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Influence of soil N availability on the difference between tree foliage and soil δ^{15}N from comparison of Mongolia and northern Japan

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Abstract

Nitrogen isotope ratios ($\delta^{15}N$) in plants and soil are widely known as indicators of the N cycle in terrestrial ecosystem. Recent studies have proposed that the difference between plant and soil $\delta^{15}N$ ($\Delta\delta^{15}N$) is a better indicator of the N cycle than plant $\delta^{15}N$ or soil $\delta^{15}N$ alone. However, the processes of the N cycle indicated by $\Delta\delta^{15}N$ are not well understood. The present study compared $\Delta\delta^{15}N$ variations between different ecosystems of northern Mongolia and northern Japan (Hokkaido) to associate the $\Delta\delta^{15}N$ characteristics with soil N availability. Needles of Siberian larch (*Larix sibirica* Ledeb.) in Mongolia, Todo-fir (*Abies sachalinensis* (F.Schmidt) Mast.) in Hokkaido, and mineral soils from both regions were acquired for determination of $\Delta\delta^{15}N$ values. $\Delta\delta^{15}N$ showed similar large variations (8‰) in the two regions with no significant correlations to climate factors. On the other hand, the relationship between $\Delta\delta^{15}N$ and soil $\delta^{15}N$ was opposite between the two regions with a positive correlation in Mongolia ($r_s = 0.504$) and a negative correlation in Hokkaido ($r_s = -0.600$). Moreover, total inorganic N (total amount of NH_4^+ and NO_3^-) contents were up to 20 times higher in Hokkaido than in Mongolia. $\Delta\delta^{15}N$ showed significant correlation with the fraction of NO_3^- relative to total inorganic N in the 0-10 cm soil layer in Hokkaido. These results indicate that $\Delta\delta^{15}N$ variation in Hokkaido can be explained by progression of nitrification in soil, which is different in Mongolia where $\Delta\delta^{15}N$ variation is explained by microbial N immobilization. Our findings suggest that soil N availability affects $\Delta\delta^{15}N$ indicator owing to changes in the N cycle process, which are reflected in the relationships of foliage $\delta^{15}N$ or soil $\delta^{15}N$ with $\Delta\delta^{15}N$.

Keywords

Larch; Fir; Foliage $\delta^{15}N$; Soil $\delta^{15}N$; Mongolia; Hokkaido

1. Introduction

Nitrogen (N) is a key element controlling the species composition, diversity, dynamics, and functioning of many terrestrial ecosystems (Vitousek et al. 1997). The N cycle in terrestrial plant-soil system is complex, with recycling of N between plant and soil through litterfall, decomposition and microbial production of biologically available N (NH_4^+, NO_3^-, and amino acids) which is taken up by the plant again, as well as N input as N_2-fixation and atmospheric deposition, and output as leaching and gas emission (Ågren and Andersson 2012). To understand the N cycle processes which are difficult to measure directly, the ratio of two stable isotopes of nitrogen (^{15}N: ^{14}N) in plant and soil ($\delta^{15}N$) have been proposed as an useful indicator (Meyers et al. 2016; Robinson 2001). Each process of the N cycle described above offers an opportunity for isotopic fractionation, which results in reaction products that are depleted in ^{15}N relative to substrates. For example, nitrate (NO_3^-) produced through nitrification has been shown to be depleted in ^{15}N, while residual ammonium (NH_4^+) becomes enriched in ^{15}N because of mass balance. Differences in $\delta^{15}N$ between substrate and product sometimes decreases when substrate pools become very small, namely nearly all available N is consumed in the reaction (Nadelhoffer and Fry 1994). Plant $\delta^{15}N$ reflects those N transformation processes because plants take up available N produced by these processes (Craine et al. 2009), and soil $\delta^{15}N$ also reflects these processes through loss of available N (Hobbie and Ouimette 2009).

However, plant and soil $\delta^{15}N$ have been reported to be affected by site-specific characteristics, such as climate, land management practices, and soil age (Amundson et al. 2003). A more recent study has reported that precipitation and temperature are the main factors regulating N availability and the $\delta^{15}N$ variation in plants with altitude regardless of C3 or C4 plants (Liu and Wang 2010). To investigate the N cycle in large spatial scales, the difference between plant and soil $\delta^{15}N$ ($\Delta\delta^{15}N$ = plant $\delta^{15}N$ – soil $\delta^{15}N$) has been recommended as a more appropriate indicator than individual plant or soil $\delta^{15}N$ when comparing $\delta^{15}N$ values among sites with different characteristics (Garten 1993; Garten and Van Miegroet 1994; Pardo et al. 2007). Because $\Delta\delta^{15}N$ normalizes spatial heterogeneity in mineral soil $\delta^{15}N$, this standardizing pattern of plant $\delta^{15}N$ for underlying variation in soil $\delta^{15}N$ removes variation in the signature of the source of $\delta^{15}N$ and reveals N cycling patterns better (Craine et al. 2015).

In previous studies, it has been proposed that three processes cause $\Delta\delta^{15}N$ variation: (1) difference in plant N source (Averill and Finzi 2011; Brearley 2013; Brenner et al. 2001; Callesen et al. 2013; Hobbie et al. 1999); (2) nitrification progression (Kang et al. 2011; Schuur and Matson 2001); and (3) N immobilization by soil microorganisms (Callesen et al. 2013; Fujiyoshi et al. 2017). In one example of the first cause, $\Delta\delta^{15}N$ ranged from -3 to 0‰ with increasing elevation in the White Mountains, USA (Averill and Finzi 2011). This $\Delta\delta^{15}N$ variation with increasing elevation was explained by a change in N form, in which plants uptake from inorganic N (which is more ^{15}N-depleted

N than organic N) to organic N with decreasing soil N availability. Another example is by Hobbie et al. (1999), who reported that $\Delta\delta^{15}N$ ranged from -1 to -6 ‰ with increasing forest succession age. This variation was explained by increase in reliance on mycorrhizal symbionts for N supply as soil N availability decreased: when plants uptake N via mycorrhizal fungi, $\Delta\delta^{15}N$ becomes larger than that caused by direct uptake from plant root due to enzymatic reactions which produces ^{15}N-depleted N. In an example of the second cause of $\Delta\delta^{15}N$ variation, $\Delta\delta^{15}N$ ranged from -8 to -3‰ with decreasing precipitation in a humid tropical forest in Hawaii (Schuur and Matson 2001). This variation was explained by an increase of nitrification rate with decreasing precipitation: when plants uptake nitrate as the sole source of N, high nitrification causes the ammonium-to-nitrate reaction to go further towards completion, which would decrease the isotope fractionation factor from ammonium to nitrate. Thus $\Delta\delta^{15}N$ becomes small and nitrate becomes less deficient in ^{15}N. For an example of the third cause of $\Delta\delta^{15}N$ variation, Fujiyoshi et al. (2017) observed a $\Delta\delta^{15}N$ range from -8 to -2‰ along forest-grassland ecotone in northern Mongolia. The larger $\Delta\delta^{15}N$ in the forest was explained by ^{15}N-enriched N immobilization by soil microorganisms, such as bacteria and mycorrhizal fungi under low soil N availability.

In the present study, different ecosystems of northern Mongolia and northern Japan

(Hokkaido) were compared for variations in $\Delta\delta^{15}$N. Mongolia and Hokkaido are both classified as cold climate in Köppen-Geiger climate classification that are similar in temperature, but differ in precipitation. The distinct characteristics in N cycle reported at forests in Hokkaido and Mongolia is N loss. In Hokkaido, N loss as leaching (as the form of NO_3^-) from soil occurs throughout the year (Ozawa et al. 2001; Fukuzawa et al. 2006; Nagasaka et al. 2015), whereas N loss due to leaching is negligible in taiga forests in northern Mongolia (Shugalei and Vedrova 2004). We hypothesize that a difference in soil N availability in the two regions influenced by climate leads to a difference in $\Delta\delta^{15}$N, because a difference in soil N availability is expected to link with a difference in the processes which causes $\Delta\delta^{15}$N variation from the previous studies above.

