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The effect of collembolan grazing on fungal activity in differently managed upland pastures: A microcosm study

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Abstract. Laboratory microcosms containing litter from three tussock grasslands were used to assess the impact of grazing by a collembolan, *Onychiurus procampatus*, on the abundance, nutrient release, and respiration of the saprotrophic fungus, *Phoma exigua*. The fungal biomass and respiration rate were significantly reduced only when Collembola were present in excess of mean field densities but perhaps more typical of spatial aggregations in the soil. A high efficiency of nutrient immobilization by *P. exigua* was demonstrated but nutrient release was not significantly affected by the fauna. Problems associated with the use of microcosms in the simulation of field conditions are discussed.

Key words: Fungal biomass – Respiration – Nutrient cycling – Microcosms – Grass litter – *Onychiurus procampatus* – *Phoma exigua* – Collembola

It is widely accepted that the grazing activities of soil fauna have an important regulatory effect on the processes of decomposition and nutrient cycling, through their influence on the activities and composition of soil microbial communities (Seastedt 1984; Visser 1985; Moore et al. 1988; Lussenhop 1992). Field populations of certain soil fauna feed preferentially on fungal hyphae (Anderson and Healey 1972; Newell 1984a, b). During their digestion and metabolism, nutrients such as N and P are mineralized and excreted into the soil environment (Anderson et al. 1981) and made available for plant uptake (Clarholm 1985; Ingham et al. 1985). In addition, the soil fauna may indirectly influence nutrient availability by stimulating or reducing microbial biomass and activity (Hanlon 1981; Faber et al. 1992).

In a field study of upland grassland sites, Bardgett et al. (1993a) hypothesized that observed, simultaneous trends of decreasing numbers of a fungal-feeding collembolan *O. procampatus* Gisin, and increasing abundance of fungal mycelium, along a gradient of reduced sheep management intensity, could be explained in part by interactions between the biota. Follow-on studies from the same sites (Bardgett et al. 1993b) showed that the abundant collembolan *O. procampatus* fed preferentially on a commonly isolated fungus. *P. exigua* Desm. In the present study, two microcosm experiments were carried out to observe whether the changes in fungal biomass identified by Bardgett et al. (1993a) could be explained in part by the grazing activities of the collembolan *O. procampatus* on the fungus *P. exigua*. The impact of this interaction on N and P release and retention and on respiration of the test fungus was also studied.

Materials and methods

The soil collembolan *O. procampatus* and the fungus *P. exigua*, derived from the field sites, were used in two experiments.

In April 1990, grass litter was collected from three adjacent upland grassland sites under different intensities of farm management, in lower Dentdale, south-east Cumbria (national grid reference SD 6590). The three grassland sites, described in detail by Bardgett et al. (1993a), were: (1) heavily grazed (5–8 ewes ha^{-1}) *Agrostis–Festuca* spp. grassland which was limed, and supplied with annual applications of NPK fertilizer (40 kg ha^{-1}; 20:10:10 NPK); (2) moderately grazed (3–5 ewes ha^{-1}) *Agrostis–Festuca* spp. grassland which was limed but not fertilized; and (3) lightly grazed (1 ewe ha^{-1}) *Nardus* sp. dominated grassland, neither limed nor fertilized. The C:N ratios of the litter from these three grassland treatments were 26, 28, and 36, respectively. The C:P ratios were 241, 358, and 565, respectively.

Grass litter from each site was air-dried and chopped into lengths of less than 1 cm. Samples of 3 g (air-dry weight) were placed in heat-sealed plastic bags and sterilized by γ irradiation (30 kGy), and stored for 2 weeks to allow free enzyme activity to subside. Random subsamples of litter were checked for sterility by plating onto 2% malt extract agar to test for the presence of fungi, and on tryptone soya agar to test for bacteria.

The experiments were carried out in microcosms, as described by Anderson and Ineson (1982), which were sterilized by γ irradiation (30 kGy). Samples of 3 g sterile litter from each grassland site were asep-

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tically transferred to the microcosm and rehydrated by leaching with 100 ml sterile distilled water. Half the litter samples were subsequently inoculated with a 10-ml homogenate of the test fungus, *P. exigua*, prepared from a liquid culture of 150 ml 2% malt extract inoculated with a 7-mm disc of the fungus grown on 2% malt extract agar at 24 °C for 10 days. The remaining litter samples were left sterile. The sealed microcosms were arranged in a randomized block design and incubated at 18 °C for 35 days to permit establishment of the test fungus on the litter. Collembola were collected by gentle heat extraction from large soil and litter samples taken from the field sites, and were then stored in Petri dishes with a base of moist plaster of Paris and fed on mycelium of the test fungus. Before addition to the microcosms, the Collembola were "cleaned" by three successive transfers onto plates of plaster of Paris, made up with sterile distilled water, at 3-h intervals (Newell 1980).

