



## RELATIONSHIP OF SOIL ERGOSTEROL CONCENTRATION AND FUNGAL BIOMASS

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**Summary**—We studied the relationship between amounts of fungi and ergosterol in soil to further evaluate the use of ergosterol as an indicator of soil fungal biomass. Soils from seven sites in central Iowa were analyzed for soil ergosterol content, total fungal hyphal length (living and non-living, using calcofluor as stain), and living fungal hyphal length (using fluorescein diacetate as stain) to: 1) determine how soil ergosterol concentration correlates with total fungal hyphal length and living fungal hyphal length; and 2) determine an approximate value for ergosterol concentration in living fungal biomass present in soil. Correlation significance tests and analysis of variance indicated highly significant positive correlation between soil ergosterol content and both measures of hyphal length, but regression analysis demonstrated only a moderate degree of linear correlation between these variables (coefficients of linear correlation,  $r = 0.638$  to  $0.874$ ). Calculated values for ergosterol concentration in living fungal biomass present in the soils examined ranged from 5 to 31 mg ergosterol  $\text{g}^{-1}$  living fungal biomass. Data indicate that the wide range in specific ergosterol content of living fungal biomass in soils is related to the total amount of fungal hyphae (living and non-living) in a soil. We propose a method to estimate living fungal biomass from soil ergosterol content which compensates for the variability in fungal ergosterol concentrations by accounting for this relationship. A preliminary evaluation of this approach using independent data from the literature provides support for this method, in that a high correlation ( $r^2 = 0.999$ ) between predicted and measured living fungal biomass was observed. Copyright © 1996 Elsevier Science Ltd

### INTRODUCTION

Fungi are extremely important contributors to terrestrial ecosystem function (Christensen, 1989). These organisms, as a group, are the heterotrophs primarily responsible for the decomposition of organic residues (Paul and Clark, 1989), represent large nutrient pools (Whittaker, 1975), participate in mycorrhizal symbioses with the majority of land plants (Harley, 1971), are critically involved in soil food webs (Hunt *et al.*, 1987), and serve to stabilize soils through their contribution to soil aggregation (Tisdall and Oades, 1982). In fact, fungal biomass in terrestrial environments may be surpassed only by that of plants, but problems in accurate quantification of fungi make this hypothesis difficult to test.

A number of factors contribute to the complexity in measuring fungal presence in soil, the most important habitat for these organisms. One is a characteristic they share with many other microorganisms in that they cannot readily be seen or separated from the soil matrix. Another problem is that the fungi are a highly diverse group of organisms with major morphological and physiological disparities which make it difficult to quantify their biomass with a single assay or measurement.

A variety of techniques have been described to quantify fungi in litter and soil (reviewed by Frankland *et al.*, 1990 and Newell, 1992) but serious sources of error exist in all of these techniques. The most widely accepted and commonly used method for measuring fungal presence in soil is direct microscopic counting of hyphae. This procedure allows for visual observation of mycelium in soil and can be used to estimate total or living mycelial volume (Jones and Mollison, 1948; Hanssen *et al.*, 1974; West, 1988). Direct microscopic techniques tend to underestimate the amount of fungi (Frankland *et al.*, 1978; Parkinson, 1982), however, and the results obtained by these methods may be influenced by a high degree of observer subjectivity (Domsch *et al.*, 1979; Stahl *et al.*, 1995).

Biochemical methods for assay of fungal marker molecules such as chitin (more properly, glucosamine) and ergosterol may involve less observer-associated variability, but entail other problems such as variable content in fungal tissue and specificity to fungi. A number of drawbacks to the glucosamine technique are leading to a decline in its use (Newell, 1992). The ergosterol method, which has been proposed as a measure of fungal biomass in soil more recently (Grant and West, 1986; West *et al.*, 1987), is gaining in popularity but has not yet been evaluated thoroughly. Ergosterol may be a particularly useful

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index of fungal presence (Davis and Lamar, 1992) because it is endogenous only to fungi (Weete, 1989) and certain green microalgae (Newell *et al.*, 1987). One of the problems with this method is in converting soil ergosterol concentrations to fungal biomass estimates. Ergosterol content of fungal tissue is known to vary with species and physiological state of the fungus (Huang *et al.*, 1985; Newell *et al.*, 1987) and a few groups of fungi do not produce ergosterol at all (Weete and Weber, 1980). For these reasons, it is important to examine the relationship between the amounts of ergosterol and fungi in soil to better understand and evaluate this method. Although a number of studies have reported ergosterol concentrations for fungi grown in pure culture, data for fungi growing in a natural soil habitat are limited. West *et al.* (1987) correlated ergosterol concentration in soil with mycelial volume and surface area and reported an estimate of the relationship between ergosterol and fungal surface area of fungi growing in soil of  $0.16 \mu\text{g ergosterol cm}^{-2}$  fungal surface area. Scheu and Parkinson (1994) related soil ergosterol content to fungal biomass as measured by substrate-induced respiration in forest soils and obtained an overall mean value of  $11 \text{ mg ergosterol g}^{-1}$  fungal biomass carbon.

