Pedobiologia 58 (2015) 211–218

Contents lists available at ScienceDirect

Pedobiologia - Journal of Soil Ecology

journal homepage: www.elsevier.de/pedobi

Effect of substrate quality on the N uptake routes of soil microorganisms in different soil depths

Johanna Pinggera[a,b], Daniel Geisseler[a,c], Hans-Peter Piepho[d], Rainer Georg Joergensen[b], Bernard Ludwig[a,*]

[a] Department of Environmental Chemistry, University of Kassel, Nordbahnhofstr. 1a, 37213 Witzenhausen, Germany
[b] Soil Biology and Plant Nutrition, University of Kassel, Nordbahnhofstr. 1a, 37213 Witzenhausen, Germany
[c] Department of Land Air & Water Resources, University of California Davis, One Shields Avenue, Davis, CA, 95616, USA
[d] Institute of Crop Science, University of Hohenheim, Biostatistics Unit, Fruwirthstr. 23, 70599 Stuttgart, Germany

ARTICLE INFO

Article history:
Received 16 February 2015
Received in revised form 1 October 2015
Accepted 20 October 2015

Keywords:
Amino acid mineralization
Direct route
MIT route
Gross N mineralization
Protease activity
Soil depth

ABSTRACT

A few studies have indicated that substrate quality is an important factor affecting the N uptake route of soil microorganisms, but less is known about the effect of soil depth on the N uptake route under different nutrient conditions. Objectives were to investigate (i) the effects of corn residues with different C to N ratios in the presence and absence of mineral N and (ii) the effects of soil depth on the N uptake route of soil microorganisms. An incubation experiment with surface soils (0–5 cm, C/N = 10) and subsoils (30–40 cm, C/N = 9) from three German loess sites was carried out for 21 days at 20 °C and 60% of their water-holding capacity. The following treatments were used: no addition (control), addition of corn residues with a C/N ratio of 20, 40, and 40 + $(NH_4)_2SO_4$. To distinguish between the N uptake routes, the mineralization rate of amino acids was determined using ^{15}N-labeled amino acids. In the control surface soil and subsoil the direct uptake of organic N was favored with no significant ($p < 0.05$) differences between depths despite significantly higher microbial activity, protease activity, gross N mineralization rate and availability of inorganic N in the surface soil, suggesting that N availability relative to C was similar at both depths. Substrate additions resulted in significantly increased protease activities at both depths after 3–7 days. Addition of corn residue with a high C/N ratio resulted in an increased direct uptake (97% and 94% in the surface soil and subsoil, respectively), compared with addition of corn residue with a low C/N ratio or addition of corn residue and inorganic N (79 to 91% direct uptake). This suggests that the enzyme system involved in the direct uptake was slightly repressed under conditions of sufficient mineralizable N (C/N of 20) or increased concentrations of NH_4^+. Substrate additions resulted in an initial significantly higher increase in the direct uptake in the surface soil than in the subsoil.

1. Introduction

Nitrogen is an essential nutrient for plants and soil microorganisms. The distribution of total N in humic substances and soils has been reported to consist of about 40% proteins and peptides, and about 35% of heterocyclic N compounds (Schulten and Schnitzer, 1998). Therefore, proteinaceous material plays a central role in N transformation processes. However, proteins cannot be utilized directly by soil microorganisms. They first have to be depolymerized by proteases (Magasanik, 1993; Wanek et al., 2010), extracellular enzymes that are secreted into the soil by microorganisms such as bacteria and fungi (Glenn, 1976; Mrkonjic Fuka et al., 2008). Proteases can break down large polymeric compounds and release soluble amino acids or small peptides, which are important sources of N and C for the microorganisms (Rothstein, 2010). Protease production is generally induced by the presence of substrate in the medium (Haab et al., 1990).

