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Short communication

Determinants of fungal growth and activity in botanically diverse haymeadows: effects of litter type and fertilizer additions

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Previous studies have shown that intensification of management of botanically diverse haymeadows, and principally the application of nitrogenous fertilizers, result in changes in the structure of their soil microbial communities (Bardgett et al., 1997; Bardgett and McAlister, 1999; Donnison et al., 1999). In a study of a range of submontane haymeadow sites in northern England and north Wales, Donnison et al. (1999) showed that although the size of the soil microbial biomass did not change in response to intensification of management, there were significant reductions in amounts of fungal biomass and the proportion of fungi relative to bacteria within the soil microbial community. Since these two microbial groups make up some 90 to 95% of the total heterotrophic metabolism of soils (Petersen and Luxton, 1982), it should be investigated whether such changes will have functional implications with respect to the efficiency of organic matter decomposition and nutrient cycling in meadow grasslands.

The mechanisms responsible for such changes in soil microbial communities remain largely unknown. However, it has been suggested that changes, in particular the reduction in fungal biomass, are related to direct effects of inorganic fertilizers on the fungal community or to an indirect effect of changes in plant community productivity and composition, leading to quantitative and qualitative changes in litter inputs to soil (Donnison, L.M., 1998. Unpub. Ph.D. thesis, University of Wales; Donnison et al., 1999). Although some studies have shown that fungal communities can be directly influenced by inorganic fertilizer additions (e.g. Arnebrandt et al., 1990), most evidence tends to point to the latter suggestion, that changes in plant community productivity and species composition, in particular changes in litter type, are responsible for these changes in the soil microbial community (Wardle and Lavelle, 1997; Wardle et al., 1997; Bardgett and Shine, 1999). Despite this recognition, no studies have examined this question in relation to decomposer fungi, and in particular those of botanically diverse haymeadows where the fungi make up such a large proportion of the soil microbial community (Bardgett et al., 1997; Donnison, loc cit; Donnison et al., 1999). In this study we specifically tested the hypothesis of Donnison et al. (1999) that changes in litter type, associated with management intensification, are a greater determinant of fungal growth and activity than are fertilizer additions. To test this hypothesis, we used culture- and microcosm-based studies of the growth and activity of commonly isolated fungi of haymeadows (Donnison et al., 1999) under different litter and fertilizer treatments, similar to those encountered in the field.

Six commonly isolated fungal inhabitants of haymeadow litter and soil were selected for the study (Donnison et al., 1999). These were *Fusarium culmorum* (W.G.Sm.) Sac., *Acremonium* sp., *Phoma* sp., *Phoma exigua* Desm., *Mucor* sp. and *Absidia* sp. The isolation frequencies and functional capabilities of these fungi are given by Donnison et al. (1999). The effect of inorganic fertilizers on fungal growth rates was determined in laboratory culture experiments.

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Table 1
Mean ($n = 4$) linear extension rate (mm d^{-1}) of fungi grown on 5 media: 1 (intensive soil medium), 2 (traditional medium), 3 (traditional medium and NPK 250 kg ha^{-1} yr^{-1} equivalent), 4 (NPK 500 kg ha^{-1} yr^{-1} equivalent), 5 (NPK 1500 kg ha^{-1} yr^{-1} equivalent)[a]

Species	Media					ANOVA *F*-value
	1	2	3	4	5	
F. culmorum	7.8ab (0.03)	7.0a (0.06)	9.5b (0.02)	9.3b (0.03)	9.1b (0.04)	7.8**
Phoma sp.	3.6 (0.00)	3.6 (0.00)	3.5 (0.01)	3.6 (0.01)	3.8 (0.01)	2.2 n.s.
Acremonium sp.	4.4 (0.00)	4.3 (0.00)	4.2 (0.01)	4.3 (0.01)	4.5 (0.01)	2.2 n.s.
P. exigua	3.6 (0.00)	3.6 (0.01)	3.6 (0.01)	3.6 (0.01)	3.6 (0.01)	0.03 n.s.
Absidia sp.	3.8a (0.01)	4.3ab (0.10)	4.7b (0.01)	4.6b (0.03)	4.8b (0.05)	7.1**
Mucor sp.	9.0ac (0.02)	9.7abc (0.02)	8.8a (0.01)	10.0bc (0.03)	9.8bc (0.02)	5.7**

[a] Standard error of the mean is given in parenthesis. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ or nonsignificant (n.s.) medium differences. Extension rates denoted by different letters are significantly (Tukey's H.S.D.) different within each type of media.

