Short-term plant-decomposer feedbacks in grassland plants

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ABSTRACT

Plant species differ in their effects on ecosystem productivity and it is recognised that these effects are partly due to plant species-specific influences on soil processes. Until recently, however, not much attention was given to the potential role played by soil biota in these species-specific effects. While soil decomposers are responsible for governing the availability of nutrients for plant production, they simultaneously depend on the amount of carbon provided by plants. Litter and rhizodeposition constitute the two basal resources that plants provide to soil decomposer food webs. While it has been shown that both of these can have effects on soil decomposer communities that differ among plant species, the putative significance of these effects for plant nitrogen (N) acquisition is currently understudied.

My PhD work aimed at clarifying whether the species-specific influences of three temperate grassland plants on the soil microfood-web, through rhizodeposition and litter, can feed back to plant N uptake. The methods and approach used (^{15}N labelling of plant litter in microcosm experiments) revealed to be an effective combination of tools in studying these feedbacks. Plant effects on soil organisms were shown to differ significantly between plant species and the effects could be followed across several trophic levels. The labelling of litter further permitted the evaluation of plant acquisition of N derived from soil organic matter.

The results show that the structure of the soil microfood-web can have a significant role in plant N acquisition when its structure is experimentally manipulated, such as when comparing systems consisting of microbes to those consisting of microbes and their grazers. However, despite this, the results indicate that differences in N uptake from soil organic matter between different plant species are not related to the effects these species exert on the structure of the soil microfood-web. Rather, these differences in N uptake seem to be determined by other species-specific traits of live plants and their litter. My results thus indicate that different resources provided by different plant species may not induce species-specific decomposer feedbacks on plant N uptake from soil organic matter. This further suggests that the species-specific plant effects on soil decomposer communities may not, at least in the short term, have significant consequences on plant production.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which in the text are referred to by their Roman numerals:

I. Saj, S., Mikola, J. and Ekelund, F. (2007) Root-induced decomposer growth and plant N uptake are not positively associated among a set of grassland plants. *Applied Soil Ecology* **37**, 215-222.

II. Saj, S., Mikola, J. and Ekelund, F. Effects of live plant and plant litter on soil decomposers differ among species of grassland plants, but do they predict plant N uptake from the litter? *(submitted)*

III. Ekelund, F., Saj, S., Vestegård, M. and Mikola, J. The "soil microbial loop" is not needed to explain the positive effect of protozoan stimulation on plants. *(submitted)*

IV. Saj, S., Mikola, J. and Ekelund, F. Legume defoliation affects rhizosphere decomposers, but not the uptake of organic matter N by a neighbouring grass. (*submitted)*

AUTHORS CONTRIBUTION

For I, II and IV, SS designed the study with JM, established the experiment, and carried out all samplings and measurements, except nematode identification (done by Prof. Iuliana Popovici) and ^{15}N analyses (done at Iso-analytical Ltd). SS planned the statistical analyses with JM, performed the data analyses, interpreted the results and wrote the first draft of the manuscript, which was then completed in cooperation with JM and FE.

For III, SS designed and established the experiment together with FH, JM and FE. SS and FH planned and carried out all samplings and measurements, except for protozoa counts (FE), hormone-producing bacteria measurements (MV) and FISH (JB). SS and FE planned and performed the statistical analyses. SS wrote the first sketch of the paper with FE and JM. The paper was then finished by FE in cooperation with SS, JM and MV.

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ABBREVIATIONS

ANOVA = analysis of variance

BR = microbial basal respiration

C = carbon

C:N ratio = carbon to nitrogen ratio

CFU = colony forming unit

SOM = soil organic matter

IAA = indole-3-acetic acid

N = nitrogen

NPP = net primary production

SIR = microbial substrate-induced respiration, relative measure of microbial biomass

CONCEPTS USED IN THE THESIS

Diffusion = passive transfer of compounds resulting from their concentration gradient between two compartments.

Excretion = active release of compounds deemed to facilitate internal metabolism of the plant (e.g. respiration).

Exudation = secretions + excretions + diffusates.

Feedback = supply of an input to some process or system as a function of its output.

Microbial efficiency = ratio between basal respiration (BR) and substrate-induced respiration (SIR).

Microfood-web = microflora + microfauna and their interactions.

Microfauna = organisms with a body size inferior to 100 μm.

Microflora = microbes = bacteria + fungi.

Prosthecals = bacteria's cytoplasmic extrusion often forming a distinct appendage.

Rhizodeposition = root exudation + sloughed cells from roots.

Secretion = active release of compounds deemed to facilitate external processes (e.g. nutrient acquisition).

Short-term plant-decomposer feedbacks in grassland plants

1. INTRODUCTION

All terrestrial ecosystems, including grasslands, consist of two sub-compartments, whose sustainability is highly dependent on one another: the primary producers and the soil decomposers. The former provide basal resources to decomposer food webs and the latter governs the availability of nutrients for plant productivity. However, properly evaluating the mechanisms behind these biotic interactions has proved to be highly challenging. Indeed, the soil is a very complex milieu including billions of individuals from several thousand species per cm^3 (May, 1988; Torsvik et al. 2002). Most of these species are either not known to science and/or uncultivable (Klopatek et al., 1992; Coleman and Crossey, 1995; Ovreas, 2000). Therefore, the study of soil biotic interactions is arduous (Tunlid, 1999). Furthermore, grasslands are composed of an array of plant species from different functional groups. Even though grassland plant species are less numerous, better described and their interactions better characterised than those of soil organisms, this adds another complex group of organisms to be integrated into the study of aboveground - belowground biotic interactions. Nevertheless, thanks to an increasing effort of the scientific community, plant-soil biotic relationships are gradually revealing their subtle but also tight mechanisms.

1.1. The intimate links between plants and soil organisms

Live roots alter the structure of the soil they are foraging in and, by releasing diffusates, excretions, secretions and sloughed cells into the soil matrix (in general called rhizodeposition) they build up a unique habitat for soil organisms: the rhizosphere (Hiltner, 1904). Similarly, input of plant litter into the soil creates an environment different from bulk soil (Jones et al., 1994). Soil food webs in turn respond readily to the presence of plant material. Bacterial communities living in the rhizosphere are far denser and more active than those of bulk soil (Youssef et al., 1989) and were shown to be qualitatively different from non-rhizosphere communities (Hozore and Alexander, 1991). Bacterial feeders (Christensen et al., 1992; Griffiths, 1994) and other upper trophic level organisms (Lussenhop and Fogel, 2007) were reported to be more abundant in the vicinity of roots as well. Finally, litter patches in soil were also found to support different communities than bulk soil (Griffiths and Caul, 1993; Bengtsson et al., 1994; Hall and Hedlund, 1999).

