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Responses of plant traits of four grasses from contrasting habitats to defoliation and N supply

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Abstract The objective of the study was to identify specific plant traits determining adaptation of grass species to defoliation and N availability, and thus having a major impact on species dynamics, primary productivity, and on nutrient cycling in grassland ecosystems. It was specifically examined whether the response of species to defoliation is related to their plasticity in leaf growth and in leaf growth zone components, and whether the response of species to nitrogen is related to their plasticity in root morphology and subsequent N acquisition, and to N losses through leaf senescence. The study was conducted on *L. perenne and D. glomerata*, two grazing tolerant species from fertile habitats, and on *F. arundinacea* and *F. rubra,* two less grazing tolerant species from less fertile habitats. Plants were subjected to repeated defoliation at three cutting heights under both high N and low N supply. Biomass allocation, leaf elongation, characteristics of the leaf growth zone (height and relative growth rate), and root morphology, N uptake and N losses through leaf senescence were evaluated. Under high N supply, *L. perenne* and *D. glomerata* showed the greatest tolerance to defoliation, due to a large plasticity in the height of the leaf growth zone and due to compensatory growth, either within the leaf growth zone or between growing leaves. Under low N supply, *F. rubra* was the only species with the ability to develop a more branched root system and a greater length of tertiary roots than under high N. As a consequence, under low N supply *F. rubra* had a higher specific N uptake and a higher growth rate than the other species. This slow growing species also showed a higher nitrogen allocation to dead leaves and subsequently a higher potential N loss to leaf litter.

Keywords Defoliation · *Dactylis glomerata* · *Festuca arundinacea* · *Festuca rubra* · Grass · Leaf growth · *Lolium perenne* · Morphology · Nitrogen · Root · Topology

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Introduction

Fertility and management are major factors determining species dynamics of grassland vegetation (Balent 1991; Duru et al. 1998, 2005), impacting upon species composition, primary productivity, and on nutrient cycling in grassland ecosystems. Under fertile conditions, fast growing species, such as *Lolium perenne* and *Dactylis glomerata*, are dominant and highly productive, while under low fertility, slower growing species like *Festuca rubra*

and *Anthoxanthum odoratum* become dominant (Williams 1978; Elberse et al. 1983; Grime et al. 1988; Ellenberg et al. 1991; Elberse and Berendse 1993). Additionally, defoliation intensity and frequency can determine major changes in vegetation composition (Briske 1996). While some species are tolerant to defoliation, such as *Lolium perenne* or *Dactylis glomerata*, others are less tolerant, like *Festuca* species (Davidson and Milthorpe 1966; Davies et al. 1972; Davies 1988). However, the specific traits allowing plant adaptations to such factors are still uncertain. Therefore, an overall objective of the present study was to determine morphological and functional traits which determine plasticity and adaptation of grass species to defoliation and to nitrogen availability.

Plants respond and adapt to defoliation by compensatory photosynthesis, by rapid leaf replacement or by alteration in biomass allocation patterns between and within organs (Trlica and Rittenhouse 1993; Rosenthal and Kotanen 1994; Richards 1993). In general, species responses vary depending upon growth form, morphology, genetic capabilities, and physiology. Grasses are well adapted to defoliation during vegetative development since their leaf meristematic zone, located close to ground level, allows the ability to rapidly regenerate new leaf tissue after defoliation (Volenec and Nelson 1983; Briske 1991, 1996). Nevertheless, defoliation may cause a rapid decrease in leaf elongation rate (Davidson and Milthorpe 1966), attributed in part to a decrease in the height of the leaf growth zone and in part to a decrease in the relative growth rate of leaf segments within the leaf growth zone (Schäufele and Schnyder 2000). A large variability in height of the leaf growth zone and in relative growth rate of leaf segments within the leaf growth zone has been shown between species (Arredondo and Schnyder 2003). It can be postulated that although grasses are generally well adapted to defoliation and that most species have the capacity for altering biomass allocation between organs, some species have more ability than others to alter the height of the leaf growth zone and to compensate in terms of relative elemental growth rate in response to defoliation height. Such ability would be a positive advantage in grazed swards.

Plants also exhibit a wide range of responses to nutrient limitation, which can increase their nutrient use efficiency. Plants under nitrogen limitation proportionally allocate more biomass and nitrogen to roots. In general, a higher plasticity in allocation is found in faster growing species (Robinson and Rorison 1988; Campbell and Grime 1989). Root morphology also responds to nitrogen availability. Fast growing species are characterised by a high degree of plasticity in root proliferation and adjustment in root weight ratio (Lambers and Poorter 1992). Moreover, root systems of fast and slow growing species differ in their architecture. Slow growing species tend to have 'herringbone' root topologies, allowing effective exploration and exploitation of mobile soil resources, while faster growing species have a more 'dichotomous' topology (Fitter 1987; Fitter et al. 1988).

Adaptation to low fertility may also be determined by the nutrient strategy of the species. Fast growing species generally show a strategy of nutrient investment under fertile conditions, while slow growing species have a more conservative nutrient strategy (Aerts 1999; Aerts and Chapin 2000). Nutrient resorption during leaf senescence (or conversely nutrient losses from dead leaves to soil litter) is a major trait related to the conservation versus investment nutrient strategy of plant species (deAldana et al. 1996). Changing root morphology and adopting a more conservative nutrient strategy may both contribute to increased efficiency of nutrient use under low nutrient supply, but their relative importance as adaptative traits is still uncertain (Aerts and Chapin 2000).

A growth cabinet experiment was set up (i) to test the hypothesis that within shoots, plasticity of the leaf growth zones, and more specifically plasticity of the two main components of leaf elongation, length of the leaf growth zone and relative elemental growth rate of elongating tissues, are two determinant traits influencing grass species adaptation to defoliation, (ii) to evaluate the extent to which the responses of root morphology and N losses from dead leaves (conversely N resorption through leaf senescence) to N supply, are two major traits contributing to the adaptation of species to N fertility.

A complementary objective of the experiment was to verify that the plasticity of biomass allocation between shoots and roots in response to N supply, is a response which operates additionally to the previous specific shoot and root responses.

