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**The interaction effect of soil organisms and plant intraspecific  
diversity on ecosystem functions**

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## Foreword

This dissertation is a cumulative work of manuscripts, either published or submitted for publication at international peer-reviewed journals. This thesis is based on the following articles:

I. **Guo, X.**, J. S. Petermann, C. Schittko, and S. Wurst. 2015. Independent role of belowground organisms and plant cultivar diversity in legume-grass communities. *Applied Soil Ecology*. 95:1-8. doi: 10.1016/j.apsoil.2015.05.010. (Submitted: 23 February 2015; Accepted 25 May 2015, Published online: 9 June 2015)

II. **Guo, X.**, J. S. Petermann, C. Schittko, and S. Wurst. Root knot nematodes modify the positive plant intraspecific diversity-productivity effect on red clover in clover-grass communities. *Applied Soil Ecology*. (Submitted)

III. **Guo, X.**, J. S. Petermann, P. Latour, and S. Wurst. Plant cultivar-specific responses to parasitic nematodes mediate the effect of plant intraspecific diversity on productivity. *Plant Ecology*. (Submitted)



## Summary

Biological diversity and soil biota interactions are both well known to play a dominant role for ecosystem functions. Functionally dissimilar soil organisms are increasingly studied and their beneficial or detrimental impacts on ecosystem functions (e.g. plant productivity) are widely recognized. Soil organisms have been found to drive the plant diversity-ecosystem functions relationship at plant species level, while their effects on the relationship at the plant intraspecific diversity level are poorly documented. To improve the understanding of independent and interactive effects of plant intraspecific diversity and functionally dissimilar soil organisms on ecosystem functioning, two field experiments in a temperate grassland ecosystem and one greenhouse experiment were designed and conducted for the purposes of this PhD research.

In the first field experiment (Chapter 2), I investigated whether the addition of beneficial soil organisms, arbuscular mycorrhizal fungi (AMF) and anecic earthworms (*Lumbricus terrestris* L.), and plant intraspecific diversity (manipulated by sowing cultivar mixtures of *Trifolium pratense* L. and *Lolium perenne* L.) interactively influence plant productivity, aboveground herbivory, diversity of aboveground herbivores, and weed establishment. The results showed that the addition of AMF increased aboveground biomass of plant community and tended to decrease the diversity of aboveground herbivores. In addition, weed biomass significantly decreased in plant communities with increased plant intraspecific diversity. Earthworms attenuated the positive effect of AMF treatment on AMF root colonization rate, but had no effect on aboveground biomass or herbivore diversity. However, no significant interactions between plant intraspecific diversity and the beneficial soil organisms were found. The results indicate the independent roles and additive positive effects of AMF and plant intraspecific diversity on ecosystem functions such as productivity and resistance against weeds in the field.

The second experiment (Chapter 3) was closely connected to the previous chapter. A detrimental soil organism (plant-parasitic nematodes *Meloidogyne hapla* Chitwood) was introduced to the field plots to investigate the effects of its interactions with beneficial soil organisms (AMF and earthworms) and plant intraspecific diversity on plant productivity, aboveground herbivory, and floral visitation rate. The results showed a positive effect of plant intraspecific diversity on total aboveground biomass, contributed mostly by *T. pratense*. The positive plant intraspecific diversity-productivity effect on *T. pratense* was mediated by the nematodes, i.e. aboveground biomass of *T. pratense* was increased by higher plant intraspecific diversity solely in the nematode treatment.

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Further, the average floral visitation length by pollinators on *T. pratense* was decreased by earthworm addition, and the abundance of pollinators tended to be increased by high plant intraspecific diversity. The aboveground biomass of *L. perenne* and leaf damage of *T. pratense* were not influenced by the treatments. These results suggest that plant intraspecific diversity and soil organisms can interactively or independently modify ecosystem functions such as aboveground productivity and pollination in the field. Specifically, the positive plant intraspecific diversity effect appears to emerge only under conditions of stress (high nematode abundance). This result indicates that plant intraspecific diversity may be important for the maintenance of ecosystem functions, especially in an agricultural context, where pest pressure can be high and can have large detrimental consequences.

Based on the results that I found in the field, I conducted a greenhouse experiment (Chapter 4) with plant-parasitic nematodes *M. hapla* and different manipulated levels of plant intraspecific diversity. The aim was to elucidate the mechanisms behind the potential effect of plant-parasitic nematodes modifying the plant intraspecific diversity-ecosystem functions relationship. To investigate the plant diversity-productivity relationship on the intraspecific level, the diversity was set to four levels using two-, four-, eight-, and sixteen-cultivar mixtures of *T. pratense* and *L. perenne*. The results showed that the presence of plant-parasitic nematodes mediated the effect of plant intraspecific diversity on aboveground biomass of *T. pratense* on the intermediate level (the four-cultivar mixture). The evenness of the community decreased with increasing plant intraspecific diversity, while it was not affected by nematodes. The biomass of each plant individual was measured and the data showed that the biomass of the *T. pratense* cultivars was influenced by nematodes or plant intraspecific diversity. Specifically, the biomass of the *T. pratense* cultivar Violetta was significantly increased by nematodes, while the other *T. pratense* cultivars and *L. perenne* cultivars did not significantly respond to the nematode addition. Aboveground biomass of half of the *T. pratense* and *L. perenne* cultivars were influenced by plant intraspecific diversity and the effect size and direction depended on cultivar identity. I would suggest that the positive response of certain *T. pratense* cultivars to plant-parasitic nematodes as well as the dissimilar performance of plant cultivars with regard to plant intraspecific diversity contributed to the observed plant intraspecific diversity-productivity effects. These results highlight the influence of belowground parasitic nematodes on the effect of plant intraspecific diversity on productivity, suggesting that future biodiversity-ecosystem functioning research should give extra consideration to the effect of soil organisms.

## German summary (Zusammenfassung)

Es ist bekannt, dass Pflanzendiversität und Interaktionen von Bodenorganismen eine dominierende Rolle für Ökosystemfunktionen spielen. Funktionell unterschiedliche Bodenorganismen werden zunehmend untersucht, und deren vorteilhafte oder nachteilige Auswirkungen auf Ökosystemfunktionen (z.B. die Produktivität) sind weithin anerkannt. Es wurde gezeigt, dass Bodenorganismen das Verhältnis von Pflanzenvielfalt und Ökosystemfunktionen auf Artenebene bestimmen. Auf der Ebene intraspezifischer Pflanzendiversität ist die Wirkung von Bodenorganismen auf dieses Verhältnis hingegen schlecht dokumentiert. Um das Verständnis der individuellen und kombinierten Effekte von intraspezifischer Pflanzendiversität und funktionell unterschiedlichen Bodenorganismen auf Ökosystemfunktionen zu verbessern, wurden im Rahmen dieser Doktorarbeit zwei Feldversuche in einem gemäßigten Grünland und ein Gewächshausversuch konzipiert und durchgeführt.

In einem manipulativen Feldexperiment (Kapitel 2) untersuchte ich, ob die Zugabe von nützlichen Bodenorganismen (arbuskuläre Mykorrhizapilze (AMF) und Regenwürmer) und verschiedene Stufen der Pflanzensortendiversität interaktiv die Produktivität, die Etablierung von Fremdarten (Unkräutern), den Blattschaden durch Herbivore und Pathogene, und die Diversität oberirdischer Herbivore in Klee-Gras-Gemeinschaften beeinflussen. Die Zugabe von AMF führte zu einer Erhöhung der oberirdischen Pflanzenproduktivität und hatte einen negativen Einfluss auf die Diversität von oberirdischen Herbivoren. Die Biomasse von Unkräutern war geringer in Pflanzengemeinschaften mit hoher, im Vergleich zu geringer Sortendiversität. Regenwürmer reduzierten die Kolonisierung der Wurzeln durch AMF, wenn AMF zugegeben wurde, hatten aber keinen Effekt auf die Pflanzenproduktivität und Diversität der Herbivoren. Interaktive Effekte der beiden Bodenorganismen und der Sortendiversität wurden nicht gefunden. Unsere Ergebnisse zeigen die unabhängigen Rollen und die additiven, positiven Effekte von AMF und Sortendiversität auf Funktionen wie Produktivität und Resistenz gegen Fremdarten.

Auf Basis dieser Ergebnisse wurde ein zweiter Feldversuch (Kapitel 3) durchgeführt. Ein schädlicher Bodenorganismus (pflanzenparasitären Nematoden *Meloidogyne hapla* Chitwood) wurde auf den Feldflächen eingeführt, um den Einfluss seiner Wechselwirkung mit nützlichen Bodenorganismen (AMF und Regenwürmer) und Pflanzensortendiversität auf die Produktivität der Flächen, oberirdische Herbivorie und Blütenbesuchsrate zu untersuchen. Die Ergebnisse zeigten eine positive Wirkung von Pflanzensortendiversität auf die oberirdische Primärproduktion, die

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größtenteils von *T. pratense* gebildet wurde. Der Effekt der Pflanzensortendiversität auf die Produktivität von *T. pratense* wurde durch die Nematoden vermittelt, d.h. die Pflanzensortendiversität erhöhte die oberirdische Primärproduktion ausschließlich in Flächen, in denen Nematoden zugefügt worden waren. Ferner wurde die durchschnittliche Blütenbesuchsdauer von Bestäubern auf *T. pratense* durch Zugabe von Regenwürmern verringert, und die Abundanz der Bestäuber durch die Pflanzensortendiversität tendenziell erhöht. Die oberirdische Primärproduktion von *L. perenne* und Blattschäden von *T. pratense* wurden nicht von den Behandlungen beeinflusst. Diese Ergebnisse legen nahe, dass intraspezifische Pflanzendiversität und Bodenorganismen interaktiv oder unabhängig Ökosystemfunktionen modifizieren können, wie beispielsweise oberirdische Primärproduktion und Bestäubung. Genauer gesagt, scheint der positive Effekt der intraspezifischen Pflanzendiversität nur unter Bedingungen von Stress (hohe Nematoden-Abundanz) aufzutreten. Dieses Ergebnis zeigt, die Wichtigkeit intraspezifischer Pflanzendiversität für den Erhalt von Ökosystemfunktionen, vor allem in einem landwirtschaftlichen Kontext, in dem Schädlingsdruck hoch sein und nachteilige Folgen haben kann.

Basierend auf den Ergebnissen der Feldversuche führte ich einen Gewächshausversuch (Kapitel 4) mit pflanzenparasitären Nematoden, *M. hapla*, und unterschiedlichen intraspezifischen Pflanzendiversitäten durch. Ziel war es, die Mechanismen des Effekts pflanzenparasitärer Nematoden auf die Wechselwirkung zwischen intraspezifischer Pflanzendiversität und Ökosystemfunktionen aufzuklären. Um diese Wechselwirkung auf innerartlicher Ebene zu untersuchen, wurden vier Pflanzensortendiversitäten mit zwei, vier, acht oder sechzehn Sorten von *T. pratense* und *L. perenne* etabliert. Das Ergebnis zeigte, dass die Anwesenheit von pflanzenparasitären Nematoden den Effekt der Pflanzensortendiversität auf die Primärproduktion von *T. pratense* bei mittlerer Diversität (vier Kultivar-Gemisch) vermittelt. Die Evenness nahm mit zunehmender Pflanzensortendiversität ab, wurde aber nicht von Nematoden beeinflusst. Die Biomasse der Einzelpflanzen wurde bestimmt, und die Daten zeigten, dass die Primärproduktion der *T. pratense* Sorten von Nematoden oder Pflanzensortendiversität abhing. Im einzelnen wurde die Primärproduktion der *T. pratense* Sorte ‚Violetta‘ durch die Zugabe von Nematoden erhöht, während die anderen *T. pratense*- und *L. perenne* Sorten nicht signifikant auf die Nematoden Behandlung reagierten. Die Hälfte der *T. pratense*- und *L. perenne* Sorten wurden von der Pflanzensortendiversität beeinflusst, wobei Größe und Richtung der Effekte von der Identität der Sorte abhing. Ich nehme an, dass die positive Reaktion bestimmter *T. pratense* Sorten auf Nematoden sowie die unterschiedliche Reaktion der Pflanzensorten auf die Pflanzensortendiversität zu den beobachteten Wechselwirkungen zwischen intraspezifischer Pflanzendiversität und Ökosystemfunktionen beitrugen. Diese Ergebnisse unterstreichen den Einfluss bodenlebender Nematoden auf die Wirkung intraspezifischer Pflanzendiversität auf die

Produktivität, was darauf hindeutet, dass Bodenorganismen in zukünftiger Forschung zu Wechselwirkungen von Biodiversität und Ökosystemfunktionen besonders berücksichtigt werden sollten.



# Chapter 1

## General introduction

Our earth is extraordinary for its biological diversity: the terrestrial and marine ecosystems provide habitat for around 2.3 million species of plants, animals, protists, and fungi (Hinchliff et al. 2015). As natural processes, species gains and species losses are always happening. In recent decades, the vast majority of the world's nations have declared that biodiversity loss is accelerating at an alarming rate because of human activities that affect other species (Butchart et al. 2010). The main cause of the biodiversity loss can be summarized in the following key points: 1) natural areas alteration, urbanization, and habitat destruction; 2) occupation of invasive species; 3) environment pollution; 4) overexploitation of resources (e.g. overhunting, overfishing); 5) industrial agricultural expansion; 6) climate change (Millennium Ecosystem Assessment 2005). The changes in biodiversity affect the functioning of various ecosystems, e.g. terrestrial ecosystems and marine ecosystems (Loreau et al. 2001, Hooper et al. 2005, Stachowicz et al. 2007, Tilman et al. 2014, Lefcheck et al. 2015).

Ecosystem functions are affected by ecosystem structure, including the abiotic and biotic constituents of ecosystems (Loreau et al. 2001, Brussaard 2012, Wagg et al. 2014, Lefcheck et al. 2015). The widely-studied plant-mediated ecosystem functions include plant productivity, plant community succession, aboveground and belowground interaction, decomposition, nutrient cycling, nutrient retention, etc. Ecosystem services, mainly including supporting, provisioning, regulating, and cultural services, derive from ecosystem functions (Loreau et al. 2002, Naeem et al. 2009). Recent studies have shown that biodiversity and ecosystem functions are mainly positively linked (Hooper et al. 2005, van Ruijven and Berendse 2005, Tilman et al. 2014, Lundholm 2015). Biodiversity loss has become a global concern as the loss of species will negatively alter ecosystem functions and their ability to provide human beings with goods and services (Hooper et al. 2005, Naeem et al. 2009, Cardinale et al. 2011, Cardinale 2012). Therefore, understanding and preserving biodiversity is of great importance for maintaining valuable ecosystem services (Reaka-Kudla et al. 1997, Millennium Ecosystem Assessment 2005).

Because of the rapid loss of biodiversity and its important implications for ecosystem functions and services, biodiversity research has become one of the most active fields in ecology in the last

decades (Naeem 2002, Cardinale 2012, Naeem et al. 2012, Reich et al. 2012). Biodiversity loss and its impact on ecosystem functions have been widely investigated in agricultural ecosystems, and other terrestrial ecosystems (Swift et al. 2004, Cardinale et al. 2011, Cardinale 2012). A number of well-controlled experiments in grassland ecosystems, such as the Cedar Creek biodiversity experiment in the USA (Tilman et al. 1997, Tilman et al. 2001), the European Biodepth experiment (Hector et al. 1999, Spehn et al. 2005), and the Jena experiment in Germany (Roscher et al. 2004), were conducted to improve understanding of the relationship between biodiversity and ecosystem functions.

### **Plant diversity and ecosystem functions**

The increased research on plant diversity-ecosystem functions relationships over the past decade has demonstrated that plant diversity can influence plant productivity (Hector et al. 1999, Tilman et al. 2001, Cardinale et al. 2013), plant interaction with herbivores (Crowder and Jabbour 2014, Loranger et al. 2014) and pathogens (Latz et al. 2012, Hantsch et al. 2014), plant invasion (Maron and Marler 2007, Chang and Smith 2012), as well as the performance of pollinators and other arthropods of higher trophic levels (Ebeling et al. 2008, Bangert et al. 2005).

Plant diversity within an ecosystem consists of diversity of genes, species, functional types, and communities. Plant species diversity and its effect on ecosystem functions is the most studied type (Tilman et al. 2001, Jiang et al. 2009, Lundholm 2015, Yuan et al. 2015). The previous studies concerning the relevance of plant diversity to ecosystem multi-functionality have demonstrated that the more ecosystem processes were considered, the more species were found to influence overall ecosystem functions (Lundholm 2015, Lefcheck et al. 2015). Thereafter, ecologists proposed that the effect of biodiversity on ecosystem functions might be caused by plant community composition (Hooper and Vitousek 1997, Breulmann et al. 2011) or functional composition (Jiang et al. 2007, Marquard et al. 2009). Fundamentally, plant identity and plant functional traits might contribute the most to ecosystem processes (Wardle et al. 2003, Breza et al. 2012, Roscher et al. 2012, Schittko et al. 2014, Latz et al. 2015).

Plant productivity is generally recognized as an integrating indicator of ecosystem functions and commonly used in evaluating the effect of plant diversity (e.g. Tilman et al. 2001, Mulder et al. 2004, Tilman et al. 2012). There is a growing amount of evidence that different plant diversity-productivity relationships have been found at the plant species level, such as positive (Hector et al. 1999, Tilman et al. 2001, HilleRisLambers et al. 2004, van Ruijven and Berendse 2009, Cardinale et al. 2013) or idiosyncratic (Hector et al. 1999, Huston et al. 2000, Yuan et al. 2015), suggesting no

one simple explanation. Generally, high plant productivity is one of the main advantages reported for high plant diversity in most studies (e.g. Isbell et al. 2009, Cardinale et al. 2013). The most important processes explaining why high diverse plant communities perform better than low diverse ones are ‘selection effect’ and ‘complementary effect’ (Loreau and Hector 2001, van Ruijven and Berendse 2005). Firstly, the high diversity plant communities may perform better because they have a higher possibility of containing high yield species (i.e. selection effect; Loreau and Hector 2001, Fox 2005). Secondly, another contrasting explanatory model is the complementary effect (Loreau and Hector 2001, Poisot et al. 2013, Zuppinger-Dingley et al. 2014). The different species use resources in different niches complementarily (i.e. niche partitioning; Loreau and Hector 2001); also, certain species combinations may facilitate each other in growth (Huston 1997). Previous studies (Fargione et al. 2007, Marquard et al. 2009) have shown that in long term experiments the niche complementary effect is more conveyed than the selection effect in the positive diversity-ecosystem functions relationship.

Increasing investigations have shown a positive effect of plant diversity on productivity of plant communities on multiple levels, for instance, with biodiversity measured taxonomically on the species level, functionally on the functional traits level, and genetically on the intraspecific level. Recent studies have demonstrated that plant intraspecific diversity shares similar positive effect as plant species diversity in influencing ecosystem functions (e.g. plant productivity; Crutsinger et al. 2006, Cook-Patton et al. 2011). Also, positive effects of resisting plant pathogens and aboveground herbivores have been shown in the studies at both plant species (Latz et al. 2012, Crowder and Jabbour 2014, Hantsch et al. 2014) and plant intraspecific levels (Zhu et al. 2000a, Tooker and Frank 2012, Zeller et al. 2012, Shoffner and Tooker 2013). Furthermore, plant invasion has been found to be decreased by both enhanced plant species diversity (Knops et al. 1999, Maron and Marler 2007, Liao et al. 2015) and intraspecific diversity (Crutsinger et al. 2008a).

The form of the plant intraspecific diversity-ecosystem functions relationship has recently become a hot research topic. However, the relationship between plant intraspecific diversity and ecosystem functioning is not well-established. A great deal of empirical evidence suggests that plant primary production and intraspecific diversity are often positively related (Kotowska et al. 2010, Tooker and Frank 2012); and some researchers have also demonstrated that there is no (or weakly) positive relationship between plant intraspecific diversity and productivity (Prieto et al. 2015, Schöb et al. 2015). The widely accepted point is that the productivity of a high plant intraspecific diversity community is often higher and more stable than that of a mono-cultivar (see Tooker and Frank 2012 for a review). Furthermore, plant intraspecific diversity can also influence other ecosystem functions on different trophic levels, e.g. enhancing resistance to plant antagonists (i.e.

aboveground and belowground herbivores, pathogens, and plant invaders) (Crutsinger et al. 2008a, Ostfeld and Keesing 2012, Yang et al. 2012, Shoffner and Tooker 2013), and also shifting the effect of accompanying plant species diversity (Schöb et al. 2015).

At plant intraspecific level, the selection effect and niche complementarity were also mostly used to explain the positive effect of plant diversity on productivity (Cook-Patton et al. 2011, Tooker and Frank 2012). Since increasing research has focused on belowground processes recently, the role of soil organisms in driving the positive species diversity-productivity relationship has also found to be dominant (Schnitzer et al. 2010, Maron et al. 2011, Eisenhauer 2012, Eisenhauer et al. 2012a). This has raised the question whether soil organisms are important driving factors in the positive effect of diversity on productivity at a specific diversity level.

### **Soil biota and ecosystem functions**

Aboveground ecosystem processes have been studied intensively over the past couple of decades, whereas how belowground biota may affect ecosystem processes and ecosystem functions have only received scientific attention in recent decades (Wardle 2002, Wurst et al. 2012, Bardgett and van der Putten 2014). Soil ecosystems also represent the largest reservoir of biological diversity and provide a crucial habitat for a large amount of soil organisms, including a variety of prokaryotes and eukaryotes. Each individual plant is exposed to belowground microorganisms such as fungi and bacteria, as well as a potentially large variety of soil fauna, mostly nematodes, earthworms, insects, and microarthropods (Jeffery et al. 2010). Potentially, a large proportion of soil bacteria, fungi, and nematode species are not well known (Coleman et al. 2004, Wall et al. 2012). Recent evidences have shown the vital importance of soil biota in ecosystem functions (Turbé et al. 2010, Wurst et al. 2012, Bardgett and van der Putten 2014, Nielsen et al. 2015). Soil biota manipulates key processes belowground (e.g. soil organic matter, nutrient cycling, and soil structure maintenance) and plays a significant role in ecosystem stability and a broad range of ecosystem services provisions, e.g. it improves water and food quality, controls erosion, attenuates pollutants and degradation, etc. (Brussaard 2012, Thiele-Bruhn et al. 2012, Nielsen et al. 2015, Pascual et al. 2015, Rodriguez and Sanders 2015).

