Nitrogen use efficiency in six perennial grasses from contrasting habitats

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Running headline: Nitrogen use efficiency in grass species

Summary

1. We studied the nitrogen use efficiency (*NUE*) in six perennial grasses adapted to a wide range of nutrient availability. The glasshouse experiment was carried out in pots containing nutrient solution, with two fertility treatments. Nitrogen use efficiency was considered as the product of nitrogen productivity and mean residence time of the nitrogen in the plant (calculated using ^{15}N pulse labelling).
2. The species investigated are characteristic of habitats ranging from very nutrient-rich to extremely nutrient-poor, in the following order: *Lolium perenne*, *Arrhenatherum elatius*, *Festuca rubra*, *Anthoxanthum odoratum*, *Festuca ovina* and *Molinia caerulea*.
3. *L. perenne* (adapted to nutrient-rich habitats) had higher nitrogen productivity (*A*) than *M. caerulea* (species adapted to nutrient-poor habitats) but lower than that of *F. rubra* (from habitats with an intermediate availability of nutrients).
4. In the low fertility treatment, species with the lowest nitrogen use efficiency had the lowest N productivity and the highest mean nitrogen residence time (*MRT*); however, although species with the highest nitrogen use efficiency had the highest N productivity they did not have the lowest *MRT*. In all species the nitrogen use efficiency decreased with increasing N supply. The two components of the *NUE* (*A* and *MRT*) are inversely correlated along gradients of nutrient availability, but not at very high levels of nutrient availability.
5. The nitrogen use efficiency of species at constant levels of nutrient supply tends to increase with increasing nutrient availability in their preferred habitat, according to the Clausman nutrient index, up to a certain nutrient availability and then decreases. The results support the contention that species from nutrient-poor sites

are not necessarily adapted by a high nitrogen use efficiency, but by low nutrient loss rates (high mean residence time of N in the plant).

Key-words: Mean residence time of nitrogen, nitrogen losses, nitrogen productivity, plant strategies

Introduction

Part of the success of a perennial plant population is determined by the amounts of carbon or nutrients assimilated versus those lost from the population. The importance of nitrogen has been investigated in many studies because in many natural environments the supply of this nutrient does not meet the demand from plants. Plants may adapt to environments where nutrient supply limits plant growth by following one of two routes: maximizing assimilation of nutrients (Tilman 1988) or reducing losses (Grime 1979; Chapin 1980; Berendse & Elberse 1989). These two adaptive strategies of perennial plants with respect to the nutrient availability can be summarized in the index of nitrogen use efficiency (*NUE*) proposed by Berendse & Aerts (1987). They redefined nitrogen use efficiency as the product of nitrogen productivity (*A*: rate of dry matter production per unit N in the plant) and mean residence time (*MRT*) of the nitrogen in the plant, and postulated that due to evolutionary tradeoffs, genotypically determined plant characteristics promoting rapid growth are inversely correlated with characteristics that reduce nutrient losses. Thus, nutrient-poor habitats will be dominated by slow-growing species (low *A*) with low nutrient loss rates (long *MRT*) and nutrient-rich habitats by fast-growing species (high *A*) with high rates of nutrient loss (short *MRT*). This

definition of nitrogen use efficiency has been used or proposed previously by other authors (e.g. Hirose 1975; Boerner 1984). However these definitions have not been based on a theoretical background.

