Impacts of Insect Herbivory and Nitrogen Eutrophication on Grassland Ecosystems

Pernilla Borgström
Faculty of Natural Resources and Agricultural Sciences
Department of Ecology
Uppsala

Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2017

Acta Universitatis agriculturae Sueciae
2017:22

Cover: Field experiment at Krusenberg. Illustration by Matilda Borgström.

ISSN 1652-6880
ISBN (print version) 978-91-576-8817-0
ISBN (electronic version) 978-91-576-8818-7

Print: SLU Service/Repro, Uppsala 2017

Dedication

To the insects (you weird and wonderful little beasts).

There are flings and pulls in every direction at every moment. The world is a wild wrestle under the grass; earth shall be moved.
Annie Dillard

You have to be where you are to get where you need to go.
Amy Poehler

Impacts of Insect Herbivory and Nitrogen Eutrophication on Grassland Ecosystems

Abstract

Nitrogen (N) eutrophication causes severe species richness declines in grassland ecosystems worldwide. Species interactions, for example between plants and herbivores, are likely to mediate the grassland's response to increased N availability. However, attempts to investigate how herbivores might modify ecosystem-level N impacts have so far focused near-exclusively on the aboveground subsystem. Such approaches overlook the importance of the great number of belowground interactions that occur among plants, herbivores and other organisms. A great challenge in global change ecology is to understand how the above- and belowground subsystems interact to determine the ultimate, ecosystem-level impact of N eutrophication and other global change drivers. In this thesis, I present controlled experiments that were carried out to explore the role of above- and belowground insect herbivory in mediating interactions among plant species, and how the interaction between above- and belowground herbivory determines the response of the grassland to N eutrophication.

In a field experiment, I found that above- and belowground herbivory determined the impact of N eutrophication on the grassland ecosystem, in terms of plant community composition, ecosystem functioning, and the soil food web. N and herbivory synergistically conferred a competitive advantage to forbs over grasses, as the former strongly benefited from the presence of both herbivory types at elevated N. Nutrient turnover displayed inverted responses to above- and belowground herbivory at contrasting N levels. Additionally, the shifts in the plant community extended to shifts in the functional composition of the soil nematode community. In a parallel plant competition experiment, I found that belowground herbivory mitigated an increased competitive asymmetry caused by aboveground herbivory.

I conclude that insect herbivores can be important mediators of the impact of N eutrophication on grasslands, and that their mediating role will depend on whether both above- and belowground herbivores are present. Therefore, the ecosystem-level impact of herbivory above ground should, whenever possible, be studied alongside that of herbivory below ground, as the omission of either herbivory type could lead to erroneus conclusions about the role of the other.

Keywords: global change ecology; above-belowground linkages; foliar herbivory; root herbivory; grassland plant communities; ecosystem functioning; soil nematodes.

Author's address: Pernilla Borgström, SLU, Department of Ecology,
P.O. Box 7044, 750 07 Uppsala, Sweden
E-mail: pernilla.borgstrom@ slu.se

Contents

List of Publications

This thesis is based on the work presented in the following papers, referred to by Roman numerals in the text:

I **Borgström, P**., Strengbom, J., Marini, L., Viketoft, M., Bommarco, R. (2017). Above- and belowground insect herbivory modifies the response of a grassland plant community to nitrogen eutrophication. *Ecology* 98:545-554.

II **Borgström, P.**, Strengbom, J., Viketoft, M., Bommarco, R. (2016). Aboveground insect herbivory increases plant competitive asymmetry, while belowground herbivory mitigates the effect. *PeerJ* 4:e1867.

III **Borgström, P.**, Bommarco, R., Viketoft, M., Strengbom, J. Nitrogen eutrophication inverts the interaction between above- and belowground insect herbivory effects on grassland ecosystem functioning. (*manuscript*).

IV **Borgström, P.**, Bommarco, R., Strengbom, J., Viketoft M. Above- and belowground insect herbivory mediates the impact of nitrogen eutrophication on soil nematode communities in a grassland ecosystem. (*manuscript*).

Papers I-II are reproduced with the permission of the publishers.

The contribution of Pernilla Borgström to the papers included in this thesis was as follows:

I Planning and carrying out the experiment with co-authors, analysing and interpreting the results with co-authors, first author of the paper.

II Planning and carrying out the experiment with co-authors, analysing and interpreting the results with co-authors, first author of the paper.

III Planning and carrying out the experiment with co-authors, analysing and interpreting the results with co-authors, first author of the paper.

IV Planning and carrying out the experiment with co-authors, analysing and interpreting the results with co-authors, first author of the paper.

Abbreviations

ANPP	Aboveground net primary production
N	Nitrogen
NCR	Nematode Channel Ratio
NPP	Net primary production
RY	Relative yield

1 Introduction

The natural world is a complicated, messy and massively interlinked jumble of species. They compete with one another for resources, eat (or try to avoid being eaten by) one another, and indirectly influence one another by altering the environment. In recent decades, this profoundly complex global interaction network has been put under increasing pressure from various human-related activities. This pressure is collectively termed *global change*, and incorporates all the major environmental and climate change effects caused by humans (Vitousek 1994).

A major challenge in ecological research today is how to increase our understanding of the consequences of global change, so that we can reliably predict and counteract potential negative effects on for example biodiversity. At the heart of such predictive efforts and countermeasures lies the need for a solid empirical understanding of the ecosystems we are trying to protect. In recent decades, researchers have become increasingly aware that such an understanding requires us to not only consider the species that we can see, but also those that we *can't* see. More specifically, we need to expand the picture to include the world below ground.

In grassland ecosystems[1], a considerable portion of the species interactions that build the ecosystem are effectively invisible to us, as everything that goes on in the soil is hidden from view. Grasslands are severely affected by global change, in terms of current and projected future biodiversity loss (Sala et al. 2000). The majority of studies assessing the impact of global change on grasslands have focused on shifts in the aboveground compartment of the plant

---

[1]. Broadly defined as ecosystems where grasses dominate the plant community and there is little or no tree cover. The vast majority of European grasslands are *semi-natural*, i.e. they contain natural plant communities that have been strongly shaped by long-term agricultural practices, and are currently to some degree managed by grazing or mowing (e.g. hay meadows).

community. There is, however, increasing awareness of the necessity to expand our observations to also include belowground processes (Eisenhauer et al. 2012).

Interactions between the above- and belowground components of an ecosystem are likely to determine the ultimate impact of global change drivers (Van der Putten et al. 2009), but the studies that adopt an above-belowground perspective when addressing these questions are exceedingly rare. It is possible that functionally similar organisms in the above- and belowground subsystems have different – maybe even opposing – roles in determining the global change outcome. In this thesis, I outline these possibilities, and their ecosystem-level consequences, in the context of insect herbivory under nitrogen (N) eutrophication – one of the most severe drivers of global change. I make two general claims: *i*) that insect herbivores are mediators of the impact of N eutrophication on grassland ecosystems, and *ii*) that the mediating role of herbivory will ultimately depend on whether above- or belowground herbivores, or both, are present and abundant. I will make the further claim that effects of herbivory above ground should, whenever possible, be studied alongside effects of herbivory below ground, because the omission of one can lead to misleading conclusions about the role of the other.

Figure 1. A plant community: what we see and what we don't see. Illustration: Matilda Borgström.

2 Background

2.1 Nitrogen eutrophication and biodiversity in grasslands

Nitrogen (N) eutrophication[2] is one of the most severe drivers of global change effects on biodiversity in grasslands, as it can substantially change the composition and productivity of plant communities (Vitousek et al. 2002, Bobbink et al. 2010, Stevens et al. 2010), impacting on other trophic levels as well (Tylianakis et al. 2008). N eutrophication often results in a decreased species richness in both the plant and the insect community (Haddad et al. 2000, Stevens et al. 2004). However, the variation in documented ecosystem responses to N eutrophication is considerable. This variation is probably in part caused by modification of the N effect by biotic drivers, such as herbivory (Neff et al. 2002, Knorr et al. 2005).

The composition of the plant community (herein defined as the relative abundances of the component species) is determined by the interplay between resource availability and consumer pressure (Gruner et al. 2008). N eutrophication confers an increased resource availability for the plants, and can shift competitive hierarchies among plant species (Vallano et al. 2012). Herbivores also shift competitive relationships in the plant community (Carson and Root 2000, Kim et al. 2013), and therefore have the potential to modify the ultimate impact of N eutrophication. Such a modification has so far mainly been assessed with regards to vertebrate herbivory. Specifically, there is evidence indicating that grazing by large mammals can counteract the negative effect that N eutrophication has on plant species diversity (Borer et al. 2014). However, we know little about whether insect herbivores might play a similar role.

---

[2] Human-induced increases in the amount of plant-available N in natural systems, caused by for example fossil fuel combustion, and the extensive use of mineral fertilizers in agriculture (Vitousek et al. 1997).

Insect herbivory was formerly suggested to have considerably less of an ecosystem-level impact than vertebrate herbivory (e.g. Crawley 1989), and research on insect herbivory effects in grasslands has long been eclipsed by that on large grazing mammals. This accords poorly with the abundance and ubiquity of herbivorous insects worldwide (Schoonhoven et al. 2005), and the broad consensus that they are central determinants of process rates in many ecosystems (Weisser and Siemann 2004). In some cases, the impact of insect herbivores on plant communities can be greater than that of vertebrate herbivores (La Pierre et al. 2015). Simply put: reliable predictions regarding the ecological consequences of N eutrophication in grasslands require a better understanding of the trophic control that insect herbivores provide.

2.2 Linking the above- and belowground subsystems

The trophic control of grasslands is made more complex when we consider the fact that these ecosystems, and the plant communities that sustain them, exist both above and below ground. The potential for interactions between above- and belowground consumers add a considerable layer of complexity to the already intricate problem of mapping indirect interactions within an ecosystem (Sotomayor and Lortie 2015). Nonetheless, adopting such a whole-system perspective might prove to be critical for advancing global change research. There can, for example, be differences between the above- and belowground subsystems in how fast they respond to change (van der Putten et al. 2004).

The influence of combined above- and belowground insect herbivory on plant populations has been subject to some investigation (Mullerscharer and Brown 1995, Maron 1998), but little is known about their impact at the level of the plant community or the whole ecosystem. Plant community-level effects have been examined by means of insecticide application (e.g. Brown and Gange 1989, Stein et al. 2010), but manipulations of herbivore densities and species by adding herbivores, for example with the use of experimental, enclosed ecosystems, are rare. A notable exception is van Ruijven et al. (2005), who found non-additive effects of above- and belowground herbivory on plant species diversity in their experimental mesocosms. The authors attribute the results to differences in feeding preference between the above- and belowground herbivores, and argue that such differences would be masked when insecticides are used to manipulate herbivory. They conclude that there are complex shifts in indirect interactions within the plant community when subjected to simultaneous above- and belowground herbivory, and that such shifts are impossible to deduce from the separate impacts of each herbivory type.

