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Root morphological plasticity and nutrient acquisition of perennial grass species from habitats of different nutrient availability

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Abstract We studied the root foraging ability and its consequences for the nutrient acquisition of five grass species that differ in relative growth rate and that occur in habitats that differ widely in nutrient availability. Foraging responses were quantified, based on the performance of the plants in homogeneous and heterogeneous soil environments of the same overall nutrient availability. Although all species tended to produce a significantly higher root length density in a nutrient-rich patch, this response was significant only for the faster-growing species. The increased root length density resulted from small, though not significant, changes in root biomass and specific root length. The effectiveness of root proliferation was determined by quantifying the total amount of nutrients (N and P) accumulated by the plants over the course of the experiment. Plants acquired more N in a heterogeneous environment than in a homogeneous environment, although the total nutrient availability was the same. The ability to acquire nutrients (N or P) in the heterogeneous environment was not related to the ability of species to increase root length density in response to local nutrient enrichment. In contrast to other studies, our results suggest that the role of morphological plasticity of roots in acquiring patchily distributed resources is limited. Possible reasons for this discrepancy are discussed.

Key words Foraging · Morphological plasticity · Nutrient heterogeneity · Perennial grasses · Root proliferation

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Introduction

Spatial and temporal resource heterogeneity is ubiquitous in natural ecosystems (Caldwell and Pearcy 1994). Nutrients are patchily distributed in the soil at scales relevant to individual plants (Kotliar and Wiens 1990; Jackson and Caldwell 1993a,b; Stuefer 1996). Plants have developed foraging mechanisms that enable them to acquire adequate amounts of resources in these heterogeneous environments (see Hutchings and de Kroon 1994 and references therein). Foraging in plants is accomplished by morphological changes in response to environmental conditions, and may result in the selective placement of resource-acquiring structures (leaves and roots) within the environment (Grime et al. 1986; Hutchings and Slade 1988; Hutchings and de Kroon 1994; de Kroon and Hutchings 1995).

Several studies have shown the ability of plants to proliferate roots in nutrient-rich patches, i.e. to produce high root length density in nutrient-rich patches (Drew 1975; Crick and Grime 1987; Jackson and Caldwell 1989; Gross et al. 1993; Pregitzer et al. 1993; Larigauderie and Richards 1994; Bilbrough and Caldwell 1995). The degree of root proliferation is nutrient specific (Drew 1975; Jackson and Caldwell 1989), modulated by soil nutrient concentration (Jackson and Caldwell 1989) and plant nutrient demand (Caldwell 1994), but is also species specific (Crick and Grime 1987; Jackson and Caldwell 1989; Caldwell et al. 1991; Robinson 1994).

To explain this latter variation, it has been hypothesised that root foraging characteristics differ among species from habitats of different successional stage or nutrient status (Grime et al. 1986; Fitter 1994; Grime 1994; Hutchings and de Kroon 1994). Root foraging characteristics of fast-growing species from nutrient-rich habitats will be characterised by high levels of morphological plasticity which allow an extension from the localised nutrient depletion zones that are a consequence of the high nutrient uptake achieved by these plants (Grime 1994).

In contrast, slow-growing species from nutrient-poor habitats are assumed to depend on a large long-lived root system which remains viable under prolonged conditions of nutrient depletion (Grime 1994; Hutchings and de Kroon 1994). In particular, the ability to reduce nutrient losses is an important feature determining the success of species in nutrient-poor habitats (Berendse 1994; Berendse and Elberse 1990). Slow-growing species are therefore assumed to respond to environmental heterogeneity primarily by physiological plasticity (Grime 1994; Hutchings and de Kroon 1994). Physiological plasticity is the enhancement of the nutrient uptake capacity per unit root length in response to localised soil enrichment (Jackson et al. 1990; Jackson and Caldwell 1991; Robinson 1994; Van Vuuren et al. 1996). These differential responses to nutrient heterogeneity between fast- and slow-growing species may explain the documented changes in species composition during succession in natural plant communities.

In this study we test this hypothesis by examining the root foraging abilities of a range of grass species that differ in relative growth rate (RGR), and that occur along a gradient of soil nutrient availability. By using species of a single plant family, confounding effects of gross differences in growth form and phylogeny (Felsenstein 1985; Harvey et al. 1995) are avoided. Root morphological changes in response to nutrient heterogeneity are assessed as local root biomass production and as changes in specific root length (SRL, root length per unit root dry weight). Root proliferation in nutrient-rich patches is measured as root length density (RLD, root length per unit soil volume). The consequences of root foraging for the nutrient acquisition of plants (total N and P taken up) in spatially heterogeneous environments are also determined. We hypothesise that (1) faster-growing species from more nutrient rich habitats will increase root biomass and SRL in nutrient-rich patches, resulting in a higher RLD in nutrient-rich compared to nutrient-poor patches; (2) nutrient acquisition in spatially heterogeneous environments is positively correlated with the ability to generate a high RLD in the nutrient-rich patches.

