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# Plant species and nitrogen effects on soil biological properties of temperate upland grasslands

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**Summary**

**1.** The aim was to assess the extent to which the microbial biomass and activity, and community structure of fertilized upland grasslands are directly related to changes in soil N availability or indirectly related to individual plant species effects caused by changes in plant species composition and dominance. We investigated the short-term interactive effects of dominant plant species (*Lolium perenne, Agrostis capillaris, Holcus lanatus* and *Festuca rubra*) and nitrogen (N) amendment using an N-limited upland grassland soil.

**2.** In soils planted with different grass species, soil microbial biomass, and to some extent microbial activity, were determined by temporal changes in plant productivity. Variations in the way that individual plants influenced soil microbial biomass and activity were highly inconsistent over time, and largely independent of N-additions and differences in plant productivity. At the final sample date, those grass species which co-dominate the total plant biomass of intermediate fertility (*H. lanatus*) and semi-improved grasslands (*A. capillaris* and *F. rubra*) had a beneficial effect on the soil microbial biomass. In contrast, the dominant plant species of improved grasslands, *L. perenne*, had zero or a negative effect on soil microbial biomass. Two plant species (*A. capillaris* and *H. lanatus*) increased the proportion of fungi relative to bacteria in the soil microbial community, relative to the unplanted control soil and the other plant species. *Lolium perenne* and *A. capillaris* reduced the evenness of microbial PLFAs, suggesting negative effects of these plant species on the diversity of the soil microbial community.

**3.** The addition of N had no consistent effect on measures of soil microbial biomass or activity, but significantly altered the structure of the microbial community in favour of fungi. The lack of effects of N-addition on microbial biomass and activity were despite the finding that nitrogen addition reduced root biomass in all plant species and increased rhizosphere acidity.

**4.** The results suggest that in the short term, the abundance and activity of soil micro-organisms in upland grasslands are regulated more by plant species traits than by a direct effect of nitrogen. These effects are likely to be related to variations amongst plant species in root exudation patterns and/or efficiency of nutrient aquisition.

**5.** Our study provides evidence that the functional characteristics of dominant plant species are important determinants of soil biological properties, and hence ecosystem functioning in temperate upland grasslands.

*Key-words:* Grasslands, microcosm, nitrogen, soil, soil microbial biomass, upland

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## Introduction

There is presently much interest in understanding the factors which regulate community organization and function in terrestrial ecosystems. Central to this goal



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is the study of relationships between environment and community organization, and in particular the patterns of performance of communities along environmental gradients (Austin 1990). There has been much recent interest in understanding the ways in which below-ground microbial communities and their functions change along environmental gradients, in particular in agricultural systems where the manipulation of soil organisms to promote reliance on soil biological processes of nutrient turnover is central to sustainable production (Altieri 1991; Yeates *et al.* 1997). Such an objective is of particular relevance in managed grassland systems which owing to a high turnover of shoot and root material, and consequent large pool of organic carbon at the soil surface, support a uniquely large and active soil microbial community (Bardgett & Cook 1998).

A common finding in studies of temperate grasslands, along soil fertility gradients, is that the size and activity of the soil microbial community is higher under low-fertility conditions than under high-fertility conditions maintained by regular nitrogen (N) additions (Lovell, Jarvis & Bardgett 1995; Bardgett *et al.* 1997; Yeates *et al.* 1997). Broad-scale shifts in the soil community are often associated with such biomass changes with high soil fertility, or N fertilization, favouring bacterial pathways of decomposition and low soil fertility promoting fungal pathways of decomposition. (Bardgett, Frankland & Whittaker 1993; Bardgett, Hobbs & Frostegård 1996; Yeates *et al.* 1997; Bardgett & McAlister 1999). Various mechanisms have been put forward to explain these changes in soil microbial communities of managed grasslands. For example, it has been suggested that fertilizer N application has a direct inhibitory effect on the soil community, in particular the decomposer (Arnebrant, Bååth & Soderstrom 1990) and mycorrhizal fungi (Sparling & Tinker 1978; Hamel *et al.* 1994), through the repression of enzyme activity and the buildup of recalcitrant and toxic compounds (Fog 1988). However, other studies hypothesize that the effects of fertilizers on the soil microbial community are largely indirect, through a change in the species composition of the vascular plant community (Bardgett & McAlister 1999; Bardgett & Shine 1999). In semi-natural upland grasslands, one such change indirectly linked to fertilization is a shift in composition from a sward co-dominated by indigenous plant species, such as *Agrostis capillaris* L. and *Festuca rubra* L., towards one which is dominated by the more productive species *Lolium perenne* L. (Bradshaw *et al.* 1964; Rodwell 1992). Such changes in plant composition and species dominance are likely to exert strong selective pressures on the soil microbial community through plant-specific differences in the release of root exudates into the rhizoshere (Vancura 1964; Klein *et al.* 1988), which represent an high-quality nutrient source for micro-organisms (Whipps & Lynch 1983; van Veen *et al.* 1989), and through alterations in nutrient competition between plants and rhizosphere micro-organisms. Changes in plant productivity, in particular root biomass which often declines in long-term N-fertilized grasslands (Ennik, Gillet & Sibma 1980), are also likely to influence strongly the soil microbial community by altering root exudation patterns and the supply of root C to soil.

Evidence is accumulating to support the notion that the indirect effects of fertilization, namely a change in the species composition and dominance of the plant community, may be an important determinant of soil biological properties of grassland soils. For example, recent studies in the field (Wardle *et al.* 1999) and laboratory (Wardle & Nicholson 1996; Grayston *et al.* 1998; Wardle *et al.* 1998; Bardgett & Shine 1999) have shown that individual grassland plant species can greatly influence the size and activity of the microbial biomass, and that these effects are often closely related to various plant ecophysiological traits (Wardle *et al.* 1998). Such findings are supportive of the emerging view in ecology that individual plant species effects are important determinants of ecosystem properties of organic matter decomposition and nutrient recycling (Hooper & Vitousek 1997; Wardle *et al.* 1997; Grime 1998).

