutrients, tough and long-lived leaves from fast growing species with high leaf nutrient contents, soft and short-lived leaves. It is indicated by several traits, particularly specific leaf area (SLA), leaf dry matter content (LDMC) and leaf nitrogen contents. The leaf economics spectrum traits predict community shifts in response to environmental variation, in particular a shift towards fast species in fertile, nitrogen rich environments and therefore an increase in the mean SLA and a decrease in the mean LDMC of communities (Allan et al. 2015; Lavorel and Grigulis 2012). Community functional composition is typically quantified as a community weighted mean (CWM), i.e. the mean trait value across species in the community, weighted by species abundances. In addition to predicting responses to the environment, the leaf economics spectrum traits have strong effects on a number of ecosystem functions. In particular, slow growing species (with low SLA and high LDMC) produce less aboveground biomass and are associated with slow biogeochemical cycling but potentially high belowground biomass production due to different resource allocation patterns between resource-use strategies (see Freschet et al. 2015; Mahaut et al. 2019). In contrast, fast growing species (high SLA and low LDMC) produce more biomass and promote fast cycling. N enrichment shifts community composition from slow to faster growing plant communities and could do so via a direct increase in resources. However, N enrichment can also indirectly affect ecosystems via changing diversity (Allan et al. 2015) or by altering consumer groups (Tylianakis et al. 2008) and these indirect effects could also alter community functional composition.

Shifts in community functional composition (CWM) with environmental change can occur through three mechanisms: changes in species identity, a shift in the relative abundances of species with particular traits (hereafter abundance shift) or an intraspecific shift in traits between individuals (intraspecific shift) (Lepš et al. 2011). Intraspecific shifts can occur either through plastic changes in trait expression or through genetic changes (Geber and Griffen 2003). It is commonly assumed that intraspecific shifts will have a smaller effect than abundance shifts on community mean trait values (Siefert et al. 2015; Garnier et al. 2001a; Albert et al. 2011b). However, experiments have revealed a large variation in certain functional traits among individuals of the same species (Violle et al. 2012; Jung et al. 2010; Albert 2015), particularly for specific leaf area (SLA) (Mitchell et al. 2017; Derroire et

al. 2018). How the intraspecific shift and abundance shift affect community weighted mean traits in different growth conditions is still unclear (Roscher et al. 2018).

Nitrogen enrichment can directly and indirectly affect functional composition. Nitrogen addition typically increases community SLA by favouring the establishment and growth of faster growing species (Wright et al. 2004), but also because individual species produce leaves with higher SLA (Siefert and Ritchie 2016; Shipley and Almeida-Cortez 2003). Nitrogen enrichment could also indirectly affect intraspecific trait expression and indirectly result in abundance shifts. Nitrogen enrichment causes a loss of plant diversity and changes in consumer abundance, which might modify functional composition. A loss of species richness might reduce intraspecific SLA values due to lower light competition in species poor communities (Lipowsky et al. 2015). A change in the abundance of foliar fungal pathogens with nitrogen enrichment (Dordas 2008) might also affect plant traits. By attacking high nutrient leaves, fungal pathogens could reduce community SLA either by reducing the relative abundance of fast growing species or by causing species to shift trait expression towards lower SLA. The direct and indirect effects of N on functional composition could also differ between fast and slow communities if trait variability differs between the two types of species. High SLA species which are more competitive for light might show higher plasticity and intraspecific variation in SLA (Crick and Grime 1987; Freschet et al. 2015). However, the amount of intraspecific variation in SLA along the leaf economics spectrum is not well known. Testing how intra and abundance shifts in traits relate to environmental factors, and how this depends on the initial functional composition of the community, requires experimental manipulation of communities differing in leaf economic traits.

Shifts in community functional composition can have large effects on a range of ecosystem functions (Allan et al. 2015; Lavorel and Grigulis 2012; Ratcliffe et al. 2017; Díaz et al. 2007 and the two other chapters of this thesis). However, many studies looking at the effects of changes in functional composition on ecosystem function calculate functional shifts using literature or monoculture data, which ignores intraspecific variation (Violle et al. 2012). Many other studies use in situ measured traits but do not test how important intraspecific changes are relative to compositional or abundance changes in affecting ecosystem functioning (Roscher et al. 2018). It is known that intraspecific shifts can explain a large proportion of community trait variation, however, the importance of intraspecific changes in affecting ecosystem functioning is still poorly known and under debate (Albert et al. 2011b). Intraspecific changes in traits might not have the same effect on ecosystem functioning as interspecific shifts in abundance or composition because the strong interspecific correlations between leaf economic traits break down within communities (Anderegg et al. 2018; Messier et al. 2017), which could be due to lower variation in leaf life span within communities (Funk and Cornwell 2013). As functional composition has a large effect on ecosystem functioning it is crucial to understand how

environmental change alters the components of functional composition - intraspecific and abundance shifts - and how this translates into changes to ecosystem functioning.

To quantify the extent of intraspecific shifts in response to the direct and indirect effects of N and the consequences of these shifts for ecosystem functioning, we conducted a field experiment manipulating N enrichment, species richness, functional composition and fungicide application. We therefore manipulated direct effects of N and two indirect effects (loss of diversity and changes in consumer communities). We also independently manipulated the mean SLA of communities by changing species composition, which also created a large gradient in initial LDMC. Communities were initially sown with species at equal abundance, but abundances and intraspecific trait expression could shift freely within and between communities. We can therefore quantify the contribution of shifts in abundance and intraspecific trait expression to changing community mean traits, how large this variation is relative to the compositional variation between plots and whether the degree of intraspecific and abundance shifts depends on the initial (sown) trait composition. We fitted two structural equation models for SLA and LDMC, first to quantify the intraspecific and abundance shifts due to N enrichment, fungicide and species richness, depending on the initial trait composition, and how these contribute to variation in community weighted mean traits between communities. We then fitted a second pair of SEMs linking intra and abundance shifts to above and belowground biomass production. To test the effect of intraspecific shift on biomass production, previous studies calculated the effect for individual species of intraspecific shift on biomass (Liancourt et al. 2015), or compared models fitting community weighted mean calculated using traits of different origins (Roscher et al. 2018). Our approach has the advantage of jointly estimating the relative response and effect of each component of traits shifts.

Within this framework, we tested the following hypothesis:

1) Intraspecific shifts in trait expression and shifts in the relative abundance of species are similarly important in determining the overall variation in community weighted mean traits between communities
2) N enrichment, species richness and fungicide application increase SLA and decrease LDMC
3) Both intraspecific shifts in trait expression and shifts in relative abundances affect ecosystem functioning

Material and Methods

Field site

We conducted the study in the PaNDiv experiment, a large grassland experiment located in München-buchsee, Switzerland. The PaNDiv experiment started in autumn 2015 and manipulated plant functional composition, species richness, N enrichment and fungicide application in a full-factorial design. We assembled plant communities using a pool of 20 species common in central European grasslands. We divided the species into two groups according to their SLA and leaf N content. The two groups contained both herbs and grasses, and were classified as having a slow growing (low SLA, low N content), or fast growing strategy (high SLA, high N). Experimental communities contained one, four or eight species and at the four and eight species levels, communities contained either slow or fast growing species, or a mix of both (monocultures could contain only one functional strategy). The species in each community were randomly drawn from the particular species pool (i.e. all species, slow or fast growing), for a total of 50 different combinations. This created a large gradient in mean SLA values (15 to 29 m^2 kg^{-1}) and mean LDMC values (208 to 290 mg g^{-1}) between communities, which is comparable to trait values along a central European land use intensity gradient (SLA: 13-32 m^2 kg^{-1}, LDMC: 220-420 mg g^{-1}, Breitschwerdt et al. 2018). Each combination of species was grown four times; in control conditions, with N enrichment (100 kg N ha^{-1} y^{-1} as urea twice a year in April and June), with fungicide application ("Score Profi", 24.8 % Difenoconazol 250 $g.L^{-1}$, four times during the growing season) and with both N and fungicide together. Communities were sown on 2x2m plots with equal numbers of seeds per species, corrected for germination rates (total 1000 seedlings m^{-2}). The plots were randomly distributed in four blocks, each containing every species combination, each time with a randomly assigned treatment.

The site was weeded and ploughed before the start of the experiment. To maintain the different diversity levels, we weeded each plot three times a year in April, July and September, enabling us to keep total weed abundance to only 5% of total cover on average. The whole field was mown in mid June and late August and the biomass was removed, which simulates extensive to intermediate grassland management in the Swiss lowlands. Further information about the design of PaNDiv and field characteristics can be found in Pichon et al. 2019 (the third chapter of this thesis).

We harvested above ground biomass production before mowing in August 2017 on two quadrats of 20x50 cm in the centre of each plot, clipping vegetation above 5 cm. The samples were dried at 65°C

for 48h and weighed. Percentage cover of target species, weeds and bare ground, was recorded at the same time. Aboveground biomass production was corrected for weed cover by multiplying the biomass by the proportion of target (non-weed) species. Belowground biomass was measured in autumn 2017 by taking two cores per plot to 20 cm depth (440 cm3 of soil). We homogenised the two samples and used a subset of 40g fresh soil in which we washed and sorted out the roots. We then dried the roots at 65°C for 48 hours. To calculate belowground biomass per g of dry soil, we estimated soil bulk density by weighing 40g of soil from the same plot before and after drying for 24 hours at 105°C. Aboveground biomass data were square-root transformed and belowground biomass data were log-transformed in order to meet the models assumptions described in the analysis sub-section.

