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The measurement of soil fungal:bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands

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Abstract There is much interest in the development of agricultural land management strategies aimed at enhancing reliance on ecosystem self-regulation rather than on artificial inputs such as fertilisers and pesticides. This study tested the usefulness of measures of soil microbial biomass and fungal:bacterial biomass ratios as indicators of effective conversion from an intensive grassland system, reliant mainly on fertilisers for crop nutrition, to a low-input system reliant mainly on self-regulation through soil biological pathways of nutrient turnover. Analysis of soils from a wide range of meadow grassland sites in northern England, along a gradient of long-term management intensity, showed that fungal:bacterial biomass ratios (measured by phospholipid fatty acid analysis; PLFA) were consistently and significantly higher in the unfertilised than the fertilised grasslands. There was also some evidence that microbial biomass, measured by chloroform fumigation and total PLFA, was higher in the unfertilised than in the fertilised grasslands. It was also found that levels of inorganic nitrogen (N), in particular nitrate-N, were significantly higher in the fertilised than in the unfertilised grasslands. However, microbial activity, measured as basal respiration, did not differ between the sites. A field manipulation trial was conducted to determine whether the reinstatement of traditional management on an improved mesotrophic grassland, for 6 years, resulted in similar changes in the soil microbial community. It was found that neither the cessation of fertiliser applications nor changes in cutting and grazing management significantly affected soil microbial biomass or the fungal:bacterial biomass ratio. It is suggested that the lack of effects on the soil microbial community may be related to high residual fertility caused by retention of fertiliser N in the soil. On the basis of these results it is recommended that following the reinstatement of low-input management, the measurement of a significant increase in the soil fungal:bacterial biomass ratio, and perhaps total microbial biomass, may be an indicator of successful conversion to a grassland system reliant of self-regulation.

Key words Grasslands · Management · Microbial biomass · Bacteria · Fungi · Nitrogen

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Introduction

There is much interest in the development of agricultural land management strategies aimed at enhancing reliance on ecosystem self-regulation, rather than on artificial inputs such as fertilisers and pesticides (Altieri 1991). A key requirement of these systems is the need to manipulate soil biota to increase reliance on natural soil biological processes, such as organic matter decomposition and nutrient mineralization, for provision of available plant nutrients for crop growth. In achieving this goal, it is becoming evident that management regimes that encourage a soil community that bears the closest resemblance to natural ecosystems are most likely to require fewer inputs because of greater reliance on ecosystem self-regulation (Altieri 1991; Wardle et al. 1995; Yeates et al. 1997).

A key feature of natural ecosystems is a soil community that is dominated by fungal pathways of decomposition (Bardgett 1996). In view of this, a recent study of agricultural grasslands by Yeates et al. (1997) proposed that, during a change from intensive to organic grassland farming, an indicator of successful conversion to a system reliant on soil biological processes was a shift in the soil biotic community towards fungal dominance (including fungal-feeding fauna and decomposer fungi).

This proposal was based on the finding that in a range of grassland sites in mid-Wales, soil food-webs of intensive systems were found to be consistently dominated by bacterial pathways of decomposition, whereas soils of long-term organic systems were dominated by fungal pathways (Yeates et al. 1997). An additional feature of the organically managed grassland soils was a high total microbial biomass, relative to soils of intensive grassland systems (Yeates et al. 1997). These findings are broadly consistent with those of other studies of various temperate grassland systems which have shown that both total microbial biomass and fungal:bacterial biomass ratios are higher in long-term unfertilised than in fertilised grassland soils (Bardgett et al. 1993,1997a; Lovell et al. 1995; Bardgett 1996).

It is possible, therefore, that in managed grassland systems the detection of a substantial increase in soil microbial biomass and fungal:bacterial biomass ratio, from a defined baseline, represents a suitable indicator of effective conversion from an intensive system, reliant mainly on fertiliser N for crop nutrition, to a low-input system reliant mainly on self-regulation through soil biological pathways of nutrient turnover. The aim of this study was largely to test the validity of this proposal, first by measuring soil microbial biomass and activity, and fungal:bacterial biomass ratios in a wider range of established meadow grasslands along a gradient of management intensity in northern England, and secondly to determine the effectiveness of various combinations of grazing and cutting treatments, and the cessation of fertiliser applications, imposed for 6 years, on achieving this goal in a previously improved mesotrophic meadow. An additional aim was to determine whether changes in soil microbial biomass and fungal:bacterial biomass ratios were related to concurrent changes in soil mineral nitrogen (N) availability. In particular, we predicted that the fungal:bacterial biomass ratio will be lower in fertilised than in unfertilised soils, since fungi are known to be adversely affected by high amounts of mineral N (Bardgett 1996).

