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NITROGEN LOSSES FROM PERENNIAL GRASS SPECIES

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ABSTRACT

Nitrogen losses from plants may occur through a variety of pathways, but so far, most studies have only quantified losses of nutrient by above-ground litter production. We used ^{15}N pulse labelling to quantify total nitrogen losses from above- and below-ground plant parts. Using this method we were able to include also pathways other than above-ground litter production. To test the hypothesis that species from nutrient-poor habitats lose less nitrogen than species from more fertile soils six perennial grasses from habitats with a wide range of nutrient availability were investigated: Lolium perenne, Arrhenatherum elatius, Anthoxanthum odoratum, Festuca rubra, Festuca ovina and Molinia caerulea. The results of an experiment carried out in pots in a greenhouse at two fertility treatments show that statistically significant losses occur through pathways other than litter production. In the low fertility treatment, most (70%) losses from L. perenne occurred by litter production, but in A. elatius, F. rubra, F. ovina and M. caerulea, more than 50% of labelled N losses took place by root turnover, leaching or exudation from roots. When nutrient supply increased, the ^{15}N losses in above-ground dead material increased in all species and in A. elatius, A. odoratum and F. rubra the ^{15}N losses via other pathways decreased. Ranked according to decreasing turnover coefficient the sequence of species was: L. perenne, A. odoratum, F. rubra, F. ovina, A. elatius, M. caerulea. These results show that species adapted to sites with low availability of nutrients lose less nitrogen (including above- and below-ground losses) than species adapted to more fertile soils.

Key words: grasses, litter production, nitrogen loss, ^{15}N pulse labelling

INTRODUCTION

Plants lose an important part of the nutrients they assimilate. These losses may occur through different pathways: mortality of plant parts (abscission of leaves and flowers, turnover of roots); removal by animals (grazing) or by microorganisms; leaching from leaves (losses of water-soluble ions); root exudation; production of seeds and pollen; gaseous losses (nitrogenous compounds) and other processes such as fire and soil erosion (Berendse 1985). Perennial species are able to translocate nutrients from senescing tissues to underground organs for storage (Morton 1977; Thornton and Millard 1993). The ability to reduce nutrient losses may be especially valuable on nutrient-poor sites (Berendse et al. 1987; Escudero et al. 1992; Li et al. 1992). Removal of large amounts of nutrients from senescent plant organs results in low concentrations of nutrients in litter and low nutrient loss rates. It seems that plants growing in habitats with low nutrient availability are most likely to have an efficient mechanism for nutrient conservation (Grime 1979; Berendse and Elberse 1989).

So far, most studies have focused on the loss of nutrients from the plant by litter production (Pastor et al. 1984; Aerts and Caluwe 1994) as a process related to input of nutrients to the soil. Other studies have focused on the loss of nutrients through grazing, simulated by clipping (Berendse et al. 1992). The use of ^{15}N pulse labelling allows the total N loss from above-ground and below-ground parts to be estimated and two different pathways of plant nitrogen losses to be discriminated: losses due to above-ground litter production (that can easily be measured by collecting the dead material produced) and other losses (e.g. root mortality, leaching and root exudation). Using this technique the labelled N can be followed in different plant parts while plants continue to grow and to take up (unlabelled) nitrogen. The amount of ^{15}N in the total plant was measured after different time intervals. Using this method we estimated nitrogen losses in six perennial grasses from habitats with a wide range of nutrient availabilities. First, we estimated nitrogen losses through the two different pathways. Second, we compared nitrogen losses among species, to test the hypothesis