There is mounting evidence that soil organisms have crucial effects on regulating plant productivity, community structure as well as the performance of aboveground antagonists and mutualists (Wurst et al. 2012, Bardgett and van der Putten 2014). 1) Multiple soil organisms interacting with plants may result in antagonistic or synergistic effects on plant growth. On the one hand, positively, beneficial soil organisms (e.g. earthworms, mycorrhizal fungi, and plant growth-promoting bacteria)

can increase access to limited nutrients in the soil through their feeding, moving, and excreting activities, which often leads to a positive impact on plant growth (Javaid 2009, Lambers et al. 2009, Lavelle 2011, Kuzyakov and Xu 2013). Moreover, soil organisms themselves are also nutrient sources after their death. On the other hand, negatively, the accumulation of detrimental soil organisms (e.g. parasites, pathogens, and herbivores) can decrease plant primary production through removing carbon and nutrients from the host plant and reducing root uptake capacity (Brown and Gange 1989, Bardgett 2005, Eisenhauer et al. 2010a, Mendes et al. 2013). 2) Soil organisms play a dominant role in regulating plant community structure and dynamics (De Deyn et al. 2003, Reynolds et al. 2003). For instance, the biotic interactions of plant and soil microorganisms in the rhizosphere can lead to the different performances of plant species in early and later stages of ecological succession and further change the composition and dynamics of plant communities (Reynolds et al. 2003). 3) At a higher trophic level, soil biota can also influence the performance of aboveground antagonists or mutualists of plants. Aboveground herbivores can be affected by soil organisms through inducing changes to the phenotypical and physiological characteristics of the host plants (Bezemer and van Dam 2005, Bennett et al. 2006, Fontana et al. 2009, Kempel et al. 2010, Jung et al. 2012), or through improving the performance of the antagonists of herbivores (Hempel et al. 2009, Pineda et al. 2010, Soler et al. 2012, Ueda et al. 2013). For example, the presence of root pathogens and root-feeding herbivores can inhibit the performance of aboveground herbivores and change the structure of aboveground higher trophic communities (Bezemer and van Dam 2005, Rudgers and Clay 2008). Further, the activity of soil organisms can also affect mutualists, such as pollinators and parasitoids. For instance, Poveda et al. (2005) showed that the flower visitation rate of pollinators can be improved by root herbivory. Cardoza et al. (2012) also found that the vermicomposting of earthworms improves pollinator attraction and visit length.

The diversity of soil organisms is profuse, and plants directly and indirectly interact with most of the soil organisms (Coleman et al. 2004, Jeffery et al. 2010). Concerning the interaction of soil biota and aboveground food web, the vital and commonly studied subset of soil biota includes rhizosphere microorganisms, earthworms, nematodes, etc. Arbuscular mycorrhiza fungi (AMF) represent a crucial mutualist of soil microorganisms and are found to associate with more than 80% of terrestrial plant families (Smith and Read 2008). Earthworms represent a key decomposer and macrofauna in the soil and are generally recognized as ecosystem engineers (Edwards and Bohlen 1996). Plant-parasitic nematodes are broadly distributed herbivores that continually attack a wide range of plants and inducing significant losses in plant growth and commercial production (Evans et al. 1993). In this study, I use these functionally beneficial (AMF and earthworms) and detrimental soil organisms (plant-parasitic nematodes) to study their potential roles in the effect of

plant intraspecific diversity on ecosystem functions (e.g. productivity).

### *Arbuscular mycorrhiza fungi*

A mycorrhiza is a symbiotic association between plant and fungi in and on the root system of a host plant that benefits both organisms (Smith and Read 2008). AMF, the most common group of mycorrhizal fungi, form mutualistic symbioses in root systems (Smith and Read 2008). Through the symbiosis, the mycorrhizal fungus provides the host plant with a better capacity for absorbing mineral nutrients (particularly phosphate) and water from the soil in exchange for carbon sources from the host plant (Javaid 2009, Willis et al. 2013). AMF can also promote soil aggregation through the growth and excretion of external hyphae (Rillig and Mummey 2006, Willis et al. 2013), which may lead to improved plant growth. It is worth mentioning that even within one AMF species (e.g. *Rhizophagus irregularis*) there can be high genetic diversity (Koch et al. 2004, Croll et al. 2008, Borstler et al. 2010). Depending on the identity and composition, AMF species and fungal diversity can have different effects on plant growth (Klironomos 2000, Klironomos 2003, Munkvold et al. 2004). Furthermore, AMF have also been shown to affect plant coexistence and competition (Zhu et al. 2000b, Hart et al. 2003, van der Heijden et al. 2003, Scheublin et al. 2007, Barto et al. 2010, Klabi et al. 2014). The mutualistic symbionts show different levels of effects on certain plant species and thus may alter the structure and diversity of the plant community (van der Heijden et al. 1998, Wurst et al. 2011). For instance, AMF fungal hyphae can alter the performance of host plants and further influence plant diversity and ecosystem stability (Wurst et al. 2011, Wurst and Rillig 2011, Liang et al. 2015).

Accumulating studies have pointed out that AMF fungal hyphae can assist host plants in handling both abiotic (Campagnac and Khasa 2014) and biotic stresses (Khaosaad et al. 2007, Elsen et al. 2008, Sikes et al. 2009, Liang et al. 2015). For example, AMF colonization has been found to affect aboveground herbivory (Gehring and Bennett 2009, Hartley and Gange 2009, Koricheva et al. 2009, Kempel et al. 2010). The authors suggest that AMF affect plant resources allocation and further influence plant defense against herbivory. Similar results were also found on the effects of AMF on plant resistance to soil pathogens (Elsen et al. 2008, Wehner et al. 2010). The effect size and direction of AMF on herbivory depends on the diet breadth and feeding mode of the herbivore and also on the identity of the AMF and the plant (Koricheva et al. 2009, Sikes et al. 2009). Each plant may benefit most from a unique fungal isolate, and variation in the mycorrhizal composition may influence plant growth and nutrient status (Klironomos 2000, Klironomos 2003, van der Heijden et al. 2003, Koch et al. 2006, Roger et al. 2013), which could thereafter influence the abundance of related herbivores and pathogens above- and belowground (Elsen et al. 2007, Elsen et al. 2008, Wooley and Paine 2011, Barber et al. 2013) and also influence the performance of

pollinators (Gange and Smith 2005).

### *Earthworms*

Through decomposing soil organic matter and leaf litter in the soil and casting behavior, earthworms can accelerate the transformation and circulation of soil organic matter and soil nutrients (N, P, K, etc.) and promote nutrient availability in soil (Lavelle 1988, Blouin et al. 2013). Earthworms can also affect soil structure through producing stable macropores and aggregates, which can facilitate the infiltration of water, air, and nutrients into the soil (Lavelle 1988, Edwards and Bohlen 1996). Moreover, earthworms can promote the activity of rhizosphere microorganisms via enhanced C levels in soil, and further enhance soil biological fertility which will facilitate plant growth in return (Cao et al. 2015a, Huang et al. 2015). Thus, plant growth can be promoted by the activities of earthworms (e.g. feeding, burrowing, transporting, and casting) through improving soil water, air, and nutrient availability in soil. Furthermore, it is increasingly recognized that earthworms have crucial effects on the structure and diversity of plant communities (Forey et al. 2011, Zaller et al. 2011a). Depending on the plant species, earthworms can strongly influence the seed dispersal and seeds germinating through selective seed feeding and burial (Drouin et al. 2014), and change plant production and competition (Eisenhauer et al. 2008, Zaller et al. 2013), which will further alter the composition and structure of plant communities.

Recent studies have also demonstrated that earthworms can either increase the abundance of aboveground aphid (Poveda et al. 2005), or increase plant defense levels and decrease aboveground and belowground herbivory (Wurst 2010, 2013). Earthworms were found to influence aboveground herbivore performance by changing the distribution of plant resources between growth and defense and altering concentrations of chemical cues (primary and secondary metabolites) (Scheu 2003, Wurst et al. 2003, Wurst 2010). Earthworms were also reported to enhance the tolerance of grasses to root-feeding nematodes and counteract the negative effects of soil microorganisms on the evenness of the plant community (Wurst et al. 2008).

Earthworms (Annelida: Oligochaeta), differing in their behaviors, activity zones, and food preferences within the soil, were divided into three functional groups: vertically burrowing anecics, endogeics or horizontally burrowing endogeics, and surface dwelling epigeics (Edwards and Bohlen 1996). I used the vertically burrowing anecic earthworm species *Lumbricus terrestris* L. in my experiments because of the active behavior, common distribution, and the relatively clear background information on them (Fründ et al. 2010).

*L. terrestris* is considered to be a predominant ecosystem engineer in temperate soils (Ransom 2012). *L. terrestris*, commonly known as the nightcrawler, is a large annelid and typically ranges from 8-15 cm in length (Sims and Gerard 1999). In Europe, the deeper burrows mostly belong to *L. terrestris* (Sims and Gerard 1999, Canti 2003). *L. terrestris* lives in deep vertical burrows of 1-3 m deep and generally only emerges to feed, mainly on organic litter on the soil surface (Schulman and Tuinov 1999). Functioning as ecosystem engineer, *L. terrestris* can affect plant growth by altering nutrient mineralization and enhancing soil water permeability (Schulman and Tuinov 1999, Tiunov and Scheu 1999). The activity of *L. terrestris* incorporating large amounts of organic matter into mineral soil layers can also create a favorable microhabitat for the activity of soil microorganisms (Tiunov and Scheu 1999). Moreover, *L. terrestris* can also influence above- and belowground herbivory. For example, it has been shown in previous greenhouse experiments that *L. terrestris* can increase the random aphid infestation of grass plants (Eisenhauer and Scheu 2008), facilitate dispersal of nematodes through transporting (Shapiro et al. 1993), or decrease nematodes abundances (Boyer et al. 2013). Furthermore, *L. terrestris* can also positively influence seed transportation (Thompson et al. 1994) and *L. perenne* seedlings establishment (Aira and Pearce 2009). It has been documented that the casts of *L. terrestris* are safe places for germination of some plant species, e.g. *Trifolium dubium* (Thompson et al. 1993).

### *Nematodes*

Soil nematodes, the most widely spread multicellular animals in nature, are an abundant and species-rich animal phylum. Depending on their feeding type, soil-inhabiting nematodes can be classified into plant-parasitic nematodes, fungivorous nematodes, bacterivorous nematodes, predatory nematodes, and omnivore nematodes (Freckman 1982). To date, accumulating studies (Weisser and Siemann 2004, Soler et al. 2012, Johnson and Rasmann 2015) have stressed the significance of different belowground herbivores on ecosystem processes and functions. Root-knot nematodes (*Meloidogyne spp.*) are the plant parasitic herbivores that cause the most damage in many crops worldwide (Evans et al. 1993). The nematodes can influence their plant food resources (Yeates et al. 2009), related microbial activity (Poll et al. 2007), plant community composition and succession (Verschoor 2001), and may also cause trophic cascades (Yeates 2010, Soler et al. 2012). Herbivory by plant parasitic nematodes can disrupt cell structure in root systems, remove nutrients from the wounds, and alter the genetic expression of plants (Davis et al. 2000, Williamson and Gleason 2003). Further, herbivory by plant parasitic nematodes can also induce resistance of plants to aboveground herbivory (see Bezemer and van Dam 2005 for a review).

I used *Meloidogyne hapla* Chitwood (northern root-knot nematode) in my study. The commonly seen species *M. hapla* has a wide host range, including clover and other vegetable crops such as

onions, carrots, celery, beans, lettuce, and potatoes (Stanelis 2004). The legume plant *Trifolium pratense* was widely reported to be parasitized by root-knot nematodes (Yeates et al. 1973, Willis 1981, Stanelis 2004). Root systems that are severely infected by *M. hapla* generally show the production of small galls (also known as root knots), which restrain the provision of water and nutrients necessary for ordinary plant growth, resulting in the wilting of plants (Yeates et al. 2009). However, depending on the density of plant-parasitic nematodes, their effects on plant growth can be positive at a low level of herbivory. A low level of root feeding by these nematodes can improve carbon and nutrient leaching from the damaged root systems (Yeates et al. 1999a, Yeates et al. 1999b). The accessible C in the rhizosphere represents an important energy source for soil food webs (Kramer et al. 2012, de Vries et al. 2013). The carbon and nutrient release may further improve the rhizosphere bacterial activity, which could also indirectly increase the nutrient availability and further increase plant growth (Yeates et al. 1998, Poll et al. 2007). Because of the damage caused by *M. hapla*, increasing studies have been working on depressing the negative effect of nematodes biologically (Mojtahedi et al. 1991, Chen et al. 2000, Adam et al. 2014).

#### *The interactions of AMF, nematodes, and earthworms*

Increased investigation into the interactions of soil biota has demonstrated that the separated effects of functionally dissimilar soil organisms can either support (Eisenhauer et al. 2010b), mitigate (Kohler-Milleret et al. 2013), counterbalance (Wurst et al. 2008, Liang et al. 2015), or not influence each other (Eisenhauer et al. 2009a, Wurst et al. 2011, Wurst and Rillig 2011).

Earthworms can directly influence AMF root colonization and AMF biomass in the soil either negatively, through selectively feeding on fungal hyphal (Ortiz-Ceballos et al. 2007, Salem et al. 2013), or positively, through dispersing AMF spores (Reddell and Spain 1991, Gange 1993, Gormsen et al. 2004). It has been documented that the interaction between earthworms and AMF can influence soil physical properties, as well as increase nutrient availability and plant growth (Li et al. 2012, Li et al. 2013, Cao et al. 2015b, Paudel et al. 2015). For instance, Kohler-Milleret (2013) found that the soil physical characteristics and properties can be improved by endogenic earthworms (*Allolobophora chlorotica*) and AMF (*Glomus intraradices* N.C. Schenck & G.S. Sm.), and the individual effects of earthworms and AMF can either add up together or mitigate each other.

Further, AMF and earthworms can also independently or interactively influence other soil organisms and ecological processes belowground. Accumulating evidence shows the effect of AMF on decreasing occurrence and impact of soil pathogens and root herbivores, as well as on inducing bioprotection against these soil organisms, e.g. oomycetes and pathogenic fungi, and plant-parasitic

nematodes (Kesba and Al-Sayed 2005, Elsen et al. 2007, Khasa et al. 2009, Veresoglou and Rillig 2012). Reversely, nematode can influence plant symbiotic relationships with AMF through promoting sporulation (Kesba and Al-Sayed 2005). Previous studies have also shown the inhibition of earthworms on plant parasitic nematode abundance, as earthworms can ingest and digest large amounts of soil and litter and simultaneously a large number of plant-parasitic nematodes (Senapati 1992, Boyer et al. 2013). Wurst et al. (2008) reported that earthworms can cancel out the negative effects of plant-parasitic nematodes on the grasses and further influence the plant community structure. Besides, the dispersal of nematodes can also be enhanced by earthworms (e.g. *L. terrestris*) via their direct contacting and the movement of earthworms (Shapiro et al. 1993). Recent studies have shown that the soil microbial biomass and activity (Zarea et al. 2009, Aghababaei et al. 2014) and the community structure of different groups of soil nematodes (Hua et al. 2010) can be influenced by the interaction of earthworms and AMF.

The interaction between earthworms and AMF is an important mediator in plant diversity, community structure, and functions of grassland ecosystems (Zaller et al. 2011a). Mammitzsch et al. (2012) illustrated that the effects of earthworms and AMF depend on the successional stage of the plant community. Specifically, the effect of AMF on plant production depends on the successional stage of the plant community, which may be altered by earthworms. Furthermore, on a higher trophic level, the feeding behavior of aboveground herbivores in grassland communities can also be affected by the interaction of earthworms and AMF (Trouvé et al. 2014). Yet, some other studies (Eisenhauer et al. 2009a, Wurst et al. 2011, Wurst and Rillig 2011) have also demonstrated the non-interactive relationships of AMF and earthworms. For example, AMF and endogeic earthworms were found to have independent effects on plant diversity and plant invader establishment (Wurst et al. 2011). Eisenhauer et al. (2009a) also reported that the impacts of earthworms (*Aporrectodea caliginosa* Savigny and *L. terrestris*) and AMF (*G. intraradices*) on the performance of plants (*T. pratense*, *Lolium perenne* L. and *Plantago lanceolata* L.) are not interrelated. The effects of earthworms and AMF on plant performance are not always consistent (see Paudel et al. 2015 for a review).

The interaction of soil organisms is complex. Depending on the context (e.g. plant identity and community composition), the results of the interaction effects of functionally dissimilar soil organisms have shown to be inconsistent (e.g. Paudel et al. 2015). AMF, earthworms, and nematodes have been independently reported to influence ecosystem functions (e.g. plant growth, plant community composition and diversity, or aboveground herbivores). However, the interaction among all of these functionally dissimilar soil organisms (earthworms, nematodes, and AMF) and their potential effects on plant growth, aboveground herbivory, and ecological processes at higher

trophic levels (e.g. pollination) are not well studied, especially in natural conditions. The knowledge regarding the interaction effects of functionally dissimilar soil organisms on ecosystem functions is insufficient and has been gained mostly through studies in controlled environments (Olson et al. 2008; Ladygina et al. 2010; Johnson et al. 2012; Kostenko et al. 2012; Singh et al. 2014). My studies attempt to investigate the interaction effects of earthworms, nematodes, and AMF on ecosystem functions in the field.

### **The effect of soil organisms on plant diversity-ecosystem functions relationship**

At the plant species level, plant diversity and soil organisms have been shown the potential influencing each other in the increasing studies over recent decades. Firstly, plant competition and the composition of grassland plant communities can be influenced by soil organisms through their effects on the nutrient acquisition and performance of plant individuals (Sabais et al. 2012). Previous studies have demonstrated that AMF and other soil microorganisms (e.g. N-fixing bacteria and soil borne pathogens) can affect the diversity, structure, and functioning of plant communities (Reynolds et al. 2003, Zaller et al. 2011a). For instance, soil organisms, e.g. root herbivores or earthworms, can modulate the diversity (Körner et al. 2014) and invasibility of plant communities in terrestrial ecosystems (Eisenhauer et al. 2008, Eisenhauer and Scheu 2008, Whitfeld et al. 2014). Gange and Brown (2003) proposed that the plant community structure can be influenced by the interactions of soil subterranean invertebrates (e.g. earthworms, nematodes, mites, or insects) and AMF through their modifying effects on the seedling establishment.

Secondly, accumulating evidence (Kostenko et al. 2015, Lange et al. 2015, Prober et al. 2015) has demonstrated that plant species diversity is a major determinant of the biomass, distribution, and community structure of soil organisms in grassland ecosystems. Increasing plant species diversity may influence soil biota through increasing root morphological characteristics and root chemical properties, e.g. litter types, tissues, and root exudates (Mueller et al. 2012, Ravenek et al. 2014, Mommer et al. 2015). For example, a recent study (Thakur et al. 2015) has shown that enhanced plant species diversity significantly increases biomass of soil microbial communities with the strongest effects shown in long-term experiments. Depending on the season and temporal scale, earthworm density and community structure were also found to be influenced by the declining plant species diversity in a long term Jena experiment (Eisenhauer et al. 2009c). Furthermore, Johnson (2003) has shown that soil microbial activity and diversity can be influenced by different plant functional types. De Deyn et al. (2004) showed that plant identity is more important than plant species diversity in affecting the diversity of soil nematodes. The long term grasslands experiment on the effects of plant species diversity and composition on soil nematode communities at the Biodepth site also indicated that plant species identity had a more important effect on

nematode community composition than plant species diversity (Viketoft et al. 2009). Similar results have also been found in other studies (Viketoft and Sohlenius 2011, Kostenko et al. 2015), which showed that both plant species identity and diversity can influence the soil nematode fauna of different nematode types. A recent field study by Kostenko et al. (2015) demonstrated that even when soil nematodes belong to the same feeding guild, the plant species diversity and identity effects on them can be different. Besides, the study of the effect of plant species diversity on soil biota in temperate grassland communities (Gastine et al. 2003) demonstrated complex interactions among different trophic levels of soil organisms, which may counterbalance and buffer the effect of plant species diversity manipulations on soil biota.

Soil organisms, which extensively interact with plants and drive important processes belowground, potentially play a prominent role in biodiversity and ecosystem functions relationships. Previous studies on plant species diversity illustrated that soil biota can mediate the species diversity-productivity relationship (Schnitzer et al. 2010, Maron et al. 2011, Eisenhauer 2012, Eisenhauer et al. 2012a). AMF, earthworms, and plant pathogens have all been reported to alter the effect of plant species diversity on ecosystem functions. Eisenhauer (2012) reviewed the role of functionally dissimilar soil organisms in grassland biodiversity experiments and demonstrated that soil biota can drive both negative and positive complementary effects between plant species through a multitude of mechanisms. He proposed that aboveground-belowground interactions affect complementary effects through four main processes: enlarging biotope space, mediating legume effects, increasing plant community resistance, and maintaining plant species diversity (Eisenhauer 2012). It is worth mentioning that, the detrimental soil organisms were more stressed in determining the species diversity-productivity relationship than the other organisms such as AMF (Schnitzer and Klironomos 2011). The low-diversity plant community has been shown to be more inhibited by host-specific pathogens or parasites, while the high-diversity plant community is more resilient to the disturbance by these soil pathogens (Schnitzer et al. 2010).

At the plant intraspecific level, plant intraspecific diversity can also affect soil organisms. A previous study showed that a litter-based arthropod community was weakly influenced by the plant genotypic diversity (Crutsinger et al. 2008b). Cadet et al. (2006) found that nematodes can be decreased by increasing within-field intraspecific diversity of sugarcane (*Saccharum officinarum* L.). A previous study has also shown that the different plant genotypes react differently to AMF colonization (Elsen et al. 2005). Yet studies on the effect of soil biota on the effect of diversity on ecosystem functions at the plant intraspecific level are lacking. Therefore, it is of crucial significance to account for the role of soil biota in the biodiversity study (especially at plant specific diversity level), as soil biota may not only influence the plant performance, but also

interact with plants-associated food webs and potentially alter important ecosystem functions.

### **Clover-grass plant community**

Legumes and grasses mixtures are generally grown together and are commonly recognized to be effective mixtures because compared to a single functional group, they possess several advantages: improved weed suppression ability, more efficient N fixation, improved C:N ratio, and lower seed costs (Sollenberger et al. 1984, Moorby et al. 2009). To date, the widely cultivated and diverse crops and forage cultivars provided us a great opportunity to investigate the effect of plant intraspecific diversity on ecosystem functions. The mixture of red clover and perennial ryegrass, commonly used in agriculture, was used in my experiments.

#### *Red clover (Trifolium pratense L.)*

Red clover (*Trifolium pratense* L.) is nowadays used as a farming crop and spread over many temperate zones worldwide, including Europe, central and eastern Asia, America, and Oceania (Taylor and Quesenberry 1996). *T. pratense* is a dicotyledon plant that belongs to the Fabaceae (Leguminosae) family. As a tap-rooted species, the mature taproot of *T. pratense* can reach up to 3 m depth in well-drained soils, but the depth relies on soil density, humidity, growth type, competition status, and other microenvironment factors (Taylor and Quesenberry 1996, Vleugels 2013). *T. pratense* has been suffering from nematode pests, including root-knot nematodes (*Meloidogyne* spp.), root lesion nematodes (*Pratylenchus penetrans*), clover cyst nematodes (*Heterodera trifolii*), and clover stem nematodes (*Ditylenchus dipsaci*) (Taylor and Quesenberry 1996). The pollination of *T. pratense* flowers is mostly conducted by bumblebees and honeybees, and also by some wasps and butterflies (Taylor and Quesenberry 1996, Vleugels 2013).