Escudero *et al.* (1992) have reported that a prolonged retention time of nutrients in the leaf biomass is one way of increasing nutrient use efficiency in conditions of low nutrient availability. Various researches have investigated the change in nitrogen use efficiency in a single species at different levels of nutrient supply. It has been shown that the efficiency of nitrogen use decreases after nutrient availability to a single species has been enhanced (Birk & Vitousek 1986; Lajtha & Klein 1988; Gutschick 1993; Aerts & de Caluwe 1994). For species with different growth forms there are comparative studies on nutrient use efficiency, being greater in evergreen species than in deciduous species (Gray 1983; Chapin & Shaver 1989). However, it is not clear how nitrogen use efficiency (*NUE*) changes in species characteristic of contrasting habitats along a natural gradient of nutrient availability. Therefore, we decided to use the *NUE* index defined by Berendse & Aerts (1987) to study the nitrogen use efficiency in six perennial grasses when growing at two different levels of nutrient supply. The species are characteristics of a wide range of nutrient availabilities from very nutrient-rich to extremely nutrient-poor environments in the following order: *Lolium perenne*, *Arrhenatherum elatius*, *Festuca rubra*, *Anthoxanthum odoratum*, *Festuca ovina* and *Molinia caerulea*. *L. perenne* is characteristically dominant in heavily fertilized pastures, *A. elatius* is dominant in lightly shaded and fertile environments, *A. odoratum* and *F. rubra* are characteristic from soils of intermediate fertility, *F. ovina* occurs in uplands and unproductive grasslands, and *M. caerulea* is characteristic from wet,

acidic and nutrient-poor habitats (Grime *et al.* 1988). We used ^{15}N pulse labelling to determine the mean residence time of the nitrogen in the plant by estimating the total N loss from above- and below-ground plant parts.

We set out to answer two questions: How does *NUE* change among species that are characteristic for a gradient of nutrient availability? And how are the components of *NUE* related in species growing at the same level of nutrient supply although adapted to environments with different nutrient availability?

Materials and methods

The measurements were performed as a part of the same experiment described by Vázquez de Aldana *et al.* (1996). Monocultures of six perennial grasses *Lolium perenne*, *Arrhenatherum elatius*, *Festuca rubra*, *Anthoxanthum odoratum*, *Festuca ovina*, and *Molinia caerulea* were grown in a glasshouse in pots containing nutrient solution (3 litres). These species are characteristic of a wide range of nutrient availability. The experiment contained five replicated blocks. Two fertility treatments were applied after ^{15}N labelling the plants. The experiment was carried out in a glasshouse with temperature controlled under natural light intensity from April 1993 to April 1994.

GROWTH AND LABELLING OF PLANT MATERIAL

From 22 to 26 April 1993, seeds of *Lolium perenne*, *Arrhenatherum elatius*, *Festuca rubra*, *Anthoxanthum odoratum*, and *Festuca ovina* were germinated on a 1:2 mixture of poor sandy soil and pure sand (1% organic matter content in the mixture) in a glasshouse. Germination tests showed that *Molinia caerulea* did not

germinate under the given conditions. On 20 April 1993, *Molinia caerulea* plant material was collected from the field and transplanted into pots containing identical substrates than the other species. From 27 May to 2 June 1993, plants of equal size from each species were selected and placed on pots (capacity 3 litres) containing nutrient solution (four plants per pot). Until pulse labelling with ^{15}N , all plants were grown in the same concentration of nutrient solution. This solution was a 1:16 (low fertilizer) dilution of the solution described by Hoagland & Snyder (1938) cited in Hewitt (1966). The solution supplied contained $0.31 \text{ mmol l}^{-1} \text{ KNO}_3$; $0.31 \text{ mmol l}^{-1} \text{ Ca}(\text{NO}_3)_2$; $0.12 \text{ mmol l}^{-1} \text{ MgSO}_4$; $0.06 \text{ mmol l}^{-1} \text{ KH}_2\text{PO}_4$; $46 \text{ } \mu\text{mol l}^{-1} \text{ H}_3\text{BO}_3$; $9.1 \text{ } \mu\text{mol l}^{-1} \text{ MnCl}_2$; $0.3 \text{ } \mu\text{mol l}^{-1} \text{ CuSO}_4$; $0.7 \text{ } \mu\text{mol l}^{-1} \text{ ZnSO}_4$; $0.5 \text{ } \mu\text{mol l}^{-1} \text{ H}_2\text{MoO}_4$. On 28 June 1993 all pots were supplied with $4 \text{ mg } ^{15}\text{N}$ ($^{15}\text{NH}_4^{15}\text{NO}_3$, 95 atom % ^{15}N). The nutrient solution in the pots was replaced by tap water, 48 hours before labelling with ^{15}N . The ^{15}N pulse labelling took 24 hours. Immediately afterwards the root systems were rinsed in tap water and the plants were transferred to unlabelled nutrient solutions. The first series of pots (6 species $\times$ 5 replicates) was harvested at this time. The remaining pots were divided into two series: one series was treated with low fertilizer nutrient solution (LF) as described, and the other was treated with high fertilizer nutrient solution (HF) (1:4 dilution Hoagland). The high fertilizer nutrient solution contained: $1.25 \text{ mmol l}^{-1} \text{ KNO}_3$; $1.25 \text{ mmol l}^{-1} \text{ Ca}(\text{NO}_3)_2$; $0.5 \text{ mmol l}^{-1} \text{ MgSO}_4$; $0.25 \text{ mmol l}^{-1} \text{ KH}_2\text{PO}_4$ 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 of solution in the pots was maintained with demineralized