Soil microorganisms are able to take up a wide range of N compounds: ammonium (NH_4^+), nitrate (NO_3^-), and organic molecules, such as amino acids or small peptides (Merrick and Edwards, 1995; Marzluf, 1997) and have developed several mechanisms for N uptake (Barak et al., 1990; Barraclough, 1997; for a review see Geisseler et al., 2010). They can take up simple organic molecules directly into the cell (direct route) or they can

* Corresponding author at: Department of Environmental Chemistry, University of Kassel, Nordbahnhofstr. 1a, 37213 Witzenhausen, Germany. Fax: +49 5542 981633.
E-mail address: bludwig@uni-kassel.de (B. Ludwig).

http://dx.doi.org/10.1016/j.pedobi.2015.10.002
0031-4056/

first mineralize the molecules to NH_4^+ before uptake via the mineralization-immobilization-turnover (MIT) route (Hadas et al., 1992). An advantage of the direct uptake of small organic molecules is energy conservation, required for synthesizing C structural components (Geisseler et al., 2010). In the MIT route, the deamination of organic material is carried out by extracellular enzymes and all N is mineralized to NH_4^+ before assimilation (Manzoni and Porporato, 2007).

Microorganisms produce a wide range of enzymes for the direct uptake and transformation of organic N. For example, the uptake of amino acids into cells is catalyzed by functionally specific transport systems. Laboratory experiments with cultivated microorganisms in growth media indicated that the activation of the synthesis of amino acid transport systems occurs by an internal lack of C, N, or S and is repressed by NH_4^+ and high intracellular concentrations of amino acids (Payne, 1980; Geisseler et al., 2010). Thus, Geisseler et al. (2010) suggested that the MIT route is favored in the presence of NH_4^+ at high concentrations and that the direct route may be generally important in aerated soils due to nitrification and plant uptake. The studies available for soil incubations after addition of different plant residues indicate that both uptake routes were active simultaneously, with the direct route being preferred (Luxhøi et al., 2006; Geisseler et al., 2009). However, the MIT route may be an important alternative when N is severely limiting (Geisseler et al., 2012).

Subsoils differ from surface soils in a number of factors: soil organic carbon (SOC) and N contents, bulk density, and activity of soil microorganisms generally decline markedly with depth, with the decline being more pronounced for fungi than for bacteria (Agnelli et al., 2004; Ekschmitt et al., 2008). In contrast, the changes in the microbial biomass C/SOC ratio and different enzyme activities with depth were variable (Lavahun et al., 1996; Agnelli et al., 2004; Enowashu et al., 2009). However, information on depth-related changes of the N uptake route in soils is not available.

Objectives were to investigate (i) the effects of corn residues with different C to N ratios in the presence and absence of mineral N and (ii) the effects of soil depth on the N uptake route of soil microorganisms. We hypothesized that N availability is higher in surface soils (0–5 cm) and that the MIT route is thus more prevalent there than in subsoils (30–40 cm) and that addition of corn residue with a high C/N ratio results in an increased relevance of the direct uptake route.

2. Material and methods

2.1. Experimental sites

The current study was carried out with soil from three fields under no-till (direct drilling) at different sites located in arable loess-regions of eastern Germany (Koch et al., 2009). The sites differ in their soil texture and SOC content (Table 1).

Table 1
Soil organic carbon (SOC), total nitrogen (N_{tot}), pH and soil texture are mean values with standard errors in brackets ($n = 3$ samples per site).

Site	Depth [cm]	SOC [g kg^{-1} soil]	N_{tot}	pH [$CaCl_2$]	Clay [%]	Silt [%]	Sand [%]
Friemar	0–5	24.0 (0.4)	2.43 (0.06)	6.7	29 (2)	66 (2)	5 (1)
	30–40	6.9 (0.3)	0.72 (0.02)	7.0	37 (7)	57 (5)	6 (2)
Lüttewitz	0–5	18.5 (0.7)	1.84 (0.04)	7.3	17 (1)	79 (1)	4 (0)
	30–40	2.7 (0.2)	0.34 (0.01)	7.1	17 (4)	78 (3)	5 (1)
Zschortau	0–5	14.2 (0.1)	1.40 (0.01)	7.3	13 (0)	59 (1)	28 (1)
	30–40	5.8 (0.5)	0.61 (0.05)	7.3	13 (1)	57 (1)	30 (1)