Depending on their genotype, the plant characteristics of various *T. pratense* cultivars are more or less pronounced, e.g. yield, maturity type, resistance to disturbances, color of inflorescence, etc. (Muntean 2006, Tucak et al. 2013, Vleugels 2013, Jacob et al. 2014). For example, tetraploid cultivars are generally superior in primary production and persistence, more resistant to disease than diploid cultivars, less branched, and often producing fewer but larger flowers (Taylor and Quesenberry 1996, Vleugels 2013). The inflorescence of *T. pratense* falls within a color ranges from white to purple, with pink flower head as the most common (Saviranta et al. 2008, Vleugels 2013). Further, depending on the maturity type of *T. pratense* cultivars, the *T. pratense* harvest rate ranges from one to five cuts per year (Taylor and Quesenberry 1996). Early cultivars, known as double cut cultivars, develop early in spring and recover quickly after a cutting (Taylor and Quesenberry 1996). Medium and late types, referred to as single cut cultivars, develop in late

spring and their regrowth is slower; therefore, they only produce one growth flush and the flowering is usually postponed and limited (Taylor and Quesenberry 1996, Vleugels 2013).

*T. pratense* can produce a high yield without fertilizer input with relatively high protein content and good digestibility for livestock (Taylor and Quesenberry 1996). As legume plants, *T. pratense* can form nodules with rhizobia bacteria, allowing *T. pratense* crops to fix nitrogen in the air, which can also be partly transferred to the rhizosphere or to companion species (Taylor and Quesenberry 1996). As a legume pasture, *T. pratense* is commonly sown with timothy (*Phleum pratense* L.) or Italian ryegrass (*Lolium multiflorum* L.), or with perennial ryegrass (*L. perenne*) in arable land.

#### *Perennial ryegrass (Lolium perenne L.)*

*Lolium perenne* L., common name perennial ryegrass or English ryegrass or winter ryegrass, is a perennial bunchgrass from the family Poaceae (Jung et al. 1996). It is native to Europe, Asia, and northern Africa, and is now widely-cultivated, naturalized, and best adapted to mild climate areas for livestock grazing and for fodder (as hay or silage) because of its high yield potential, high quality, palatability, and digestibility (Brock and Fletcher 1993, Smith et al. 1998, Wims et al. 2013). The plant produces a highly branched root system and tillers (Ledebøer 2009). The majority of its root system is replaced annually in the spring time (Ledebøer 2009). *L. perenne* also possesses the capability of rapid germination and development (Jung et al. 1996, Wacker et al. 2009). Earthworms tend to turn the senescing foliage of *L. perenne* into a muddy mess (Edwards and Bohlen 1996, Schulman and Tuinov 1999). Besides, endophytic fungi have been shown to affect the growth of *L. perenne* and confer resistance to insect pests and enhance its performance in competition with other grass (Latch et al. 1985, Clay et al. 1993). Also, a previous experiment by Hartwig et al. (2002) demonstrated that AMF colonization (*G. intraradices*) shows the potential to promote the growth of *L. perenne*.

Modern breeding programs, considering yield, quality, cold tolerance, growth rate, and resistance to common diseases and herbivores, have led to the development of new cultivars (Wilkins 1991, Lee et al. 2012). Forage types of *L. perenne* are either diploid ( $2n = 14$ ) or tetraploid ( $4n = 28$ ). The *L. perenne* cultivars can differ in terms of morphological and physiological traits (Wilkins et al. 2000, Roldán-Ruiz et al. 2001, Flores-Lesama et al. 2006). For instance, compared to diploids, tetraploids normally have fewer but larger tillers, wider leaves, and a slower growth rate (Gilliland et al. 2002); whilst diploids are more winter-hardy and more persistent than tetraploids (Gilliland et al. 2002, Wilkins and Humphreys 2003). Besides, the major herbivores of *L. perenne* are grass grubs, the European crane fly (*Tipula paludosa* Meigen), sod webworms, billbugs, chinch bugs, and major diseases such as *Rhizoctonia* and *Pythium* (Jung et al. 1996, Wilkins and Humphreys 2003).

## **The connection of antecedent and present research**

The knowledge on plant-mediated aboveground-belowground interactions plays a vital role in sustainable ecological agriculture. Soil organisms, which are closely related to plant root systems, can have positive or negative effects on plants. Functionally dissimilar soil organisms are increasingly studied for their beneficial or detrimental impacts on plant productivity and their ecosystem functions are widely recognized (Wall et al. 2012). AMF represents a generally recognized beneficial biological resource in relation to plant production and quality (Smith and Read 2008). Earthworms have numerous beneficial effects on overall soil quality for crop production (Lavelle 2011). Plant-parasitic nematodes, such as the root-knot nematode *M. hapla*, are severe pests in agroecosystems worldwide (Freckman 1982, Evans et al. 1993). AMF, earthworms, and nematodes co-occur in all terrestrial ecosystems worldwide (Coleman et al. 2004, Bardgett 2005). However, the interaction effect of AMF, earthworms, and nematodes is rarely investigated, and their interaction with plant diversity has only recently garnered attention.

Furthermore, plant diversity and soil organisms both play vital roles in influencing plant communities and their associated aboveground organisms; however, little is known about their interactions and combined effects. Plant species diversity has received a lot of attention, whereas plant intraspecific diversity has only recently come into the focus of community ecology (e.g. see Tooker and Frank 2012 for a review). The increasing number of studies on species diversity effect (Schnitzer et al. 2010, Maron et al. 2011, Eisenhauer 2012, Eisenhauer et al. 2012a, Eisenhauer et al. 2012b) reporting significant soil biota effects on plant species diversity-productivity relationships suggest that the functional importance of belowground soil organisms in the study of biodiversity may have been underestimated and overlooked so far. Plant intraspecific diversity also plays a predominant role in regulating ecosystem functions (Tooker and Frank 2012), and the role of soil biota in these processes is largely untested. To the best of my knowledge, the interactive effect of functionally dissimilar soil organisms and plant intraspecific diversity has never been addressed. Thus far, no study has experimentally investigated the effect of soil organisms on the effect of plant intraspecific diversity on ecosystem functions in natural assemblages. Thus, more research in this area is needed.

To increase understanding of the individual and interactive effects of functionally dissimilar soil organisms and plant intraspecific diversity on ecosystem functions in sustainable ecological agriculture, I designed and conducted two field experiments in a temperate grassland ecosystem and one greenhouse experiment using clover and grass plant community. As a dominant reservoir of terrestrial soil carbon stock (Hungate et al. 1997, Trumper et al. 2009), the grassland ecosystem

is a crucial model ecosystem in the study of the interaction of plant diversity and functionally dissimilar soil organisms.

### **Thesis outline and research objectives**

In the first experiment, I asked: How do beneficial soil organisms (earthworms and AMF) interact and affect a plant community and associated herbivores in the field? Do plant communities of different genetic diversity react differently to the soil organisms? Therefore, a fully-factorial field experiment considering intraspecific diversity of the plant community (low/high) and multi-species beneficial soil organism treatments (AMF and earthworms) was designed to evaluate their effects on plant productivity, and plant resistance to herbivores. I hypothesized that the beneficial soil biota would have positive effects on the performance of plants, and negative effects on the performance of aboveground herbivores.

In the second experiment, I addressed the following questions: Will the beneficial soil organisms (earthworms and AMF) counterbalance the side effects of detrimental soil organisms (plant-parasitic nematodes) in the field and hence influence the ecosystem functions, such as the plant community with its associated herbivores and pollinators in the field? Will the plant intraspecific diversity influence the interactions of different functional groups of belowground biota? I hypothesized that beneficial soil biota (AMF and earthworms) may counterbalance the side effects of detrimental soil organism (plant-parasitic nematodes) and hence affect ecosystem functions, such as plant productivity, resistance of the plant community to plant herbivores, and pollination.

In the third experiment, I investigated the mechanisms behind the interaction effect of plant-parasitic nematodes and plant intraspecific diversity on ecosystem functions in a clover-grass plant community. I asked: is there a positive linear plant intraspecific diversity-productivity relationship? How do the detrimental soil organisms affect the positive effect of plant intraspecific diversity on plant productivity? Plant-parasitic nematodes were used and studied for their role in mediating the plant diversity-productivity effect on an intraspecific level. I hypothesized that the plant productivity increases with plant intraspecific diversity, and the effect of plant intraspecific diversity on plant productivity is influenced by plant-parasitic nematodes.

## Chapter 2

### **Independent role of belowground organisms and plant cultivar diversity in legume-grass communities**

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## **Chapter 3**

### **Root knot nematodes modify the positive plant intraspecific diversity-productivity effect on red clover in clover-grass communities**

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## **Root knot nematodes modify the positive plant intraspecific diversity-productivity effect on red clover in clover-grass communities**

### **Abstract**

Plant diversity and soil organisms are both widely studied for their contributions to ecosystem functions. It has been recently shown that soil biota may drive the plant species diversity-function relationship in grasslands, while so far studies on plant intraspecific diversity are lacking. To improve the understanding of how ecosystem functions may be influenced by plant intraspecific diversity, soil organisms, and their interactions, we used a field setting to examine the effects of plant intraspecific diversity and functionally dissimilar soil organisms (manipulated by the addition of plant-parasitic nematodes, earthworms, and arbuscular mycorrhizal fungi) on aboveground primary production, pollination and shoot herbivory. Plant intraspecific diversity was manipulated by sowing different seed mixtures of *Trifolium pratense* L. and *Lolium perenne* L. cultivars. We found a positive effect of plant intraspecific diversity on total aboveground biomass, contributed mostly by *T. pratense*. The plant intraspecific diversity-productivity effect on *T. pratense* was mediated by plant-parasitic nematodes, i.e. aboveground biomass was increased by intraspecific diversity only in the nematode treatment. Further, the average floral visitation length was decreased by earthworm addition, while the abundance of pollinators tended to be increased by high plant intraspecific diversity. Aboveground biomass of *L. perenne* and leaf damage of *T. pratense* were not influenced by the treatments. We conclude that plant intraspecific diversity and soil organisms can interactively or independently modify functions such as aboveground primary production and pollination of *T. pratense* in the field. Specifically, the positive plant intraspecific diversity effect appears to only emerge under conditions of stress (high nematode abundance). This result indicates that plant intraspecific diversity may be important for the maintenance of ecosystem functions, especially in an agricultural context, where pest pressure can be high and can have large detrimental consequences.

### **Introduction**

Changes in plant species diversity have been reported to affect ecosystem functions and processes, such as plant productivity, vegetation dynamics and insect food web interactions (Hector et al. 1999, Cardinale et al. 2011, Tilman et al. 2012). Previous studies have shown that the effect of plant species diversity on aboveground primary production can be modified by soil organisms, such as arbuscular mycorrhizal fungi (AMF) or plant pathogens (van der Heijden et al. 2008, Schnitzer et al. 2010, Maron et al. 2011, Eisenhauer et al. 2012a). While numerous biodiversity-ecosystem functions studies have focused on plant species diversity (Hooper and Vitousek 1997, Grime 1998,

Hector et al. 1999, Loreau and Hector 2001), increasing attention has been paid to the possible consequences of plant genotypic diversity for ecosystem functions (Zhu et al. 2000a, Crutsinger et al. 2006, Crawford and Rudgers 2012, Tooker and Frank 2012, Shoffner and Tooker 2013). Some recent studies have indicated a comparable importance and similar influence of genotypic diversity and plant species diversity for increasing plant productivity (Crutsinger et al. 2006, Cook-Patton et al. 2011, Roger et al. 2012).

Changes in plant intraspecific diversity have been shown to influence not only plant productivity (Himanen et al. 2013), but also community stability (Prieto et al. 2015), stress tolerance, and the resistance of plant community to herbivores and plant invaders (Kotowska et al. 2010, Tooker and Frank 2012, Yang et al. 2012, Shoffner and Tooker 2013). In previous studies, the positive effect of plant intraspecific diversity on plant productivity was usually explained by two main processes, the selection effect and the complementary effect (Hughes et al. 2008, Cook-Patton et al. 2011, Tooker and Frank 2012). First, high intraspecific diversity plots have greater odds of containing more productive and pathogen resistant cultivars (selection effect) (Fox 2005, Tooker and Frank 2012). Second, complementary traits and functions of cultivars may result in increased plant productivity (complementary effect) (Loreau and Hector 2001, Poisot et al. 2013). Besides the effects on plant productivity, it has been proposed that plant intraspecific diversity may have effects on soil biota communities, albeit sometimes idiosyncratic ones (Cadet et al. 2006, Crutsinger et al. 2008b). For example, intraspecific genetic structure and community composition of soil microorganisms have been reported to be influenced by plant genotypes (Schweitzer et al. 2008, Gehring et al. 2013) or by plant cultivars (Dalmastri et al. 1999, İnceoğlu et al. 2010). However, the role of soil biota in modifying the impact of plant genotypic diversity on ecosystem functions is not well understood.

Previous studies show that aboveground ecosystems in general can be influenced by soil biota in a variety of ways (Clay and Holah 1999, Klironomos 2002, Wardle et al. 2004). The effect of soil organisms on plant performance ranges from beneficial to detrimental (Wagg et al. 2011, Bardgett and van der Putten 2014). Functionally dissimilar soil organism groups are widely known to be of prime importance for soil processes such as organic matter turnover, nutrient cycling and uptake, and engineering of soil physical properties (Wurst et al. 2012). Earthworms, for example, the most recognized soil engineering organisms, can modify soil structure and enhance the availability of soil nutrients for plants through burrowing, casting and bioturbation (Edwards and Bohlen 1996). AMF can improve plant uptake of nutrients (mainly phosphorous) by forming a network of fine extra-radical hyphae (Smith and Read 2008). Plant-parasitic nematodes, known as pest organisms in agriculture, can inhibit plant growth through their direct root feeding; yet, they can also indirectly increase nutrient mineralization through increased carbon and nutrient leaching from the

damaged roots, which promotes soil microbial activity and hence plant nutrient uptake (Bardgett et al. 1999). Both, belowground mutualists and antagonists of plants, have been reported to influence aboveground herbivory (Wurst et al. 2004, Leimu and Koricheva 2006, Núñez-Farfán et al. 2007, Wurst and van der Putten 2007) and floral visitation rates by pollinators (Gange and Smith 2005, Poveda et al. 2005, Wolfe et al. 2005, Cahill et al. 2008, Becklin et al. 2011). Furthermore, it has been proposed that individual effects of soil organisms may modify the impact of other groups when combined (Bradford et al. 2002, Ladygina et al. 2010, Paudel et al. 2015). However, the multiple interactions between functionally dissimilar soil organism groups and their influence on grassland ecosystems have rarely been explored.

In most studies on the effects of multiple, potentially interacting drivers on ecosystem functions (e.g. soil organisms and plant intraspecific diversity), effects on plant performance and aboveground higher trophic levels are largely measured in greenhouse experiments and during the first few months of the lifespan of the plants. However, different results may be expected under field conditions, since a more diverse set of soil organisms may interact in the field (Jiang et al. 2009), potentially resulting in complex interactions above- and below-ground (Bradford et al. 2002, Pineda et al. 2010, Johnson et al. 2012, Sabais et al. 2012). Therefore, the aim of the present study was to elucidate the individual and combined impacts of functionally dissimilar soil organisms and plant intraspecific diversity on ecosystem functions of a clover-grass community in a field setting. Soil organisms such as earthworms, AMF and plant-parasitic nematodes were added to natural background soil communities, without using pesticide as control, since pesticides might also influence non-target soil organisms (Ekelund 1999, Yang et al. 2011, Al-Assiuty et al. 2014, Pelosi et al. 2014).

Overall, we predicted that: 1) higher plant intraspecific diversity increases aboveground primary production because of functional complementarity among cultivars, with detrimental soil organisms modifying the effect of plant intraspecific diversity on aboveground primary production; 2) floral visitation rate is promoted by the beneficial soil organism treatments (i.e. AMF and earthworms) because of the nutrient-mediated improvement of floral quantity or quality, and by higher plant intraspecific diversity because of a higher diversity of floral characteristics; 3) aboveground herbivory is affected by the added soil organisms through plant-mediated indirect effects. Aboveground herbivory might be decreased by detrimental soil organisms (i.e. the nematodes) through systemically induced plant defenses, and counterbalanced by beneficial soil organisms and plant intraspecific diversity through compensatory growth.

## **Materials and methods**

## Experimental setup

On the campus of Freie Universität Berlin, Germany, a fully-factorial field experiment with four different categorical treatments was set up on a former cornfield, consisting of 64 1 m × 1 m plots, separated by 1 m of a grass-sown path (*Festuca rubra* L.). The plots were established in April 2012 (see also Chapter 2 for a detailed description of the plot characteristics). The crossed, fully-factorial design included an intraspecific diversity treatment of the plant community (low/high), AMF addition (no/yes), earthworm addition (no/yes), and nematode addition (no/yes) with four replicates each treatment.

Plant intraspecific diversity was manipulated by sowing seed mixtures of clover and grass cultivars with one or four cultivars of *Trifolium pratense* L. (Red clover) and one or four cultivars of *Lolium perenne* L. (Perennial ryegrass) representing the low and high diversity treatment, respectively. To minimize the sampling effect on the results, the clover and grass cultivars in the low diversity treatment were randomly chosen and paired (Appendix Table S3.1). The *T. pratense* cultivars ‘Milvus’ ‘Larus’ ‘Diplomat’ and ‘Taifun’ and the *L. perenne* cultivars ‘Lipresso’ ‘Lacerta’ ‘Licampo’ and ‘Sures’ were used. Clover and grass seeds (*L. perenne*: 660 seeds m<sup>-2</sup>, *T. pratense*: 1350 seeds m<sup>-2</sup>) were sown in the same total numbers in the low and high plant intraspecific diversity treatments.

For the AMF addition treatment 730.0 ± 0.5 g sand with commercial AMF inoculum (from INOQ GmbH; Schnega, DE) was added to half of the plots in April 2012 and 2013. The same amount of pure sand without AMF inoculum was added to the control plots. The inoculum consisted of three AMF species *Claroideoglossum etunicatum* W.N. Becker & Gerd., *Glomus multisubstansum* Mukerji, Bhattacharjee & J.P. Tewari and *Rhizophagus irregularis* (Błaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler comb. nov., and contained 220 mycorrhizal units (including AM hyphae and spores) per cm<sup>3</sup> substrate.

The vertical-burrowing anecic earthworm species *Lumbricus terrestris* L. (Klages Angelköder, Brieskow-Finkenheerd, DE) was selected as our experimental species. In May 2013, seven similar-sized individuals were cleaned, weighed, and added to holes (15cm depth, dug with a shovel) into each earthworm treatment plot weekly for four weeks. In May 2012, fifteen adult earthworms *L. terrestris* were added to the plots. Because the fences are not effective in preventing the escape of earthworms (Eisenhauer et al. 2009a, b, Eisenhauer pers. comm.), we chose to add the earthworms in the plots repeatedly instead. The earthworm control plot was treated the same way, i.e. holes of the same size were dug and covered again without an addition of earthworms.

To achieve increased levels of root herbivory (Freckman 1982, Gugino et al. 2006), northern root knot nematodes *Meloidogyne hapla* Chitwood (provided by HZPC Research & Development, Metslawier, NL) were mixed with 1L tap water and evenly poured around plant roots in each nematode treatment plot ( $937,500 \pm 500$  juveniles  $m^{-2}$ ) in May 2013. The same volume of tap water without nematodes was evenly poured onto the control plots. The plots were kept moist by irrigating them regularly over the next three days to facilitate the establishment of nematodes.

### Sampling

To measure aboveground primary production, we harvested aboveground plant biomass with 20 cm  $\times$  40 cm frames in the center of each plot, in June and October 2013. To maintain the vitality of plants, all aboveground shoots in the frames were cut at 5 cm above the ground level. Sampled shoots were then separated into species and oven-dried at 50°C and weighed. Weeds were not included when measuring aboveground primary production as the biomass of weeds account for a very small percentage of total plot biomass ( $1.7\% \pm 0.7\%$ ). The measured biomass can only be related to the respective cultivar of *T. pratense* or *L. perenne* in the low diversity treatment, while the distinction between cultivars of clover or grass was not possible in the high diversity treatment.

At the second harvest in October 2013, we extracted soil samples with four medium sized soil cores (5 cm diameter  $\times$  10 cm deep) at the same positions of each 1 m  $\times$  1 m plot, placed on the diagonal line in 35 cm distance to each corner. To determine how root biomass was influenced by the treatments, all roots were extracted from the soil samples, washed to remove soil particles, and weighed after being dried at 50°C. The roots could not be sorted by plant cultivars or species.

To evaluate the efficiency of the earthworm addition treatment, earthworms samples of all plots were extracted by electroshocking in October 2013, using an octet device (DEKA 4000, Deka Gerätebau, Marsberg, DE) (Thielemann 1986). In each plot, the earthworm extraction was performed for 40 minutes, increasing the voltage from 250 V (5 min) to 300 V (5 min), 400 V (5 min), 500 V (5 min) and 600 V (10 min). The extracted earthworms were counted and weighed. Earthworm individuals were separated into adults and juveniles, the adults were then identified to species level. To measure AMF root colonization rate, 30 random sub-samples (1.5 cm each) of fine roots per plot were cleared with 10% KOH and stained with 0.05% Trypan blue in lactoglycerol. The percentage root colonization by AMF (arbuscules, vesicles and hyphae) was calculated under the microscope at 200  $\times$  magnification (at 100 intersects along the root) using the magnified intersections method (McGonigle et al. 1990). To ascertain the efficacy of the nematode

addition treatment, all root knots induced by *M. hapla* on all sampled roots were counted to check the colonization rate by nematodes. The number of root knots per plot was then standardized with regard to dry root weight.

To assess the percentage of leaf damage by shoot herbivores, 50 randomly selected leaves of *T. pratense* per plot were chosen at both harvests. All the sampled leaves per plot were scanned with an Epson Perfection 4990 photo scanner (Seiko Epson Corporation, Hino, Tokyo, JP) and the leaf area consumed by shoot herbivores was estimated with the WinFolia leaf area analysis software (Regent Instruments, Sainte-Foy, CA) by reconstructing the leaf area before damage and calculating the proportion consumed. The leaf damage of *L. perenne* by shoot herbivores was not monitored as no evidence of leaf damage was observed in the experimental plots.

All the observations of floral visitors were performed randomly among plots for 10 minutes per plot on sunny days from 22-25 July 2013 between 10: 00 and 14: 00 hours, when the *T. pratense* was at the stage of maximum flowering and pollinators were most active at the site. A “visit” was defined as a single pollinator staying on one flower longer than two seconds. A reference collection of pollinators was established by catching flower visitors at the site before the recording of pollinator visits. Within the bees, our groupings included large-bodied bumble bees (*Bombus* spp.), honey bees (*Apis* spp.) and “other pollinators” including wasps, butterflies, hoverflies and muscid flies. We followed all pollinators within the plots and used handheld digital voice recorder to record the pollinator taxa, their visits, and time spent per visit in seconds. The number of open flowers in the plots was counted before the observations of floral visitors. We then analyzed the abundance of pollinators, total frequency of pollinator visits, and average floral visitation length per visit by the pollinators.