We have studied the relationship between the amounts of fungi and ergosterol in soil to further evaluate the use of ergosterol as a measure of fungal biomass. Our specific objectives were to 1) determine how soil ergosterol concentration correlates with total fungal hyphal length (living and non-living) and with living fungal hyphal length in several soils and 2) determine an approximate value for ergosterol concentration in endogenous soil fungi.

#### METHODS

Soil samples were collected from seven sites in central Iowa during the first two weeks of June 1994 (Table 1). Eight soil cores (2.5 cm dia) were taken randomly from within a 10 m circular area at each site. That portion of the core representing soil depth from 5 to 15 cm was placed in small plastic bag and immediately returned to the laboratory and placed in the refrigerator (4°C). To prepare samples for analysis, each core

was broken up and thoroughly homogenized. Five grams of each sample were added to 15 ml cold (2°C) methanol and stored at 2°C for analysis of ergosterol content. One gram of each sample was used for examination of total hyphal length and 1 g was used for analysis of living hyphal length. Finally, a 5 g subsample was used for gravimetric determination of soil moisture content. All subsamples were prepared within 24 h of sample collection.

Direct microscopic examination of fungal hyphae in soil was conducted using the membrane filter method (Hanssen *et al.*, 1974) with calcofluor M2R white for observation of total fungal hyphal length and, separately, fluorescein diacetate (FDA) as a stain to assess the amount of living (metabolically-active) fungal hyphae (Soderstrom, 1977, 1979; Ingham and Klein, 1984). Calcofluor-stained slides were prepared as described by Stahl *et al.* (1995) except that prepared filters were immediately mounted on slides with one drop of immersion oil (as opposed to being allowed to dry for a few seconds). FDA-stained slides were prepared by dispersing a one g soil subsample into 450 ml nanopure filtered water in a Waring blender at highest speed for 1 min. Fifty ml of FDA stain stock solution (2 mg FDA  $\text{ml}^{-1}$  acetone) was then added to the blender and allowed to stand for 3 min with occasional mixing. The contents of the blender were then mixed again for a few seconds to resuspend the subsample and a 1 ml aliquot was immediately removed and passed through a 25 mm dia  $0.4 \mu\text{m}$  mesh polycarbonate membrane filter. The remainder of the procedure is as described by Stahl *et al.* (1995) except that the filter was not rinsed. Each FDA-stained slide was examined immediately after preparation. All slides were prepared within 24 h of sample collection. For each site, eight slides were made (one slide per soil core or sample) as was indicated as an efficient method by the results of our previous study (Stahl *et al.*, 1995).

Slides were examined with a Nikon Microphot-SA epifluorescent microscope equipped with a high intensity mercury light source. Observations were made using a dry  $40\times$  objective,  $10\times$  eyepiece, and a  $1.5\times$  light path magnifier (total magnification:  $600\times$ ). A Nikon UV-1A filter cube was used for

Table 1. Sites sampled

Site	Soil	Description
1. Ridgetop, corn-soybean field	Clarion loam (Typic Hapludoll)	Eroded, ridgetop site in a conventionally-tilled field managed in an alternate year rotation of corn and soybeans (Cambardella <i>et al.</i> , 1994).
2. Pothole, corn-soybean field	Canisteo silty clay loam (Typic Haplaquoll)	Same field as Site 1, but in a depression in which soil is accumulating from eroded areas. Higher organic content than Site 1 (Cambardella <i>et al.</i> , 1994).
3. No-till cornfield	Clarion loam	Site continuously managed for no-till corn production (Typic Hapludoll) for past 12 years (Radke and Berry, 1993).
4. Mcfarland prairie	Lester loam (Mollic Hapludalf)	Restored upland tallgrass prairie site, restoration initiated in 1980.
5. Doolittle prairie	Kossuth silty clay loam (Typic Haplaquoll)	Never tilled, native prairie; mesic site vegetated by mixed grasses and forbes (DeLuca and Keeney, 1993).
6. Immature forest	Lester loam (Mollic Hapludalf)	Successional woodland site, oldest trees ca. 25 years old, dense understory.
7. Mature forest	Lester loam (Mollic Hapludalf)	Old growth woodland site, oldest trees > 100 years old, sparse understory.

