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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 $CaSO_4$,

0.002 M $MgSO_4$, 0.005 M $Ca(H_2PO_4)_2$, 0.0025 M K_2SO_4) (Stanford and Smith, 1972) and excess water was removed under vacuum (60 kPa). There were three treatments: (1) non-amended control soil (CK); (2) soil + glucose; and (3) soil + $(^{14}NH_4)_2SO_4$ (N). One milliliter of substrate solution (glucose or $(^{14}NH_4)_2SO_4$) was added to the soil microcosms after each leaching. The amount added each time was 0.1 mg N or 1.0 mg C g^{-1} soil. Each treatment was in triplicate and totally 90 samples were incubated. The soil samples were successively leached at 0, 1, 2, 4, 6, 8, 12, 16, 22 and 30 weeks, respectively, prior to each sampling. The leachates were collected and prepared for the determination of inorganic N. The soil was sampled at the same weeks as above and then air-dried and sieved <0.25 mm prior to determination of amino acids.

2.4. Analytical methods

2.4.1. Soil amino acids analysis and ^{15}N enrichment measurement

The hydrolysis, purification, and derivatization of soil amino acids were done according to Amelung and Zhang (2001) and Hou et al. (2009). Briefly, after the soil samples were hydrolyzed with 6 M HCl for 12 h, solutions were filtered, and the hydrolyte purified before derivatization to remove interfering organic compounds and metal ions in the soil matrix (Amelung and Zhang, 2001). The pure amino acids were transformed into 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) derivatives for better peak separation and quantification via UV detector (He et al., 2011). L-2-aminobutyric acid was added as an internal standard before hydrolyzes (Hou et al., 2009).

The 15 individual amino acid derivatives were separated on a XTerra^R MS C_{18} column (3.5 μm, 150 mm × 2.1 mm I.D., Waters Corporation, USA) maintained at 35 °C, and the ^{15}N incorporation into individual amino acids was determined by HPLC/MS (Waters 2695 HPLC/MS systems, USA) (He et al., 2011). The instrument was equipped with four pumps, a Waters 996 photodiode array detector for ultraviolet detection (Waters Corporation, USA), and an ion trap mass spectrometer with an electrospray ionization source (Waters micromass ZQ4000, USA). The mobile phase consisted of three solvents: 1.5 mM ammonium formate in water (pH 3.0, titrated with formic acid), acetonitrile, and water. The gradient profile was set according to Hou et al. (2009), and the mobile phase flow rate was 0.2 ml min^{-1}. Ultraviolet detection was set at 248 nm and a 50 min running time. The signal intensity of m/z $[M+H]^+$ for each amino acid on the positive ion mode was maximized under the following conditions: capillary voltage, 3000 V; cone voltage, 25 V; extractor and RF lens voltage, 4 and 0.4 V, respectively; ion source temperature, 105 °C; desolvation temperature, 120 °C; desolvation gas flow (N_2), 2.5 L min^{-1}; and cone gas flow (N_2), 1.7 L min^{-1}. MassLynx 3.5 software was used for data analysis (He et al., 2011).

Individual amino acids were quantified with an ultraviolet detector, and the isotope ratio calculated by MS with selective ion storage (SIS) mode. The intensity of the N-containing molecule ion (M) and also the corresponding M plus 1 fragment ([M+1]) were measured for those individual amino acids containing only 1 N atom. For arginine, histidine and lysine, the intensity of [M+4], [M+3], [M+2] and [M+1] was measured, because there are 4, 3, and 2 N atoms in these amino acids, respectively (He et al., 2011). Thereafter, the isotope ratio was calculated by the peak area of [M+1] divided by the peak area of [M] for the amino acids contained 1 N atom, and the sum of the isotope peaks from [M+1] to [M+n] (n means the N atom number in the molecular ion) divided by the peak area of [M] for arginine, histidine and lysine, respectively. Accordingly, the ^{15}N enrichment in each amino acid is expressed as atom percentage excess (APE) and calculated as follows:

$$APE = (R_e - R_c)/[1 + (R_e - R_c)] \times 100\%$$

where R_e is the isotope ratio of labeled samples and R_c represents the corresponding ratio obtained from original soil (before labeling) analyzed on the same HPLC/MS assay (He et al., 2011).

The content of each ^{15}N-labeled amino acid was calculated by multiplying the concentration of the amino acid and the APE (He et al., 2011). The total ^{15}N-amino acid contents were the sum of 15 individual ^{15}N-amino acids.

2.4.2. Analysis of inorganic N in the leachates

Total inorganic N (nitrate and ammonium) in the leachates was determined by heat distilling the samples with magnesium oxide and Devarda's alloy (Lu, 2000). Subsequently, the atom% ^{15}N in the acidified aqueous distillate was measured using an isotope ratio mass spectrometer (Finnigan Mat model 251, USA). All the operation procedure was carried out from lower to higher atom% ^{15}N to avoid cross-contamination.

2.5. Calculation

The soil newly synthesized and original amino acid pool changes were calculated from: [measured soil amino acid contents at time 0 minus the soil amino acid contents measured at time T (1–30 weeks)].

2.6. Statistical analysis

The effect of substrate amendment on the change of the soil newly synthesized and original amino acids pools was analyzed using a one-way analysis of variance (ANOVA) and the Tukey method at a 95% confidence level ($P < 0.05$). Significance was considered at $P < 0.05$. All statistical analyses were performed using a SPSS 13.0 software package (SPSS Inc., Chicago, USA).