Two series of experiments with different numbers of Collembola were set up. The numbers of Collembola added to each micrososm in the first experiment were based on mean field densities recorded from each of the three grassland sites in July 1988 (Table 1). Combinations of fungal mycelium and Collembola within the microcosms are shown in Table 2. Each combination was replicated three times. In the second experiment, designed to simulate field aggregations, a sequence of increasingly higher collembolan numbers in excess of mean field densities was added to microcosms containing grass litter from the moderately grazed grassland site and inoculated with the test fungus as above. Collembola were added at rates of 0, 10, 20, 30, 50, 75, and 100 per microcosm. Each microcosm was replicated three times. Microcosms from both experiments were incubated at 18 °C.

Fourteen days after the introduction of Collembola, nutrients were leached from each microcosm by adding 125 ml sterile distilled water, soaking the litter for 1 min, and drawing the water out of the chamber into collecting bottles under negative pressure. This process was immediately repeated three times using the same water. The leachates were filtered through Whatman No. 44 filter paper, stored at 5 °C, and measured for (NH_4^+-N, NO_3^--N and PO_4^{3-}-P on a Skalar continuous flow autoanalyser. Respiration of the leached litter in each microcosm was estimated by measuring CO_2 evolution using an Infra Red Gas Analyser 225 MK3 (Analytical Development Co. Ltd. UK). In order to achieve a stable respiration reading, and to eliminate any pulse-type wetting response, the CO_2 evolution was measured three times over a 24-h period. In the first experiment, leaching and measurement of respiration were repeated every 14 days, over a period of 84 days, whereas the second experiment was ended after the first leaching.

Table 1. Mean field densities of the collembolan *Onychiurus procampatus* and fungal hyphal length in the surface 3 cm soil of the three grassland sites at Dentdale (from Bardgett et al. 1993a)

Site	*O. procampatus* (no. $m^{-2} \pm SE \times 10^{-3}$)	Fungal hyphal length ($m\,g^{-1}$ dry soil ± SE)
Heavily grazed	48 ± 11	1100 ± 200
Moderately grazed	8 ± 1	1240 ± 40
Lightly grazed	11 ± 2	2200 ± 120

Table 2. Combinations of *Phoma exigua* mycelium and Collembola in microcosm chambers for three simulated field sites

Litter	Collembola added (no.)								
	Heavily grazed			Moderately grazed			Lightly grazed		
	0	M	H	0	M	H	0	M	H
Inoculated	0	10	20	0	2	4	0	3	6
Sterile	0	10	20	0	2	4	0	3	6

Density of Collembola: 0, none; M, medium (simulated mean field population density); H, high (twice M)

At the end of the first experiment Collembola were recovered from each microcosm by flotation and checked for viability. In both experiments, total and fluorescein diacetate-active hyphal lengths in the litter were measured using the membrane-filter technique (Hanssen et al. 1974) as modified by Bardgett (1991).

Statistical analyis

In the first experiment, data for respiration, nutrient release, and fungal biomass, from the different collembolan and grass litter treatments, were analysed by standard analysis of variance and Tukey's highest significant difference comparison of means. Similar data from the second experiment were analysed by regression analysis.

Results

Survival of Collembola

Despite the expected mortality at moulting, and by physical trapping in the litter, the recovery of Collembola from microcosms in the first experiment, expressed as a percentage of the total added, was generally greater than 60%, and never less than 40% (Table 3). There was no significant difference in the mortality of Collembola in microcosms with and without the fungus.

Respiration

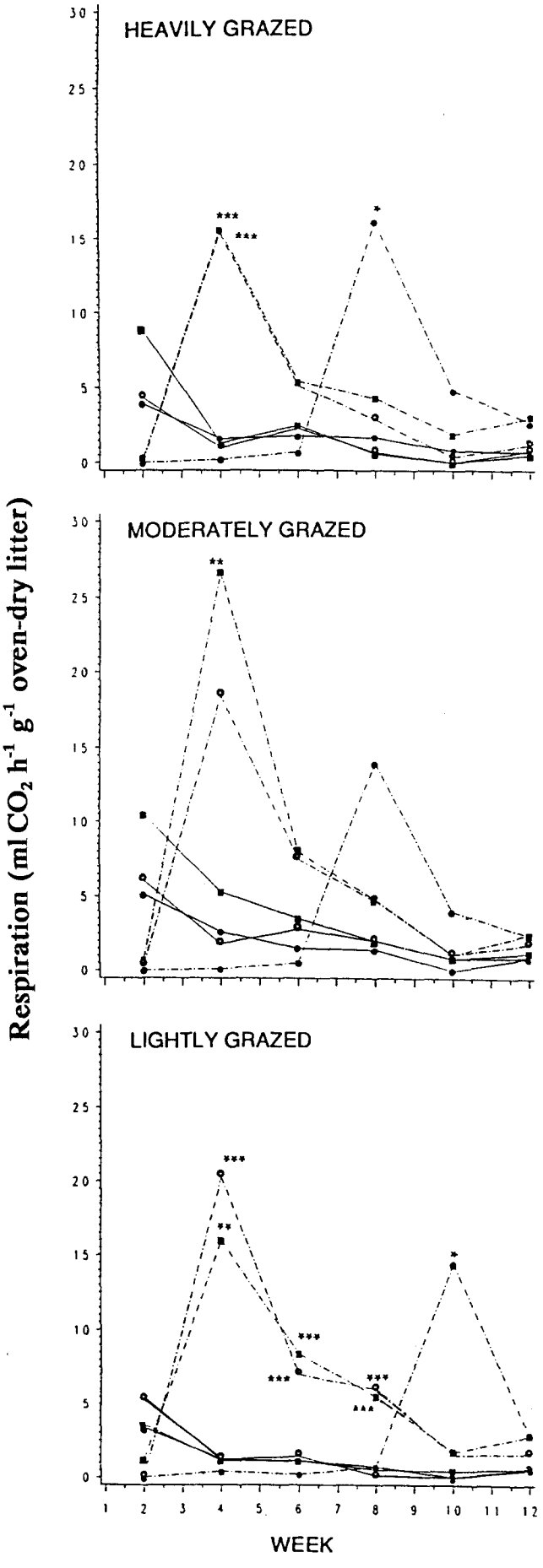
In the first experiment, the addition of numbers of Collembola based on mean field densities to microcosms containing litter inoculated with the test fungus had no significant effect on the respiration rate in any of the three microcosm treatments (Fig. 1). However, in the second experiment, the addition of increasingly higher numbers of Collembola to microcosms containing grass litter from the moderately grazed grassland site, inoculated with the test fungus, resulted in a significant ($P < 0.01$, $r^2 = 0.703$) linear decrease in the respiration rate (Fig. 2).