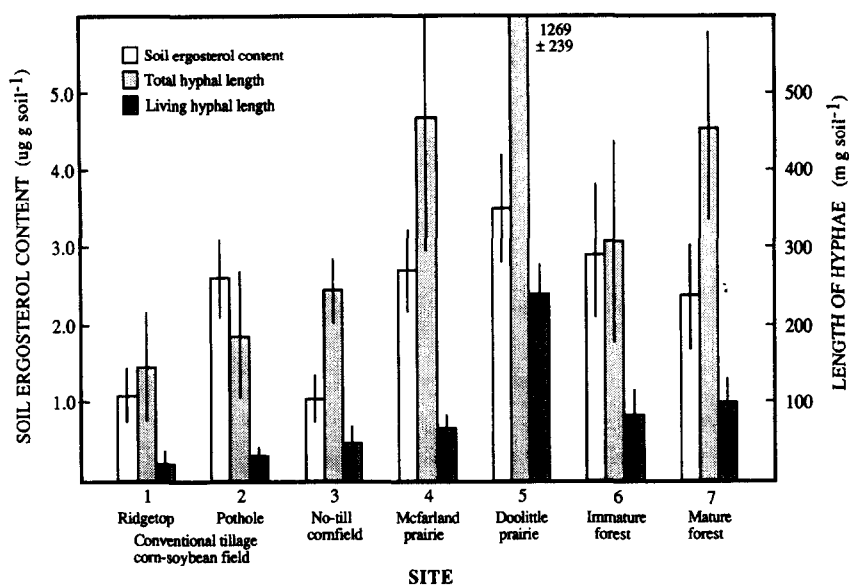


Fig. 1. Soil ergosterol content, total hyphal length (living and non-living), and living hyphal length in each of the soils examined. Vertical bars indicate  $\pm 1$  standard deviation.

examination of calcofluor-stained slides and a Nikon B-2H filter cube was used for FDA-stained slides. Twenty-five randomly-chosen fields of view were counted on each slide.

Ergosterol was extracted from soil subsamples and quantified as described by Eash *et al.* (1995) with the following modification. To transfer ergosterol from the methanol phase to the hexane phase during the extraction procedure, tubes were rotated end-over-end for 10 min using a mechanical rotating device rather than by hand and this step was not repeated. Extraction efficiency based on analysis of soil samples spiked with ergosterol standards was 82%.

Estimates of live fungal biomass were calculated from living hyphal lengths using the equation given by Paul and Clark (1989):

$$B_f = \pi r^2 L e S_c$$

where  $B_f$  is fungal biomass,  $r$  is hyphal diameter (using an average value of  $3.0 \mu\text{m}$  based on observation and measurement of hyphae from the soil samples),  $L$  is hyphal length ( $\text{cm g}^{-1}$  soil),  $e$  is hyphal density ( $1.3 \text{ g cm}^{-3}$ ), and  $S_c$  is solids content (0.3). Ergosterol concentration in living fungal biomass present in the soils examined was estimated by dividing soil ergosterol concentration ( $\mu\text{g g}^{-1}$  dry soil) by living fungal biomass content of soil ( $\text{mg g}^{-1}$  dry soil, calculated as described above).

Simple linear regression, analysis of variance, and correlation significance tests were used to investigate the relationships between variables examined (Sokal and Rohlf, 1981; SYSTAT, 1992).

## RESULTS

Mean values for soil ergosterol content, total hyphal length, and living hyphal length for each of

the seven sites examined are given in Fig. 1. Total and living hyphal lengths ranged from 146 and  $20 \text{ m g}^{-1}$  dry soil, respectively, at the ridgetop site in the conventionally-tilled corn-soybean field to 1269 and  $239 \text{ m g}^{-1}$  dry soil, respectively, at the undisturbed Doolittle prairie site. Ratios of the amount of living to total hyphae varied from 0.14 at the ridgetop in the corn/soybean field and Mcfarland prairie to 0.27 at the immature forest site. Soil ergosterol content was lowest at the no-till cornfield and ridgetop corn-soybean field ( $1.03$  and  $1.07 \mu\text{g g}^{-1}$  dry soil, respectively) and greatest at the Doolittle prairie site ( $3.49 \mu\text{g g}^{-1}$  dry soil). All three measures of fungal presence in soil were lower in cultivated soils than in prairie or forest soils.