The nutrient solution used was a 1:16 dilution (at the low fertilized treatment) of the solution described by Hoagland and Snyder (1933) cited in Hewitt (1966). The solution supplied contained $0.31 \text{ mmol l}^{-1} \text{ KNO}_3$; $0.31 \text{ mmol l}^{-1} \text{ Ca(NO}_3)_2$; $0.12 \text{ mmol l}^{-1} \text{ MgSO}_4$; $0.06 \text{ mmol l}^{-1} \text{ KH}_2\text{PO}_4$; $46 \text{ } \mu\text{mol l}^{-1} \text{ H}_3\text{BO}_3$; $9.1 \text{ } \mu\text{mol l}^{-1} \text{ MnCl}_2$; $0.3 \text{ } \mu\text{mol l}^{-1} \text{ CuSO}_4$; $0.7 \text{ } \mu\text{mol l}^{-1} \text{ ZnSO}_4$; $0.5 \text{ } \mu\text{mol l}^{-1} \text{ H}_2\text{MoO}_4$. After labelling, two treatments were differentiated: one series was treated with low fertilizer (LF) nutrient solution as previously described, and the other was treated with high fertilizer (HF) nutrient solution (1:4 dilution). The high fertilizer nutrient solution contained $1.25 \text{ mmol l}^{-1} \text{ KNO}_3$; $1.25 \text{ mmol l}^{-1} \text{ Ca(NO}_3)_2$; $0.5 \text{ mmol l}^{-1} \text{ MgSO}_4$; $0.25 \text{ mmol l}^{-1} \text{ KH}_2\text{PO}_4$ and micronutrients in the same amount as described for the LF treatment.

The nutrient solution was aerated continuously and replaced with 3 litres fresh solution every two weeks. The pH of the nutrient solution was adjusted to 5.5 by addition of H_2SO_4 . The volume in the pots was maintained with demineralized water (twice a week). The subblocks on the tables were rotated once a week, to achieve homogeneity in the light intensity and temperature experienced by the different plants. Two weeks after the first harvest (T_0) wire cages 50 cm high were placed on top of A. elatius to keep the plants upright. Note that M. caerulea is a deciduous species that dies off above-ground almost completely at the end of the growing season. Between weeks 24 and 32 of the experiment the only living organs were the basal internodes with attached buds and root systems.

Labelling and harvesting of plants

On 28 June all the pots were labelled with ^{15}N . The nutrient solution in the pots was replaced by tap water, 48 h before adding ^{15}N . The labelled N was added as $^{15}\text{NH}_4^{15}\text{NO}_3$ (95 atom % ^{15}N), supplying 4 mg ^{15}N to each pot. The ^{15}N pulse labelling took 24 hours. Immediately afterwards the root systems were rinsed in water and the plants were transferred to unlabelled nutrient solutions. The first series of pots (6 species x 5 replicates) was harvested (T_0) at this time. The remaining pots were

divided into two series: one series was supplied with low nutrient solution (LF), and the other with high nutrient solution (HF), at the concentrations described above.

Ten pots (2 treatments x 5 replicates) of each species, chosen randomly, were destructively harvested after 8 (T₁), 16 (T₂), 24 (T₃), 32 (T₄) and 40 (T₅) weeks. At each harvest, plant material was divided into: roots, above-ground living parts and above-ground dead material. No root mortality was observed during the experiment. Dry weights were determined after oven drying at 70 °C for 48 h. The total N and ¹⁵N concentrations in samples were determined using an Isotope Ratio Mass Spectrometer (ANCA-IRMS). The absolute amount of labelled N was obtained using the following equation: $C = 15 \times A \times B / (1400 + B)$, where A = weight of the total N in the subsample analysed; B = ¹⁵N atom % in the sample; C = weight of ¹⁵N in the subsample analysed.

Statistical analysis

The data were statistically analysed using GENSTAT. The two fertility treatments were started after labelling; consequently, at the first harvest (24 hours after labelling) there were no treatments. A three-way ANOVA with harvest, species and treatments as factors was applied for plant dry weight and ¹⁵N contents. This analysis only included data from the second to the sixth harvest. A two-way ANOVA with species and harvest as factors was carried out for each fertilization treatment separately to test the effect of species and all sampling dates on several variables.

RESULTS

Biomass

A three-factor ANOVA (with harvest, species and treatments as factors) showed statistically significant effects of sampling date, species, fertility treatments and their interaction on total biomass per sampling period ($P < 0.001$). In the low nutrient supply treatment L. perenne and A. elatius had the largest amount of dry matter at the first harvest (24 hours after labelling). This pattern changed over time, because in the later

period nutrients were limiting the growth of the plants. At the end of the experiment F. rubra had the highest total biomass (Figure 1a). Biomass increased more strongly in A. elatius and A. odoratum than in F. ovina and M. caerulea at the high nutrient supply treatment (Figure 1b).

