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Defoliation-induced changes in carbon allocation and root soluble carbon concentration in field-grown *Lolium perenne* plants: do they affect carbon availability, microbes and animal trophic groups in soil?

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Summary

1. It is hypothesized that defoliation-induced changes in plant carbon allocation and root soluble C concentration modify rhizosphere C availability and, further, the abundance and activity of soil microbes and their grazers. To test this hypothesis, field-grown *Lolium perenne* swards were defoliated twice during their second growing season at two nitrogen availabilities (added N or no added N). Plant, soil and microbial attributes were measured 2 and 4 days after the last defoliation, and nematode abundance was measured 6 days after the last defoliation.

2. Defoliation decreased shoot production in plots where N was added, but had no significant effect in plots where N was not added. Root biomass and the ratio of root mass to shoot production were not affected.

3. Defoliation increased root soluble C concentration by 26% at the first harvest (2 days after defoliation) and by 18% at the second harvest (4 days after defoliation). Leaf N concentration was 27% lower in defoliated than in non-defoliated swards at the first harvest, while that of stems was 14% higher in defoliated swards at both harvests, and that of roots was not affected. Defoliation increased root C : N ratio, decreased stem C : N ratio, and did not have a statistically significant effect on leaf C : N ratio.

4. Soil attributes (soil soluble C concentration and soil C and N concentrations) were not affected by defoliation. Similarly, microbial attributes such as microbial C and N content, bacterial abundance in rhizosphere soil, and diversity of C sources utilized by the rhizosphere microbial community, did not differ between defoliated and non-defoliated swards.

5. Among nematode trophic groups, defoliation reduced the abundance of fungivorous and herbivorous nematodes by 70 and 47%, respectively, but did not affect the abundance of bacterivorous, omnivorous and predatory nematodes.

6. Although defoliation altered plant C allocation and root soluble C concentration, these changes did not influence C availability, soil microbial growth or the abundance of bacteria-feeding nematodes in the plant rhizosphere. Instead, the effects on root- and fungus-feeding nematodes suggest that the effects of defoliation on soil communities were propagated not through the effects of root-released C on bacteria and bacterial grazers, but through effects of root quality on root-feeders and possibly through effects of mycorrhizal fungi on fungus-feeders.

Key-words: grassland, nematodes, root-released carbon, soil community, soil microbes

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Introduction

Removal of photosynthetic shoot tissues (defoliation) by grazing animals may have major effects on the structure and functioning of grassland ecosystems. In addition to affecting the composition, shoot production and root turnover of plant communities (McNaughton 1983; McNaughton *et al*. 1998), defoliation alters photosynthetic rates and carbon allocation within plants: the flow of energy in the plant–soil system (Painter & Detling 1981; Richards 1993; Morvan-Bertrand *et al*. 1999). It is well established that, immediately after defoliation, C is remobilized from reserves to sustain the needs of regrowing shoot biomass, whereas thereafter regrowth mainly relies on current assimilates (Welker *et al*. 1985; Briske & Richards 1995; De Visser *et al*. 1997). Although remobilized C reserves in grasses originate mainly from remaining leaf and stem rather than root tissues (Richards & Caldwell 1985; Briske & Richards 1995), root growth (Davidson & Milthorpe 1966; Richards 1993) and, with some delay, nitrogen uptake (Jarvis & Macduff 1989; Macduff *et al*. 1989) may also be depressed after defoliation due to the decreased availability of photoassimilates in roots.

While defoliation-induced changes in plant ecophysiology and C allocation are well documented, there is no consistent picture of how these changes may further affect C availability and the abundance of soil organisms in the plant rhizosphere. For instance, defoliation has been reported to have positive (Holland *et al*. 1996; Paterson & Sim 1999; Murray *et al*. 2004); neutral (Todorovic *et al*. 1999); and negative (Mikola & Kytöviita 2002; Dilkes *et al*. 2004) effects on the amount of C released from roots to the rhizosphere. These differences may have several reasons: for example, different plant species have different strategies of regrowth and C allocation (Guitian & Bardgett 2000); or different fractions of assimilated C are used to represent root-released C (Mikola & Kytöviita 2002). Defoliation has also been reported to affect organisms living in the plant rhizosphere, such as microbes (Guitian & Bardgett 2000; Hamilton & Frank 2001) and their grazers (Hokka *et al*. 2004), but it is not clear how widely these effects can be explained by changes in root C release. In addition to affecting root C exudation, defoliation can also change root quality (Seastedt 1985; Merril *et al*. 1994, Hokka *et al*. 2004) and root mortality (Jarvis & Macduff 1989; Mikola *et al*. 2001), but the relative importance of the different mechanisms is not well known. Nevertheless, it is becoming clear that defoliation-induced changes in grassland soils may have wide-ranging consequences in grassland ecosystems as they can feed back to plant growth, plant N uptake and plant N allocation (Hamilton & Frank 2001; Mikola *et al*. 2005a).

To obtain a comprehensive picture of the effects of defoliation on C transfer from plants to soil, we established a field experiment in which *Lolium perenne* swards were either not defoliated, or defoliated twice during their second growing season. We determined *L. perenne* growth and C allocation; rhizosphere C availability; and the abundance, activity and C content of the soil microbial community in the *L. perenne* rhizosphere 2 and 4 days after the last defoliation. To examine whether the effects of defoliation would propagate further through upper trophic levels of the soil food web, we also measured the abundance of different nematode trophic groups in the *L. perenne* rhizosphere 6 days after the last defoliation. Moreover, as the availability of nutrients, especially that of N, may affect the release of organic material from roots (Hale *et al*. 1971; Grayston *et al*. 1996), we investigated the effects of defoliation in swards of two different N availabilities. We tested the hypothesis that defoliation-induced changes in plant C allocation (Holland *et al*. 1996) and root soluble C concentration (Paterson & Sim 1999) lead to changes in rhizosphere C availability and, further, to changes in the abundance and activity of soil microbes and their grazers.