Materials and methods

Study sites and soils

For the study of long-term, established grasslands, three individual fields within four grassland types of the Agricultural Development Advisory Service (ADAS) Environmentally Sensitive Area (ESA) Botanical Monitoring Scheme (ADAS 1996) were selected. The grassland sites were taken to represent a typical gradient of declining management intensity, characterised by long-term reductions in fertiliser use and livestock density, and were classified as: (1) improved meadow (National Vegetation Classification [NVC] MG6a/7b; Rodwell 1992; ADAS Group A); (2) very modified meadow (NVC MG3a/MG7c; ADAS group B); (3) slightly modified meadow (NVC MG3a; ADAS group E); and (4) unmodified meadow (MG3b; ADAS group F). All sites were located within the Yorkshire Dales ESA within approximately 50 km radius of one another, and are typical of other grassland systems within the western United Kingdom. All soils were classified as brown earths and were over Carboniferous limestone. Details of the vegetation and location of the individual sample sites are given in Table 1. Soils were sampled over two days in August 1996. At each site, three 25 m^2 quadrats were randomly located and marked out. Within each quadrat, ten random 3.5-cm-diameter cores (10 cm deep) were taken. Soils from each quadrat were bulked, passed through a 6-mm sieve and stored at 4 °C until use. Soil pH significantly ($P<0.001$) differed between the sites ranging from 5.0 to 6.6; however, there were no consistent trends in soil pH between grassland types. Since the bulk densities of individual grasslands did not differ significantly, all measured values are expressed on a dry weight basis (105 °C for 24 h).

The field experiment was carried out at Colt Park meadows on the Ingleborough National Nature Reserve (National Grid Reference SD 775782), within the Yorkshire Dales ESA, on a *Lolium perenne–Cynosurus cristatus* grassland (NVC MG6; ADAS group A). As with the study of long-term grasslands, the soil at Colt Park was classified as a brown earth soil over Carboniferous limestone. In September 1990 an experimental management regime was introduced in nine 12×36 m contiguous plots arranged in a block in the 2.75 ha meadow. Factorial treatments combinations were applied in a split-split-plot format to determine main and interactive effects of fertiliser, grazing and cutting treatments, and to mimic the preceding intensive management (control), characterised by mineral fertiliser application, autumn and spring grazing and an early annual cutting date (14 June), and the traditional management regime of no mineral fertilisers, autumn and spring grazing and a late cutting dates (21 July). Three

Table 1 Site details and location of the grassland sites from the ADAS Botanical Monitoring Scheme (ADAS 1996) under different long-term management regimes, in northern England

Grassland (ADAS)	Fertiliser N (kg N ha^{-1} $year^{-1}$)	Grassland type (NVC)	Dominant plant species	Location	Altitude (m)
A. Intensive	200–300	MG6a/7b	*Lolium perenne* – *Cynosurus cristatus*	West Allendale	353
				Rawthey	150
				Wharfedale	191
B. Very modified	100–200	MG3a/MG7c	*L. perenne* – *Poa trivialis*	Weardale	290
				Bishopdale	285
				Wensleydale	270
E. Slightly modified	<100	MG3a	*Dactylis glomerata* – *Geranium sylvaticum*	Teesdale	248
				Balderdale	268
				Swaledale	274
F. Unmodified	Zero	MG3b	*Anthoxanthum odoratum* – *Geranium sylvaticum*	Dentdale	160
				Deepdale	260
				Ravenstonedale	280

grazing treatments, replicated three times, were randomly allocated to three 12×36 m (0.043 ha) blocks. The grazing treatments, which allowed free access to the plots whilst grazing the surrounding and adjacent meadows, were (1) autumn and spring grazing, consisting of 30 beef cattle grazed intensively (10.9 cattle ha^{-1}) during four short periods from mid-July to late-August, 10 beef cattle throughout October (0.9 cattle ha^{-1}), 150 cross-bred lambs throughout August (7.5 lambs ha^{-1}) 40 hoggs (sheep less than 1 year old) (2 hoggs ha^{-1}) from October to mid-March, 200 ewes (6.9 ewe ha^{-1}) from mid-April to mid-May lambed in the meadows and pastures; (2) spring grazing, consisting of the above treatment from 1 January until 15 May then no grazing until 1 January next year; and (3) autumn grazing, consisting of treatment 1 from early September to 1 January then no grazing until early-September.