Respiration from the initially sterile microcosms, in all three treatments, was generally greater than that from the microcosms inoculated with the test fungus. However, this varied significantly ($P < 0.001$), with the number of Collembola added and with time. In the initially sterile microcosms to which Collembola had been added, irrespective of the number, respiration rates peaked at the second measurement (week 4), indicating that the litter had been contaminated by introduced microorganisms at an early stage. However, in the initially sterile microcosms containing no animals, respiration rates peaked later, at

Table 3. Recovery of live *Onychiurus procampatus* from microcosms, representing three field treatments, expressed as a percentage of total number added, after a 12-week experimental period

Litter	Collembola recovered (no.)								
	Heavily grazed			Moderately grazed			Lightly grazed		
	0	M	H	0	M	H	0	M	H
Inoculated	0	57	72	0	67	75	0	67	61
Sterile	0	63	65	0	50	42	0	66	50

For explanation of collembolan density, see Table 2



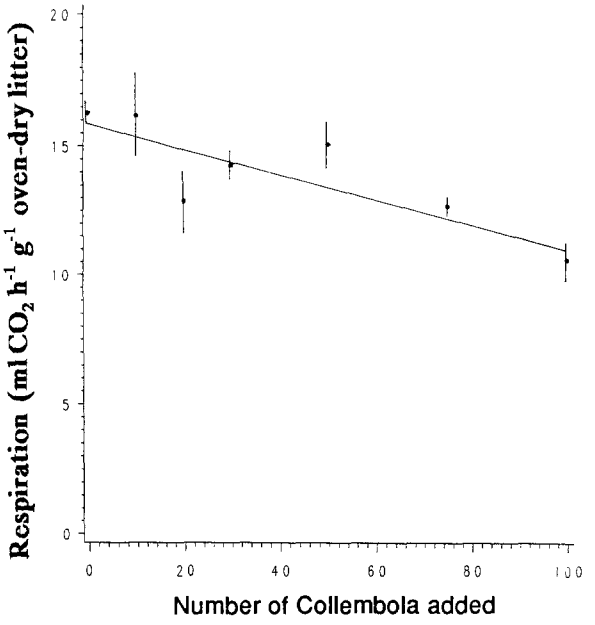


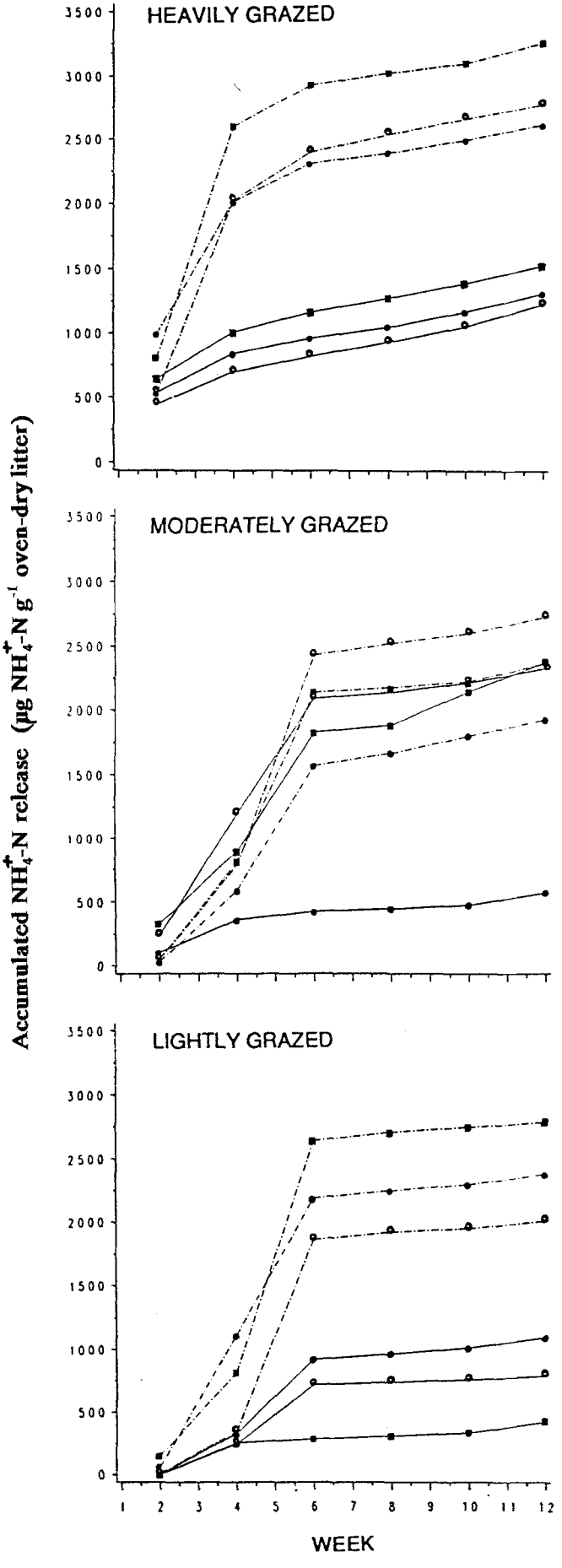
Fig. 2. Relationship between number of Collembola added to microcosms containing grass litter from the moderately grazed treatment inoculated with *Phoma exigua*, and respiration rate. *Bars* represent SE; $r^2 = 0.703$; $P < 0.001$

week 8 in the microcosms with litter from the heavily and moderately grazed grassland and at week 10 in microcosms with that from the lightly grazed grassland, indicating a delayed response in the litter to introduced microorganisms. In all contaminated microcosms, some fungal growth was observed. No peaks in respiration rates in the inoculated microcosms were recorded, although falling rates over the time period of a measurement suggest that the fungal population was more active before the measurement period. Respiration by Collembola alone in microcosms were undetectable.

Nutrient release

The addition of different numbers of Collembola to the three microcosm treatments of the first experiment had no significant effect on the release of nutrients (NH_4^+-N, NO_3^--N, PO_4^{3-}-P) from either the inoculated or the initially sterile litter (Figs. 3–5). Likewise, the addition of increasingly higher numbers of Collembola to litter from the moderately grazed grassland site, inoculated with the test fungus, had no significant effect on nutrient release

Fig. 1a–c. Effect of Collembola grazing activities on respiration in grass litter from three field treatments in the presence and absence of *Phoma exigua*. **a** Heavily grazed: ●, 0 Collembola; ○, 10 Collembola; ■, 20 Collembola. **b** Moderately grazed: ●, 0 Collembola; ○, 2 Collembola; ■, 4 Collembola. **c** Lightly grazed: ●, 0 Collembola; ○, 3 Collembola; ■, 6 Collembola. Mean respiration rates are shown for inoculated (———) and uninoculated (– – –) microcosms containing different numbers of Collembola. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$



(Fig. 6). The release of nutrients was generally greater from the microcosms with litter from heavily grazed grassland and least from the microcosms with litter from lightly grazed grassland (experiment 1). Within each microcosm treatment, nutrient release was generally greatest from the initially sterile litters; however, this varied significantly (NH_4^+-N, $P<0.001$; PO_4^{3-}-P, $P<0.001$; NO_3^--N, NS) with treatment and time. In the heavily grazed treatment, the release of all nutrients was greatest in the initially sterile microcosms; however, the release of PO_4^{3-}-P in the moderately and lightly grazed treatments and the release of NO_3^--N in the lightly grazed treatment were similar.

