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Spatial changes of soil fungal and bacterial biomass from a sub-alpine coniferous forest to grassland in a humid, sub-tropical region

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Abstract Fungal and bacterial biomass were determined across a gradient from a forest to grassland in a sub-alpine region in central Taiwan. The respiration-inhibition and ergosterol methods for the evaluation of the microbial biomass were compared. Soil fungal and bacterial biomass both significantly decreased ($P<0.05$) with the shift of vegetation from forest to grassland. Fungal and bacterial respiration rates (evolved CO_2) were, respectively, 89.1 μl CO_2 g^{-1} soil h^{-1} and 55.1 μl CO_2 g^{-1} soil h^{-1} in the forest and 36.7 μl CO_2 g^{-1} soil h^{-1} and 35.7 μl CO_2 g^{-1} soil h^{-1} in the grassland surface soils (0–10 cm). The fungal ergosterol content in the surface soil decreased from the forest zone (108 μg g^{-1}) to the grassland zone (15.9 μg g^{-1}). A good correlation ($R^2=0.90$) was exhibited between the soil fungal ergosterol content and soil fungal CO_2 production (respiration) for all sampling sites. For the forest and grassland soil profiles, microbial biomass (respiration and ergosterol) declined dramatically with depth, ten- to 100-fold from the surface organic horizon to the deepest mineral horizon. With respect to fungal to bacterial ratios for the surface soil (0–10 cm), the forest zone had a significantly ($P<0.05$) higher ratio (1.65) than the grassland zone (1.05). However, there was no fungal to bacterial ratio trend from the surface horizon to the deeper mineral horizons of the soil profiles.

Keywords Bacteria · Biomass · Ergosterol · Fungal respiration

Introduction

Fungi and bacteria often occupy separate ecological niches in the soil environment and thus play different roles in nutrient cycling. Hence, it is important to quantify the soil biomass in terms of distinct fungal and bacterial components (West 1986). Numerous physiological methods have been developed in order to quantify the total soil microbial (bacterial and fungal) biomass. These methods have the advantage of being more rapid than observational and biochemical methods, but most of them provide no information other than total biomass figures (Parkinson and Coleman 1991).

The selective respiratory inhibition technique is important for determining fungal and bacterial biomass when it is coupled with selective inhibitors (e.g. cycloheximide and streptomycin for fungi and bacteria, respectively) applied at predetermined optimal concentrations (Anderson and Domsch 1975). Differences in respiration rates of antibiotic-treated and untreated soil samples allow the calculation of the relative proportions of fungi and bacteria in the total microbial biomass (Parkinson 1994).

Another faster approach than direct observational methods to estimate fungal biomass is to measure the concentration of specific biochemical components. Ergosterol is a more sensitive and reliable indicator of fungal biomass than other biochemical indices. Ergosterol is endogenous only in fungi and certain green microalgae (Zelles and Alef 1995) and can be recovered from soils by extraction with methanol, saponification and re-extraction with hexane (Grant and West 1986). However, little information is available regarding the relationship between the ergosterol and selective respiratory inhibition techniques used to determine microbial activities.

Sub-alpine and alpine regions are critical for studies of global change because they are sensitive and fragile ecosystems and thus allow the monitoring of ecological change. However, few studies on soil biomass have been conducted in such areas. The objectives of this study were to evaluate the spatial change of soil bacterial and fungal biomass across a gradient from a forest to grassland in a sub-alpine, sub-tropical region by comparing and correlating the two techniques de-

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scribed above. The paper looks at microbial biomass in both the surface soil (0–10 cm) and throughout the whole profile (vertical distribution).

Materials and methods

Study sites

The study was conducted at Tatachia, in the saddle of Jade Mountain, central Taiwan (23°28′N, 120°54′E). Tatachia has an elevation of 2700 m, mean annual precipitation of 4100 mm and temperature of 9.5 °C. The area has been selected as a representative long-term ecological study site of sub-alpine forest ecosystems in Taiwan. The study area geologically consists of metamorphosed sedimentary rock (Miocene epoch) comprising sandstone and shale.

The coniferous forest is dominated by Chinese hemlock (*Tsuga chinensis*). Less dominant species include Taiwan false cypress (*Chamaecyparis formosensis*), spruce (*Picea morrisonicola*) and Armand's pine (*Pinus armandi*). The grassland is dominated by dwarf bamboo (*Yushania niitakayamensis*) and alpine silver grass (*Miscanthus transmorrisonensis*).