2.2. Soil sampling and incubation

Soil samples were taken in October 2013 at each site. The field size ranged from 7 to 8 ha. Three subsamples were taken per site at a distance of 150 m in average. Each subsample consisted of a composite sample from three soil cores, taken with a core sampler of 8 cm diameter. Samples were taken from 0–5 cm and 30–40 cm. Soil samples were sieved (<2 mm) and stored at 4 °C in the dark before processing.

Field-moist samples equivalent to 8 g oven dry soil were weighed into 50 mL glass vials. De-ionized (DI) water was added to bring the soil to 60% of its water holding capacity. Water holding capacity was determined by draining water-saturated soil samples for 30 min (the remaining moisture content is referred to as 100% water holding capacity) followed by drying the soil samples at 105 °C for 24 h (Forster, 1995). Four glass vials were placed into 1 L glass jars and incubated at 20 °C for 21 days in the dark.

Ground dried corn leaves differing in their C/N ratios were added to the soil samples. The following treatments were carried out, each in triplicate for each site and depth:

(I) control, no addition of residues;
(ii) addition of corn residues with a C/N ratio of 20. The amount of N and C added were 0.1 mg g^{-1} and 2 mg g^{-1}, respectively;
(iii) addition of corn residue with a C/N ratio of 40. The amount of N added was 0.1 mg g^{-1} and the amount of C added was thus 4 mg g^{-1}; and
(iv) addition of corn residue with a C/N ratio of 40 and $(NH_4)_2SO_4$. The amounts added were 0.05 mg organic N g^{-1}, 0.05 mg NH_4^+–N g^{-1} and 2 mg C g^{-1}.

To get C/N ratios of 20 and 40, residues from young and old corn leaves were mixed. The amount of 100 mg N kg^{-1} soil was added to treatments II, III, and IV. In treatment IV, half of the N was added with the residue and the other half with $(NH_4)_2SO_4$.

Total C and N contents in soil and corn leaves were analyzed after drying and grinding with a CN analyzer (Heraeus Elementar Vario EL, Hanau, Germany). Since no carbonates were found in the soil, total C corresponds to SOC.

2.3. Basal respiration, net-N mineralization and microbial biomass

CO_2 production was determined using NaOH traps (Coleman et al., 2002). Cups containing 10 mL 1 M NaOH were added to the 1 L glass jars, which contained 4 glass vials (each containing 8 g of soil). After 3, 7, 14, and 21 days the traps were replaced and the jars aerated. The accumulated CO_2 was determined by titration (Alef, 1995).

For the extraction of NH_4^+ and NO_3^-, 40 mL of 0.5 M potassium sulfate (K_2SO_4) were added to moist soil corresponding to 8 g dry weight (Kuderna et al., 1993). Samples were shaken on a reciprocal shaker for 1 h and the suspension was filtered to determine the concentrations by a continuous flow analyzer (Evolution II auto-analyzer, Alliance Instruments, Salzburg, Austria).

The microbial biomass was determined by using the chloroform fumigation extraction method (Brookes et al., 1985; Vance et al., 1987). After 0, 3, 7 and 21 days, samples corresponding to 8 g dry weight were fumigated at 25 °C for 24 h with ethanol-free $CHCl_3$. Forty ml of 0.5 M K_2SO_4 were added to extract the fumigated and non-fumigated samples by shaking them on a reciprocal shaker at 175 rpm for 1 h. The suspensions were filtered through a filter paper and the organic C and N in the extracts analyzed by a multi N/C analyzer (Analytik Jena 2100S, Jena, Germany). Microbial biomass C was calculated with a k_{EC} value of 0.45 (Joergensen, 1996) and microbial biomass N with a k_{EN} value of 0.54 (Brookes et al., 1985).