The biomass in dead parts was greater in all species ($P < 0.001$) in the treatment with high nutrient supply. There was a significant interaction ($P < 0.001$) between the effects of harvest and species. At the end of the experiment the produced dry weight of dead parts was greater in L. perenne and A. odoratum than in F. rubra and M. caerulea. Note that L. perenne had the largest amount of litter production but not the largest total biomass.

Biomass distribution over root and shoots

Biomass distribution was calculated as the proportion of the biomass of one plant part in the total dry matter of the whole plant. Analysis of variance revealed statistically significant effects of sampling dates, species and nutrient supply ($P < 0.001$) on the biomass production of different plant parts. In all species there was a significant effect of the fertility treatment on the proportional biomass allocation to shoots ($P < 0.001$) and roots ($P < 0.001$). Biomass allocation to shoots of plants growing in the low fertilized treatment was less than that of plants in the high fertilized treatment (data not shown). By contrast, biomass allocation to the roots decreased with increasing nutrient supply. The effect of fertility treatment was not statistically significant in M. caerulea after 24 weeks.

Percentage biomass allocation to shoots and roots differed statistically significantly between species ($P < 0.001$). At the end of the experiment A. elatius had the highest percentage biomass allocation to roots followed by F. rubra and then F. ovina.

Nitrogen uptake

The total nitrogen (unlabelled) absorption was estimated as the accumulation of nitrogen (mg pot^{-1}) in the whole plant including dead plant parts. Note that this estimation does not include losses other than those by above-ground mortality. In the low fertilized treatment, the differences in the N total absorbed between L. perenne and A. odoratum, and between A. elatius and F. rubra at the end of the experiment were not statistically significant (using two-way ANOVA with species and harvest as factors). M. caerulea absorbed 46% of the total amount of nitrogen supplied (Figure 2a) and the other species values ranked between 75% and 82%. In the high nutrient supply treatment the difference between L. perenne and A. elatius was not statistically significant at the end of the experiment. L. perenne, A. elatius, A. odoratum and F. rubra absorbed between 72% and 80% of the total amount of nitrogen supplied (Figure 2b). F. ovina absorbed 56% and M. caerulea 14%. In both fertility treatments the total amount of nitrogen absorbed by M. caerulea did not further increase after 24 weeks because in this period the leaves and part of the stems of M. caerulea died. The data (not shown) revealed that nitrogen was translocated to basal internodes and roots for storage.

Absorption and distribution of labelled N in the plant

After 24 hours labelling the ^{15}N absorbed ranged from 58% in M. caerulea to 91% in F. rubra (as a percentage of the amount supplied: 4 mg). The analysis of variance (three-way) revealed that harvest date, species, and treatment ($P < 0.01$) had a statistically significant effect on the amount of ^{15}N in shoots and roots. In the low fertility treatment A. elatius had the highest ^{15}N content in roots and M. caerulea the lowest (Figure 3a). More ^{15}N was contained in the roots of A. elatius and L. perenne than in the two Festuca species, but at the high nutrient supply treatment the differences between L. perenne and F. rubra were not statistically significant. When nutrient supply increased the amount of ^{15}N decreased significantly in roots in all

species except M. caerulea (Figure 3b; effect of the interaction treatment x species: $P < 0.001$).

In the low fertility treatment the ^{15}N content (mg pot^{-1}) was higher in shoots than in roots in all species except in A. elatius (Figure 3a). The differences in ^{15}N content between shoots and roots became greater with increasing nutrient supply.

Losses of labelled N

We assumed that nitrogen losses had two components: losses via above-ground litter production and other losses including root mortality, leaching and root exudation.

During the experiment we did not observe any shift in color of roots that could indicate root mortality. Nitrogen losses through above-ground litter production were calculated as the amount of ^{15}N in the above-ground dead material (leaf, sheaths, stems). Note that all produced dead material could be collected for each pot separately except fallen seeds and shed panicles. Nitrogen losses from other causes were calculated as the difference between the amount of ^{15}N present in the whole plant at the end of the experiment and at the beginning (24 hours after labelling). Since at 24 hours after labelling there was no detectable amount of ^{15}N in dead material, the ^{15}N present in the living parts at this time was considered as the total amount absorbed per pot.