Materials and methods

EXPERIMENTAL DESIGN

We established three experimental grass swards (each 4×4 m) by sowing *L. perenne* cv. Canasta seeds (25 kg ha^{-1}) into clay-loamy soil at the experimental station of INRA-SAD at Mirecourt, France (6°81′ E, 53°6′ N) in September 2002. In February 2003 the grass swards were trimmed to 5 cm above the soil surface to even out plant shoot mass in different swards before the beginning of the second growing season. Each sward was then divided into two plots (2×4 m), and each plot further split into two subplots (1×4 m). To enhance later sampling of plant roots and rhizosphere soil, four *L. perenne* individuals were randomly selected at each subplot and the soil beneath the plants was isolated from surrounding soil using PVC cylinders (area 50 cm^2, depth 20 cm). The experimental design consisted of two treatments: N addition and defoliation. Nitrogen addition was applied at plot level: one randomly chosen plot at each sward received additional N, while the other received no additional nutrients. Defoliation, in turn, was applied at subplot level: one randomly chosen subplot at each plot was subjected to defoliation, while the other was left intact. The defoliation treatment included two trimmings, on 30 April and 19 May. For measuring total shoot production, trimmed material was collected, lyophilized and weighted. Nitrogen was added immediately after the February and April trimmings: at each sward, one of the main plots received 50 kg N ha^{-1} as NH_4NO_3 solution after both trimmings, while the other plot received water only.

SAMPLING PROCEDURE

The grass swards were sampled three times, 2, 4 and 6 days after the last trimming in May. At first and second samplings, two PVC cylinders, including growing *L.*

perenne individuals, were removed from each subplot for measuring plant, soil and soil microbial attributes. Timing of these samplings was determined based on earlier findings, suggesting that defoliation-induced changes in root C release last for 4–5 days (Paterson & Sim 1999, 2000). At the third sampling, two soil cores (area 9·6 cm^2, depth 10 cm) were taken from each subplot beneath two randomly selected *L. perenne* individuals for examination of the nematode community. This sampling combines the responses of nematodes to both April (25 days before sampling) and May (6 days before sampling) trimming, as while some nematode genera can respond to defoliation within a week, others require 3–4 weeks (Mikola *et al*. 2005b). At each sampling, soil cores and the plants attached to them were kept intact until further analysis in the laboratory.

At first and second samplings, plant shoots were first removed and sorted into leaf and stem mass. Roots were then picked from the soil by hand and the rhizosphere soil (soil adhering to roots) was detached using a brush. After washing roots with 0·5 M $CaCl_2$ isotonic solution, all plant samples were frozen in liquid N and stored at –30 °C. Rhizosphere soil was sieved (2 mm) and one half was frozen in liquid N, stored at –30 °C and later used for analysing soil C and N concentrations, while the other half was used fresh for analysing soluble C and microbial attributes.

PLANT AND SOIL ANALYSES

For measuring plant and soil C and N concentrations, frozen samples were lyophilized, ground to fine powder, and analysed using a C and N auto-analyser (Flash EA 1112 Serie, Thermo Finnigan, Les Ulis, France). Before grinding, lyophilized plant samples were weighted to obtain the dry mass of different plant parts. The concentration of water-soluble carbohydrates in roots (including free sugars, oligosaccharides, polysaccharides and their derivatives) was then measured using the phenol method (Dubois *et al*. 1956). Briefly, 40 mg subsamples of dry roots were extracted with 2 ml 80% ethanol at 80 °C for 15 min. The extract (2 ml), 1 ml 5% phenol and 5 ml of concentrated H_2SO_4 were then mixed and incubated for 20 min at 25 °C. The light absorption of the mixture was measured at 490 nm and the carbohydrate concentration expressed as hexose equivalent (mol kg^{-1} roots).

The concentration of hot-water-extractable C (HWC) in soil, a measure of soluble organic C composed of microbial and other sources of C (Sparling *et al*. 1998), was determined using the method of Haynes & Francis (1993). Subsamples of fresh soil were first dried at 37 °C for 12 h. Of the dried soil, 5 g was mixed with 25 ml distilled water in a vial and left in an 80 °C water bath for 18 h. At the end of the extraction period, the vial was shaken to ensure the HWC released from the soil was fully suspended in water. Suspension was then filtered through Whatman paper no. 42, and total organic C (TOC) was quantified using a TOC analyser.

MICROBIAL ANALYSES

Microbial C and N contents were estimated from fresh soil samples using the chloroform fumigation–extraction method (Vance *et al*. 1987), and the C and N contents of the K_2SO_4 solution were measured using a TOC analyser (TOC-VCSH CSH/CNS, Shimadzu, Champs-sur-Marne, France) connected online to an N analyser (TNM-1, Shimadzu).