For cutting treatments, each plot was divided along its length into three 12×12 m sub-plots and three cutting date treatments (14 June, 21 July, 1 September) randomly allocated to them. The herbage was cut each year using a mower with a 1.3-m-wide reciprocating cutter bar. The cut herbage was dried on the sub-plot and then removed. Each sub-plot was divided into two 6×12 m sub-sub-plots and two fertiliser treatments (no fertiliser or 25 kg ha^{-1} N plus 12.5 kg ha^{-1} phosphorus and potassium) randomly allocated to them. The fertiliser was an established 20:10:10 NPK product, spread by hand in early May each year. Data on the vegetation responses to the treatments are given by Smith et al. (1997).

Soils were sampled in June 1996 by taking ten random 3.5-cm-diameter cores (10 cm deep) from each of 108 treatment plots. After removal of surface vegetation, soil cores from individual plots were bulked and sieved (<6 mm), and fine material was stored at 4 °C until use. At the time of sampling soil moisture contents ranged from 39% to 49% (dry weight basis) and soil pH ranged from 5.3 to 6.1. There were no consistent trends between the management treatments and soil pH or moisture. As the bulk density of individual treatment plots did not differ significantly, all values are expressed on a dry weight basis.

Total microbial biomass and activity

Microbial extractable C was measured using the fumigation-extraction technique (Vance et al.1987). Organic C in filtered extracts was determined by the acid dichromate oxidation method (Tate et al. 1988). Microbial C (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 measured by determining the rate of CO_2 evolution from samples of 1 g dry weight equivalent soil incubated for 24 h at 30 °C in McCartney bottles 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 Ltd, Series 225, Mk3).

Fungal:bacterial biomass ratios

Measures of fungal:bacterial biomass ratios were made using phospholipid fatty acid analysis (PLFA), a technique previously shown to be sensitive to changes in microbial community structure in grasslands soils subjected to different agricultural management (Bardgett et al. 1996, 1997a,b; Yeates et al. 1997). Briefly, 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 as nmol g^{-1} dry soil.

Fatty acid nomenclature was used as described by Frostegård et al. (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 was used as an indicator of fungal biomass (Federle 1986). The ratio of 18:2ω6:bactPLFAs was taken to represent the ratio of fungal:bacterial biomass in soil (Bardgett et al. 1996; Yeates et al. 1997). Total PLFA was used as a measure of the active microbial biomass; due to their rapid turnover in soils after biomass phospholipids released upon cell death are used as substrate by living micoorganisms and within minutes to hours are metabolized to diglyceride and PO_4^{3-} (White et al. 1979).

Exchangeable NH_4^+-N and NO_3^--N

Soil samples (10 g fresh weight) were shaken with 1 M KCl (100 ml) on an orbital shaker for 1 h. The suspension was filtered through Whatman No. 1 paper and concentrations of NO_3^--N and NH_4^+-N in the extracts were determined colorimetrically (Lovell et al. 1995). Values are expressed as mg 100 g^{-1} dry soil.

Statistical analysis

Data were tested for normality and untransformed data of soil microbial biomass, activity and PLFA were analysed by factorial analysis of variance (ANOVA) and Fisher's least significant difference comparison of means (LSD). For the study of long-term grassland sites, the main factors taken to be grassland type, as defined by ADAS (1996) and individual site. In the field experiment the three factors of the analysis were fertiliser application ($df=1$), cutting date ($df=2$) and grazing ($df=2$). All values are expressed as means ± standard errors. Samples were considered significant if $P<0.05$; however, higher levels of significance were recorded.