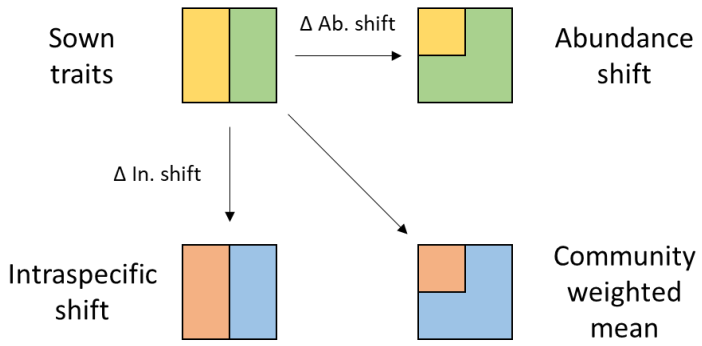


Figure 1:

Schematic description of the components of community weighted mean traits, for a community of two species, represented by coloured rectangles. Shifts in community weighted mean traits are due to intraspecific shifts (Δ in. shift, represented by a change in colours) and to shifts in species relative abundances (Δ ab. shift, represented by a change in the size of the rectangles).

Leaf sampling

We collected leaf samples on the 200 plots over two weeks in August 2017, taking five leaves per species per plot. Specific leaf area and leaf dry matter content were measured following the protocol of Garnier et al. (2001b). We measured leaf fresh weight and recorded the leaf area with a leaf area meter (LI-3000C, LI-COR 253 Biosciences) after overnight rehydration in the dark. We dried the samples at 65°C for two days and measured their dry weight. We calculated SLA (area/dry weight) and LDMC (dry/fresh weight) per species per plot by averaging the values of the five samples. Two species, *Anthriscus sylvestris* and *Heracleum sphondylium* had extremely low rates of germination across the whole field. Because of a lack of plants for these two species, we excluded them completely from the community trait calculations.

In order to characterise community functional composition and compare the changes in traits due to shifts in species relative abundance or in intraspecific trait values, we calculated a set of community trait metrics (Figure 1). 1) The **sown trait** value per plot = $\sum_i^n x_{i0}/n$; with x_{i0} being the trait value of species *i* in the control monocultures and n the number of species per plot. We use this as the baseline measure, as this indicates the trait value in control (unfertilised, no fungicide) conditions and for species experiencing intraspecific competition only. By comparing realised trait values with those expected from control monocultures we can assess the effects of nitrogen, fungicide, diversity and the functional composition of interspecific competitors on trait values. 2) The **abundance shift** per plot = $\sum_i^n p_i\, x_{i0}$; with p_i being the relative abundance of species *i* in a given plot, and x_{i0} the trait value of species *i* in the control monoculture. 3) The **intraspecific shift** per plot = $\sum_i^n x_i/n$; with x_i being the trait value of the species *i* measured in a particular plot and n the number of species in a given plot. 4) The overall **community weighted mean** = $\sum_i^n p_i\, x_i$; with p_i the relative abundance of the species *i* and x_i the trait value of species *i* per plot. In the following analyses we investigated the effects of our experimental treatments on shifts in community traits due to abundance only, i.e. the abundance shift (Δ abundance shift = abundance shift - sown), and due to intraspecific shifts only (Δ intraspecific shift = intraspecific shift - sown). Therefore, this dataset did not include monocultures, as the Δ were equal to 0 by definition in a monoculture.

Seven species did not establish or were present at a very low percentage in 36 plots, and traits of these species could not be sampled (44 of 716 data points). Although this did not influence abundance shift and community weighted mean calculations, we replaced these values with monoculture data in the intraspecific shift calculations and therefore made the conservative assumption that the intraspecific shift would have been 0 for these species.

Analysis

We tested how our different factors influenced the variation in community mean SLA and LDMC, and how it translated into ecosystem functions by fitting different models: first a set of linear mixed effect models, then a set of structural equation models (Grace 2006). All analysis were conducted in R (Bates et al. 2015; R Core Team 2019).

Interactions between all of the experimental treatments are plausible, however, incorporating large numbers of interactions in SEMs is challenging. As there is no theory to predict which interactions should be most important, we used linear mixed effects models to determine which interactions

should be fitted in the SEMs. We fitted four linear mixed models, testing the effect of nitrogen enrichment, fungicide, plant species richness, sown mean traits, and all two-fold interactions, as fixed effects, on the trait abundance shifts (Δ abundance shift in SLA and LDMC) and intraspecific shifts (Δ intraspecific shift in SLA and LDMC). The models contained block and species composition (the randomly assembled sets of species) as random terms. The data were not transformed because the errors were normally distributed and the variance homogenous. We simplified the initial models using likelihood-ratios to drop terms that did not significantly improve overall model fit.

We fitted two different structural equation models for each trait. We first tested the relative importance of intraspecific and abundance shifts in determining community weighted mean traits and then tested the effect of the two shifts on ecosystem functions. In the first set of models, we fitted direct paths from nitrogen enrichment, species richness, fungicide and sown traits to the Δ abundance shift and the Δ intraspecific shift, with the hypothesis that nitrogen, richness and fungicide would increase the two Δ SLA shifts and decrease the two Δ LDMC shifts. We added interactions between sown SLA and the three other factors, following the lmer output (Table S1), to test how the treatment effects differed between plots dominated by species with different growth strategies. To assess the relative importance of community composition, abundance shifts and intraspecific shifts in determining overall functional composition, we then fitted direct paths from the sown trait values, and the two Δ, to the community weighted mean trait. The second set of SEMs tested the effect and relative importance of the different factors and aspects of functional composition on above and belowground biomass. The structure was similar, but we fitted paths to above ground and belowground biomass rather than community weighted mean traits. We added paths from nitrogen, fungicide and species richness to aboveground and belowground biomass, to include direct (hypothesised positive) effects of these factors that would not occur through changes in SLA or in LDMC.

Results

We observed large intraspecific trait and abundance changes, which altered community weighted mean trait values, depending on the treatments. However, the observed intraspecific trait shifts did not translate into changes in ecosystem functioning.

Trait responses to the experimental treatments

Community weighted mean (CWM) values of SLA and LDMC were affected by compositional variation (the sown community values) but also by intraspecific and abundance shifts (Figure 2 and Table S2 and 3). For both traits, intraspecific shifts affected the final CWM traits to a similar extent as abundance shifts, which means that there was substantial intraspecific variation in these two plant traits. For LDMC the intraspecific shifts even had a similar magnitude of effect on the CWM as the interspecific compositional variation between the plots (path coefficient of 0.6±0.05 for sown LDMC and 0.53±0.05 for intraspecific shifts), while for SLA the compositional variation had the same effect as intra and abundance shifts together (path coefficient for sown SLA of 0.98±0.04, intraspecific shift 0.44±0.03 and abundance shift 0.54±0.04).

Plant species richness, nitrogen addition and fungicide application, in interaction with sown SLA, all caused intraspecific shifts in SLA (Figure 2a) but had no effect on intraspecific shifts in LDMC (Figure 2b). In slow plots, those with low sown SLA, species on average increased their SLA compared to monoculture values. In fast plots, N enrichment resulted in intraspecific shifts towards higher SLA and fungicide towards lower SLA, while N and fungicide had no significant effect in plots sown with slow species (Figure 3). These intraspecific shifts increased community SLA overall, but in plots without N and with fungicide application, intraspecific shifts resulted in a homogenisation of community towards intermediate values of SLA (Figure 4). Intraspecific shifts towards higher SLA in the slow growing communities only occurred in 8 species plots, in plots with 4 species intraspecific shifts slightly increased SLA regardless of the sown SLA.

Abundance shifts tended to decrease mean SLA and LDMC of communities, particularly at high sown values of the traits. Communities sown with generally high SLA species became more strongly dominated by the species with a lower SLA, leading to a reduction in CWM SLA in communities with high sown SLA. This also caused convergence towards lower SLA values between

