Soil Biology & Biochemistry 65 (2013) 22–32

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Microbial use of ^{15}N-labelled maize residues affected by winter temperature scenarios

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ARTICLE INFO

Article history:
Received 26 November 2012
Received in revised form
13 May 2013
Accepted 15 May 2013
Available online 1 June 2013

Keywords:
Freeze–thaw cycles
Decomposition
CO_2
Microbial biomass C and N
Ergosterol
Extractable C and N
Soil total N
$\delta^{13}C$ and $\delta^{15}N$
Priming

ABSTRACT

A 56-day incubation experiment was carried out to investigate decomposition and microbial use of ^{15}N-labelled maize (*Zea mays* L.) residues incubated under four winter temperature scenarios. The residues were mixed to mesocosms equivalent to 1.2 mg C and 42.5 μg N g^{-1} dry soil, after which the samples were incubated at a constant temperature of +4 °C, a constant −3 °C, and under multiple and single freeze–thaw conditions. A constant +4 °C was most favourable for microbial substrate use, with 4- and 6-fold higher total and maize-C mineralization, respectively, in comparison with constant frost. The cumulative maize mineralization was not determined by the frequency of freeze–thaw events, but regulated by the overall time of frost and thaw conditions. The decomposition of maize straw significantly increased soil organic C mineralization (in all scenarios) and incorporation into microbial biomass (in the freeze–thaw scenarios only). The positive priming effects observed were equivalent to an additional loss of total soil organic C of between about 0.2 (continuous frost) and 0.8% (single freeze–thaw). Microbial biomass was significantly increased after maize straw amendment, with constant frost and freeze–thaw scenarios not having any negative effect on microbial biomass C compared with constant +4 °C. Highest fungal biomass was found after constant frost without fresh substrates and also after extended frost followed by a warm period when fresh plant residues were present. On average, 50% of the added maize N were recovered in the soil total N after 56 days of constant 4 °C and in the freeze–thaw scenarios, with the strongest effect after single freezing and thawing.

1. Introduction

Although it is predicted that climate warming will be most pronounced at high latitudes (Houghton et al., 2001; IPCC, 2007), temperate soils may also be affected as they remain close to freezing point throughout the winter (Henry, 2008). In fact, trend analysis has shown an increase in mean annual temperature for Germany of about 0.8–1.1 °C from 1901 to 2000, with marked increases in winter precipitation (Schönwiese and Janoschitz, 2008). A positive trend for the mean annual temperature (increase of 1.3 °C from 1951 to 2005) and increased winter precipitation as well as a decrease in the number of days with minimum temperatures below 0 °C was also found by Haberlandt et al. (2010) for Lower Saxony, which is a large temperate area (357,000 km^2) in the north of Germany. Winter climate change may have stronger effects on microbial activity and C and N dynamics in fallow arable soils, due to a lack of protective plant cover in comparison with grassland or forest systems.

It is assumed that climate warming reduces the snow pack thickness, thus leading to lower soil temperatures or to higher frequencies of freezing and thawing, i.e. colder soils in a warmer world (Isard and Schaetzl, 1998; Groffman et al., 2001). Increased soil freezing can cause leaching losses of C and N (Groffman et al., 2001; Fitzhugh et al., 2001) as well as lower winter soil respiration (Monson et al., 2006). In addition, it has been suggested that soil freezing and thawing disrupts soil aggregates (Oztas and Fayetorbay, 2003; Six et al., 2004), plant material (Mellick and Seppelt, 1992; Harris and Safford, 1996) and microbial cells (Skogland et al., 1988; Yanai et al., 2004; Larsen et al., 2002). This enhances microbial activity upon thawing, due to increased availability of substrates or easily decomposable organic matter (Edwards and Cresser, 1992; Schimel and Clein, 1996; Lipson et al., 2000; Grogan et al., 2004; Sharma et al., 2006). Microorganisms are the main drivers of soil organic matter decomposition and nutrient