### **Statistical analyses**

All statistical analyses were implemented using the software ‘R 3.1.3’ (R Core Team 2013). We analyzed the treatment effects on aboveground biomass and leaf damage by fitting linear mixed-effects models with the treatments (plant intraspecific diversity, AMF addition, earthworm addition and nematode addition) as fixed effects and the plot as a random effect using the function `lme()` in the *nlme* package (Pinheiro et al. 2015). Factorial ANOVAs were calculated to test the effect of the treatments and their interactions on root biomass, average floral visitation length and number of root knots per gram root biomass. The normal distribution of errors (visual residues analysis, Shapiro-Wilk test) and homogeneity of variances (Levene’s test) were used to evaluate the qualification of the data for ANOVA. Response variables were transformed when necessary to achieve normality and homoscedasticity. Root biomass and the number of root knots per gram root

biomass were log transformed. Generalized linear models (GLMs) with a quasibinomial error distribution (Bolker et al. 2009) were used for the analysis of AMF root colonization rate. For the dataset on the number of open *T. pratense* flowers, the abundance of pollinators, total frequency of pollinator visits and total floral visitation length we used GLMs with a quasipoisson error distribution (Guisan et al. 2002). The effects of the treatments and their interactions on the floral visitation rates (i.e. the abundance of pollinators, total frequency of pollinator visits, as well as total and average floral visitation length) were analyzed with the number of open flowers as a covariate, and with plot as a random effect. Since the abundance of *L. terrestris* was both highly overdispersed and zero-inflated, we analyzed the effect of earthworm addition on the abundance of *L. terrestris* using a zero-inflated poisson model with the *zeroinfl* function in the *pscl* library in R (Zeileis et al. 2008).

## Results

### Soil organism treatments

AMF root colonization rate was not significantly different in the plots with an addition of AMF compared with the control (AMF treatment:  $4.8 \pm 1.4\%$ ; Control:  $5.2 \pm 1.3\%$ ), but was increased by the addition of earthworms from  $2.8 \pm 0.6\%$  (Control) to  $7.2 \pm 1.7\%$  (Earthworm treatment) (Table 3.1). We generally found low numbers of *L. terrestris* in the earthworm addition plots at harvest, yet the numbers were higher compared with control plots (Earthworm treatment:  $0.97 \pm 0.17$  individuals, Control:  $0.50 \pm 0.15$  individuals;  $df_1 = 1$ ,  $df_2 = 62$ ,  $z = -2.15$ ,  $P = 0.032$ ). The number of root knots per gram root biomass was increased by 145.5 % by the nematode treatment, from  $11.5 \pm 3.3$  root knots  $g^{-1}$  (Control) to  $28.2 \pm 5.6$  root knots  $g^{-1}$  (Nematode treatment) (Table 3.1).

### Aboveground primary production

The total aboveground biomass of the plant community was increased by the high plant intraspecific diversity treatment, and this effect was marginally influenced by the nematode addition treatment (Fig. 3.1, Table 3.2). The clover-grass community was dominated by *T. pratense* in the end ( $96.7 \pm 1.2\%$ , Fig. 3.2). The aboveground biomass of *T. pratense* was significantly increased by 51.4% in the high plant intraspecific diversity treatment, only within the nematode addition treatment (Fig. 3.1, Table 3.2). We further found that the aboveground biomass of the plant community and the aboveground biomass of *T. pratense* were both marginally influenced by a four-way interaction of all treatments (Table 3.2), indicating that the interaction between the nematode treatment and plant intraspecific diversity was more pronounced in plots where earthworms and

**Table 3.1** Results of linear models testing the effects of plant intraspecific diversity (Div), arbuscular mycorrhizal fungi (AMF), earthworm (E) and nematode (Nem) treatments on total dry root biomass (log transformed), the number of root knots per gram root biomass, and the average floral visitation length per visit (in seconds); and of generalized linear models testing the effects of all treatments on AMF root colonization rate, the number of open *T. pratense* flowers, the total frequency of pollinator visits and the abundance of pollinators at the stage of maximum flowering.

	df	AMF root colonization rate		Root biomass		Number of root knots		Number of open flowers		df	Abundance of pollinators		Total frequency of pollinator visits		Average floral visitation length	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
NF	-	nc	nc	nc	nc	nc	nc	nc	nc	1,47	<b>14.379</b>	<b>0.000</b>	<b>18.386</b>	<b>0.000</b>	<b>2.970</b>	<b>0.092</b>
Div	1,48	0.310	0.581	0.736	0.395	0.000	0.987	0.233	0.631	1,47	<b>3.859</b>	<b>0.055</b>	1.817	0.184	0.154	0.696
AMF	1,48	0.039	0.844	2.506	0.120	2.645	0.110	2.191	0.145	1,47	0.020	0.887	1.092	0.301	<b>3.109</b>	<b>0.085</b>
Nem	1,48	0.054	0.817	0.880	0.353	<b>15.634</b>	<b>0.000</b>	0.297	0.588	1,47	0.874	0.355	2.292	0.137	0.003	0.955
E	1,48	<b>6.419</b>	<b>0.015</b>	0.911	0.345	2.073	0.156	<b>6.143</b>	<b>0.017</b>	1,47	0.024	0.879	1.354	0.251	<b>5.271</b>	<b>0.026</b>
Div:AMF	1,48	1.714	0.197	0.193	0.663	0.038	0.847	0.261	0.612	1,47	0.053	0.820	0.865	0.357	0.353	0.555
Div:Nem	1,48	0.705	0.405	0.464	0.499	1.155	0.288	0.144	0.706	1,47	1.458	0.233	0.596	0.444	0.057	0.812
AMF:Nem	1,48	0.020	0.888	0.454	0.504	0.085	0.772	0.461	0.500	1,47	0.236	0.630	0.003	0.955	2.492	0.121
Div:E	1,48	0.007	0.935	0.014	0.908	0.001	0.973	0.025	0.875	1,47	0.010	0.922	0.010	0.922	0.001	0.980
AMF:E	1,48	0.319	0.575	2.295	0.136	2.528	0.118	0.779	0.382	1,47	0.342	0.561	1.268	0.266	0.238	0.628
Nem:E	1,48	0.087	0.770	0.715	0.402	0.175	0.677	<b>2.857</b>	<b>0.097</b>	1,47	0.068	0.796	0.001	0.982	0.167	0.685
Div:AMF:Nem	1,48	0.004	0.949	1.450	0.234	1.286	0.262	0.418	0.521	1,47	0.745	0.393	0.063	0.803	0.328	0.570
Div:AMF:E	1,48	0.142	0.708	0.193	0.662	0.046	0.832	0.454	0.504	1,47	1.046	0.312	0.260	0.613	0.651	0.424
Div:Nem:E	1,48	0.620	0.435	1.437	0.237	1.999	0.164	0.581	0.450	1,47	0.016	0.900	0.004	0.953	0.001	0.969
AMF:Nem:EW	1,48	1.039	0.313	0.389	0.536	0.749	0.391	0.019	0.891	1,47	0.708	0.404	0.254	0.616	0.482	0.491
Div:AMF:Nem:E	1,48	0.091	0.764	0.007	0.932	0.704	0.406	1.235	0.272	1,47	0.447	0.507	0.398	0.531	0.007	0.934

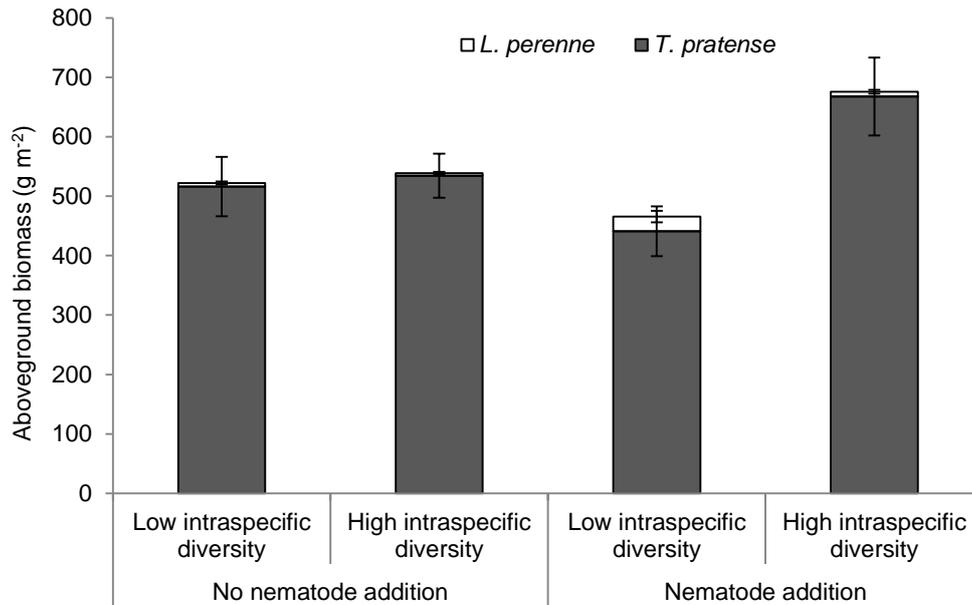
Number of open flowers (NF) was used as a covariate in the analysis on abundance of pollinators, total frequency of pollinator visits and average floral visitation length.

<sup>a</sup>Significant *P*-values ( $P < 0.05$ ) are printed in bold and marginally significant *P*-values ( $0.05 < P < 0.1$ ) are printed in bold and italics. For each treatment:  $n = 4$ , degree of freedom = 1, 62. nc: not calculated

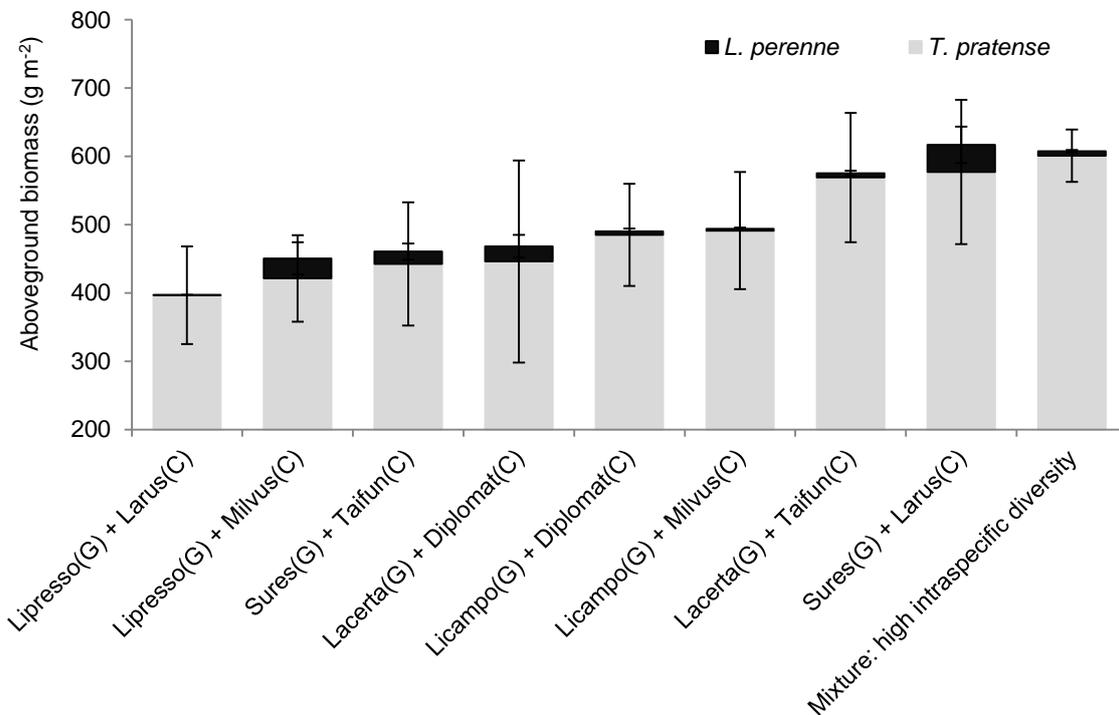
AMF were added (Fig. S3.1).

**Table 3.2** Results of linear mixed models testing the effects of plant intraspecific diversity (Div), arbuscular mycorrhizal fungi (AMF), earthworm (E) and nematode (Nem) treatments on aboveground biomass of *Trifolium pratense* and *Lolium perenne*, and leaf damage of *T. pratense* [%]. Significant *P*-values ( $P < 0.05$ ) are printed in bold and marginally significant *P*-values ( $0.05 < P < 0.1$ ) are printed in bold and italics. For each treatment:  $n = 8$ , degrees of freedom = 1, 111

	Total aboveground biomass		Aboveground biomass of <i>T. pratense</i>		Aboveground biomass of <i>L. perenne</i>		Leaf damage [%]	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Div	<b>5.071</b>	<b>0.029</b>	<b>5.974</b>	<b>0.018</b>	2.085	0.155	0.008	0.929
AMF	0.273	0.604	0.328	0.569	0.144	0.706	0.716	0.402
Nem	0.643	0.427	0.336	0.565	<b>3.168</b>	<b>0.081</b>	1.422	0.239
E	0.396	0.532	0.305	0.584	0.394	0.533	0.150	0.701
Div: AMF	0.281	0.599	0.321	0.574	0.075	0.786	0.921	0.342
Div: Nem	<b>3.697</b>	<b>0.061</b>	<b>4.316</b>	<b>0.043</b>	1.343	0.252	0.025	0.875
AMF: Nem	1.786	0.188	1.458	0.233	1.131	0.293	0.419	0.521
Div:E	0.000	0.984	0.013	0.910	1.124	0.294	1.688	0.200
AMF:E	0.671	0.417	0.736	0.395	0.080	0.779	0.484	0.490
Nem:E	0.723	0.399	0.912	0.345	0.635	0.430	2.703	0.107
Div:AMF:Nem	2.301	0.136	2.383	0.129	0.026	0.874	0.010	0.921
Div:AMF:E	0.502	0.482	0.510	0.479	0.000	0.987	0.635	0.429
Div:Nem:E	0.148	0.702	0.161	0.690	0.015	0.903	0.290	0.593
AMF:Nem:E	0.029	0.865	0.060	0.808	0.333	0.567	0.812	0.372
Div:AMF:Nem:E	<b>3.209</b>	<b>0.080</b>	<b>3.236</b>	<b>0.078</b>	0.000	0.999	0.000	0.997



**Figure 3.1** The effect of plant intraspecific diversity and of the addition of nematodes on aboveground biomass of *T. pratense* and *L. perenne*. Mean values and standard errors are shown for each treatment.  $n = 32$

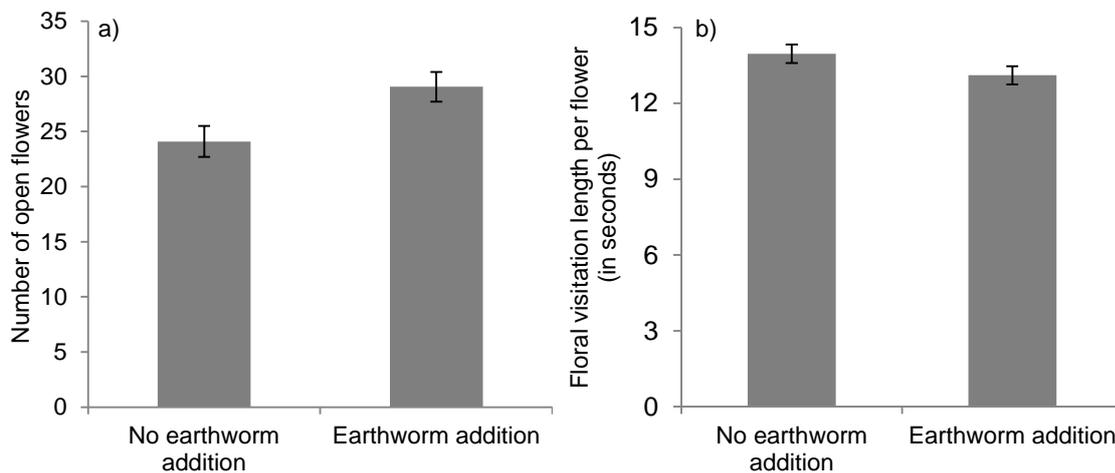


**Figure 3.2** Aboveground biomass of cultivar mixtures. Aboveground biomass of the *T. pratense* cultivars (Milvus, Larus, Diplomat and Taifun) and the *L. perenne* cultivars (Lipresso, Lacerta, Licampo and Sures) in the low intraspecific diversity plant community ( $n = 8$ ) and in the high intraspecific diversity plant community ( $n = 64$ ). G: *L. perenne* cultivar, C: *T. pratense* cultivar. Mean values and standard errors are shown for each treatment

The high plant intraspecific diversity community outperformed most cultivar mixtures in the low diversity treatment. Certain combinations of cultivar mixtures in the low plant intraspecific diversity treatment had similar aboveground biomass as the high plant intraspecific diversity treatment (e.g. the combination of clover cultivar Larus and grass cultivar Sures, see Fig. 3.2). The aboveground biomass of *L. perenne* and the belowground biomass of the plant community were not influenced by any treatment (Table 3.1). The overall leaf damage of *T. pratense* was also not influenced by any treatment (Table 3.2).

### Flowers and pollination

The number of open flowers of *T. pratense* at the stage of maximum flowering was increased by the addition of earthworms (Fig. 3.3a), whereas it was not changed by the other treatments (Table 3.1). The abundance of pollinators was marginally higher in the plots with high plant intraspecific diversity ( $32.2 \pm 1.3$  individuals) compared to the plots with low plant intraspecific diversity ( $27.9 \pm 1.5$  individuals) (Table 3.1). Furthermore, the addition of earthworms decreased the average floral visitation length per visit by all pollinators (Fig. 3.3b, Table 3.1). Both, the total frequency of pollinator visits and the total floral visitation length by all pollinators, were not influenced by any treatment (Table 3.1).



**Figure 3.3** The effect of earthworm addition on a) the number of open *T. pratense* flowers at the stage of maximum flowering; and b) the average floral visitation length per visit (in seconds) by pollinators. Mean values and standard errors are shown for each treatment.  $n = 32$

## Discussion

Explicitly designed experiments in temperate grasslands have only recently started to be used for exploring the effects of plant intraspecific diversity on multiple ecosystem functions. Our field study was the first to combine the investigation of plant intraspecific diversity effects with the study of effects of functionally dissimilar soil biota to demonstrate their respective and interacting roles on ecosystems functions, e.g. aboveground primary production, floral visitation by pollinators as well as herbivory.

In our experiment we found that plant-parasitic nematodes significantly mediated the effect of plant intraspecific diversity on plant primary production of *T. pratense* under field conditions. This finding is in line with previous results suggesting that positive plant diversity-productivity relationships are often found in legume plants (including *T. pratense*) (HilleRisLambers et al. 2004, Spehn et al. 2005, Marquard et al. 2009). Further, *L. perenne* was shown to be a non-host for the plant-parasitic nematode *M. hapla* (Knight et al. 1997); *T. pratense*, instead, is a common host (Mojtahedi et al. 1988, Stanelis 2004) and its cultivars have been shown to differ in resistance and/or tolerance against herbivorous nematodes including *M. hapla* (Willis 1981, Taylor and Quesenberry 1996). Disturbance (e.g. herbivory) has previously been found to be crucial in influencing the effect of plant intraspecific diversity on ecosystem functions (Hughes and Stachowicz 2004, Burton et al. 2014, Cook-Patton et al. 2014). The increased disturbance level on *T. pratense* by the nematode herbivory might consequently influence the effect of plant intraspecific diversity on productivity of *T. pratense*.

The role of nematodes in influencing the effect of plant intraspecific diversity on productivity of *T. pratense* is partially consistent with studies reporting that plant antagonists (soil pathogens) may mediate the positive plant species-productivity relationship (Schnitzer et al. 2010, Maron et al. 2011.). Previous studies have proposed that the effects of plant species diversity on plant productivity can be mediated by soil biota such as soil microbes and pathogens (van der Heijden et al. 2008, Schnitzer and Klironomos 2011, Eisenhauer 2012). Studies have also shown that belowground herbivores can alter plant community structure (De Deyn et al. 2003, Wallinger et al. 2014) and influence the plant diversity-productivity relationship (Eisenhauer 2012). Maron et al. (2011) showed that the positive effect of plant species diversity on aboveground primary production in their study was driven by soil pathogens, through a suppression of the productivity of assemblages with low species diversity and the maintenance of the productivity of assemblages with high species diversity. Correspondingly, testing the effect of plant intraspecific diversity instead of species diversity, we found that the plant-parasitic nematodes mediated the positive effect of plant intraspecific diversity on aboveground primary production of *T. pratense*. However,

the pattern of the interaction was different: nematodes had no effect on the primary production in the low plant intraspecific diversity treatment, while they increased the primary production in the high plant intraspecific diversity treatment.

There are different possible mechanisms for the role of the plant-parasitic nematodes in influencing the positive effect of plant intraspecific diversity on productivity of *T. pratense*. First, the clover cultivars differed in their primary production and in their resistance to nematode herbivory. Depending on the level of nematode herbivory, plant growth can either be negatively or positively affected by nematodes. Although generally recognized as pest, low levels of root herbivory by plant-parasitic nematodes can promote plant growth indirectly through enhancing the leaching of nutrients from damaged root systems to the rhizosphere (Yeates et al. 1998, 1999a, 1999b). The higher possibility of containing cultivars with specific characteristics (e.g. high yield or positive response to nematode herbivory) in the plant communities with high intraspecific diversity might have led to the positive intraspecific diversity–productivity effect on *T. pratense*. Furthermore, positive cultivar interactions or complementarity between cultivars in regard to resource allocation might also contribute to the increase of primary production in plant community with high plant intraspecific diversity. The higher aboveground primary production under high plant intraspecific diversity and nematode herbivory may also be attributed to the specific high-yield cultivars combination (Mytton 1975, Huston 1997).

Second, clover cultivars of the low diversity treatment generally had lower primary production compared to the high intraspecific diversity treatment, which indicates that complementary effects (Loreau and Hector 2001) might be a potential explanation for the positive effect of plant intraspecific diversity on productivity of *T. pratense*. In a recent review, Tooker and Frank (2012) ascribed greater plant productivity in genotypically diverse populations to varietal complementarity, i.e. niche partitioning (Crutsinger et al. 2006, Kotowska et al. 2010). Previous studies have shown that cultivars exploited different micro-niches for light, nutrient and water resources (Smithson and Lenne 1996) and differed in productivity and quality (Dear et al. 1993). In our case, we propose that the complementary effect can be altered by nematode herbivory. Herbivory from plant-parasitic nematodes might facilitate nutrient flow (Bardgett et al. 1999); and communities with high plant intraspecific diversity may possess higher abilities of utilizing these nutrients. Resource limitation is believed to be a key factor of plant productivity in response to diversity (Hooper et al. 2005, Tooker and Frank 2012). Bardgett et al. (1999) summarized the beneficial roles of infestations of root nematodes in temperate grassland ecosystems and described, for example, facilitation of soil microbial activity by high carbon supply, as well as enhanced nutrient mineralization and plant nutrient uptake. Enhanced root exudation and soil microbial

activity in the *rhizosphere* mediated by nematode feeding can contribute to the improvement of plant productivity. Thus, we assume that in our study communities with high plant intraspecific diversity were able to exploit more of the resources potentially made available by nematodes than low plant intraspecific diversity communities. Moreover, the positive plant intraspecific diversity effect on aboveground primary production of *T. pratense* mediated by the nematode addition treatment tended to be more pronounced in the earthworm treatment and the AMF treatment. Functionally dissimilar soil organisms might strengthen or counterbalance each other's effects on plant growth depending on their identities or functional roles (Wurst et al. 2008, Eisenhauer et al. 2010b, Ladygina et al. 2010). We assume that in our case the nematode effect might be strengthened by the enhanced nutrient availability with the addition of beneficial soil organisms. Further research is needed to elucidate the mechanisms behind the observed effect of soil organisms on the plant intraspecific diversity-productivity effect.

