PERGAMON

Soil Biology & Biochemistry 32 (2000) 253–263

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Management influences on soil microbial communities and their function in botanically diverse haymeadows of northern England and Wales

Louise M. Donnison[a, b], Gwyn S. Griffith[a], John Hedger[b, 1], Phil J. Hobbs[c], Richard D. Bardgett[d,*]

[a]*Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Ceredigion SY23 3EB, UK*
[b]*Institute of Biological Sciences, University of Wales, Aberystwyth, Ceredigion SY23 13DA, UK*
[c]*Institute of Grassland and Environmental Research, North Wyke, Okehampton, Devon EX20 2SB, UK*
[d]*Department of Biological Sciences, Institute of Environmental and Natural Sciences, University of Lancaster, Lancaster LA1 4YQ, UK*

Accepted 9 September 1999

Abstract

The effects of management intensification on the size, activity and structure of soil microbial communities in botanically diverse haymeadows were examined. Paired traditionally managed and intensively managed haymeadows, at three submontane regions in northern England and north Wales, were sampled over four seasons. Management intensification had no significant effect on soil nutrient status, soil microbial biomass and soil microbial activity. Management intensification did influence soil microbial community structure, resulting in a significant reduction in soil fungal biomass, measured as soil ergosterol content, and a decline in the proportion of fungi relative to bacteria in the soil microbial community. Fungi of the genera *Fusarium*, *Mucor*, *Absidia*, *Cladosporium*, *Trichoderma*, *Acremonium*, *Zygorhynchus*, *Phoma* and *Paecilomyces* were commonly isolated from litter and soil of both the traditionally and intensively managed haymeadows of the site tested. Management had a significant effect on the relative isolation frequency of these fungi at this site. All commonly isolated species had proteolytic and urease activity and approximately half had cellulolytic and lignolytic activities. These findings were taken to suggest that although management improvements to submontane haymeadows will induce changes in the size and composition of the fungal community, they do not necessarily influence the functioning of the soil microbial community with respect to soil ecosystem-level processes of organic matter decomposition and nutrient cycling. We suggest that changes in soil microbial communities are related primarily to changes in plant productivity and composition or the form and quantity of fertiliser applied to the site.

Keywords: Haymeadow; Management intensification; Soil; Ergosterol; Phospholipid fatty acid; Decomposer fungi; Fungal community; Fertilizer

1. Introduction

Traditionally managed haymeadows within submontane regions of northern England and north Wales support, perhaps, the largest diversity of plants of all temperate grasslands (Rodwell, 1992). However, over the last 40 yr some 95% of these traditional flower rich haymeadows have been subjected to intensive grassland agriculture (Porter, 1994), leading to increases in plant productivity, but marked declines in plant species diversity from some 40 plus species to, typically, around 10–15 species m^2 (Rodwell, 1992; Smith and Rushton, 1994). Such considerable changes in plant productivity and species composition are likely to strongly influence soil microbial community dynamics, and hence the efficiency of ecosystem func-

* Corresponding author. Tel.: +44-1524-594-856; fax: +44-1524-843-854.
E-mail address: r.bardgett@lancaster.ac.uk (R.D. Bardgett).
[1] Present address: School of Biosciences, University of Westminster, London W1R 8AL, UK.

0038-0717/00/$ - see front matter
PII: S0038-0717(99)00159-5

Table 1
Characteristics of traditionally and intensively managed grasslands at three sites

Property	Teesdale		Ravenstonedale		Dolgellau	
Grid reference	NY817338		NY736033			NY803225
Altitude (m. a. s. l.)	450		280		180	
Soil type	brown earth		brown earth		brown earth	
Rock base	carboniferous limestone		carboniferous limestone		igneous rock/ boulder clay	
Management regime	traditional	intensive	traditional	intensive	traditional	intensive
Number plant species	33	27	26	14	22	16
pH	5.9	5.5	5.6	5.7	5.5	5.5
Soil NH_4^+ ($\mu g\ g^{-1}$)	11.7	14.0	13.4	14.1	12.8	12.1
Soil NO_3^- ($\mu g\ g^{-1}$)	12.3	8.4	21.8	30.2	21.0	19.1
Manure regime	8 t $ha^{-1}y^{-1}$	–	13 t $ha^{-1}y^{-1}$	a good coat	thick coat	–
NPK regime (kg ha^{-1} y^{-1})	–	50	–	100–150	–	250
Lime regime t ha^{-1} over last 15 yr	5	–	–	–	2.5	7.4
Herbage utilisation	hay	hay	hay	hay	hay	hay/silage
Date closed off for hay growth	mid May	end May	start May	start May	end April	end May
Date hay cut	late July	late July	late June	late June	end July	start July
Cattle grazing	no	after hay cut	after hay cut	March	after hay cut	after hay cut
Sheep grazing	for all fields and sites through out year					
Vegetation	MG3[a]	MG6[b]	MG3[c]	MG6[d]	MG5[e]	MG6[fg]

[a] *Alchemilla glabra*, *Alchemilla* sp., *Bellis perennis*, *Caltha palustris*, *Cardamine pratensis*, *Carex nigra*, *Centaurea fontanum*, *Crepis paludosa*, *Euphrasia* spp., *Leontodon autumnalis*, *Montia fontana*, *Myosotis discolor*, *Plantago lanceolata*, *Ranunculus acris*, *Ranunculus repens*, *Rhinanthus* spp., *Rumex acetosa*, *Sagina procumbens*, *Taraxacum officinale*, *Trifolium pratense*, *T. repens*, *Trollius europaeus*, *Viola* sp., *Agrostis stolonifera*, *Anthoxanthum odoratum*, *Bromus* spp., *Cynosurus cristatus*, *Festuca pratensis*, *F. rubra*, *Holcus lanatus*, *Phleum pratense*, *Poa annua*, *P. pratensis* & *P. trivialis*.

[b] *Alchemilla* sp., *B. perennis*, *C. palustris*, *C. pratensis*, *C. nigra*, *C. fontanum*, *M. fontana*, *M. discolor*, *P. lanceolata*, *R. acris*, *R. repens*, *Rhinanthus* spp., *R. acetosa*, *T. officinale*, *T. repens.* sp., *A. stolonifera*, *Alopecurus pratensis*, *A. odoratum*, *Bromus* spp., *C. cristatus*, *Festuca ovina*, *F. pratensis*, *F. rubra*, *H. lanatus*, *Lolium pratensis*, *P. pratense*, *P. pratensis* & *P. trivialis*.

[c] *Achillea millefolium*, *A. glabra*, *Anthriscus sylvestris*, *B. perennis*, *C. fontanum*, *Filipendula ulmaria*, *Geranium sylvaticum*, *P. lanceolata*, *R. acris*, *Rhinanthus* spp., *R. acetosa*, *Sagina* sp., *Sanguisorba officinalis*, *Stellaria* sp., *T. officinale*, *T. pratense*, *T. repens.* sp., *Viola* sp., *Agrostis canina*, *Alopercurus geniculatus*, *A. pratensis*, *A. oderatum*, *C. cristatus*, *Dactylis glomerata*, *L. pratensis*, *P. pratense* & *P. trivialis*.