water (twice a week). Ten pots (2 treatments $\times$ 5 replicates) of each species, chosen randomly, were destructively harvested after 8, 16, 24, 32 and 40 weeks on 24 August, 19 October, 14 December, 8 February and 5 April respectively.

At each harvest the plant material was divided into roots, stems including sheaths, leaves, flowers and above-ground dead material. No root mortality was observed during the experiment. Dry weighs were determined after oven drying at 70 °C for 48 h. The nitrogen content and ^{15}N concentration of labelled plant material were determined using an Isotope Ratio Mass Spectrometer (ANCA-IRMS).

CALCULATIONS

The absolute amount of ^{15}N present in the sample was obtained using the following equation: $B = (15 \times C \times D) / (1400 + D)$, where C = weight of the total N in the sample analysed; D = ^{15}N atom % in the sample; B = weight of ^{15}N in the sample analysed. Values of D were corrected for the natural abundance of ^{15}N in the plant material by subtracting the natural abundance ^{15}N (0.366%) from laboratory determinations.

We used the relative nitrogen requirement (L_n ; $\text{mg } ^{15}\text{N mg}^{-1} ^{15}\text{N 40 weeks}^{-1}$) as nitrogen turnover variable (Berendse, Oudhof & Bol 1987). It was calculated as the ratio between the total amount of ^{15}N lost from above- and below-ground plant parts at the end of the experiment (40 weeks) and the amount of ^{15}N in the total plant biomass initially absorbed (24 hours after labelling). Nitrogen losses were quantified by the decrease in total labelled N during the experiment. As no dead

material was found at the first harvest (24 hours), the N lost quantified at the end of the experiment was considered as the total loss.

The mean residence time of the nitrogen in the plant (*MRT*: 40 weeks) was calculated as the inverse of the relative nitrogen requirement, according to Berendse & Aerts (1987).

Nitrogen productivity was calculated for each sampling interval (8 weeks) as the ratio of dry matter production to mean amount of N present in the plant during that period according to the following equation:

$$A(t) = \frac{B(t) - B(t-1)}{\frac{N(t) + N(t-1)}{2}}$$

where *B* is total (living + dead) biomass and *N* the amount of nitrogen in the living biomass. The average of the N productivity (*A*) was calculated across the estimates within all sampling intervals within the whole experiment (*A*(1), *A*(2), *A*(3), *A*(4), *A*(5)).

Nitrogen use efficiency (*NUE*) was calculated as the product of the nitrogen productivity average (*A*) and the mean residence time (*MRT*).

The data were analysed statistically using Statgraphics (1991) for analysis of variance. Multiple comparisons among pairs of means were made using Tukey range test.

