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Nitrogen dynamics in the intact grasses *Poa trivialis* and *Panicum maximum* receiving contrasting supplies of nitrogen

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Abstract

The C$_3$ grass *Poa trivialis* and the C$_4$ grass *Panicum maximum* were grown in sand culture and received a complete nutrient solution with nitrogen supplied as 1.5 mol m^{-3} NH$_4$NO$_3$. ^{15}N tracer techniques were used to quantify the relative use of root uptake and mobilization in supplying nitrogen to growing leaves in intact plants which either continued to receive nitrogen or which received the complete nutrient solution without nitrogen. The allocation of both ^{15}N-labelled nitrogen uptake and unlabelled mobilized nitrogen indicated that, under their conditions of growth, the sink strength of growing leaves was relatively greater in *P. maximum* than *P. trivialis*. The supply of nitrogen by mobilization to side tillers of *P. trivialis* was completely stopped as the external nitrogen supply was reduced, whilst in *P. maximum* some allocation of mobilized nitrogen to side tillers, roots and growing leaves was maintained. In both plant species receiving an uninterrupted supply of nitrogen the allocation pattern of mobilized nitrogen differed from that of nitrogen derived from root uptake. Differences exist in the degree to which *P. trivialis* and *P. maximum* utilized uptake and mobilization to supply nitrogen to the growing leaves. In *P. trivialis* roots were always a net sink of mobilized nitrogen, irrespective of the external nitrogen supply. In *P. maximum*, roots were a net sink of mobilized nitrogen when external nitrogen was withdrawn, but exhibited both source and sink behaviour when nitrogen supply was continued.

Introduction

Nitrogen is often one of the most limiting nutrient elements in many ecosystems (Hopkins, 2000). Grasses, along with other plant species, have evolved several mechanisms to use nitrogen in an efficient manner. The ability to store, mobilize and, subsequently, to reuse nitrogen has been suggested to contribute to the competitive fitness of grasses, especially under nitrogen-limited conditions (de Aldana and Berendse, 1997). In agricultural systems, understanding how grasses mobilize and reuse nitrogen may help in the development of more efficient nitrogen fertilization strategies with reduced deleterious effects on the environment.

Using ^{15}N tracers, many studies have shown that grasses mobilize nitrogen from both roots and remaining shoot structures towards growing leaves in response to defoliation (see reviews by Volenec *et al*., 1996; Schnyder *et al*., 2000; Thornton *et al*., 2000). Such defoliation-induced nitrogen mobilization is dependent on many factors. Several studies have observed an increase in the relative use of mobilization to supply nitrogen to growing leaves as nitrogen supply was reduced (Millard *et al*., 1990; Ourry *et al*., 1990; Thornton *et al*., 1994), although contrasting results have also been reported (Skinner *et al*., 1999). Different grass species have also been shown to differ in the extent to which they rely on the mobilization of nitrogen to supply growing leaves following defoliation

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(Thornton *et al*., 1993, 1994). The optimum leaf nitrogen concentration for photosynthesis is greater in C_3 than C_4 species (Sinclair and Horie, 1989); this may be expected to result in differences in the nitrogen allocation patterns, both in response to defoliation and nitrogen deprivation. However, a comparison of the response of a C_3 grass *Pascopyrum smithii* (Rydb.) A. Löve with the C_4 grass *Bouteloua gracilis* (Kunth) Lag. ex Steud. showed that, in the initial 7–10 d following defoliation, both species remobilized a similar percentage of the available nitrogen pools towards growing leaves (Skinner *et al*., 1999).

Fewer studies have utilized the ^{15}N labelling technique to quantify the contribution of root uptake and mobilization in supplying nitrogen to leaf growth in intact grasses, those which do exist have concentrated on C_3 grasses. In contrast to defoliated grasses, in intact plants of the C_3 grasses *Agrostis capillaris* (L.), *Festuca rubra* (L.) and *Lolium perenne* (L.) roots were a sink of mobilized nitrogen (Schulte auf'm Erley *et al*., 2000; Bausenwein *et al*., 2001*a*). Relatively more of the nitrogen available to be mobilized was directed towards the roots as the external nitrogen supply was reduced (Schulte auf'm Erley *et al*., 2000). As with defoliated grasses, species differences exist in the allocation of mobilized nitrogen in intact grass species. Whilst *F. rubra* always directed a large proportion of nitrogen from older leaves towards new tiller growth irrespective of nitrogen supply, *L. perenne* only mobilized nitrogen towards new tillers when nitrogen supply was plentiful (Schulte auf'm Erley *et al*., 2000).

The following hypotheses were tested: (1) that differences exist in the degree to which the C_3 grass *Poa trivialis* (L.) and the C_4 grass *Panicum maximum* (Jacq.) utilize root uptake and mobilization to supply nitrogen to growing leaves of intact plants, and (2) in both *P. trivialis* and *P. maximum* roots are a net sink of mobilized nitrogen irrespective of external nitrogen supply. The grasses, *P. trivialis* and *P. maximum*, both considered relatively fast-growing species (Grime *et al*., 1988; Jank *et al*., 1994; Santos *et al*., 1999), were grown in sand culture and received a complete nutrient solution containing nitrogen supplied as 1.5 mol m^{-3} NH$_4$NO$_3$. ^{15}N tracer techniques coupled with destructive harvesting were used to quantify the relative use of root uptake and the mobilization of stores in supplying nitrogen to growing leaves, in intact plants which either continued to receive nitrogen at 1.5 mol m^{-3} NH$_4$NO$_3$ or which received the complete nutrient solution without nitrogen.