On the other hand, soil organisms and their interactions in soil communities influence plants. Several plant attributes, and notably plant productivity, shoot to root ratio and tissue nutrient concentrations are affected by the presence of soil macro-, meso- and micro-fauna (see Mikola et al., 2002). These effects are likely to be due (1) to alteration of soil physical and chemical structure by ecosystem engineers and litter transformers and (2) interactions occurring between soil microflora and microfauna – which, in tandem, are ultimately responsible for organic matter breakdown and nutrient release (reviewed by Wardle, 2002). However, until relatively recently, the appraisal of plant effects on soil N cycling scarcely included soil decomposer communities (Bever et al., 1997). Soil decomposer activity is high in soil sites where plant material deposition occurs, and presumably, is a key-factor in controlling plant nutrient availability and growth (Alphei et al., 1996; Bonkowski et

al., 2000). Consequently, a better understanding of the many mechanisms underlying relationships between plants, soil and nutrients relies on an understanding of the significance of soil decomposer responses to plant material deposition and their feedback on plant growth (Andren et al., 1999; Osler and Sommerkorn, 2007).

1.2. Species-specific plant traits: a driving force of decomposer communities

Plants possess traits that vary greatly between species. This variability can be noticed, for instance, in biomass productivity and quality as well as in nutrient acquisition strategies and nutrient demand (Olff et al., 1994; Dawson et al., 2003; Schimel and Bennett, 2004). The extent to which plants influence nutrient cycling and soil food webs rely on these traits and can thus be species-specific.

Interestingly, plant species differ in their effects on soil nutrient status and soil biota in a manner that cannot be exclusively explained by productivity (Wheatley et al., 1990; Wardle and Nicholson, 1996; Bardgett et al., 1999). Hence, other factors like differences in resource quality provided to the soil and/or nutrient uptake abilities must significantly affect the soil biota. Plants provide resources to soil via addition of dead plant material (litter) and rhizodeposition. Species-specific differences in these inputs are likely to induce different decomposer activity, which can be reflected in soil nutrient availability and, further, induce species-specific feedback on plant growth.

In the two next sections, I will examine more closely the effects that each input type has on soil decomposer communities and the putative mechanisms by which the nutrient feedback on plant growth could occur. In the last section, I shall give some examples of other species-specific plant traits that may also play a role in this issue.

1.2.1. Root exudation and soil biota

Plant species and even ecotypes vary with respect to quantity and quality of exudates they release into the soil (Vančura and Hanzlikova, 1972; Rovira et al., 1974; Cieslinski et al., 1997; Brimecombe et al., 2001), and the quality of these compounds may strongly influence bacterial composition and activity in the rhizosphere (Chan et al., 1963; Rovira, 1965). Communities of microbial-feeders living in the root vicinity may likewise respond to differences between plant species (Griffiths et al., 1992; Wasilewska, 1995; Bardgett et al., 1999; Wardle et al. 2003; Innes et al., 2004). Hence, the soil microfood-web (including the primary decomposers, bacteria and fungi, and their feeders, protozoa and nematodes) may significantly respond to differences between plant species and, the processes driven by the microfood-web may exhibit plant species-specific patterns.

Root exudation is thought to enhance plant nutrient uptake by promoting soil organic matter mineralisation (Clarholm, 1985; Raynaud et al., 2006). In the rhizosphere, plant exudation fuels bacterial production with compounds having high C:N ratio. Since bacteria have much lower C:N ratio than exudates, they need to mineralise soil organic matter to cover their N demands. Protozoan grazing on these bacteria should eventually release the N immobilised by the bacteria and make it available for plant uptake. Protozoan grazing is also assumed to select for bacteria that can release beneficial compounds for plant root growth (Jentschke et al., 1995; Bonkowski, 2004). Both of these mechanisms should promote further exudation and constitute

the basic wheel of a virtuous circle beginning with plant germination: the so-called soil microbial loop.

Such a beneficial interaction with the soil microfood-web could act as a selective force for plants, and some species may have developed specific exudation features to improve the efficiency of the soil microbial loop. Complementarily, there is evidence that microbial biomass in general promotes root exudation (Přikryl and Vančura, 1980; Brimecombe et al., 2001) and that some metabolites produced by particular bacterial species induce an increase in root exudation of some plant species only (Meharg and Killham, 1995). The soil microbial loop would consequently depend on a multitude of tight species-specific interactions between the plant and the soil community. The soil microbial loop theory remains however controversial. For instance, some theoretical models indicate that root-induced N mineralisation is not quantitatively significant in relation to plant requirements (Griffiths and Robinson, 1992). Other studies argue that although release of simple C compounds may promote microbial growth, it may not induce production of microbial enzymes needed for enhanced decomposition of soil organic matter (Fontaine et al., 2003).

1.2.2. Plant litter deposition and soil biota

Of the factors that control the N cycle, litter deposition is among the most extensively investigated and, indeed, litter is a major source of OM to soil communities. Plant species differ with respect to the quality of litter they produce and decomposition rates of leaf litter reflect plant ecophysiological traits. In earlier studies, leaf palatability (Grime et al., 1996), tissue strength (Cornelissen and Thompson, 1997), nutrient use efficiency (Aerts, 1997) and plant growth rate, size or longevity (Wardle et al., 1998) were found to be significantly related to litter mineralisation patterns. Hence, sets of specific plant traits are likely to promote particular soil decomposer communities when dead plant material is returned to soil, which could further feed back to soil N status and plant nutrition (Wardle, 2002).

There is evidence that different litter types may induce development of different decomposer communities (Bardgett and Shine, 1999; Wardle et al., 2006) and species-specific traits, especially C:N ratios and concentrations of structure materials, were distinguished on the basis of their putative effect on soil food webs (Coleman et al., 1983; Moore and Hunt, 1988). It is now generally recognised that fast-growing plant species allocate most of their C to rapid growth, generously manufacture foliage of high photosynthetic capacity and produce easily decomposable litter that is rich in nutrients. This favours fast-growing bacterial biomass and, further, soil food webs that permit rapid nutrient turnover in the soil (the so-called bacterial-based energy channel). In contrast, slow-growing species manufacture recalcitrant compounds (e.g. lignin and phenolics) that accumulate in sparse and less photosynthetically efficient leaves, which, in turn, form litter that is poor in nutrients and difficult to decompose. This favours soil decomposer communities that are able to break down complex compounds, i.e. those dominated by fungi, and ultimately leads to slow turnover of nutrients in the soil (the so-called fungal-based energy channel).

Thus, the quality of litter produced may act as a selective force for plants and some species may display specific features in their litter that select for particular decomposer communities (Wardle, 2002;

Ayres et al., 2006). However, although there is a profuse literature on species-specific rates of litter decomposition and N mineralisation, the characterization of the decomposer food webs involved in these processes is still mostly lacking.