Individual and combined impacts of above- and belowground trophic interactions on plant communities are likely to change when N levels increase (e.g. Lu et al. 2015), but experiments specifically addressing this issue are rare. Given the vulnerability of plant communities to N eutrophication, and the potential for above- and belowground herbivores to dramatically shift indirect interactions among plants, an experimental approach addressing the impact of herbivory at contrasting N levels is warranted.

2.3 Insect herbivores as mediators of N effects

By far, the most common response of a plant community to N eutrophication is a reduction in plant species richness (e.g. Suding et al. 2005, Stevens et al. 2010) and an increase in productivity (Bobbink et al. 2010). There is, however, a great degree of variation in the magnitude of these responses, which indicates that the environmental and biotic context are important determinants of the ultimate N impact (Cleland and Harpole 2010). Herbivory is one such potentially important aspect of the biotic context.

To understand the mechanisms underlying these N-driven shifts in plant species abundances, and the potential for herbivores to mediate them, we need to explore the effect of N eutrophication on competition among plant species.

2.3.1 N eutrophication and competitive asymmetry among plants

The average grassland plant community includes plant species that fall along a spectrum of optimal N availability; some species in the community will perform best when N availability is high, others when it is low. As N levels rise, fast-growing species that are well-adapted to conditions of high N availability (e.g. many grasses) will be released from N limitation, and start to shade and outcompete their slower-growing neighbours (Sala et al. 2000). N-induced reductions in species richness are therefore generally a consequence of increased competition for light, as the slower-growing species suffer competitive exclusion (Hautier et al. 2009). More specifically, an increase in N increases the *size-asymmetry* of competition (DeMalach et al. 2017). Competitive size-asymmetry simply means that a large plant will have a disproportionately large competitive advantage, as it will partially shade its smaller neighbours and thereby intercept more light per size unit than they do.

Any driver that modifies this increased competitive asymmetry can be an important determinant of the ultimate impact of N eutrophication. Aboveground herbivory can both intensify (Bentley and Whittaker 1979, Kim et al. 2013) and relax (Van et al. 1998) interspecific competition, which demonstrates its potential to mediate N effects on competition. However, few studies have specifically explored the effect of insect herbivory on competitive asymmetry, and to my knowledge there are none that have incorporated both above- and belowground herbivory.

2.3.2 Herbivory as a mediator of competitive asymmetry during N eutrophication

If an aboveground herbivore preferentially feeds on slow-growing species, herbivory can amplify competitive asymmetry, possibly accelerating competitive exclusions and the consequent decline in species richness. By contrast, if the herbivore prefers fast-growing species, competitive asymmetry might be reduced and competitive exclusion delayed. Herbivores often preferentially feed on fast-growing, competitive species (Huntly 1991), which makes the second scenario the most likely for aboveground herbivory effects. Such an offset of the N eutrophication effect on competitive asymmetry has, for example, been demonstrated for vertebrate herbivores in grasslands (Borer et al. 2014) and gastropod grazers in kelp forests (Ghedini et al. 2015), but no similar investigations of insect herbivory have been made. Possibly, the importance of aboveground herbivory in mediating competition increases under N eutrophication (*Fig. 2* illustrates this further).

The influence of belowground herbivory on competition has received comparatively little attention. Belowground competition is likely to be stronger when N availability is low (Cahill 1999), so belowground herbivory might have its main impact on plant competition at low N levels. Additionally, belowground herbivory might influence the effect of aboveground herbivory. If the belowground herbivores feed on the same plant species as their aboveground counterparts, they are likely to further decrease the competitive potential of that species. If they instead feed on a competitor to the species on which the aboveground herbivore is feeding, they might have a balancing effect on competition.

At the population level, simultaneous above- and belowground herbivory can have additive effects on overall fitness (Maron 1998) and biomass production (He et al. 2014), but little is known about how this translates to the competitive ability of the species relative to a population of another species. As van Ruijven et al. (2005) demonstrated, plant species can respond differently to simultaneous above- and belowground herbivory, which in turn can yield non-additive effects of above- and belowground herbivory on plant community composition.

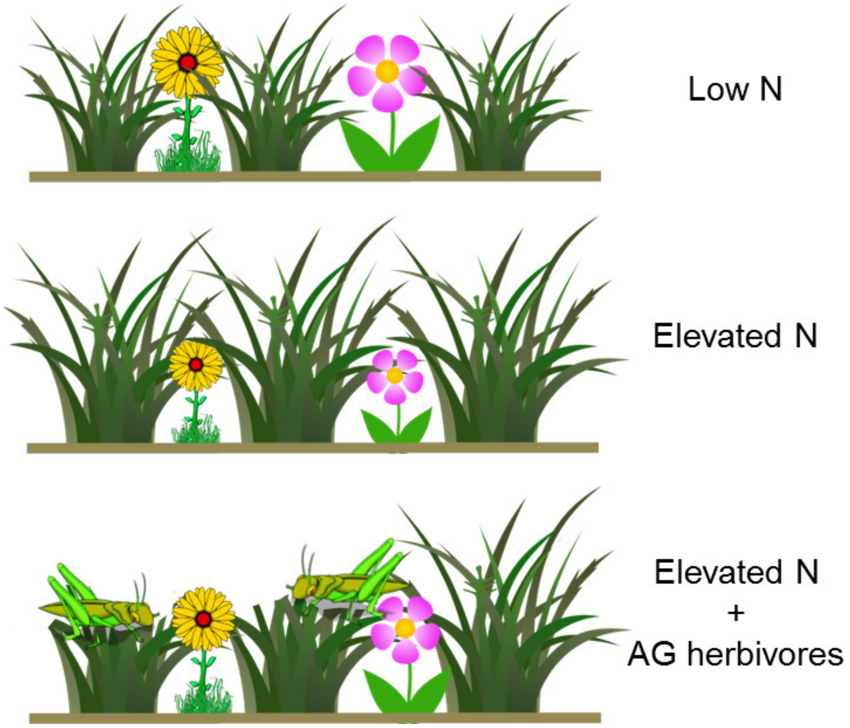

Figure 2. Scenario in which aboveground (AG) herbivory counteracts increased competitive asymmetry caused by N addition. In the topmost panel, N availability is low and competitive ability is relatively even among plant species. In the middle panel, N eutrophication has given a competitive advantage to fast-growing grasses, which have started shading their slower-growing neighbours. This scenario can, with time, lead to competitive exclusion of the slower-growing species and reduced species richness. In the bottom panel, aboveground herbivores preferentially feed on the fast-growing grasses, reducing their foliage and consequently reducing their shading effect. Thus, the slower-growing species can intercept more light and catch up with the grasses, and species richness is maintained.

A better understanding of above- and belowground herbivory effects on plant competition will likely shed light on the mechanisms that underlie plant community shifts under N eutrophication. An important extension of this is to investigate how such plant community shifts might propagate to functioning of the entire ecosystem.

2.4 Impacts on ecosystem functions

The preceding sections have outlined how insect herbivory can drastically shift plant community composition, and how it might therefore play a part in determining the ultimate impact of N eutrophication on the plant community.

However, the sheer volume of biomass that insects consume means that they not only alter competitive interactions among plants, but also total productivity in the plant community (Weisser and Siemann 2004). How this interacts with the general boost in productivity caused by increased N is largely unknown. Moreover, any joint impact of herbivory and N on the plant community is likely to have consequences for ecosystem functions that are driven by the above- and belowground food webs, since plants are the basal resource in both.

2.4.1 Primary production

When it comes to insect herbivory effects on ecosystem functions, by far the best-studied function is aboveground net primary production (ANPP). Aboveground herbivores consistently reduce ANPP in temperate herbaceous plant communities (Coupe and Cahill 2003). Belowground herbivores can also have a substantial impact on ANPP (Masters et al. 1993). When combined, the effects of above- and belowground herbivores on ANPP appear to be additive (van Ruijven et al. 2005, Stein et al. 2010). In other words, combined above- and belowground herbivory should have a stronger suppressive effect than either herbivory type does on its own, but their combined effect is likely not greater than the sum of the two effects.

N eutrophication generally increases ANPP (Zavaleta et al. 2003, Bobbink et al. 2010), but might in the long term (20+ years) also reduce total productivity as plant species are lost from the community (Isbell et al. 2013). Here, herbivory could play an important mediating role: when above- and belowground herbivores alter the rate of compositional change in the plant community, they can either decelerate or accelerate such a reduction in ANPP. The interaction between herbivory and resource control of ANPP is quite poorly studied in terrestrial systems; in particular, there has been a lack of controlled experiments (Gruner et al. 2008). One possibility is that biomass loss due to herbivory counteracts the increase in ANPP caused by N eutrophication (Throop and Lerdau 2004), giving a net neutral combined effect, but so far there is no experimental support for this hypothesis.

2.4.2 Nutrient turnover

Insect herbivory can have a strong impact on the speed of nutrient turnover[3] (Belovsky and Slade 2000, Schädler et al. 2004). The impact runs through several pathways, for example alteration of litter quality (e.g. caused by changes in relative abundances of plant species) and litter quantity (through

---

[3] In this thesis, I use nutrient turnover as a collective term for N mineralization and decomposition of soil organic matter, two processes important for determining the speed at which nutrients are returned to the soil and made available to the plants.

alteration of plant biomass production rates) (Hunter 2001). This means that insects not only affect ANPP by consuming plant biomass, but also by influencing the amount of plant-available nutrients that are present at any given time (Bardgett and Wardle 2010). Such concurrent, dual impacts of herbivory highlight that the concept of resource versus consumer regulation of ecosystems is anything but straightforward.

There is evidence that N eutrophication can speed up nitrification rates (Matson et al. 2002) and decomposition processes in the soil (Neff et al. 2002). Given the multiple roles that herbivores can have in modifying the plant community and nutrient turnover, it is likely that they can act as mediators not only of N effects, but of global change effects in general (Bardgett and Wardle 2010). However, little empirical work has specifically addressed these questions, and the relative importance of above- and belowground consumers in controlling nutrient turnover is largely unknown.