Materials and methods

Experimental materials and design

Seedlings of four grass species: *Lolium perenne* cv Vigor (Lp), *Dactylis glomerata* cv Lutetia (Dg), *Festuca arundinacea* cv Clarine (Fa) and *Festuca rubra* cv Agio (Fr) were planted at a density of one seedling per pot into 0.95 l cylinders (7.5 mm diameter and 21.5 cm depth) containing pure fine quartz sand (grain size 0.2–0.7 mm). The seedlings were grown in a greenhouse for 60 days under natural daylight (preconditioning period). They were then transferred into a growth cabinet under a photoperiod of 14 h daylength, an average PPFD of 510 μmol m^{-2} s^{-1} at the level of the pot surface provided by metallic iodine lamps (HQI, Osram, France), an air temperature of 18.5°C and a relative humidity of 65–75%.

During the greenhouse pre-growth period, the plants were grown undefoliated and supplied a nutrient solution containing 1.9 mM KNO_3, 0.55 mM $Ca(NO_3)_2$, 2.5 mM NO_3NH_4, 0.5 mM $CaCl_2$, 0.1 mM NaCl, 0.5 mM $MgSO_4$, 0.4 mM KH_2PO_4 and 0.3 mM K_2HPO_4 plus full micronutrients. At the time of transfer of the plants from the greenhouse to the growth room, the nitrogen and defoliation treatments were initiated. Nitrogen treatments included a high N supply regime (HN), consisting of the 8 mM N nutrient solution delivered during the pregrowth period, and a low N supply regime (LN) given by a modified 0.1 mM N nutrient solution, containing 0.01 mM KNO_3, 0.015 mM $Ca(NO_3)_2$, 0.03 mM NO_3NH_4, 0.1 mM $NaSO_4$, 1.45 mM $CaCl_2$, 0.1 mM NaCl, 0.5 mM $MgSO_4$, 0.35 mM K_2SO_4, 1.05 mM KH_2PO_4 and 0.8 mM K_2HPO_4. For both N supply regimes, the nutrient solution was delivered by an automatic irrigation system to each pot, six times per day with a total daily amount of 100 ml per plant.

The plants were defoliated at three cutting heights (3, 6, and 9 cm above shoot base). Four successive defoliation events, 12 days apart, were imposed from the day of transfer from greenhouse to growth cabinet. Plant growth and partitioning were studied immediately before and in the 12 days following the 4th defoliation. Defoliation of the plants was performed with all leaves held erect, allowing that all the tillers of the plants were cut at the same length, thus avoiding a possible effect of tiller orientation on residual tiller length. The plant base was carefully cleaned from the sand to allow a precise localisation of shoot base during cutting. This ensured that residual tiller height was properly determined by reference to the shoot base and not by reference to the surface of the sand. Nitrogen and defoliation treatments were combined, giving 6 treatments per species (2 nitrogen regimes × 3 cutting heights). Plants were arranged in a 4 replicate block design.

Biomass allocation between organs

One set of four plants per treatment was harvested immediately before the 4th defoliation (i.e. 12 days following the 3rd defoliation), and another set of four additional plants per treatment was harvested 12 days following the 4th defoliation event. At each harvest, plants were washed free from the sand over a 1 mm mesh and all loose root material was retained. The number of tillers and number of primary root axes were counted. The shoots were removed from the roots at the stem base and the root system was carefully washed free of sand. Shoots were separated into leaf blade and leaf sheath above cutting height, leaf blade and leaf sheath below cutting height, and dead leaf material, on a fresh weighed shoot subsample, which represented approximately half the entire plant shoot material. Leaf area was determined with a leaf area meter (Li-3100, Licor, Lincoln, Nebraska). Root and shoot samples were placed in an oven at 70°C for 48 h and then reweighed.

Leaf elongation rate and leaf growth zone determination

Leaf length was measured immediately after the 4th defoliation and later at day 6 and at day 12 on all leaves on a random selection of 6 tillers per treatment. Leaf length measurements were used to calculate overall leaf elongation rate per tiller (LER) during the intervals of days 0–6 and 6–12 after the 4th defoliation, in the 3 cm and 6 cm cutting height treatments. Leaf elongation per tiller was not calculated on the 9 cm cutting height treatment due to significant uncertainties in ligule position and therefore in lamina length under this cutting height. Partitioning of leaf elongation per tiller between the successively visible growing leaves of the tillers was calculated as the ratio of elongation of individual growing leaf to total leaf

elongation of the tiller. Leaf 1 (L1) refers to the first leaf which grew after the 4th defoliation, and leaves 2 and 3 (L2 and L3, respectively) refer to leaves which started to elongate later on. An additional subsample of tillers was randomly selected, leaf length measured at days 9 and 10 to calculate LER, and tillers were then used to evaluate the spatial distribution of elongation within the growing zone of the leaf, following Schnyder et al. (1990), on the high N treated plants only. In grasses, leaf growth occurs through division and elongation of epidermal cells at the base of the growing leaf, inside the sheath of mature leaves (Volenec and Nelson 1983). On each tiller, the base of the rapidly growing leaf was exposed by carefully removing the sheath of mature leaves. A drop of a 4% solution of polyvinylformaldehyde (Formvar) in chloroform was spread on the surface of the leaf base. After evaporation of the solvent, the formvar film was removed with adhesive tape and transferred to a microscope slide. The length of 20 epidermal cells was measured at each 3 mm location along the whole leaf base. For each leaf imprint, a Richard's curve was fitted between average cell length and position from leaf base on each replicate. Height of the leaf growth zone was defined as the height from the leaf base at which 95% of the final cell length had been achieved. Relative elemental growth rate of leaf segments (REGR, increase in length of 1 mm long leaf segments within the growth zone, per hour) was derived from epidermal cell length profiles, according to Schnyder et al. (1990).