We did not find a positive effect of AMF addition on aboveground primary production as in a related study the year before (Guo et al. 2015), probably due to the widespread dispersal of AMF by plant roots or mobile soil organisms (e.g. earthworms) with time. The abundance of added earthworms was doubled and they were added four times instead of once. Likely for this reason, our results show more effects of earthworm addition than in the first year (Guo et al. 2015). For instance, in the present study, we found a positive effect of earthworm addition on AMF root colonization rate of the plants in the plots, whereas this effect was not observed in the study the year before (Guo et al. 2015). Previous studies investigating the effects of earthworms on AMF dispersal reported inconsistent results. Some studies found a positive relationship between earthworm activity and AMF root colonization rate or AMF biomass (Reddell and Spain 1991, Gange 1993, Gormsen et al. 2004), while others showed an idiosyncratic (Pattinson et al. 1997, Bonkowski et al. 2000, Lawrence et al. 2003, Ortiz-Ceballos et al. 2007) or no response (Tuffen et al. 2002). We assume that in our case the addition of earthworms facilitated the dispersal of AMF propagules in the soil and hence stimulated the colonization of plant roots.

In our study, above- and below-ground primary production were found to be unaffected by the earthworm addition treatment, however, the number of open flowers responded positively to the addition of earthworms. Although earthworms have been generally reported to modify soil structure, mineralize soil nutrients, induce hormone production and alter soil microbial community structure, their effects on plant growth can be inconsistent, which might be attributed to plant or earthworm identity, soil properties and the influence of other soil organisms (Scheu 2003, Wurst et al. 2003, Aira and Pearce 2009, Laossi et al. 2010, Zaller et al. 2011a). Cardoza et al. (2012) found that earthworm compost amendment enhanced soil quality and hence affected plant-pollinator

interactions on *Cucumis sativus* L.. The earthworm compost influenced the nutrition and overall performance of flowers, such as producing larger and more active ovaries with better nutritional quality, which subsequently increased visit length and reduced the initial discovery time by pollinators (Cardoza et al. 2012). In contrast, the floral visitation length per visit of all pollinators was reduced by the addition of earthworms in our study. It seems that the pollinators decided to stay shorter on an individual flower in the earthworm treatment plots. Earthworm addition may have influenced both the number of *T. pratense* flowers and floral characteristics such as nectar quality of *T. pratense*. Further studies examining floral characteristics, visits by pollinators and reproductive outcome of plants in relation to earthworm activity are necessary to further understand their impact on mutualists and on higher trophic levels.

In addition to this, the abundance of pollinators tended to be higher in communities with higher plant intraspecific diversity. Similar results have been reported in previous studies concerning species diversity loss and the pollinator crisis (Kearns et al. 1998, Kevan 1999, Hajjar et al. 2008). Distinct cultivars of *T. pratense* differing in their floral morphologies, maturity date and primary metabolites (Muntean 2006, Saviranta et al. 2008, Tucak et al. 2013, Vleugels 2013), likely attracted more pollinators in plant community of high plant intraspecific diversity (Ghazoul 2006).

In general, research on soil organisms is becoming increasingly important for sustainable agriculture, since modern agricultural practices may threaten the diversity of soil biota (Turbé et al. 2010). The present study is the first study investigating the influence of soil organisms on the effect of within-species diversity on ecosystem functions and should inspire future diversity-ecosystem functions research. The results showed that the positive effect of plant intraspecific diversity on plant productivity in *T. pratense* is mediated by soil organisms, suggesting that soil organisms play a vital role in the effect of plant intraspecific diversity on plant primary production in agroecosystems. Further, functionally dissimilar soil organisms can separately or interactively influence aboveground plant mutualists in the field. Future research should pay more attention to soil organisms as potential driving factors of diversity-ecosystem functions relationships at plant intraspecific level.

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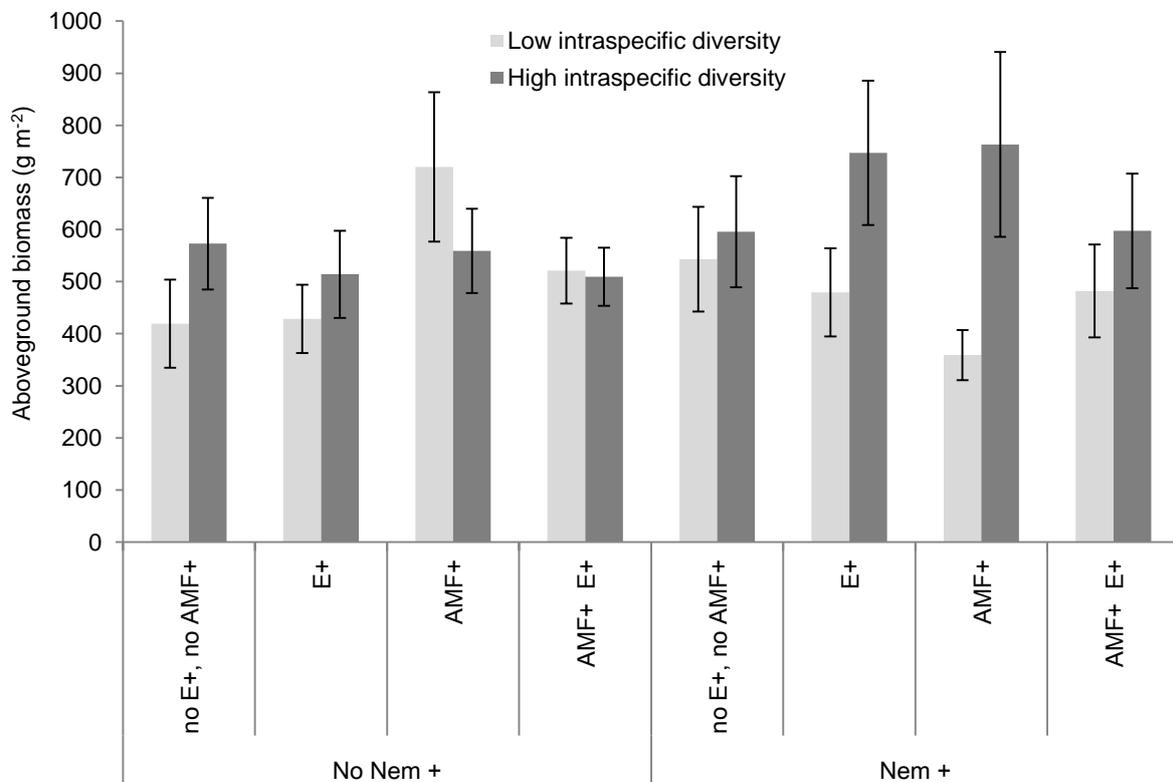
*Appendix B. Supplementary Material*

### Appendix B: Supplementary Material to Chapter 3

**Table S3.1** The experimental design including the low and high plant intraspecific diversity treatment

Species	Cultivars	Low cultivar diversity								High cultivar diversity	
		Mixture 1	Mixture 2	Mixture 3	Mixture 4	Mixture 5	Mixture 6	Mixture 7	Mixture 8	Mixture 9	
<i>Lolium perenne</i>	Lipresso	1	1								1
	Lacerta			1	1						1
	Licampo					1	1				1
	Sures							1	1		1
<i>Trifolium pratense</i>	Milvus	1				1					1
	Larus		1					1			1
	Diplomat			1			1				1
	Taifun				1				1		1
Number of cultivars		2	2	2	2	2	2	2	2		8
Replicates		4	4	4	4	4	4	4	4		32

**Figure S3.1** The effect of plant intraspecific diversity and of the addition of soil organisms, i.e. earthworms (E), AMF and plant-parasitic nematodes (Nem), on total aboveground biomass of *T. pratense* and *L. perenne* in the field experiment. Mean values and standard errors are shown for each treatment.  $n = 8$



## **Chapter 4**

### **Cultivar-specific responses to plant-parasitic nematodes drive the effect of intraspecific diversity on productivity**

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## **Cultivar-specific responses to plant-parasitic nematodes drive the effect of intraspecific diversity on productivity**

### **Abstract**

Soil organisms can influence the plant diversity-productivity relationship. However, very little is known about the role of soil organisms in driving the effect of plant intraspecific diversity on plant productivity. In the present greenhouse experiment, we investigated the effects of different levels of plant intraspecific diversity (manipulated by using two-, four-, eight- and sixteen-cultivar mixtures of *Trifolium pratense* L. and *Lolium perenne* L.) and plant-parasitic nematodes (*Meloidogyne hapla* Chitwood) on primary production, nutrient uptake and community evenness. We found that the presence of plant-parasitic nematodes mediated the effect of plant intraspecific diversity on primary production in the intermediate level of the intraspecific diversity, the four-cultivar mixture. The primary production of the *T. pratense* cultivars was influenced by nematodes or plant intraspecific diversity. Specifically, the primary production of the *T. pratense* cultivar Violetta was significantly increased by the addition of nematodes, while the *L. perenne* cultivars did not respond to the nematode treatment. Half of the *T. pratense* and *L. perenne* cultivars were influenced by plant intraspecific diversity, and the size and direction of the effects depended on cultivar identity. The evenness of the plant community was decreased with higher plant intraspecific diversity, while the evenness of the plant community was not affected by nematodes. We conclude that the positive response of certain *T. pratense* cultivars to plant-parasitic nematodes as well as the difference performance of plant cultivars in regard to plant intraspecific diversity contributed to the observed plant intraspecific diversity-productivity effects. This study highlights the influence of belowground parasitic nematodes on the effect of plant intraspecific diversity on productivity, suggesting that future biodiversity-ecosystem functions research should consider the effect of soil organisms.

### **Introduction**

Biodiversity is of crucial importance for the maintenance of ecosystem functions. Plant productivity is frequently measured in biodiversity studies as one of the most important ecosystem functions and plant diversity has been shown to influence ecosystem functions, primarily by improving plant productivity (e.g. Hooper and Vitousek 1997, van Ruijven and Berendse 2005, Schnitzer et al. 2010, Tilman et al. 2014). The positive effect of plant diversity on productivity was commonly explained by the processes selection effect and niche complementarity (Loreau and Hector 2001, van Ruijven and Berendse 2005, Hughes et al. 2008). Specifically, under high plant diversity the possibility is high that high-yield species are present (i.e. selection effect) (Fox 2005);

and high plant diversity is able to enhance resource utilizing via niche partitioning (i.e. niche complementarity) (Loreau and Hector 2001, Zuppinger-Dingley et al. 2014). Further, increased investigations considering also the belowground compartment have demonstrated that the plant diversity-productivity relationship can also be influenced by belowground processes, i.e. plant-soil feedback (Kulmatiski et al. 2012, Jing et al. 2015) and the activity of soil biota (Schnitzer et al. 2010, Maron et al. 2011, de Kroon et al. 2012, Eisenhauer 2012, Eisenhauer et al. 2012a). Thus, it is of crucial importance to consider the influence of belowground processes in biodiversity-ecosystem functions studies.

Recent studies have underlined that soil biota (e.g. arbuscular mycorrhizal fungi, decomposer, and soil pathogens) may play a predominant role in driving the positive plant diversity-productivity relationship (van der Heijden et al. 2008, Schnitzer et al. 2010, Maron et al. 2011, Eisenhauer et al. 2012b). Soil organisms can alter nutrient cycling, and further positively and negatively influence plant primary production, as well as plant community structure and dynamics (Reynolds et al. 2003, Zaller et al. 2011a, Sabais et al. 2012, Wurst et al. 2012, Körner et al. 2014). By comparing the effect of functionally dissimilar soil organisms (e.g. plant mutualists, pathogens, parasites, etc.), previous studies (Schnitzer and Klironomos 2011, Eisenhauer 2012) have stressed the predominant role of plant pathogens or parasites in driving the plant diversity-productivity relationship.

Plant intraspecific diversity, a substantial part of biodiversity, is receiving increasing interest as a crucial determinant in influencing plant primary production (Kotowska et al. 2010, Tooker and Frank 2012, Himanen et al. 2013, Bukowski and Petermann 2014), resistance against plant invaders (Crutsinger et al. 2008a, Guo et al. 2015) and herbivores or diseases (Tooker and Frank 2012, Yang et al. 2012, Zeller et al. 2012, Shoffner and Tooker 2013), as well as soil community structure (Cadet et al. 2006, Crutsinger et al. 2008b). Recent studies have shown similar results regarding the effects of plant intraspecific diversity and plant species diversity on increasing plant productivity, as well as maintaining plant community structure (Crutsinger et al. 2006, Cook-Patton et al. 2011, Roger et al. 2012).

Previous studies commonly explained the plant intraspecific diversity-productivity relationship by selection effects and complementarity effects (Crutsinger et al. 2006, Cook-Patton et al. 2011, Kotowska et al. 2010, Tooker and Frank 2012). It has been suggested that under high plant intraspecific diversity the possibility is high that cultivars with specific characteristics (e.g. high yield, high competitiveness) are present (i.e. selection effects); and that high plant intraspecific diversity can increase resource exploitation through niche partitioning (i.e. niche complementarity) (Loreau and Hector 2001, Tooker and Frank 2012). We propose that belowground processes

mediated by soil organisms might be overlooked within this relationship. Soil organisms can directly and indirectly influence plant growth and plant population dynamics (Reynolds et al. 2003, Coleman et al. 2004, Wurst et al. 2012) and different plant cultivars might differ in their response to soil organisms (de Oliveira et al. 2006, Alves et al. 2014, Rodríguez-Blanco et al. 2015). Besides, similar to the effect of plant species diversity on the activity of soil organisms and the belowground community structure (Viketoft et al. 2009, Viketoft and Sohlenius 2011, Hacker et al. 2015, Kostenko et al. 2015), plant intraspecific diversity have also been found to affect soil communities (Cadet et al. 2006, Crutsinger et al. 2008b). Considering the driving role of soil pathogens in the species diversity-ecosystem functions relationship (Schnitzer et al. 2010, Maron et al. 2011, Eisenhauer 2012, Eisenhauer et al. 2012a, b), it may not be surprising when detrimental soil organisms have also the potential to influence diversity-productivity at the intraspecific plant diversity level. However, relevant experimental tests regarding the impact of soil organisms on ecosystem functions under different plant intraspecific diversity are lacking.

Since we recently found evidence that plant-parasitic nematodes modified the effect of plant intraspecific diversity on productivity of *T. pratense* (Chapter 3), we aimed to investigate the mechanisms behind the effect of plant-parasitic nematodes (*Meloidogyne hapla* Chitwood) on the plant intraspecific diversity-productivity relationship. Therefore, we set up a greenhouse experiment using four different levels of plant intraspecific diversity of a clover-grass community as well as a plant-parasitic nematode treatment. The clover-grass community was manipulated by sowing cultivar mixture of *Trifolium pratense* L. (red clover) and *Lolium perenne* L. (perennial ryegrass), which are widely used as forage crops in agricultural systems because of their ability to produce large quantities of high quality forage (Jung et al. 1996, Taylor and Quesenberry 1996, Moorby et al. 2009). Nowadays, breeding programs in agriculture provides us with many cultivars with various characteristics (e.g. yield, quality, competitiveness, resistance to their natural enemies, etc.) to study the effect of plant intraspecific diversity (Wilkins and Humphreys 2003, Vleugels 2013, Fu 2015). The cultivars of *T. pratense* and *L. perenne* differ in their yield, quality as well as resistance to herbivores and pathogens (Yeates et al. 1973, Smit et al. 2005, Tucak et al. 2013, Wims et al. 2013, Jacob et al. 2014). Further, plant-parasitic nematodes *M. hapla* is a very abundant species of multicellular soil organisms and one of the most prevalent pests in agricultural systems (Evans et al. 1993). *L. perenne* is not a host to *M. hapla*, and *T. pratense* is well studied regarding its interactions with *M. hapla* (Yeates et al. 1973, Mojtahedi et al. 1988, Knight et al. 1997, Kouamé et al. 1997). We hypothesized that 1) plant primary production increases with plant intraspecific diversity; 2) the effect of plant intraspecific diversity on plant primary production is influenced by plant-parasitic nematodes, specific plant cultivar(s), or their interactions; 3) The growth of specific cultivars might be influenced by nematodes and plant intraspecific diversity, and

the performance of different cultivar identity may affect the diversity effect.

## Materials and methods

In January 2014, we established a greenhouse experiment using soil from an old agricultural site (Albrecht-Thaer-Weg, Freie Universität Berlin, Germany). Prior to use, the soil was sieved and steamed (24 h, 100°C). The loamy soil contained 1.42 g total N kg<sup>-1</sup>, 18.2 g organic C kg<sup>-1</sup>, 325 mg available P kg<sup>-1</sup>, 194 mg available K kg<sup>-1</sup>, with pH 7.3 (LUFA, Rostock, Germany). Plant communities were established in pots in the greenhouse using this soil. In each pot (diameter 25 cm, height 15 cm) eight individuals of *T. pratense* and eight individuals of *L. perenne* were sown (Appendix Table S4.1). The pots were assigned to one of four intraspecific diversity treatments: two clover-grass cultivars (containing one *T. pratense* cultivar and one *L. perenne* cultivar;  $n = 16$  replicates), four clover-grass cultivars (containing two *T. pratense* cultivars and two *L. perenne* cultivars;  $n = 8$  replicates), eight clover-grass cultivars (containing four *T. pratense* cultivars and four *L. perenne* cultivars;  $n = 8$  replicates), and sixteen clover-grass cultivars (containing eight *T. pratense* cultivars and eight *L. perenne* cultivars;  $n = 6$  replicates). To minimize the potential confounding effects between cultivar identity and plant intraspecific diversity (Huston 1997), all cultivars were assigned to each treatment with same frequency (see details of cultivars selection in Appendix Table S4.1). Seeds were sown into pots at marked positions.

Half of the pots received a nematode treatment. For this treatment, 50 ml tap water with  $550 \pm 25$  juveniles of the northern root-knot nematode *M. hapla* (provided by HZPC Research & Development, Metslawier, NL) were evenly poured on the soil surface in each nematode treatment pot ( $n = 38$ ), seven weeks after sowing. The same volume of tap water without nematodes was added to the control pots. Pots were kept moist by watering thoroughly for the next two days to facilitate the establishment of the nematodes. Overall, 76 pots were used in the present experiment (Appendix Table S4.1). The experiment lasted for 16 weeks. Day length was maintained at 12 h with supplementary light (sodium lamps, 400 W). Temperature ranged between 15°C (night) and at maximum 26°C (day). The pots were watered every second or third day, each with the same amount of tap water to avoid effects of different water availability. The position of the experimental pots was randomized twice a week to minimize the influence of microclimate differences in the greenhouse.

After 16 weeks shoots of plant individuals were harvested separately, dried at 50°C and weighed. To determine the C and N content of each *T. pratense* cultivar, subsamples of leaves were collected for each pot and analyzed with a CN analyzer (Euro EA, HEKAtech GmbH, Germany). The leaves

of cultivars from two, four and eight clover-grass mixtures were evenly milled per pot before the C/N analyses. Roots were cleaned, dried at 50°C for three days and weighted. Roots were not sorted by cultivars or species because of the indivisibility of the mixed *T. pratense* and *L. perenne* root systems. In order to measure realized nematode abundances, 100g fresh weight soil samples were immediately collected with a soil core (2 cm diameter) after clipping the plant shoots from five randomly chosen locations of the top 10 cm of the experimental pots. The samples were sealed in plastic bags, transported to the laboratory in a cooling box, and stored at 4°C. Nematodes were extracted using a modified Cobb's method (van Bezooijen 1999). The soil was put on a moist double cotton/non-woven filter (Boeco, Germany) on plastic sieves for 72h and nematodes were allowed to pass through and collected in the water. Nematodes were extracted from the water suspension through a 5 µm pore filter. The extracted nematodes were compiled per pot, concentrated in 1 ml, heat killed (60°C, 2 min) and fixed in 4 ml of 4% formaldehyde. All nematodes in 5 ml samples were counted under low magnification (50×). Numbers were expressed per 100 g of soil fresh weight.

All analyses were conducted using 'R 3.1.3' (R core team 2013). The data on aboveground biomass was analyzed using linear models with the explanatory variables 'nematodes' and 'plant intraspecific diversity'. To examine the linear relationship of plant intraspecific diversity and productivity and to evaluate how the relationship might be altered by nematodes, the plant intraspecific diversity was analyzed as linear ( $Div_{linear}$ ) and categorical ( $Div_{cat}$ ) factors, respectively (Milcu et al. 2008, Eisenhauer et al. 2012b). All two-way interactions with nematodes were included. Normality was checked for using the Kolmogorov-Smirnov (Liliefors) test and homogeneity of variances using Levene's test. Data on individual or total aboveground biomass of *T. pratense* and *L. perenne*, and belowground biomass were log transformed to improve normality and homogeneity of variances. As we did not find a significant effect of nematodes and plant intraspecific diversity on the primary production of *L. perenne* or the whole plant community, we focused the analysis on *T. pratense* and the potential effect of certain *T. pratense* cultivars.

To test whether the primary production of plant cultivars differed in the nematode and plant intraspecific diversity ( $Div_{cat}$ ) treatments, we used linear mixed effects models (function `lme()` in the `nlme` package of R) (Pinheiro et al. 2015) with the aboveground biomass of each individual as a response variable and 'pot' as an random effect, because the measurements on cultivar individuals in a pot are not independent from each other. The analysis on the effect of cultivar identity on leaf C:N ratio was also conducted using mixed-effects models with pot as an random effect. To determine how the leaf C:N ratio of each *T. pratense* cultivar might be influenced by nematodes and plant intraspecific diversity, we used a generalized linear models (GLMs) with a Gamma error

distribution (Crawley 2007). The evenness of the plant community (E) was calculated using the aboveground biomass of each individual with the formula:

$$E = (1 / \sum_{i=1}^s P_i^2) / S$$

, where  $P_i$  is the proportion of biomass of cultivar  $i$ , and  $S$  is the total number of cultivars in the pot (Mulder et al. 2004). The plant-parasitic nematode abundance and evenness of the plant community were analyzed in generalized linear models (GLMs) with a quasipoisson and quasibinomial error distribution (Guisan et al. 2002), respectively, using plant intraspecific diversity and nematode treatments as explanatory variables. Comparisons of the effect of nematodes and plant intraspecific diversity on response variables were based on Tukey's HSD tests ( $P < 0.05$ ). Means  $\pm$  SE presented in text, tables and figures were calculated using non-transformed data.

## Results

### Plant primary production

Aboveground biomass of the community (average weight: 30 165  $\pm$  404 mg) was not influenced by either plant intraspecific diversity or nematodes (Appendix Table S4.2). We did not find a clear positive linear relationship of plant intraspecific diversity on the aboveground biomass of *T. pratense* (average weight: 650  $\pm$  36 mg), but the aboveground biomass of *T. pratense* significantly differed between the four plant intraspecific diversity levels (Table 4.1). The aboveground biomass of *T. pratense* was highest in the four clover-grass cultivars mixture (Fig. 4.1). Importantly, we found the aboveground biomass of *T. pratense* significantly increased with the plant-parasitic nematodes addition (Control: 562  $\pm$  36 mg; Nematodes: 730  $\pm$  61 mg), and the effect size depended on the intraspecific diversity level of the plant community (Table 4.1). The aboveground biomass of *T. pratense* was significantly increased by 105.0  $\pm$  26.4% by the nematode addition in the four clover-grass cultivars mixture, and marginally increased by 38.9  $\pm$  11.7% in the eight clover-grass cultivars mixture (Fig. 4.1). Yet, the aboveground biomass of *L. perenne* (average weight: 29 515  $\pm$  396 mg) was neither affected by plant intraspecific diversity, nor by nematodes (Table 4.1, Appendix Table S4.2).

