

Phenotypic plasticity in response to nitrate supply of an inherently fast-growing species from a fertile habitat and an inherently slow-growing species from an infertile habitat

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Abstract. The aim of the present study was to investigate possible differences in plasticity between a potentially fast-growing and a potentially slow-growing grass species. To this end, *Holcus lanatus* (L.) and *Deschampsia flexuosa* (L.) Trin., associated with fertile and infertile habitats, respectively, were grown in sand at eight nitrate concentrations. When plants obtained a fresh weight of approximately 5 g, biomass allocation, specific leaf area, the rate of net photosynthesis, the organic nitrogen concentration of various plant parts and the root weight at different soil depths were determined. There were linear relationships between the morphological and physiological features studied and the ln-transformed nitrate concentration supplied, except for the specific leaf area and root nitrogen concentration of *H. lanatus*, which did not respond to the nitrate concentration. The root biomass of *H. lanatus* was invariably more evenly distributed over the soil layers than that of *D. flexuosa*. However, *D. flexuosa* allocated more root biomass to lower soil depths with decreasing nitrate concentration, in contrast to *H. lanatus*, which did not respond. The relative response to nitrate supply, i.e. the value of a character at a certain nitrate level relative to the value of that character at the highest nitrate supply, was used as a measure for plasticity. For a number of parameters (leaf area ratio, root weight ratio, root nitrogen concentration, vertical root biomass distribution and rate of net photosynthesis per unit leaf weight) the potentially slow-growing *D. flexuosa* exhibited a higher phenotypic plasticity than the potentially fast-growing *H. lanatus*. These findings are in disagreement with current literature. Possible explanations for this discrepancy are discussed in terms of differences in experimental approach as well as fundamental differences in specific traits between fast- and slow-growing grasses.

Key words: Biomass allocation – Nitrogen supply – Phenotypic plasticity – Photosynthesis – Root distribution

Nitrogen is a major plant nutrient, often limiting plant productivity in (semi-)natural communities (Bradshaw et al. 1964; Berendse and Elberse 1989). When nitrogen is limiting for growth, relatively more biomass and nitrogen are allocated to the roots (Brouwer 1963; Boot et al. 1992; Fichtner and Schulze 1992), and photosynthesis per unit leaf weight (Chapin 1988; Evans 1989), shoot respiration per unit shoot weight (Waring et al. 1985; Lambers and Poorter 1992) and root respiration per unit root weight (Lambers et al. 1981; Van der Werf et al. 1992) are reduced. These responses to nitrogen supply reflect a plant's plasticity.

Plasticity is defined as the ability of organisms to alter their morphology and/or physiology in response to varying environmental conditions (Bradshaw 1965; Schlichting 1986). Hereby the definition of phenotypic plasticity does not necessarily imply any functional significance (Schlichting 1986). Nevertheless, Grime et al. (1986) hypothesized that plasticity of particular traits is of vital importance for optimizing the acquisition and use of resources for vegetative growth. Furthermore, it has been suggested that plasticity is predictably related to habitat productivity (Grime 1979; Grime et al. 1986; Chapin 1980, 1988). That is, productive species from nutrient-rich habitats tend to show a higher degree of physiological and morphological plasticity than species from nutrient-poor habitats.

Some findings agree with the contention that inherently fast-growing species from productive habitats show a greater morphological plasticity, when grown at different levels of nitrogen availability, than inherently slow-growing species from unproductive sites (Grime 1979; Lambers and Poorter 1992 and references therein). Other authors however, found no difference in morphological plasticity, or even a higher degree of plasticity for certain

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plant features for the inherently slow-growing species (Robinson and Rorison 1987, 1988; Boot and Mensink 1990, 1991). For example, Robinson and Rorison (1988) compared the response of plant features of a number of grass species from contrasting habitats to the nitrogen supply at different levels of organization. They found that the fast-growing species exhibited a higher degree of plasticity of biomass allocation, whereas the inherently slow-growing one showed a more prominent response in length and density of root hairs. Thus, any conclusions on variation in plasticity drawn from comparative studies, are partly determined by the particular features studied in these experiments (Robinson and Rorison 1988; Robinson 1991).