3. Results

3.1. Change of the soil newly synthesized and original amino acids pool after the addition of N and glucose

3.1.1. The newly synthesized soil amino acids (^{15}N-amino acids)

The total loss of soil ^{15}N-amino acids was rapid in CK and N treatments during the first week, and thereafter showed a slower rate over the incubation time (Fig. 1a). There were no significant differences in the total loss of soil ^{15}N-amino acids between N and CK treatments ($P > 0.05$) during the first 16 weeks (Fig. 1a). At 22 and 30 weeks, the N addition caused a 17.0% increase in the loss of ^{15}N-amino acids ($P < 0.05$) compared to the CK treatment. With glucose addition, the loss of ^{15}N-amino acids increased during the first 4 weeks, and then decreased from 5 to 20 weeks. In comparison to CK treatment, the addition of glucose decreased the loss of soil ^{15}N-amino acids by 98.4% during the whole incubation ($P < 0.05$) (Fig. 1a).

3.1.2. The original soil amino acids (^{14}N-amino acids)

The loss of soil ^{14}N-amino acids in CK and N treatments was rapid in the first 2 weeks and then showed a slower rate with increasing incubation time (Fig. 1b). Compared with the CK treatment, N addition decreased the loss of ^{14}N-amino acids by 19.9% over the whole incubation ($P < 0.05$), although ^{14}N-amino acids in the N addition treatment were also affected by de-novo formed amino acids from the added $(^{14}NH_4)_2SO_4$. At the end of the incubation, the total loss in CK and N treatments were 513 and 463 mg kg^{-1}, respectively. Addition of glucose decreased the loss of

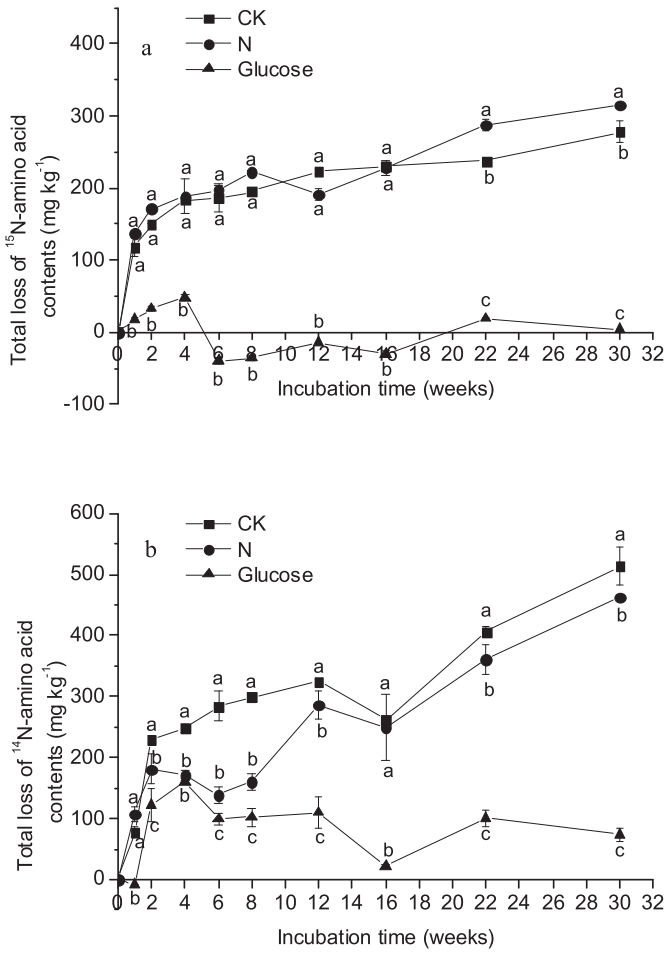

Fig. 1. The total loss of soil amino acid pool in the CK, N and glucose treatments. a: ^{15}N-amino acids; b: ^{14}N-amino acids. CK, non-amended control soil; Glucose, soil + glucose; N, soil + $(^{14}NH_4)_2SO_4$. Different letters at each sampling date indicate significant difference between different treatments at $P < 0.05$.

soil ^{14}N-amino acids compared to the CK treatment during the whole incubation (71.3%) ($P < 0.05$).

3.1.3. *The change percentages of the newly synthesized and original amino acids in soil*

The loss percentages of either newly synthesized or originally present amino acids compared to their concentrations at 0 week increased with time in the CK and N treatments (Fig. 2a, b). The relative loss percentage of ^{15}N-amino acids was greater than ^{14}N-amino acids throughout the entire incubation in both the CK and N treatments. At the end of the incubation (30 weeks), the loss percentage of ^{15}N-amino acids had increased to 51.5% and 58.3%, while the ^{14}N-amino acids were only 28.8% and 26.0% in CK and N treatments, respectively. With glucose addition (Fig. 2c), the loss percentage of ^{15}N-amino acids was smaller than 10%, and even negative between 6 and 16 weeks. Overall, for the glucose amended treatment, the loss percentage of ^{14}N-amino acids was higher than the ^{15}N-amino acids.

3.2. *Cumulative contents of inorganic ^{15}N and ^{14}N leached from soil*

3.2.1. *Inorganic ^{15}N*

The content of inorganic ^{15}N leached from soil in all the treatments increased cumulatively, but was influenced by the different treatments ($P < 0.05$, Fig. 3a). The content of inorganic ^{15}N in both N