Results

BIOMASS DISTRIBUTION IN THE PLANT

The distribution of biomass (g dry matter per pot) in different plant parts at the end of the experiment at both fertility treatments is presented in Fig. 1. In the low

fertility treatment, the biomass in roots of *A. elatius* significantly ($P<0.001$) exceeded that of the other species, with *M. caerulea* having the lowest root biomass. *F. rubra* had the highest biomass in living leaves ($P<0.001$). *L. perenne*, *A. odoratum* and *F. ovina* had the highest biomass in stems ($P<0.001$). The total biomass of *M. caerulea* is lower than that of the other species. In terms of proportional allocation of dry matter, *M. caerulea* allocated more biomass to the roots and less to the leaves than *L. perenne*.

At increasing nutrient supply, the biomass in all plant parts increased significantly ($P<0.001$). In all species, dry matter allocation in roots decreased and allocation to stems and to living leaves increased.

There was a significant effect of the interaction species $\times$ treatments on the total biomass at the end of the experiment ($P<0.001$). *F. rubra* and *A. odoratum* had the highest total biomass in the low fertility treatment and *A. elatius* had the highest in the high fertility treatment (Fig. 1).

ABSORPTION OF LABELLED NITROGEN AND RELATIVE NITROGEN REQUIREMENT

The absorption of ^{15}N (24 h after labelling) as percentage of the amount supplied ranged from 57.5% in *M. caerulea* to 91.9% in *F. rubra* (Table 1).

Analysis of variance revealed that species differed significantly ($P<0.05$) in their relative nitrogen requirement (Table 1). Differences between treatments were not statistically significant. In both fertility treatments, the relative nitrogen requirement of *L. perenne* and *A. odoratum* significantly ($P<0.05$) exceeded that of *M. caerulea*. This shows that species from nutrient-rich habitats lose more ^{15}N per

unit of ^{15}N absorbed than do species characteristic of nutrient-poor habitats. However, *A. elatius* had lower losses than expected for a species adapted to nutrient-rich habitats. In the high fertility treatment *A. elatius* had a lower relative nitrogen requirement than *M. caerulea* (Table 1).

NITROGEN PRODUCTIVITY

In all species nitrogen productivity at each sampling interval decreased with plant size until a certain moment and then increased (Fig. 2). The nitrogen productivity decrease was stronger in the low fertility treatment than in the high fertility treatment.

The nitrogen productivity average (A) and the nitrogen productivity at the first time interval ($A(1)$) are shown in Table 2. There was a significant effect of species and treatment and their interaction on the two nitrogen productivity estimates (A , $A(1)$) ($P < 0.01$). In the low fertility treatment, *A. elatius*, *F. rubra*, *A. odoratum* and *F. ovina* showed no statistically significant differences in the nitrogen productivity during the first sampling interval ($A(1)$) and *F. rubra* and *A. odoratum* had the highest A . In the high fertility treatment *A. elatius* had the highest A , $A(1)$ and A_{tot} . In both fertility treatments *M. caerulea* had the lowest A and $A(1)$. This comparison of N productivity (in any of the two estimates) among species shows that when growing in low fertility treatment (LF), species adapted to nutrient-rich habitats, such as *L. perenne*, have a higher N productivity than species adapted to nutrient-poor habitats (*M. caerulea*) but a lower N productivity than species from habitats with an intermediate availability of nutrients (*F. rubra*). The low N productivity in *M. caerulea* is attributable to its low biomass production. It must be remembered that

M. caerulea is deciduous and died down almost completely at the end of the growing season (between weeks 24 and 32 of the experiment). At this time, nitrogen was translocated into the basal internodes and roots resulting in small differences between *M. caerulea* and the other five species in the total nitrogen present in the plant (Vázquez de Aldana *et al.* 1996). However, there were large differences in total plant biomass between *M. caerulea* and the other species.