1.2.3. Examples of other species-specific plant traits influencing soil biota

Plant species differ with respect to nutrient uptake per root mass unit. This may be due to different ability to compete for nutrients with soil microorganisms (Griffiths et al., 1994; Kaye and Hart, 1997, Hodge et al., 1998) and/or different intrinsic nutrient uptake efficiency (Aerts and Chapin, 2000). Whatever the relative significance of each of these factors, they can be expected to influence soil food webs and nutrient availability in the root vicinity, and thus provoke a range of feedbacks across plant species.

Plant species also vary in their root morphology. The spatial foraging patterns in the soil reflect different adaptations to soil conditions (e.g. Campbell et al., 1991) and can quantitatively influence plant-induced soil biota activities *per se*. Plants living in crowded environments, such as grasslands, are under strong selective pressure to develop nutrient uptake strategies that could give them a competitive advantage. Many plant species were reported to proliferate roots into nutrient-rich patches (e.g. Grime, 1994; Robinson and van Vuuren, 1998; Fransen et al., 1999). Further, the difference between plant species with respect to how fast they colonize litter patches is thought to be a key factor in plant-plant competition (Hodge et al., 1999). Hence, the ability of a plant species to colonize soil zones of high decomposer activity (e.g. litter patches) can lead to specific soil communities in these hotspots – affecting the processes these communities are sustaining.

Many grassland plants form symbiotic associations with mycorrhizal fungi. There is some evidence that AM fungi can acquire N from both organic (Hodge et al., 2001) and inorganic N (Govindarajulu et al., 2005) sources. Once assimilated, nutrients can be provided via the fungal hyphae to the host plant root. In turn the plant provides the fungi with carbohydrates (see Martin et al., 2001 for more details). Thus, in ecosystems that comprise high plant densities such as grasslands, the species-specific interactions with mycorrhizal fungi can potentially alter N cycling and influence soil biota. Moreover, since plants can be linked together via a common mycorrhizal network, these species-specific interactions can potentially reduce the impact upon heterogeneous supplies of N in the environment and plant community structure.

Finally, particular attention should also be given to the role of microorganisms that proceed to N transformations in the soil and thus affect nitrogen cycling. For example, it is debated whether certain grass species and/or ecotypes could influence nitrifying bacteria (e.g. Lata et al., 2004). Moreover, terrestrial ecosystems comprise only a few species of organisms that are able to fix atmospheric N_2 and these provide a very significant part of ecosystem nitrogen (Cleveland et al., 1999). N_2-fixers living free in the soil as well as plant symbionts constitute therefore a pivot in plant-soil biota relationships. For instance, Witty et al. (1979) found that free-living cyanobacteria contributed significantly to the maintenance of ecosystem fertility in prairies, i.e. grasslands dominated by graminaceous plants. Grasslands also comprise plants species that are able to live in symbiotic association with either

Rhizobia or *Frankia* bacteria. Legumes form specialized organs on roots, i.e. the nodules, where they host *Rhizobia*. Plants provide carbohydrates to the *Rhizobia*, whereas the latter fuel the plant with amino acids from reduced N. These species-specific relations between plant and bacteria are highly significant for the legume nutrition strategy and physiology. Legumes exhibit high N concentrations in their tissues, which is manifested in their rhizo- and litter deposition and which affects soil food webs living in their rhizosphere or on their litter. Since the plant-rhizobium interaction significantly alters the availability of N in terrestrial ecosystems (Walker, 1993), its effect on soil food webs is likely to be as considerable as it is specific.

1.3. Implications of plant-decomposer interactions at the plant community level

In terrestrial ecosystems, soil organic matter (SOM) is the largest pool of N and accounts for more than 90% of total ecosystem N content (Knops et al., 2002). Plants are unable to exhibit sustainable growth if a significant part of this SOM is not mineralised continuously and, thus, rely on the activity of decomposer biota to meet their N needs (Lee and Pankhurst, 1992; Sparling, 1994). Such a tight dependence is likely to keep plants under selection pressure for developing features that could alter soil decomposer communities in a way that would enhance plant N acquisition. As discussed in the above sections, this could be achieved at the level of plant modules in several ways. Plants may increase their ability to compete with soil microbes for nutrients, enhance their intrinsic nutrient uptake capacity, promote symbiotic associations, modify root morphology or regulate the quantity and quality of deposits. These changes can all influence soil biota, and it is likely that the effects on the soil biota not only affect the plant module itself, but also the neighbouring plants. Plant species-specific effects on soil biota could thus potentially feed back on plant growth at the plant community level – as it is briefly illustrated in the following paragraphs.

Wardle (2002) stressed that input of dead plant material (litter) to soil affects soil food webs over longer time scales than input from live plants (rhizodeposition). Rhizodeposition only occurs when plants are alive and roots are growing actively (Přikryl and Vančura, 1980). It is a continuous process, whose intensity correlates with root growth and diminishes after flowering stage (Keith et al., 1986). Litter deposition is a more discrete process, whose intensity often peaks at plant death or seasonal senescence. In addition, the duration of the assumed effects of the two inputs on soil biota differs highly.

The quality and quantity of litter material can significantly affect the soil organic matter content and the decomposer food web. Sometimes the effects are so pronounced that they have been distinguished according to which energy channel they would promote (see 1.2.2). It has been shown that these litter effects can feed back to plant communities and last for several years after the actual deposition, partly because of effects on nutrient mobilisation (Facelli and Pickett, 1991). Because rhizodeposition occurs only when plants are alive, its direct effect on soil decomposers and the eventual feedback on plant community cannot exceed plant death that much. However, rhizosphere organisms are responsive to plant exudates and, in turn, have been shown to significantly affect several plant attributes, such as productivity or leaf nutrient content (see 1.2.1). Hence, it is possible that rhizodeposition effects last longer than

plant life span by influencing plant growth and thus indirectly affecting litter deposition patterns.

Grasslands typically possess high density of roots in relatively shallow soil layers (Sun et al., 1997), where plant rhizospheres could be considered as a continuum. Hence, both rhizodeposits and leaf litter deposits can affect comparable soil surface in grasslands. Yet, they differ with regard to their vertical distribution in the soil. Leaf litter occurs mainly at low soil depth. Rhizodeposition can occur at various soil depths and depends on plant species-specific root foraging patterns. These differences are at the source of the patchy distribution and activity of soil organisms and, thus, can participate to the spatial distribution of plants within the community.

2. OBJECTIVES OF THE STUDY

Links between above- and below-ground compartments of grassland ecosystems have been an actively studied topic for more than a decade now. But, despite a growing body of knowledge, much effort is still needed to more accurately assess the feedbacks that exist between plants and soil organisms.

The plant and decomposer subsystems are tightly connected, each carrying out some of the processes required for the maintenance of the other. Hence, plants are responsible for the amount of carbon entering the decomposer subsystem, which in turn, is accountable for governing the availability of nutrients for plant productivity. Since the rise of agricultural practices, plant species have been known to differ in their effects on soil fertility and ecosystem productivity. It is now recognized that these empirical observations are partly due to plant species-specific influences on soil processes and especially on nutrient cycling. Until recently, however, not much attention was given to the role of soil biota in such effects. Yet, soil decomposers are a necessary "channel" through which nutrients have to pass if to be continuously available to plants, and since decomposers are likely to discriminate between different resources, they potentially represent a major determinant of nutrient availability.

