Effects of plant species on nitrogen mineralization in grassland ecosystems

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Proefschrift

Ter verkrijging van de graad van doctor
op gezag van de rector magnificus van Wageningen Universiteit,
dr. ir. L. Speelman,
in het openbaar te verdedigen
op vrijdag 1 december 2000
des namiddags te vier uur in de Aula.

door

Anthonia Johanna van der Krift
geboren op 13 december 1969, te IJsselstein

ISBN: 90-5808-320-9

Cover design: Tanja van der Krift, Jaap Slot
Cover photo: Tanja van der Krift

Printed by Ponsen & Looijen bv, Wageningen

The research presented in this thesis was carried out at the Nature Conservation and Plant Ecology Group, Department of Environmental Sciences, Wageningen University, Bornsesteeg 69, 6708 PD Wageningen, The Netherlands.

Stellingen

I. Resultaten van wetenschappelijk onderzoek zijn nooit in tegenspraak met de natuur, maar alleen met wat we ervan begrijpen.

II. In aanvankelijk gelijke bodems kunnen snel groeiende plantensoorten een hogere stikstofmineralisatie veroorzaken dan langzaam groeiende soorten.
- Dit proefschrift –

III. De levensduur van wortels is positief gecorreleerd met hun maximale dikte.
- Dit proefschrift –

IV. De kwaliteit van dode wortels bepaalt in welke mate levende planten de afbraaksnelheid van deze wortels kunnen versnellen.
- Dit proefschrift –

V. Over "dood" valt te twisten.

VI. In Nederland is geen natuur in de exacte betekenis van het woord. Daarom zou de term "Natuurbeheer" voor gebieden in Nederland moeten worden vervangen door "Landschapsbeheer".

VII. De mogelijkheid geven aan promovendi om parttime te werken is een nobel streven. Echter, wanneer de hoeveelheid werk per week voor een promovendus niet omlaag kan worden gebracht, zal het altijd bij een streven blijven.

VIII. De leukste ideeën komen voort uit tegendraadse gedachten.

IX. Zolang het gras elders groener is, is de kans groot dat (potentiële) promovendi ook elders wortel schieten.

X. Roken moet alleen toegestaan worden in daarvoor aangewezen luchtdicht afgesloten kamers, zodat niet-rokers niet ongewild de rook van rokers in hoeven ademen.

XI. Het is jammer dat veel mensen pas door een ingrijpende ziekte leren genieten van het leven.

XII. Lachen is als een kogelvrij vest; het beschermt je tegen de verwoesting door negatieve emoties (Norman Cousins).

Stellingen behorend bij het proefschrift "Effects of plant species on nitrogen mineralization in grassland ecosystems" van Tanja van der Krift.

Wageningen, 1 december 2000.

Abstract

Van der Krift A.J. (2000) Effects of plant species on nitrogen mineralization in grassland ecosystems. Ph.D. thesis, Wageningen University, Wageningen, The Netherlands.

In many ecosystems, the nutrient supply is an important factor that determines plant species composition. Plant species have developed different characteristics, which make them successful competitors in either nutrient-poor or more fertile environments. These plant characteristics could, in turn, have important consequences for soil fertility. The research described in this thesis set out to investigate different plant characteristics of species from habitats that differ in nitrogen availability, to assess their possible consequences for soil nitrogen mineralization. Compared to species from nutrient-poor habitats, species from fertile habitats were expected to stimulate the N mineralization because they produce larger quantities of rhizodeposits and litter, which decompose better.

Overall, the results described in this thesis support this hypothesis. Plant species from high fertility habitats increased soil N mineralization more than species from low fertility habitats. Living plants of species from high fertility habitats produced more root biomass and consequently more rhizodeposits. Moreover, species from high fertility habitats had a shorter root lifespan than low fertility species. As a result, they added greater amounts of dead roots to the soil, but the decomposability of these dead roots was not related to the fertility of the habitat that they preferred. The effect of N availability on the plant characteristics studied was striking. When N supply decreased, root biomass declined, especially for the species from high fertility habitats, and as a result the rhizodeposition decreased. Moreover, for all species lower N supplies had a negative effect on rhizodeposit and dead root decomposition. Living plants stimulated dead root decomposition but the degree of stimulation depended on the C:N ratio of the decomposing roots. Overall, the differences in stimulation or inhibition of the N mineralization as a result of different levels of soil fertility seemed to be greater in species from high fertility habitats than in species from low fertility habitats.

Keywords: decomposition, nitrogen mineralization, perennial grasses, rhizodeposition, root lifespan, soil fertility.

Contents

1. General Introduction

Plant species and nutrient availability

In both agricultural and natural ecosystems, plant growth is most frequently limited by nitrogen (Chapin et al. 1987; Aerts and Chapin 2000). The species composition and species diversity in these ecosystems reflects the availability of nitrogen (Olff and Bakker 1991; Wedin and Tilman 1996), because the plant species present have developed different traits through natural selection which make them successful competitors in either nutrient-poor or more fertile habitats. In nutrient-poor environments, for example, it has been shown that selection favors plant species with traits increasing nutrient use efficiency, like low loss rates of nutrients and biomass (Berendse and Aerts 1987; Berendse and Elberse 1990; Aerts and van der Peijl 1993). By contrast, in nutrient-rich environments, selection favors plant species with traits increasing their growth rate, like high rates of photosynthesis and large specific leaf areas (Poorter et al. 1995). Because of their contrasting traits, species from nutrient-poor and nutrient-rich environments mutually exclude them from each other's habitats (Berendse 1994; Aerts 1999). As a result, nutrient-poor habitats will be dominated by species with a low potential growth rate and low nutrient loss rates and fertile habitats by species with a high potential growth rate and high rates of nutrient loss (Grime and Hunt 1975; Berendse and Elberse 1990; Berendse 1994; Aerts 1999; Aerts and Chapin 2000).

Given that environment exerts selection pressure, via nutrient availability, on plant species, perhaps the traits that characterize the species adapted to a particular nutrient scenario will themselves be influential on the environment. Could they, for example, have consequences for carbon and nitrogen cycling in the ecosystem? This thesis attempts to answer this question. It describes experiments that investigated different plant characteristics of species from habitats that differ in nitrogen availability; the aim was to assess the possible

consequences of these characteristics for the nitrogen availability in the habitat of the species concerned.

It is well documented and generally accepted that the rate of nitrogen mineralization is regulated by both abiotic factors (Swift et al. 1979) and biotic factors (Van Breemen and Finzi 1998; Wardle et al. 1998; Aerts and Chapin 2000). The most important abiotic factors affecting nitrogen mineralization are temperature and soil moisture content (Swift et al. 1979; Sierra 1997). The soil moisture content affects the availability of water and oxygen to the growing microorganisms, leading to a humped shaped relationship between soil moisture and soil microbial activity (Sierra 1997).

The effects of dominant plant species, however, can be equally important in controlling ecosystem fertility (Wedin and Tilman 1996; Berendse 1998; Van Breemen and Finzi 1998; Wardle et al. 1998; Aerts and Chapin 2000). Living plant species can affect the carbon and nitrogen cycle in the soil by the activity of their living roots (Clarholm 1985; Nicolardot et al. 1995; Brevedan et al. 1996; Cadisch and Giller 1997) and by the production of dead plant litter (Aerts et al. 1992; Van Vuuren et al. 1993; Bloemhof and Berendse 1995; Brevedan et al. 1996). The production of roots by living plants is quantitatively important and in many ecosystems exceeds aboveground productivity (Eissenstat and Yanai, 1997; Gorissen and Cotrufo 2000). A very important influence of growing roots is their influence on soil microbial activities. By water uptake and transpiration, living roots can desiccate the soil and thereby alter soil microbial activity (Jenkinson 1977; Sparling et al. 1982). Moreover, living plants supply substantial amounts of simple carbon compounds like exudates, mucilages and lysates to the soil through their roots (rhizodeposition) (Rovira 1969). Rhizodeposition stimulates microbial activity and the microbial degradation of dead roots and soil organic matter by supplying an additional energy source (Helal and Sauerbeck 1989). The microbial use of these rhizodeposited carbon substrates has a major influence on soil nutrient availability (Grayston et al. 1996).

In addition, living plants add significant amounts of carbon and nutrients to the soil through the senescence of their root biomass. The nutrients input into the soil due to root turnover are organic nitrogen and phosphorus compounds derived from the dead root material. Most plants, however, obtain most of their nitrogen by absorbing the inorganic ions ammonium and nitrate (Chapin et al. 1987). The supply of inorganic nitrogen depends largely on nitrogen mineralization, the microbial mediated conversion of organic nitrogen to the

inorganic forms (NO_3^- and NH_4^+). So, the decomposition of plant litter and rhizodeposits and the final mineralization of nutrients are decisive processes in the nutrient cycle (Cadisch and Giller 1997).

Soil microorganisms have a key role in regulating the availability of plant nutrients through the mineralization of soil organic matter and the solubilization of soil minerals (Grayston 1996). Microbial growth in soil is carbon limited and carbon can enter the soil from plants as litter, dead roots and rhizodeposits (Grayston 1996). The rate at which the decomposing plant material decomposes and releases nutrients depends on its quality (Wedin and Tilman 1990; Cotrufo et al. 1994; Cadisch and Giller 1997). A variety of predictive equations have been proposed to calculate litter decomposability, mainly using various ratios of carbon (C), nitrogen (N), lignin and polyphenols (Cadisch and Giller 1997). The C:N ratio is accepted as a general index of quality (Cadisch and Giller 1997). However, the C:N ratio is not a consistently accurate predictor of organic matter decomposition because it does not take into account the quality of the C (for example, the lignin concentration). Organic matter with similar C:N ratios can decompose at very different rates simply because of differences in C quality (Entry 2000). Good predictors for the decomposition rate have been found to be the lignin concentration or lignin:N ratio in litter (Berg and Staaf 1980; Melillo et al. 1982). The C:N ratio of the decomposable resources in general determines whether nitrogen mineralization or nitrogen immobilization takes place (Berendse et al. 1987; Robinson et al. 1989; Cadisch and Giller 1997). The theoretically optimal C:N ratio of litter for microbial growth is about 25 (Swift et al. 1979; Cadisch and Giller 1997; Seneviratne 2000) but it differs depending on the type of soil organism (Robinson et al. 1989). Materials with C:N ratios lower than 20 decompose rapidly, often with a net release of inorganic nitrogen, because organic nitrogen compounds are metabolized as C source (Cadisch and Giller 1997). Nitrogen immobilization occurs if soil microbes consume organic matter with a higher C:N ratio than their own, after respiratory C demands have been taken into account (Robinson et al. 1989).

Several authors have stated that biomass turnover rate (Berendse et al. 1987; Aerts et al. 1992; Schlapfer and Ryser 1996) and litter decomposability (Berendse et al. 1989b; Van Vuuren et al. 1993; Cornelissen 1996) can have significant effects on the changes in nitrogen mineralization in the soil. However, plant species vary greatly in the amount of litter they produce and its decomposability (Aber et al. 1990; Berendse et al. 1989b; Van Vuuren et al.

1992; Cornelissen 1996; Wardle et al. 1998). It has been demonstrated that species from habitats of different nutrient availability also differ in biomass production, biomass turnover and decomposition rate of the litter (Berendse et al. 1989b; Aerts et al. 1992; Van Vuuren et al. 1993; Brevedan et al. 1996; Jensen 1996; Aerts and De Caluwe 1997; Cadisch and Giller 1997; Gorissen and Cotrufo 2000). Such differences between plant species seem likely to have important consequences for soil organic matter dynamics and nutrient mineralization, especially when plant species composition changes during succession. If individual plant species can affect the nitrogen mineralization, this can lead to positive feedbacks between plant species replacement and changes in nitrogen availability.

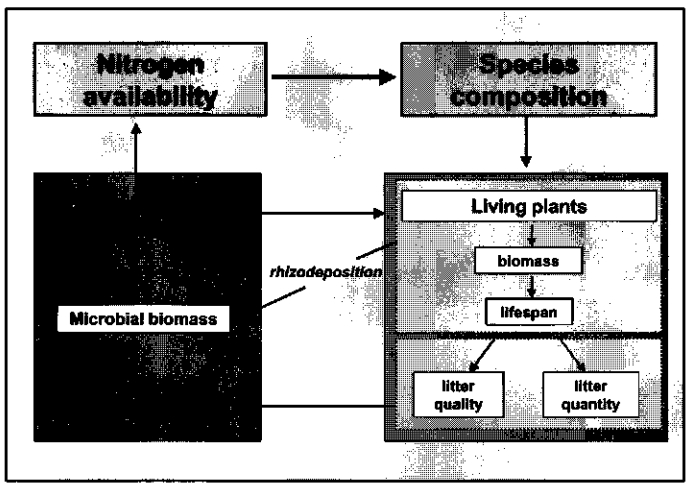

Figure 1. Schematic view on the influence of plant species on the nitrogen cycle and, vice versa, the influence of nitrogen availability on the plant species composition.

The species used in this study

Various studies have shown that there is a species-level effect on nitrogen mineralization (Vitousek et al. 1987; Berendse 1990; Wedin and Tilman 1990; Van Vuuren et al. 1992; Hobbie 1992; Stelzer and Bowman 1998). For example, experiments with *Erica tetralix* and *Molinia caerulea* showed that the nitrogen mineralization was correlated with the amounts of organic nitrogen in the soil and the decomposability of the above-ground litter (Van Vuuren and Berendse 1993; Van Vuuren et al. 1993). These comparative experiments and most others only compared species that differed in growth form and belonged to different families. Much of the variation between species may be associated with phylogenetic constraints. Hence, to

avoid confounding effects of differences in growth form and phylogeny, in the research described in this thesis mainly species of one family (Gramineae) were compared.

The study focussed on *Lolium perenne*, *Holcus lanatus*, *Arrhenatherum elatius*, *Festuca rubra*, *Anthoxanthum odoratum*, *Festuca ovina*, *Nardus stricta* and *Molinia caerulea* and investigated their influence on the nitrogen mineralization (table 1).

Table 1. Grass species used in the experiments, life form and main habitat

Species	Life form	Habitat
Lolium perenne L.	perennial	humid – dry, nutrient-rich grassland
Holcus lanatus L.	perennial	wet – humid, moderately fertile – nutrient-rich grassland, ruderal
Arrhenatherum elatius (L.) J. & C. Presl	perennial	humid, moderately fertile – nutrient-rich grassland
Festuca rubra L.	perennial	humid – dry, nutrient-poor – moderately fertile grassland
Anthoxanthum odoratum L.	perennial	nutrient-poor – moderately fertile grassland
Festuca ovina L.	perennial	acid, dry, nutrient-poor sand
Nardus stricta L.	perennial	acid – moderate, humid – dry, nutrient-poor grassland
Molinia caerulea (L.) Moench	perennial	acid – moderate, wet – humid, nutrient-poor, forest, grassland or heathland

These grass species occur in environments with different nitrogen availabilities. *L. perenne*, *H. lanatus* and *A. elatius* grow in nutrient-rich environments, *F. rubra* and *A. odoratum* grow in environments with moderate nutrient availability and *F. ovina*, *N. stricta* and *M. caerulea* prefer a nutrient-poor environment (table 1). Moreover, if nitrogen availability changes, these species can replace each other. It has been demonstrated that a dramatic change in nitrogen supply has extremely important effects on the species composition of the community (Tilman 1984; Berendse and Elberse 1990). And in grasslands that are withdrawn from agriculture for the purpose of restoring the plant species diversity, species composition also changes. When the fertilization of these grasslands is stopped and the organic matter is removed every year after mowing, there is a marked decline in mineralization and productivity (Bakker 1989; Olff et al. 1994). One of the areas where the reversed succession of grasslands has been studied is the Drentse Aa in north-east Netherlands. Here, the pasture species *L. perenne* was replaced by *H. lanatus* shortly after fertilization was stopped. (Olff et al. 1990; Olff and Bakker 1991). After c. 10 years *F. rubra*

became one of the dominant species. *A. odoratum* became dominant after c. 15 years and *N. stricta* appeared when the soil had become nutrient-poor (Bakker 1989; Olff and Bakker 1991).

Thesis synopsis

The aim of the research presented in this thesis was to answer the question: Do grass species from habitats that differ in nitrogen availability differ in certain adaptive traits, like root lifespan, litter decomposability and rhizodeposition, and do these different traits affect the nitrogen mineralization rate?

Whether grass species from habitats with different nitrogen availabilities really have a different effect on the nitrogen mineralization was tested in a long-term garden experiment (chapter 2). In this experiment grass species with different potential growth rates which are adapted to habitats with different nutrient supplies were planted in monocultures. The species studied were assigned to three groups on the basis of the soil fertility in their preferred habitat: high, intermediate or low soil fertility. The nitrogen mineralization and nitrification was measured in soil samples taken from and incubated in the plots in order to analyse the long-term effects of the species. To test whether the influence of these different groups of grass species on nitrogen mineralization corresponds with the influence of species of other families, I compared the nitrogen mineralization in soil in which these grass species were growing with the nitrogen mineralization in soil in which different groups of dicot species were growing. These dicot species were also divided into high, moderate and low fertility species, corresponding to the nutrient availability in their optimal habitat.

The next aspect investigated was the plant trait that can bring about changes in nitrogen mineralization. To date, much has been found out on the effects of aboveground litter production, but very little is known about the subterranean biomass turnover of plant species. Eissenstat and Yanai (1997) suggested that plant roots have important effects on carbon and nitrogen cycling. In the next four chapters of this thesis I focus on the possible subterranean impact of plant species.

In a garden experiment I and my colleagues measured during three years the root lifespans of four grass species, *L. perenne*, *A. elatius*, *N. stricta* and *M. caerulea*, with a similar growth

form but with a contrasting ecological response to nutrient availability (chapter 3). Minirhizotrons were used to assess root dynamics, because this technique enables individual roots to be monitored from "birth" to "death" in successive observations. I postulated that during aging of the roots the root diameter would decrease as a result of the death of epidermal and cortical cells. This decrease in root diameter was determined to assess biomass and nutrient losses during root senescence prior to complete root death.

The dead root material that enters the soil due to root senescence contains organic nitrogen and phosphorus. To be available for plant uptake, this litter must decompose and its nutrients must be mineralized. Chapter 4 describes a study of the differences in ^{15}N-labelled root decomposability of three grass species, *H. lanatus*, *F. rubra* and *F. ovina*, and the subsequent N and ^{15}N availability for the growing plants of *H. lanatus* and *F. ovina* over a period of 6 weeks. This experiment also examined the influence of these growing plants on the decomposition of dead roots and the nitrogen mineralization.

Relatively moderately fertile soil was used for the experiments in chapter 2, 3 and 4, because under these conditions the relative effects of plant species on nitrogen availability are greater and therefore easier to detect. Moreover, Poorter et al. (1995) found that most parameters related to growth and chemical composition differ between high and low fertility species and are affected by nitrogen supply, but that the differences between species at high nitrogen availability persist at low nitrogen supply. However, to test whether differences in the investigated plant characteristics occur both in nutrient-poor and in nutrient-rich conditions, the studies in chapters 5 and 6 were done at two levels of nitrogen availability.

Chapter 5 considers the influence of nitrogen availability on rhizodeposition and on decomposition of rhizodeposits, dead roots and old soil organic matter. To examine whether there is interspecific variation in these processes, four grass species, *H. lanatus*, *F. rubra*, *A. odoratum* and *F. ovina* were studied. Plants of these species were homogeneously labelled with $^{14}CO_2$ during their growth at two nitrogen levels. Using this method, it was possible to measure the carbon release to the soil through rhizodeposition during growth at the end of a growing period. Moreover, the influence of nitrogen availability on the four species was studied and how these species differed in the decomposition rate of 1) the labelled rhizodeposits in the soil in which the plants had been growing and of 2) the labelled dead roots incubated in fresh soil, during 69 days.

A study investigating whether and how growing plant species regulate the litter decomposition rate during one growing season (May-November) is described in chapter 6. I tested whether living plants of *H. lanatus* and *F. ovina* affect the decomposition of ^{14}C-labelled dead roots of *H. lanatus*, *F. rubra*, *A. odoratum* or *F. ovina* and whether this effect is species-specific. In the same experiment I studied the influence of nitrogen availability during root growth on root decomposability and whether this has an affect on the growing plants.

Van der Krift A.J. and Berendse F. The effect of plant species on soil nitrogen mineralization. *Submitted for publication.*

2. The effect of plant species on soil nitrogen mineralization

Abstract. To ascertain the influence of different plant species on N cycling, we performed a long-term garden experiment with 14 species with different potential growth rates, which are adapted to habitats with different nutrient supplies. We planted monocultures of 6 grass species (*Lolium perenne*, *Arrhenatherum elatius*, *Festuca rubra*, *Anthoxanthum odoratum*, *Festuca ovina* and *Nardus stricta*) and 8 dicotyledonous species (*Urtica dioica*, *Rumex obtusifolius*, *Anthriscus sylvestris*, *Centaurea pratensis*, *Achillea millefolium*, *Succisa pratensis*, *Calluna vulgaris* and *Erica tetralix*) and measured in situ N mineralization and nitrification of the soil during the fourth year of the experiment.

In this study we focussed on the effects of the different species on (1) annual net N mineralization; (2) the seasonal pattern of N mineralization; and (3) the fraction of the total N mineralization that is nitrified. Our hypothesis was that plant species of nutrient-rich habitats enhance the N mineralization compared to species of nutrient-poor habitats.

The results demonstrate a strong influence of the species on the net N mineralization and net nitrification. The N mineralization and nitrification fluctuated strongly during the year. Overall, species from high fertility habitats increased N mineralization and nitrification more than species from low fertility habitats. About 90% of the mineralized ammonium was oxidized to nitrate. There was no significant difference in this proportion in the plots of species from nutrient-rich, moderate and nutrient-poor habitats.

Keywords: grasses, dicots, monocultures, nitrogen mineralization, nitrification

Introduction

The species composition, species diversity and primary productivity of terrestrial ecosystems are strongly affected by the rates at which limiting nutrients such as nitrogen are supplied (Chapin 1980; Berendse 1983; Wedin and Tilman 1990; Olff et al. 1994). The supply rate of N depends largely on N mineralization, the microbial mediated conversion of organic N to inorganic forms (NO_3^- and NH_4^+). In turn, the rate of N mineralization is regulated by abiotic factors (Swift et al. 1979) and biotic factors (e.g. plants, soil animals and microorganisms) (Van Breemen 1993).

Earlier studies showed that the effects of dominant plant species could be as important as abiotic factors in controlling ecosystem fertility (Berendse 1990; Wedin and Tilman 1990; Van Vuuren et al. 1992). There is great inter-species variation among plants in the amount of litter they produce and its decomposability (Melillo et al. 1982; Melillo and Aber 1984; Aber et al. 1990; Berendse et al. 1989b; Van Vuuren et al. 1992). Such differences between plant species will likely have important consequences for soil organic matter dynamics and nutrient mineralization when plant species composition changes during succession. Various studies have indeed demonstrated the influence of the dead organic material plants produce on N mineralization (Olson 1958; Berendse 1990; Wedin and Tilman 1990; Bloemhof and Berendse 1995). Moreover, various observations suggest species level effects on N mineralization (Vitousek et al. 1987; Berendse 1990; Wedin and Tilman 1990; Van Vuuren et al. 1992; Hobbie 1992; Stelzer and Bowman 1998). However, most of these studies have only compared clearly divergent species (Vitousek et al. 1987; Berendse 1990; Van Vuuren et al. 1992; Stelzer and Bowman 1998).

Some of the studies done so far report conflicting results. Wedin and Tilman (1990) found that N mineralization was higher in plots with early successional grasses than in plots with grasses from late-successional grasslands. Van Vuuren et al. (1992 and 1993) found that litter from a different set of early successional species with low potential growth rates decomposed less easily than litter from late successional species with a high potential growth rate. They contended that it was this increase in decomposability, in combination with an increase in biomass turnover, that resulted in an accelerated increase in N mineralization during succession

(cf. Berendse 1998). These examples illustrate that the interpretation of observational studies can be confounded by the choice of the species.

It is difficult to interpret the results of descriptive field studies on the relation between species composition and soil nutrient mineralization. Not only plant species composition does affect the release of nutrients, but also vice-versa the soil fertility has a major impact on the plant community composition. To elucidate this we carried out a long-term garden experiment with 6 grass species and 8 dicotyledonous species with different potential growth rates and which were adapted to habitats with different nutrient supplies. The species studied were assigned to three groups on the basis of the nutrient level of their preferred habitat: high, intermediate or low soil fertility, respectively. The species were planted in monocultures in garden plots to ensure that the initial soil conditions were identical for each of the species. The nitrogen mineralization and nitrification was measured in soil samples taken from and incubated in these plots in order to analyse the long-term effects of the species. We focussed on the effects of the test species on (1) annual net N mineralization; (2) the seasonal pattern of N mineralization; and (3) the fraction of the total N mineralization that is nitrified. Our hypothesis was that different plant species have different effects on nitrogen mineralization and that plant species of nutrient-rich habitats enhance the N mineralization compared to species of nutrient-poor habitats.

Materials and Methods

GARDEN EXPERIMENT

The long-term garden experiment was started in June 1993. In this experiment 14 different plant species were planted at the same time in monocultures. The monocultures were arranged according to a randomized block design with 5 replicates. Plots of 1 by 1 m were cut out to a depth of 50 cm, where the yellow subsoil was present. Each block contained two rows with seven plots each. The distance between the rows was 1 m and the distance between the plots in one row was 50 cm. The plots were subsequently replenished with an intermediate fertile sandy soil with 6.6% organic matter, 2.0 g/kg total N (26.5 NO_3 mg/kg, 6.07 NH_4 mg/kg), and pH of

5.6. First the soil was sieved in order to remove old roots. All plots were surrounded by 50 cm deep sheets. The plots were planted with monocultures of 6 grass species, *Lolium perenne*, *Arrhenatherum elatius*, *Festuca rubra*, *Anthoxanthum odoratum*, *Festuca ovina* and *Nardus stricta*, and 8 dicotyledonous species, *Urtica dioica*, *Rumex obtusifolius*, *Anthriscus sylvestris*, *Centaurea pratensis*, *Achillea millefolium*, *Succisa pratensis*, *Calluna vulgaris* and *Erica tetralix*. 64 young tillers were planted per plot. In August 1993 dead plants were replaced by new tillers. In September 1993 35 cm high shadow gauze was placed around the plots to keep the produced plant biomass inside the plots.

In August 1999 the whole garden experiment was harvested. The above ground biomass was clipped and dried at 70°C to a constant weight.

During the experimental period mean temperature and total precipitation were 4.2°C and 174 mm during winter (January until March), 12.3°C and 178 mm during spring (April until June), 16.2°C and 215 mm during summer (July until September) and 6.3°C and 230 mm during autumn (October until December) (Meteorological data, Wageningen University).

CLASSIFICATION OF THE SPECIES

The species were divided into three groups according the Clausman N index parameter (Melman et al. 1985). This index is a ranking parameter, varying from 1 to 9, that characterizes the relative soil N availability at which the species involved is most frequently found. The first group contained species of nutrient-rich habitats: their Clausman N index was higher than 5.5 (Table 1). The grasses of this group were *Lolium perenne* and *Arrhenatherum elatius* and the dicots were *Rumex obtusifolius*, *Urtica dioica* and *Anthriscus sylvestris*. Species of the second group occur in moderately fertile habitats. They were the grass species *Festuca rubra* and *Anthoxanthum odoratum* and the dicot species *Achillea millefolium* and *Centaurea pratensis*. Species of the third group occur in nutrient-poor habitats and have a Clausman N index lower than 3. They were the grasses *Festuca ovina* and *Nardus stricta* and the dicots *Succisa pratensis*, *Calluna vulgaris* and *Erica tetralix*.

Table 1. Species used in the garden experiment. The Clausman N index parameters are given in parenthesis.

Fertility groups	Grasses	Dicots
Group 1	*Lolium perenne* (9.1)	*Rumex obtusifolius* (9.0)
(> 5.5)	*Arrhenatherum elatius* (5.6)	*Urtica dioica* (6.5)
		Anthriscus sylvestris (6.2)
Group 2	*Festuca rubra* (3.8)	*Achillea millefolium* (5.1)
(5.5 > x > 3.0)	*Anthoxanthum odoratum* (3.3)	*Centaurea pratensis* (3.2)
Group 3	*Festuca ovina* (2.3)	*Succisa pratensis* (1.1)
(< 3)	*Nardus stricta* (1.5)	*Calluna vulgaris* (1.1)
		Erica tetralix (1.1)

N MINERALIZATION

In 1996, after the experiment had been running for three years, we measured net N mineralization in the monocultures of the grass species and the dicot species from 26 March 1996 until 25 March 1997. We took soil core samples and incubated them in situ for 4 periods of 8 weeks in the period from 26 March until 5 November, one period of 13 weeks from 5 November until 11 February and one period of 6 weeks from 11 February until 25 March.

At the start of the incubation period, a paired sample of the first 10 cm of the soil was taken under or near an individual plant in each monoculture, using preweighed polyvinyl chloride tubes (internal diameter 2.8 cm, length 15 cm and wall thickness 2 mm). These tubes were pushed through the loose litter into the soil to a depth of 10 cm. Then the tubes were removed and their ends closed with caps of low-density polyethene. One of each pair of samples (initial sample) was transported to the laboratory in a cooled box and stored overnight at 5°C. The mineral N was extracted the day after collection. The other tube (incubated sample) was returned to its original position in the soil and left there in order to measure the accumulation of mineral N during the subsequent incubation period. The incubated soil tube had four holes of 4 mm diameter in the part that remained above the soil surface, to allow for the passage of air. The lids prevented water entering the tubes. At the end of the incubation period the

incubated samples were also transported to the laboratory in a cooled box and stored overnight at 5°C, and the mineral N was extracted the following day. The followed method was similar to earlier N mineralization measurements (Berendse 1990).