Soil sampling

Soil profiles were taken from the forest and grassland zones and classified into horizons according to the international soil classification system (Soil Survey Staff 1994). The soils are Spodosol-like with low base saturation. Soil samples were collected from each horizon and transported to the laboratory. Visible material, such as roots and litter, was manually removed prior to sieving though a 2-mm sieve. Soil was stored at 4 °C in the dark until use.

In addition, in order to examine the spatial change of microbial biomass, surface soil (0–10 cm deep) was collected from the O horizon of three different merging vegetation zones (i.e. grassland, transition zone and forest) in 1999. Each vegetation zone was divided into four replicate blocks (sub-sites) transecting each other, so in total there were 12 sampling sites. Three sub-samples were randomly collected from each site with a soil auger before being bulked. The core of each sub-sample was 8 cm in diameter and 10 cm deep.

Ergosterol content

The soil ergosterol content was determined according to Rossner's (1996) modification of the method of Zelles et al. (1987). Sieved (2 mm) field-moist soil (2 g) was weighed into small centrifuge tubes. Twenty millilitres of methanol, 5 ml ethanol and 2 g KOH was added to each tube. Ergosterol (8 μg) was added to every second tube to form spiked replicates so as to determine ergosterol loss occurring during the experimental procedure. The mixture was heated at 70 °C in a water bath before being shaken vigorously. When cooled to room temperature, 5 ml distilled water was added and the samples were again shaken before being centrifuged at 8,000 r.p.m. for 20 min. The supernatant was kept while the residue was washed with 20 ml methanol and re-centrifuged again as before. Then the residue was discarded and the supernatant combined with that above. Hexane (30 ml) was added and the samples were again shaken vigorously before being separated. The upper hexane phase was eluted into a rotary evaporator tube. The samples were then rotary evaporated at 40 °C to dryness.

The ergosterol was redissolved in 2 ml methanol and the solution filtered though a membrane filter (0.4 μm). Samples (100 μl) were injected into a Spectra-Physics SP 8800 high performance liquid chromatograph. A reverse-phase analytical column was used (Hypersil RPC-18, 5 μm, 150 × 4.6 mm). The flow rate was set at 1.5 ml min^{-1} and the UV detector set at 282 nm. The mobile phase was 97% methanol to 3% pure water.

CO_2 measurement

Soil fungal and bacterial respiration was measured according to the procedures of West (1986) and Henrot and Robertson (1994) as follows. Soil stored in the refrigerator at 4 °C was pre-incubated for 3 days at 25 °C in plastic bags closed with rubber bands to prevent moisture loss but to allow air flow. About 1.2 g fresh soil was weighed out into 150-ml gas space-capacity Erlenmeyer flasks. Distilled water (2 ml) was added which contained dissolved glucose (30 mg ml^{-1}) and antibiotics (streptomycin or cycloheximide). The flasks were left open to the atmosphere at 25 °C for at least 30 min, then swirled for about 5 s by hand to equilibrate CO_2 in the liquid and gaseous phases. The flasks were then closed with double-seal rubber stoppers and further incubated at 25 °C. Headspace gas (1 ml) was sampled with a gas-tight syringe at the start and end of the incubation (5 h). The CO_2 concentration was estimated using a Hitachi 263–30 gas chomatograph with a 2 m x 2 mm stainless steel column, thermal conductivity detector and integrator. Both the respiration rate and above-mentioned ergosterol content were converted on an oven-dried basis for the calculations.

Determination of antibiotic concentrations for respiration experiments

The inhibitor concentrations selected for the respiration experiment were determined by a series of preliminary experiments. Both grassland and forest soils were tested. Streptomycin concentrations tested included 0, 16, 32, 64 and 128 mg ml^{-1}, while cycloheximide concentrations used were 0, 8, 16, 32 and 64 mg ml^{-1}. It was found that the optimum inhibition concentrations for both soils were 32 mg ml^{-1} for streptomycin and 64 mg ml^{-1} cycloheximide. Hence these inhibitor concentrations were used in further experiments. Four treatments were administered to each soil [i.e. control (no antibiotics), 32 mg ml^{-1} streptomycin, 64 mg ml^{-1} cycloheximide and 32 mg ml^{-1} streptomycin combined with 64 mg ml^{-1} cycloheximide]. Two replicates were prepared for each soil treatment.