To date, no-one has attempted to determine the relative role of changes in soil nutrient availability and plant species composition in the regulation of soil microbial communities and their function in temperate grasslands soils. The aim of this study, therefore, was to determine the extent to which previously observed differences in soil biological properties (microbial biomass and activity, and community structure) in grasslands of contrasting fertility, may be directly related to the addition of fertilizer N, or indirectly related to individual plant species effects caused by changes in plant species composition and dominance. Specifically, we tested the hypothesis that soil biological properties of grasslands are regulated more by indirect effects of fertilization, i.e. changes in individual traits of dominant plant species, than by direct effects of nitrogen amendments on soil nutrient availability. To examine these questions we conducted a soil microcosm experiment using a range of grass species which dominate the total plant biomass of temperate upland grasslands of contrasting fertility, which were grown with or without added N. The ultimate aim of the experiment was to assess the importance of dominant plant species in determining soil biological properties in grasslands.

## Materials and methods

### SOIL AND MICROCOSMS

The soil used in this study was collected from the surface 15 cm (Ah horizon) of a semi-natural *Agrostis–Festuca* grassland (National Vegetation Classification [NVC] U4a; Rodwell 1992) at the University of Bangor Experimental Farm, Aber-

gwyngregyn, North Wales (National Grid Reference SH 656726). The soil had an average pH of 4·7 ($H_2O$ 1:2·5 w:v), an organic carbon content of 0·15 g $g^{-1}$ dry mass, and a bulk density of 0·755 g $cm^{-3}$. The soil represents that of a typical 'semi-improved' sheep-grazed grassland of 'sheep-walk' of western upland United Kingdom and received no NPK inputs or lime. The soil was sampled in September 1995, brought back to the laboratory, sieved (< 6 mm) and kept at 4 °C until used.

Soil microcosms were prepared by weighing 100 g dry mass equivalent soil (30% moisture content) into foil covered polyvinyl-chloride specimen containers (5 cm i.d. × 10 cm) with pierced bottoms to allow the free drainage of water. Microcosms were planted with three seedlings of either *L. perenne*, *A. capillaris*, *F. rubra* or *Holcus lanatus* L., or left unplanted, and incubated in a randomized block design in a constant environment growth chamber (temperature 18 °C, light dark cycle 16/8 h) for 40 days until the plants had become well established. The grasses examined were selected as being representative and dominant species of a successional sequence of grassland communities which commonly occurs in upland regions of the United Kingdom. *Lolium perenne* is a dominant species of improved grassland (*L. perenne–Cynosurus crisatus*: NVC MG6; Rodwell 1992) receiving regular inputs of fertilizer nitrogen and lime, *H. lanatus* is commonly a dominant species of grasslands of intermediate fertility (NVC U4b), whereas *A. capillaris* and *F. rubra* are co-dominants of semi-natural upland grasslands (*Agrostis–Festuca–Galium*: NVC U4b and U4a) receiving no inputs of fertilizer nitrogen.

Soil was maintained at 30% moisture content (60% field capacity) by the daily addition of distilled water. At day 0, ammonium nitrate ($NH_4NO_3$) at a rate equivalent to 100 kg $ha^{-1}$ was applied to half the microcosms, the remainder receiving distilled water. 24 h after N-application, and subsequently on days 15, 56, 78 and 107, six replicate microcosms per treatment were destructively sampled by carefully removing the soil and plants from the plastic chamber and manually separating the soil from the plants. Soils were subsequently passed through a 6 mm sieve and measurements of soil pH, organic matter content, microbial respiration, acid phosphatase activity and β-glucosidase activity were made. In addition on days 1, 78 and 107 measurements were made of the phospholipid fatty-acid profiles of the different soils to determine microbial biomass and community structure. Plant roots were separated from shoots, and the roots were washed with distilled water. Fresh masses of both roots and shoots were measured before samples were dried for 24 h at 70 °C and dry masses determined.

### MICROBIAL ACTIVITY

Microbial activity was measured as basal respiration. Briefly, samples of soil (1 g dry-mass equivalents) were incubated for 24 h at 30 °C in universal bottles sealed with a No. 37 Subaseal cap. The concentration of $CO_2$ in a 1 ml sample of headspace gas was subsequently measured using an infra-red gas analyser (Analytical Development Co., Hoddesdon, UK, Series 225, Mk. 3). Microbial activities were also assessed by analysis of enzyme activities. β-glucosidase activity was measured using the method of Eivazi & Tabatabai (1988) except that analyses were carried out on 0·5 g soil and the concentration of p-nitrophenyl-β-D-glucopyranoside was increased to 50 mM. Acid phosphatase activity was determined based on the methods of Tabatabai & Bremner (1969) and Eivazi & Tabatabai (1977).

### MICROBIAL COMMUNITY STRUCTURE–PHOSPHOLIPID FATTY-ACID ANALYSIS

Changes in rhizosphere microbial community structure were assessed by analysing the ester-linked phospholipid fatty-acid (PLFA) composition of the soil, because certain groups of micro-organisms have different 'signature' fatty acids (Tunlid & White 1992). Specifically, the technique was used to measure the relative abundance of active fungi and bacteria (Bardgett *et al.* 1996), which constitute some 90–95% of total heterotrophic metabolism in most soils (Petersen & Luxton 1982). Lipids were extracted from soil, fractionated and quantified using the procedure described by Bardgett *et al.* (1996), which is based on the method of Bligh & Dyer (1959) as modified by White *et al.* (1979). Separated fatty-acid methyl-esters were identified by chromatographic retention time and mass spectral comparison using standard qualitative bacterial-acid methyl-ester mix (Supelco) that ranged from C11 to C20. For each sample, the abundance of individual fatty-acid methyl-esters was expressed on a dry-mass basis per unit area ($m^{-2}$).

Fatty-acid nomenclature was used as described by Frostegård, Bååth & Tunlid (1993). The fatty acids i15:0, a15:0, 15:0, i16:0, 17:0, i17:0, cy17:0, cis18:1ω7 and cy19:0 were chosen to represent bacterial PLFAs (bactPLFAs) (Federle 1986; Tunlid *et al.* 1989; Frostegård *et al.* 1993) and 18:2ω6 and 10Me:18:0 were used as an indicators of fungal biomass and actinomycete (Federle 1986). The ratio of 18:2ω6:bactPLFAs was taken to represent the ratio of fungal:bacterial biomass in soil (Bardgett *et al.* 1996; Frostegård & Bååth 1996). To assess the degree of dominance, or evenness, of the soil microbial community, we calculated a Shannon–Weiner evenness index using the above PLFAs known to be signatures of different groups of micro-organisms.

