Effects of severity of defoliation on root functioning in grasses.

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Abstract

Grass shoots after defoliation can be supplied with the nitrogen required for regrowth by either root uptake or remobilization of stores. Whilst it is accepted that after a single defoliation inhibition of root uptake and remobilization from roots occurs, it has not been established how the capability of roots to supply nitrogen by uptake and from storage is affected with differing severities of regular defoliation, as experienced by grazed swards. The objective was to examine this question using *Agrostis castellana* Boiss et Reut., *Festuca rubra* L., *Lolium perenne* L. and *Poa trivialis* L., grasses associated with sites of differing fertility, grown in sand culture and defoliated weekly at a height of either 4 or 8 cm. Nitrogen was supplied as NH_4NO_3 in a complete nutrient solution. The use of ^{15}N as a tracer allowed the nitrogen supplied to the shoot by root uptake and remobilization to be discriminated over a 35 day period. An increased severity of defoliation resulted in decreased root mass, and increased nitrogen uptake per unit root weight for all species. Increased severity of defoliation did not affect uptake on a per plant basis for *A. castellana*, 0.54 mg N (plant) $^{-1}$ (week) $^{-1}$ and *F. rubra*, 0.40 mg N (plant) $^{-1}$ (week) $^{-1}$, whilst mg N (plant) $^{-1}$ (week) $^{-1}$ decreased from 0.54 to 0.14, and 0.54 to 0.34 for *L. perenne* and *P. trivialis* respectively. For plants clipped at 4 or 8 cm, over 88% and 77% respectively of uptake appeared in the shoot. Nitrogen was remobilized from roots to the shoot for *A. castellana* and *F. rubra* when clipped at 4 cm, and for *A. castellana*, *L. perenne* and *P. trivialis* when clipped at 8 cm. Uptake by roots was more important than remobilization from roots in supplying nitrogen to the shoot. The ability to maintain the supply of nitrogen by uptake and remobilization to the shoot with increased severity of defoliation was species dependant.

Key Words: clipping height, pasture species, root uptake, remobilization, nitrogen

Grasses are often defoliated repeatedly by grazing herbivores (Curll and Wilkins 1982). Carbohydrate reserves are mobilized in response to defoliation and play a role in regrowth of plants (Bahrani et al. 1983, Gonzalez et al. 1989, Danckwerts 1993). However, carbohydrate reserves are not always correlated to regrowth ability after defoliation (Davidson and Milthorpe 1966a, Richards and Caldwell 1985) and nitrogen availability is consid-

ered to be at least equally important (Ourry et al. 1994). After defoliation, roots of grasses can potentially supply nitrogen for laminae regrowth by uptake from the soil solution and remobilization from stores. On defoliation of previously uncut grass, nitrogen is remobilized from roots (Ourry et al. 1988, 1990, Thornton and Millard 1993), however root uptake is inhibited (Clement et al. 1978). What is less clear is how the capability of roots to supply nitrogen by absorption or mobilization from storage alters with differing height of repeated defoliation, as experienced under differing grazing intensities (Curll and Wilkins 1982).

We selected 4 grass species for this work, *Lolium perenne* L. and *Poa trivialis* L. which are associated with higher fertility sites, and *Agrostis castellana* Boiss et Reut and *Festuca rubra* L. which are usually found on poorer sites (Grime et al. 1988). We used ^{15}N to discriminate root uptake from remobilization of nitrogen. The hypothesis that an increased severity of repeated defoliation reduces N availability for shoot growth, from both uptake and from storage, was then tested.

Materials and Methods

Sixty pots, 8-cm diameter, each containing a mixture of sand and limestone chips, were sown with seed of either *Agrostis castellana* Boiss et Reut. cv. Highland; *Festuca rubra* L. cv. Boreal; *Lolium perenne* L. cv. Aurora or *Poa trivialis* L. cv. Erte, and germinated in environmental cabinets, as described by Thornton et al. (1993), with a 16-hour photoperiod ($250 \mu\text{mol m}^{-2} \text{s}^{-1}$) beginning 7 days after germination. The seedlings were watered to field capacity 3 times each week with a complete nutrient solution containing $3 \text{ mol m}^{-3} \text{NH}_4\text{NO}_3$ at natural abundance. Details of the solution used are given in Thornton et al. (1993). Pots were thinned to 20 seedlings 14 days after germination. Plants in 30 pots for each species were clipped to 4-cm height, and the remaining plants were clipped to 8-cm height initially 22 days after germination and weekly thereafter.