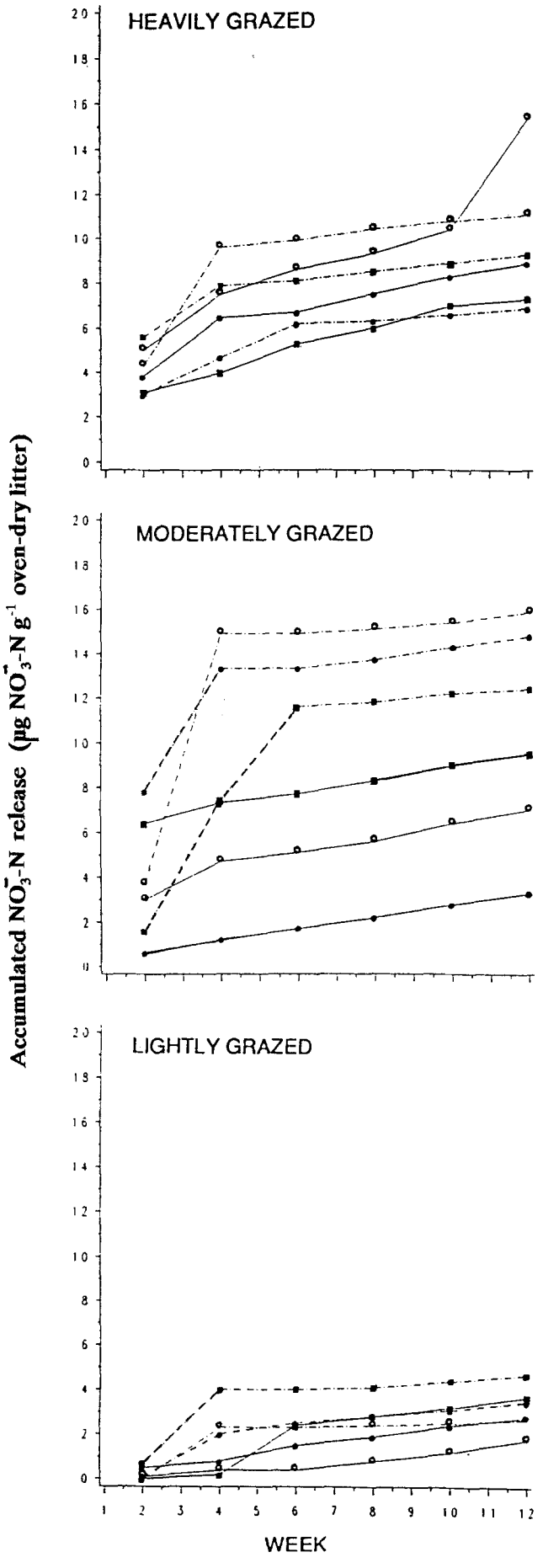
Total and fluorescein diacetate-active hyphal lengths

The abundance of total and fluorescein acetate-active fungal hyphae on grass litter from the microcosms of each of the three treatments, at the end of the 12-week period, are shown in Fig. 7. In the heavily grazed treatment of the first experiment, the addition of 20 Collembola to inoculated microcosms significantly reduced the quantity of total ($P<0.001$) and fluorescein diacetate-active ($P<0.01$) hyphae on the litter. In the initially sterile microcosms of the heavily grazed treatment, hyphal lengths increased with increasing Collembola numbers. The quantity of total fungal mycelium was significantly ($P<0.05$) greater in the microcosms containing 20 Collembola than in those were none was present. Changes in fungal biomass in other treatments and in the second experiment (Fig. 8) were not significantly different.

Discussion

This study demonstrated that collembolan numbers in excess of mean field densities can reduce the total and fluorescein diacetate-active fungal biomass and respiration rate of a common grass litter fungus with which it is in a position to