Spatial comparison of δ^{15}N across ecosystems at regional and even global scales have the potential to provide insights into patterns of and controls on N cycling across ecosystems (Pardo and Nadelhoffer 2010), which may in turn be indicative of the response of ecosystems to increased N deposition or other forms of disturbance in future (Amundson et al. 2003). Although $\Delta\delta^{15}$N can be a potent indicator for spatial comparison of N cycle, each study above was limited to single ecosystems. Therefore, to the best of our knowledge, no studies have evaluated $\Delta\delta^{15}$N utility based on comparison among different ecosystems, particularly regarding different climate and soil N availability conditions.

2. Materials and Methods

2.1. Site description

The Pinaceae forests of northern Mongolia and Hokkaido were selected for study (Fig. 1). In northern Mongolia, six areas were observed where Siberian larch (*Larix sibirica* Ledeb.) is dominant (Fig. 1a). The soil in this region is cryosol. The climate is cold continental climate with dry winter (Dwc in Köppen-Geiger climate classification), and mean annual temperature (MAT) ranges from -5.9°C to 0.1°C, and mean annual precipitation (MAP) ranges from 201 mm to 353 mm (Table 1). In Hokkaido, eight areas were observed where Todo-fir (*Abies sachalinensis* (F.Schmidt) Mast.) is dominant (Fig. 1b). The soil in this region is brown forest soil. The climate is humid continental climate with warm summer (Dfb in Köppen-Geiger climate classification), and MAT ranges from 3.7°C to 6.9°C, and MAP ranges from 818 mm to 2213 mm (Table 1). Sampling was conducted at each site within each area (Table 1). The observation period was from June to August, 2004-2012 in Mongolia, whereas the sites in Hokkaido was observed from June to September, 2011-2013 (Table S1). To compare Mongolia with Hokkaido, we compiled new and published data (Fujiyoshi et al. 2017) for Mongolia. Among observation areas in Mongolia, needle and soil $\delta^{15}N$ in Terelj (TR) and Mongonmorit (MM) areas have been reported in Fujiyoshi et al. (2017).

2.2. Samplings

2.2.1. Needle samples

Needles of *L. sibirica* in Mongolia and *A. sachalinensis* in Hokkaido were collected. Both species studied are non-N_2-fixing ectomycorrhizal plants (Brundrett 2009). We collected needles from mature tree whose height approached or reached the canopy. DBHs (Diameter at breast height) ranged 20 to 40 cm for *Abies sachalinensis* (F.Schmidt) Mast. in Hokkaido, and 3 cm to 40 cm for *Larix sibirica* Ledeb. in Mongolia. We collected needles from previous year shoots of several main branches on a tree. Generally, more than three trees were sampled at each site, but some sites were sampled from only one to two trees due to limited number of trees. Needle samples were oven-dried at 60°C and ground to a fine powder for analysis.

2.2.2. Soil samples

Soil samples were collected at the same sites where needles were sampled. A small pit (0.5 m × 0.5 m × 0.5 m deep) was prepared, and one to three cores (1.5 cm diameter, 4.5

cm length) of bulk soil were taken from the cross section of the pit every 10 cm down to 50 cm depth or until a rock appeared. The organic layer was also sampled by collecting the surface of bulk soil. The fresh soil samples were sieved with a 2 mm mesh to remove gravel and roots, and these samples oven-dried at 105°C for more than 24 hours, and ground for analysis. Samples of the organic layer were dried at 70°C for more than 24 hours and ground for analysis. Fresh soil samples collected from two areas in Mongolia (TR and MM) in 2012, and six areas in Hokkaido (TU, AS, NI, HI, UR, and ER) in 2011 and 2012 were measured for KCl-extractable nitrogen [dissolved organic nitrogen (DON), NH_4^+, and NO_3^-]. In addition, ion exchange water (IEW)-extractable inorganic nitrogen (NH_4^+, and NO_3^-) was measured from soil samples collected from three areas (TU, NI, and UR) in Hokkaido.

2.3. Analysis

2.3.1. N isotopic ratio and concentration

The $\delta^{15}N$ value and N concentrations were analyzed 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:

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

where R_{sample} is the isotope ratio (^{15}N/^{14}N) of a sample, and R_{std} is the isotope ratio (^{15}N/^{14}N) of atmospheric N_2. Analytical errors were at 0.3‰ and 0.1% for δ^{15}N and N concentration, respectively.

2.3.2. KCl and IEW-extractable nitrogen

Dissolved N in soil (DON, NH_4^+, and NO_3^-) was extracted from 4 g of fresh sieved soil with 40 ml of 2M KCl after 1 hour shaking and filtration. The extracts were kept in coolers during fieldwork and stored in a freezer in the laboratory until analysis. Concentrations of NO_3^-, NH_4^+, and total dissolved nitrogen (TDN) were analyzed colorimetrically using a continuous flow nutrient analyzer (QuAAtro; BRAN+LUEBBE, Hamburg, Germany). Thereafter, the concentration of DON was calculated by subtracting total inorganic N (NO_3^- and NH_4^+) from TDN. The concentration of nitrite (NO_2^-) was also analyzed, but not detected in any samples. As for soil samples collected in 3 areas in Hokkaido (TU, NI, and UR), extraction of dissolved N with 40 ml of IEW was also conducted.

2.3.3. Calculation of average values at each site

To obtain needle N concentration and δ^{15}N at each site, data were first averaged for all trees in each sampling period. Thereafter, the average for all sampling periods at each site was calculated. Soil $\delta^{15}N$ of each site was calculated by weighted mean of $\delta^{15}N$ in the 0-20 cm soil layer for the sites in Mongolia, whereas it was calculated as the weighted mean of the 0-40 cm soil layer for the sites in two areas (AS and HI) and the 0-50 cm soil layer for the other areas in Hokkaido. The $\Delta\delta^{15}N$ value was calculated by subtracting soil $\delta^{15}N$ from needle $\delta^{15}N$ at each site. Temporal changes in needle $\delta^{15}N$ and soil $\delta^{15}N$ during sampling period were negligible.

2.3.4. Statistical analysis

The spatial relationships between the variables were evaluated by Spearman's rank correlation coefficient at $p < 0.05$ (two-tailed).

3. Results

3.1. Vertical profiles of needle, organic layer, and bulk soil $\delta^{15}N$ and N concentration

Typical profiles of needle, organic layer, and bulk soil $\delta^{15}N$ and N concentrations in Mongolia and Hokkaido are shown in Fig. 2. The sites where the maximum and minimum $\Delta\delta^{15}N$ were observed in Mongolia and Hokkaido were plotted. A difference in soil $\delta^{15}N$ profile was observed between the sites in Mongolia and those in Hokkaido (Fig. 2a). In Mongolia, soil $\delta^{15}N$ reached a maximum above the 20-30 cm layer for all sites, whereas soil δ^{15}N continued to increase below 20-30 cm layer for most of the sites in Hokkaido (14 sites out of 17 sites). The sites with maximum $\Delta\delta^{15}$N (TR1n in Mongolia and UR1sw in Hokkaido) increased 8‰ from needle δ^{15}N to soil δ^{15}N of 0-10 cm layer, whereas the sites with minimum $\Delta\delta^{15}$N (MM5s in Mongolia and ER2 in Hokkaido) increased less than 1‰ (Fig. 2a). The N concentration gradually deceased from needle to deeper soil at all sites in Mongolia and Hokkaido (Fig. 2a). The N concentration of needle was lower for *Abies sachalinensis* (F.Schmidt) Mast. in Hokkaido (1.5%) than *Larix sibirica* Ledeb. in Mongolia (more than 2%), whereas the N concentration of deeper soil was higher in Hokkaido (0.2%) than in Mongolia (less than 0.1%) (Fig. 2b). The difference of needle δ^{15}N between the sites of maximum and minimum $\Delta\delta^{15}$N was 8‰ in Mongolia but much smaller (3‰) in Hokkaido (Fig. 2a). Similarly, the difference of needle N concentration between the sites of maximum and minimum $\Delta\delta^{15}$N was 0.7% in Mongolia but negligible in Hokkaido (Fig. 2b).