No pattern of relationship between the three measures of fungal biomass in soil was obvious from the data presented in Fig. 1. Differences in the length of fungal hyphae in soil among the seven sites examined were much greater than were amounts of ergosterol; values for hyphal lengths (total and living) varied slightly under 10-fold whereas soil ergosterol contents ranged slightly over three-fold. Additionally, average coefficients of variation were higher for total and living hyphal length measurements (33 and 36%, respectively) than for soil ergosterol determinations (27%).

Scatterplots showing soil ergosterol values plotted against total hyphal length and living hyphal length for all samples are given in Fig. 2. Although correlation between soil ergosterol content and both measures of fungal hyphae were not exceptionally strong (Fig. 1; Table 2), they were, nevertheless, highly significant as indicated by a correlation significance test and analysis of variance (Table 2 and Table 3). For both the relationships between soil ergosterol content and total hyphal length as well as

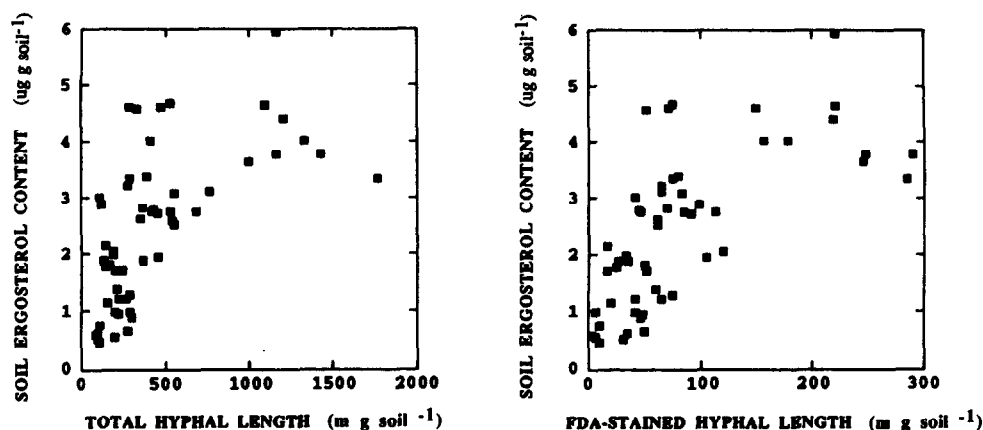


Fig. 2. Scatterplot showing soil ergosterol content values plotted against total hyphal length and FDA-stained (living) hyphal length values for all soil samples.

soil ergosterol content and living hyphal length, coefficients of linear correlation ( $r$ ) were greater for the model which included no  $y$ -intercept ( $y = bx$ , Table 2). Standard error of the estimate, however, was lower in both cases for the linear model which did include a  $y$ -intercept ( $y = a + bx$ ). Coefficients of linear correlation were slightly higher and standard errors of estimates slightly lower for both regressions of living hyphal length and soil ergosterol content than for total hyphal length and soil ergosterol content (Table 2).

Estimates of living fungal biomass at each site, based on measurements of length of living hyphae, varied from 0.06 to 0.66 mg g<sup>-1</sup> dry soil (Table 4). Further, calculated values for ergosterol concentration in living fungal biomass present in the soils examined ranged from a low of 5 mg ergosterol g<sup>-1</sup> living fungal biomass at the Doolittle prairie to a high of 31 mg ergosterol g<sup>-1</sup> living fungal biomass at the pothole site in the conventionally-tilled corn-soybean field. The average value for ergosterol concentration of living fungal biomass in soil, based on these estimates, was 14 mg ergosterol g<sup>-1</sup> living fungal biomass.

#### DISCUSSION

The assay of total hyphal length in soil using calcofluor for stain, in theory, is a measure of all mycelial fungi regardless of metabolic state or taxonomic affiliation. Because calcofluor stains cell walls composed of chitin or cellulose, all types of filamentous fungi can be detected and cell contents do

not affect results. Fungal tissue that may not be detected by this method includes non-filamentous forms such as yeast cells and reproductive structures or resting bodies such as sclerotia. Quantification of FDA-stained hyphal length, theoretically, is a measure of living mycelial fungi because FDA fluoresces within enzymatically-active hyphae (Soderstrom, 1977). There are a number of studies that show, however, that this method significantly underestimates the amount of living hyphae due to problems of hyphal disintegration during release from substrate with homogenization (Soderstrom, 1979; Ingham and Klein, 1982) and failure of all living hyphae to fluoresce after FDA incubation (Carroll *et al.*, 1980).