In all species except *M. caerulea*, nitrogen productivity decreased significantly ($P<0.01$) with increasing nutrient supply. Contrary to dry matter production, interspecific differences in *A* were greater under low nutrient supply than under high nutrient supply.

MEAN RESIDENCE TIME

The mean residence time (estimated from ^{15}N labelling) differed significantly ($P<0.05$) among species but not between fertility treatments. In the low fertility treatment, *L. perenne* had the highest *MRT* and *M. caerulea* the lowest *MRT* (Table 3). *M. caerulea*'s retention long of a unit of ^{15}N indicates that this species loses less nitrogen through mortality of plant parts and other pathways (exudation from the roots, leaching). Because of its seasonal dynamics, *M. caerulea* translocates most of the nitrogen to basal internodes and roots before dying down above-ground. This shows that *M. caerulea* has an efficient mechanism for nutrient conservation and retains nutrients for longer. In the HF treatment, *A. elatius* and *F. rubra* had the highest *MRT*. In both fertility treatments, *L. perenne* and *A. odoratum* had the lowest *MRT*.

The results show that when grown in the LF treatment, the species with the lowest dry matter production, *M. caerulea*, had the highest *MRT* (lowest turn-over rate). However, the species with the highest dry matter production in our experiment, *F. rubra*, did not have the lowest *MRT* (highest turn-over rate).

NITROGEN USE EFFICIENCY

There was a significant effect of species and fertility treatments ($P < 0.01$) on the nitrogen use efficiency. In the low fertility treatment, *A. elatius*, *F. rubra* and *F. ovina* had much higher *NUE* values than *L. perenne* and *M. caerulea* (Table 3). In the high fertility treatment *A. elatius* and *F. rubra* had the highest *NUE* and *L. perenne* and *M. caerulea* the lowest *NUE*. The results showed that in the low fertility treatment, species with the lowest *NUE* had the lowest N productivity (*A*) and the highest *MRT*; however, species with the highest *NUE* had the highest *A* but did not have the lowest *MRT*. In all species *NUE* decreased with increasing N supply (Table 3). In all species except *M. caerulea*, this was due to the declining *A*.

Discussion

We measured the nitrogen use efficiency in perennial grasses by distinguishing two components: nitrogen productivity (*A*) and mean residence time (*MRT*) according to the *NUE* index of Berendse & Aerts (1987). This approach allows for total biomass production (above- and below-ground). That is substantial when comparing species since allocation of biomass to roots may differ among species and roots comprise an important fraction of total biomass (e.g. Fig. 1). Our

calculations of the mean residence time of the nitrogen are based on ^{15}N pulse labelling technique that includes total nitrogen losses from the plant (litter production, exudation, leaching...). These are important issues to consider in the concept of nitrogen use efficiency (Grubb 1989). The component *A* (nitrogen productivity) of the *NUE* was calculated as average of the nitrogen productivity estimates at each sampling interval within the whole experiment. The *NUE* index of Berendse & Aerts (1987) was applied to field populations that were supposed to have a steady-state biomass. Since that is not the case in our experiment, we used the average of the *N* productivity (as indicated in Material and Methods). There was a positive significant correlation between the the nitrogen productivity average (*A*) and the *N* productivity at the first time interval (*A*(1)) ($r=0.961$, $n=12$, $P<0.0001$). This makes that using the *N* productivity average (*A*), the results of species comparisons and relationships between *NUE* and its components, which are dealt further below, are similar than when *A*(1) is considered.