Root morphology analysis

One randomly selected root axis was subsampled from the entire fresh root system on the second harvest date only. It was placed in a plastic bag in a freezer at −20°C. Later the root axis was thawed out and immersed for 1 h at 4°C in a 0.01% methyl violet and glycerol solution (1:1 v/v). The intact stained root axis was then rinsed in deionised water and carefully spread out on 200 cm^2 glass plates, 3 mm thick. A thin layer of glycerol was added using a Pasteur pipette to prevent dehydration of the root. A sheet of transparent acetate, the same dimensions as the glass plate, was placed over the root and taped in place. The root axis was scanned at a resolution of 150 d.p.i. as a black and white image using a flat bed scanner, an Apple Mac computer and *Photoshop* (version 4.0) software. The image was edited using *NIH image* (version 1.61) software to remove shadows, loops and to ensure contiguous pixelation. The digitised image was then analysed for total root axis length, altitude, magnitude, and total pathlength using *Branching* (version 6.4) software (Berntson 1992). On a separate axis, which was also subsampled from the root system, the roots were stained with Schiffs reagent to identify sites of lateral primordia development on the end section of the primary root axis using a dissecting microscope as described in Bingham and Stevenson (1993).

N uptake and allocation to dead leaves

In order to evaluate N uptake and allocation during the 12 days of growth following the 4th defoliation, the pots of the same previously described experiment were carefully washed with deionised water immediately after the 4th defoliation, and plants were then supplied for the next 12 days with high or low N nutrient solutions identical to those during their previous growth except that all N was enriched with ^{15}N to 2 atom %, together on nitrate and ammonium ions. At harvest, plant samples were dried, weighed and milled. The total N and ^{15}N content of the milled samples was determined using a TracerMAT continuous flow mass spectrometer (Finnigan MAT Ltd, Hemel Hempsted, England). Root N uptake of the plants from the start of the labelling period, and relative allocation of labelled and unlabelled N to dead leaves (respectively allocation to dead leaves of N taken up during the ^{15}N labelling period as percent of labelled N taken by the entire plant, and allocation to dead leaves of N taken up prior to N labelling as percent of unlabelled N taken by the entire plant), were calculated from the ^{15}N content data according to equations described in Millard and Neilsen (1989).

Data analysis

The data was assessed for effect of position in the growth room in relation to the location of the source nutrient reservoirs and distance from this source. Regression analyses were used to model the data, fitting the spatial effects (rows, columns and pipe positions) as factors, and the other fitting the spatial effects as smoothed variates. As there was no significant effect of the spatial term for all variates

presented, the data was analysed as a randomised block design with 4 replicate blocks using ANOVA (Genstat 5, version 4.1) with species, defoliation and nitrogen supply as factors, both before and after transformation using $\log_e$ and angular transformations to normalise the variance for selected variables. A time comparison on biomass and allocation parameters between harvest at 0 days and 12 days was performed and allowed to calculate plant relative growth rate (RGR), as the difference of Log values of biomass over time. Means, least significant differences at the 5% level (LSD < 0.05) and p values are quoted. As the 6 cm defoliation height generally had an intermediate effect compared to the 3 and 9 cm defoliation heights, the data for 6 cm height are not presented unless specified.

Growth and biomass partitioning were evaluated on independent plant individuals at two occasions (3rd and 4th defoliations). In addition, plant growth and the defoliation and nitrogen supply treatments were repeated in a second experiment, during which the major plant parameters followed in the first experiment were again evaluated (biomass partitioning, leaf elongation rate, leaf growth zone components). Conclusions in this second experiment were similar to conclusions drawn in the first experiment, therefore the data from the second experiment are not presented.

Results

Growth and biomass allocation

After 4 cycles of defoliation, growth had stabilised in all treatments, with no significant difference in the biomass of the clipped material produced in the 12 days following the 3rd and 4th defoliation ($P < 0.05$). Consequently, only data from the harvest 12 days after the 4th defoliation are presented. Whole plant biomass was significantly greater for Lp and Dg than Fr and Fa (3.1, 2.9, 2.0, and 1.3 g plant^{-1} respectively, LSD species of 0.32, $P < 0.05$). *Lolium perenne* and Dg plants established more rapidly during the early growth period, reaching a higher biomass at the onset of clipping and nitrogen treatments, which partly explains their higher biomass at day 12 after the 4th defoliation. The relative growth rate (RGR, relative growth increment in the 12 days following the 4th defoliation) was considered to be a more relevant growth measurement for species comparison. There was no significant effect of defoliation height on RGR ($P > 0.05$). Consequently, the overall defoliation means only are presented. Relative growth rate was strongly affected by N supply (Fig. 1). At high N, RGR was similar for Lp, Dg and Fr and was lower for Fa. At low N, RGR was significantly higher for Fr compared with Fa, and intermediate for Dg and Lp.

Lolium perenne and Dg showed the greatest effect of N on whole plant biomass, particularly at the 9 cm cut level (Table 1). The root to shoot ratio was significantly higher under low N than under high N supply for all species (Table 1). The effect of N supply on the root to shoot ratio was more marked in Lp and Dg than in Fr and Fa. Under high N supply, the root to shoot ratio was lower in Lp and Dg than Fa, particularly at the 3 cm cutting height. Conversely under low N supply, the root to shoot ratio was significantly lower in Fa and Fr than the other two species, particularly at the 3 cm cutting height. The proportion of biomass allocated to above the cutting height was significantly greater in Fr and Lp under high N supply, and was greater in Fr only at low N (Table 1). *Festuca rubra* had a significantly higher biomass allocation to dead shoot material than the three other species, at both N levels (Table 1).