Results

Soil microbial biomass and activity

Analysis of soils from the long-term grassland sites (Table 2) showed that soil microbial biomass-C differed significantly between the grasslands (ANOVA, $P<0.05$). However, the only difference revealed by the LSD test was between the improved meadow (ADAS group A) and the very modified meadow (ADAS group B), being higher in the latter. Although not significant, microbial biomass-C was also lower in the improved meadow receiving regular dressings of fertiliser N than in the two unfertilised meadows (ADAS group E and F). Total PLFA, a measure of total active microbial biomass, also significantly differed between sites (ANOVA, $P<0.05$), being lowest in the improved meadows, and highest in the two unfertilised meadows (ADAS groups E and F). However, when differences in total PLFA between individual sites were compared by the LSD test, only the improved meadow (ADAS group A) was found to be significantly different from the slightly modified meadow (ADAS group E). Soil microbial activity, measured as basal respiration, did not differ between the grassland types.

In the field manipulation trial at Colt Park (Tables 3, 4) soil microbial biomass-C was lowest in spring grazed plots, but was unaffected by fertiliser and cutting treat-

Table 2 Differences in soil microbial characteristics and amounts of mineral N (mean ± SE, $n=9$) between different meadow types from the ADAS Botanical Monitoring Scheme (ADAS 1996). Values in a given line with the same letter are not significantly different at $P<0.05$

	Improved Mg6a/MG7b (Group A)	Very modified Mg3a/Mg7c (Group B)	Slightly modified Mg3a (Group E)	Unmodified Mg3b (Group F)	Signi-ficance
Microbial biomass-C (μg C g^{-1} soil)	1189 ± 163^{a}	2101 ± 322^{b}	1673 ± 486^{ab}	1317 ± 98^{ab}	*
Basal respiration (μg CO_2-C g^{-1} soil h^{-1})	1.17 ± 0.06^{a}	1.10 ± 0.10^{a}	0.97 ± 0.13^{a}	0.93 ± 0.07^{a}	NS
Total PLFA (nmol g^{-1} soil)	58 ± 6^{a}	73 ± 10^{ab}	97 ± 8^{b}	83 ± 9^{ab}	*
Bact PLFA (nmol g^{-1} soil)	21.7 ± 2.2^{a}	27.1 ± 5.5^{a}	25.9 ± 3.5^{a}	24.5 ± 4.2^{a}	NS
Fungal PLFA (nmol 18:2ω6 g^{-1} soil)	0.2 ± 0.1^{a}	0.3 ± 0.1^{a}	0.6 ± 0.2^{b}	0.5 ± 0.1^{b}	**
Fungal:bactPLFA (10^2)	0.92^{a}	1.11^{a}	2.32^{b}	2.04^{b}	*
Extractable NH_4^+-N (mg 100 g^{-1} soil)	4.3 ± 1.6^{a}	2.4 ± 0.4^{a}	2.4 ± 0.4^{a}	2.6 ± 0.4^{a}	NS
Extractable NO_3^--N (mg 100 g^{-1} soil)	8.5 ± 1.5^{a}	6.2 ± 1.1^{a}	5.1 ± 1.1^{a}	5.0 ± 0.6^{b}	NS

*, **, ***, $P<0.05$, $P<0.01$, $P<0.001$, respectively; *NS* not significant

Talbe 3 Main effects of grazing (G) ($df=2$), fertiliser (F) ($df=1$) and cutting date (CD) ($df=2$) and their two-way interactions on siol microbial characteristics and N levels after 6 years at Colt Park. Summary statistics from ANOVA. Three-way interactions were not significant and hence are not shown

	Main treatment effects			Two-way interactions		
	G	F	CD	G*F	G*CD	F*CD
Microbial biomass-C	*	NS	NS	NS	NS	NS
Basal respiration	***	*	*	NS	NS	NS
Total PLFA	*	NS	NS	NS	NS	NS
Bact PLFA	***	NS	NS	NS	NS	NS
Fungal PLFA	NS	NS	NS	NS	NS	NS
Ratio fungal:bactPLFA	NS	NS	*	NS	NS	NS
Extractable NH_4^+-H	NS	NS	NS	NS	NS	NS
Extractable NO_3^--N	***	NS	NS	NS	NS	NS

*, **, ***, $P<0.05$, $P<0.01$, $P<0.001$, respectively; *NS* not

ments. Total PLFA was also affected by grazing, again being lowest in the spring grazed plots. Total PLFA was unaffected by fertiliser and cutting date treatments. Microbial activity was significantly affected by grazing (ANOVA, $P<0.001$) and to a lesser degree fertiliser application (ANOVA, $P<0.05$) and cutting date (ANOVA, $P<0.05$). Microbial activity was significantly affected by grazing (ANOVA, $P<0.001$), being highest in plots that were spring grazed. Microbial activity was also affected by fertiliser and cutting treatments (ANOVA, $P<0.05$) being higher in the fertilised and the June cut plot (early cut).

