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Spatial variations in larch needle and soil $\delta^{15}\text{N}$ at a forest-grassland boundary in northern Mongolia

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Spatial variations in larch needle and soil $\delta^{15}\text{N}$ at a forest-grassland boundary in northern Mongolia

The spatial patterns of plant and soil $\delta^{15}\text{N}$ and associated processes in the N cycle were investigated at a forest-grassland boundary in northern Mongolia. Needles of *Larix sibirica* Ledeb. and soils collected from two study areas were analysed to calculate the differences in $\delta^{15}\text{N}$ between needle and soil ($\Delta\delta^{15}\text{N}$). $\Delta\delta^{15}\text{N}$ showed a clear variation, ranging from -8‰ in the forest to -2‰ in the grassland boundary, and corresponded to the accumulation of organic layer. In the forest, the separation of available N produced in the soil with ^{15}N -depleted N uptake by larch and ^{15}N -enriched N immobilization by microorganisms was proposed to cause large $\Delta\delta^{15}\text{N}$, whereas in the grassland boundary, small $\Delta\delta^{15}\text{N}$ was explained by the transport of the most available N into larch. The divergence of available N between larch and microorganisms in the soil, and the accumulation of diverged N in the organic layer control the variation in $\Delta\delta^{15}\text{N}$.

Keywords: nitrogen isotope ratio; larch; soil; forest-grassland boundary; Mongolia; organic layer

1. Introduction

Nitrogen (N) is one of the key elements in biological processes. The N cycle in plant-soil system includes the decomposition of soil organic matter, the uptake of biologically available N (e.g. NH_4^+ and NO_3^-) by plants and soil microorganisms, and the return of litterfall to the soil as an internal processes, as well the N input as atmospheric deposition and/or biological N_2 fixation, and the loss by leaching and/or gaseous emission as external processes [1]. All these processes result in variations in plant and soil $\delta^{15}\text{N}$, which are caused by differences in the $\delta^{15}\text{N}$ of sources for plants [2], N loss, such as nitrate (NO_3^-) leaching [3] or gaseous emission (N_2O , N_2) via nitrification and denitrification [4], decomposition processes, such as mineralization and nitrification[5], mycorrhizal fungi [6], and physiological processes within the plant [7]. As a result, plant and soil $\delta^{15}\text{N}$ serve as useful indicators of the N cycle in the plant-soil system [8].

The $\delta^{15}\text{N}$ values of plant and soil in forest ecosystem have been investigated and various characteristics have been reported [9]. The plant $\delta^{15}\text{N}$ values of co-occurring species in boreal forest showed differences among species, which were attributed to the different N source for each species in terms of N form and/or the depth of N uptake [10].

On the other hand, it has been shown that soil $\delta^{15}\text{N}$ generally increases with depth, from organic layer which is typically observed in forest, to mineral soil [11]. Previous studies also showed that the organic layer contributes as significant nitrogen source for trees [12, 13]. Furthermore, recent global syntheses in $\delta^{15}\text{N}$ values have shown the spatial trends in $\delta^{15}\text{N}$ values of plant and soil along climate gradient [14–16].

The difference between plant and soil $\delta^{15}\text{N}$ ($\Delta\delta^{15}\text{N} = \text{plant } \delta^{15}\text{N} - \text{soil } \delta^{15}\text{N}$), which is an apparent enrichment factor, allows the comparison of different sites by normalizing the spatial heterogeneity in soil $\delta^{15}\text{N}$ [17]. $\Delta\delta^{15}\text{N}$ has been widely applied, and variations in $\Delta\delta^{15}\text{N}$ have been attributed to various causes such as differences in N forms that plant uptake (DON , NH_4^+ , or NO_3^-) [17–22] and changes in $\delta^{15}\text{N}$ of each N form affected by nitrification [17,18,23,24]. Mycorrhizal fungi have also been reported to affect $\delta^{15}\text{N}$ through ^{15}N -enrichment in the fungal body [25], although the degree of enrichment is still controversial [26]. Not only these internal processes within the plant-soil system, but also atmospheric N deposition that serve as the N source for plants [27], has been reported to cause variations in $\Delta\delta^{15}\text{N}$. Additionally, the strategy of field observations often complicates the interpretation of $\Delta\delta^{15}\text{N}$. Previous studies set wide spatial scales (region, country, or globe) along the gradient of specific environmental variables (climate, topography, soil age, and parent material), which hampered the interpretation

of $\Delta\delta^{15}\text{N}$, due to the needed comparison among different plant species with different degrees of isotopic fractionation within the plant body [7], and the co-existence of variables that affect plant and soil $\delta^{15}\text{N}$ [14].