Soils cores (3.5 cm dia, 10 cm deep) were collected from adjacent intensively and traditionally managed haymeadows at a site in north Wales, near Dolgellau (National Grid Reference NY 803225), in October 1996. Details of this site, its vegetation and the management regimes of both meadows have been given by Donnison et al. (1999). Soils were bulked, sieved (6 mm^2 mesh) and soil extract agars prepared as described by Gams et al. (1987). Soil extract agar was amended with four different amounts of NPK inorganic fertilizers. Final concentrations of NH_4NO_3, KH_2PO_4 and KOH were: 0, 0 and 0 g l^{-1}; 0.9, 0.24 and 0.06 g l^{-1}; 1.8, 0.48 and 0.12 g l^{-1}; 5.4, 1.44 and 0.36 g l^{-1}. These concentrations were approximately equivalent to field application rates of 0, 250, 500 and 1500 kg NPK ha^{-1} yr^{-1}, respectively. Petri dishes containing the four nutrient rates in soil extract agar were inoculated centrally with 10 mm-dia plugs of agar and mycelium taken from the growing margins of fungal colonies growing on 2% potato dextrose agar (four replicates per fungus per agar type). Plates were then sealed with parafilm and incubated in the dark at 15°C. Colony diameters were measured at 24 or 48 h intervals (depending on their growth rates). For each fungus treatment combination, mean colony extension was plotted against time since inoculation. Mean rates of linear extension were then estimated from the linear portion of the growth curves.

Microcosm experiments were carried out to determine the effects of litter type and inorganic fertilizer addition on fungal activity. Soil was collected from the unfertilized, traditionally managed hay meadow (from now on referred to as traditional soil) at the Dolgellau field site in November 1996. At the same time a bulk sample of plant litter was collected from a traditionally and adjacent intensively managed meadow. Glass bottles (250 ml) were filled with 50 g dry weight equivalent of traditional soil. Litter (0.5 g, cut up into 1 cm lengths) from either traditionally or intensively managed meadows was placed in a layer on top of the soil in each bottle. Microcosms were then sterilized by gamma irradiation (29 kGy). After irradiation some microcosms were sampled and litter and soil plated onto 2% PDA to check sterility. Irradiated microcosms were stored for 21 d to allow free enzyme activities to subside.

High or low doses of NPK inorganic fertilizer were then added to some microcosms. The high fertilizer dose was 432.0, 28.8 and 115.2 mg per bottle of NH_4NO_3, KH_2PO_4 and KOH, respectively (approximately equivalent to a field application rate of 1500 kg NPK h^{-1} yr^{-1}). The low dose was 72.0, 4.8 and 19.2 mg per bottle of NH_4NO_3, KH_2PO_4 and KOH, respectively (approximately equivalent to a field application rate of 250 kg NPK h^{-1} yr^{-1}). This generated 6 treatments: (1) traditional litter with no fertilizer; (2) traditional litter with a low dose of fertilizer; (3) traditional litter with a high dose of fertilizer; (4) intensive litter with no fertilizer; (5) intensive litter with a low dose of fertilizer; and (6) intensive litter with a high dose of fertilizer. Microcosms were inoculated with 10 mm dia plugs of agar and mycelium taken from the growing margins of 14 d old fungal colonies growing on unamended soil extract agar (three replicates per fungus per treatment). Some microcosms were not inoculated. These served as controls. Microcosms were sealed hermetically with sterile rubber bungs (Subaseals) and incubated in the dark at 16°C. Every 3 d during the first 21 d, and 7–10 d thereafter, the headspace in each microcosm was sampled and CO_2 concentrations using an infra red gas analyzer (IRGA; Analytical Development Co. Ltd, Series, mk 3) calibrated with a known standard before and after each set of measurements. Cumulative production of CO_2 in each microcosm was then calculated. When CO_2 concentrations in microcosms exceeded 10%, they were purged with filter sterilized air (filter pore size 0.2 μm) for 1 min. Immediately after purging CO_2 concentrations in microcosms were measured to determine how much CO_2 remained. At

Table 2
Mean ($n = 9$) total carbon dioxide production (ml) from each fungus (*F.culmorum*, sp.3, *Acremonium* sp., *P.exigua*, *Mucor* sp. 1 and *Absidia* sp) on either intensive (I) or traditional (T) litter soil microcosms. Mean ($n = 6$) carbon dioxide production (ml) from each fungus at three different rates of NPK addition: 0 (0 NPK), 1 (250 kg ha^{-1} yr^{-1} equivalent), 2 (1500 kg ha^{-1} yr^{-1})[a]