Fungal:bacterial biomass ratios

In the long-term sites, bacterial PLFA was found to be unaffected by grassland management (Table 2). Fungal PLFA, however, was significantly (ANOVA, $P<0.01$) higher in soils of the unimproved meadows (groups E and F) than the fertilised, improved sites (groups A and B). Likewise, the ratio of fungal:bacterial PLFA was significantly (ANOVA, $P<0.05$) higher (>2.0) in the soils of the more unimproved meadows (groups E and F) than the improved sites (groups A and B).

In the field manipulation trial (Tables 3, 4) bacterial PLFAs were significantly (ANOVA, $P<0.001$) affected by grazing, being most abundant in the autumn grazed plots, and least abundant in the spring grazed plots. The other management treatments had no effect on bacterial PLFAs (Tables 3, 4). Although not significant, fungal PLFA, a measure of active fungal biomass, was also found to be least abundant in the spring grazed plots (Tables 3, 4). Fungal PLFA was unaffected by fertiliser application, but was found to be most abundant in the September cut plots (ANOVA, $P<0.05$), and

Table 4 Main treatment effects of grazing, fertiliser, cutting date and seeding on soil microbial characteristics and amounts of mineral N at Colt Park. Values are means with standard errors in parenthesis. Values with the same letter are not significantly different at $P<0.05$

	Grazing ($n=36$)			Fertiliser ($n=54$)		Cutting date ($n=36$)		
	Autumn + Spring	Spring	Autumn	−	+	June	July	Sept
Microbial biomass-C (μg C g^{-1} soil)	2358 ±145^{a}	1923 ±94^{b}	2293 ±145^{ab}	2210 ±101^{a}	2174 ·116^{a}	2196 ±143^{a}	2298 ±119^{a}	2081 ±137^{a}
Basal respiration (μg CO_2-C g^{-1} soil h^{-1})	1.22 ±0.07^{a}	1.6 ±0.07^{b}	1.29 ±0.9^{a}	1.46 ±0.08^{a}	1.29 ±0.05^{b}	1.48 ±0.1^{a}	1.23 ±0.06^{b}	1.43 ±0.07^{ab}
Total PLFA (nmol g^{-1} soil)	116 ±5^{ab}	114 ±4^{ab}	129 ±4^{a}	118 ±4^{a}	121 ±4^{a}	118 ±5^{a}	119 ±4^{a}	121 ±4^{a}
Bact PLFA (nmol g^{-1} soil)	26.8 ±1.2^{ab}	23 ±1.1^{a}	32.6 ±1.8^{b}	26.7 ±1^{a}	28.2 ±1.5^{a}	27.0 ±1.8^{a}	26.9 ±1.4^{a}	28.4 ±1.4^{a}
Fungal PLFA (nmol 18:2ω6 g^{-1} soil)	0.13 ±0.01^{a}	0.11 ±0.01^{a}	0.13 ±0.01^{a}	0.12 ±0.01^{a}	0.13 ±0.01^{a}	0.12 ±0.01^{ab}	0.11 ±0.01^{a}	0.14 ±0.01^{b}
Ratio fungal:bactPLFA (10^2)	0.5^{a}	0.5^{a}	0.4^{a}	0.5^{a}	0.5^{a}	0.5^{ab}	0.4^{a}	0.5^{b}
Extractable NH_4^+-N (mg 100 g^{-1} soil)	3 ±0.3^{a}	3.2 ±0.4^{a}	2.9 ±0.3^{a}	3.2 ±0.3^{a}	2.9 ±0.2^{a}	3.3 ±0.3^{a}	2.6 ±0.3^{a}	3.2 ±0.2^{a}
Extractable NO_3^--N (mg 100 g^{-1} soil)	6.2 ±0.3^{a}	4.8 ±0.2^{b}	6.7 ±0.3^{a}	5.8 ±0.3^{a}	6 ±0.2^{a}	5.9 ±0.3^{a}	6 ±0.3^{a}	5.8 ±0.3^{a}

least in the July cut plots. The fungal:bacterial PLFA ratio was unaffected by management.