2.5 Impacts on the soil food web

The composition of the soil food web (broadly defined as the relative abundances of different taxonomic groups of soil organisms) can be tightly linked to ecosystem functioning, e.g. rates of N and carbon cycling (de Vries et al. 2013). Plant species identity and abundance can strongly shape the soil food web (Wardle et al. 2003), so if N eutrophication and herbivory interact to change the plant community, they are likely to also have knock-on effects on the soil food web (De Deyn et al. 2004). N enrichment has been shown to lead to simplified soil food webs, with lower species richness (Eisenhauer et al. 2012), but little is known about how herbivory might modify this effect by modifying the impact on the plant community.

One part of the soil food web that has great functional importance is the soil nematode community, as nematodes are central contributors to nutrient turnover (Yeates 2003). Soil nematodes can easily be classified into distinct feeding groups based on their mouthparts. Since the abundances of the different feeding groups reflect abundances of other soil biota, nematodes are useful indicators of soil health and processes (Neher 2001). For example, a high abundance of bacterial-feeders relative to fungal-feeders suggests that bacterial decomposition dominates in the soil (Yeates 2003), while a high abundance of omnivorous and predatory nematodes could indicate a more stable soil food web, with a high buffering potential against seasonal shifts in for instance nutrient availability (Bongers and Bongers 1998).

An important aspect of potential herbivore-driven shifts in the soil nematode community is that it might lead to feedbacks on total grazing pressure. Belowground insect herbivory has been shown to increase the abundance of root-feeding nematodes (De Deyn et al. 2007). Root-feeding nematodes can themselves be significant soil herbivores (Ingham and Detling 1990). Insect herbivore-induced changes in the plant community that in turn alter the abundance and subsequent grazing pressure of root-feeding nematodes can therefore have implications for the ecosystem, for example in terms of ANPP.

3 Motivation and Aims

In this thesis, I present factorial experiments carried out in order to investigate *i*) how the interaction between above- and belowground insect herbivory shapes a grassland ecosystem, and *ii*) the role this interaction plays in determining the ultimate impact of N eutrophication on a grassland ecosystem. This is done in the following stages.

1. In **Paper I**, I address the role of above- and belowground herbivory in modifying N effects on the plant community. The plants serve as a main communication channel between the above- and belowground subsystems, and compositional changes in the plant community can yield considerable knock-on effects on other ecosystem processes and characteristics. Plant community responses are therefore a relevant starting point to assess ecosystem-level effects of N eutrophication and herbivory.
2. Impacts of both herbivory and N on the plant community can in part be attributed to changes in the magnitude and direction of competition between plants. In **Paper II**, I present an assessment of how competition between two functionally similar plant species changes under above- and belowground herbivory at contrasting N levels.
3. In **Paper III**, I explore how the response of the plant community in **Paper I** can extend to effects on ecosystem functions, with a focus on rates of nutrient turnover.
4. Insect herbivores above and below ground can drive feedbacks between the above- and belowground subsystems, for example by altering the abundance and grazing pressure of other invertebrate herbivores. In **Paper IV**, I examine how the changes in the plant community outlined in **Paper I** can associate with changes in the soil food web, by investigating an important indicator group: soil nematodes.

4 Methods

The questions were addressed using two experimental approaches. The data for papers I, III and IV were collected in a field experiment, while the data for paper II was collected in a greenhouse experiment.

4.1 Field experiment (**Papers I**, **III** and **IV**).

To investigate the role of above- and belowground herbivory in modifying ecosystem-level N effects, I established a field experiment at Krusenberg, approximately 10 km south of Uppsala in central Sweden, in the spring of 2013. The experiment consisted of 64 enclosures placed in an 8×8 formation (see *Fig. 3* for enclosure design). The belowground part of the enclosure consisted of a metal frame (1×1 m) which was filled with soil from the field, to a depth of 0.5 m. The frame had a bottom made of fine-mesh net (mesh size 0.2×0.4 mm, Anti-aphid net 20/12, Artes Politecnica, Italy) to prevent added herbivores from escaping. Above ground, the enclosure consisted of a wooden frame (height 2 m), covered with a net of the same mesh size and make as the bottom of the enclosure.

Plants were sown in the greenhouse in late March 2013 (see Table 1 for the selected species), and replanted in the enclosures in late May. The plant species were selected to represent three main plant groups (grasses, non-leguminous forbs, and legumes) that were expected to display different responses to the N treatment. The chosen species commonly co-exist in European grasslands. They were planted at equal abundances, and their placement randomized within each individual enclosure.

The N treatment consisted of mineral fertilizer (NH_4NO_3) applied at 40 kg/ha at three times: in mid-July 2013, late June 2014, and early July 2015.

The aboveground herbivory treatment consisted of the predominantly graminivorous grasshopper *Chorthippus albomarginatus* De Geer

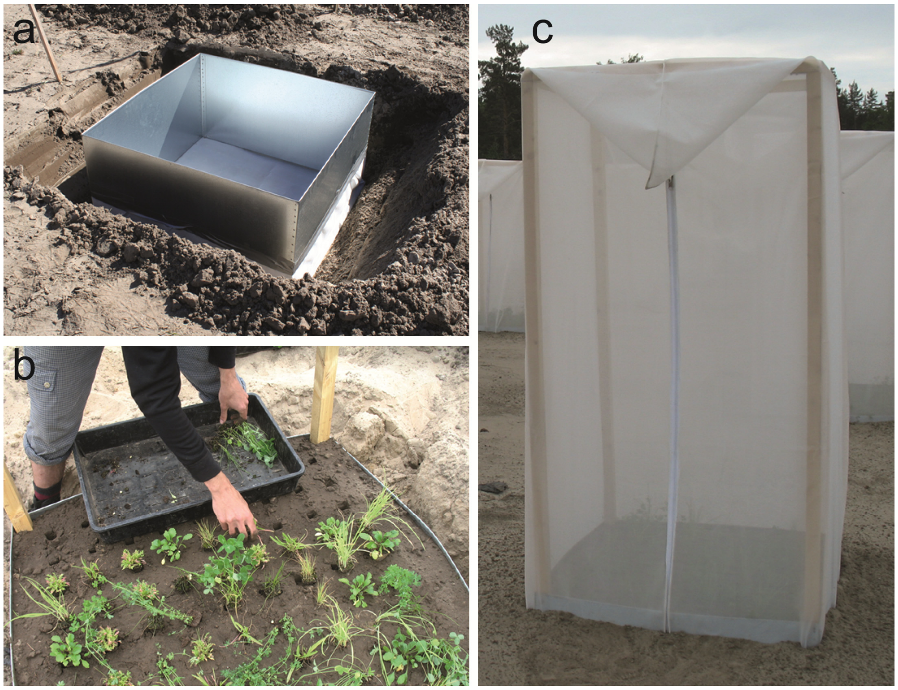

Figure 3. Establishment of the field experiment: a) the sheet metal base frame with a mesh net bottom, b) planting of the experimental plant communities, c) the aboveground compartment of the enclosure.

(Picaud et al. 2003). Adult individuals of *C. albomarginatus* were collected in fields near Uppsala and added to enclosures at a density of 10 individuals (5 females and 5 males) per enclosure. The grasshopper density was monitored in the following years.

The belowground herbivory treatment consisted of 10 wireworms, which is the common name for the larval stage of click beetles of the genus *Agriotes* spp. L. They are common generalist root feeders in European grasslands, and mainly feed on the species that have the most abundant roots in the soil (Sonnemann et al. 2012). The wireworms were acquired commercially (Applied Plant Research, PPO, Wageningen UR, Wageningen, Netherlands).

4.1.1 Plant community response (**Paper I**)

The plant community was assessed on four occasions: mid-September 2013, mid-June 2014, mid-September 2014, and mid-September 2015. Each harvest corresponded to the peak of standing biomass, apart from the mid-June harvest, which was conducted to simulate the management of a semi-natural grassland. A similar harvest was not conducted in 2015, as I wanted the plants to go to seed for a parallel study on plant reproduction.

Table 1. *The nine plant species included in the field experiment.*

Name	Functional group
Agrostis capillaris L.	Grass
Dactylis glomerata L.	Grass
Festuca rubra L.	Grass
Lolium perenne L.	Grass
Achillea millefolium L.	Non-leguminous forb
Leucanthemum vulgare Lam.	Non-leguminous forb
Plantago lanceolata L.	Non-leguminous forb
Lotus corniculatus L.	Legume
Trifolium pratense L.	Legume

At harvest, plants were cut 5 cm above the soil surface and sorted by species. They were then oven-dried at 65°C for 48 hours and weighed. In 2015, I assessed belowground biomass by collecting soil cores from each enclosure, sieving the soil to obtain the roots, drying and weighing them.

4.1.2 Ecosystem function response (**Paper III**)

In 2015, I assessed ecosystem functioning in four ways: aboveground net primary production (ANPP), decomposition rate, N mineralization, and litter decomposition.

ANPP was measured in September 2015, as described in the "Plant community response" section, i.e. by harvesting, drying and weighing the plants.

Decomposition rate was measured using tea bags (a method introduced by Keuskamp et al., 2013). This method presents a way to obtain standardized decomposition rate estimates that are comparable between environments. Bags of red and green tea were dug into the soil in mid-June 2015 and left for 90 days before being dug up again. The bags were weighed before being placed in the field, and were upon retrieval weighed again to determine how much of the tea had decomposed.

Nitrogen mineralization rate was measured using the buried bag technique (Eno 1960). A 300 g soil sample was taken from each enclosure in mid-June 2015, to a depth of 10 cm. The soil from each enclosure was split into two equal subsamples that were each placed in a polyethylene bag. One bag was buried in the soil in the middle of the enclosure, the other taken back to the lab for storage in a freezer (-20°C) until analysis. After 90 days, the buried bags were recollected and also frozen. All control bags and buried bags were then analysed for inorganic N content (g/kg of NO_3/NO_2 and NH_4 respectively) using 2M KCl extraction (Agrilab AB, Uppsala, Sweden). The N mineralization rate was obtained by subtracting the amount of nitrate and ammonium in the control bags from that in the buried bags.

4.1.3 Soil nematode community response (**Paper IV**)

Smaller soil cores were taken concurrently with the harvests in mid-September 2013 and 2014, using a small soil corer (⌀11 mm). Cores were taken next to each of the nine plant species, ca 1 cm from the base of two randomly selected individuals per plant species, and to a depth of 10 cm. The 18 cores were combined into one larger sample per enclosure and stored at 4°C until nematode extraction took place. A subsample of 20 g was then used for nematode extraction, which was performed using a modified Baermann method for 24 hours (Viketoft et al. 2005). The nematodes were counted under a microscope (200× magnification) and sorted by trophic group; root-feeders, roothair-feeders, bacterivores, fungivores, and omnivores.