### STATISTICAL ANALYSIS

Data were tested for normality and analysed by analysis of variance (ANOVA) to determine effects of the three factors of plant species, nitrogen addition and

time on soil microbial and plant characteristics. All data are presented as means with standard errors, on a dry-mass basis, and data of interactions between factors were presented when their significance was greater than the main effect. Regression analysis was used to examine relationships between root and shoot biomass and measures of microbial biomass and activity.

## Results

### SHOOT AND ROOT BIOMASS

Most of the variation in shoot and root biomass was attributed to sampling date, owing to the growth of plants over time ($F$ = 320, $P < 0{\cdot}001$ and $F$ = 40·5, $P < 0{\cdot}001$, respectively). The addition to N to soils significantly increased shoot biomass only in *A. capillaris* ($F$ = 7·3, $P < 0{\cdot}001$ for the plant–nitrogen interaction), but decreased root biomass in all species (mean dry mass of 2·3 and 1·8 g pot$^{-1}$ for – and + N, respectively) ($F$ = 5·9, $P < 0{\cdot}05$; Fig. 2). Both shoot and root biomass differed significantly between plant species ($F$ = 55·8, $P < 0{\cdot}001$ and $F$ = 18·6, $P < 0{\cdot}001$, respectively) and were highest in *A. capillaris* and lowest in *F. rubra* over the experimental period, other than at day 78 when shoot biomass of *L. perenne* was highest (Fig. 1). The shoot:root biomass ratio was mostly affected by N-addition, being higher in all grass species amended with N ($F$ = 25·0, $P < 0{\cdot}001$; Fig. 3). This effect accounted for some 38% of the total variance in the data. The shoot:root ratio also differed significantly between grass species in increasing order from *A. capillaris* < *L. perenne* < *F. rubra* < *H. lanatus* ($F$ = 12·7, $P < 0{\cdot}001$; Fig. 3). This effect differed with time, however, the interaction between plant species and sample date only accounted for some 4% of the variance ($F$ = 2·4, $P < 0{\cdot}05$ for the plant–time interaction.

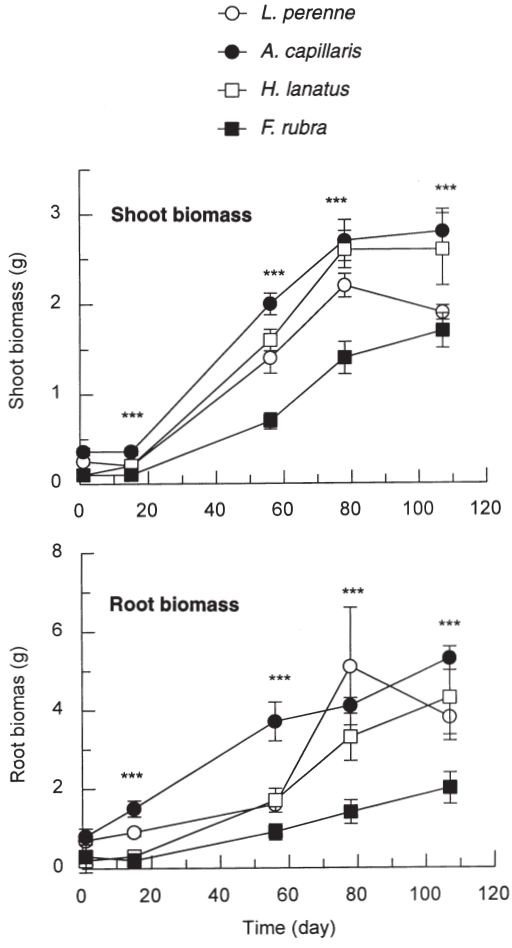


**Fig. 1.** Inter-species differences in shoot and root biomass (g) over the experimental period. ***$P < 0{\cdot}001$.

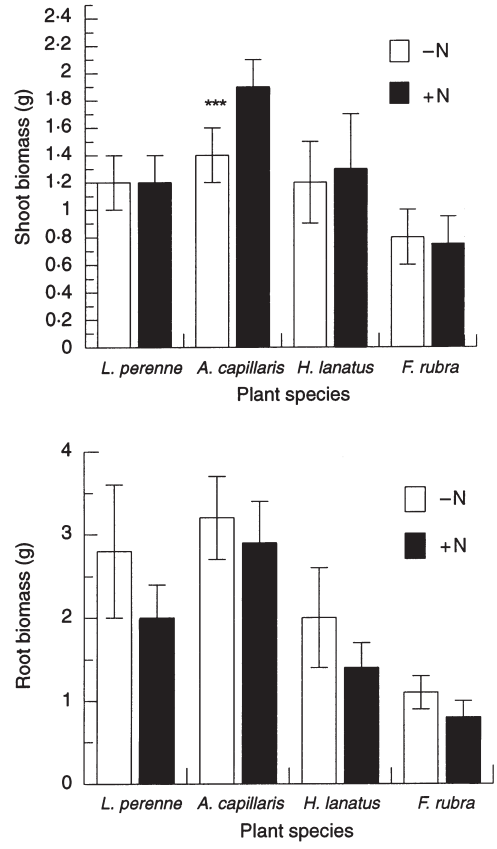


**Fig. 2.** Effect of N-addition on shoot and root biomass (g). ***$P < 0{\cdot}001$.

### SOIL PH

Soil pH was most affected by N-addition, accounting for some 46% of the total variance ($F$ = 181, $P < 0{\cdot}001$). With all plant species, soil pH was significantly reduced as a consequence of N-addition (Fig. 4). However, the addition of N to the unplanted control had no effect on soil pH (Fig. 4).