Our results showed that the species characteristics from nutrient-poor habitats did not have the highest *NUE* but they had the highest *MRT* and the lowest *A*. This agrees with the hypothesis of Berendse & Aerts (1987) that selection in nutrient-poor habitats is not necessarily on a high *NUE* but on features that reduce nutrient loss (high *MRT*) (cf. Grime 1979; Sibly & Grime 1986). A high *MRT* in the plant suggests a prolonged retention time of nitrogen and consequently reduced nitrogen losses. Our experiment showed that in the low fertility treatment, species from nutrient-rich habitats had the lowest *MRT* but not the highest *A*. This does not agree with the high *N* productivity postulated by Berendse & Aerts (1987) for

species characteristic of nutrient-rich habitats. But other aspects need to be considered when interpreting these results.

In the low fertility treatment, *L. perenne*, *A. elatius* and *F. rubra* did not differ statistically significantly in total nitrogen content (Vázquez de Aldana *et al.* 1996) but the total biomass in *F. rubra* was statistically significantly greater than that in *L. perenne* and *A. elatius*. Therefore *F. rubra* has a faster rate of dry matter production per unit of N present in the plant. Similarly, in the high fertility treatment, the differences in N productivity between *L. perenne* and *A. elatius* are attributable to differences in total biomass, since differences in total N absorbed were not statistically significant. Plants found in nutrient-poor habitats generally have lower relative growth rates than those preferring more fertile sites (Grime & Hunt 1975; Poorter & Remkes 1990). In short term experiments (12 weeks), it has been reported that in nutrient-poor habitats species with a high potential growth rate also produce more biomass than species with a low potential growth rate (Boot & den Dubbelden 1990). However, our results showed that in the low fertility experimental conditions (LF), *L. perenne*, a species with a higher potential growth rate than *F. rubra*, produced less biomass than *F. rubra*. Species adapted to nutrient-rich habitats seem to be more nutrient-limited than species from nutrient-poor habitats, and the N productivity of species from nutrient-rich sites was not the highest.

In all species, except *M. caerulea*, nitrogen productivity decreased after enhanced nutrient supply. This phenotypic response has been reported previously (Birk & Vitousek 1986; Gutschick 1993; Aerts & de Caluwe 1994; Bowman 1994). The fact that in *M. caerulea* the N productivity (A) did not increase significantly

after enhanced nutrient supply could be a consequence of its inability to take up the available nitrogen. Ågren (1983) who showed mathematically that N productivity, calculated from values at the end of the growing season, in a plant decreases as biomass increases. Our results showed that N productivity for each sampling interval decreases with plant size until a certain moment and then increases (Fig. 2). The N productivity increase in the last sampling interval can be explained by the light intensity conditions since N productivity increases with light intensity (Ågren 1985; Hirose & Werger 1987). Our experiment run under natural light conditions (no extra light) and the N productivity increase occurs in the last sampling interval (8 February - 5 April) when the light intensity increases. The results show a variation of N productivity with plant size that seems to follow a hyperbola which according to Ågren (1985) would be similar to the photosynthesis-light response curve.

Looking at total ^{15}N losses from above- and below-ground plant parts, we found that *M. caerulea* had the highest *MRT* and *L. perenne* the lowest. This implies that under low fertility conditions, species adapted to nutrient-rich habitats have a higher N turnover rate than species from nutrient-poor habitats. This does not agree with the results found by Aerts & de Caluwe (1994) in three *Carex* species. Contrary to their expectations, they found that the species with the lowest maximum growth rate (*Carex diandra*) had the lowest *MRT*, whereas the species with the highest maximum growth rate (*Carex acutiformis*) had the highest *MRT*. Our results showed that when grown under the same conditions of low nutrient availability, species from contrasting habitats exhibit the same interspecific differences in *MRT* as those expected when grown at the level of nutrient

availability prevailing in their natural habitat. This agrees with the contention that poorly productive species from nutrient-poor habitats have lower nutrient turn-over rates (high *MRT*) than very productive species from nutrient-rich habitats (Chapin 1980; Berendse & Aerts 1987). The mean residence time of nitrogen in the plant, which is an index of the nitrogen turn-over rate, can be considered to be an inherent characteristic of species that explains the adaptation of species to natural gradients of nutrient availability.