interact. Despite this, collembolan grazing had no significant effect on the release of nutrients immobilized by the fungus. Although not representative of the mean field densities observed by Bardgett et al. (1993a) on a soil volume basis, high numbers of Collembola added to microcosms may more realistically relate to the actual field situation in two ways. Firstly, high numbers within microcosms may simulate the effects of spatial aggregations of Collembola in soil. Secondly, they may reflect more realistically the actual ratio of collembolan–fungal abundance recorded in the field, because the micrososms contained an unrealistically high fungal biomass (Bardgett et al. 1993a).

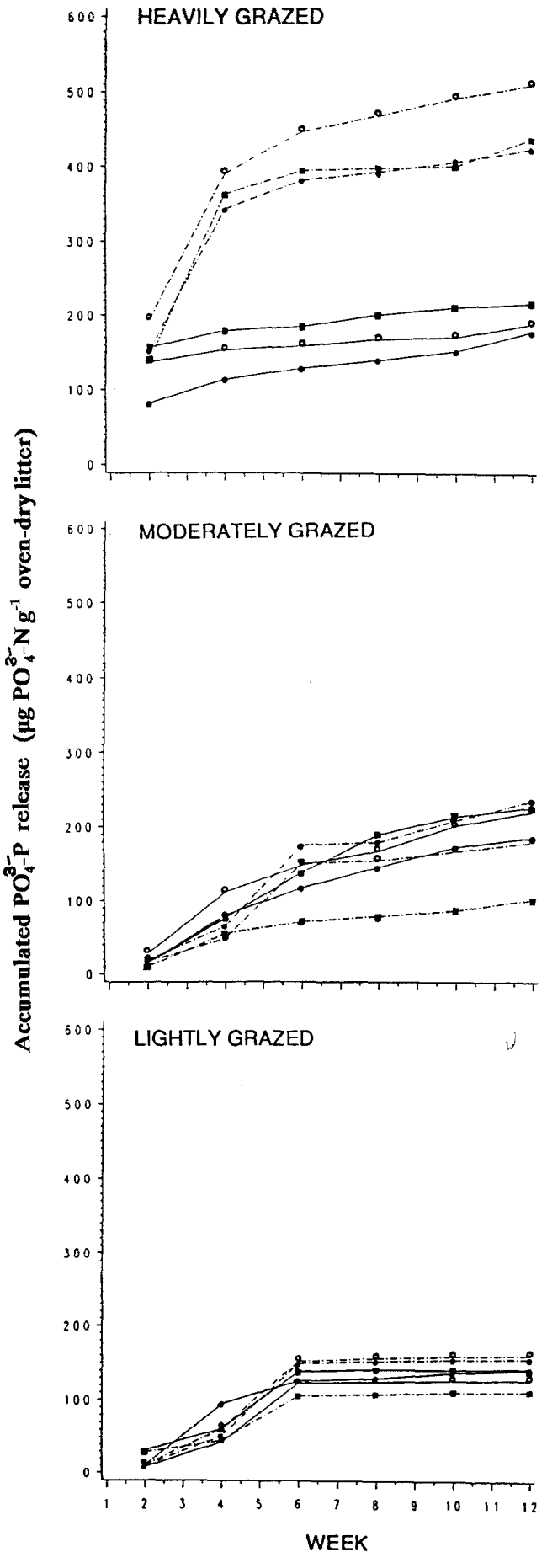
Fig. 3a–c. Effect of Collembola grazing activities on accumulated release of NH_4^+-N from grass litter of three field treatments in the presence and absence of *Phoma exigua*. See Fig. 1 for explanation of symbols. Mean accumulated release of NH_4^+-N is given for inoculated (———) and uninoculated (– – –) microcosms containing different numbers of Collembola. No significant animal effects were found in either inoculated or sterile microcosms