Litter and rhizodeposition constitute the two basal resources that plants provide to soil decomposer food webs and it has been shown that both can induce species-specific soil communities. However, the putative significance of these different soil communities for plant N-acquisition is currently understudied. My PhD work aimed at clarifying whether plant species-specific influences on the soil microfood-web, either through rhizodeposition or litter, could feed back to plant N uptake.

Using greenhouse experiments, I aimed at acquiring better knowledge on (1) whether soil decomposers promoted by different plant species differ in their ability to provide N from dead organic matter (added leaf litter in my experiments) for plant uptake; (2) whether different litter types induce species-specific decomposer communities that in turn affect N-uptake of live plants; (3) whether rhizosphere C-release affects the soil microfood-web and whether this has consequences for plant N uptake; and (4) whether defoliation (i.e. removal of shoot tissue) can affect root-induced soil decomposer communities and thus indirectly affect plant uptake of N from dead organic matter.

3. MATERIAL AND METHODS

3.1. Plants and soil

All the experiments were carried out with soil originating from a former agricultural field, abandoned more than ten years ago and since then turned into grassland (Planken Wambuis, 52° 04' 5° 04', Netherlands). The soil was shipped to the laboratory and stored at 3-6°C before further use (see Table 1 for details of the soil). The plant species used – the grass *Holcus lanatus L.*, the herb *Plantago lanceolata L.* and the leguminous herb *Lotus corniculatus L.* – co-exist in the site of the soil origin. The soil and the species of plants were chosen as common test material in the multi-national project "Biotic interactions in the rhizosphere as structuring forces for plant communities", of which this study is a part. The ^{15}N-labelled plant litter used in the experiments was produced by growing the same three species and *Lolium perenne L.* in quartz sand culture and using $^{15}NH_4{}^{15}NO_3$-enriched nutrient solution prepared according to Ingestad (1979). After "mimicking" winter season by dark and cold conditions, the aboveground ^{15}N-labelled biomass was removed, dried, ground and stored for further use.

3.2. Microcosms and growth conditions

All experiments, except III, were performed in a greenhouse using microcosms containing 678 to 1307 g soil (dry weight equivalent) in plastic pots. Each pot also included one (II) or two plant specimens (I, IV) and 0.5 g (IV) or 0.7 g (I, II) ^{15}N-labelled litter. Before mixing and adding to pots, the soil was passed through a 1-cm sieve (I and II) or hand sorted (IV) to remove big organic matter particles and stones. No organisms were removed from or added to the soil, which allowed persistence of diverse and

Table 1: Planken Wambuis site - soil analysis

	original soil	autoclaved soil
clay (< 2µm) [1]	60	63
silt fine (2-20 µm) [1]	50	51
silt coarse(20-50 µm) [1]	32	30
sand fine (50-200 µm) [1]	139	138
sand coarse (200-2000 µm) [1]	717	718
organic carbon [1]	21.3	19.3
total nitrogen [1]	1.27	1.19
C/N	16.7	16.2
organic matter [1]	36.8	33.4
pH	6.26	6.22
phosphorus (P_2O_5) [1]	0.334	0.2
potassium (K_2O) [1]	0.527	0.519
potassium (K) [1]	0.437	0.431
ammonium (NH_4) [2]	1.6	16.4

[1]: g kg^{-1} ; [2]: mg kg^{-1}

natural soil communities. Seedlings were raised from seeds sown in vermiculite. Litter was either mixed with the soil before addition to the pot (IV) or added into the soil by pulling out soil cores, mixing the litter with the core soil and reintroducing the mixture back into the holes caused by coring (I, II). In the greenhouse, the microcosms were placed on a plastic tray within five (I, II) or seven (IV) replicate blocks. The microcosms were watered regularly with tap water and supplementary light was provided via 400 W daylight lamps for 16 hours per day. The density of photosynthetic photon flux varied between 130 and 330 μmol m^{-2} s^{-1} at the height of plant shoots depending on outdoor weather and position on the tray. To equalize the amount of radiation for each microcosm, the blocks were relocated and the microcosms rearranged within the blocks each week. The temperature varied between 10°C at night and 26°C in the daytime, but peaked at 32°C during a couple of days (II).

Experiment III was performed in a growth cabinet using microcosms composed of a plastic pot, 975 g soil (dry weight equivalent), one plant specimen and 0.4 g ^{15}N-labelled litter. The soil was sieved (4 mm), autoclaved, rinsed and dried twice, mixed with the litter, rewetted before adding to the pots and finally autoclaved once more. For seedling production, *H. lanatus* seeds were surface sterilized and potential microbial contamination was checked while germinating them on sterile agar plates. After sowing the seedlings, the microcosms were placed in the growth cabinet under 16 h of light with a density of photosynthetic photon flux of 860 μmol m^{-2} s^{-1} for 8 hours in the middle of the day and 355 μmol m^{-2} s^{-1} in the remaining day hours. The temperature was 20°C for 12h, centred at the time of highest light intensity, and 15°C the remaining day hours. Pots were closed with a lid that was perforated with a tube having hydrophobic cotton clogging on its top. Plant leaves grew first within this tube, but when they attained approx. 7cm, the tubes were removed and sterile hydrophobic cotton was placed at the bottom of plant leaves (Fig. 1 in III). The microcosms were watered twice a week with autoclaved tap water to 70% of the soil water-holding capacity.

3.3. Experimental designs and treatments

The experiment I comprised of two treatment factors: (1) live plant combination and (2) litter addition (Table 2). Two three-week old seedlings of either *H. lanatus*, *L. corniculatus* or *P. lanceolata* were planted into each pot to produce three monocultures and three two-species combinations. In addition, five microcosms were set up without plants to be able to test the general plant effect on soil decomposers. After five weeks of plant growth, ^{15}N-labelled *Lolium perenne* shoot litter was added into half of the replicates of each of the six plant combinations. The microcosms were destructively harvested 30 days after litter addition.

Experiment II involved two treatment factors: (1) species of live plant and (2) species of plant litter, in a fully factorial design (Table 2). The live plant factor consisted of four levels, i.e. no seedling, or one seedling of either *H. lanatus*, *P. lanceolata* or *L. corniculatus*. Similarly, the plant litter factor consisted of four levels: i.e. no plant litter, or litter of either *H. lanatus*, *P. lanceolata* or *L. corniculatus* added to the microcosm soil. Seedlings were raised from seeds sown in vermiculite and were transferred to the microcosms when three weeks old. Four weeks later, litter was added to the twelve out of sixteen combinations that needed litter amendment. The microcosms were

destructively harvested 30 days after litter addition.