Before the analysis, the loose litter was removed from the soil core and discarded. The soil samples were homogenized after removing the roots. A 20 g sample of field-moist soil was taken from each tube and extracted with 50 ml of 1 mol KCl l^{-1}. Thereafter the NH_4^+ and the NO_3^- content were determined using a Skalar autoanalyser San^{plus} system. Soil moisture contents were determined in all samples by drying the remaining part of the field-moist soil overnight at 105°C. The volumetric soil moisture content was calculated relative to the volumetric amount of dry soil. The bulk soil density of the 0-10 cm soil layer at each site was calculated from the average amount of dry soil per initial sample.

Net N mineralization was calculated as the increase in $N\text{-}NH_4^+$ plus $N\text{-}NO_3^-$ whereas net nitrification was calculated as the increase in $N\text{-}NO_3^-$ in the incubated samples relative to their paired initial samples. For each period net N mineralization and net nitrification was multiplied by the bulk density of the 0-10 cm soil layer to obtain results per unit area. Annual net N mineralization and annual net nitrification was calculated as the total of net mineralization and net nitrification during the different periods from 26 March 1996 – 25 March 1997. Relative nitrification was calculated as the fraction of the annual released inorganic nitrogen that is oxidized to nitrate.

STATISTICAL ANALYSES

All data were analysed using analysis of variance (General Linear Model; SPSS 7.0, 1995) with life form (dicot or grass) and fertility group as fixed factors and block as random factor. When variances increased with the means, the data were logarithmically transformed. Tukey's Studentized range tests were used to test for differences among means. Because the morphology of dicots and grass species is different, we separated these two growth forms and the GLM-procedure was used to ascertain whether the three groups to which species had been assigned had different effects on mineralization and nitrification.

Results

The nitrogen mineralization and nitrification per unit area fluctuated strongly during the year (Figs 1 and 2). Both N mineralization and nitrification were low during autumn and winter (November until March). During March and April the N mineralization and nitrification peaked in the plots with species characteristic of relatively fertile soils. In this period the N mineralization and nitrification in the plots of grass species from nutrient-rich habitat (group 1) were three times greater than in the plots of grass species from intermediate and nutrient-poor habitats (groups 2 and 3). This pattern was more pronounced in the dicot species: the N mineralization and nitrification in the plots of dicot species from groups 1 or 2 were up to seven times greater than in the plots of species from group 3.

The three species groups had different effects on the annual net nitrogen mineralization. Figure 3 shows that for both the grass species and the dicot species the N mineralization in the plots of species form nutrient-rich habitats (group 1) was almost double that in the plots of species from nutrient-poor habitats (group 3). This difference was significant in both cases (Table 2). For the grass species, N mineralization in the plots with species of group 2 was intermediate between the plots with the species of groups 1 and 3. For the dicot species, the N mineralization in plots with group 2 species was not different from the N mineralization in plots with high nutrient species (group 1) and was significantly higher than in plots with species from low fertility habitats (group 3).

Table 2. F values calculated by analysis of variance, using GLM, for annual N mineralization (Nmin), nitrification ($N\text{-}NO_3$) and relative nitrification (rel. NO_3) from 26 March 1996 – 25 March 1997 and net living aboveground biomass (g m^{-2}) in August 1999 in plots with grass and dicot species (life form).

Source	df	Nmin	$N\text{-}NO_3$	rel. NO_3	biomass
Life form (lf)	1	0.82^{ns}	1.63^{ns}	1.92^{ns}	3.40^{ns}
Groups (g)	2	9.02^{***}	9.34^{***}	2.36^{ns}	7.61^{***}
Block	4	2.45^{ns}	1.58^{ns}	2.05^{ns}	0.76^{ns}
lf x g	2	0.39^{ns}	0.51^{ns}	0.33^{ns}	1.68^{ns}

*P<0.05, ** P<0.01, *** P<0.001.

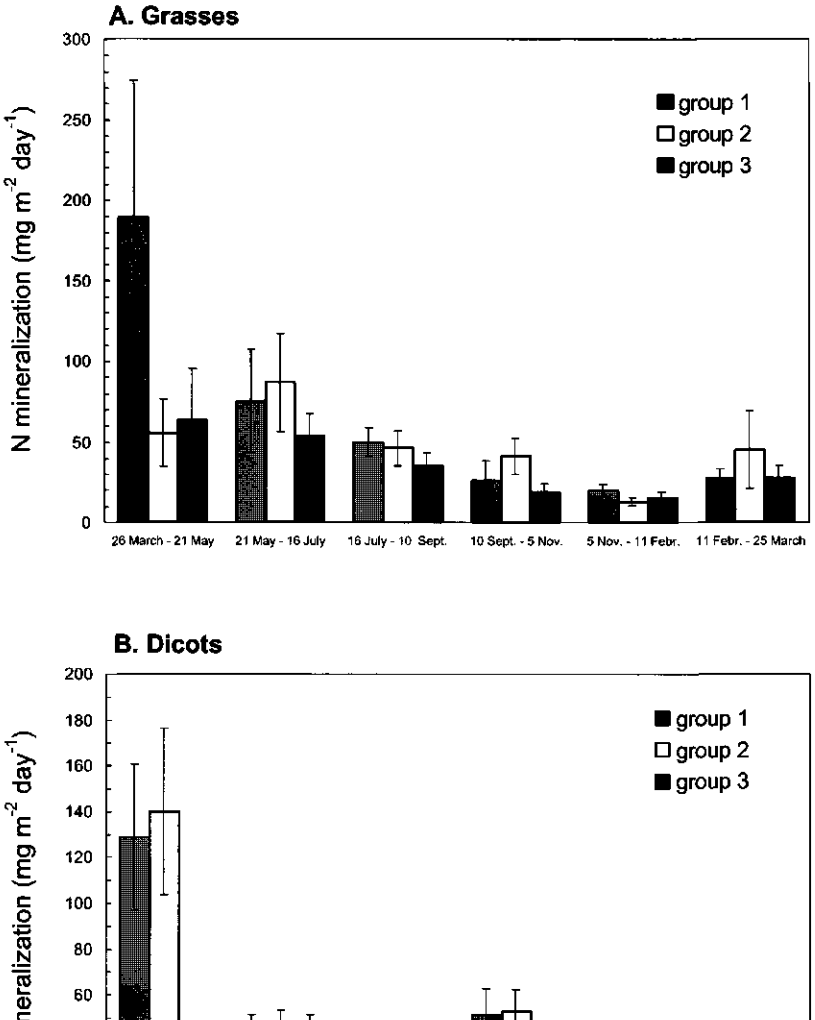

Figure 1. Net N mineralization (mg m^{-2} day^{-1}) measured in situ in three-year-old monocultures with 6 grass species (A) and 8 dicot species (B) during different periods of the year. Species are divided into 3 groups; high fertility species (group 1), intermediate fertility species (group 2) and low fertility species (group 3). Values are means ± se.

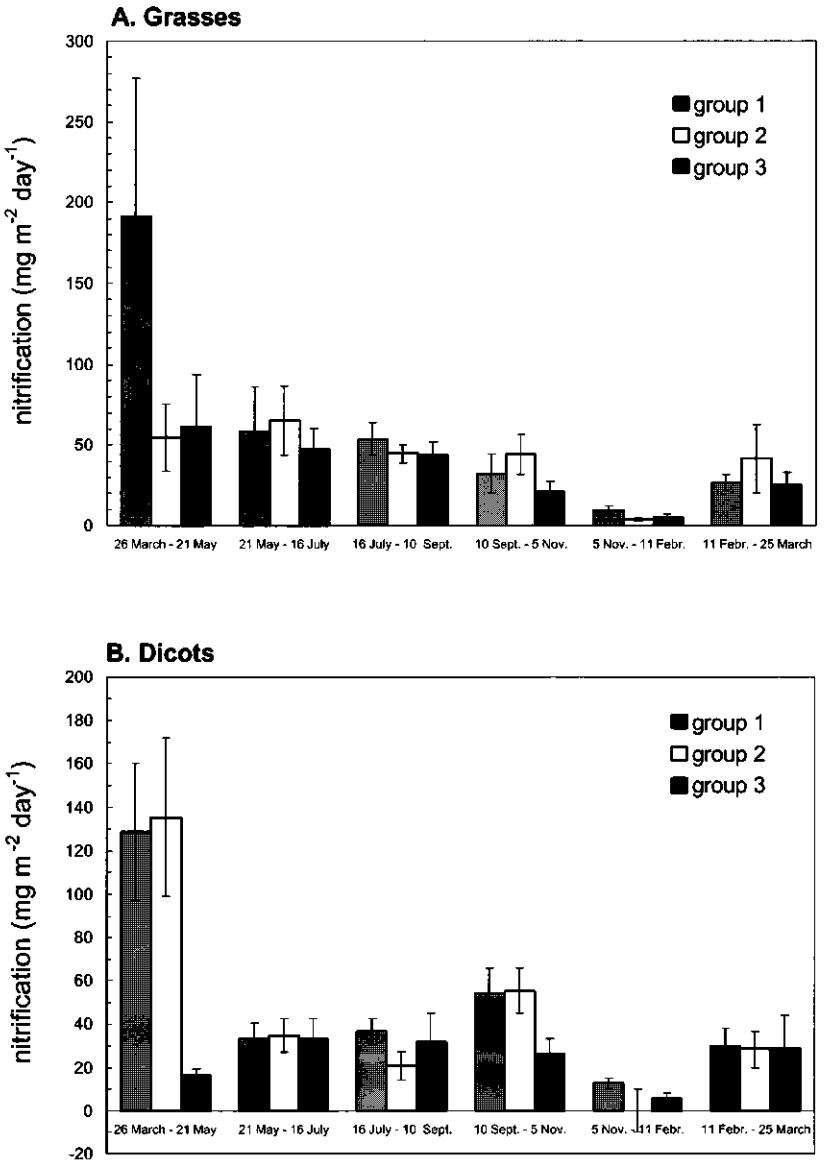

Figure 2. Net nitrification (mg $N\text{-}NO_3$ m^{-2} day^{-1}) measured in situ in three-year-old monocultures with 6 grass species (A) and 8 dicot species (B) during different periods of the year. Species are divided into 3 groups; high fertility species (group 1), intermediate fertility species (group 2) and low fertility species (group 3). Values are means ± se.

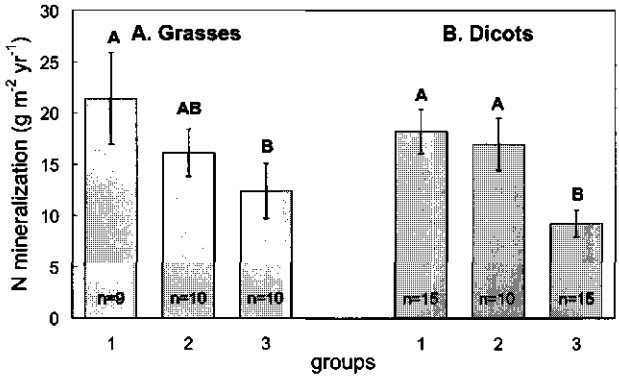

Figure 3. Annual net N mineralization (g N m^{-2} yr^{-1}) from 26 March 1996 – 25 March 1997 in monocultures with 6 grass species and 8 dicots divided into 3 groups; high fertility species (group 1), intermediate fertility species (group 2) and low fertility species (group 3). Values are means ± se.

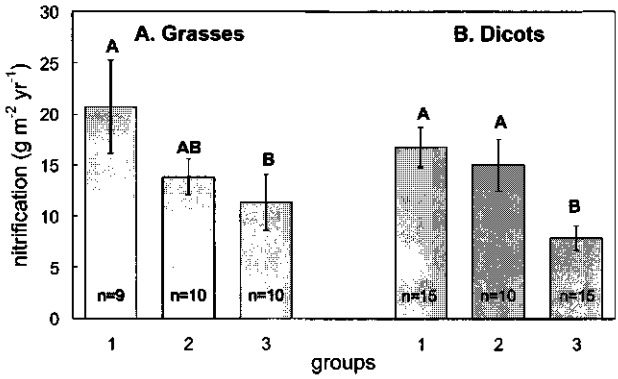

Figure 4. Annual net nitrification (g $N\text{-}NO_3$ m^{-2} yr^{-1}) from 26 March 1996 – 25 March 1997 in monocultures with 6 grass species and 8 dicots divided into 3 groups; high fertility species (group 1), intermediate fertility species (group 2) and low fertility species (group 3). Values are means ± se.

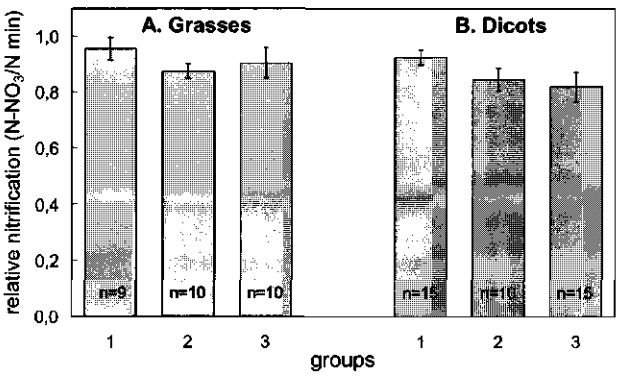

Figure 5. Annual relative nitrification (g $N\text{-}NO_3$ / g N mineralization) from 26 March 1996 – 25 March 1997 in monocultures with 6 grass species and 8 dicots divided into 3 groups; high fertility species (group 1), intermediate fertility species (group 2) and low fertility species (group 3). Values are means ± se.

The nitrification showed the same pattern as the N mineralization (Fig. 4). The nitrification in the plots of group 1 species was twice as high as in the plots of group 3 species and this difference was significant. For both the grass species and the dicot species, the nitrification in the group 2 plots was lower compared with the plots of the group 1 species, but this difference was not significant.

There were no significant differences between the 3 groups of grass species and dicot species in the fraction of mineralized N that was converted into nitrate (Table 2). The average fraction that was nitrified was 0.91 g $N\text{-}NO_3$ per g N mineralized in plots with grass species and 0.87 $N\text{-}NO_3$ per g N mineralized in plots with dicot species (Fig. 5).

At the end of the experiment, the living aboveground biomass in the plots with the grass species and dicot species was not significantly different (P=0.07, table 2 and 3). The amount of biomass in plots with species of group 3 was significantly higher than in plots with species of group 1 and 2.

Table 3. Net living aboveground biomass (g m^{-2}) in monocultures with grass and dicot species in August 1999. The grass species and 8 dicots are divided into 3 groups; high fertility species (group 1), intermediate fertility species (group 2) and low fertility species (group 3). Values are means ± se. Different uppercase letters indicate significant differences between the means ($P < 0.05$).

	Growth form		
group	Grasses	Dicots	means
1	171.1 ± 42.6	269.1 ± 58.4	**228.2 ± 39.0^{B}**
2	217.7 ± 53.8	526.2 ± 155.7	**371.9 ± 88.2^{B}**
3	379.4 ± 88.3	1677.1 ± 291.3	**1205.2 ± 230.1^{A}**
means	**248.4 ± 38.0**	**864.4 ± 157.5**	

Discussion

The differences we found after four years in the net N mineralization and nitrification rates in initially identical soils, comparing the three species groups, clearly show that plant species can affect soil N mineralization rates. These soil N mineralization rates were negatively related to the living biomass of the species in August 1999. From earlier studies we know that the biomass production and the turnover rate is much greater for high fertility species than for low fertility species (Grime 1994; Berendse et al. 1998). The lower amount of living biomass of the high and moderate fertility species was expected to be the result of these higher turnover rates. The dead leaves supply large quantities of litter for decomposition resulting in an increased N mineralization rate.

Within pattern of soil N mineralization rates there was a strong seasonality. The seasonal patterns of N mineralization and nitrification can be largely attributed to the variation in temperature and soil moisture content (Swift et al. 1979; Sierra 1997). The rates of N mineralization and nitrification were low during winter (November until March) in response to the low temperature and high soil moisture content. The rise in temperature during spring (March until May) enabled an increase in microbial activity and more inorganic N to be released (Sierra 1997). There were some exceptions to this general pattern. The N mineralization in the plots with the dicot species of groups 1 and 2 and the grass species of group 1 was higher during spring (March until May) but in the plots with the species from the other groups it was not. In autumn too (September until November) the N mineralization rate and nitrification rate of the dicots of groups 1 and 2 was higher, with the species of group 1, characteristic of fertile soils, having a high biomass production and a high biomass turnover (Berendse et al. 1998). The dead leaves of the high fertility dicots (which begin senescing in late summer) supply large quantities of litter for decomposition in autumn. By contrast, most grass species remain green until the winter. It therefore seems likely that the timing of senescence of the plant biomass and the amount of litter produced were responsible for the higher net mineralization of the high and moderate fertility dicots during autumn and spring and for high fertility grass species during spring.

The fraction of mineralized N that was nitrified was close to 1 for all three groups of both the dicots and the grasses. Almost all the mineralized nitrogen was oxidized to NO_3^-. The N

mineralization rate in monocultures with species from high fertility habitats was 18 g N m^{-2} yr^{-1} for dicots and 21 g N m^{-2} yr^{-1} for grasses. In monocultures with species from low fertility habitats the N mineralization was 9 g N m^{-2} yr^{-1} for dicots and 12 g N m^{-2} yr^{-1} for grasses. The differences we found in N mineralization rates between high and low fertility species showed the same order of magnitude as the differences in N mineralization in early "nutrient-rich" (17.6 g N m^{-2} yr^{-1}) and late "nutrient-poor" (6.1 g N m^{-2} yr^{-1}) fields during reversed grassland succession (Olff et al. 1994).

Other authors have reported that changes in N supply have important effects on species replacement (Chapin 1980; Berendse 1983; Wedin and Tilman 1990; Olff et al. 1994). We found evidence that species replacement during succession might also have major effects on the N cycle. The species from fertile habitats caused an increase in N mineralization whereas species from much less fertile habitats had a relatively negative effect on the N release from the soil. The differences in N mineralization and nitrification in our experiment were not simply a function of the net plant biomass production. Most likely other factors like biomass turnover rate (Berendse et al. 1987; Aerts et al. 1992; Schläpfer and Ryser 1996) and litter decomposability (Berendse et al. 1989b; Van Vuuren et al. 1993; Cornelissen 1996) are responsibly for the changes in N mineralization. We postulate that high fertility species can accelerate and low fertility species can slow down the N cycle and the feedbacks from such species effects could accelerate or slow down the changes in species composition during succession. We must conclude that to obtain a deeper insight in the effects that different plant species have on nutrient mineralization, more knowledge about the below ground carbon and nutrient flows from plant to the soil is needed.

Acknowledgements

We thank J. Blijenberg, H. Klees, F. Möller and J. van Walsem and the numerous summer workers for the assistance in this project and we also appreciate the valuable comments on earlier drafts of the manuscript provided by J. Burrough.

Van der Krift A.J., Möller F and Berendse F. Root lifespan of four grass species from habitats differing in nutrient availability. *Submitted for publication.*

3. Root lifespan of four grass species from habitats differing in nutrient availability

Abstract Root lifespan and root diameter were studied by observations in minirhizotrons placed in monocultures of *Lolium perenne*, *Arrhenatherum elatius*, *Molinia caerulea* and *Nardus stricta*. These grass species are from habitats differing in nitrogen availability. The initial soil conditions in the monocultures were identical for the four species. Root lifespan was 14 weeks in *L. perenne*, 40 weeks in *A. elatius*, 53 weeks in *M. caerulea* and 58 weeks in *N. stricta*. There was a significant negative correlation between root lifespan and the nitrogen availability of the ecologically optimal habitats (N-index) for the four species. Root lifespan of species from fertile habitats was significantly shorter than the root lifespan of species from low fertility habitats. In addition, the root lifespan of the four species was positively correlated to root diameter. This root diameter decreased during aging.

This study shows that there are great differences in root lifespan and root diameter between grass species from habitats with different nitrogen availability. The data presented in this article indicate that species from fertile habitats add more carbon and nutrients into the soil system as a result of a shorter root lifespan than species from less fertile habitats. We suggest that these differences in root lifespan together with the known differences in decomposability of the dead roots have an important effect on nutrient cycling in ecosystems.

Keywords: minirhizotron, nitrogen cycling, root diameter, perennial grass species, root lifespan

Introduction

The dead organic material produced by plants has an important influence on N mineralization (Berendse 1990; Wedin and Tilman 1990; Van Vuuren et al. 1992; Bloemhof and Berendse 1995). Plants add significant amounts of carbon and nutrients to the soil through the senescence of their roots. Root production, senescence and decomposition have been found to be key processes in the carbon and nitrogen dynamics of ecosystems (Aerts et al. 1992; Van Vuuren et al. 1993; Brevedan et al. 1996; Aerts and De Caluwe 1997).

The amount of root litter added to the soil, and therefore the importance of root turnover for nutrient cycling, differs strongly between species (Aerts et al. 1989; Aerts et al. 1992; Stelzer and Bowman 1998). Species differ in turnover rate depending on their optimal habitat (Aerts 1999). This has been most clearly demonstrated in studies on plant leaves. In leaves, a long lifespan increases nutrient conservation and nutrient use efficiency, whereas characteristics associated with short lifespans, such as large Specific Leaf Area and low biosynthesis costs are thought to be important for rapid growth (Chapin III 1980; Berendse and Aerts 1987; Poorter and Remkes 1990; Reich et al. 1992; Grime 1994). These different traits make species successful competitors in either nutrient-poor or nutrient-rich habitats. It has been suggested that root lifespan may be linked to suites of traits similar to the ones found in leaves (Grime 1994; Eissenstat and Yanai 1997), and that the mass and energy involved in the growth and death of roots may be at least as great as that involved in the growth and death of leaves (Eissenstat and Yanai 1997).

In the study described here we compared the root lifespans of species with a similar growth form but with a contrasting ecological response to nutrient availability. The species used in this study, *Lolium perenne*, *Arrhenatherum elatius*, *Nardus stricta* and *Molinia caerulea*, are grass species characteristic of soils with different nutrient supplies. *L. perenne* and *A. elatius* are fast growing species typical of nutrient-rich habitats, whereas *N. stricta* and *M. caerulea* are characteristic of nutrient-poor habitats. We hypothesized that the roots of the slower growing species (*N. stricta* and *M. caerulea*) live longer than the roots of the faster growing species, *L. perenne* and *A. elatius*. There is some evidence that species with thin roots have a shorter lifespan than those with coarse roots (Eissenstat and Yanai 1997). Therefore, we set out to determine whether there was a link between root lifespan and root diameter.

During aging of the roots, the early death of epidermal and cortical cells is an important phenomenon (Deacon 1987). After the cortex has died, the stele remains alive and presumably still functions in conduction. In addition, root diameter may decrease, because nutrients have been reabsorbed by the plant (Gordon and Jackson 2000). To assess biomass and nutrient losses during root senescence preceding complete root death, we measured the change in root diameter during the lifespan of the roots. Assuming that the species from fertile habitats would have a shorter lifespan, we expected that their root diameter would decrease faster than that of species from less fertile habitats.

Earlier studies on root lifespan of grass species were based on core sampling (Troughton 1981; Schläpfer and Ryser 1996). In our study we measured root lifespans in minirhizotrons, which provide more detailed information on the root dynamics because individual roots can be monitored by repeated observations and root death can be observed (De Ruijter et al. 1996).

Materials and Methods

GARDEN EXPERIMENT

The long-term garden experiment started in June 1993. In this experiment four different grass species were planted in monocultures arranged in a randomised block design with five replicates. Plots of 1 by 1 m were cut out to a depth of 50 cm, at which the yellow sandy subsoil was appeared. The plots were subsequently replenished with sandy soil with 6.6% organic matter, 2.0 g/kg total N (26.5 NO_3 mg/kg, 6.07 NH_4 mg/kg), and pH of 5.6, that had been sieved to remove old roots. All plots were separated by 50 cm deep underground sheets surrounding the plots. The plots were planted with monocultures of four grass species, *Lolium perenne* L., *Arrhenatherum elatius* L., *Nardus stricta* L. and *Molinia caerulea* L., chosen because they have a different Clausman N index parameter (Melman et al. 1985). This index is a ranking parameter, varying from 1 to 9, that characterises the relative availability of soil N in the habitat where the species in question is most frequently found. The Clausman N indexes for *L. perenne*, *A. elatius*, *N. stricta* and *M. caerulea* are respectively 8.1, 4.9, 1.5 and 1.1.

Per plot, 64 young tillers were planted. In August 1993 dead plants were replaced by new tillers. In September 1993 shading gauze was erected around the plots to a height of 35 cm, to keep the produced plant biomass inside the plots.

OBSERVATIONS IN MINIRHIZOTRONS

The minirhizotron technique used to measure root lifespans and root diameters was developed by Van Noordwijk et al. (1985) at the Institute of Soil Fertility in the Netherlands; for a detailed description, see Vos and Groenwold (1983). The technique allows the same roots to be observed repeatedly under conditions that are the best possible approximation of natural growing conditions. In contrast to Vos and Groenwold (1983) the minirhizotrons we used were round tubes made of 3 mm transparent acrylate and with a diameter of 6 cm and a length of 60 cm (outside measurements). In January 1995 one tube was inserted in the ground in each monoculture, at an angle of 45° to the soil surface. In each tube a roll of insulation material was placed to prevent condensation on the inside of the tubes and to keep the inside of the tubes dark. The tubes were capped to prevent light penetrating the root environment (figure 1).

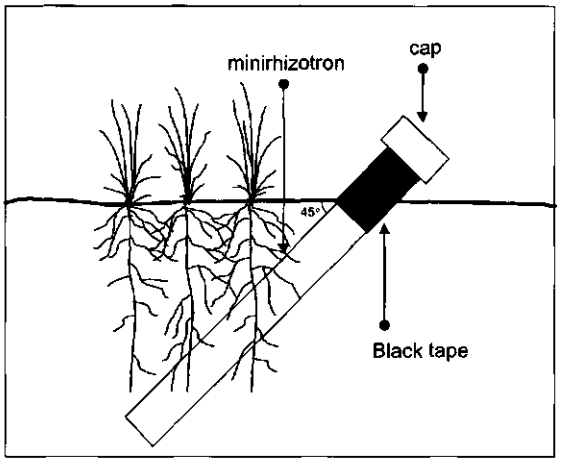

Figure 1. Schematic presentation of the minirhizotron observation tube in the monoculture.

The observations in the minirhizotrons started in February 1995. They continued at 2-week intervals until September 1997. On each observation date, colour slides were made in each tube at fixed positions 12.4 and 23.0 cm below soil surface, using fibreglass optics. Each root

observation position consisted of an area of ∅ 26.3mm. At the end of the observation period we selected roots for further analyses. The selection criteria were: roots had to be absent on the first observation date and they had to be in the centre of the slide so that they could be followed easily during their life.

DETERMINATION OF ROOT LIFESPAN

The minirhizotron slides were digitized and contrast and brightness were optimized for each slide with slide analysis software (Image-Pro Plus 2.0). Because of the large overlap in color levels between roots and soil particles, the roots had to be traced manually. The successive minirhizotron observations on the same roots allowed us to measure root lifespan and changes in root diameter. One problem we had to overcome was the definition of root death. When roots are a few weeks old, cortical cells in the roots already begin to die whereas the stele is still active (Deacon 1987). Moreover, roots may continue to absorb water and nutrients after the epidermic and cortical cells have died (Eissenstat and Yanai 1997). Even portions of roots whose entire epidermis and cortex has sloughed may still provide important transport functions (Spaeth and Cortes 1995). Therefore roots were only classified as dead when we could no longer see them.

ANALYSES AND STATISTICS

We determined root lifespan in two ways. First, we determined root survival by monitoring the presence of the roots of the initial root cohort that appeared on the minirhizotron slides some time after the first observation date (February 1995) and up to 4 July 1995. We estimated root survival curves and determined the differences in root survival between the four species, using a Gehal-Wilcoxon nonparametric test (SPSS 7.0 1995).

The second way we determined root lifespan was by monitoring the presence of roots from their first appearance on the minirhizotron slides until they were no longer visible. The statistical analyses were to ascertain whether the root lifespan was significantly different between the four species. We used a General Linear Model (SPSS 7.0 1995). Tukey's Studentized range tests were used to test for differences among means.

Root diameter was determined by measuring the relative root diameter every two weeks at one particular point during the period from appearance on the minirhizotron slides until the roots were no longer visible. Per species, mean root diameter was calculated for each age

group. Pearsons Correlation coefficients were calculated for root lifespan and root diameter and for the mean root diameter and root age (SPSS 7.0 1995). A covariance analyses was performed to determine differences in the decline of root diameter over time between the four species, with age as covariate (GLM procedure, SPSS 7.0 1995). GLM procedure was also used to determine differences between the four species in mean root diameter over the whole period.

The Pearsons Correlation coefficient of the root lifespan versus the Clausman N index was calculated (SPSS 7.0 1995).

Results

Root lifespan

Most (76-92%) of the roots visible in the minirhizotrons appeared during spring and summer; up to 8% appeared during the winter. The lifespan of these roots was determined in two ways. First, the overall lifespan of the roots that emerged during the whole year was measured. Second, the survival of one root cohort in each of the four grass species was analysed.

The overall root lifespan differed between the four species, varying from 14 weeks for *L. perenne* roots to 58 weeks for *N. stricta* roots. The overall lifespan of *L. perenne* roots was significantly shorter than the lifespan of *A. elatius*, *M. caerulea* and *N. stricta* roots ($p<0.001$) (figure 2). The lifespan of *N. stricta* roots was significantly longer than the lifespan of the *L. perenne* and the *A. elatius* roots ($p<0.001$).