Leaf area remaining below the cut is potentially a major determinant of regrowth following defoliation. Leaf area below the cutting height was greatest in Dg, intermediate in Lp and lowest in Fr and Fa, at both N levels and at all cutting heights (Fig. 2). As expected,

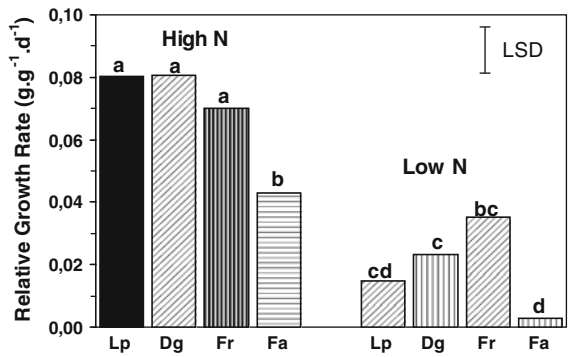

Fig. 1 Relative growth rate of the four species *Lolium perenne* (Lp), *Dactylis glomerata* (Dg), *Festuca rubra* (Fr) and *Festuca arundinacea* (Fa), in relation to N supply (averaged over all defoliation treatments) (LSD at 5%)

Table 1 Biomass allocation and tiller and root axis number in the four species *Lolium perenne* (Lp), *Dactylis glomerata* (Dg), *Festuca rubra* (Fr) and *Festuca arundinacea* (Fa), as affected by nitrogen supply (High N and low N supply, HN and LN, respectively) and by defoliation height (3 and 9 cm)

	Root biomass (g pot^{-1})	Whole plant biomass (g pot^{-1})	Root/ shoot	Proportion allocated to cut	Proportion allocated to dead	Tiller number (tiller pot^{-1})	Root axes number (axis pot^{-1})	No of root axes per tiller
Lp High N 3 cm	0.63	2.18	0.50	0.44	0.09	96	117	1.43
Lp High N 9 cm	1.62	6.42	0.49	0.24	0.10	98	191	1.96
Lp Low N 3 cm	0.57	1.04	1.33	0.10	0.17	35	76	2.25
Lp Low N 9 cm	1.20	2.68	0.90	0.05	0.21	28	89	2.50
Dg High N 3 cm	0.68	2.28	0.44	0.40	0.05	87	102	1.21
Dg High N 9 cm	1.50	6.44	0.41	0.19	0.03	70	120	1.75
Dg Low N 3 cm	0.64	1.21	1.39	0.08	0.14	21	52	2.62
Dg Low N 9 cm	1.03	2.33	0.97	0.04	0.12	21	61	3.30
Fr High N 3 cm	0.25	0.85	0.58	0.37	0.19	77	53	0.76
Fr High N 9 cm	0.58	2.56	0.40	0.29	0.22	112	101	1.06
Fr Low N 3 cm	0.28	0.62	0.94	0.16	0.21	51	47	1.07
Fr Low N 9 cm	0.78	1.70	0.95	0.07	0.24	65	76	1.28
Fa High N 3 cm	0.54	1.29	0.81	0.29	0.08	16	39	2.66
Fa High N 9 cm	1.24	4.06	0.57	0.28	0.04	19	79	4.24
Fa Low N 3 cm	0.42	0.85	1.13	0.12	0.20	10	27	3.07
Fa Low N 9 cm	0.78	1.84	0.86	0.07	0.15	28	61	2.98
LSD (5%)	0.31	0.79	0.17	0.05	0.05	21	23	0.85

leaf area below cutting height significantly increased with defoliation height. Similar results were obtained when leaf area below cut was expressed as a proportion of leaf area per plant (data not shown). Conversely, the percentage of leaf area allocated above the cutting height was greater for Fa and Fr than the other two species, particularly at the low N supply (overall means for the species were 75, 90, 94, and 81%, respectively for Dg, Fr, Fa, and Lp, respectively; LSD interaction 5.9). Specific area of lamina and sheath was evaluated in order to relate the biomass and leaf area allocation data above and below cutting height. The specific area of the lamina was significantly greater ($P < 0.01$) in Lp (315 cm^2 g^{-1}) and Dg (278 cm^2 g^{-1}), and significantly lower in Fr (239 cm^2 g^{-1}) and in Fa (231 cm^2 g^{-1}). The specific area of the sheath was significantly greater in Fr (740 cm^2 g^{-1}), intermediate in Lp (532 cm^2 g^{-1}) and lower in Fa (431 cm^2 g^{-1}) and in Dg (386 cm^2 g^{-1}). Therefore, the specific area of Fr contrasted with the specific area of the other species: despite having the lowest specific lamina area, Fr had the highest specific sheath area. The high specific area and thus the very thin sheath of Fr probably explains, at least in part, its high proportion of leaf biomass above, and conversely the low proportion below the cutting height.

Leaf elongation and leaf growth zone

Leaf elongation rate was significantly different at the two levels of N ($P < 0.001$). Under high N supply, leaf elongation rates per tiller over the 12 days regrowth period, were unaffected by the severity of defoliation in Lp and Dg, whereas the rates for Fa and Fr were significantly reduced under the 3 cm defoliation height (Fig. 3). Under low N, Fr had the highest rate of leaf elongation per tiller compared to Dg, which had the lowest rate.

The four species showed differing strategies of partitioning of leaf elongation between the successively visible growing leaves. Between days 0 and 6 under high N and at 6 cm cutting height, total leaf elongation per tiller predominantly occurred on leaf 1, but was more evenly partitioned between leaf 1 and leaf 2 for Dg and Lp (Fig. 4). Between days 6 and 12, total leaf elongation per tiller was generally partitioned between leaf 1 and leaf 2, except for Dg and for Lp, for which leaf 3 elongated significantly.

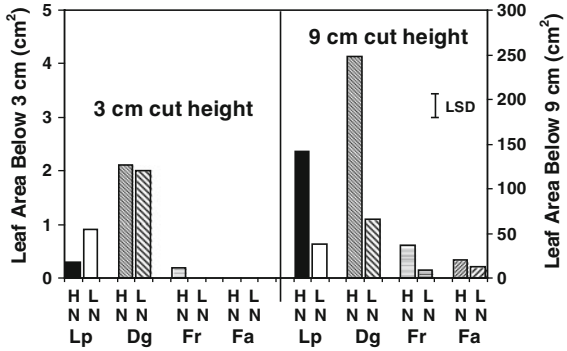

Fig. 2 Leaf area below defoliation height of the four species *Lolium perenne* (Lp), *Dactylis glomerata* (Dg), *Festuca rubra* (Fr) and *Festuca arundinacea* (Fa), grown under high and low N supply (HN and LN, respectively), and defoliated at 3 and 9 cm height (LSD at 5%)