3.2. Needle and soil δ^{15}N values, and $\Delta\delta^{15}$N of all sites

Needle and soil δ^{15}N, and $\Delta\delta^{15}$N of all sites in Mongolia and Hokkaido are shown in Fig. 3. Needle δ^{15}N varied by 10‰ in Mongolia from -5.4 ± 0.2‰ to +4.3 ±0.5‰. This variation was twice that observed in Hokkaido, which ranged from -4.9 ±2.4‰ to -0.95

±0.34‰. On the other hand, soil $\delta^{15}N$ varied by 5‰ from +2.0 ±0.1‰ to +6.8‰ in Mongolia, and a similar variation of 4‰ was observed in Hokkaido from +0.17‰ to +4.6 ±0.4‰. $\Delta\delta^{15}N$ also showed similar variation between Mongolia and Hokkaido (Fig. 3b). $\Delta\delta^{15}N$ varied by 7‰ from -8.4 ±0.7‰ to -1.8 ±0.1‰ in Mongolia, and $\Delta\delta^{15}N$ varied 6‰ from -8.3 ±0.4‰ to -2.7 ±0.4‰ in Hokkaido. The average needle and soil $\delta^{15}N$ of each area showed significant correlations with MAP ($r_s = -0.537$ for needle $\delta^{15}N$ and $r_s = -0.709$ for soil $\delta^{15}N$) and with elevation ($r_s = 0.568$ for needle $\delta^{15}N$ and $r_s = 0.696$ for soil $\delta^{15}N$). The average soil $\delta^{15}N$ of each area also showed significant correlation with MAT ($r_s = -0.631$). In contrast, the average $\Delta\delta^{15}N$ of each area did not show any correlation with MAP, MAT, or elevation.

3.3. DON, NH_4^+, NO_3^- and their relationship with $\Delta\delta^{15}N$

The KCl-extractable N (DON, NH_4^+, and NO_3^-) in each area in Mongolia and Hokkaido are shown in Fig. 4. The NH_4^+ and NO_3^- concentrations were higher in Hokkaido than those in Mongolia. The NH_4^+ concentration in Hokkaido was four to eight times higher in the 0-10 cm layer (Fig. 4a) and two to four times higher in the 0-50 cm layer (Fig. 4b) than those in Mongolia. Similarly, the NO_3^- concentration in Hokkaido was four to 20 times higher in the 0-10 cm layer and 10 to 20 times higher in the 0-50 cm layer than those in Mongolia. Furthermore, the fraction of total inorganic N (NH_4^+ and NO_3^-) to TDN was clearly different between Mongolia and Hokkaido. In Hokkaido, the fraction was 57-84% in the 0-10 cm layer and 30-55% in the 0-50 cm layer, whereas the fraction was less than 14% in both layers in Mongolia. In Hokkaido, the fraction of NO_3^- relative to total inorganic N in the 0-10 cm soil layer showed significant rank correlation with $\Delta\delta^{15}N$ ($r_s = 0.687$) (Fig. 5).

On the other hand, the inorganic N differed between KCl-extraction and IEW-extraction (Fig. S1). For three areas in Hokkaido, NH_4^+ in IEW-extraction was less than 40% of that measured by KCl-extraction, whereas NO_3^- in IEW-extraction was more than 60% of that measured by KCl-extraction.

3.4. Relationships among needle $\delta^{15}N$, soil $\delta^{15}N$, and $\Delta\delta^{15}N$

The relationships among needle $\delta^{15}N$, soil $\delta^{15}N$, and $\Delta\delta^{15}N$ for all the sites in Mongolia and in Hokkaido are shown in Fig. 6. Positive correlation between soil $\delta^{15}N$ and needle $\delta^{15}N$ was found in Mongolia ($r_s = 0.795$), whereas no correlation was observed in Hokkaido (Fig. 6a). In addition, the relationship between soil $\delta^{15}N$ and $\Delta\delta^{15}N$ was also different between Mongolia and Hokkaido, with positive correlation in Mongolia ($r_s = 0.504$) but negative correlation in Hokkaido ($r_s = -0.600$) (Fig. 6c). On the other hand, needle $\delta^{15}N$ showed positive correlation with $\Delta\delta^{15}N$ in both regions ($r_s = 0.908$ for Mongolia and $r_s = 0.578$ for Hokkaido).

4. Discussion

In this study, we hypothesized that a difference in soil N availability in the two regions led to a difference in $\Delta\delta^{15}N$. One contrasting result between the two regions was soil inorganic N, which was two to 20 times higher in Hokkaido than in Mongolia (Fig. 4). Moreover, a gradual increase in soil $\delta^{15}N$ from surface to deeper layers in Hokkaido than in Mongolia (Fig.2a) indicates that available N production occurs in deeper layer in Hokkaido than in Mongolia. These differences may reflect a suppressed N cycle in Mongolia under a severe climate, and indicates the difference in soil N availability in the two regions. Previous studies have also reported that in northern Mongolia including our study areas, soil is frozen in winter from November to March (Nandintsetseg et al. 2011), and soil freezing suppresses N cycle processes such as mineralization and nitrification in Inner Mongolia (Zhao et al. 2010).

When the two regions were compared for variation of $\Delta\delta^{15}N$, similar values were observed in Mongolia and Hokkaido (Fig. 3b). This result suggests that a difference in soil N availability does not associate with a difference in the variation range of $\Delta\delta^{15}$N, as we hypothesized. On the other hand, the relationships between needle δ^{15}N and soil δ^{15}N, or between soil δ^{15}N and $\Delta\delta^{15}$N, were different between these regions (Fig. 6). We expect that different N cycle processes between Mongolia and Hokkaido are reflected in those relationships. Interestingly, the fraction of NO_3^- to total inorganic N showed a significant rank correlation with $\Delta\delta^{15}$N in Hokkaido (Fig. 5). Because the fraction of NO_3^- to total inorganic N can be a proxy for the fraction of NH_4^+ nitrified in the soil (Spoelstra et al. 2007), an increase in the NO_3^- fraction implies progression of nitrification. Therefore, the correlation between the fraction of NO_3^- to total inorganic N and $\Delta\delta^{15}$N in Hokkaido indicates that $\Delta\delta^{15}$N becomes small with the progression of nitrification in the soil. Generally, ammonification causes very little fractionation, but nitrification process strongly fractionates against δ^{15}N, and the δ^{15}N of NO_3^- can be approximately 20‰ more negative than the δ^{15}N of soil organic N (Högberg 1997). However, as nitrification progresses and the conversion of NH_4^+ to NO_3^- proceeds towards completion in a closed system, the δ^{15}N of NO_3^- increases and approaches that of soil organic N, and the apparent fractionation between NO_3^- and NH_4^+ (or soil organic N) decreases (Högberg 1997). Therefore, the observed relationship between a high fraction of NO_3^- and small $\Delta\delta^{15}$N in Hokkaido can be explained by the change in δ^{15}N of NO_3^- in soil, dependent on the progression of nitrification. Furthermore, the observed negative correlation between $\Delta\delta^{15}N$ and soil $\delta^{15}N$ in Hokkaido (Fig. 6c) can be explained by that the sites with smaller $\Delta\delta^{15}N$, where nitrification is progressed, may have prominent NO_3^- leaching, which increases soil $\delta^{15}N$ over a long time-scale due to loss of ^{15}N-depleted NO_3^- from soil. We premise uptake of NO_3^- by *Abies sachalinensis* (F.Schmidt) Mast. as the sole N source for this explanation. Because the dominant form of inorganic N in soil water was NO_3^- in Hokkaido (Fig. S1), it is reasonable to expect that *A. sachalinensis* takes up NO_3^- from the soil. The alternative explanation is based on the preferential uptake of NO_3^- by *A. sachalinensis*. Although there is no previous study on selective uptake of NO_3^- by *A. sachalinensis*, one study reported that growth of *A. sachalinensis* was promoted when NO_3^- was the sole N source (Sato and Yamaguchi 1939). Another study reported that trees of genus *Abies* showed higher growth rate with increasing NO_3^- content in N source (Rothstein and Cregg 2005). These studies suggest the possibility that *A. sachalinensis* prefer NO_3^- as the primary N source.