Materials and methods

Plant species

This experiment was carried out with *Lolium perenne* L., *Holcus lanatus* L., *Festuca rubra* L., *Anthoxanthum odoratum* L. and *Nardus stricta* L., all common perennial grasses with a wide distribution in western Europe (Weeda 1994). *L. perenne* and *H. lanatus* are fast-growing species, *F. rubra* and *A. odoratum* are species with an intermediate RGR and *N. stricta* is a slow-growing species (Grime and Hunt 1975).

The plant material used in the experiment originated from fields along the Anlooër Diepje, a brook in the 'Drentsche Aa' Nature Reserve (53°N, 6°40′E). The management in these former agricultural grasslands changed from cutting twice a year and fertilising (100–200 kg N ha^{-1} $year^{-1}$) to cutting once a year (July) without fertilisation (Bakker 1989). Fertiliser application was stopped in different years (Olff et al. 1990). Hence, fields in which the management changed only recently are still relatively nutrient rich whereas fields in which the management changed a long time ago are nutrient poor (Olff et al. 1994). Fields with different management duration represent different stages in a reversed successional gradient (Olff et al. 1994). As a consequence of the decline in nutrient availability during this reversed succession, the species composition of the fields changed. The pasture species *L. perenne* is replaced by *H. lanatus* shortly after fertilisation stops, and *H. lanatus* is gradually replaced by *F. rubra*, *A. odoratum* and *N. stricta* (Olff et al. 1990; Olff and Bakker 1991).

The original plants (i.e. a group of tillers) of each species were collected from several fields along the Anlooër Diepje in March 1995 and cloned in a common garden in Wageningen. Plants taken from different fields are genotypically different. In August 1995, young tillers of four genotypes of each species were isolated from the garden plants and grown individually in the greenhouse to ensure homogeneous starting conditions. At the start of the experiment in September 1995, tillers were randomly assigned to either an initial harvest or to the experiment. In the experiment, each genotype was used in both treatments.

Experimental treatments and growing conditions

The experiment consisted of a homogeneous treatment and a heterogeneous treatment which were replicated four times. In both treatments, the plants were grown individually in root boxes (22 dm^3; Mechalectron B.V., The Netherlands). The perforated walls of the root boxes were covered with black plastic in this experiment. The root boxes were divided in half by a water-tight PVC partitioning that was sealed to the bottom and the walls of the root box with plasticine (Rhiwa-Hartomex B.V., The Netherlands).

In the homogeneous treatment, both halves of the root box were filled with a homogeneous mixture of humus-rich black soil and sand (ratio 1:3.5 v/v), hereafter referred to as nutrient-poor soil. The root box was filled to a depth of 40 cm with a bulk soil density of 1.4 kg dm^{-3}.

In the heterogeneous treatment, the same total amounts of humus-rich black soil and sand were used. One half of the root-box in this treatment was filled with the same nutrient-poor soil that was used in the homogeneous treatment. In the other half, a nutrient-rich patch was created by filling a central PVC cylinder (diameter 7 cm, depth 25 cm) with a soil mixture in which the ratio humus-rich black soil:sand was 4:1 (v/v), hereafter referred to as nutrient-rich soil. The remaining part of this half of the root-box was filled with the remaining humus-rich black soil and sand in a ratio 1:5 (v/v). After filling, the PVC cylinder was removed, and hence no barrier existed between the nutrient-rich soil in the patch and the surrounding nutrient-poor soil. Roots could therefore freely penetrate the nutrient-rich patch. Both the nutrient-rich patch as well as the rest of the half were filled with the same bulk density of 1.4 kg dm^{-3} as the nutrient-poor soil. In this way, 30% of the humus-rich black soil was concentrated in 10% of the volume of this half of the root box. The total amounts of humus-rich black soil and sand used in the experiment were equal in both halves and in both treatments.