To evaluate the rhizosphere availability of simple C sources, which are typically released by roots into the soil, we measured the response of soil microbes to the addition of microquantities of labelled ^{14}C-glucose (Nguyen & Guckert 2001). It has been shown that the proportion of assimilated ^{14}C stored by microbes increases, and the proportion of assimilated ^{14}C respired by microbes decreases, with decreasing soil C availability (Nguyen & Henry 2002). The ratio of respired to stored ^{14}C therefore indicates the availability of C in the soil: the higher the ratio, the higher the C availability. Moreover, respired ^{14}C gives a relative measure of soil microbial activity (Nguyen & Henry 2002). In the measurement, two glass vials were first filled with 4 g fresh soil (one to be fumigated later with chloroform, the other not) and set in a plastic jar containing 25 ml 1 M NaOH to trap evolving $^{14}CO_2$. In each vial, 100 µl ^{14}C-glucose solution (37 kBq ml^{-1}; 0·038 µg C ml^{-1}, a negligible amount of C compared with the total pool of soluble C in the soil) was spread over the soil surface and the plastic jar was closed immediately. After 6 days' incubation in the dark at 22 °C, the respired ^{14}C trapped in NaOH was counted by liquid scintillation (Tri-Carb 2100 Tr, Packard, Perkin-Elmer, Courtaboeuf, France). Soil from one vial was then fumigated with chloroform vapour and the amount of stored ^{14}C calculated as a difference of ^{14}C extracted by 0·5 M K_2SO_4 from the fumigated and the non-fumigated soil.

To measure bacterial abundance and to evaluate the catabolic profile of the microbial community in the rhizosphere soil, a dilution series of microbial suspensions was produced by mixing 5 g fresh soil with 50 ml phosphate buffer (7·2 g NaCl, 2·8 g N_2HPO_4, 0·4 g KH_2PO_4 l^{-1} water, pH 7·2). The mixture was placed on an orbital shaker (40 r.p.m.) for 10 min and centrifuged (750 r.p.m.) for another 10 min, and the supernatant was used to produce a dilution series of 10^{-2}–10^{-5}. Dilutions from 10^{-3} to 10^{-5} were spread onto agar plates (Tryptone Soy Agar, Difco, Becton-Dickinson, Le Pont-de-Claix, France) to estimate the number of culturable bacterial cells, measured as colony-forming units (CFU). Two replicates were prepared for each dilution, the agar plates were incubated in the dark at 27 °C, and the number of CFU was recorded after 2 days' incubation.

The catabolic profile of the microbial community was characterized using Biolog GN microplates with 95 wells of different C sources (Biolog Inc., Hayward, CA, USA). Each microplate well was inoculated with 150 µl 10^{-3} dilution, plates were incubated in the dark at 27 °C, and colour development in wells

was recorded automatically after 1, 2, 6, 24, 48 and 72 h incubation at 550 nm using a microplate reader (MR 7000 Dynatech, Issy Les Moulineaux, France). Absorbance values for the wells with C sources were blanked against the control well, and all negative absorbance values were set to zero. In order to measure the diversity of C sources utilized, the area under the colour development curve was calculated for each C source. The diversity of utilized C sources was then expressed as Shannon's diversity index:

$$H = -\sum_{i=1}^{95} P_i \log_{10} P_i,$$

where P_i is the proportion of the area under the colour development curve in well i to the sum of areas in all 95 wells (Preston-Mafham *et al*. 2002).

NEMATODE COMMUNITY ANALYSIS

To evaluate the response of soil microfauna to the treatments, nematodes were extracted from 30 g (fresh mass) rhizosphere soil using a wet funnel device (Sohlenius 1979). The total number of live individuals was counted; later, using preserved samples, 150 nematodes per sample were identified to genus and allocated into trophic groups according to Yeates *et al*., 1993).

STATISTICAL ANALYSES

The mean of the two samples from each subplot was calculated for each variable. The effects of defoliation and N addition on the variables measured were then analysed with the SPSS statistical package (SPSS 2001) using a nested ANOVA modified to engage the two harvests made for plant, soil, and soil microbial attributes. In this mixed-model ANOVA, (1) N addition, defoliation, harvest time and their second- and third-order interactions were treated as fixed effects; (2) the plot effect, nested within N addition, and its interactions with the fixed effects, were treated as random effects; and (3) random effects were used as error terms for the tests of fixed effects. The nematode data with only one harvest were analysed using nested ANOVA. Homogeneity of variances was tested using Levene's test, and to satisfy the assumption of homogeneity of variances a logarithmic transformation was applied to dependent variables whenever necessary. When the mixed-model ANOVA resulted in a significant interaction between harvest time and defoliation, or between harvest time and N addition, the effects of defoliation and N addition were tested separately within each harvest using a nested ANOVA. When a significant interaction was found between N addition and defoliation, the factors were fixed one by one, and effects of the other factor were analysed within the levels of the fixed factor using a mixed-model ANOVA.

Results

PLANT GROWTH

The effects of N addition and defoliation on shoot production were dependent on each other (Table 1; Fig. 1a). Nitrogen addition promoted 3·3-fold plant shoot production in non-defoliated plots ($F = 479$, $P < 0{\cdot}001$),

Table 1. *F* statistics from mixed-model ANOVA for the effects of defoliation (no trimming, or trimming twice during second growing season); nitrogen addition (no added N, or N added after trimmings); and harvest time (2 or 4 days after last trimming) on plant attributes in field-grown *Lolium perenne* swards