The absence of significant collembolan effects on nutrient release and respiration in the first experiment may also be related to the presence of relatively large quantities of fungal mycelium within microcosms. As with Visser et al. (1981), the levels of collembolan grazing activity, although based on field data, may have been insufficient to make an impact on the high fungal densities, and hence on nutrient release. This suggestion is supported by the observation that only at high grazing intensities in the microcosms with litter from the heavily grazed grassland did the Collembola have any significant effect on the quantity of total and fluorescein-active fungal mycelium growing on litter. In the second experiment, however, although the addition of much higher numbers of Collembola led to decreased respiration, it had no apparent effect on the quantity of total or fluorescein diacetate-active fungal mycelium growing on the litter (Fig. 8). It is possible that the microscopic measurement of total fungal hyphal length may have masked the effects of collembolan grazing at a microsite level (Faber et al. 1992). In addition, any morphological changes in mycelial growth associated with collembolan grazing (Hedlund et al. 1991) would not have been detected by the method used for examining the hyphae.

Although the animals added were "cleaned", it is likely that the increase in respiration rates in microcosms containing initially sterile litter with Collembola added was partly due to the colonization of litter by contaminant microorganisms that had been introduced via the bodies and faeces of the Collembola. Where no Collembola had been added to sterile litters, increased respiration rates, which occurred at a later date, may have been due to the introduction of contaminant microorganisms during the experimental procedure, particularly during the measurement of respiration when sterile conditions in microcosm chambers could not be maintained. This may have also contributed to increased respiration rates in microcosms where Collembola had been added. Faber et al. (1992) suggested that the length of the establishment period for the microbial population may strongly influence the impact of collembolan grazing activities on microbial biomass and activity. Their results suggested that the initial microbial population colonizing pine litter was faster-growing, causing higher respiration rates, and in the later stages slower-growing microorganisms prevailed. Therefore, in the present study it is likely that Collembola were added to pre-inoculated litter during a later stage of fungal development characterized by slow growth and low activity. During this phase, the response by the fungus to collembolan grazing will, we suggest, have been minimal. In the initially sterile microcosms, however, the colonizing microbial population that had been intro-

Fig. 4a–c. Effect of Collembola grazing activities on accumulated release of NO_3^--N from grass litter of three field treatments in the presence and absence of *Phoma exigua*. See Fig. 1 for explanation of symbols. Mean accumulated release of NO_3^--N is given for inoculated (———) and uninoculated (– – –) microcosms containing different numbers of Collembola. No significant animal effects were found in either inoculated or sterile microcosms



duced by the animals could have been faster-growing and more active, hence explaining the high respiration rates. Despite this, the addition of different numbers of Collembola to the litter types had no effect on respiration or nutrient release.

In the first experiment, a smaller release of nutrients from microcosms containing inoculated litter, compared to initially sterile but apparently contaminiated microcosms, demonstrated the high efficiency of the fungus in immobilizing nutrients, particularly NH_4^+-N (1–2 mg NH_4^+-N g^{-1} oven-dry litter in all treatments after 12 weeks; (Figs. 3–5). This finding indicates that the presence of large quantities of fungal mycelium in both experiments may have masked the effects of the Collembola due to rapid immobilization of nutrients released by the fungus. N and P limitations in the litter could further promote immobilization due to continual autolysis and re-use of nutrients by the fungus (Dighton and Boddy 1989). The lower C:N and C:P ratios of the litter from heavily and moderately grazed grassland, compared to that from the lightly grazed grassland, would also explain the general increase in the release of N and P from these microcosm treatments, irrespective of the fungal–collembolan combination.