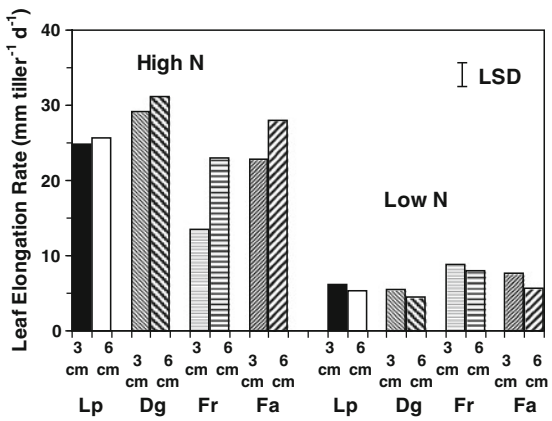

Fig. 3 Leaf elongation rate per tiller of the four species *Lolium perenne* (Lp), *Dactylis glomerata* (Dg), *Festuca rubra* (Fr) and *Festuca arundinacea* (Fa), grown under high and low N supply, and defoliated at 3 and 6 cm height (LSD at 5%)

However, in the case of Dg and between days 6 and 12, leaf growth occurred on the 3 leaves L1 to L3, whereas for Lp, L1 had ceased to elongate with leaf elongation only being observed on leaves L2 and L3. Therefore, under high N supply, leaf elongation of Dg tillers was achieved by the simultaneous elongation of 2–3 visible leaves, while only 1–2 visible leaves were elongating for the other species. Under low N supply, elongation was confined to leaves L1 (days 0–6) and to leaves L1 and L2 (days 6–12), without significant differences between species according to nitrogen and defoliation treatments (data not shown).

In grasses, leaf growth occurs over a few centimetres at the base of the leaf, and relative elemental growth rate (REGR) of growing leaf segments

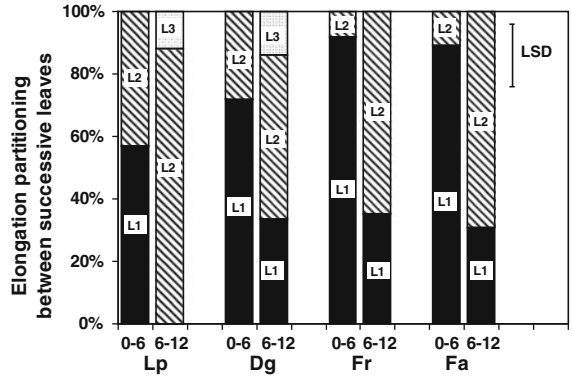

Fig. 4 Partitioning of leaf elongation between the successive visible leaves (L1, L2, and L3) of individual tillers, in the four species *Lolium perenne* (Lp), *Dactylis glomerata* (Dg), *Festuca rubra* (Fr) and *Festuca arundinacea* (Fa). Data obtained under high N supply and 6 cm defoliation height, between days 0–6 and 6–12 of the regrowth period following defoliation (LSD at 5%)

displays a bell shaped curve (Fig. 5), with a maximum value located in the middle part of the growth zone (Schnyder et al. 1990). The height of the leaf growth zone was significantly reduced by an increasing severity of defoliation, particularly in Lp and Dg (Fig. 5; Table 2). *Dactylis glomerata* had the shortest length of leaf growth zone, always located below the cutting height ($P < 0.05$). In contrast, Fr had the longest leaf growth zone. The leaf growth zone of Fr cut at 3 cm was longer (33 mm) than the cutting height, implying that in this situation part of the leaf elongation zone was removed by the cut. *Lolium perenne* showed the greatest plasticity in response to defoliation, characterised not only by a large reduction in the length of its growth zone under 3 cm cutting height, but also by a large compensative increase in REGR. In comparison, Dg also showed a large decrease in length of its growth zone but a more limited ability to compensate in terms of REGR. In contrast to the large plasticity of Lp and Dg, a very limited plasticity in response to defoliation height was observed in Fr and to a lesser extent in Fa, both in terms of length and REGR.

Root architecture

Lolium perenne had the greatest number of root axes per plant (Table 1). High N supply significantly increased the number of root axes per plant in all species. The number of root axes also significantly

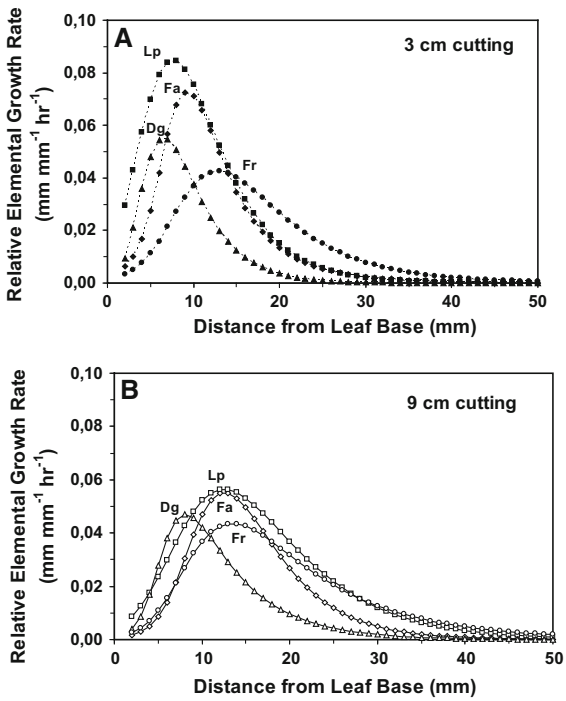

Fig. 5 Spatial distribution of relative elemental growth rate in the growth zone of expanding leaves. The four species *Lolium perenne* (Lp, *square*), *Dactylis glomerata* (Dg, *triangle*), *Festuca rubra* (Fr, *circle*) and *Festuca arundinacea* (Fa, *losange*) were defoliated at 3 cm (**a**, *dark symbols*) and 9 cm (**b**, *open symbols*) height. Data obtained under the high N treatment

Table 2 Effect of defoliation height on the length of the growth zone of rapidly elongating leaves