In this study, we investigated a forest-grassland boundary (ecotone) ecosystem in northern Mongolia, in which vegetation changes from Siberian larch (*Larix sibirica* Ledeb.) forest to temperate grassland within a very short distance of a few kilometres. Investigation of this ecotone offered the following advantages: (1) existence of a single plant species (*L. sibirica* Ledeb.) that allowed comparisons along the forest-grassland gradient, and (2) exclusion of variables such as climate, soil age, and parent material which were considered similar at all sites within this region. Therefore, this study aimed to clarify the spatial patterns and underlying mechanisms that control $\Delta\delta^{15}\text{N}$ which arise only from the differences between forest and grassland ecosystems.

2. Materials and methods

2.1. Site description

Two study areas, Terelj (TR) and Mongonmorit (MM), were chosen for sampling at a forest-grassland boundary in northern Mongolia (Figure 1(a)). Information on site

locations and positions is presented in Table 1. The forest consists of Siberian larch (*Larix sibirica* Ledeb.) and white birch (*Betula platyphylla* Sukach.) in some places [28,29]. This region corresponds to the southern boundary of permafrost [29], which coincides with the distribution of boreal forest. The forest dominates the north-facing slopes, whereas temperate grassland dominates the south-facing slopes, dry valleys, and flat plains [29]. The soil in this region is cryosol, and the climate is cold continental climate with dry winters, according to Köppen-Geiger climate classification [30]. The mean annual temperature (MAT) is -3.6°C in TR area and -2.9°C in MM area, and average temperature from May to September is 10.4°C in TR area and 11.2°C in MM area. The mean annual precipitation (MAP) is 353 mm in TR area and 272 mm in MM area, whereas 90% of precipitation occurs during the growing season of larch trees (May to September) [28].

In this study, samplings were conducted along the forest-grassland gradient, and the sampling sites were classified as forest or boundary as described by Tuhkanen [31]. Forest site was defined as the site in the continuous forest, whereas boundary site was defined as the site between the edge of continuous forest and grassland. In TR area, sampling was conducted along a transect from the north-facing slope to the south-facing slope over a valley at 11 sites (TR1n to TR2s) (Figure 1(b)), whereas in MM area, two

transects were set on the south-facing slope and southwest-facing slope and sampling was conducted at 10 sites (MM1sw to MM7s) (Figure 1(c)). Among the 21 sites in total, 6 sites in TR area (TR1n to TR6n) and 6 sites in MM area (MM1sw to MM3s) were forest sites, and the rest were boundary sites, except for patchy forest (TR1s) and grassland site with no trees (TR2s) in TR area. Both TR and MM areas included forest-grassland gradient, however MM area had more boundary sites with sparser tree distribution on south-facing slope. Observations at those sites in these two areas covered all range of forest-grassland gradient, and also wide range of conditions regarding organic layer accumulation (litter, fermentation, and humus), which was relatively rich in forest sites (mor-type) to poor in boundary sites (mull-type) [32].

[Figure 1 and Table 1 near here]

2.2. Sampling

2.2.1. Foliar samples

Larch needles were collected during the growing season (May to August) from 2004 to 2012. Needles from three to four branches at a height of 1 to 5 m were taken from each tree. More than three trees were usually sampled at each site, but only one or two trees in boundary sites due to their limited number. Needles were also collected from several

trees at three sites in TR area (TR3n, TR6n, and TR1s) and two sites in MM area (MM2s and MM1sw) during the growing season of 2004 and 2005 to evaluate temporal variations. Needle samples were oven-dried at 60°C, milled, and wrapped in tin cups for analysis.

2.2.2. *Soil samples*

Soil samples were collected at the same sites as needle samples. A small pit (0.6 m × 0.6 m × 0.6 m deep) was made, and one to three cores (1.5 cm diameter, 4.5 cm length) of bulk soil were collected from the cross section of the pit every 10 cm from 0 cm (the top of mineral soil) down to 50 cm depth or until a rock appeared. The organic layer was also sampled by collecting the organic matter above the mineral soil. The fresh soil samples were sieved with a 2 mm mesh to remove gravel and living roots, oven-dried at 105°C for more than 24 h, and used for isotope analysis. Fresh soil samples collected in 2012 were also used for KCl-extractable N (DON, NH₄⁺, and NO₃⁻) analysis.

2.3. *Analysis*

2.3.1. *C/N isotopic ratio and concentrations*

The δ¹³C, δ¹⁵N, and C, N concentrations were analysed using Conflo system with DELTA V Plus and FlashEA 1112 (Thermo Fisher Scientific) at the Graduate School of

Environmental Science, Hokkaido University, Japan. The isotope ratio was expressed using the δ notation:

$$\delta^{15}\text{N} \text{ (or } \delta^{13}\text{C}) = \left(\frac{R_{\text{sample}}}{R_{\text{std}}} - 1 \right) \times 1000 \text{ (‰)}$$

where R_{sample} is the isotope ratio ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) of a sample, and R_{std} is the isotope ratio ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) of Vienna Pee Dee Belemnite (VPDB) or atmospheric N_2 for C and N. Analytical errors were at 0.2‰ for $\delta^{13}\text{C}$, 0.3‰ for $\delta^{15}\text{N}$, 0.5% for the bulk C concentration, and 0.1% for the bulk N concentration.