Materials and methods

Growth of plant material

Twenty-five pots each of 15 cm and 23 cm diameter, were filled with coarse sand (1–10 mm diameter) to a depth of 1 cm then the remaining space filled with fine sand (0.25–0.7 mm diameter). The total volume of sand used was 1.25 l in the 15 cm pots and 5.40 l in the 23 cm pots. A disc of Tygan mesh (Bradley Lomas Electrolok Ltd, Eckington, UK) covered by a single layer of Whatman No. 1 filter paper at the base of the pots prevented sand loss through the drainage holes. All filled pots were completely flushed with deionized water three times. Thirty seeds of *P. trivialis* (L.) (unknown cultivar obtained from Emorsgate Seeds, King's Lynn, UK) were placed on the sand of the 15 cm pots and 15 seeds of *P. maximum* (Jacq.) cv. Tanzania placed in the 23 cm pots. The pots of each species were then placed in separate controlled environment rooms (Conviron, Winnipeg, Canada). The position of the 25 pots within each room was block randomized. Seeds were allowed to germinate for 6 d in the dark at 25 °C for *P. trivialis* and for 3 d in the dark at 30 °C for *P. maximum*, and with a relative humidity of 90%. During this period the sand was kept moist with deionized water at all times.

After germination, plants of both species were grown with a 12 h photoperiod of 500 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR) at plant height and a constant relative humidity of 60%. Plants were grown with light:dark temperatures of 20:16 °C for *P. trivialis*, and 30:26 °C for *P. maximum*. A decision was taken to grow each species at or near its optimum temperature of growth rather than at one common temperature that preferentially favoured one species. Two weeks after germination, pots containing *P. trivialis* were thinned to 10 germinated seedlings and those containing *P. maximum* to three germinated seedlings. Throughout growth, pots were watered to field capacity three times a week with a complete nutrient solution identical to that used by Thornton *et al*. (1993) except that nitrogen was supplied as 1.5 mol m^{-3} NH$_4$NO$_3$.

Labelling and harvesting of plants

On each plant, small rings made from coloured plastic coated wire were placed over individual leaves to allow their identification. The first leaf produced was designated as leaf 1, the second leaf produced designated as leaf 2 etc. After the appearance of leaf 5 on plants of *P. trivialis* and leaf 7 on plants of *P. maximum*, leaf lengths were measured every 2 d as described by Davies (1993). Leaves were considered fully expanded when more than 50% of plants showed a reduction in elongation rate; leaf elongation rapidly decreased to zero beyond this point. Five replicate pots of each species were then destructively harvested, for *P. trivialis* this was when leaf 5 was fully expanded and for *P. maximum* when leaf 7 was fully expanded. Ten plants of *P. trivialis* and three plants of *P. maximum* contributed to each replicate pot. Concurrent with the first harvest of each species, all nutrient solution was washed from the remaining pots with four changes of deionized water, 0.25 dm^3 each for *P. trivialis* and 1.0 dm^3 each for *P. maximum*. Then for each given species, half the pots were flushed with four changes of a nutrient solution identical to that used for growth, except that all nitrogen was enriched with ^{15}N to 5.01 atom% abundance. The remaining pots were similarly flushed with a nutrient solution similar to that used for growth, but which contained no nitrogen. Plants were then fed with the appropriate solution, either ^{15}N labelled (+N treatment) or containing no nitrogen (zero-N treatment) three times each week.

Five replicate pots of *P. trivialis* plants were harvested when leaves 6 and 7 were fully expanded, whilst *P. maximum* plants were harvested when leaves 8 and 9 were fully expanded. At these later harvests, only plants which continued to receive nitrogen were used to determine the time of full leaf expansion. However, a lack of any interaction between nitrogen treatment and harvest for the dry mass of the pertinent leaves (Tables 1, 2) indicated that leaf expansion was still synchronous for both nitrogen treatments. At harvest, the roots were washed free from the sand over a 1 mm-mesh sieve with deionized water, resulting in minimal root loss, then blotted dry. Plants of both species were separated into roots, side tillers, stem, leaves 1–3, leaf 4, leaf 5, leaf 6, and leaf 7. Additionally, *P. trivialis*

Table 1. *Dry mass (mg plant⁻¹) of individual plant compartments for* P. trivialis

Plant compartment	1st harvest	2nd harvest		3rd harvest		Contrasts[a]			
		+N	Zero-N	+N	Zero-N	1st harvest× others	2nd×3rd harvests	+N×Zero-N	N×harvest
Side tillers	23.1	68.7	42.2	153.6	52.6	***	***	***	*
Roots	41.9	76.6	67.8	149.7	103.1	***	***	NS	NS
Stems	1.7	2.4	2.1	3.5	2.3	**	**	**	NS
Leaves 1–3	3.2	3.4	3.0	2.9	2.8	NS	NS	NS	NS
Leaf 4	3.4	3.1	3.1	3.6	3.1	NS	NS	NS	NS
Leaf 5	4.9	4.8	5.2	5.5	5.3	NS	NS	NS	NS
Leaf 6	4.1	6.3	6.5	7.7	6.5	**	NS	NS	NS
Leaf 7	1.3	5.5	4.2	9.5	4.6	***	*	**	NS
Leaves 8–11	0.1	2.7	0.7	12.7	1.6	***	***	***	*

[a] NS=P >0.05; * P <0.05; ** P <0.01; *** P <0.001.