The nitrogen use efficiency in the six perennial grasses changed with the N productivity. There was a positive significant correlation between N productivity (*A*) and *NUE* of the six species in the low fertility treatment ($r=0.79$, $P<0.05$) and in the high fertility treatment ($r=0.82$, $P<0.05$). The mean residence time of the nitrogen and *NUE* were positively correlated in the HF treatment ($r=0.79$, $P<0.05$) but not in the LF treatment.

We found that the two components of *NUE* (*A* and *MRT*) were inversely correlated ($r=-0.87$, $P<0.10$, Spearman's rank order correlation) in all the species except *L. perenne* (Fig. 3). The species from the extremely nutrient-poor habitats had the highest *MRT* and the lowest *A*. However, the species from the extremely nutrient-rich sites had the lowest *MRT* but not the highest *A*. This suggests that the two components of the *NUE* proposed by Berendse & Aerts (1987) are inversely correlated along gradients of nutrient availability, but not at very high levels of nutrient availability. It seems that species characteristic of very nutrient-rich habitats growing in conditions of low nutrient availability do not necessarily have a high *A* but always have a low *MRT*. The inherently low *MRT* of these species implies that they have a high nitrogen turnover rate.

We ranked the species according to the nutrient index proposed by Clausman (1985) which is based on several correlative field studies on the relation between nutrient availability and the frequency of species occurrence. When the nitrogen use efficiency of the six species was plotted against their Clausman nutrient index (Fig. 4), it was observed that at uniform levels of nutrient supply (the same fertility treatment), the *NUE* of species tends to increase in the same order as the Clausman nutrient index, up to a certain nutrient availability and then to decrease. This means that the *NUE* of species characteristic of a gradient of nutrient availability increases with increasing nutrient availability of their preferred habitat, up to a certain level. It should be distinguished between these interspecific differences and the decrease of *NUE* of each species after enhanced nutrient supply (phenotypic response). In all species except *M. caerulea*, the decrease of *NUE* with increasing nutrient supply was due to the declining *A*.

In conclusion, the results show that species characteristics from very nutrient-rich sites growing in conditions of low nutrient availability have lower nitrogen productivity than species from habitats with an intermediate availability of nutrients. Thus, the components of the *NUE* index (*A* and *MRT*) (Berendse & Aerts 1987) are inversely correlated along gradients of nutrient availability, but not at very high levels of nutrient availability as indicated by *L. perenne*. It seems that a long mean residence time of nitrogen in the plant (and not an high nutrient use efficiency) is an advantageous characteristic of species growing in nutrient-poor sites.

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↓ an earlier draft of this manuscript.

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Table 1. Absorption of ^{15}N (24 hours after labelling) as percentage of the amount supplied and relative nitrogen requirement per unit of ^{15}N taken up at the start of the experiment (L_n : $\text{mg } ^{15}\text{N}$ total losses $\text{mg}^{-1} ^{15}\text{N}$ taken up 40weeks^{-1}) of *Lolium perenne*, *Arrhenatherum elatius*, *Festuca rubra*, *Anthoxanthum odoratum*, *Festuca ovina*, and *Molinia caerulea* when grown in the low fertility treatment (LF) and high fertility treatment (HF). Values are means of five replicates $\pm$ SE.

species	^{15}N absorption	L_n	
		LF	HF
<i>L. perenne</i>	85.1 $\pm$ 1.2	0.47 $\pm$ 0.02	0.50 $\pm$ 0.03
<i>A. elatius</i>	76.7 $\pm$ 2.0	0.33 $\pm$ 0.04	0.29 $\pm$ 0.02
<i>F. rubra</i>	91.9 $\pm$ 2.2	0.37 $\pm$ 0.02	0.31 $\pm$ 0.01
<i>A. odoratum</i>	83.3 $\pm$ 1.7	0.44 $\pm$ 0.02	0.42 $\pm$ 0.02
<i>F. ovina</i>	78.5 $\pm$ 3.2	0.35 $\pm$ 0.05	0.43 $\pm$ 0.05
<i>M. caerulea</i>	57.5 $\pm$ 2.3	0.28 $\pm$ 0.05	0.36 $\pm$ 0.02