Soil mineral-N status

In the long-term sites (Table 2) soil NO_3^--N levels were significantly affected by management, showing a trend of declining levels from the improved (group A) through to the unimproved (group F) meadows. In contrast, levels of NH_4^+-N were unaffected by management. In all meadow types, the mineral N was largely in the form of NO_3^--N.

In the manipulation trial at Colt Park (Tables 3, 4), soil NO_3^--N levels were significantly (ANOVA, $P<0.001$) affected by grazing regime, being lower in the spring grazed plots. Levels of NH_4^+-N were unaffected by management. In all treatments, mineral N was largely in the form of NO_3^--N.

Discussion

Soil microbial communities under long-term grassland management

In this study, measurements were made on soils from a wide range of long-established grasslands which were assumed to have chemical, physical and biological properties that were in 'equilibrium' with their present management and environmental conditions. The particular sites were selected to represent a gradient of management intensity, ranging from an intensively managed, fertilized, high producing grassland (ADAS group A) to an unfertilized traditionally managed hay-meadow (ADAS group F). Intermediate sites were either moderately or slightly modified, receiving intermediate and low levels of fertilizer N application, respectively. Measures of soil microbial communities were made at one sample date only, since previous studies by Bardgett et al. (1997a) have shown that the magnitude of differences in fungal:bacterial biomass ratios between grasslands is seasonally consistent.

The PLFA data from these long-term grassland sites confirm the findings of Yeates et al. (1997) that soil biotic communities of lightly or unfertilised grasslands are characterised by a higher fungal biomass and fungal:bacterial biomass ratio than soils of intensive, fertilised grassland systems. There was also some evidence, although not significant, to suggest that total microbial biomass was higher in the less intensively managed grasslands; measures of microbial biomass were lowest in the intensively fertilised grasslands. However, these changes in microbial communities were not associated with changes in microbial activity, measured as basal respiration. In the present study, fungal:bacterial biomass ratios were 2.32 and 2.04 in the slightly modified and unmodified meadow grasslands, respectively. These values were approximately twice as high as the biomass ratios recorded for the two intensively managed grassland types receiving large quantities of artificial fertiliser inputs. These findings suggest that the soil microbial community of low input grasslands is perhaps larger and has a greater proportion of fungi relative to bacteria than those of more intensively managed systems. In making this assessment it is important to note

that the fatty acid 18:2ω6, used as a measure of fungal biomass, also occurs in high concentrations in plant cells and especially in plant roots (Tunlid et al. 1985). Differences in the abundance of this fatty acid may, therefore, be in part attributed to a change in plant litter input and/or root turnover in the soils from the different treatments. However, since soils were sieved and root material was removed (as much as possible by eye) it is unlikely that fatty acids from plant litter or roots contributed significantly to the changes in PLFA biomass ratios reported here.

The finding that fungal:bacterial biomass ratios were higher in long-term unfertilised than in fertilised grasslands is in agreement with other studies of managed grasslands, under a wide range of environmental conditions (Table 5). This also tends to agree with studies of soil fauna by Siepel (1996) which showed that low-input grasslands were dominated by fungivorous microarthropods, and presumably a fungal dominated microbial community. Since increases in soil fungal:bacterial biomass ratios along natural successional gradients are thought to reflect enhanced ecosystem efficiency and food web complexity (Sakamoto and Oba 1994; Wardle et al. 1995), our findings may provide some support for the thesis that biotic communities that develop in less intensively managed soils have more resemblance to those of natural ecosystems, and hence may require fewer management inputs due to greater reliance being placed on ecosystem self-regulation (Altieri 1991; Wardle et al. 1995; Yeates et al. 1997). On this basis it is suggested that a significant increase in fungal:bacterial biomass, from a defined baseline, may be indicative of successful conversion from an intensive system, previously reliant on fertilisers applications, to a low-input or organic system reliant mainly on soil biological processes of nutrient cycling for plant nutrition. However, to establish such linkages it is recognised that further temporal testing of these indicators and their relationship to soil microbial processes are required before acceptable recommendations or thresholds can be made for use in practical agriculture.