On the other hand, in Mongolia, variation of $\Delta\delta^{15}N$ was explained by 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. This caused a large $\Delta\delta^{15}N$, whereas transport of the most available N into larch caused small $\Delta\delta^{15}N$ (Fujiyoshi et al. 2017). Our results suggest that a difference in soil N availability between Mongolia and Hokkaido associates with a difference in N cycle processes which determine $\Delta\delta^{15}N$, and the difference in the relationships of foliage and soil $\delta^{15}N$ with $\Delta\delta^{15}N$, which might be originally influenced by climate.

5. Conclusion

$\Delta\delta^{15}N$ showed similar large variations in Mongolia and Hokkaido, which suggests that a difference in soil N availability does not associate with a difference in the variation range of $\Delta\delta^{15}N$. On the other hand, differences observed in the relationships of foliage and soil $\delta^{15}N$ with $\Delta\delta^{15}N$ might indicate differences in N cycle processes between the two regions. Our study suggests that a difference in soil N availability associates with changes in N cycle processes, which are reflected in the relationships of foliage and soil $\delta^{15}N$ with $\Delta\delta^{15}N$. When applying $\Delta\delta^{15}N$ as an indicator of N cycle among different ecosystems, not only $\Delta\delta^{15}N$ but also soil N availability should be considered together.

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References

Ågren, G.I., Andersson, F.O., 2012. Terrestrial ecosystem ecology: principles and applications. Cambridge, Cambridge University Press.

Amundson, R., Austin, A.T., Schuur, E.A.G., Yoo, K., Matzek, V., Kendall, C., Uebersax, A., Brenner, D., Baisden, W.T., 2003. Global patterns of the isotopic composition of soil and plant nitrogen. Global Biogeochemical Cycles, 17(1). doi:10.1029/2002gb001903

Averill, C., Finzi, A. C., 2011. Increasing plant use of organic nitrogen with elevation is reflected in nitrogen uptake rates and ecosystem delta N-15. Ecology, 92(4), 883-891. doi:10.1890/10-0746.1

Brearley, F. Q., 2013. Nitrogen stable isotopes indicate differences in nitrogen cycling between two contrasting Jamaican montane forests. Plant and Soil, 367(1-2), 465-476. doi:10.1007/s11104-012-1469-z

Brenner, D. L., Amundson, R., Baisden, W. T., Kendall, C., Harden, J., 2001. Soil N and N-15 variation with time in a California annual grassland ecosystem. Geochimica Et Cosmochimica Acta, 65(22), 4171-4186. doi:10.1016/s0016-7037(01)00699-8

Brundrett, M. C., 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant and Soil, 320(1-2), 37-77. doi:10.1007/s11104-008-9877-9

Callesen, I., Nilsson, L. O., Schmidt, I. K., Vesterdal, L., Ambus, P., Christiansen, J. R., Hogberg, P., Gundersen, P., 2013. The natural abundance of N-15 in litter and soil profiles under six temperate tree species: N cycling depends on tree species traits and site fertility. Plant and Soil, 368(1-2), 375-392. doi:10.1007/s11104-012-1515-x

Craine, J. M., Brookshire, E. N. J., Cramer, M. D., Hasselquist, N. J., Koba, K., Marin-Spiotta, E., Wang, L. X., 2015. Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. Plant and Soil, 396(1-2), 1-26. doi:10.1007/s11104-015-2542-1

Craine, J.M., Elmore, A.J., Aidar, M.P.M., Bustamante, M., Dawson, T.E., Hobbie, E.A., Kahmen, A., Mack, M.C., McLauchlan, K.K., Michelsen, A., Nardoto, G.B., Pardo, L.H., Penuelas, J., Reich, P.B., Schuur, E.A.G., Stock, W.D., Templer, P.H., Virginia, R.A., Welker, J.M., Wright, I.J., 2009. Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. New Phytologist, 183(4), 980-992. doi:10.1111/j.1469-8137.2009.02917.x

Emmett, B. A., Kjonaas, O. J., Gundersen, P., Koopmans, C., Tietema, A., Sleep, D., 1998. Natural abundance of N-15 in forests across a nitrogen deposition gradient. Forest Ecology and Management, 101(1-3), 9-18. doi:10.1016/s0378-1127(97)00121-7

Fujiyoshi, L., Sugimoto, A., Tsukuura, A., Kitayama, A., Lopez Caceres, M. L., Mijidsuren, B., Saraadanbazar, A., Tsujimura, M. (2017). Spatial variations in larch needle and soil δ15N at a forest–grassland boundary in northern Mongolia. Isotopes in environmental and health studies, 53(1), 54-69

Fukuzawa, K., Shibata, H., Takagi, K., Nomura, M., Kurima, N., Fukazawa, T., Satoh, F., Sasa, K., 2006. Effects of clear-cutting on nitrogen leaching and fine root dynamics in a cool-temperate forested watershed in northern Japan. Forest Ecology and Management, 225(1-3), 257-261. doi:10.1016/j.foreco.2006.01.001

Garten, C. T., 1993. VARIATION IN FOLIAR N-15 ABUNDANCE AND THE AVAILABILITY OF SOIL-NITROGEN ON WALKER BRANCH WATERSHED. Ecology, 74(7), 2098-2113. doi:10.2307/1940855

Garten, C. T., Vanmiegroet, H., 1994. RELATIONSHIPS BETWEEN SOIL-NITROGEN DYNAMICS AND NATURAL N-15 ABUNDANCE IN PLANT FOLIAGE FROM GREAT SMOKY MOUNTAINS NATIONAL-PARK. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, 24(8), 1636-1645. doi:10.1139/x94-212

Hobbie, E. A., Macko, S. A., Shugart, H. H., 1999. Insights into nitrogen and carbon dynamics of ectomycorrhizal and saprotrophic fungi from isotopic evidence. Oecologia, 118(3), 353-360. doi:10.1007/s004420050736

Hobbie, E. A., Ouimette, A. P., 2009. Controls of nitrogen isotope patterns in soil profiles. Biogeochemistry, 95(2-3), 355-371. doi:10.1007/s10533-009-9328-6

Hogberg, P., 1997. Tansley review No 95 - N-15 natural abundance in soil-plant systems. New Phytologist, 137(2), 179-203. doi:10.1046/j.1469-8137.1997.00808.x

Kang, H. Z., Liu, C. J., Yu, W. J., Wu, L. L., Chen, D. M., Sun, X., Ma, X.P., Hu, H.B., Zhu, X. L., 2011. Variation in foliar delta N-15 among oriental oak (Quercus variabilis) stands over eastern China: Patterns and interactions. Journal of Geochemical Exploration, 110(1), 8-14. doi:10.1016/j.gexplo.2011.02.002

Liu, X. Z., Wang, G., 2010. Measurements of nitrogen isotope composition of plants and surface soils along the altitudinal transect of the eastern slope of Mount Gongga in southwest China. Rapid Communications in Mass Spectrometry, 24(20), 3063-3071.

Meyers, L. M., Nahlik, A. M., DeKeyser, E. S., 2016. Relationship between the natural abundance of soil nitrogen isotopes and condition in North Dakota wetlands. Ecological Indicators, 60, 394-401. doi:10.1016/j.ecolind.2015.06.042

Nadelhoffer, K.J., Fry, B., 1994. Nitrogen isotope studies in forest ecosystems, in: Lajtha, K., Michener, R.H. (Eds.), Stable isotopes in ecology and environmental science. Oxford: Blackwell Scientific, pp. 22–44.

Nagasaka, A., Nagasaka Y., Ishikawa Y., 2015. Export of stream dissolved organic carbon and inorganic nitrogen at two headwater catchments with different forest types in central Hokkaido. Bulletin of the Hokkaido Forestry Research Institute, No.52.

Ozawa, M., Shibata, H., Satoh, F., Sasa, K., 2001. Annual element budget of soil in snow-dominated forested ecosystem. Water Air and Soil Pollution, 130(1-4), 703-708. doi:10.1023/a:1013877316949

Pardo, L. H., Hemond, H. F., Montoya, J. P., Pett-Ridge, J., 2007. Natural abundance N-15 in soil and litter across a nitrate-output gradient in New Hampshire. Forest Ecology and Management, 251(3), 217-230. doi:10.1016/j.foreco.2007.06.047

Pardo, L. H., Nadelhoffer, K.J., 2010. Using Nitrogen Isotope Ratios to Assess Terrestrial Ecosystems at Regional and Global Scales, in: West, J.B., et al (Eds.), Isoscapes: Understanding Movement, Pattern, and Process on Earth Through Isotope Mapping. Springer Science+Business Mdedia B.V.2010, pp. 221–249.