Cohort survival analyses of the first root cohorts that appeared between February and July 1995 showed the same differences. After 4 months all *L. perenne* roots in the cohort had died, but the roots of the *A. elatius*, *M. caerulea* or *N. stricta* cohorts died after 17-22 months (figure 3).

Root diameter

Root lifespan increased significantly with increasing diameter of the young root (young = no more than 2 weeks old) ($r^2=0.213$, $p<0.001$, $n=74$). The root diameters of the four species differed (figure 4). Overall, roots of N. stricta had a greater diameter than roots of *L. perenne*,

A. elatius and *M. caerulea* ($p<0.001$). *L. perenne* roots had a significantly smaller diameter than the roots of the other three species ($p<0.001$). The root diameters of *A. elatius* and *M. caerulea* were not different ($p=0.94$). In the older roots the root diameter decreased significantly in all species (*L. perenne* $r^2=0.236$*; *A. elatius* $r^2=0.605$***; *M. caerulea* $r^2=0.767$***; *N. stricta* $r^2=0.181$**). Root diameter decreased more slowly in *N. stricta* than in *A. elatius* ($p=0.02$) and *M. caerulea* ($p=0.004$).

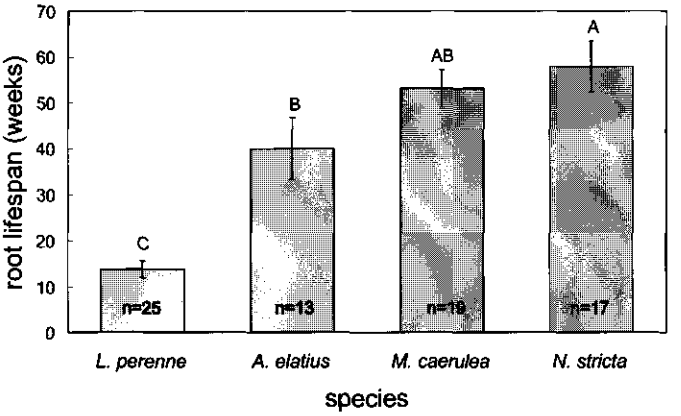

Figure 2. Root lifespan (weeks) for *L. perenne*, *A. elatius*, *M. caerulea* and *N. stricta* measured in minirhizotrons in monocultures of these species. Values are means ± se. Different uppercase letters indicate significant differences between living plants of these species ($P < 0.05$).

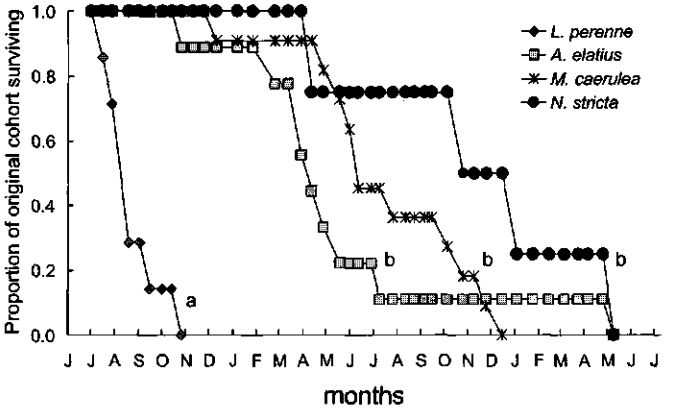

Figure 3. Root survival curves for the root cohort produced before 4 July 1995 in monocultures of *L. perenne* (n=7), *A. elatius* (n=9), *M. caerulea* (n=11) and *N. stricta* (n=4). Lines with the same letters are not significantly different (Gehal-Wilcoxon test, $P>0.05$).

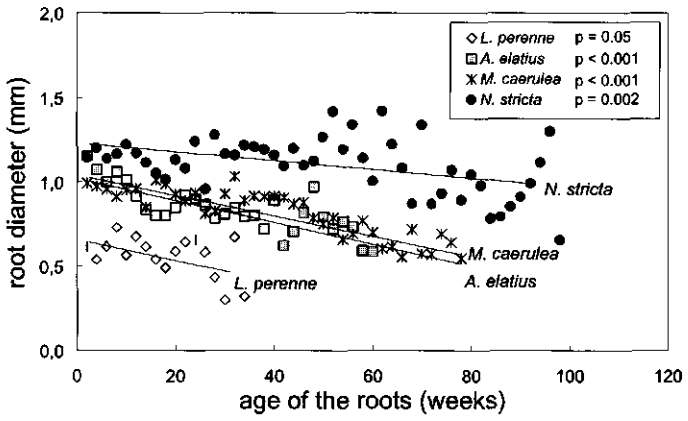

Figure 4. Root diameter (mm) of *L. perenne*, *A. elatius*, *M. caerulea* and *N. stricta* roots measured in minirhizotrons plotted against the age of the roots. The value of significance for the correlation between root diameter and age of the roots is given in the top right corner.

Discussion

Using the minirhizotron method we succeeded in measuring the root lifespan of the four grass species and its relation with the initial root diameter. The species clearly differed in their root longevity. The species from N-rich habitats, *L. perenne*, had a significantly shorter root lifespan than the species from N-poor habitats, *M. caerulea* and *N. stricta*. Our decision to classify roots as dead only when they were no longer visible meant that we may have slightly overestimated the absolute root lifespan. This did not affect the differences between the four species, however. Our results confirm earlier studies on root turnover (Aerts et al. 1989; Aerts et al. 1992; Stelzer and Bowman 1998). However, those studies used species differing in growth form and from different families. Much of the variation between species may be associated with phylogenetic constraints. It was therefore to avoid any confounding effects of differences in growth form and phylogeny that we compared species from one family, Gramineae (see also, Fransen 1999). Even for species as closely related as those we used, there was a difference in root longevity between the slower growing species (*N. stricta* and *M. caerulea*) and the faster growing species, *L. perenne*. Moreover, the differences in root lifespan between the four species were significantly negatively related with the N index of the habitats for the four species (r^2=0.95 and p=0.03; figure 5).

In nutrient-poor environments the long lifespan of roots provide an important mechanism for nutrient conservation (Eissenstat and Yanai 1997). Conversely, when plants from nutrient-rich habitats have a greater root turnover rate, they add more nutrients to the soil through dead

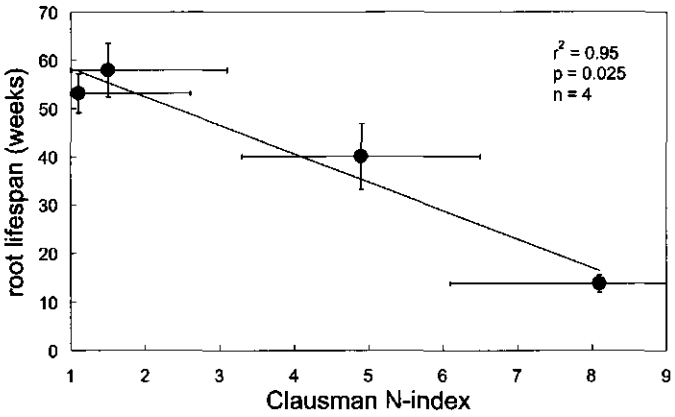

Figure 5. Root lifespan (weeks) for *L. perenne*, *A. elatius*, *M. caerulea* and *N. stricta* roots versus the Clausman N index of these species (Melman et al., 1985). Values are means ± se. The coefficient of determination (r^2) and the significance of the correlation are given in the top right corner.

root tissues and thereby activate the nutrient cycle (Aerts et al. 1992; Van Vuuren et al. 1993).

Our study also provides evidence that in grass species too, root lifespan is linked to root diameter. Our finding that the species from N-rich habitats, *L. perenne*, had a significantly lower root diameter than the species from N-poor habitats, *N. stricta*, agrees with earlier studies showing that thin roots often die sooner than coarse roots (Eissenstat 1992). Note however, that these comparisons were based on studies performed under different environmental conditions. A possible reason for the earlier death of thinner roots may be the costs of carbon allocation for the plant. If, per gram root, the C costs are the same for fine and coarse roots, then thin roots will be more efficient in nutrient uptake than coarse roots, because of the importance of root length and root surface area in root uptake (Yanai et al. 1995). However, the optimal root diameter will change when C costs of fine roots are higher than of coarse roots as a result of an increasing risk of herbivory (Eissenstat and Yanai 1997). Under nutrient-poor conditions it is especially advantageous for plant species to increase the mean residence time of nutrients in the plant (Berendse and Aerts 1987). This could explain why the roots of species of nutrient-poor habitats are coarser than the roots of species of nutrient-rich habitats when we compare closely related species. Moreover, species of nutrient-poor habitats can reduce the risk of herbivory and parasitism by investing in a wide range of recalcitrant compounds, like phenolics, which may increase the costs of biosynthesis. As a result these species can increase the lifespan of their roots (Eissenstat and Yanai 1997). On the other hand, under relatively nutrient-rich conditions, low biosynthesis costs may be

favorable for plant species (Chapin III 1980; Berendse and Aerts 1987; Poorter and Remkes 1990). Such plant species are able to respond rapidly to increased nutrient availability. Under nutrient-rich situations the root surface area might be more important than the risk of herbivory, which is why thin roots are more efficient than coarse roots.

The decrease in root diameter with root aging was expected to be the consequence of the early death of root cortex cells (Deacon 1987) and of the reabsorption of nutrients by the plant (Gordon and Jackson 2000). Robinson (1990) argued that root cortical death (RCD) could be beneficial to plants if mineral nutrients are remobilized from senescing cells, but no-one has found any evidence for this. However, the rapid loss of root cortical cells is responsible for important nutrient losses from the plant. Our finding that root diameter decrease was less in *N. stricta* than in *A. elatius* and *M. caerulea* means that the decrease in root diameter was not related to the N index of the habitat of the species.

It should be noticed that the nutrient input into the soil from root turnover refers to organic N and P contained in the dead root material. To be available for plant uptake again the litter must be decomposed and nutrients must be remineralized. Species differ substantially in their litter decomposability and N release from this litter (Berendse et al. 1989b; Van Vuuren et al. 1993; Jensen 1996), and in another paper we will report on the differences in root decomposability and the subsequent N mineralization between grass species (chapter 4 and 5). The differences between species in dead root decomposability and the subsequent nutrient mineralization, probably enhance the differences in nutrient input to the soil that result from root turnover.

In conclusion, the data presented in this article indicate that species from fertile habitats lose more biomass and nutrients by root turnover and input more carbon and nutrients into the soil system than species from less fertile habitats.

Acknowledgements

We thank R. Geerts, H. Klees, and J. van Walsem and the numerous summer workers for the assistance in this project and we also appreciate the valuable comments on earlier drafts of the manuscript provided by J. Burrough, M.M.P.D. Heijmans, E. Jongejans, W.J. Roem, J. van Ruijven and G.W.W. Wamelink.

Van der Krift A.J., Gioacchini P., Kuikman P.J. and Berendse F. (2000) Effects of high and low fertility plant species on dead root decomposition and nitrogen mineralization. *Soil Biology & Biochemistry*, in press.

4. Effects of high and low fertility plant species on dead root decomposition and nitrogen mineralization

Abstract The influence of growing grass species *Holcus lanatus* and *Festuca ovina* on the decomposition of dead roots of *H. lanatus*, *F. rubra* and *F. ovina* and on the nitrogen (N) mineralization from these residues was studied in a greenhouse experiment. These three species are typical of soils with high, intermediate and low fertility, respectively. Dead roots of high fertility species were expected to decompose faster compared to those of low fertility species and living roots of high fertility species were expected to accelerate the rate of decomposition of dead roots more than low fertility species. To test this hypothesis, decomposition of ^{15}N-labelled roots of these three species was measured after a 6-week incubation period in soil planted with either *H. lanatus* or *F. ovina* plants. After this period, plant biomass and carbon (C), N and ^{15}N distribution among plant, soil and dead roots was determined. The decomposition rate of dead roots from the three plant species was not significant different. However, *H. lanatus* dead roots caused a lower N uptake by the growing plants (18.3 mg N) compared to *F. ovina* (21.5 mg N) and *F. rubra* (21.9 mg N) dead roots, possibly as a result of a higher N immobilization in soil with *H. lanatus* dead roots. This N immobilization resulted in a higher ^{15}N retention in soil with *H. lanatus* dead roots (43.7% ^{15}N) compared to soil with *F. ovina* (31.3% ^{15}N) and *F. rubra* (35.4% ^{15}N) dead roots. The presence of growing plants stimulated dead root decomposition, N mineralization and N availability. *H. lanatus* plants took up more N and ^{15}N (26.3 mg N and 0.30 mg ^{15}N) than *F. ovina* plants (14.1 mg N and

0.17 mg ^{15}N). However, the final N concentration in *H. lanatus* (9.4 mg N/g) was lower than in *F. ovina* (25.5 mg N/g), which suggests a stronger nitrogen limitation for *H. lanatus*. Contribution of ^{15}N to total N uptake by the growing plants, Ndfr, was not different between *H. lanatus* and *F. ovina*. Our results indicate that growing plants stimulate nitrogen transfer from plant residues to the soil solution and thereby facilitate their own growth. This effect was stronger for high fertility species than for low fertility species. On the other hand, dead roots of high fertility species immobilized more N and this resulted in a lower N availability to the growing plants. This study shows that there is both positive and negative feedback between soil fertility and plant species.

Keywords: decomposition, grass species, growing plants, ^{15}N, N-uptake, dead roots

Introduction

Decomposition of plant litter is a key process in the nutrient cycle of most terrestrial ecosystems (Aerts and De Caluwe 1997; Cadisch and Giller 1997). Both quantity and quality of decomposing plant litter determine the rate of nitrogen mineralization in soils. Plant species differ widely in the quantity and quality of the litter they produce. N concentration (Witkamp 1966; Taylor et al. 1989; Tian et al. 1992), P concentration (Vitousek et al. 1994), lignin concentration or lignin:N ratio (Berg and Staaf 1980; Melillo et al. 1982) in litter have been found to be good predictors for the decomposition rate. In general, litter of plant species from nutrient-poor environments is more resistant to decomposition than litter of plant species from nutrient-rich environments due to its lower N concentration and higher concentration of decay-resistant chemical compounds (Berendse 1990; Wedin and Tilman 1990). In addition, species from nutrient-rich habitats generally have a higher biomass turnover than species from nutrient-poor habitats and so add more organic matter to the soil (Berendse et al. 1987).

In our study, we focussed on roots because root turnover provides most dead organic matter in the soil in grassland ecosystems where the aboveground biomass is removed by grazing or haymaking. Turnover and decomposition of roots determines to a large extent the

cycling of carbon and nutrients in ecosystems but little data are available on these processes (Raich and Nadelhoffer 1989; Aerts et al. 1992; Wedin and Pastor 1993).

Plants can affect the decomposition of organic matter not only through litter production, but also the by activity of living roots (Reid and Goss 1982; Nicolardot et al. 1995). Plants supply substantial amounts of simple carbon compounds to the soil through their living roots (Rovira 1969). These compounds may stimulate microbial activity and the microbial degradation of dead roots and soil organic matter by supplying an additional energy source. However, no hard evidence exists for active regulation by plants of these processes in soil. Some authors say that high fertility species promote nitrogen mineralization compared with low fertility species (Janzen and Radder 1989; Van Veen et al. 1993; Berendse et al. 1998), whereas others believe that the influence of plant growth on nitrogen mineralization depends on soil N availability (Fog 1988; Bremer and Kuikman 1997).

In this study, we focussed on the influence of dead and living roots on N availability. Decomposition of roots of three species that are characteristic for either high, intermediate or low fertility habitats and the influence of growing plants on the decomposition and nitrogen mineralization from dead roots was measured. The species appear in subsequent stages of grassland succession in the Netherlands.

They have different potential relative growth rates, i.e. *Holcus lanatus* 225 mg $g^{-1}day^{-1}$, *Festuca rubra* 176 mg $g^{-1}day^{-1}$ and *Festuca ovina* 140 mg $g^{-1}day^{-1}$ (Van der Werf et al. 1998). We hypothesed that 1) Dead roots of species from nutrient-rich habitats decompose more rapidly than of species from nutrient-poor habitats resulting in higher N mineralization rates, 2) Root decomposition rate depends on the N and lignin concentration. Roots with a high lignin content and a low N content decompose more slowly, 3) Living roots accelerate the decomposition of the dead roots by the production of exudates that act as an additional energy source for the decomposing organisms, and 4) Living root activity of high fertility species accelerates the rate of decomposition of decaying roots and N mineralization more than low fertility species, because they produce more root biomass and, consequently, more exudates.

Materials and methods

LABELLING OF ROOTS

Three grass species were selected, *H. lanatus*, *F. rubra* and *F. ovina*, which characterize decreasing levels of soil fertility. During grassland succession, after the cessation of fertilizer application, *H. lanatus* appears during the first years of mowing management. After approximately 10 years, *F. rubra* becomes one of the dominant species (Olff and Bakker 1991; Oomes and Van der Werf 1996). *F. ovina* appears when the soil has become really nutrient-poor.

In May 1997 seeds of *H. lanatus*, *F. rubra* and *F. ovina* were sown on sand without organic matter and germinated in a greenhouse. The temperature during daytime (07:00 – 21:00) was 20°C and during nighttime (21:00 – 07:00) 16°C. Relative air humidity was 75% and there was no additional light. After 6 weeks the seedlings were transplanted to pots (13 cm diameter and 15 cm height) with sand (four plants per pot) and grown for 6 weeks. Each pot had a dish underneath to prevent water-loss. During this growing period of 6 weeks, N was supplied in five parts at 10, 15, 20, 25 and 30% of the total amount supplied from week 1-5. The total amount N added per plant was 24 mg and was enriched with ^{15}N at 15%. Nutrients were added as solution containing: $MgSO_4 \cdot 7H_2O$ (474 mg ml^{-1}); KH_2PO_4 (134 mg ml^{-1});K_2SO_4 (155 mg ml^{-1}); H_2SO_4 1 N (1,23 ml); $Ca(NO_3)_2 \cdot 4H_2O$ (897 mg ml^{-1}); KNO_3 (433 mg ml^{-1}) pH 6,5. The nutrient solution was added to the surface of the pots. To avoid nutrients leaching from the soil, water was added not at the surface of the soil but to the dish: 100 ml after planting and subsequently 50 ml every 3-4 days.

The ^{15}N-labelled plants were harvested after 6 weeks. Roots and sand were carefully separated. Then the shoots were separated from the roots. The roots were washed twice in tap-water in two different bottles containing 1 l of water each, to remove the soil. Plant material was dried at 60°C to constant weight. Root and shoot dry weight was determined per pot with four plants. The dried roots were cut into 2 cm lengths, mixed and stored in glass vials. A sub-sample was taken and analysed for total C, total N, and ^{15}N, using an ANCA-MS (Europa Scientific Ltd, Crewe, UK). In addition, lignin was determined in the root samples following Kemp et al. (1994).

INCUBATION OF DEAD ROOTS IN SOIL

The decomposition experiment was carried out in the greenhouse. It consisted of a complete randomized design with five replicates (see table 1). The number of replicates for labelled roots of *F. rubra* and *F. ovina* was four and three respectively, due to an insufficient amount of labelled roots. Labelled dead roots were incubated in a mixture of black soil and sand (1:1). Before mixing, the black soil was sieved through a 4 mm sieve. The soil mixture had the following characteristics: 5.4 $pH_{(H2O)}$, 0.67 g kg^{-1} total N, 0.27 g kg^{-1} total P, C:N ratio of 17.03, and 2.1% organic matter. Pots (13 cm diameter and 15 cm height) were filled with 500 g of soil mixture. Then the dead roots were mixed with 250 g of soil mixture and added in the next layer. The last 250 g of soil mixture was used to cover this layer. N added in dead roots was equal for the different species, which contained 5.0-5.5 mg N per pot with a ^{15}N enrichment of 13%. These quantities of dead root N per pot corresponded to 480, 328 and 319 mg dry weight of biomass for *H. lanatus*, *F. rubra* and *F. ovina*, respectively. Each pot was placed on a dish to prevent water loss.

In each pot, three seedlings of *H. lanatus* or *F. ovina* were planted that were germinated, as described previously, 3-4 weeks earlier. Each species was planted in pots with dead roots of its own species or with dead roots of another species. Roots of the three species were also incubated in pots without living plants. The surface of the soil was covered with a 1 cm layer of white plastic grains to prevent growth of algae and reduce water loss by evaporation.

Table 1. Number of replicated pots per treatment.

		Dead roots		
		H. lanatus	*F. rubra*	*F. ovina*
Growing plants	*H. lanatus*	5	5	4
	F. ovina	5	4	4
	no plants	5	4	3

GROWTH OF PLANTS IN THE DECOMPOSITION PERIOD, HARVEST AND ANALYSIS

To make sure that N was the only limiting nutrient, 15 ml of nutrient solution without N and 15 ml of water was added to each pot at day 1, 11 and 21 after planting. Water use was estimated by weekly weighting the pots and watering three times a week when necessary. During the experiment the pots with *H. lanatus* plants lost more water than the pots with *F. ovina* plants or the control pots. During the first 14 days of the decomposition period the soil moisture of the pots was maintained between 16% and 18%. After this period the soil moisture was maintained between 9% and 18%.

The plants were grown for 6 weeks. During the last week, no water was added in order to reduce soil moisture to approximately 5% at harvest. The shoots were clipped off and the living roots were carefully separated from the soil mixture. It was particular easy to differentiate between added dead roots and the growing roots by color and root structure. In addition, the growing plants were very young and therefore root death of the growing plants was not expected. Subsequently, the living roots were washed in bottles by shaking them for 15 min with 250 ml of tap-water. No detectable nitrogen remained in the tap water after washing. The remaining soil mixture was sieved (1 mm mesh) to separate dead roots from the soil. With this method it is possible that root particles smaller than 1 mm where also considered as soil. This could give an overestimation of the amount of labelled soil organic matter and of the decomposition rate of the dead roots. However, it is virtually impossible to sieve the soil mixture at a lower mesh size. Dead roots were cleaned by hand. Plant material was dried at 60°C. Soil material was dried at 40°C.

Shoots, roots, soil and residues of the labelled dead roots were ground and the amount of C, N, and ^{15}N in the samples was determined using an ANCA-MS (Europe Scientific Ltd, Crewe, UK). The ^{15}N recovery in the living plants, soil, and dead roots was expressed as % of the total amount of ^{15}N added to the soil. The contribution of N added with dead roots to the total N uptake by the plant, "Nitrogen derived from residues" (Ndfr), was calculated as:

$$Ndfr\ (\%) = 100 * E_{pl} / E_{dr}$$

where E_{pl} is the % ^{15}N in excess in the growing plant and E_{dr} is the initial % ^{15}N in excess in the dead roots.

STATISTICAL ANALYSES

Statistical analysis was used to determine "the effect of growing plant species" and "the effect of the decomposing dead root species" on the decomposition rate of the labelled dead roots. Furthermore, differences in recovery of the N released from the decomposing dead roots by the different species were calculated. Correlation between ^{15}N lost from dead roots and the produced root biomass was tested by Pearson correlation (SPSS 7.0 1995). Data were analysed using analysis of variance (GLM-procedure; SPSS 7.0 1995) for a completely randomized factorial design model. Within the GLM procedure, different numbers of replicates are compensated. The data were checked for deviations from normality and homogeneity of variance prior to analysis and transformed where necessary. *A posteriori* comparisons were carried out with Tukey's honest significant difference test where appropriate (Norusis 1993; SPSS 7.0 1995).

Results

PRODUCTION OF ^{15}N-LABELLED DEAD ROOTS

During the ^{15}N labelling phase *H. lanatus* plants produced 1.3 times the amount of plant biomass produced by *F. rubra* plants and 4.2 times the amount of plant biomass produced by *F. ovina* plants (table 2).

The shoot-to-root ratio ranged between 1.78 and 2.09 and was lowest for *H. lanatus*, intermediate for *F. rubra*, and highest for *F. ovina* (table 2). So *Holcus* plants allocated more biomass to its roots than the two *Festuca* species.

The percentage N in the roots was lower in *H. lanatus* (1.15%) than in *F. ovina* (1.76%) and intermediate in *F. rubra* (1.55%). The C:N ratio in the roots was higher in *H. lanatus* (33.3) than in *F. ovina* (18.1) and intermediate in *F. rubra* (22.3). The % ^{15}N in excess decreased slightly from 13.6% to 13.2% in the order *H. lanatus* > *F. rubra* > *F. ovina* (table 2). Roots of *H. lanatus* had the lowest lignin concentration (8%). Higher concentrations were found in *F. rubra* and *F. ovina* (16% and 17%, respectively).

Table 2. Characteristics of the labelled plants used for the decomposition experiment. Shoot and root dry mass production (DM) per pot, shoot-to-root ratio (S/R), nitrogen concentration (%N), carbon concentration (%C), C:N ratio, ^{15}N in excess (%) and lignin concentration (%) in *Holcus lanatus*, *Festuca rubra* and *Festuca ovina* roots grown for 6 weeks to produce ^{15}N-labelled residues. Values of root characteristics are means (n = 3) with standard errors in parentheses.

	Shoots DM (g/pot)	Roots DM (g/pot)	S/R	Roots % ^{15}N in excess	%N	%C	C:N	%lignin
H. lanatus	2.14	1.20	1.78	13.56(0.04)	1.15(0.01)	38.1(0.3)	33.3(0.3)	8(1)
F. rubra	1.65	0.84	1.97	13.51(0.01)	1.55(0.01)	34.5(0.3)	22.3(0.1)	16(1)
F. ovina	0.54	0.26	2.09	13.18(0.01)	1.76(0.06)	31.7(0.8)	18.1(0.4)	17(3)

ROOT DECOMPOSITION

Mass loss of dead roots ranged from 59 to 91% (Fig. 1a). There were no significant differences in mass loss rate between dead roots of *H. lanatus*, *F. rubra* and *F. ovina*. The presence of growing plants species stimulated root decomposition significantly, *H. lanatus* plants having a stronger positive effect than *F. ovina* plants. The average mass loss of the dead roots was 85% in the presence of *H. lanatus* plants, 67% in the presence of *F. ovina* plants, and 61% without plants (Fig. 1a).

Nitrogen loss from dead roots showed a similar pattern. The presence of growing plants increased the nitrogen loss from the dead roots significantly. The nitrogen loss rate of the dead roots was not significantly different between the residue species (Fig. 1b). The percentage nitrogen loss ranged from 37 to 87% and was highest for *H. lanatus* dead roots in the presence of growing *H. lanatus* plants (Fig. 1b). Nitrogen loss of the three residue species was significantly higher in the presence of growing *H. lanatus* plants compared to the presence of *F. ovina* plants or no plants.

The percentage ^{15}N loss of the dead roots depended again on the growing plant species (Fig. 1c). ^{15}N loss was significantly higher in the presence of growing *H. lanatus* plants (89%) compared with *F. ovina* plants (71%) or no plants (64%). The percentage ^{15}N loss from the dead roots was significantly lower without growing plants. The percentage ^{15}N loss showed a significant positive correlation ($r = 0.9$) with the root biomass of the growing plants (Fig. 2).

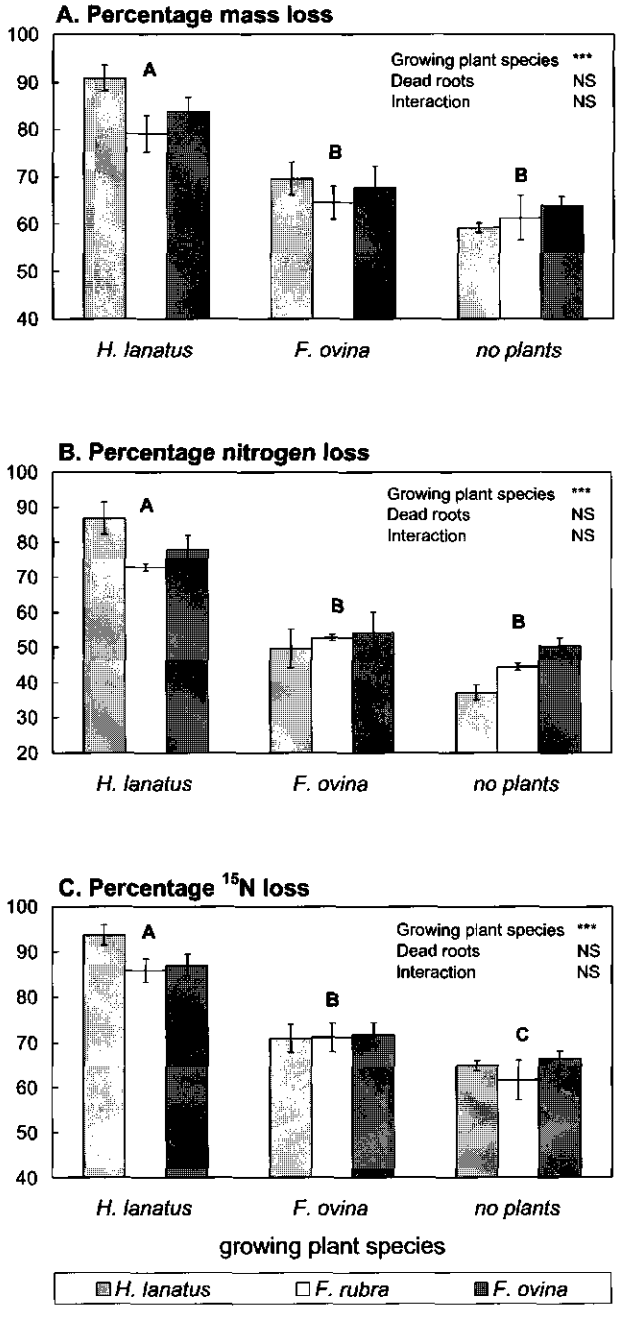

Figure 1. A. Mass loss, **B.** nitrogen loss, and **C.** ^{15}N loss from dead roots of *Holcus lanatus*, *Festuca rubra*, or *Festuca ovina* as a percentage of the initial amount after 6 weeks of incubation in the presence of growing plants of *Holcus lanatus* or *Festuca ovina*, or no plants. Values are means ± standard errors. Different uppercase letters above sets of bars indicate significant differences between living plant species ($P < 0.05$). The results of the GLM procedure are in the right corner of the graph; NS, not significant ($P > 0.05$); $P < 0.05$*; $P < 0.01$**; $P < 0.001$***.