The different outcomes of the morphological response to nitrogen availability of inherently fast- and slow-growing species may also be the result of experimental design. When plants are compared over a relatively narrow range of nutrient availability, the response of species may not reflect the full capacity of the plant's plasticity. Even when a wide range of nitrogen supply is used, but with large intervals of concentration, variation in response between species remains unclear, especially when the response is non-linear. Furthermore, when plants with varying potential growth rates are analyzed, the duration of the experiment may also affect the outcome. For example, changes in biomass allocation will only become manifest when plants increase in size. Since it takes inherently slow-growing species a longer period to gain a similar increase in size, compared to fast-growing species, it will also take them longer to manifest their potential plasticity in biomass allocation. So far, differences in morphological responses between species from productive and unproductive habitats have not been analyzed for plants grown at a number of nitrogen levels, distributed over a wide range of nitrogen availability and compared at similar weights. Additionally, little experimental information is available on differences in physiological plasticity between inherently fast- and slow-growing species in relation to nutrient availability.

The aim of this study was to compare the morphological and physiological plasticity of species from productive and unproductive sites, of comparable total weight, to a number of widely differing concentrations of nitrate. To this end, *Holcus lanatus* (L.), an inherently fast-growing grass species occurring on relatively nutrient-rich soils and *Deschampsia flexuosa* (L.) Trin., an inherently slow-growing grass species occurring on nutrient-poor acidic soils (Grime et al. 1988), were grown in sand with eight concentrations of nitrate. Both morphological (leaf weight ratio, root weight ratio, leaf area ratio and specific leaf area), physiological (rate of photosynthesis) and biochemical characteristics (leaf nitrogen concentration, root nitrogen concentration and plant nitrogen concentration) were determined. Additionally, the root biomass at different soil depths was analyzed.

Materials and methods

Plant material, growth conditions and nitrogen treatment

Seeds of *Holcus lanatus* (L.) were commercially obtained (Kieft, Blokker, the Netherlands), while seeds of *Deschampsia flexuosa* (L.) Trin. were collected from the heathlands of Uddeler Buurtveld (The Netherlands). Seeds of *H. lanatus* and *D. flexuosa* were germinated on wet filter paper in Petri dishes in a growth cabinet for 9 and 15 days, respectively, the latter after a stratification for 4 weeks at 4 °C. Conditions were – day: 12 h, quantum flux density $100 \pm 10 \mu\text{mol m}^{-2} \text{s}^{-1}$, temperature 25 °C; night: 12 h, temperature 15 °C. After germination, seedlings were planted in trays filled with washed sand. Each tray was supplied to field capacity with half strength of the following modified Hoagland solution: 795 μM KNO_3 , 603 μM $\text{Ca}(\text{NO}_3)_2$, 270 μM MgSO_4 , 190 μM KH_2PO_4 , 41 μM Fe-EDTA, 20 μM H_3BO_3 , 2 μM MnSO_4 , 0.85 μM ZnSO_4 , 0.25 μM Na_2MoO_4 , 0.15 μM CuSO_4 . Trays were placed in a growth chamber with the following conditions: day length, 14 h; photosynthetic quantum flux density at mean plant height, $320 \pm 30 \mu\text{mol m}^{-2} \text{s}^{-1}$; day and night temperature: $20 \pm 0.5^\circ \text{C}$; relative humidity day and night: 70 \pm 5%.

After 2–3 weeks, plants were transferred to plastic tubes (PVC, height = 30 cm, diameter = 10.6 cm), filled with washed quartz sand (grain size = 0.7–1.3 mm). The water-holding capacity of the tubes filled with sand was about 400 ml. The bottom of the tubes was covered with a perforated lid to allow unrestricted water replacement in the tube. Synthetic gauze was placed on the inside of the lid, to prevent sand from washing out. Each of the tubes received a full-strength solution as described above, with the exception of nitrate, which was supplied as $\text{Ca}(\text{NO}_3)_2$ at 4000, 2000, 1200, 667, 300, 150, 67 or 30 μM . In the lower nitrogen treatments $\text{Ca}(\text{NO}_3)_2$ was substituted by CaSO_4 , to avoid calcium deficiency. (The amount of CaSO_4 added for each treatment was such that Ca^{2+} concentrations were kept at 603 μM Ca^{2+} .) Soil solution in all tubes was replenished every 2nd day by flushing with 450 ml of the nutrient solution. There were five tubes per treatment. Two seedlings were planted per tube and thinned to one, prior to the start of the experiment, to obtain a uniform population. Tubes were rotated every other day to minimize the effect of spatial variation in environmental parameters within the growth chambers.

