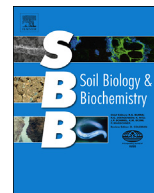
Soil Biology & Biochemistry 76 (2014) 22–33

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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



Medium-term response of microbial community to rhizodeposits of white clover and ryegrass and tracing of active processes induced by ^{13}C and ^{15}N labelled exudates

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ARTICLE INFO

Article history:
Received 12 December 2013
Received in revised form
30 April 2014
Accepted 3 May 2014
Available online 16 May 2014

Keywords:
Root exudates
Rhizosphere processes
Plant–microbial interactions
Labelling approaches
Rhizodeposition

ABSTRACT

Rhizodeposition affects the microbial community in the rhizosphere, and microbial composition and activity may therefore differ in soil depending on plant species. We hypothesised that these differences increase over the plant growth period because roots occupy larger soil volumes and release more rhizodeposits. We tested how such medium-term responses of the microbial community can be explained by the short-term utilisation of root exudates. To test this we analysed ^{15}N incorporation into microbial biomass, phospholipid fatty acid (PLFA) composition and ^{13}C incorporation into the PLFAs of specific microbial groups in soil under white clover (*Trifolium repens* L.) and ryegrass (*Lolium perenne* L.) following leaf-labelling with ^{13}C-bicarbonate and ^{15}N-urea. In this way microbial N and ^{15}N and the composition of PLFAs reflect the medium-term (two months) response of microorganisms to rhizodeposits, whereas the ^{13}C-label of the PLFAs reflects the short-term (one week) utilisation of root exudates following labelling of shoots. In the medium term, microbial biomass N and ^{15}N were greater under the ryegrass, whereas total PLFA was higher under white clover. The relative abundance of fungi and actinomycetes was unaffected by plant species, but pool of Gram-negative and Gram-positive bacteria was greater under white clover at the 10 percent significance level. In the short term, microorganisms more actively utilised fresh exudates (^{13}C-labelled) of ryegrass than of white clover. We expected ryegrass exudates initially to be incorporated into bacterial PLFA and into fungi over time, but surprisingly fungi had the highest utilisation of ryegrass-derived C over the week. At 0–5 cm soil depth, white clover exudates were utilised only by bacteria, whereas fungi dominated at 5–15 cm. This reflects differences in the quality of white clover exudates or differences in the microbial community composition at the two depths. We conclude that despite clear short-term differences in microbial response to the exudates of white clover and ryegrass, this is only to a limited extent transferred into medium-term defects on the composition of the microbial communities under the two plant species. Hence, our study showed that different short-term C utilisation patterns may lead to similar medium-term responses of the microbial community.

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1. Introduction

Improving the use efficiency of legume-derived N is a means of improving the sustainability of agricultural production. The ability of legumes to biologically fix atmospheric N and hence improve soil fertility via rhizodeposition (Høgh-Jensen and Schjoerring, 2000) is widely recognised. Of the forage legumes, white clover is one of the most commonly used in temperate grasslands. However, the knowledge of root–microbial interactions in white-clover-based grasslands is particularly limited.

The main input of N and C to the rhizosphere is via functional (excretions, secretions) and non-functional (diffusates, root debris) rhizodeposition in the form of carbohydrates, amino acids, fatty acids, enzymes, proteins, etc. (Badri et al., 2009). Root effects on the microbial community may differ in the short and medium term since rhizodeposits may alter bacterial and fungal communities (Jones et al., 1998; Zak et al., 2000) as well as change their activities (Blagodatskaya et al., 2009). Microbial communities play a key role in nutrient transformation and storage in the rhizosphere (Petersen et al., 2002) and in the mobilisation and/or mineralisation of soil

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http://dx.doi.org/10.1016/j.soilbio.2014.05.003
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organic matter (SOM) (Churchland et al., 2012). Short-term shifts in the activity of fungi and bacteria in the rhizosphere compared to the root-free soil are related to the quality (C/N ratio, lignin content, recalcitrance) (Grayston et al., 2001; Bai et al., 2012), diversity (Paterson et al., 2008) and quantity (De Vries et al., 2006) of exudates. Bacteria actively respond to low molecular weight compounds with a low C/N ratio (Ding et al., 2011) and show fast response to fresh exudates (Bell et al., 2003; Dungait et al., 2011). Fungi have the ability to decompose both easily-degradable substrates (De Graaff et al., 2010) and more recalcitrant compounds (Poll et al., 2008; Bai et al., 2012). However, high inputs of mineral N (Bardgett et al., 1996) at low levels of soil organic carbon (Petersen et al., 2002) negatively affect the fungal community. Previous studies show that the composition and activity of the microbial biomass vary depending on management (Clegg et al., 2003; Haubert et al., 2009; Bird et al., 2011), grassland plant species composition (Bardgett et al., 2003; Butler et al., 2003), grazing intensity (Hafner et al., 2012), water regime (Tian et al., 2013), root density (Helal and Sauerbeck, 1986), and soil depth (Petersen et al., 2002; Zhang et al., 2012). However, it is not clear whether short-term (hours to days) effects on microbial activities persist over time and structure the microbial community in the medium term (weeks to months). Therefore, we investigated, how the utilisation of root exudates over the short term corresponds to the medium-term composition of the microbial community.

