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Another bottleneck for nitrogen mineralization in temperate forest soils: Arginine metabolism in microorganisms

Kazumichi Fujii[a,b,*], Takahiro Yamada[a], Chie Hayakawa[a], Asami Nakanishi[a], Shinya Funakawa[a]

[a] Graduate School of Agriculture, Kyoto University, Kyoto, 606-8502, Japan
[b] Forestry and Forest Products Research Institute, Tsukuba, 305-8687, Japan

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ABSTRACT

Soil nitrogen (N) mineralization is generally limited by microbial N assimilation when microorganisms are exposed to substrates with high carbon-to-nitrogen (C/N) ratios. We hypothesized that microbial N release is also limited by repression of arginine-degrading activity in forest soils with the high C/N ratios. We analyzed the microbial assimilation and mineralization rates of ^{14}C-labeled amino acid mixture, arginine, ornithine, and urea added to a variety of forest and cropland soils. The proportions of amino acid mixture derived ^{14}C incorporated into microbial biomass (MB^{14}C) in a 24-h incubation (3.7–20.4%) increased with soil C/N ratio and an increase in MB^{14}C retards inorganic N release in the forest soils. Arginine mineralization displays a contrary pattern to amino acid derived MB^{14}C and decreased with increasing soil C/N ratio. The reduced arginine-degrading activity in the forest soils with the high C/N ratios is consistent with general microbial N assimilation associated with growth, but it also correlates with the enhanced fungi-specific N preservation (*e.g.*, recycling of ammonium in urea-ornithine cycle and accumulation of arginine or ornithine in vacuoles). Arginine or ornithine degradation is one of slower amino acid degradation pathways and potentially retards N mineralization in N-limited forest soils.

1. Introduction

Nitrogen (N) is an essential nutrient for plants and the mineralization of soil organic N is a key process regulating plant productivity and ecosystem N biogeochemistry (Lambers et al., 1998). Slow N mineralization in soil can limit primary production in some temperate coniferous forests (Reich et al., 1997), but there is wide variability among the values and factors regulating N mineralization rates (Kemmitt et al., 2006; Högberg et al., 2007).

Apparent (net) N mineralization rates are dependent on the balance between gross N mineralization and N assimilation (immobilization) of the soil microbial community (Geisseler et al., 2010). Due to the complexity of and interactions between the many enzyme reactions involved, the gross and net N mineralization rates are variable, depending on climate (temperate *vs*. tropical) and substrate quantity (soil N concentration) and quality (C/N ratios) (Booth et al., 2005; Urakawa et al., 2016). Even in the laboratory, various factors affect the gross and net N mineralization, including soil physicochemical properties [pH, clay content, and carbon-to-nitrogen (C/N) ratio] (Colman and Schimel, 2013) and microbial community composition (fungi/bacteria ratio) (Cookson et al., 2007).

Soil N mineralization involves several steps: the depolymerization of proteins into amino acids by proteases; the uptake of amino acids by the microbial community; and amino acid degradation in microbial biomass (ammonification) (Geisseler et al., 2010). The overall N flow is usually limited by amino acid production via depolymerization (Schimel and Bennett, 2004; Jan et al., 2009), while amino acid metabolism in microbial biomass can act as another N mineralization bottleneck in some cases (Knowles et al., 2010; Jones and Kielland, 2012). Net N mineralization in soil can be limited in substrates with high C/N ratios due to the dominance of microbial N assimilation (Schimel and Weintraub, 2003). The greater N demand for microbial growth can lead to a tight N cycle in microbial biomass under N limitation [or carbon (C) enrichment relative to N] by limiting microbial enzyme activities that promote N mineralization (Harder Nielsen et al., 1998; Dippold and Kuzyakov, 2013).

Arginine degradation is one of amino acid degradation pathways that excrete excess NH_4^+ from microbial biomass most efficiently in agricultural soils (Roberts et al., 2009). On the other hand, arginine utilization by microbial communities might be different in acidic forest soils, where fungal activity is relatively high (Fujii et al., 2012). Under N limitation, fungal community can preserve N through recycling of ammonium-N through urea–ornithine cycle (Harder Nielsen et al., 1998), accumulation of ornithine or arginine in vacuole (luxury uptake;

* Corresponding author. Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki, 305-8687, Japan.
E-mail address: fjkazumichi@gmail.com (K. Fujii).