Species	Defoliation height	Height of leaf growth zone (mm)
Dg	3 cm	16
Dg	9 cm	21
Fa	3 cm	21
Fa	9 cm	26
Fr	3 cm	33
Fr	9 cm	36
Lp	3 cm	21
Lp	9 cm	33
LSD (5%)	(Species × height)	4

Data from high N supply

increased with increasing cutting height. *Festuca arundinacea* had the greatest number of root axes produced per tiller and Fr the least. *Dactylis glomerata* showed the greatest plasticity in the response of

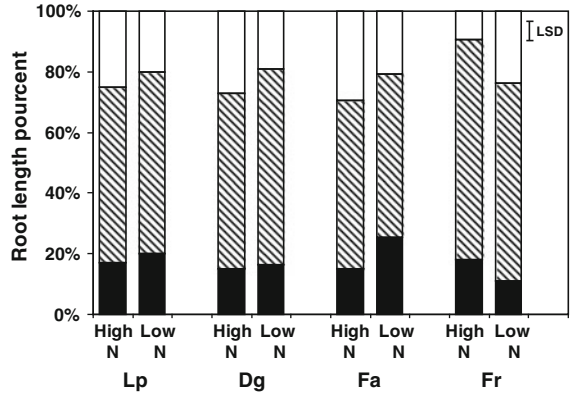

Fig. 6 Relative distribution of root length between primary (*dark area*), secondary (*hatched area*) and tertiary (*light area*) root axes in the four species *Lolium perenne* (Lp), *Dactylis glomerata* (Dg), *Festuca arundinacea* (Fa) and *Festuca rubra* (Fr), grown under high and low N supply (data averaged over the defoliation treatments, LSD 5%)

number of root axes per tiller to nitrogen supply and to defoliation height.

When root morphology was characterised at the axes level, it was observed that the individual weight of root axes was significantly greater ($P < 0.01$) in Fa than in the other three species (0.028 mg for Fa compared to 0.017, 0.015, and 0.015 mg for Dg, Fr and Lp, respectively). Specific root length (length/mass) was not significantly affected by defoliation and N treatments, but was significantly greater ($P < 0.05$) for Fr (180 m g^{-1}) than for Fa (59 m g^{-1}), reflecting the thinner roots of Fr compared to those of Fa. The percentage of the root length present as a primary axis was increased under low N supply for Fa and decreased for Fr (Fig. 6). Correspondingly, the percentage of the root system as tertiary increased for Fa with increased N supply, while it decreased in Fr. The root axis structure for the high N treatment under the 9 cm cutting height was more branched in Fa and more herringbone in Fr. Overall, the log altitude/log magnitude ratio was significantly higher in Fr (0.91) and significantly lower ($P < 0.01$) in Fa (0.82), where a value of 1 represents a herringbone structure, with branching confined to the main axis. Values for the ratio log pathlength/log magnitude, where a value of 1.92 represents a herringbone structure, were again significantly higher ($P < 0.001$) in Fr (1.77) and lower in Fa (1.68). The 3 cm defoliation height log pathlength/log magnitude ratio overall was significantly higher than the 9 cm cut (1.74 and 1.70,

respectively; $P < 0.05$), with higher values representing a more herringbone branching structure.

Nitrogen uptake and allocation to dead leaves

In order to evaluate whether the plasticity of root traits observed in response to N supply would have a significant impact on N acquisition, N uptake was compared between species. Due to the significant differences in plant weight and plant size between the species, the comparison of the absolute value of N uptake between species was not appropriate and therefore specific N uptake (N uptake per g of N accumulated in the plant) was evaluated. Under high N supply, specific N uptake was significantly higher in Lp and Dg than in the two Festuca species, Fr having the lowest value (Fig. 7). In contrast, under low N supply, specific N uptake was significantly higher for Fr than for the three other species.

Relative allocation of nitrogen to dead leaves, which represents the loss of nitrogen from living shoots and thus potential N deposition to shoot litter, was also derived from the ^{15}N content of the plants. Relative allocation of labelled N (N taken up following ^{15}N labelling; Fig. 8a) and relative allocation of unlabelled N (N taken up prior to N labelling and partly remobilised within the plant; Fig. 8b) showed a significant species effect, in interaction with cutting height ($P < 0.001$). Relative allocations of labelled and unlabelled N to dead leaves were both significantly lower for Dg and significantly higher for Fr, particularly under the 9 cm cutting height.

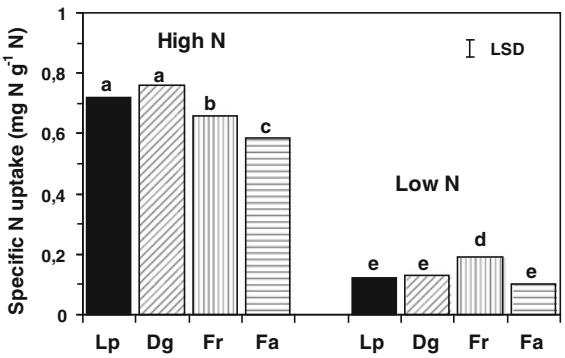

Fig. 7 Specific nitrogen uptake of the four species *Lolium perenne* (Lp), *Dactylis glomerata* (Dg), *Festuca rubra* (Fr) and *Festuca arundinacea* (Fa), grown under high and low N supply (data averaged over the defoliation treatments, LSD and mean comparison at 5%)