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http://dx.doi.org/10.1016/j.soilbio.2013.05.008

cycles (Swift et al., 1979), with saprotrophic fungi being most important in decomposing plant residues in arable soils (Bowen and Harper, 1990; Cheshire et al., 1999). Contradictory impacts of freeze–thaw events on microorganisms have been reported, with either decreasing (Lipson et al., 1999; Pesaro et al., 2003) or no effects (Grogan et al., 2004; Sharma et al., 2006) on microbial biomass. If temperature drops below 0 °C, shifts in microbial substrate use occur (Schimel and Mikan, 2005), which may be accompanied by shifts in microbial community composition from bacteria towards fungi (Lipson et al., 2002; Schadt et al., 2003; Lipson and Schmidt, 2004; Sjursen et al., 2005).

In order to follow microbial C and N dynamics around the freezing point, we combined the natural $\delta^{13}C$ value of maize straw, which is usually different from soil organic matter (Ryan and Aravena, 1994; Rochette et al., 1999; Potthoff et al., 2005), and an artificial enrichment in $\delta^{15}N$. To our knowledge, this is the first attempt to simultaneously follow microbial respiration and C and N sequestration into different fractions, such as microbial biomass, CO_2, particulate organic matter, extractable C and N, and soil total C and N, during the decomposition of a complex organic substrate at low temperatures. This incubation study addressed the following questions: (1) Do freeze–thaw scenarios accelerate the decomposition of straw-derived C and N in comparison with constant temperatures around 0 °C? (2) Is the microbial use, i.e. mineralization and immobilization, of straw-derived organic matter regulated by the frequency of freeze–thaw cycles?

2. Material and methods

2.1. Soil and plant material

The arable soil used for the experiment was taken from the upper 10 cm of an experimental site in Neu-Eichenberg near Witzenhausen (51°23′ N, 9°55′ E, Northern Hessia, Germany) in September 2009. The site is located at 240 m above sea level with a mean annual precipitation and temperature of 670 mm and 8.7 °C. The soil is classified as a Haplic Luvisol (FAO-WRB, 2006) with the following characteristics: 3.3% sand, 83.4% silt, 13.3% clay, a water holding capacity of 55%, a pH ($CaCl_2$) of 6.3, 1.4% total C, a $\delta^{13}C$ value of $-26.4 \pm 0.1‰$, 0.14% N and a $\delta^{15}N$ value of 7.8 ± 0.3. Plant tissue, insects and stones were removed by hand and the soil was sieved (<2 mm). The soil was incubated at 3 °C for 4 weeks before the experiment started. For ^{15}N labelling, maize was grown for six months in the greenhouse and fertilised once with 278 ml fertilizer solution containing 160 mg l^{-1} NH_4NO_3 enriched with 10 atom-% ^{15}N. Maize leaf residues were harvested in October 2009, air dried, chopped into pieces of 0.5 cm × 1 cm and stored in a paper bag at room temperature. The maize residues contained 43.7% (±0.3) C with a $\delta^{13}C$ value of −12. ‰, 1.8% (±0.01) N with a $\delta^{15}N$ value of 594.8‰ and had a C/N ratio of 24.7.

2.2. Incubation procedure

The incubation experiment was carried out in 2 L glass jars with 5 replicates per treatment, each containing soil with a water content of 20% on a dry weight basis (corresponding to 36% of the water holding capacity), equivalent to 300 g dry soil. After equilibration at 3 °C, maize residues were mixed thoroughly into half of the soil samples at amounts of 1.2 mg C and 42.5 μg N g^{-1} dry soil, the remaining samples serving as non-amended controls. All samples were then incubated simultaneously by temperature treatment in two climate cabinets (MV 600, LinTek, Germany) for 56 days. To simulate winter climate regimes, four different temperature treatments were applied: (1) a constant +4 °C ($+4_{CON}$), (2) a constant −3 °C (-3_{CON}), (3) multiple freeze–thaw cycles of 48 h at +4 and −3 °C, respectively ($+4/-3_{MULTIPLE}$), and (4) a single freeze–thaw cycle of four weeks at −3 °C between two warm periods of two weeks each at +4 °C ($+4/-3_{SINGLE}$).

