Seasonal variations in the soil amino acid pool and flux following the conversion of a natural forest to a pine plantation on the eastern Tibetan Plateau, China

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A B S T R A C T

Although the importance of amino acids to plant nitrogen (N) nutrition in most terrestrial ecosystems is being increasingly recognized, the availability and seasonal variations of soil amino acids in subalpine coniferous forests are poorly understood. Few studies have investigated the differences in the soil amino acid pool and the cycling between forest types caused by land-use changes. In this study, we quantified the concentrations and fluxes of soil amino acids in a dragon spruce plantation and an adjacent natural forest (NF) on the eastern Tibetan Plateau, China. Soil samples from organic and mineral soil horizons were collected in May, July, September, and December of 2015 and March of 2016. The pools and fluxes of soil amino acids in the plantation converted from NF decreased significantly during the growing season and increased during the non-growing season. Meanwhile, greater pools and fluxes of soil amino acids were observed in the organic horizon than in the mineral soil horizon in both forest types. Our results indicate that distinct nutrient economies were likely formed in the plantation compared with the NF because the quality of the input litter decreased in the plantation. In addition, the importance of soil amino acids in the subalpine coniferous forest ecosystem was evaluated based on published literature. The ratios of amino acids to dissolved inorganic N (DIN) in the soils from the two studied forests were generally much lower than the range of ratios reported for other ecosystems. However, the relative high amino acid: DIN ratio in the plantations during the non-growing season suggests that the plants in the plantation may have the potential to modify their strategies for acquiring N due to the changed relative dominance between the DIN and amino acids in the soils.

1. Introduction

Nitrogen (N) is an essential element for plant growth and has an important impact on net primary productivity in terrestrial ecosystems (Reich et al., 1997; Elser et al., 2007). Any change in the soil N availability can markedly influence plant productivity and consequently affect ecosystem functions (Esser et al., 2011), especially in N- limited regions. Studies of soil N pools and cycling have largely focused on inorganic N forms (i.e., NH₄⁺ and NO₃⁻) (Burton et al., 2007a; Fagott et al., 2012; Li et al., 2014), partly due to the belief that trees can only take up inorganic N. However, recent studies have indicated that some plant species (with or without associated mycorrhiza) that occur in a wide range of ecosystems have the physiological capacity to directly compete with soil microbes for organic N by assimilating amino acids (Nasholm et al., 2009; Warren, 2012; Hill et al., 2013). Previous studies have also shown that amino acids generally comprise approximately 20–60% of the total soil N pool, both as free and bound amino acids (Amelung et al., 2006; Bol et al., 2008), and represent an important potential N source and sink in terrestrial ecosystems. These observations have led to the hypothesis that amino acids and...
inorganic N may play equally critical roles in soil N cycling (Jones et al., 2004; Weigelt et al., 2005). The phenomenon that plants can acquire N from multiple sources has also caused the re-evaluation of the N cycling model of terrestrial ecosystems (Schimel and Bennett, 2004; Jones et al., 2005a).

Soil N pools and cycles are microbially and abiotically mediated, which means they are affected by various factors, including vegetation coverage, soil microbial community composition, the soil microclimate, and soil conditions (Templer et al., 2003; Cheng et al., 2011; Butterbach-Bahl et al., 2013; Li et al., 2014). The soil amino acid pool has been demonstrated to be significantly influenced by the temporal variations involved with root exudation and turnover, the activity of extracellular enzymes responsible for amino acid production, and litter inputs (Abuarghub and Read, 1988; Lipson and Nasholm, 2001; Schimel and Bennett, 2004; Jones et al., 2005b). Previous studies have also indicated that the amino acid pool and the functions of soil enzymes in amino acid production are closely related to soil temperature (Warren and Taranto, 2010; Brzostek et al., 2012). Thus, seasonal shifts accompanied by soil temperature changes may largely affect the size of the soil amino acid pool by influencing the microbial and enzymatic activities. However, most previous studies of soil amino acids that have been conducted are limited to the growing season, and few studies have attempted to resolve the seasonal dynamics of the soil amino acid pool, especially during the non-growing season (Burton et al., 2007a; Werdin-Pfisterer et al., 2009; Wang et al., 2013; Xue et al., 2013). This may cause partial quantification of the soil amino acid pools, because even in the same ecosystem completely different results could be observed regarding the relative pool sizes of the inorganic N and amino acids (Kielland, 1995; Nordin et al., 2004). Therefore, a thorough evaluation of the soil amino acid pool and its proportions relative to inorganic N during the growing season and non-growing season would help create a better understanding of the roles of amino acids in the soil N cycle and forest nutrient supply.