Biomarker techniques are powerful tools to investigate microbial groups and hence get insight into root–microbial interactions (Ding et al., 2011). Since phospholipid fatty acids (PLFAs) are components of living cells and rapidly decompose after cell death, they are useful biomarkers of living microbial groups (Fry, 2006). PLFA biomarkers reflect the composition of microbial groups including fungi, Gram-positive and Gram-negative bacteria, and actinomycetes (Abraham et al., 1998; Fang et al., 2001; Treonis et al., 2004).

Stable dual $^{13}C/^{15}N$ labelling in combination with analysis of biomarkers of soil microorganisms enables estimations of the contribution of various types of root exudates to C and N dynamics in soil. The ^{13}C analyses of PLFAs have been used to investigate: 1) linkages between plants and microorganisms (Dungait et al., 2011) and 2) the effects of individual substances on microorganisms (Dungait et al., 2011; Apostel et al., 2013). However, previous investigations with, for example, sieved soil (harming the fungal hyphae) may not fully reflect *in-situ* conditions. Therefore, we investigated in our study the plant–microbial interactions under undisturbed field conditions.

The objective of the study was to examine the incorporation of ^{15}N in the microbial community and the ^{13}C uptake by microbial groups through analysis of PLFA composition in soil under white clover and ryegrass leaf-labelled with ^{15}N and ^{13}C. As root–microbial interactions are highly dynamic (Bardgett et al., 2005), we investigated N and C dynamics over a timescale of days to weeks.

This study examined the medium-term (two months) effects of rhizodeposits of white clover and ryegrass on the microbial community in the rhizosphere by examining the PLFA composition and ^{15}N incorporation in the microbial community, and the short-term (seven days) utilisation of ^{13}C-enriched exudates by microbial groups. We hypothesised:

- a greater incorporation of root-released N into the microbial community under ryegrass compared to white clover because of stronger N limitation under ryegrass and because of its higher release of energy-rich root-derived C stimulating microbial growth and turnover in the rhizosphere;
- a greater incorporation of released exudates into microorganisms in the uppermost layer (0–5 cm) compared to the deeper layer due to higher root density resulting in more available substrate for microbial growth;
- a lower incorporation of root-released C into fungal PLFA under white clover than under ryegrass because legumes exudate inorganic (NH_4^+) and organic N compounds with a low C/N ratio (de Neergaard et al., 2002), while fungi are more C-dependent;
- finally, we expected that bacteria incorporate C immediately after its release into the rhizosphere with a temporal shift of C to fungi in later stages.

2. Materials and methods

2.1. Experimental site and conditions

The experiment was conducted on a sandy loam at Foulumgård Experimental Station, Viborg, Denmark (55°28′N, 09°07′E). Since 1987 the site has been under intensive dairy farming with grassland-arable crop rotations (Eriksen et al., 1999, 2014). The soil is classified as a typic hapladult with 6.4% clay, 8.5% silt, 44% fine sand and 39% coarse sand. Soil contained 1.8% C at 0–5 cm and 1.6% C at 5–15 cm, and the N content was 0.18% at 0–5 cm and 0.15% at 5–15 cm. The field experiment was established in the beginning of April 2012 by installing 8 cm diameter and 20 cm high PVC cylinders (90 in total) in 2nd year perennial ryegrass (*Lolium perenne* L., 28 kg ha^{-1}) and white clover (*Trifolium repens* L., 6 kg ha^{-1}) pure stands in a randomised block design with four replicates of each plant type for each sampling date. The rainfall was 28 mm and 84 mm in May and June 2012, respectively, and the mean temperature was 12° C. The mean annual temperature was 8°°C and precipitation 738 mm in 2012.

2.2. Leaf $^{15}N/^{13}C$ labelling

Leaf labelling with ^{15}N-urea (99.6 atom% ^{15}N, 0.5% w/v) (Høgh-Jensen and Schjoerring, 2000) and $Na_2^{13}CO_3$ (99.9%, 0.01 M) (Rasmussen et al., 2013a) was conducted with five tubes per cylinder. White clover or ryegrass leaves were inserted into a 2-ml tube filled with 1 ml of the labelling solution and sealed with an inert plastic material (UNIGUM Sanitary putty, Unipak A/S, Galten, Denmark) to avoid $^{15}N/^{13}C$ losses (Høgh-Jensen and Schjoerring, 2000). Labelling was started in the morning on May 1st and continued for 48 h. After the labelling was terminated, labelled leaves were wiped off with a paper towel to avoid soil contamination with $^{15}N/^{13}C$ after the tubes were removed. We assumed that more than 90% of the labelling solution was taken up by plants (Rasmussen et al., 2007).

2.3. Sampling times and initial sample preparation

Sampling of the soil-plant system was done 2 days before the labelling and 0 h, 1 d, 2 d, 4 d, 7 d, 14 d, 28 d and 2 months after labelling was terminated. At sampling, cylinders were excavated and placed in a cooling box and immediately transported to the lab and placed in a room at 2°°C. Samples were processed within 4 h after the excavation and within half an hour of the removal from the cylinder. First, plant shoots were cut at the soil surface and the soil removed from the cylinder. The soil core was then sliced at two depths to give 0–5 cm and 5–15 layers. The soil/root sample was shaken carefully to separate roots from the soil that was passed through a 2-mm sieve. Plant leaves and washed plant roots were oven-dried at 60°°C to constant weight. The soil sample was thoroughly mixed and divided into four subsamples for the following analyses: water content (oven-dried at 110°°C), $^{15}N/^{13}C$ bulk analyses (oven-dried at 60°°C), ^{13}C-PLFA (frozen at −18°°C)

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