The lower soil microbial biomass and fungal:bacterial biomass ratios recorded in intensively managed, fertilised grasslands are likely to be related to high levels of mineral N availability. In the present study, there was a general trend of increasing soil NO_3^--N levels, and total soil mineral N, as management intensity and fertiliser use increased. This suggestion is consistent with the findings of Fog (1988) who in an extensive review cited 60 papers demonstrating reductions in microbial biomass and activity following the addition of N to decomposing organic matter. Reductions in microbial biomass in fertilised soils have been discussed extensively by Lovell et al. (1995) and have been attributed to changes in substrate quality and root growth (Ennik et al. 1980; Hassink 1992; Lovell et al. 1995), changes in microbial competition and community structure, 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 oxide) in soil (Christie and Beattie 1989). However, in the present study no differences in soil pH between the grassland sites were recorded.

Few studies have attempted to explain differences in fungal:bacterial biomass ratios between fertilised and unfertilised grasslands and the mechanisms at play are likely to be very complex. However, shifts in microbial community structure linked to management have been attributed to changes in resource availability, particularly root exudates (Mawdsley and Bardgett 1997), quantitative and qualitative changes in the input of organic substrates, including plant litter and animal faeces

Table 5 Microbial community responses to changes in the intensity of grassland management

Reference	System	Methodology	Response of soil microbial community
Yeates et al. (1997)	Comparison of long-term organic and conventional grassland systems in lowland mid-Wales	PLFA	Total microbial biomass and fungal:bacterial biomass ratio significantly higher in organic than fertilised grassland soils
Bardgett et al. (unpublished)	Different intensities of long-term grassland management in Devon, SE England	PLFA	Total microbial biomass and fungal:bacterial biomass ratio significantly higher in long-term unfertilised than fertilised grasslands (280 kg N ha^{-1} an^{-1})
Bardgett et al. (1997a)	Adjacent fertilized and unfertilized meadows in northern England and Wales	PFLA, ergosterol analysis	Total microbial biomass, fungal biomass and fungal:bacterial biomass ratio consistently higher in unfertilised than adjacent fertilised upland meadows
Bardgett et al. (1996)	Productive upland grasslands under extensive management (3 years) in South Wales	PLFA	Cessation of fertiliser applications and liming resulted in significant increase in the soil fungal:bacterial biomass ratio
Bardgett et al. (1993)	Established upland grasslands in Dentdale, Cumbria	Membrane filtration	Total fungal biomass (hyphal length) was greater in unfertilised than adjacent fertilised grasslands

(Holland and Coleman 1987; Yeates et al. 1997), changes in the composition of the soil fauna, in particular fungal-feeding animals (Yeates et al. 1997) and differences in soil nutrient availability (Lovell et al. 1995). It is also known that fertilisers adversely affect production of fungal fruitbodies in forest soils (Garbaye and Le Tacon 1982) and vesicular-arbuscular mycorrhizal (VAM) fungi are known to be sensitive to applications of inorganic fertilisers, particularly phosphate (Sparling and Tinker 1978). In the present study, reduced fungal populations in fertilised soils may also have been a reflection of shifts in the species composition of the fungal community (Bardgett et al. 1993). Further studies are required to determine the factors affecting microbial communities, and particularly fungi in fertilised grassland soils. Studies are also required to determine the functional significance of these changes in grassland ecosystems with respect to decomposition and nutrient turnover.

The influence of short-term changes in management on fungal:bacterial biomass ratios

The data from the Colt Park field experiment showed that few changes in management imposed on the improved mesotrophic grassland, for 6 years, had a significant effect on the various measures of soil microbial community structure. In particular, none of the unfertilised management regimes, on previously fertilised grassland, resulted in changes in soil microbial biomass or fungal:bacterial biomass ratios. Likewise, the cessation of fertiliser applications and other changes in management from the preceding intensive regime had no effect on potential soil microbial activity, measured as basal respiration. These findings suggest that over the 6-year period, a shift to a system reliant on self-regulation through soil biological processes had not occurred in these unfertilised grasslands.