Measurements

CO_2 exchange was measured differentially with an infra-red gas analyzer (ADC, model 225 MK3, Hoddesdon, UK) in an open system. For *H. lanatus* the middle parts of the youngest, fully expanded leaves were used. Due to their small size, the middle parts of the five youngest, fully expanded leaves of *D. flexuosa* were required. The rate of net photosynthesis was calculated according to Von Caemmerer and Farquhar (1981), after correction of the IRGA output as suggested by Bunce and Ward (1985).

The experimental design was such, that it allowed plants of all treatments to increase substantially in biomass, enabling them to exhibit their plasticity. To this end, plants were harvested when they had an estimated fresh weight of approximately 5 g. Accordingly, growth period ranged from 25 to 122 days after the start of the treatments. Average dry weight in grams, with SE in brackets, of *H. lanatus* was 2.29 (0.26), 0.63 (0.05), 0.83 (0.07), 0.83 (0.12), 0.74 (0.04), 0.71 (0.05), 0.44 (0.03), 0.27 (0.05) for 4000, 2000, 1500, 667, 300, 150, 67 and 30 μM nitrate treatment respectively and those of *D. flexuosa* were 0.40 (0.03), 0.69 (0.09), 0.21 (0.01), 0.36 (0.03), 0.38 (0.07), 0.72 (0.08), 0.56 (0.07), 0.37 (0.03), respectively. The total biomass had increased substantially in all nitrogen treatments, allowing plants to exhibit their plastic response.

At each harvest, the above-ground plant parts were separated into live and dead leaf blades and leaf sheaths. Immediately after harvesting, leaf area was determined. Leaf area (one-sided) of *H. lanatus* was measured with a leaf area meter (TFDL, Wageningen,

The Netherlands), whereas half of the total area of the (almost cylindrical) leaves of *D. flexuosa* was calculated as: leaf blade length \times average leaf blade diameter $\times \pi/2 \times 1.1$ (Poorter and Remkes 1990). Roots were carefully washed out and split into three levels of soil depth, 0–10, 10–20, 20–30 cm. There were no data on root distribution for the 67 and 2000 μM nitrate treatments. Dry weights of the shoot and root fractions were measured after 48 h at 70° C.

Chemical analysis

Organic nitrogen was determined on dried plant material with a modified Kjeldahl method using concentrated sulphuric acid and Na_2SO_4 , K_2SO_4 and Se in a ratio of 62:1:1 (w/w) as a catalyst. The diluted digests were analyzed colorimetrically on a continuous flow analyzer (Skalar, Breda, The Netherlands).

Statistical analyses

Data were analyzed with the SPSS/PC+ statistical package. Species and treatment effects were tested in a two-way analysis of variance (ANOVA). A significant treatment term indicates plasticity for a character (Schlichting 1986). However, in the present study treatment is a continuous variable and therefore regression analysis is more appropriate for analyzing a treatment effect. Therefore, response to treatment was analyzed by regression analysis, using the ln-transformed nitrate concentrations as the independent factor, thus yielding insight into the direction and magnitude of the response (Schlichting 1986). This was also done for the relative response, which was obtained by expressing the mean values of features at different treatment levels, as a percentage of the value at 4000 μM nitrate (Hunt and Nicholls 1986). Differences in plasticity between the two species were tested by comparing the slopes of the relative response with a *t*-test. As a measure for the unevenness in vertical root biomass distribution, we calculated for each individual plant the standard deviation of the percentage root biomass at the three soil depths. This parameter was accordingly used as the dependent variable in the tests described above.

Table 1. Left: Two-way analysis of variance (ANOVA) of the effect of 8 nitrate treatments on LAR ($\text{m}^2 \text{kg}^{-1}$), SLA ($\text{m}^2 \text{kg}^{-1}$), LWR (g g^{-1}), RWR (g g^{-1}), PS (photosynthesis, $\text{nmol CO}_2 \text{g}^{-1} \text{s}^{-1}$), LNC, RNC and PNC (mmol g^{-1}) of *Deschampsia flexuosa* (Df) and *Holcus lanatus* (Hl); right: outcome of linear regression analysis