Ergosterol is an important membrane sterol in almost all eumycotic fungi (Weete and Weber, 1980) and has been postulated to be a good indicator of living fungal biomass in soil (West *et al.*, 1987). An important disadvantage of this fungal marker compound is that it is not produced by oomycetous fungi (e.g. *Pythium*, *Saprolegnia*, etc.) and a number of yeasts, both of which may be important in some soils. Another potential drawback of this method is the variability of ergosterol content of fungi in natural soil environments (Newell, 1992). Both of these factors contribute to the difficulty in estimating fungal biomass in a soil based upon its ergosterol content.

Soil from each site we examined had a unique combination of soil ergosterol content, total hyphal length and living hyphal length. All three measures indicate that soil from the Doolittle prairie site had,

Table 2. Coefficients of linear correlation ( $r$ ) and standard error of estimates for regressions of soil ergosterol content with total hyphal length and living hyphal length

Variables (x,y)	Model	Standard error of estimate	$r$ value	$P$ value
Total hyphal length, soil ergosterol content	$y = 1.48 + 0.0022 (x)$	1.05	0.638	0.000
	$y = 0.00413 (x)$	1.42	0.863	0.000
Living hyphal length, soil ergosterol content	$y = 1.43 + 0.0124 (x)$	1.00	0.677	0.000
	$y = 0.02213 (x)$	1.37	0.874	0.000

Table 3. Summary of ANOVA results

Variables (x, y)	Model	Source	D.F.	S.S.	M.S.	F	P
Total hyphal length, soil ergosterol content	$y = 1.48 + 0.0022 (x)$	Regression	1	39.910	39.910	36.363	0.0000
		Residual	53	58.170	1.097		
	$y = 0.00413 (x)$	Regression	1	321.622	321.662	158.050	0.0000
		Residual	54	109.887	2.035		
Living hyphal length, soil ergosterol content	$y = 1.413 + 0.0124 (x)$	Regression	1	44.953	44.953	44.846	0.0000
		Residual	53	53.127	1.002		
	$y = 0.002213 (x)$	Regression	1	329.469	329.469	174.356	0.0000
		Residual	54	102.040	1.889		

by far, the greatest amount of fungal biomass. It is also apparent from Fig. 1 that the non-cultivated soils all contained more fungal biomass than did the cultivated soils. That is, all three indicators were greater in the non-cultivated sites than in the cultivated sites with one exception; soil ergosterol content at the pothole site in the conventionally-tilled corn-soybean field was slightly greater than at the mature forest site.

The data in Fig. 1 also give indications as to the relative amounts of living and non-living fungal biomass at each site. For example, at the immature forest site, soil ergosterol content and living hyphal length are relatively high compared to the total hyphal length, suggesting that a larger proportion of the fungal biomass is living. In contrast, soil from the Doolittle prairie has an extremely large amount of total hyphae in relation to ergosterol content and living hyphae, suggesting that a large amount of fungal biomass at this site is non-living. Thus, not only does Doolittle prairie soil contain the greatest total amount fungal biomass, but it also appears to have the largest amount of living fungal biomass, and the highest ratio of non-living-to-living biomass.

Another point of interest is the much broader range in values among sites for both total and living hyphal lengths than in soil ergosterol content values. There is approximately three times more spread in the range of hyphal lengths than in ergosterol (Fig. 1, Table 4). First, regarding the relationship between living hyphae and soil ergosterol content, we would have hypothesized that their range in values would be

similar because living hyphal length and soil ergosterol are both measures of components of the living fungal biomass. These ranges in values are generally similar at all but the Doolittle prairie site, which has a very large amount of living hyphae but not a proportionately high ergosterol content (Fig. 1, Table 4). This may be an indication that filamentous fungi that do not produce ergosterol (i.e. oomycetes) make up a higher portion of the total fungal biomass than they do at the other sites. This is quite possible in that the Doolittle prairie site is much more mesic than the others and may be a good habitat for these organisms. Cultural or molecular studies would be required to verify this conjecture. As for the relationship between total hyphal length and soil ergosterol content, we would hypothesize that they are not necessarily closely related because most of the fungal hyphae in a soil are non-living and, presumably, contain no ergosterol. Additionally, the dynamics of production and decomposition of ergosterol and chitin are quite different which would lead to disparate patterns of occurrence and distribution in soil.