2.3.2. *KCl-extractable N*

Soil N pools (DON , NH_4^+ , and NO_3^-) were extracted from 4 g of fresh soil with 40 ml of 2M KCl after 1 h of shaking and filtration. The extracts were kept in coolers, transported to the laboratory, and stored in a freezer until analysis. The concentration of NO_3^- , NH_4^+ , and total dissolved N (TDN) was analysed colorimetrically using a continuous flow nutrient analyser (QuAatro; BRAN+LUEBBE, Hamburg, Germany). Then, the concentration of dissolved organic N (DON) was calculated by subtracting total inorganic N (NO_3^- and NH_4^+) from TDN. The concentration of nitrite (NO_2^-) was also analysed, but not detected in any samples.

2.3.3. *Calculation of average values at each site*

To obtain needle N concentration, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ at each site, data were first averaged

for all trees in each sampling period excluding those obtained in May, and then averaged for all sampling periods at each site. Needle $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and N concentrations observed on all sampling dates at each site are shown in Figure S1. For bulk soil $\delta^{15}\text{N}$ at each site, the weighted mean of $\delta^{15}\text{N}$ in the 0-20 cm soil layer was calculated from all the available data. The mean N concentration and the C/N ratio in the 0-20 cm soil layer at each site were also calculated from all the available data. $\Delta\delta^{15}\text{N}$ was calculated as (needle $\delta^{15}\text{N}$ – soil $\delta^{15}\text{N}$) at each site.

2.3.4. *Isotope mass balance on available N*

To interpret the processes reflected in the variation of $\Delta\delta^{15}\text{N}$, we applied the mass balance of biologically available N. Assuming that the available N pool is at a steady state, the following equations are established:

$$F_{\text{input}} + F_{\text{a}} = F_{\text{p}} + F_{\text{m}} + F_{\text{leach}} + F_{\text{gas}} \quad (1)$$

and for $\delta^{15}\text{N}$,

$$F_{\text{input}} \times \delta_{\text{input}} + F_{\text{a}} \times \delta_{\text{a}} = F_{\text{p}} \times \delta_{\text{p}} + F_{\text{m}} \times \delta_{\text{m}} + F_{\text{leach}} \times \delta_{\text{leach}} + F_{\text{gas}} \times \delta_{\text{gas}} \quad (2)$$

where F_{input} is the N derived from atmospheric N deposition and biological N_2 fixation, and F_{a} is the N produced in the soil through the decomposition of soil organic matter. F_{p} and F_{m} are the fluxes of available N taken up by plants and immobilized by soil microorganisms, respectively. F_{leach} and F_{gas} are the N lost due to leaching and gaseous

emission, respectively. The values of δ_{input} , δ_a , δ_p , δ_m , δ_{leach} , and δ_{gas} are the $\delta^{15}\text{N}$ of input, produced N from soil organic matter, plant uptake, immobilization by soil microorganisms, leaching, and gaseous emission of available N, respectively. We assumed that larch was the only plant species (i.e. $\delta_p = \text{needle } \delta^{15}\text{N}$), and that the $\delta^{15}\text{N}$ of available N produced in the soil was the same as that of the soil (i.e. $\delta_a = \text{soil } \delta^{15}\text{N}$). Generally, available N produced in the soil has similar $\delta^{15}\text{N}$ to that of soil organic matter, if all produced N remains in the N pool without any fractionated loss [33]. The schematic representation of each process is shown in Figure S2.

2.3.5. *Statistical analysis*

The temporal variation of tree components was evaluated by Wilcoxon signed-rank test at $p < 0.05$ (two-tailed). The spatial relationships between the variables were evaluated by Spearman's rank correlation coefficient at $p < 0.05$ (two-tailed).

3. Results

3.1. *Temporal variations in $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and N concentration of larch needles*

Temporal variations in needle N concentration, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ of nine individual trees were observed during the growing season of 2004 and in July 2005 at three sites in TR

area (TR3n, TR6n, and TR1s) (Figure 2). Needle N concentrations were significantly higher in May 2004 than the following months in 2004 and July in 2005 ($p < 0.05$) (Figure 2(a)). Temporal changes or inter-annual variations were not observed for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (Figure 2(b) and 2(c)). The same temporal variations were also observed in MM area (data not shown). When the data collected in May were excluded, the standard deviations of individual trees during the observed period were 0.4%, 0.5‰, and 0.9‰ for N concentration, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$, respectively.