After the sixth clipping plants were transferred to a thermostatically controlled greenhouse with additional lighting of $140 \mu\text{mol m}^{-2} \text{s}^{-1}$ at plant height from high pressure sodium lamps (G.E.C. HPS.U11, 400W) to provide a 16-hour photoperiod. The temperature of the greenhouse (day or night) remained in the range $20 \pm 5^\circ \text{C}$ except for a few occasions at night when temperatures down to 10°C were recorded. Pots were laid out in a randomized block arrangement of 5 replicate blocks with treatments randomly applied within each block.

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Immediately after the fifteenth weekly clipping, the unlabelled nutrient solutions were washed from the pots with 2 additions of 75 cm³ deionized water, then 3 additions of 75 cm³ nutrient solution, identical except that the nitrogen was labelled with ¹⁵N enriched to 5 atom %. Pots were then watered with the ¹⁵N labelled solution 3 times weekly.

Five pots, 1 from each block, of each of the 8 treatment combinations of 4 species × 2 clipping heights, were destructively harvested immediately (day 0) and after 7, 14, 21, 28, and 35 days. At harvest plants were clipped to the appropriate height. The material removed by clipping after day 0 was referred to as leaf top. The root system was washed free from the sand limestone mixture onto a series of 1-mm mesh sieves resulting in minimal root loss. Plants were then separated into leaves plus pseudostem below the clipping height, referred to as leaf base, and root material. Unharvested pots were clipped to the appropriate height as usual on days 7, 14, 21, and 28.

All plant material was weighed fresh and after freeze drying, then milled prior to analysis. The total N and ¹⁵N concentrations in all samples were determined using an ANA-SIRA mass spectrometer (VG Isogas, Middlewich, Cheshire, England). The ¹⁵N calculations used have been described previously in Millard and Neilsen (1989). Briefly, the weight of ¹⁵N present in the subsample analysed by the mass spectrometer C (μg) was calculated using the equation $C = 15AB / (1400 + B)$ where A = weight of total N in subsample analysed (μg) and B = ¹⁵N atom per cent in the sample. Values of C were corrected for the natural abundance of ¹⁵N in the plant material which was taken to be 0.37 atom per cent. The weight of N in a tissue derived from uptake of labelled solution from day 0, W (mg), was then calculated from $W = CED/A$ where D = N content of the tissue (mg) and E = fertilizer equivalent (gN g⁻¹ ¹⁵N). Subtraction of the root uptake from the total N content gave a measure of unlabelled N content, assumed to be that present in the leaf base and roots on day 0.

The total N content of clippings, and the labelled N uptake into the whole plant (including material removed by clipping) provided estimates of nitrogen losses and total N uptake respectively. Relative nitrogen loss over the 35-day labelling period was calculated as defined by Berendse et al. (1992), i.e. nitrogen loss as a percentage of total nitrogen uptake.

Analysis of variance (ANOVA) was conducted to assess whether differences were significant using Genstat 5 release 2.2 (Lawes Agricultural Trust, Rothamsted Experimental Station, England). Analyses were performed after log transformation of dry weight data and angular arcsine transformation of percentage data, in order to stabilize the variance.

Results

At the second destructive harvest clipping to 4 cm had reduced both the biomass of root and leaf base of all grass species, compared with those clipped to 8 cm ($P < 0.001$ in each case, Table 1). When clipped to 4 cm *L. perenne* produced less root material than the other species, but plants produced similar amounts of root material when clipped to 8 cm ($P < 0.05$). The amount of leaf base produced decreased in the species order *A. castellana*, *F. rubra*, *P. trivialis*, and *L. perenne* when clipped to 4 cm, however when clipped to 8 cm only *A. castellana* produced significantly more than any other species ($P < 0.001$). For leaf tops an interaction between species and clipping height ($P < 0.001$) meant that clipping at 4 compared to 8 cm reduced the dry weight of *L. perenne* and *P. trivialis* plants only (Table 1).

Uptake of labelled N per unit root weight was greater in all species for plants clipped at 4 compared to 8 cm ($P < 0.001$; Fig. 1A). Clipping height did not affect N uptake (plant)⁻¹ by *A. castellana* and *F. rubra*, but *L. perenne* and *P. trivialis* plants took up more N when clipped to 8 cm than to 4 cm (Fig. 1B). The increased uptake per dry weight root with the more severe defoliation (Fig. 1A) was, therefore, sufficient to mitigate the effect of increased defoliation on root mass (Table 1) for *A. castellana* and *F. rubra*, but not for *L. perenne* and *P. trivialis*. For plants clipped to 4 cm, 88% of labelled N taken up by the plant subsequently appeared in the shoot, compared with 77% in plants clipped to 8 cm (Figs. 1B, 1C). The effects of increasing severity of defoliation upon N uptake and translocation to the shoot and upon total N uptake per plant were similar, except for *F. rubra* where the amount of N translocated to the shoot was greater ($P < 0.05$) at the more severe defoliation (Fig. 1C).