At the start of the experiment, plants were placed on top of the divider separating both halves of the root-box. Around each plant, a buffer zone (nutrient-poor soil: 1:3.5 v/v humus-rich black soil: sand) was created to ensure equal soil conditions on both sides of the plant to enable it to equally distribute its roots over both halves of the root-box (Fig. 1). To diminish evaporation, the soil surface was covered with a 1-cm-thick layer of white plastic grains.

The experiment was carried out in a greenhouse. During the experiment (September–December), the plants were supplemented with light from high-pressure sodium lamps (Philips SON-T Plus 400 W) giving a photoperiod of 16 h. Temperature was kept constant at 20°C (day) and 15°C (night) and relative air humidity was kept at 70%. The plants were watered three times a week and the soil water content was monitored regularly with a Frequency Domain probe (IMAG-DLO, The Netherlands) and kept constant.

Harvest

In the initial harvest, ten plants per genotype of each species were harvested, and shoot and root biomass were determined. At the final harvest, shoots were removed from the plants and soil cores (diameter 5 cm, depth 0–10 cm and 10–20 cm) were taken in both treatments in both halves of the root boxes. The centre of all soil cores taken was equivalent to the position of the centre of the nutrient-rich patch in the heterogeneous treatment, i.e. 8.5 cm from the shoot base (Fig. 1). The soil cores were collected and the roots in these soil cores were washed clean of soil particles, frozen and used later for the measurement of the root morphological parameters. The rest of the root system in either half of the root box was washed clean at harvest, removed from the shoot base, frozen and stored.

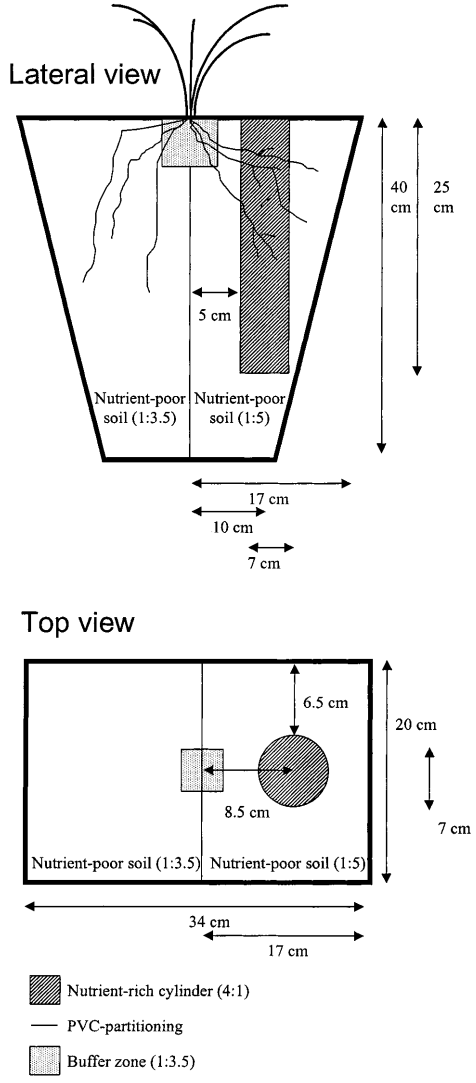

Fig. 1 Experimental set-up of the root boxes in the heterogeneous treatment. The root boxes (22 dm^3) were divided in half by a water-tight PVC partitioning. One-half was filled with a nutrient-poor soil mixture of humus-rich black soil and sand (ratio 1:3.5 v/v). In the other half, a nutrient-rich patch was created by filling a central patch with a humus-rich black soil:sand mixture (ratio 4:1 v/v). The rest of this half was filled with the remaining humus-rich black soil and sand in a 1:5 (v/v) ratio. The roots could freely grow into the nutrient-rich soil in the patch; there was no barrier. The root boxes in the homogeneous treatment were also divided by a PVC partitioning, but both halves were filled with the 1:3.5 nutrient-poor soil mixture. The total amount of humus-rich black soil and sand used in the experiment was equal in both halves and in both treatments. In both treatments, a buffer zone ($4 \times 4 \times 4$ cm; nutrient-poor soil 1:3.5 v/v humus-rich black soil:sand ratio) was created around each plant to ensure identical conditions at the start of the experiment

Comparisons among species have generally been conducted at common points in time or at a common plant age, but plants growing in different environments are likely to grow at different rates and will be of different sizes and developmental stage at a given time or age (Coleman et al. 1994; Coleman and McConnaughay 1995). Apparent differences in morphology may then be the result of ontogenetic drift rather than expressions of an adaptation to environmental heterogeneity (Coleman et al. 1994). For this reason, the grass species were harvested sequentially to reduce size differences among species, and to enable a functional interpretation of the differences in foraging mechanisms. Following the rank of their relative growth rates, *H. lanatus*, *L. perenne*, *F. rubra*, *A. odoratum* and *N. stricta* were harvested after 8, 9, 10, 11 and 12 weeks, respectively.