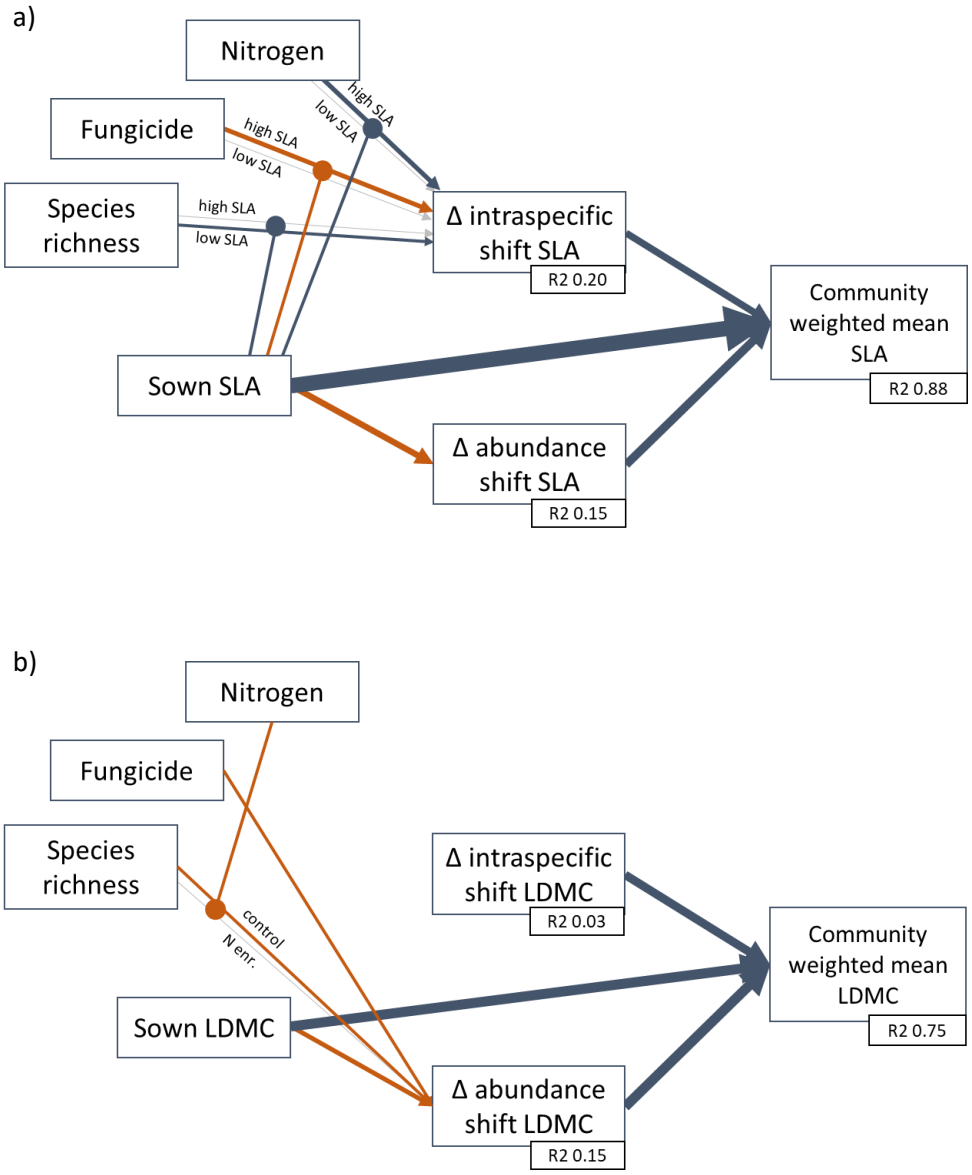


Figure 2: Structural equation models. Effect of nitrogen, fungicide, species richness and sown trait mean on community weighted mean trait values through a shift in species abundance (Δ abundance shift) or in intraspecific trait values (Δ intraspecific shift). a) Final model for specific leaf area (SLA), model fit: Pvalue = 0.999, Chisq = 14.865, Df = 35, RMSEA = 0.000. b) Final model for leaf dry matter content (LDMC), model fit: Pvalue 0.458; Chisq 22.034; Df 22; RMSEA 0.000. The interactions are plotted as partial plots in figure 3. The effect of nitrogen, fungicide and species richness on the intraspecific shift SLA depended on the initial sown community, and the effect of richness on abundance shift LDMC depended on nitrogen. These interactions are represented here by round-headed arrows. Blue shows a positive effect, red a negative effect. The size of the arrows is proportional to the path coefficient.

communities (Figure 4). In communities sown with high LDMC species, it was also the low LDMC species that dominated (Figure 3). Whereas the abundance shifts that altered SLA could not be explained by any of the other treatments, fungicide application led to abundance shifts that reduced mean LDMC. This indicates that species with a relatively low LDMC dominated in plots where foliar fungal pathogens were removed. In addition, species richness led to abundance shifts reducing LDMC in unfertilised plots, whereas N dampened this effect.

We noticed that the R^2 for community weighted mean SLA and LDMC were 0.88 and 0.75 respectively. The reason why they were not equal to one is that there are interactions between the different components on the community trait shifts. Including an interaction between Δ interspecific and Δ abundance shift increased the R^2, still not until one. The remaining variation could be due to other, possibly higher order interactions between the factors.

Effects of trait shifts on ecosystem function

Whereas intraspecific trait shifts explained the same amount of variation in CWM trait values as abundance shifts, this did not translate into an effect of intraspecific shifts on ecosystem functioning (Figure 5-6 and Table S4-5). Only abundance shifts affected above and belowground biomass. Shifts in species abundances towards higher SLA increased aboveground biomass production and decreased belowground production, indicating contrasting effects of SLA on these two ecosystem functions (Figure 6a and b). Compositional variation was also important as a high sown SLA further decreased belowground biomass, although sown SLA had no effect on aboveground biomass production, after taking the abundance shifts into account. Similarly, LDMC only affected functioning through shifts in abundance. Shifts in species abundances towards higher mean LDMC increased belowground biomass (Figure 6c and d), indicating that plots dominated by slow growing species had higher root production, which agrees with the SLA effect. High values of sown LDMC also increased belowground biomass. None of the measures of LDMC affected aboveground biomass and in the LDMC SEM, aboveground biomass was only directly affected by nitrogen enrichment. Species richness had no direct effect on functioning, however, this is probably because we only included data from the 4 and 8 species plots, which means there was very low variation in species richness.

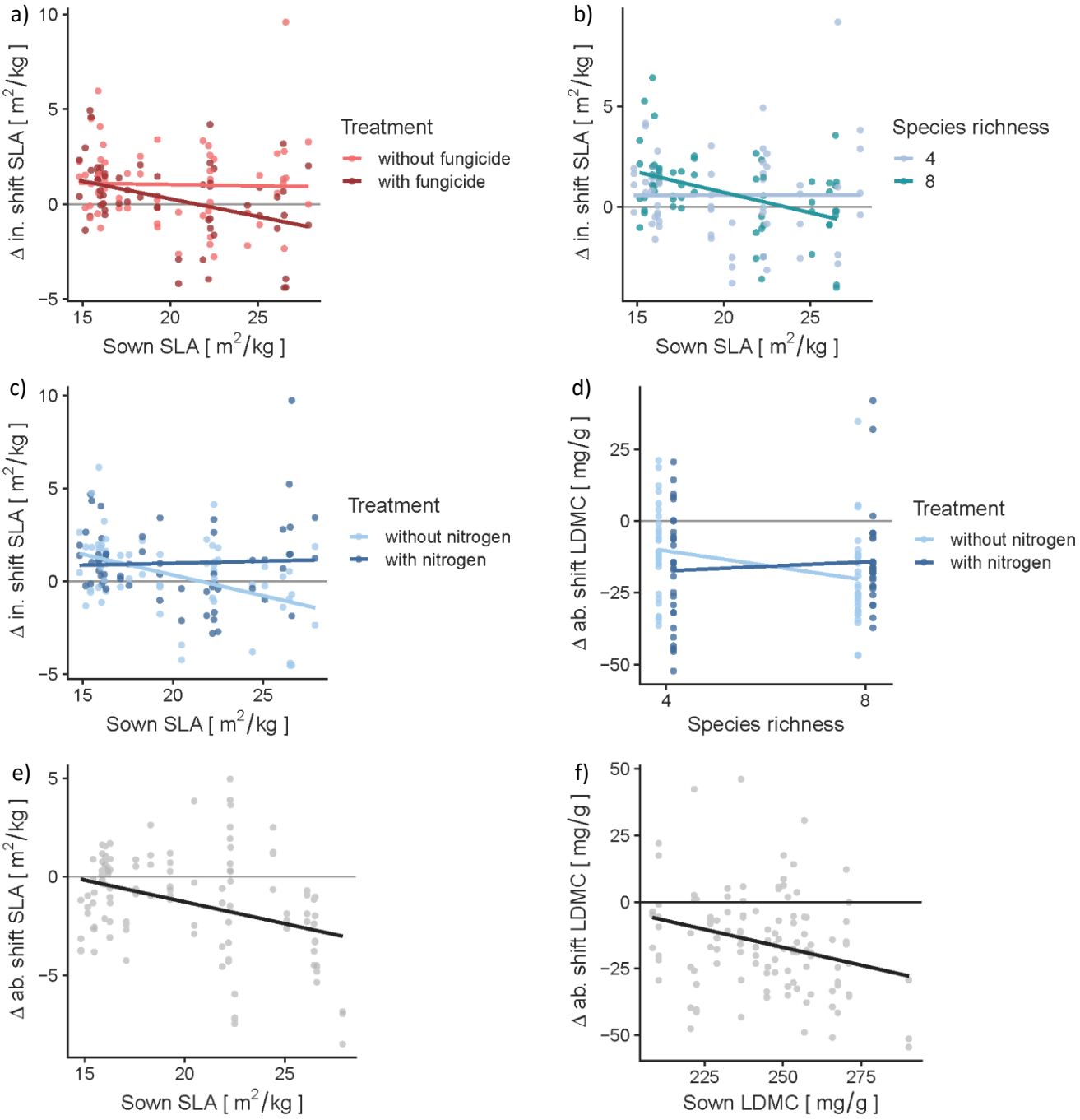


Figure 3: Partial plots visualising the Structural equation models output (Figure 2). Effect of sown specific leaf area (SLA) on the difference between intraspecific shift and sown SLA (Δ in. shift SLA) depending on **a)** fungicide, **b)** species richness and **c)** nitrogen. **d)** Effect of species richness on the difference between abundance shift and sown LDMC (Δ ab. shift LDMC) depending on nitrogen enrichment. Effect of sown trait on the difference between abundance shift and sown trait (Δ ab. shift) **e)** for SLA and **f)** for LDMC. The 0 line indicates no changes from the sown values. X-axis units are back-transformed values, y-axis are back-transformed residuals of the target explanatory variables on the remaining explanatory variables.