	Source of variation						
Dependent variable	Defoliation	N addition	Harvest time	D × N	D × H	N × H	D × N × H
Plant growth							
Shoot production	0·05	35·9**	0·09	16·5*	0·14	0·36	<0·01
Root mass	5·24	0·83	3·52	5·06	0·11	1·84	0·16
Ratio root mass : shoot production	2·88	24·4**	0·83	2·64	0·26	1·31	0·64
Plant C concentration							
Leaf C concentration	58·0**	1·32	0·48	0·05	2·77	0·34	0·02
Stem C concentration	9·97*	8·05*	0·16	0·09	7·07	0·02	0·01
Root C concentration	2·50	9·75*	8·50*	<0·01	0·68	0·80	3·66
Root soluble C concentration	31·5**	0·07	1·83	2·66	10·3*	0·55	0·59
Plant N concentration							
Leaf N concentration	9·27*	336***	11·1*	0·05	9·92*	1·19	0·12
Stem N concentration	16·3*	204***	0·78	2·65	0·40	0·31	0·69
Root N concentration	3·25	13·5*	7·81*	0·12	0·06	1·92	0·48
Plant C : N ratio							
Leaf C : N ratio	0·37	152***	17·8*	0·01	10·3*	0·02	2·22
Stem C : N ratio	105***	204***	0·95	4·09	0·62	0·02	0·10
Root C : N ratio	21·2**	10·7*	3·55	<0·01	0·03	1·25	1·14

Degrees of freedom: 1,4 for each source of variation.
*, **, ***, Treatment effect significant at $P < 0{\cdot}05$, 0·01 and 0·001, respectively.

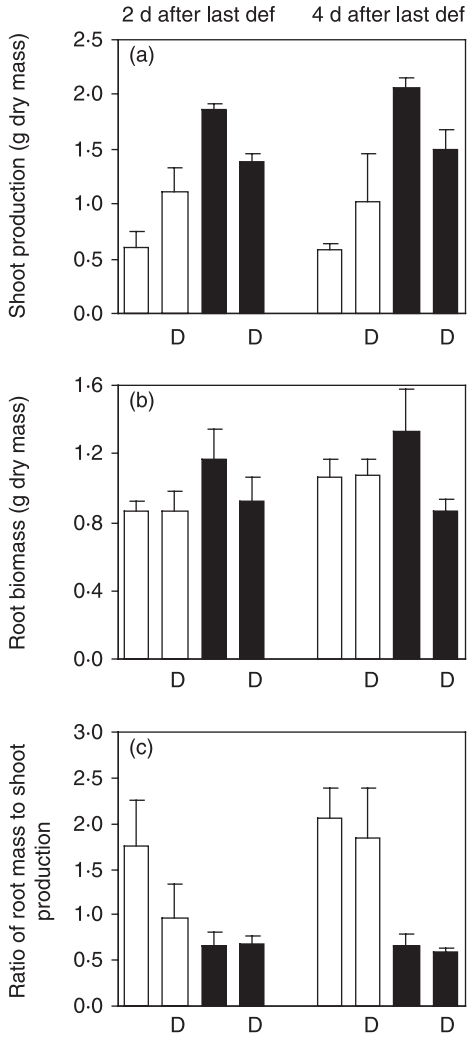

Fig. 1. Plant growth (mean + 1 SE, $n = 3$) in field-grown *Lolium perenne* swards in response to defoliation (def) and nitrogen addition 2 and 4 days after the last defoliation: (a) shoot production (defoliated and harvested shoot mass); (b) root biomass; (c) ratio of root biomass to shoot production. Black and white bars represent swards with and without added N, respectively; D, defoliated swards.

but had no effect in defoliated plots ($F = 2{\cdot}07$, $P = 0{\cdot}223$). Defoliation, in turn, decreased shoot production by 27% in plots where N was added ($F = 41{\cdot}8$, $P = 0{\cdot}023$), but had no statistically significant effect in plots where N was not added ($F = 4{\cdot}17$, $P = 0{\cdot}178$) (Fig. 1a). In contrast to shoot production, N addition and defoliation did not affect harvested root biomass (Table 1, Fig. 1b). The ratio of root mass to shoot production was reduced by N addition on average by 61%, but was not affected by defoliation (Table 1; Fig. 1c).

PLANT CARBON AND NITROGEN CONCENTRATIONS

Defoliation reduced leaf and stem C concentrations on average by 12 and 7%, respectively, but did not affect root C concentration, whereas N addition increased root and stem C concentrations on average by 7 and 9%, respectively, but did not affect leaf C concentration (Table 1; Fig. 2a–c). Nitrogen addition had a clear positive effect on plant N concentrations, with 111, 101 and 38% higher leaf, stem and root N concentrations, respectively, found in plots with N addition (Table 1; Fig. 2d–f). Defoliation reduced leaf N concentration by 27% at the first harvest (2 days after defoliation) ($F = 13{\cdot}2$, $P = 0{\cdot}022$), but had no effect at the second harvest (4 days after defoliation) ($F = 0{\cdot}35$, $P = 0{\cdot}585$), increased stem N concentration at both harvests on average by 14%, and did not affect root N concentration (Table 1; Fig. 2d–f). The C : N ratios of different plant parts were significantly decreased by N addition, whereas defoliation increased the ratio in roots, decreased the ratio in stems, and had no statistically significant effect on the ratio in leaves (Table 1; Fig. 2g–i).

Defoliation increased root carbohydrate concentration by 26% at the first harvest ($F = 61{\cdot}5$, $P = 0{\cdot}001$) and by 18% at the second harvest ($F = 13{\cdot}8$, $P = 0{\cdot}021$), while N addition had no effect (Fig. 3).

SOIL CARBON AND NITROGEN CONCENTRATIONS

Soil soluble C concentration (Fig. 4a) and total soil C and N concentrations (data not shown) were not affected by defoliation or N addition (Table 2).