[d] *C. fontanum*, *R. acris*, *R. acetosa*, *Sagina* sp., *Stellaria* sp., *T. repens*, *A. canina*, *Agrostis capillaris*, *Bromus mollis*, *C. cristatus*, *D. glomerata*, *L. pratensis* & *P. trivialis*.

[e] *Alchemilla* sp., *B. perennis*, *Centaurea nigra*, *C. fontanum*, *Euphrasia* spp., *Heracleum sphondylium*, *L. autumnalsis*, *P. lanceolata*, *R. repens*, *Rhianthus* spp., *R. acetosa*, *T. repens.* sp., *A. capillaris*, *A. odoratum*, *Arrhenatherum elatius*, *Bromus hordeaceus*, *C. cristatus*, *D. glomerata*, *F. rubra*, *L. pratensis*, *P. pratense*, *P. pratensis*, *P. trivialis*.

[f] *A. millefolium*, *Alchemilla* sp., *B. perennis*, *C. fontanum*, *L. autumnalsis*, *P. lanceolata*, *R. repens*, *R. acetosa*, *T. repens.* sp., *A. capillaris*, *A. odoratum*, *B. hordeaceus*, *F. rubra*, *L. perenne*, *P. pratensis* & *P. trivialis*.

[g] The traditional meadow at Dolgellau was classified as *Cynosurus cristatus–Centaurea nigra* (MG5a). All fields under improved management (improved fields) were classed as MG6 (Rodwell, 1992).

tions, such as organic matter and nutrient recycling. For example, increases in plant productivity associated with fertilisation are likely to increase the amount of carbon added to the soil through roots and litter, and possibly root exudates, thereby increasing microbial growth and activity (Zak et al., 1994; Bardgett et al., 1998). Reductions in the diversity of plants and litter inputs, and changes in litter quality associated with management intensification, may also influence the soil microbial biomass and activity through the removal of certain synergistic interactions between plant roots and litters of different plant species (Wardle and Nicholson, 1996; Wardle et al., 1997; Bardgett and Shine, 1999). Such effects of plant composition or diversity on the soil microbial community, however, are likely to be highly unpredictable in these grasslands (Wardle and Nicholson, 1996; Wardle et al., 1997) and dependent on the traits of the plant species in question (Wardle et al., 1998).

The application of fertilisers to meadows may also directly influence soil microbial community dynamics. For example, studies of grazed, lowland grassland by Lovell et al. (1995) showed that the application of nitrogen fertiliser significantly reduced the size and activity of the soil microbial biomass. It has been suggested that fertiliser N application can have a direct inhibitory effect on the soil microbial community, in particular the decomposer (Arnebrandt et al., 1990; Bardgett et al., 1993) and mycorrhizal fungi (Sparling and Tinker, 1978; Hamel et al., 1994), through the repression of enzyme activity and the build up of recalcitrant and toxic compounds (Fog, 1988). Reductions in microbial biomass in

fertilised grasslands have also been attributed to the acidifying effect of nitrogenous fertilisers (microbial production of nitric acid) in soil (Christie and Beattie, 1989). Information on the relative importance of direct (nutrient availability) and indirect (plant response) effects of fertilisation on grassland soil microbial communities is scarce, although microcosm studies by Bardgett et al. (in press) suggest that changes in plant composition of grasslands may have a greater influence on soil microbial communities than do changes in nutrient availability. Likewise, studies by Wardle and Nicholson (1996) and Wardle et al. (1998) suggest that plant species effects (productivity and species composition) are fundamental in shaping the soil microbial community of grasslands.

Although some research has addressed how changes in agricultural management can affect soil microorganisms (Lovell et al., 1995; Bardgett and Leemans, 1995; Bardgett et al., 1996), little work has considered the effects of management in grasslands of such high botanical diversity, where changes in the plant community in response to management, and hence soil carbon and other nutrient inputs, are so large. One study that has been done on these botanically diverse haymeadows in northern England provides evidence, from a range of sites along gradients of management intensity, that high intensity farming reduces the proportion of fungi relative to bacteria in the soil microbial community (Bardgett and McAlister, 1999); an effect that appears to be common to other temperate grassland systems (Bardgett et al., 1999). The aim of this study was also to confirm that long-term intensive management of haymeadows decreases fungal biomass and the proportion of fungi relative to bacteria within the soil microbial community. However, our principal aim was to see whether observed changes are associated with shifts in fungal community structure and hence the functional capability of the soil with respect to enzyme production. To achieve these aims, we compared measures of soil microbial biomass, fungal-to-bacterial biomass ratios and fungal community structure, and fungal enzyme activities, in adjacent traditionally and intensively managed submontane haymeadows, at different locations in the United Kingdom. Donnison et al. (1999) consider the mechanisms for changes in soil microbial communities with respect to the direct and indirect effects of fertilisers on the soil microbial community, through changes in nutrient availability or plant composition, respectively.

2. Materials and methods

2.1. Study sites and soils

Sites (Table 1) were selected to allow direct comparisons of different management systems at different biogeographical locations in the United Kingdom. Plant species composition was assessed by quadrat sampling. Botanical names in Table 1 follow Clapham et al. (1987). Soils at all sites were sampled in May, July and October 1995, and January 1996. These dates were taken to broadly represent the prevailing climatic conditions of spring, summer, autumn and winter, respectively. In addition soils and surface plant litter at the Dolgellau site in north Wales were sampled in July 1996 to determine the composition of resident fungal communities.

Due to the unique nature of these haymeadows, which are protected under the UK Sites of Special Scientific Interest (S.S.S.I.) legislation, only one grassland of each type was present at each location. Therefore, we were limited to using three permanently located 1 m^2 quadrats as replicates within each grassland type, at each site. We were confident that these replicates covered the range of variability expected for such grasslands for any given site; previous studies suggest that within field variability in soil conditions and microbial properties is as high as that found between fields within any given location (Bardgett and McAlister, 1999). Soil was sampled for all analyses by taking five randomly located 3.5 cm dia soil cores (10 cm deep) from each quadrat at each sample date. After removal of surface vegetation, soil cores from the same quadrats were bulked and sieved (<6 mm), plant material was removed, and the soil was stored at 4°C (for no more than 24 h) until analyses. Surface plant litter was also stored at 4°C until use.

Soil pH, organic matter (loss on ignition), plant available NO_3^- and NH_4^+ (determined in May and July 1995 and January 1996 only) and total nutrient content (Mg, Ca, N, Na, P and K; determined in October 1995 only) were measured by standard methods (Allen, 1989). Olsen's bicarbonate extractable P (PO_4^{3-}) was also measured (Olsen et al., 1954; Allen, 1989; in October 1995 and January 1996 only). Results were expressed on a dry weight basis except for the total soil nutrient analysis where values were expressed as meq% element. Average values for pH and plant available NO_3^- and NH_4^+ are given in Table 1.