Respiration and fungal to bacterial ratios were calculated according to West (1986) as follows:

1. Eukaryote (fungal) respiration (B) = total (non-inhibited, control) respiration (A)–respiration after addition of streptomycin.
2. Prokaryote (bacterial) respiration (C) = A-respiration after addition of cycloheximide.
3. Residual respiration (D) = Respiration remaining after addition of combined antibiotics.
4. Eukaryote to prokaryote ratio = B/C.
5. Evaluation ratio = $(B + C)/(A\text{-}D)$.

The respiration rate was converted into microbial biomass C assuming that for incubations at 22 °C, a substrate-induced maximal respiration rate of 1 ml CO_2 h^{-1} corresponds to about 40 mg microbial C (Beck et al. 1996).

Statistical analysis

Statistical analysis of measurements included ANOVA. Analysis was carried out using the statistical package SigmaStat for Windows version 2.0 (SPSS 1997). Since the data was not normally distributed it was converted by log transformation for statistical analysis. Statistically significant differences ($P < 0.05$) between each treatment were compared by using Duncan's multiple range test. Regression analysis was also carried out to determine correlations between respiration rate and ergosterol content.

Results

Microbial biomass in surface soils.

Table 1 shows the results of the soil respiration and ergosterol contents in the surface soil (0–10 cm) of the different vegetation zones. Both the grassland and transition zones had significantly lower fungal and bacterial respiration (evolved CO_2) rates than the forest zone. In comparison, the grassland and transition zones had significantly lower ergosterol contents than the forest. Compared to the other two soils the forest surface soil had a significantly higher fungal to bacterial ratio.

When converted to amount per square meter, the forest surface soil again contained a significantly higher level of biomass than the grassland surface soil (see Table 1) despite the grassland soil having a higher bulk density than the forest soil (0.347 cf. 0.087 g cm^{-3}).

Microbial biomass throughout the soil profiles

Results of the respiration and ergosterol experiments which examined different horizons in the soil profiles are shown in Table 2. With respect to the forest zone profile, the two surface organic horizons Oe1 and Oe2 showed the highest rates of fungal respiration and bacterial respiration, as expected. Fungal and bacterial respiration decreased as soil depth increased. The deepest mineral horizons examined, E and Bt, both showed the lowest fungal and bacterial respiration. The soil fungal ergosterol content also decreased uniformly down the forest soil profile.

For the grassland zone profile, again the two surface organic horizons Oa1 and Oa2 showed the highest rates of fungal and bacterial respiration. Fungal and bacterial respiration both decreased as soil depth increased. The lowest mineral horizons studied, E and Bt, showed the lowest fungal and bacterial respiration rates. For this profile there was again no fungal to bacterial ratio trend from the surface soil to the lowest horizon, and evaluation ratios were also variable. The soil fungal ergosterol content also decreased uniformly down the profile. There was a strong positive correlation ($R^2 = 0.90$) between fungal ergosterol and fungal respiration for all soils (Fig. 1). In fact, correlations between all the different microbial biomass parameters were strong (see Table 2).

Discussion

Few soil microbial biomass studies have been carried out under sub-alpine forest and grassland ecosystems in humid, sub-tropical regions. Hence it is only possible to compare the results of this investigation with studies undertaken in forests and grasslands of temperate regions.

Stahl and Parkin (1996) found that prairie sites in Iowa contained between 3.49 and 2.71 μg ergosterol g^{-1} soil compared to only 2.36 μg g^{-1} for a mature forest. Djajakirana et al. (1996) found that grassland and forest surface soils (0–10 cm) in Germany contained about the same quantities of ergosterol, i.e. 5.52 μg ergosterol g^{-1} soil and 5.45 μg ergosterol g^{-1} soil, respectively. These levels of ergosterol are much lower than those found in this study. In this investigation, the forest soil contained 108 μg ergosterol g^{-1} soil whilst the grassland had 15.9 μg g^{-1}. Frostegärd and Bååth (1996) also discovered, as with this study, that a grassland surface soil possessed lower ergosterol levels (between 9 and 69 μg ergosterol g^{-1} soil) than forest soils.