Table 2. *Dry mass (mg plant⁻¹) of individual plant compartments for* P. maximum

Plant compartment	1st harvest	2nd harvest		3rd harvest		Contrasts[a]			
		+N	Zero-N	+N	Zero-N	1st harvest× others	2nd×3rd harvests	+N×Zero-N	N×harvest
Side tillers	13.1	128.8	36.3	399.6	104.2	***	***	***	NS
Roots	168.0	414.6	387.3	686.2	445.9	***	*	NS	NS
Stems	12.5	22.0	15.6	50.1	28.3	***	***	***	NS
Leaves 1–3	8.4	8.2	6.6	7.2	6.6	NS	NS	NS	NS
Leaf 4	15.1	13.6	11.0	16.6	14.1	NS	NS	NS	NS
Leaf 5	26.6	29.0	23.2	32.7	32.1	NS	NS	NS	NS
Leaf 6	51.9	53.9	45.9	64.0	64.4	NS	**	NS	NS
Leaf 7	80.0	87.0	76.9	100.8	101.9	NS	**	NS	NS
Leaf 8	54.0	111.4	90.1	142.7	144.8	***	***	NS	NS
Leaves 9–13	5.3	96.6	50.8	382.0	157.8	***	***	***	NS

[a] NS=P >0.05; * P <0.05; ** P <0.01; *** P <0.001.

was separated into leaves 8–11, and *P. maximum* separated into leaf 8 and leaves 9–13. The stem was defined as that material remaining at the base of the shoot after all leaves had been removed. All plant material was weighted fresh and after oven-drying at 65 °C, and then ball-milled (Retsch, Haan, Germany) prior to analysis.

^{15}N determinations and calculations

The total N and ^{15}N concentrations of the samples were determined using a TracerMAT continuous flow mass spectrometer (Finnigan MAT, Hemel Hempstead, UK).

For the +N treatment, ^{15}N enrichment was used to calculate the uptake of nitrogen from the ^{15}N-labelled nutrient solution using equations described earlier (Millard and Nielsen, 1989). The difference between the total and labelled nitrogen content was designated unlabelled nitrogen and was assumed to be the nitrogen present within the plants at the time of the first harvest. For the zero-N treatment, the unlabelled nitrogen content was equivalent to the total nitrogen content. Any increase in the unlabelled nitrogen content of a plant compartment with time represented mobilization of nitrogen to the compartment from other plant parts; similarly, a decrease in unlabelled nitrogen represented mobilization out of the compartment.

Statistical analysis

All statistical analysis were performed using the SAS system (SAS Institute Inc, 1990). There was one missing observation for *P. trivialis* leaves 1–3. One value each of the unlabelled nitrogen content of the roots and whole plant of *P. maximum* were classified

as outliners, these values were subsequently treated as missing observations in order to adjust the model. Analysis of variance (ANOVA) was conducted to assess whether differences were significant and orthogonal contrasts were used to compare treatment means. Data were transformed prior to analysis whenever the assumptions of ANOVA were violated. Because the transformation did not affect the interpretation of the results, untransformed data are presented for clarity.

Results

Both plant species, receiving both the +N and zero-N treatments achieved increases in whole plant mass throughout the harvests (P <0.001, Fig. 1a, b). The increases in whole plant mass were greater for plants that continued to receive N (Fig. 1a, b, P <0.001 for *P. trivialis*, P <0.01 for *P. maximum*). In both *P. trivialis* and *P. maximum* the mass of side tillers, stems and expanding leaves (leaves 7–11 in *P. trivialis* and leaves 9–13 in *P. maximum*) all increased with an increased N supply, whilst root mass was unaffected (Tables 1, 2). The increased whole plant mass of +N compared with zero-N plants was therefore primarily achieved through increased shoot growth.
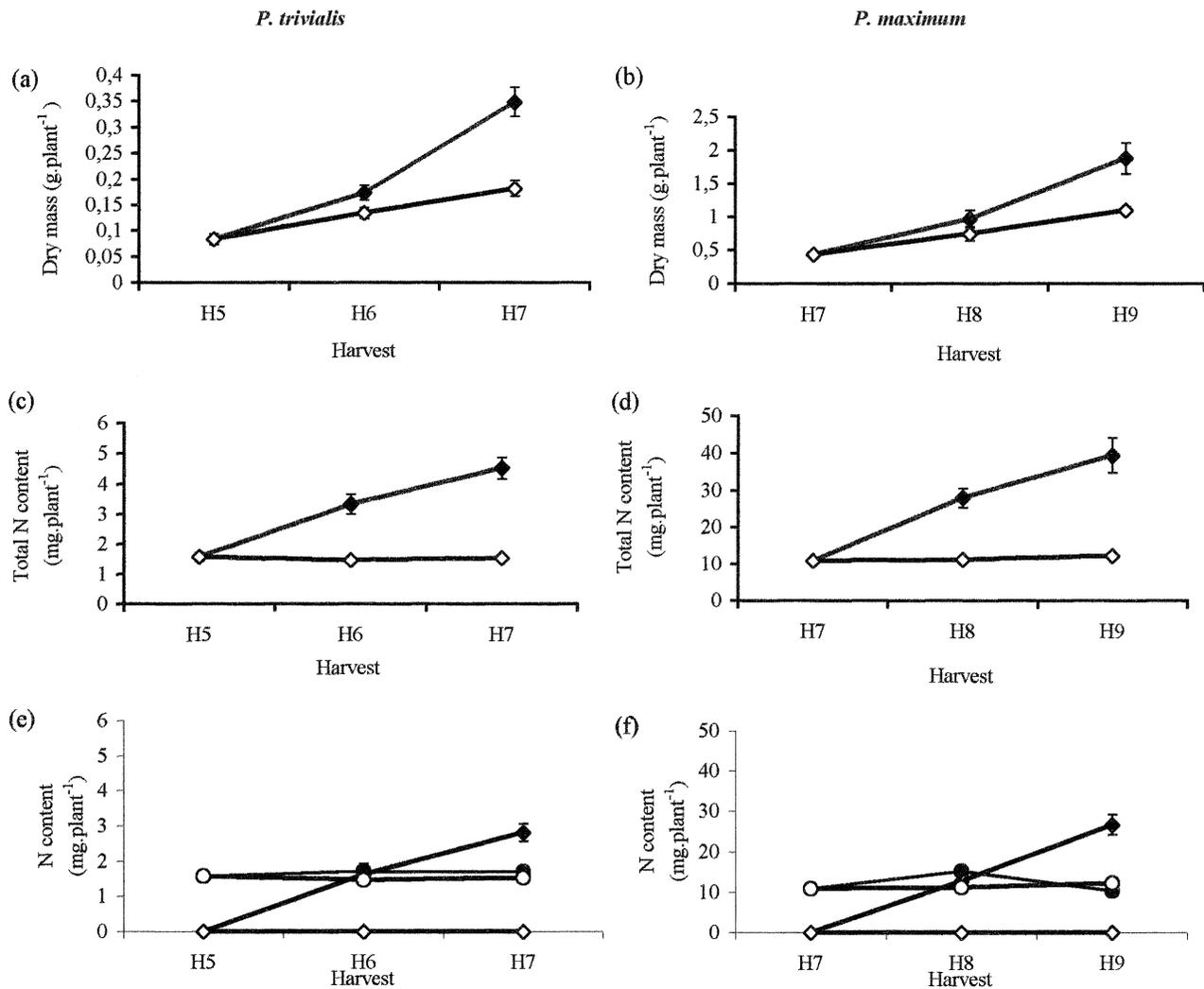
Fig. 1. (a, b) Dry mass; (c, d) total N content; and (e, f) labelled (closed diamonds, open diamonds) and unlabelled (closed circles, open circles) N content of whole plant at the time of harvest of *P. trivialis* and *P. maximum*. Closed symbols represent +N treatment and open symbols represent zero-N treatment. Values are means of five replicates (except for unlabelled N of *P. maximum* on the second harvest, where $n=4$). Bars represent the mean standard error.