	ANOVA			Regression								
	Species	Nitrate level	Species * Nitrate level	r^2		Slope						
				Df	Hl	Absolute			Relative			
						Df	Hl	P	Df	Hl	P	
LAR	***	***	***	0.72***	0.68**	1.13	1.10	ns	12.0	7.61	*	
SLA	***	ns	*	0.27***	0.03 ^{ns}	0.734	-0.126	*	3.70	-0.35	*	
LWR	***	***	***	0.77***	0.75***	0.042	0.032	*	8.93	7.75	ns	
RWR	***	***	***	0.80***	0.73***	-0.060	-0.043	*	-20.3	-12.2	*	
PS	***	***	**	0.60***	0.43***	19.1	27.2	ns	13.2	7.48	*	
PNC	ns	***	**	0.87***	0.73***	0.199	0.127	*	13.9	10.1	ns	
LNC	**	***	ns	0.90***	0.87***	0.253	0.227	ns	13.6	11.3	ns	
RNC	ns	***	***	0.70***	0.04 ^{ns}	0.124	-0.016	*	14.3	-3.35	*	

LAR, leaf area ratio; SLA, specific leaf area; LWR, leaf weight ratio; RWR, root weight ratio; PS, photosynthesis; PNC, plant nitrogen concentration; LNC, leaf nitrogen concentration; RNC, root nitrogen concentration

Results

Two-way analysis of variance revealed inherent differences in all morphological features analyzed (Table 1). The leaf area ratio (LAR, leaf area:total plant weight), specific leaf area (SLA, leaf area:leaf weight) and root weight ratio (RWR, root weight:total plant weight) were on average higher for *H. lanatus* than for *D. flexuosa*, while the reverse was found for the leaf weight ratio (LWR, leaf weight:total plant weight) (Fig. 1). LAR, the product of SLA and LWR, increased significantly with increasing nitrogen concentration for both species (Fig. 1A), mainly due to an increase in LWR (Fig. 1C), while SLA was virtually unaffected (Fig. 1B). RWR of both species decreased significantly with increasing nitrogen supply (Table 1, Fig. 1D).

In terms of absolute response to nitrate supply, significant differences between species were found in the slopes of the SLA (Table 1, Fig. 1B). However, quantitatively, the absolute changes of SLA were negligible. Substantial differences in absolute response were found in dry matter partitioning between roots and shoots. *D. flexuosa* showed a 31% and 40% greater response in its LWR and RWR, respectively, than *H. lanatus*. The difference between species in the response of LAR was only 3% and not significant.

Plasticity of LAR (i.e. the relative response) was 58% greater for *D. flexuosa* than for *H. lanatus* (Table 1). While plasticity of the LWR was the same for the two species, the RWR of *D. flexuosa* revealed a 66% greater decrease with increasing nitrogen supply than that of *H. lanatus*.

Photosynthesis per unit leaf weight differed significantly between the species and increased for both species with increasing nitrogen supply (Fig. 2A). The absolute

of the response to nitrate supply after ln-transformation of the nitrate supply. The r^2 is given for each regression, and concerns both the absolute and relative slopes. Relative slopes were obtained by expressing the mean values of features at different treatment levels, as a percentage of the mean value at 4000 μM nitrate

Levels of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, ns = not significant