2.2. Assessment of soil microbial biomass and activity

Extractable microbial C was measured using the fumigation–extraction technique (Vance et al., 1987). Briefly, soil samples were fumigated with $CHCl_3$ for 24 h at 25°C. After removal of the $CHCl_3$, soluble C was extracted from fumigated and unfumigated samples with 0.5 M K_2SO_4 for 30 min on an end-over-end shaker (soil-to-solution ratio 1:4 w/v). Total organic C in filtered extracts was determined by the acid dichromate oxidation method (Tate et al., 1988). Microbial C flush

(difference between extractable C from fumigated and unfumigated samples) was converted to microbial biomass C using a K_{EC} factor of 0.35 (Sparling et al., 1990).

Total soil microbial activity was determined by measuring the rate of CO_2 production from 1 g d.w. equivalent of soil, incubated for 2 h at 30°C in a McCartney bottle sealed with a No. 41 Subaseal. Headspace concentrations of CO_2 (1 ml sample) were measured using an infra red gas analyser (Analytical Development Co., series 225, Mk3). Results are expressed on a dry weight basis. Since no substrates were added to soil we were measuring basal respiratory rates.

Total soil microbial activity was also estimated by determining soil dehydrogenase activity. This was measured as the production of formazan from iodonitrotetrazolium violet (INT) as described by von Mersi and Schinner (1991).

2.3. Assessment of the relative contribution of fungi and bacteria to soil microbial biomass

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 and 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 weight basis.

Fatty acid nomenclature was used as described by Frostegård et al. (1993). The fatty acids i15:0, a15:0, i16:0, 17:0, i17:0, cy17:0, 18:1ω7 and cy19:0 were chosen to represent bacterial PLFAs (bactPLFAs) (Federle, 1986; Tunlid and White, 1992; Frostegård et al., 1993; Bardgett et al., 1996) and 18:2ω6 was used as an indicator of fungal biomass (Federle, 1986). These values were used to calculate the ratio of 18:2ω6:bactPLFAs, which was taken to represent the ratio of fungal-to-bacterial biomass in soil (Bardgett et al., 1996).

2.4. Assessment of fungal abundance and community composition

Ergosterol was extracted from 1 g dry weight soil using the procedure described by Frostegård and Bååth (1996). Dried ergosterol samples were dissolved in 1 ml methanol (40°C for 15 min; sonicated for 1 min), centrifuged (9000 rev min^{-1}; 5 min) and supernatants filtered (0.45 mm pore size teflon syringe; Merck, UK). Ergosterol content of extracts was quantified by high performance liquid chromatography with methanol as the mobile phase and a reverse phase C18 column (Highchrom, Reading, UK).

Saprotrophic fungi were isolated from soil and surface plant litter collected from the Dolgellau site in July. Fungi were isolated from litter by a root-washing technique (Harley and Waid, 1955) adapted for isolating fungi from plant litter (Bardgett et al., 1993). Briefly litter was cut into 1–2 mm^2 fragments and washed for 5 min in five changes of sterile distilled water. Fragments were then plated out on Czapex Dox agar with 1:15,000 w/v rose Bengal (five fragments per plate; a total of 750 fragments per site) and incubated at 15°C for 1–3 weeks.

Saprotrophic soil fungi were isolated using the dilution plate technique. Soil dilutions were mixed with molten Czapex Dox agar with 1:15,000 w/v rose Bengal in Petri dishes. Plates were incubated at 15°C for 3–7 d. From each replicate sample, 250 colonies were randomly picked (1500 colonies in total). Fungal isolates were identified to genus and if possible to species level. The relative isolation frequency of taxa isolated was recorded as percentage occurrence.

The diversity of the litter and soil fungal communities at each site was assessed by calculating values for the Shannon-Wiener function (H; Margalef, 1958):

$$H = \sum_{i=1}^{s} p_i \log_2 p_i,$$

where s is the number of species and p_i is the proportion of the total sample belonging to the ith species. This function takes two components of species diversity into account, number of species and evenness of allotment of individuals among the species (Lloyd and Ghelardi, 1964).

2.5. Determination of enzymatic capacity of commonly isolated fungi

Commonly isolated fungi (isolation frequency $>5\%$) and some incidentals were screened for their enzymatic capabilities. Protease, urease, ligninase and beta-glucosidase activity were tested as described by Paterson and Bridge (1994). Cellulase was tested by the ability of an isolate to clear cellulose medium (Eggins and Pugh, 1962) and release dye from cellulose azure dye (Paterson and Bridge, 1994). Phenoloxidase activity was tested as described by Poppe and Welvaert (1983). Esterase activity was tested as described by Paterson and Bridge (1994) and Stalpers (1978). Production of extracellular peroxidase, laccase naphthol, laccase guaiacol and acid phosphatase activity were tested as described by Stalpers (1978).

Table 2
Summary of MANOVAs performed on soil measurement data in May, July, October and January. Overall significant effects of site treatment and site × treatment interaction are marked as $^{*}(P < 0.05)$, ** $(P < 0.01)$, $^{***}(P < 0.001)$ and nonsignificant differences as (ns) for Wilk's Lambda statistic. The number of variables used in the MANOVA computation is given, – indicates no MANOVA computed

Date	Site	Treatment	Site × treatment	Number of variables
May	14.24**	9.912*	6.7*	12
July	ns	ns	ns	8
October	7**	8.64*	–	14
January	ns	ns	ns	12

2.6. Statistical analysis

Statistical analysis was carried out with SAS software (SAS Institute, Cary, NC). Due to the large number of variates measured and the possibility of correlations between these variates, MANOVA analyses were employed. Where these indicated significant overall differences between managements or sites, univarate ANOVAs and Tukey tests were performed to confirm the source of these differences. Data for each season were analysed separately because incorporating season as an element in the MANOVA analysis would have resulted in an excessive number of degrees of freedom. In addition, experimental limitations, as discussed in the Materials and methods section, meant that our replicate plots within each grassland type were not truly independent with respect to time. Management effects revealed by MANOVA were confirmed by examining the consistency of responses over time at the three sites. Due to the unbalanced nature of the data sets (missing values), general linear models procedures were adopted.

The χ^2 test was used to confirm the null hypothesis that for each method, management practice had no effect on fungal community structure. Tukeys tests (SAS) were used to confirm which individual species were effected by management practice.

3. Results

In May and October, site, management and site management interactions had significant overall effects on soil measurements (Table 2).