Measurements

The nutrient concentrations and the pH of the initial soil mixtures and of the soil at the end of the experiment were determined by extracting 20 g of fresh soil with 50 ml KCl (1 M) for 2 h. Soil pH was determined within the extract. Simultaneously, the soil water content was determined after drying soil at 105°C for at least 24 h. The extracts were analysed colorimetrically for NH_4^+, NO_3^- and PO_4^- with a continuous-flow analyser (SKALAR, The Netherlands). The total available soil nitrogen and phosphorus contents of the root boxes in the homogeneous and heterogeneous treatments were calculated.

To determine the root morphological parameters, defrosted roots from the soil cores were stained by submerging them in 50 mg (i.e. 50 mgl^{-1}) methylene blue for at least 12 h, after which they were spread out in water trays and scanned with a 3D scanner (TRU-VEL TZ-3, Vidar System Corporation, Herndon, USA). Roots were stained to increase contrast and excessive dye was rinsed off with water before scanning. The total length of a root sample was determined by analysing the scanned images with the interactive image analysis package TCL-Image V4.6 (TNO Institute of Applied Physics, Delft, The Netherlands). For details of the procedure see Smit et al. (1994). After scanning, the dry weight of the root sample was determined and the SRL and the RLD were calculated.

Leaves, shoot base and the remaining roots of both the initial harvest and the final harvest were dried at 70°C for at least 48 h prior to weighing and nutrient analyses. The dried plant material was digested with sulphuric acid, selenium and salicylic acid (Novozamsky et al. 1983). Nitrogen and phosphorus concentrations were measured colorimetrically using a continuous-flow analyser (SKALAR).

Statistical analyses

The initial soil characteristics were analysed with one-way ANOVA. The total amount of available nutrients in the root boxes in the homogeneous and the heterogeneous treatments was analysed with the Student's *t*-test.

The experiment had a split-plot design (Sokal and Rohlf 1981). The root morphological parameters, (i.e. root biomass production, SRL and RLD) were analysed using a mixed-model ANOVA (Genstat5 version 3.1), with species and treatments randomised over the root boxes (four replicates, 30 *df* for error term) with the following block structure: half (nested within root box, two halves, 30 *df* for error term) and depth (nested within half, two depths, 59 *df* for error term). Genotypic effects could not be considered in the analyses, since the genotypes were nested within species and the number of degrees of freedom was insufficient to allow for a nested analysis of genotype. Prior to analyses, the root morphological

data were $Y' = Y^{1/3}$ transformed, satisfying best the conditions of normality and homogeneity of variance.

Total plant nutrient content, plant biomass and plant RGR were analysed with a two-factor ANOVA with five species and two treatments (homogeneous, heterogeneous). Prior to analyses, nutrient and biomass data were ln-transformed to satisfy the conditions of normality and homogeneity of variance. RGR data were not transformed prior to analysis. Plant RGR is calculated per genotype as: $RGR = [\ln(dw_2) - \ln(dw_1)]/t$, where dw_2 is the final plant dry weight, dw_1 is the initial plant dry weight and t is the length of the growth period of the plant. The initial plant dry weight is the mean dry weight ($n = 10$) of the plants harvested per genotype in the initial harvest.

Results

Soil characteristics

The initial ammonium, nitrate and phosphate concentrations of the soil in the nutrient-rich patch in the heterogeneous treatment differed significantly ($P < 0.05$) from the concentrations of the nutrient-poor soil (Table 1). The total amount of ammonium ($t_s = 2.5$), nitrate ($t_s = 1.24$) and phosphate ($t_s = 1.45$) available in the root boxes did not differ ($t_{0.05[2]} = 4.303$) between the treatments. During the experiment, the ammonium and nitrate concentrations in the nutrient-rich soil declined significantly. The average soil water content during the experiment was 5.7 ± 0.1% ($n = 250$) in the nutrient-poor (1:3.5) soil mixtures and 10.1 ± 0.2% ($n = 105$) in the nutrient-rich (4:1) soil mixture, corresponding with soil water potentials of‘ −0.01 MPa and −0.02 MPa, respectively.