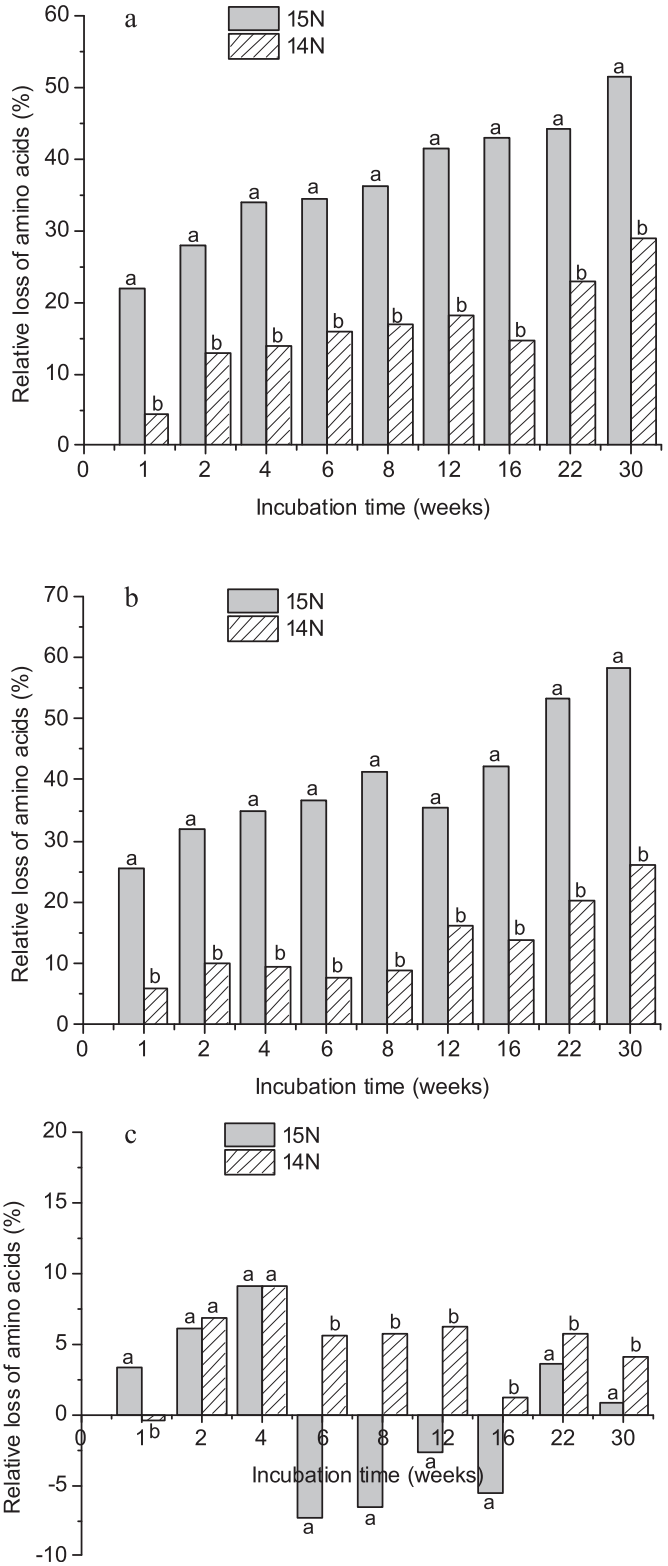

Fig. 2. The percentages of ^{15}N- and ^{14}N-amino acid loss to their initial amino acid contents (0 weeks). a: CK treatment; b: N treatment; c: Glucose treatment (^{15}N stands for the newly synthesized amino acids and ^{14}N stands for the original amino acids). Different letters at each sampling date indicate significant difference between the percentage of the newly synthesized and the original amino acids at $P < 0.05$.

and glucose treatments was significantly smaller than CK treatment throughout ($P < 0.05$). By the end of the incubation, the cumulative

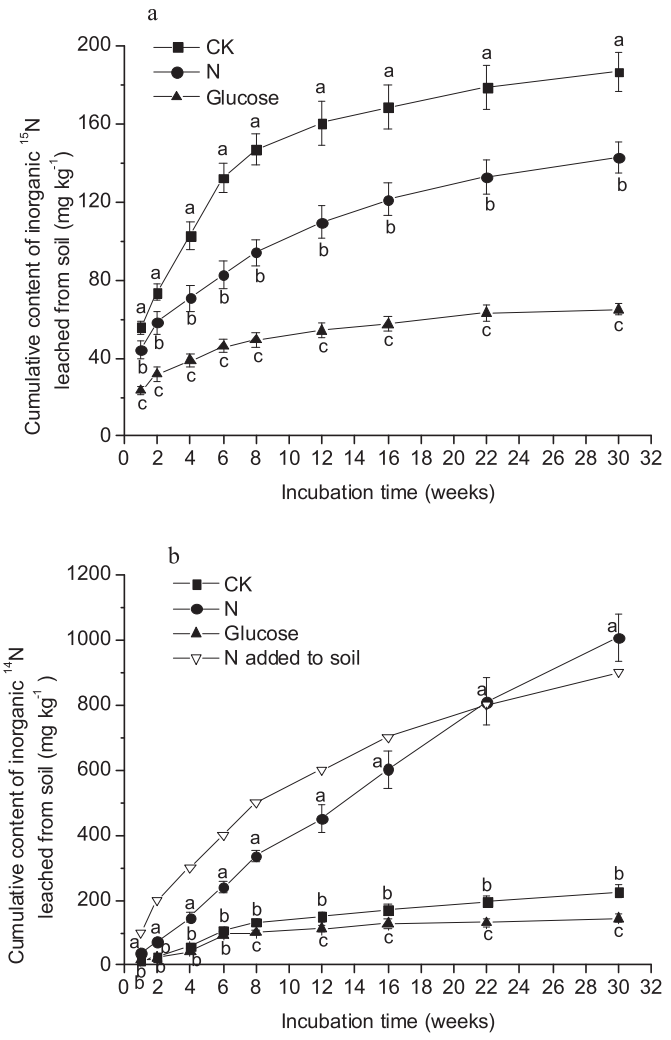

Fig. 3. The cumulative content of inorganic N leached from soil in the CK, glucose and nitrogen treatments ("N added to soil" means the inorganic N content added to soil during the incubation). a: inorganic ^{15}N; b: inorganic ^{14}N. CK, non-amended control soil; Glucose, soil + glucose; N, soil + $(^{14}NH_4)_2SO_4$. Different letters at each sampling date indicate significant difference between different treatments at $P < 0.05$.