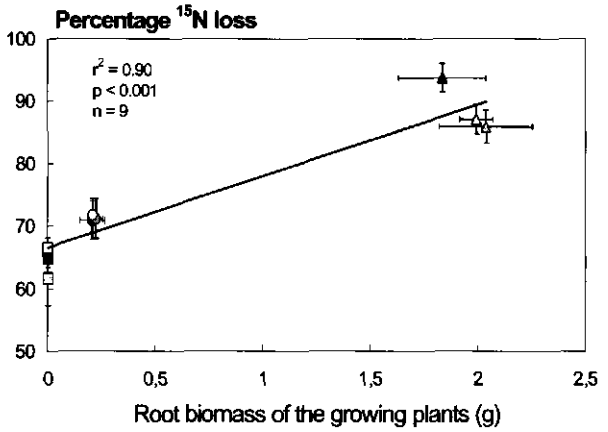

Figure 2. ^{15}N loss from dead roots of *Holcus lanatus* (closed symbols), *Festuca rubra* (grey symbols) or *Festuca ovina* (open symbols) versus root biomass production in pots with living plants of *Holcus lanatus* (▲) or *Festuca ovina* (●), or without plants (■) during a 6-week period (r = 0.95). Values are means ± standard errors.

So the difference in the stimulating effects of *H. lanatus* ad *F. ovina* plants may result from the difference in living root biomass and 90% of the variability is explained by this linear relationship.

The ^{15}N enrichment of the dead roots decreased during the experiment from 13.6 - 13.2% to 6.5 - 9.4% (tables 2 and 3). The ^{15}N enrichment of the dead roots at the end of the decomposition period was significant lower in the presence of growing *H. lanatus* plants (7.1%) compared with *F. ovina* plants (8.2%) or no plants (8.6%) (table 3). The ^{15}N enrichment decreased significantly more in *H. lanatus* dead roots than in *F. rubra* or *F. ovina* dead roots. So, growing plants of *H. lanatus* stimulated N mineralization more than growing plant of *F. ovina*, while dead roots of *H. lanatus* released and immobilized more N than root materials of *F. rubra* and *F. ovina*.

SOIL

Growing plants significantly affected the amount of ^{15}N that was transferred from the dead roots to the soil (table 4). Also, there was a significant effect of the dead root species on the amount of ^{15}N that was recovered in the soil. No significant interaction between growing plant species and dead root species on ^{15}N content of the soil was found.

Significantly more ^{15}N was transferred to the soil from *H. lanatus* dead roots (44%) compared with dead roots of *F. rubra* (35%) or *F. ovina* (31%). There was no significant

Table 3. ^{15}N enrichment (^{15}N in excess/total N * 100%) in dead roots of *Holcus lanatus*, *Festuca rubra* and *Festuca ovina* after 6 weeks of incubation in the soil, in the presence of growing plants of *H. lanatus*, *F. ovina* or no plants. Values are means ± standard errors. Different superscript letters indicate significant differences between the means ($P < 0.05$).

		Dead roots			
		H. lanatus	*F. rubra*	*F. ovina*	
Growing plant species	*H. lanatus*	6.5 ± 0.1	7.2 ± 0.5	7.8 ± 0.1	**7.1**^{b}
	F. ovina	8.0 ± 0.1	8.4 ± 0.4	8.4 ± 0.4	**8.2**^{a}
	No plants	7.8 ± 0.1	9.4 ± 0.2	9.0 ± 0.2	**8.6**^{a}
		7.4^{b}	**8.2**^{a}	**8.4**^{a}	

Table 4. The amount of ^{15}N transferred from dead roots to the soil, during 6 weeks of incubation of the dead roots, expressed as a percentage of the initial amount of ^{15}N in the added dead roots. The ^{15}N in the soil originates from decomposing dead roots of *Holcus lanatus*, *Festuca rubra* or *Festuca ovina*. These dead roots were incubated in the presence of growing plants of *H. lanatus* or *F. ovina*, or no plants. Values are means ± standard errors. Different superscript letters indicate significant differences between the means ($P < 0.05$).

		Dead roots			
		H. lanatus	*F. rubra*	*F. ovina*	
Growing plant species	*H. lanatus*	38.2 ± 1.9	28.3 ± 2.2	21.3 ± 0.8	**29.8**^{b}
	F. ovina	40.2 ± 2.6	33.9 ± 3.2	32.0 ± 4.0	**35.7**^{b}
	No plants	52.6 ± 5.5	45.8 ± 3.0	43.6 ± 1.5	**48.1**^{a}
		43.7^{a}	**35.4**^{b}	**31.3**^{b}	

difference in the amount of ^{15}N in the soil between pots with dead roots of *F. rubra* and *F. ovina*. So, *H. lanatus* dead roots released more ^{15}N to the soil than *F. rubra* and *F. ovina* dead roots.

The amount of ^{15}N in the soil was significant higher in pots without growing plants (48%) compared to pots with growing plants (30% and 36%). This difference is attributed to the ^{15}N uptake by the growing plants so that soil organic matter can immobilize less inorganic ^{15}N.

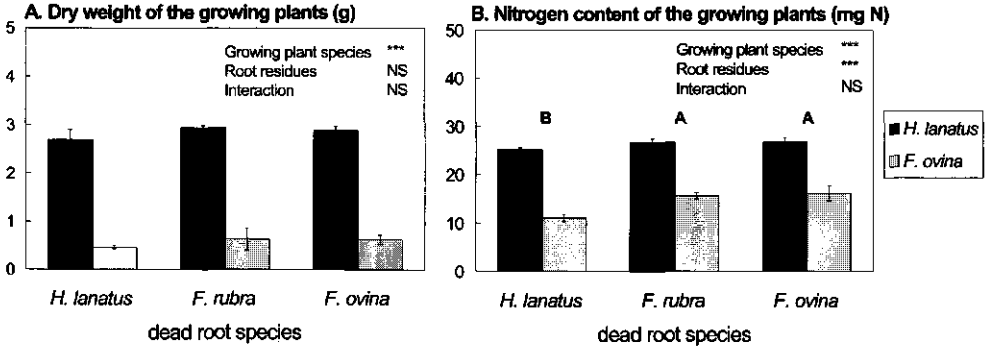

Figure 3. A. Dry weight (g) and **B.** nitrogen content (mg) of *Holcus lanatus* or *Festuca ovina* plants grown for 6 weeks in pots with dead roots of *Holcus lanatus*, *Festuca rubra* or *Festuca ovina*. Values are means ± standard errors. Different uppercase letters above sets of bars indicate statistical differences between living plant species ($P < 0.05$). The results of the GLM procedure are in the right corner of the graph; NS, not significant ($P > 0.05$); $P < 0.05$*; $P < 0.01$**; $P < 0.001$***.

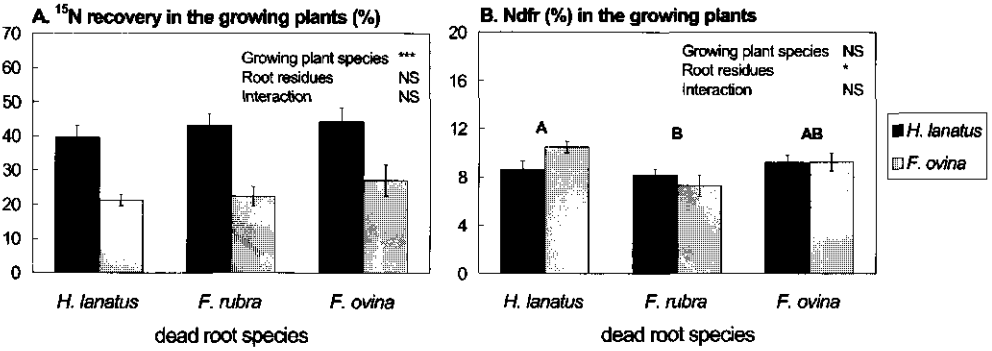

Figure 4. A. ^{15}N recovery (%) and **B.** Ndfr (%) in the growing plants of *Holcus lanatus* or *Festuca ovina* grown for 6 weeks in pots with dead roots of *Holcus lanatus*, *Festuca rubra* or *Festuca ovina*. Values are means ± standard errors. Different uppercase letters above sets of bars indicate statistical differences between living plant species ($P < 0.05$). The results of the GLM procedure are in the right corner of the graph; NS, not significant ($P > 0.05$); $P < 0.05$*; $P < 0.01$**; $P < 0.001$***.

PLANT GROWTH AND N UPTAKE IN THE DECOMPOSITION EXPERIMENT

H. lanatus produced five times more plant biomass than *F. ovina* (Fig. 3a). There was no effect of the dead root species on the total biomass production.

Average N accumulation in *H. lanatus* plants (26 mg/pot) was significantly higher than in *F. ovina* plants (14 mg/pot) (Fig. 3b). The dead root species significantly affected N accumulation in the growing plants. N accumulation was significant lower (18 mg/pot) in plants growing in pots with *H. lanatus* dead roots than in plants growing in pots with *F. rubra* or *F. ovina* dead roots (22 mg/pot). There was no significant interaction between the effect of the dead root species and the growing plant species on the N accumulation.

The ^{15}N recovery in *H. lanatus* plants was significantly higher than in *F. ovina* plants (42% and 23%, respectively) (Fig. 4a). The dead root species did not affect the ^{15}N recovery by the growing plants.

The fraction of the nitrogen content in the growing plants that was derived from the dead roots (Ndfr) was calculated on the basis of the ^{15}N content and the N content of the growing plants and the ^{15}N enrichment of the added residues (see materials and methods). The Ndfr in *H. lanatus* plants was not different from that in *F. ovina* plants but the Ndfr in plants grown on *H. lanatus* dead roots (9.6%) was significantly higher than in the plants grown on *F. rubra* dead roots (7.4%) (Fig. 4b).

^{15}N BUDGET

A ^{15}N budget was calculated using the amount of ^{15}N recovered in the soil, the growing plants and the remaining parts of the dead roots and comparing this with the initial amount of ^{15}N in the dead roots (Fig. 5). Approximately 9% to 23% (on average 15%) of the applied residue-^{15}N was lost during the experiment, probably due to denitrification. There was no significant effect of the growing plant species or dead root species on the total ^{15}N recovery.

The distribution of ^{15}N over the different pools depended on the growing plant species. *H. lanatus* plants contained 42% of the total added ^{15}N, 30% was in the soil and only 11% was still in the dead roots (Fig. 5). In contrast, *F. ovina* plants contained only 23% of the total added ^{15}N, 36% was in the soil and 29% was still in the dead roots. Without growing plants, 48% of the added ^{15}N was in the soil and 36% was still in the dead roots.

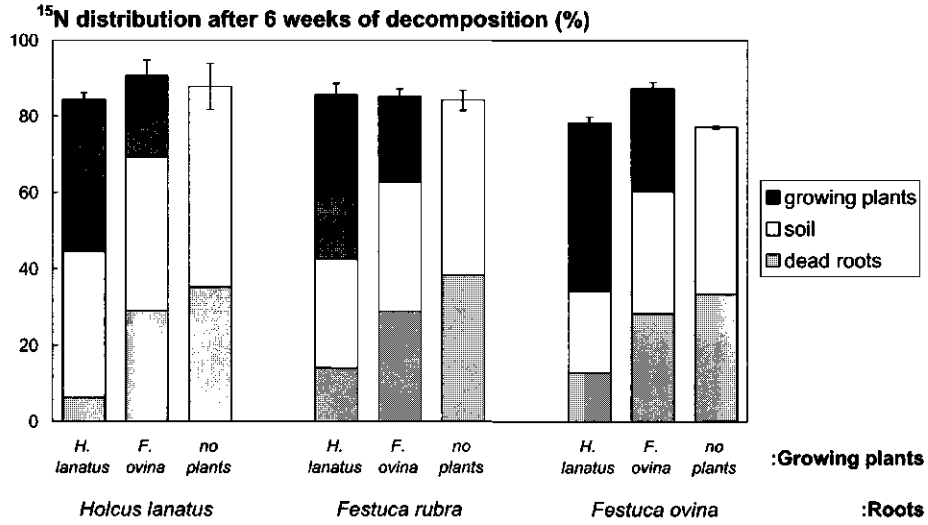

Figure 5. ^{15}N recovery for the nine treatments expressed as percentage of the total amount of ^{15}N added with the dead roots. Values are means ± standard errors.

On average, plants grown on pots with *H. lanatus* dead roots contained 31% of the added ^{15}N, whereas plants grown on *F. rubra* or *F. ovina* dead roots contained 34% and 36%, respectively, of the added ^{15}N.

Discussion

Influence of decomposing roots on N mobilization

The decomposition was expected to be positively correlated with the N concentration (Witkamp 1966, Taylor et al. 1989, Tian et al. 1992) and negatively with the lignin concentration of the decomposing material (Berg and Staaf 1980; Melillo et al. 1982). However, in this study initial chemical characteristics of the dead roots did not reflect the decomposition rates observed. The three grass species did not differ in decomposition rate despite different initial lignin concentrations, %C, %N and C:N ratio between the dead roots of the three species. The lignin concentrations in the plant material were relatively low (*H.*

lanatus 8%, *F. rubra* 16% and *F. ovina* 17%). When lignin concentrations are low, the lignin:N ratio is not as strong a predictor of decay rates as the C:N ratio (Berg 1986; Taylor et al. 1989). Moreover, according to observations by Taylor et al. (1989), lignin is a better predictor for decomposition during the second to fourth month of decay than during the first month, suggesting that lignin was not of great importance for the decomposition rate in our experiment which lasted only 1½ month. Also, the %N and C:N ratio was different between the three species but the differences were apparently too small to cause measurable differences in decomposition rate. Other authors stated that plant species can affect N availability by producing litter with different decomposability (Berendse et al. 1989a; Wedin and Tilman 1990; Hobbie 1992; Van Vuuren et al. 1992; Stelzer and Bowman 1998). The results of the presented study do not confirm this expectation.

Although there were no differences in decomposition rate between the dead roots of the three species, N immobilization by the decomposing roots of *H. lanatus* caused a lower N uptake by the growing plants compared with the dead roots of *F. rubra* or *F. ovina*. The observed differences in N uptake by the growing plants can be explained by the C:N ratio of the decomposing roots. The C:N ratio of *H. lanatus* dead roots was higher compared to the C:N ratio of *F. rubra* or *F. ovina* dead roots. The fraction of mineralized organic N is linearly related to the C:N ratio for equally decomposable materials and, when the C:N ratio of decomposing material is higher than the critical C:N ratio, N immobilization reduces the N availability for the growing plants (Janssen 1996). This N immobilization also explains larger amounts of N in soils from the decomposing roots of *H. lanatus* than of *F. rubra* or *F. ovina* dead roots.

At the end of the decomposition period, the decrease in ^{15}N enrichment was greater in *H. lanatus* dead roots than in *F. ovina* or *F. rubra* dead roots. This can be explained by the attachment of soil microbes to the decomposing roots. The higher C:N ratio of the *H. lanatus* dead roots could have resulted in more microbial immobilization of unlabelled soil N (Janssen 1996) compared to the microbes associated to the *F. rubra* and *F. ovina* dead roots. This would result in a lower ^{15}N enrichment of the remaining dead roots (including the microbes) of *H. lanatus* than of *F. rubra* or *F. ovina* as we have observed.

Despite the lower N availability for the growing plants in pots with dead roots of *H. lanatus*, there was no significant effect of the dead root species on the ^{15}N uptake by the

growing plants. This resulted in a higher contribution of dead root N to the total N uptake (%Ndfr) for plants grown on *H. lanatus* dead roots compared to plants grown on *F. rubra* and *F. ovina* dead roots.

INFLUENCE OF GROWING PLANTS ON N MINERALIZATION

There is no clear evidence that living plants affect nutrient availability (Van Veen et al. 1993). Some studies found that root growth suppressed the decomposition and transformation of dead roots or soil organic matter (Reid and Goss 1982; Sparling et al. 1982; Nicolardot 1995). Other studies found that the presence of living roots increased decomposition (Clarholm 1985). The results of the presented study confirm a stimulating effect of the growing plants on dead root decomposition and N mineralization. In addition, this study shows that different plant species have different effects on decomposition and N availability. This difference results from the variation in root biomass that the plant species produced during the experiment. *H. lanatus* stimulated dead root decomposition more than *F. ovina*. The influence of *F. ovina* plants on the decomposition rate compared to bare soil was not significant but the percentage ^{15}N lost from dead roots affected by growing *F. ovina* plants was higher than the ^{15}N lost from residues in bare soil.

Reid and Goss (1982) proposed several mechanisms which could explain how plant-cover affected decomposition of labelled roots and the N mineralization from plant remains in our study:

a) Competition between living roots and soil microbes for the labelled nitrogen in the dead substrate;

b) Predation around roots of microbes that might otherwise have utilized the labelled substrate;

c) The stimulating effect on soil microbes of fresh materials released from living roots;

d) Production by the root and/or rhizosphere microbes of compounds which inhibit the activity of microbes attempting to use the labelled substrate.

The first hypothesis could explain the different utilization of N in dead roots produced by fast and slow growing species. The higher root decomposition rate and higher dead root N uptake by *H. lanatus* growing plants compared to *F. ovina* growing plants supports the third hypothesis. The high fertility species (*H. lanatus*) in our study produced more root biomass

than the low fertility species (*F. ovina*). Root biomass production is positively correlated with the amount of rhizodeposition (unpublished results) and rhizodeposition is a significant source of carbon for the microbial biomass (Helal and Sauerbeck 1989). This addition of carbon may stimulate microbial degradation of dead roots and old soil organic matter (Rovira 1969). As a result, the degradation of old soil organic matter and dead roots will increase resulting in higher nitrogen availability. Earlier studies showed that high fertility species promote nitrogen mineralization compared with low fertility species (Janzen and Radder 1989; Van Veen et al. 1993; Berendse et al. 1998). In our study, total nitrogen and dead root N uptake by *H. lanatus* plants was almost twice as high as the nitrogen uptake by *F. ovina* plants. This agrees with the observation that *H. lanatus* plants stimulated the decomposition rate of the dead roots more than the *F. ovina* plants.

Dead root decomposition rate and ^{15}N uptake by the growing plants in this study may have been overestimated. If dead roots get attached to living roots it is difficult to separate them and so the living roots may have been contaminated with decomposing roots. However, the decrease of ^{15}N enrichment in the decomposing roots was greater in pots with *H. lanatus* growing plants compared to pots with *F. ovina* or *F. rubra* growing plants (see table 3). This suggests higher microbial activity in dead roots with *H. lanatus* growing plants and explains the increased dead root decomposition rate and ^{15}N uptake by the growing plants observed.

Conclusion

The purpose of this study was to investigate whether high and low fertility species have different effects on nitrogen mineralization from dead roots. The results revealed a new perspective on how plant species can influence nitrogen cycling between decomposing roots, soil, and growing plants. Other authors have already shown that, in the field, shifts in species composition of vegetation or in the physiological status of dominant species follow changes in soil nutrient availability (Berendse 1990; Hobbie 1992; Wedin and Tilman 1990). We found that, in addition to the effects of the production of different quantities or quality of litter, living plant roots may also have a significant effect on nutrient mineralization from soil organic matter. Moreover, our data show that living plants of high fertility species can increase the

nutrient supply rate by stimulating nutrient turnover and thereby facilitate their own growth. On the other hand, dead roots of high fertility species caused N immobilization resulting in a lower N availability for the growing plants. Such species effects, when combined with differences in competitive ability, could provide feedback between processes controlling species composition and ecosystem processes, such as N cycling. This feedback can lead to stable states, but could also accelerate succession processes in the ecosystem.

Acknowledgements

We thank A. van der Werf for his helpful advice on this experiment and G. Jansen, F. Möller and J. van Walsem for their technical assistance on this project. We also appreciate the valuable comments on earlier drafts of the manuscript provided by W. Braakhekke.

Van der Krift A.J., Kuikman P.J., Möller F and Berendse F. (2000) Plant species and nutritional-mediated control over rhizodeposition and root decomposition. *Plant and Soil*, in press.

5. Plant species and nutritional-mediated control over rhizodeposition and root decomposition

Abstract This study focuses on the influence of nitrogen (N) availability and species on rhizodeposition and on decomposition of rhizodeposits, roots and soil organic matter. Four perennial grass species were studied that are characteristic of grassland habitats that differ in nutrient availability. These perennial grass species, *Holcus lanatus* L., *Festuca rubra* L., *Anthoxanthum odoratum* L. and *Festuca ovina* L., were homogeneously labelled with $^{14}CO_2$. Plants were grown on soil without N addition and with N addition (14 g N m^{-2}). After 8 weeks, plants were harvested and root production and the remaining amount of rhizodeposits in the soil were measured. ^{14}C-labelled roots were incubated in fresh soil. Decomposition was measured of 1) the labelled rhizodeposits in the soil in which the plants had been growing and 2) the labelled dead roots incubated in fresh soil, by trapping the evolved $^{14}CO_2$, over 69 days.

In general, decomposability of both roots and rhizodeposits increased when nitrogen availability increased. Moreover, the species differed in their response to N. Higher N supply increased total rhizodeposition of *H. lanatus* and the decomposability of rhizodeposited carbon compounds of this high fertility species was greater than of the low fertility species *F. ovina*, but lower than of *A. odoratum*. The presented study gives no evidence for a relation between root decomposition rate and the nutrient availability of the habitat of the four species. Overall, we suggest on the basis of the results that species can affect nutrient cycling by differences in rates of rhizodeposition and litter production. This offers a mechanism whereby species can influence species replacement during succession.

Keywords: decomposition, grass species, C-labelling, nitrogen-level, rhizodeposition, roots

Introduction

Plant species can have important effects on nutrient cycles and soil fertility (Vitousek et al. 1987; Berendse et al. 1989b; Wedin and Tilman 1990; Van Vuuren et al. 1992; Berendse et al. 1998). Such effects of individual plant species on ecosystem processes may impact on the dynamics of species composition of a plant community. The significance of individual species for ecosystem processes can be understood from the response of species to environmental factors and, conversely, from how species can affect their environment (Wedin and Tilman 1990; Vinton and Burke 1995; Wardle et al. 1998). Plant species can affect carbon and nitrogen (N) cycles by rhizodeposition through their living roots (Grayston et al. 1996) and by the production of litter and dead roots (Van Vuuren and Berendse 1993; Bloemhof and Berendse 1995; Brevedan et al. 1996; Aerts and De Caluwe 1997). Moreover, soil N supply affects rates and amounts of rhizodeposition and litter production and consequently the effect of plants on the N cycle (Janzen and Radder 1989; Van Veen et al. 1993; Bremer and Kuikman 1997).

Living plant roots supply substantial amounts of carbon to the soil (Grayston et al. 1996). The release of organic substances from roots (rhizodeposition) like exudates, mucilages and lysates, is a significant source of carbon for the microbial biomass (Helal and Sauerbeck 1989; Grayston et al. 1996). Rhizodeposits have an important stimulatory effect on microbial growth and activity because they are readily assimilated (Grayston et al. 1996). Therefore, microbial use of rhizodeposited carbon substrates has a major influence on nutrient availability (Grayston et al. 1996). It is important to gain more knowledge on the factors affecting the release of carbon components by living plants, to understand their effect on the microbial activity and N mineralization. There is clear evidence that species that naturally grow on soil with high N availability (high fertility species) promote N mineralization compared to species that naturally grow on soil with low N availability (low fertility species) (Berendse et al. 1998). However, no clear view yet exists on any active regulation of N mineralization in soil by living plants (Heal et al. 1997).

Root death might be equally important for carbon flow as rhizodeposition (Swinnen 1994). Generally, litter of low fertility plant species is more difficult to decompose than litter of high fertility species because of higher concentrations of decay resistant plant compounds (Taylor et al. 1989; Berendse et al. 1989b; Wedin and Tilman 1990; Janssen 1996). Due to these interspecific differences in decomposability, species are expected to either accelerate or slow down the nutrient cycling in their respective habitats. However, not only the interspecific variation but also phenotypic responses to changes in soil N availability may alter litter chemistry and thereby the litter decomposition rate and the release of nutrients (Aerts and De Caluwe 1994; Vitousek et al. 1994; Aerts and De Caluwe 1997). It is not clear whether N availability during the growth of the plant has different effects on litter decomposability of high fertility and low fertility species. If this is true, changes in N availability during succession may accelerate as a result of different responses in high fertility species and low fertility species to the soil nutrient supply. As a consequence, a shift in plant species composition may be driven by plant induced changes in N mineralization. In hay meadows, where the aboveground plant material is removed regularly, the major flow of carbon and other nutrients from the plant to the soil occurs through root turnover and through the activity of living roots. The aims of our study were: (1) to test if there is significant variation among species in rhizodeposition of carbon and in the decomposability of rhizodeposits and roots; and (2) to study the influence of N availability on rhizodeposition and the decomposability of rhizodeposits and dead roots.

For litter decomposition measurements the litterbag method is a frequently applied technique (Andren 1987; Berendse et al. 1989b; Bloemhof and Berendse 1995; Aerts and De Caluwe 1997; Wardle et al. 1997). This technique was developed to elucidate decomposition in undisturbed soil systems with a litter layer. However, litterbag studies of buried substrates appear to provide an incomplete measure of mass loss and N dynamics of buried litter in grassland soils (Seastedt et al. 1992; Tian et al. 1992). Consequently, we measured decomposition rates in our study by trapping evolved $^{14}CO_2$ from ^{14}C-labelled root materials.

Material and Methods

We selected four perennial grass species, *Holcus lanatus* L., *Festuca rubra* L., *Anthoxanthum odoratum* L. and *Festuca ovina* L., common in hay meadows and with a wide distribution in Western Europe (Weeda 1994). The four grass species are characteristic of different levels of soil fertility and which have different maximum relative growth rates. Moreover, these species appear successively in hay meadows that are withdrawn from agricultural use for the purpose of restoration of plant species diversity. In these hay meadows application of fertilization is stopped and the annual removal of the organic matter after mowing results in a decline in N mineralization and productivity. Under such condition, where the nitrogen outputs exceed the inputs, reversed succession takes place (Olff and Bakker 1991; Olff et al. 1994). During reversed grassland succession *H. lanatus* appears during the first years of mowing management. After c. 10 years *F. rubra* becomes one of the dominant species. *A. odoratum* becomes dominant after c. 15 years and *F. ovina* appears when the soil has become nutrient-poor (Olff and Bakker 1991; Oomes and Van der Werf 1996). Moreover, *H. lanatus* (225 mg $g^{-1}day^{-1}$) grows faster than *F. ovina* (140 mg $g^{-1}day^{-1}$) whereas *A. odoratum* (208 mg $g^{-1}day^{-1}$) and *F. rubra* have an intermediate potential relative growth rate (176 mg $g^{-1}day^{-1}$) (Van der Werf et al. 1998).

Labelling of the plants

Single 10 to 20-day-old seedlings of *H. lanatus, F. rubra, A. odoratum* and *F. ovina* were set in PVC-tubes (4.6 cm in diameter and 22 cm in length). The bottom of the tubes was sealed with 30 μm gauze to prevent roots from growing out and to allow water to enter the tubes. A layer of 3 cm silversand (1.4 g cm^{-3}) was added at the bottom of the tubes. The tubes were filled with a soil mixture of sandy soil and sand (2:1, 1.4 g cm^{-3}) and covered with grit. Each tube received 15 ml nutrient solution without N (N-) or with N (N+; 24 mg N per tube). The nutrient solution contained KNO_3 (only for N+ treatment; 11.55 mg ml^{-1}); KH_2PO_4 (1.34 mg ml^{-1}); $MgSO_4 \cdot 7H_2O$ (4.73 mg ml^{-1}); K_2SO_4 (1.54 mg ml^{-1}); $MnSO_4 \cdot H_2O$ (20.00 μg ml^{-1}); H_3BO_3 (26.90 μg ml^{-1}); $ZnSO_4 \cdot 7H_2O$ (25.06 μg ml^{-1}); $CuSO_4 \cdot 5H_2O$ (0.78 μg ml^{-1}); $Na_2MoO_4 \cdot 2H_2O$ (1.26 μg ml^{-1}); pH 6.5. The tubes were placed next to one another in a tray with felt at the bottom. Tubes without plants were included. The plant were grown in the

ESPAS (Experimental Soil Plant Atmosphere System; Gorissen et al. 1996) growth chambers in a continuously labelled $^{14}CO_2$ atmosphere (specific activity 0.70 kBq mg^{-1} C). During the growth period, light and dark periods were 14 and 10 hours respectively. During the light period, the temperature was 18° C, relative air humidity was 70%, CO_2 concentration was 350 PPM and Photosynthetic Active Radiation (PAR) was 350 µmol m^{-2} s^{-1} at plant level. During the dark period the temperature was 14° C, relative air humidity was 80% and CO_2 concentration was 350 PPM. Plants were watered by adding water to the tray once or twice per week after weighing individual columns and measuring water loss.