The experiment III was set up in a fully factorial design with (1) two levels of biota addition - a bacterial community without protozoa vs. a bacterial community with a mixture of three flagellates, and (2) two levels of carbon (C) addition - none and addition of 40 mg of glucose (Table 2). All microcosms were first inoculated with protozoa-free bacteria suspension, and three days later, half were inoculated with flagellates. Another 72 hours later, each microcosm received three-day old *H. lanatus* seedlings. After plants had grown for four weeks in the cabinet, 4 x 1 mL glucose solution (10g L^{-1}) was added to half of the replicates of both biota levels. The microcosms were destructively sampled 1, 3, 9 and 32 days after glucose addition.

In the experiment IV, nine-day old pairs of seedlings of the grass *H. lanatus* and the legume *L. corniculatus* were planted into each pot (Table 2). Plants were allowed to grow for four weeks before part of the leaves of *L. corniculatus* were clipped in half of the replicates. The systems were destructively sampled 1, 3, 9 and 30 days after the last clipping event. The clipping treatment included removal of three, six and nine halves of leaves 72, 48 and 24 h, respectively, before the first sampling.

3.4. Plant and soil variables measured

In all studies, plant shoot variables measured included dry weight, N and ^{15}N concentrations (Iso Analytical Ltd, UK, performed the isotope analyses) (Table 2). Dry root weight was measured either for the whole microcosm (I, IV) or individually for each plant (II, III). Total activity and biomass of soil microbes (i.e. bacteria and fungi) were in all experiments determined as described by Wardle (1993), based on the microbial basal respiration (BR) and substrate induced respiration (SIR) approach by Anderson and Domsch (1978). Prior to the microbial analyses, all visible root material was removed from the soil samples by hand. Nematodes (I, II, IV) were extracted from the soil using wet funnels (Sohlenius, 1979). They were counted live and later, using preserved samples, up to 150 nematodes per sample were identified to genus and allocated to trophic group according to Yeates et al. (1993). The number of protozoa was estimated using the most probable number method (Rønn et al., 1995).

A more comprehensive set of soil and microbial variables was measured for the experiment III. Soil suspensions used to extract protozoa were employed to determine the number of Colony Forming Units (CFU) of bacteria. The proportion of bacterial colonies producing indole-3-acetic acid (IAA) was determined according to Bric et al. (1991) using 50 colonies from each CFU sample. Soil concentrations of nitrate and ammonia were determined calorimetrically (Milton Roy Spectronic 301, Bie & Berntsen, Rodovre, Denmark) after incubation and were used to determine net N mineralisation. Bacterial community composition was estimated using FISH (Bertaux et al., 2007) at the last sampling (abundances of ten groups were assessed).

3.5. Data analyses

The data were statistically analysed either with the SPSS statistical package (I, II and IV; SPSS 12.0) or with SAS Enterprise Guide (III; Statistical Analysis System Institute, V.9.1.3). Treatment effects were tested using analysis of variance (ANOVA). When an interaction was detected between treatment factors, the factors were fixed one by one and the

Table 2: Overview of experimental designs and analyses

Corresponding manuscript		I	II	III	IV
Live plant species		▪ *Holcus lanatus* ▪ *Lotus corniculatus* ▪ *Plantago lanceolata*	▪ *Holcus lanatus* ▪ *Lotus corniculatus* ▪ *Plantago lanceolata*	▪ *Holcus lanatus*	▪ *Holcus lanatus* ▪ *Lotus corniculatus*
Litter species (g)		▪ *Lolium perenne (0,7)*	▪ *Holcus lanatus (0,7)* ▪ *Lotus corniculatus (0,7)* ▪ *Plantago lanceolata (0,7)*	▪ *Holcus lanatus (0,4)*	▪ *Lotus corniculatus (0,5)*
Soil handling		▪ Sieving ▪ Addition of litter into soil cores	▪ Sieving ▪ Addition of litter into soil cores	▪ Sieving, drying, washing ▪ Addition of litter ▪ Sterilizing ▪ Reinoculation of bacterial wash	▪ Sorting ▪ Addition of litter
Treatment		▪ Plant combination: Monocultures (HH, PP, LL) Bi-cultures (HL, HP, LP) ▪ Litter addition	▪ Live plant species (H, L, P) ▪ Litter species (h, p, l)	▪ Protozoa addition (P+, P-) ▪ Glucose addition (G+, G-)	Defoliation of *Lotus corniculatus*
Timing (weeks)		12	11	9	10
Sampling (days after last treatment)		30	30	1,3,9,32	1,3,9,30
Common measured variables	**Plant**	shoot dry weight, %N, ^{15}N content			
	Soil	BR, SIR, protozoa abundance, nematode abundance and allocation to trophic groups and genera			

Live plant: H = *Holcus lanatus*; L = *Lotus corniculatus*; P = *Plantago lanceolata*; Litter: h = *Holcus lanatus*; l = *Lotus corniculatus;* p = *Plantago lanceolata*
Protozoa added: P+; Protozoa non added: P-; Glucose added: G+; Glucose non added: G-

effect of the other factor was analyzed within the levels of the fixed factor using one-way ANOVA. Following ANOVA, Student-Neuwman-Keuls test was used to find the statistically significant differences between treatment level means. Homogeneity of variances was tested using Levene's test and when necessary, the data were logarithmically transformed to meet the homogeneity assumption of ANOVA. If this assumption was not met, even after transformation, the data were analysed using non-parametric Kruskal-Wallis test in combination with an appropriate post-hoc test.

4. RESULTS AND DISCUSSION

4.1. General plant effects on soil microfood-web

4.1.1. Live plant

The two experiments (I and II), where systems with and without live plants were contrasted, showed that live plants significantly affected the soil decomposer community. In experiment I, plant presence increased the abundance of decomposer organisms at three consecutive trophic levels (Table 3), which is consistent with earlier experiments (Wardle et al., 2003). Microcosms containing plants had higher microbial biomass (SIR) and microbial activity (BR) and higher abundances of protozoa and nematodes than those without plants. In contrast, in experiment II, presence of plants was not that beneficial to either microflora or microfauna and decreased microbial efficiency (*sensu* Wardle and Ghani, 1995). In that experiment, microcosms containing plants had higher microbial activity, but displayed lower nematode abundances than those without plants (Table 3). Negative effects of live plants on decomposer growth have also been reported earlier (Bardgett et al., 1999; Guitian and Bardgett, 2000; Mikola et al. 2005a) and, since the soil used is relatively poor in N (see Table 1), these negative effects could be due to the low soil fertility (Innes et al., 2004).

