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Significance of organic nitrogen uptake from plant residues by soil microorganisms as affected by carbon and nitrogen availability

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ABSTRACT

Soil microorganisms can use a wide range of nitrogen (N) compounds. When organic N sources are degraded, microorganisms can either take up simple organic molecules directly (direct route), or organic N may be mineralized first and taken up in the form of mineral N (mineralization–immobilization–turnover [MIT] route). To determine the importance of the direct route, a microcosm experiment was carried out. Two types of wheat residue were added to soil samples, including younger residue with a carbon (C) to N ratio of 12 and older residue with a C to N ratio of 29. Between days 1 and 4, the gross N mineralization rate reached 8.4 and 4.0 mg N kg^{-1} dry soil day^{-1} in the treatment with younger and older residue, respectively. During the same period, there was no difference in protease activity between the two residue amended treatments. The fact that protease activity was not related to gross N mineralization, even though the products of protease activity are the substrates for N mineralization, suggests that not all organic molecules released from residue or soil N passed through the soil mineral N pool. In fact, when leucine and glycine were added, only 10 and 53% of the amino acid-N, respectively, was mineralized. The fraction of N taken up via the direct route was estimated to be 55 and 62% for the young and older residue, respectively. After 28 days of incubation, the proportion of amino acid-N mineralized had increased especially in the soil amended with older residue, suggesting that the MIT route became increasingly important. This result is supported by an increase in the activities of enzymes responsible for the intracellular assimilation of ammonium (NH_4^+). Our results suggest that in contrast to what is proposed by many models of soil N cycling, both the direct and MIT routes were operative, with the direct route being the preferred route of residue N uptake. The direct route became less important over time and was more important in soil amended with older residue, suggesting that the direct route is favored by lower mineral N availabilities. An important implication of these findings is that when the direct route is dominant, gross N mineralization underestimates the amount of N made available from the residue.

1. Introduction

Nitrogen is essential for the growth and activity of plants and soil microorganisms. Soil microorganisms can use a wide range of N compounds. These include inorganic compounds such as NH_4^+ and nitrate (NO_3^-), as well as organic molecules such as amino acids and small peptides (Merrick and Edwards, 1995; Marzluf, 1997). Therefore, when organic N sources are degraded, there are two possible mechanisms for N assimilation by soil microorganisms. First, the direct route in which simple organic molecules are taken into the cell, deaminated and only the surplus N is released into the soil NH_4^+ pool. Second, the mineralization–immobilization–turnover (MIT) route in which deamination occurs outside the cell with all N mineralized before assimilation (Barraclough, 1997).

In living organisms most organic N is in the form of proteins (Kögel-Knaber, 2006). However, proteins, like many other organic compounds released into soil after the death of organisms, are polymers and cannot be taken up directly by decomposers (Allison and Vitousek, 2005). They are first hydrolyzed to peptides and amino acids by extracellular proteases. Since the products of protease activity are the substrates for N mineralization, protease activity and gross N mineralization should be related when the MIT route is dominant.

The interpretation of gross N mineralization rates as measured by isotope dilution methods directly depends on which of these two routes is dominant. If the MIT route is dominant, all the N passes through the NH_4^+ pool. Therefore, gross N mineralization represents the total amount of bioavailable N. If in contrast the

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direct route is dominant, gross N mineralization determined by isotope pool dilution only measures the surplus N released from cells. The proportion of N released depends on the ratio of available C to N. The route of N uptake also has implications for the competition for N between plants and microorganisms. When the MIT route is dominant, plants and microorganisms compete for mineral N (Manzoni and Porporato, 2007). On the contrary, when the direct route is dominant, microorganisms meet their N requirements with organic N compounds and plants face reduced competition from microorganisms for mineral N.

Results from several studies indicate that the direct route may be important in soil ecosystems (Barak et al., 1990; Hadas et al., 1992; Barraclough, 1997; Gibbs and Barraclough, 1998). However, little is known about the factors that determine the relative importance of the two routes. Especially the availability of C and N may have an effect, as microorganisms may take up N containing organic molecules not only because of the need for N, but also to meet their C and energy requirements.

When mineral N serves as a N source, it is assimilated as NH_4^+ via two different pathways, their relative importance depending on C and N availability. One pathway is catalyzed by glutamate dehydrogenase (GDH) and the other involves two enzymes, glutamine synthetase (GS) and glutamate synthase (glutamine:2-oxoglutarate aminotransferase; GOGAT). The GS/GOGAT enzyme system has the same overall characteristics as GDH with the additional cost of 1 mol of ATP per mole of NH_4^+ assimilated (Brown, 1980). The expenditure of energy is compensated for by the fact that GS has a much higher affinity for NH_4^+ than GDH and therefore enables organisms to scavenge NH_4^+ at low concentrations (Merrick and Edwards, 1995; Schmid et al., 2000; Silberbach et al., 2005). In contrast, GDH usually functions efficiently only at high NH_4^+ concentrations. When NO_3^- serves as the N source, it is reduced and assimilated in the form of NH_4^+ (McCarty, 1995). Therefore, the activity of GDH and GS is a measure of the importance of mineral N assimilation, while the ratio of the two activities is an indicator of mineral N and energy availability.