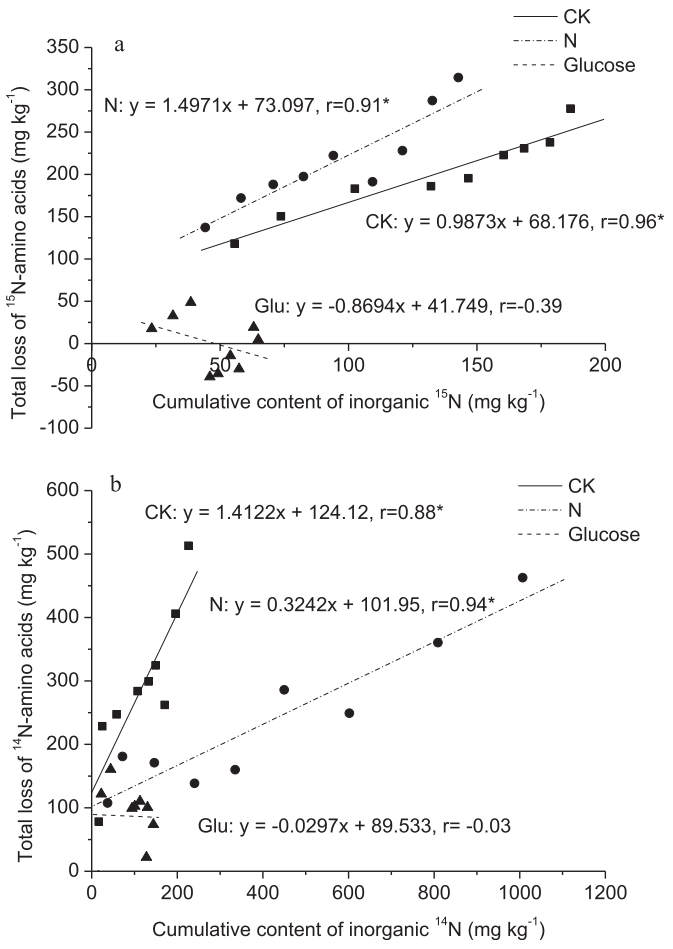

Fig. 4. The relationship between the total loss of amino acids and the cumulative content of inorganic N. a: ^{15}N; b: ^{14}N. CK, non-amended control soil; Glucose, soil + glucose; N, soil + $(^{14}NH_4)_2SO_4$.

content of inorganic ^{15}N in CK, N and glucose treatments were 186, 143 and 65 mg kg^{-1}, respectively.

The loss of ^{15}N-amino acids pool was significantly and positively correlated with the cumulative content of inorganic ^{15}N in CK and N treatments (r = 0.96 and 0.91), respectively ($P < 0.05$) (Fig. 4a). There was no statistically significant correlation with glucose at $P > 0.05$, despite the apparent negative trend.

3.2.2. Inorganic ^{14}N

Similar to the dynamic of inorganic ^{15}N, the cumulative content of inorganic ^{14}N gradually increased with time in all treatments and was affected by N or glucose addition ($P < 0.05$, Fig. 3b). In the N treatment, the inorganic ^{14}N value may be attributed to the mineralization of both the "old" and the "de-novo formed" organic N. If the previously added inorganic ^{14}N content was subtracted from the cumulative content of inorganic ^{14}N, the results were even negative before 20 weeks, and became significantly lower than the CK treatment after 20 weeks (Fig. 3b). Glucose addition significantly decreased the inorganic ^{14}N concentration ($P < 0.05$). By the end of incubation, the content of inorganic ^{14}N in CK and glucose treatments was 226 and 144 mg kg^{-1}, respectively.

The loss of ^{14}N-amino acids pool was significantly and positively correlated with the cumulative content of inorganic ^{14}N in CK and N treatments (r = 0.88 and 0.94), respectively ($P < 0.05$) (Fig. 4b). However, no significant correlation was found in the glucose treatment ($P > 0.05$).

4. Discussions

4.1. Differentiation between the mineralization of newly synthesized and original amino acids in soil under incubation conditions

Mineralization of soil organic N is mediated by microorganisms, but the mineralization rate in soil is dependent on the accessibility of organic N (Killham et al., 1993; Six et al., 2002). Compared to original SOM, newly immobilized N is readily accessible to microorganisms for growth, and thus, easier to be mineralized (Hansen et al., 2005). The more physically or chemically protected the SOM, the less accessible to microorganisms the nutrients become (Olk and Cassman, 1995). In this study, we found significant increases in loss percentages of ^{15}N-labeled amino acids compared to the original compounds in the CK treatment. Consequently, these newly synthesized amino acids were more easily mineralized than the original amino acids. Rasul et al. (2009) reported the mineralization rate of freshly formed microbial residues was more than twice that of older native SOM, which is consistent with our result.