In addition to analysing treatment effects on the abundances of each feeding group, I calculated the Nematode Channel Ratio (NCR), which compares the abundances of fungal- and bacterial-feeding nematodes to give an indicator of which decomposition pathway (fungal or bacterial decomposition) dominates in the soil (Yeates 2003).

4.2 Greenhouse experiment (**Paper II**)

In the background section, I outlined how increased competition for light is a major factor underlying N-induced shifts in plant community composition. Possibly, the importance of aboveground herbivory in mediating plant competition increases with increasing N availability, as aboveground herbivores have the potential to modify light competition. Contrastingly, belowground herbivory might have its strongest influence on competition at low N availability. I tested these predictions in a greenhouse experiment, using two commonly co-existing grass species: *Dactylis glomerata* L., which is considered a strong competitor on nutrient-rich soils, and *Festuca rubra* L., considered a strong competitor on nutrient-poor soils.

4.2.1 Experimental set-up

I used a replacement design (De Wit 1960) to investigate competition between two grass species. A replacement design compares the growth of a species when grown with conspecific neighbours to when it is grown with another species (Cousens 1996). I cultivated monocultures and 1:1 mixtures of *F. rubra* and *D. glomerata.* A monoculture contained four stands of one species, while a mixture pot contained two stands of each species in a mixture. One experimental unit consisted of a triplet of one mixture pot, and two monoculture pots (one of each species). *Fig. 4* shows the experimental set-up.

4.2.2 Treatments

I applied three treatments (aboveground herbivory, belowground herbivory, and N) in a fully factorial design, 2.5 weeks after seedlings were replanted into experimental pots. The aboveground herbivory treatment consisted of larvae of the noctuid moth *Spodoptera littoralis*, the belowground herbivory treatment consisted of wireworms, and the N treatment consisted of ammonium nitrate corresponding to 17 kg/ha (the mid-range of N deposition levels to which European grasslands are currently exposed; Stevens et al. 2010). Each experimental triplet was assigned to one of the eight treatment combinations, and each treatment combination was replicated eight times, which gave a total of 64 replicates.

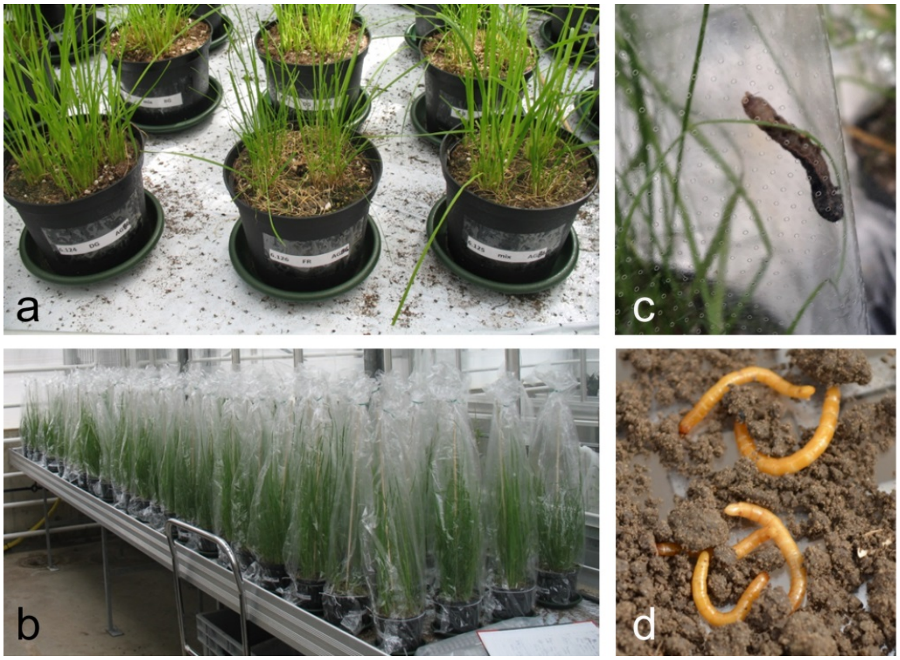

Figure 4. The greenhouse experiment: a) an experimental triplet of monocultures and mixture of the two species, b) the experimental pots enclosed in perforated plastic to stop herbivores from escaping, c) larva of *Spodoptera littoralis*, the aboveground herbivore, d) larvae of *Agriotes* spp., the belowground herbivore.

4.2.3 Measurements

A first harvest was conducted three weeks after treatment application. Plants were cut 2 cm above the soil surface and their biomass was dried for 48 h at 65°C and then weighed. All *S. littoralis* individuals were removed from aboveground herbivory-treated pots after the first harvest. Belowground herbivores were not removed, as they could not be extracted from the soil without causing damage to the plant roots. Plants were allowed to regrow without aboveground herbivores after the first harvest. They were then cut, dried and weighed after three and six more weeks.

4.2.4 Calculations

I estimated competitive asymmetry using the aggressivity index (McGilchrist and Trenbath 1971). This index takes the relative yield (henceforth RY) of each species as a measure of the strength and direction of a two-species competitive interaction. RY is in turn calculated by dividing the yield (Y) of a species grown in mixture with that of the same species when grown in monoculture. The respective RY values can be used as indicators of competitive asymmetry (*sensu* Keddy and Shipley 1989). The equations below detail this calculation for the yield (Y) of a hypothetical species *a* that is grown in monoculture and in mixture with another species, denoted *b.*

$$\mathrm{RY(a)} = \frac{Y(ab)}{Y(aa)}$$

$$\text{Aggressivity } = RY(a) - RY(b)$$

5 Results & Discussion

Plant community productivity and composition, plant competitive asymmetry, nutrient turnover, and the soil nematode community were all affected by the interactions between above- and belowground herbivory. In many cases, this interplay determined how that specific variable responded to N addition. Here, I present and discuss the main results from the mesocosm and greenhouse experiments. All responses and effects discussed below display statistical significance at $p \leq 0.05$.

5.1 Plant community productivity and composition

5.1.1 Aboveground net primary production (ANPP)

The response of ANPP to N addition depended on both above- and belowground herbivory. At ambient N, above- and belowground herbivory combined to reduce ANPP (*Fig. 5*), which is in line with the consistently suppressive effect that insects have on plant biomass production in temperate herbaceous plant communities (Coupe and Cahill 2003).

At elevated N, aboveground herbivory reduced ANPP, while belowground herbivory mitigated this negative effect (*Fig. 5*). Previously, above- and belowground herbivory effects on ANPP have

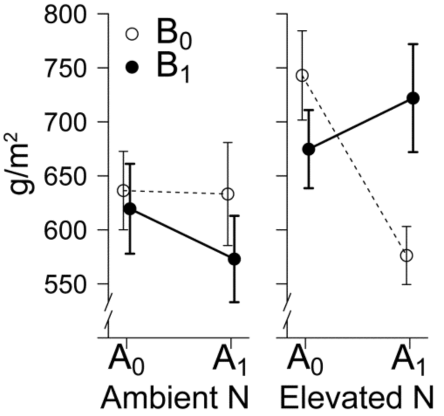

Figure 5. Response of ANPP (measured as biomass dry weight per m^2) to aboveground (A) and belowground (B) herbivory at contrasting N levels.

been shown to be additive (Brown and Gange 1989) or minor (Stein et al. 2010) at ambient nutrient availability. **Paper I** demonstrates that N addition can cause interactive effects between above- and belowground herbivory. One possible explanation is that above- and belowground herbivory both increased the rate of nutrient turnover (Belovsky and Slade 2000, Masters 2004), which when coupled with N addition caused a synergistic acceleration of shoot biomass production. N eutrophication most often increases ANPP (Zavaleta et al. 2003, Bobbink et al. 2010). I show here that above- and belowground herbivory can produce considerable variation in such ANPP responses, something which may complicate predictions of global change impacts.

5.1.2 Plant group responses

The response of ANPP to the treatments was mainly driven by contrasting responses of grasses and forbs (**Paper I**). At ambient N, grass biomass decreased under aboveground herbivory (*Fig. 6a*). Forbs appeared to benefit from this, possibly due to reduced aboveground competition from grasses (*Fig. 6b*). At elevated N, aboveground herbivory still reduced grass biomass, but only had an effect on forb biomass when combined with belowground herbivory. Legume biomass decreased at elevated N, but was unaffected by above- and belowground herbivory (*Fig. 6c*).

The shifts in the plant community caused by aboveground herbivory can be attributed to the feeding preference of the aboveground herbivores, which are predominantly graminivorous (Picaud et al. 2003). This corresponds with recent evidence that herbivore preference can alter plant community composition for instance by giving well-defended, unpalatable plant species a chance to catch up with their less-defended, faster-growing neighbours (Kempel et al. 2015). These herbivory-driven plant community shifts can in turn be linked to species-specific herbivore traits, such as bite strength (Deraison et al. 2015). A next step should therefore be to assess how diversity in the above- and belowground herbivore communities might change the impact of herbivory.

It was not possible in our field experiment to separate roots of the different plant species. The role of belowground herbivory is therefore impossible to assess in comparable detail to that of aboveground herbivory. However, a possible explanation for the strong competitive advantage that forbs received when both above- and belowground herbivores were present at elevated N (*Fig. 6b*, right-hand panel) is that their advantage above ground was amplified by decreased competition from grasses below ground.

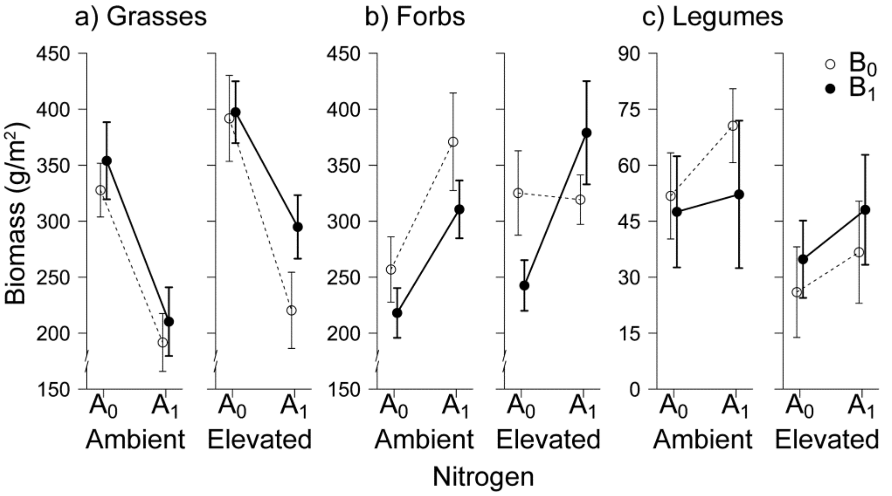

Figure 6. Aboveground biomass (expressed as grams of dry weight per m^2) of the different plant groups at the September 2015 harvest. The graphs show treatment averages ± s.e for enclosures without (A_0) and with (A_1) aboveground herbivory, and without (B_0) and with (B_1) belowground herbivory.

