

Soil Biology & Biochemistry 38 (2006) 912-922

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Microbial immobilisation and turnover of ¹⁵N labelled substrates in two arable soils under field and laboratory conditions

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Received 10 April 2003; received in revised form 20 June 2005; accepted 25 July 2005 Available online 9 September 2005

Abstract

Microbial biomass N dynamics were studied under field and laboratory conditions in soils of high yield (HY) and low yield (LY) areas in an agricultural field. The objective of the study was to determine the size and activity of soil microbial biomass in the soils of the different yield areas and to compare these data obtained under field and laboratory conditions. Soils were amended with ¹⁵N labelled mustard (*Sinapis alba*) residues (both experiments) and labelled nitrate (laboratory only) at 30 μ g N g⁻¹ dry soil. Soil microbial biomass (SMB) N, mineral N (N_{min}) and total N content was monitored both in the field and in the laboratory. N₂O efflux was additionally measured in laboratory treatments. Isotope ratios were determined for SMB in both experiments, for all other parameters only in the laboratory treatments. In the laboratory less amounts of added substrate N were immobilised by the SMB in HY soils compared to LY soils, whereas in the field immobilisation of added N by SMB was higher in HY soils initially and slightly lower after 40 days of incubation. Calculated turnover times in the laboratory nitrate, laboratory mustard and field mustard amendments were 0.18, 0.27 and 0.74 years (HY) and 0.22, 0.61 and 1.01 years (LY), respectively. The turnover times of added substrate N always showed the trend to be faster in HY soils compared to LY soils. A faster turnover of nutrients in the HY soils may involve a better nutrient supply of the plants, which coincides with the higher agricultural yield observed in these areas.

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Keywords: ¹⁵N; Nitrogen turnover; Immobilisation; Isotope pool dilution; Microbial Biomass

1. Introduction

Soil microbial biomass (SMB) represents only 1–5% of the total C- and N-pool in soil organic matter (SOM) (Jenkinson and Ladd, 1981; Sparling, 1985; Smith and Paul, 1990). However, it plays an important role as a dynamic source and sink of nutrients, and it is the driving force behind SOM transformations (Jawson et al., 1989; Smith, 1994; Burger and Jackson, 2003). N transformation by SMB occurs at rates capable of turning over the inorganic N pool several times a day (Coyne et al., 1998). Soil microbial N immobilisation may play an important role in regulating the soil N retention capacity (Bengtsson and Bergwall, 2000) and may thus prevent nitrogen loss through leaching of NO_3^- (Bengtsson et al., 2003). Singh et al. (1989) showed

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that SMB can be a source of plant nutrients in nutrient-poor tropical soils, where it acted as a sink of nutrients during the dry period (high biomass, low turnover) and as a source during the monsoon period of plant growth (low biomass, high turnover). N flux through SMB has been shown to be sufficient to supply plant N demand (Lethbridge and Davidson, 1982; Paul and Voroney, 1984).

The turnover time of N through the microbial biomass can be estimated by the ¹⁵N isotope dilution technique (Powlson and Barraclough, 1993; Hart and Myrold, 1996). Soils are amended with a labelled substrate, which is immobilised by the SMB and the change of biomass ¹⁵N is monitored subsequently. Turnover rates can be estimated from changes of label in the SMB pool by first-order kinetics (Chaussod et al., 1988). This simple model can give a very good fit to the data, but may underestimate turnover rates because it does not take the recycling of labelled N into account (Jenkinson and Parry, 1989).

Most of the recent research in soil ecology has been done in laboratory microcosm studies (Kampichler et al., 2001). In spite of the importance of combining laboratory and field

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studies (Carpenter, 1996; Verhoef, 1996), this combination has very rarely been realised in soil-ecological research (Kampichler et al., 2001).

In our approach we want to follow the pathways of added labelled N through the microbial biomass, including immobilisation and turnover through the SMB. The use of labelled substrate may give a better insight into the role of SMB as a sink and source of nutrients. For an investigation of the sink-source aspect, we compared two soils from high and low yield areas of an agricultural field. The experiments were conducted under laboratory and field conditions, which gives the opportunity to compare in how far the laboratory estimations agree with those under natural field conditions.