Peterson, B. J., Fry, B., 1987. STABLE ISOTOPES IN ECOSYSTEM STUDIES. Annual Review of Ecology and Systematics, 18, 293-320. doi:10.1146/annurev.ecolsys.18.1.293

Popova, A. S., Tokuchi, N., Ohte, N., Ueda, M. U., Osaka, K., Maximov, T. C., Sugimoto, A., 2013. Nitrogen availability in the taiga forest ecosystem of northeastern Siberia. Soil Science and Plant Nutrition, 59(3), 427-441. doi:10.1080/00380768.2013.772495

Robinson, D., 2001. delta N-15 as an integrator of the nitrogen cycle. Trends in Ecology & Evolution, 16(3), 153-162. doi:10.1016/s0169-5347(00)02098-x

Rothstein, D. E., Cregg, B. M., 2005. Effects of nitrogen form on nutrient uptake and physiology of Fraser fir (Abies fraseri). Forest Ecology and Management, 219(1), 69-80. doi:10.1016/j.foreco.2005.08.043

Sato, Y., Yamaguchi, S., 1939. Relationships between seedling growth in water culture and nitrogen source and hydrogen concentration (in Japanese). RESEARCH BULLETINS OF THE COLLEGE EXPERIMENT FORESTS, HOKKAIDO IMPERIAL UNIVERSITY, 11(1), 1-45.

Schuur, E. A. G., Matson, P. A., 2001. Net primary productivity and nutrient cycling across a mesic to wet precipitation gradient in Hawaiian montane forest. Oecologia, 128(3), 431-442. doi:10.1007/s004420100671

Shugalei, L. S., Vedrova, E. F., 2004. Nitrogen pool in northern-taiga larch forests of central Siberia. Biology Bulletin, 31(2), 200-208. doi:10.1023/B:BIBU.0000022477.99224.fb

Spoelstra, J., Schiff, S. L., Hazlett, P. W., Jeffries, D. S., Semkin, R. G., 2007. The isotopic composition of nitrate produced from nitrification in a hardwood forest floor. Geochimica Et Cosmochimica Acta, 71(15), 3757-3771. doi:10.1016/j.gca.2007.05.021

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D., 1997. Human alteration of the global nitrogen cycle: Sources and consequences. Ecological Applications, 7(3), 737-750. doi:10.2307/2269431

Table 1

Location, elevation, mean annual temperature (MAT), and mean annual precipitation (MAP) of all study areas in Mongolia and Hokkaido. The climate data of each area was obtained from the nearest meteorological station. The number of sampling sites in each area was varied from one to eight.

Region	Area code [a]	Number of site	Latitude °N	Longitude °E	Elevation m (a.s.l)	MAT °C	MAP mm
Mongolia	TG	5	51.35	99.30	1641-1702	-5.9	215
	HG	2	50.42	100.17	1700-1705	-3.8	304
	AB	2	49.87	99.52	2046-2074	-4.4	201
	MR	2	49.65	100.35	1701-1744	0.1	240
	TR[b]	8	47.97	107.42	1587-1791	-3.6	353
	MM[b]	8	48.35	108.66	1525-1623	-2.9	272
Hokkaido	TA	2	44.20	143.07	213-221	5.6	917
	TU	3	43.37	144.02	498-501	5.8	818
	AS	1	43.38	143.32	398	3.7	1403
	NI	3	42.38	143.18	183-189	6.9	1731
	HI	1	42.37	143.05	370	6.9	1731
	UR	3	42.27	143.02	348-354	6.4	1717
	SA	2	42.17	142.97	67-71	6.4	1717
	ER	2	42.08	143.27	65-283	NA[c]	2213

[a] Area code was based on the abbreviation of the location’s name.

[b] Needle and soil $\delta^{15}N$ in TR and MM areas have been previously reported in Fujiyoshi et al. (2017).

[c] NA: not available

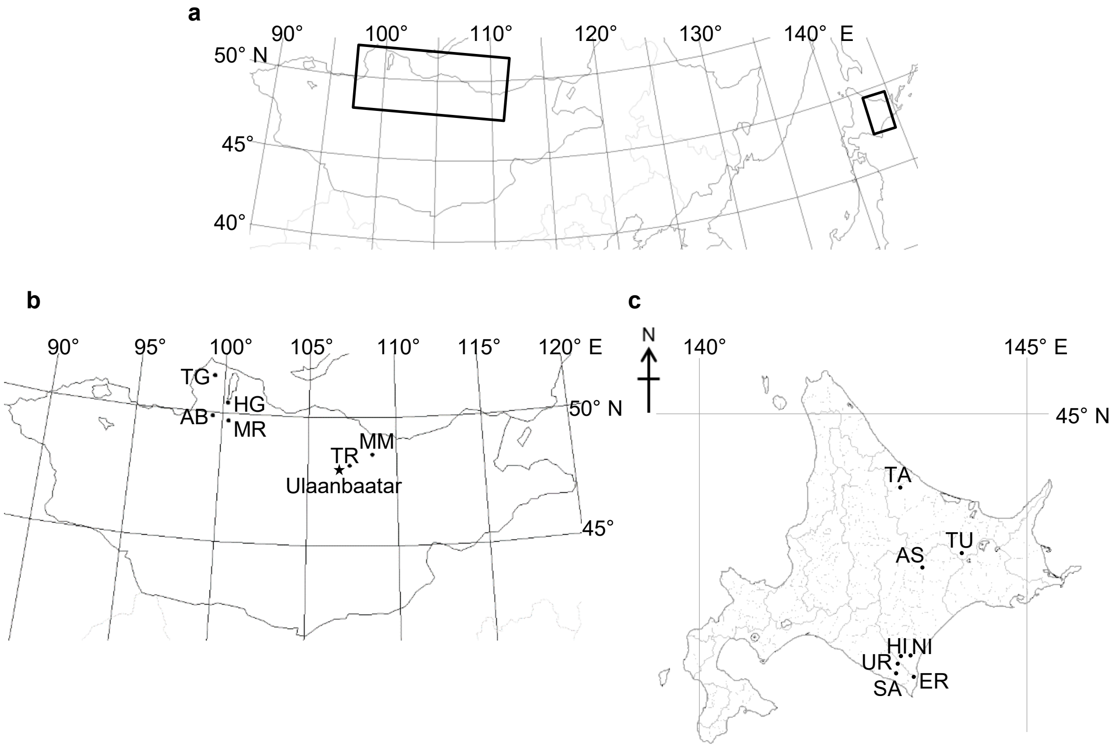

Fig. 1. Maps of observation regions in Mongolia and Hokkaido shown by black square (a), 6 observation areas with capital Ulaanbaatar in Mongolia (b), and 8 observation areas in Hokkaido (c). In Mongolia, samplings were conducted in the western region [TG (Tsagaanuur), HG (Hatgal), AB (Arbulag), MR (Murun)], and the eastern region [TR (Terelj), MM (Mongonmorit)]. In Hokkaido, samplings were conducted in the southern region [ER (Erimo), SA (Samani), UR (Urakawa), HI (Hiroo), NI (Nishihiroo)], and the eastern region [AS (Ashoro), TU (Tsubetsu), TA (Takinoue)].