Towards the end of the growth period no water was added to allow the soil to dry and the plants to die off. Once soil moisture content was < 4% the shoots were clipped. Intact soil columns were left to dry until soil moisture content was < 1% and then stored for 1 year in closed containers until the start of the decomposition experiment.

DECOMPOSITION EXPERIMENT

The decomposition experiment was started in August 1997 and carried out in a climate chamber in a complete randomized design with four replicates. We measured decomposition of (1) ^{14}C-labelled dead roots incubated in fresh soil and of (2) the labelled rhizodeposits in the soil on which the plants had been growing in the ESPAS.

To separate the root from the soil, the root/soil core was pushed out of the PVC tube onto a 2 mm sieve. The upper 4 cm and the lower 4 cm of the core were removed. Roots were separated and cleaned carefully by hand. The soil was sieved on a 2 mm and a 1 mm sieve to further remove any visible root fragments. Root samples were divided into two subsamples for chemical analyses and for use in the decomposition experiment. Roots for the decomposition experiment were clipped into 1.5 cm pieces, and two subsamples of 50 mg each were mixed with 50 g of pre-incubated fresh soil. Soil without roots had been pre-incubated in 125 cm^3 plastic cups (17-18% w/v moisture) two months before the start of the experiment to ensure a stable state in microbial activity in the soil at the beginning of the decomposition experiment. Cups were closed to prevent water loss and stored at 14°C. The soil was a mixture of sandy soil from an arable field in the "Gelderse Vallei" in The Netherlands (particle size < 2 mm) and sand (2:1). The soil mixture had the following characteristics: 5.3 $pH_{(H2O)}$, 0.98 g total N kg^{-1}

soil, 0.47 g total P kg^{-1} soil, C:N ratio of 11.45 and 26 g organic matter kg^{-1} soil. At the start of the experiment the moisture content of the soil was still 17-18% (w/v).

Duplicate samples of 41 g sieved soil from the ESPAS tubes with and without plants were incubated in empty 125 cm^3 plastic cups, after adjusting to 17-18% moisture content. The cups were incubated in a 1.5 l jar with 10 ml water at the bottom to maintain a high humidity, and with a 25 ml cup with 10 ml of 0.5 M NaOH to capture released CO_2. The jars were closed air-tight and incubated in a climate chamber at 14°C. The NaOH traps were replaced once a week.

For each of the four species from N+ or N- soil, duplicate samples of the roots and duplicated samples of the ESPAS soil with their rhizodeposits were incubated in jars. So, this gave in total 64 jars (2 nutrient levels x 4 species x 4 replicates x 2 jars) with decomposing roots and 64 jars with ESPAS soil including decomposing rhizodeposits. Furthermore, 8 jars with pre-incubated soil without roots and 8 jars with ESPAS soil without rhizodeposits from tubes on which no plants had been growing were used as a control.

After 69 days, the soils were harvested. Cups with soil and roots were dried at 40°C and then roots were collected from the soil by sieving subsequently through a 2 mm and a 1 mm sieve. Soil without roots was also sieved. Soil was removed manually from roots as well as possible.

ANALYSES

Roots and soil were analysed at the start of the incubation experiment and at the final harvest after 69 days. At the start of the decomposition experiment, roots were shaken in 250 ml water to remove soil particles and then dried for four days at 40°C and grounded. The C concentration and N concentration of the initial roots was measured with an element analyser (Fisons Instruments, EA 1108). The ^{14}C content in roots and soil was determined by scintillation counting after chemical destruction with $K_2Cr_2O_7$ (100 g/l). Rhizodeposited C was calculated from the measured activity (Bq) in the sieved soil from the ESPAS-tubes and the specific activity of the plants (Bq/mg C). After incubation, the absorbed CO_2 in the NaOH was determined by titration of the remaining NaOH with HCl after precipitation HCO_3^-/CO_3^{2-} by excess of $BaCl_2$. The $^{14}CO_2$ in the NaOH was calculated from the measured activity (Bq) by scintillation counting using a liquid scintillation counter (Packard, TRI-CARB 2100TR).

STATISTICAL ANALYSES

Data were analysed ANOVA to determine whether there was a treatment effect on the decomposition rate of the dead roots and of the soil organic matter. This effect was split into "the effect of N availability" and "the effect of the different species". Analyses of variance were performed with Genstat (531) applied to a complete split plot design. Percentages were angular transformed and Tukey's HSD tests were used to test for significant differences among means.

Pearsons correlation coefficients were calculated for 1) the amount of rhizodeposition versus root biomass production, 2) root C evolution as CO_2 versus rhizodeposition and 3) root decomposition versus initial N and C concentration (SPSS 7.0 1995).

Results

ROOT GROWTH AND RHIZODEPOSITION

There was a significant interaction between species and N effects on the amount of root biomass production ($F=9.05^{***}$). N application had only a significant effect on the root biomass production of the *H. lanatus* plants ($p<0.05$). With N addition root biomass was significantly higher in *H. lanatus* (2.68 mg/g soil) than in the three other species. *F. rubra* and *A. odoratum* produced intermediate amounts of root biomass (1.70 and 1.79 mg/g soil respectively) and *F. ovina* produced a significantly ($p<0.05$) lower amount of root biomass (0.51 mg/g soil). Without N addition, root biomass production was similar in *H. lanatus*, *F. rubra* and *A. odoratum* (1.22, 1.37 and 1.56 mg/g soil respectively) but the root biomass production in the *F. ovina* plants was significantly lower (0.36 mg/g soil). The N and C concentrations in the roots of *H. lanatus*, *F. rubra*, *A. odoratum* and *F. ovina* were 0.7-1.7% and 48-51% and the C:N ratio was 30-77 (Table 1).

There was also a significant interaction between species and N effects on the amount of rhizodeposited C in the soil ($F=8.36^{***}$). At high N, net rhizodeposition had been significantly higher in *H. lanatus* (0.39 mg C/g soil) than in the three other species. *F. rubra* and *A. odoratum* produced intermediate amounts of rhizodeposits (0.17 and 0.28 mg C/g soil

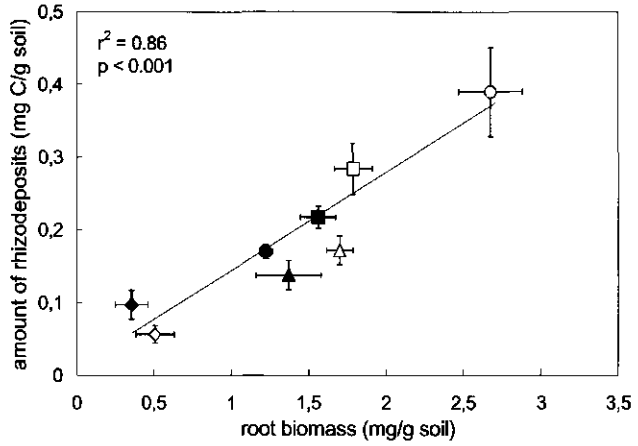

Figure 1. Rhizodeposited C present in the soil (mg root C/g soil) of the species *F. ovina* (◆), *A. odoratum* (■), *F. rubra* (▲) or *H. lanatus* (●) versus root biomass at the end of the growing period (mg roots/g soil). The plants were grown at 2 nutrient levels: fertilizer with N (open dots) and fertilizer without N (closed dots). Values are means ± standard errors.

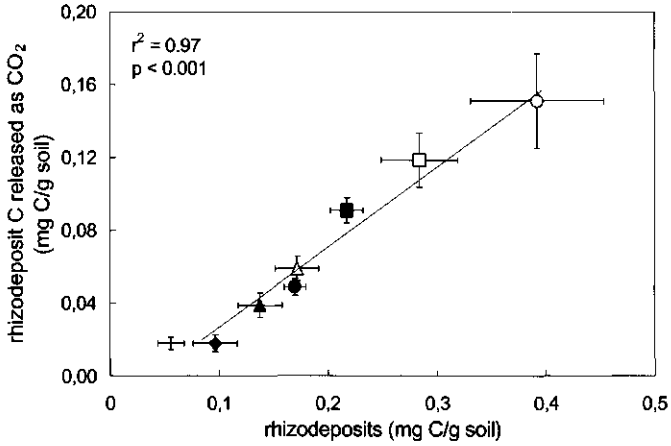

Figure 2. CO_2 evolution (mg C/g soil) from rhizodeposits in soil produced by *F. ovina* (◆), *A. odoratum* (■), *F. rubra* (▲) or *H. lanatus* (●) versus the amount of rhizodeposited C present in the soil at the end of the growing period (mg C/g soil). The plants were grown at 2 nutrient levels: fertilizer with N (open dots) and fertilizer without N (closed dots). Values are means ± standard errors.

Table 1. Nitrogen (N) and Carbon (C) concentration (g/100g) and C:N ratio at the end of the growing period in the ESPAS of *Holcus lanatus*, *Festuca rubra*, *Anthoxanthum odoratum* and *Festuca ovina* roots used for the decomposition experiment. The plants were grown at 2 nutrient levels: fertilizer with N (N+) and fertilizer without N (N-). Values are means (SE).

Nutrient level	Species	N	C	C:N ratio
N+	*H. lanatus*	0.92 (0.05)	48.59 (0.09)	53.13 (2.51)
	F. rubra	0.95 (0.05)	49.69 (0.22)	52.99 (2.76)
	A. odoratum	0.77 (0.06)	48.14 (0.14)	62.98 (3.94)
	F. ovina	1.71 (-)	50.88 (-)	29.75 (-)
N-	*H. lanatus*	0.84 (0.08)	49.96 (0.16)	60.77 (5.44)
	F. rubra	1.12 (0.25)	50.08 (0.43)	50.57 (9.26)
	A. odoratum	0.65 (0.09)	47.58 (0.33)	76.80 (8.36)
	F. ovina	0.93 (-)	50.15 (-)	54.09 (-)

respectively) and *F. ovina* produced a significantly ($p<0.05$) lower amount of root biomass (0.06 mg C/g soil). At low N, net rhizodeposition was not different between *H. lanatus*, *F. rubra* and *A. odoratum* (0.17, 0.14 and 0.22 mg C/g soil respectively), but the difference between *A. odoratum* and *F. ovina* (0.09 mg C/g soil) plants was significant ($p<0.05$). N fertilization had a significant effect only on the rhizodeposition of the *H. lanatus* plants ($p<0.05$). The amount of rhizodeposits was positively correlated with root biomass (Fig. 1).

DECOMPOSITION OF RHIZODEPOSITS

CO_2 evolved from rhizodeposits increased linearly with the amount of rhizodeposits in the soil (Fig. 2). *H. lanatus* and *A. odoratum* grown at a high N level produced a large root biomass and a large amount of rhizodeposits (Fig. 1) resulting in an increased CO_2 evolution from the rhizodeposits (Fig. 2). *F. ovina* produced a small root biomass and a small amount of rhizodeposits resulting in a lower rate of CO_2 evolution.

The decomposition of the rhizodeposits, calculated as the percentage of the amount of rhizodeposits present at the start of the decomposition experiment, was different between species (Table 2). Rhizodeposits of *A. odoratum* decomposed faster than the rhizodeposits of the other three species. *H. lanatus* rhizodeposits decomposed faster than those of *F. ovina* did.

Table 2. The percentage of the original amount of rhizodeposited ^{14}C that is decomposed and released as $^{14}CO_2$ during a period of 69 days. The rhizodeposits were produced by roots of *Festuca ovina*, *Anthoxanthum odoratum*, *Festuca rubra* or *Holcus lanatus* during a preceding growing period at 2 nutrient levels N+ (fertilizer with N) and N- (fertilizer without N) during a previous growing period in the ESPAS. Values are means ± standard errors. Different superscript letters indicate statistical differences between the means ($p<0.05$).

		Species				
		F. ovina	*A. odoratum*	*F. rubra*	*H. lanatus*	mean
Nutrient level	N-	20.35 ± 4.12	42.65 ± 2.70	27.65 ± 2.68	28.89 ± 2.61	**29.88^{B}**
	N+	33.30 ± 4.33	42.61 ± 4.37	36.17 ± 4.99	41.19 ± 6.19	**38.32^{A}**
	mean	**26.82^{C}**	**42.63^{A}**	**31.91^{BC}**	**35.04^{B}**	

Table 3. Total dead root decomposition measured as the amount of ^{14}C released as $^{14}CO_2$ during the experimental period of 69 days as percentage of initial amount of ^{14}C in dead roots from *Festuca ovina*, *Anthoxanthum odoratum*, *Festuca rubra* or *Holcus lanatus* at the start. Dead roots originated from plants that had been growing at 2 nutrient levels N+ (fertilizer with N) and N- (fertilizer without N) during a previous growing period in the ESPAS. Values are means ± standard errors. Different superscript letters indicate statistical differences between the means ($p<0.05$).

		Species				
		F. ovina	*A. odoratum*	*F. rubra*	*H. lanatus*	mean
Nutrient level	N-	12.27 ± 1.80	25.51 ± 2.28	14.77 ± 1.72	11.04 ± 0.61	**16.27^{B}**
	N+	12.86 ± 1.21	25.30 ± 1.96	20.36 ± 0.70	16.41 ± 1.47	**19.13^{A}**
	mean	**12.59^{C}**	**25.41^{A}**	**17.56^{B}**	**13.72^{C}**	

Table 4. Root ^{14}C remaining in the soil after 69 days as percentage of initial amounts of dead roots from *Festuca ovina*, *Anthoxanthum odoratum*, *Festuca rubra* or *Holcus lanatus*. Dead roots originated from plants that had been growing at 2 nutrient levels N+ (fertilizer with N) and N- (fertilizer without N) during a previous growing period in the ESPAS. Values are means ± standard errors. Different superscript letters indicate statistical differences between the means ($p<0.05$).

		Species				
		F. ovina	*A. odoratum*	*F. rubra*	*H. lanatus*	mean
Nutrient level	N-	6.09 ± 0.53	13.88 ± 1.92	9.22 ± 1.63	8.72 ± 1.01	**9.83**
	N+	6.61 ± 0.83	11.01 ± 0.78	11.04 ± 1.04	9.66 ± 1.30	**9.78**
	mean	**6.37^{C}**	**12.45^{A}**	**10.13^{B}**	**9.19^{B}**	

In general, rhizodeposits of plants grown at a high N level decomposed faster than rhizodeposits of plants grown at a low N level.

Neither of the four species had a significant effect on the decomposition of soil organic matter ($p>0.05$)).

DECOMPOSITION OF DEAD ROOTS

Root decomposition ranged from 11-26% (Table 3). *A. odoratum* dead roots decomposed faster than dead roots of *F. rubra* and dead roots of *F. rubra* decomposed faster than those of *F. ovina* or *H. lanatus*. Roots grown on N rich soil decomposed faster than roots from N poor soil. There was no significant interaction between species and N-treatment.

Root residue decomposition was not related to the initial percentage N or C:N ratio of the roots. However, despite the small variation in the initial percentage C of the grass roots, root residue decomposition was negatively related to this initial percentage C (Fig. 3). This significant negative correlation was the result of the lower initial percentage C in *A. odoratum* roots and their higher decomposition compared to the other three species.

The amount of root ^{14}C still present in the soil after 69 days of decomposition, calculated as the percentage of the initial root ^{14}C amount, differed between species (Table 4). Soil with *F. ovina* dead roots had the lowest percentage root C after 69 days. The percentage root C in the soil was intermediate in soils with *H. lanatus* and *F. rubra* dead roots. Soils with *A. odoratum* roots contained the highest percentage root C after 69 days. The N supply rate had no effect on the root ^{14}C content of the soil. The more root-derived C was in the soil, the more root-derived C was respired.

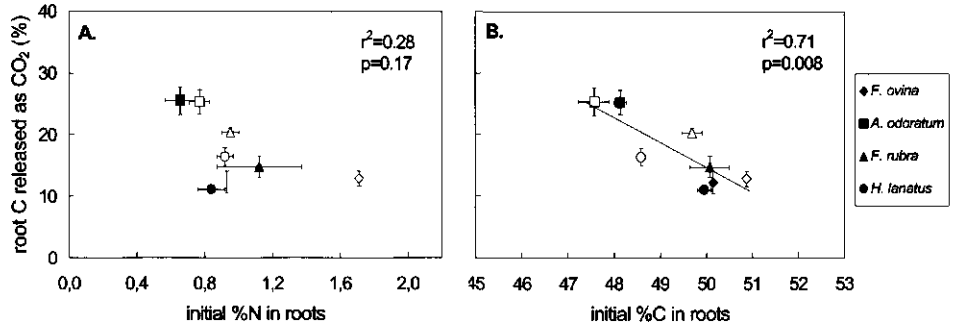

Figure 3. A. The initial percentage N and **B.** the initial percentage C of *Festuca ovina*, *Anthoxanthum odoratum*, *Festuca rubra* or *Holcus lanatus* labelled dead roots versus the C loss as CO_2 from these roots during a 69-days decomposition period. Open dots refer to the N+ treatment and closed dots to the N- treatment. Values are means ± standard errors.

Discussion

RHIZODEPOSITION

Rhizodeposition during the growing period is defined as the addition of root C to the soil either as a result of exudation or root death. Rhizodeposition differed among species and the amount of rhizodeposits was positively correlated with root biomass accumulation. Our data fit with recent studies that postulated that a higher root mass production would result in a greater rhizodeposition of carbon compounds (Cotrufo and Gorissen 1997; Haynes and Beare 1997). Moreover, as a general trend, the high and moderate fertility species *H. lanatus*, *F. rubra* or *A. odoratum*, deposited more C in the rhizosphere than the low fertility species *F. ovina*. Earlier studies have suggested a major impact of nutrient availability on rates of plant exudation (Van Veen et al. 1993; Grayston et al. 1996) but have not described interspecies differences. Different effects of soil nutrient supply on high fertility species versus low fertility species could accelerate decrease in N depletion during reversed grassland succession. N availability increased rhizodeposition of *H. lanatus*, a high fertility species, as a result of its increased root mass (Fig. 1), but had negligible effects on rhizodeposition of the intermediate (*F. rubra* and *A. odoratum*) and low fertility (*F. ovina*) species. So, at an increased N supply, rhizodeposition

rates are higher in the high fertility species *H. lanatus* compared to the low fertility species *F. ovina* because of the larger root growth response in this high fertility species.

DECOMPOSITION OF RHIZODEPOSITS

There are two possible causes of differences in the measured amounts of rhizodeposited carbon compounds. An increase in the amount of rhizodeposits can be caused either by greater rhizodeposition, including root death, during growth or by greater resistance to decomposition among the root-derived carbon compounds (Grayston et al. 1996). Since the amount of deposited carbon compounds was measured at the end of the ESPAS growing period, we could not assess turnover of root-derived C within the first 8 weeks. We also did not assess the chemical characteristics of the rhizodeposited compounds. However, the decomposability of the rhizodeposited carbon compounds should reflect their chemical characteristics (Heal et al. 1997). We expected the rhizodeposits of the high fertility species to decompose faster than those of the low fertility species (Taylor et al 1989; Berendse et al. 1989b; Wedin and Tilman 1990; Janssen 1996). On this basis we predicted that *H. lanatus* would decompose faster than *F. ovina* and that *F. rubra* and *A. odoratum* would decompose at an intermediate rate. The decomposition rate of the deposited organic compounds of the four species increased with the potential growth rate of each species. Rhizodeposits from the high fertility species *H. lanatus* decomposed at a faster rate than rhizodeposits from the low fertility species *F. ovina*, but at a slower rate than the rhizodeposits of *A. odoratum*. Moreover, the four grass species used in our study had no significant effect on the decomposition rate of soil organic matter.

Root derived organic compounds in N-rich soil mineralized faster than those derived in N poor soil, so higher N supplies during growth of the plants increased the decomposition of their rhizodeposits. Several explanations are possible for the increased decomposition of rhizodeposits. Species could respond to changes in nutrient availability by depositing not only different quantities of organic compounds but also organic compounds of a different quality, thereby altering decomposition rates (Grayston et al. 1996). Another explanation is that higher soil N content can increase the activity of soil microbial biomass (Van Ginkel et al. 1997), resulting in increased decomposition rates. Therefore, the measured amount of final rhizodeposits at the end of the growth period may underestimate total rhizodeposition. When the amounts of measured rhizodeposits increase, the decomposability also increases. This

further supports our conclusion that living plants of high fertility species deposited more C in the rhizosphere than those of low fertility species. In addition the rhizodeposits of high fertility species mineralized faster, so these species likely activate the nutrient cycling more than low fertility species. However, when the N availability declines, this effect of high fertility species becomes less significant, as we learned from the response of *H. lanatus* on N fertilization and the absence of such a response of *F. ovina*.

DECOMPOSITION OF DEAD ROOTS

In our study, reduced N availability during plant growth resulted in a lower decomposition rate of the dead roots of all four species (Table 3). Measured amounts of root C incorporated in the soil organic matter after 69 days of incubation showed the same pattern. We therefore conclude that species respond to lower soil fertility by a lower decomposability of their residues. This will result in a lower turnover rate of nutrients. Such a process will likely alter nutrient dynamics in the soil and species replacement during succession. Other authors have already found that changes in N availability are an important cause of changes in plant species composition during succession in N-limited ecosystems (Berendse and Elberse 1990; Olff and Bakker 1991). In this study, we found evidence that, the opposite is also true: species can affect the N availability and thereby have an important impact on the progress of the succession.

In addition, plant species differed in dead root decomposition rates. The presented study provided no evidence for the hypothesis that the decomposition rate of dead roots is related to the potential relative growth rate of the four species. Species differed in dead root decomposability, but the decomposability was higher in *A. odoratum* compared to *H. lanatus* and the decomposability in *F. rubra* was intermediate. Moreover, there was no significant difference in decomposition rate between dead roots of *H. lanatus* and *F. ovina*.

Our results contradict earlier research, which indicate that litter of low fertility plant species is more difficult to decompose than litter of high fertility species because of higher concentrations of decay resistant plant compounds (Taylor et al. 1989; Berendse et al. 1989b; Wedin and Tilman 1990). Differences in the quality of plant litter, expressed as C:N ratio, have been suggested to be responsible for differences in decomposition rates (Berg and Staaf 1980; Taylor et al. 1989; Janssen 1996; Ball and Drake 1997). However, in our study, differences in

the decomposition rate of dead roots could not be explained by differences in their N content and C:N ratio. This agrees with other recent observations on C:N ratio and decomposability (Van Ginkel et al. 1996; Franck et al. 1997; Gorissen and Cotrufo 2000). Our results indicate that quality factors other than N content and C:N ratio may determine the decomposition rate. The variation in decomposability among species was related to the initial C concentrations in the roots although the differences between these initial C concentrations were very small. When the C content of plant biomass increases, the form of C could change in more recalcitrant components, like lignins and phenols (Heal et al. 1997) and other authors found a negative relation between these plant compounds and the decomposition rate (Berg and Staaf 1980; Melillo et al. 1982; Taylor et al. 1989). However, the differences in C concentration in the presented study are too small to expect different form of C compounds. The relationship between litter quality characteristics and decomposition rates are largely empirical and there is still insufficient understanding of the interactions of the biochemical constituents of litter at the cellular level where microbial decomposition takes place (Heal et al. 1997).

The most significant effect of N availability in ecosystems on decomposition processes in grasslands is its effect on primary productivity. When N availability changes during succession the total amount of produced root biomass can change depending on the species (Cotrufo and Gorissen 1997). From this information combined with our results, we expect that an increase in nutrient supply will enhance the addition of dead roots to the soil and the decomposition of these roots to a different degree in different species. This could have important effects on the nutrient cycle.

Acknowledgement

We thank Wim Didden, Henk van Ginkel and Ton Gorissen for their advice related to this experiment, and Roy Geerts and Jan van Walsem for their technical assistance. We also appreciate the valuable comments on earlier drafts of the manuscript provided by Catharina de Kat-Reijnen, Adrie van der Werf and several PhD-students of our department.

Van der Krift A.J., Kuikman P.J. and Berendse F. The effect of living plants on root decomposition of four grass species. *Submitted for publication.*

6. The effect of living plants on root decomposition of four grass species

Abstract In the presented study we focussed on one of the possible pathways through which plant species can regulate soil nutrient supply. We tested whether living plants of *Holcus lanatus* and *Festuca ovina* can affect the decomposition rate of dead roots of *Holcus lanatus*, *Festuca rubra*, *Anthoxanthum odoratum* and *Festuca ovina* and whether this effect is species-specific and dependent on nitrogen (N) availability. The selected perennial grass species are typical of grassland habitats in a range from high to low nitrogen availability. Seedlings of *H. lanatus*, *F. rubra*, *A. odoratum* and *F. ovina* were homogeneously labelled with $^{14}CO_2$ for eight weeks. Plants were grown on soil at two nitrogen levels: one without additional nitrogen and one with nitrogen addition (14 g N m^{-2}). After 8 weeks plants were dried and stored for 2 years.

At the start of the decomposition experiment ^{14}C labelled roots were separated from soil and incubated in litterbags (mesh width 1mm) in fresh soil. These ^{14}C labelled roots were left to decompose for 19 weeks in an open greenhouse in soil planted with *H. lanatus* or *F. ovina* and in unplanted soil. After the incubation period, the decomposition of the ^{14}C labelled roots of the four species was measured. The loss of mass and of ^{14}C from the dead roots were calculated and the living plant biomass and C, N and P contents of the live plants were determined.

Root decomposition rates differed among the species. We found no evidence to support our hypothesis that dead roots of high fertility species (i.e. *H. lanatus* and *F. rubra*) decompose faster than dead roots of low fertility species (i.e. *A. odoratum* and *F. ovina*). In unplanted soil, the mass loss and total ^{14}C loss of *A. odoratum* dead roots were higher than those of *H.*

lanatus, *F. rubra* and *F. ovina* dead roots. Dead roots of *F. ovina* lost less mass and total ^{14}C than dead roots of *H. lanatus*. Dead roots of *F. ovina* decomposed faster in the presence of living *F. ovina* plants but, dead *A. odoratum* roots from the N fertilized treatment decomposed slower in the presence of living *F. ovina* plants. It seems likely that living plants like *F. ovina* exude carbon compounds that could stimulate the growth rate of soil microbes and thereby increase dead root decomposition and mineralization. The results indicate that whether plant exudates stimulate dead root decomposition depends on the quality of these dead roots. These effects of plant species on nutrient cycling processes could be an important regulating mechanism promoting ecosystem stability.

Keywords: ^{14}C, C:N ratio, nitrogen availability, root decomposition

Introduction

The cycling of carbon (C) and nitrogen (N) is an important ecosystem process. Dominant plant species can have important effects on this (Berendse 1990; Wedin and Tilman 1990; Van Vuuren et al. 1992). The importance of the effects of plant species on ecosystem processes lies in the potential for feedback to develop between the effects of plant species and the effects of ecosystem processes (Wedin and Tilman 1990; Vinton and Burke 1995; Berendse 1998; Wardle et al. 1998). This could have important consequences for ecosystem functioning and plant species composition (Berendse et al. 1998).

Living plants can enhance microbial degradation of dead roots and old soil organic matter and N mineralization by adding easily metabolizable carbon compounds to the soil by rhizodeposition (Clarholm 1985; Robinson et al. 1989; Swinnen et al. 1994a and 1994b). However, evidence is contradictory: both negative and positive effects have been observed (Reid and Goss 1982; Sparling et al. 1982; Clarholm 1985; Van Veen et al. 1993; Nicolardot 1995; Bremer and Kuikman 1997). Living plant species not only affect the carbon and nitrogen cycle by the activity of their living roots (Clarholm 1985; Nicolardot et al. 1995; Brevedan et al. 1996; Cadisch and Giller 1997), but also by shoot and root turnover (Berendse et al. 1989; Aerts et al. 1992; Van Vuuren et al 1993; Brevedan et al. 1996). Whether N mineralization or N immobilization takes place depends on the C:N ratio in the decomposable

resources produced by the plant (Robinson et al. 1989; Cadisch and Giller 1997; Sakala et al. 2000).

In addition, stimulation or inhibition of the microbial growth by plant species depends on the availability of N in the ecological habitat of the species. Soil nitrogen supply may affect both rhizodeposition and litter production and hence influence the effect of plants on the nitrogen cycle (Liljeroth et al. 1990; Van Veen et al. 1993; Grayston et al. 1996; Bremer and Kuikman 1997). Moreover, when soil nitrogen availability changes litter chemistry and the release of nutrients may be altered. The degree of change in litter decomposability and release of nutrients depends on species and on phenotypic responses in litter chemistry to changes in nitrogen availability (Aerts and De Caluwe 1994; Vitousek et al. 1994; Aerts and De Caluwe 1997). Earlier studies have shown that compared with species adapted to less fertile conditions, species adapted to fertile conditions promote nitrogen mineralization (Janzen and Radder 1989; Van Veen et al. 1993; Berendse et al. 1998). If plants were to have an advantage from regulating nutrient fluxes in the rhizosphere, this would occur through conservation of nutrients when they are in excess, or through enhanced mineralization of nutrients when they are deficient (Van Veen et al. 1993).

In the study presented here we tested whether living plant species can affect the decomposition of dead roots and whether these effects are species-dependent. We also studied the effect of nitrogen availability during the production of the roots on their subsequent decomposability and whether this would impact the growth rate and N uptake of the plants that were grown in substrate containing the decomposing root material. In this study, we focussed on the effect of growing plants of two grass species on the decomposition of the dead roots of four different grass species. We hypothesized that: 1) Living plants enhance dead root decomposition and species requiring fertile habitats accelerate the rate of decomposition more than those from low fertility environments, because they produce more root biomass and consequently more exudates.

2) N availability during the growing period of the dead roots has an important influence on the quality of the dead roots. As a result, an increased N availability during the production period increases the decomposability of the plant material and thereby the N mineralization.

3) Dead roots of species from nutrient-rich habitats decompose more rapidly than those of species from nutrient poor-habitats.