SOIL MICROBES

When we analysed in detail the three-way interaction between the effects of N addition, defoliation and harvest time on microbial C content (Fig. 4b; Table 2), a significant interaction between defoliation and N addition was found at the first harvest ($F = 11{\cdot}2$, $P = 0{\cdot}044$), but none of the treatments had statistically significant main effects. Microbial N content was not affected by defoliation or N addition (Table 2).

In the case of short-term utilization of ^{14}C-labelled glucose by soil microbes, the proportion of added ^{14}C respired by microbes was decreased by N addition at first harvest ($F = 15{\cdot}06$, $P = 0{\cdot}018$), but no other effects of defoliation or N addition on the amount of respired ^{14}C, stored ^{14}C or their ratio were observed (Fig. 4c–e; Table 2). The number of culturable cells of bacteria in rhizosphere soil (Fig. 4f) and the diversity of C sources utilized by the rhizosphere microbial community (data not shown) were not affected by defoliation or N addition (Table 2).

NEMATODES

Defoliation decreased the abundance of fungivorous and herbivorous nematodes on average by 70 and 47%, respectively, but did not have a statistically significant effect on the abundance of bacterivorous, omnivorous and predatory nematodes (Table 3; Fig. 5). None of the nematode trophic groups was affected by N addition (Table 3; Fig. 5).

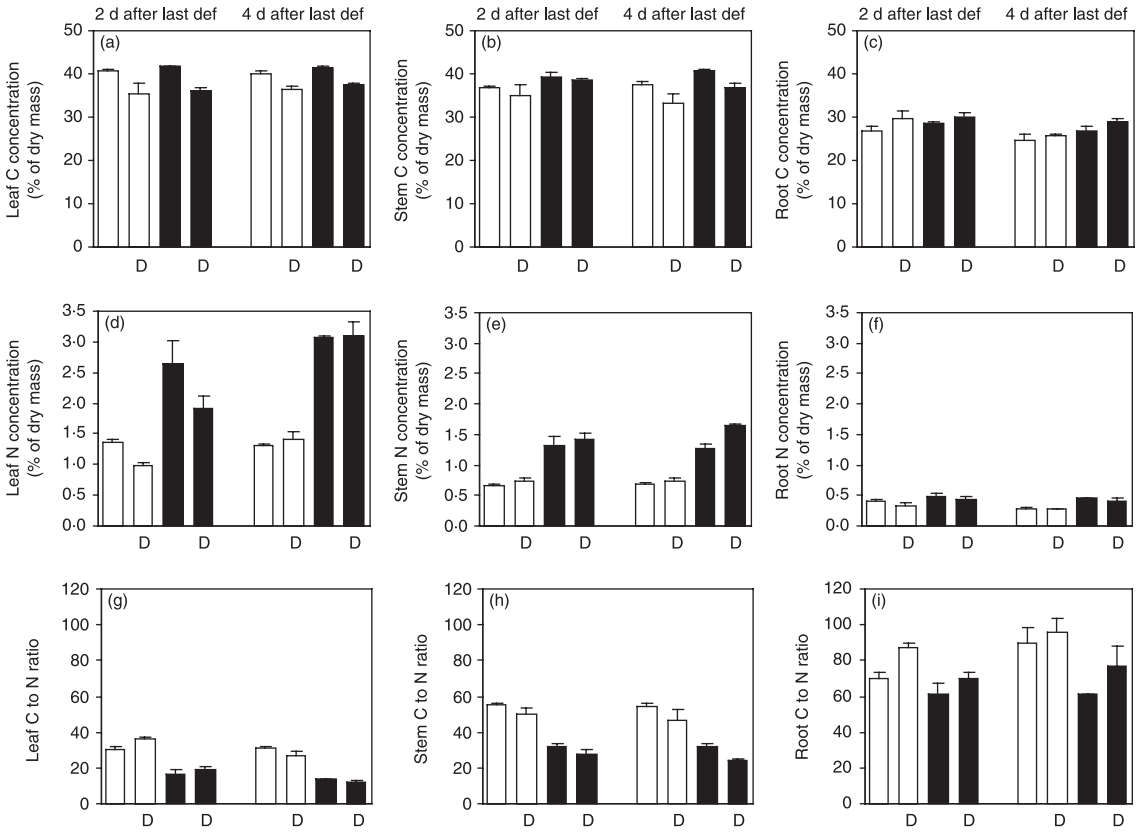

Fig. 2. Plant carbon and nitrogen concentrations (mean + 1 SE, $n = 3$) in field-grown *Lolium perenne* swards in response to defoliation (def) and N addition 2 and 4 days after the last defoliation: (a–c) leaf, stem and root C concentration; (d–f) leaf, stem and root N concentration; (g–i) leaf, stem and root C : N ratio. Treatment symbols as for Fig. 1.

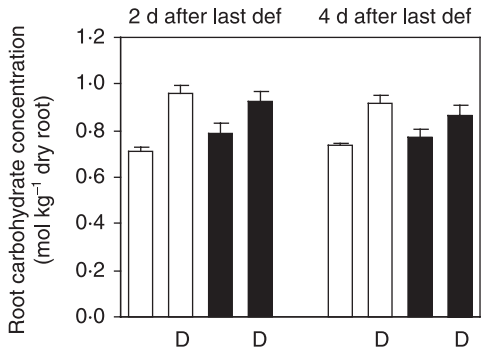

Fig. 3. Root soluble carbon concentration (mean + 1 SE, $n = 3$) in field-grown *Lolium perenne* swards in response to defoliation (def) and nitrogen addition 2 and 4 days after the last defoliation. Treatment symbols as for Fig. 1.