[Figure 2 near here]

3.2. Vertical profile of larch needle, organic layer, and bulk soil $\delta^{15}\text{N}$

The $\delta^{15}\text{N}$ value increased vertically from needle, organic layer to soil with depth, and also differed between forest and boundary sites, as seen in the example for TR area (Figure 3). At the forest site (TR1n) and the boundary site (TR7n), soil $\delta^{15}\text{N}$ increased up to 20-30 cm soil depth. This pattern in soil $\delta^{15}\text{N}$ was common at all sites in TR and MM areas. However, changes in the $\delta^{15}\text{N}$ of needle, organic layer, and soil were different between the two sites; a gradual increase in $\delta^{15}\text{N}$ was observed at TR1n, whereas a slight increase was observed at TR7n. Similarly, in MM area, a gradual increase in the $\delta^{15}\text{N}$ from needle, organic layer to soil was observed at the forest site

(MM2sw), whereas slight increase was observed at the boundary sites, especially at MM5s, MM6s and MM7s (data not shown). [Figure 3 near here]

3.3. *Spatial variations along the forest-grassland gradient*

Characteristic spatial patterns in $\delta^{15}\text{N}$ values, needle $\delta^{13}\text{C}$, and needle N concentration were observed along the forest-grassland gradient in TR and MM areas (Figure 4). In TR area, needle $\delta^{15}\text{N}$ increased gradually from -3.9‰ at the forest site (TR1n) to +3.3‰ at the boundary site (TR8n) on the north-facing slope, whereas on the south-facing slope, needle $\delta^{15}\text{N}$ at TR1s in the patch forest (+1.2‰) was similar to that at TR6n and slightly lower than that at TR8n. In contrast to needle $\delta^{15}\text{N}$, soil $\delta^{15}\text{N}$ slightly varied from +2.9‰ at TR2n to +5.3‰ at TR7n on the north-facing slope, whereas it was higher on the south-facing slope at TR1s (+5.8‰) and TR2s (+7.4‰). The values of $\Delta\delta^{15}\text{N}$ showed the same pattern as needle $\delta^{15}\text{N}$ on the north-facing slope (Figure 4(b)); $\Delta\delta^{15}\text{N}$ was larger in the forest (-8‰ at TR1n) and smaller in the boundary (-3‰ at TR7n) on the north-facing slope, whereas $\Delta\delta^{15}\text{N}$ at TR1s on the south-facing slope (-5‰) was similar to that at TR5n. On the north-facing slope, needle $\delta^{13}\text{C}$ increased from -27.8‰ to -26.2‰ from the forest site (TR1n) to the boundary site (TR8n), and needle N concentration also increased from 2.0% to 2.7%, respectively. On the

252 south-facing slope, needle $\delta^{13}\text{C}$ at TR1s (-26.0‰) was as high as that at TR8n, and
253 needle N concentration at TR1s (2.4%) was similar to that at TR7n (Figure 4(c)).
254 KCl-extractable N (DON , NH_4^+ , and NO_3^-) pools in the 0-20 cm soil layer were
255 observed at two forest sites on the north-facing slope (TR2n and TR5n) in August 2012
256 (Table S1). At both sites, DON was more than one-order of magnitude larger than NH_4^+
257 and NO_3^- pools. Additionally, NH_4^+ pool was larger than NO_3^- pool with lower NO_3^- to
258 NH_4^+ ratios (< 0.2).

259 In MM area, needle $\delta^{15}\text{N}$ values were higher on the south-facing slope (+2.7‰ to
260 +4.3‰) where most sites were located in the boundary, than those on the
261 southwest-facing slope (+0.34‰ to +2.0‰) where all sites were located in the forest
262 (Figure 4(d)). In contrast, soil $\delta^{15}\text{N}$ slightly varied (+5.1 to +6.8‰) in MM area. The
263 $\Delta\delta^{15}\text{N}$ slightly changed from -4‰ to -2‰ at the sites on the south-facing slope, which
264 were smaller than those on the southwest-facing slope (-6‰) (Figure 4(e)). Needle $\delta^{13}\text{C}$
265 and needle N concentration were higher at the sites on the south-facing slope (-26.2‰
266 to -24.6‰) than those on the southwest-facing slope (-27.5‰ to -26.2‰) (Figure 4(f)).
267 KCl-extractable N (DON , NH_4^+ , and NO_3^-) pools in the 0-20 cm soil layer were
268 observed at one forest site (MM3s) and three boundary sites (MM4s, MM5s, and
269 MM6s) on the south-facing slope in August 2012 (Table S1). The DON pool was more

than one-order of magnitude larger than NH_4^+ and NO_3^- pools at all sites, just as observed in TR area. However, unlike forest sites in TR area, NO_3^- pool size was similar to that of NH_4^+ at boundary sites (MM4s, MM5s, and MM6s), namely high NO_3^- to NH_4^+ ratios (close to 1).

[Figure 4 near here]

3.4. Relationships between $\delta^{15}\text{N}$ values and other parameters

Significant correlations between different variables of all the sites in TR and MM areas were observed (Figure 5). The correlation was observed between needle $\delta^{13}\text{C}$ and needle $\delta^{15}\text{N}$ ($r_s = 0.877$) (Figure 5(a)), soil $\delta^{15}\text{N}$ and needle $\delta^{15}\text{N}$ ($r_s = 0.718$) (Figure 5(b)), needle N concentration and $\Delta\delta^{15}\text{N}$ ($r_s = 0.591$) (Figure 5(c)), and C/N ratio of bulk soil and $\Delta\delta^{15}\text{N}$ ($r_s = -0.541$) (Figure 5(d)). Data in MM area showed small $\Delta\delta^{15}\text{N}$ (-5‰ to -2‰), high needle $\delta^{15}\text{N}$ (+0.34‰ to +4.3‰), high soil $\delta^{15}\text{N}$ (+5.1‰ to +6.8‰), whereas in TR area showed larger variations in those values.