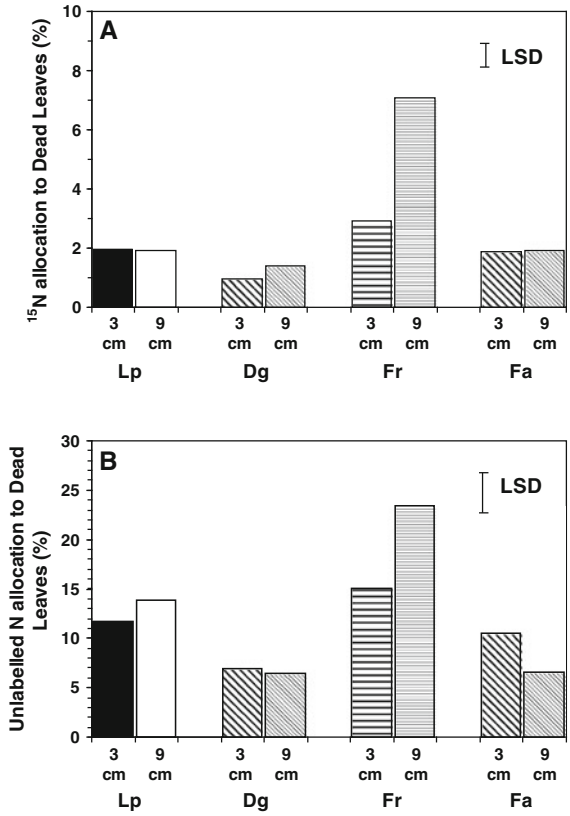

Fig. 8 Relative allocation of nitrogen to dead leaves in the four species *Lolium perenne* (Lp), *Dactylis glomerata* (Dg), *Festuca rubra* (Fr) and *Festuca arundinacea* (Fa), defoliated at 3 and 9 cm height (data averaged over the high and low N supplies). **a** Relative allocation of labelled nitrogen taken up during the labelling and **b** relative allocation of unlabelled nitrogen (nitrogen accumulated in the plant before the labelling date). LSD at 5%

Discussion

Growth rate and biomass allocation in relation to N uptake

Although Dg, Lp and Fr had a similar relative growth rate under high N supply, Fr had the highest relative growth rate under low N supply (Fig. 1). The higher relative growth rate of Fr under low N supply was likely mainly determined by its higher specific N uptake capacity (Fig. 7), in turn related to the ability of this species to develop finer and more branching root structure than the three other species under low N conditions (Fig. 6). The higher specific N uptake of Fr resulted into a significantly higher N concentration

in root and shoot tissues of this species (data not shown), and as a consequence into a higher leaf elongation rate (Fig. 3) and hypothetically into a higher leaf photosynthetic potential.

Relative biomass allocation to roots decreased with increasing N supply for the four species (Table 1), in agreement with Brouwer (1962; 1983) and many other studies (Gastal and Saugier 1986). More importantly for the present study, Dg and Lp had a higher plasticity in biomass allocation between roots and shoots with changing N supply than had Fr and Fa. Currently the lowest root/shoot ratio was observed for Dg under high N supply in agreement with Chapin (1980) and the highest for Dg and Lp under low N supply. Elberse and Berendse (1993) and Boot and Mensink (1990) previously observed species from nutrient rich environments allocate more dry matter to roots than species from nutrient poor environments. Whereas all plants invest relatively more biomass in roots under N limiting conditions, species differ in the size of their response (Boot 1989).

In the present study with repeated defoliation, a higher root biomass allocation was generally found under the low cutting height (Table 1). This result contrasts with other studies (Brouwer 1962; Ryle and Powell 1975; Gastal and Saugier 1986; Richards 1993; Lestienne et al. 2006), showing root growth is more restricted than shoot growth following defoliation. However, in these studies plants were only submitted to a single defoliation. Our plants had time to exhibit adaptive responses to defoliation, potentially explaining the fact that the greatest change in biomass allocation to roots for Lp and Dg in response to N supply, was observed at the 3 cm cutting height.

The four species also showed significant differences in biomass and leaf area allocation above and below the cutting height (Table 1). Grass growth following defoliation depends on water soluble carbohydrates accumulated in leaf bases (Fulkerson and Slack 1994, 1995; Donaghy and Fulkerson 1997), on remaining leaf area (Davies 1988; Richards 1993) and on leaf area restoration in the following days (Schnyder et al. 2000). *Dactylis glomerata* allocated less biomass to leaf tissue above and hence more biomass to leaf tissue below the cutting height (except at 3 cm, high N). Correspondingly, Dg also had the highest leaf area remaining below cutting height. Sheath length was not measured precisely in the present experiment. However, considering the large correlation reported between sheath length and length of the leaf growth zone (Arredondo and Schnyder 2003), and given that Dg had the shortest leaf growth zone, it may be hypothesised that Dg had a shorter sheath length and a lower ligule position, therefore explaining its higher residual lamina area remaining after cutting. In contrast, Fr allocated relatively more biomass to above the cutting height than the other species (except at 3 cm, high N). The contrast between its low specific area of lamina, generally observed in slow growing species (Lambers and Poorter 1992), and its surprisingly high specific area of sheath, probably explains its higher above cut biomass allocation. A generalisation made by Elberse and Berendse (1993) that faster growing species are taller and leave less photosynthetic tissue remaining after cutting than slower growing species from nutrient poor environment, does not always hold. The role of other traits influencing plant development, such as sheath height, tissue mass density (Ryser and Lambers 1995) and leaf thickness (Ryser and Eek 2000), is also important to maintain a plastic response in relation to environmental constraints.

Plasticity of leaf growth and leaf growth zone components in response to defoliation

Defoliation generally decreases biomass yield by reducing growth per tiller more than the number of tillers per plant or per unit ground area (Volenec and Nelson 1983; Davies 1988; Matthew et al. 2000). In the present study, under high N supply, leaf elongation rate per tiller was unaffected by defoliation in Lp and Dg (Fig. 3), whilst it was significantly reduced in Fa and Fr under the severest defoliation treatment. Thus, Fa and Fr appeared less tolerant to defoliation. Leaf elongation per tiller is determined by the elongation rate of individual leaves and by the number of leaves growing simultaneously. The four species differed in the number of growing leaves (Fig. 4). Over the 12 days of measurement, leaf elongation occurred over 2 successive leaves for Fr and Fa, and over 3 successive leaves for Dg and Lp. This is in accordance with Ryle (1964) who showed Dg and Lp had faster leaf appearance rates than Fa.