Adsorption to mineral surfaces was a major process in the preservation of OM in soils. Usually there is no homogenous sorption of OM to minerals, instead, it is patchy (Kaiser and Guggenberger, 2003; Mikutta et al., 2004; Mödl et al., 2007). Additional sorption could follow this patchiness, and a multiple sorption layer theory showed that sorption occurs at places where organic matter is already accumulated (Kleber et al., 2007). A new study using NanoSIMS indicated that a preferential attachment of new OM to organo-mineral clusters with rough surfaces containing pre-existing OM (Vogel et al., 2014). A position-specific labeling of amino acids isotope analysis technique also showed that the newly formed microbial products can be absorbed at the multiple sorption sites (Dippold et al., 2014). Therefore, the newly synthesized amino acids that were in the outer organo-mineral complexes may mineralize faster than the older amino acids which were in the inner layer. In addition, there are other possible mechanisms of protection for original soil amino acids compared with newly synthesized ones, including entrapment in mesopores which are too small for enzyme access, interaction with other recalcitrant organic materials, and encapsulation in organic matter (Friedel and Scheller, 2002; Kleber et al., 2007). As a result, the mineralization rate of newly synthesized and original amino acids may differ.

In order to test the differences in stability between the newly synthesized and original amino acids, a non-linear model, allometric curve fitting was applied to calculate the turnover time of both amino acid fractions for the CK treatment. The functions were as follows: $y = 125.83571x^{0.22271}$ for the newly synthesized amino acids ($r = 0.98$, $P < 0.001$); $y = 135.29298x^{0.35959}$ for the originally present amino acids ($r = 0.89$, $P < 0.001$). Where y means the loss of amino acids pools, and x means the time.

The simulated turnover time of the newly synthesized and originally present amino acids was about 13 years and 25 years, respectively, which indicated that the newly synthesized amino acids could be mineralized 2 times faster than the original ones. In a field experiment, Lü et al. (2013) have studied the incorporation and allocation of inorganic fertilizer N to different organic soil N fractions, and found that amino acids could serve as a transitional pool of available N in a soil-crop system. Our study further indicated that the newly derived amino acids from N fertilizers may serve as a fast transitional pool for N supply, while soil original amino acids may serve as a slow one.

4.2. The effect of added substrates on the degradation of newly synthesized and original amino acids under incubation conditions

The temporal pattern of the mineralization of newly synthesized amino acids observed during incubation with N, compared with the CK treatment, could be divided into two phases (i.e. the first 16 weeks and later 16 weeks) in the full course of incubation. During the first phase, the addition of N did not significantly affect the mineralization of newly synthesized amino acids. However, in the second phase, the addition of N significantly increased the mineralization of newly synthesized amino acids. We interpreted that both C and N were not limited in the first phase, while C became limited in the second phase where amino acids were decomposed additionally for C supply. As also suggested by McFarland et al. (2002), when N was added to the soil, the C source and energy would be exhausted, and some of the immobilized N might be used in enzyme synthesis and then released as extracellular enzymes to decompose more recalcitrant organic substrates for C acquisition.

Moreover, the cumulative content of inorganic ^{15}N in our study further verified our findings that amino acids were primarily used as C and energy sources when N was abundant. Jan et al. (2009) added uniformly ^{14}C-labeled soluble protein to soil and determined the rate of $^{14}CO_2$ evolution over 30 days. The addition of inorganic N to soil was found to increase the rate of protein-C mineralization, indicating that the protein was utilized as a C source when N was abundant (Jan et al., 2009). Correspondingly, McFarland et al. (2010) showed that in low C/N soil environments, free amino acids were used primarily as a C source by soil microorganisms. Other studies have shown that low-molecular-weight (LMW) organic compounds, including free amino acids, play an important role in sustaining the short-term energy balance of microorganisms involved in the decomposition of SOM (De Nobili et al., 2001; Mondini et al., 2006). In inorganic N-rich soils, the uptake of amino acids as part of LMW-dissolved organic N by soil microorganisms may primarily provide them with C to fuel respiration, rather than to satisfy their internal N demand (Jones et al., 2004). Knowles et al. (2010) also showed the similar decoupling effect of C and N metabolism in microorganisms on a molecular/metabolic scale. These studies have mainly focused on the free amino acids, however, protein and/or peptides were the largest and most reliable source of free amino acids (Schulten and Schnitzer, 1998), so our findings indicated that the newly synthesized protein and/or peptide amino acids were used primarily as C and energy sources when the N was sufficient in soil.

In addition, the different mineralization rate of ^{15}N-amino acids with incubation time in N and CK treatments may indicate that newly synthesized amino acids could be divided into two parts: the rapid decomposition phase and the relative slower decomposition phase. Others have hypothesized that the new amino acids may stabilize gradually with time through humification (Springob and Kirchmann, 2003). In most instances, soil amino acids may persist for longer periods by adsorption to mineral components of soil (Gonod et al., 2006) or chemical inclusion into humic substances (Kuzyakov and Galitsa, 1993). Humics can also bind to proteins, potentially interfering with proteolysis (Rejsek et al., 2010). Moreover, studies have shown that the time that a compound remains in a soil affects its biodegradability. Aging increased the resistance of compound to biodegradation (Hatzinger and Alexander, 1995; Ciglasch et al., 2008). These reasons may lead to a slower mineralization rate of newly synthesized amino acids during longer incubations. It has been shown before that SOM degrades in two phases, i.e., freshly added compounds likely dissipate faster (Dalal and Mayer, 1986; Amelung et al., 2002), as observed here for the labeled amino acids.

During the incubation, the mineralization of the newly synthesized soil amino acids in the CK and N treatments occurred in tandem with the original amino acids. However, the effect of N on the mineralization of original amino acids was different from the newly synthesized ones in our study. Specifically, N addition significantly decreased the mineralization of the soil original amino acids and the percent mineralization of original amino acids was lower than the ^{15}N-fraction in the N treatment. However, since we have added inorganic $(^{14}NH_4)_2SO_4$ into the soil in the N treatment, the added ^{14}N was obviously not just remaining as inorganic N in the system, but that it can get immobilized and later on mineralized. Thus, we cannot differentiate whether the measured amount of mineral ^{14}N was from manually-added inorganic ^{14}N or from the inorganic ^{14}N which was derived from original soil N. By subtracting the added inorganic ^{14}N, the inorganic ^{14}N in N treatment was lower than the inorganic ^{14}N amount in the CK treatment. Therefore, it is confident to say that the inorganic N addition decreased the soil N mineralization. The findings indicated that the humified protein or peptides were relatively recalcitrant to microbial attack due to chemical and physical protection (Schulten and Schnitzer, 1998), which may lead to different responses of the amino acid fractions.