[Figure 5 near here]

4. Discussion

In this study, we observed large spatial variations in needle $\delta^{15}\text{N}$ and the difference between needle and soil $\delta^{15}\text{N}$ ($\Delta\delta^{15}\text{N}$) at each area, whereas relatively small variation in soil $\delta^{15}\text{N}$ (Figure 5(b)). The range of $\Delta\delta^{15}\text{N}$ observed in this study (6‰) (Figure 5(b)) accounts for a half of the global latitudinal variation [14], despite the same plant species (*L. sibirica* Ledeb.), soil age, land use history, and climate. The spatial variation in $\Delta\delta^{15}\text{N}$ indicates that the N cycle changes along the forest-grassland gradient. Here, the isotope mass balance of available N in the soil was applied to understand the mechanism of variation in $\Delta\delta^{15}\text{N}$.

First, we simplify the mass balance equations by deleting some terms in the equations (1) and (2). Assuming a steady state condition of plant and soil N pools in the system, the flux of input equals to that of loss ($F_{\text{input}} = F_{\text{leach}} + F_{\text{gas}}$), and then the equations (1) and (2) can be simplified as follows:

$$F_a = F_p + F_m \quad (3)$$

$$\delta_a = \delta_s = f \times \delta_p + (1-f) \times \delta_m \quad (4)$$

where δ_s is soil $\delta^{15}\text{N}$, and f is the fraction of available N which is transported to the plants ($f = F_p/F_a$). Namely, under a steady state condition, available N produced in the soil is transported to plants and microorganisms. We assume that larch is the only plant species (i.e. $\delta_p = \text{needle } \delta^{15}\text{N}$), and also $\delta^{15}\text{N}$ of available N produced in the soil is the

same as that of the soil (i.e. $\delta_a = \delta_s$) as described in the Materials and methods. We focus on the 0-20 cm soil layer to apply this mass balance, since all sites showed significant increase in soil $\delta^{15}\text{N}$ at this layer (Figure 3), in which an active decomposition of soil organic matter occurs [33].

From these equations, two processes could explain the small difference in $\delta^{15}\text{N}$ between larch needle and soil ($\Delta\delta^{15}\text{N} \cong 0$) observed at boundary sites (Figure 5(b)): (1) available N is transported to larch and/or microorganisms without significant fractionation ($\delta_a = \delta_p = \delta_m$), or (2) almost all available N is transported to larch ($f \cong 1$). However, N immobilization by soil microorganisms (F_m) might not be a suitable explanation, because it is generally accepted that immobilization is associated with significant isotope fractionation, causing higher $\delta^{15}\text{N}$ in microorganisms than that of substrate organic N [34,35]. Therefore, it is reasonable to consider that the observed small $\Delta\delta^{15}\text{N}$ at boundary sites show that available N produced in the soil is mostly transported to larch without significant fractionation. In contrast, large difference in $\delta^{15}\text{N}$ between larch needle and soil ($\Delta\delta^{15}\text{N} < 0$) in forest sites (Figure 5(b)) suggests that the ^{15}N -depleted part of available N is transported to larch, whereas the ^{15}N -enriched part of available N is immobilized in soil microorganisms ($\delta_p < \delta_m$). This process is consistent with previously observed ^{15}N enrichment in soil microorganisms during

immobilization [34,35], or with ^{15}N enrichment in the mycorrhizal fungal body and ^{15}N -depletion in the host plant [36,37]. In fact, the observed magnitude of $\Delta\delta^{15}\text{N}$ at TR1n (8‰) was within the range of isotopic fractionation caused by ectomycorrhizal fungi (5‰ to 9‰) [38], although such large enrichment is not always observed [26]. Interpretations of $\Delta\delta^{15}\text{N}$ described above are also supported by other observed data. At boundary sites, a high needle N concentration, a low C/N ratio of bulk soil, and a high needle $\delta^{13}\text{C}$ were observed (Figure 5(a), 5(c), and 5(d)). Foliar N concentration and $\delta^{13}\text{C}$ have been frequently used as indicators of N availability for trees [39] and light and moisture conditions for plants [40], whereas the soil C/N as an indicator of the decomposition rate of soil organic matter [41]. Therefore, these parameters as well as the high NO_3^- to NH_4^+ ratios at boundary sites in MM area (Table S1) indicate a rapid decomposition of soil organic matter and high N availability for larch under sunny and dry conditions. On the other hand, at forest sites forest, a low needle N concentration, a high C/N ratio of bulk soil, and a low needle $\delta^{13}\text{C}$ were observed (Figure 5(a), 5(c), and 5(d)), indicating the slow decomposition of soil organic matter and low N availability for larch under shady and relatively wet conditions. Our results suggest that the quantitative importance of the immobilization of available N, which is in agreement with a previous study that showed the severe competition of available N between plant

and soil microorganisms in taiga forest ecosystem [42].