4) When N availability increases, living plants of species from fertile habitats will increase their growth more than species from low fertility habitats. So, when grown in substrate containing dead roots from N-rich soil the decomposition-stimulating effect of high fertility species will increase more than the effect of low fertility species, than when the species are grown on substrate containing dead roots from N-poor soil.

To test these hypotheses we conducted a decomposition experiment with ^{14}C labelled roots for one growing season. By using the carbon tracer ^{14}C a distinction could be made between native-soil carbon (i.e. the carbon present in the soil) and carbon derived from the dead roots. We used the litterbag method, which is a technique frequently applied for litter decomposition measurements (Wardle et al. 1997). We calculated decomposition rates on the basis of the ^{14}C loss from ^{14}C-labelled dead root materials in litterbags with a large mesh width (1 mm). The litterbags enabled us to easily recover the remaining dead roots after the decomposition period. The large mesh width of the litterbags optimized the contact and interactions of the dead roots with the soil and allowed the roots of living plants to grow through the litterbags.

Materials and Methods

PLANT LABELLING

In 1996 seedlings 10-20 days old of *Holcus lanatus* L., *Festuca rubra* L., *Anthoxanthum odoratum* L. and *Festuca ovina* L., were labelled with ^{14}C by being grown for 8 weeks in a continuously ^{14}C-labelled atmosphere in the ESPAS (Experimental Soil Plant Atmosphere System; Gorissen et al. 1996). The individual plants were grown in PVC tubes (4.6 cm in diameter and 22 cm length). The bottom of the tubes was closed with 30 μm gauze to prevent roots growing out and to allow water to enter the tubes. A layer of 3 cm silversand (1.4 kg dm^{-3}) was put in the base of the tubes before filling the tubes with a mixture of sandy soil and sand (2:1) (v/v 14% 1.4 kg dm^{-3}). Each tube received 15 ml nutrient solution without N (N-) or with N (N+; 24 mg N per tube). The nutrient solution contained; KH_2PO_4 (1.34 mg ml^{-1}); $MgSO_4 \cdot 7H_2O$ (4.73 mg ml^{-1}); K_2SO_4 (1.54 mg ml^{-1}); $MnSO_4 \cdot H_2O$ (20.00 μg ml^{-1}); H_3BO_3 (26.90 μg ml^{-1}); $ZnSO_4 \cdot 7H_2O$ (25.06 μg ml^{-1}); $CuSO_4 \cdot 5H_2O$ (0.78 μg ml^{-1}); $Na_2MoO_4 \cdot 2H_2O$ (1.26 μg ml^{-1}); pH 6.5. In addition, the solution for the N+ treatment contained 11.55 mg KNO_3 ml^{-1}. The soil was mulched with plastic granules. The tubes were placed next to each

other in a tray lined with felt. Each tube contained a single plant or was plantless. The light and dark periods during the growing period were 14 and 10 hours respectively. During the light period the temperature was 18° C, relative humidity was 70%, CO_2 concentration was 350 PPM and Photosynthetic Active Radiation (PAR) was 350 µmol m^{-2} s^{-1} at plant level. During the dark period the temperature was 14° C, relative humidity was 80% and CO_2 concentration was 350 PPM. Plants were watered by adding water to the tray once or twice per week after weighing individual containers and measuring water loss.

After a growth period of 8 weeks, no water was added, to allow the soil to dry. When soil moisture content dropped below 4%, the shoots were clipped. The intact soil containers were left to dry to soil moisture content below 1% and then stored in closed containers until the start of the decomposition experiment.

Before the start of the decomposition experiment, a sub-sample of the dead roots was taken. These roots were rinsed from soil by shaking them in a bottle with water. Then, the roots were dried at 60°C to constant weight and the C and N concentrations were measured using an element analyser (Fisons Instruments, EA 1108). The percentage N in the roots grown in soil with N addition was 0.9% for *H. lanatus*, 0.9% for *F. rubra*, 0.8% for *A. odoratum* and 1.7% for *F. ovina*. For roots grown in soil without N addition the figures were respectively 0.8%, 1.1%, 0.6% and 0.9%.

DECOMPOSITION EXPERIMENT

The decomposition experiment started on 22 May and lasted until 2 November 1998. It was carried out in an open greenhouse in a completely randomized design. First, small containers (10 cm diameter and 31 cm height) were filled with 2670 g of a mixture of sandy soil and sand (1:1) (v/v 14% and 1.4 kg/dm^3). Before mixing, the sandy soil was sieved through a 4 mm sieve. The soil mixture had the following characteristics: 5.9 $pH_{(H_2O)}$, 1.17 g total N kg^{-1} soil, 0.43 g total P kg^{-1} soil, C:N ratio of 16.6 and 30 g organic matter kg^{-1} soil. The litterbags (5 cm x 5 cm, mesh width 1 mm) were filled with 0.06-0.37 g labelled dead roots of *H. lanatus*, *F. rubra*, *A. odoratum* or *F. ovina* and placed on the soil in the containers and than covered with 600 g soil mixture.

In each container, 3 seedlings of *H. lanatus* or *F. ovina* were planted, aged respectively 9 weeks and 11 weeks old. Each species was planted in containers with dead roots of its own species or with dead roots of another species. Roots of the 4 species were also incubated in

containers without living plants. In general, there were 8 replicates for labelled roots. Because insufficient labelled root material was available, however, there were only 4 or 5 replicates for N- dead roots of *F. ovina*, 3 or 4 replicates for N+ dead roots of *F. ovina* and 5 replicates for N- dead roots of *H. lanatus*. The soil surface was mulched with 50 g (1 cm) of white plastic granules to prevent growth of algae and reduce water loss from evaporation.

GROWTH OF PLANTS IN THE DECOMPOSITION PERIOD, HARVEST AND ANALYSIS

The water content of the containers was kept at 14% w/w by weighing the containers 3 times a week and than refilled them with water to their original weight to compensate for their water losses. During the experiment the containers with *H. lanatus* plants lost more water than the containers with *F. ovina* plants or the control containers without growing plants (Fig. 1).
The decomposition experiment was harvested at the end of the growing season (2 November). At that time approximately 50% of the shoot biomass of *H. lanatus* was yellow. Shoots were clipped from the soil containers. The soil was carefully pushed out of the containers and separated into 4 layers; 1) the upper 5 cm above the litterbags (0-5 cm depth), 2) the 5 cm below the litterbags (5-10cm depth), 3) 10-15 cm depth and 4) 15-27 cm depth. Soil and roots were separated. Shoots, roots and soil were dried at 70°C.

CHEMICAL ANALYSES

At the start of the decomposition experiment, roots were shaken in 250 ml water to remove soil particles. The roots were dried for 4 days at 40°C and analysed. The C and N concentration of the initial roots were measured with the element analyser (Fisons Instruments, EA 1108). After chemical destruction with $K_2Cr_2O_7$ (100 g/l) the activity (kBq/g root) of the roots was determined by scintillation counting, using a liquid scintillation counter (Packard, TRI-CARB 2100TR).

At the end of the decomposition experiment, the remaining amount of the labelled dead root ^{14}C (kBq) in the litterbags and the amount of dead root ^{14}C (kBq) in the soil and the living roots were determined by scintillation counting, using a liquid scintillation counter after chemical destruction with $K_2Cr_2O_7$ (100 g/l). The percentage dead root ^{14}C recovery was calculated as the ^{14}C recovered in the litterbag (kBq) divided by the amount of the dead root ^{14}C (kBq) at the start of the decomposition period, times 100. The percentage dead root ^{14}C recovery in soil was calculated as the final amount of ^{14}C (kBq) in the soil, divided by the

total amount of ^{14}C (kBq) in the ^{14}C-labelled dead roots in the litterbags at the start of the decomposition experiment, times 100. The latter method was also used to calculate the percentage dead root ^{14}C attached to the living roots at the end of the decomposition experiment. The amount of dead root ^{14}C attached to the roots of the living plants was negligible (less than 0.7% of the amount in the dead roots at t=0). It was equal for the *H. lanatus* and the *F. ovina* roots and also not different for the four decomposing root species.

Shoots and roots of the growing plants were dried at 70°C for 48 hours and organic matter content (550°C) and total C and N concentrations (element analyser) were measured. The N and P contents of the shoots and the roots were determined using a continuous flow analyser (Skalar autoanalyser San^{plus} system) after destruction with sulphuric acid, selenium and salicylic acid.

STATISTICAL ANALYSES

Statistical analysis was used to determine "the effect of growing plant species", "the effect of the decomposing dead root species" and "the effect of the N availability during the production of the decomposing roots" on the decomposition rate of the labelled dead roots. Data were analysed using analysis of variance (GLM procedure; SPSS 1995) for a completely randomized design factorial model. The GLM procedure compensates for different numbers of replicates. When variances increased with the means, the data were logarithmically transformed. Where appropriate, *a posteriori* comparisons were carried out with Tukey's honest significant difference test.

Results

Throughout the experiment (22 May 1998 – 30 October 1998) gravimetric water contents (percentage w/w) of the containers were determined by weighing the containers before watering. The water content of the containers fluctuated, especially at the beginning of the experiment (Fig. 1). The decline in water content was caused by the increase in plant transpiration. The water content of the containers with *H. lanatus* plants decreased dramatically once during the 26th week, because of strongly increased transpiration.

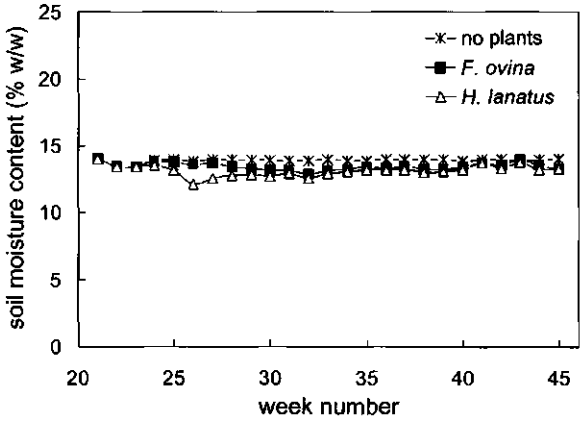

Figure 1. Water content (percentage w/w) of the soil columns containing the decomposing roots and with either living *H. lanatus* or *F. ovina* plants, or no plants, during the experiment. The experiment started in week 21 (May 1998) and ran until week 45 (end October 1998).

THE AMOUNT OF DEAD ROOTS REMAINING IN THE LITTERBAGS

The relative mass loss and the recovery of the ^{14}C in the initial roots in the litterbags at the end of the decomposition period were determined. The effects of growing plant species on the percentage mass recovery differed for the four decomposing root species (table 1). Without growing plants, the remaining mass of decomposing roots of *A. odoratum* was significantly lower than that of the decomposing roots of *F. ovina*, *F. rubra* and *H. lanatus*. The remaining mass of roots was greater in *F. ovina* than in *F. rubra* and *H. lanatus*. In all the root species the presence of growing plants increased the percentage of remaining root mass significantly (Fig. 2). Moreover, in the presence of living plants the remaining amount of *F. ovina* dead roots was significantly greater than that of the other three species. This difference between *F. ovina* and the other species was greater than in the treatment without living plants. Overall, the N availability during the production of the decomposing plant species had no effect on the percentage mass recovery after the decomposition period (table 1). When data were analysed separately for the four decomposing root species, N availability during growth had a significant stimulating effect on the decomposition rate of the *F. rubra* roots (Fig. 2c), but not on the roots of the three other species (Fig. 2 a, b and d).

In addition, at the end of the decomposition period the percentage of dead root ^{14}C recovery in the litterbags was determined. Overall, the ^{14}C recovery in decomposing roots of *A. odoratum* was significantly lower than in decomposing roots of *F. ovina*, *F. rubra* and *H. lanatus* and more ^{14}C was recovered from *F. ovina* roots than from *H. lanatus* roots.

Table 1. Analyses of variance (GLM) using a complete split plot design for the percentage of the root mass remaining in litterbags, dead root ^{14}C recovery from litterbags and soil, and total dead root ^{14}C loss. The ^{14}C-labelled dead roots are from four species: *H. lanatus*, *F. rubra*, *A. odoratum* and *F. ovina*. Root species, living plant species and nitrogen level were used as main factors.

		F-values of recovery (%)			F-values of loss (%)
Effects	df	mass in litterbags	^{14}C in litterbags	^{14}C in soil	total ^{14}C loss
Dead root species (R)	3	55.25^{***}	29.84^{***}	9.37^{***}	19.07^{***}
Growing plant species (P)	2	80.78^{***}	16.42^{***}	9.29^{***}	3.20^{*}
Nitrogen level (N)	1	3.66^{NS}	20.80^{***}	19.48^{***}	5.21^{*}
R x N	3	1.91^{NS}	9.16^{***}	2.49^{NS}	7.25^{***}
R x P	6	2.82^{*}	3.42^{**}	2.56^{*}	3.16^{**}
N x P	2	1.24^{NS}	3.11^{*}	0.04^{NS}	0.95^{NS}
R x P x N	6	0.86^{NS}	1.64^{NS}	2.55^{*}	1.70^{NS}

NS = not significant ($P > 0.05$) *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Both the nitrogen availability during growth and the growing plant species had a significant effect on the percentage ^{14}C recovery and this effect differed for the four decomposing root species (table 1). Compared to the presence of *F. ovina* plants, the presence of living *H. lanatus* plants significantly increased the percentage ^{14}C recovery from decomposing roots of *F. ovina* (Fig. 3a). Moreover, living *H. lanatus* plants significantly increased the percentage ^{14}C recovery from decomposing *H. lanatus* roots, and both *H. lanatus* and *F. ovina* living plants increased the ^{14}C recovery from decomposing *F. rubra* roots compared to the treatment with no plants (Fig. 3c and d). More ^{14}C was recovered from dead roots of *A. odoratum* in the presence of living plants than in the treatment without plants, but there was a significant interaction with the N availability during the period in which these roots had been grown (Fig. 3b). The N availability during this period had no effect on the ^{14}C recovery from dead roots of *A. odoratum* in the treatment with living *H. lanatus* plants or without plants, but had a positive effect on the ^{14}C recovery in the treatment with living *F. ovina* plants (Fig. 3b). N availability during growth had a significant stimulating effect on the decomposition rate of the *F. ovina* and *F. rubra* roots (Fig. 3a and c), but had no effect on the decomposition rate of the *H. lanatus* (Fig. 3d).

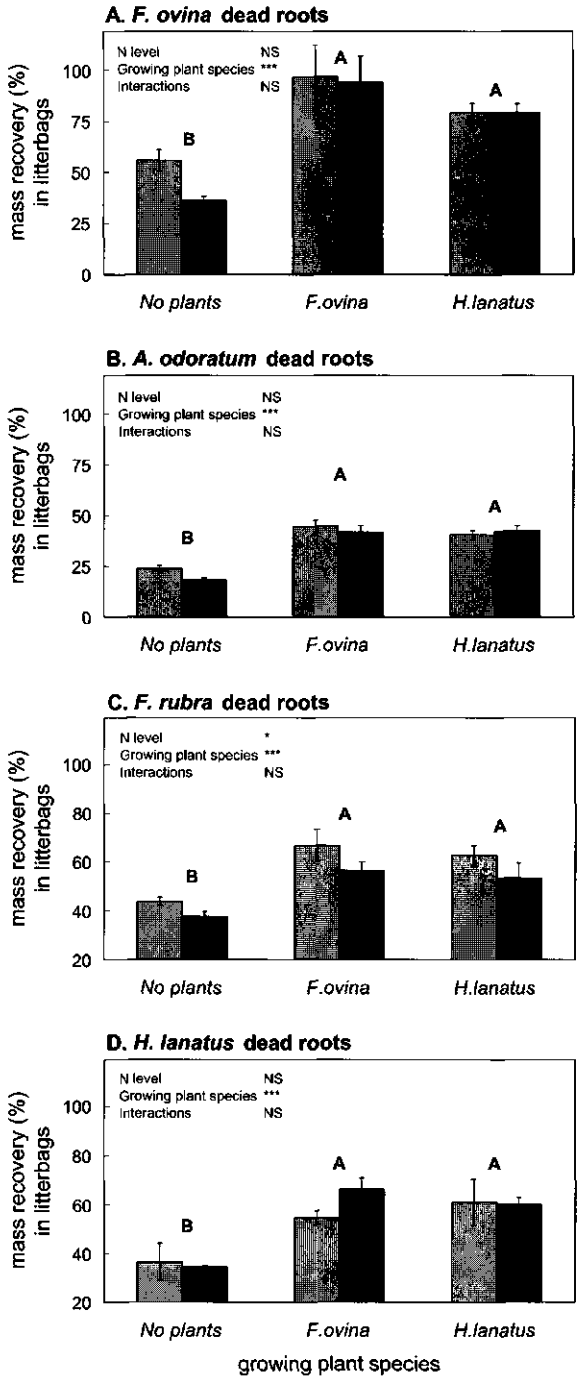

Figure 2. Root mass recovery of **A.** *F. ovina* dead roots, **B.** *A. odoratum* dead roots, **C.** *F. rubra* dead roots and **D.** *H. lanatus* dead roots as the percentage of the initial root mass in the litterbags. The dead roots are from plants grown at a high level of nitrogen availability (dark bars) or at a low nitrogen availability (hatched bars). The dead roots decomposed in columns without growing plants, with growing plants of *H. lanatus* or with growing plants of *F. ovina*. Data are means ± SE. Bars with the same letter are not significantly different (Tukey-HSD, $P>0.05$). *$P<0.05$, **$P<0.01$, ***$P<0.001$, NS = not significant.

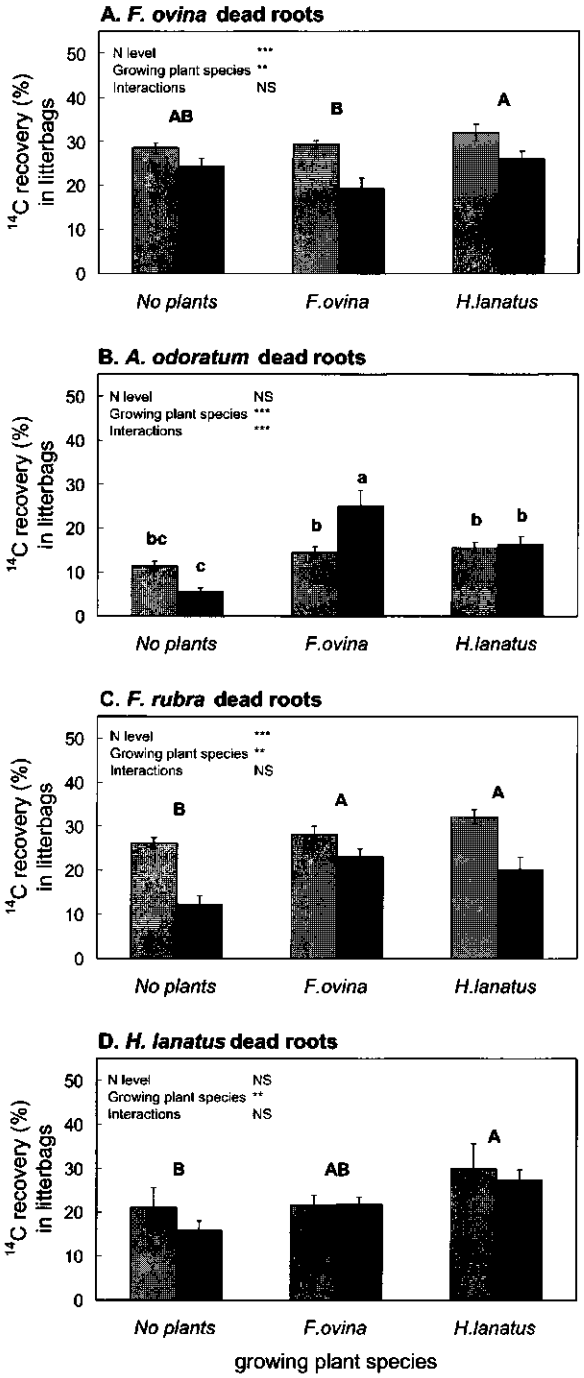

Figure 3. Recovery of ^{14}C from **A.** dead *F. ovina* roots, **B.** dead *A. odoratum* roots, **C.** dead *F. rubra* roots and **D.** dead *H. lanatus* roots as the percentage of the initial amount of dead root C in the litterbags. The dead roots are from plants grown at a high level of nitrogen availability (dark bars) or at a low nitrogen availability (hatched bars). The dead roots decomposed in columns without growing plants, or with growing plants of *H. lanatus* or with growing plants of *F. ovina*. Data are means ± SE. Bars with the same letter are not significantly different (Tukey-HSD, P>0.05). *P<0.05, **P<0.01, ***P<0.001, NS = not significant.

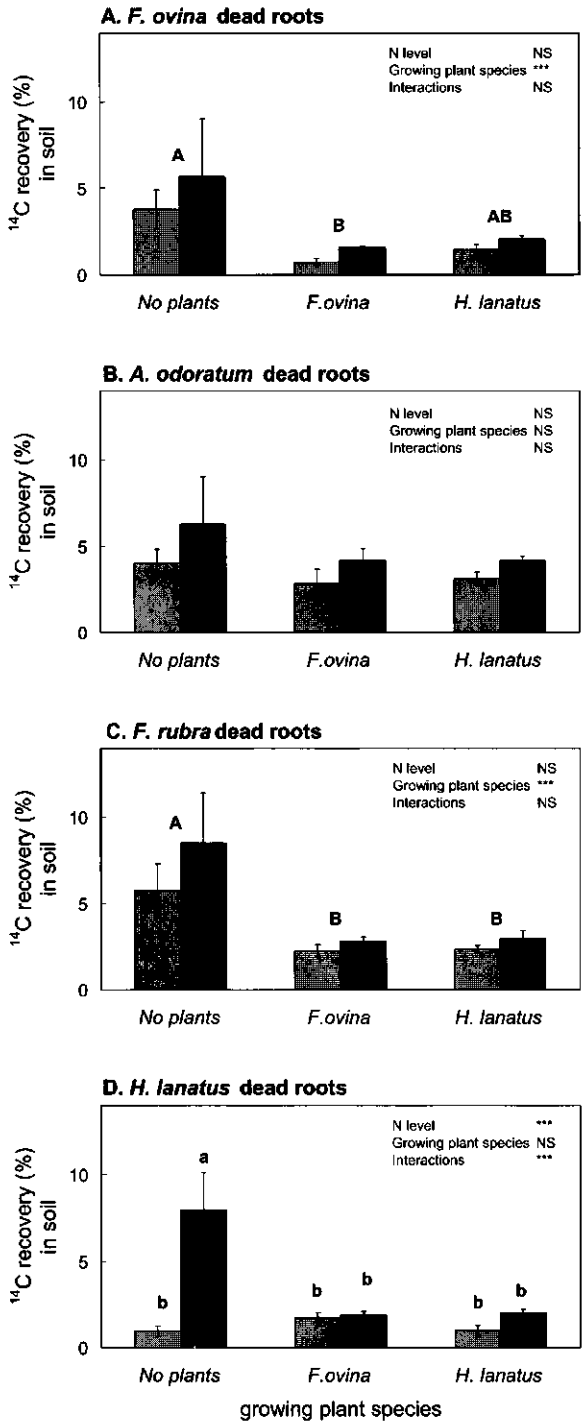

Figure 4. Root ^{14}C recovered from soil containing **A.** dead roots of *F. ovina* , **B.** dead roots of *A. odoratum*, **C.** dead roots of *F. rubra* and **D.** dead roots of *H. lanatus* as the percentage of the initial amount of dead root C in the litterbags. The dead roots are from plants grown at a high level of nitrogen availability (dark bars) or at a low nitrogen availability (hatched bars). The dead roots decomposed in columns without growing plants, with growing plants of *H. lanatus* or with growing plants of *F. ovina*. Data are means ± SE. Bars with the same letter are not significantly different (Tukey-HSD, P>0.05). *P<0.05, **P<0.01, ***P<0.001, NS = not significant.

PERCENTAGE DEAD ROOT ^{14}C IN SOIL

Both nitrogen availability during root growth and the presence of living plant species had a significant effect on the percentage of dead root ^{14}C that was recovered from the soil. This effect differed for the four decomposing root species (table 1). The presence of growing plants decreased the percentage ^{14}C from decomposing roots of *F. ovina* and *F. rubra* that was recovered from the soil (Fig. 4a and c). In the treatment without growing plants, decomposing *H. lanatus* roots grown at high N availability (N+) resulted in increased recovery of ^{14}C from the soil (Fig. 4d). This effect did not occur in the treatments with growing plants. Neither N availability during growth nor the living plants had any effect on the amount of ^{14}C from decomposing roots of *A. odoratum* that was recovered in soil (Fig. 4b).

In the treatment without living plants, the percentage dead root ^{14}C recovered from the soil did not differ for the four decomposing root species. However, in the presence of living plants there was a significant difference in the ^{14}C recovery from soil in which the four different species had been grown (table 1). In the presence of living *F. ovina* plants, the percentage ^{14}C from *A. odoratum* roots recovered from soil was significantly higher than ^{14}C from soil with decomposing *H. lanatus* and *F. ovina* roots. With growing plants of *H. lanatus*, the ^{14}C recovered from soil containing decomposing roots of *A. odoratum* and *F. rubra* was significantly higher than the ^{14}C recovered from soil containing decomposing *H. lanatus* and *F. ovina* roots.

PERCENTAGE TOTAL DEAD ROOT ^{14}C LOSS

Overall, the presence of living plants and N availability during the period in which the dead roots were grown had a significant effect on the percentage total dead root ^{14}C loss during the decomposition period. This effect differed for the four decomposing root species, however (table 1). The effect of the living plants was only significant for decomposing roots of *A. odoratum* and *F. ovina*. The percentage total ^{14}C loss from decomposing roots of *F. ovina* increased significantly in the presence of living *F. ovina* plants (Fig. 5a). Moreover, the presence of living *F. ovina* plants had a significant inhibiting effect on the total ^{14}C loss from dead *A. odoratum* roots that were grown on soil with high N availability (N+) (Fig. 5b). N availability during growth also had a significant effect on the total ^{14}C loss from *F. ovina* and *F. rubra* roots (Fig. 5a and c), but not on that of decomposing roots from *H. lanatus* (Fig. 5b).

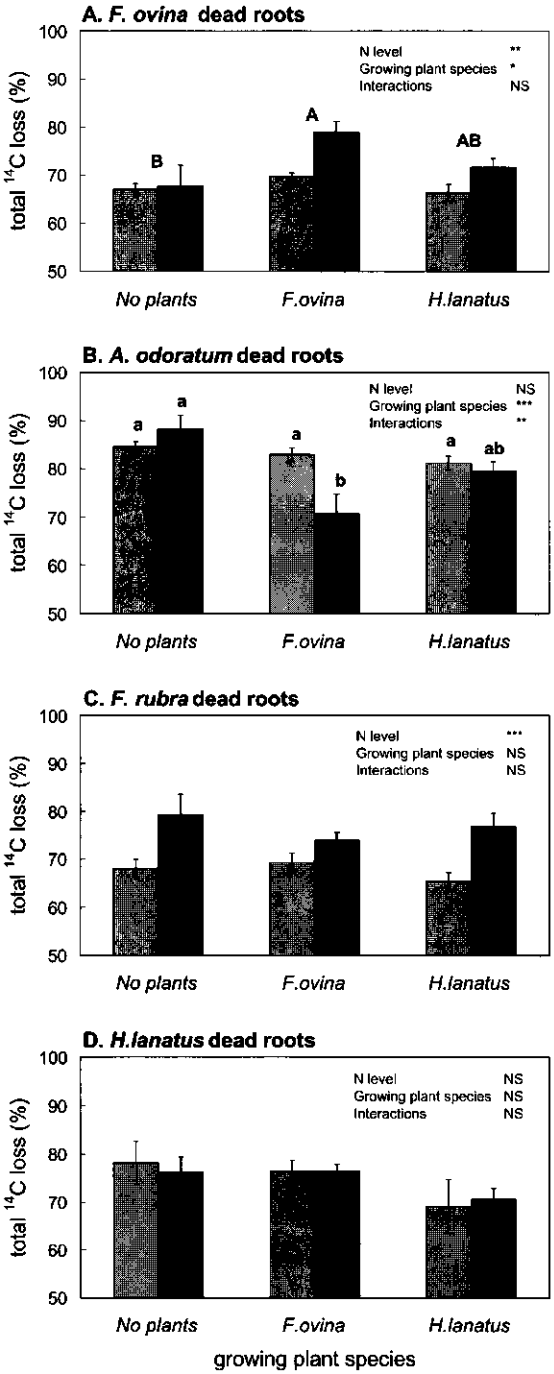

Figure 5. Total root ^{14}C loss origin of **A.** *F. ovina* dead roots, **B.** *A. odoratum* dead roots, **C.** *F. rubra* dead roots and **D.** *H. lanatus* dead roots as the percentage of the initial amount of dead root C in the litterbags. The dead roots are from plants grown at a high level of nitrogen availability (dark bars) or at a low nitrogen availability (hatched bars). The dead roots decomposed in columns without growing plants, with growing plants of *H. lanatus* or with growing plants of *F. ovina*. Data are means ± SE. Bars with the same letter are not significantly different (Tukey-HSD, P>0.05). *P<0.05, **P<0.01, ***P<0.001, NS = not significant.

