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Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland

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Abstract In this study we examined the effect on soil fungal:bacterial biomass ratios of withholding fertiliser, lime, and sheep-grazing from reseeded upland grassland. The cessation of fertiliser applications on limed and grazed grassland resulted in a reduction in soil pH from 5.4 to 5.1. The cessation of fertiliser applications and liming on grazed grassland resulted in a fall in pH from 5.4 to 4.7, whereas withholding fertiliser and lime and the removal of grazing resulted in a further reduction to pH 4.5. Substrate-induced respiration was reduced in the unfertilised grazed (21%; $P<0.01$) and unfertilised ungrazed (36%; $P<0.001$) treatments. Bacterial substrate-induced respiration and bacterial fatty acids were unaffected by the treatments. The relative abundance of the fungal fatty acid 18:2ω6 increased by 39 and 72% ($P<0.05$) in the limed grazed and unfertilised grazed treatments, respectively. Fungal substrate-induced respiration increased in the limed grazed (18%) and unfertilised grazed (65%; $P<0.05$) treatments. The ratio of 18:2ω6: bacterial fatty acids was correlated with the ratio of fungal:bacterial substrate-induced respiration ($r=0.69$; $P<0.001$).

Key words Phospholipid fatty acids · Substrate-induced respiration · Fungi · Bacteria · Sheep-grazing · Fertiliser · Lime · Microbial biomass · Soil

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Introduction

In a recent study of a sheep-grazed upland grassland, Bardgett and Leemans (1995) showed that withholding management inputs, such as fertilisers, liming, and grazing, resulted in significant reductions in both total microbial biomass and activity in the surface soil. The soil microbial biomass is largely composed of fungi and bacteria, and it is likely that quantitatively they both respond to such management perturbations in different ways. An indication of this was provided in a study by Bardgett et al. (1993), which showed that reductions in management intensity and the short-term cessation of sheep-grazing resulted in increases in total fungal hyphal length (live and dead) in the surface soil.

One objective of the present study was to observe the effect on the fungal:bacterial biomass ratio of changes in upland grassland management that resulted in increases in soil acidity. In particular, this study was designed to test the hypothesis that a reduction in soil pH results in an increase in the relative abundance of fungi concomitant with the reduced microbial biomass observed by Bardgett and Leemans (1995). Another objective was to compare two methods of estimating fungal:bacterial biomass ratios in soil.

Materials and methods

Details of the study site, located at Bronydd Mawr, Wales (370–390 m altitude) are given by Bardgett and Leemans (1995). Briefly, the grassland site was reseeded with *Lolium perenne* in 1966–1967 and limed (5 t ha^{-1}) in 1984. Since then it has received moderate inputs of NPK fertiliser (50 kg N, 25 kg P, 25 kg K ha^{-1} an^{-1}). The soil is a typical brown earth (Milford series) over Devonian sandstone. Four grassland treatments in a randomized complete block design with three replicates were studied: (1) CaNPK, grazed, fertilised (150 kg N, 25 kg P, 50 kg P ha^{-1} $year^{-1}$) and limed (5 t ha^{-1} in 1990); (2) no fertiliser, but limed; (3) no inputs; (4) no inputs and ungrazed. Treatments 1, 2, and 3 were grazed by sheep to a sward height of 4 cm. Treatments 1–4 are referred to as limed NPK grazed, limed grazed, unfertilised grazed and unfertilised ungrazed, respectively. The limed grazed treatment resulted in a reduction in soil pH

from 5.4 (pH of limed NPK grazed) to 5.1, the unfertilised grazed to 4.7, whereas the unfertilised ungrazed treatment resulted in a further reduction to pH 4.5.

Soils for phospholipid fatty acid analysis were sampled on 4 February 1994 by taking 10 random 3.5-cm diameter cores (15 cm deep) from each treatment replicate plot. To measure substrate-induced respiration and fungal:bacterial ratios, the soils were sampled as above on 8 July 1994. Soil cores from each treatment were bulked and sieved (<6 mm), the roots were removed by hand-sorting, and the fine material was analysed immediately.