Ohtonen and Väre (1998) obtained higher ergosterol contents in their study of Boreal Scots pine forest soils in Finland, which ranged from 30.8 μg g^{-1} to 34.4 μg g^{-1}. Scheu and Parkinson (1994), in accordance with our results, found high ergosterol contents of >100 mg g^{-1} in the L and F layers of *Populus tremuloides* forest soils in the Canadian Rocky Mountains.

In this study, the soil fungal ergosterol content also decreased uniformly down the forest soil profile, with the surface Oe horizon (0–15 cm) having 71 μg ergosterol g^{-1} dry soil and the lowest Bt horizon having 1.3 μg ergosterol g^{-1} dry soil. The ergosterol concentrations obtained in different horizons of the forest profile of

Table 1 Comparisons of respiration and ergosterol contents and fungal to bacterial ratios in surface soils (0–10 cm) among three different vegetation zones. Within columns, any two means followed by the *same letter* are not significantly different at the 5% level of significance. *SIR* Substrate-induced respiration

Vegetation zone	Total respiration (SIR) (μl CO_2 g^{-1} h^{-1})	Total microbial biomass C[a] (mg g^{-1} h^{-1})	Fungal respiration (μl g^{-1} h^{-1})	Bacterial respiration (μl g^{-1} h^{-1})	Respiration with both cycloheximide and streptomycin (μl g^{-1} h^{-1})	Evaluation ratio[b]	Fungal to bacterial ratio	Ergosterol content	
								(μg g^{-1})	(mg m^{-2})
Forest	156 a[c]	6.18 a	89.1 a	55.1 a	45.4 a	1.45 a	1.62 a	108 a	956 a
Transition	83.9 b	3.32 b	37.2 b	35.5 b	33.7 a	1.87 a	1.05 b	23.4 b	796 ab
Grassland	75.0 b	2.97 b	36.7 b	35.7 b	32.5 a	1.90 a	1.05 b	15.9 b	544 b

[a] A substrate-induced maximal respiration rate of 1 ml CO_2 h^{-1} corresponds to about 40 mg microbial C (Beck et al 1996)

[b] Evaluation ratio $= (B + C)/(A\text{-}D)$, where A is control respiration, B is fungal respiration, C is bacterial respiration and D is residual respiration

[c] Data were log transformed for statistical analysis

Table 2 Comparisons of respiration and ergosterol contents and fungal to bacterial ratios among two soil profiles under different vegetation zones. Within columns, any two means followed by the *same letter* are not significantly different at the 5% level of significance

Vegetation zone	Soil horizon	Soil depth (cm)	Total respiration (SIR) (μl CO_2 g^{-1} h^{-1})	Total microbial biomass C[a] (mg g^{-1} h^{-1})	Fungal respiration (μl g^{-1} h^{-1})	Bacterial respiration (μl g^{-1} h^{-1})	Residual respiration (μl g^{-1} h^{-1})	Evaluation ratio[b]	Fungal to bacterial ratio	Ergosterol content (μg g^{-1})	Ergosterol content (mg m^{-2})
Forest	Oe1	0–15	200 a[c]	7.92 a	86.1 a	78.4 a	63.0 a	1.72 a	1.10 a	71.4 a	814 a
	Oe2	15–20	95.5 b	3.78 b	38.8 b	26.1 b	46.4 ab	3.12 a	1.46 a	38.4 b	437 b
	Oa	20–25	65.3 bc	2.59 bc	29.0 bc	24.6 b	24.5 bc	1.90 a	1.29 a	19.7 c	563 b
	A	25–30	25.2 c	1.00 c	12.0 c	8.43 c	10.0 c	1.97 a	1.51 a	4.60 d	368 b
	E	30–39	2.82 d	0.11 d	1.21 d	2.01 c	1.75 c	1.93 a	0.88 a	0.60 de	72 c
	Bt	39–52	6.64 d	0.26 d	3.17 d	3.34 c	2.60 c	1.81 a	0.99 a	1.25 de	150 c
Grassland	Oa1	0–10	113 b	4.47 b	40.4 b	48.3 b	40.3 a	1.96 a	0.91 a	28.2 b	979 a
	Oa2	10–15	38.7 c	1.53 c	12.4 c	14.7 c	16.2 b	2.32 a	0.86 a	9.44 c	318 b
	Oa3	15–20	18.9 c	0.75 c	9.13 c	3.20 c	9.46 b	2.70 a	3.12 a	2.99 d	103 c
	Oa4	20–25	15.1 cd	0.60 cd	6.09 cd	7.02 c	5.11 bc	2.01 a	1.18 a	1.41 d	48 c
	OA	25–32	6.48 cd	0.26 cd	2.37 cd	2.43 c	2.30 bc	2.08 a	1.36 a	1.20 de	54 c
	A	32–37	3.22 d	0.13 d	2.12 d	0.97 c	0.67 c	1.34 a	2.47 a	0.96 de	77 c
	E	37–45	5.09 cd	0.20 cd	3.36 d	2.44 c	0.61 c	1.00 a	1.28 a	0.57 e	100 c
	Bt	45–55	4.15 cd	0.16 cd	1.64 d	1.73 c	1.44 c	2.27 a	1.00 a	0.55 e	69 c