3.1. Soil nutrient status and physical properties

Site and management had little or no effect on soil pH, plant available NH_4^+ and NO_3^- (not assessed in October) and total soil N and K (assessed in October only). Site had an effect on soil Na concentrations which were lowest at Dolgellau (Table 3). Site–management interactions had effects on soil Ca, Mg, P and PO_4^{3-} levels (Table 3; Olsen's PO_4^{3-} assessed in January and October, other elements in October only). Amounts of Ca and Mg were lowest at Dolgellau and elevated under traditional management at Teesdale. Concentrations of PO_4^{3-} and P were highest under intensive management at Ravenstonedale. Site management interactions also had effects on soil organic matter content in May (Table 4). At this time soils from Teesdale contained higher amounts of organic matter than soils from other sites. Soil organic matter content was slightly elevated under traditional management at Teesdale and Dolgellau but slightly reduced under traditional management at Ravenstonedale.

3.2. Soil microbial biomass and activity

Site management interactions had effects on measures of soil microbial biomass and activity. In both May and October the basal respiration, dehydrogenase activity and microbial C content of soils were

Table 3
Nutrient status of soil in October 1995. Values are given as means ($n = 3$), standard error of the mean is given in parentheses

Site/treatment	Na[a] (meq%)	Ca[b] (meq%)	Mg[b] (meq%)	K (meq%)	N (μg g^{-1})	P[b] (μg g^{-1})	Olsens P[c] (g dry $soil^{-1}$)
Teesdale							
Traditional	0.18 (0.03)	20.60 (1.00)	7.40 (0.70)	0.30 (0.03)	0.98 (0.07)	11.93 (0.32)	21.30 (2.10)
Intensive	0.17 (0.01)	13.60 (0.19)	2.87 (0.02)	0.30 (0.02)	0.50 (0.22)	5.80 (0.57)	18.00 (0.80)
Ravenstonedale							
Traditional	0.17 (0.01)	12.00 (1.00)	3.79 (0.07)	0.33 (0.43)	0.24 (0.20)	11.13 (2.13)	25.80 (1.40)
Intensive	0.18 (0.02)	14.10 (0.75)	3.12 (0.13)	0.70 (0.17)	0.47 (0.21)	42.90 (3.67)	59.50 (10.40)
Dolgellau							
Traditional	0.15 (0.15)	8.60 (0.70)	0.94 (0.01)	0.30 (0.03)	0.61 (0.27)	0.80 (0.46)	24.30 (0.27)
Intensive	0.12 (0.02)	8.10 (1.14)	1.34 (0.35)	0.30 (0.14)	0.68 (0.03)	3.60 (0.41)	30.60 (1.75)

[a] Univariate ANOVA indicated a significant ($P < 0.05$) effect of site.
[b] Univariate ANOVA indicated significant ($P < 0.01$) differences between means.
[c] Univariate ANOVA indicated significant ($P < 0.001$) differences between means.

Table 4
Mean and standard errors ($n = 3$) for dehydrogenase activity and percentage loss on ignition of soils sampled in May, July, October and January from traditionally and intensively managed haymeadows at Teesdale, Ravenstonedale and Dolgellau

Site/treatment	Percentage loss on ignition				Dehydrogenase activity (nmol INTF $h^{-1}g^{-1}$dry soil)			
	May[a]	July	October[b]	January	May[c]	July	October[a]	January
Teesdale								
Traditional	23 (2.3)	20 (0.6)	22 (0.7)	20 (1.1)	1286 (62)	1133 (150)	2530 (159)	1096 (118)
Intensive	18 (0.9)	22 (1.1)	21(1.3)	15 (0.7)	902 (9)	1463 (58)	2297 (122)	1167 (107)
Ravenstonedale								
Traditional	9 (1.1)	22 (1.6)	22 (1.1)	11 (0.9)	883 (133)	1184 (118)	1562 (25)	749 (21)
Intensive	15 (0.6)	21 (1.1)	19 (0.4)	12 (1.0)	704 (86)	1754 (159)	1677 (101)	826 (27)
Dolgellau								
Traditional	165 (1.2)	21 (1.4)	19 (0.2)	19 (0.9)	766 (20)	1025 (101)	1216 (260)	767 (100)
Intensive	10 (1.6)	20 (0.9)	23 (0.9)	16 (2.4)	643 (62)	1157 (39)	1467 (185)	801 (102)

[a] Univariate ANOVA indicated significant differences between means ($P < 0.001$).
[b] Univariate ANOVA indicated significant differences between means ($P < 0.01$).
[c] Univariate ANOVA indicated significant differences between means ($P < 0.05$).

highest at Teesdale and in May dehydrogenase activity and microbial C content were elevated in the traditionally managed site at Teesdale (Table 4; Fig. 1). Basal respiration showed a marked seasonal trend, being greatest at all sites in late spring and apparently declining thereafter (Fig. 1).

3.3. Soil microbial community

Ergosterol contents of soils under traditional management were always greater than those under intensive management (Fig. 2). This trend was statistically significant in October. In May, site management inter-