Hence, live plants were shown to alter significantly the soil decomposer community and these effects could be followed up to tertiary consumer level. That the effects on the soil microfood-web differed across experiments was, however, surprising (Table 3). Since these studies were run in the same facilities, of approximately same duration, with seeds coming from the same collection and the soil treated in an identical way (before the experiments and at sampling), this difference merits some examination. With regard to nutrient acquisition, plants compete with one another (Aerts and Chapin 2000), but also with microbes (Kaye and Hart, 1997; Schimel and Bennett, 2004) and some grassland plants were recently shown to efficiently compete for N with soil biota (Harrison et al., 2008). Thus, the discrepancies observed in the response of the soil decomposer community to plant presence could possibly result from a difference in the competitive balance between plants and soil microbes in the two experiments.

4.1.2. Litter amendment

Soil decomposer food webs were further significantly affected by litter amendment (I and II). Adding litter into bare soil (II) promoted basal and substrate-induced respiration, but decreased abundances of bacterivorous and predatory nematodes - other variables being not responsive (Table 4). Adding litter to planted soil (I and II) also promoted substrate-induced respiration but decreased

Table 3: General live plant effect on soil variables in comparison to bare soil (I and II)

		I	II
basal respiration		+	+
substate induced respiration		+	0
BR : SIR		0	+
protozoa		0	0
nematodes	**bacterivores**	+	-/0
	hyphal feeders	0	-
	omnivores	+	-
	predators	+	-
	plant feeders	0	0

results from constrast tests; + = positve effect; 0 = no effect; - = negative effect

abundances of bacterivorous and predatory nematodes - other variables being not responsive (Table 4). Adding litter to planted soil (I and II) also promoted substrate-induced respiration, but seemed to benefit bacterivorous fauna more in the experiment I than experiment II (Table 4).

Litter deposition represents a basal resource for soil microbes and the fact that microbial biomass responded positively to litter reflects its bottom-up regulation. Increased microbial growth is, in turn, likely to affect organisms that feed on microbes and especially those that are mainly bottom-up regulated. Although fungal feeders are considered to be more bottom-up regulated than bacterial-feeders (Wardle, 2002), fungal feeders did not show any significant response. Since only the nematode abundance was assessed, this does not preclude other fungal feeders being possibly affected (e.g. mites, collembolans; see Lenoir et al., 2007). However, it is more probable (1) that fungal activity *per se* in the soil was not very high because the short duration of the experiments did not allow an efficient colonisation of litter patches by fungi after significant soil disturbance by transportation, sieving and mixing, and/or (2) that the high-quality litter favoured the development of a bacterial-based decomposer system (Coleman et al., 1983; Moore and Hunt, 1988).

Indeed, bacterial grazers were significantly affected by litter addition - but also exhibited differential responses among them. In microcosms without plants, the abundance of bacterivorous nematodes was adversely affected, primarily due to a decrease in numbers of *Mesorhabditis* and *Rhabditis* (Fig. 3d,e in II; Table 4). In planted soils, protozoa were promoted in both experiments (Fig. 1c in I and Fig. 2d in II; Table 4), but bacterivorous nematodes in experiment I only (Fig. 1c in I; Table 4). Besides showing that organisms at the same trophic level can have different responses to increased resource availability (cf. discussion in II), these results suggests that the response can depend on plant presence *per se* (II; bare soil vs. planted soil) and potentially on plant growth features (I vs. II).

Finally, the fact that the abundance of predatory nematodes was affected by litter addition (positively in I and

Table 4: General litter addition effect on soil variables (I and II)

		I	II	
		planted soil	bare soil *	planted soil *
basal respiration		+	+	0
substate induced respiration		0/+	+	+
BR : SIR		0	0	0
protozoa		+	0	0/+
nematodes	**bacterivores**	+	-	0
	hyphal feeders	0	0	0
	omnivores	0	0	0
	predators	+	-	-
	plant feeders	0	0	0

* results from contrast test; + = positve effect; 0 = no effect; - = negative effect

negatively in II) demonstrates, as for live plant effects, that addition of basal resources into soil is echoed up at least to tertiary consumer level.

4.2. Protozoa interactions with microflora and their effects on live plants

Protozoan grazing on bacteria is assumed to increase soil N mineralisation by liberating N that is immobilised into bacterial biomass. This phenomenon is further recognised for its importance in the turnover of organic matter and stimulation of plant growth. Moreover, it is increasingly believed that the positive effects of protozoa on plant growth involve complex interactions between bacteria and protozoa in the plant rhizosphere (Bonkowski, 2004). The significant effects of live plants and litter addition on microflora and protozoa observed in the experiments I and II indicated that the activity and structure of the soil microfood-web is affected by plant material entering the soil. The purpose of experiment III was to test how differences in the structure of the soil microfood-web can affect plant performance.

The results from experiment III show that protozoan grazing had significant effect on bacterial abundance and functioning. CFU counts and respiration measurements showed that bacteria were top-down controlled by protozoan grazing in our systems (Fig. 3b,c in III). The presence of protozoa also resulted in an enhanced ammonium production in the soil (Fig. 3a in III), and plant growth greatly benefited from the better N availability since plants doubled their biomass (Fig. 2a in III). Further, the percentage of total shoot N coming from ^{15}N-labelled litter was higher when protozoa were present (Fig. 2d in III). This clearly indicates that protozoa participated actively to the turnover of SOM. Interestingly, in addition to having smaller total biomass, bacteria revealed a change in the community composition of the potentially active bacteria, with Verrucomicrobia and Actinobacteria groups being relatively more abundant in presence than in absence of protozoa (Fig. 1; n=27; F=5.358; p=0.033 and F=10.623; p=0.005, respectively). The Verruco-microbia group is numerically abundant in soils (Buckley and Schmidt, 2001) and comprises of small-sized, non-motile,

prosthecals-forming bacteria (Hedlund et al., 1997). These particular characteristics could enhance the ability of bacteria to avoid predator grazing (as suggested by Buckley and Schmidt, 2001) and therefore could explain the relative increase of Verrucomicrobia among potentially active bacteria. The Actinobacteria group can also be abundant in grassland soil (Singh et al., 2007), and includes types that are able to form branching filaments and/or spores and/or to produce antibiotics. Further, it has been suggested that Actinobacteria, like other gram-positive bacteria, are poor quality food for protozoa (Bjørnlund et al., 2006). These characteristics could explain their apparent resistance to protozoan grazing.