Subalpine coniferous forest ecosystems play a critical role in the terrestrial ecosystems in China (Zhang et al., 2011). Meanwhile, the subalpine zone of southwestern China is considered an important ecological zone of defence for the Upper Changjiang River Valley. During the last century, natural coniferous forests in southwestern China were deforested to meet the increasing demands of the timber market for fuel materials and other forest products. After deforestation, a monoculture of dragon spruce (Picea asperata Mast.) was immediately planted on the cut area. Currently, over one million hectares of dragon spruce plantation exists in Western Sichuan, accounting for approximately 50% of the forest area in this region (Xu et al., 2010, 2012b). The long-term sustainability and productivity of forest plantations depends on the soil nutrient (mainly N) supply (Burton et al., 2007b). N limitations were previously reported to occur in the plantations in this region because the pools and turnover rates of soil available inorganic N significantly decline following the conversion of natural forests (NFs) to plantations (Xu et al., 2010, 2014). However, little research has been conducted on the impacts of this type of land-use change on the cycling of N in another important N pool, soil amino acids. Therefore, as a special forest type on the eastern Tibetan Plateau, it is important to assess the pools and cycling of soil amino acids in plantations relative to their adjacent NFs to understand the nutrient acquisition strategies of the plantation plants when soil inorganic N is limiting.

In this study, we quantified the soil amino acid pools and fluxes along with their importance relative to the soil inorganic N pools in plantation forests and NFs during the growing and non-growing seasons. Our aim was to determine whether plantations derived from land-use change from NFs have the potential to modify their strategies for acquiring N to compensate for severely limited N availability. In addition, to evaluate whether soil amino acids are a N source for plants in this study area, the pool of amino acid N and the ratio of the amino acid pool to the inorganic N pool were compared with those measured in other ecosystems. We hypothesized that (1) compared with NFs, the plantation forest would have a larger soil amino acid pools and a greater turnover rates in response to the lower inorganic N availability; (2) the amino acid N would dominate the soil N pools available for plants uptake during the non-growing season because N mineralization is constrained in cold climates; and (3) amino acid N could be an important potential N source in subalpine coniferous forest ecosystems.

2. Materials and methods

2.1. Study sites

This research was conducted on two sites that were within approximately 300 m distance of each other. Both experimental sites are located in the Miyaluo Experimental Forest in Lixian County on the eastern Tibetan Plateau in Sichuan, China (31°35’N, 102°35’E, and 3150 m a.s.l.). One site consists of a spruce-fir-dominated NF (c. 200 years old) and the other site is a dragon spruce plantation (c. 70 years old). The spruce plantation originated from NF that was clear-cut in the 1950s, and since then, there has not been any management practice, such as forest tending and thinning, performed in this region. The understory of the NF is dominated by Acer mona, Loniceria ssp., and Betula alba-sinensis, occurrence of the herb Anemone rivularis and the sedge Carex capilliformis. In the plantation, less vegetation is present, with Festuca ovina, Deyeuxia arundinacea, and Carex capilliformis mainly growing under the plantation canopy. The two study sites provide a natural platform for assessing the differences of the soil N pool and N cycling between the forest types caused by land-use change. The climate at the study site is hilly monsoon, with a mean annual precipitation of 800 mm and a mean annual temperature of 6.8 °C (the mean temperatures in January and July are −8 °C and 12.6 °C, respectively). The growing season lasts approximately six months, extending from late-April to late-October. Snow cover is typical from late-November to mid-February. The soils at both sites are typical brown forest soils and are classified as a Cambic Umbrisols according to the IUSS Working Group (2007). The basic soil properties at both sites were determined in July 2015 and are shown in Table 1.