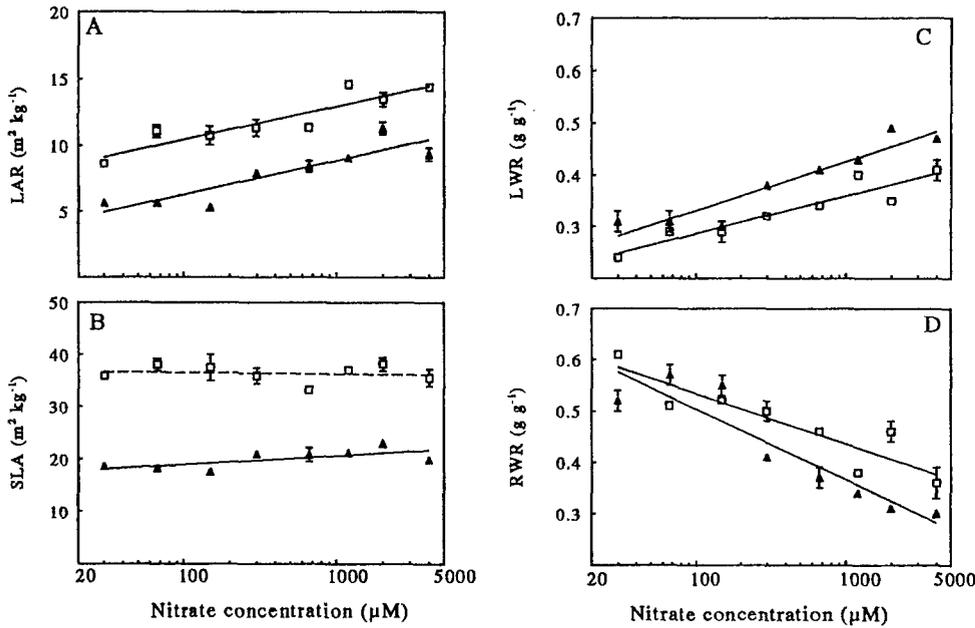


Fig. 1. **A** Leaf area ratio LAR, **B** specific leaf area SLA, **C** leaf weight ratio LWR and **D** root weight ratio RWR of *Deschampsia flexuosa* (\blacktriangle) and *Holcus lanatus* (\square) plotted against the ln-transformed nitrate concentration; mean values \pm SE ($n=5$). The *continuous straight line* indicates a significant linear regression ($P<0.05$) of the parameter with the ln-transformed nitrate supply. The *dashed line* indicates a non-significant relation. When SE is not shown it lies within the sign

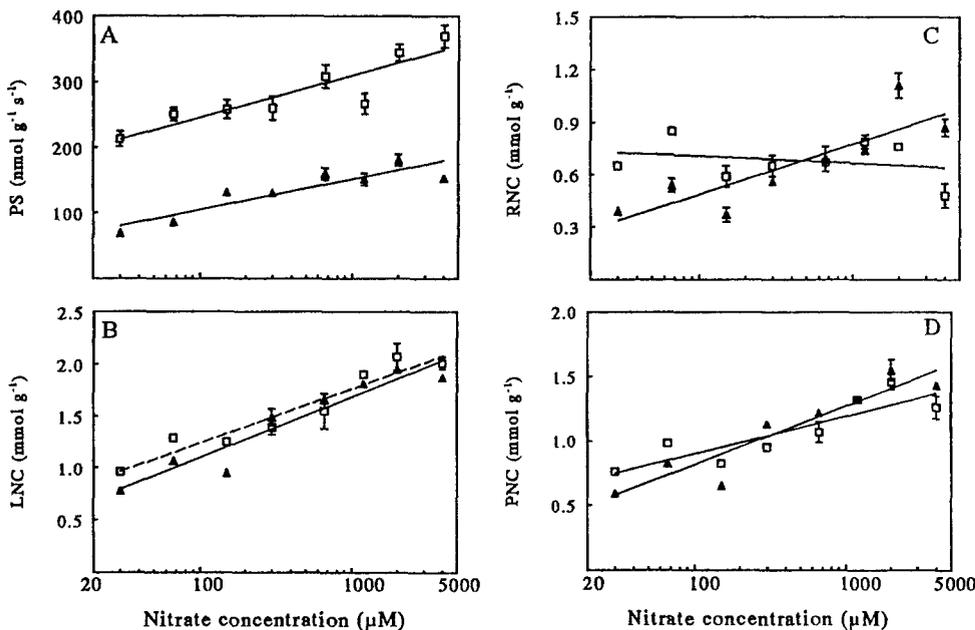


Fig. 2. **A** Rate of photosynthesis per unit leaf weight PS, **B** leaf nitrogen concentration LNC, **C** root nitrogen concentration RNC and **D** plant nitrogen concentration PNC of *Deschampsia flexuosa* (\blacktriangle) and *Holcus lanatus* (\square) plotted against the ln-transformed nitrate supply; mean values \pm SE ($n=5$). The *continuous line* indicates a significant linear regression ($P<0.05$) of the parameter with the ln-transformed nitrate supply. The *dashed line* indicates a non-significant relation

increase of the rate of photosynthesis, although 42% greater for *H. lanatus*, did not differ significantly between the two species. Plasticity (i.e. the relative response) of the photosynthetic rate of *D. flexuosa* revealed a 76% greater response than that of *H. lanatus* (Table 1, $P<0.05$).