Air samples for CO_2 measurement were taken on days 1, 4, 8, 10, 16, 18, 22, 36, 43, 46, 50, 52, 54 and 56 after the incubation period started. For this purpose, two evacuated gas containers (50 ml) were connected to one of two ports on the PVC lid of each glass jar, which was attached with two rubber bands to achieve an airtight seal. The second port was used to connect a 50 L gas bottle with CO_2-free synthetic air (synthetic air 5.0, ≥99.999 vol% purity, Air Liquide, Germany, at 20.5 ± 0.5% O_2 in 79.5 ± 0.5% N_2). Each glass jar and the connections of the attached gas containers were flushed with synthetic air for about 2 min to remove the CO_2 before the first sampling. The headspace volume of each jar was homogenized by a fan affixed to the inside of the PVC lid. To take the temperature differences between the synthetic air and the refrigerators/freezers into account, the synthetic air was first cooled in a styrofoam box by passing it through a 10-m flexible silicon tube covered with ice. After the flushing procedure, the first air sample (A_{T0}) was taken. The second air sample (A_{T1}) was taken after an accumulation period of 24 h. CO_2 concentrations were measured using an automated gas chromatograph with an electron capture detector according to Loftfield et al. (1997). The volume of CO_2 in the headspace-volume of each jar under standard conditions for temperature and pressure was calculated as:

$$CO_{2_{Headspace}}(ml) = V_{Net} \times \Delta CO_2 \times \frac{p_n \times T_n}{p_n \times (T_n + IT)} \quad (1)$$

where V_{Net} is the headspace volume (ml), ΔCO_2 is the difference between the CO_2 concentrations A_{T1} and A_{T0} (expressed as %), p_n is the standard atmospheric pressure (hPa), T_n is the standard temperature (K) and *IT* is the temperature during the incubation (°C). Soil respiration expressed as CO_2–C was then calculated by the following equation:

$$CO_2 - C\left(\mu g\ g^{-1} soil\ d^{-1}\right) = \frac{CO_{2_{Headspace}} \times M_{CO_2}}{V_{m0}} \times 0.2729 \times \frac{1000}{ds} \times \frac{24}{\Delta t} \quad (2)$$

where $CO_{2\ Headspace}$ is the volume of CO_2 (ml) under normal conditions related to the respective incubation temperature, M_{CO_2} is the molar mass of CO_2 (mg), V_{m0} is the volume of one mol of a gas under normal conditions (ml), 0.2729 is the mass fraction of C in CO_2, ds is the dry weight of the incubated soil sample (g) and Δt is the time between A_{T0} and A_{T1} (*h*). Air samples for $\delta^{13}C$ analysis of evolved CO_2 were taken with a syringe (35 ml) directly after A_{T1} and stored in 12 ml Labco Exetainer vials (Labco Limited, UK). CO_2–$\delta^{13}C$ analyses were performed on a Delta plus IRMS (Thermo Scientific, Bremen, Germany). Soil sub-samples for analysis were taken at the end of the incubation on day 56 after all visible particles of maize straw residues had been removed from the amended soil samples by sieving (2 mm).

2.3. Analytical procedures

Microbial biomass C and N were estimated by fumigation extraction (Brookes et al., 1985; Vance et al., 1987). A sub-sample of 20 g moist soil was taken and separated into two portions of 10 g. One portion was fumigated at 25 °C with ethanol-free $CHCl_3$, which was removed after 24 h. Fumigated and non-fumigated samples were extracted for 30 min with 40 ml of 0.05 M K_2SO_4 (Potthoff et al., 2003) by horizontal shaking at 200 rev min^{-1} and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany). Organic C