Despite the limitations of the experimental procedure, as mentioned above, the results of this study suggest that the localized grazing activities of high numbers of Collembola could influence fungal abundance and activity in upland grassland soils. Further studies more representative of the actual faunal and microbial complexity and spatial heterogeneity of upland soils are required to substantiate these findings. It is likely, however, that the observed changes in the relative abundance of Collembola and fungi along a gradient of sheep management intensity (Bardgett et al. 1993a) are related largely to changes in soil conditions and fertilizer application. Several studies have shown that the abundance of soil microarthropods, particularly Collembola, are strongly influenced by levels of sheep stocking intensity and fertilizer application (King et al. 1976; Hutchinson and King 1980; King and Hutchinson 1980). Similarly, fungal abundance is known to be sensitive to changes in soil conditions, particularly pH (Alexander 1977). The effects of soil conditions on collembolan and fungal abundance in relation to this study have been discussed in detail by Bardgett et al. (1993a).

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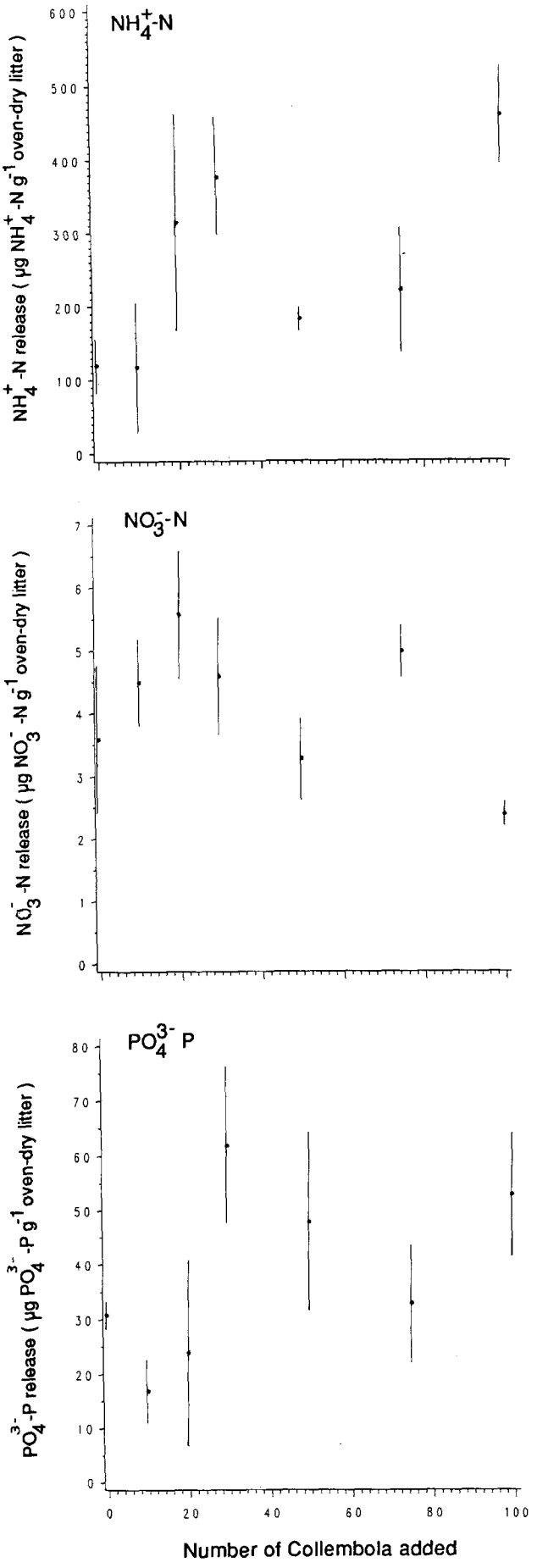
Fig. 5a–c. Effect of Collembola grazing activities on accumulated release of PO_4^{3-}-P from grass litter of the three field treatments in the presence and absence of *Phoma exigua*. See Fig. 1 for explanation of symbols. Mean accumulated release of PO_4^{3-}-P is given for inoculated (———) and uninoculated (– – –) microcosms containing different numbers of Collembola. No significant animal effects were found in either inoculated or sterile microcosms