Differences between *H. lanatus* and *D. flexuosa* in nitrogen concentration and allocation were not as evident as those of the above-mentioned plant features (Fig. 2B, C, D). On average, plant nitrogen concentrations (PNC) and root nitrogen concentrations (RNC) did not differ between species, while the leaf nitrogen concentration (LNC) was slightly, but significantly, higher for *H. lanatus*. Both species responded to an increasing

nitrogen supply with a higher PNC, in which the response was 57% greater for *D. flexuosa*. Plasticity of the PNC, although 38% greater for *D. flexuosa*, did not differ significantly. The LNC of both species responded in a similar manner to the nitrogen supply. A large difference in response was found for the RNC: that of *H. lanatus* was not significantly affected, whereas the RNC of *D. flexuosa* showed a large increase with increasing nitrate supply.

Root biomass of *H. lanatus* was much more equally distributed in the soil than that of *D. flexuosa* ($P<0.001$, Fig. 3). Figure 3 also shows that the vertical root distribution of *D. flexuosa* responded more strongly to the nitrogen supply than that of *H. lanatus*. Vertical root

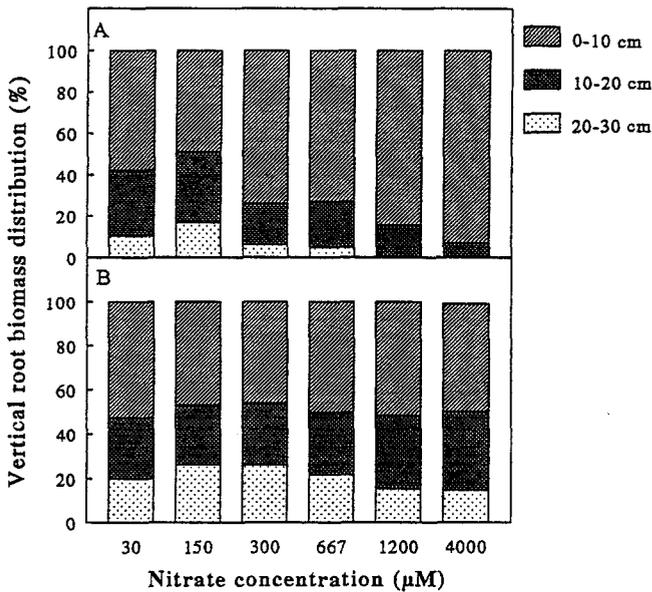


Fig. 3A, B. Root biomass distribution in three layers of the pot (0–10, 10–20, 20–30 cm) as a percentage of the total root biomass for A *Deschampsia flexuosa* and B *Holcus lanatus*

biomass of *D. flexuosa* became more evenly distributed with decreasing nitrate concentration ($P < 0.001$), whereas no significant response was found for *H. lanatus*.

Discussion

The inherent differences between *H. lanatus* and *D. flexuosa* at high nitrogen availability correspond with those found by Robinson and Rorison (1988), Boot (1989) and Poorter and Pothmann (1992). LAR, SLA and RWR were significantly higher for *H. lanatus*, while LWR was higher for *D. flexuosa*. Photosynthesis per unit leaf weight was also higher for *H. lanatus*, as was LNC, again in agreement with earlier observations (Poorter and Pothmann 1992). However no differences were found in PNC and RNC, in contrast with Robinson and Rorison (1988), who found higher values of RNC for *D. flexuosa*, and Poorter and Pothmann (1992), who found higher PNC values for *H. lanatus*. In addition, the values for PNC and LNC of both species, as found by the aforementioned authors, were twice as high as the present ones. The growth conditions in the experiments of Robinson and Rorison (1988) differed in many respects from the present ones. Compared with the growth conditions in the experiments of Poorter and Pothmann (1992), the only difference was that we grew our plants in sand, rather than hydroponically. This suggests that our plants receiving the highest nitrogen level (4000 µM nitrate every other day), did not yet experience the nitrate supply as given by Poorter and Pothmann (1992) growing their plants in a continuously stirred nutrient solution at 2000 µM nitrate.