A two-way ANOVA (with species and harvest dates as factors) showed a significant reduction in the amount of labelled N present in the whole plant including the dead plant parts ($P < 0.001$) with time. This indicates that there was a net loss of labelled N from the species studied by other pathways than that by litter production. Figure 4 shows these nitrogen losses for the different species. The differences in the amounts of ^{15}N lost through above-ground mortality at the end of the experiment were statistically significant between all species ($P < 0.01$; using two-way ANOVA with species and harvest as factors) except between A. elatius and F. rubra (Table 1). The amounts ^{15}N lost through pathways other than litter production at the end of the experiment differed statistically significantly ($P < 0.05$) between most of the species as well. In the low nutrient supply treatment, losses of ^{15}N from L. perenne mostly (70%

of the total losses) occurred via litter production. However, in A. elatius, F. rubra, F. ovina and M. caerulea more than 50% of the losses of labelled N took place by leaching or root exudation. A two-way ANOVA (with species and treatments as factors) showed that at the end of the experiment there were significant differences between treatments in the amount of labelled N lost via above-ground mortality ($P < 0.01$) and via other pathways ($P < 0.05$). When the nutrient supply increased, the ^{15}N losses by above-ground mortality increased in all species, and the ^{15}N losses by other pathways decreased in A. elatius, A. odoratum and F. rubra. A two-way ANOVA showed that at the end of the experiment there were no statistically significant interactions between the effects of species and fertilization treatments on the total amount of ^{15}N lost.

The total amount of labelled N lost (as a percentage of the amount of ^{15}N absorbed at the start of the experiment) is defined as turnover (TO) according to the following equation:

$$TO = \frac{{}^{15}\text{NWS} - {}^{15}\text{NLE}}{{}^{15}\text{NWS}} = \frac{{}^{15}\text{NWS} - {}^{15}\text{NWE}}{{}^{15}\text{NWS}} + \frac{{}^{15}\text{NWE} - {}^{15}\text{NLE}}{{}^{15}\text{NWS}} \quad (1)$$

where: ^{15}NWS = ^{15}N content in the whole plant at the start of the experiment; ^{15}NWE = ^{15}N content in the whole plant at the end of the experiment; ^{15}NLE = ^{15}N content in living parts at the end of the experiment. In the low nutrient supply treatment the sequence of species in order of decreasing turnover coefficient was: L. perenne, A. odoratum, F. rubra, F. ovina, A. elatius, M. caerulea. In the high fertility treatment the sequence was: L. perenne, A. odoratum, F. ovina, F. rubra, A. elatius, M. caerulea.

The amount of ^{15}N in the above-ground parts decreased statistically significantly ($P < 0.01$) throughout the experiment. The net reduction of labelled N in above-ground living parts was partly attributable to losses through the death of plant parts but probably also to translocation to roots and in some species to abscission of flowers. A. elatius produced flowers after 8 weeks but those samples contained

insufficient material for analysis. M. caerulea produced panicles after 8 weeks and the abscission of spikelets and flowers led to significant N losses.

