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Differentiating the mineralization dynamics of the originally present and newly synthesized amino acids in soil amended with available carbon and nitrogen substrates

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ABSTRACT

Newly synthesized amino acids are the principle compounds created after inorganic nitrogen (N) is rapidly immobilized into microbial tissues. However, little is known about the mineralization kinetics of these newly synthesized amino acids compared to the amino acids originally present in the soil, and how substrate availability controls their mineralization. With ^{15}N isotope tracing, the newly synthesized (^{15}N-labeled) amino acids can be differentiated from the amino acids originally present (unlabeled) in soil, making it possible to evaluate the mineralization of the newly synthesized amino acids in tandem with the original amino acids. As amino acids can serve as both N and carbon (C) sources for microorganisms, the mineralization dynamics of amino acids may be manipulated by the availability of extraneous C and N. In this study, an aerobic 30-week intermittent leaching experiment was conducted, using glucose as C source and $(^{14}NH_4)_2SO_4$ as N source, following separate additions to soil. The newly synthesized amino acids were determined by an isotope-based high performance liquid chromatography/mass spectrometry (HPLC/MS). The newly synthesized soil amino acids mineralized faster than the original ones, which indicated more rapid cycling of N in the newly synthesized soil amino acids pool. Glucose addition significantly decreased the mineralization of both the newly synthesized and the original amino acids. However, when inorganic N was abundant, the newly synthesized amino acids decomposed rapidly, and preferentially as a C source and energy, while N addition inhibited the mineralization of the original amino acids in the soil. We conclude that the presence of readily degradable C (e.g. glucose) and inorganic N controls the mineralization of newly synthesized and original amino acid pools in soil differently, which is a crucial mechanism in adjusting the N supply and sequestration processes in soil ecosystems.

1. Introduction

Nitrogen (N) mineralization has been a major focal point in ecosystem studies of soil N cycling (Schimel and Bennett, 2004). Accurate evaluation of soil N mineralization in agricultural soils is critical because the process influences crop yields, soil sustainability, and environmental pollution (Aber and Melillo, 2001). As an important constituent of soil organic matter (SOM), soil N is found in many forms differing in function with distinct consequences for terrestrial N cycling.

Amino acids have been identified as major soil organic N compounds, accounting for 20–50% of the soil N pool (O'Dowd et al., 1999; Muruganandam et al., 2009). They are closely associated with microbial metabolism, serving as both an important storage pool for the immobilized N (Amelung, 2003; Lü et al., 2013) and a main source of available N for soil microorganisms and plants (Werdin-Pfisterer et al., 2009). Therefore, amino acid turnover in

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soil is an important part of terrestrial N cycles (Dippold and Kuzyakov, 2013) and more accurate characterization of their mineralization dynamics is needed to better understand carbon (C) and N cycling in terrestrial ecosystems (Nasholm et al., 1998, 2001; Lipson and Nasholm, 2001; Henry and Jefferies, 2003; Berthrong and Finzi, 2006).

In soil, the hydrolyzed amino acids include both free and bound (proteins and peptides) forms (Paul and Clark, 1996). The contents of free amino acids are generally less than 4 μg g^{-1} soil (Monreal and McGill, 1985), accounting for a very small portion of total amino acids (500–16,000 μg g^{-1} soil, Amelung and Zhang, 2001). Therefore, the mineralization dynamics of amino acids have been mainly determined by amino acids bound in peptides and proteins. The mineralization of free amino acids is usually rapid (Jan et al., 2009) and the direct use of free amino acids as a N source by both plants and soil microbes has also been well documented (Jones and Hodge, 1999; Owen and Jones, 2001; Vinolas et al., 2001). However, the mineralization dynamics of soil amino acids bound in peptides and proteins remains unclear, because it is difficult to determine the portion of amino acids bound to soil minerals or stabilized by other mechanisms (Zhang et al., 2007; Rejsek et al., 2010). In soil, microorganisms can take up a variety of mineral and organic molecules to satisfy their N requirements (Geisseler and Horwath, 2014) and amino acids bound to peptides and proteins are the principle compounds synthesized during this process (Paul and Clark, 1996). Moreover, this newly synthesized amino acids (including both free and peptide-/protein-bound amino acids, but mainly as peptide-/protein-bound forms) could gradually be added and accumulate in soils over time during humification (Paul and Clark, 1996), which makes it difficult to assess different amino acid transformation pathways.

The mineralization of the newly synthesized amino acids and of those already present in soil may occur at different rates, and this dissimilarity may play a significant role in the biogeochemical cycling of C and N. To date, the differences between mineralization dynamics of newly synthesized and the original amino acids present in soil remain elusive. Studies on the mineralization dynamics of soil amino acids can improve the understanding to the origin and transformation of amino acids in soils.