Considering that the three measures of fungal biomass we quantified all measure different components of the total fungal biomass in soil and are subject to disparate sources of error, it is not surprising that there was only a moderate degree of linear correlation between soil ergosterol content and hyphal lengths in soil. Two statistical tests did, with a high degree of significance, confirm a relationship between soil ergosterol content and both measures of

Table 4. Estimation of ergosterol concentration of living fungal biomass in soil

Site	Soil ergosterol content ( $\mu\text{g g}^{-1}$ soil)	Length of living fungal hyphae in soil ( $\text{m g}^{-1}$ soil)	Estimated living fungal biomass in soil ( $\text{mg g}^{-1}$ soil)	Estimated ergosterol concentration of living fungal biomass in soil ( $\text{mg g}^{-1}$ fungal biomass)
1. Ridgetop, conventionally-tilled corn-soybean field	1.07	20	0.06	19
2. Bottom, conventionally-tilled corn-soybean field	2.61	31	0.09	31
3. No-till corn field	1.03	48	0.13	8
4. McFarland prairie	2.71	64	0.18	15
5. Doolittle prairie	3.49	239	0.66	5
6. Immature forest	2.89	83	0.23	13
7. Mature forest	2.36	98	0.27	9

fungus hyphal length (Table 2 and Table 3). As mentioned above, soil ergosterol content would be expected to correlate more closely with living than total hyphal length in soil. Results of regression analysis, however, indicated only slightly different coefficients of linear correlation for regressions of soil ergosterol content with living hyphal length and total hyphal length (Table 2). These results may be an indication of the error involved with the FDA-staining method or that the ergosterol method may be just as good an indicator of fungal biomass in soil as both direct count methods. Unfortunately, no definitive method exists to quantify fungal biomass in soil and calibrate the techniques we used.

In a study of biochemical components of soil microbial biomass, West *et al.* (1987) examined the relationship between direct microscopic counts of total soil mycelia (both fungi and actinomycetes, using calcofluor for staining) and soil ergosterol content. They report a coefficient of linear correlation of 0.70 for the regression of soil ergosterol values and mycelial surface area values using data from all three soils examined in their study and a coefficient of 0.84 using data from two of those soils in which, they hypothesize, the mycelia present are all fungal (West, 1986). In their work on microbial biomass in two forest soils, Scheu and Parkinson (1994) obtained a coefficient of linear correlation of 0.69 for the regression of soil ergosterol content and fungal biovolume as measured by direct microscopic counting using calcofluor as stain. The regression models used by both West *et al.* (1987) and Scheu and Parkinson (1994) are linear and include both a slope and  $y$ -intercept ( $y = a + bx$ ). The coefficient of linear correlation values reported in those two studies are very similar to what we obtained ( $r = 0.638$ ) using the same model (Table 2). Scheu and Parkinson (1994) also found soil ergosterol content to be much more closely correlated with fungal biomass as measured by the substrate inhibition method (coefficient of linear correlation = 0.91) than by direct microscopy. This is consistent because the substrate inhibition method is an estimator of living biomass whereas direct microscopic counting as conducted by Scheu and Parkinson (1994) using calcofluor as stain is a measure of both living and non-living fungal hyphae.

The estimated concentration of ergosterol in fungi growing in the soils we examined ranged from 5 to 31 mg g<sup>-1</sup> fungal biomass (Table 4), with an average value of 14 mg g<sup>-1</sup> fungal biomass. This average value is within the range of ergosterol concentrations reported for various fungi grown on a variety of media (Weete and Weber, 1980; Newell, 1992; Gessner and Chauvet, 1993; Davis and Lamar, 1992) but is higher than most observations. West *et al.* (1987) report a mean value for ergosterol content of fungi in the soils they studied of 0.16  $\mu\text{g cm}^{-2}$  fungal surface area which converts to a value of 5.5 mg g<sup>-1</sup> fungal biomass, assuming a mean hyphal diameter of 3.0  $\mu\text{m}$ . This value is closer to those reported in Weete