2. Material and methods

2.1. Soils

The soils studied under field and laboratory conditions originated from a conventionally treated field at the experimental farm at Scheyern, which is located 40 km northeast of Munich, Germany (N 48°30.0', O 11°20.7'). The mean temperature in Scheyern is 7.4 °C and the annual precipitation amounts to 833 mm (Auerswald and Kainz, 1990). Yield data of this agricultural field collected over 4 years showed distinct areas of higher and lower yield. The high-yield areas are situated in the lower parts of the field, while the low yield areas are localised on a rounded hilltop. Soils in the high yield (HY) part were characterised as typic Udifluvent with sand, silt and clay fractions of 36, 49 and 15%, respectively. HY areas have an organic C content of 1.7%, a total N content of 0.17% and a pH 5.9 in the upper 10 cm. Soils in the low yield (LY) area were described as dystric Eutrochrept with sand, silt and clay fractions of 29, 51 and 20%. Organic C and total N content in 0-10 cm depth were 1.4 and 0.15%, respectively, and soil pH was 6.1.

In September 1999 maize was harvested followed by the sowing of winter wheat in the beginning of November. Fertiliser was applied the first time in March 2000 $(50 \text{ kg N ha}^{-1})$ after finishing the last sampling.

2.2. Experimental design

2.2.1. Field experiment

The field experiment was started in October 1999, after harvest of maize. Two plots of 4×4 m², one amended with ¹³C- and ¹⁵N-labelled white mustard (*Sinapis alba*) and another not amended control plot, were set up each in the HY and the LY area. The mustard material had a ¹³C content of 101.1‰ PDB and a ¹⁵N-content of 18 at.% of dry matter. Maize residues were removed from the plot areas and their vicinity. The soil in the plots was removed to a depth of 15 cm and sieved (15 mm) through a compost sieving machine. Mustard material was chopped to 6 mm. Mustard and soil material were thoroughly mixed with a ratio of 1.37 g mustard kg⁻¹ dry soil (corresponding to 500 μ g C g⁻¹ dry soil and 30 μ g N g⁻¹ dry soil) and returned into the plots. Soil in the control plots was treated accordingly, without the addition of mustard straw. Within each plot, four adjacent stripes of 0.75 m width were defined as replicates. A margin of 0.5 m width was spared from sampling as a transition to the untreated areas of the field. Sampling started one day after amendment and was continued with a decreasing frequency from daily to twice a month until the first application of N fertiliser at the end of March 2000. Soil samples were taken from the upper 12 cm with a soil corer of 3 cm diameter and were processed at the same day without sieving. On each sampling date soil microbial biomass (SMB) N, mineral N (N_{min}) and total N content were analysed. Isotope ratios were determined for SMB and total N on 10 selected sampling dates. CaCl₂ and K₂SO₄ extracts were stored at -18 °C until analysis (Section 2.3).

2.2.2. Laboratory experiment

Soil from HY and LY areas was sampled from the upper 12 cm in April 2000 (winter wheat), before the first application of pesticides. The soil was sieved (<5 mm) and stored air-dry until preparations for the experiment were finished after 3 months. The experiments with LY and HY soil were run in two stages, using the LY soil first while keeping the HY soil stored air-dry. Before the incubation, the soil water content was adjusted to 40% WHC and the soils were kept at 22°C for 2 weeks.

Maximum Water holding capacity (WHC) of the soil material was determined according to Nehring (1960) using glass containers with a permeable bottom. WHC was expressed as percentage weight.

Batch incubations took place in PE-tubes (height 25 cm, Ø 4.5 cm), at a water content increased to 50% WHC at 14 °C for a period of 98 days. Soil was treated in three variants: (1) Control; (2) white Mustard (¹³C/¹⁵N labelled, ground mustard); (3) Glucose/nitrate solution (same C/N ratio and isotope labelling as mustard). Mustard and glucose/nitrate solution were added in amounts corresponding to the field experiment mentioned above, i.e. $500 \ \mu g \ C \ g^{-1}$ dry soil and $30 \ \mu g \ N \ g^{-1}$ dry soil. For each sampling date, three PE tubes were filled as replicates for each variant, making up a total of 90 tubes. During the incubation period samples were taken on 10 sampling dates, 0, 2, 6, 13, 20, 34, 55, 76 and 98 days after the amendment. At each sampling date SMB N, N_{min} and total N content including the respective ¹⁵N isotope ratios were determined. CaCl₂ and K₂SO₄ extracts were stored at -18 °C until analysis (Section 2.3). Additionally N₂O emissions were quantified.

2.3. Biomass N and N_{min} measurements

Biomass N was measured by fumigation extraction (FE) (Brookes et al., 1985). Fumigated and non-fumigated soil

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