For C substrate addition studies, glucose serves as both a highly available energy and C source for soil microorganisms. It can trigger soil microbial activity and growth (De Nobili et al., 2001; Blagodatskaya et al., 2009; Blagodatsky et al., 2010), which increases the immobilization of inorganic N and has a lower N mineralization rate (Chander and Joergensen, 2001; Dilly and Nannipieri, 2001; Vinten et al., 2002; Hamnér and Kirchmann, 2005; Rasul et al., 2009). In the present study, the significantly lower mineralization speed of newly synthesized amino acids in the glucose treatment versus CK treatment indicated that the addition of glucose inhibited the mineralization of newly synthesized amino acids, presumably due to immobilization (Vinten et al., 2002; Hamnér and Kirchmann, 2005; Rasul et al., 2009). The temporal dynamics of ^{15}N-amino acids in the glucose treatment indicated that there was an immobilization of ^{15}N after 6 weeks. With high C availability, organic and inorganic N are both used by microorganisms (Coyle et al., 2009) for subsequent uptake and re-synthesis into microbial proteins (Miltner and Zech, 1999). Thus, the cumulative inorganic ^{15}N was lower in the glucose treatment than in the CK treatment during the entire experiment, which further verified the inhibitory effect of glucose on the mineralization of newly synthesized fractions.

The effect of glucose on decreasing mineralization of both ^{14}N and ^{15}N-amino acids was similar. However, the rate of mineralization of ^{14}N-amino acids was faster than the ^{15}N-amino acids with glucose, which showed the opposite trends in CK treatment, possibly due to the re-utilization of a portion of ^{15}N-amino acids by microbes to synthesize the new ^{15}N-amino acids to compensate their loss. This study indicated that the addition of glucose changed the mineralization dynamics of both soil amino acid fractions.

4.3. Conclusions

This study has improved our understanding of the mineralization of newly synthesized amino acids compared to original amino acids in soil. When there was no C and N addition, the newly synthesized amino acids were more readily mineralized than the original fraction, which indicated that newly synthesized amino acids are more accessible than the original portion when there is no additional C and N supply. The newly synthesized soil amino acids may serve as a potential fast transitional pool for N supply, while original ones likely serve as a relatively slow N pool. Interestingly, the mineralization rate of newly synthesized amino acids and soil original fractions were regulated differently by external C and N availability; thus, the mineralization dynamics were substrate-dependent. External C supply (glucose addition) decreased the mineralization of both amino acid fractions. However, when N was abundant, the newly synthesized amino acids were preferentially mineralized to provide additional C and energy, while N addition inhibited the mineralization of soil original amino acids. Our findings are very significant for the N management in crop-soil systems. Available C addition (such as crop residue return) should be the key approach in regulating the mineralization of both N fertilizer-derived amino acids and soil original ones. Adjusting N availability by N fertilizers may balance the process of N supply and sequestration. Yet, further research is needed to verify this mechanism under field conditions.