DISCUSSION

Various authors (Berendse et al. 1992; Escudero et al. 1992) have quantified nitrogen losses from plants by measuring only litter production when demonstrating differences between species. Our results show that an important part of the absorbed nitrogen is lost through pathways other than mortality of above-ground plant parts. In our experiment other losses even exceeded those by mortality of above-ground plant parts in some species. These other pathways include: leaching from leaves and roots, root exudation, turnover of roots and abscission of flowers. We were unable to distinguish between these different kinds of losses. Note that due to reabsorption from the nutrient solution, underestimation of the amounts of ^{15}N that are lost may occur. The decrease of labelled N in the living plant parts may be due to: mortality, leaching, and losses of plant material by abscission of flowers. We quantified losses via litter production from the above-ground parts and observed losses by shedding of flowers in A. elatius and M. caerulea. Leaching from leaves becomes an important pathway for nitrogen loss at the time of autumn rainfall (Chapin and Moilanen 1991). Experiments with synthetic rainwater resulted in leaching 2-8% of the total foliar nitrogen in deciduous tree species (Boerner 1984). However, nitrogen losses by leaching from leaves in grasslands are generally negligible (Clark 1977; Woodmansee et al. 1981). In our experiment that was carried out in a greenhouse plants did not experience any rainfall and so leaching from leaves can be excluded. It can therefore be inferred that losses other than those by litter production from the whole plant should be attributed to root exudation or leaching from roots in L. perenne, A. odoratum, F. rubra and F. ovina, whereas in A. elatius and M. caerulea also losses by shedding of flowers occurred. Our results show that the amounts of ^{15}N lost through pathways other than mortality decreased in most of species with increasing nutrient supply. In contrast, Li et al. (1992) observed a small percentage of ^{15}N in the nutrient solution leached from the

roots in a high fertility treatment though not under low N supply rates. Their experiment was short (6 weeks), and it is important to remember that as plants develop, the transfers of nutrient through decomposition of moribund tissues become more important than exudation by diffusion (Rovira 1969, cited by Wetselaar and Farquhar 1980). On the other hand, variation in root exudation appears to be mainly explained by differences in root growth, rather than by the nitrogen concentration in the nutrient solution (Liljeroth et al. 1990).

In both fertility treatments nitrogen losses via the non-litter pathways were greater in species found in nutrient-poor habitats. These differences may be related to differences in root system morphology, root length, percentage of fine roots or total root length (Boot and Mensink 1990). The roots of *F. rubra* and *F. ovina* are fine and were therefore more susceptible to shedding as a result of possible physical damage during the experiment (continuous aeration of nutrient solution, replacing of the nutrient solution). Under experimental conditions it has been demonstrated that even slight agitation of the root system increases the amount of amino-acids released into the nutrient solution (Hale et al. 1971). It is possible that differences in root growth account for the differences in nitrogen losses from the roots between treatments. Given that the percentage of fine roots increases with decreasing nutrient supply (Boot and Mensink 1990), it is feasible that the larger losses of labelled N in the low nutrient supply treatment reflect a higher percentage of fine roots susceptible to damage.

In *M. caerulea* it is important to consider that part of the non-litter losses are attributable to plant material lost through abscission of flowers; 38% of the labelled N found in the above-ground plant parts was in panicles. But it must also be remembered that *M. caerulea* dies down at the end of the growing season. After 32 weeks of labelling we found that 70% of the ^{15}N present in *M. caerulea* was in roots and basal internodes.

We measured important differences in nitrogen losses (through above-ground mortality and other losses) between species in this experiment. Our results show that in both the fertilized and unfertilized treatments species adapted to nutrient-rich sites lose

more nitrogen than species from less fertile soils . These differences between species also occurred in losses due to litter production in both fertility treatments. This finding confirms Chapin (1980) and the results obtained by Berendse and Elberse (1989) and Berendse et al. (1992). However, the losses we found in A. elatius were lower than would be expected from a species adapted to environments in which nutrients are readily available. Nitrogen losses by litter production in A. elatius were small (Table 1), but this can be explained in relation to the high biomass and nitrogen allocation to the roots (60%). Furthermore, litter production included leaves and leaf sheaths; after 8 weeks of labelling A. elatius had produced culms (real stems) and we observed that the leaf sheaths had a lower turnover rate in the culm than in pseudostems.

When for these reasons A. elatius is left out of consideration there appears to be a significant positive correlation ($r=0.893$; $P<0.05$) between relative ^{15}N total losses at the end of the experiment under low fertilizer conditions and the growth during the first 8 weeks after labelling under the high fertility treatment (Figure 5). It was assumed that the growth at the high level of nutrient supply during the first weeks measures the potential growth rates of the different species.

These results suggest that there is a positive relationship between potential growth rate and total nitrogen loss in a series of species characteristic of a gradient of nutrient availabilities. This significant relationship vanishes when A. elatius is taken into account as well. The observed relationship suggests the two strategies (slow potential growth rate and small losses versus fast potential growth rate and large losses) that correspond with the stress-tolerant and competitive strategies distinguished by Grime (1979). We can conclude that the total N loss from above- and below-ground plant parts is an important feature to explain the mechanisms of adaptation of species to habitats with contrasting nutrient supplies.