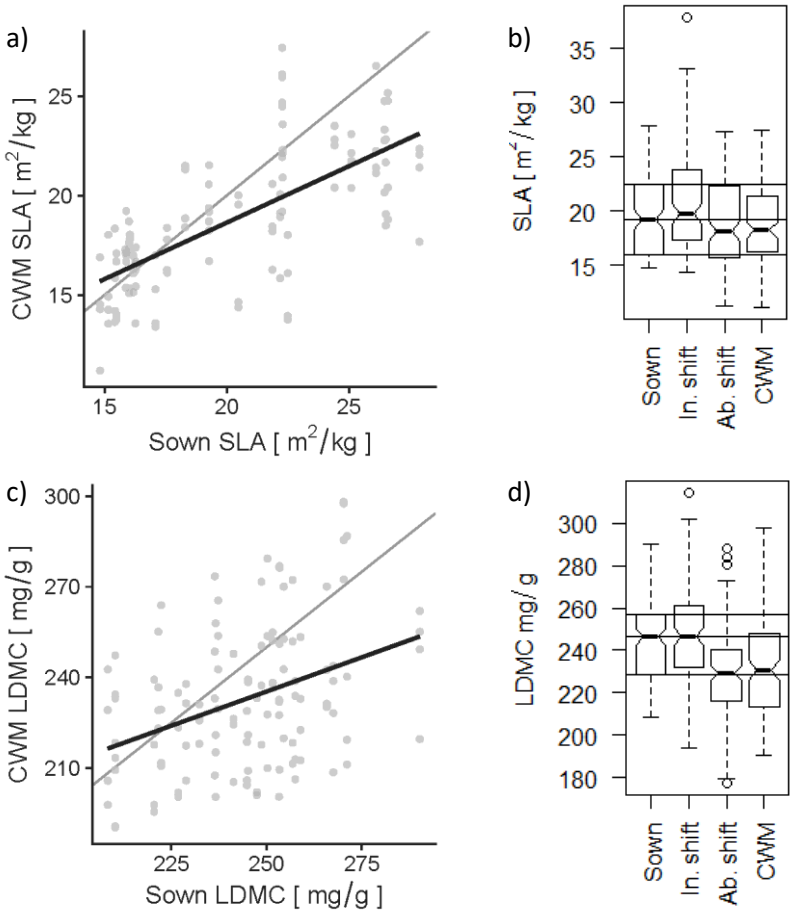


Figure 4: Effect of sown trait on the community weighted mean (CWM) trait, **a)** for specific leaf area (SLA) and **c)** for leaf dry matter content (LDMC). The 1:1 line is pictured in light grey. The amount of variation in community mean (**b**) SLA and (**d**) LDMC is represented by the adjacent boxplots, showing the difference in community mean when calculated using sown values, intraspecific shift, abundance shift or community weighted mean values.

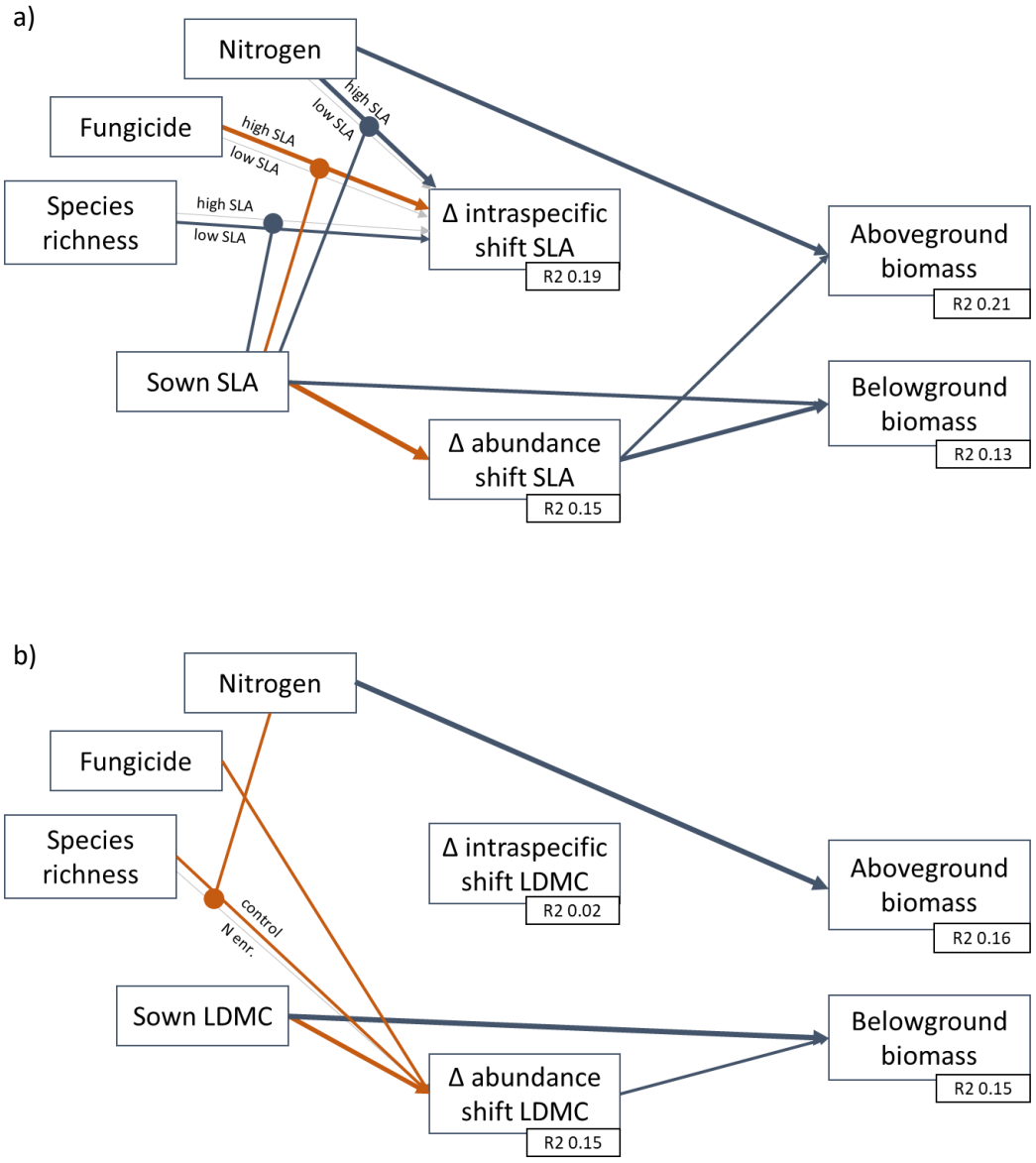


Figure 5: Structural equation models. Effect of nitrogen, fungicide, species richness and sown trait mean on aboveground and belowground biomass. Effects are direct or through a shift in species abundance (Δ abundance shift) or in intraspecific trait values (Δ intraspecific shift). a) Final model for specific leaf area (SLA), model fit: Model fit: Pvalue = 1.000, Chisq = 7.7733, Df = 33, RMSEA = 0.000. b) Final model for leaf dry matter content (LDMC), model fit: Pvalue = 1.000, Chisq = 1.479, Df = 12, RMSEA = 0.000. The interactions are plotted as partial plots in figure 3. The effect of nitrogen, fungicide and species richness on the intraspecific shift SLA depended on the initial sown community, and the effect of richness on abundance shift LDMC depended on nitrogen. These interactions are represented here by round-headed arrows. Blue shows a positive effect, red a negative effect. The size of the arrows is proportional to the path coefficient.