Independent of N-addition, soil pH varied significantly between plant species showing a general trend of declining pH in order: *A. capillaris*, *L. perenne*, *H. lanatus* and *F. rubra* ($F$ = 77·0, $P < 0{\cdot}001$; Fig. 4). This effect accounted for 19% of the total variance, and was weakly dependent on time of sampling with the

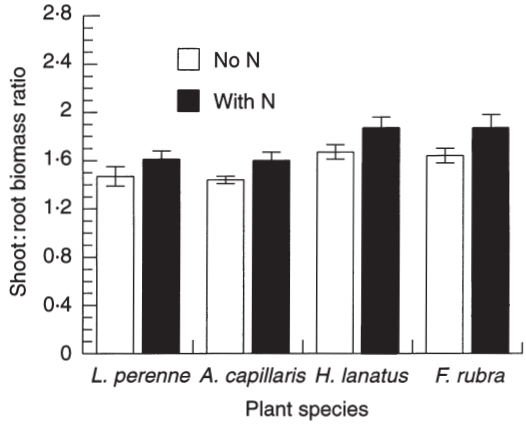


**Fig. 3.** Effect of N-addition on shoot:root biomass ratios.

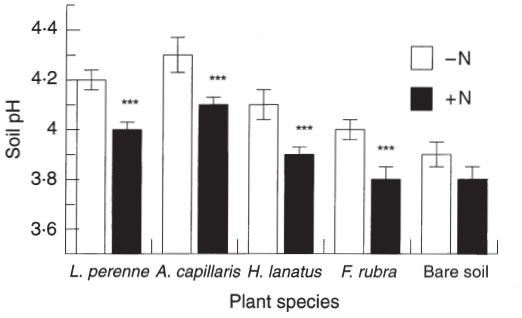


**Fig. 4.** Effect of N-addition on the pH of soils grown with different plant species. ***$P < 0{\cdot}001$.

plant–time interaction accounting for only 1% of the total variance ($F = 4{\cdot}8$, $P < 0{\cdot}001$). None of the soil microbial assessments were correlated with soil pH.

MICROBIAL ACTIVITY

Microbial activity, measured as basal respiration, was most affected by time which accounted for 40% of the total variance ($F = 6{\cdot}0$, $P < 0{\cdot}01$). Basal respiration differed significantly in soils growing different plant species, however, this effect was dependant on sample date, being significant at days 15 and 56 only ($F = 2{\cdot}6$, $P < 0{\cdot}01$ for the plant–time interaction; Table 1). This interaction between plant species and time accounted for 18% of the total variance. Although respiration was lower at day 15 in planted soils than the unplanted controls, this effect was not consistent over time (Table 1).

For acid-phosphatase and β-glucosidase activity the main factor influencing the variance in the data was sample date, accounting for 42% of the variance in both cases ($F = 19{\cdot}6, P < 0{\cdot}001$ and $F = 10{\cdot}0, P < 0{\cdot}01$, respectively). Both β-glucosidase and acid phosphatase activity in the rhizosphere differed significantly between plant species ($F = 9{\cdot}6$, $P < 0{\cdot}001$ and $F = 3{\cdot}9$, $P < 0{\cdot}01$, respectively) (16% and 21% of the total variance, respectively), however, these differences were dependent on time of sampling ($F = 2{\cdot}8$, $P < 0{\cdot}001$ and $F = 2{\cdot}5$, $P < 0{\cdot}01$, respectively, for the plant–time interaction; Table 1). For β-glucosidase activity, plant differences were significant at days 1, 15 and 56 only; there were no consistent trends between planted and unplanted controls at these sample dates (Table 1). At days 78 and 107 there were no significant differences in β-glucosidase activity between the planted and unplanted control (Table 1). For phosphatase activity, plant differences were significant at days 56 and 78 (Table 1). At day 56, phosphatase activity was lower in the rhizosphere soil of

**Table 1.** Interaction between plant species and time (days) for soil microbial activity measures. Significant differences are shown as *, ** and *** for $P < 0{\cdot}05$, $P < 0{\cdot}01$ and $P < 0{\cdot}001$, respectively; NS, not significant

| | *L. perenne* | *A. capillaris* | *H. lanatus* | *F. rubra* | Bare soil | LSD ($P < 0{\cdot}05$) | *P*-value |
|---|---|---|---|---|---|---|---|
| BASAL RESPIRATION ($\mu l\ CO_2\text{-}C\ g^{-1}\ h^{-1}$) | | | | | | | |
| Day 1 | 4·0 (0·5) | 4·5 (0·3) | 4·7 (0·6) | 4·8 (0·5) | 5·2 (0·6) | 1·5 | NS |
| Day 15 | 2·4 (0·2) | 2·1 (0·1) | 3·0 (0·2) | 3·1 (0·2) | 4·3 (0·4) | 0·9 | *** |
| Day 56 | 6·3 (1·1) | 4·5 (0·7) | 3·1 (0·4) | 3·2 (0·2) | 3·8 (0·7) | 2·0 | * |
| Day 78 | 4·5 (0·9) | 3·5 (0·7) | 3·2 (0·3) | 6·4 (1·3) | 4·3 (0·9) | 2·7 | NS |
| Day 107 | 3·1 (0·3) | 4·4 (0·6) | 4·2 (0·3) | 3·6 (0·6) | 3·5 (0·6) | 1·4 | NS |
| PHOSPHATASE ACTIVITY ($\mu g\ p\text{-nitrophenol}\ g^{-1}\ s^{-1}$) | | | | | | | |
| Day 1 | 289 (23) | 342 (26) | 326 (13) | 361 (16) | 299 (24) | 53 | NS |
| Day 15 | 243 (15) | 312 (51) | 241 (13) | 219 (8) | 247 (20) | 67 | NS |
| Day 56 | 231 (49) | 373 (23) | 321 (23) | 238 (8) | 333 (14) | 72 | ** |
| Day 78 | 404 (19) | 419 (24) | 343 (22) | 322 (15) | 319 (16) | 55 | ** |
| Day 107 | 297 (16) | 324 (18) | 247 (11) | 266 (21) | 283 (31) | 54 | NS |
| β-GLUCOSIDASE ($\mu g\ saligenin\ g^{-1}\ h^{-1}$) | | | | | | | |
| Day 1 | 69 (7) | 74 (7) | 94 (5) | 81 (7) | 67 (3) | 17 | * |
| Day 15 | 61 (6) | 68 (6) | 87 (4) | 75 (6) | 65 (4) | 15 | * |
| Day 56 | 70 (10) | 58 (4) | 79 (7) | 64 (3) | 90 (6) | 18 | * |
| Day 78 | 92 (7) | 73 (8) | 85 (3) | 95 (5) | 82 (5) | 18 | NS |
| Day 107 | 64 (4) | 62 (10) | 62 (1) | 62 (5) | 65 (3) | 14 | NS |