In the mass balance equation, we assumed that the available N pool was theoretically at a steady state condition. Strictly speaking, it is not always true, however, the input and loss or their imbalance might not affect our interpretations described above.

Generally, recycled N within the plant-soil system provides the primary source of N for biological activities in most terrestrial ecosystems [43]. Although there is not enough data to evaluate the N budget at our study sites, we assume that recycled N is the dominant flux compared with the input and loss fluxes in the plant-soil system. In taiga, the annual N demand of larch has been reported at 1,500 mg N m⁻² yr⁻¹ near at Tura in central Siberia [44], and 850 to 3,100 mg N m⁻² yr⁻¹ at Yakutsk in northeastern Siberia [42]. The estimated N loss as leaching (< 10mg N m⁻² yr⁻¹) at Tura [44] and N input as deposition (48 mg N m⁻² yr⁻¹) at Yakutsk [42] were much smaller than the N demand.

In TR area, N deposition has been observed to be 96 to 289 mg N m⁻² yr⁻¹ [45], much smaller than the forest N demand estimated in central and northeastern Siberia.

Furthermore it is unlike that N deposition causes spatial variation in $\delta^{15}\text{N}$ observed at such small spatial scale (less than 2 km) in each area, because the amount and isotopic composition of N deposition may be similar at all sites in each area. Although an uptake of atmospheric deposited N by forest canopy has been reported to be a significant N

source for trees in the region where atmospheric pollution is severe [46], it may not affect our results for similar reasons described above.

Leaching (F_{leach}) seems to be insignificant in the boundary, since no runoff has been reported at the grassland in TR area; whereas it may occur on the north-facing slope in the forest [47]. However, leaching leads to loss of ^{15}N -depleted N (such as NO_3^-), which causes ^{15}N enrichment in available N especially inorganic N in the soil [48]. This process is opposite to our interpretation to bear large $\Delta\delta^{15}\text{N}$ in the forest (i.e. larch uptake ^{15}N -depleted N which exists in the soil). The loss of N through N_2O gas emission seems to be very small, as shown in the temperate steppe (17 to $28 \text{ mg N m}^{-2} \text{ yr}^{-1}$) [49]. Therefore, both N input and loss processes may not be suitable to explain the variation in $\Delta\delta^{15}\text{N}$ observed in this study.

In the above mass balance, we assumed that only the 0-20 cm soil layer provided available N to larch; however, the organic layer may also provide available N [50]. The organic layer showed lower $\delta^{15}\text{N}$ than the bulk soil (Figure 3), suggesting that the $\delta^{15}\text{N}$ of available N produced in the organic layer might be lower than that in the 0-20 cm soil layer [51]. In the forest, where thick organic layer accumulates on the mineral soil layer, larch uptake available N not only from the mineral soil layer, but also from the organic layer; therefore, this additional uptake of lower $\delta^{15}\text{N}$ of available N from the organic

layer might contribute to large $\Delta\delta^{15}\text{N}$.

Recently, global syntheses of $\delta^{15}\text{N}$ values of plant and soil have been done by comparing the $\delta^{15}\text{N}$ data with climate variables such as mean annual temperature (MAT) and precipitation (MAP). Amundson et al. [14] compiled the $\delta^{15}\text{N}$ values from previous studies, performed regression analysis, and successfully formulated plant and soil $\delta^{15}\text{N}$ and their difference ($\Delta\delta^{15}\text{N}$) with MAT and MAP. In addition, Craine et al. [15] compiled foliar $\delta^{15}\text{N}$ and showed that foliar $\delta^{15}\text{N}$ increased with MAT at a rate of $0.23\text{‰ }^{\circ}\text{C}^{-1}$ for ecosystems with $\text{MAT} > -0.5^{\circ}\text{C}$, and decreased by 2.6‰ for every order of magnitude increase of MAP. Similarly, Craine et al. [16] conducted an analysis with compiled soil $\delta^{15}\text{N}$, and reported that soil $\delta^{15}\text{N}$ increased with MAT at a rate of $0.18\text{‰ }^{\circ}\text{C}^{-1}$ for ecosystems with $\text{MAT} > 9.8^{\circ}\text{C}$, showed no with MAT below 9.8°C , and decreased at a rate of 1.78‰ for every order of magnitude increase of MAP. Plant $\delta^{15}\text{N}$ values calculated for the MAT and MAP of TR and MM areas with the multi regression equation by Amundson et al. [14] derive -1.1‰ for TR area and -0.8‰ for MM area, and those values are close to the average for all sites in this study ($+1.5 (\pm 2.2)\text{‰}$). Observed range in needle $\delta^{15}\text{N}$ (-3.9‰ to $+4.3\text{‰}$) also drops to the ranges of foliar $\delta^{15}\text{N}$ for both MAT and MAP as shown by Craine et al. [15]. Similarly, soil $\delta^{15}\text{N}$ values were calculated to be $+3.2\text{‰}$ and $+3.4\text{‰}$ with MAT and MAP for both TR and MM areas by