Species	Litter		NPK			ANOVA *F*-value		
	I	T	0	1	2	L	NPK	L∗NPK
F. cumorum	172 (18)	152 (20)	153 (22)	166 (19)	168 (18)	1.8 n.s.	0.4 n.s.	1.7 n.s.
Phoma sp.	159 (20)	195 (18)	194 (24)	166 (22)	171 (19)	5.7*	3 n.s.	1.5 n.s.
Acremonium sp.	191 (20)	183 (19)	215a (15)	177ab (15)	168b (17)	0.2 n.s.	4.8*	2.5*
P. exigua	193 (21)	187 (20)	191ab (17)	218a (19)	161b (11)	0.1 n.s.	6.4**	2.3 n.s.
Mucor sp.1	121 (24)	115 (19)	137a (16)	140a (13)	76b (8)	0 n.s.	1384***	464**
Absidia sp.	106 (20)	125 (18)	99 (8)	119 (24)	129 (21)	1.5 n.s.	1.3 n.s.	1.1 n.s.

[a] Standard error of the mean is given in parenthesis. ANOVA marking significant $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ or nonsignificant (n.s.) *F*-values for litter (L), NPK and, NPK litter interaction (L∗NPK). Carbon dioxide production values denoted by different letters are significantly (Tukey's H.S.D.) different within each type of litter or NPK rate.

the end of the experiment samples of soil and litter from each microcosm, including controls, were plated onto 2% PDA to determine whether they had been contaminated with other microorganisms. Further samples of litter and soil from each microcosm were ground together (blender full speed 1 min) for pH measurements (Allen, 1989). The treatment effects on each fungus were assessed by ANOVA analysis.