**Table 2.** Relationships between measures of plant growth of the different grass species and various soil microbial parameters. Values are correlation coefficients and their level of significance is shown as *, **, *** for $P < 0{\cdot}05$, $P < 0{\cdot}01$ and $P < 0{\cdot}001$, respectively. Non significant values are shown as NS. ($n = 30$ for respiration and enzyme activities, whereas $n = 18$ for PLFA measures)

| | Basal respiration | Phosphatase | Total β-glucosidase | Bacterial PLFA | Fungal PLFA (B) | PLFA (F) | Ratio F:B |
|---|---|---|---|---|---|---|---|
| *L. perenne* | | | | | | | |
| Root biomass | NS | NS | NS | 0·713*** | 0·712*** | 0·603*** | NS |
| Shoot biomass | NS | NS | NS | 0·771*** | 0·802*** | 0·743*** | NS |
| Shoot:root | NS | – 0·381* | NS | NS | NS | NS | NS |
| *A. capillaris* | | | | | | | |
| Root biomass | NS | NS | 0·504** | 0·803*** | 0·827*** | 0·733*** | – 0·624** |
| Shoot biomass | NS | NS | 0·508** | 0·775*** | 0·805*** | 0·732*** | – 0·632** |
| Shoot:root | NS | NS | NS | NS | NS | NS | NS |
| *H. lanatus* | | | | | | | |
| Root biomass | NS | NS | NS | 0·769*** | 0·784*** | 0·562* | NS |
| Shoot biomass | NS | NS | NS | 0·888*** | 0·899*** | 0·776*** | NS |
| Shoot:Root | 0·46* | – 0·552** | NS | NS | NS | 0·542* | NS |
| *F. rubra* | | | | | | | |
| Root biomass | 0·413* | NS | NS | 0·679** | 0·728*** | 0·716*** | – 0·457* |
| Shoot biomass | 0·369* | NS | NS | 0·787*** | 0·822*** | 0·835*** | – 0·506* |
| Shoot:root | NS | 0·552* | 0·396* | 0·621** | 0·625** | 0·615** | NS |

*L. perenne* and *F. rubra* than in all other treatments, whereas at day 78 phosphatase activity was higher with *L. perenne* and *A. capillaris* than in the unplanted soil (Table 1). At the end of the experiment there was no difference in phosphatase activity in the soil of the planted and unplanted pots (Table 1). The addition of N had no effect β-glucosidase activity in the rhizosphere soil of any of the planted treatments (Table 1). Acid-phosphatase activity was affected by N-addition (8% of the total variance) only with *A. capillaris*, being greater in the N-amended than the no-N treatment over the entire study ($F = 5{\cdot}7$, $P < 0{\cdot}001$ for the plant–nitrogen interaction; Table 1). There were few significant relationships between measures of plant productivity and microbial activity. However, shoot and root biomass of *F. rubra* were positively, but weakly, correlated with microbial activity, measured as basal respiration. (Table 2). Root and shoot biomass of *A. capillaris* were also positively correlated with β-glucosidase activity (Table 2).

### MICROBIAL BIOMASS AND COMMUNITY STRUCTURE

Most of the variance in measures of total PLFA ($F = 136$, $P < 0{\cdot}001$), bacterial PLFA ($F = 251$, $P < 0{\cdot}001$) and fungal PLFA ($F = 180$, $P < 0{\cdot}001$) (95%, 97% and 94%, respectively) were attributed to sample date (Table 3). These effects were primarily

**Table 3.** Interactions between plant and time for measures of microbial community structure. Values are means with standard errors in parenthesis. Significant differences are shown as *, ** and *** for $P < 0{\cdot}05$, $P < 0{\cdot}01$ and $P < 0{\cdot}001$, respectively. NS, not significant. When significant interactions were observed (i.e. day 107), values with the same letter are not significantly different

| | *L. perenne* | *A. capillaris* | *H. lanatus* | *F. rubra* | Bare soil | LSD ($P < 0{\cdot}05$) | P-value |
|---|---|---|---|---|---|---|---|
| Total PLFA (nmol $g^{-1}$ soil) | | | | | | | |
| Day 1 | 32·1 (2·8) | 24·4 (1·9) | 26·8 (3·2) | 30·2 (1·3) | 29·2 (1·7) | 6·7 | NS |
| Day 78 | 110·3 (6·5) | 105·0 (6·4) | 88·6 (11·7) | 98·2 (5·6) | 83·3 (17·2) | 21·6 | NS |
| Day 107 | 62·1 (2·2)a | 79·6 (3·7)b | 80·1 (4·5)b | 85·6 (6·4)b | 75·7 (3·9)b | 10·2 | * |
| Bacterial PLFA (nmol $g^{-1}$ soil) | | | | | | | |
| Day 1 | 13·1 (1·6) | 8·2 (1·6) | 10·2 (1·8) | 13·1 (1·1) | 12·1 (1·3) | 4·0 | NS |
| Day 78 | 51·3 (2·6) | 50·5 (2·8) | 42·5 (5·9) | 46·5 (2·1) | 48·3 (2·8) | 10·4 | NS |
| Day 107 | 29·8 (2·3)a | 39·2 (2·5)b | 41·1 (2·4)b | 44·2 (3·3)b | 39·3 (1·9)b | 7·9 | * |
| Fungal PLFA (nmol $g^{-1}$ soil) | | | | | | | |
| Day 1 | 0·6 (0·4) | 0·6 (0·0) | 0·6 (0·0) | 0·6 (0·0) | 0·7 (0·0) | 0·1 | NS |
| Day 78 | 2·1 (0·1) | 2·1 (0·1) | 2·0 (0·3) | 1·9 (0·1) | 2·1 (0·1) | 0·5 | NS |
| Day 107 | 1·0 (0·0)a | 1·5 (0·0)b | 1·5 (0·0)b | 1·7 (0·2)b | 1·3 (0·1)c | 0·2 | * |