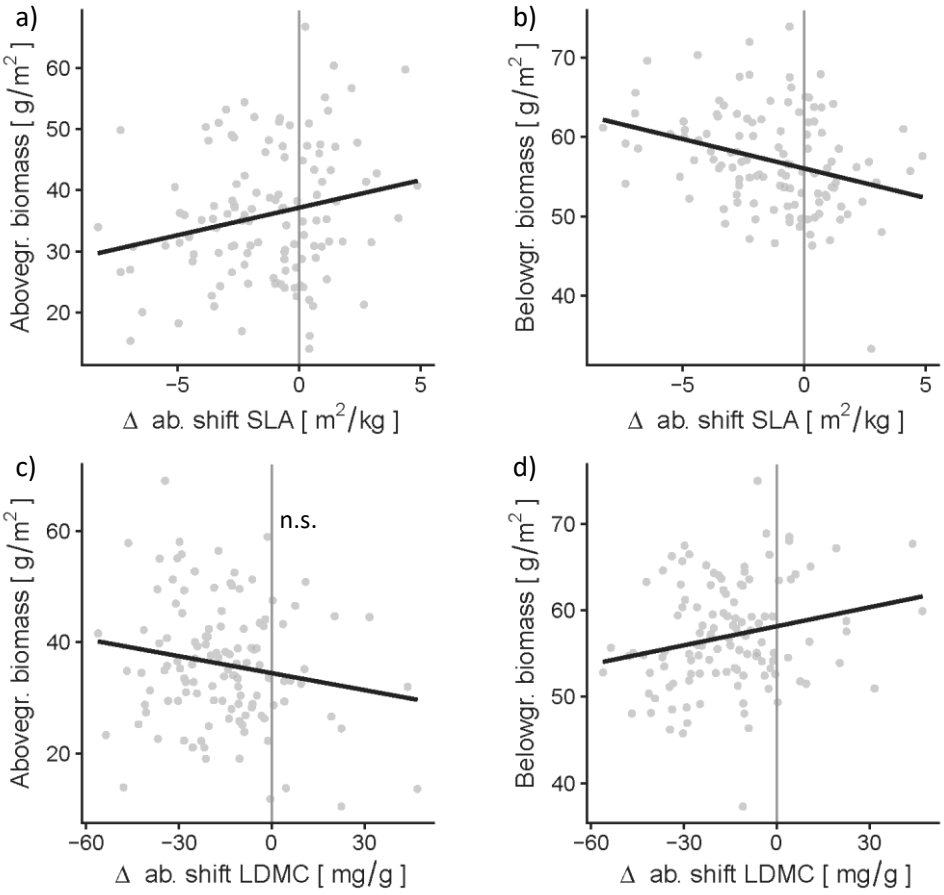


Figure 6: Partial plots visualising the Structural equation models output (Figure 5). Effect of the difference between abundance shift and sown specific leaf area (Δ ab. shift SLA) on **a)** aboveground biomass and **b)** belowground biomass. Effect of the difference between abundance shift and sown leaf dry matter content (Δ ab. shift LDMC) on **c)** aboveground biomass (non-significant) and **d)** belowground biomass. The 0 line indicates no changes from the sown values. X-axis units are back-transformed values, y-axis are back-transformed residuals of the target explanatory variables on the remaining explanatory variables.

Discussion

Intraspecific trait shifts have large effects on overall functional composition

Our results showed that changes in functional composition due to abundance and intraspecific shifts were of similar importance in explaining overall community weighted mean trait measures. This agrees with an increasing volume of literature on the importance of intraspecific variation in SLA (Lepš et al. 2011; Violle et al. 2012; Albert et al. 2011b), but also for LDMC, which is often considered less plastic than SLA (Garnier et al. 2001b). Considering the small scale at which we measured traits, on only 20 species all growing within the same field, the large contribution of intraspecific, compared to abundance, shifts to overall community weighted means could be expected (Petruzzellis et al. 2017; Cordlandwehr et al. 2013). However, we experimentally created a gradient in mean leaf traits, which is similar to the variation between communities occurring along a large gradient in land-use intensity (Breitschwerdt et al. 2018) and deliberately selected a pool of species to cover a large range in SLA, from slow to fast growing plants. Therefore, even relative to this large initial variation in trait composition, intraspecific trait shifts explained a large amount of overall functional variation. This large contribution of intraspecific variation challenges the idea that within species shifts in traits only matter at small scales. Our findings suggest that assessment of functional composition should use field measured trait data rather than only database trait values.

The abundance and intraspecific shifts caused large changes in functional composition relative to the originally sown communities. Shifts in species abundances decreased both mean SLA and LDMC across the whole field. In our experiment, grasses and herbs differ strongly in LDMC and high mean LDMC values indicates a larger relative cover of grasses (Figure S1). The decrease in both SLA and LDMC due to abundance shifts therefore indicates that slow growing herbs (low SLA, low LDMC) dominated the experimental communities. Intraspecific shifts resulted in overall higher SLA in slow and fast communities. Intraspecific shifts quantify changes in SLA from monoculture to mixtures. An overall increase in intraspecific shift SLA could therefore be due to higher competition for light in mixtures (Lipowsky et al. 2015). The intraspecific change in SLA was however modulated by N enrichment, fungal pathogen presence and species richness, as we will discuss further below. Opposing intraspecific and abundance shifts led to an overall convergence in community weighted mean SLA towards intermediate values. This could reflect the fairly fertile but water limited conditions on our field site. All of the traits were measured in high summer after the first cut of the experiment, which could explain the reduction in abundance shift SLA, as the dry and warm conditions would favour

species or individuals with low SLA values (Poorter et al. 2009). These results show that intraspecific trait shifts can cause large changes in functional composition but that changes due shifts in abundance and intraspecific trait values can be decoupled.

The environmental conditions causing trait shifts

Intraspecific trait shifts occurred in both fast and slow dominated communities. In their analysis, Freschet et al. (2015) hypothesised that acquisitive (fast) species should be more plastic than conservative (slow) ones in response to short-term environmental variation. However, we found that both fast and slow species communities changed their intraspecific SLA values, although they did so to different extents depending on the environmental conditions. Contrary to SLA, intraspecific shifts in LDMC were not influenced by any of our experimental treatments. The total amount of intraspecific variation in LDMC was equivalent to variation due to composition and to abundance shifts but we could not explain any of the intraspecific variation with our treatments. Intraspecific shifts in LDMC could have been driven by variation in microclimatic conditions, like local water availability, between plots. Intraspecific shifts in both traits could be driven by both, genetic differences between individuals in the different experimental plots and by plastic changes in trait expression (Geber and Griffen 2003). A large amount of initial genetic variation in LDMC could therefore explain the variation in LDMC between communities, if random processes led to differential establishment of genotypes in the plots. However, it seems unlikely that genetic variation alone could explain such large intraspecific variation, and different environmental factors driving different plastic shifts in the various plots might be a more plausible explanation (Siefert et al. 2015; Lajoie and Vellend 2018). Intraspecific shifts in traits occurred in both fast and slow communities showing that intraspecific trait variation is high across many different species.

Exclusion of fungal pathogens affected intraspecific shifts in SLA. In communities sprayed with fungicide, SLA values converged towards intermediate values due to an increase in slow communities and a decrease in fast communities. The intraspecific shifts reflected the change in SLA from monocultures to mixtures and therefore the trait changes associated with a shift from intra to interspecific competition. Mayfield and Levine (2010) suggested that competition could lead to either trait divergence when traits are linked to niche differences, or trait convergence when they are linked to fitness differences. The presence of fungal pathogens can reduce competitive interactions between species in plant communities (Chesson 2000; Mordecai 2011) and previous studies have suggested that SLA is linked to fitness rather than niche differences (Kraft et al. 2015). Removing pathogens might

therefore have increased competition in our communities and led to convergence in SLA in fungicide sprayed communities.

Fungicide spraying further decreased LDMC. This result is in line with the growth-defence trade-off (Heckman et al. 2019). Fungicide favoured the establishment and growth of low LDMC species (fast growing strategy along the leaf economics spectrum) because their energy was invested more in growth than in defence. The reason why we observe this abundance shift in LDMC and not in SLA could be because LDMC reflects structural components of the leaves that contribute physical defence against herbivores and pathogens, and might therefore correlate more with defence-associated costs than SLA (Descombes et al. 2017; Ibanez et al. 2013). Fungicide therefore contributes to the overall reduction in CWM traits by decreasing both SLA and LDMC due to intraspecific shifts and abundance shifts respectively.

Nitrogen addition led to intraspecific shifts in SLA but did not favour high SLA species in general. Although N enrichment directly increased above ground biomass, it did not increase mean SLA through shifts in abundance, contrary to our expectations (Lavorel and Grigulis 2012). This result indicates that N enrichment did not favour fast species in particular, suggesting further limitations than N availability, or that SLA responded to overall fertility but not to particular nutrients. Due to the dry conditions of the field in high summer, we could hypothesise that fast growing plots were further limited by water availability and did not establish better under the N treatment. Nitrogen enrichment and diversity caused intraspecific trait shifts and affected the degree of convergence in trait values between fast and slow communities. Nitrogen only increased SLA in fast growing communities, in slow growing communities SLA increased regardless of whether communities were fertilised or not. Nitrogen addition therefore reduced the convergence in SLA between communities and led to general increases in SLA. This suggests that fast species reduced their SLA when growing in mixtures compared to monocultures, but that N enrichment dampened this effect by increasing SLA. This increase in SLA could also be due to a shift of optimal values towards higher SLA under N enrichment. We also found that trait convergence occurred only in eight species communities, not in plots with only four species. Lipowsky et al. (2015) also found greater intraspecific shifts in SLA at higher species richness. The greater convergence at higher species richness could have been caused by greater trait variation and therefore more opportunities for trait shifts in eight species communities. This would be a type of sampling effect, where eight species communities are more likely to contain species with high genetic variation in traits or with high trait plasticity. The convergence in SLA due to intraspecific shifts was dampened by N and increased by species richness, but these mechanisms were not reflected in community composition due to abundance shift.

Effect on biomass

Although intraspecific shift in SLA and LDMC had a large effect on community weighted mean values, this did not translate into a change in above or in belowground biomass production. This result runs counter to the study by Liancourt et al. (2015) showing an effect of plastic response of SLA on biomass production. However, this study looked at species level biomass production in a naturally occurring community under different treatments, which makes it difficult to compare with overall community production. Roscher et al. (2018) showed no significant difference in model fits when calculating CWM effect on biomass using traits measured in monocultures or in mixtures. The model fit even decreased when CWM was calculated with traits measured on individuals. Their findings suggest, in line with ours, that abundance shifts related more to biomass production than intraspecific shifts. This result tends to indicate a decoupling of leaf traits and resource use strategy within species, in line with recent studies (Anderegg et al. 2018; Messier et al. 2017). The leaf economics spectrum relies on trade-offs between high photosynthetic rates due to high leaf area per unit of invested mass, related to exploitative high SLA strategies, and a long leaf lifespan that can compensate in time for lower photosynthetic rates, related to conservative low SLA strategies (Wright et al. 2004). In their study, Funk and Cornwell (2013) showed that the importance of leaf traits in relating to resource use strategies depends on the amount of variation in leaf lifespan within a community. Therefore, the relationship would break down at a certain scale. We show here that even within a large gradient in trait variation and between different nutrient, pathogen presence and richness treatments, the changes in SLA due to intraspecific shifts did not affect functioning. Accounting for changes in leaf traits due to intraspecific shift reduced the link between community trait mean and the considered functions.