Root responses

Overall, the species produced more root biomass in the soil cores in the heterogeneous treatment than in the soil cores in the homogeneous treatment ($P < 0.01$; Table 2). However, when compared within species, no differences could be shown ($P > 0.05$; Fig. 2A) between the root biomass production within the different soil cores of both treatments.

Treatment did not affect the SRL of the species (Table 2). However, the SRL of *L. perenne* and *N. stricta* was significantly higher ($P < 0.05$) in the

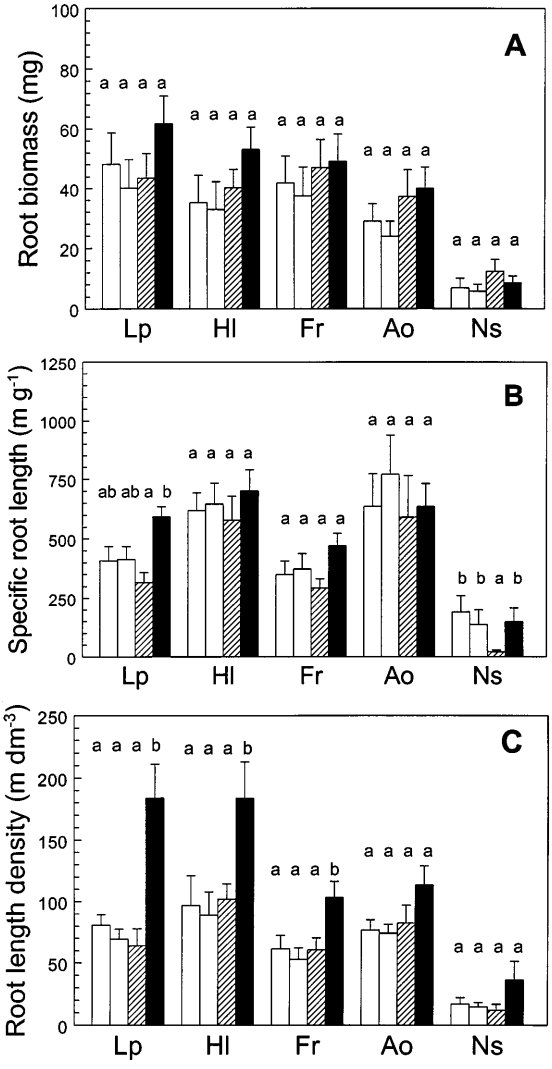

Fig. 2A–C Root morphological parameters of *Lolium perenne* (*Lp*), *Holcus lanatus* (*Hl*), *Festuca rubra* (*Fr*), *Anthoxanthum odoratum* (*Ao*) and *Nardus stricta* (*Ns*). Data are means ± SE ($n = 8$) from the roots sampled from soil cores taken within both nutrient-poor sides of the homogeneous treatment (□), the nutrient-poor side in the heterogeneous treatment (▨), and within the nutrient-rich patch in the heterogeneous treatment (■). *Bars with the same letter within species* are not significantly different (LSD test; $P > 0.05$). Note that *H. lanatus*, *L. perenne*, *F. rubra*, *A. odoratum* and *N. stricta* were harvested after 8, 9, 10, 11 and 12 weeks, respectively. The actual root length density of *N. stricta* is multiplied by 10 in the figure

Table 1 Soil characteristics of the nutrient-poor (1:3.5) and nutrient-rich (4:1) humus-rich black soil:sand mixtures at the beginning and at the end of the experiment. *Means with the same superscript* are not significantly different (least-significant-difference test ($P = 0.05$) after one-way ANOVA) (*DW* dry weight)

	Nutrient-poor soil		Nutrient-rich soil	
	Initial ($n = 3$)	End ($n = 24$)	Initial ($n = 3$)	End ($n = 8$)
pH	4.9^{a}	5.18^{b}	4.6^{c}	4.72^{c}
NH_4^+ (mg N kg^{-1} DW)	0.15^{a}	0.27^{a1}	1.25^{b}	0.59^{a}
NO_3^- (mg N kg^{-1} DW)	8.20^{a}	0.60^{b2}	48.05^{c}	2.16^{b}
PO_4^- (mg P kg^{-1} DW)	0.22^{a}	0.20^{a}	0.54^{b}	0.48^{b}

[1] Denominator = 17
[2] Denominator = 12

Table 2 Analysis of variance of the effect of species, treatment, half and depth on root biomass allocation, specific root length (*SRL*) and root length density (*RLD*). The experiment was analysed as a split-plot design with species and treatment randomised over root boxes, half nested within root box and depth nested within half. Data were $Y' = Y^{1/3}$ transformed prior to analyses (x = 57 for DW, 56 for SRL, 59 for RLD)