In soil, the dynamics of amino acid mineralization are regulated by substrate availability (Geisseler et al., 2009). Highly labile substrate C, such as glucose, stimulates soil microbial growth and activity, resulting in an enhanced N demand (Brant et al., 2006; Mondini et al., 2006; Schneckenberger et al., 2008). Alternatively, microorganisms may be C limited when ammonium (NH_4^+) is in excess (McFarland et al., 2002). Therefore, the availability of both C and N may have an effect on the mineralization of amino acids because amino acids serve not only as a principal N source, but also as a C and energy source for microorganisms (McFarland et al., 2002; Adour et al., 2006). While research on the effects of substrate addition on microbial activity is abundant, the effect of extraneous C and N on the mineralization of newly synthesized and original amino acids remains unknown. The use of isotope tracing techniques may elucidate this by allowing for the differentiation of newly synthesized amino acids from the fractions originally present in soil (Zhang et al., 2007; He et al., 2011). Recently, a high performance liquid chromatography/mass spectrometry (HPLC/MS) combined with isotope labeling incubation technique was developed to differentiate ^{15}N-labeled and unlabeled amino acids in soils (He et al., 2011). This approach offers an opportunity to investigate the mineralization dynamics of different soil amino acid fractions.

In this paper, we investigate the mineralization dynamics of the newly synthesized and the original amino acids in soil, as well as the effects of extraneous C and N addition on this mineralization process. The stability of the newly synthesized and the original amino acids is likely to be different due to the varying extent that the compounds are protected through physical and chemical mechanisms (Killham et al., 1993; Six et al., 2002; Vogel et al., 2014). We hypothesized that the mineralization characteristics of the newly synthesized amino acids will differ from those in the older, humified soil fractions. Additionally, we hypothesized that substrate addition will alter mineralization patterns of the newly synthesized amino acids relative to the original amino acids. To test this, an aerobic intermittent leaching incubation (Stanford and Smith, 1972) was conducted using soils containing newly synthesized amino acids obtained by pre-incubation with $(^{15}NH_4)_2SO_4$ for 10 weeks. The mineralization and transformation of the soil newly synthesized amino acids (^{15}N-amino acids) and the fractions originally present in soil (unlabeled-amino acids) were investigated by HPLC/MS. To our knowledge, this is the first study that investigates the mineralization dynamics of newly synthesized and original amino acids in soil simultaneously.

2. Materials and methods

2.1. Soil samples

A bulk surface soil sample (0–20 cm), classified as a Mollisol (Typic Hapludoll; Soil Survey Staff, 2003), was collected from Gongzhuling, Jilin Province of China (124°48′E, 43°30′N). The samples were air-dried and sieved (<2 mm). The soil pH was 6.07 (soil:water ratio = 1:2.5). The contents of soil organic C and total N were 15.56 g kg^{-1} and 1.47 g kg^{-1}, respectively.

2.2. Preparation of the newly synthesized soil amino acids

An incubation experiment was carried out to prepare for the soils that contained newly synthesized amino acids (^{15}N-amino acids) and then was used for the mineralization study. Soils (100 g air-dried) were weighed into plastic containers, to 2 cm depth. A mixed solution, 4 ml of $(^{15}NH_4)_2SO_4$ (^{15}N, 98%, 11.8 mg ml^{-1}) and glucose (62.5 mg ml^{-1}) were added weekly, to provide 0.1 mg N and 1.0 mg C g^{-1} soil. The incubation time was intentionally set as 10 weeks, since with this time length, the total amino acids content were maximized when glucose and $(^{15}NH_4)_2SO_4$ were added in the same manner in a former test. A solution (8.77 mg ml^{-1} KH_2PO_4, 10 ml) containing 0.2 mg phosphorus (P) and 0.25 mg potassium (K) g^{-1} soil was added at the beginning of the incubation to ensure adequate supply of P and K. Soil moisture was maintained at 20% of air-dry soil weight at 25 °C. After 10 weeks of incubation, the prepared soils were mixed and air-dried at room temperature for 7 days for the mineralization study.

2.3. Mineralization experiment

The mineralization experiment was conducted using the procedure of Stanford and Smith (1972) with minor modifications. Briefly, 50 g air-dried soil (prepared as described in Section 2.2) was mixed with 50 g 100-mesh clean quartz sand. The incubation was conducted with 100 ml leaching tubes. The soil samples were then preincubated at 25 °C with 20% water for 1 week to adjust the same initial conditions before mineralization incubation. After preincubation, the incubation temperature for mineralization was maintained at 30 °C and the moisture at 20% on an air-dry soil basis. We designed the experiment so that the mineral N could be leached to decrease the re-immobilization of mineralized N and to avoid feedback effects on the mineralization process (Bloem et al., 2006). Leaching was conducted using 100 ml 0.01 M $CaCl_2$ followed by a 25 ml wash with a N-free nutrient solution (0.002 M $CaSO_4$,