The data from experiment III do not support the other mechanisms that protozoa are suggested to sustain as well (Bonkowski, 2004). Glucose addition did not enhance net N mineralisation and plant N uptake, which is in contrast to the soil microbial loop hypothesis suggested by Clarholm (1985). Yet, the bacterial community responded significantly to the pulse of sugar and these effects were still visible one month after glucose application (Fig. 3c in III). In microcosms containing only bacteria, addition of glucose, which mimicked root exudation, was expected to lead to N immobilisation. However, this expectation was not fulfilled in plant- or microbial-related variables assessed. This indicates that plant N availability probably remained unaltered in our systems. Reasons for this could be that the glucose-induced increase of bacterial biomass was too slender to immobilise significant amounts of soil N and/or that the turnover rate of bacterial biomass was high enough to prevent significant N immobilisation at a time scale relevant for plant growth. In microcosms containing protozoa, the glucose-induced peak of respiration did not propagate into any of the other variables assessed and particularly not to protozoa abundance and litter-N uptake. Subsequently, it appeared that despite a measurable response of the bacterial community to glucose addition, the

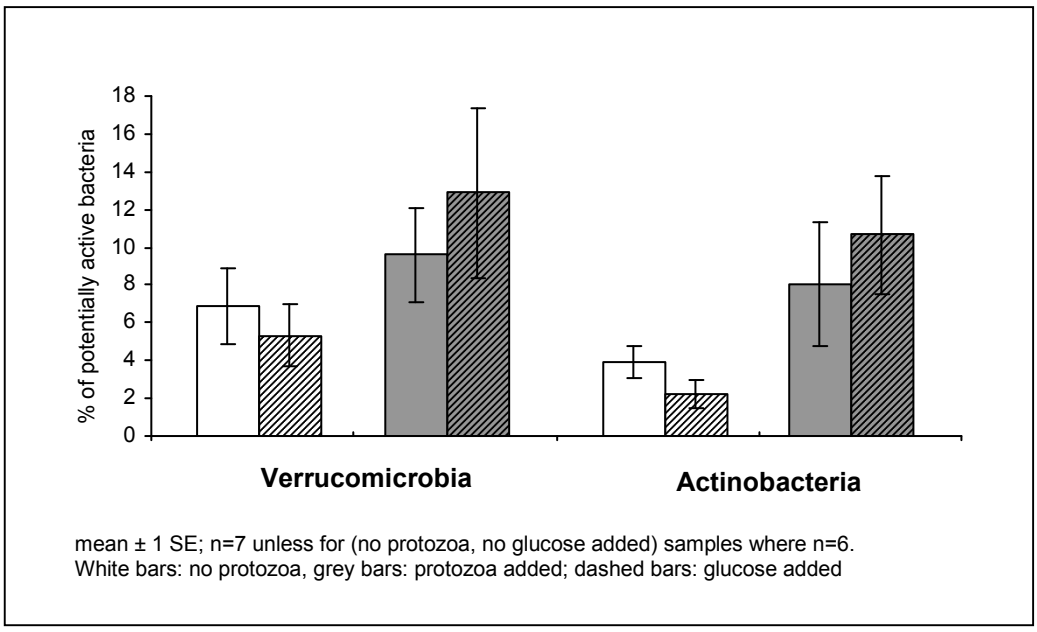

Figure 1 - Relative abundance of Verrucomicrobia and Actinobacteria in active bacterial community (III)

bottom-up effect exerted by the simple sugar did not promote N mineralisation through our soil microfood-web.

Finally, although protozoan grazing decreased bacterial biomass, in the remaining biomass the proportion of auxin producers was not significantly increased (Fig. 4 in III). Moreover, no effects were detected among the root variables assessed (e.g. Fig. 5 in III). These results indicate that the plant growth promotion observed was not a result of an IAA effect on root morphology. Hence, even though protozoa affected the relative abundance of several bacterial groups, protozoan grazing-pressure did not select for auxin producing bacteria.

4.3. Entering the species-specific feedbacks

After having stressed the effects of litter and live plant addition on the soil decomposer communities, as well as the effects of protozoan-bacterial interaction on plants, I will go further into the data dealing with species-specific interactions and feedbacks of plants *H. lanatus*, *L. corniculatus* and *P. lanceolata* and the soil decomposer microfood-web.

4.3.1. Features of live plant species

The average ratio of litter-N to total N recovered in plant shoots ranged from 2.2% in *H. lanatus* to 0.89% in *P. lanceolata* and 0.12% in *L. corniculatus*. No significant increase in plant biomass production was detectable after litter amendment (I, II). The quantity of litter added accounted for approximately 2% of total organic matter content of the microcosm soil (cf. Table 1), and thus, did not seem to induce "green manuring". Further, as different types of litter had different effects on the soil attributes in experiment II, litter addition seemed to represent a proper tool for investigating the species-specific feedbacks mediated by the microfood-web on plant N uptake.

Differences between the species remained constant in many features from one experiment to another, but some features appeared to depend on experimental conditions as well (Table 5). Shoot N concentration was highest for *L. corniculatus*, intermediate for *P. lanceolata* and lowest for *H. lanatus*. Shoot N content was higher for *L. corniculatus* than for the other species whilst litter-N uptake and proportion of litter-N in total shoot N were higher for *H.*

Table 5: Comparison of plant specific features (I and II)

	I *	II
shoot weight	H = L = P	L > H > P
root weight	H = P > L	L > P > H
shoot N concentration	L > P > H	L > P ≥ H
amount of N in shoot	L > H = P	L > H = P
litter-N shoot concentration	H > P = L	H > P > L
amount of litter-N in shoot	H > P = L	H > P > L

Live plant: H = *Holcus lanatus*; L = *Lotus corniculatus*; P = *Plantago lanceolata*

* monocultures

lanatus than for the other species. In monocultures of experiment I, shoot biomass production did not differ significantly between species. In experiment II, shoot biomass production was highest for *L. corniculatus*, intermediate for *H. lanatus* and lowest for *P. lanceolata*.

4.3.2. Species-specific litter effects on decomposers and potential feedbacks

When compared to microcosms where no litter was added (II), the tested litter types consistently promoted microbial biomass (Fig. 2b in II) and decreased numbers of predatory nematodes (Fig. 2i in II). Litter of *L. corniculatus* and *P. lanceolata* also increased significantly protozoan abundance (Fig. 2d in II). Each of the litter types decreased the abundance of bacterivorous nematodes, but *H. lanatus* had a stronger effect than the other litter types. Thus, while each litter type addition enhanced primary decomposers, showing that microbial growth was bottom-up regulated, the promotion of microbial grazers depended on the type of the litter and the group of grazers.

The fact that *L. corniculatus* and *P. lanceolata* litter significantly promoted protozoa abundance indicates increased bacterial production (Christensen et al., 1996, 2007), which could be assumed to be associated with faster decomposition of *L. corniculatus* and *P. lanceolata* litter in comparison to that of *H. lanatus*. Yet, this was not reflected in plant litter-N uptake (Fig. 1c in II) and, as discussed in manuscript II, litter-specific chemistry traits rather than litter-induced decomposer growth appeared to predict plant litter-N uptake. Lower N uptake from *L. corniculatus* litter in comparison to *H. lanatus* litter was possibly due to condensed tannins, which are known to decrease N mineralisation by inhibiting ammonification and by increasing microbial immobilisation of N (Kraus et al., 2004). Similarly, lower N uptake from *P. lanceolata* litter may be due to iridoid glycosides, which although not being toxic to soil microbes (Meyer et al., 2006), are defence compounds (Biere et al., 2004) and therefore could decelerate microbial growth.