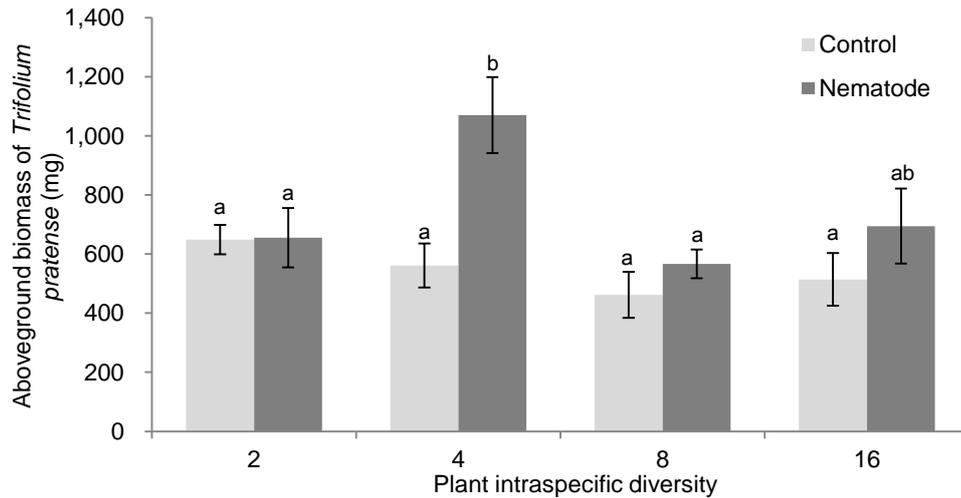
**Table 4.1** Linear model and generalized linear model results of the effects of plant intraspecific diversity (Div) and nematodes (Nem) on aboveground and belowground biomass of *T. pratense* and *L. perenne*, proportion of *T. pratense* aboveground biomass in clover-grass communities, evenness of the plant communities and nematode abundance. The plant intraspecific diversity was analyzed as linear (Div<sub>linear</sub>) and categorical (Div<sub>cat</sub>) factors, respectively. Significant *P*-values ( $P < 0.05$ ) are printed in bold

	df	Aboveground biomass of <i>T. Pratense</i>		Aboveground biomass of <i>L. perenne</i>		Total aboveground biomass of community		Total belowground biomass		Proportion of <i>T. pratense</i> biomass in community		Evenness of community		Nematode abundance	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Nem	1, 64	<b>4.233</b>	<b>0.043</b>	0.962	0.330	1.374	0.245	1.930	0.169	<b>4.402</b>	<b>0.040</b>	1.773	0.188	<b>31.678</b>	<b>0.000</b>
Div <sub>cat</sub>	3,64	<b>2.745</b>	<b>0.0497</b>	1.734	0.168	1.632	0.190	0.760	0.521	<b>3.730</b>	<b>0.015</b>	<b>18.265</b>	<b>0.000</b>	0.419	0.740
Div <sub>Linear</sub>	1, 64	1.603	0.210	2.936	0.091	3.220	0.077	0.559	0.457	0.596	0.443	<b>53.422</b>	<b>0.000</b>	0.778	0.381
Nem:Div <sub>cat</sub>	3, 64	<b>2.880</b>	<b>0.042</b>	1.691	0.177	1.935	0.132	0.547	0.652	1.498	0.223	1.992	0.123	0.021	0.996
Nem:Div <sub>Linear</sub>	1, 64	1.148	0.288	2.809	0.098	2.834	0.097	0.659	0.420	0.109	0.742	0.046	0.830	0.002	0.969

**Table 4.2** Results of linear mixed effects models testing the effects of plant intraspecific diversity (Div<sub>cat</sub>) and nematodes (Nem) on aboveground biomass of eight *T. pratense* cultivars (Violetta, Titus, Nemaro, Lucrum, Milvus, Larus, Diplomat and Taifun). Significant *P*-values ( $P < 0.05$ ) are printed in bold and marginally significant *P*-values ( $0.05 < P < 0.1$ ) are printed in bold and italics

	df	Violetta		Titus		Nemaro		Lucrum		Milvus		Larus		Diplomat		Taifun	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Div <sub>cat</sub>	3,20	1.023	0.404	<b>32.624</b>	<b>&lt;0.001</b>	1.426	0.265	<b>6.210</b>	<b>0.003</b>	0.355	0.786	<b>3.187</b>	<b>0.046</b>	<b>2.726</b>	<b>0.071</b>	<b>5.459</b>	<b>0.007</b>
Nem	1,20	<b>4.425</b>	<b>0.048</b>	0.379	0.545	2.815	0.109	2.703	0.115	0.400	0.534	0.059	0.811	0.012	0.913	<b>3.740</b>	<b>0.067</b>
Div <sub>cat</sub> :Nem	3,20	0.366	0.778	1.680	0.203	1.275	0.310	2.053	0.138	0.789	0.514	1.194	0.337	1.009	0.409	1.013	0.408

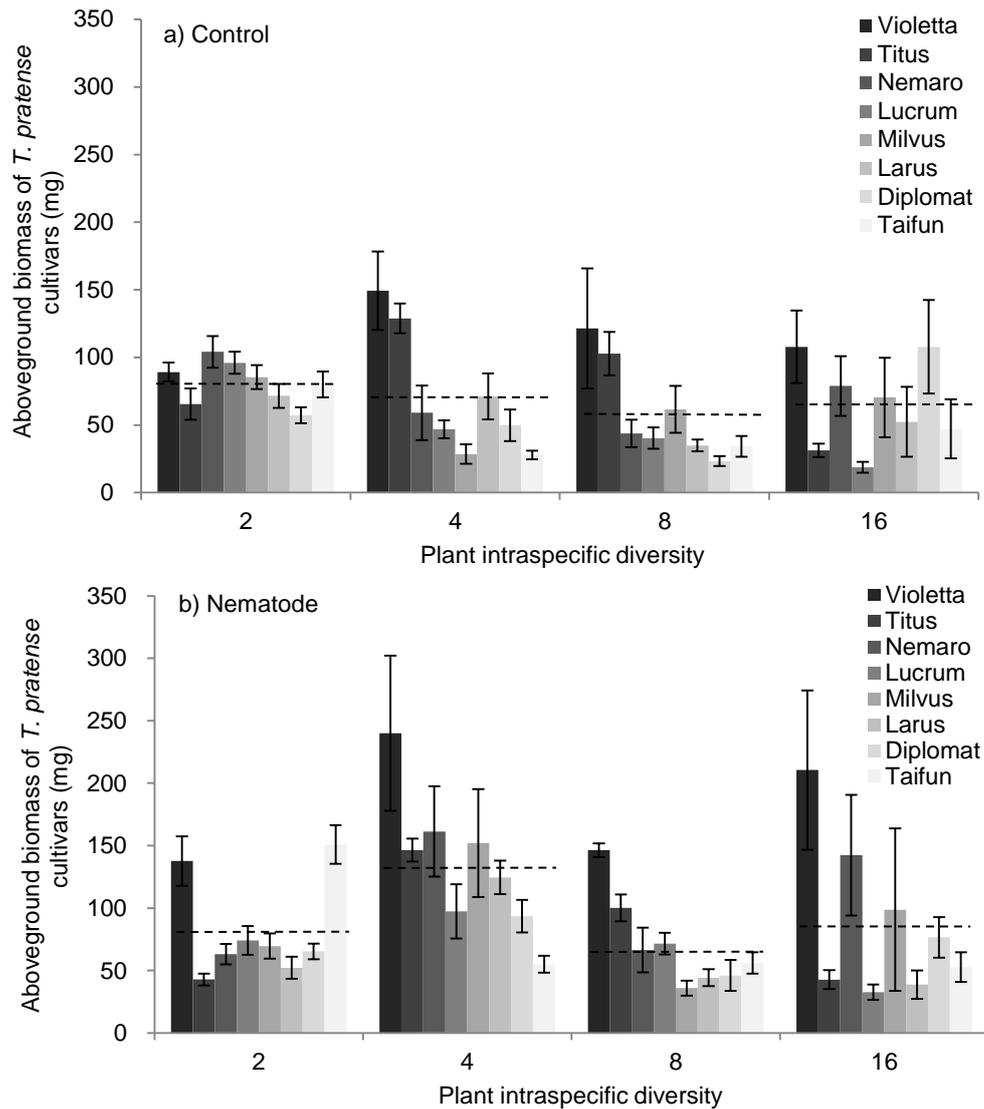
The interaction of nematodes and plant intraspecific diversity



**Figure 4.1** The effect of plant intraspecific diversity and nematodes on aboveground biomass of *T. pratense*. Different letters on the columns indicate significant differences between the treatments ( $P < 0.05$ , Tukey's HSD test). Values are means  $\pm$  SE

**Cultivar identity effect and the influence of nematodes and plant intraspecific diversity**

Cultivars of both, *T. pratense* and *L. perenne* differed in their aboveground biomass in our experiment (Fig. 4.2, Appendix Fig. S4.1-2). Across the experiment, the *T. pratense* cultivars Violetta (aboveground biomass:  $142 \pm 12$  mg) and Larus (aboveground biomass:  $62 \pm 5$  mg), as well as the *L. perenne* cultivars Valerio (aboveground biomass:  $4\,494 \pm 173$  mg) and Noah (aboveground biomass:  $2\,715 \pm 129$  mg) had the biggest and lowest aboveground biomass of each species, respectively (Appendix Fig. S4.1). Further, the leaf C/N ratio also differed between *T. pratense* cultivars (lme:  $F = 4.909$ ,  $P < 0.001$ ; Appendix Table S4.3): *T. pratense* cultivar Larus (average value:  $21.643 \pm 1.010$ ) had the highest C/N ratio, while Violetta (average value:  $16.696 \pm 0.841$ ) and Nemaro (average value:  $16.783 \pm 0.572$ ) had lower C/N ratios than other cultivars. Only the C/N ratio of *T. pratense* cultivar Lucrum increased with plant intraspecific diversity ( $F_{3,24} = 8.331$ ,  $P < 0.001$ ), while in the other *T. pratense* cultivars the C:N ratio was not affected (Appendix Table S4.4). The C:N ratio of any *T. pratense* cultivar was not affected by the nematodes.



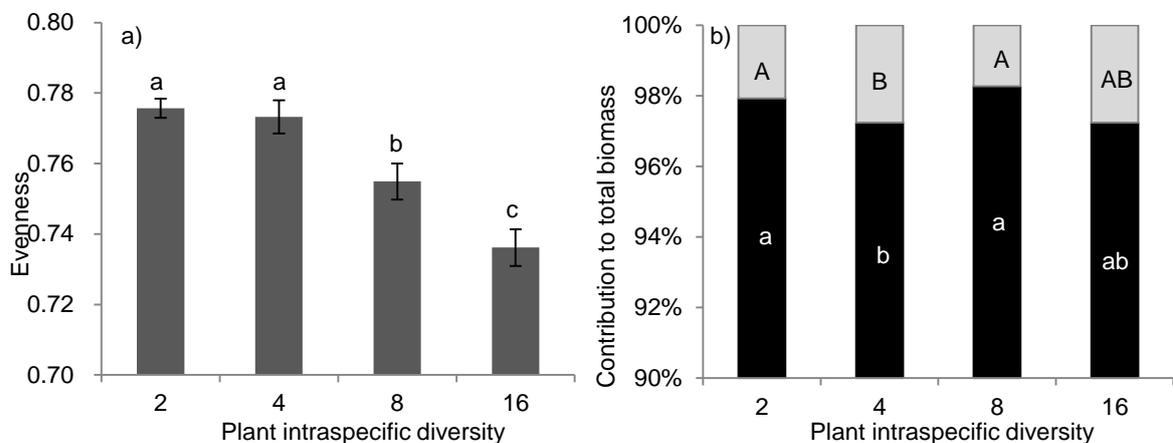
**Figure 4.2** The effect of the plant intraspecific diversity on aboveground biomass of eight *T. pratense* cultivars (Violetta, Titus, Nemaro, Lucrum, Milvus, Larus, Diplomat and Taifun) in a) nematodes control and b) nematodes addition pots. The dashed line represents the averaged aboveground biomass of *T. pratense* per cultivar per mesocosm. Values are means  $\pm$  SE

The aboveground biomass of specific *T. pratense* cultivars was also affected by plant intraspecific diversity, and/or nematodes, but not affected by their interaction (Table 4.2, Fig 4.2). The aboveground biomass of the high-yield *T. pratense* cultivars, Violetta and Taifun, was increased by the addition of nematodes by  $87.6 \pm 19.4\%$  (significantly) and  $133.8 \pm 34.7\%$  (marginally), respectively (Table 4.2, Appendix Fig. S4.1a). Cultivars did not always have a consistent aboveground biomass in different intraspecific diversity levels (Fig. 4.2). Specifically, half of the *T. pratense* cultivars (Titus, Lucrum, Larus and Taifun) and half of the *L. perenne* cultivars (Picaro, Valerio, Noah and Licampo) were significantly influenced by intraspecific diversity (Table 4.2, Appendix Table S4.5). Two *T. pratense* cultivars (Lucrum and Taifun) and three *L. perenne*

cultivars (Picaro, Noah and Licampo) performed better when planted alone, rather than planted with other cultivars of the same species. On the contrary, the cultivar Valerio performed better when planted with other cultivars of *L. perenne*.

### Evenness of community and proportion of *T. pratense*

The evenness of the community linearly decreased with increasing plant intraspecific diversity, while it was not influenced by nematodes or the interaction between diversity and nematodes (Table 4.1, Fig. 4.3a). The mean aboveground biomass production of *L. perenne* (aboveground biomass:  $29\,515 \pm 396$  mg) was on average over 45 times higher than that of *T. pratense* (aboveground biomass:  $646 \pm 36$  mg). Both, plant intraspecific diversity and nematodes had significant effects on the contribution of *T. pratense* and *L. perenne* species to total plant community biomass (Table 4.1). *T. pratense* contributed most to overall biomass in the four clover-grass cultivar mixtures, and its proportional contribution was significantly higher than in the mixtures with two and eight clover-grass cultivars (Fig. 4.3b). The nematode addition significantly increased the proportion of *T. pratense* in the plant community from  $1.9 \pm 0.1\%$  to  $2.4 \pm 0.2\%$  (Table 4.1, Fig. 4.3b).



**Figure 4.3** a) The evenness of the clover-grass plant community. The evenness was calculated from the biomass of each plant cultivar. b) The effect of plant intraspecific diversity on the contribution of *Trifolium pratense* and *Lolium perenne* to total community biomass production. *T. pratense* (light grey), *L. perenne* (dark). Columns marked by the same letter are not significantly different ( $P < 0.05$ ; Tukey's HSD test). Values are means  $\pm$  SE

### Nematode abundance and belowground biomass

The data on nematode abundance at harvest showed an efficient establishment of the nematode

treatment in the greenhouse experiment (Nematode treatment:  $35.5 \pm 8.0$  per 100g fresh soil; Control:  $2.5 \pm 0.7$  per 100g fresh soil). Soil nematode abundance was not influenced by plant intraspecific diversity (Table 4.1). Root biomass (average weight:  $15\,169 \pm 375$  mg) was neither influenced by nematodes nor by plant intraspecific diversity (Table 4.1).

## Discussion

Inconsistence to our first hypothesis, our results did not show a positive cultivar diversity-productivity relationship. However, we found partial support for our second hypothesis that plant-parasitic nematodes (*M. hapla*) may influence the effect of plant intraspecific diversity on aboveground biomass in *T. pratense*, as the diversity effect in a specific plant intraspecific level depended on nematodes. In the following, we will focus on *T. pratense*, on which we found the interaction effect of plant-parasitic nematodes and plant intraspecific diversity on its aboveground biomass.

We found that the effect of plant intraspecific diversity on productivity of *T. pratense* only emerged when nematodes were present in one specific plant intraspecific diversity level. In a field study (Chapter 3) we found that aboveground biomass of *T. pratense* was only enhanced by the nematode addition in plant communities with high compared to low intraspecific diversity. Yet, in the present greenhouse study, we used more levels of plant intraspecific diversity and tracked the performance of each cultivar individually. Similar results can also be found in studies of the effects of plant species diversity, which have demonstrated a crucial role of soil microbes (especially soil pathogens and parasites) in the diversity-productivity relationship (van der Heijden et al. 2008, Schnitzer et al. 2010, Maron et al. 2011, Schnitzer and Klironomos 2011). We propose that cultivar identity, and the characteristics of plant cultivars (i.e. the response of cultivars to nematodes, their performance when planted alone or with other cultivars), played crucial roles in the observed interaction effect of nematodes and plant intraspecific diversity on aboveground biomass.

The diversity-productivity relationship has previously been well explained by functional traits at both species diversity (Roscher et al. 2012, Roscher et al. 2013, Liu et al. 2014, Schittko et al. 2014) and intraspecific diversity levels (Kotowska et al. 2010, Breza et al. 2012). Plant cultivars differing qualitatively in their functional traits (e.g. root depth and canopy structure) have been reported to use water, light and other nutrients in a complementary way (Kotowska et al. 2010, Tooker and Frank 2012). A combination of cultivars with complementary functional traits may lead to niche partitioning and the plant aboveground biomass may increase (Loreau and Hector 2001, Kotowska et al. 2010, Zuppinger-Dingley et al. 2014). In the present study, resource

complementarity could be an explanation for the differing plant aboveground biomass in different cultivar mixtures, as the C/N ratio difference of *T. pratense* cultivars (Appendix Table S4.3) indicated that there might be a complementary effect in their traits.

The increase in aboveground biomass of *T. pratense* with the addition of nematodes in the four clover-grass cultivars mixtures was mainly due to the positive biomass responses of certain *T. pratense* cultivars (e.g. Violetta) to nematode herbivory. *T. pratense* and *L. perenne* cultivars in Europe are selected for a number of characteristics, in particular yield and resistance to certain diseases and pests. Previous studies (Willis 1981; Taylor and Quesenberry 1996, Jacob et al. 2014) have shown a high level of discrepancy in the resistance of various *T. pratense* cultivars to plant pathogens and parasitic nematodes. We also found that the *T. pratense* cultivars responded inconsistently to plant-parasitic nematodes with respect to their aboveground biomass. Depending on plant identity or cultivar identity, plant-parasitic nematodes can either inhibit through their damage feeding activities, or promote plant growth indirectly through promoting nutrient availability and microbial activity via carbon and nutrient leaching from the damaged roots (Bardgett et al. 1999, Yeates et al. 1999a, 1999b). As we did not grow pure monocultures of only one cultivar, it is not possible for us to partition the selection effect and complementary effect on plant primary production (Loreau and Hector 2001). Yet, our results indicate that certain cultivars might have contributed more than others to the increase of primary production of *T. pratense*, and that the response of cultivars to nematodes plays a predominant role in driving the increase in primary production.

Moreover, the cultivars did not have the same performance when grown in different plant intraspecific diversity levels. The difference in the aboveground biomass of cultivars in high and low intraspecific diversity treatments can also be found in previous studies (Zhu et al. 2000a, Kotowska et al. 2010, Aguirre and Marshall 2012). We propose that the difference in primary production of plant cultivars in different intraspecific diversity levels could be their unique characteristics which imply their ability to deal with stress from neighbor competition (Harris et al. 1980, Fridley et al. 2007). In a previous study on plant species diversity, it has been shown that certain plant species can change their characteristics when grown in a community (Zuppinge-Dingley et al. 2014). Similarly by using plant intraspecific diversity, we found depending on cultivar identity, the growth of certain cultivars to be inhibited by the competition with other cultivars (Hakala and Jauhiainen 2007). Yet, one cultivar (*L. perenne* cultivar Valerio) performed better in higher intraspecific diversity communities which may be attributed to the higher competitive ability of the cultivar compared to the other cultivars. The competition between different cultivars for resources (water, light, and nutrients) in the communities with high

intraspecific diversity may have led to the variance in their growth, and ultimately reduced in the evenness of the plant community with high intraspecific diversity. Moreover, the cultivar-specific responses to plant intraspecific diversity might contribute to the negative effect of plant intraspecific diversity on evenness of clover-grass community. The low evenness of communities with high plant intraspecific diversity indicates the dominance of one or more cultivars in communities with higher intraspecific diversity (Wilsey et al. 2005, Lamb 2008). Therefore, the different performance of cultivars when grown in monocultures and mixtures could be an important characteristic, which should be considered in future biodiversity studies and agricultural application.

Generally, previous studies have demonstrated controversial results regarding the effect of plant intraspecific diversity on plant productivity, either positive (Zhu et al. 2000a, Kotowska et al. 2010, Tooker and Frank 2012, Himanen et al. 2013) or neutral (Fridley and Grime 2010, Burls et al. 2014, Avolio et al. 2014, Guo et al. 2015, Prieto et al. 2015). We have not found a general positive linear relationship of plant intraspecific diversity on plant aboveground biomass but an increase in the presence of nematodes in a certain diversity level instead. Indeed, in a short greenhouse experiment a positive linear diversity effect may not easy to be found as it may take time to develop as shown in several long-term experiments (Tilman et al. 2001, van Ruijven and Berendse 2005, Marquard et al. 2009, Eisenhauer et al. 2012a). Root depth increase induced stronger complementary effects (Marquard et al. 2009, Mueller et al. 2012, Ravenek et al. 2014, Mommer et al. 2015), or delayed soil biota effects over time (Eisenhauer et al. 2012a) may contribute to the delayed diversity effect. Moreover, genetic diversity of plant community can also change over time due to genetic flow or selection processes (Nestmann et al. 2011), which might also contribute to the temporal increases in complementary effects. Further, previous studies proposed that species diversity-productivity relationship might also depend on experimental scale (Ricklefs 1987, Hector et al. 1999, Venail et al. 2010) and the environmental conditions (Jiang et al. 2009). These factors could also affect the diversity effect at the intraspecific level.

Further, we did not find effects of plant-parasitic nematodes on aboveground biomass of *L. perenne*, but significant effects on *T. pratense*. This can be attributed to plant identity, as plant species respond differently to nematodes. In term of host preference of plant-parasitic nematodes, previous studies have shown that *T. pratense* is preferred over *L. perenne* by *M. hapla* (Knight et al. 1997, Stanelis 2004). Moreover, a previous study has shown that grass species can benefit from plant-parasitic nematode infestation on legume roots through absorbing the leaking N (Dromph et al. 2006). We assume that in our study the leaking of N from *T. pratense* was limited, and might not

have been enough to make a difference in the growth of *L. perenne* in the relatively nutrient rich soil.