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References

Aber, J., Melillo, J., 2001. Terrestrial Ecosystems, second ed. Harcourt Academic Press, San Diego, California, USA.
Adour, L., Aziza, M., Couriol, C., Amrane, A., 2006. Amino acids as carbon, energy and nitrogen sources for Penicillium camembertii. Journal of Chemical Technology and Biotechnology 81, 573–579.
Amelung, W., 2003. Nitrogen biomarkers and their fate in soil. Journal of Plant Nutrition and Soil Science – Zeitschrift Fur Pflanzenernahrung Und Bodenkunde 166, 677–686.
Amelung, W., Lobe, I., Du Preez, C.C., 2002. Fate of microbial residues in sandy soils of the South African Highveld as influenced by prolonged arable cropping. European Journal of Soil Science 53, 29–35.
Amelung, W., Zhang, X., 2001. Determination of amino acid enantiomers in soils. Soil Biology and Biochemistry 33, 553–562.
Berthrong, S.T., Finzi, A.C., 2006. Amino acid cycling in three cold temperate forests of the northeastern USA. Soil Biology and Biochemistry 38, 861–869.
Blagodatskaya, E.V., Blagodatsky, S.A., Anderson, T.-H., Kuzyakov, Y., 2009. Contrasting effects of glucose, living roots and maize straw on microbial growth kinetics and substrate availability in soil. European Journal of Soil Science 60, 186–197.
Blagodatsky, S., Blagodatskaya, E., Yuyukina, T., Kuzyakov, Y., 2010. Model of apparent and real priming effects: linking microbial activity with soil organic matter decomposition. Soil Biology and Biochemistry 42, 1275–1283.
Bloem, J., Hopkins, D.W., Benedetti, A., 2006. Microbiological Methods for Assessing Soil Quality. CABI Publishing, Wallingford, UK.
Brant, J.B., Sulzman, E.W., Myrold, D.D., 2006. Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation. Soil Biology and Biochemistry 38, 2219–2232.
Chander, K., Joergensen, R.G., 2001. Decomposition of ^{14}C glucose in two soils with different amounts of heavy metal contamination. Soil Biology and Biochemistry 33, 1811–1816.
Ciglasch, H., Busche, J., Amelung, W., Totrakool, S., Kaupenjohann, A., 2008. Field aging of insecticides after repeated application to a Northern Thailand Ultisol. Journal of Agricultural and Food Chemistry 56, 9555–9562.
Coyle, J.S., Dijkstra, P., Doucett, R.R., Schwartz, E., Hart, S.C., Hungate, B.A., 2009. Relationships between C and N availability, substrate age, and natural abundance ^{13}C and ^{15}N signatures of soil microbial biomass in a semiarid climate. Soil Biology and Biochemistry 41, 1605–1611.
Dalal, R.C., Mayer, R.J., 1986. Long term trends in fertility of soils under continuous cultivation and cereal cropping in southern Queensland. II. Total organic carbon and its rate of loss from the soil profile. Australian Journal of Soil Research 24, 281–292.
De Nobili, M., Contin, M., Mondini, C., Brookes, P.C., 2001. Soil microbial biomass is triggered into activity by trace amounts of substrate. Soil Biology and Biochemistry 33, 1163–1170.
Dilly, O., Nannipieri, P., 2001. Response of ATP content, respiration rate and enzyme activities in an arable and a forest soil to nutrient additions. Biology and Fertility of Soils 34, 64–72.
Dippold, M., Biryukov, M., Kuzyakov, Y., 2014. Sorption affects amino acid pathways in soil: implications from position-specific labeling of alanine. Soil Biology and Biochemistry 72, 180–192.
Dippold, M.A., Kuzyakov, Y., 2013. Biogeochemical transformations of amino acids in soil assessed by position-specific labelling. Plant and Soil 373, 385–401.
Friedel, J.K., Scheller, E., 2002. Composition of hydrolysable amino acids in soil organic matter and soil microbial biomass. Soil Biology and Biochemistry 34, 315–325.
Geisseler, D., Horwath, W.R., 2014. Investigating amino acid utilization by soil microorganisms using compound specific stable isotope analysis. Soil Biology and Biochemistry 74, 100–105.
Geisseler, D., Horwath, W.R., Doane, T.A., 2009. Significance of organic nitrogen uptake from plant residues by soil microorganisms as affected by carbon and nitrogen availability. Soil Biology and Biochemistry 41, 1281–1288.
Gonod, L.V., Jones, D.L., Chenu, C., 2006. Sorption regulates the fate of the amino acids lysine and leucine in soil aggregates. European Journal of Soil Science 57, 320–329.
Hamnér, K., Kirchmann, H., 2005. Net nitrogen immobilization in soil induced by small additions of energy sources. Acta Agriculturae Scandinavica, Section B – Plant Soil Science 55, 177–185.
Hansen, J.P., Eriksen, J., Jensenl, L.S., 2005. Residual nitrogen effect of a dairy crop rotation as influenced by grass-clover ley management, manure type and age. Soil Use and Management 21, 278–286.
Hatzinger, P.B., Alexander, M., 1995. Effect of aging of chemicals in soil on their biodegradability and extractability. Environmental Science and Technology 29, 537–545.