Discussion

Our results show that defoliation altered plant C allocation and root soluble C concentrations in *L. perenne* swards. However, against our hypothesis, these changes did not influence C availability, soil microbial growth or the abundance of bacteria-feeding nematodes in the plant rhizosphere. Instead, we found negative effects of defoliation on root-feeding and fungus-feeding nematodes. This suggests that the effects of defoliation were propagated not through the effects of root C release on bacteria and bacterial grazers, but through effects of root quality on root-feeders and possibly through effects of mycorrhizal fungi on fungus-feeders.

Although defoliation had no effect on biomass allocation in the *L. perenne* swards (the ratio of root biomass to shoot production did not differ between defoliated and non-defoliated plants), C allocation was altered. Defoliation reduced leaf and stem C concentration as well as stem C : N ratio, but increased root C : N ratio and root carbohydrate concentration, which suggests that defoliated plants allocated relatively more C to roots and had more water-soluble C in their roots than non-defoliated plants. This does not support the common view that plants tolerate defoliation by mobilizing C into leaf meristems at the expense of roots after defoliation (Caldwell *et al*. 1981; Miller & Rose 1992; Strauss & Agrawal 1999), but is consistent with the hypothesis that plants increase C allocation to roots after defoliation to create C stores less accessible for above-ground grazers (Dyer *et al*. 1991; Holland *et al*. 1996). It has been suggested that increased allocation of photosynthate to roots after defoliation (Holland *et al*. 1996) and amplified root concentrations of soluble C in defoliated plants (Paterson & Sim 1999) can lead to increased C exudation from roots. Our results do not support these hypotheses: although concentrations of water-soluble C in *L. perenne* roots were increased by defoliation, and relatively more C appeared to be

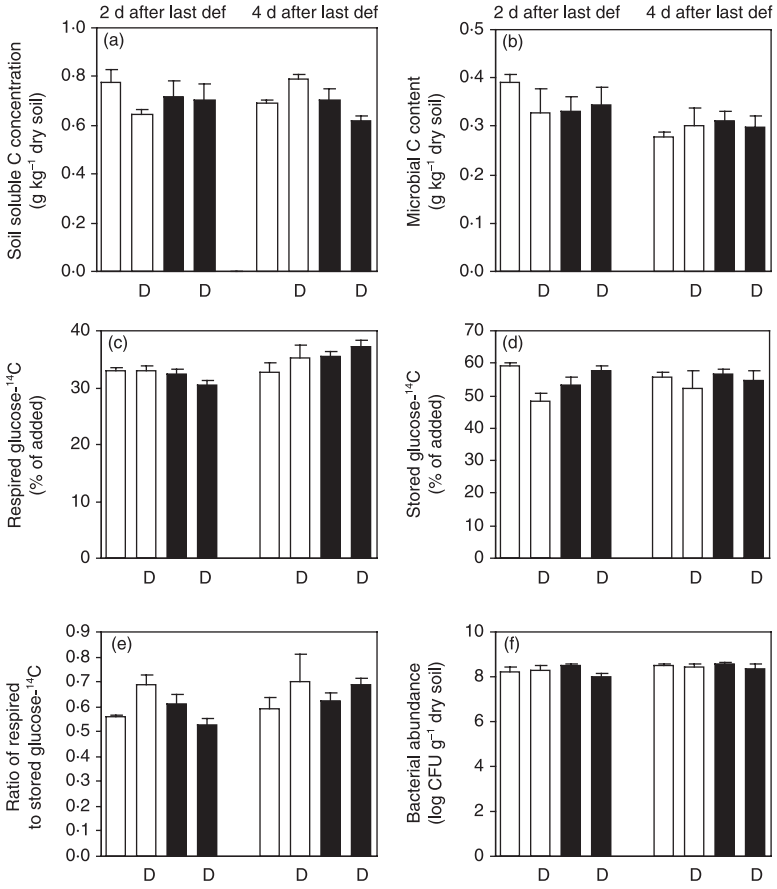

Fig. 4. Soluble carbon concentration and microbial attributes of rhizosphere soil (mean + 1 SE, $n = 3$) in field-grown *Lolium perenne* swards in response to defoliation (def) and nitrogen addition 2 and 4 days after the last defoliation: (a) soil soluble C concentration; (b) microbial C content; (c,d) respired and stored proportion of added glucose-^{14}C; (e) ratio of respired to stored glucose-^{14}C; (f) bacterial abundance, measured as colony-forming units on agar plates. Treatment symbols as for Fig. 1.

allocated below ground in defoliated than in non-defoliated plants, none of the soil, microbial or animal attributes indicated increased C availability in the rhizosphere soil. For instance, defoliation did not affect soluble C concentration or microbial utilization of ^{14}C-labelled glucose in the rhizosphere soil, which suggests that the availability of labile C compounds in the rhizosphere was not affected by defoliation. Similarly, Murray *et al*. (2004) recently reported no effect of defoliation on overall root C exudation in field-grown *Agrostis capillaris* plants. In other studies with graminoids, root C release has been found to increase (Holland *et al*. 1996; Paterson & Sim 1999, 2000; Paterson *et al*. 2003) or decrease (Mikola & Kytöviita 2002; Nguyen & Henry 2002; Dilkes *et al*. 2004; Macdonald *et al*. 2004) after defoliation, suggesting that the influence of defoliation on root C release may be difficult to predict. However, studies differ in terms of the plant species used, type of defoliation and fraction of assimilated C measured in the rhizosphere (Mikola & Kytöviita 2002), and more trials may be needed before predictable patterns emerge. What is noticeable in our study is that we did not find a link between the amount of C available in roots and the amount of C released from roots, which has been recorded in some earlier studies (Mikola & Kytöviita 2002; Dilkes *et al*. 2004; Macdonald *et al*. 2004). A similar discrepancy was reported by Paterson *et al*. (2003), who found that defoliation decreased soluble C concentrations in *Festuca rubra* roots but increased root C exudation.