2.2. Sample collection

At each site, four 20 m × 20 m plots separated by more than 50 m were delineated. The plots were sampled during the growing season (i.e., 10 May, 19 July, and 18 September of 2015), and the non-growing season (25 December in 2015 and 1 March in 2016). On each sample date, five sampling points were randomly chosen in each plot. The sampling points were >5 m apart, and the organic horizon was sampled at each site by obtaining a 10 × 20 cm monolith. The mineral soil was sampled directly beneath the organic horizon to a depth of 15 cm by using a 5-cm diameter soil sampler. The samples from the five points chosen in each plot were mixed thoroughly, and then the mixed samples were immediately brought to the laboratory (with ice bags). Each composite sample was passed through a 2-mm sieve and then visible living plant materials and rocks were manually removed from the samples. Subsamples of each sieved soil sample were immediately frozen at −80 °C for later extracellular enzyme activity assays. All extractions (except net fluxes) were completed within 36 h of sample collection. Proteolytic rates were analysed within 48 h of sample
collection. Subsamples of each soil were air dried to determine the total phenolic concentrations in the soils because phenols are considered important for regulating soil N availability.

2.3. Soil analysis

2.3.1. Soil extractable N pools and fluxes

The pools and net fluxes of amino acids and dissolved inorganic N (DIN) (the sum of NH$_4^+$-N and NO$_3^-$-N) were quantified for each soil sample for each sampling date. Organic and mineral soil samples of 15 and 30 g, respectively, were extracted in 100 mL of 2 M KCl. The rates of net N mineralization and net amino acid production were extracted in 100 mL of 2 M soil sample for each sampling date. Organic and mineral soil samples were filtered through Whatman #1 filter paper. The total dissolved N (TDN) contents in the extracts were measured using a C/N analyzer (Multi N/C 2100, Analytik Jena, Germany). The DON content was calculated as follows: DON = TDN - DIN.

2.3.2. Gross proteolysis

The rate of proteolysis was measured under ambient (= ‘native’) conditions and in the presence of added casein (= ‘potential’) using a method modified from Lipson et al. (1999). Because casein is a labile substrate for proteolytic enzymes, we added it to samples to determine if proteolysis was substrate-limited. To perform this assay under ambient conditions, two subsamples (initial and incubated) of soil (2–3 g) were placed in 50 mL centrifuge tubes, respectively. Then, 10 mL of 0.05 M sodium citrate buffer (pH = 6.0, which matched the soil pH in this study) was added to the tube, followed by a small volume of toluene (400 μL), which was used as a bacteriostatic agent to inhibit microbial uptake. The incubated subsamples were incubated at room temperature (25 °C) on a reciprocal shaker at 120 rpm for 4 h, and the initial subsamples were immediately received 3 mL of a 0.11 M trichloroacetic acid solution (TCA) to terminate the extracellular proteolytic enzyme activity in the soil. To measure the potential proteolysis rate, 10 mL of 0.3% casein in 0.05 M sodium acetate buffer was added to each subsample before following the procedures described above. Immediately after the incubation of the native and potential subsamples, 3 mL of the TCA solution was added to stop proteolytic enzyme activity. All the subsamples were then centrifuged and filtered through Whatman #1 filter paper (Finzi and Berthrong, 2005). The proteolytic rates were calculated as the difference between the amino acid concentrations in the incubated and initial subsamples. The amino acid concentrations were quantified using the OPAME method described above (Jones et al., 2002).