Lipids were extracted from soil using the procedure described by Frostegård et al. (1991), based on the method of Bligh and Dyer (1959). Extracted fatty acid methyl-esters were analysed on a Hewlett-Packard 5890 II gas chromatograph equipped with a 5972A mass selective detector (MSD II). Separation of the fatty acid methyl-esters was performed on a 30-m db-225 column with an internal diameter of 0.25 mm, a 0.25-μm bonded film of methyl phenyl cyanopropyl silicone (J&W Scientific, Folsom, Calif.), and a flow rate of 0.74 ml min^{-1}. A mass flow controller was used to offset the effect of increasing pressure resistance with increasing gas chromatography column temperature to reduce peak broadening. An Optic temperature programmable injector (Analytical Instruments, Cambridge) was operated as follows: 50°C, increased to 230°C for 1 min at a rate of 16°C s^{-1} with the gas chromatography–mass spectrometry interface at 280°C. The gas chromatography was set initially at a temperature of 80°C, and was then increased to 180°C at 20°C min^{-1}, and to 220°C at 3°C min^{-1}. The mass spectrometer operated from 50 to 350 mass units every 0.2 s.

The relative abundance of individual fatty acid methyl-esters was expressed as the proportion (mol%) of the sum of all fatty acids. 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, 18:1ω7, and cy19:0, were chosen to represent bacterial phospholipid fatty acids (Federle 1986; 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 (Frostegård and Bååth 1995).

Substrate-induced respiration was assayed using the methodology of West and Sparling (1986), except that the rate of glucose amendment was 20 mg ml^{-1} (minimum required for maximal soil respiratory activity). Fungal:bacterial ratios were determined by the selective respiratory inhibition method of Anderson and Domsch (1975) with modifications suggested by West (1986), except that the rates of amendment (mg ml^{-1}) were 20 glucose, 60 streptomycin sulphate, and 40 cyclohexamide. Concentrations of the inhibitors were optimized for maximum selective respiratory inhibition. For both total substrate-induced respiration and antibiotic-inhibited substrate-induced respiration, rates of CO_2 evolution were measured at 30 and 150 min using an Infra Red Gas Analyzer (Analytical Development Co Ltd, Series 225, MK3). Inhibition of substrate-induced respiration by streptomycin sulphate was taken to represent the metabolically active bacterial biomass of the soil, whereas inhibition by cyclohexamide was taken as related to the metabolically active fungal biomass. Fungal and bacterial substrate-induced respiration are expressed as percentages of total substrate-induced respiration and as the ratio of fungal:bacterial substrate-induced respiration.

Results

The results of the fatty acid and substrate-induced respiration analyses are shown in Table 1. The relative abundance of the fungal fatty acid 18:2ω6 increased by 39 and 72% ($P<0.05$) in the limed grazed and unfertilised grazed treatments, respectively. However, the unfertilised ungrazed treatment had no effect on the fatty acid 18:2ω6. The relative abundance of bacterial fatty acids was unaffected by the treatments. The ratio of 18:2ω6 bacterial fatty acids increased by 83% ($P<0.05$) in the unfertilised grazed treatment. The limed grazed treatment also resulted in an increase in the ratio of 18:2ω6 bacterial fatty acids, but this effect was not significant. The ratio was unaffected by the unfertilised ungrazed treatment.

The cessation of fertiliser applications and liming, with and without grazing, resulted in a significant reduction in total substrate-induced respiration. In the unfertilised grazed treatment, total substrate-induced respiration was reduced by 21% ($P<0.01$), whereas in the unfertilised ungrazed treatment total substrate-induced respiration was reduced by 36% ($P<0.001$). The limed grazed treatment had