In accordance with the measurements of rhizosphere C availability, we found no indication of effects of defoliation on soil microbes. Microbial activity –

Table 2. *F* statistics from mixed-model ANOVA for the effects of defoliation (no trimming, or trimming twice during second growing season); nitrogen addition (no added N, or N added after trimmings); and harvest time (2 or 4 days after last trimming) on soil and soil microbial attributes in the rhizosphere of field-grown *Lolium perenne* swards

	Source of variation						
Dependent variable	Defoliation	N addition	Harvest time	D × N	D × H	N × H	D × N × H
Soil C and N content							
Soil C concentration	0·65	0·10	12·5*	7·01	0·58	1·91	0·02
Soil soluble C concentration	0·50	0·92	0·01	0·02	1·08	2·42	3·98
Soil N concentration	<0·01	2·38	6·02	1·92	1·48	0·01	0·86
Microbial C and N content							
Microbial C content	0·14	0·01	7·80*	0·14	1·76	0·62	14·3*
Microbial N content	2·64	0·02	18·9**	4·51	1·02	11·9*	1·87
Microbial utilization of added ^{14}C glucose							
Proportion of respired ^{14}C	0·26	0·76	23·9**	0·35	2·51	1·06*	0·13
Proportion of stored ^{14}C	3·33	1·16	0·02	6·00	<0·01	<0·01	1·65
Ratio of respired to stored ^{14}C	2·01	1·36	4·93	2·87	0·46	1·45	0·76
Bacterial abundance							
Number of colony-forming units	2·14	<0·01	7·65	2·31	0·06	<0·01	0·64
Microbial community catabolic profile							
Diversity of utilized C compounds	0·01	0·22	11·4*	0·62	0·04	0·27	1·62

Degrees of freedom: 1,4 for each source of variation.
Treatment effect significant at $*P < 0·05$, $**P < 0·01$ and $***P < 0·001$.

Table 3. *F* statistics from nested ANOVA for the effects of defoliation (no trimming, or trimming twice during second growing season) and nitrogen addition (no added N, or N added after trimmings) on abundance of nematode trophic groups in rhizosphere soil of field-grown *Lolium perenne* swards 6 days after last trimming

	Source of variation		
Dependent variable	Defoliation	N addition	D × N
Bacterivore abundance	0·02	2·74	1·45
Fungivore abundance	11·1*	4·01	0·65
Herbivore abundance	10·5*	0·08	3·22
Omnivore abundance	6·28	0·09	2·12
Predator abundance	1·00	1·18	5·67

Degrees of freedom: 1,4 for each source of variation.
*, **, ***, Treatment effect significant at $P < 0{\cdot}05$, 0·01 and 0·001, respectively.

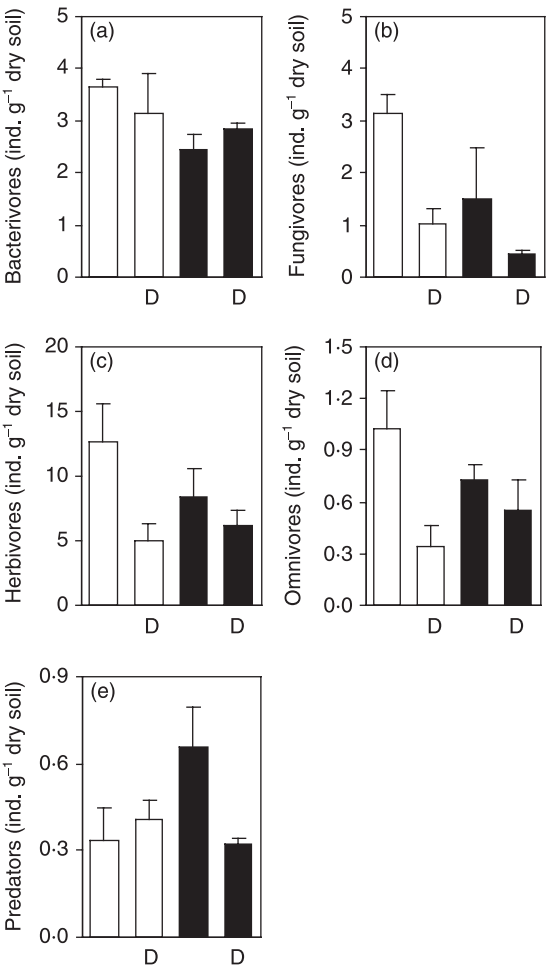

Fig. 5. Nematode abundance in rhizosphere soil (mean + 1 SE, $n = 3$) in field-grown *Lolium perenne* swards in response to defoliation and nitrogen addition 6 days after the last defoliation: (a) bacterivores; (b) fungivores; (c) herbivores; (d) omnivores; (e) predators. Treatment symbols as for Fig. 1.

