ELSEVIER

Soil Biology & Biochemistry 39 (2007) 2173–2177

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Short communication

Fungal biomass production and turnover in soil estimated using the acetate-in-ergosterol technique

Johannes Rousk*, Erland Bååth

Department of Microbial Ecology, Lund University, Ecology Building, SE-223 62 Lund, Sweden

Received 1 February 2007; received in revised form 14 March 2007; accepted 21 March 2007
Available online 18 April 2007

Abstract

We report the first attempt to estimate fungal biomass production in soil by correlating relative fungal growth rates (i.e., acetate incorporation into ergosterol) with fungal biomass increase (i.e., ergosterol) following amendments with dried alfalfa or barley straw in soil. The conversion factor obtained was then used in unamended soil, resulting in fungal biomass productions of 10–12 $\mu g\,C\,g^{-1}$ soil, yielding fungal turnover times between 130 and 150 days. Using a conversion factor from alfalfa-treated soil only resulted in two times higher estimates for biomass production and consequently lower turnover times. Comparing fungal biomass production with basal respiration indicated that these calculations overestimated the former. Still, the turnover times of fungal biomass in soil were in the same range as turnover times estimated in aquatic systems. The slow turnover of fungal biomass contrasts with the short turnover times found for bacteria. The study thus presents empirical data substantiating the theoretical division of bacteria and fungi into a fast and a slow energy channel, respectively, in the soil food web.

Keywords: Ergosterol; ^{14}C acetate; Fungi; Biomass production; Soil

Methods of assessing the presence, growth and activity of bacteria and fungi are prerequisites when studying the soil ecosystem. Several methods of differentiating between the biomass of these groups exist, including microscopy (Bölter et al., 2002), substrate-induced respiration combined with specific inhibitors for fungi and bacteria (Anderson and Domsch, 1973; Bailey et al., 2003; Wallenstein et al., 2006) and the use of indicator substances. Fungal biomass can, for example, be estimated using the PLFA 18:2ω6,9 or the fungal-specific lipid ergosterol (Frostegård and Bååth, 1996). These biomass-based methods can be used to study microbial growth in situations with increasing biomass, e.g., after the addition of fresh substrate, but cannot be used in this way under situations where microbial growth is balanced by predation and death, that is, with no biomass increase.

Quantifying the growth rate and the production of each microorganism group separately has thus been difficult. The thymidine and leucine incorporation techniques, originally developed for aquatic systems (Fuhrman and Azam, 1982; Kirchman et al., 1985) and subsequently adapted to soil (Bååth, 1992, 1994; Bååth et al., 2001), have partly overcome this problem for bacteria. Although these two methods have been mainly used as relative measures of bacterial activity in soil, attempts have been made to quantify both production and turnover rates. Bacterial production in soil, at similar levels as those for respiration, has been calculated (Bååth, 1994), with biomass turnover times between 2.3 and 33 days (thymidine incorporation) and 2.1 and 13.1 days (leucine incorporation) (Bååth, 1998).

A corresponding tool to estimate fungal growth has hitherto proved more elusive. A method of estimating fungal growth, originally devised for aquatic habitats (Newell and Fallon, 1991) was, however, recently adapted to soil (Pennanen et al., 1998; Bååth, 2001). The method is based on the incorporation of radioactively labelled acetate into the fungal-specific lipid ergosterol. The acetate-in-ergosterol (Ac-in-erg) method has been applied to

*Corresponding author. Tel.: +46 46 2223762.
E-mail address: johannes.rousk@mbioekol.lu.se (J. Rousk).

0038-0717/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2007.03.023

determine relative levels of fungal growth in differently treated soils (Pennanen et al., 1998; Rajapaksha et al., 2004; Pietikäinen et al., 2005; Tobor-Kapłon et al., 2005).

No estimate of fungal biomass production or turnover rate has, however, been presented in native, untreated soil. The present study used data from a larger study, designed to compare fungal and bacterial responses with different substrates and levels of N amendment over time (J. Rousk and E. Bååth, unpublished), to achieve such an estimate. First we determined a conversion factor between Ac-in-erg incorporation and the increase in fungal biomass (ergosterol content) after adding different plant materials. This conversion factor was then used in a separate experiment to estimate fungal biomass production and turnover rates and to compare these with respiration rates.

A garden sandy loam soil (organic matter content = 17%, pH = 5.9) was used. Dried alfalfa ($C/N = 15$) and barley straw ($C/N = 75$) were ball-milled, sieved (<0.25 mm) and applied at 2 mg C g^{-1} soil (d.w.). NH_4NO_3 was applied at concentrations of 0, 0.05, 0.10 and 0.15 mg N g^{-1} soil (d.w.). Two control treatments, with no substrate addition and 0 or 0.15 mg N g^{-1} soil were also used resulting in 10 treatments, which were duplicated. Thus, 20 jars containing 100 g soil were incubated at room temperature without light. These were sampled regularly until there was no further increase in ergosterol content in the amended soils (21 days). The repeat experiment was identical except that fewer N levels were used for the alfalfa treatment (0 and 0.15), resulting in eight duplicated treatments, and only 8 days' incubation. Basal respiration was also measured in this experiment.