5.1.3 Plant community evenness

Calculating plant community evenness is useful for detecting if an environmental driver is conferring a competitive advantage to a certain species or group of species. Grasses generally benefit considerably from N addition, and an increased dominance of grasses is often an underlying cause of N-induced decreases in plant community evenness (Bobbink et al. 2010).

At ambient N, evenness among abundances of the three plant groups was higher when aboveground herbivores were present (*Fig. 7*). This corresponds well with the finding that herbivory consistently increases plant community evenness across ecosystems (Hillebrand et al. 2007). At elevated N, the positive effect of herbivory was weaker. The hypothetical scenario depicted in *Fig. 2*, in which aboveground herbivores counteract the N-induced plant species evenness reduction, does therefore not appear to be true in our system. Possibly, the insects were unable to relax the increased interspecific competition for light that N eutrophication causes (Hautier et al. 2009). This would mark a contrast to vertebrate herbivory, which may help to maintain plant community evenness under N eutrophication, specifically by alleviating light competition (Borer et al. 2014).

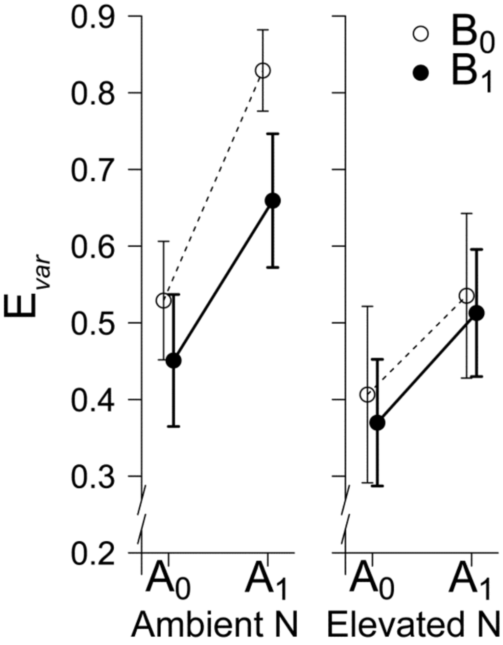

Figure 7. Plant community evenness among plant groups, as measured by the E_{var} index, at the September harvest in 2015. The graph shows treatment averages ± s.e for enclosures without (A_0) and with (A_1) aboveground herbivory, and without (B_0) and with (B_1) belowground herbivory.

5.1.4 Effects on interspecific plant competition

In **Paper II**, I investigated the potential of above- and belowground herbivores to act as mediators of the increased competitive asymmetry that N eutrophication causes. As outlined in the introduction, if herbivores increase competitive asymmetry, they might exacerbate N-induced competitive asymmetry and accelerate species loss. If herbivores instead alleviate competitive asymmetry, they might decelerate species loss.

I found that aboveground herbivory strongly influenced the competitive symmetry between two functionally similar plant species, the grasses *F. rubra* and *D. glomerata*. Specifically, it caused an increase in the aggressivity index, which was in turn driven by an increase in the competitive ability (measured as RY) of *D. glomerata* and a reduction in that of *F. rubra* (see **Paper II** for figures). The aboveground herbivores showed a marked preference for *F. rubra*, so these results are in line with both theory (Kim et al. 2013) and empirical evidence (Bentley and Whittaker 1979) of herbivore preference driving an increase in competitive asymmetry.

Belowground herbivory increased aggressivity as well, but the combined effect of the two herbivory types was smaller than their individual effects would predict. In other words, the herbivore-induced increase in competitive asymmetry was alleviated when both herbivory types were present. A possible explanation for this is that above- and belowground herbivores had different feeding preferences. It was not possible to monitor the feeding preference of the belowground herbivores, but wireworms sometimes avoid feeding on *F. rubra* if other alternatives are available (Hemerik et al. 2003, Roubíčková et al. 2012), so in our experiment they might have preferred *D. glomerata*. Such a contrast in feeding preference of the above- and belowground herbivores could explain why they counteracted each other's effects.

N addition did not alter the effect of herbivory, nor did it have any main effect on competition. This was somewhat surprising, considering that N increase is known to cause shifts in both competitive and trophic relationships (Tylianakis et al. 2008, Hoover et al. 2012). However, it is possible that interactions between effects of N and herbivory occur mainly at higher rates of N eutrophication, such as those I applied in **Paper I**, **III** and **IV**. Alternatively, N might have had an influence on belowground growth and competition, and the experiment was too short in duration to capture feedbacks of belowground effects on aboveground processes.

5.2 Effects on ecosystem functioning

5.2.1 Decomposition and N mineralization

Nutrient turnover displayed inverted responses to above- and belowground herbivory at contrasting N levels (**Paper III**). The responses of red tea decomposition and N mineralization were strikingly similar (*Fig. 8*), which indicates that the patterns I observed were, in fact, due to consistent effects on processes related to nutrient turnover. These results will therefore be discussed as overall effects on nutrient turnover.

At ambient N, nutrient turnover was higher when belowground herbivores were present, but only in the absence of aboveground herbivores. At elevated N, nutrient turnover only increased when both above- and belowground herbivores were present (*Fig. 8*). This result plainly illustrates how complex the context-dependency of herbivory effects can be. Schmitz (2008) famously showed that higher trophic levels can modify the impact of herbivory on nutrient cycling. I demonstrate here that belowground herbivores can do the same.

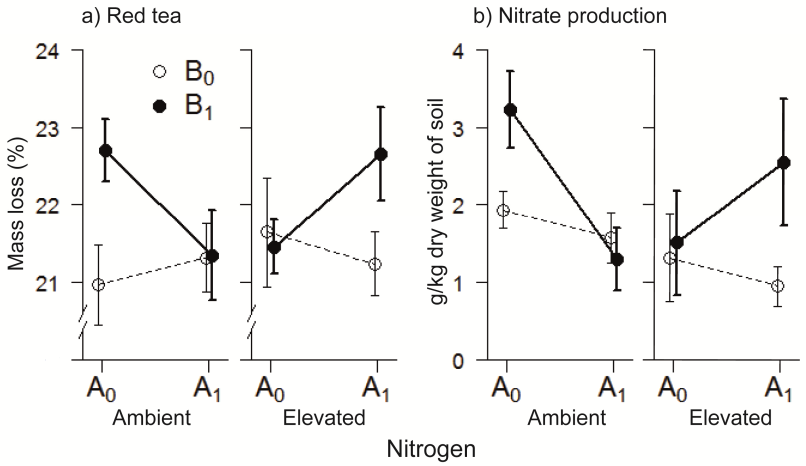

Figure 8. Effects of aboveground (A) and belowground (B) herbivores on nutrient turnover at ambient and elevated N. The graphs show treatment averages ± s.e for enclosures without (A_0) and with (A_1) aboveground herbivory, and without (B_0) and with (B_1) belowground herbivory.

Importantly, our results highlight that biotic components of ecosystems can play a central role in determining the ecosystem-level impact of global change. N eutrophication has previously been demonstrated to accelerate nitrification rates (Matson et al. 2002) and decomposition processes in the soil (Neff et al. 2002). In our experiment, it was the presence of herbivory, and more precisely the combination of above- and belowground herbivory, that appeared to accelerate nutrient turnover at elevated N.

A critical aspect of above- belowground linkages is the potential for feedbacks between the two subsystems. For instance, if herbivory accelerates N mineralization rates, plant productivity might be boosted to a degree that matches or exceeds the amount of biomass lost to herbivory (Bardgett and Wardle 2003). That ANPP increased when above- and belowground herbivory were combined at elevated N in our experiment may support this (compare *Figs. 5* and *8*). Our results suggest that interacting effects of above- and belowground herbivory can generate feedback mechanisms on grassland nutrient cycling.

5.2.2 Fungal vs. bacterial decomposition pathways

We can infer treatment effects on decomposition pathways by comparing the relative abundances of bacterial- and fungal-feeding nematodes (**Paper IV**). The relative abundance of fungal-feeders increased when aboveground herbivores were present, but only at elevated N. This indicates that aboveground herbivory and N in combination caused the decomposition pathway to shift towards being more fungal-based. This shift was concurrent with an increased forb biomass in the plant community. Our results therefore corroborate the finding that high forb abundance promotes the fungal-based energy channel in the soil (Wardle et al. 2003). Decomposition processes dominated by fungi are suggested to lead to increased soil carbon storage (Six et al. 2006), with possible implications for carbon cycling in grasslands.

5.3 The soil nematode community

The abundances of the different nematode feeding groups shifted in the presence of above- and belowground herbivores, and these shifts were in turn dependent on the N level (**Paper IV**). Notably, the response of root-feeders to N addition was strongly dependent on the two herbivory treatments such that they increased under both above- and belowground herbivory, but only when each herbivory type was applied separately. When they were applied together, they appeared to cancel one another's impact (*Fig. 9*). Most likely, this result is linked to species abundance shifts in the plant community (Veen et al. 2010, Vandegehuchte et al. 2017). Treatment combinations that produced low root-

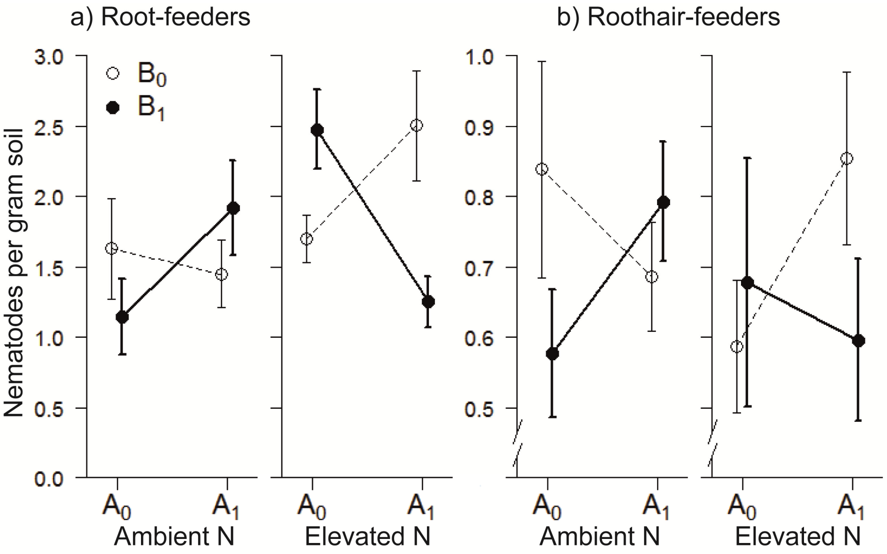

Figure 9. Abundances of the two groups of root-feeding nematodes in the mesocosm experiment. N = nitrogen, A = aboveground herbivory, B = belowground herbivory. Plots display treatment averages ± s.e.

feeder abundances were those that yielded high biomass of *Plantago lanceolata* and *Achillea millefolium*: two plant species that have been shown to correlate negatively with root-feeding nematodes (Wardle et al. 2003, De Deyn et al. 2004, Viketoft et al. 2005). In terms of possible facilitative versus inhibitory interactions among insect and nematode herbivores, our results suggest that N eutrophication causes both above- and belowground insect herbivores to facilitate root-feeding nematodes, which could lead to an increased total grazing pressure. However, this facilitation appears to happen only when one herbivory type is present; when both types are present, they appear to counteract one another's effect.