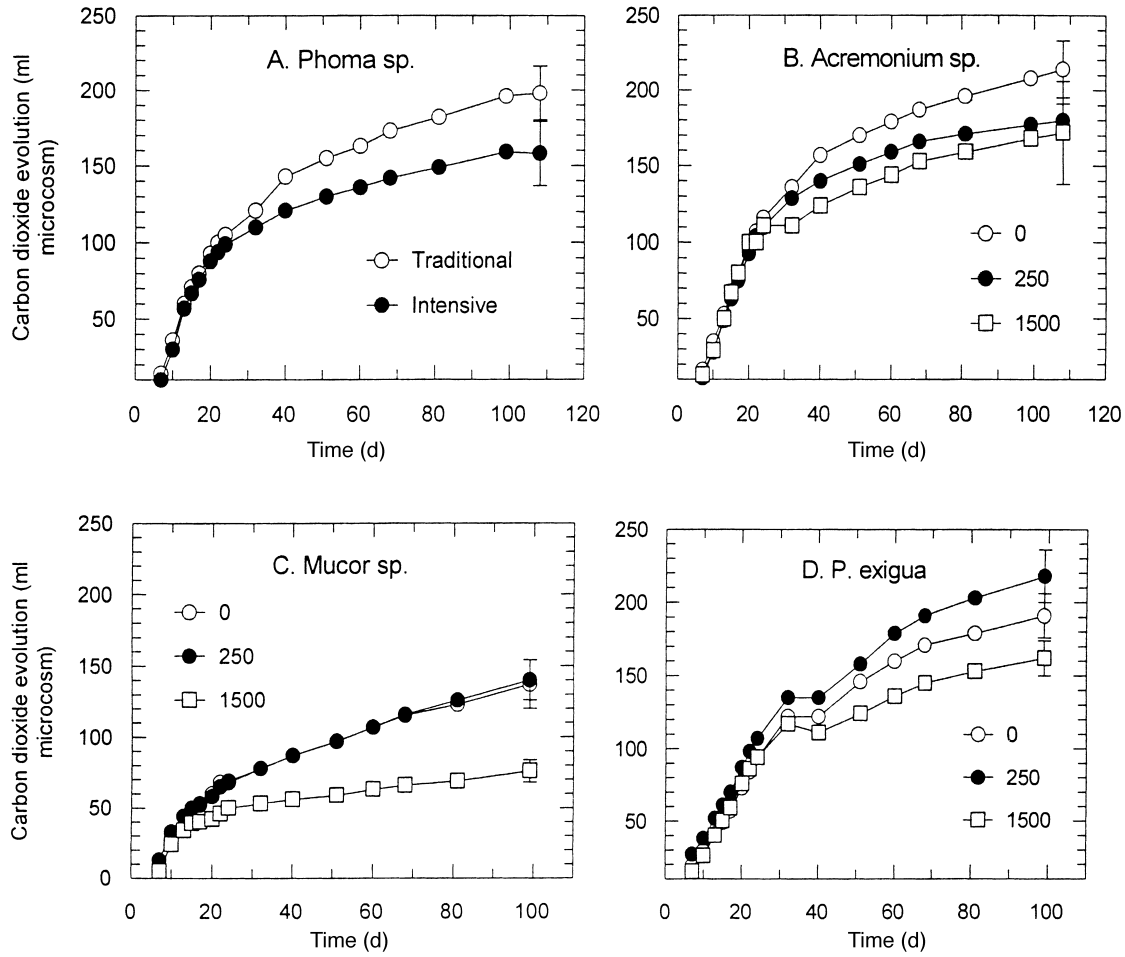

Fig. 1. Cumulative carbon dioxide evolution (ml) of fungi over time from microcosms varying in litter (traditional or intensive) and NPK additions (0, 250 or 1500 kg ha^{-1} yr^{-1} equivalent), from *Phoma* sp. (A), *Acremonium* sp. (B), *Mucor* sp. (C) and *P. exigua* (D). The maximum standard error bar for any time point or treatment is given.

Table 3
Mean ($n = 9$) microcosm pH for each fungus (*F. culmorum*, sp.3, *Acremonium* sp., *P. exigua*, *Mucor* sp. 1 and *Absidia* sp) on either intensive (I) or traditional (T) litter soil microcosms. Average ($n = 6$) pH from each fungus at three different rates of NPK addition: 0 (0 NPK), 1 (250 kg ha^{-1} yr^{-1} equivalent), 2 (1500 kg ha^{-1} yr^{-1})[a]

Species	Litter		NPK			ANOVA *F*-value		
	I	T	0	1	2	L	NPK	L∗NPK
F. culmorum	5.5 (0.05)	5.5 (0.05)	5.6 (0.04)	5.5 (0.06)	5.5 (0.04)	0.4 n.s.	2.7 n.s.	2.4 n.s.
Phoma sp.	5.5 (0.05)	5.5 (0.06)	5.6a (0.03)	5.5b (0.05)	5.4c (0.03)	1.6 n.s.	19***	14***
Acremonium sp.	5.7 (0.20)	5.8 (0.14)	6.1a (0.03)	5.7b (0.05)	5.5c (0.05)	0.4 n.s.	105***	118***
P. exigua	5.6 (0.10)	5.6 (0.06)	5.7a (0.04)	5.7a (0.09)	5.4b (0.03)	0.04 n.s.	12***	7**
Mucor sp.	5.4 (0.10)	5.4 (0.08)	5.5a (0.03)	5.4ab (0.09)	5.3b (0.07)	0.1 n.s.	6*	4*
Absidia sp.	5.3 (0.06)	5.4 (0.02)	5.4 (0.04)	5.3 (0.02)	5.3 (0.07)	2.5 n.s.	2.5 n.s.	2 n.s.

[a] Standard error of the mean is given in parenthesis. At the end of the table are *F*-values from the ANOVA marking significant $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ or nonsignificant (n.s.) difference for litter (L), NPK and NPK litter interaction (L∗NPK). pH values denoted by different letters are significantly (Tukey's H.S.D.) different within each type of litter or NPK level.

Generalized linear model procedures were adopted. Differences between treatment means were assessed by Tukey tests. SAS software (SAS Institute, Cary, NC) was used for all statistical analysis.

Inorganic fertilizer application had a greater direct effect on the growth and activity of some commonly isolated fungi, than did changes in litter type. However, the effects of inorganic fertilizer additions on fungi were highly species-specific; our soil extract culture experiments showed that rates of fertilizer application common to intensively managed meadows significantly increased mycelial growth rates of *F. culmorum* and *Absidia* sp. (by ca. +30 and +10%, respectively; Table 1), but significantly decreased mycelial growth rates of *Mucor* sp. on media amended with the lowest amounts of fertilizer (by ca. −10%; Table 1). In microcosm studies, we also found that most of the variation was a result of the effect of fertilizer addition (Table 2). Metabolic activities of *Acremoniu*m sp., *P. exigua* and *Mucor* sp. 1 were significantly affected by the addition of doses of inorganic fertilizer to microcosms (Table 2; Fig. 1). Activity of *Acremonium* sp. was inhibited (ca. −20% decrease in total CO_2 production) at both low and high doses of inorganic fertilizer. Activity of *P. exigua* was enhanced (ca. +15% increase in total CO_2 production) at low doses, but inhibited at high doses (ca. −15%) of fertilizer. Activity of *Mucor* sp. was unaffected by low doses but markedly inhibited (ca. −45% reduction in CO_2 production) by high doses.