2.4. Statistical analysis

A repeated measures ANOVA was used to determine the effects of forest type, soil horizon, sampling date, and their interactions on the amino acid N, DIN, DON, and protease pools, along with the related N transformation rates (i.e., net mineralization and net amino acid production). For a specific sampling date, Student’s t-tests were used to assess the differences of all response variables for a given soil horizon between the two forest types. Before this
The results of the repeated measures ANOVA showing the interaction effects all had significant effects on the net amino acid N production and net N mineralization rates (Table 2). During the growing season, net consumption of amino acid N occurred in both forest types and soil horizons, and the rates of net consumption were greater in the planation than in the NF. In contrast, during the non-growing season, amino acid N still showed a net consumption tendency in the NF, while there was a net production in the planation (Fig. 2a). Regardless of the seasonal pattern and forest type, the rates of amino acid production and consumption were significantly faster in the organic horizon than in the mineral soil horizon (P < 0.05, Fig. 2a). The average rates of net N mineralization during the growing season were much greater in the NF (3.40 and 1.45 on average) than in the plantation (1.44 and 0.49 on average) (F = 2.60, P = 0.019; F = 1.42, P = 0.003) in the organic and the mineral soil horizons, respectively. In comparison, the net N mineralization rates were not significantly different between the NF and plantation across the soil horizons during the non-growing season (Fig. 2b). In addition, the average rate of net N mineralization in both soil horizons in the NF decreased significantly from the growing season to the non-growing season when compared with the plantation (Fig. 2b).

### 3.3. Gross proteolysis in the soil

In the organic horizon, the rate of gross proteolysis rare under ambient conditions (i.e., ‘native’ in Table 3) in the NF was generally higher than that in the plantation during the growing season, but significant effects were only observed in May and July of 2015. In contrast, this trend was reversed during the non-growing season. No significant differences between the two forest types in the mineral soil horizon were observed across all sampling dates. When cassein was added, the gross rates of proteolysis increased in both forest types (i.e., ‘potential’ in Table 3). The potential proteolysis rates in the organic horizon doubled in the plantation but increased by 3–4 times in the NF when compared with the native proteolysis rates. Gross proteolysis was more substrate-limited in the NF, which was indicated by the relatively lower ratio of native: potential proteolysis at the NF site than at the plantation site, although significant effects were only found in the organic horizon (average values of 0.42 and 0.25 in the plantation and NF, respectively) (Table 3). The statistical analyses also showed that the effect of forest type on the native proteolysis was dependent on the soil horizon and sampling season (Table 2). Moreover, the total phenol content was strongly and negatively correlated with the native proteolysis during the growing season across all soil horizons and forest types ($R^2 = 0.69, P < 0.001$; Fig. 3), while no marked correlation between the proteolysis rates and total phenol content was observed for the non-growing season ($R^2 = 0.04, P > 0.05$; Fig. 3).

### 3.2. Net fluxes of amino acid and inorganic N in the soil

The forest type, soil horizon, sampling date, and their interactions all had significant effects on the net amino acid N production and net N mineralization rates (Table 2). During the growing season, net consumption of amino acid N occurred in both forest types and soil horizons, and the rates of net consumption were greater in the planation than in the NF. In contrast, during the non-growing season, amino acid N still showed a net consumption tendency in the NF, while there was a net production in the planation (Fig. 2a). Regardless of the seasonal pattern and forest type, the rates of amino acid production and consumption were significantly faster in the organic horizon than in the mineral soil horizon (P < 0.05, Fig. 2a). The average rates of net N mineralization during the growing season were much greater in the NF (3.40 and 1.45 on average) than in the plantation (1.44 and 0.49 on average) (F = 2.60, P = 0.019; F = 1.42, P = 0.003) in the organic and the mineral soil horizons, respectively. In comparison, the net N mineralization rates were not significantly different between the NF and plantation across the soil horizons during the non-growing season (Fig. 2b). In addition, the average rate of net N mineralization in both soil horizons in the NF decreased significantly from the growing season to the non-growing season when compared with the plantation (Fig. 2b).