The unlabelled N content on day 0 and day 35 was similar in whole plants, which included clipped material removed over the

Fig. 1. Labelled N contents, over a 35 day period from changing to nutrient solutions containing labelled N, including material removed by clipping. A: Total plant content mg N (g dry wt root)⁻¹. B: Total plant content mg N (plant)⁻¹. C: Shoot content mg N (plant)⁻¹. Unhatched histograms are plants clipped to 4 cm, and hatched histograms are plants clipped to 8 cm. Values are means of 5 replicates ± SE, except for *L. perenne* clipped to 4 cm which is the mean of 3 replicates.

Table 1. Dry weight of the root, leaf base and leaf top of plants 7 days after changing to labelled N nutrient solutions.

Tissue	Clipping height	<i>Agrostis. castellana</i>	<i>Festuca rubra</i>	<i>Lolium perenne</i>	<i>Poa trivialis</i>
	(cm)-				
Root	4	25.3 (4.3) ^a	36.0 (18.5)	5.3 (0.8)	20.0 (6.2)
Root	8	68.5 (16.0)	86.0 (24.0)	60.7 (7.4)	76.8 (13.6)
Leaf base	4	100.6 (10.8)	49.4 (7.0)	7.4 (1.9)	26.7 (4.6)
Leaf base	8	172.1 (14.7)	113.4 (8.5)	85.0 (6.8)	107.9 (18.1)
Leaf top	4	9.1 (0.8)	7.3 (1.1)	1.0 (0.4)	5.5 (0.6)
Leaf top	8	9.0 (1.7)	6.9 (0.5)	10.7 (0.4)	11.4 (0.8)

^aMean (SE) of 5 replicates.

35-day period beginning when labelling was initiated ($P > 0.05$, Table 2). This indicates there was no carry-over of unlabelled N when the N supply was changed to labelled N, and there were no significant losses of unlabelled N by root turnover or exudation. Loss of unlabelled N from roots over the 35-day period, therefore, represents remobilization from these structures to shoot material. The unlabelled N content of roots on day 0 at the start of the labelling period was greater for plants clipped at 8-cm height than with those clipped at 4-cm ($P < 0.001$, Fig. 2). The reduction of unlabelled N in the roots with time was significant when considered over all species and clipping heights ($P < 0.001$, Fig. 2). An interaction indicated that significant reductions from day 0 to day 35 ($P < 0.05$) only occurred for *A. castellana* and *F. rubra* when clipped at 4 cm, and for *A. castellana* and *P. trivialis* when clipped at 8 cm. Unlabelled N was also reduced in roots of *L. perenne* plants clipped at 8 cm, though in this instance it was from days 7 and 14 to day 28 (Fig. 2).

Fig. 2. Unlabelled N contents (mg plant⁻¹) remaining in root material of plants clipped to 4 cm □, or 8 cm ■. Values are means of 5 replicates $\pm$ SE, except 4 cm *L. perenne* on day 35 which is a mean of 3 replicates.

Table 2. The unlabelled N content of whole plants over a 35-day period from the time of changing to nutrient solutions containing labelled N, including material removed by clipping where appropriate.

Clipping height	Unlabelled N content	
	Day 0	Day 35
	----- (mg / plant) -----	
4 cm		
<i>A. castellana</i>	1.73 (0.06) ^a	1.67 (0.06)
<i>F. rubra</i>	1.42 (0.08)	1.21 (0.05)
<i>L. perenne</i>	0.27 (0.02)	0.39 (0.08) ^b
<i>P. trivialis</i>	0.73 (0.03)	0.81 (0.08)
8 cm		
<i>A. castellana</i>	2.64 (0.11)	2.43 (0.14)
<i>F. rubra</i>	2.42 (0.16)	2.34 (0.19)
<i>L. perenne</i>	1.70 (0.07)	1.76 (0.08)
<i>P. trivialis</i>	2.07 (0.08)	2.08 (0.08)

^aMean (SE) of 5 replicates except b which is a mean of 3 replicates.

The relative N loss for all species was greater when clipped at 4 compared with 8 cm ($P < 0.001$, Fig. 3). An interaction between species and defoliation height ($P < 0.01$) indicated that when clipped at 4 cm the relative N loss of *L. perenne* was only greater than that of *F. rubra*, whilst when clipped at 8 cm it was greater than *P. trivialis* and *A. castellana*, which in turn were greater than *F. rubra* (Fig. 3).