For both *P. trivialis* and *P. maximum* receiving +N, the increases in whole plant dry mass were concomitant with increases in the N content of the whole plants (Fig. 1a, b, c, d, $P <0.001$ for each species). However in zero-N plants the observed increases in whole plant mass throughout the harvests was achieved despite having no change in their total N content over this period (Fig. 1a, b, c, d).

For *P. trivialis*, there was no change in the unlabelled N contents of the whole plant, receiving either N treatments, during the experimental period ($P >0.05$, Fig. 1e). The unlabelled N in these plants could, therefore, be considered to be a closed system with no significant loss of N (e.g. from root turnover or exudation) in the time scale of the experiment. Plants of *P. maximum* receiving zero-N also showed no change in unlabelled N content of the whole plant with harvest ($P >0.05$, Fig. 1f). However, for *P. maximum* receiving +N, the unlabelled N content of

whole plants was greater at the second harvest ($P <0.01$, Fig. 1f). As there was no difference in the unlabelled N content between the first and the third harvests ($P >0.05$), the increase at the second harvest was not considered to be a systematic change throughout the harvests. Two approaches were used to 'correct' the unlabelled N content of +N *P. maximum* plants at the second harvest. In the first approach, it was assumed that no uptake of unlabelled N had occurred between the first and second harvest. The unlabelled N contents of all compartments of individual plants were then reduced by the proportion (mean whole plant unlabelled N content at first harvest)/(mean whole plant unlabelled N content at second harvest). In the second approach, it was assumed that uptake of unlabelled N had somehow occurred between the first and second harvests. The mean increase in unlabelled N from the first to the second harvest was then considered as uptake by
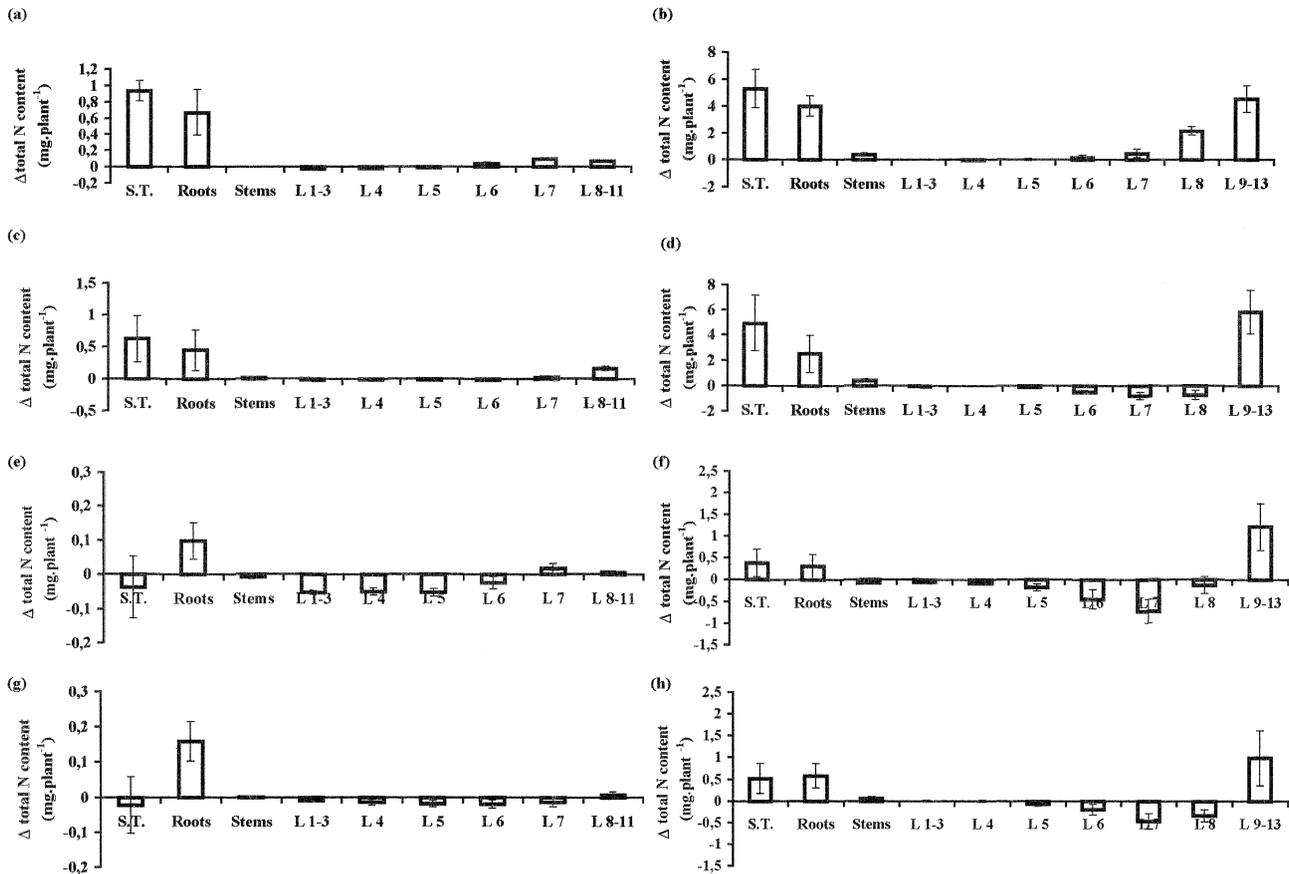
Fig. 2. Change in total N content of *P. trivialis* and *P. maximum* plants. Compartments: side tillers (S.T.), roots, stems, leaves 1–3 (L 1–3), leaf 4 (L4), leaf 5 (L5), leaf 6 (L6), leaf 7 (L7), and leaves 8–11 (L 8–11) (*P. trivialis*) or leaf 8 (L8) and leaves 9–13 (L 9–13) (*P. maximum*). (a, b) +N treatment between first and second harvests; (c, d) +N treatment between second and third harvests; (e, f) zero N treatment between first and second harvests; (g, h) zero N treatment between the second and third harvests. Values represents the means of five replicates (except for leaves 1–3 of *P. trivialis* on first harvest, where *n*=4). Bars represent standard error of the difference.