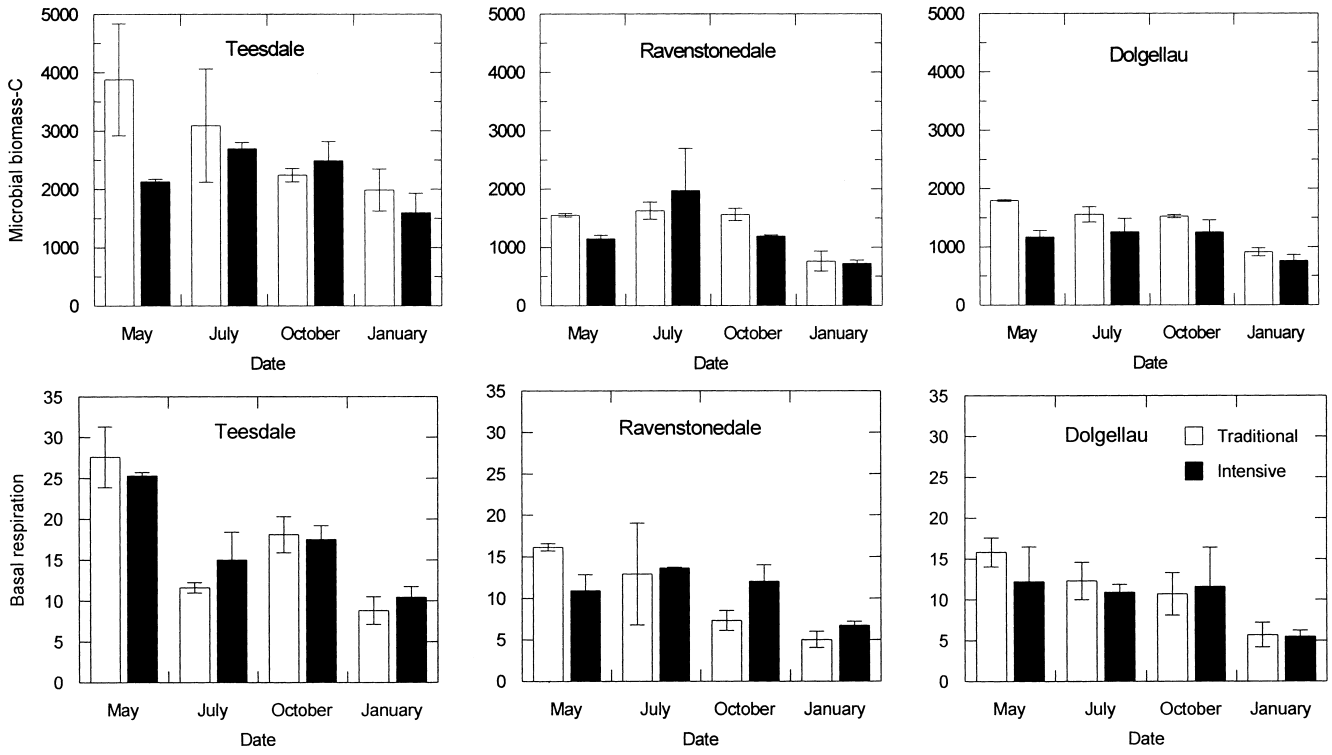

Fig. 1. A comparison of mean and standard errors ($n = 3$) for microbial biomass (μg C g^{-1} d.w. soil) and basal respiration (μl CO_2 g^{-1} d.w. soil h^{-1}) of soils sampled in May, July, October and January from traditional (clear bars) and intensive (full bars) soils at Teesdale, Ravenstonedale and Dolgellau. For microbial biomass C and basal respiration univariate ANOVAs for individual sample dates indicated significant differences between means ($P < 0.001$ in May and $P < 0.01$ in October for microbial biomass C and $P < 0.01$ in May and $P < 0.05$ in October for basal respiration).

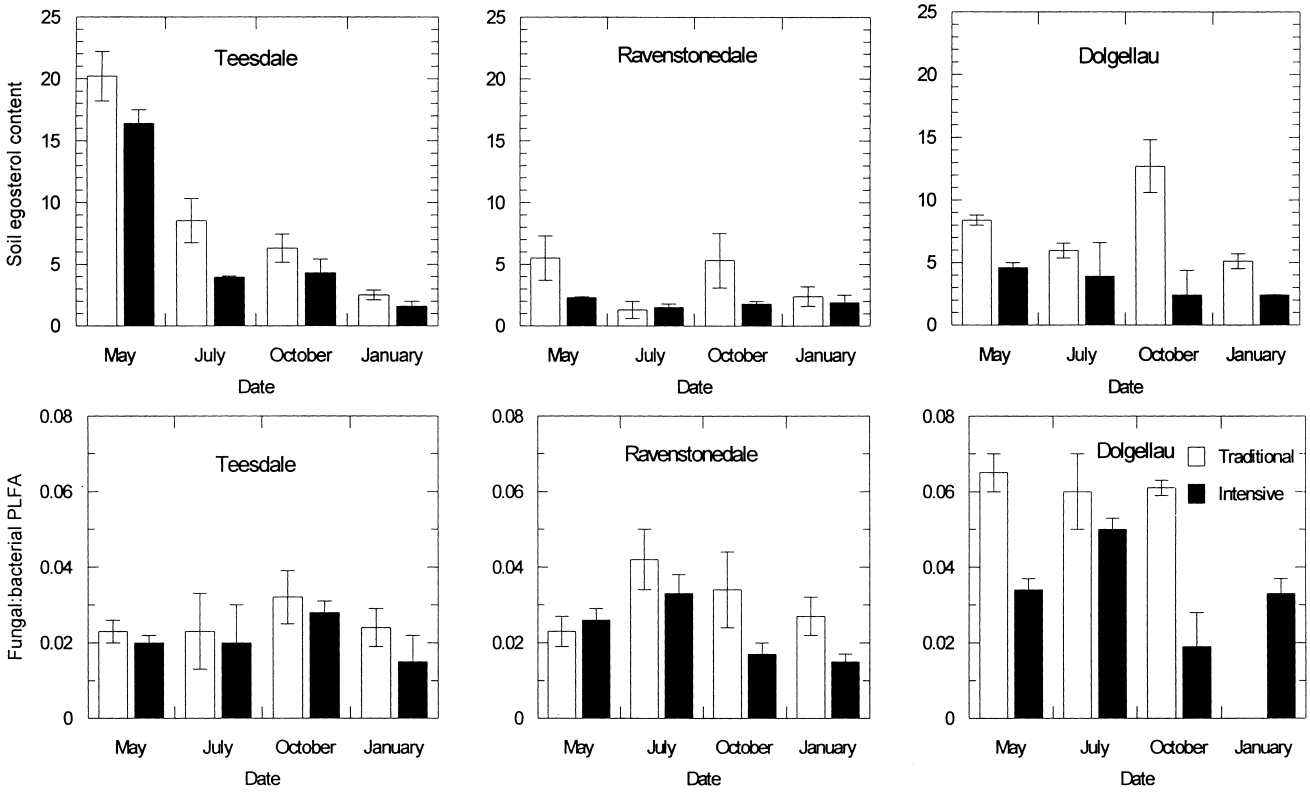

Fig. 2. A comparison of mean and standard errors ($n = 3$) for soil ergosterol content (μg g^{-1} d.w. soil) and fungal-to-bacterial PLFA ratios of soils sampled in May, July, October and January from traditional (clear bars) and intensive (full bars) soils at Teesdale, Ravenstonedale and Dolgellau. For ergosterol and fungal-to-bacterial PFLA ratios univariate ANOVAs for individual sample dates indicated significant ($P < 0.001$) differences between means in May and a significant ($P < 0.01$) effect of treatment in October.