Discussion

Defoliating plants reduces root growth (Davidson and Milthorpe 1966b, Chapin and Slack 1979) which can be manifested as reduced root number (Richards 1984, Karl and Doescher 1991). A reduction in roots can potentially affect both root uptake and storage capabilities. In the present study root growth was reduced with increasing severity of defoliation.

Fig. 3. Relative nitrogen loss (nitrogen loss in clippings as a percentage of total nitrogen uptake) over a 35 day period from the time of changing to nutrient solutions containing labelled N. Unhatched histograms are plants clipped to 4 cm and hatched histograms are plants clipped to 8 cm. Values are means of 5 replicates $\pm$ SE, except for *L. perenne* clipped to 4 cm which is the mean of 3 replicates.

A single defoliation caused a rapid, almost complete cessation of NO_3^- uptake by *L. perenne* roots, lasting at least 2 days (Clement et al. 1978). In contrast, Polley and Detling (1989) observed increased N uptake on a root weight basis in repeatedly defoliated *Agropyron smithii* Rydb. compared with undefoliated control plants. With a high N supply the increased uptake post defoliation per unit of root in *A. smithii* failed to offset a decrease in uptake caused by concomitant decline in root biomass. With a low N supply increased N uptake per unit of root balanced decreases in uptake caused by defoliation-induced decline in root biomass (Polley and Detling 1989). We found that N uptake increased on a root weight basis in response to an increased severity of repeated defoliation in all the species we studied. Increased N uptake per unit of root offset the effect of root mass decreases on plant uptake in *A. castellana* and *F. rubra* but not in *L. perenne* or *P. trivialis*. Mechanisms of this response are unknown. In the sedge *Eriophorum vaginatum* L., Chapin and Slack (1979) found both a linear decrease in root weight per unit length with increasing number of defoliations and an increased uptake of phosphate on a root weight basis with defoliation. Root hairs are thought to facilitate root uptake by increasing the area available for uptake (Robinson and Rorison 1983). Species from infertile sites respond to a decreased N supply by increasing root hair density and length to a greater degree than species from fertile sites (Boot and Mensink 1990). The possibility exists that *A. castellana* and *F. rubra*, associated with lower fertility (Grime et al. 1988), respond to increased severity of defoliation with greater changes in root morphology than *L. perenne* and *P. trivialis*.

Alternatively, species differences in canopy architecture may have led to the observed uptake differences. Uptake of NO_3^- requires active transport (Macklon et al. 1990) and depends on the level of radiation incident on the shoot in *L. perenne* (Clement et al. 1978). In *Lolium multiflorum* Lam. Hansen (1980) found a close relationship between the diurnal variations of NO_3^- uptake and root respiration, suggesting NO_3^- uptake was in part dependant on photosynthate. In the present study *L. perenne* and *P. trivialis* were the more erect species which would have lost proportionally more shoot material with the more severe clipping regime (Table 1). In turn, this may have restricted their ability to maintain NO_3^- uptake to a greater extent than *A. castellana* or *F. rubra*.

After a single defoliation of *L. perenne*, stubble material provided the major source of remobilized N for several days. However, remobilization from roots occurs (Ourry et al. 1988, 1990). Decreases in soluble protein and increases in peptidase enzyme activity of both stubble and roots after a single defoliation also indicate both compartments supply N (Ourry et al. 1989). Remobilization of N from roots of *L. perenne* plants regularly defoliated to 6-cm height has previously been reported (Millard et al. 1990). We found remobilization from roots of regularly clipped *L. perenne* is dependant on the severity of defoliation, and the effect of defoliation severity differs between grass species. Our finding that repeatedly defoliated grass species, uptake by roots was more important in supplying N to the shoot than remobilization from roots, agrees with previous work on *L. perenne* receiving a single defoliation (Ourry et al. 1990).

In the short term, immediately following defoliation, remobilization and root uptake are independent of each other, as the total amount of N remobilized is unaffected by changing the N supply (Ourry et al. 1990). In the long term, the 2 processes are obviously related, since stored N must ultimately be derived from root

uptake. In this context it is worth noting that both *A. castellana* and *F. rubra* were able to maintain whole plant N uptake with increased severity of defoliation and remobilize N from roots at the more severe defoliation. Also, with the less severe defoliation, *F. rubra* was the only species not utilizing remobilization from roots, and had the lowest relative N loss. In this instance, remobilization from stubble alone and/or root uptake may have been sufficient to supply N for regrowth.

In conclusion, uptake by roots was more important in supplying N to the shoots of repeatedly defoliated grasses than remobilization from roots. The hypothesis that increased severity of repeated defoliation reduces the supply of N from both root uptake and remobilization to the shoot was proved to be incorrect, both sources increased or decreased dependant on the species. This species dependency will have implications for grass plants subject to grazing in the field.

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