The present data correspond with previous findings on the effect of an increased nitrogen availability on the plant's allocation pattern and photosynthesis, in that

LAR increased, due to a higher allocation of biomass to the shoot (LWR), while there was little effect on the SLA. Furthermore, the rate of photosynthesis was increased, due to a higher nitrogen concentration in the leaves (LNC) (Bradshaw 1964; Chapin 1980, 1988; Lambers and Poorter 1992; but see Boot et al. 1992). The RNC of *H. lanatus* however did not respond to an increasing nitrogen supply. Since the RNC of *D. flexuosa* was substantially higher at a higher nitrate supply, this was the only plant character for which a different pattern of response was found between the two species. The response of the parameters analyzed could be described by a linear equation after ln-transformation of the supplied nitrate concentration, except for the SLA, RNC and vertical root biomass distribution of *H. lanatus*, which were not significantly affected by treatment.

Inherent differences in phenotypic plasticity have been analyzed in various ways and all have operational or conceptual difficulties (for review see Schlichting 1986). Hunt and Nicholls (1986) defined plasticity as the variation in a plant feature relative to its value under non-limiting growth conditions. Therefore, the ratio between these values represents the extent of a species' plasticity for a particular character when grown under the environmental conditions studied. For example, Robinson and Rorison (1988), applying this definition, measured plasticity by relating phenotypic variation to the state of the plant growing at maximum relative growth rate. Accordingly, we compared the value of a parameter to its value at the highest nitrate supply. Since the relative response could be described by a linear equation, we used the slopes of the regression lines to analyze the differences in response (Schlichting 1986).

The plastic response in LAR of *D. flexuosa*, calculated by regression analysis, was 58% higher than that of *H. lanatus* (Table 1). The fact that we found this higher degree of plasticity for *D. flexuosa* as well as a higher degree of plasticity in RWR (66% higher for *D. flexuosa*), is at variance with statements of previous authors that fast-growing species exhibit a stronger plastic response in dry matter allocation between roots and shoots than slow-growing ones (Grime 1979; Grime et al. 1986; Lambers and Poorter 1992). However, analyzing plasticity as a relative response has its draw-backs when it comes to complementing ratios as LWR and RWR. For example, if a plant's LWR decreases in time from 0.8 to 0.7, and RWR increases from 0.1 to 0.2, this may erroneously suggest that allocation to the leaves is less plastic than allocation to the roots. Nevertheless, when the absolute response is considered, the present data reveal an outcome similar to that for the relative response: a greater change in LWR and RWR for *D. flexuosa* than for *H. lanatus* (Table 1).

The decrease in the rate of net photosynthesis at a low nitrate availability was also stronger for *D. flexuosa* than for *H. lanatus*. This again disagrees with the general finding that slow-growing species tend to maintain a similar, relatively low, rate of net photosynthesis over a wide range of nitrogen availability (Chapin 1980, 1988; Grime 1979). The same applies to the plant nitrogen concentration and nitrogen partitioning. Chapin (1980,

1988) suggests that slow-growing species retain relatively constant nutrient concentrations in their roots and shoots, independent of nutrient availability, whereas high-nutrient-adapted species show a large decrease when nitrogen becomes limiting. We however found no difference in plasticity of LNC, while the RNC of *D. flexuosa* revealed a much higher degree of plasticity than that of *H. lanatus*. However, our data do agree with results of Robinson and Rorison (1988), who found a greater plasticity in RNC for *D. flexuosa* than for *H. lanatus*, while differences in the nitrogen concentration of the shoot were smaller. A greater decrease in RWR accompanied by a higher increase in RNC of *D. flexuosa* at high N availability and no difference in response of LNC and LWR for the two species resulted in a similar response of the PNC.

Finally, Fig. 3 reveals that *D. flexuosa* showed a greater response to nitrogen supply in vertical root distribution than *H. lanatus*. This is at variance with data of Robinson and Rorison (1985) who found a greater response for *H. lanatus*. This variance is likely to be due to the longer growth period for the low treatments of *D. flexuosa* in the present experiment, which gave our plants more time to proliferate their roots into deeper zones than in Robinson and Rorison's experiments.

How can the variation in outcome of the present plasticity data be reconciled with the results of the previously mentioned authors who found a greater plasticity for the inherently fast-growing species? Firstly, as stated earlier, analysis of plasticity data in a relative or an absolute way may have different outcomes. However, even if the absolute response to nitrate availability, rather than the relative one, is considered, our data reveal either no difference in response or a greater response for *D. flexuosa* (Table 1).