Root biomass production increased in communities at the slow end of the leaf economics spectrum. Sown SLA and abundance shift SLA decreased belowground biomass, meaning that communities sown with fast growing species produced less root biomass than those sown with slow species, and that communities dominated by faster species additionally produced less root biomass. The effect of SLA on root biomass production is usually not assessed or depends a lot on the species or ecosystem, as root and leaf economics spectra tend not to be coupled (Mommer and Weemstra 2012; Bergmann et al. 2017). We observe here that slow growing species invested more in roots than fast growing ones. Plants adapted to resource poor environments would be expected to invest more resources belowground, as this would give them an advantage under dry or low nutrient conditions (Freschet et al. 2015). Slow species investing more in roots would also explain the positive effects of sown and abundance shift LDMC on root biomass, as LDMC reflects slow strategies. In addition, due to the

correlation between LDMC and the grass percentage cover in our field, this also reflects a higher root production by grasses (Gastine et al. 2003; Ravenek et al. 2014). Therefore, an overall reduction in community SLA and LDMC due to abundance shifts had contrasting effects on belowground biomass production.

Aboveground biomass production was affected by SLA but abundance shift and compositional variation had opposing effects. Where plots became dominated by higher SLA species (positive abundance shift), above ground biomass production increased. However, sown community SLA had a negative indirect effect on biomass (by reducing SLA through abundance shifts) and we could see some evidence for a negative direct effect (marginally significant, see Table S4). This means that sown SLA decreased aboveground biomass production, which is opposite to expectations (Lavorel and Grigulis 2012). The effect is likely due to the better establishment of slow species at the beginning of the experiment. Slow growing, low SLA species established better in the first year when the plots were very open, with large fractions of bare soil leading to strong water limitation, probably because of their higher investment in root biomass. This initial advantage for slow species means that plots with only fast species present (high sown SLA) still produce less biomass overall. However, the communities that were increasingly dominated by faster growing species, i.e. in which SLA increased due to abundance shift, produced higher aboveground biomass. These contradictory results draw attention to the importance of initial establishment conditions (as in Mahaut et al. 2019 for instance) and suggest that relationships between effect traits and functions may change during community reassembly.

Conclusion

Intraspecific changes in resource economics traits had large effects on overall functional composition. Intraspecific shifts had as large effect on community weighted mean traits as abundance changes, showing that measuring traits in situ is important to accurately measure functional composition. Intraspecific trait shifts tended to lead to convergence in functional composition between diverse communities, however, the degree of convergence depended on resource levels and enemy abundance. Interspecific competition therefore seems to select for particular strategies. Although large, this intraspecific trait shift did not translate into an effect on above or belowground biomass production. Intraspecific trait variation may therefore have different effects on function compared to interspecific trait differences. The lack of a functional effect of intraspecific trait changes, cautions that response and effect trait correlations may differ between and within species.

Supporting information

Table S1: For specific leaf area (SLA) and leaf dry matter content (LDMC), lmer outputs testing the presence of interactions between nitrogen (N), species richness (SR), fungicide (Fng) and sown traits on the difference between sown trait and intraspecific shift mean (Δ intraspecific shift), and sown trait and abundance shift mean.

Δ intraspecific shift SLA				
Factor	**Estimate**	**Std.Error**	**Pr(Chi)**	
(Intercept)	0.004	0.258		
Nitrogen	0.217	0.072		
Sown SLA	-0.186	0.088		
Species richness	0.028	0.088		
Fungicide	-0.180	0.071		
Sown SLA x SR	0.186	0.071	0.009	**
Sown SLA x SR	-0.191	0.088	0.028	*
Sown SLA x Fng	-0.181	0.071	0.011	*

Δ abundance shift SLA				
Factor	**Estimate**	**Std.Error**	**Pr(Chi)**	
(Intercept)	0.000	0.156		
Sown SLA	-0.359	0.156	0.021	*
Species richness	-0.070	0.156		
Fungicide	0.052	0.047		
SR x Fungicide	0.098	0.047	0.039	*

Δ intraspecific shift LDMC				
Factor	**Estimate**	**Std.Error**	**Pr(Chi)**	
(Intercept)	0.000	0.234		
Fungicide	0.150	0.078	0.05	.

Δ abundance shift LDMC				
Factor	**Estimate**	**Std.Error**	**Pr(Chi)**	
(Intercept)	0.000	0.131		
Nitrogen	-0.029	0.076		
Sown LDMC	-0.286	0.111	0.011	*
Species richness	-0.096	0.111		
Fungicide	-0.159	0.076	0.034	*
N x SR	0.176	0.076	0.020	*

Table S2: Structural equation model output: effect of N enrichment, species richness (SR), fungicide (Fng) and sown specific leaf area (Sown SLA) on the shift of community mean SLA due to abundance shift (Δ ab. shift) and to intraspecific shift (Δ in. shift), and of sown SLA and the Δs on community weighted mean (CWM) SLA.

Model fit: Pvalue = 1.000, Chisq = 9.522, Df = 32, RMSEA = 0.000.

Response	Predictor	Estimate	Std.Err	P(>|z|)	
CWM SLA	Sown SLA	0.975	0.036	0.000	***
	Δ in. shift SLA	0.436	0.034	0.000	***
	Δ ab. shift SLA	0.538	0.035	0.000	***
Δ in. shift SLA	N	0.144	0.082	0.077	
	SR	0.028	0.082	0.736	
	Fng	-0.170	0.082	0.037	
	Sown SLA	-0.186	0.082	0.023	
	Sown SLA x N	0.228	0.082	0.005	**
	Sown SLA x SR	-0.191	0.082	0.020	*
	Sown SLA x Fng	-0.161	0.082	0.050	*
Δ ab. shift SLA	N	0.058	0.084	0.490	
	SR	-0.070	0.084	0.408	
	Fng	0.052	0.084	0.535	
	Sown SLA	-0.359	0.084	0.000	***
	SR x Fng	0.097	0.084	0.249	

Covariances		Estimate	Std.Err	P(>|z|)
Sown SLA	Sown SLA x N	0	0.09	1
	Sown SLA x SR	0.034	0.09	0.709
	Sown SLA x Fng	0	0.09	1
N	Sown SLA x N	0	0.09	1
SR	Sown SLA x SR	-0.001	0.09	0.994
Fng	Sown SLA x Fng	0	0.09	1
SR	Fng	0		Fixed
N	SR	0		Fixed
	Fng	0		Fixed
Sown SLA	N	0		Fixed
	Fng	0		Fixed
SR x Fng	SR	0	0.09	1
	Fng	0	0.09	1

R square	
CWM SLA	0.876
Δ in. shift SLA	0.201
Δ ab. shift SLA	0.150

Table S3: Structural equation model output: effect of N enrichment, species richness (SR), fungicide (Fng) and sown specific leaf area (Sown LDMC) on the shift of community mean LDMC due to abundance shift (Δ ab. shift) and to intraspecific shift (Δ in. shift), and of sown LDMC and the Δs on community weighted mean (CWM) LDMC.

Model fit: Pvalue = 0.999, Chisq = 14.865, Df = 35, RMSEA = 0.000.

Response	Predictor	Estimate	Std.Err	P(>|z|)	
CWM LDMC	Sown LDMC	0.599	0.048	0.000	***
	Δ in. shift LDMC	0.528	0.046	0.000	***
	Δ ab. shift LDMC	0.626	0.048	0.000	***
Δ in. shift LDMC	N	-0.045	0.090	0.616	
	SR	-0.070	0.090	0.439	
	Fng	0.124	0.090	0.168	
	Sown LDMC	-0.080	0.090	0.376	
Δ ab. shift LDMC	N	-0.016	0.084	0.851	
	SR	-0.096	0.084	0.25	
	Fng	-0.168	0.084	0.045	*
	Sown LDMC	-0.286	0.084	0.001	**
	N x SR	0.184	0.084	0.029	*

Covariances		Estimate	Std.Err	P(>|z|)
Sown LDMC	Sown LDMC x N	0	0.088	1
	Sown LDMC x SR	-0.231	0.092	0.013
	Sown LDMC x Fng	0	0.088	1
N	Sown LDMC x N	0	0.09	1
SR	Sown LDMC x SR	0.011	0.088	0.9
Fng	Sown LDMC x Fng	0.000	0.090	1
SR	Fng	0		Fixed
N	SR	0		Fixed
	Fng	0		Fixed
Sown LDMC	N	0		Fixed
	Fng	0		Fixed
N x SR	SR	0	0.09	1
	N	0	0.09	1

R square	
CWM LDMC	0.754
Δ in. shift LDMC	0.029
Δ ab. shift LDMC	0.154

Table S4: Structural equation model output: effect of N enrichment, species richness (SR), fungicide (Fng) and sown specific leaf area (Sown SLA) on the shift of community mean SLA due to abundance shift (Δ ab. shift) and to intraspecific shift (Δ in. shift), and of sown SLA, N enrichment, SR, Fng and the Δs on aboveground and belowground biomass production.