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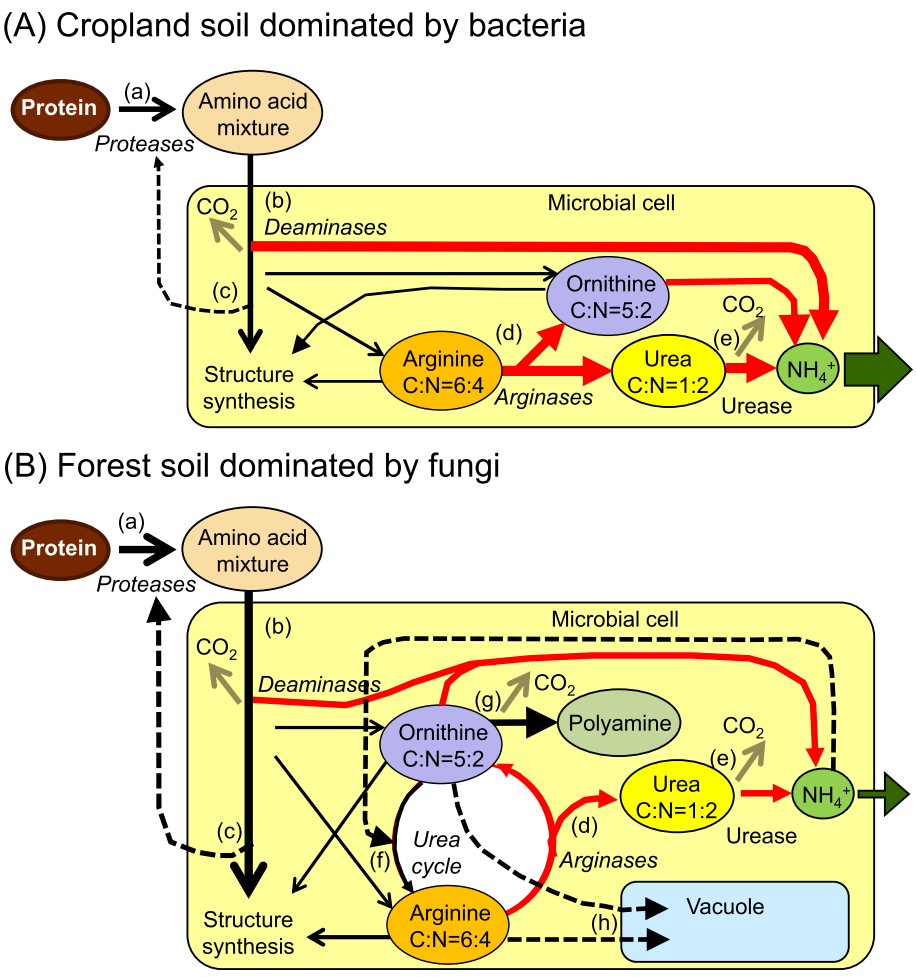

Fig. 1. Hypothetical diagram of amino acid degradation and N flow in cropland soil dominated by bacteria (A) and forest soil dominated by fungi (B). In cropland soil (A), production of amino acids by proteases (a) is a rate-limiting step for N mineralization. After microbial uptake, some amino acids are mineralized by deaminases (b). Some N is incorporated into amino acid or protein synthesis (c), while the other C or N is further decomposed. Arginine is typically decomposed by arginases to ornithine and urea (d). Urea is further decomposed to NH4+. In forest soils dominated by fungi (B), N mineralization can be retarded by fungi-specific processes: recycling of NH4+ and ornithine in ornithine cycle (f), polyamine formation from ornithine that precede fungal growth (g), and accumulation in vacuole (h). The red or black arrows indicate the processes that promote or retard N mineralization, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fog, 1988), and formation of polyamine from ornithine for fungal growth (Hoyt and Davis, 2004) (Fig. 1). These processes related to arginine, ornithine, and urea degradation can potentially affect the rates of N mineralization by soil microbial community. Microorganisms can regulate arginine-degrading activity (production of arginases) depending on surrounding environments (e.g., C/N ratio) (Abdelal, 1979). We hypothesize that arginine-degrading enzyme activity can be repressed and affect the N release from amino acid mineralization in N-limited forest soils to minimize the loss of N from microbial biomass in forest soils.

To test whether the arginine-degrading activities and microbial N release are limited in forest soils with high C/N ratios, we traced the fate of amino acids added in soils and compared microbial activity of N mineralization among a variety of soils using arginine, ornithine, and urea.

2. Materials and methods

2.1. Sampling forest floor material

Sites were selected to include temperate coniferous forest soils with the high C/N ratios, temperate and tropical broad-leaved forest soils with the low C/N ratios, and temperate and tropical cropland soils with the low C/N ratios. Soils samples were collected in August 2010 from the surface horizon (0–10 cm) at six forest sites in Iwate and Kyoto and three cropland sites in Kyoto and Osaka, Japan, and one forest site and one cropland site in Chiang-Rai, Thailand (THF and THC, respectively) (Table S1). The forest sites in Japan included a fir forest on acidic soil (APF), beech forests on volcanic soil in Appi (APB) and Morioka (MRB) in Iwate, cypress forests located on upper and lower slopes in the Kamigamo Experimental Station (KMU and KML, respectively), and an evergreen oak forest on acidic soil on Mt. Yoshida (KTO) in Kyoto. The cropland sites include two soybean fields in Kyoto (KTS) and Osaka (OSS) and one paddy field (OSP) in Osaka. The forest sites were selected to ensure variability in soil pH and C/N ratios, while the cropland sites consistently have a less acidic pH and narrow C/N ratios (Table 1). Within forests, the soils at APF, KMU, KML, and KTO have a lower pH than the volcanic soils (APF and MRB) and tropical soil (THF). The soil C/N ratios of the three coniferous forests (APF, KMU, and KML) were higher than those of the broad-leaved forests.

2.2. Experimental design and incubation condition

The composite soil sample collected from three subplots in each site was passed through a 4-mm sieve and used for microbiological analyses and incubation studies. All experiments were carried out using a composite soil sample in three technical replicates. The soil samples were pre-incubated at 25 °C for 7 days. The soil water content was adjusted to 60% of the water holding capacity (WHC) by adding distilled water (Öhlinger, 1995). Under the same incubation conditions, we measured soil microbiological properties (section 2.3.), the mineralization rates (C and N) and microbial biomass incorporation of amino acids in soil samples amended with an amino acid mixture (section 2.4.), and the mineralization rates of arginine, ornithine, and urea (section 2.5.). The sorption experiments were conducted using chloroform fumigated soil samples without pre-incubation (section 2.6.).