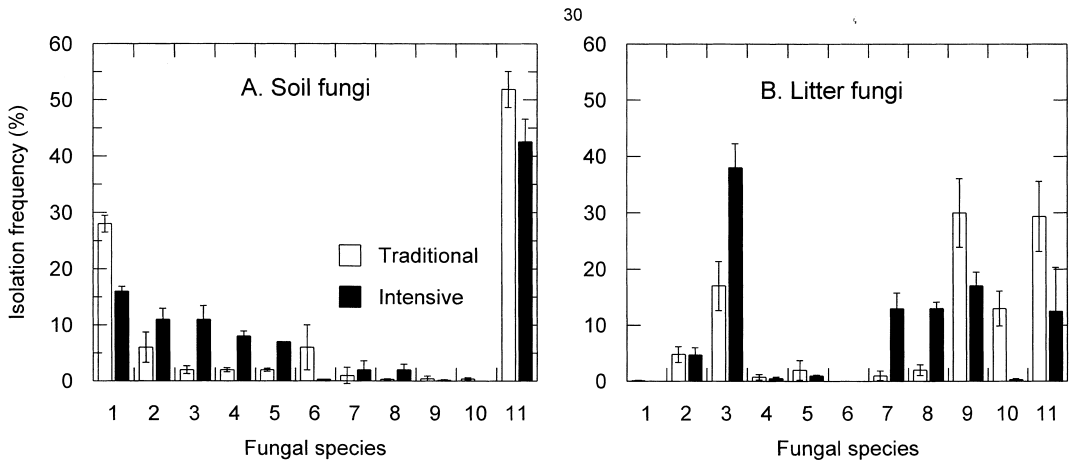

Fig. 3. A comparison of the average ($n = 3$) isolation frequency of 1. *Paecilomyces* sp., 2. *Cladosporium* spp. (mainly *Cladosporium cladosporiodes* (Fres.) de Vries), 3. *Fusarium culmorum* (W.G.Sm.) Sac., 4. *Plectosphaerella cucumerina* (Lindf.) Gams, 5. Unidentified sp. 1, 6. *Zygorhynchus* sp., 7. *Mucor* sp. 1 8. *Phoma exigua* Desm., 9. *Acremonium* sp., 10. *Phoma* sp. and 11. incindentals (*Mucor* sp. 2, *Absidia* sp., *Epicoccum nigrum* Link, *Gliocladium roseum* (Link) Bainier, *Geocladium* sp., *Michoidichium nivale* (Fr.) Samuels and Hallett, *Trichoderma* spp., *Sclerotinia* spp., *Verticillium* spp. & unidentified spp.) sampled from traditional and intensive fields at Dolgellau and isolated from: (A) soil and (B) surface plant litter, bars indicate one S.E.

Table 5
Positive (+) or negative activites (−) of common (isolation frequency <5%) and incidental fungi (isolation frequency <1%) for cellulase (C), protease (P), esterase (E), phenoloxidase (Ph), laccase guiacol (G), laccase napthol (N), peroxidase (Pe), ligninase (L), acid phosphatase (Ps) and aesculine (A)

	C	P	E	Ph	G	N	Pe	L	Ps
Paecilomycese sp.	+	+	−	+	−	−	+	−	−
Cladosporium spp.	+	+	−	+	−	+	+	+	−
F.culmorum	+	+	−	+	−	−	−	−	−
P.cucumerina	+	+	−	+	+	+	+	−	−
Und.1	+	+	+	+	−	−	+	−	−
Zygorhynchus sp.	+	+	−	−	−	−	−	−	−
Mucor sp. 1	+	+	−	−	−	−	−	−	−
P.exigua	+	+	−	+	+	+	−	−	−
Acremonium sp.	+	+	−	−	−	−	−	−	−
Phoma sp.	+	+	−	+	+	+	+	−	−
Incidentals[a]	72	94	6	50	39	22	44	0	0

[a] a *Mucor* sp. 2, *Absidia* sp., *E. nigrum*, *G. roseum*, *Geocladium* sp., *M. nivale*, *Trichoderma* spp., *Sclerotinia* spp., *Verticillium* spp. and six unidentified spp. Figures are percentage of isolates scoring positive.

actions had significant effects on soil ergosterol contents. Concentrations were highest at the Teesdale site and, as in October, amounts were always highest under traditional management (Fig. 2).

With the single exception of the Ravenstonedale site in May fungal-to-bacterial PLFA ratios were always greatest in soils under traditional management (Fig. 2). This trend was statistically significant in October. In May, site management interactions had significant effects on the soil fungal-to-bacterial PLFA ratios. The ratios were greatest at Dolgellau, particularly in soils under traditional management (Fig. 2).

3.4. Fungal community structure at the Dolgellau site

Soils from the traditionally and intensively managed meadows contained 81 and 73 fungal species, respectively. The values for the Shannon–Wiener function for the diversity of these respective fungal communities were 4.81 and 4.84. Litter from the traditionally and intensively managed field contained 62 and 40 fungal species, respectively. The values for the Shannon–Wiener function for these respective communities were 3.63 and 2.91.

Fungal communities of soils and litter differed significantly under the two management systems (χ^2, $P < 0.001$; Fig. 3). In particular *Paecilomyces* sp., *Phoma* sp., Unidentified sp. 1 and incidental sp. were significantly more common under traditional management (Tukey's, $P < 0.05$; Fig. 3), whereas, *Fusarium culmorum*, *Plectosphaerella cucumerina, Phoma exigua* and *Mucor* sp 1. were significantly more common under intensive management (Tukey's, $P < 0.05$; Fig. 3).

3.5. Enzymatic activity of fungi

None of the fungi screened positive for phosphatase and aesculin, whereas all fungi screened positive for urease and protease (except an incidental) (Table 5). Some fungi screened positive for cellulase and the enzymes involved in lignin degradation including ligninase, phenoloxidase, peroxidase and laccase (Table 5).

4. Discussion

Our temporal data provides evidence that, although the size and metabolic activity of the soil microbial biomass and total amounts of soil nutrients may not be affected by long-term intensification of haymeadow management, there are large changes in the structure or make-up of the soil microbial community. In particular, intensification of management decreased soil ergosterol content, a sterol specifically found in higher fungi (Newell, 1992), and reduced the fungal-to-bacterial PLFA ratio; an effect that was particularly pronounced in early autumn. It has been proposed that changes in the relative abundance of fungal and bacterial PLFAs can be used as indicators of relative changes in the biomass of these two microbial groups (Frostegård and Bååth, 1996; Bardgett et al., 1996), which constitute some 90–95% of the total heterotrophic metabolism in most soils (Petersen and Luxton, 1982). Therefore, together, these findings (ergosterol and PLFA) provide evidence that intensification of haymeadow management reduces fungal biomass and the proportion of fungi relative to bacteria within the soil microbial community. Similar effects of grassland intensification on the soil microbial community — an increase in the proportion of bacteria relative to fungi — have also been reported by Bardgett et al. (1999) and Bardgett and McAlister (1999) in studies of temperate grasslands in southwest and northern England, respectively. These authors suggested that decomposition processes may be driven more by fungal-pathways in low-input or unfertilised grassland systems.

Reductions in soil fungal biomass due to intensive management, at the Dolgellau site, were associated with shifts in the species composition, but not the diversity of the saprotrophic fungal community. In particular, we found that *Paecilomyces* sp., *Phoma* sp., Unidentified sp. 1, and an unidentified sp., were more common under traditional management, whereas, *F.culmorum. P. cucumerina, P. exigua and Mucor* sp 1. were more common under intensive management. The finding that the composition of the fungal community changed in response to management is consistent with studies of other ecosystems, including grasslands (Bardgett et al., 1993), forests (Arnebrandt et al.,

1990), moorland (Widden et al., 1986) and arable soils (Guillemat and Montègut, 1960; Joffe, 1967; Popova, 1993). The mycoflora recorded in this study is typical of British grasslands and has many genera in common with other studies. For example, *Fusarium* spp., *Mucor* spp., *Absidia* spp., *Zygorhyncus* spp., *Phoma* spp. and *Trichoderma* spp. were all recorded in either alluvial grasslands (Apinis, 1958), Welsh pastures (Griffiths, 1955), or neutral grasslands in Britain (Warcup, 1959). This study also recorded many genera common to studies of temperate grasslands world-wide (Orpurt and Curtis, 1957; Thornton, 1960; Clarke and Christensen, 1981). This supports the view of Christensen (1981) that despite geographical barriers, grassland mycofloras are similar world-wide.

