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Soil Biology and Biochemistry 31 (1999) 1999–2014

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Comparison of substrate induced respiration, selective inhibition and biovolume measurements of microbial biomass and its community structure in unamended, ryegrass-amended, fumigated and pesticide-treated soils

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Received 18 June 1999

Abstract

Two UK grassland soils, one from Rothamsted (24% clay) and the other from Woburn (8% clay) were incubated at 25°C, unamended or amended with ryegrass followed by fumigation 20 d later followed by a further 20 d incubation. Other portions of the Rothamsted grassland soil were treated separately with a fungicide (Captan), a bacteriocide (Bronopol), or a herbicide (Dinoseb). The substrate-induced respiration (SIR) method coupled with use of antibiotics (selective inhibition) and biovolume measurements by direct microscopy were used to comparatively measure total microbial biomass and the proportions of fungal and bacterial biomass in these two treated soils. Both methods gave similar estimates of total microbial biomass and the proportions of bacteria and fungi in the two soils. The different treatments did not significantly change the proportions of bacteria and fungi in the soil microbial biomass. It was concluded that both SIR and biovolume measurements are equally valid in measuring total biomass as are selective inhibition and biovolume measurements in measuring the proportions of fungi and bacteria in soils which are either unamended or undergoing rapid changes in metabolism due to substrate amendment, fumigation or biocidal treatments.

Keywords: Substrate-induced respiration; SIR; Selective inhibition; Biovolume measurements; Microbial biomass; Community structure

1. Introduction

Increasing attention has been paid to the development of techniques to quantify bacterial and fungal biomass in soil. The techniques developed so far may be divided into four groups: (1) viable counts on agar plates (Martin, 1950; Williams and Davies, 1965), (2) direct microscopy (Jones and Mollison, 1948; Babiuk and Paul, 1970; Jenkinson et al., 1976; Söderström, 1977), (3) specific cellular components such as diaminopimelic acid (Work and Dewey, 1953; West et al., 1987), chitin (Donald and Mirocha, 1977), adenosine 5′-triphosphate (Jenkinson and Oades, 1979), ergosterol (Seitz et al., 1977, 1979; Grant and West, 1986) and fatty acids (Zelles et al., 1992), and (4) physiological approaches (Anderson and Domsch, 1973a,b, 1975; West and Sparling, 1986).

Viable counts invariably measure only a small portion (typically 1-3%) of the total soil microbial biomass (Skinner et al., 1952) because of the diversity of nutrients and growth conditions that they require. The measurements of many specific constituents of bacterial and fungal cells have limitations in soil microbial studies since soil may contain a large amount of these compounds in exocellular forms (Durska and Kaszubiak, 1980, 1983) and their concentrations in microorganisms may vary depending, for example,

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0038-0717/99/$ - see front matter
PII: S0038-0717(99)00122-4

upon the age of the cells, nutrient supply and other factors (Sharma et al., 1977).

Microscopy is the only direct approach for studying the community structure of the soil microbial biomass. Usually a thin soil-agar film, a soil smear or a membrane filter is prepared. The microorganisms in this thin film are identified, sized, and then counted. The agar-film method is commonly used to count the bacteria and fungal spores (spherical) and fungal hyphae (cylindrical) (Jones and Mollison, 1948; Jenkinson et al., 1976; Brookes et al., 1986; Beare et al., 1990; Vance et al., 1987). An apparently simpler method to estimate the proportions of bacterial and fungal biomass in soil is the selective inhibition method. This method was developed by Anderson and Domsch (1973a,b, 1975) and is based upon the measurements of the selective inhibition of glucose-induced respiration (SIR) by a bacterial antibiotic (usually streptomycin) and a fungal antibiotic (usually cycloheximide).

There is therefore a considerable body of literature describing different techniques to estimate the community structure of the soil microbial biomass. However, very few data are available to show the relationships between the different methods to measure the proportions of bacterial and fungal biomass in the soil. Probably the most widely used method to measure microbial community structure (or more specifically, the proportions of fungi and bacteria) is selective inhibition. It is simple, cheap and precise. However the accuracy of selective inhibition, quite a different thing, is much more difficult to determine, particularly in soils undergoing intense microbial activity. Nevertheless, there is increasing evidence of a research need to compartmentalise the 'black-box' of the microbial biomass into its key components (i.e. fungi, bacteria, protozoa, etc) and then into microbial species and even specific genes (Ritz et al., 1994). We therefore need simple accurate methods to make this possible.

Here we compare SIR, in conjunction with selective inhibition, with direct microscopy in a silty clay loam (24% clay) and a sandy loam soil (8% clay) given a range of treatments prior to analysis. These include incubation without amendment; amendment with ryegrass followed by incubation (20 d) then fumigation and a further incubation (20 d); and other sets of soils treated with Captan (a fungicide), Bronopol (a bacteriocide) or Dinoseb (a herbicide) then incubation (20 d). The aim was to attempt to change the community structure and activity of the microbial biomass by these treatments and then see how the very different techniques of selective inhibition and direct microscopy compared in detecting these differences, if they occurred.

Remarkably, despite the fact that selective inhibition was developed more than 20 years ago, there has been practically no other comparative work of this nature. West (1986) reported bacterial-to-fungal ratios measured by selective inhibition in two grassland soils of 1-to-1 but of 1-to-2 when the same communities were measured by microscopy. The selective inhibition measurements failed in the arable soil but he obtained a microscopic ratio of 1 bacteria-to-3 fungi. Beare et al. (1990) reported that SIR and selective inhibition measurements agreed well with community structure measurements of fungi and bacteria made by microscopy. However, since they did the measurements on isolated plant residues it would not be wise to extrapolate these results to soil. Scheu and Parkinson (1994) measured changes in microbial communities, including bacterial and fungal biomass (by SIR) and biovolume in the air-dried and rewetted four soil layers (L, F, H and Ab) of an aspen forest and the F/H layer of a pine forest. Both methods of measuring bacterial and fungal biomass were strongly correlated throughout a 40 d incubation after air drying and rewetting. The only other relevant work we could find was that of Velvis (1997). He found higher fungal-to-bacterial ratios with selective inhibition (0.50 to 0.60) than by biovolume measurements (0.19 to 0.46) in acid (pH 3.8 to 5.8) agricultural Dutch soils. He also stressed the need for simultaneous approaches for the quantitative measurements of fungal and bacterial biomasses in soil.

In view of the very small amount of work done previously, some of which was contradictory, as reviewed above, and our interest in measuring the community structure of the soil microbial biomass, we decided to carry out the experiments outlined above with the aim of further determining relationships between selective inhibition and biovolume fungal-to-bacterial ratios in perturbed and incubated soils.

2. Materials and methods

2.1. Soils

Two soils were used. One, a clay loam soil (Batcombe Series, 24% clay) was from a Permanent Grassland Plot of the Highfield Ley-Arable experiment. The soil contained 4.08% organic C, 0.364% total N and had a pH of 4.8. The other was a sandy loam soil (Cottenham Series, 8% clay) from a first-year ley of the Woburn Ley-Arable Experiment. This soil contained 1.21% organic C, 0.12% total N and had a pH of 6.4. The two field-moist soils were sieved (<2 mm), soil moisture adjusted to 40% water-holding capacity (WHC) and then incubated at 25°C for 7 d. Plant residues and visible animals (e.g. earthworms and nematodes) in the soils were removed manually.