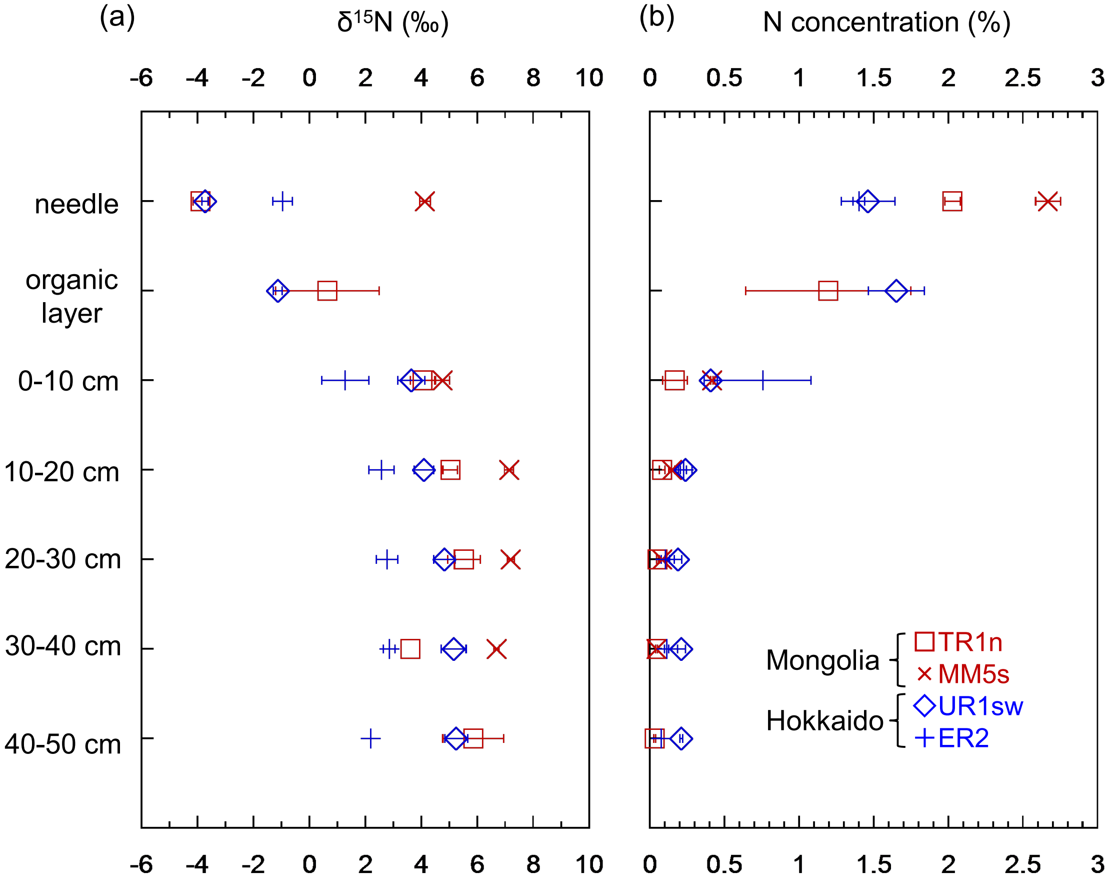

Fig. 2. Typical vertical profiles of needle, organic layer, and bulk soil $\delta^{15}N$ (a) and N concentration (b) in Mongolia and Hokkaido.

The sites that showed maximum $\Delta\delta^{15}N$ (TR1n in Mongolia and UR1sw in Hokkaido) and minimum $\Delta\delta^{15}N$ (MM5s in Mongolia and ER2 in Hokkaido) were plotted. $\Delta\delta^{15}N$ is defined as [needle $\delta^{15}N$ – soil $\delta^{15}N$]. Bars represent standard error of the mean.

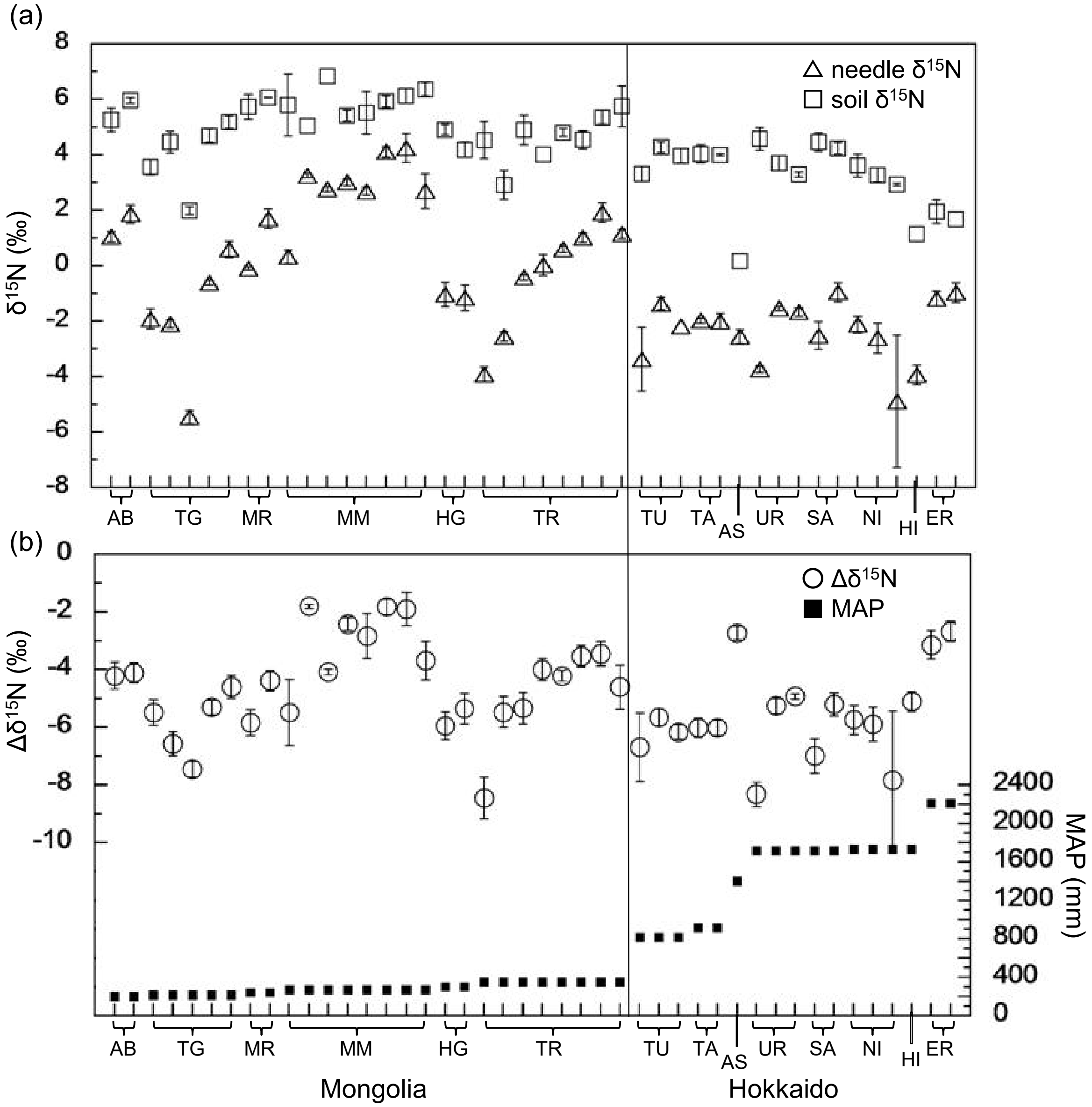

Fig. 3. Needle $\delta^{15}N$ and soil $\delta^{15}N$ (a), and the difference between needle $\delta^{15}N$ and soil $\delta^{15}N$ ($\Delta\delta^{15}N$) and mean annual precipitation (MAP) (b) of all areas in Mongolia and Hokkaido.
The values of each site in each area are plotted. Bars represent standard error of the mean.