### Table 2

The results of the repeated measures ANOVA showing the P-values for soil amino acid (AA), dissolved inorganic N (DIN), AA/DIN, DON, MBN, net mineralization, net amino acid production, and native proteolysis in different forest types (F), soil horizons (H), and sampling dates (D). P-values smaller than 0.05 are in bold.

| Factor     | AA     | DIN     | AA/DIN | DON     | Net mineralization | Net AA production | Native proteolysis |
|------------|--------|---------|--------|---------|--------------------|-------------------|-------------------|
| Forest type| 0.046  | < 0.001 | < 0.001| < 0.001 | < 0.001            | 0.003             | 0.035             |
| Horizon    | < 0.001| < 0.001 | 0.217  | < 0.001 | < 0.001            | 0.001             | < 0.001           |
| Date       | < 0.001| < 0.001 | < 0.001| < 0.001 | < 0.001            | < 0.001           | < 0.001           |
| F × H      | 0.021  | 0.510   | 0.082  | 0.001   | 0.001              | 0.002             | 0.102             |
| F × D      | < 0.001| < 0.001 | 0.005  | 0.009   | < 0.001            | < 0.001           | < 0.001           |
| H × D      | 0.009  | < 0.001 | 0.668  | 0.073   | 0.001              | 0.001             | 0.002             |
| F × H × D  | < 0.001| 0.543   | 0.044  | 0.013   | 0.022              | 0.003             | < 0.001           |
4. Discussion

The organic N (amino acids in particular) is increasingly recognized as a critical component of the terrestrial N cycle (Schimel and Bennett, 2004; Werdin-Pfisterer et al., 2009; Quinta et al., 2015). The knowledge of the variations of soil amino acid (as an important N pool) dynamics following the conversion of NF to plantation forest, may provide a novel insights for understanding the processes of N cycling. In this study, two adjacent sites were selected that were located at the same position on slopes and had the same vegetation coverage before reforestation with dragon spruce plantation. In addition, the soils at both sites developed from the same parent material. Therefore, the differences of the N pools and soil N transformations observed between the two sites were assumed to reflect the ecological consequences of the land-use change from NFs to plantations.

4.1. Differences of soil amino acid cycling between the plantation and NF

In contrast with our previous hypothesis, the sizes of the DON and amino acid pools in the plantation were generally smaller than those in the NF in all soil horizons during the growing season (Fig. 1a and b), which was in agreement with the changing tendency of DIN observed in this study (Fig. 1c) and in our previous study (Xu et al., 2010). This finding indicated that the availability of N (both inorganic and organic N) in the plantation soil decreased markedly following the conversion from NF. Land-use changes accompanied by shifts in vegetation coverage may directly impact the quality and quantity of organic matter derived from leaf litter and root exudates (Burton et al., 2007a), consequently influencing the availability and cycling of soil N (Patra et al., 2006; Ste-Marie and Houle, 2006). High quality litter is often characterized by lower C: N and lignin: N ratios, and can decompose faster, resulting in rapid C and N mineralization rates compared with low quality litter (Sanchez, 2001; Phillips et al., 2013). Our previous studies have demonstrated that the leaf litter from the dominant tree species in the spruce plantation has a higher C: N ratios than the litter from the multi-species NF (Xu et al., 2012a, 2015). The difference in litter quality is also reflected in the soil C: N ratio (Table 1). Soils with a wide C: N ratio typically have low net mineralization rates because microbes are more N limited than C limited (Finzi and Berthrong, 2005; Mooshammer et al., 2014), which contributes to the more restrained inorganic N availability in the plantation soils. However, amino acids in the plantation soils seemed not to be immobilized by soil microbes for their N content, since not much of them was retained in the microbial biomass (Fig. S1), which is inconsistent with the findings of previous studies (Finzi and Berthrong, 2005; Berthrong and Finzi, 2006). In contrast, the amino acids taken up by the microbes in the NF soils with...
narrow C:N ratios were likely assimilated for their C content, resulting in the increase of NH$_4^+$ to the soil (that is, a high N mineralization rate) (Wanek et al., 2010). The difference in the soil amino acid pools between the plantation and NF also potentially resulted from a discrepancy in the chemical composition of the leaf litter (an indicator of litter quality) from the dominant tree species in the two types of forest. Generally, the leaf litter from coniferous tree species contains more recalcitrant compounds, such as hydrophobic aromatics, while the leaf litter from hardwood tree species contains more labile and hydrophilic compounds (e.g., sugar and fatty acids) (Hongve et al., 2000; Kaiser et al., 2001; Kalbitz et al., 2003; Shirato and Yokozawa, 2006). Polyphenols are recognized as an important class of plant secondary metabolites that are involved in regulating the chemical composition of the leaf litter from coniferous tree species.