Our data suggest that changes in management had little effect on the overall metabolic activity, measured as basal respiration of the soil microbial community. We also found no consistent differences in the enzymatic capabilities of fungi commonly isolated under the different management regimes. For example, all the fungi screened positive for protease activity, suggesting a high degree of functional overlap with respect to protein utilisation. This finding is consistent with Clark and Paul (1970) who emphasised the importance of studying extracellular proteases, since much of the N in grassland systems can be in the form of protein, which fungi are capable of breaking down into amino acids. Our data also revealed that about two-thirds of the isolates displayed cellulolytic ability; again suggesting a high degree of functional overlap with respect to cellulose decomposition. However, about half of the isolates (from both traditional and intensive management) had some of the enzymes involved in lignin degradation. This suggests that although lignin degradation is normally considered a Basidiomycete dominated sphere (Hammel, 1997), some of the decomposer fungi in this study were also contributing to it. None of the fungi screened positive for phosphatase and aesculin activity, and only one species had esterase activity, perhaps suggesting that these enzymatic functions are not important in these haymeadows. These findings suggest that, despite large changes in microbial community structure and fungal species composition, there are few differences in the functional capabilities of the soil microflora of haymeadows under different management regimes. However, it should be borne in mind that the ability of fungi to actually decompose substrates in soil are not always well correlated with the results of plate assays.

The mechanisms for changes in the soil microbial community, and in particular the fungal component, are not known. However, the responses of the fungi are likely to be related to direct effects of fertilisers on the fungal community or to an indirect effect of changes in plant community productivity and species diversity, leading to quantitative and qualitative changes in detrital and non-detrital carbon inputs to soil. Although studies have demonstrated that fertilisers may directly influence the soil fungal community (Arnebrandt et al., 1990) most work tends to point to a greater role of species changes in the plant community. For example, a microcosm study by Bardgett et al. (in press) suggested that changes in plant composition of grasslands were a more important determinant of soil microbial community structure than were changes in soil nitrogen availability through fertiliser additions. Studies by Wardle and Nicholson (1996) and Wardle et al. (1998) also suggest that plant species effects (productivity and diversity) are fundamental in shaping the soil microbial community of grasslands. One factor that can be ruled out, however, is that the observed changes in soil microbial communities were pH driven; management intensification had no effect on soil pH. This finding is consistent with Bardgett et al. (1996) who also found that changes in fungal biomass and fungal-to-bacterial biomass ratios in grasslands soils were not related to soil pH.

In conclusion, this temporal study has demonstrated that management intensification did not greatly alter the size and total metabolic activity of the soil microbial biomass, but it had an effect on the structure and species composition of the soil microbial and fungal community of haymeadows. The observed microbial community shifts were likely to be related to changes in the productivity and species composition of the plant community, however, further studies are required to substantiate this claim. We have shown that several enzyme activities are ubiquitous to fungi that occur in both traditional and intensively managed haymeadows. Therefore, we provide no evidence to suggest that changes in the species composition of the fungal community will alter the functional capability of the soil microbial community with respect to soil ecosystem-level functions of organic matter decomposition and nutrient recycling.

Acknowledgements

We thank Juliet Frankland and the late Raven Frankland of Bowberhead, Ravenstonedale, Mr Beton of Upper Teesdale and Mr Lewis of Dolgellau for allowing access to their remarkable meadow lands. We also thank Andrew Jones for helping with vegetation analysis. Thanks to Dr. D. Brayford and others at I.M.I. for confirming the identity of some of our fungal isolates. This research was supported by a research studentship awarded to Louise Donnison, funded by IGER and the Institute of Biological Sciences, University College of Wales, Aberystwyth.

References

Allen, S.E., 1989. Chemical Analysis of Ecological Materials. Blackwell, Oxford.

Apinis, A.E., 1958. Distribution of microfungi in soil profiles of certain alluvial grasslands. Angew. Pflanzensoziol. 15, 83–90.

Arnebrandt, K., Bååth, E., Söderström, B., 1990. Changes in microfungal community structure after fertilisation of Scots pine forest soil with ammonium nitrate or urea. Biol. Fertil. Soils 22, 309–312.

Bardgett, R.D., Leemans, D.K., 1995. The short-term effects of fertiliser applications, liming and grazing on microbial biomass and activity in a reseeded upland grassland soil. Biol. Fertil. Soils 19, 148–154.

Bardgett, R.D., Hobbs, P.J., Frostegård, A., 1996. Changes in soil fungal-to-bacterial ratios following reductions in the intensity of management of an upland grassland. Biol. Fertil. Soils 22, 261–264.

Bardgett, R.D., Lovell, R.J., Hobbs, P.J., Jarvis, S.C., 1999. Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. Soil Biol. Biochem. 31, 1021–1030.

Bardgett, R.D., McAlister, E., 1999. The measurement of soil fungal-to-bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. Biol. Fertil. Soils 29, 282–290.

Bardgett, R.D., Mawdsley, J.L., Edwards, S., Hobbs, P.J., Rodwell, J.S., Davies, W.J. (in press). Plant species and nitrogen effects on soil biological properties of temperate upland grassslands. Functional Ecology, in press.

Bardgett, R.D., Shine, A., 1999. Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. Soil Biol. Biochem. 31, 317–321.

Bardgett, R.D., Wardle, D.A., Yeates, G.W., 1998. Linking aboveground and below-ground food webs: how plant responses to foliar herbivory influence soil organisms. Soil Biol. Biochem. 30, 1867–1878.

Bardgett, R.D., Whittaker, J.B., Frankland, J.C., 1993. The effects of agricultural practises on the soil biota of some upland grasslands. Agric. Ecosyst. Environ. 45, 25–45.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Christensen, M., 1981. Species diversity and dominance in fungal communities. In: Wicklow, T., Caroll, G.C. (Eds.), The Fungal Community. Marcel Dekker, New York, pp. 201–233.

Christie, P., Beattie, J.A.M., 1989. Grassland soil microbial biomass and accumulation of potentially toxic metals from long term slurry application. J. Appl. Ecol. 26, 597–612.

Clapham, A.R., Tutin, T.G., Moore, D.M., 1987. Flora of the British Isles. Cambridge University Press, Cambridge.

Clark, F.E., Paul, E.A., 1970. The microflora of the grassland. Adv. Agron. 22, 375–435.

Clarke, D.C., Christensen, M., 1981. The soil microfungal community of a South Dakota grassland. Can. J. Bot. 59, 1950–1960.