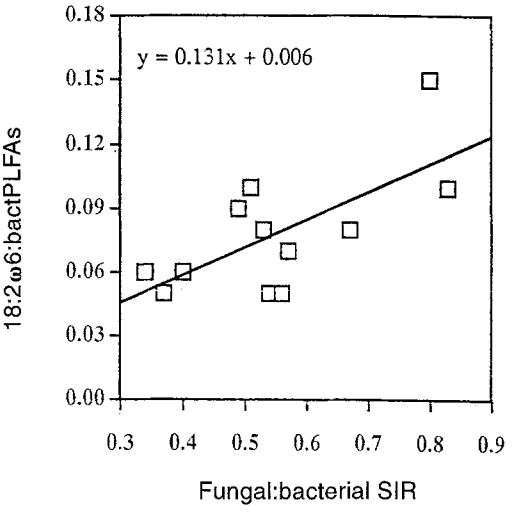

Fig. 1 Relationship between the ratio of fungal:bacterial substrate-induced respiration (*SIR*) and the ratio of 18:2ω6 bacterial phospholipid fatty acids (*bactPLFAs*) in differently managed upland grassland soils $n=12$, $r=0.69$; $P<0.001$)

Table 1 Effects of cessation of fertilizer inputs, liming, and grazing on the relative abundance of bacterial phospholipid fatty acids (*bactPLFAs*), the fungal PLFA 18:2ω6, the ratio 18:2ω6:bactPLFAs, total substrate-induced respiration (*SIR*), fungal and bacterial SIR, the ratio fungal:bacterial SIR, and soil pH in differently managed upland grassland soils (Nil no fertiliser or lime) Significant treatment differences were detected using analysis of variance (Genstat) and Student's *t*-test: * $P<0.05$, ** $P<0.01$, *** $P<0.001$, versus fertilised limed and grazed

Treatment	BactPLFAs (% total)	Fungal PLFA (18:2ω6) (% total)	Ratio18:2ω6: bactPLFAs	Total SIR (μl CO_2C g^{-1} h^{-1})	Fungal SIR (% total)	Bacterial SIR (% total)	Ratio fungal: bacterial SIR	Soil pH
CaNPK, grazed	43.32±2.78	2.62±0.59	0.06	17.69±1.41	19.98±5.43	40.52±3.34	0.49	5.4
Ca, grazed	42.26±1.13	3.65±0.53	0.09	16.66±0.38	23.60±3.95	44.93±5.57	0.52	5.2
Nil, grazed	40.60±0.50	4.51±1.38*	0.11**	13.98±0.79**	32.92±3.95	42.73±0.66	0.77**	4.7
Nil, ungrazed	42.53±3.60	2.46±0.40	0.06	11.37±0.40***	17.64±11.38	38.81±6.23	0.43	4.5

no effect on total substrate-induced respiration. Fungal substrate-induced respiration (% of total) significantly ($P<0.05$) increased in the unfertilised grazed treatment, but was unaffected by the other treatments (limed grazed and unfertilised ungrazed). Bacterial substrate-induced respiration (% of total) was unaffected by the treatments. The ratio of fungal:bacterial substrate-induced respiration increased by 47% ($P<0.01$) in the unfertilised grazed, but was unaffected by the other treatments. Figure 1 shows that the ratio of 18:2ω6 bacterial fatty acids was significantly correlated with the ratio of fungal:bacterial substrate-induced respiration ($n=12$, $r=0.69$; $P<0.01$).

Discussion

In agreement with findings reported by Bardgett and Leemans (1995), this study shows that the microbial biomass, measured as total substrate-induced respiration, was significantly reduced following the removal of fertiliser applications and liming with and without sheep-grazing, while the removal of fertiliser applications from limed and sheep-grazed grassland had no significant effect on the total microbial biomass. These reductions are likely to have been due in part to reductions in soil pH in these treatments, since several studies have shown that low microbial biomass values are associated with acid soils (Anderson and Domsch 1993).

The relative abundance of soil bacteria, measured as bacterial substrate-induced respiration and bacterial fatty acids were unaffected by any of the treatments. In contrast, the relative abundance of soil fungi, measured as the fungal fatty acid 18:2ω6 and fungal substrate-induced respiration, increasesd in the limed grazed and unfertilised grazed treatments. These changes were also reflected in an increase in the ratio of 18:2ω6: bacterial fatty acids and fungal:bacterial substrate-induced respiration in both the limed grazed and unfertilised grazed treatments, suggesting that the proportion of fungi relative to bacteria increases as management becomes less intensive (particularly after the cessation of fertiliser and lime) on grazed upland grassland. These findings appear to be in agreement with those by Bardgett et al. (1993), who showed that reductions in management intensity increased soil acidity, and resulted in an increase in the total fungal hyphal length (live and dead) in the surface soil.