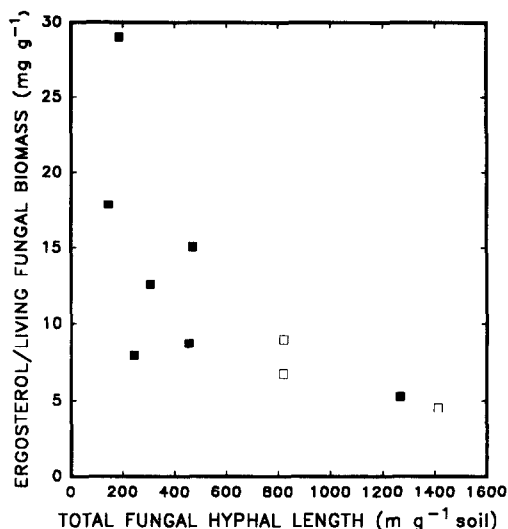


Fig. 3. Scatterplot showing ratio of soil ergosterol concentration to living fungal biomass plotted against total fungal hyphal length values. Solid squares represent data collected in our study, open squares represent data from Scheu and Parkinson (1994).

and Weber (1980) and Newell (1992), but is calculated from total fungal hyphae (calcofluor-stained) in soil as opposed to live (FDA-stained) fungal hyphae, as we used. Scheu and Parkinson (1994) calculated ergosterol content of endogenous soil fungi based on an estimate of living fungal biomass obtained from the substrate-induced respiration method and obtained a value of 11 mg ergosterol g<sup>-1</sup> live fungal biomass carbon. Assuming that fungi are approximately 45% carbon, this figure converts to a value of about 5 mg ergosterol g<sup>-1</sup> live fungal biomass. Our calculated value for ergosterol concentration in fungi growing in soil is probably too high because the FDA staining method underestimates the amount of living hyphae in soil, whereas the value obtained by West and Grant (1987) may be too low because it is based on total fungal hyphae in soil (both living and non-living). Conflicting reports on the accuracy of the substrate-induced respiration method (Frankland *et al.*, 1990) make it difficult to evaluate the precision of soil fungal biomass measurements based on this technique.

Examination of our data led us to the observation that in those soils with the lowest amounts of total (living and non-living, calcofluor-stained) fungal hyphae, the ergosterol concentration in living fungal biomass was higher than in soils with greater amounts of total hyphae (Table 4; Fig. 1). This led us to examine more closely the relationship between the total hyphal length in a soil and the concentration of ergosterol in living fungi present in that soil. Using data from both our study and that of Scheu and Parkinson (1994), we plotted calculated fungal ergosterol concentration values and total hyphal length values for 10 soils. Results (Fig. 3) suggest that the amount of ergosterol in the living fungal biomass

pool of a soil is related to the total hyphal length in that soil. We believe it is highly significant that data from two independent studies using different methods to determine living fungal biomass support this premise. Possible explanations for this observation are described below.

The relationship between the specific ergosterol content of the living fungal biomass and the total hyphal length in a soil may be influenced by 1) the metabolic state of the fungal community, 2) the species composition of the fungal community, and 3) the turnover rate of ergosterol. A number of studies on different fungi have demonstrated that ergosterol concentration is significantly greater in older and stationary phase mycelia than in young or growth phase mycelia (Nout *et al.*, 1987; Newell *et al.*, 1987; Schnurer, 1992) and current models of fungal growth propose that actively growing fungi preferentially allocate resources to hyphal wall synthesis over cytoplasmic constituents (the location of ergosterol) which can be translocated within the mycelium (Jennings, 1976; Paustian and Schnurer, 1987; Zinser *et al.*, 1991). Partial mycelial autolysis can also drastically reduce ergosterol content of residual mycelial mass (Newell *et al.*, 1987). Soils with large, dynamic populations of fungi may contain large amounts of young, actively growing hyphae as well as significant amounts of mycelia in a state of partial autolysis with low ergosterol concentrations. Additionally, the greater the amount of total fungal hyphae in a soil, the higher the likelihood that a portion of the living hyphae was formed by non-ergosterol producing filamentous oomycete fungi, further reducing the ratio of soil ergosterol content to living fungal biomass. Soils with low fungal biomass may also contain relatively small amounts of total microbial biomass resulting in slow decomposition and reduced rates of ergosterol turnover in soil.