In soil without growing plants, the total ^{14}C loss from *F. ovina* roots was lower compared with that from *H. lanatus* and *A. odoratum* roots and the total ^{14}C loss from *A. odoratum* roots was higher than that from *H. lanatus*, *F. rubra* and *F. ovina* roots. The differences in ^{14}C loss between the four species in the treatments without growing plants were the same as the differences in ^{14}C loss for the four species grown with low N availability in the presence of growing *H. lanatus* plants. In the treatments with growing *F. ovina* plants, however, the differences in ^{14}C loss from the N- roots became smaller. Moreover, when the dead roots had been grown on soil with high N availability, there were no significant differences in ^{14}C losses between the four species of decomposing roots in the treatments with living plants of *H. lanatus* and *F. ovina*.

PLANT GROWTH IN THE DECOMPOSITION EXPERIMENT

At the end of the experiment, the total plant biomass production did not differ between *F. ovina* and *H. lanatus* plants and was not affected either by the decomposing roots or by the N level (Fig. 6a and table 2). Shoot-to-root ratio was higher for *H. lanatus* plants than for *F. ovina* plants (Fig. 6b), because *H. lanatus* plants had a smaller root biomass compared to *F. ovina* plants. In the living *F. ovina* plants the total amount of N and the N concentration were higher than in the living *H. lanatus* plants (Fig. 6c and d). The total amount of N and the N concentration in the living plants were not affected by the decomposing roots or by the N level (table 2). However, living plants of *F. ovina* grown on soil with decomposing roots that had been grown in N+ soil had a significantly higher N-to-P ratio than *F. ovina* plants grown on decomposing roots grown in N- soil or living *H. lanatus* plants (Fig. 6e). The C:N ratio of living *H. lanatus* plants grown on soil with decomposing roots that had been grown in N+ soil was significantly lower than the C:N ratio of the other living plants (Fig. 6f and table 2). The C:N ratio of living *F. ovina* plants grown on soil with decomposing roots that had been grown in N+ soil was significantly higher than the C:N ratio of the living *H. lanatus* plants, but did not differ from that of living *F. ovina* plants grown on decomposing roots that had been grown on N- soil (Fig. 6f).

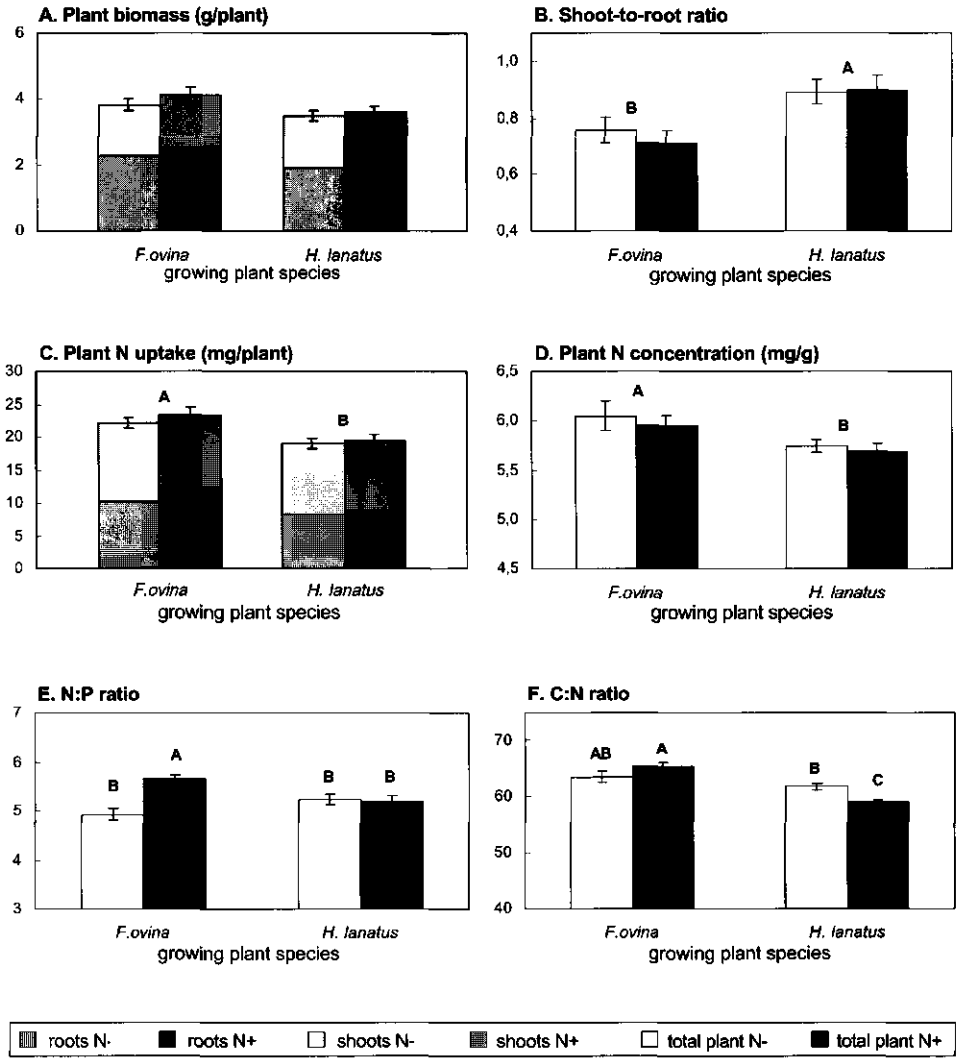

Figure 6. A. The total biomass production, **B.** the shoot-to-root ratio of the plant, **C.** the total N uptake by the plant, **D.** the N concentration in the total plant, **E.** the N:P ratio and **F.** the C:N ratio of the total living plant species *H. lanatus* and *F. ovina* grown on soil with decomposing roots of the 4 species *H. lanatus*, *F. rubra*, *A. odoratum* and *F. ovina* after a growing period of 22 weeks. The decomposing roots are from plants grown at a high level of nitrogen availability (dark bars) or at a low nitrogen availability (light bars). Data are means ± SE. Bars with the same letter are not significantly different (Tukey-HSD, P>0.05).

Table 2. F-values of the analyses of variance using a complete split plot design for the total plant biomass production, the shoot:root ratio of the plant, the total N uptake by the plant, the N concentration in the total plant, the N:P ratio and the C:N ratio of the total living plant species *H. lanatus* and *F. ovina* grown on soil with decomposing roots of the four species *H. lanatus*, *F. rubra*, *A. odoratum* and *F. ovina* after a growing period of 22 weeks. Root species, living plant species and nitrogen level were used as main factors.

Effects	df	biomass (g)	S:R ratio	N uptake (mg)	N concentration (mg/g)	N:P ratio	C:N ratio
Root Species (R)	3	0.55^{NS}	0.11^{NS}	1.67^{NS}	0.76^{NS}	1.43^{NS}	1.18^{NS}
Plant Species (P)	1	3.82^{NS}	6.07^{*}	12.37^{***}	8.53^{**}	0.33^{NS}	38.74^{***}
Nitrogen level (N)	1	1.06^{NS}	0.97^{NS}	0.94^{NS}	0.67^{NS}	9.84^{**}	0.49^{NS}
R x N	3	0.29^{NS}	0.89^{NS}	0.22^{NS}	1.52^{NS}	0.72^{NS}	0.24^{NS}
R x P	3	1.89^{NS}	1.63^{NS}	1.59^{NS}	0.62^{NS}	1.39^{NS}	1.09^{NS}
N x P	1	0.10^{NS}	0.15^{NS}	0.23^{NS}	0.01^{NS}	14.32^{***}	12.71^{***}
R x P x N	3	0.29^{NS}	0.16^{NS}	0.29^{NS}	0.41^{NS}	1.32^{NS}	2.97^{*}

NS = not significant (P > 0.05) *P < 0.05, **P < 0.01, ***P < 0.001.

Discussion

In this study we focussed on one of the possible pathways through which plant species can regulate soil nutrient supply. We tested whether living plants can affect the decomposition of dead roots and whether this effect differs between plant species. We found that living plant species enhanced the decomposition rate of *F. ovina* decomposing root litter but had no positive effects on the decomposition of root litter of the other species. We even found a negative effect on dead roots of *A. odoratum* from the N fertilized treatment. *F. ovina* is a low fertility species with a low relative growth rate. Such species often produce biomass with a wide range of recalcitrant compounds, such as phenolics, to reduce the risk of herbivory and parasitism (Eissenstat and Yanai 1997). It is possible that these phenolic compounds strongly reduce the microbial activity in the dead roots of these species. It seems that the living *F. ovina* plants could accelerate the decomposition of dead roots of *F. ovina* by activating the microbial biomass with their rhizodeposits.

Total ^{14}C loss is a good approximation of the total decomposition and mineralization of the organic material into an inorganic form. Prior to total mineralization, it is possible for decomposing litter to be transformed into different forms of organic matter and still be present

in the soil (Cadisch and Giller 1997). Therefore, in this study both dead root recovery (mass recovery and dead root ^{14}C recovery in litterbags) and dead root organic matter in the soil (^{14}C recovery in soil) were measured. The results for mass recovery and ^{14}C recovery in litterbags ran counter to the results for total ^{14}C losses. In contrast to the total ^{14}C losses, the data on mass recovery and ^{14}C recovery in the litterbags indicate that living plant species inhibit dead root decomposition. This contrast can be explained by the amount of ^{14}C recovered from the soil. The more dead root ^{14}C was lost from the litterbags, the more ^{14}C was recovered from soil. This indicates that conclusions about decomposition from litterbag experiments have to be considered carefully. Our results suggest that decomposition measurements with ^{14}C labelled roots are far more accurate than simple mass loss measurements.

The expected higher root decomposition in the presence of living plants of *H. lanatus* than in the presence of living *F. ovina* plants, did not occur. Our hypothesis was based on the expectation that *H. lanatus* plants would produce more root biomass than *F. ovina*, because *H. lanatus* plants grow faster than *F. ovina* (225 mg g^{-1} day^{-1} versus 140 mg g^{-1} day^{-1}). The amount of rhizodeposits produced that could stimulate microbial degradation of dead roots (Clarholm 1985; Robinson et al. 1989) is related to the root biomass production (Cotrufo and Gorissen 1997; Haynes and Beare 1997). In an earlier experiment we also measured greater rhizodeposition by *H. lanatus* than by *F. ovina* plants (Chapter 5). In the present experiment, however, the root biomass of living *H. lanatus* plants was even lower than that of *F. ovina* plants. This could account for the greater stimulating effect on *F. ovina* dead root decomposition of *F. ovina* plants compared to living *H. lanatus* plants.

The results for total ^{14}C loss in our study contradict those of other experimental studies where root growth suppressed the decomposition and transformation of dead roots or soil organic matter (Reid and Goss 1982; Sparling et al. 1982; Nicolardot et al. 1995), but agree with another study where the presence of living roots increased decomposition rates (Clarholm 1985). There are several mechanisms that could explain how plants affect the decomposition of labelled roots and the N mineralization from plant remains. Here we would like to discuss the possible mechanisms affecting the decomposition in our experiment and the possible explanations for the discrepancies with other studies.

One important process affecting the decomposition process is the drying and rewetting cycle of soils as a result of water uptake by plants. Such cycles may lead both to activation of the microbial biomass (Van Schreven 1967) or to a retarded decomposition of plant material

(Magid et al. 1999). Earlier studies have shown that in field experiments, decomposition rates were reduced in the presence of plant cover (Jenkinson 1977; Sparling et al. 1982). These authors suggested that the living plants could inhibit the microbial activity by the desiccation of the soil as a result of the water uptake and transpiration (Jenkinson 1977; Sparling et al. 1982). In our study we eliminated the water effect as much as possible. The mean water content of the containers fluctuated between 12% and 14%. We do not expect such small variations in water content to have significant effects on microbial activity (Bremer and Kuikman 1997).

Another possible mechanism whereby living plants can affect the microbial activity is when soil micro-organisms prefer to use materials released from the living roots instead of the compounds from dead roots (Reid and Goss 1982; Nicolardot et al. 1995). This mechanism accounts for living plants having an inhibiting effect on dead root decomposition. In their "preferential substrate utilization concept" Van Veen et al. (1993) stated that rhizodeposits provide an energy-rich basis for energy and biosynthesis processes for microbes and that microbes prefer these root-released carbon compounds over native soil organic matter. When insufficient supplies of nutrients are available for microbial metabolism, for example as a result of N uptake by the living plants, microbes will also utilize other soil organic compounds, like dead roots, in order to secure a sufficient nutrient supply (Van Veen et al. 1993). Perhaps this increased utilization of dead roots results in a positive effect and this might explain the greater total ^{14}C loss from *F. ovina* dead roots in the presence of living *F. ovina* plants.

Finally, another possible mechanism is competition for N between plants and soil microbes. The hypothesis we tested was based on an earlier study with the same species, where we also found that living plants activated root decomposition (Chapter 4). However the stimulating effect of the living plants in that study was much stronger than in this study. A possible reason for this is the characteristics of the decomposing plant material: the C:N ratio in the present study was almost double the C:N ratio in the earlier study (table 3). The C:N ratio of the decomposable resources determines whether N mineralization or N immobilization takes place (Robinson et al. 1989; Cadisch and Giller 1997; Sakala et al. 2000). The C:N ratio of the decomposing roots in the present study was above the critical C:N ratio for the decomposer organisms (Cadisch and Giller 1997; Seneviratne 2000). This means that soil microorganisms need to immobilize inorganic nitrogen to decompose the dead roots.

However, growing plants also need N for their growth. This leads to competition for nitrogen between growing plants and microbes, which may have reduced the positive effects of living plants on ^{14}C loss in this study compared to the earlier study (Chapter 4).

Table 3. C:N ratio of the dead roots of four grass species, *H. lanatus*, *F. rubra*, *A. odoratum* and *F. ovina*, used in two decomposition experiments; the present study and an earlier study (Chapter 4).

Species	present study		Chapter 4
	0 mg N/8 weeks	24 mg N/8 weeks	24 mg N/6 weeks
H. lanatus	61	53	33
F. rubra	51	53	22
A. odoratum	77	63	-
F. ovina	54	30	18

Our second hypothesis was that N availability during growth could increase the decomposability of dead roots and thereby the N mineralization. The addition of N during the period in which the roots had been growing increased total ^{14}C losses and ^{14}C losses from the litterbags in the case of decomposing *F. ovina* and *F. rubra* roots but did not increase the ^{14}C loss of dead *H. lanatus* roots. This could partly be explained by the C:N ratio of the roots. The decrease in the C:N ratio of *F. ovina* roots as a result of more addition N during their growth was greater than that for the other species (54 versus 30; table 3); this seems to have resulted in a faster decomposition rate. The C:N ratio of dead *H. lanatus* roots also decreased if N had been added during their growth, but this had no effect on the ^{14}C loss. It seems that the effects on the C:N ratio for *H. lanatus* were too small to affect the decomposition rate. The C:N ratio of the dead roots of *F. rubra*, however, did not change if they had been grown with N addition, but the ^{14}C loss of these roots increased. This may be because factors other than the C:N ratio were possibly also affected by the N availability; for example, the production of phenolic components such as lignin (Taylor et al. 1989; Gorissen and Cotrufo 2000). In contrast, N addition during the growth of *A. odoratum* roots decreased the decomposition rate of these roots in the treatment with *F. ovina* plants.

Compared with the other three species the dead roots of *A. odoratum* decomposed at a significantly faster rate, but also had a higher C:N ratio. Obviously, the interaction mechanism

between plant species and soil organisms is complex and cannot be explained merely by the C:N ratio of the decomposing material.

The third hypothesis was that dead roots of species from fertile habitats decompose more rapidly than dead roots of species from nutrient-poor habitats. In the present study, plant species differed in dead root decomposition rates but there was no evidence for the hypothesis that dead roots of high fertility species decompose faster than dead roots of low fertility species. In bare soil, the decomposability of *A. odoratum* dead roots was higher than that of *H. lanatus*, *F. rubra* and *F. ovina* dead roots. This confirms the results of earlier studies on the same grass species (Chapters 4 and 5).

Finally, we hypothesized that the effect of living plants on the decomposition rate of dead roots depends on the decomposing root species, because dead roots of species from nutrient-rich habitats decompose more rapidly than those of species from nutrient-poor habitats. This higher decomposition rate results in a higher N mineralization rate, which stimulates plant biomass production more in high fertility species, such as *H. lanatus*, than in low fertility species, such as *F. ovina* (Chapin 1980, Lambers and Poorter 1992). We found that the effect of plant growth on dead root decomposition rate was related to the N availability during growth period of the dead roots. If there had been low N availability during root growth, differences in decomposition rate in the treatment with growing plants were comparable with the differences in the treatment without plants. However, if there had been high N availability during root growth, differences in root decomposition rate between species disappeared in the treatment with living plants, as a result of an increased decomposition rate of dead roots of *F. ovina*, *F. rubra* and *H. lanatus*. This effect was stronger for living plants of *H. lanatus* than for living plants of *F. ovina*.

An intriguing question is whether the decomposing roots had an effect on the growth and the N uptake of the living plant species. In the present study, added amounts of N in the dead roots were very small (1.54 mg N) compared to the total amount of N in the soil organic matter (3.29 g N per container). Nevertheless, in the presence of decomposing roots, *F. ovina* plants took up more N. It is striking that the overall N uptake by the *F. ovina* plants exceeded the N uptake by *H. lanatus* plants. It is possible that living plants of *F. ovina* not only stimulated the dead root decomposition, but that they could also take advantage of this effect, whereas the living plants of *H. lanatus* could not. However, the differences in N uptake of *F. ovina* and *H. lanatus* plants is not solely attributable to the N release from litterbags (max. 1

mg N/litterbag = 0.3 mg N/plant). So, possibly *F. ovina* plants can also increase the mineralization of the soil organic matter. More research is necessary on this.

Conclusion

The main question in this study was whether living plant species actively regulate the decomposition of litter. We tested whether living plant species can affect the decomposition of dead roots and whether this effect is species-specific. Other authors have already stated that in the field shifts in species composition of vegetation or in the physiological status of dominant species follow changes in soil nutrient availability (Berendse 1990; Wedin and Tilman 1990; Hobbie 1992). We found that in addition to the effects of differences in biomass production, biomass turnover time and litter decomposition (Berendse et al. 1989; Aerts et al. 1992; Van Vuuren et al. 1993; Brevedan et al. 1996; Aerts and De Caluwe 1997; Cadisch and Giller 1997) living plant roots may also have significant effects on the decomposition of litter. Our data show that living plants can accelerate dead root decomposition and subsequent mineralization. We conclude that living plants probably release carbon compounds that could stimulate the growth of soil micro-organisms (Reid and Goss 1982; Van Veen et al. 1993; Nicolardot et al. 1995).

Acknowledgements

We thank Wim Didden, Henk van Ginkel and Ton Gorissen for their useful advice related to this experiment, and Roy Geerts, Frans Möller and Jan van Walsem for their technical assistance. We also appreciate the valuable comments on earlier drafts of the manuscript provided by Eelke Jongejans, Wieger Wamelink, Wilma Roem and Jasper van Ruijven. Joy Burrough we thank for her advice on the English.

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Summary

In many ecosystems an important factor that determines plant species composition is nutrient availability. Plant species have developed different characteristics, which make them successful competitors in either nutrient-poor or more fertile environments. It has been proposed that these different plant characteristics can in turn have significant consequences for soil nutrient availability. If this is true, changes in species composition could have important consequences for ecosystem functioning.

The study presented in this thesis focussed on the possible effects of different species on the nitrogen (N) mineralization. In agricultural grasslands in the Netherlands N is often the limiting nutrient for plant growth. In the grasslands that have been withdrawn from agricultural use, for the purpose of restoration, fertilization is stopped and the aboveground biomass is removed by haymaking once or twice a year. Under these conditions N mineralization declines and fast-growing plant species are replaced by slower-growing plant species (reversed succession). This raises the question of what in turn the influence of these plant species is on the change in N mineralization. My hypothesis was that as result of their adaptive traits, species from fertile habitats stimulate the N mineralization more than species from nutrient-poor habitats; they produce more litter that decomposes more easily. Because in a management strategy involving haymaking all of the aboveground biomass is removed, in this thesis I focussed on the effects of the subterranean plant parts.

The question this thesis addressed was, therefore: Do grass species from habitats that differ in N availability have different adaptive traits, like root lifespan, litter quality and rhizodeposition, and do these affect the N mineralization? To answer this question, my colleagues and I measured root lifespan, litter decomposability and rhizodeposition in perennial grass species that are characteristic of habitats ranging from inherently nutrient-poor to nutrient-rich. Mainly species of one family (Gramineae) were compared, to minimize confounding effects of differences in growth form and phylogeny.

The first and most important question to be answered was whether grass species from habitats with different N availability do indeed have different effects on the N mineralization (chapter 2). This was tested in a long-term garden experiment in which six grass species (*Lolium perenne*, *Arrhenatherum elatius*, *Festuca rubra*, *Anthoxanthum odoratum*, *Festuca*

ovina and *Nardus stricta*) which are adapted to habitats with different nutrient supplies were planted in monocultures. The species studied were assigned to three groups on the basis of the nutrient level of their preferred habitat: high, intermediate or low soil fertility. During the fourth year of the experiment, the N mineralization and nitrification were measured in soil samples taken from the plots in order to analyse the long-term effects of these species. These species did indeed have different effects on the net N mineralization and net nitrification. Species from high fertility habitats (*L. perenne* and *A. elatius*) increased N mineralization and nitrification more than species from low fertility habitats (*F. ovina* and *N. stricta*). To test whether the influence of these different groups of grass species on N mineralization corresponded with the influence of species of other families, the N mineralization in soil of these grass species was compared with N mineralization in soil with a diverse group of dicot species (*Urtica dioica*, *Rumex obtusifolius*, *Anthriscus sylvestris*, *Centaurea pratensis*, *Achillea millefolium*, *Succisa pratensis*, *Calluna vulgaris* and *Erica tetralix*). It was found that for the dicot species too, N mineralization and nitrification in the plots with the species from high fertility habitats (*U. dioica*, *R. obtusifolius*, *A. sylvestris*) was significantly higher than in the plots with the species from low fertility habitats (*S. pratensis*, *C. vulgaris* and *E. tetralix*).

Next we investigated the plant traits that can bring about changes in N mineralization. In chapters 3 to 6 of this thesis I focus on the plant features that possibly determine the subterranean impacts of the grass species: root lifespan, root decomposability and rhizodeposition.

In a garden experiment (chapter 3), root lifespan was studied for three years by observations in minirhizotrons placed in monocultures of two species from nutrient-rich habitats (*L. perenne* and *A. elatius*) and two species from nutrient-poor habitats (*M. caerulea* and *N. stricta*). The minirhizotron technique was used because this technique enables individual roots to be monitored from "birth" to "death" in successive observations. There was a significant negative correlation between the root lifespan and the N availability of the ecologically optimal habitats (N-index) for the four species. The root lifespan of species from fertile habitats was significantly shorter than that of species from low fertility habitats: 14 weeks in *L. perenne*, 40 weeks in *A. elatius*, 53 weeks in *M. caerulea* and 58 weeks in *N. stricta*. These data indicate that species from fertile habitats add more carbon and nutrients to the soil system as a result of a shorter root lifespan than species from less fertile habitats.

However, the nutrient input into the soil from root senescence is the organic N and P contained in the dead root material. So, for these to be available for plant uptake again, the litter must be decomposed and nutrients must be mineralized. In a greenhouse experiment, differences in root decomposability of three grass species, *H. lanatus*, *F. rubra* and *F. ovina* (which are typical of soils of high, intermediate and low fertility, respectively) and the subsequent N availability for the growing plants of *H. lanatus* and *F. ovina* were measured (chapter 4). This entailed measuring the decomposition of ^{15}N-labelled roots of the three species after a 6-week incubation period in soil planted with *H. lanatus* or *F. ovina* plants or with no plants. After this period, the decomposition rate of dead roots from the three plant species was not significantly different. However, dead roots of *H. lanatus* led to a lower N uptake by the growing plants compared to dead roots of *F. ovina* and *F. rubra*, possibly because the higher C:N ratio in the decomposing *H. lanatus* roots resulted in more N immobilization. When the C:N ratio of decomposing material exceeds the critical C:N ratio for soil microorganisms, the latter immobilize N to compensate for the low N content of the organic matter. This N immobilization reduces the N availability for the growing plants.

In this study (chapter 4) the influence of growing grass species *H. lanatus* and *F. ovina* on the decomposition of dead roots of *H. lanatus*, *F. rubra* and *F. ovina* and on the N mineralization from these residues was also studied. The presence of growing plants stimulated dead root decomposition and N mineralization. This effect was stronger for *H. lanatus* plants than for *F. ovina* plants. Also, *H. lanatus* plants took up more N and ^{15}N than *F. ovina* plants. However, the final N concentration in *H. lanatus* was lower than in *F. ovina*, which points to a stronger N limitation for *H. lanatus*. These results indicate that growing plants stimulate N transfer from plant residues to the soil solution and thereby facilitate their own growth. This effect was stronger for the high fertility species than for the low fertility species.

The next question was how living plants can stimulate dead root decomposition. Can the difference in stimulating effect between plant species of high and low fertility habitats be explained by differences in the amount of rhizodeposits that the plants produce? And what is the effect of N availability on the plant characteristics investigated? The conclusions of the first part of this thesis are based on experiments that were carried out at one fertility level. To test whether differences in the investigated plant characteristics occur both under nitrogen-

poor and in nitrogen-richer conditions, the studies in chapters 5 and 6 were done at two levels of N supply.

To examine the interspecific variation in rhizodeposition and decomposition of rhizodeposits and dead roots, four grass species, *H. lanatus*, *F. rubra*, *A. odoratum* and *F. ovina* were studied (chapter 5). Plants of these species were homogeneously labelled with $^{14}CO_2$ during their growth at two N levels (no mineral N addition and 14 g N m^{-2} addition). Using this method, it was possible to measure the carbon release to the soil through rhizodeposition during growth. The amount of rhizodeposits in the soil was measured after a growing period of 8 weeks. Rhizodeposition differed among species and the amount of rhizodeposits was positively correlated with root biomass accumulation. Moreover, as a general trend, the high and moderate fertility species *H. lanatus*, *F. rubra* or *A. odoratum*, deposited more C in the rhizosphere than the low fertility species *F. ovina*. Higher N availability increased rhizodeposition of *H. lanatus*, a high fertility species, as a result of its increased root mass, but had negligible effects on rhizodeposition of the intermediate (*F. rubra* and *A. odoratum*) and low fertility (*F. ovina*) species.

Subsequently, decomposition rates of the rhizodeposits and the ^{14}C-labelled dead roots were measured. The ^{14}C-labelled roots were incubated in fresh soil and, by trapping the evolved $^{14}CO_2$ during a period of 69 days, we measured the decomposition of 1) the labelled rhizodeposits in the soil in which the plants had been growing and of 2) the labelled dead roots incubated in fresh soil. In general, the decomposition rate of the rhizodeposited organic compounds of the four species increased with the potential growth rate of each species. The rhizodeposits of high fertility species mineralized faster, which suggests that these species activate the nutrient cycling more than low fertility species. When the N availability increased, the decomposition rate of the rhizodeposits also increased. In addition, the decomposability of the dead roots increased when more N was available during growth. The species differed in dead root decomposability, but the decomposability was higher in *A. odoratum* than in *H. lanatus*, with *F. rubra* being intermediate. There was no significant difference in decomposition rate between dead roots of *H. lanatus* and *F. ovina*. So these results did not support the idea that the decomposition rate of dead roots is related to the fertility of their optimal habitat.

The study described in chapter 6 investigated the possible active regulation by plants of the litter decomposition during a period longer than the studies reported in chapters 4 and 5. I

tested whether living plants of *H. lanatus* and *F. ovina* could affect the decomposition of ^{14}C-labelled dead roots of *H. lanatus*, *F. rubra*, *A. odoratum* or *F. ovina* during one growing season (May-November) and whether this effect was species-dependent. In addition, I investigated the influence of the N availability during the growing period on the long-term decomposability of the roots and its effect on the influence of the growing plants. Like the study described in chapter 5, seedlings of *H. lanatus*, *F. rubra*, *A. odoratum* and *F. ovina* were homogeneously labelled with $^{14}CO_2$ for eight weeks. Plants were grown on soil at two N levels: one without additional N and one with N addition (14 g N m^{-2}). At the start of the decomposition experiment ^{14}C-labelled roots were incubated in litterbags (mesh-width 1mm) in fresh soil. In an open greenhouse, ^{14}C-labelled roots decomposed in soil either planted with *H. lanatus* or *F. ovina* or in unplanted soil. In this experiment too, root decomposition rates differed among plant species but again there was no evidence to support the hypothesis that dead roots of high fertility species (i.e. *H. lanatus* and *F. rubra*) decompose faster than dead roots of low fertility species (i.e. *A. odoratum* and *F. ovina*). In unplanted soil, differences in mass loss and total ^{14}C loss between the dead roots of the four species were comparable to the differences in decomposition rates in the study described in chapter 5. Dead roots of *F. ovina* decomposed significantly faster when influenced by living *F. ovina* plants. But living plants had no stimulating effect on the dead root decomposition of the other three species. This may have been caused by the high C:N ratio of the dead roots, which made it necessary for soil microbes to immobilize N in order to decompose and mineralize the dead root organic compounds. The stimulation of the microbial degradation of dead roots as a result of rhizodeposition by the living plants might have been reduced as a result of N limitation caused by the N uptake by the living plants. The results suggest that whether plant exudates stimulate dead root decomposition depends on the quality of these dead roots and the N availability in soil.

In conclusion, the results described in this thesis support the hypothesis that grass species from high fertility habitats increase the N availability more than species from low fertility habitats. It seems that living plants of species from high fertility habitats can increase the N mineralization compared to species from poor habitats as a result of their higher root biomass production and consequently greater rhizodeposition. Moreover, species from high fertility habitats have a shorter root lifespan than low fertility species so they add larger amounts of dead roots to the soil. However, the decomposability of the dead roots was not related to the

fertility of their optimal habitat. Nevertheless, the overall effect is that plant species from high fertility habitats are able to stimulate the N mineralization more than plant species from poor habitats, as indicated by the N mineralization data of the garden experiment. The effect of N availability on the plant traits studied is striking. When N availability decreased, root biomass declined, especially for the species from high fertility habitats, and as a result the rhizodeposition decreased. Moreover, for all species lower N availability had a negative effect on rhizodeposit and dead root decomposability.