Fungal growth was assessed using the Ac-in-erg method adapted for soil by Bååth (2001). Briefly, 1 g w.w. of soil was transferred to test-tubes to which 0.025 ml 1,2-[^{14}C]acetic acid (sodium salt, 2.04 GBq $mmol^{-1}$, 7.4 MBq ml^{-1}, Amersham), 0.475 ml 1 mM unlabelled acetate (pH = 6) and 1.5 ml distilled water had been added, resulting in an acetate concentration of 0.2 mM. The resulting soil slurry was incubated at room temperature (22 °C) for 24 h, after which 1 ml 5% formalin was added to terminate growth. The test tubes were centrifuged 1000 × g for 5 min and the supernatant with non-incorporated acetate was discarded. Ergosterol of the soil was then extracted in 5 ml 10% KOH in methanol, sonicated for 15 min followed by 90 min heat treatment at 70 °C, and partitioned twice with 2 ml cyclohexane. The combined cyclohexane phases were evaporated to dryness at 40 °C under N_2. The samples were then dissolved in 200 µl methanol, heated at 40 °C for 15 min, filtered through a 0.45 µm filter, and analysed using HPLC with a UV detector (282 nm). The ergosterol peak was collected and the amount of incorporated radioactivity was determined using a scintillator. Respiration was analysed on 1 g w.w. samples for 24 h using gas chromatography.

In the first experiment, the cumulative increase in fungal growth using Ac-in-erg incorporation was correlated to fungal biomass production as increased ergosterol content in amended soils (ergosterol and Ac-in-erg incorporation in control soils were subtracted) using type-II major axis regression (JMP IN version 5.1, SAS Institute Inc., Cary, NC, USA). A significant linear regression was found when combining both substrates (Fig. 1, $R^2 = 0.72$, $p < 0.001$). There was no obvious effect of nitrogen amendment. Assuming 5 mg ergosterol g^{-1} of fungal biomass (Joergensen, 2000; Ruzicka et al., 2000) and 45% C content, a conversion factor where 1 mg fungal biomass-C was equivalent to 54×10^3 DPM of incorporated Ac was determined. Treating the applied substrates separately, there was no significant difference between barley straw and the combined substrates, while alfalfa resulted in a conversion factor where 1 mg fungal biomass-C was equivalent to 22×10^3 DPM ($R^2 = 0.54$, $p < 0.001$). The conversion factor obtained from the alfalfa treatment thus resulted in a fungal biomass production about twice that of the combined treatment. Henceforth, calculations will be based on the conversion rate obtained from the combined substrates, unless explicitly stated otherwise.

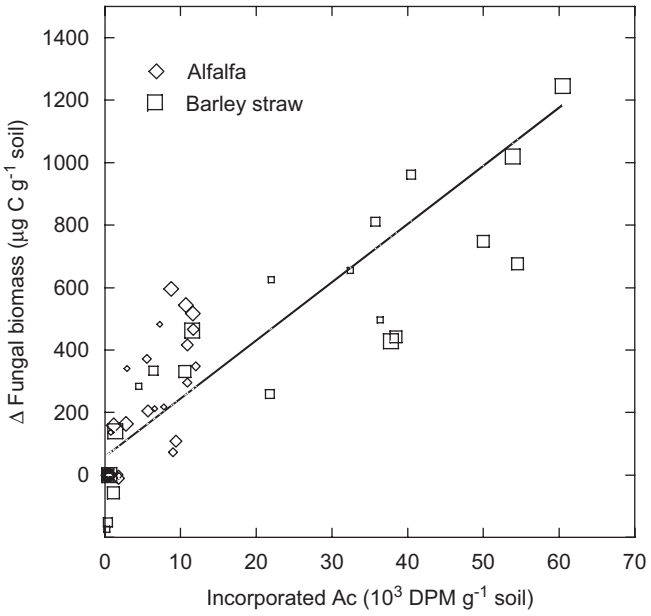

Fig. 1. Cumulative fungal biomass production (Ac-in-erg) and biomass increase (based on ergosterol content) following the addition of alfalfa (diamonds) and barley straw (squares). Values for unamended control soils have been subtracted. Increasing symbol size denotes increasing levels of nitrogen amendment (0, 0.05, 0.10 and 0.15 mg N g^{-1} soil). $R^2 = 0.72$, $p < 0.001$.

The conversion factor obtained was applied in the repeat experiment (Fig. 2). The fungal biomass production clearly reacted to the substrate additions, with the highest production in the straw-amended soils and the lowest in the unamended controls. The two higher N concentrations of the straw treatments gave slightly higher values than the two lower N concentrations, while the N additions had no effect on the alfalfa or control treatments. Most importantly, an estimate of the fungal biomass production rate of the control treatments could be calculated, amounting to 12 ± 0.8 µg fungal biomass-C day^{-1} g^{-1} soil during 8 days