The abundance of omnivorous nematodes increased by 51% when belowground herbivores were present. This pattern did not correlate with any obvious response in the plant community (**Paper I**). Belowground herbivory might instead have stimulated the abundance of omnivorous nematodes by increasing root exudations, which in turn stimulated bacterial growth (Denton et al. 1998). The diet of omnivorous nematodes includes bacteria and bacterial-feeding amoeba and flagellates, so an increased bacterial abundance could increase the food supply for omnivores (Freckman and Caswell 1985, Hunt et al. 1987). The fact that there was no corresponding effect on bacterial-feeders does not necessarily contradict this possibility, as the bacterial-feeder abundance might have been kept in check by competition and predation from omnivores. The result was not dependent on the N level. However, such a stimulating effect on the soil food web might have implications for the simplifying effect that N eutrophication is predicted to have on the soil food web (Eisenhauer et al. 2012).

6 Conclusion

My results show that insect herbivory can be instrumental in determining the impact of N eutrophication on grassland ecosystems, both in terms of plant community composition and ecosystem functioning. Moreover, they show that a robust understanding of this trophic control requires that the often neglected belowground consumers are studied alongside their aboveground counterparts. Including both herbivory types, rather than just one, can evidently invert the response of the ecosystem to N eutrophication. Considering only one subsystem might lead to erroneous conclusions being drawn regarding the impact of global change drivers. The results presented here can guide future research endeavours concerning the role of above-belowground feedback mechanisms as drivers of ecosystem dynamics in grasslands, and inform about how this role might change in an ecosystem subjected to N eutrophication.

7 Future challenges

7.1 From outcomes to mechanisms

The results presented here provide information about the outcome of applying certain N and herbivory conditions on the ecosystem. Documenting outcomes is a necessary first step to guide future experimental initiatives. A next step is to understand the mechanisms that lead to these outcomes. This requires experiments that target the complex interplay of plant and microbial ecology, with the aim of understanding how N and herbivory effects on the plant community are mediated by – or themselves mediate – microbial processes. The microbial community in the soil is the hub that drives and regulates ecosystem functioning (Hallin et al. 2009), but our understanding of the role it plays in mediating N and herbivory effects is limited at best. In recent years there has been great progress in the development of molecular tools that can help us identify and understand microbial communities better, something that will hopefully also help us understand the roles they play in biogeochemical cycling (Schlesinger 2006).

7.2 The plant community below ground

A central challenge in above- belowground ecology is how to examine the above- and belowground compartments of the plant community on equal terms. The major reasons for why belowground communities have been insufficiently studied for so long are methodological. While we can fairly straightforwardly follow changes in plant species abundances above ground, it is nearly impossible to acquire the same level of detail for relative abundances belowground. Belowground plant community responses have consequently most often been limited to measurements of total root biomass. DNA-based approaches could prove to be a powerful tool for advancing our understanding of plant coexistence below ground (Jones et al. 2011), as they allow mapping of belowground species richness (e.g. Hiiesalu et al. 2012), and might also be able to provide approximations of species abundances (Mommer et al. 2010).

7.3 Concurrent global change drivers

Global change drivers rarely act in isolation. Determining how multiple, concurrent drivers amplify or mitigate one another's impact is arguably the greatest challenge in global change research (Tylianakis et al. 2008). The biodiversity loss caused by N eutrophication is suggested to be exacerbated by climate change (i.e. increased temperature and changed precipitation patterns) but few empirical assessments exist of this (Greaver et al. 2016). Throop and Lerdau (2004) highlight CO_2 increase as another important global change driver to study alongside N enrichment effects on herbivory. This is attributed to the fact that both N and CO_2 can have strong, possibly counteracting effects on the plant as a food source for herbivores. N addition often increases the N content of foliar tissues, with implications for feeding rates and herbivore population development (Throop and Lerdau 2004), while CO_2 generally reduces the nutritional quality of plant tissues (Bezemer and Jones 1998).

Questions about how interplaying global change drivers will combine with trophic interactions to shape ecosystems are compelling avenues of future research. The results from my thesis can be useful for building hypotheses about under what conditions, and to what degree, N eutrophication will interact with other global change drivers to alter grassland ecosystems.

References

Bardgett, R. D., and D. A. Wardle. 2003. Herbivore-mediated linkages between aboveground and belowground communities. Ecology 84:2258–2268.

Bardgett, R. D., and D. A. Wardle. 2010. Ecosystem-level significance of aboveground consumers. Page Aboveground-Belowground Linkages: Biotic Interactions, Ecosystem Processes and Global Change. Oxford University Press, New York.

Belovsky, G. E., and J. B. Slade. 2000. Insect herbivory accelerates nutrient cycling and increases plant production. Proceedings of the National Academy of Sciences of the United States of America 97:14412–7.

Bentley, S., and J. B. Whittaker. 1979. Effects of grazing by a chrysomelid beetle, Gastrophysa viridula, on competition between Rumex obtusifolius and Rumex crispus. Journal of Ecology 67:79–90.

Bezemer, T. M., and T. H. Jones. 1998. Plant-insect herbivore interactions in elevated atmospheric CO2: quantitative analyses and guild effects. Oikos 82:212–222.

Bobbink, R., K. Hicks, J. Galloway, T. Spranger, R. Alkemade, M. Ashmore, S. Bustamante, S. Cinderby, E. Davidson, F. Dentener, B. Emmett, J. Erisman, M. Fenn, A. Gilliam, A. Nordin, L. Pardo, and W. De Vries. 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity : a synthesis. Ecological Applications 20:30–59.

Bongers, T., and M. Bongers. 1998. Functional diversity of nematodes. Applied Soil Ecology 10:239–251.

Borer, E. T., E. W. Seabloom, D. S. Gruner, W. S. Harpole, H. Hillebrand, E. M. Lind, P. B. Adler, J. Alberti, T. M. Anderson, J. D. Bakker, L. Biederman, D. Blumenthal, C. S. Brown, L. a Brudvig, Y. M. Buckley, M. Cadotte, C. Chu, E. E. Cleland, M. J. Crawley, P. Daleo, E. I. Damschen, K. F. Davies, N. M. DeCrappeo, G. Du, J. Firn, Y. Hautier, R. W. Heckman, A. Hector, J. HilleRisLambers, O. Iribarne, J. a Klein, J. M. H. Knops, K. J. La Pierre, A. D. B. Leakey, W. Li, A. S. MacDougall, R. L. McCulley, B. a Melbourne, C. E. Mitchell, J. L. Moore, B. Mortensen, L. R. O'Halloran, J. L. Orrock, J. Pascual, S. M. Prober, D. a Pyke, A. C. Risch, M. Schuetz, M. D. Smith, C. J. Stevens, L. L. Sullivan, R. J. Williams, P. D. Wragg, J. P. Wright, and L. H. Yang. 2014. Herbivores and nutrients control grassland plant diversity via light limitation. Nature 508:517–20.

Brown, V. K., and A. C. Gange. 1989. Differential effects of above- and below-ground insect herbivory during early plant succession. Oikos 54:67–76.

Cahill, J. F. 1999. Fertilization effects on interactions between above- and belowground competition in an old field. Ecology 80:466–480.

Carson, W. P., and R. B. Root. 2000. Herbivory and plant species coexistence: community regulation by an outbreaking phytophagous insect. Ecological Monographs 70:73–99.

Cleland, E. E., and W. S. Harpole. 2010. Nitrogen enrichment and plant communities. Annals of the New York Academy of Sciences 1195:46–61.

Coupe, M. D., and J. F. Cahill. 2003. Effects of insects on primary production in temperate herbaceous communities: a meta-analysis. Ecological Entomology 28:511–521.

Cousens, R. 1996. Design and interpretation of interference studies: Are some methods totally unacceptable? New Zealand Journal of Forestry Science 26:5–18.

Crawley, M. J. 1989. Insect Herbivores and Plant Population Dynamics. Annual Review of Entomology 34:531–562.

DeMalach, N., E. Zaady, and R. Kadmon. 2017. Light asymmetry explains the effect of nutrient enrichment on grassland diversity. Ecology Letters 20:60–69.

Denton, C. S., R. D. Bardgett, R. Cook, and P. J. Hobbs. 1998. Low amounts of root herbivory positively influence the rhizosphere microbial community in a temperate grassland soil. Soil Biology and Biochemistry 31:155–165.

Deraison, H., I. Badenhausser, L. Börger, and N. Gross. 2015. Herbivore effect traits and their impact on plant community biomass: an experimental test using grasshoppers. Functional Ecology 29:650–661.

De Deyn, G. B., C. E. Raaijmakers, J. van Ruijven, F. Berendse, and W. H. van der Putten. 2004. Plant species identity and diversity effects on different trophic levels of nematodes in the soil food web. Oikos 106:576–586.

De Deyn, G., J. van Ruijven, C. Raaijmakers, P. de Ruiter, and W. van der Putten. 2007. Above-and belowground insect herbivores differentially affect soil nematode communities in species-rich plant communities. Oikos 116:923–930.

Eisenhauer, N., S. Cesarz, R. Koller, K. Worm, and P. B. Reich. 2012. Global change belowground: impacts of elevated CO2, nitrogen, and summer drought on soil food webs and biodiversity. Global Change Biology 18:435–447.

Eno, C. 1960. Nitrate production in the field by incubating the soil in polyethylene bags. Soil Science Society of America Journal 24:277–279.

Freckman, D. W., and E. P. Caswell. 1985. The ecology of nematodes in agroecosystems. Annual Review of Phytopathology 23:275–296.

Ghedini, G., B. D. Russell, and S. D. Connell. 2015. Trophic compensation reinforces resistance : herbivory absorbs the increasing effects of multiple disturbances. Ecology Letters 18:182–187.