roots and added to the labelled N contents, assuming it was partitioned between different plant compartments identically to the uptake of labelled N over the same time period. As the interpretation of the results was not affected by the approach chosen, only data using the second approach are presented.

Changes in the net total N content of individual plant compartments occurred between the harvests (Fig. 2). For plants receiving the +N treatment these changes represent the overall change in N due to both uptake and its allocation and remobilization of N, whilst for plants receiving zero-N the changes represent remobilization of N only. The remobilization which occurred in plants receiving +N is given by the net change in unlabelled N (Fig. 3). In *P. trivialis* receiving +N, the total N content of side tillers, roots and to a lesser extent leaves 8–11 increased between harvests (Fig. 2a, c). However, when N supply was removed, only the root compartment showed any substantial increase in total N content between harvests (Fig. 2e, g). As with *P. trivialis*, in plants of

P. maximum receiving +N the side tillers and roots were net sinks of N between harvests (Fig. 2b, d). However, the total N content of the youngest leaves of *P. maximum* also considerably increased between harvests (Fig. 2b, d), indicating that, over this period, the growing leaves of *P. maximum* were a relatively greater sink for N than in *P. trivialis*. When the N supply to *P. maximum* was removed, side tillers, roots and the youngest leaves all continued to increase their total N contents between harvests (Fig. 2f, h), though these increases were understandably not as great as in plants which continued to receive N.

Between the first and second harvest, N remobilization in *P. trivialis* receiving +N was from older leaves mainly directed towards the side tillers and roots, though leaf 7 and leaves 8–11 were also smaller sinks of remobilized unlabelled N (Fig. 3a). Later, between the second and third harvests, the N remobilization to side tillers, roots and leaf 7 was reduced whilst the amount of N mobilized to leaves 8–11 was maintained (Fig. 3c). In *P. maximum* plants
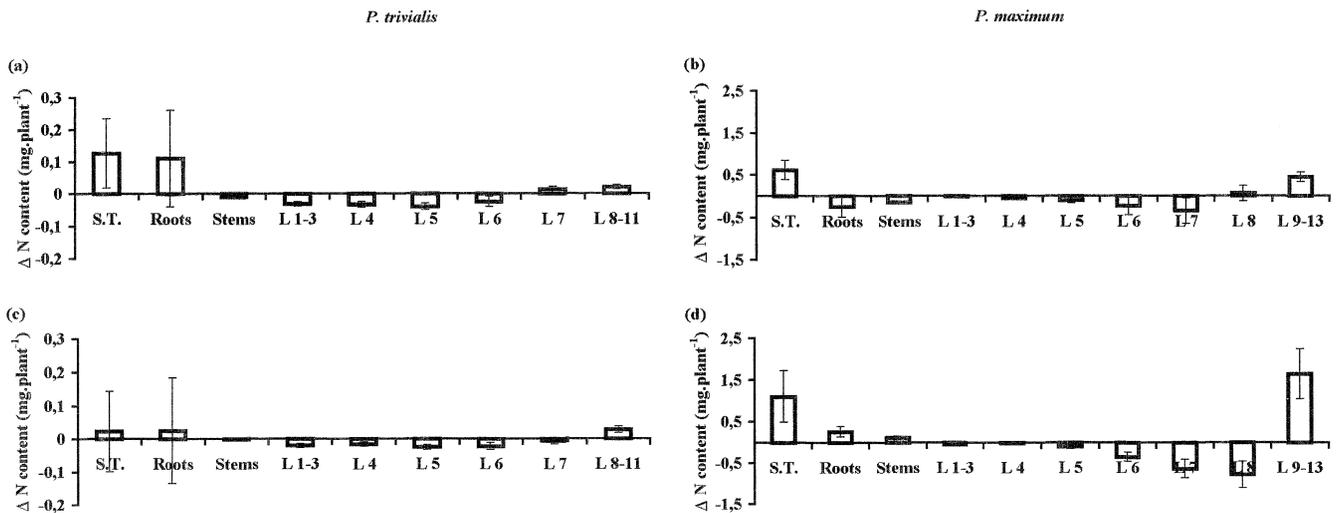
Fig. 3. Change in unlabelled N content of *P. trivialis* and *P. maximum* plants. Compartments on +N treatment: side tillers (S.T.), roots, stems, leaves 1–3 (L 1–3), leaf 4 (L4), leaf 5 (L5), leaf 6 (L6), leaf 7 (L7), and leaves 8–11 (L 8–11) (*P. trivialis*) or leaf 8 (L8) and leaves 9 to 13 (L 9–13) (*P. maximum*). (a, b) Changes in N content between first and second harvests; and (c, d) between second and third harvests. Values represent the means of five replicates (except for leaves 1–3 of *P. trivialis* on the first harvest and for roots of *P. maximum* on the second harvest, where $n=4$). Bars indicate the standard error of the difference.