The rates of native and potential proteolysis (µg N g$^{-1}$ dry soil h$^{-1}$) and their ratio in the organic horizon and the top 15-cm of the mineral soil in the plantation and the natural forest.

| Months   | May     | July    | September | December | March    |
|----------|---------|---------|-----------|----------|----------|
|          | P       | N       | P         | N        | P        |
| Organic horizon |         |         |           |          |          |
| Native proteolysis | 0.40 (0.04)b | 1.44 (0.20)a | 0.48 (0.09)b | 0.80 (0.04)a | 0.52 (0.08) | 0.60 (0.05) | 0.36 (0.02)a | 0.18 (0.02)b | 0.42 (0.07)a | 0.15 (0.03)b |
| Potential proteolysis | 1.05 (0.36)b | 4.35 (0.56)a | 1.26 (0.40)b | 3.68 (0.44)a | 1.08 (0.04)b | 2.22 (0.34)a | 0.80 (0.05) | 0.75 (0.06) | 1.10 (0.10) | 0.78 (0.04) |
| Native potential | 0.38 (0.05) | 0.33 (0.03) | 0.41 (0.05)a | 0.22 (0.02)b | 0.48 (0.05)a | 0.27 (0.02)b | 0.45 (0.01)a | 0.24 (0.02)b | 0.41 (0.08)a | 0.19 (0.03)b |
| Mineral soil horizon |         |         |           |          |          |
| Native proteolysis | 0.24 (0.04) | 0.28 (0.02) | 0.20 (0.01) | 0.28 (0.02) | 0.14 (0.04) | 0.22 (0.16) | 0.11 (0.01) | 0.10 (0.02) | 0.11 (0.02) | 0.07 (0.03) |
| Potential proteolysis | 1.52 (0.34) | 1.95 (0.08) | 0.76 (0.16)a | 1.47 (0.16)a | 1.32 (0.15) | 2.12 (0.37) | 1.50 (0.06) | 1.89 (0.02) | 1.19 (0.08) | 1.18 (0.16) |
| Native potential | 0.18 (0.02) | 0.14 (0.02) | 0.26 (0.04) | 0.19 (0.03) | 0.02 (0.01) | 0.02 (0.01) | 0.07 (0.01) | 0.06 (0.01) | 0.09 (0.02) | 0.05 (0.02) |

Each value is the mean ($\pm$ SE) of samples from four replicate plots per site. Followed letters within a row denote significant differences between two forest types at $P<0.05$. P: plantation; N: Natural forest.
soil N cycling due to their ability to complex with protein (Talbot and Finzi, 2008). Polyphenol-protein complexes (PPC), which are resistant to most decomposing organisms, are created when polyphenols bind with proteins in the soil (Hattenschwiler and Vitousek, 2000). Therefore, the formation of PPCs can decrease the availability of the protein substrate in the soil and subsequently constrain the proteolysis rate (Northup et al., 1995; Berthrong and Finzi, 2006), which is considered to be the most important process in soil amino acid production (Schimel and Bennett, 2004). In this study, the rates of gross proteolysis in the plantation were generally lower than those in the NF during the growing season (Table 3) and were stimulated less by the addition of casein in the NF, which indicated that the indigenous protein substrate in the plantation soils can be consumed more slowly. The limited process of soil amino acid production in the plantation was also evidenced by the significantly negative correlation between the proteolysis rate and total phenol content during the growing season and the much higher extractable total phenol concentrations in the organic horizon in the plantation soil (Fig. S4; Fig. 3).