## **Conclusion**

Our results show a significant interaction effect of soil organisms (i.e. plant-parasitic nematodes) and plant intraspecific diversity on the aboveground biomass of *T. pratense*, indicating that plant-parasitic nematodes can influence the effect of plant intraspecific diversity on aboveground biomass in some cases. We also found that plant intraspecific diversity affected the evenness of the community. These findings indicate that important characteristics of cultivars, especially how cultivars respond to soil pathogen stress and their competitive ability with different neighbors, are of crucial importance to understand the effect of plant intraspecific diversity on ecosystem functions. Therefore, the careful choice of plant cultivars is important when a high productivity is to be maintained, especially in agricultural ecosystem in the presence of plant-parasitic nematodes and other soil pathogens. Further analysis investigating the resistance of different plant cultivars to nematodes and pathogens and the competitive ability of plant cultivars will provide information needed to choose the most appropriate cultivars for the establishment of successful clover-grass mixtures. Future biodiversity studies on the relationship between biodiversity and ecosystem functions need to account for the interaction of plant intraspecific diversity and identity with soil organisms, especially root antagonists.

## **Acknowledgements**

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*Appendix C. Supplementary Material*



**Table S4.2** The effect of plant intraspecific diversity and nematode treatments on the aboveground biomass of *Lolium perenne* and the total aboveground biomass of the clover-grass community. Mean  $\pm$  SE

Plant intraspecific diversity	n	Aboveground biomass of <i>Lolium perenne</i> (g)		Aboveground biomass of community (g)	
		Nematode	Control	Nematode	Control
2	16	29.939 $\pm$ 0.938	30.966 $\pm$ 0.817	30.594 $\pm$ 0.992	31.615 $\pm$ 0.819
4	8	29.898 $\pm$ 0.519	27.444 $\pm$ 1.566	30.968 $\pm$ 0.563	28.005 $\pm$ 1.537
8	8	29.768 $\pm$ 1.486	29.091 $\pm$ 0.860	30.335 $\pm$ 1.505	29.553 $\pm$ 0.840
16	6	29.932 $\pm$ 0.974	26.581 $\pm$ 1.286	30.626 $\pm$ 0.885	27.095 $\pm$ 1.319

**Table S4.3** The data on the effect of plant intraspecific diversity on average C/N ratios of eight *Trifolium pratense* cultivars (Violetta, Titus, Nemaro, Lucrum, Milvus, Larus, Diplomat and Taifun). Mean  $\pm$  SE

Plant intraspecific diversity	Treatment	n	Violetta	Titus	Nemaro	Lucrum	Milvus	Larus	Diplomat	Taifun
2	Control	2	18.202 $\pm$ 3.592	17.65 $\pm$ 1.029	14.703 $\pm$ 0.240	15.127 $\pm$ 0.842	16.310 $\pm$ 1.140	17.650 $\pm$ 0.268	15.022 $\pm$ 0.035	17.040 $\pm$ 0.797
	Nematode	2	14.715 $\pm$ 0.032	18.109 $\pm$ 0.097	19.766 $\pm$ 0.898	16.228 $\pm$ 1.145	16.724 $\pm$ 1.299	21.220 $\pm$ 3.368	20.743 $\pm$ 2.857	14.864 $\pm$ 0.246
4	Control	2	17.734 $\pm$ 3.438	16.553 $\pm$ 0.051	16.481 $\pm$ 1.951	18.962 $\pm$ 3.688	19.169 $\pm$ 2.201	20.206 $\pm$ 3.384	20.544 $\pm$ 2.628	17.833 $\pm$ 2.020
	Nematode	2	14.963 $\pm$ 0.216	17.700 $\pm$ 0.586	14.024 $\pm$ 0.360	14.773 $\pm$ 0.141	16.563 $\pm$ 1.621	17.267 $\pm$ 1.274	14.993 $\pm$ 0.299	18.666 $\pm$ 0.657
8	Control	4	18.916 $\pm$ 3.766	16.709 $\pm$ 1.307	15.598 $\pm$ 0.283	17.158 $\pm$ 0.855	18.470 $\pm$ 2.831	20.852 $\pm$ 1.315	17.303 $\pm$ 1.139	19.585 $\pm$ 1.185
	Nematode	4	15.332 $\pm$ 0.565	20.356 $\pm$ 2.483	17.160 $\pm$ 1.518	16.566 $\pm$ 1.189	18.385 $\pm$ 1.731	19.993 $\pm$ 3.143	15.932 $\pm$ 0.353	16.247 $\pm$ 0.663
16	Control	6	17.582 $\pm$ 2.613	20.443 $\pm$ 1.039	18.577 $\pm$ 2.007	22.996 $\pm$ 2.119	19.765 $\pm$ 2.995	26.586 $\pm$ 3.310	15.898 $\pm$ 1.692	20.033 $\pm$ 1.402
	Nematode	6	15.631 $\pm$ 1.257	20.051 $\pm$ 1.136	16.248 $\pm$ 0.791	22.805 $\pm$ 2.296	22.571 $\pm$ 2.786	21.735 $\pm$ 0.819	17.364 $\pm$ 0.555	21.218 $\pm$ 4.610
Overall mean		28	16.696 $\pm$ 0.841	18.973 $\pm$ 0.559	16.783 $\pm$ 0.572	19.282 $\pm$ 0.926	19.249 $\pm$ 1.011	21.643 $\pm$ 1.010	16.968 $\pm$ 0.545	18.844 $\pm$ 1.055

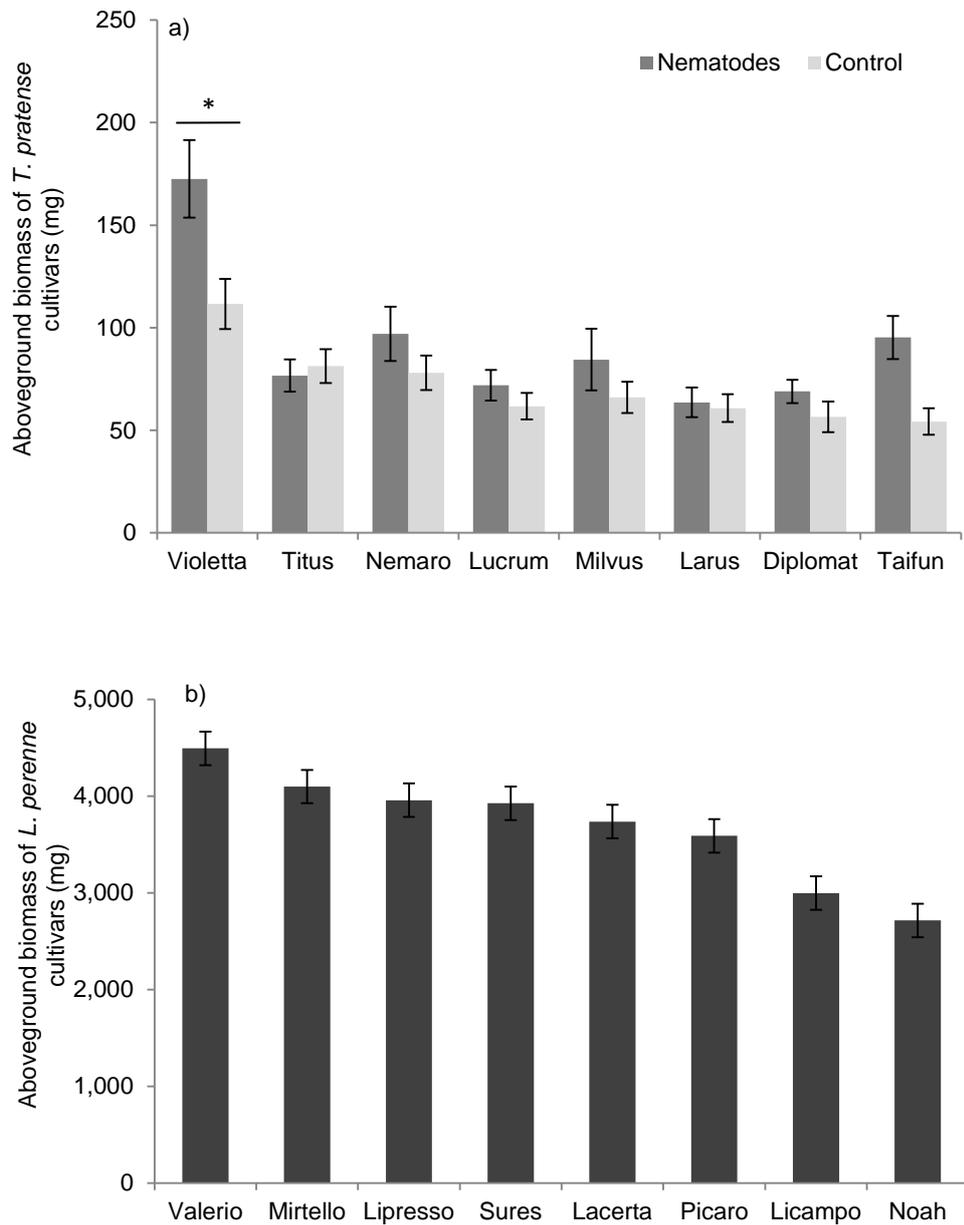
**Table S4.4** Results of generalized linear models testing the main effects of nematodes (Nem) and plant intraspecific diversity (Div) on the C/N ratio of eight *T. pratense* cultivars (Violetta, Titus, Nemaro, Lucrum, Milvus, Larus, Diplomat and Taifun). Significant *P*-values ( $P < 0.05$ ) are printed in bold

	df	Violetta		Titus		Nemaro		Lucrum		Milvus		Larus		Diplomat		Taifun	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Nem	1,68	2.896	0.102	1.065	0.313	0.023	0.880	0.246	0.625	0.195	0.663	1.481	0.236	0.051	0.823	0.097	0.758
Div	3,68	0.041	0.989	1.664	0.203	0.579	0.635	<b>6.922</b>	<b>0.002</b>	1.102	0.368	2.012	0.140	0.290	0.832	0.938	0.438

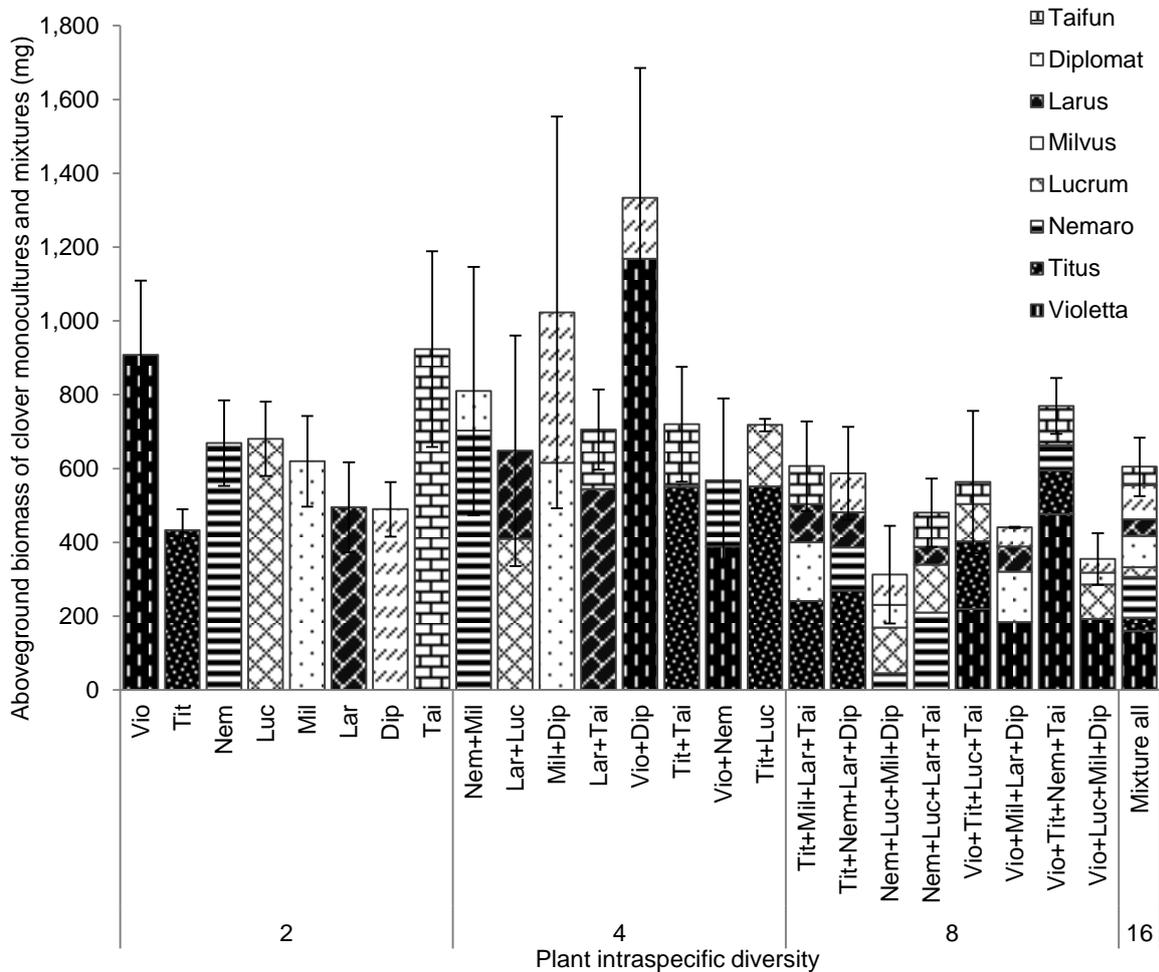
**Table S4.5** Results of linear mixed effects models testing the effects of plant intraspecific diversity (Div<sub>cat</sub>) and nematode (Nem) on aboveground biomass of eight *L. perenne* cultivars (Violetta, Titus, Nemaro, Lucrum, Milvus, Larus, Diplomat and Taifun). Significant *P*-values ( $P < 0.05$ ) are printed in bold

	df	Mirtello		Picaro		Valerio		Noah		Lipresso		Lacerta		Licampo		Sures	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Div <sub>cat</sub>	3,20	1.207	0.333	<b>9.734</b>	<b>&lt;0.001</b>	<b>4.508</b>	<b>0.014</b>	<b>18.082</b>	<b>&lt;0.001</b>	1.976	0.150	0.485	0.697	<b>10.598</b>	<b>&lt;0.001</b>	0.511	0.679
Nem	1,20	0.030	0.863	0.127	0.725	0.949	0.342	0.297	0.592	0.763	0.393	0.001	0.973	1.848	0.189	0.642	0.433
Nem:Div <sub>cat</sub>	3,20	0.248	0.862	1.550	0.232	1.309	0.299	1.344	0.288	2.321	0.106	0.048	0.986	0.090	0.965	0.450	0.720

**Figure S4.1** a) The effect of nematodes and the *Trifolium pratense* cultivar identity on aboveground biomass production. The asterisk indicates a significant difference of the nematode effect on biomass in each cultivar ( $P < 0.05$ ; Tukey's HSD test).  $n = 38$  per bar. b) Average aboveground biomass of each *Lolium perenne* cultivars (Valerio, Mirtello, Lipresso, Sures, Lacerta, Picaro, Licampo and Noah) from the experiment. Values are means  $\pm$  SE.  $n = 76$  per bar



**Figure S4.2** Aboveground biomass of *Trifolium pratense* cultivars in two-, four-, eight- and sixteen-cultivar mixtures of *T. pratense* and *Lolium perenne*. *T. pratense* cultivars: Violetta (Vio), Titus (Tit), Nemaro (Nem), Lucrum (Luc), Milvus (Mil), Larus (Lar), Diplomat (Dip) and Taifun (Tai). Sample size for the different diversity levels:  $n_{1,2,4,8} = 4, 2, 2, 12$  respectively. Values are means  $\pm$  SE. The SE is given for the mixtures of the *T. pratense* cultivars in four, eight and sixteen cultivar mixtures



## Chapter 5

### General discussion

The study of the interaction between soil organisms and plant intraspecific diversity and its effect on ecosystem functions is a relatively novel area of ecology. Soil organisms have only in recent decades been intensively studied for their influence on plant performance and ecosystem functions. Plant intraspecific diversity and soil organisms have significant influences on ecosystem processes and functions, and both can potentially influence each other. In this thesis, I studied the interactive effect of soil organisms and plant diversity on ecosystem functions at plant intraspecific level by manipulating experiments using functionally dissimilar soil organisms and different levels of plant cultivars mixture. I examined the effect of soil biota by focusing on the representative species of the crucial functional groups of soil organisms (AMF, earthworms, and nematodes). In the field experiment (Chapter 2), I found that AMF increased aboveground biomass of plant community and tended to decrease aboveground herbivore diversity, even without using fungicides as a control. I found high intraspecific diversity plant communities to be more resistant to weeds. In the second year field experiment (Chapter 3), my results showed that the aboveground biomass of *T. pratense* was improved by high plant intraspecific diversity only with the addition of plant-parasitic nematodes. In the greenhouse experiment (Chapter 4), I had a similar finding that plant-parasitic nematodes have on influencing the effect of plant intraspecific diversity on the aboveground biomass of *T. pratense*, although only at a specific (intermediated) intraspecific diversity level; however, I did not find a linear relationship between plant intraspecific diversity and community productivity. Importantly, my studies showed the interactive effect of plant intraspecific diversity and plant parasitic nematodes on aboveground biomass of *T. pratense*, which highlighted the importance of plant parasitic nematodes in shaping the relationship between plant diversity and productivity at plant intraspecific level.

#### **Plant-parasitic nematodes mediated the effect of plant intraspecific diversity on the aboveground biomass of *T. pratense***

Following the widely used approach of evaluating the strength of ecosystem functions (Tilman et al. 2001, van Ruijven and Berendse 2005, Tooker and Frank 2012, Schöb et al. 2015), I primarily concentrated on aboveground biomass. Even with the different indications of plant species' dominance (see Chapter 3 & 4), both results showed that the nematodes mediated intraspecific

diversity effect was only found on the aboveground biomass of *T. pratense*, which indicated the importance of plant species identity to the observed plant intraspecific diversity effect. *T. pratense* is a mid-successional species while *L. perenne* is a fast-growing early-successional species (Wacker et al. 2009). It is reasonable that *L. perenne* dominated the plant community in the early stage of the field experiment (Chapter 2) and in the short term greenhouse experiment (Chapter 4). Eventually *T. pratense* competed over *L. perenne* and became the dominant species, as observed in the later stage of the field experiment (Chapter 2 & 3). Because of the high proportion of *T. pratense* in the community (Chapter 3), I propose that the interaction effect of plant intraspecific diversity and nematodes treatment on the total aboveground biomass of the plant community was mainly contributed by their effect on *T. pratense*. Moreover, *T. pratense* is one of the typical legumes and legumes plants were often reported to play a key role in the positive plant diversity-productivity relationship (HilleRisLambers et al. 2004, Spehn et al. 2005, Marquard et al. 2009, Eisenhauer 2012). Legumes and grasses differed in the decomposition of fine roots and nutrient turnover rates (Bezemer et al. 2006). In particular, the presence of legumes can also enhance the microbial decomposition processes and affect community composition of soil fungi and bacteria (Breulmann et al. 2011). It is known that the legume-grass competition relationship can also be influenced by soil organisms (Wurst and van Beersum 2009). In line with these findings, I found that the biomass proportion of *T. pratense* in the community was increased by nematodes (see Chapter 4). Furthermore, previous studies found that *T. pratense* is a host for *M. hapla*, while *L. perenne* is not (Yeates et al. 1973, Knight et al. 1997, Stanelis 2004). As discussed in Chapter 3, the host preferences might also explain the distinct effects of nematodes on the primary production of different plant species, as the disturbance (e.g. herbivory) can be a key driver of the effect of plant intraspecific diversity on ecosystem functions (Hughes and Stachowicz 2004, Cook-Patton et al. 2014).

Previous studies have focused on explaining the effect of plant intraspecific diversity on productivity with regard to selection effect and complementary effect (Kotowska et al. 2010, Cook-Patton et al. 2011, Tooker and Frank 2012, Zeller et al. 2012, Bukowski and Petermann 2014). First, high intraspecific diversity plots have greater odds of containing cultivars with superior characteristics, e.g. high productivity, pathogen resistant ability, or competitive ability (i.e. a selection effect) (Dear et al. 1993, Tucak et al. 2013, Jacob et al. 2014). Second, high intraspecific diversity allows for a greater ability to utilize resources due to the partitioning niches among plant niches (i.e. niche partitioning) (Crutsinger et al. 2006, Cowger and Weisz 2008, Kotowska et al. 2010, Cook-Patton et al. 2011, Tooker and Frank 2012). A combination of diverse characteristics (e.g. height, leaf area, maturity stage, flowering duration, root depth, etc.) in high intraspecific diversity plant communities can lead to niche complementarity (Poisot et al. 2013, Zuppinger-

Dingley et al. 2014). For example, the complementary pattern of foliar or root architecture under high intraspecific diversity has been found to use light, water, and nutrients more efficiently (e.g. Fang et al. 2014). Nevertheless, my results indicated the importance of soil organisms' activities in influencing the effect of diversity on productivity at plant intraspecific level. My studies showed the influence of root herbivory on the effect of diversity on productivity at the intraspecific level in a field experiment (Chapter 3) and a controlled experiment (Chapter 4). In the first year of the field experiment (Chapter 2), the nematodes were not introduced and there was no effect of plant intraspecific diversity on aboveground biomass; when the plant-parasitic nematodes were added in the second year, I found that the aboveground biomass of *T. pratense* under high intraspecific diversity conditions was increased only in the nematodes addition plots, indicating that the enhanced root herbivory by plant-parasitic nematodes in soil can be a predominant factor in the positive effect of plant intraspecific diversity on productivity.

Similarly, at the plant species diversity level, the detrimental soil organisms were stressed for their crucial importance in driving the diversity-productivity relationship (Maron et al. 2011, Schnitzer and Klironomos 2011, Eisenhauer 2012). Belowground and aboveground herbivores and plant pathogens are important drivers of plant community structure and species diversity (Klironomos 2002, Schnitzer and Klironomos 2011, Cook-Patton et al. 2014). In a recent review, Eisenhauer (2012) compared the role of soil organisms (e.g. AMF, plant pathogen, plant parasitic, etc.) in the plant species diversity-productivity relationship and suggested that detrimental soil organisms might play a more predominant role in mediating the diversity-productivity relationship than other organisms like AMF. The previous study by Schnitzer et al. (2010) also demonstrated the negative effects of detrimental soil organisms at low species diversity, but no effect at high species diversity level.

Differently, as discussed in Chapter 2, my study extended to the intraspecific level and I found the effects tended toward a different direction: the increase of aboveground biomass was due to nematodes in the high intraspecific diversity assemblage, but nematodes had no effect in the low intraspecific diversity assemblage. I propose that different soil organisms might affect the diversity-productivity relationship through different mechanisms. As discussed in Chapter 3, the positive effect of nematodes in a high intraspecific diversity plant community might be attributed to: 1) the selection of cultivars with prominent characteristics (e.g. high yield or positive response to root herbivory) (Fox 2005); 2) a certain combination of cultivars (Mytton 1975, Huston 1997, Hooper et al. 2005); 3) enhanced level of nematode herbivory might improve the nutrient level in soil; and 4) the high intraspecific diversity plant community is more efficient in using these nutrients through complementary effects. Therefore, to better understand the potential mechanisms behind the

findings, I set up a greenhouse experiment with different levels of plant intraspecific diversity and the same nematode species (Chapter 4).