receiving the +N treatment, between the first and second harvest N was remobilized from leaves of mid age, in particular leaves 6 and 7, and in addition from root and stem material. Mobilization was directed towards side tillers and the youngest leaf material (leaves 9–13) (Fig. 3b). The mobilization of N towards side tillers and leaves 9–13 continued between the second and third harvests, additionally roots and stems were a net sink of mobilized N over this period whilst leaf 8 had become the major source of mobilized N (Fig. 3d). By contrast to *P. trivialis*, more N was mobilized in +N *P. maximum* plants between the second and third harvests than between the first two harvests (Fig. 3).

Uptake of N by roots of *P. trivialis*, receiving the +N treatment, between the first two harvests was mainly allocated to side tillers and roots, with smaller amounts allocated to younger leaves (leaves 5–11) (Fig. 4a). This allocation pattern was essentially repeated between the second and third harvests (Fig. 4c) although uptake to the youngest leaf age category (8–11) was increased as the growth of leaves 8–11 became more rapid (Table 1). Uptake of N by roots of *P. maximum*, receiving the +N treatment was also allocated towards side tillers and roots (Fig. 4b, d). Compared with *P. trivialis*, plants of *P. maximum* allocated relatively more of their total N uptake towards the youngest leaves (Fig. 4), suggesting that, in *P. maximum*, the youngest leaves on the main tiller represent a relatively more important sink compared with leaves on side tillers. Whilst *P. maximum* leaves 6–8 were a sink for labelled N between the first two harvests, they ceased to be so between harvests two and three.

Uptake was the main nitrogen source for new growth in both *P. trivialis* and *P. maximum* during the whole experiment (Table 3). Between the first and the second harvest, the relative use of uptake for root development was higher for *P. maximum* than for *P. trivialis*, while the percentage of remobilized nitrogen used for new tiller and leaf growth was almost the same for both species (Table 3). Later, between the second and the third harvest, the relative use of uptake for new leaves, roots and side tillers development was higher for *P. trivialis* than for *P. maximum*. When considering the whole period (between first and third harvests) the relative contribution of uptake for new tillers and leaf development was higher for *P. trivialis* than for *P. maximum* while for roots the opposite was observed (Table 3).

Discussion

The C_3 grass *P. trivialis* is found in temperate areas (Grime *et al.*, 1988), whereas by contrast, *P. maximum* is naturally found in tropical areas (Savidan *et al.*, 1990). In the present study a decision was taken to grow the different plant species at temperatures appropriate for their optimum growth rather than at a common temperature. Differences also existed between the two species in available rooting volume and planting density. Direct comparisons between the two species are, therefore, restricted to being under the stated different conditions of growth.

The current study with *P. trivialis* supports the results of Schulte auf'm Erley *et al.* (2000) and Bausenwein *et al.* (2001*a*), who observed, with other C_3 grass species, that, in
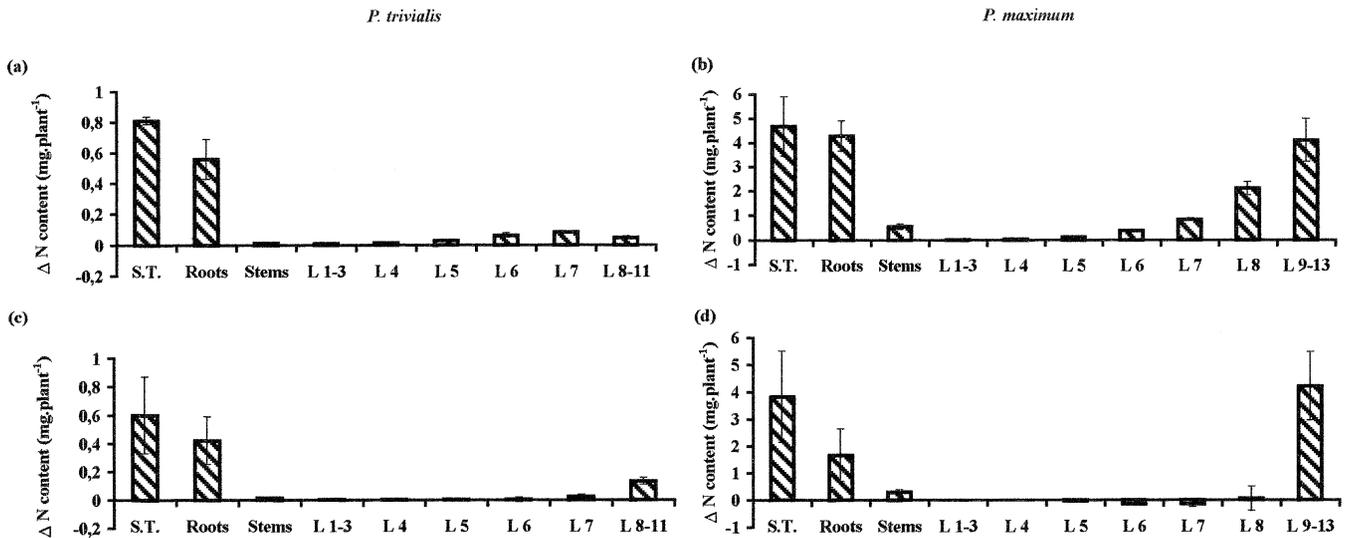
Fig. 4. Change in labelled N content of *P. trivialis* and *P. maximum* plants. Compartments on +N treatment: side tillers (S.T.), roots, stems, leaves 1–3 (L 1–3), leaf 4 (L4), leaf 5 (L5), leaf 6 (L6), leaf 7 (L7), and leaves 8–11 (L 8–11) (*P. trivialis*) or leaf 8 (L8) and leaves 9–13 (L 9–13) (*P. maximum*). (a, b) Changes in labelled N content between first and second harvests; and (c, d) between second and third harvests. Values represent the means of five replicates (except for leaves 1–3 of *P. trivialis* on the first harvest, where *n*=4). Bars indicate the standard error of the difference.