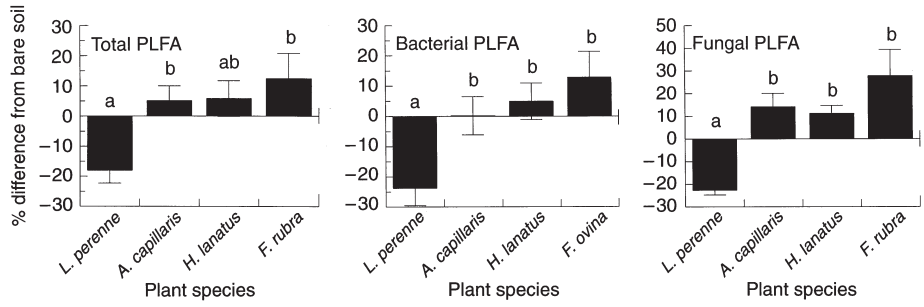


**Fig. 5.** The response of the soil microbial community to different plant species (relative to the bare soil) at the final sample date. Bars with the same letter are not significantly different.

related to temporal increases in plant productivity over the experiment; for all grass species, there were strong positive relationships between measures of plant productivity (shoot and root biomass) and total PLFA, bacterial PLFA and fungal PLFA (Table 2). N-addition had no effect on total PLFA, bacterial PLFAs or the fungal PLFA 18:2ω6 in the planted or unplanted soils.

At day 107, the total amount of PLFAs ($F$ = 1·8, $P$ < 0·05 for the plant–time interaction), bacterial PLFAs ($F$ = 2·6, $P$ < 0·05 for the plant–time interaction) and fungal PLFAs ($F$ = 2·4, $P$ < 0·05 for the plant–time interaction) differed significantly in soils planted with different plant species (Table 2). In comparison to the unplanted control at day 107, total PLFA, bacterial PLFA and fungal PLFA with *L. perenne* were reduced significantly by 18%, 24% and 22%, respectively (Fig. 5). With other plant species, these measures were increased relative to bare soil, particularly with *F. rubra* which stimulated total, bacterial and fungal PLFA by 12%, 13% and 28%, respectively, (Fig. 5). These responses, however, accounted for only a small proportion of the total variance in the data, and were not consistent over time. The ratio of fungal:bacterial PLFA differed significantly between plant species ($F$ = 2·5, $P$ < 0·05) being higher in soils planted with *A. capillaris* and *H. lanatus* than with other plants or the unplanted control (Fig. 6a); relative to the unplanted control, these two plant species increased the ratio by 26% and 20%, respectively. However, a larger proportion of the total variance (27%) in the ratio of fungal:bacterial PLFA was attributed to N-addition; at days 1 and 78 the ratio significantly increased in all soils in response to N-addition ($F$ = 8·5, $P$ < 0·001 for the nitrogen–time interaction; Fig. 7). N-addition had no effect on the fungal:bacterial PLFA ratio at day 107 (Fig. 7). For *A. capillaris* and *F. rubra* there was a negative relationship between plant productivity (shoot and root biomass) and the fungal:bacterial PLFA biomass ratio (Table 2). This relationship was particularly strong for *A. capillaris* suggesting that increases in the growth of this species, over time, had a greater effect on the fungi than the bacteria. The evenness index of the soil microbial community, measured using selected PLFA, was significantly affected by plant species ($F$ = 2·74, $P$ < 0·04), being lower with *L. perenne* and *A. capillaris* than in the bare-soil control (Fig. 6b). N-addition had no effect on the evenness index of selected PLFA. These responses appear to be related to significant increases in the proportion of the fatty acids cy19:0 and i16:0 in soils planted with *L. perenne* and *A. capillaris*, respectively (Table 4).

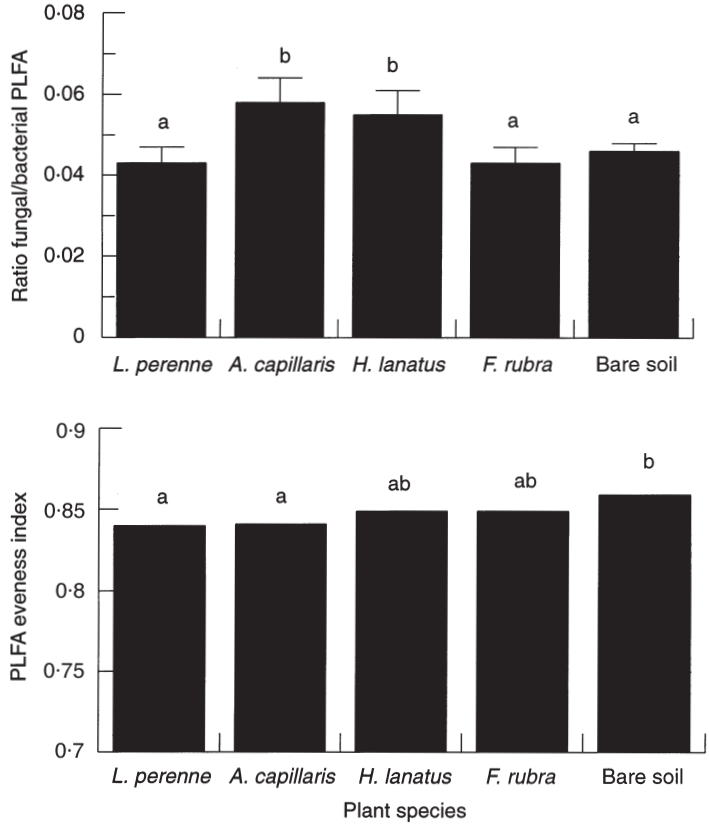


**Fig. 6.** (a) Effects of individual plant species on the fungal:bacterial PLFA ratio ($F$ = 2·5, $P$ < 0·05) and (b) evenness index of selected PLFAs. Values with the same letter are not significantly different.