measured as the proportion of added ^{14}C-labelled glucose respired by microbes; microbial C content; abundance of bacteria in the rhizosphere soil; and diversity of C sources utilized by the microbial community – was not affected by defoliation. Moreover, the unaffected abundance of bacteria-feeding nematodes in defoliated *L. perenne* swards further suggests that bacterial growth was not affected by defoliation. Microbe-feeding nematodes are good indicators of microbial growth in soil (Sohlenius 1990; Christensen *et al*. 1992; Mikola & Setälä 1998), and their response to defoliation has been shown to be most clear a few weeks after defoliation (Mikola *et al*. 2005b). In the present study, we sampled nematodes 6 days after the May trimming and 25 days after the April trimming, suggesting that we should have observed the response of bacteria-feeding nematodes had it occurred. Like the effects of defoliation on root C release, effects on soil microbes (Garcia & Rice 1994; Guitian & Bardgett 2000; Hamilton & Frank 2001) and microbe-feeding animals (Stanton 1983; Todd *et al*. 1992; Todd 1996; Mikola *et al*. 2001) differ from positive to negative in earlier studies. Our finding that bacterial growth and abundance of bacterial grazers do not respond to defoliation when such a response is not observed in root C release suggests that part of this discrepancy may be accounted for by differences in root C release after defoliation.

In grasses, defoliation typically increases N concentration in shoot and root biomass (Wilsey *et al*. 1997; Fahnestock & Detling 1999; Green & Detling 2000; Hokka *et al*. 2004), which has been suggested to result from improved N availability in soil after defoliation (Holland & Detling 1990). Supporting this idea, Hamilton & Frank (2001) recently showed how increased C release from *Poa pratensis* roots after defoliation stimulated soil microbial growth, increased N availability in the plant rhizosphere, and finally led to elevated shoot N concentration in defoliated plants. In our study, defoliation reduced leaf N concentration 2 days after defoliation by 27%, did not affect root N concentration, and increased stem N concentration by 14%. In comparison with 129 and 56% increases in shoot and root N concentrations, respectively, recorded by Hokka *et al*. (2004) in defoliated *Phleum pratense* plants, defoliation appeared to have little impact on plant N uptake in our study. This is in good agreement with our finding that defoliation did not stimulate root C release and the growth of rhizosphere organisms in our grass swards, and suggests that rhizosphere N availability was not enhanced by defoliation in our study.

In contrast to the response of bacteria-feeding nematodes to defoliation, we found that defoliation reduced the abundance of root- and fungus-feeding nematodes. However root biomass was not significantly reduced by defoliation, suggesting that root mass did not determine the abundance of root-feeding nematodes. Instead, the lower quality of roots (higher C : N ratio) appears to account for the decreased abundance of root-feeding nematodes found in the rhizosphere of defoliated plants, suggesting that changes in root quality after defoliation can substantially affect the

abundance of root-feeding animals. Defoliation typically enhances root quality in terms of increasing root N concentration and decreasing root C : N ratio (Seastedt 1985; Seastedt *et al*. 1988; Hokka *et al*. 2004), but the effects may vary from positive to negative depending on the growth phase of defoliated plants (Ilmarinen *et al*. 2005), and our results show, in contrast to earlier observations, that defoliation can also increase the C : N ratio of grass roots. That fungus-feeding nematodes had a similar negative response to defoliation appears unexpected considering we did not find any indication of reduced microbial activity after defoliation. However, we did not separately measure the response of fungi to defoliation, and in bacteria-dominated agricultural soil their response may have gone unnoticed. Also, we did not measure whether defoliation affected the abundance of arbuscular–mycorrhizal (AM) fungi in soil. In earlier studies, negative effects of defoliation on the root colonization rate of AM fungi are most common (reviewed by Gehring & Whitham 1994), although neutral (Ilmarinen *et al*. 2005; Mikola *et al*. 2005a) and positive (Hokka *et al*. 2004) effects in grass roots have also been found. Finally, the classification of root-feeding and fungus-feeding nematodes is problematic, and it is possible that some genera classified as fungus-feeders in this study also feed on roots, and *vice versa* (Yeates *et al*. 1993; Okada *et al*. 2005). On the other hand, earlier studies in which the same classification of nematode genera (Yeates *et al*. 1993) is applied have reported different responses of root-feeding and fungus-feeding nematodes to plant ageing and defoliation (Mikola *et al*. 2001; Ilmarinen *et al*. 2005; Mikola *et al*. 2005b). Nevertheless, our results show clearly that, even though no effects of defoliation on bacteria and their grazers are found, effects on root- and fungus-feeders may still appear. Whether these effects also have significant consequences for further plant growth and nutrient uptake remains to be clarified.

We anticipated that soil N availability affects the response of plants and soil organisms to defoliation, but found evidence of this only in the case of plant shoot production. Defoliation reduced shoot production in plots where N was added, but did not affect plant growth in non-fertilized plots. Defoliation is known to reduce root nutrient uptake (Jarvis & Macduff 1989; Donaghy & Fulkerson 1998) and, as N was added immediately after trimming, the reduced growth of defoliated plants in fertilized plots may result from their reduced capability to utilize the additional N. This is supported by our finding that N addition did not significantly increase shoot production when plants were defoliated, but had a substantial positive effect on the shoot production of non-defoliated plants. That N addition had little direct influence on soil microbes was expected, as microbial growth in soils is usually constrained by C rather than N availability (Schnürer *et al*. 1985; Zak *et al*. 1994). However, nor did N addition appear to have much indirect influence on soil microbes through altered C availability, despite having positive effects on plant C assimilation (manifested by increased shoot production in non-defoliated plots) and root C concentration. The response of microbes to N addition neither supports results from studies with woody plants which have shown that, due to increased fine root turnover, C input to soil increases when plants are fertilized (King *et al*. 2002).

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