4.3.3. Species-specific live plant effects on decomposers and potential feedbacks

In experiment II, the presence of plants significantly increased microbial biomass for each of the plant species introduced. The effects on microbial activity were depending on the species of live plant. When compared to bare soil, *H. lanatus* and *P. lanceolata* significantly decreased basal respiration, whilst *L. corniculatus* tended to increase it (Fig. 2a in II). Consequently, microbial efficiency was highest under *L. corniculatus*, intermediate under *P. lanceolata* and lowest under *H. lanatus* live plants (Fig. 2c in II). Moreover, live plants had species-specific effects on several nematode trophic groups. Root-feeding nematodes were more abundant under *L. corniculatus* (Fig. 2g in II). Bacterial-feeding nematode *Cephalobus* was more abundant under *H. lanatus* than *P. lanceolata* (Fig. 3b in II), whilst the opposite was true for bacterial-feeding nematodes *Eucephalobus* and *Aporcelaimellus* (Fig. 3c,g in II). However, none of these live plant effects did appear to explain the species-specific differences in litter-N uptake (Table 5). As discussed in paper II, other plant traits, such as plant species-specific abilities of root proliferation (Hodge et al., 1999) or competitiveness for soil N (Dunn et al., 2006), are clearly needed to explain the differences in litter-N uptake among plants.

In experiment I, plant presence significantly increased microbial activity and biomass as well as abundance of bacterivorous, omnivorous and predatory nematodes - regardless of the plant combination introduced (Fig. 2 in I). When comparing species monocultures, microbial activity was highest under *H. lanatus* (Fig. 2a in I), whilst microbial biomass and the abundance of bacterial-feeding nematodes were highest under *L. corniculatus* (Fig. 2b,d,f in I). When comparing the effects of single species on soil decomposers to those of two-species mixtures, plant combination also significantly affected the microfood-web. *Holcus lanatus* x *L. corniculatus* combinations had higher microbial biomass in comparison to *H. lanatus* monocultures (Fig. 1b in I). Similarly, *P. lanceolata* x *L. corniculatus* combinations had higher microbial biomass and abundance of bacterivorous nematodes in comparison to *P. lanceolata* monocultures (Fig. 1b,d in I). The original hypothesis was that those monocultures and species combinations, which have highest microbial biomass and abundance of bacterial feeders, would also have highest litter-N mineralisation and litter-N uptake (I and II). Yet, this was not the case and, as discussed in manuscripts I and II, differences among plant species in litter-N uptake apparently need explanation from other species-specific plant traits than those that affect the soil decomposer miorofood-web. Moreover, the beneficial effects of sharing the soil matrix with *L. corniculatus* on plant shoot N content of *H. lanatus* and *P. lanceolata* seemed to be related to the mineralisation of the fixed N leaching from *L. corniculatus* roots or to worse competitive ability of *L. corniculatus* for soil N.

Altogether my results show that soil microfood-webs are significantly affected by plants and that these effects differ among plant species. Further, these species-specific effects differ with regard to the resource type added (live plant vs. leaf litter) and also appear to be context-dependent (I vs II). However, at least among those plant species used in my studies, the effects of plants on the decomposer community do not feed back to plant uptake of N from SOM.

4.4. Adding dynamics and complexity to the system

Community and ecosystem processes above and below ground do not occur in isolation. For instance, aboveground herbivores are able to consume up to 60% of grassland net aboveground primary production (McNaughton et al., 1989). This vegetation removal may affect the structure and functioning of soil food webs and thus feed back to the remaining plants and/or affect succession (e.g. Verhoef and Brussaard 1990; Bardgett and Wardle 2003; Mikola et al., 2005b). For grassland productivity, legume-grass interactions are often vitally important and depend on nitrogen-based competitive trade-offs (Thorney et al., 1995). The purpose of experiment IV was therefore to examine the legume-grass interaction and the role of the soil decomposer food web in a situation, where the legume, *L. corniculatus*, is defoliated and the grass, *H. lanatus*, is able to react to the potential changes in decomposer growth and litter-N availability. The results show that the physiology of the legume responded rapidly to a rather restricted alteration of its integrity due to leaf removal (Fig. 1a,b,d in IV). Following the clipping of the legume, protozoan abundance readily increased (Fig. 2d in IV), whereas other soil variables assessed did not show significant responses. Despite the significant increase in protozoan numbers after *L. corniculatus* clipping, no feedback on grass litter-N uptake occurred. These

results suggest that if aboveground defoliation of legumes is found to be a significant factor affecting grass N nutrition in grasslands, this is more likely to be due to a direct transfer of fixed N (Ayres et al., 2007).

Microbial biomass and activity as well as abundance of decomposers were in general higher in experiment IV than in experiments I and II. This shows again that, despite having almost equal experimental systems, soil decomposer abundances can be significantly affected by experimental conditions. I recognize four main factors that could, at least partly, explain such differences. First, spatial heterogeneity is a major feature of soils and even though coming from the same field site, the soil could have varied in its biological patterns. Second, the soil of experiment IV was collected a year later than that used for experiment I and II and differences in soil decomposer abundances and activity could reflect their temporal variability. Third, the soil was sorted in experiment IV whilst it was sieved in experiment I, which can be harmful to some of the soil biota. Finally, the way plants were watered differed between experiments and the moisture content of microcosms was maintained on average at a higher level in experiment IV (≈ 15% at last harvest) than in experiment I and II (≈ 10% at harvest), which could have led to better development of microfauna in IV, as in the study by Christensen et al. (2007).

5. CONCLUSIONS AND PERSPECTIVES

The approach (microcosms + ^{15}N tracing) I used in my studies revealed to be an effective tool in studying the interactions between plants and the soil microfood-web. Live plant and litter addition effects could be followed across several trophic groups of soil organisms and these effects were shown to differ significantly across plant species. The use of ^{15}N-labelled litter permitted to evaluate plant uptake rates of N derived from SOM, which also appeared to be plant species-specific.

The crucial role of the structure of the soil microfood-web for plant uptake of SOM-derived N was demonstrated in experiment III. However, despite this finding, the plant-induced species-specific effects on the structure of the soil microfood-web did not appear to explain the amount of litter-N taken up by the plants. To predict these amounts, other species-specific plant traits are apparently needed (I and II). In a recent study Kemmit et al. (2008) claim that mineralisation of native soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass. Although I did not measure the mineralisation of the ^{15}N-labeled litter *per se*, my experiments point to the same direction.

My results advocate that, for those plant species tested, the species-specific effects of litter and rhizodeposits on decomposers cannot predict the live plant uptake of SOM-derived N. This further indicates that the species-specific effects on soil decomposer communities may not affect plant productivity in the short term.

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