Collectively, the decrease in the availabilities of N in both the amino acids and DIN pools in the plantation following its conversion from NF suggests that distinct nutrient economies (i.e., nutrient use strategies) may exist between the two forest types as a result of differences in the quality of litter input. We assumed that the plants in the plantation forest prefer to increase soil organic N retention by creating stable complexes (e.g., PPC) rather than by stimulating the production of amino acids to compensate for the low DIN availability during the growing season after being converted from NF (Fig. 4). The occurrence of a ‘preserved’ nutrient economy in the plantation may reflect its adaption to the soil environment (Wurzburger and Hendrick, 2009). Therefore, although the plants in the plantation influence soil N dynamics due to the quality of their litter, whether the plants themselves are favoured by their influences on the soil environment remains unclear and warrants further study.

4.2. Seasonal variations of soil amino acid cycling

Seasonal changes had significant effects on the soil DON and amino acid pools (Table 2), with relatively higher pools of soil DON and amino acids observed in the plantation than in the NF during the non-growing season (Fig. 1a and b). These results agree with the previous studies, which found that pools of soil amino acid and soil organic N pools fluctuate widely with season (Weintraub and Schimel, 2005; Farrell et al., 2011; Wang et al., 2013). The variations in temperature resulting from seasonal shifts may explain the inconsistent results observed for the soil amino acid pools and the fluxes between the growing season and non-growing season because the soil temperature likely alters the soil amino acid pool by influencing the balance between productive processes (e.g., proteolysis) and consumptive processes (i.e., microbial assimilation, N mineralization, and sorption to soil colloids) (Wang et al., 2013). The decrease of the soil amino acid concentrations in the NF during the non-growing season may result from the relatively lower amino acid production than consumption. However, during the non-growing season, the productive processes in the plantation exceed the consumptive processes, resulting in the accumulation of more amino acids in the soil (Fig. 2a).

It was noteworthy that the relatively higher amino acid production in the plantation during the non-growing season did not result in an increase in the microbial biomass N (MBN) as well (Fig. 51). Meanwhile, the significantly greater ratio of amino acid N to MBN in the plantation than in NF (Fig. S2) also suggested that the soil microbes in the plantation with a wide C : N ratio were not prone to assimilate amino acids for their N content. This observation agrees with previous studies and suggests that the uptake of low-weight organic N compounds may occur for C uptake rather than for N uptake (Jones and Murphy, 2007; Farrell et al., 2014). Therefore, further investigations of the behaviours of amino acids in soils and their assimilation by microbes using isotope labelling techniques should be conducted to verify this explanation.
Considering the severe decrease in DIN concentration and the relatively higher amino acid to DIN ratio in the plantation soil during the non-growing season (Fig. 1c), we propose that the ‘preserved’ nutrient economy of the plantation assumed in the growing season is likely to be changed by seasonal temperature variations. When the availability of DIN in the soil decreased to the threshold concentration that could no longer meet the fundamental N supply due to extremely limited soil N mineralization under cold conditions, the plants in the plantation would be forced to increase their amino acid production by releasing the organic N preserved in the complex organic substrates (Fig. 4). This assumption is consistent with the previously hypothesized conceptual models of terrestrial N cycling that have viewed amino acids as a substantial contributor to plant-available pools of N only in soils with very low nutrients (Schimel and Bennett, 2004). Unfortunately, there is no quantitative information exists about the soil organic N complexes in these two forest types due to methodological difficulties. Further examination of the potential availability of N derived from complex organic N compounds and their response to environmental changes would be a worthwhile focus of future studies.