## Discussion

This study set out to determine the relative effects of changes in soil N availability and plant composition, resulting from fertilization, on various soil biological

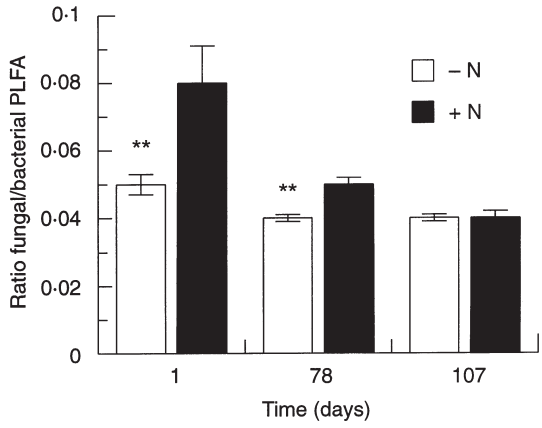


**Fig. 7.** Effects of N-addition on the ratio of fungal:bacterial PLFA over the experimental period. $^{**}P < 0{\cdot}01$.

properties of semi-natural upland grassland soils. In agreement with other laboratory (Wardle & Nicholson 1996) and field (Zak *et al.* 1994) studies, our data suggest that the main determinant of soil microbial biomass, and to some extent microbial activity, are temporal changes in plant productivity, an effect that was independent of N-additions to soil. For all plant species, we report strong positive correlations between plant productivity, measured as changes in root and shoot biomass over time, and total PLFA, a measure of the total 'active' microbial biomass of the soil (Tunlid & White 1992). We also report significant relationships between the productivity of different plant species and the abundance of bacterial and fungal PLFAs, which are thought to be indicators of bacterial and fungal biomass (Federle 1986; Frostegård & Bååth 1996). A few significant relationships between plant productivity and measures of soil microbial activity were detected, however, these effects tended to be statistically weak, and inconsistent for different plant species. We detected no effects of inorganic N-addition on measures of soil microbial biomass and activity, and furthermore, these soil biological properties were, on the whole, unrelated to indirect effects of N fertilization, such as reduced soil pH and increased shoot:root biomass ratios.

Our data also show that individual plant species, which dominate the plant community of different grasslands, had markedly different effects on various soil biological properties over the experimental period. Interestingly, these effects were independent of N-additions and were unrelated to differences in plant productivity between the plant species, which were quite marked over the experiment. At the final sample date we found that most grass species, and especially those which dominate the total plant biomass of the intermediate fertility (*H. lanatus*) and semi-natural grasslands (*A. capillaris* and *F. rubra*), had beneficial effects, of a similar scale, on microbial biomass and the abundance of bacterial and fungal PLFAs in soil. In contrast, *L. perenne*, the dominant grass of improved pasture, had a negative effect on these measures relative to the unplanted control. Although this negative effect was probably related to this species being pot-bound, as indicated by the reduction in its root biomass at the last sample date, we report no significant beneficial effects of this species on soil biological properties throughout the experiment. This finding agrees with Wardle & Nicholson (1996) who found that, relative to less productive dicotyledonous species, *L. perenne* had little effect on various soil biological properties of a grassland soil. Together, these findings may suggest a lack of coupling of *L. perenne* with the biological properties of grassland soils. In contrast, the dominant grasses of intermediate and low-fertility semi-natural grasslands demonstrate a degree of coupling with the soil biological community.

We also found that measures of soil microbial activity showed greater responses to individual plant species than to N-additions, again an effect that appeared to be unrelated to interspecies differences in plant productivity. These effects were highly variable with respect to their direction and were highly inconsistent over time; we report both positive and negative effects of individual plant species on basal respiration,

**Table 4.** Effects of plant species on the relative abundance of signature PLFAs (% of total bacterial, fungal and actinomycete fatty acids). Values with different suffix letters are significantly different at the $P < 0{\cdot}05$ level. Values are means and standard errors ($n = 18$)

| PLFA | *L. perenne* | *A. capillaris* | *H. lanatus* | *F. rubra* | Bare soil |
|---|---|---|---|---|---|
| i15 : 0 | 25·9 (1·7)a | 22·2 (2·5)a | 25·9 (2·2)a | 25·9 (1·7)a | 26·6 (2·0)a |
| a15 : 0 | 12·8 (0·8)a | 12·6 (1·3)a | 13·2 (1·1)a | 13·9 (0·7)a | 14·2 (0·7)a |
| 15 : 0 | 2·4 (0·1)ab | 1·8 (0·3)a | 2·3 (0·3)ab | 2·7 (0·1)b | 2·7 (0·1)b |
| i16 : 0 | 15·1 (1·1)ab | 18·6 (1·7)a | 15·6 (1·8)ab | 14·7 (1·5)ab | 12·4 (1·5)b |
| 17 : 0 | 7·0 (0·9)a | 8·3 (1·3)a | 8·1 (1·5)a | 6·9 (1·1)a | 7·4 (1·0)a |
| i17 : 0 | 6·3 (0·4)a | 7·1 (0·9)a | 6·9 (0·4)a | 6·6 (0·5)a | 6·9 (0·4)a |
| cy17 : 0 | 0·9 (0·2)a | 0·9 (0·2)a | 0·8 (0·2)a | 0·9 (0·2)a | 0·9 (0·2)a |
| 10 Me 18 : 0 | 4·0 (0·6)a | 5·0 (1·1)a | 4·6 (1·0)a | 3·2 (0·5)a | 4·1 (0·5)a |
| cis18 : 1ω 7 | 8·5 (1·6)a | 10·1 (1·5)a | 8·9 (1·7)a | 11·2 (1·4)a | 11·3 (1·1)a |
| 18 : 2ω 6 | 3·9 (0·3)a | 5·0 (0·6)a | 4·8 (0·6)a | 4·0 (0·3)a | 4·1 (0·3)a |
| cy19 : 0 | 13·2 (1·3)a | 8·3 (1·3)b | 9·3 (1·3)b | 9·9 (0·9)ab | 9·3 (1·2)b |

phosphatase activity and β-glucisodase activity at different sample dates. The highly variable nature of these plant responses, and their apparent non-coupling with plant productivity, suggests that the reported individual plant species effects on soil biological properties (microbial biomass and activity) were related to interspecies variations in root exudation patterns and/or efficiency of nutrient aquisition. Variations amongst the plant species in the quantity and quality of compounds lost by rhizodeposition (Vancura 1964; Klein *et al.* 1988), which represent a high quality nutrient source for micro-organisms (Whipps & Lynch 1983; van Veen *et al.* 1989), vary markedly as plants develop and age (Miller *et al.* 1990; Bolton, Fredricksen & Elliott 1992), perhaps providing an explanation for the highly inconsistent nature of individual plant species effects on the soil microbial community. Variations amongst the plant species in their efficiency of nutrient aquisition are also likely to have a strong influence on the soil microbial community through altering of the nature of nutrient competition between plants and microbes; the grasses used in this experiment are known to vary greatly in their N requirements (Ellenberg 1952) and ability to take-up mineral N from soil (Bradshaw *et al.* 1964; Davies 1971; Luxmore & Millington 1971).