Secondly, experimental design may affect the degree to which plants reveal their plasticity. For example, inherent and environmentally induced differences in relative growth rate result in differences in plant size between species and treatments. When large fast-growing plants are grown in relatively small volumes of soil, nutrient depletion may occur to a greater extent as compared to smaller slow-growing individuals. Therefore, when no allowance is made for the size of the plant, a size-by-nitrogen interaction may occur, which may interfere with the outcome of plasticity analyses (Boot and Mensink 1990). The present study has dealt with these shortcomings by using large containers and a high frequency of nutrient supply.

Furthermore, when plasticity is considered as the response to a change within a short period, fast-growing species will show a higher degree of plasticity in biomass allocation since they have higher carbon gain and nutrient absorption rates, while slow-growing species cannot react that quickly, due to their low RGR. Thus the latter need a longer period to reveal their potential plasticity. Since we compared plants of similar weight this may offer an explanation for the higher degree of plasticity of *D. flexuosa* in the present study. This is supported by data of Berendse and co-workers who found large differences in RWR between species from nutrient-rich

and nutrient-poor soils at the beginning of their experiment, while this difference had disappeared 16 weeks later (Berendse and Elberse 1989; Berendse et al. 1992).

Thirdly, intrinsic characteristics of plants may influence the outcome of plasticity analysis. Conceptually, a plant's response to nutrient availability in terms of biomass allocation can be separated into two, more or less independent processes. The first is the capacity of the roots to acquire nutrients, the second is the response in allocation as dependent on the internal nutrient status of the plant (cf. Van der Werf et al. 1993). Nutrient acquisition is determined by root distribution, architecture and nutrient uptake characteristics. Figure 3 shows that a larger fraction of root biomass of *H. lanatus* was found in the deeper layers of the soil when compared to *D. flexuosa*, in agreement with data of Robinson and Rorison (1988). Consequently, *D. flexuosa* did not have access to the same amount of nitrogen as *H. lanatus*. This may have induced *D. flexuosa* to invest even more biomass in its roots and therefore likely contributed to the unexpectedly greater plasticity in RWR and RNC of *D. flexuosa*.

Another characteristic that may differ between species is the nutrient uptake rate per unit root weight. At high nutrient availability the uptake of fast growing species is higher than that of slow growing ones (Chapin 1980; Poorter et al. 1991). At low concentrations of soil nutrients, uptake is more determined by mass flow and diffusion than by the characteristics of the uptake system. As *H. lanatus* had similar rates of transpiration per unit leaf area as *D. flexuosa* (data not shown), but a higher leaf area: root weight ratio, we expect *H. lanatus* to induce a much higher mass flow to the roots. This may have resulted in a higher nutrient flow into the plant per unit root weight. Therefore, when nitrogen becomes limiting, a similar increase in RWR causes the fast-growing species to obtain a higher absolute increase in nitrogen uptake than that of slow-growing species. Consequently, if slow-growing species were to compensate for a decrease in nitrogen supply they would have to invest relatively more in root biomass to attain sufficient nitrogen for growth. This may cause slow-growing species to reveal a higher degree of plasticity in biomass allocation.

The response in allocation in relation to the internal nutrient status of the plant can be studied by adding nutrients exponentially (Ingestad 1982). In this way interference of above mentioned differences between species in nitrogen uptake capacity, plant-size interactions, root distribution and root architecture with the internal response to a low nutrient status is avoided. Van der Werf et al. (1993) accordingly compared five inherently slow- and fast-growing monocotyledonous species grown in nutrient solution at a range of relative nitrogen addition rates. They found that biomass and nitrogen allocation of the inherently fast-growing species responded to a greater extent than that of the inherently slow-growing ones. If this applies to the two species investigated here as well, it would imply that the higher degree of plasticity in parameters related to biomass allocation for *D. flexuosa*, as observed in the present data may be due

to the differences in factors related to nutrient acquisition.

We postulate that more insight into the mechanisms of plasticity can only be accomplished by accounting for the three above-mentioned factors.

Conclusions

Of the two grass species investigated, the slow-growing one was phenotypically more plastic than the fast-growing one. When considering plasticity of parameters related to growth of inherently fast- and slow-growing species at different nutrient supply, factors such as the interaction of plant size and nitrogen, the roots' capacity to acquire nutrients, root distribution and architecture, and duration of growth may affect the outcome of plasticity analysis.

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