Surprisingly, the greenhouse study (in Chapter 4) showed that the presence of plant-parasitic nematodes mediated the effect of intraspecific diversity on aboveground biomass only under intermediate levels of intraspecific diversity. I propose that the selective use of cultivars with various characteristics (e.g. yield, cultivar-specific responses to plant-parasitic nematodes, and competitive ability) might contribute to the distinct interactive effects of plant intraspecific diversity and nematodes on aboveground biomass in Chapter 3 & 4 (Crutsinger et al. 2008a). As was shown and discussed in Chapters 3 and 4, I found that plant cultivars differed in their aboveground biomass, resistance to *M. hapla*, and in their competitiveness with other cultivars. Previous studies have also shown that *T. pratense* cultivars have different performance in plant growth (Tucak et al. 2013) and resistance to plant pathogens (Vleugels 2013, Jacob et al. 2014) and plant parasitic nematodes (Yeates et al. 1973, Taylor and Quesenberry 1996). The aboveground biomass of plant cultivars differed under different plant intraspecific diversity levels, as plant diversity and composition can influence the growth of plant individuals (Schmidtke et al. 2010). The intertwining of the roots of the cultivars mixture can modify the overall abiotic and biotic environment belowground and may also modify the intraspecific competition between cultivars (Semere and Froud-Williams 2001, Fetene 2003). Therefore, plant cultivars with better performance in yield, response to plant parasitic nematodes, and competitive ability can contribute to the positive effect of plant intraspecific diversity on aboveground biomass through selection effect. In conclusion, the different characteristics of plant cultivars (e.g. yield, cultivar-specific responses to plant-parasitic nematodes, and competitive ability) can contribute to the selection effect and may further influence the effect of plant intraspecific diversity on productivity on different plant intraspecific diversity levels.

Furthermore, the role of beneficial soil organisms is also worth mentioning in regard to the plant intraspecific diversity effect on productivity. The marginally significant four-way interaction effect of plant intraspecific diversity and the soil organism treatments on aboveground biomass of *T. pratense* and of the plant community (Chapter 3) showed that the effect of nematodes on the diversity effect tends to be more pronounced with the addition of earthworms. I suggest that the beneficial soil organisms (e.g. earthworms) can improve the availability of soil nutrients and water resources (Lavelle 2011), and the plant community with high intraspecific diversity is better able to utilize the enhanced soil resources and has higher productivity. The mechanisms beyond the multi-interactions of functionally dissimilar soil organisms and plant diversity are unclear so far and need to be further investigated.

Yet, I have not found the positive linear plant intraspecific diversity-productivity relationship (Chapter 4). The major difference between plant intraspecific diversity and plant species diversity is the smaller morphological and genetic variance with plant intraspecific diversity. I propose that the factors which can influence the diversity-productivity relationship at the plant species level may also influence the diversity effect at the plant intraspecific level. As I have discussed in Chapters 2-4, temporal scale can be a limitation for investigating the biodiversity-ecosystem functions relationship. Most former studies at the plant species level have explored the mechanisms for the delayed ecosystem responses to plant species diversity in long term experiments and demonstrated that the effect of plant diversity on aboveground biomass generally increases with time (van Ruijven and Berendse 2005, Eisenhauer et al. 2012a). Due to the slow accumulation of available resources in the soil over time, the longer term experiment generally has better productivity performance (Eisenhauer et al. 2012a). Results from the long term experiments showed that high diversity plant communities can increase complementary resource utility through a spatially and temporally increased root system with time (Fargione et al. 2007, van Ruijven and Berendse 2009, Ravenek et al. 2014, Mommer et al. 2015). Moreover, Hooper and Dukes (2004) suggested that plant functional traits (e.g. resource capture characteristics) and environment conditions (particularly water availability) might also change the dominance of particular plant species over time, which could influence the structure and even the performance of a plant community. Relatedly, van Ruijven and Berendse (2005) also ascribed the strengthening diversity-productivity relationships over time to increased input of nitrogen by legumes. Besides, previous studies have also demonstrated that the effect of soil organisms (e.g. soil microorganisms and earthworms) on plant growth could be stronger in long term experiments (Laossi et al. 2011, Eisenhauer et al. 2012a). As I have discussed in Chapters 2-4, the plant diversity-ecosystem functions can also be affected by plant species identity or composition (Hooper and Vitousek 1997, Cadotte 2013, Kostenko et al. 2015, Latz et al. 2015), plant-soil feedback (Kulmatiski et al. 2012, Jing et al. 2015), soil organisms (Schnitzer et al. 2010, Maron et al. 2011, Eisenhauer 2012), experiment scale (Jiang et al. 2009, McBride et al. 2014), climate (Guo and Ren 2014), latitude (Gillman et al. 2015), and other disturbances (e.g. grazing) (Cook-Patton et al. 2014). Studies on plant intraspecific diversity have been limited so far, and studies on the limiting factors influencing the diversity effect are even rare. I assume that these biotic and abiotic parameters can also affect the diversity effect at the intraspecific diversity level.

Extending the diversity to the plant intraspecific level, I assume the effect of intraspecific diversity on productivity would be more pronounced in longer term experiments because of the enhanced complementary effect with the increased rooting depth of the plants, the increased nutrient level by

legumes, and the enhanced soil organisms' activity with time. Besides, the experimental type (e.g. greenhouse or field experiment) can also influence the effect of plant intraspecific diversity on ecosystem functions, as the field environment is more complex in terms of the biotic and abiotic disturbances (Jiang et al. 2009, Guo and Ren 2014). Further, the higher aboveground biomass in the high intraspecific diversity plant community could also be attributed to the particular cultivar mixture (Mytton 1975, Huston 1997, Hooper et al. 2005), as a positive interaction among cultivars may also result in increased plant productivity. Yet, due to the difficulty of using all possible combinations of plant cultivars mixtures for the diversity treatment, it is not easy to evaluate the specific effects of the cultivar mixture. Even being unaware of these potential hidden effects (Huston 1997), I showed in both a field and a greenhouse study the mediating effect of plant-parasitic nematodes on the effect of plant intraspecific diversity on aboveground biomass of *T. pratense*. Future biodiversity-ecosystem function studies should consider the influence of soil organisms, especially of detrimental soil organisms.

### **The effect of plant intraspecific diversity on above and belowground organisms**

I showed that plant communities with high intraspecific diversity performed better in terms of resistance to weeds (Chapter 2), much like species-rich plant communities against plant invaders (Elton 1958, Knops et al. 1999). Previous studies have shown that plant communities of enhanced genetic diversity generally possess better competitive ability, which can suppress the growth and propagation of plant invaders (Crutsinger et al. 2008a, Kaut et al. 2008). For example, Crutsinger et al (2008a) studied the effect of intraspecific diversity on plant invaders and found the enhanced intraspecific diversity limiting colonization dynamics of plant invaders. Owing to a combination of genotypic diversity and identity effects, stem density of native plants was enhanced and hence restricted the colonization dynamics of plant invaders (Crutsinger et al. 2008a). Also, plant cultivars responded differently to weeds invasion (Hucl 1998, Mason et al. 2008), and the dominant genotype can play a dominant role in resisting plant invasion (Crutsinger et al. 2008a). In Chapter 4, I also showed that the *T. pratense* and *L. perenne* cultivars significantly differed in their competitive ability. Therefore, cultivar identity may also explain the effect of plant cultivar diversity on resistance to weeds.

I showed the positive tendency of plant intraspecific diversity on abundance of pollinators (Chapter 3). Previous studies have demonstrated that genotypic diversity may increase abundance of flowers and facilitate pollination services by pollinators (Genung et al. 2010, Tooker and Frank 2012). Enhanced levels of plant intraspecific diversity leads to greater flower characteristics and tends to attract more pollinators. The identity of plant cultivars may also contribute to this, as the inflorescences might be different among various cultivars, and the multiple inflorescences can

influence the behavior of pollinators and further plant propagation (Genung et al. 2010, Liao and Harder 2014). Diverse plant cultivars mixtures are expected to have more morphological traits and higher trait diversity (Taylor and Quesenberry 1996, Saviranta et al. 2008, Vleugels 2013) and this may in turn result in the observed tendency of more pollinators in plant communities with high intraspecific diversity (Chapter 3).

I did not find significant effects of plant intraspecific diversity on above and belowground herbivores, but I did observe a tendency toward *T. pratense* plants being more damaged by both herbivores and fungal pathogens in the high intraspecific diversity plant communities compared to those in low intraspecific diversity plant communities (Chapter 2). Previous studies on the effect of high plant intraspecific diversity on plant responses to pathogens and herbivores have revealed inconsistent results, either positive (Lively 2010, King and Lively 2012, Tooker and Frank 2012, Shoffner and Tooker 2013), negative (Kotowska et al. 2010) or neutral (Crawford and Rudgers 2012). As was discussed in Chapter 2, I propose that the effects of plant intraspecific diversity on leaf damage by herbivores and pathogens might depend on the chosen cultivars. Besides, I have not found any significant effect of plant intraspecific diversity either on the abundance of earthworms or on AMF root colonization. Even in the studies on plant species diversity, some evidences (Gastine et al. 2003, Salamon et al. 2004) also showed the neutral effect of plant diversity on soil biota. I assume that the plant intraspecific diversity treatment might have no strong effect on the abundance or activity of the soil organisms. Future studies should focus more on elucidating the mechanisms behind the effect of plant intraspecific diversity on the performance of aboveground herbivores and fungal pathogens. In conclusion, the foreknowledge of plant cultivars' defense against aboveground herbivores and fungal pathogens and of their competitive ability with weeds might be helpful in establishing a plant community with a high potential of resisting its antagonists.

### **The independent and interactive effects of soil organisms**

The results on AMF root colonization showed that the AMF treatment was well-established in the first year (Chapter 2) and may not be well-established in the second year (Chapter 3). This is probably due to the widespread dispersal of AMF in the soil over time. I assume that a wide spreading of AMF by hyphae, plant roots, or mobile soil organisms (such as earthworms) might increase the amount of AMF in control plots. Therefore, fewer effects of AMF being found in the second year might be attributed to the weaker AMF treatment. Concerning the intention of enhancing the earthworm effect, the amount and the adding frequency were doubled in the second year; correspondingly, I found more effects of earthworms in the second year than in the first year. Although I cannot distinguish the long term effect of the addition effect of earthworms, my results still highlighted the significance of earthworms on AMF root colonization and floral visitation rate

in the field (Chapter 3).

Soil organisms can induce strong independent effects on subsequent trophic interactions (De Roissart et al. 2013, Ueda et al. 2013, Wurst 2013, Tao et al. 2015). Interactions of plants with beneficial soil organisms, such as AMF or earthworms, often lead to a positive plant response (Scheu 2003, Gange and Smith 2005, Elsen et al. 2008, Barto et al. 2010, Lavelle 2011). On aboveground plant antagonists, the Shannon diversity of aboveground herbivores tended to decrease with the addition of AMF (Chapter 2). Here, a higher value of Shannon diversity index would be representative of a diverse and equally distributed community and lower value represents a less diverse community. In general AMF may induce host tolerance and/or increase host resistance to aboveground herbivores. Accumulating knowledge suggested AMF as a critical component for plant resistance to herbivory (Elsen et al. 2008, Koricheva et al. 2009, Ueda et al. 2013). As was discussed in Chapter 2, AMF addition might induce the decrease of Shannon diversity of aboveground herbivores through: 1) altering the plant defensive chemicals (e.g. antioxidants and phenolic compounds) (Bezemer and van Dam 2005, Reidinger et al. 2012) or plant quality (Koricheva et al. 2009); 2) the priming effect of plants (e.g. jasmonate-regulated defense mechanisms) (Pozo and Azcon-Aguilar 2007, Kempel et al. 2010, Jung et al. 2012); 3) changing volatile profiles to attract parasitoids (Guerrieri et al. 2004); or 4) influencing the abundance of particular groups of aboveground herbivores (Ueda et al. 2013). Regarding aboveground plant mutualists, I have found positive bottom-up effects of earthworms on pollinators (Chapter 3). The flower abundance and average floral visitation length by pollinators were increased and decreased by the addition of earthworms, respectively. As illustrated in previous studies, the flower characteristics or the performance of pollinators can be influenced by functionally dissimilar soil organisms, e.g. soil fungi (including AMF) (Wolfe et al. 2005, Becklin et al. 2011, Aguilar-Chama and Guevara 2012, Barber et al. 2013) and root-feeding herbivores (Barber et al. 2012). The research on the effect of beneficial or detrimental soil organisms on aboveground antagonists and mutualists has previously shown inconsistent results and is worthy of further attention (Wardle et al. 2004, Koricheva et al. 2009, Wurst 2010, Johnson et al. 2012). Future investigations on the mechanisms of the bottom-up effect of functionally dissimilar soil organisms on high trophic levels would be helpful for explaining the inconsistent effect of the soil organisms in different years and for better manipulating soil organisms in agricultural practice.

Furthermore, I found that the addition of earthworms can either increase AMF root colonization rate through their movement (Chapter 3) or decrease AMF root colonization rate through their feeding (Chapter 2). Previous studies investigating the effects of earthworms on AMF dispersal also reported inconsistent results. A recent review by Paudel et al (2015) suggested inconsistent

relationships between the activity of earthworms and AMF root colonization rate or AMF distribution, either positive (Reddell and Spain 1991, Gange 1993, Gormsen et al. 2004, Zaller et al. 2011a), negative (Bonkowski et al. 2000, Lawrence et al. 2003, Ortiz-Ceballos et al. 2007, Salem et al. 2013) or no response (Eisenhauer et al. 2009a, Salem et al. 2013). As was discussed in Chapter 2, I propose that the effect of AMF and earthworms on AMF root colonization depends on AMF density: the earthworms could have negative effects at high AMF abundance (in the addition treatment), but positive effects at low AMF abundance. Further, the results from the field study (Chapter 2 & 3) indicated that the effect of earthworms on AMF root colonization also changes temporally: I have not found the interactive effect of earthworms and AMF, but I did find a main positive effect of earthworm treatment on AMF root colonization rate in the second year. I assume that this may be attributed to the increased addition of earthworms in the second year. Besides, the feeding and burrowing activities of earthworms influenced AMF root colonization rate, but had no effect on plant biomass. This is reasonable, as the AMF root colonization rate is not always consistent with plant biomass production (Klironomos 2000, Smith and Read 2008). Also, previous studies illustrated that the primary production of plants is determined by plant identity, AMF identity, and AMF fungal diversity (van der Heijden et al. 1998, Klironomos 2000, Klironomos 2003).

Previous studies on the interaction effect of soil organisms showed inconsistent results. In detail, functionally dissimilar soil organisms have been found to: 1) interactively strengthen the others' effect (Salem et al. 2013, Aghababaei et al. 2014, Trouvé et al. 2014); 2) counterbalance or cancel out the effects of others (Bradford et al. 2002, Wurst et al. 2008, Liang et al. 2015); or 3) work independently and not influence each other's effects (Eisenhauer et al. 2009a, Wurst et al. 2011, Wurst and Rillig 2011). I suppose that the interactions between AMF, earthworms, and plant-parasitic nematodes depend on several factors, including both soil organism identity and host plant identity (for AMF or plant-parasitic nematodes). The interactive effects of soil organisms (e.g. earthworms and AMF) on the performance of plants (e.g. growth and nutrient uptake) vary between different plant species (Ortiz-Ceballos et al. 2007, Wurst et al. 2008, Eisenhauer et al. 2009a, Ladygina et al. 2010, Paudel et al. 2015). In the field, the environment is more complex with multiple biotic and abiotic disturbances, and I did not find much interactive effects of soil organisms on ecosystem functions. Exploring the knowledge of these interactions with different plant species is fundamental and essential for understanding the effect of soil biota on plant community and ecosystem processes in natural environments. Future investigation should pay more attention to the complex interactive effects of soil organisms on ecosystem functions.

## Future challenges

Plant intraspecific diversity can have cascading effects on the associated ecological communities and ecosystem processes, and further on ecosystem functions and important ecosystem services (Crutsinger et al. 2008a, Hughes et al. 2008, Kotowska et al. 2010, Tooker and Frank 2012, Shoffner and Tooker 2013). A great number of studies have shown that high plant intraspecific diversity can increase plant productivity (Sarandon and Sarandon 1995, Tooker and Frank 2012, Zeller et al. 2012). My field studies (Chapter 2 & 3) were the first to investigate the interactive effect of functionally dissimilar soil organisms and plant intraspecific diversity on ecosystem functions. The results highlight the importance of plant-parasitic nematodes as drivers of the plant intraspecific diversity effect on aboveground biomass of *T. pratense* in the field (Chapter 3). The aboveground biomass is higher in high diversity plots than in low diversity plots with the presence of plant-parasitic nematodes. In my greenhouse study (Chapter 4), I have shown similar findings on the intraspecific diversity effect regarding the nematodes as in the field experiment; however, in the greenhouse study, the nematodes' effect depended on the level of plant intraspecific diversity. These results indicated that the mechanisms of nematodes modifying the diversity effect on ecosystem functions can only be better understood when complex belowground processes are considered. Future studies on diversity-ecosystem functions with consideration of detrimental soil organisms may be one promising approach to revealing their influence on the diversity-ecosystem functions, both at the plant species and intraspecific levels. Much further work is also required to test the effect of other detrimental soil organisms on the plant intraspecific diversity-ecosystem function relationships and it must extend to include their interaction with dominant beneficial soil organisms, e.g. AMF and earthworms.

Understanding the characteristics of plant cultivars is also paramount for elucidating the effect of plant intraspecific diversity on ecosystem functions. In nature, intraspecific genetic variation of plants provides a basis for the adaptation of the species to the changing environment. It is expected that plant intraspecific genetic variation can influence plant morphological and physiological characteristics, e.g. plant size, resistance to herbivores, competitiveness with neighbors, floral traits, and plant quality (Dungey et al. 2000, Underwood and Rausher 2000, Hochwender and Fritz 2004, Johnson 2008). Cultivars of many crops and forage plants (including *T. pratense* and *L. perenne*) were bred to have different characteristics in their yield, competitive ability, and resistance against parasitic pathogens and herbivores (Taylor and Quesenberry 1996, Wilkins and Humphreys 2003, Lee et al. 2012, Vleugels 2013). Cultivars selected by breeders as being persistent may perform better in one or more aspects. More concrete background information on cultivars concerning their characteristics, especially their response to above- and belowground herbivores or pathogens and on their competitive ability, would be helpful in biodiversity and

agricultural studies and in maintaining vital ecosystem functions in high intraspecific diversity plant community.

Further, a better understanding of the independent role of functionally dissimilar soil organisms could also help us elucidate their combined effect on ecosystem functions in natural conditions. Knowledge regarding soil organisms could contribute to sustainable and ecological agriculture (Brussaard et al. 2007). Nowadays, this knowledge appears to be more important as increasing numbers of studies are leaning toward increasing plant productivity and solving crop diseases and pests problems by using agrochemicals or transgenic technology (Smithson and Lenne 1996, Mundt 2002, Hajjar et al. 2008, Thiele-Bruhn et al. 2012). The plant productivity can be increased by the addition of beneficial soil organisms (e.g. AMF in Chapter 2). The application of beneficial soil organisms (e.g. earthworms or AMF) might be an inexpensive and ecological way to improve plant productivity and control pests (Cameron 2010, Lavelle 2011, Thiele-Bruhn et al. 2012), yet the amount used must be evaluated to ensure their positive effect. Further, my studies (Chapter 3 & 4) also showed that the enhanced level of detrimental soil organisms (plant parasitic nematodes) can be useful for increasing plant productivity. These findings indicated that instead of using fertilizers, plant productivity can still be increased by appropriately manipulating the effect of soil organisms and using appropriate level of plant intraspecific diversity. Therefore, I suggest that to fully understand the effect of soil biota on plant community there is a need to go further than just dividing them into detrimental and beneficial groups. Future research should evaluate which functional roles or functional traits of soil organisms can influence ecosystem functions and how. A further understanding of the independent and interactive effects of soil biota, especially those important soil components (including AMF, earthworms, and nematodes), could provide useful guidance in managing agricultural ecosystems, such as enhancing plant productivity (Thiele-Bruhn et al. 2012).

Also, long term experiments are indispensable in revealing plant diversity effects on soil organisms because it was proposed that the effect of soil organisms in experimental diverse plant communities might increase with time scale (Viketoft et al. 2009, Laossi et al. 2011, Eisenhauer et al. 2012a). With the repeated addition of AMF and earthworms, I showed the effect of beneficial soil organisms' addition on ecosystem functions, yet the long term effect was not investigated. Thus, long term experiments on the interaction effect of soil organisms and plant intraspecific diversity should be carried out in future studies because the soil organisms can have different effects on the short and long term availability of nutrients (Laossi et al. 2011, Eisenhauer et al. 2012a), the root depth and structure might be changed (Mueller et al. 2012, Ravenek et al. 2014, Mommer et al. 2015), and meanwhile the complementary effect of plant intraspecific diversity may be increased

with time (Fargione et al. 2007).

As I illustrated in Chapter 2, chemical application to control soil plant-parasitic nematodes, pathogens, or weeds can induce undesired non-target effects (Ware 1980, Ekelund 1999, Pelosi et al. 2014, Gaupp-Berghausen et al. 2015), e.g. the herbicides and pesticides can directly influence the effect of earthworms *L. terrestris* and AMF and the activity and reproduction of earthworms *L. terrestris* (Yasmin and D'Souza 2010, Pelosi et al. 2014). Besides, intensive modern agricultural management practices can also negatively affect the presence and abundance of soil biota in agricultural ecosystems (Ponge et al. 2013, Tsiafouli et al. 2015). The wide application of fertilizers and agrochemicals can negatively influence soil biota and their ecosystem functions, such as carbon and nutrient cycling, and aboveground pest control (Turbé et al. 2010). The abundance, diversity, and performance of soil organisms in terrestrial ecosystems are threatened by anthropogenic activities (Turbé et al. 2010, Tsiafouli et al. 2015). Therefore, experimental studies are essential to assessing the role of soil organisms in ecosystem functions in agro-ecosystems and to developing efficient strategies for their preservation as well as manipulation in sustainable agriculture.

In the end, for the sustainability of agro-ecosystems, it is essential to develop plant disease management protocols based on biological resources (Reddy 2013). Besides the use of appropriate soil organisms, ecological approaches to controlling crop diseases also include sound crop rotation programs, selecting cultivar mixtures (Smithson and Lenne 1996, Mundt 2002, Yang et al. 2012, Zeller et al. 2012), and developing resistant plant genotypes (Wilkins 1991, Vleugels 2013). Meanwhile, practically, because of the resistance of plant intraspecific diversity or certain cultivars to plant invaders, a new ecological approach could be helpful in agricultural management with the ultimate aim of reducing herbicide application (Crutsinger et al. 2008a, Crowder and Jabbour 2014). Taking into consideration the response of plant cultivars to detrimental soil organisms and the plant cultivars' competitive ability (with plant invaders *per se*) is important in plant diversity research. The study on the effect of multiple levels of plant diversity and soil organisms on ecosystem functions can be crucial in providing predominant ecosystem services and is promising to sustainable and ecological agriculture.

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## Contribution to the manuscripts

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**Own contributions:** XG, SW and JSP conceived and designed the experiment. JSP and XG performed statistical analyses. XG performed the experiments, interpreted the data, wrote the manuscript and submitted it to *Applied Soil Ecology*. All authors reviewed the manuscript.

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## **Curriculum vitae**

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