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Fig. 1 Biomass of living plant parts and the whole plant (g dry matter pot⁻¹) in the low (a) and high (b) nutrient supply treatment in *Lolium perenne* (Lp), *Arrhenatherum elatius* (Ae), *Anthoxanthum odoratum* (Ao), *Festuca rubra* (Fr), *Festuca ovina* (Fo), and *Molinia caerulea* (Mc).

Fig. 2 Accumulated total nitrogen absorption (mg pot⁻¹) in the low (a) and high (b) nutrient supply treatments in *Lolium perenne* (Lp), *Arrhenatherum elatius* (Ae), *Anthoxanthum odoratum* (Ao), *Festuca rubra* (Fr), *Festuca ovina* (Fo), and *Molinia caerulea* (Mc).

Fig. 3 ¹⁵N content (mg pot⁻¹) of shoots and roots in the low (a) and high (b) nutrient supply treatments in *Lolium perenne* (Lp), *Arrhenatherum elatius* (Ae), *Anthoxanthum odoratum* (Ao), *Festuca rubra* (Fr), *Festuca ovina* (Fo), and *Molinia caerulea* (Mc).

Fig. 4 ¹⁵N content (mg pot⁻¹) of living parts and whole plant in the low (a) and high (b) nutrient supply treatments in *Lolium perenne* (Lp), *Arrhenatherum elatius* (Ae), *Anthoxanthum odoratum* (Ao), *Festuca rubra* (Fr), *Festuca ovina* (Fo), and *Molinia caerulea* (Mc).

Fig. 5 Relationship between ¹⁵N relative total losses (%) in the low fertility treatment and the growth during 8 weeks after labelling (mg pot⁻¹ week⁻¹) in the high fertility treatment.

Table 1. Losses of ^{15}N (mg pot^{-1}) by above-ground mortality ($^{15}\text{NWE} - ^{15}\text{NLE}$), other losses ($^{15}\text{NWS} - ^{15}\text{NWE}$) and total losses ($^{15}\text{NWS} - ^{15}\text{NLE}$) at the end of the experiment. In each case relative losses were calculated as a percentage of the ^{15}N content in the whole plant at start of the experiment (^{15}NWS), according equation 1 in text. Values in the same column with different superscript differ significantly at $P < 0.05$ (two-way ANOVA with species and treatments as factors)

Low fertility treatment						
Species	Above-ground mortality		Other losses		Total losses	
	mg pot^{-1}	relative (%)	mg pot^{-1}	relative (%)	mg pot^{-1}	relative (%)
Lp	1.12 a	33	0.48 a	14	1.60 a	47
Ae	0.47 b	15	0.56 ab	18	1.03 b	33
Ao	0.82 c	24	0.64 b	19	1.46 c	47
Fr	0.48 b	13	0.88 c	24	1.34 c	37
Fo	0.31 d	10	0.80 c	26	1.11 b	35
Mc	0.14 e	6	0.52 a	22	0.65 d	28
High fertility treatment						
Lp	1.21 a	36	0.49 a	14	1.70 a	50
Ae	0.65 b	21	0.24 b	8	0.89 b	29
Ao	1.06 c	32	0.33 bc	10	1.39 c	45
Fr	0.71 b	19	0.44 ac	12	1.15 d	31
Fo	0.60 d	19	0.76 d	24	1.35 c	43
Mc	0.21 e	9	0.63 e	27	0.83 b	36

Fig. 1

(a) Low nutrient supply treatment

(b) High nutrient supply treatment

—▲— whole plant

—○— living parts

Fig 2

(a) Low nutrient supply treatment

(b) High nutrient supply treatment

Fig 3

(a) Low nutrient supply treatment

(b) High nutrient supply treatment

—□— Shoots

—■— Roots

Fig 4

(a) Low nutrient supply treatment

(b) High nutrient supply treatment

—▲— whole plant

—○— living parts

Fig 5