[a–c] For footnotes, see Table 1

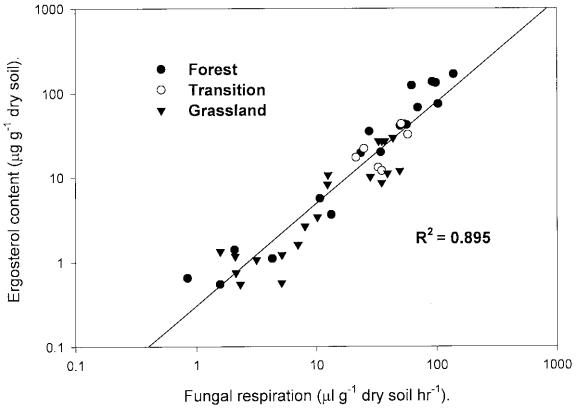

Fig. 1 Correlation between soil ergosterol content and fungal respiration for all tested soils

this study are in the range shown by Joergenson and Sheu (1999) for their German forest soil profiles.

Frostegärd and Bååth (1996) tested different soils in Sweden and found that the upper 5 cm of spruce forest soils contained between 197 and 218 μg ergosterol g^{-1} soil, while the B horizon had only 75 μg ergosterol g^{-1} soil. However, the upper 5 cm of beech forest soils contained only between 49 and 124 μg ergosterol g^{-1} soil, while the mineral horizon at 40 cm depth contained 35 μg ergosterol g^{-1} soil.

Scheu and Parkinson (1994) studied four soil layers (L, F, H and Ah) of a *Populus tremuloides* forest in the Canadian Rocky Mountains. They found ergosterol contents of >100 μg g^{-1} in the L and F horizons, between 50 and 100 μg g^{-1} in the H and F/H horizons, and <10 μg ergosterol g^{-1} soil in the Ah horizon.

Ergosterol concentrations obtained depend on the extraction technique used, fungal species present, growth temperature, fungal age, fungal reproduction stage, fungal substrate composition, nutrient availability in soil, soil pH and other environmental factors (Tunlid and White 1992). In addition, the ratio of ergosterol to fungal biomass can vary among species. Hence, it may be that one needs to identify the fungal species present when using the ergosterol technique (Newell 1992).

To test the reliability of the ergosterol technique, results have been correlated with those of the selective inhibition respiration method. Table 3 and Fig. 1 show that there was a strong positive correlation ($R^2 = 0.90$) between fungal ergosterol and fungal respiration for all the tested soils. Thus, the two methods appear to be able to reliably estimate fungal biomass in soils.

The results of Table 1 show that fungal to bacterial ratios were highest for the forest (1.62), followed by transition (1.05) and grassland (1.05) surface soils, respectively. Most results obtained using this method generally show fungi to be the dominant component of the total microbial biomass, with fungal to bacterial biomass ratios ranging from 1.3 to 9.0 (Parkinson 1994). In acid coniferous forest soils, the fungal biomass usually exceeds the bacterial biomass (Anderson and Domsch 1975; Scheu and Parkinson 1994), but exceptions have been reported (Frostegärd et al. 1993; Bååth et al. 1995). Frostegärd et al. (1993) only obtained fungal to bacterial ratios of 0.75–0.88 for their pine and spruce forest soils in Sweden. Ratios of 2.4 and 3.1 have been reported for organic layers of spruce forests by Parkinson et al. (1978) and Domsch et al. (1979), respectively, whilst Alphei et al. (1995) measured ratios ranging between 1.1 and 1.4 for beechwood soils. Blagodatskaya and Anderson (1998) obtained high fungal to bacterial ratios, using the selective inhibition technique, of 18 and 9.5, for acidic spruce forests in Germany.