the multi regression equation [14], and these values are also close to the average for all sites in this study ($+5.2 (\pm 1.0)\text{‰}$). The observed range of soil $\delta^{15}\text{N}$ ($+2.9\text{‰}$ to $+7.4\text{‰}$) is also within the range reported by Craine et al. [16]. It is worth noting that a large difference in $\delta^{15}\text{N}$ values was observed between forest and boundary sites, but average $\delta^{15}\text{N}$ values observed for all sites, nevertheless, are close to the values obtained from global syntheses.

As described above, global distributions of foliar and soil $\delta^{15}\text{N}$, and their difference ($\Delta\delta^{15}\text{N}$) depend on climate parameters [14–16]. The global map of $\Delta\delta^{15}\text{N}$ reported by Amundson et al. [14] is therefore corresponding to the vegetation that also depends on the climate [1]. $\Delta\delta^{15}\text{N}$ is larger in boreal forest and smaller in temperate grassland as seen in their global map, and this trend is exactly consistent with this study. Here we propose that the accumulation of organic layer, as a comprehensive process of N cycling in the ecosystem, can be regarded as a key process which controls $\Delta\delta^{15}\text{N}$.

5. Concluding remarks

At a forest-grassland boundary in northern Mongolia, larch needle and soil $\delta^{15}\text{N}$ were investigated to understand the mechanisms that control the spatial pattern of $\delta^{15}\text{N}$. The

difference between needle and soil $\delta^{15}\text{N}$ ($\Delta\delta^{15}\text{N}$) showed clear spatial variation along the forest-grassland gradient with large $\Delta\delta^{15}\text{N}$ in the forest (up to -8‰) and small $\Delta\delta^{15}\text{N}$ in the grassland boundary (up to -2‰). The difference in $\delta^{15}\text{N}$ between larch and mineral soil reflects the difference in the accumulation patterns of the organic layer. In the grassland boundary, litter decomposes quickly and produced available N is transported to larch without significant fractionation, whereas in the forest, larch trees uptake available N with lower $\delta^{15}\text{N}$ than that produced in the soil. Namely, the ^{15}N -depleted part is taken by larch, whereas the ^{15}N -enriched part remains in the soil, which is balanced by ^{15}N -depleted litter provided by larch. The divergence of available N between larch and microorganisms in the soil, and the accumulation of diverged N in the organic layer control the variation in $\Delta\delta^{15}\text{N}$.

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646 Table 1. Locations of sampling sites.

Area		Site No. ^a	Latitude	Longitude	Elevation
name	code	position	°N	°E	m
Terelj	TR	1n, 2n, 3n, 4n, 5n, 6n, 7n, 8n	47.97	107.42	1587-1750
		ic	47.98	107.40	1639
		1s, 2s	47.99	107.42	1651-1791
Mongonmorit	MM	1s, 2s, 3s, 4s, 5s, 6s, 7s	48.35	108.66	1525-1619
		1sw, 2sw, 3sw	48.35	108.65	1593-1623

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648 ^a Each sampling site is expressed by area code and position on a slope. The position
649 number increases along upper to lower slope. The letter after position number indicates
650 the direction of slope. For example, “n” means north-facing slope and “sw” means
651 southwest-facing slope. TRic is a site in valley.

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Figure 1. A map of the two observation areas (TR and MM) and Ulaanbaatar, the capital of Mongolia (a), and schematic figures of longitudinal cross sections in TR area (b) and MM area (c). Samplings were conducted at Terelj (TR) and Mongonmorit (MM) at the forest-grassland boundary in northern Mongolia. In TR area, sampling was conducted at 11 sites along a transect line from the north-facing slope to the south-facing slope over a valley. In MM area, two transects were set on the south-facing slope and the southwest-facing slope, and sampling was conducted at 10 sites.

Figure 2. Temporal variations in larch needle N concentration (a), $\delta^{15}\text{N}$ (b), and $\delta^{13}\text{C}$ (c) in TR area. Data from two trees at TR3n, four trees at TR6n, and three trees at TR1s are shown. Each tree is expressed by a different shape (circle, rectangle, or triangle) and colour (black or white). Trees within the same site share the same shape.

Figure 3. Vertical profiles of larch needle, organic layer, and bulk soil $\delta^{15}\text{N}$ values at the forest site (TR1n) and the boundary site (TR7n) in TR area. Bars represent standard deviation of the mean.