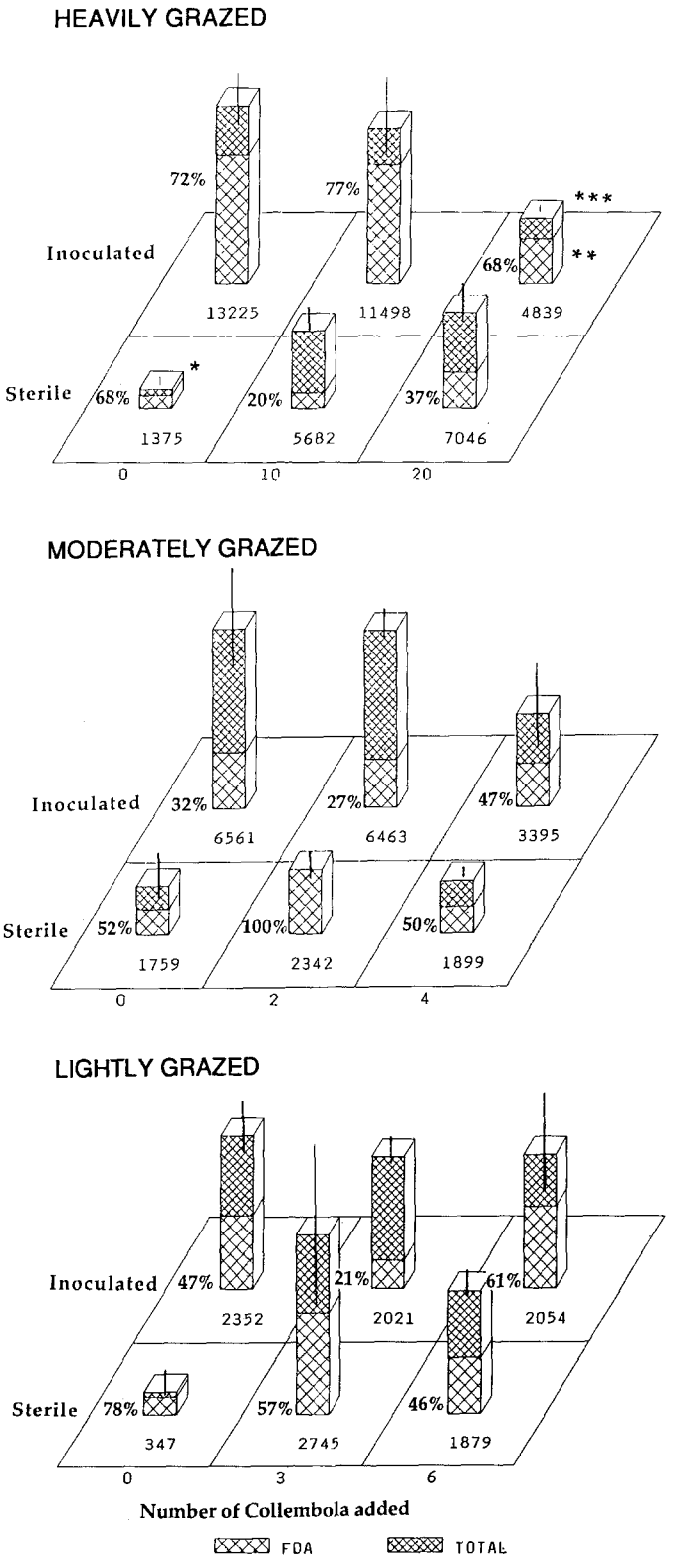


Fig. 7. Abundance of total (m g^{-1} oven-dry litter ± SE) and fluorescein diacetate (*FDA*)-active (percentage of total) fungal hyphae on grass litter from three field treatments at the end of a 12-week experimental period. $*P<0.05$; $**P<0.01$; $***P<0.001$

Fig. 6. Effect of adding increasing numbers of Collembola to microcosms containing grass litter from the moderately grazed treatment inoculated with *Phoma exigua*, on the release of NH_4^+-N, NO_3^--N, and PO_4^{3-}-P. *Bars* represent SE. No significant animal effects were found

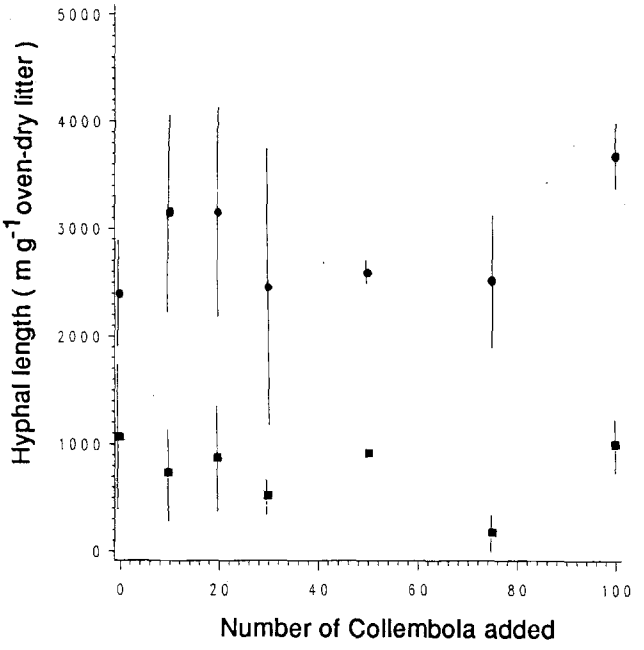


Fig. 8. Effect of adding increasing numbers of Collembola to microcosms containing grass litter from the moderately grazed treatment inoculated with *Phoma exigua*, on the abundance of total (●) and fluorescein diacetate-active (■) fungal hyphae. *Bars* represent SE. No significant animal effects were found

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