To further examine and describe the observed relationship between ergosterol content of living fungal biomass and the total hyphal length in a soil, we regressed the ratios of living fungal biomass to soil ergosterol content with total hyphal length values for only the seven soils sampled in our study (Fig. 4). Results showed a strong relationship and high degree of linear correlation between these two variables indicating that the specific ergosterol content of the living biomass pool is correlated to the total amount of fungal hyphae in that soil. The resulting regression equation (Fig. 4) describes this relationship and should be useful in calculating factors for converting soil ergosterol concentrations into estimates of living fungal biomass in soils with a range of total fungal hyphae. To use such a procedure, soil ergosterol concentration and total hyphal length (using calcofluor as stain) must be measured, followed by determination of the ratio of living fungal biomass to soil ergosterol concentration using the regression equation given in Fig. 4. Finally, soil ergosterol

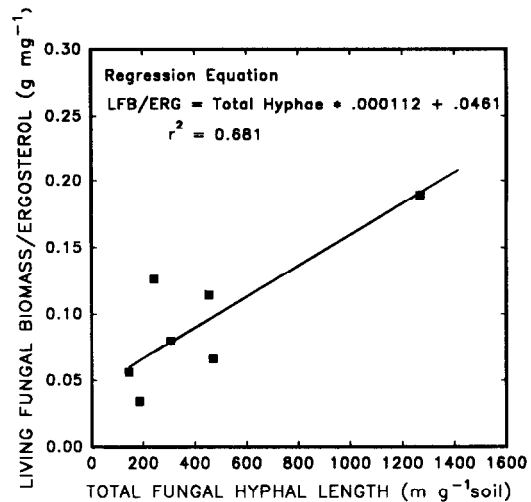


Fig. 4. Regression of ratio of living fungal biomass to soil ergosterol content values and total fungal hyphal length values. Only data from our study were used in development of the regression equation.

concentration should be multiplied by the ratio of living fungal biomass to soil ergosterol concentration to give an estimate of living fungal biomass.

We tested this procedure by comparing actual measurements of living fungal biomass to predicted living fungal biomass values using data from both our study and, for independent verification, Scheu and Parkinson's (1994). Predicted values were very close to actual measurements (Fig. 5) supporting the use of this method to estimate living fungal biomass from soil ergosterol concentration. The resulting correlation coefficient between observed and predicted

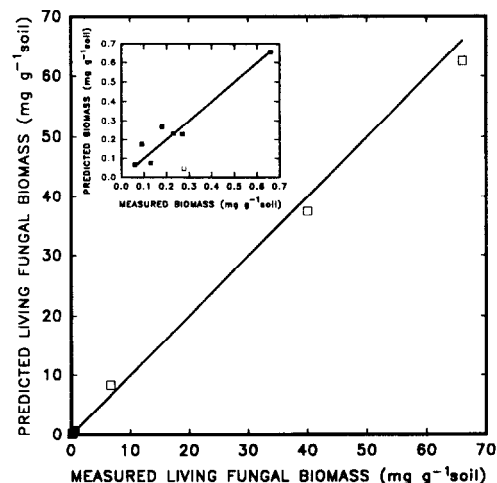


Fig. 5. Results of regression test of proposed method to estimate living fungal biomass in soil from soil ergosterol concentration and total fungal hyphal length. Inset shows results for living fungal biomass values less than  $0.7 \text{ mg g}^{-1} \text{ soil}$ . Line represents a perfect one-to-one relationship between measured and predicted values. Solid squares represent data collected in our study, open squares represent data from Scheu and Parkinson (1994).

values was 0.999 and a remarkably good fit to the independent data set of Scheu and Parkinson.

In conclusion, we believe that the results of this and other studies (West *et al.*, 1987; Davis and Lamar, 1992; Scheu and Parkinson, 1994) support the use of ergosterol as a measure of fungal biomass in soil. We found soil ergosterol concentration to be positively correlated to both total and living hyphal lengths in soil with a high degree of significance, but only a moderate degree of linear correlation. Therefore, a moderate degree of error will be involved in estimation of living fungal biomass from soil ergosterol content alone. Our results indicate, however, that the amount of error can be reduced by accounting for the relationship between total hyphal length in soil and the amount of ergosterol in the living fungal biomass pool. Additionally, we believe it is important to distinguish between the living and non-living components of fungal biomass for accurate quantification because of their different nutrient contents and functional importance. Thus, we recommend determination of soil ergosterol content as a indicator of living fungal biomass and total fungal hyphal length using calcofluor as stain as a measure of the total (living and non-living) fungal biomass in soil. To calculate an estimate of living fungal biomass from soil ergosterol content we propose using the method described above. This technique should be used critically and further examination of the relationship between soil ergosterol content, fungal community composition, and other indicators of fungal biomass is needed to fully understand and refine this method.

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