4.3. Horizontal variations of soil amino acid cycling

The concentrations and net production (or consumption) rates of amino acids were significantly higher in the organic horizons than in the mineral soil horizons (Figs. 1 and 2), which was consistent with previous studies (Finzi and Schlesinger, 2003; Berthrong and Finzi, 2006). Such a result was presumably manifested in the more labile components of the organic matter in the organic horizon than in the mineral soil horizon (Schlesinger, 1997). The leaf litter in the organic horizons contains large quantities of organic N compounds, including proteins, peptides, amino acids, amino sugars, and nucleic acids, all of which can be sources of amino acids after they are released into the soil (Finzi and Schlesinger, 2003). The larger belowground organic matter inputs derived from greater root activity (e.g., root exudation, root turnover) in the top soil layer may also result in a greater amino acid pool in the organic horizon, as it has been suggested that most functional fine roots are concentrated in the topsoil (Chen et al., 2004). In addition, the much higher microbial biomass and subsequently greater turnover of microbial cells, as well as the larger production of extracellular enzymes, could also explain the higher overall concentrations and fluxes of amino acids in the organic horizons than in the mineral soil horizons (Fig. S1 and S3).

4.4. Considerations of the importance of amino acids

An appreciable soil amino acid pool size is necessary for amino acids to serve as an important source of N in terrestrial ecosystems (Jones et al., 2005a). Thus, to assess the importance of amino acids in plant N nutrition in subalpine coniferous forest ecosystems, we collected values on the amino acid pool and the ratio of the amino acid pool to the DIN pool from previous studies (Table 4). Because different agents for extracting amino acids largely result in different amino acid concentrations (Jones et al., 2005a; Berthrong and Finzi, 2006; Warren and Taranto, 2010), studies that used KCl extraction (1 M or 2 M) were considered in this comparison. In this study, the amino acid N concentrations ranged from 1.23 to 11.88 μg N g⁻¹ dry soil, which was within the range reported in the literature (Table 4). However, the range of the amino acid: DIN ratio was much smaller than that observed in other ecosystems, especially those in which amino acids are considered to be a critical N source for plants (Finzi and Schlesinger, 2003; Berthrong and Finzi, 2006; Warren and Adams, 2007). A high proportion of the amino acids among the forms of N available to plants may enhance the uptake preference of plants for amino acids (Bardgett et al., 2003; Jones et al., 2005a). Hence, the low amino acid: DIN ratio in this study suggests that DIN

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**Fig. 4.** The assumed conceptual model of the nutrient economy of the plantation differs from that of the natural forest during the growing and non-growing seasons. In the plantation (a), the availabilities of both dissolved inorganic N (DIN) and amino acid N (AA-N) are remarkably lower during the growing season as a result of low-quality litter input and slow C and N mineralization rates, which form a ‘preserved’ nutrient economy with increased soil organic N retention compared with the natural forest (b). During the non-growing season, the plantation is hypothesized to have greater amino acid production from the release of organic N preserved in complex organic substrates due to the severely limited DIN availability compared with the natural forest. The size of the boxes indicates the relative importance of the pools. The size of the arrows indicates the relative intensity of every process. The dotted arrow indicates a negative effect.
remains the dominant form of available N for plants. Nevertheless, considering the relatively high amino acid: DIN ratio (70.5% in the organic horizon and 60% in the mineral soil horizon) in the plantation soil during the non-growing season (Fig. 1d), we presumed that amino acids may become the primary source of N compared with the dramatically reduced DIN concentration in the non-growing season, as the amino acid pool was found to be generally larger than any form of the DIN pools (i.e., NH₄⁻-N and NO₃⁻-N) in the plantation soil during the non-growing season (data not shown). Therefore, we can make an extensive assumption that the plants in the plantation during the non-growing season may modify their strategies for acquiring N following the changed relative dominance between the DIN and amino acids in the soils. We propose that further studies are needed to better understand the competition for N sources between plants and microbes and to quantitatively estimate the direct contributions of both amino acid N and DIN to the N nutrition of plants in a wide range of forest ecosystems.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.11.002

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