Model fit: Pvalue = 1.000, Chisq = 7.7733, Df = 33, RMSEA = 0.000.

Response	Predictor	Estimate	Std.Err	P(>|z|)	
Belowground biomass	N	0.112	0.087	0.197	
	SR	0.029	0.085	0.734	
	Fng	0.054	0.086	0.534	
	Δ in. shift SLA	0.120	0.091	0.187	
	Δ ab. shift SLA	-0.295	0.092	0.001	**
	Sown SLA	-0.249	0.092	0.007	**
Aboveground biomass	N	0.295	0.083	0.000	***
	SR	-0.107	0.082	0.192	
	Fng	0.065	0.083	0.430	
	Δ in. shift SLA	0.088	0.087	0.315	
	Δ ab. shift SLA	0.192	0.088	0.029	*
	Sown SLA	-0.146	0.089	0.098	.
Δ in. shift SLA	N	0.163	0.080	0.042	
	SR	0.008	0.080	0.916	
	Fng	-0.151	0.080	0.060	
	Sown SLA	-0.167	0.080	0.037	
	Sown SLA x N	0.209	0.081	0.009	**
	Sown SLA x SR	-0.173	0.081	0.032	*
	Sown SLA x Fng	-0.180	0.081	0.026	*
Δ ab. shift SLA	N	0.065	0.085	0.444	
	SR	-0.076	0.085	0.367	
	Fng	0.059	0.085	0.488	
	Sown SLA	-0.353	0.085	0.000	***
	SR x Fng	0.104	0.085	0.221	

Covariances		Estimate	Std.Err	P(>|z|)
Sown SLA	Sown SLA x N	0.008	0.09	0.927
	Sown SLA x SR	0.025	0.091	0.782
	Sown SLA x Fng	0.008	0.09	0.927
N	Sown SLA x N	0.008	0.091	0.926
SR	Sown SLA x SR	0.008	0.09	0.931
Fng	Sown SLA x Fng	0.008	0.091	0.926
N	Fng	0		Fixed
	Sown SLA	0		Fixed
Sown SLA	Fng	0		Fixed
Belowground biomass	Aboveground biomass	-0.118	0.076	0.12

R square	
Belowground biomass	0.132
Aboveground biomass	0.212
Δ in. shift SLA	0.194
Δ ab. shift SLA	0.148

Table S5: Structural equation model output: effect of N enrichment, species richness (SR), fungicide (Fng) and sown specific leaf area (Sown LDMC) on the shift of community mean LDMC due to abundance shift (Δ ab. shift) and to intraspecific shift (Δ in. shift), and of sown LDMC, N enrichment, SR, Fng and the Δs on aboveground and belowground biomass production.

Model fit: Pvalue = 1.000, Chisq = 1.479, Df = 12, RMSEA = 0.000.

Response	Predictor	Estimate	Std.Err	P(>|z|)	
Belowground biomass	N	0.112	0.085	0.188	
	SR	0.051	0.085	0.550	
	Fng	0.061	0.087	0.483	
	Δ in. shift LDMC	-0.066	0.087	0.445	
	Δ ab. shift LDMC	0.208	0.090	0.021	*
	Sown LDMC	0.351	0.089	0.000	***
Aboveground biomass	N	0.316	0.084	0.000	***
	SR	-0.146	0.085	0.086	.
	Fng	0.051	0.086	0.557	
	Δ in. shift LDMC	-0.103	0.086	0.233	
	Δ ab. shift LDMC	-0.157	0.090	0.080	.
	Sown LDMC	-0.107	0.088	0.224	
Δ in. shift LDMC	N	-0.059	0.090	0.513	
	SR	-0.057	0.090	0.529	
	Fng	0.110	0.090	0.218	
	Sown LDMC	-0.070	0.090	0.435	
Δ ab. shift LDMC	N	-0.014	0.085	0.864	
	SR	-0.098	0.085	0.248	
	Fng	-0.167	0.085	0.048	*
	Sown LDMC	-0.287	0.084	0.001	***
	N x SR	0.186	0.085	0.029	0

Covariances		Estimate	Std.Err	P(>|z|)
N	Fng	0		Fixed
Sown LDMC	SR	0.041	0.091	0.65
Belowground biomass	Aboveground biomass	-0.081	0.078	0.295
N x SR	SR	0.008	0.091	0.926
	N	-0.008	0.091	0.926

R square	
Belowground biomass	0.145
Aboveground biomass	0.159
Δ in. shift LDMC	0.024
Δ ab. shift LDMC	0.153

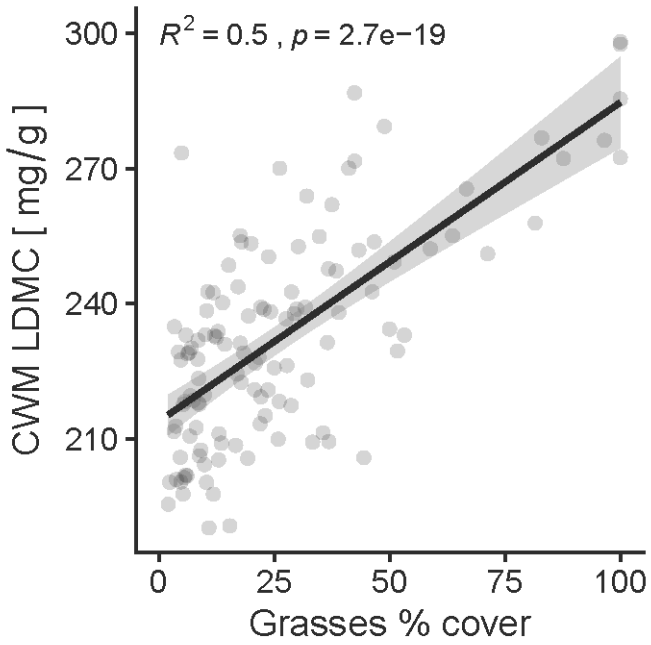


Figure S1: Sown grasses relative percentage cover compared to sown herbs in August 2017. Pearson correlation with the community weighted mean leaf dry matter content (CWM LDMC) value per plot. Correlation coefficient 0.71, R^2 0.5, pvalue <0.001.

Chapter 5

Summary and general conclusions

Nitrogen (N) enrichment, coming from direct agricultural inputs and the release of reactive N in the atmosphere, is one of the major ongoing global changes (Galloway et al. 2008; Vitousek et al. 1997). It affects ecosystem functioning directly through changes in soil abiotic conditions (Vitousek et al. 1997; Sardans et al. 2012), and indirectly through changes in the plant community (diversity loss, Stevens et al. 2004; functional shift, Lavorel and Grigulis 2012), by affecting higher trophic levels (pathogen load, Dordas 2008), and through changes in the soil community (de Vries et al. 2006; Bardgett and Wardle 2012). While the direct and indirect effects of N enrichment have been studied in experimental setups and in natural communities, their relative importance in affecting ecosystem functions is still poorly known (Allan et al. 2015). This is due to the correlation between different effects, in particular between diversity loss and functional composition shift. We therefore need experimental designs that enable us to disentangle the direct and indirect effects of N enrichment. This thesis aimed to understand the mechanisms by which N enrichment affects functioning, using a large biodiversity-ecosystem functioning experiment that factorially manipulated N enrichment, species diversity, functional composition and foliar fungal pathogens presence, and measured multiple functions in 336 sown plant communities. Within this framework we tested how these factors affected the ability of ecosystems to provide multiple functions (multifunctionality, Chapter 2). We further disentangled the effects on litter decomposition between effects on litter quality and soil biotic and abiotic conditions (Chapter 3). We then investigated how intraspecific variation drives the effect of functional shift on functioning (Chapter 4). Here, I summarise the main findings of the chapters and discuss future directions for biodiversity-ecosystem functioning research.

Relative importance of direct and indirect effects

Our results show that the direct and indirect effects of N enrichment were of similar importance in affecting ecosystem functioning, reflecting previous findings for naturally assembled communities (Allan et al. 2015; Isbell et al. 2013). We found that N, plant diversity, plant functional composition and fungicide individually affected a large set of functions and that they can have opposing effects (for

instance, a shift towards fast communities increased aboveground biomass production and decreased belowground biomass production, Chapter 2 and 4). This translated into differences in the main factors driving individual functions compared to those driving multifunctionality (Chapter 2). Functional composition was always an important driver of functioning (all chapters) and the main driver affecting decomposition rates (Chapter 3), agreeing with several studies on the large effect of plant traits in explaining decomposition (García-Palacios et al. 2016a; Cornwell et al. 2008). However, we found that the functional composition effect depended on species richness for some individual functions (e.g. plant/soil N ratio, enzymatic activity), and for multifunctionality (Chapter 2). Multifunctionality increased with species richness in the fast species communities. This interaction between richness and composition was only visible when assessing multifunctionality, suggesting that mechanisms driving multifunctionality differ from those driving individual functions. Therefore, different management practices will likely be needed depending on whether the goal is to maintain one particular function or multiple functions at a high level (Manning et al. 2018). Similarly, fungicide spraying did not affect multifunctionality, but it had effects on individual functions (e.g. herbivory, belowground biomass production). Fungicide was also a driver of community composition, as spraying shifted species composition towards faster growing strategies by changing trait relative abundance (decrease in leaf dry matter content) and shifting intraspecific trait values (increase in specific leaf area, Chapter 4). The interactive effects on functioning of the factors we manipulated reflects the complex synergies between them and underlines the need to consider multiple mechanisms by which N enrichment affects functioning.