Such effects may go some way towards explaining the shifts in fungal community structure that accompany intensification of haymeadow management (Donnison et al., 1999). For example, the fungi that showed a positive response to fertilizer, such as *F. culmorum* and *P. exigua,* have been found to be more commonly isolated from intensively managed haymeadows, whereas, *Acremonium,* which was negatively affected by fertilizer, is less commonly isolated from these sites (Donnison et al., 1999). Despite this, the inconsistent nature of the effects with respect to different fungal species suggests that, at the field scale, it is unlikely that inorganic fertilizer applications would have any predictable detrimental effect on the fungal community. Thus, on the basis of our data, the observed field-scale reductions in fungal biomass and changes in fungal community structure resulting from intensive management of haymeadows, and other grasslands (Bardgett et al., 1997; Bardgett and McAlister, 1999; Donnison et al., 1999), can probably not be explained by direct effects of inorganic fertilizers on the fungal populations.

These findings are in agreement with other studies which report that the effects of nitrate, ammonium and urea on the activity of soil microorganisms is species dependent and highly variable (Park, 1975; Jakubczyk et al., 1976; Janshekar et al., 1982; Fog, 1988). Inorganic fertilizers may inhibit or stimulate the growth and activity of certain species of fungi because nitrate or ammonium ions inhibit or promote the activity of specific extracellular enzymes. For example, carboxymethylcellulases and peroxidases are inhibited by the presence of inorganic N, whereas the activity of cellulases is promoted by this form of N (Fog, 1988). Alternatively, inorganic fertilizers may supply a nutrient that is limiting to some fungi and hence promote their growth and activity (Dickinson, 1974). For example, *Mucor* spp. are limited in their use of P, as they are often incapable of using phytate, which can comprise up to 60% of the organic pool of P (Jennings, 1995). We found that this species was more commonly isolated in an intensively-managed haymeadow that received fertilizer P and had a higher soil concentration of total and inorganic P, than in an adjacent traditionally managed haymeadow (Donnison et al., 1999).

It is possible that inorganic fertilizers may indirectly influence fungal populations through acidification of

soils. Ammonium salts applied to litters and media may have been transformed to nitrate with the excretion of excess H^+ ions by fungi (Fog, 1988). Resultant decreases in soil pH may be detrimental to some soil fungi and, to some extent, might partly explain the reduced fungal activity in some of our microcosms; in our microcosm experiment the addition of fertilizers significantly reduced the pH of soils and litters colonized by *Phoma* sp., *Acremonium* sp., *P.exigua* and *Mucor* sp. (Table 3). Although in general decreases in fungal activity were accompanied by decreases in soil and litter pH, these two effects were inconsistent and not quantitatively related (Tables 2 and 3), suggesting that changes in pH, due to fertilizer application, were not having a major effect on the commonly isolated fungi used in our study.

Although inorganic fertilizer application accounted for most of the variation in fungal growth and activity (Table 2), we present some evidence that changes in fungal activity were related to changes in litter type. One of the fungi, *Phoma* sp., showed a significant response to litter type, producing more CO_2 (ca. +20%; Fig. 1) when grown on the more diverse litter of the traditionally managed haymeadow than on the less diverse litter taken from the intensively managed meadow. This finding relates well to the field observation that this fungus was more commonly isolated from traditional haymeadows, than from adjacent intensively managed sites (Donnison et al., 1999) and suggests that, as with fertilizer effects, responses of decomposer fungi to changes in litter type are highly species-specific. The general lack of effects of litter types of contrasting diversity on fungal growth and activity, and presumably litter decomposition, might suggest that differences in the species diversity of these haymeadow litters will have little predictable effect on soil biological properties. This thesis is broadly consistent with laboratory studies of litter mixtures of grassland plants which show that differences in the species diversity of plant litters have little predictable effect on soil biological properties of grasslands, such as the biomass and activity of the decomposer community (Wardle et al., 1997; Bardgett and Shine, 1999).

In conclusion, our data suggest that fertilizer amendments have greater potential to influence the size and structure of the fungal community than do changes in litter type associated with intensive management. However, the effects of fertilizers on fungi are not unidirectional, being highly species-specific and therefore, unlikely to account for the large differences in fungal biomass that are commonly observed in the field. We provide evidence that changes in litter type account, only in a small part for some of the observed differences in fungal community structure, but not the reduction in fungal biomass commonly found in intensified meadows. In making this conclusion, however, we stress that the data from this experiment should not be generalized broadly; we considered only two contrasting litter types, albeit representative of the field, and only examined the responses of a few commonly isolated fungi, and not the nonculturables which are likely to make up the bulk of the fungal community of these grasslands.

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