TABLES7.DOC

Table 3. Nitrogen productivity average (A: g g⁻¹ 40 weeks⁻¹), mean residence time of nitrogen (MRT: 40 weeks) and nitrogen use efficiency (NUE: g g⁻¹) of *Lolium perenne*, *Arrhenatherum elatius*, *Festuca rubra*, *Anthoxanthum odoratum*, *Festuca ovina*, and *Molinia caerulea* when grown in the low fertility treatment (LF) and high fertility treatment (HF). Values are means of five replicates $\pm$ SE.

species	A		MRT		NUE	
	LF	HF	LF	HF	LF	HF
<i>L. perenne</i>	65.3 $\pm$ 2.2	37.5 $\pm$ 0.8	2.13 $\pm$ 0.11	2.00 $\pm$ 0.13	139 $\pm$ 14	75 $\pm$ 7
<i>A. elatius</i>	71.7 $\pm$ 1.7	47.8 $\pm$ 1.9	3.22 $\pm$ 0.30	3.55 $\pm$ 0.19	231 $\pm$ 18	169 $\pm$ 7
<i>F. rubra</i>	79.6 $\pm$ 2.3	39.9 $\pm$ 0.9	2.73 $\pm$ 0.15	3.41 $\pm$ 0.28	215 $\pm$ 15	136 $\pm$ 8
<i>A. odoratum</i>	78.6 $\pm$ 1.7	42.7 $\pm$ 0.6	2.34 $\pm$ 0.17	2.40 $\pm$ 0.07	184 $\pm$ 13	102 $\pm$ 3
<i>F. ovina</i>	69.6 $\pm$ 2.3	32.4 $\pm$ 1.6	3.19 $\pm$ 0.35	2.60 $\pm$ 0.30	222 $\pm$ 20	84 $\pm$ 9
<i>M. caerulea</i>	27.9 $\pm$ 1.4	21.2 $\pm$ 1.1	4.12 $\pm$ 0.40	2.83 $\pm$ 0.21	115 $\pm$ 16	60 $\pm$ 9

Fig. 1. Biomass distribution (g per pot) in different plant parts of *Lolium perenne* (Lp), *Arrhenatherum elatius* (Ae), *Festuca rubra* (Fr), *Anthoxanthum odoratum* (Ao), *Festuca ovina* (Fo) and *Molinia caerulea* (Mc), in the low fertility (LF) and high fertility (HF) treatments.

Fig. 2. Relationships between nitrogen productivity ($\text{g g}^{-1} \text{ 8 weeks}^{-1}$) for each sampling interval and plant size (g) in the low fertility (■) and high fertility (□) treatments.

Fig. 3. Relationship between nitrogen productivity ($A: \text{g g}^{-1} \text{ 40 weeks}^{-1}$) and mean residence time of the nitrogen ($MRT: 40 \text{ weeks}$) in *Lolium perenne* (Lp), *Arrhenatherum elatius* (Ae), *Festuca rubra* (Fr), *Anthoxanthum odoratum* (Ao), *Festuca ovina* (Fo) and *Molinia caerulea* (Mc), in the low fertility treatment.

Fig. 4. Nitrogen use efficiency (NUE) of *Lolium perenne* (Lp), *Arrhenatherum elatius* (Ae), *Festuca rubra* (Fr), *Anthoxanthum odoratum* (Ao), *Festuca ovina* (Fo) and *Molinia caerulea* (Mc) versus their Clausman nutrient index, in the low fertility (■) and high fertility (□) treatments.

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FIG 1

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FIG 2

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FIG 4

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