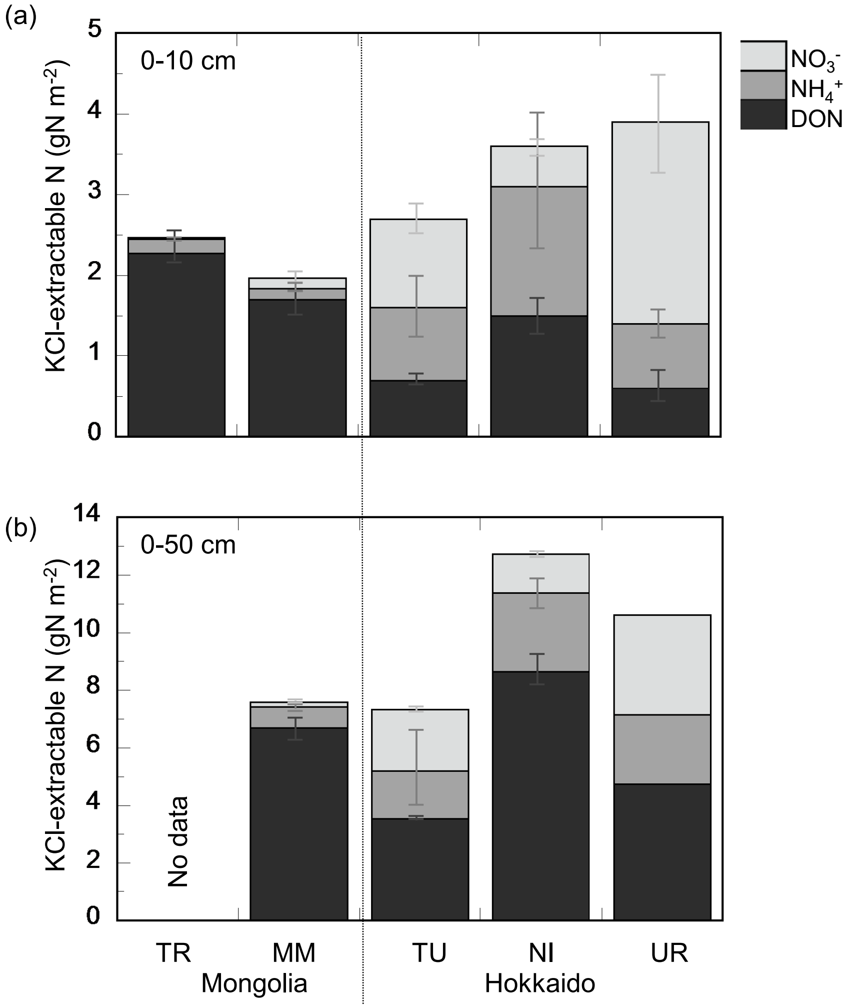

Fig. 4. KCl-extractable N (DON, NH_4^+, NO_3^-) in each area in Mongolia and Hokkaido. The values in each area are the averages of all sites which were measured in August 2012 in Mongolia and June 2012 in Hokkaido. Bars represent standard error of the mean.

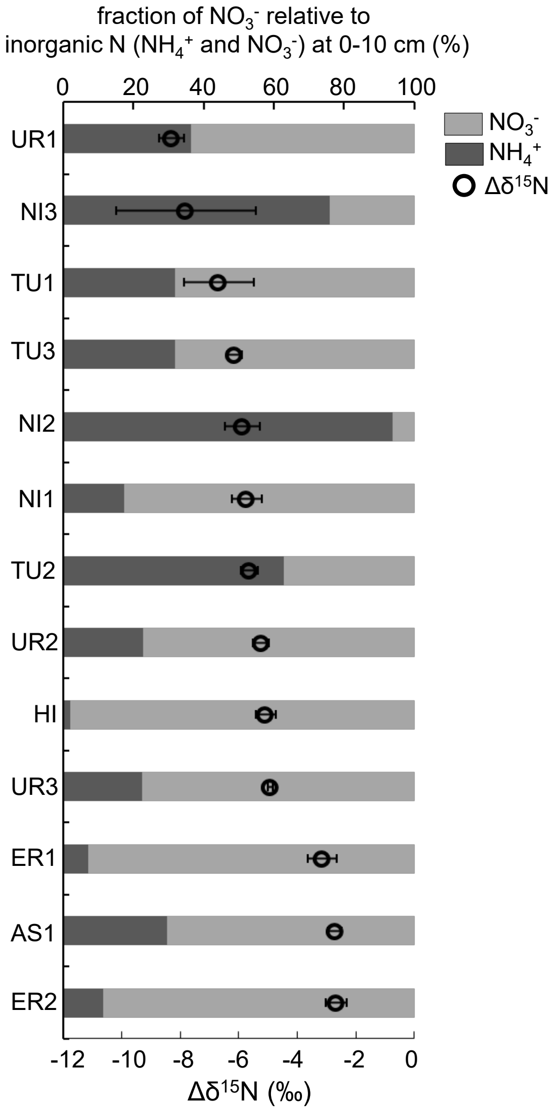

Fig. 5. The fraction of KCl-extractable NO_3^- relative to inorganic N (NH_4^+ and NO_3^-) content in 0-10 cm soil layer (bars) and $\Delta\delta^{15}N$ in Hokkaido (circle).
Correlation coefficient between the fraction of NO_3^- and $\Delta\delta^{15}N$ is $r_s = 0.687$, which is significant at $p < 0.05$. Bars in $\Delta\delta^{15}N$ represent standard error of the mean.

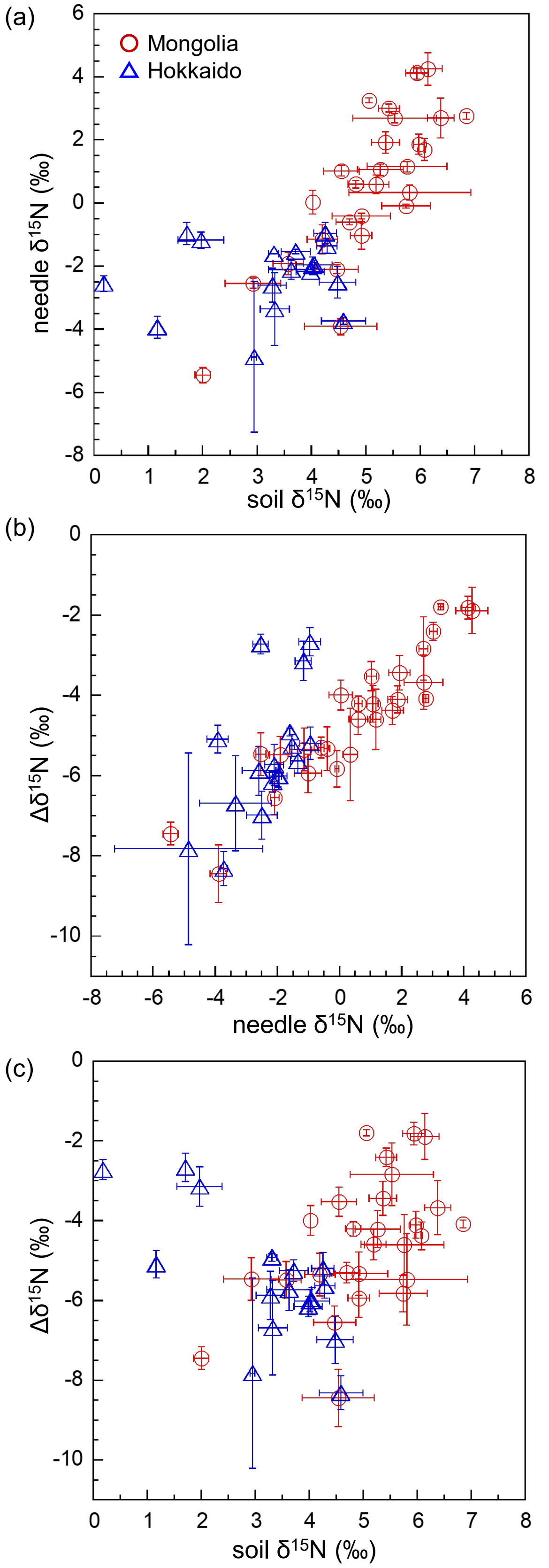

Fig. 6. Correlations between soil δ^{15}N and needle δ^{15}N (a), needle δ^{15}N and Δ δ^{15}N (b), soil δ^{15}N and Δ δ^{15}N (c) at all sites in Mongolia (circles) and Hokkaido (triangles).
Correlation coefficients are $r_s = 0.795$ in Mongolia (a), $r_s = 0.908$ in Mongolia and $r_s = 0.578$ in Hokkaido (b), and $r_s = 0.504$ in Mongolia and $r_s = -0.600$ in Hokkaido (c), which are significant at $p < 0.05$. Bars represent standard error of the mean.