This study provides information on the influence of C and N availability on the different N uptake strategies of soil microorganisms. First, we studied the relationship between protease activity and gross and net N mineralization to test our hypothesis that protease activity and gross mineralization rate are not well correlated when the direct route is dominant (hypothesis 1). Second, we determined the effect of C and N availability on the N uptake route (MIT vs. direct route), expecting the direct route to be more important when the availability of C and mineral N is low (hypothesis 2). Third, we investigated NH_4^+ uptake via the GDH and the GS/GOGAT pathway, to test the hypothesis that the activity of GS and GDH is low when the direct route is dominant due to a decreased uptake of mineral N (hypothesis 3).

2. Material and methods

2.1. Soil and residue samples

Soil samples were collected in fall 2007 after the corn harvest from the 5- to 20-cm layer of a field at the UC Davis Long-Term Research on Agricultural Systems (LTRAS) site. The soil is mapped as Rincon silty clay loam (fine, montmorillonitic, thermic Mollic Haploxeralf; Soil Survey Staff, 1997). The samples had a pH of 7.2 (determined in a 1:2 soil–water solution; Thomas, 1996) and contained 12.3 g C kg^{-1} dry soil and 1.1 g N kg^{-1} dry soil (dry combustion on a Carlo Erba CNS analyzer NA 1500 series 2; Nelson and Sommers, 1996; Bremner, 1996). Percentages of sand, silt, and clay were 15, 53 and 32%, respectively (pipet method; Gee and Bauder, 1986). The field moist soil was passed through a 4-mm sieve, spread on a paper in a thin layer, and air-dried at room temperature. The air-dry soil was stored at room temperature.

Wheat was harvested at different stages (Table 1) in spring 2006 from a field with the same soil type at the same location where wheat had been planted the previous fall as a winter cover crop. The wheat plants were cut at the base, air-dried at 40 °C and ground to pass a 1-mm screen.

2.2. Microcosm experiment

Prior to the microcosm experiment, the soil was pre-incubated for one week by adding DI water to reach 30% water holding capacity, which corresponded to 14.8% gravimetric moisture content. Water holding capacity was defined as the water content after saturated soil samples in a funnel were left to drain for an hour.

For the incubation, pre-incubated soil samples were mixed with ground wheat residue. The amount of wheat added corresponded to 0.1 mg N g^{-1} dry soil. Soil (8 g) was weighed into 50 ml centrifuge tubes and water was added to bring the soil to 24.9% gravimetric moisture content, corresponding to 50% water holding capacity. The samples were placed into glass jars and kept in the dark at 22 °C. To each jar about 30 ml of DI water was added to minimize evaporation from the soil samples during the incubation. After 1, 4, 8, 14, 20, and 27 days, four replicates per treatment were destructively sampled to determine protease activity, NH_4^+, NO_3^-, microbial biomass C and N, GS and GDH activity (see methods below). Data analysis was performed on the average values of two neighboring sampling dates. In addition, CO_2 evolution was measured frequently so that the CO_2 concentration did not exceed 1.5% in the jar headspace.

2.3. Soil and residue analyses

For soil extractions, 40 ml of 0.5 M potassium sulfate (K_2SO_4) were added to 8 g of soil (Mulvaney, 1996). Samples were shaken for 1 h on a reciprocal shaker and the suspension filtered (Fisherbrand, Q5) for the analysis of NH_4^+, NO_3^-, dissolved organic C (DOC), and total dissolved N. NO_3^- was analyzed colorimetrically using a single reagent method (Doane and Horwath, 2003). The NH_4^+ concentration was determined using the salicylate method (Verdouw et al., 1978; Foster, 1995). DOC was analyzed on a UV-Persulfate Total Organic C Analyzer (model Phoenix 8000, Tekmar Dohrmann™, Cincinnati, Ohio). Total dissolved N was determined with the alkaline persulfate oxidation method in which the filtrate was mixed with an equal amount of an oxidizing reagent (Cabrera and Beare, 1993), heated in a boiling water bath for 2 h, and analyzed for NO_3^- as described above. The total dissolved N minus the N in the form of NH_4^+ and NO_3^- was assumed to correspond to the dissolved organic N (DON).

Table 1
Properties of the wheat residue used for the laboratory incubation.

Treatment	Growth stage	DOC (g kg^{-1} dry residue)	DON (g kg^{-1} dry residue)	Cellulose (g kg^{-1} dry residue)	Lignin (g kg^{-1} dry residue)	Total C (g kg^{-1} dry residue)	Total N (g kg^{-1} dry residue)	C to N
Younger residue	Second node 32[a]	102	6.2	194	25	410	34.0	12.1
Older residue	Late milk stage 77	39	1.9	253	59	399	13.6	29.3

[a] Based on Zadoks et al. (1974).