Effect	*df*	F-values for each dependent variable		
		DW	SRL	RLD
Main effects				
Species	4,30	23.75***	43.27***	99.17***
Treatment	1,30	8.05**	0.59 NS	10.72**
Species × treatment	4,30	0.13 NS	0.72 NS	0.77 NS
Half	1,30[a]	0.43 NS	13.17***	36.26***
Species × half	4,30[a]	0.19 NS	0.61 NS	2.51 NS
Treatment × half	1,30[a]	2.61 NS	12.14**	61.31***
Species × treatment × half	4,30[a]	0.41 NS	3.04*	4.32**
Depth	1,*x*	52.32***	3.36 NS	98.66***
Species × depth	4,*x*	18.65***	12.25***	7.60***
Treatment × depth	1,*x*	0.19 NS	0.00 NS	0.75 NS
Half × depth	1,*x*	0.00 NS	0.03 NS	0.09 NS
Species × treatment × depth	4,*x*	1.71 NS	3.00*	4.16*
Species × half × depth	4,*x*	0.27 NS	0.14 NS	0.42 NS
Treatment × half × depth	1,*x*	0.41 NS	0.03 NS	1.79 NS
Species × treatment × half × depth	4,*x*	0.27 NS	0.44 NS	0.42 NS

[a] Denominator = 29 for SRL * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; *NS* not significant

nutrient-rich patch in the heterogeneous treatment than in the nutrient-poor half of that treatment. The SRL in the nutrient-rich patch did not however differ from the SRL in the nutrient-poor halves in the homogeneous treatment (Fig. 2B).

The concentration of nutrients in the nutrient-rich patch in the heterogeneous treatment significantly affected the RLD of the species (Table 2). All species (Table 2) tended to increase their RLD in response to nutrient enrichment. However, only *L. perenne*, *H. lanatus* and *F. rubra* were able to produce a significantly higher ($P < 0.05$) RLD in the nutrient-rich patch in the heterogeneous treatment compared to both the nutrient-poor half in that treatment and the nutrient-poor halves in the homogeneous treatment. Note, the RLD in the nutrient-poor half of the heterogeneous treatment was equal to the RLD in the nutrient-poor halves in the homogeneous treatment and hence was independent of the nutrient availability experienced by the plant in the nutrient-rich patch (Fig. 2C). So the difference in RLD between the nutrient-rich patch and the nutrient-poor half in the heterogeneous treatment as shown by *L. perenne*, *H. lanatus* and *F. rubra* is the result of selective root placement within the nutrient-rich patch.

Rooting characteristics also differed with soil depth (see Table 2; results not shown). In general, root biomass production decreased and SRL increased with soil depth, except for *N. stricta* which showed exactly the opposite pattern. Nevertheless, all species showed a decrease in RLD with increasing soil depth.

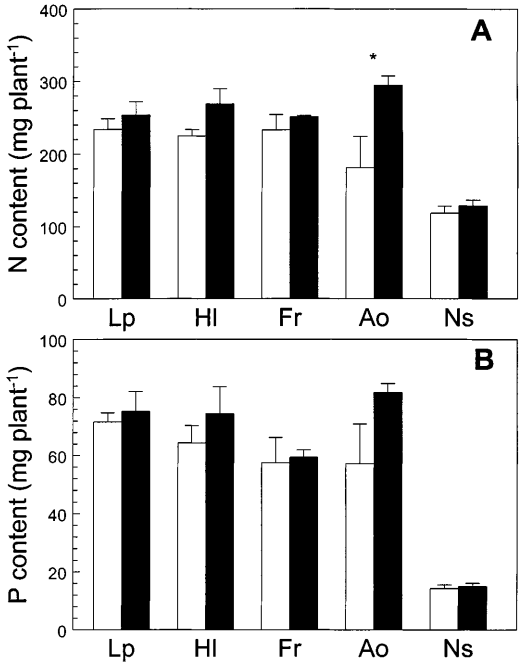

Fig. 3 Total content of nitrogen (**A**) and phosphorus (**B**) per plant of *L. perenne* (*Lp*), *H. lanatus* (*Hl*), *F. rubra* (*Fr*), *A. odoratum* (*Ao*) and *N. stricta* (*Ns*) in the homogeneous treatment (*open bars*) and heterogeneous treatment (*filled bars*). Data are means ± SE (n = 4). An *asterisk* indicates a significant difference (LSD test; $P < 0.05$) within species. Note that *H. lanatus, L. perenne, F. rubra*, *A. odoratum* and *N. stricta* were harvested after 8, 9, 10, 11 and 12 weeks, respectively

Nutrient acquisition

The total amount of nitrogen acquired per plant (Fig. 3A) differed significantly between the species ($F_{4,30}$ = 15.11, $P < 0.001$) and was affected by treatment ($F_{1,30}$ = 8.78, $P < 0.01$). Overall, the plants in the heterogeneous treatment acquired more nitrogen than the plants in the homogeneous treatment even though the total amount of available nitrogen was equal.