He, H.B., Lü, H.J., Zhang, W., Hou, S.M., Zhang, X.D., 2011. A liquid chromatographic/mass spectrometric method to evaluate ^{13}C and ^{15}N incorporation into soil amino acids. Journal of Soils and Sediments 11, 731–740.
Henry, H.A.L., Jefferies, R.L., 2003. Plant amino acid uptake, soluble N turnover and microbial N capture in soils of a grazed Arctic salt marsh. Journal of Ecology 91, 627–636.
Hou, S., He, H., Zhang, W., Xie, H., Zhang, X., 2009. Determination of soil amino acids by high performance liquid chromatography-electro spray ionization-mass spectrometry derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate. Talanta 80, 440–447.
Jan, M.T., Roberts, P., Tonheim, S.K., Jones, D.L., 2009. Protein breakdown represents a major bottleneck in nitrogen cycling in grassland soils. Soil Biology and Biochemistry 41, 2272–2282.
Jones, D.L., Hodge, A., 1999. Biodegradation kinetics and sorption reactions of three differently charged amino acids in soil and their effects on plant organic nitrogen availability. Soil Biology and Biochemistry 31, 1331–1342.
Jones, D.L., Shannon, D., Murphy, D.V., Farrar, J., 2004. Role of dissolved organic nitrogen (DON) in soil N cycling in grassland soils. Soil Biology and Biochemistry 36, 749–756.
Kaiser, K., Guggenberger, G., 2003. Mineral surfaces and soil organic matter. European Journal of Soil Science 54, 1–18.
Killham, K., Amato, M., Ladd, J.N., 1993. Effect of substrate location in soil and soil pore–water regime on carbon turnover. Soil Biology and Biochemistry 25, 57–62.
Kleber, M., Sollins, P., Sutton, R., 2007. A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. Biogeochemistry 85, 9–24.
Knowles, T.D.J., Chadwick, D.R., Bol, R., Evershed, R.P., 2010. Tracing the rate and extent of N and C flow from ^{13}C, ^{15}N-glycine and glutamate into individual de novo synthesised soil amino acids. Organic Geochemistry 41, 1259–1268.
Kuzyakov, Y.V., Galitsa, S.V., 1993. Kinetics of [2-^{14}C] glycine decomposition and its incorporation into humus fractions of Sierozem. Eurasian Soil Science 25, 39–53.
Lipson, D., Nasholm, T., 2001. The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. Oecologia 128, 305–316.
Lü, H., He, H., Zhao, J., Zhang, W., Xie, H., Hu, G., Liu, X., Wu, Y., Zhang, X., 2013. Dynamics of fertilizer-derived organic nitrogen fractions in an arable soil during a growing season. Plant and Soil 373, 595–607.
Lu, R.K., 2000. Soil and Agricultural Chemistry Analysis Methods. China Agricultural Scientech Press, Beijing.
McFarland, J.W., Ruess, R.W., Kielland, K., Doyle, A.P., 2002. Cycling dynamics of NH_4^+ and amino acid nitrogen in soils of a deciduous boreal forest ecosystem. Ecosystems 5, 775–788.
McFarland, J.W., Ruess, R.W., Kielland, K., Pregitzer, K., Hendrick, R., 2010. Glycine mineralization in situ closely correlates with soil carbon availability across six North American forest ecosystems. Biogeochemistry 99, 175–191.
Mikutta, C., Lang, F., Kaupenjohann, M., 2004. Soil organic matter clogs mineral pores: evidence from ^1H-NMR logging and N_2 adsorption. Soil Science Society of America Journal 68, 1853–1862.
Miltner, A., Zech, W., 1999. Microbial degradation and resynthesis of proteins during incubation of beech leaf litter in the presence of mineral phases. Biology and Fertility of Soils 30, 48–51.
Mondini, C., Cayuela, M.L., Sanchez-Monedero, M.A., Roig, A., Brookes, P.C., 2006. Soil microbial biomass activation by trace amounts of readily available substrate. Biology and Fertility of Soils 42, 542–549.
Monreal, C.M., McGill, W.B., 1985. Centrifugal extraction and determination of free amino acids in soil solutions by TLC using tritiated fluoro-2,4-dinitrobenzene. Soil Biology and Biochemistry 17, 533–539.
Mödl, C., Wörmann, H., Amelung, W., 2007. Contrasting effects of different types of organic material on surface area and microaggregation of goethite. Geoderma 141, 167–173.
Muruganandam, S., Israel, D.W., Robarge, W.P., 2009. Activities of nitrogen-mineralization enzymes associated with soil aggregate size fractions of three tillage systems. Soil Science Society of America Journal 73, 751–759.
Nasholm, T., Ekblad, A., Nordin, A., Giesler, R., Hogbeerg, M., Hogberg, P., 1998. Boreal forest plants take up organic nitrogen. Nature 392, 914–916.
Nasholm, T., Huss-Danell, K., Hogberg, P., 2001. Uptake of glycine by field grown wheat. New Phytologist 150, 59–63.
O'Dowd, R.W., Barraclough, D., Hopkins, D.W., 1999. Nitrogen and carbon mineralization in soil amended with D- and L-leucine. Soil Biology and Biochemistry 31, 1573–1578.
Olk, D.C., Cassman, K.G., 1995. Characterisation of two chemically extracted humic acid fractions in relation to nutrient availability. In: Lefroy, R.D.B., Blair, G.J., Craswell, E.T. (Eds.), Soil Organic Matter Management for Sustainable Agriculture: a Workshop Held in Ubon, Thailand, 24–26 August 1994. Australian Centre for International Agricultural Research Proceedings No. 56, Canberra, pp. 131–134.
Owen, A.G., Jones, D.L., 2001. Competition for amino acids between wheat roots and rhizosphere microorganisms and the role of amino acids in plant N acquisition. Soil Biology and Biochemistry 33, 651–657.
Paul, E.A., Clark, F.E., 1996. Soil Microbiology and Biochemistry, second ed. Academic Press, San Diego, CA.
Rasul, G., Khan, A.A., Khan, K.S., Joergensen, R.G., 2009. Immobilization and mineralization of nitrogen in a saline and alkaline soil during microbial use of sugarcane filter cake amended with glucose. Biology and Fertility of Soils 45, 289–296.
Rejsek, K., Formanek, P., Vranova, V., 2010. The Soil Amino Acids: Quality, Distribution and Site Ecology. Nova Science Publishers, Inc, New York.
Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85, 591–602.
Schneckenberger, K., Demin, D., Stahr, K., Kuzyakov, Y., 2008. Microbial utilization and mineralization of [(14)C]-glucose added in six orders of concentration to soil. Soil Biology and Biochemistry 40, 1981–1988.
Schulten, H.R., Schnitzer, M., 1998. The chemistry of soil organic nitrogen: a review. Biology and Fertility of Soils 26, 1–15.
Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant and Soil 241, 155–176.
Soil Survey Staff, 2003. Keys to Soil Taxonomy, ninth ed. United States Department of Agriculture and Natural Resources Conservation Service, Washington.
Springob, G., Kirchmann, H., 2003. Bulk soil C to N ratio as a simple measure of net N mineralization from stabilized soil organic matter in sandy arable soils. Soil Biology and Biochemistry 35, 629–632.
Stanford, G., Smith, S.J., 1972. Nitrogen mineralization potentials of soils. Soil Science Society of America Proceedings 36, 465–472.
Vinolas, L.C., Healey, J.R., Jones, D.L., 2001. Kinetics of soil microbial uptake of free amino acids. Biology and Fertility of Soils 33, 67–74.
Vinten, A.J.A., Whitmore, A.P., Bloem, J., Howard, R., Wright, F., 2002. Factors affecting N immobilisation/mineralisation kinetics for cellulose-, glucose- and straw-amended sandy soils. Biology and Fertility of Soils 36, 190–199.
Vogel, C., Mueller, C.W., Höschen, C., Buegger, F., Heister, K., Schulz, S., Schloter, M., Kögel-Knabner, I., 2014. Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils. Nature Communications 5, 2947.
Werdin-Pfisterer, N.R., Kielland, K., Boone, R.D., 2009. Soil amino acid composition across a boreal forest successional sequence. Soil Biology and Biochemistry 41, 1210–1220.
Zhang, X.D., He, H.B., Amelung, W., 2007. A GC/MS method for the assessment of ^{15}N and ^{13}C incorporation into soil amino acid enantiomers. Soil Biology and Biochemistry 39, 2785–2796.