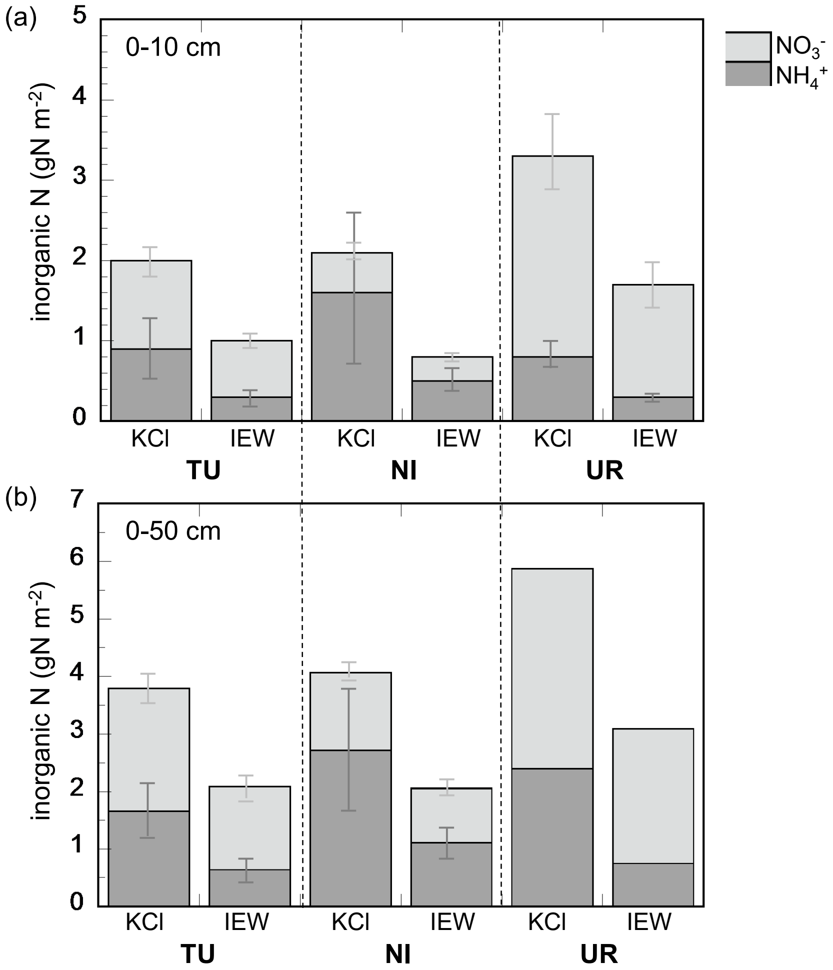

Fig. S1

Comparison of KCl-extractable and IEW-extractable inorganic N (NH_4^+ and NO_3^-) in three areas (TU, NI, and UR) in Hokkaido.

The values in each area are the averages of all sites. Bars represent standard error of the mean.

Table S1

Detailed information of study areas in Mongolia and Hokkaido, including site name and date of sampling at each site.

Region	Area name (Area code)	Site name	Latitude (°N)	Longitude (°E)	Elevation (m ASL)	Date of Sampling
Mongolia	**Tsagaannuur (TG)**	TGw1e	51.352	99.300	1684	August12, 2012
		TGw2e	51.353	99.306	1649	August10, 2012
		TGw3e	51.355	99.302	1648	August10, 2012
		TGd1e	51.340	99.308	1702	August11, 2012
		TGd2e	51.340	99.314	1641	August11, 2012
	Hatgal (HG)	HG1n	50.422	100.167	1705	August6, 2012
		HG2n	50.422	100.167	1700	August6, 2012
	Arbulag (AB)	AB1n	49.871	99.520	2074	August16, 2012
		AB2n	49.872	99.526	2046	August16, 2012
	Murun (MR)	MR1nw	49.654	100.354	1744	August17, 2012
		MR2nw	49.655	100.354	1701	August17, 2012
	Terelj* (TR)	TR1n	47.973	107.425	1750	July17, 2005
		TR2n	47.974	107.425	1683	August21-22, 2012
			47.975	107.425		August21-22, 2012
			47.975	107.425		August21-22, 2012
		TR3n	47.975	107.425	1650	June30, 2004; July4, 2004; July27, 2004; July17, 2005; July22, 2005
			47.976	107.430		June30, 2004; July4, 2004; July27, 2004; July17, 2005; July22, 2005
		TR4n	47.977	107.425	1627	August22, 2012
		TR5n	47.979	107.425	1606	July17, 2005; June25, 2010; August11, 2011; August21, 2012
		TR6n	47.979	107.425	1595	June30, 2004; July4, 2004; July27, 2004; July22, 2005; August21, 2012
		TR7n	47.980	107.425	1591	August22, 2012
		TR1s	47.991	107.414	1791	June30, 2004; July4, 2004; July27, 2004; July22, 2005; June25, 2010; August18, 2011
	Mongonmorit* (MM)	MM1s	48.351	108.658	1619	August24, 2012
		MM2s	48.350	108.657	1612	July3, 2004; July30, 2004; July20, 2005
		MM3s	48.349	108.657	1607	July20, 2005; August2, 2008; August24, 2012
		MM4s	48.347	108.656	1590	July20, 2005; August2, 2008; August24, 2012
		MM5s	48.344	108.654	1568	July20, 2005; August2, 2008; August24, 2012
		MM6s	48.341	108.654	1548	July20, 2005; August2, 2008; August24, 2012
		MM7s	48.338	108.656	1525	July20, 2005; August2, 2008; August24, 2012
		MM2sw	48.352	108.654	1615	July20, 2005
Hokkaido	**Erimo (ER)**	ER1	42.088	143.273	283	Sep19, 2011
		ER2	42.093	143.269	226	Sep19, 2011
	Samani (SA)	SA1s	42.177	142.976	71	August21, 2013
		SA2s	42.177	142.976	67	August21, 2013
	Urakawa (UR)	UR1sw	42.281	143.022	354	June13, 2012
		UR2sw	42.281	143.022	352	June13, 2012
		UR3sw	42.281	143.022	348	June13, 2012; Sep20, 2011
	Hiroo (HI)	HI1s	42.383	143.065	370	Sep20, 2011
	Nishihiroo (NI)	HIn1sw	42.398	143.187	189	June13, 2012
		HIn2sw	42.397	143.186	186	June13, 2012
		HIn3sw	42.397	143.186	183	June13, 2012
	Ashoro (AS)	ASm1	43.390	143.332	398	Sep21, 2011
	Tsubetsu (TU)	TU1nw	43.482	144.019	501	June14, 2012
		TU2nw	43.483	144.019	501	June14, 2012
		TU3nw	43.483	144.019	501	June14, 2012
	Takinoue (TA)	TA1w	44.208	143.068	221	August20, 2013
		TA2w	44.208	143.068	213	August20, 2013

* needle and soil $\delta^{15}N$ in TR and MM areas have been previously reported in Fujiyoshi et al. (2017)

Table S2

Annual precipitation from 2002 to 2015 in study areas[1]

	Mongolia				Hokkaido							
Year	**TG**	**HG**	**TR**	**MM**	**ER**	**SA**	**UR**	**HI**	**NI**	**AS**	**TU**	**TA**
2002	NA[2]	161	308	243	2161	1426	1426	1904	1904	1185	832	861
2003	NA	412	354	277	2194	1766	1766	1450	1450	1414	626	657
2004	NA	251	303	234	2087	1660	1660	1547	1547	1216	831	990
2005	NA	305	320	187	2157	1533	1533	1591	1591	1437	651	854
2006	NA	343	254	288	2223	1939	1939	1663	1663	1535	1184	1079
2007	NA	NA	259	236	1880	1501	1501	1744	1744	1169	666	636
2008	213	NA	338	311	1600	1585	1585	1185	1185	847	481	555
2009	314	NA	243	287	3034	2651	2651	2228	2228	1553	821	1003
2010	NA	NA	240	237	2187	1928	1928	1748	1748	1603	834	878
2011	NA	NA	334	311	1816	1887	1887	1499	1499	1681	782	1095
2012	NA	NA	358	352	2283	1579	1579	1882	1882	1617	904	1094
2013	NA	NA	NA	NA	2224	1756	1756	1900	1900	1435	834	931
2014	NA	NA	NA	NA	1730	1524	1524	1530	1530	1052	696	1089
2015	NA	NA	NA	NA	2269	1604	1604	1605	1605	1160	847	965

1) Annual precipitation in AB and MR area in Mongolia were not available from 2002 to 2015.

2) NA: not available