Greaver, T. L., C. M. Clark, J. E. Compton, D. Vallano, A. F. Talhelm, C. P. Weaver, L. E. Band, J. S. Baron, E. A. Davidson, C. L. Tague, E. Felker-Quinn, J. A. Lynch, J. D. Herrick, L. Liu, C. L. Goodale, K. J. Novak, and R. A. Haeuber. 2016. Key ecological responses to nitrogen are altered by climate change. Nature Climate Change 6:836–843.

Gruner, D. S., J. E. Smith, E. W. Seabloom, S. A. Sandin, J. T. Ngai, H. Hillebrand, W. S. Harpole, J. J. Elser, E. E. Cleland, M. E. S. Bracken, E. T. Borer, and B. M. Bolker. 2008. A cross-system synthesis of consumer and nutrient resource control on producer biomass. Ecology letters 11:740–55.

Haddad, N. M., J. Hoarstad, and D. Tilman. 2000. The effects of long-term nitrogen loading on grassland insect communities. Oecologia 124:73–84.

Hallin, S., C. M. Jones, M. Schloter, and L. Philippot. 2009. Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. The ISME journal 3:597–605.

Hautier, Y., P. A. Niklaus, and A. Hector. 2009. Competition for light causes plant biodiversity loss after eutrophication. Science 324:636–638.

He, M., J. Ding, and X. Lu. 2014. Increased compensatory ability of an invasive plant to above- and below-ground enemies in monocultures. Plant Ecology 215:253–260.

Hemerik, L., G. Gort, and L. Brussaard. 2003. Food preference of wireworms analyzed with multinomial logit models. Journal of Insect Behavior 16:647–665.

Hiiesalu, I., M. Opik, M. Metsis, L. Lilje, J. Davison, M. Vasar, M. Moora, M. Zobel, S. D. Wilson, and M. Pärtel. 2012. Plant species richness belowground: higher richness and new patterns revealed by next-generation sequencing. Molecular ecology 21:2004–16.

Hillebrand, H., D. S. Gruner, E. T. Borer, M. E. S. Bracken, E. E. Cleland, J. J. Elser, W. S. Harpole, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Consumer versus resource control of producer diversity depends on ecosystem type and producer community structure. Proceedings of the National Academy of Sciences of the United States of America 104:10904–9.

Hoover, S. E. R., J. J. Ladley, A. A. Shchepetkina, M. Tisch, S. P. Gieseg, and J. M. Tylianakis. 2012. Warming, CO2, and nitrogen deposition interactively affect a plant-pollinator mutualism. Ecology letters 15:227–34.

Hunt, H. W., D. C. Coleman, E. R. Ingham, E. T. Elliott, J. C. Moore, S. L. Rose, C. P. P. Reid, and C. R. Morley. 1987. The detrital foodweb in a shortgrass prairie. Biology and Fertility of Soils 3:57–68.

Hunter, M. D. 2001. Insect population dynamics meets ecosystem ecology: effects of herbivory on soil nutrient dynamics. Agricultural and Forest Entomology 3:77–84.

Huntly, N. 1991. Herbivores and the dynamics of communities and ecosystems. Annual Review of Ecology and Systematics 22:477–503.

Ingham, R. E., and J. K. Detling. 1990. Effects of root-feeding nematodes on aboveground net primary production in a North American grassland. Plant and Soil 121:279–281.

Isbell, F., P. B. Reich, D. Tilman, S. E. Hobbie, S. Polasky, and S. Binder. 2013. Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity. Proceedings of the National Academy of Sciences of the United States of America 110:11911–11916.

Jones, F. A., D. L. Erickson, M. A. Bernal, E. Bermingham, W. J. Kress, E. A. Herre, H. C. Muller-Landau, and B. L. Turner. 2011. The roots of diversity: Below ground species richness and rooting distributions in a tropical forest revealed by DNA barcodes and inverse modeling. PLoS ONE 6:e24506.

Keddy, P. A., and B. Shipley. 1989. Competitive hierarchies in herbaceous plant communities. Oikos 54:234–241.

Kempel, A., M. Razanajatovo, C. Stein, S. B. Unsicker, H. Auge, W. W. Weisser, M. Fischer, and D. Prati. 2015. Herbivore preference drives plant community composition. Ecology 96:2923–2934.

Keuskamp, J. A., B. J. J. Dingemans, T. Lehtinen, J. M. Sarneel, and M. M. Hefting. 2013. Tea Bag Index: A novel approach to collect uniform decomposition data across ecosystems. Methods in Ecology and Evolution 4:1070–1075.

Kim, T., N. Underwood, and B. Inouye. 2013. Insect herbivores change the outcome of plant competition through both inter-and intraspecific processes. Ecology 94:1753–1763.

Knorr, M., S. D. Frey, and P. S. Curtis. 2005. Nitrogen additions and litter decomposition: A meta analysys. Ecology 86:3252–3257.

Lu, X., E. Siemann, H. Wei, X. Shao, and J. Ding. 2015. Effects of warming and nitrogen on above- and below-ground herbivory of an exotic invasive plant and its native congener. Biological Invasions 17:2881–2892.

Maron, J. L. 1998. Insect herbivory above and below ground: individual and joint effects on plant fitness. Ecology 79:1281–1293.

Masters, G. J. 2004. Belowground herbivores and ecosystem processes. Pages 93–109*in* W. W. Weisser and E. Siemann, editors.Insects and ecosystem function. Springer-Verlag Berlin Heidelberg, Heidelberg.

Masters, G. J., V. K. Brown, and A. C. Gange. 1993. Plant mediated interactions between above- and below-ground insect herbivores. Oikos 66:148–151.

Matson, P., K. A. Lohse, and S. J. Hall. 2002. The globalization of nitrogen deposition: consequences for terrestrial ecosystems. Ambio 31:113–119.

McGilchrist, C. A., and B. R. Trenbath. 1971. A revised analysis of plant competition experiments. Biometrics 27:659–671.

Mommer, L., J. van Ruijven, H. de Caluwe, A. E. Smit-Tiekstra, C. A. M. Wagemaker, N. Joop Ouborg, G. M. Bögemann, G. M. van der Weerden, F. Berendse, and H. de Kroon. 2010. Unveiling below-ground species abundance in a biodiversity experiment: A test of vertical niche differentiation among grassland species. Journal of Ecology 98:1117–1127.

Mullerscharer, H., and V. K. Brown. 1995. Direct and indirect effects of above- and belowground insect herbivory on plant-density and performance of Tripleurospermum perforatum during early plant succession. Oikos 72:36–41.

Neff, J. C., A. R. Townsend, G. Gleixner, S. J. Lehman, J. Turnbull, and W. D. Bowman. 2002. Variable effects of nitrogen additions on the stability and turnover of soil carbon. Nature 419:915–917.

Neher, D. 2001. Role of nematodes in soil health and their use as indicators. Journal of Nematology 33:161–168.

Picaud, F., E. Bonnet, V. Gloaguen, and D. Petit. 2003. Decision making for food choice by grasshoppers (Orthoptera: Acrididae): comparison between a specialist species on a shrubby legume and three graminivorous species. Environmental Entomology 32:680–688.

La Pierre, K. J., A. Joern, and M. D. Smith. 2015. Invertebrate, not small vertebrate, herbivory interacts with nutrient availability to impact tallgrass prairie community composition and forb biomass. Oikos 124:842–850.

Van der Putten, W. H., R. D. Bardgett, P. C. De Ruiter, W. H. G. Hol, K. M. Meyer, T. M. Bezemer, M. A. Bradford, S. Christensen, M. B. Eppinga, T. Fukami, L. Hemerik, J. Molofsky, M. Schädler, C. Scherber, S. Y. Strauss, M. Vos, and D. A. Wardle. 2009. Empirical and theoretical challenges in aboveground-belowground ecology. Oecologia 161:1–14.

van der Putten, W. H., P. C. De Ruiter, T. M. Bezemer, J. A. Harvey, M. Wassen, and V. Wolters. 2004. Trophic interactions in a changing world. Basic and Applied Ecology 5:487–494.

Roubíčková, A., O. Mudrák, and J. Frouz. 2012. The effect of belowground herbivory by wireworms (Coleoptera: Elateridae) on performance of Calamagrostis epigejos (L) Roth in post-mining sites. European Journal of Soil Biology 50:51–55.

van Ruijven, J., G. B. De Deyn, C. E. Raaijmakers, F. Berendse, and W. H. Van der Putten. 2005. Interactions between spatially separated herbivores indirectly alter plant diversity. Ecology Letters 8:30–37.

Sala, E. O., F. S. I. Chapin, J. J. Amnesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. Huenneke, R. Jackson, A. Kinzig, R. Leemans, D. Lodge, H. Mooney, M. Oesterheld, N. Poff,

M. Sykes, B. Walker, M. Walker, and D. Wall. 2000. Global biodiversity scenarios for the year 2100. Science 287:1770–1774.

Schlesinger, W. H. 2006. Global change ecology. Trends in Ecology and Evolution 21:348–351.

Schmitz, O. J. 2008. Effects of predator hunting mode on grassland ecosystem function. Science 319:952–4.

Schoonhoven, L., J. van Loon, and M. Dicke. 2005. Insect-Plant Biology. 2nd editio. Oxford University Press, Oxford.

Schädler, M., J. Alphei, S. Scheu, R. Brandl, and H. Auge. 2004. Resource dynamics in an early-successional plant community are influenced by insect exclusion. Soil Biology and Biochemistry 36:1817–1826.

Six, J., S. D. Frey, R. K. Thiet, and K. M. Batten. 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70:555.

Sonnemann, I., H. Baumhaker, and S. Wurst. 2012. Species specific responses of common grassland plants to a generalist root herbivore (Agriotes spp. larvae). Basic and Applied Ecology 13:579–586.

Sotomayor, D. A., and C. J. Lortie. 2015. Indirect interactions in terrestrial plant communities: emerging patterns and research gaps. Ecosphere 6:art103.

Stein, C., S. B. Unsicker, A. Kahmen, M. Wagner, V. Audorff, H. Auge, D. Prati, and W. W. Weisser. 2010. Impact of invertebrate herbivory in grasslands depends on plant species diversity. Ecology 91:1639–50.

Stevens, C. J., N. B. Dise, J. O. Mountford, and D. J. Gowing. 2004. Impact of nitrogen deposition on the species richness of grasslands. Science 303:1876–1879.