Donnison, L.M., Griffith, G.S., Bardgett, R.D., 1999. Effects of litter type and inorganic fertilisers on the growth and activity of some decomposer fungi of a submontane haymeadow. Soil Biol. Biochem., submitted for publication.

Eggins, H.O.W., Pugh, G.J.F., 1962. Isolation of cellulose decomposing fungi from soil. Nature 193, 94–95.

Federle, T.W., 1986. Microbial distribution in soil: new techniques. In: Megusar, F., Gantar, M. (Eds.), Perspectives in Microbial Ecology. Slovene Society for Microbiology, Ljubljana, pp. 493–498.

Fog, K., 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biol. Rev. 63, 433–462.

Frostegård, Å., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol. Fertil. Soils 22, 59–65.

Frostegård, Å., Bååth, E., Tunlid, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biol. Biochem. 25, 723–730.

Griffiths, E., 1955. Fungi and grassland. Agric. Prog. 30, 5–11.

Guillemat, J., Montègut, J., 1960. The effect of mineral fertilisers on some soil fungi. In: Parkinson, D., Waid, J.S. (Eds.), Ecology of Soil Fungi. Liverpool University Press, Liverpool, pp. 98–111.

Hamel, C., Dalpe, Y., Lapierre, C., Simard, R.R., Smith, D.L., 1994. Composition of the vesicular–arbuscular mycorrhizal fungi population in an old meadow affected by pH, phosphorous and soil disturbance. Agric. Ecosyst. Environ. 49, 223–231.

Hammel, K.E., 1997. Fungal degradation of lignin. In: Cadisch, G., Giller, K.E. (Eds.), Driven by Nature: Plant Litter Quality and Decomposition. CAB International, Wallingford, pp. 33–45.

Harley, J.L., Waid, J.S., 1955. A method of studying active mycelia on living roots and other surfaces in the soil. Trans. Br. Mycol. Soc. 38, 104–118.

Joffe, A.Z., 1967. The mycoflora of a light soil in a citrus fertiliser trial in Israel. Mycopathol. Mycol. Appl. 32, 209–230.

Lovell, R.D., Jarvis, S.C., Bardgett, R.D., 1995. Soil microbial biomass and activity in long-term grassland: Effects of management change. Soil Biol. Biochem. 27, 969–975.

Lloyd, M., Ghelardi, R.J., 1964. A table for calculating the "equitability" component of species diversity. J. Anim. Ecol. 33, 217–225.

Margalef, R., 1958. Information theory in ecology. Gen. Syst. 3, 36–71.

von Mersi, W., Schinner, F., 1991. An improved and accurate method for determining the dehydrogenase activity of soils with idonitrotetrazolium chloride. Biol. Fertil. Soils 11, 216–220.

Newell, S.Y., 1992. Estimating fungal biomass and productivity in decomposing litter. In: Carroll, G.C., Wicklow, D.T. (Eds.), The Fungal Community, its Organization and Role in the Ecosystem, 2nd ed. Marcell Dekker, New York, pp. 521–561.

Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Dep. Cir. No. 939.

Orpurt, P.A., Curtis, J.T., 1957. Soil microfungi in relation to the prairie continuum in Wisconsin. Ecology 38, 628–637.

Paterson, R.R.M., Bridge, P.D., 1994. Biochemical Techniques for Filamentous Fungi. CAB International, Wallingford.

Petersen, H., Luxton, M., 1982. A comparative analysis of soil fauna populations and their role in decomposition processes. Oikos 39, 287–388.

Popova, L.V., 1993. Effect of fertility levels on soil microfungi. Eurasian Soil Sci. 25, 96–100.

Poppe, J., Welvaert, W., 1983. Identification of Hymenomycetes in pure culture by characterisation of their mycelia and trials for artificial fructification. Med. Fac. Landboum. Rijksuniv. Gent. 48/3.

Porter, K., 1994. Seed harvesting: a haymeadow dilemma. Enact 2, 4–5.

Rodwell, J.S., 1992. British Plant Communities Volume 3: Grasslands and Montane Communities. Cambridge University Press, Cambridge.

Smith, R.S., Rushton, S.P., 1994. The effect of grazing management on the vegetation of mesotrophic (meadow) grassland in northern England. J. Appl. Ecol. 31, 13–24.

Sparling, G.P., Tinker, P.B., 1978. Mycorrhizal infection in Pennine grassland. I. Levels of infection in the field. J. Appl. Ecol. 15, 943–950.

Sparling, G.P., West, A.W., Feltham, C.W., Reynolds, J., 1990. Estimation of microbial C by a fumigation extraction method:

use on soils of high organic matter content and reassessment of the K_{EC}-factor. Soil Biol. Biochem. 22, 300–309.

Stalpers, J.A., 1978. Identification of wood inhabiting fungi in pure culture. Stud. Mycol. 16, 1–33.

Tate, K.R., Ross, D.J., Feltham, C.W., 1988. A direct method to estimate soil microbial biomass C: effects of experimental variable and some different calibration procedures. Soil Biol. Biochem. 20, 239–335.

Thornton, R.H., 1960. Growth of fungi in some forest and grassland soils. In: Parkinson, D., Waid, J.S. (Eds.), Ecology of Soil Fungi. Liverpool University Press, Liverpool, pp. 84–91.

Tunlid, A., White, D.C., 1992. Biochemical analysis of biomass, community structure, nutritional status and metabolic activity of microbial communities in soil. In: Stotzky, G., Bollag, J.M. (Eds.), Soil Biochemistry, vol. 7. Marcel Dekker, New York, pp. 229–262.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.

Warcup, J.H., 1959. Studies on Basidiomycetes in soil. Trans. Br. Mycol. Soc. 42, 45–52.

Wardle, D.A., Barker, G.M., Bonner, K.I., Nicholson, K.S., 1998. Can comparative approaches based on plant ecophysiological traits predict the nature of biotic interactions and individual plant species effects in ecosystems? J. Ecol. 86, 405–420.

Wardle, D.A., Bonner, K.I., Nicholson, K.S., 1997. Biodiversity and plant litter: experimental evidence which does not support the view that enhanced species richness improves ecosystem function. Oikos 79, 247–258.

Wardle, D.A., Nicholson, K.S., 1996. Synergistic effects of grassland species on soil microbial biomass and activity: implications for ecosystem-level effects of enriched plant diversity. Funct. Ecol. 10, 410–416.

White, D.C., Davis, W.M., Nickels, J.S., King, J.C., Bobbie, R.J., 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40, 51–62.

Widden, P., Howson, G., French, D.D., 1986. Use of cotton strips to relate fungal community structure to cellulose decomposition rate in the field. Soil Biol. Biochem. 18, 335–337.

Zak, D.R., Tilman, D., Parmenter, R.R., Rice, C.W., Fisher, F.M., Vose, J., Milchunas, D., Martin, C.W., 1994. Plant production and soil microorganisms in late-successional ecosystems: a continental scale study. Ecology 75, 2333–2347.