However, withholding fertiliser, lime and sheep-grazing, which resulted in a large reduction in soil pH, had no effect on the relative abundance of fungi, measured either as fungal substrate-induced respiration or as fungal phospholipid fatty acids, or the ratios of fungal:bacterial biomass. This probably suggests that the increase in total hyphal abundance (using the membrane-filtration technique) observed by Bardgett et al. (1993), following the removal of sheep-grazing, may be a result of lower decomposition rates of dead fungal mycelium in the more acidic ungrazed grasslands. Since the phospholipid component of cell membranes are supposed to disappear rapidly after cell death (Tunlid et al. 1985) the method used in this study would not have detected such an accumulation of dead fungal hyphae. The selective inhibition technique is furthermore likely to assess only the active component of the soil microflora (Wardle et al. 1994).

Since the unfertilised ungrazed treatment was the most acidic (pH 4.5), these findings refute the original hypothesis that increases in the proportion of fungi relative to bacteria are related to reductions in soil pH. This supports the findings of Anderson and Domsch (1975), who reported that changes in ratios of fungal:bacterial biomass were not related to soil pH, and suggests that some other factor was responsible for the changes in microbial community structure observed in the limed grazed and unfertilised grazed treatments of the present study, and in other studies of acid soils (Frostegård and Bååth 1995). The finding that changes in grassland management alter microbial community structure in favour of soil fungi only when sheep-grazing is maintained might suggest that the input of animal faeces to soil may have an important role in encouraging the growth of fungi in such acid soils. Therefore, in the grazed grassland the increase in the proportion of fungi relative to bacteria may have been partly due to the presence of coprophilous species on sheep dung, absent in the ungrazed treatment.

It was recently reported that ratios of fungal:bacterial fatty acids can be used as an index of the relative abundance of fungi and bacteria in soils (Federle 1986; Frostegård and Bååth 1995). The finding that the ratio of 18:2ω6 bacterial fatty acids was significantly correlated with the ratio of fungal:bacterial substrate-induced respiration in these differently managed upland grassland soils further supports the use of these two measurements to estimate fungal:bacterial biomass ratios in soils. However, neither of the measurements give absolute values for biomass ratios since there are no conversion factors for phospholipid fatty acids or the inhibition of substrate-induced respiration. Therefore, changes in the ratio can only be used to indicate shifts in community structure.

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References

Anderson JPE, Domsch KH (1975) Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils. Can J Microbiol 21:314–322

Anderson TH, Domsch KH (1993) The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soil. Soil Biol Biochem 25:393–395

Bardgett RD, Leemans DK (1995) The short-term effects of cessation of fertiliser applications, liming and grazing on microbial biomass and activity in a reseeded upland grassland soil. Biol Fertil Soils 19:148–154

Bardgett RD, Frankland Juliet C, Whittaker JB (1993) The effects of agricultural practices on the soil biota of some upland grasslands. Agric Ecosyst Environ 45:25–45

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Federle TW (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

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

Frostegård Å, Tunlid A, Bååth E (1991) Microbial biomass measured as total lipid phosphate in soils of different organic content. J Microbial Methods 14:151–163

Frostegård Å, Bååth E (1995) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol Fertil Soil (in press)

Tunlid A, Baird BH, Trexler MB, Olsson S, Findlay RH, Oldham G, White DC (1985) Determination of phospholipid ester-linked fatty acids and poly β-hydroxybutyrate for the estimation of bacterial biomass and activity in the rhizosphere of the rape plant *Brassica napus* (L.). Can J Microbiol 31:1113–1119

Wardle DA, Yeates GW, Watson RN, Nicholson KS (1994) Response of soil microbial biomass and plant litter decomposition to weed management strategies in maize and asparagus cropping systems. Soil Biol Biochem 25:857–868

West AW (1986) Improvements of the selective respiration inhibition method to measure eukaryote:prokaryote ratios in soil. J Microbiol Methods 5:125–138

West AW, Sparling GP (1986) Modifications of the substrate-induced respiration method to permit measurement of microbial biomass in soils of differing water contents. J Microbiol Methods 5:177–189