Stevens, C. J., C. Dupre, E. Dorland, C. Gaudnik, D. J. G. Gowing, A. Bleeker, M. Diekmann, D. Alard, R. Bobbink, D. Fowler, E. Corcket, J. O. Mountford, V. Vandvik, P. A. Aarrestad, S. Muller, and N. B. Dise. 2010. Nitrogen deposition threatens species richness of grasslands across Europe. Environmental Pollution 158:2940–2945.

Suding, K. N., S. L. Collins, L. Gough, C. Clark, E. E. Cleland, K. L. Gross, D. G. Milchunas, and S. Pennings. 2005. Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. Proceedings of the National Academy of Sciences of the United States of America 102:4387–92.

Throop, H. L., and M. T. Lerdau. 2004. Effects of nitrogen deposition on insect herbivory: implications for community and ecosystem processes. Ecosystems 7:109–133.

Tylianakis, J. M., R. K. Didham, J. Bascompte, and D. A. Wardle. 2008. Global change and species interactions in terrestrial ecosystems. Ecology Letters 11:1351–1363.

Vallano, D. M., P. C. Selmants, and E. S. Zavaleta. 2012. Simulated nitrogen deposition enhances the performance of an exotic grass relative to native serpentine grassland competitors. Plant Ecology 213:1015–1026.

Van, T. K., G. S. Wheeler, and T. D. Center. 1998. Competitive interactions between hydrilla (Hydrilla verticillata) and vallisneria (Vallisneria americana) as influenced by insect herbivory. Biological Control 11:185–192.

Vandegehuchte, M. L., W. H. Van Der Putten, H. Duyts, M. Sch, and A. C. Risch. 2017. Aboveground mammal and invertebrate exclusions cause consistent changes in soil food webs of two subalpine grassland types , but mechanisms are system-specific. Oikos 126:212–223.

Wardle, D. A., G. W. Yeates, W. Williamson, and K. I. Bonner. 2003. The response of a three trophic level soil food web to the identity and diversity of plant species and functional groups. Oikos

102:45–56.

Veen, G. F., H. Olff, H. Duyts, and W. H. Van Der Putten. 2010. Vertebrate herbivores influence soil nematodes by modifying plant communities. Ecology 91:828–835.

Weisser, W. W., and E. Siemann. 2004. Insects and ecosystem function. First edition. Springer-Verlag Berlin Heidelberg, Heidelberg.

Viketoft, M., C. Palmborg, B. Sohlenius, K. Huss-Danell, and J. Bengtsson. 2005. Plant species effects on soil nematode communities in experimental grasslands. Applied Soil Ecology 30:90–103.

De Wit, C. T. 1960. On competition. Verslagen Landbouwkundig Onderzoek 66:1–82.

Vitousek, P. M. 1994. Beyond global warming: ecology and global change. Ecology 75:1861–1876.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications 7:737–750.

Vitousek, P. M., S. Hättenschwiler, L. Olander, and S. Allison. 2002. Nitrogen and nature. Ambio 31:97–101.

Worm, B., H. Lotze, H. Hillebrand, and U. Sommer. 2002. Consumer versus resource control of species diversity and ecosystem functioning. Nature 417:848–851.

de Vries, F. T., E. Thébault, M. Liiri, K. Birkhofer, M. a Tsiafouli, L. Bjørnlund, H. Bracht Jørgensen, M. V. Brady, S. Christensen, P. C. de Ruiter, T. d'Hertefeldt, J. Frouz, K. Hedlund, L. Hemerik, W. H. G. Hol, S. Hotes, S. R. Mortimer, H. Setälä, S. P. Sgardelis, K. Uteseny, W. H. van der Putten, V. Wolters, and R. D. Bardgett. 2013. Soil food web properties explain ecosystem services across European land use systems. Proceedings of the National Academy of Sciences of the United States of America 110:14296–301.

Yeates, G. W. 2003. Nematodes as soil indicators: functional and biodiversity aspects. Biology and Fertility of Soils 37:199–210.

Zavaleta, E. S., M. R. Shaw, N. R. Chiariello, B. D. Thomas, E. E. Cleland, C. B. Field, and H. A. Mooney. 2003. Grassland responses to three years of elevated temperature, CO2 , precipitation, and N deposition. Ecological Monographs 73:585–604.

8 Acknowledgements

Brace yourselves, this might take a while…

Riccardo: thank you so much for giving me the opportunity to go on this fun, exciting, strange journey! It's not hyperbolic to say that I can't really imagine having a better PhD experience. Thank you for always encouraging me to make the most of everything that's available, for building an inspiring working environment where there are always lots of people to discuss with and bounce ideas off of, and for being patient with my sometimes sceptical attitude towards my own results ("come on, this is *so cool!*"). I've had such a great time working with you!

Joachim, thank you for your very human approach to PhD supervision. The fact that you always make sure to ask how I'm holding up has been invaluable, from those early days of experimental design confusion ("did you remember to schedule some holidays?") up until the very end of frenetic thesis-writing. Your feedback is always excellent: friendly, constructive, and unfailingly useful. **Maria**, thanks for all your encouragement, especially for helping to motivate me towards the finish line. It was really fun working on the nematode paper with you and getting to know those fascinating little creatures!

Grazie mille a **Lorenzo** for being available for crisis management over Skype in those early days of experimental mayhem, for statistical emergency services, and for excellent co-authoring.

There are two people without whom there would not have been very much for me to write a thesis about. **Ged** and **Carol**, I know this is the sort of statement I'm supposed to save for the 'family' section but… I really don't think I could have done this without you!!! Thank you so much for all your ingenious solutions to bizarre problems, for your patience when I would come to you with the bizarre problems in question, and for your companionship in the field (the Krusenberg transportable fika station was an especial highlight). Now there's just that dreaded final task left… *disassembly.*

To all the past and present members of the ecosystem services lab group: I've been super lucky to have you all to lean on and discuss with along the way, it made these four years so much more enjoyable (and less stressful than they could have been!). Special thanks to **Alva** for the support and overall excellent role-modelling (maybe you didn't know that you're a role model… but now you do!); **Riikka**, role model #2, you're one of the best feedback-deliverers I've ever met, always constructive, kind, and making sure to highlight the good alongside the bad; **Sandra**, for being a great employer-turned-colleague, the way you approach science (enthusiastically, humbly, with empathy for the organisms you study and the people you study them with), makes you role model #3. Thanks also to the Next Generation of PhD students: **Guille**, **Chloë** and **Kate**, I'm really glad my time overlapped a bit with yours! And thanks to **Giovanni** for being one of a select few to understand the trauma of the mesocosms (although your experiences were a lot harsher than mine…!).

To everyone working in the Ecology department and VPE: I've never had a workplace I've liked as much as this one before, and that's largely thanks to all the lovely people that roam the corridors, take care of the building, figure out administrative issues, come out for fika breaks and chat about everything and nothing. Thanks to all of you for building this great working environment.

Nora Underwood and **Brian Inouye** welcomed me into their lovely lab group at Florida State University for a month of inspiring discussions. Thank you for giving me new perspectives on my work, for helping me through the hurricane (literally and figuratively), and for showing me alligators (only literally).

Big, heartfelt, emotional thanks to all my lovely Uppsala friends, without whom these four-and-something years would have been (approximately) 20% as fun and 300% as difficult. **Åsa**, jag har haft sån tur att jag haft dig att dela allt det här med! Can we find a way to be kontorskompisar forever?? **Preeti**, thanks for being a great friend, and for always agreeing that science is more fun if you can mix in some pop culture chats... I will miss our lunchtime movie talks, but let's continue them in India and Skåne! **Laura**, you magical human, thank you for all the kindness and for making me laugh when I need it the most. **Francesca**, grazie mille for being the heart of my adopted Italian family, and for travelling this confusing (but exciting!?) stretch of life with me; ci vediamo a Roma!! **Anna**, grazie for always understanding me, and for the Life Talks, and the kindness, and caring-ness (okay that's probably not a real word). **David**, your Scottish humour has kept me afloat, as has your effortless switching from super silly to super serious and back again, depending on the emotional requirements of the moment. **Jonas**, tack för alla fikapauser och lunchpauser och promenader där samtalet var kanske bara 10% jobbrelaterat, och för att du ödmjukt ställt upp som obetald musikproduktionsguru.

For aesthetic purposes, here comes a new paragraph… **Eve**, merci bien pour ton amitié, you have been an amazing support, from that first confusing week all the way through the just as confusing finish. **Davide**, thank you for all the fun times (in general) and the Tokyo fun times (in particular). Same to **Fama**, let's just go back to Asakusa shall we? **Jon**, we've collaborated and course-worked and puzzled at life over three different continents, that's a pretty good number of continents! Thanks for being a great friend and listener. **Kerstin**, tack för alla lunch- och fikapauser och för att jag fått prata av mig om stora och små saker i livet (innanför och utanför jobbet). **Audrey**, merci bien for the support and the friendship, I wish we could have worked in the same place the whole time! **Laia**, thank you for always being enthusiastic about everything that other temporary Swedes tend to be annoyed by (I think you're the only one I've heard be excited about November in Uppsala), I miss you a lot, not only for that reason but because: ets una persona fantàstica!

Till mina fina, långtida vänner hemma och borta: **Malin**, tack för att du bjudit på lite skånskt-norrländskt miljöombyte vid behov, nu vill jag komma upp oftare och hänga med er! **Ylva**, det är svårt att sätta ord på nästan ett kvarts sekels vänskap. Tack för att du alltid funnits där! Vad roligt att vi får dela på Uppsala ett tag. **Kasia**, du är en av de starkaste, coolaste och modigaste personerna jag vet. Dziękuję Ci za Twoją przyjaźń! **Torill**, du är och förblir min stora idol. Tack för ditt stöd när jag var ny och vilsen i Uppsala, och för din vänskap under så många år.

Till min älskade **Mormor**, tack för att du alltid har delat min uppspelthet över blommorna och fåglarna och insekterna och allt knasigt jag jobbar med. Snart är det vår och vi kan ut och titta på naturen tillsammans igen! Och tack till **Farmor** som förundrats över att det går att få betalt för att göra något så trevligt och konstigt som att räkna gräshoppor. När jobbet känts tungt så har det hjälpt mig att komma ihåg hur gärna ni båda nog hade jobbat med något liknande.

Och slutligen, tack till min älskade familj – ord räcker inte till. **Filippa** och **Matilda**, jag har aldrig så roligt som när vi är tillsammans; jag hoppas vi alla kan bo lite närmre varann snart. Världen är som mest spännande och fin när jag får uppleva den med er. **Mamma** och **Pappa**, tack för att ni lärde oss just det: att vi bor i en stor och fin och spännande värld, och att vi har allt att vinna på att ge oss ut i den. Ni är bäst. ♥