Table 3. *Relative use of either uptake or remobilization by main sink compartments of* P. trivialis *and* P. maximum *receiving nitrogen, between first and second, second and third, and first and third harvests*

Values are means (±SE) of 5 replicates.

Plant compartment	*P. trivialis*			*P. maximum*		
	Δ Total N (mg plant^{-1})	Uptake (%)	Mobilization (%)	Δ Total N (mg plant^{-1})	Uptake (%)	Mobilization (%)
1st–2nd harvest						
Side tillers	0.9382	86.5±9.9	13.5±9.9	5.2922	88.4±4.7	11.6±4.7
Roots	0.6689	83.3±19.0	16.7±19.0	4.0204	106.3±6.5	–6.3±6.5
New leaves	0.2002	94.9±28.9	5.1±8.9	6.7060	92.3±3.9	7.7±3.9
2nd–3rd harvest						
Side tillers	0.6227	96.3±18.7	3.7±18.7	4.9179	77.6±12.4	22.4±12.4
Roots	0.4462	94.5±33.6	5.5±33.6	1.9164	86.5±8.8	13.5±8.8
New leaves	0.1770	87.6±20.5	12.4±20.5	5.8431	71.8±9.5	28.2±9.5
1st–3rd harvest						
Side tillers	1.5609	90.4±6.7	9.6±6.7	10.2101	83.2±5.4	16.8±5.4
Roots	1.1151	87.8±5.9	12.2±5.9	5.9368	99.9±3.6	0.1±3.6
New leaves	0.3562	97.0±16.6	3.0±16.6	11.8116	88.3±7.0	11.7±7.0

intact plants, the roots do not normally act as a source of mobilized nitrogen. This was also true for the C$_4$ grass *P. maximum* when nitrogen supply was withdrawn. However, when nitrogen supply to *P. maximum* was uninterrupted, roots exhibited both source and sink behaviour regarding mobilized nitrogen. Following defoliation of C$_3$ grasses, mobilization from both remaining shoot material and roots occurs (Ourry *et al.*, 1988, 1990; Thornton and Millard, 1993). In defoliated C$_3$ grasses, mobilization of nitrogen from roots to support growing leaves may be specifically induced by defoliation. However, the mobilization from remaining older leaves to younger leaves may simply be part of the 'normal'

mobilization that occurs in undefoliated plants, the timing of which may potentially be altered by defoliation.

That roots of intact *P. maximum* can act as a source of mobilized nitrogen for the growth of new leaves may simply reflect the relative sink strengths for nitrogen of the roots compared to other plant compartments between *P. maximum* and *P. trivialis*. The allocation of both labelled nitrogen uptake and unlabelled mobilized nitrogen provides evidence that the sink strength of the growing leaves was relatively greater in *P. maximum* than *P. trivialis*. The possibility exists therefore that roots of intact plants of C$_3$ grasses may act as sources of mobilized nitrogen under some circumstances. Indeed, it has been

observed that an initial short-term response of intact *Hordeum vulgare* (L.) to nitrogen deprivation was the mobilization of nitrate from root vacuoles, which was considered in part to supply nitrogen to the shoot (van der Leij *et al.*, 1998).

The greater sink strength for nitrogen by the shoot of *P. maximum* compared with *P. trivialis* reflects the growth habit and morphology of the two species. *P. maximum* is a caespitose grass with a mean height of up to 1.3 m (Savidan *et al.*, 1990), whilst *P. trivialis* forms low growing stolons (Grime *et al.*, 1988). The percentage of soil area occupied by caespitose grasses is relatively low compared to stoloniferous or rhizomatous species. For example, Martha Júnior (1999) observed that the caespitose *Pennisetum purpureum* Schum. occupied only 50% of the soil surface area. In order to compete with its neighbours, *P. maximum* must grow tall; consequently, a reduction in main shoot development would be more prejudicial to *P. maximum* than to *P. trivialis*.