The measurement of fungal:bacterial PLFA is thought to give an indication of broad-scale changes in the relative biomass of bacteria and fungi within the soil microbial community (Frostegård & Bååth 1996; Bardgett *et al.* 1996). In this study, the fungal:bacterial PLFA ratio was significantly affected by plant species and, relative to the control, was found to be consistently higher with *A. capillaris* and *H. lanatus*. It is perhaps relevant that these plant species, in particular *A. capillaris*, are dominants of less fertile, semi-natural grasslands; soils from these grasslands have been shown to have a higher proportion of fungi relative to bacteria than in improved grasslands dominated by *L. perenne* (Bardgett *et al.* 1996). From these findings it would be tempting to conclude that the traits of dominant plant species may, in part, be responsible for these differences in the soil microbial community of these different grasslands. However, we also found that, at two sample dates, the addition of N exerted an even stronger influence on the fungal:bacterial PLFA ratio; the addition of N to the planted and unplanted microcosms increased the proportion of fungi relative to bacteria. Our results therefore indicate that although N-additions may not favour increases in the proportion fungi in the microbial community in the long term, as a consequence of increased prevalence of *L. perenne* (which had no effect on the fungal:bacterial ratio relative to the control), the short-term response is an increase in the proportion of fungi relative to bacteria within the soil microbial community.

In addition to broad-scale shifts in microbial community structure, we also found that the relative distribution, or evenness, of microbial groups (measured by PLFA) was influenced by plant species. Our data suggest that the plant species *L. perenne* and *A. capillaris* significantly reduced the evenness of the microbial community in comparison to the bare-soil control. For *L. perenne*, the reduction in microbial evenness, and hence diversity (Huston 1994), appears to be related to a significant increase in the proportion of the fatty acid cy19 : 0 within the microbial community, whereas, with *A. capillaris*, an increase in the proportion of the fatty acid i16 : 0 appears to be the casuative factor. These two fatty acids are known to be synthesized by gram-negative and gram-positive bacteria, respectively (O'Leary & Wilkinson 1988; Wilkinson 1988), suggesting that *L. perenne* and *A. capillaris* preferentially influence these microbial groups over other micro-oganisms. This also suggests that these species may have differential effects on the composition of the bacterial community of these upland soils. The implications of these changes with respect to ecosystem functioning are not known. However, some studies suggest that reductions in microbial functional diversity of soils can, in some cases, reduce their capacity to decompose organic matter (Degans 1998). However, as revealed in studies of above-ground communities (Wardle *et al.* 1997, 1999: Hooper & Vitousek 1997), the effects of changes in diversity or evenness of soil communities are likely to be highly unpredictable and related to the functional traits of the species present.

A consistent finding in this study was that although N-addition had no effect on shoot productivity, it reduced root biomass and increased the shoot:root biomass ratio of all grass species. This finding is consistent with field studies of agricultural grasslands which showed that high levels of N fertilization decreased root growth (Ennik *et al.* 1980) and with laboratory sand-culture studies which showed that controlled N-addition increased shoot:root ratios of a wide range of common upland grassland species (Bradshaw *et al.* 1964). Although these changes in plant growth appeared to have little effect on soil microbial biomass and activity, they may have contributed to the changes in microbial community structure observed in the N amended soils. For example, Liljeroth, Bååth *et al.* (1990) showed that N-additions increased bacterial numbers in the rhizoplane of barley seedlings, despite a negative effect of N-addition on root growth. The response of bacteria to N-additions in these systems was thought to be related to higher rates of root exudation in the smaller roots. Follow on studies also showed that increased soil N levels increased the efficiency of utilization of root exudates by the soil microbial community (Liljeroth, van Veen & Miller 1990). In this study, therefore, it is possible that changes in root exudation patterns in the N-amended soils may have altered C utilization by microbes, and hence the competitive advantage and relative abundance of different subsets of the microbial community.

In conclusion, our data suggest that plant ecophysiological traits, and hence indirect effects of fertilization, have a relatively more important role than do the direct effects of changes in nutrient availability, in determining the size and activity, and possibly the diversity, of the soil microbial community in upland grasslands. In particular, our results suggest a feedback mechanisms in which certain grassland plants, in particular those of intermediate and low-fertility semi-natural grasslands, may benefit biological properties of upland soils, thereby altering the supply of nutrients for plant growth. We suggest that these effects, although connected to changes in plant productivity over time, are related to variations amongst plant species in root exudation patterns. We also provide some evidence that, in the short term, soil microbial community structure is influenced by plant species traits, but also by changes in soil N availability. We stress, however, that the application of these laboratory findings to the field-scale requires a certain amount of caution. In grasslands, changes in the biomass, structure and activity of soil microbial communities are also likely to be strongly regulated by long-term changes in plant productivity (Zak *et al.* 1994), in particular the supply of C to soil via plant litter production and root turnover which did not feature in our study. Despite this caution, we present evidence to support the view (e.g. Wardle & Nicholson 1996; Wardle *et al.* 1998, 1999; Bardgett & Shine 1999) that in perennial grasslands the traits of the component plant species have an important role in determining the size and activity, and to some extent the structure, of the soil microbial community, and presumably soil ecosystem properties of decomposition and nutrient cycling. Because the plants that we examined represent dominant contributors to the total biomass of upland grasslands, our findings add support to the emerging view that the functional characteristics of dominant plant species are important determinants of ecosystem function (Hopper & Vitousek 1997; Wardle *et al.* 1997; Grime 1998).

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