The effect of living plants on dead root decomposition and, vice versa, the effect of decomposing dead roots on living plant species is complicated. The results of the studies presented in this thesis indicate that living plants can have a stimulating effect on dead root decomposition. However, this effect depends on the quality of the decomposing roots. When the C:N ratio of the dead roots is relatively low, living plants stimulate their decomposition. But when the C:N ratio of dead root increases as a result of a lower N availability during growth, the stimulating effect of living plants disappears. So, when the N availability increases, the positive effect of the living plants on N mineralization increases. However, when the N availability declines, the stimulating effect on N availability for plant growth also declines and can even inhibit the N supply. The differences in stimulation or inhibition of the N release as a result of different levels of soil fertility seem to be greater in species from high fertility habitats than in species from low fertility habitats.

The effects of plant species on nutrient cycling processes could have an important impact on ecosystem succession. If the N availability declines, as in agricultural grassland with a restoration management, the species from high fertility habitats can accelerate this decline and thereby create more favourable conditions for species adapted to low fertility habitats. The dominance of this species could reduce the N supply even faster. The results of the study presented in these thesis offers a possible mechanism whereby species can influence species replacement during succession.

Samenvatting

In veel ecosystemen is de bodemvruchtbaarheid een belangrijke bepalende factor voor de soortensamenstelling van de vegetatie. De verschillende plantensoorten hebben eigenschappen ontwikkeld die ze tot succesvolle concurrenten maken in een voedselrijke of in een voedselarme omgeving. Uit eerder onderzoek is gebleken dat planten ook zelf invloed kunnen hebben op de voedselbeschikbaarheid in de bodem. De voedselbeschikbaarheid zou beïnvloed kunnen worden door de verschillende planteneigenschappen. Als dit zo is, kunnen veranderingen in de soortensamenstelling een belangrijke invloed hebben op de voedselbeschikbaarheid en daardoor op het functioneren van een ecosysteem.

Dit proefschrift is gebaseerd op een studie naar de mogelijke effecten van verschillende soorten op de stikstofmineralisatie. Stikstof (N) is vaak de beperkende voedingsstof voor plantengroei in agrarische graslanden in Nederland. Deze beperkende voedingsstof wordt via bemesting toegevoegd om zo een maximale biomassaproductie te bereiken. Het gevolg is dat graslanden ontstaan met een weinig diverse vegetatie die voornamelijk uit snel groeiende soorten bestaat. In een aantal van deze agrarische graslanden is de bemesting gestopt en wordt de bovengrondse biomassa één tot twee maal per jaar verwijderd door te maaien. Onder deze omstandigheden neemt de N-beschikbaarheid in de bodem af met als gevolg dat de snel groeiende plantensoorten worden vervangen door langzaam groeiende soorten. Dit heeft geleid tot de vraag of de plantensoorten ook zelf invloed hebben op deze veranderingen in N-mineralisatie. De hypothese was dat soorten van voedselrijke standplaatsen door hun eigenschappen de N-mineralisatie kunnen verhogen vergeleken met soorten van voedselarme standplaatsen. De verwachting was dat soorten van rijke standplaatsen meer dood materiaal produceren dat gemakkelijker afbreekt waardoor N weer sneller beschikbaar komt. Bovendien scheiden de wortels van soorten van nutriëntenrijke omstandigheden meer organische stoffen uit, die de afbraakprocessen in de bodem door bodemorganismen kunnen versnellen. Omdat de betrokken graslanden worden gehooid en dus al het bovengrondse materiaal wordt afgevoerd, heb ik me in dit proefschrift voornamelijk gericht op de effecten van de ondergrondse plantendelen.

De hoofdvragen die ik in dit proefschrift wil beantwoorden zijn daarom: "Verschillen soorten die voorkomen op nutriëntenrijke en soorten die voorkomen op nutriëntenarme

standplaatsen in adaptieve eigenschappen, zoals wortellevensduur, afbreekbaarheid van de dode wortels en de afbreekbaarheid en kwantiteit van het organisch materiaal dat wortels uitscheiden (rhizodepositie)? Hebben deze eigenschappen een effect op de N-mineralisatie?" Om deze vragen te kunnen beantwoorden is de levensduur van wortels, de afbreekbaarheid van het dode wortelmateriaal en de rhizodepositie gemeten in een aantal perenne grassoorten. De verschillende grassoorten waren karakteristiek voor standplaatsen variërend van voedselrijk tot voedselarm. Met opzet zijn voornamelijk soorten van één familie (Gramineae) met elkaar vergeleken, omdat op deze manier effecten van uitgesproken verschillen in groeivorm en fylogenie (verwantschap) tussen soorten van verschillende families te vermijden zijn in de analyses.

De eerste en meest belangrijke vraag die beantwoord moest worden was, of grassoorten van standplaatsen met een verschillende N-beschikbaarheid werkelijk een verschillend effect hebben op de N-mineralisatie (hoofdstuk 2). Dit is getest in een langlopend proeftuinexperiment waar zes grassoorten (*Lolium perenne*, *Arrhenatherum elatius*, *Festuca rubra*, *Anthoxanthum odoratum*, *Festuca ovina* en *Nardus stricta*) in monoculturen zijn geplant. De bestudeerde soorten zijn soorten die zijn aangepast aan standplaatsen met verschillende voedselbeschikbaarheid. Voor deze studie werden ze onderverdeeld in drie groepen op basis van het nutriëntenniveau van hun natuurlijke standplaats: veel, matig veel of weinig voeding in de bodem. Om de langer termijn effecten van deze soorten te bepalen werden gedurende het vierde jaar van het experiment de N-mineralisatie en nitrificatie gemeten in bodemmonsters uit de verschillende plots. Uit de resultaten bleek dat de soorten inderdaad een verschillend effect op de netto N-mineralisatie en netto nitrificatie hadden. Soorten van voedselrijke standplaatsen (*L. perenne* en *A. elatius*) verhoogden de N-mineralisatie en nitrificatie meer dan soorten van voedselarme standplaatsen (*F. ovina* en *N. stricta*). Om te testen of de invloed op de N-mineralisatie van de verschillende groepen grassoorten overeenkomt met het effect van soorten van andere families werd de N-mineralisatie in bodem met deze grassoorten vergeleken met de N-mineralisatie in bodems beplant met verschillende dicotyle soorten (*Urtica dioica*, *Rumex obtusifolius*, *Anthriscus sylvestris*, *Centaurea pratensis*, *Achillea millefolium*, *Succisa pratensis*, *Calluna vulgaris* and *Erica tetralix*). Ook in de bodem waar deze soorten op groeiden was de N-mineralisatie en nitrificatie significant hoger in veldjes met soorten van voedselrijke standplaatsen (*U. dioica*,

R. obtusifolius en *A. sylvestris*) dan in veldjes met soorten van voedselarme standplaatsen (*S. pratensis*, *C. vulgaris* and *E. tetralix*).

Vervolgens hebben we onderzocht welke planteneigenschappen de veranderingen in N-mineralisatie kunnen veroorzaken. Hoofdstuk 3 tot en met 6 zijn gericht op de ondergrondse planteneigenschappen die mogelijk de invloed van grassoorten kunnen bepalen: wortellevensduur, de afbreekbaarheid van wortels en de uitscheiding van organische componenten door levende wortels (rhizodepositie).

Wortels van de onderzochte soorten zijn gedurende 3 jaar bestudeerd in een proeftuinexperiment door middel van observaties in minirhizotrons welke waren geplaatst in monoculturen van twee grassoorten van voedselrijke standplaatsen (*L. perenne* en *A. elatius*) en twee grassoorten van voedselarme standplaatsen (*M. caerulea* en *N. stricta*). Voor dit experiment is de minirhizotron techniek gebruikt, omdat door middel van deze techniek individuele wortels gedurende opeenvolgende observaties kunnen worden gevolgd vanaf het moment dat ze ontstaan tot het moment dat ze weer verdwijnen. Uit de observaties bleek dat er een negatief verband was tussen de wortellevensduur en de N-beschikbaarheid in de ecologisch optimale standplaats (N-index) voor deze vier soorten. Wortels van soorten van voedselrijke standplaatsen leefden significant korter dan wortels van soorten van voedselarme standplaatsen. De wortellevensduur was gemiddeld 14 weken voor *L. perenne*, 40 weken voor *A. elatius*, 53 weken voor *M. caerulea* en 58 weken voor *N. stricta* planten. Deze gegevens wijzen erop dat soorten van voedselrijke standplaatsen meer koolstof en voedingsstoffen aan het bodemsysteem toevoegen als gevolg van hun grotere wortelturnover dan soorten van voedselarme standplaatsen.

Echter, de voedingsstoffen die als gevolg van wortelsterfte in de bodem komen, zijn nog in een organische vorm. Om de voedingsstoffen weer beschikbaar te maken voor opname door de plant, moet het dode wortelmateriaal afgebroken worden en de voedingsstoffen moeten worden gemineraliseerd. Het verschil in afbreekbaarheid van dode wortels afkomstig van drie verschillende grassoorten, *H. lanatus*, *F. rubra* en *F. ovina* (respectievelijk typische soorten voor standplaatsen met een hoge, gemiddelde en lage voedselbeschikbaarheid) en de invloed van deze wortels op de N-beschikbaarheid voor levende *H. lanatus* en *F. ovina* planten is gemeten in een kasexperiment (hoofdstuk 4). In dit experiment werd de afbraak van ^{15}N gelabelde wortels van de drie soorten gemeten na een incubatie periode van 6 weken. De ^{15}N gelabelde wortels waren gedurende deze periode geïncubeerd in grond zonder planten of in

grond beplant met *H. lanatus* of *F. ovina*. Aan het einde van de incubatieperiode was er geen verschil in afbraaksnelheid tussen de dode wortels van de drie verschillende soorten. Toch veroorzaakten de dode wortels van *H. lanatus* een lagere N-opname door de levende planten vergeleken met de dode wortels van *F. ovina* en *F. rubra*. Een mogelijke verklaring hiervoor is dat de hogere C:N ratio in de afbrekende wortels van *H. lanatus* resulteerde in een hogere N-immobilisatie door bodem-microorganismen. Wanneer de C:N ratio van afbrekend materiaal boven de kritische C:N ratio ligt voor bodemmicro-organismen, immobiliseren deze micro-organismen mineraal N om het lage N gehalte in het organische materiaal te compenseren. Deze N-immobilisatie leidt tot een reductie van de N-beschikbaarheid voor de levende planten.

Ook is in dit experiment de invloed van de levende *H. lanatus* en *F. ovina* planten bestudeerd op de afbraaksnelheid van de dode wortels van *H. lanatus*, *F. rubra* en *F. ovina* en de N-mineralisatie van deze wortel residuen (hoofdstuk 4). De aanwezigheid van levende planten stimuleerde de afbraak van de dode wortels en de N-mineralisatie. Het stimulerende effect van *H. lanatus* planten was sterker dan dat van *F. ovina* planten. Ook namen de *H. lanatus* planten meer N en ^{15}N op dan de *F. ovina* planten. De uiteindelijke N-concentratie in *H. lanatus* planten was echter lager dan in *F. ovina* planten, wat wijst op een sterkere N-limitatie voor *H. lanatus* planten. Uit de resultaten bleek dat levende planten de N transfer van dood materiaal naar de bodemoplossing kunnen stimuleren en daardoor hun eigen groei kunnen bevoordelen. Dit effect was sterker voor een soort van een voedselrijke standplaats dan voor een soort van een voedselarme standplaats.

De volgende vraag was hoe levende planten de afbraak van dode wortels kunnen stimuleren. Kunnen de verschillen in het stimulerende effect tussen soorten van rijke en arme standplaats verklaard worden door verschillen in de hoeveelheid rhizodepositie die de verschillende soorten produceren? Wat is het effect van de hoeveelheid beschikbare N op de onderzochte planteneigenschappen? De conclusies van het eerste gedeelte van dit proefschrift zijn gebaseerd op experimenten die zijn uitgevoerd op één niveau van N-beschikbaarheid in de bodem. Om te testen of de verschillen in de planteneigenschappen zowel onder N-arme als N-rijkere condities voorkomen, zijn de studies in hoofdstuk 5 en 6 op twee niveaus van N-beschikbaarheid uitgevoerd.

De interspecifieke variatie in rhizodepositie en afbraaksnelheid van de rhizodepositen en dode wortels is bestudeerd voor vier grassoorten; *H. lanatus*, *F. rubra*, *A. odoratum* en *F.*

ovina (hoofdstuk 5). Planten van deze vier soorten werden homogeen gelabeld met $^{14}CO_2$ gedurende hun groei bij twee N-niveaus (zonder mineraal N of met 14 g N m^{-2}). Door de labelling met ^{14}C was het mogelijk om na een periode van groei de hoeveelheid uitgescheiden koolstof (C) naar de bodem door rhizodepositie te meten. De hoeveelheid rhizodepositie in de bodem werd gemeten na een groeiperiode van 8 weken. De hoeveelheid uitgescheiden C door de wortels verschilde tussen de soorten en was positief gecorreleerd met de productie van wortelbiomassa door de planten. Bovendien, hadden de soorten van standplaatsen met een hoge of gemiddelde voedselbeschikbaarheid, *H. lanatus*, *F. rubra* en *A. odoratum*, meer C uitgescheiden in de rhizosfeer dan de soort van voedselarme standplaatsen, *F. ovina*. Een hoger N-beschikbaarheid gedurende de groei verhoogde de rhizodepositie door *H. lanatus* planten als gevolg van de hogere productie van wortelbiomassa. Een hogere N-beschikbaarheid had verwaarloosbare effecten op de hoeveelheid rhizodepositie door *F. rubra*, *A. odoratum* of *F. ovina* planten.

Vervolgens werd de afbraaksnelheid van de rhizodepositen en de dode ^{14}C gelabelde wortels gemeten. De ^{14}C gelabelde wortels werden geïncubeerd in verse bodem. Doordat we de gerespireerde $^{14}CO_2$ gedurende een periode van 69 dagen opvingen, konden we de afbraak meten van de gelabelde rhizodepositen in de grond waar de planten op hadden gestaan en van de in verse grond geïncubeerde gelabelde dode wortels. Over het algemeen nam de afbraaksnelheid van de rhizodepositen van de vier soorten toe met de potentiële groeisnelheid van deze soorten. De rhizodepositen van de soort van een voedselrijke standplaats mineraliseerden sneller, wat aangeeft dat deze soorten de nutriëntencyclus meer activeren dan de soorten van voedselarmere standplaatsen. Wanneer meer N beschikbaar was tijdens de groei, nam zowel de afbraaksnelheid van de rhizodepositen én de afbraaksnelheid van de dode wortels toe. Ook verschilden de soorten in de afbraaksnelheid van hun wortels, maar deze afbraaksnelheid nam niet toe met de potentiële groeisnelheid van de soorten. De afbraaksnelheid van de dode wortels van *A. odoratum* was hoger dan van de dode wortels van *H. lanatus*, en *F. rubra* zat er tussenin. Er was geen significant verschil in afbraaksnelheid tussen de dode wortels van *H. lanatus* en *F. ovina*. Deze resultaten bevestigen dus niet de hypothese dat de afbraaksnelheid van dode wortels is gerelateerd aan de voedselbeschikbaarheid in de natuurlijke standplaats van de soorten.

Het in hoofdstuk 6 beschreven experiment is gericht op de mogelijke actieve regulatie van de afbraak van dood organisch materiaal door levende planten gedurende een langere periode

dan de studies in hoofdstuk 4 en 5. Dit experiment duurde één groeiseizoen (van mei tot november). In dit experiment is onderzocht of levende planten van *H. lanatus* en *F. ovina* de afbraak van ^{14}C gelabelde dode wortels van *H. lanatus*, *F. rubra*, *A. odoratum* of *F. ovina* kunnen beïnvloeden en er is onderzocht of dit effect soortafhankelijk is. Tevens hebben we het effect van N gedurende de groeiperiode op de afbraaksnelheid van de wortels over een langere periode onderzocht en het effect daarvan op de invloed van de levende planten. Zoals voor het onderzoek in hoofdstuk 5, zijn ook in dit onderzoek kiemplantjes van *H. lanatus*, *F. rubra*, *A. odoratum* en *F. ovina* homogeen gelabeld met $^{14}CO_2$ gedurende een groeiperiode van 8 weken. Tijdens deze periode groeide de planten op een bodem met additioneel N (14 g N m^{-2}) of zonder additioneel N. Aan het begin van het afbraakexperiment werden de gelabelde wortels verpakt in strooiselzakjes (1 mm maaswijdte) en geïncubeerd in kolommen met verse bodem. Deze kolommen werden beplant met *F. ovina* planten of *H. lanatus* planten of bleven onbeplant. Na één groeiseizoen werd het gehele experiment geoogst. Ook uit dit experiment bleek dat de wortelsafbraak snelheid verschilde tussen de vier verschillende soorten, maar er waren weer geen aanwijzingen voor de veronderstelling dat dode wortels van soorten van rijke standplaats (*H. lanatus* en *F. rubra*) sneller afbreken dan dode wortels van soorten van armere standplaatsen (*A. odoratum* en *F. ovina*). In de onbeplante grond waren de verschillen tussen de verschillende soorten in gewichtsverlies en ^{14}C-verlies van de dode wortels vergelijkbaar met de afbraakgegevens zoals beschreven in hoofdstuk 5. Verder bleek dat dode wortels van *F. ovina* significant sneller afbraken onder invloed van levende *F. ovina* planten. Maar de levende planten hadden geen stimulerende invloed op de afbraaksnelheid van de dode wortels van de andere drie soorten. Een verklaring hiervoor kan zijn dat de C:N ratio van de dode wortels erg hoog was, waardoor bodem-organismen N moesten immobiliseren om het dode materiaal af te kunnen breken en vervolgens te kunnen mineraliseren. De stimulatie van de microbiële degradatie van de dode wortels als gevolg van de rhizodepositie door de levende planten kan zijn afgenomen als gevolg van een N-limitatie, doordat de levende planten N opnemen. Er is geen verklaring voor het feit dat alleen de dode wortels van *F. ovina* sneller afbreken onder invloed van levende *F. ovina* planten. Het resultaat van dit experiment suggereert dat stimulatie van de afbraak van dood wortelmateriaal door plantenexudaten afhangt van de kwaliteit van het dode wortelmateriaal en van de N-beschikbaarheid in de bodem.

Uit de resultaten in dit proefschrift kan de conclusie worden getrokken dat grassoorten van voedselrijke standplaatsen inderdaad de N-beschikbaarheid in de bodem sterker kunnen verhogen dan soorten van nutriëntenarmere standplaatsen. Dit effect kan worden verklaard door de grotere wortelbiomassaproductie en de grotere rhizodepositie door soorten van nutriëntenrijke standplaatsen. Bovendien leven de wortels van soorten van rijkere standplaatsen korter en daardoor voegen deze soorten meer dode wortels aan de bodem toe. Echter, de afbraaksnelheid van de dode wortels is niet gecorreleerd met de nutriënten beschikbaarheid in de optimale standplaats van de verschillende soorten. Toch is het overall effect, dat soorten van voedselrijke standplaatsen in staat zijn de N-mineralisatie in de bodem meer te stimuleren dan soorten van voedselarme standplaatsen, zoals duidelijk is geworden uit de resultaten van het proeftuinexperiment. Opvallend is het effect van de N-beschikbaarheid op de onderzochte planteneigenschappen. Wanneer de N-beschikbaarheid afneemt, neemt de wortelbiomassa productie ook af. Dit geldt vooral voor de soorten van voedselrijke standplaatsen. Als gevolg van deze afname in wortelbiomassaproductie neemt de hoeveelheid rhizodepositie ook af. Tevens had een afname in N-beschikbaarheid een negatief effect op de afbraaksnelheid van de dode wortels en de rhizodeposieten voor alle onderzochte soorten.

Het effect van levende planten op de afbraak van dode wortels en, omgekeerd, het effect van afbrekende dode wortels op levende planten is gecompliceerd. De resultaten van de studies die worden gepresenteerd in dit proefschrift geven aan dat levende planten de afbraak van dode wortels kunnen stimuleren. Echter, dit effect is afhankelijk van de kwaliteit van het dode wortelmateriaal. Wanneer de C:N ratio van dode wortels relatief laag is, kunnen levende planten de afbraak stimuleren, maar wanneer de C:N ratio hoger wordt als gevolg van een lagere N-beschikbaarheid gedurende de groei, verdwijnt het stimulerende effect van levende planten. Dus, als de N-beschikbaarheid toe neemt, neemt het positieve effect van levende planten op de N-mineralisatie ook toe. Maar als de N-beschikbaarheid afneemt, neemt het stimulerende effect op de N-beschikbaarheid ook af en dit kan zelfs leiden tot een verdere afname van de N-beschikbaarheid. De verschillen in de mate van stimulatie en in de mate van remming van de N-mineralisatie door het effect van planten, als gevolg van verschillende niveaus in bodemvruchtbaarheid, lijken groter te zijn voor soorten van voedselrijke standplaatsen dan voor soorten van voedselarme standplaatsen.

Het effect van plantensoorten op de kringloop van nutriënten kan belangrijke consequenties hebben voor de successie van ecosystemen. Als de beschikbaarheid van

mineraal N in de bodem afneemt, zoals het geval is in agrarische graslanden waar een herstelbeheer wordt uitgevoerd, kunnen soorten van voedselrijke standplaatsen de afname van N versnellen en daardoor betere condities scheppen voor soorten die zijn aangepast aan nutriëntenarme standplaatsen. Als deze soorten van nutriëntenarme standplaatsen dominant worden in de vegetatie, kunnen zij een nog grotere afname veroorzaken van de minerale N-beschikbaarheid in de bodem. De resultaten van de studie die gepresenteerd wordt in dit proefschrift bieden een mogelijk mechanisme met betrekking tot de wijze waarop dominante soorten de vervanging van soorten tijdens de successie kunnen beïnvloeden.

Dankwoord

Het werk is gedaan en het proefschrift is af. De afgelopen zes jaar waren zwaar en het was mij niet gelukt zonder de hulp van anderen. Het meest duidelijk is de hulp van collega's, maar de hulp van vrienden en familie is zeker niet minder belangrijk.

Allereerst wil ik drie personen speciaal bedanken die vanaf het begin direct bij het project waren betrokken, mijn promotor Frank Berendse, mijn co-promotor Peter Kuikman en mijn onderzoeksassistent en paranimf Frans Möller.

Beste Frank, ik wil je bedanken voor je vertouwen en steun gedurende de afgelopen 6 jaar. Zonder jou had ik dit proefschrift niet kunnen schrijven; jij hebt immers het projectvoorstel geschreven. Van je kritische houding ten opzichte van de experimenten en de uiteindelijke resultaten heb ik ontzettend veel geleerd. Je inhoudelijke opmerkingen en je soms minuscule verbeteringen hebben me gedwongen goed na te denken en heel precies te formuleren. Jij hebt ervoor gezorgd dat dit proefschrift is geworden zoals het nu is.

Beste Peter, jij was mijn externe begeleider. Van jou heb ik geleerd dat het streven naar perfectie qua tijdsinvestering niet de optimale manier is. Je hebt mij wegwijs gemaakt bij het AB-DLO en me gesteund bij het werken met isotopen en het verwerken van de resultaten. Over bodem-ecologie, een vakgebied waar ik niets vanaf wist, heb je me veel na laten denken. Als ik met jou gepraat had over een manuscript, had ik achteraf altijd meer vragen dan vooraf, maar hierdoor kwam ik steeds een stap verder.

Beste Frans, graag wil ik je bedanken voor de intensieve samenwerking in de afgelopen 6 jaar. Je stond altijd voor mij en mijn experimenten klaar. Ik heb enorme bewondering voor hoe je altijd heel volgzaam mijn protocollen uitvoerde. Zonder jou had ik mijn experimenten nooit uit kunnen voeren. Jij hebt het grootste gedeelte van het praktische werk overgenomen en ik was blij dat ik af en toe kon helpen. Het was altijd gezellig en ik heb ontzettend van je manier van werken en je humor genoten.

Naast direct betrokkenen wil ik ook anderen bedanken die op een of andere manier een bijdrage hebben geleverd bij het tot stand komen van dit proefschrift. Jan van Walsem en Herman Klees wil ik bedanken voor de hulp bij mijn vaak omvangrijke proeven. Jan, je wist altijd een passende oplossing als er weer wat mis ging. En, dank je voor het, volgens jou, eindeloos bewaren van mijn monsters (ze mogen nu weg).

Voor al het werk dat achter de schermen is gebeurd wil ik Gerda en Roelfina bedanken. Afspraken maken, geld declareren, verlofbriefjes, spullen bestellen, enzovoort, enzovoort; zonder jullie had ik alle ingewikkelde regeltjes en formulieren nooit begrepen.

Anita van Mierlo, Bart Fransen, Carolien de Kovel en Wilma Roem waren vanaf het begin mijn naaste AIO collega's. Graag wil ik ze bedanken voor de opbeurende gesprekken en hun opbouwende kritiek. Anita was de AIO in de sollicitatie commissie en degene die me wegwijs heeft gemaakt bij onze vakgroep. Anita, ik heb veel steun aan je gehad. Wilma was de beste kamergenoot die ik me kon wensen. Wilma, de gesprekken met jou ga ik erg missen. Ik hoop dat we elkaar in de toekomst nog vaak zien en spreken. Met Carolien kon ik rustig over mijn perikelen binnen de vakgroep praten, je kende het goed maar was niet meer van Wageningen afhankelijk. Tot slot Bart. Bart, we kenden elkaar al langer, maar in Wageningen leerden we elkaar beter kennen; zowel de positieve als de negatieve kanten. Gelukkig konden we dit ook eerlijk tegen elkaar zeggen. Tijdens mijn onverwachte afwezigheid pakte jij mijn experimenten op en zorgde ervoor dat ze goed werden afgerond. Bovendien waren wij de enige die aan wortels werkten. Aangezien volgens velen dit soort onderzoek het daglicht niet kan verdragen, was ik blij dat ik een mede lotgenoot had. Ik ben heel blij dat je mijn paranimf wilt zijn.

Natuurlijk wil ik ook alle andere AIO's en OIO's en verdere collega's bedanken voor jullie goede opmerkingenen, ideeën en de leuke gesprekken.

Werk kan alleen leuk blijven als je buiten het werk vrienden hebt die op gezette tijden je dwingen om ook met andere dingen dan werk bezig te zijn. Arjan en Corina, Carolien en Laurens, Jan en Coby, Karin, Sylvia en Bart, dank jullie voor de interesse, hulp en nodige ontspanning.

Naast vrienden heb ik ook een grote steun gehad aan mijn familie. Vooral van mijn ouders heb ik veel steun gehad, ze waren altijd in de beurt als ik ze nodig had, letterlijk en figuurlijk. Lieve pa en ma, ik weet dat jullie soms niet goed begrepen waar ik nu mee bezig was. Ik hoop dat dit proefschrift het wat duidelijker maakt. Pa en ma Slot, Manon, Jan Gerrit en Aarjen, wil ik bedanken voor de belangstelling en de daaruit voortvloeiende vragen en discussies die me altijd weer aan het denken zette.

Natuurlijk wil ik ook mijn levensmaatje bedanken. Lieve Jaap, jij hebt me van het begin tot het einde van mijn onderzoek gesteund. Vanaf de eerste sollicitatie in Wageningen heb je me bijgestaan. Je hebt me gebracht en gehaald wanneer het nodig was. Gedurende de afgelopen 6

jaar stond je niet alleen klaar om me door moeilijke perioden te helpen, maar had je zelfs ook inhoudelijke inbreng in het onderzoek. Bovendien was ik dankzij jou een AIO zonder geldzorgen. Kortom, zonder jou was ik niet zo ver gekomen.

Tot slot wil ik mijn trouwe vriend Donar bedanken voor al zijn troostende en opbeurende likken en pootjes. Donar, je dwong me met mijn werk te stoppen als je vond dat ik ook mijn energie eens aan andere dingen moest besteden; aan jou dus. Bovendien werden met jou mijn verplichte "rust" dagen leuk en door jou is mijn conditie met sprongen vooruitgegaan. Donar, bedankt!

Tanja van der Krift
Zeist, september 2000

Curriculum vitae

Tanja van der Krift werd geboren op 13 december 1969 te IJsselstein. In 1988 behaalde zij het VWO diploma aan het Oosterlicht College te Nieuwegein. In september 1988 begon zij aan de studie Biologie aan de Universiteit te Utrecht. Tijdens haar doctoraalfase voerde zij, in het kader van het hoofdvak Landschaps-oecologie, een onderzoek uit naar de invloed van sulfaat en chloride op de fosfaatbeschikbaarheid in veenbodem. Voor het afstudeervak Natuur en Milieu-educatie maakte zij een lespakket voor de basisschool over watervervuiling tijdens een stage bij Stichting Reinwater. Het gedrag van angelloze bijen heeft zij bestudeerd tijdens het afstudeervak Ethologie van Sociale Insecten.

In juni 1994 studeerde zij af en in november 1994 begon zij met haar promotieonderzoek bij de leerstoelgroep Natuurbeheer en Plantenecologie van de Wageningen Universiteit. In deze periode onderzocht zij de invloed van plantensoorten op de stikstofmineralisatie in grasland-ecosystemen. De resultaten van dit onderzoek zijn vastgelegd in dit proefschrift.