In grasses, leaf growth is confined to the intercalary meristem, at the base of the tiller (Volenec and Nelson 1983). Schäufele and Schnyder (2000) showed that in *Lolium perenne*, a 5 cm cutting height

resulted in a transitory reduction in leaf elongation rate and in the length of the leaf growth zone. The elongation rate of individual leaves is determined by its two components, length of the leaf growth zone and relative elemental growth rate of expanding leaf tissues. In the present study, Dg had the shortest leaf growth zone, with a maximum length of 21 mm, always below any cutting height. In contrast, the length of the leaf growth zone of Fr was evaluated to be 36 mm under high N and a 9 cm cutting height, and was 33 mm under the 3 cm cutting treatment. Therefore, the 3 cm defoliation must have resulted in physical damage of the upper part of the leaf growth zone. In addition, exposure to light of the proximal end of the leaf growth zone, as a result of sheath removal, may also lead to a reduction in length of leaf growth zone and in leaf elongation rate (Casey et al. 1999), contributing to the overall observed reduction in leaf elongation rate.

Lolium perenne, which also had a relatively large leaf growth zone under the 9 cm cutting height, showed the largest plasticity in reducing this length under the 3 cm cutting treatment and at the same time in compensating its relative elemental growth rate (REGR) of expanding leaf tissues, leading to the maintenance of leaf elongation rate. Such plasticity of Lp, in terms of leaf growth zone components, agrees with Schäufele and Schnyder (2000). *Dactylis glomerata* exhibited a different strategy to maintain leaf elongation rate under the lowest cutting height. In this species, the reduction in the length of the growth zone of the rapidly elongating leaf under the 3 cm cutting treatment, was not accompanied by a so large compensation in REGR, but was accompanied by maintaining more visible leaves elongating simultaneously, compared with the other 3 species. In this respect, Dg was the most plastic of the studied species, though it should be noted this was only observed under high N conditions.

Plasticity of root morphology, nitrogen acquisition and nitrogen losses from dead leaves in response to nitrogen supply

Increased root growth is a strategy for plants subject to low nutrient availability, increasing the contact area between roots and soil (Norby et al. 1986). Defoliation often reduces root length and root elongation rate (Evans 1971; Brouwer 1983; Richards 1984; Jarvis and Macduff 1989). An increased severity of defoliation can increase the time taken to resume root growth (Donaghy and Fulkerson 1998). Indeed Davidson and Milthorpe (1966) found that defoliating Dg to a height of 25 mm caused an immediate and complete cessation in root elongation. In the present study, root biomass was generally reduced with the increasing severity of defoliation for all four species (Table 1), agreeing with previous findings (Ennik and Hoffman 1983; Halland and Detling 1990; Matthew et al. 1991; Mawdsley and Bardgett 1997; Lestienne et al. 2006). The greatest reduction in root biomass between the 9 cm and 3 cm cutting treatments was observed for Lp and Dg, while the least for Fr. *Festuca rubra* was also the only species which did not show a significant reduction in root biomass with reduced N supply, at both cutting heights.

The four species also exhibited contrasting root morphologies and differing responses in this variable to defoliation and nitrogen. *Festuca rubra* had the largest specific root length (SRL) and Fa the smallest, in agreement with earlier studies showing that species from nutrient poor environment generally have a higher SRL and relatively more fine roots (Berendse and Elberse 1990; Boot and Mensink 1990). Arredondo and Johnson (1998) found that root branching was unaffected by defoliation in a grazing tolerant species, while it increased the number of laterals in a grazing sensitive species. None of the current species showed increased branching with increased defoliation intensity, possibly because they were already well adapted to the repeated defoliation procedure. However, the four species showed a relatively unbranched root architecture, which offers effective exploration and exploitation of the soil (Fitter 1987). Under nutrient limitation, plants tend to have a fine root system and a high SRL (Boot 1989). In the present study, Fr appeared to be the most plastic in terms of root architectural response to N supply, since it was the only species showing increased length of tertiary roots under low supply (Fig. 6), thus having a more favourable topology to capture N. This plasticity may reflect the greater ability of this species to compete in a low N environment. The impact of root proliferation on N uptake is still a matter of debate (Robinson 2001). That Fr appeared to be the most plastic species in terms of root architectural response to N supply, and

was the species with the highest specific N uptake under low N supply, strongly suggests these two parameters may be linked, in the present study.

Allocation of biomass and nitrogen to leaves which subsequently die represents a potential carbon and nitrogen loss as litter, a trait often considered in relation to conservation versus investment nutrient strategy of plant species (Aerts 1999). Although it is generally considered that slower growing species follow a more conservative nutrient strategy than faster growing species, this was not the case in the present experiment. Relative allocation of biomass and nitrogen to dead leaves (nitrogen taken up both prior to and following ^{15}N labelling), were higher in the slow growing Fr species than in the fast growing Dg species (Fig. 8). Aerts and Chapin (2000) noted nutrient resorption and conversely nutrient losses, do not seem to explain the distribution of growth forms over habitats differing in fertility.

Conclusion

The present study identified a number of species specific traits which explain, at least partly, the ecological behaviour of grass species.

The greater ability of Dg and Lp to maintain leaf elongation under the lowest defoliation height confirmed the greater defoliation tolerance of these two species compared with the two Festuca species. This was determined by plasticity of their leaf growth zone. The height of leaf growth zone was more largely reduced by severe defoliation in Lp and Dg than in Fa and Fr, allowing better protection of the leaf meristem. Maintenance of leaf elongation rate was allowed by a greater relative growth rate of leaf segments within the leaf growth zone for Lp, and by a greater number of leaves elongating simultaneously in the case of Dg. In contrast, these adaptations were more limited in the two festuca species. Although it is known that grass species differ in leaf growth zones characteristics, the present study brings new insight into the relation between these characteristics in contrasted species and their response to defoliation.

The species Fr, whose root architecture was the most plastic with N supply potentially conferring it a higher ability to capture nitrogen with reduced supply, maintained a higher growth rate than the two fast growing species Dg and Lp. *Festuca rubra* also showed a higher biomass and nitrogen allocation to dead leaves than the other species under low N supply The present study therefore suggests adaptation of root structure to have significantly more impact than adoption of a nutrient conservative strategy in slower growing species under low N availability.

Whereas all species responded to N supply by favouring biomass allocation to roots, the size of the response appeared species dependant, with Dg and Lp showing a larger root/shoot response to N supply than Fr. The present study therefore shows that allocation between root and shoots is also an adaptative trait in addition to within shoot and within root traits.

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