Figure 4. Spatial variations in larch needle (triangle) and soil (square) $\delta^{15}\text{N}$ (a),

678 differences in $\delta^{15}\text{N}$ between needle and soil ($\Delta\delta^{15}\text{N}$) (b), and needle $\delta^{13}\text{C}$ (open-triangle)
679 and N concentration (filled-triangle) (c) in TR area, and the same as (d), (e), and (f) in
680 MM area. Bars represent standard deviation of the mean.

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682 Figure 5. Correlations between larch needle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ($r_s = 0.877$) (a), needle and
683 soil $\delta^{15}\text{N}$ ($r_s = 0.718$) (b), needle N concentration and $\Delta\delta^{15}\text{N}$ ($r_s = 0.591$) (c), and C/N
684 ratio of bulk soil and $\Delta\delta^{15}\text{N}$ ($r_s = -0.541$) (d) at all sites in TR area (circle) and MM area
685 (triangle). Dotted lines in (b) indicate $\Delta\delta^{15}\text{N}$ values at 0‰, -4‰ and -8‰. Correlation
686 coefficients are significant at $p < 0.05$. Bars represent standard deviation of the mean.

Figure 1.

A map of the two observation areas (TR and MM) and Ulaanbaatar, the capital of Mongolia (a), and schematic figures of longitudinal cross sections in TR area (b) and MM area (c). Samplings were conducted at Terelj (TR) and Mongonmorit (MM) at the forest-grassland boundary in northern Mongolia. In TR area, sampling was conducted at 11 sites along a transect line from the north-facing slope to the south-facing slope over a valley. In MM area, two transects were set on the south-facing slope and the southwest-facing slope, and sampling was conducted at 10 sites.

Figure 2.

Temporal variations in larch needle N concentration (a), $\delta^{15}\text{N}$ (b), and $\delta^{13}\text{C}$ (c) in TR area. Data from two trees at TR3n, four trees at TR6n, and three trees at TR1s are shown. Each tree is expressed by a different shape (circle, rectangle, or triangle) and colour (black or white). Trees within the same site share the same shape.

Figure 3.

Vertical profiles of larch needle, organic layer, and bulk soil $\delta^{15}\text{N}$ values at the forest site (TR1n) and the boundary site (TR7n) in TR area. Bars represent standard deviation of the mean.

Figure 4.

Spatial variations in larch needle (triangle) and soil (square) $\delta^{15}\text{N}$ (a), differences in $\delta^{15}\text{N}$ between needle and soil ($\Delta\delta^{15}\text{N}$) (b), and needle $\delta^{13}\text{C}$ (open-triangle) and N concentration (filled-triangle) (c) in TR area, and the same as (d), (e), and (f) in MM area. Bars represent standard deviation of the mean.

Figure 5.

Correlations between larch needle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ($r_s = 0.877$) (a), needle and soil $\delta^{15}\text{N}$ ($r_s = 0.718$) (b), needle N concentration and $\Delta\delta^{15}\text{N}$ ($r_s = 0.591$) (c), and C/N ratio of bulk soil and $\Delta\delta^{15}\text{N}$ ($r_s = -0.541$) (d) at all sites in TR area (circle) and MM area (triangle). Dotted lines in (b) indicate $\Delta\delta^{15}\text{N}$ values at 0‰, -4‰ and -8‰.

Correlation coefficients are significant at $p < 0.05$. Bars represent standard deviation of the mean.

Figure S1.

Larch needle N concentration (a), $\delta^{15}\text{N}$ (b), and $\delta^{13}\text{C}$ (c) in TR area, and the same as (d), (e), and (f) in MM area observed on all sampling dates at each site. Bars represent standard deviation of the mean.

Figure S2.

The schematic representation of mass balance of biologically available N in plant-soil system.

Assuming that the available N pool is at a steady state, the following equations are established:

$$F_{\text{input}} + F_a = F_p + F_m + F_{\text{leach}} + F_{\text{gas}} \quad (1)$$

and for $\delta^{15}\text{N}$,

$$F_{\text{input}} \times \delta_{\text{input}} + F_a \times \delta_a = F_p \times \delta_p + F_m \times \delta_m + F_{\text{leach}} \times \delta_{\text{leach}} + F_{\text{gas}} \times \delta_{\text{gas}} \quad (2)$$

where F_{input} is the N derived from atmospheric N deposition and biological N_2 fixation, and F_a is the N produced in the soil through the decomposition of soil organic matter. F_p and F_m are the fluxes of available N taken up by plants and immobilized by soil microorganisms, respectively. F_{leach} and F_{gas} are the N lost due to leaching and gaseous emission, respectively. The values of δ_{input} , δ_a , δ_p , δ_m , δ_{leach} , and δ_{gas} are the $\delta^{15}\text{N}$ of input, produced N from soil organic matter, plant uptake, immobilization by soil microorganisms, leaching, and gaseous emission of available N, respectively. We assumed that larch was the only plant species (i.e. $\delta_p = \text{needle } \delta^{15}\text{N}$), and that the $\delta^{15}\text{N}$ of available N produced in the soil was the same as that of the soil (i.e. $\delta_a = \text{soil } \delta^{15}\text{N}$).