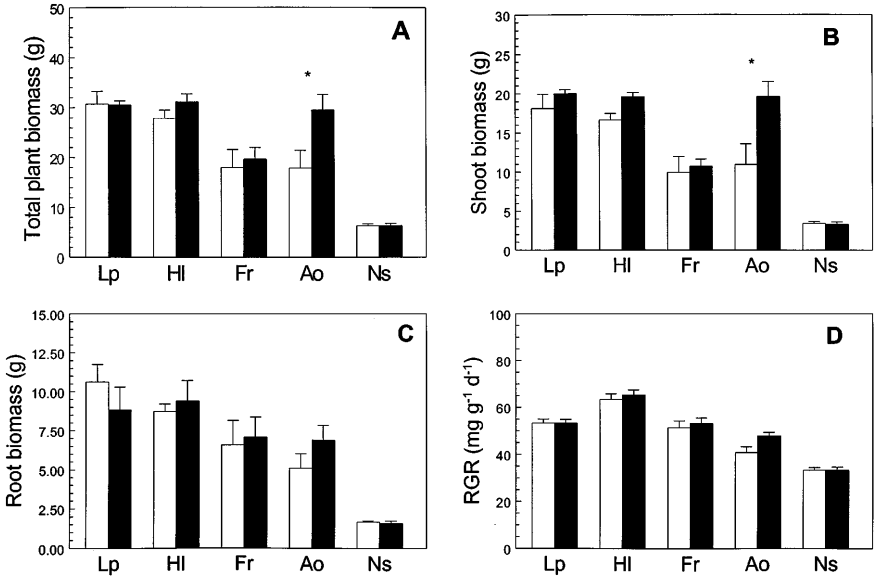

Fig. 4 Plant total biomass (**A**), shoot biomass (**B**), root biomass (**C**) and relative growth rate (*RGR*) (**D**) in the homogeneous treatment (*open bars*) and the heterogeneous treatment (*filled bars*) for *L. perenne* (*Lp*), *H. lanatus* (*Hl*), *F. rubra* (*Fr*), *A. odoratum* (*Ao*) and *N. stricta* (*Ns*). Data are means ± SE ($n = 4$). An *asterisk* indicates a significant difference (LSD test; $P < 0.05$) within species. Note that *H. lanatus*, *L. perenne*, *F. rubra*, *A. odoratum* and *N. stricta* were harvested after 8, 9, 10, 11 and 12 weeks, respectively

Table 3 Analysis of variance of shoot biomass, root biomass and total plant biomass. Data are means ± SE ($n = 4$). Data were ln-transformed prior to analysis

Effect	*df*	F-values for each dependent variable		
		Leaves	Roots	Plant
Species	4,30	58.83***	38.17***	55.48***
Treatment	1,30	6.40*	0.19 NS	4.35*
Species × Treatment	4,30	2.15 NS	0.79 NS	1.70 NS

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; *NS* not significant

However, least-significant-difference tests revealed that *A. odoratum* was the only species that acquired significantly ($P < 0.05$) more nitrogen in the heterogeneous than in the homogeneous treatment.

The total amount of phosphorus acquired by the plants (Fig. 3B) differed significantly between the species ($F_{4,30} = 54.40$, $P < 0.001$), but was not affected by treatment ($F_{1,30} = 3.59$, $P > 0.05$).

Biomass production

Treatment affected total plant biomass production although the total amount of available nutrients was equal in both treatments. The species produced overall more plant biomass in the heterogeneous treatment (Table 3), but in a posteriori tests only *A. odoratum* showed a significantly higher ($P < 0.05$) plant biomass production in the heterogeneous treatment (Fig. 4A). The difference in total plant biomass between the two treatments was mainly due to the higher above-ground biomass production in the heterogeneous treatment (Table 3, Fig 4B); root biomass of the species was not affected by treatment (Table 3, Fig. 4C). The RGR of the species in the experiment differed significantly ($F_{4,30} = 63.59$, $P < 0.001$) and coincides with their ranking within the successional sequence in the field (Fig. 4D), except for *L. perenne*.