The finding that the cessation of fertiliser applications from meadows for 6 years had no effect on the soil microbial community is in agreement with several other studies of temperate pastures in New Zealand (Sarathchandra et al. 1988; Perrott et al. 1992) and the United Kingdom (Bardgett and Leemans 1994). It has been suggested that this lack of effects of removing fertilisers in the short-term is due in part to the long history of fertilisation and accumulation of nutrient reserves in the soil before the various experiments began (Sarathcahndra et al. 1988; Bardgett and Leemans 1994). Therefore, it is likely that the effects of removing fertiliser applications on soil microorganisms will not be evident until accumulated inorganic nutrient reserves have been depleted. In the present study, the finding that changes in management had no effect on soil mineral N would support this thesis, suggesting that nutrient levels at Colt Park were still too high to allow an increase in total and fungal biomass in these soils. Further support for this claim comes from the finding that mineral N levels at Colt Park (9–10 mg N $100\,g^{-1}$ soil) were higher than those found in the long-term slightly modified and unmodified grasslands (treatments 3 and 4; ADAS group E and F) (approximately 7.5 mg N $100\,g^{-1}$ soil; Table 2). They were also higher than those reported by Lovell et al. (1995) for long-term unfertilised pastures in south-west England (mineral N levels in the region of 6–7 mg $100\,g^{-1}$ soil). Further tests on soil nutrient dynamics in the treatments, including P, are required to substantiate these findings.

The lack of effects of changes in management, in particular the cessation of fertiliser application, may also be related to the observation of Smith et al. (1997) that in the same sites there were few changes in the composition of the vegetation over the 6 years. It is generally considered that different plants will exert strong selective pressures on rhizosphere populations since the quantity and variety of compounds lost by rhizodeposition varies from plant to plant (Klein et al. 1988). In addition, inter-species differences in litter quality are also likely to exert a selective pressure on the soil microflora, in particular the decomposer fungi (Donnison 1998). Therefore, it is likely that changes in the soil microbial community, as found in the long-term grasslands, will become apparent only when a significant shift in the composition of the sward has occurred. The view that soil microbial communities are regulated indirectly by changes in vegetation, as opposed to a direct effect of N addition, is supported by the laboratory finding of Bardgett and Shine (1999) that high levels of plant litter diversity, associated with the less intensively managed meadow grasslands (Smith 1997), positively influences microbial biomass in these same grassland soils. Likewise, recent studies by Bardgett et al. (unpublished data) showed that grassland species prevalent in low-input grasslands (e.g. *Agrostis capillaris*) positively influence the soil microbial biomass, whereas those of intensive systems (e.g. *L. perenne*) have little effect on the soil microbial community.

The only treatment that did significantly affect the soil microbial community and soil N dynamics was animal grazing. In particular, it was found that total microbial biomass-C and bacterial biomass (bacterial PLFA) were lowest in the spring grazed plots. The reasons for this effect is not clear; however, it is of interest that levels of NO_3^--N were also lowest in this treatment, presumably due to low levels of nitrification. These effects were also found to be irrespective of whether fertilisers were applied or not, suggesting that changes in soil N dynamics were related directly to the effects of livestock grazing. These effects are presumably due to either differences in the quantity of animal faeces returned to soils under the grazing regimes, or due to indirect effects of grazing regimes on plant nutrient uptake (and hence plant-microbial competition for nutrients) and growth, in particular root growth and exudation patterns. Despite these effects grazing regimes had no effect on soil fungal biomass or fungal:bacterial biomass ratios and hence ecosystem self regulation.

Conclusion

This study of a wide range of meadow grasslands in northern England provides evidence that measures of soil microbial community structure, in particular fungal:bacterial biomass ratios, offer potential as indicators of ecosystem self-regulation in managed grasslands under a shift from intensive to low input or organic farming. However, it is evident from the field manipulation trial that the imposition of management regimes on improved grassland, such as the removal of fertiliser applications or changes in grazing pressure, have little effect on these indicators, and hence reliance on ecosystem self-regulation, over a period of 6 years. This lack of effects is consistent with other studies of temperate grasslands and appears to be related to the long history of fertilisation and accumulation of mineral nutrient reserves in the soil and/or the absence of significant changes in the composition of the vegetation. These results indicate that depletion of mineral nutrients in these soils (or an increase in organic nutrient pools) and an associated change in the composition of the plant community is a necessary prerequisite to the development of a soil biotic community that is better adapted to biological pathways of nutrient cycling and plant nutrition, and hence ecosystem self-regulation.

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