Multiple diversity metrics

Diversity is an important driver of ecosystem functioning and of multifunctionality (Cardinale et al. 2006; Lefcheck et al. 2015). The results of Chapter 2 showed that multiple diversity metrics were important in assessing the supply of multiple functions, in line with Le Bagousse-Pinguet et al. (2019). Species richness increased multifunctionality (an effect modulated by functional composition), and functional diversity also increased multifunctionality, while N dampened this positive effect. The most functionally diverse communities were even slightly better at providing multiple functions than the low diversity fertilised communities. This suggests that managing for diversity can provide not only an increase in productivity (Weigelt et al. 2009) but multiple benefits compared to intensive systems. However, we did not find a positive effect of functional diversity on decomposition (Chapter 3), as it influenced neither litter quality nor soil biotic and abiotic conditions. We suggest that functional

diversity might only be important for decomposition in the presence of legumes (Handa et al. 2014). We might have therefore underestimated the functional diversity effect by excluding legumes from the experimental design (Milcu et al. 2008). Finally, we found that the effect of functional diversity on multifunctionality was not linked to species abundance, but rather to species presence. This suggests that locally rare species play a large role in the positive effect of diversity, but also that evenness is an important metric to consider as it might reduce the diversity effect, as observed in biocrust systems (Maestre et al. 2012). In our experiment, communities with the highest multifunctionality level were functionally diverse, dominated by fast, high functioning related species, and included a few complementary ones at low abundance. These results overall underline the importance of considering several diversity metrics and the presence of locally rare species in affecting diversity-functioning relationships.

Multiple traits

The functional composition of plant communities can be assessed using the leaf economics spectrum (LES), which distinguishes fast, acquisitive strategies on one end, and slow, conservative strategies on the other end (Díaz et al. 2015; Wright et al. 2004; Reich 2014). The LES is often determined using a few functional traits as proxies: specific leaf area (SLA), leaf N content and/or leaf dry matter content (LDMC). We saw however that different traits might be of importance in explaining functioning. For instance, SLA had no large effect on decomposition (Chapter 3), but leaf N and micronutrients such as calcium and magnesium increased decomposition rates through an increase of litter quality. LDMC reflects structural components of the plant material, which affected litter decomposability (Grigulis et al. 2013, Chapter 3). We found that both LDMC and fibre content increased litter recalcitrance, which means that fibre content added a complementary dimension not taken into account by LDMC, such as stem structure. This adds ups to the idea that functional composition refers to a range of possible trait combinations, and that different traits might be important to consider in addition to the typical LES traits to assess composition effect on functioning. We also found that functional composition was driven to a large extent by intraspecific shifts in traits (when considering SLA and LDMC, Chapter 4), for species at both ends of the LES. These shifts were influenced by fungal presence, plant species richness and N enrichment (Chapters 3 and 4). However, this large variation among species did not reflect changes in functioning, suggesting that the effect of traits on function may differ between and within species. Therefore, assessing functional community and its effect on functioning may require

to look at multiple traits, and to distinguish the variation due to inter or intraspecific shifts under N enrichment.

Insight into some of the mechanisms

Complementarity - Our results shed light on some of the mechanisms behind N direct and indirect effects on functioning. For instance, in Chapter 2, we show that N enrichment dampens the positive effect of functional diversity on multifunctionality. This might be due to trade-offs between functions increased by N enrichment and those increased by diversity. We also show in this chapter that species richness increased multifunctionality in fast species communities only, suggesting that fast species were complementary in the set of functions they provided, whereas slow ones were more redundant. Because complementarity within slow and fast groups occurred for individual functions, it suggests that complementarity for multifunctionality is another mechanism than complementarity for individual functions.

Competition - In Chapter 4, the SLA of species in mixed communities converged towards more intermediate values, which could reflect an increase in competition (Mayfield and Levine 2010). N enrichment cancelled this effect by shifting fast species SLA towards higher values, possibly due to an increase of the optimum SLA under N enrichment. This convergence in SLA was also visible in the communities not sprayed with fungicide, suggesting that fungal pathogens reduced competition between species, which modified functional composition. Pathogens further affected composition by increasing the abundance of fast growing, low LDMC, communities, therefore LDMC captured the trade-off between investment of energy in growth or in defence (Blumenthal et al. 2009; Heckman et al. 2019).

Soil communities - Finally, the results of Chapter 3 show that N indirect effects on soil communities occurred mainly through changing the microclimatic conditions in our experiment. However, the activity of soil organisms increased with litter inputs of higher quality, in particular due to an increase of macrofauna activity. Simultaneously, soil communities receiving high quality litter were less efficient in decomposing recalcitrant material, which could be explained by a change in soil enzymatic activity (Carreiro et al. 2000). These findings contributed to a better mechanistic understanding of the complex interactions between N enrichment direct and indirect effects, but also raised a number of unsolved points, such as indirect effects occurring belowground.

Further disentangling considerations

In Chapter 2, we observed that fast communities contributed more to multifunctionality related to aboveground functions, whereas slow communities contributed more to multifunctionality related to belowground functions. Although we assessed part of the changes in soil communities by looking at decomposition (Chapter 3), we have only just started to understand how N directly and indirectly affects soil functioning. This is also because we manipulated plant richness, functional composition and the presence of pathogens, but N direct effects in our experimental design included all indirect ones occurring through soil community changes (see Giling et al. 2019). As a manipulation of soil communities is close to impossible at the large scale of the PaNDiv experiment, this suggests that experimental setups would need to switch to smaller scales to further disentangle these mechanisms. This might be also necessary in order to assess finer scale mechanisms such as accounting for fine root decomposition or root exudates on functioning (Eisenhauer et al. 2017; Fornara et al. 2009). Additionally, we still lack a complete picture of the N cycle in our communities. It would be interesting to look at the remaining fluxes such as nitrification rates or leaching which should be included in multifunctionality measures to assess the nutrient use efficiency in the ecosystems. Two interesting additions to this work would be to assess plant-mycorrhizae interactions, how important they are in driving community changes and functioning (van der Heijden et al. 1998), as well as the effect of moss communities, which have been ignored here and are likely to drive a large part of the belowground functions by increasing humidity and affecting soil communities activity and composition (Cornelissen et al. 2007). There is still a lot to investigate to disentangle N effects, and the PaNDiv experiment provided an ideal design to start this process. These first results provided useful insights into future directions of the biodiversity-ecosystem functioning field.

Time-scale dependency

One of the constants in the results we presented here is that the effects of species establishment in spring 2016 was still visible in the following years, translated by a low biomass production in the fast communities (Chapters 3 and 4), although a shift towards faster species abundance increased biomass production (Chapter 4). This establishment effect could have large implications on how diversity affects ecosystem functioning (Delory et al. 2019). We also suggested in Chapter 3 that the small effect of soil biotic and abiotic conditions in driving decomposition could result from the recent setup of the experiment, and that more time is needed to see a response of soil conditions to N direct and indirect

effects (Eisenhauer et al. 2011; Boeddinghaus et al. 2019). In addition, the effect of the drivers we manipulated could be time-scale dependent (Santonja et al. 2019; Lepš and Wan 2014; Cardinale et al. 2007). We would therefore need to put these results in perspective within a larger time-scale, to see how species interactions, communities, drivers' effects potentially change with time. In this sense, the next phase of the PaNDiv experiment is a great opportunity to look at the time-scale dependency of the mechanisms we highlighted during the initial years.

Final conclusions

Nitrogen enrichment affects ecosystem functioning directly, and indirectly through plant diversity loss and a shift in plant communities towards fast growing strategies. It also affects the abundance and effect of higher trophic levels, and the soil community. We disentangled the direct and indirect effects of N in a large grassland experiment by factorially manipulating plant species richness, functional composition, N enrichment and fungicide spraying. We assessed the individual and interactive effects of these drivers on multifunctionality, and further disentangled the effects by focusing on litter decomposition. We then tested how N enrichment shifts functional composition through inter and intraspecific shifts, and the relative importance of these shifts in driving functioning. We found that the indirect effects of N enrichment were as important in driving functioning as direct effects, and that the main drivers, their direction, and their interactions depended on the functions considered. We were able to assess for the first time that species richness and functional composition effects were not independent but rather, enhanced each other. Our results underline the importance of considering multiple diversity metrics like species richness, functional diversity and evenness. We also showed the importance of considering different traits to measure the effect of functional composition, and of distinguishing the effects driven by inter and intraspecific trait variation. Overall, this thesis contributes to a mechanistic understanding of N direct and indirect effects on functioning and underlines the importance of considering these effects and their interactions simultaneously, particularly in the current context of multiple global changes.

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Declaration of consent

on the basis of Article 30 of the RSL Phil.-nat. 18

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I declare herewith that this thesis is my own work and that I have not used any sources other than those stated. I have indicated the adoption of quotations as well as thoughts taken from other authors as such in the thesis. I am aware that the Senate pursuant to Article 28. RSL Phil.-nat. 05 is authorized to revoke the title awarded on the basis of this thesis.
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