Table 3 Correlation coefficients between total, fungal, bacterial and residual respiration (*resp.*) and ergosterol content for all tested soils. All data were log transformed

	Total resp.	Fungal resp.	Bacterial resp.	Residual resp.
Total resp.	1.00			
Fungal resp.	0.98	1.00		
Bacterial resp.	0.99	0.96	1.00	
Residual resp.	0.97	0.93	0.95	1.00
Ergosterol content	0.91	0.90	0.85	0.83

Few studies have been conducted on the vertical distribution of microflora in the soil profile (Berg et al. 1998). Nevertheless, Scheu and Parkinson (1994) found overall means of fungal to bacterial ratios to be 6.0, 2.2, 1.3, 1.0 and 1.5 in their *Populus tremuloides* L, F, H and Ah soil horizons, and *Pinus contorta* F/H soil horizon, respectively, in the Rocky mountains of Canada. In the mineral soil of a pine forest, Bewley and Parkinson (1984) found a fungal to bacterial ratio of 1.6. In contrast, in this study there were no fungal to bacterial ratio trends from the surface soil to the lowest horizon for both soil profiles. Fungal to bacterial ratios were variable throughout the profile and ranged from 0.86 to 3.12 (Table 2).

The evaluation ratios $(B+C)/(A\text{-}D)$ in Table 1 ranged from 1 to almost 2, which indicates that the activities of the antibiotics with respect to eukaryotic and prokaryotic soil organisms overlapped (West 1986). Hence, the antibiotics may have affected a fraction of the non-target population as well as the target population. An alternative explanation for a ratio >1 given by West (1986) is that these antibiotics when combined do not work as effectively as when applied alone, i.e. the sum of the effects of the individual components exceeds that of the antibiotics administered together.

Several factors combine to reduce the effectiveness of the antibiotics. They may be sorbed to soil organic matter and minerals, and they are rapidly degraded in soil. The passage of the antibiotics through the soil is difficult, meaning that some organisms may not come into contact with the antibiotic or may encounter only low concentrations (Alef and Sparling 1995). Soil microorganisms may show resistance to them, and soil factors such as pH affect the solubility, rate of diffusion and adsorption of antibiotics. Consequently, there is rarely any absolute specificity for an antibiotic; this is usually soil and concentration dependent (Alef and Sparling 1995).

The respiration-inhibition method should be used with caution when estimating fungal to bacterial ratios throughout soil profiles. Wardle and Parkinson (1990) and Bolan et al. (1996) suggested that the selective inhibition-respiration technique can only be used to calculate the relative activity of bacteria and fungi for those organisms undergoing protein synthesis following glucose addition. Since both antibiotics inhibit protein synthesis (peptide elongation and translation) they may only inhibit the active population undergoing protein synthesis. Therefore, this technique probably measures only part of the fungal or bacterial biomass. It may be possible to define the respiration resistant to both antibiotics as that of the inactive population. Hence, the fungal or bacterial population estimated by the inhibition method may be considered as biomass indices of only the active population.

For instance, for some mineral horizons located at lower depths of the soil profiles (Table 2), the respiration resistant to both antibiotics was larger than fungal or bacterial respiration. This was not the case for the surface soil experiment. When respiration resistant to both antibiotics was larger than fungal or bacterial respiration, this was probably mainly due to the action of clay minerals, which sometimes block the contact of antibiotic with microorganisms. Unequal antibiotic distribution throughout the soil, organism resistance to antibiotics, and soil factors may have added to the variation.

So, it can be tentatively concluded that, as found in this study, forest surface soils tend to have both higher levels of microbial biomass and ratios of fungal biomass to bacterial biomass than grassland soils. It can also be concluded that both the fungal and bacterial biomass decreases from the surface horizon to the deeper horizons in forest and grassland soils. However, the fungal to bacterial ratio showed no sequential change throughout the forest or grassland soil profiles. The ergosterol technique gave a reliable estimate of fungal biomass, although it may have been more appropriate to correlate the results of this method with those of direct observation methods, rather than the respiration-inhibition method, when testing mineral soils. There were some limitations with the respiration-inhibition method. This method was successfully used to determine fungal to bacterial ratios in forest and grassland surface soils but was not suitable for the lower mineral horizons of these soils, which have low biomass contents.

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