Discussion

Only the faster-growing species, i.e. *L. perenne*, *H. lanatus* and *F. rubra*, produced significantly higher RLDs in the nutrient-rich patch than in the nutrient-poor soil, although all species showed a quantitatively similar response. The increases in RLD were the result of small (insignificant) increases in root biomass production and SRL in response to nutrient enrichment. Remarkably, the acquisition of both nitrogen and phosphorus in the heterogeneous treatment was not related to the root proliferation ability of the species.

Root proliferation can be generated by a local increase in root biomass and/or SRL. In contrast to our study, numerous experiments have shown that species are able to produce significantly more root biomass in nutrient-rich patches (see Robinson 1994 for review). The discrepancy with these studies may be caused by the depletion of the nutrient-rich patch in our experiment. The nitrogen availability in the nutrient-rich patch declined drastically during the experiment (Table 1). In other studies (Drew 1975, Drew and Saker 1975, 1978; Crick and Grime 1987; Granato and Raper 1989; Campbell et al. 1991), the nutrient concentrations applied to the roots were more constant as a result of continuous replenishment. The response shown by the species in our study might reflect a more realistic re-

sponse of species to nutrient-rich patches in natural habitats. Species may initially allocate more root biomass to such enriched microsites, but when depletion of the local soil environment occurs, root biomass production in the patch may stall. Such a flexible biomass allocation mechanism will enable species to reduce the risk of biomass and resource losses when patches become unprofitable.

Generally, enhanced root growth in nutrient-enriched patches may occur at the expense of root growth elsewhere in the root system (Gersani and Sachs 1992; Fitter 1994; Hutchings and de Kroon 1994; Robinson 1994). However, in our experiment, the increased RLD of *L. perenne*, *H. lanatus* and *F. rubra* in the nutrient-rich patch did not compromise the RLD in the nutrient-poor half of the root box. Robinson (1994) showed that root growth in nutrient-poor areas is only loosely correlated with root growth in nutrient-rich areas. Correlative growth among different parts within a root system will predominantly occur in young plants that do not have nutrient storage pools. These plants have to use the acquired nutrients immediately for growth and maintenance, and stimulated root growth in a nutrient-rich area results inevitably in a growth reduction in other parts of the root system. Large perennial plants with a considerable accumulation of nutrients are probably able to maintain growth in both nutrient-rich and nutrient-poor areas due to their ability to remobilise stored nutrients.

The significantly enhanced root proliferation in the nutrient-rich patch, as shown by *L. perenne*, *H. lanatus* and *F. rubra*, did not result in a significantly enhanced acquisition of nitrogen and phosphorus in the heterogeneous treatment. The only species that acquired significantly more nitrogen in the heterogeneous treatment, i.e. *A. odoratum*, did not produce significantly more roots in the nutrient-rich patch than in the nutrient-poor soil. There are reasons to assume that the increased nitrogen acquisition by *A. odoratum* is the result of physiological plasticity. A stimulated nutrient inflow rate in roots in nutrient-rich areas can result from increased uptake kinetics in response to nutrient enrichment, but can also simply result from the higher nutrient concentration in the soil solution in these areas (Caldwell et al. 1992; Jackson and Caldwell 1996). However, if the latter was true in our study, all species would have shown increased nutrient acquisition in the heterogeneous treatment.

Our results suggest that the ecological significance of root morphological plasticity for the acquisition of heterogeneously distributed nutrients is limited. In other studies also, physiological plasticity appears to be more important than morphological plasticity for the acquisition of nutrients (Caldwell et al. 1992; Jackson and Caldwell 1996; Robinson 1996; Van Vuuren et al. 1996). However, physiological plasticity may only be more beneficial when plants are grown individually or when soil water content allows high nutrient diffusion rates. When plants are grown in competition or when ions are immobile, the ability to rapidly reach and fill nutrient-rich patches may be more significant for nutrient capture (cf. Robinson 1994).

In conclusion, our results show that although all species tended to respond, only faster-growing species were able to produce significantly higher RLDs in the nutrient-rich patch. These increased RLDs were due to small, insignificant, increases in root biomass and SRL. In contrast to our expectations, the response of RLD did not correlate with an increased nutrient acquisition in the heterogeneous treatment. For the one species for which nitrogen acquisition was particularly high, these benefits were probably due to physiological plasticity.

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