In both grass species, the number of side tillers initiated during the experimental period was less when nitrogen supply was withdrawn (data not shown). Rather than absolute concentrations or contents of carbon and nitrogen solutes, the C:N ratio of substrate pools has been suggested as an important signal for the overall regulation of plant metabolism (Lemaire and Millard, 1999). In the sedge *Carex rostrata* (Stokes.), the initiation of new shoots was shown to be related to the ratio of total non-structural carbohydrates (TNC) to free amino acids (FAA) (Saarinen and Haansuu, 2000); the lower the TNC:FAA ratio the greater the number of new shoots initiated. Current results support this view as, at the end of the experiment, plants of both *P. trivialis* and *P. maximum*, when nitrogen supply was withdrawn, had greater dry mass:total N content ratios and fewer new tillers, compared with plants which continued to receive an uninterrupted supply of nitrogen.

As nitrogen supply was reduced, *P. trivialis* was more plastic in the allocation of mobilized nitrogen than *P. maximum*. The supply of nitrogen by mobilization to side tillers of *P. trivialis* was completely stopped as the external nitrogen supply was reduced. However, in *P. maximum* at least some of the allocation of mobilized nitrogen to side tillers, roots and growing leaves, that occurred with the adequate supply of external nitrogen, was maintained with the reduced nitrogen supply. The ability of plants to store, mobilize and, subsequently, to reuse nitrogen *per se* has been suggested as an adaptation to lower fertility environments (de Aldana and Berendse, 1997). The current results demonstrate that species differences exist in the plasticity of allocation of mobilized nitrogen with a changing nitrogen supply. An ability to alter the allocation of mobilized nitrogen with a changing external nitrogen supply may also be advantageous for plants in low fertility situations. Although both *P. trivialis* and *P. maximum* are associated with relatively fertile

habitats (Grime *et al.*, 1988; Vieira and Kichel, 1995), as nitrogen supply is reduced, *P. trivialis* will invade into swards of *Lolium perenne* (Morrison, 1979), whilst *P. maximum* tends to be invaded by other species (Vieira and Kichel, 1995).

[15]N tracers were used to distinguish between current root uptake of nitrogen and the mobilization of stored nitrogen. As the length of the experimental period is increased, the usefulness of the tracer to discriminate clearly between these two possible sources of nitrogen becomes blurred. Over longer time periods, nitrogen taken up by the roots may be transported to one plant compartment and subsequently mobilized from that compartment to another. In comparing the allocation pattern of uptake with mobilized nitrogen, discussion will be restricted to the results obtained between the first two harvests only.

In both plant species receiving an uninterrupted supply of nitrogen, the allocation pattern of mobilized nitrogen differed from that of nitrogen derived from root uptake, though this difference was far more pronounced in *P. maximum* than with *P. trivialis*. Some compartments such as the roots and leaf 7 of *P. maximum* continued to receive nitrogen directly from uptake concomitant with nitrogen losses by mobilization. Analogous nitrogen dynamics have been observed in the taproot of the forb *Rumex acetosa* (L.) in spring (Bausenwein *et al.*, 2001*b*), mobilization of nitrogen out of the taproot occurring simultaneously with partitioning of root nitrogen uptake into the taproot. Currently, in roots and leaf 7 of *P. maximum*, root nitrogen uptake partitioned into these compartments more than compensated for the nitrogen losses by mobilization and the compartments had a net increase in nitrogen content. By contrast, in the taproot of *R. acetosa*, nitrogen uptake partitioned to the taproot was insufficient to account for the losses by mobilization and taproots exhibited a net loss of nitrogen content (Bausenwein *et al.*, 2001*b*). Differential partitioning of nitrogen derived from current root uptake and the mobilization of stores has also previously been observed in trees of *Picea sitchensis* (Proe and Millard, 1994). Differential partitioning of nitrogen derived from current root uptake and mobilization therefore occurs in grasses, forbs and trees (Proe and Millard, 1994; Bausenwein *et al.*, 2001*b*; this study). This differential partitioning suggests that, in a wide range of species of contrasting morphology, the pools of nitrogen derived from root uptake and mobilization of stores do not fully mix with each other, at least until they have reached the sink tissues.

In the current study no attempt was made to separate the root system into possible functionally different components, i.e. new versus old roots or meristem versus non-meristem tissue. A single grass leaf can also show all the stages of development, from the most immature at the meristem through fully functional and differentiated tissue to senescing and finally dead tissue at the tip (Dale, 1992).

This range of development within both the roots and leaves may, in part, explain the different allocation patterns of root uptake compared with mobilization in *P. maximum*. For example, nitrogen may be mobilized out of the older mature regions of the roots and leaf 7 of *P. maximum* to other parts of the plant, whilst root uptake supplies nitrogen to the meristems of these tissues.

In plants which continued to receive nitrogen, more nitrogen was mobilized by *P. trivialis* between the first two harvests while *P. maximum* mobilized more nitrogen between the second and third harvests. Though the potential problems in the use of ^{15}N tracers to unequivocally discriminate nitrogen uptake and mobilization at later time intervals must be considered pertinent. The rate of nitrogen mobilization has been shown to be related to the size of the available pool (Walker *et al.*, 2001). In *P. maximum* leaf length increases with the level of insertion until a maximum size is reached (Wilson, 1976; Carvalho *et al.*, 1999). Results indicate that the source of mobilized nitrogen in *P. maximum* was from leaves of a higher level of insertion at the later time interval. Assuming that the nitrogen pool available for mobilization also increases with level of insertion, this may explain the increased mobilization of nitrogen with time in this species.

Conclusions

In conclusion, differences did exist in the degree to which *P. trivialis* and *P. maximum* utilized uptake and mobilization to supply nitrogen to growing leaves. In *P. trivialis*, roots were always a net sink of mobilized nitrogen, irrespective of the external nitrogen supply. In *P. maximum*, roots were also a net sink of mobilized nitrogen when external nitrogen was withdrawn, but exhibited both source and sink behaviour when nitrogen supply was continued.

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