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

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

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

50

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

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54

55 **1. Introduction**

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

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

72 On the other hand, it has been shown that soil $\delta^{15}\text{N}$ generally increases with depth, from
73 organic layer which is typically observed in forest, to mineral soil [11]. Previous studies
74 also showed that the organic layer contributes as significant nitrogen source for trees
75 [12, 13]. Furthermore, recent global syntheses in $\delta^{15}\text{N}$ values have shown the spatial
76 trends in $\delta^{15}\text{N}$ values of plant and soil along climate gradient [14–16].

77 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
78 an apparent enrichment factor, allows the comparison of different sites by normalizing
79 the spatial heterogeneity in soil $\delta^{15}\text{N}$ [17]. $\Delta\delta^{15}\text{N}$ has been widely applied, and variations
80 in $\Delta\delta^{15}\text{N}$ have been attributed to various causes such as differences in N forms that plant
81 uptake (DON , NH_4^+ , or NO_3^-) [17–22] and changes in $\delta^{15}\text{N}$ of each N form affected by
82 nitrification [17,18,23,24]. Mycorrhizal fungi have also been reported to affect $\delta^{15}\text{N}$
83 through ^{15}N -enrichment in the fungal body [25], although the degree of enrichment is
84 still controversial [26]. Not only these internal processes within the plant-soil system,
85 but also atmospheric N deposition that serve as the N source for plants [27], has been
86 reported to cause variations in $\Delta\delta^{15}\text{N}$. Additionally, the strategy of field observations
87 often complicates the interpretation of $\Delta\delta^{15}\text{N}$. Previous studies set wide spatial scales
88 (region, country, or globe) along the gradient of specific environmental variables
89 (climate, topography, soil age, and parent material), which hampered the interpretation

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

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

102

103

104 **2. Materials and methods**

105 **2.1. Site description**

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

108 locations and positions is presented in Table 1. The forest consists of Siberian larch
109 (*Larix sibirica* Ledeb.) and white birch (*Betula platyphylla* Sukach.) in some places
110 [28,29]. This region corresponds to the southern boundary of permafrost [29], which
111 coincides with the distribution of boreal forest. The forest dominates the north-facing
112 slopes, whereas temperate grassland dominates the south-facing slopes, dry valleys, and
113 flat plains [29]. The soil in this region is cryosol, and the climate is cold continental
114 climate with dry winters, according to Köppen-Geiger climate classification [30]. The
115 mean annual temperature (MAT) is -3.6°C in TR area and -2.9°C in MM area, and
116 average temperature from May to September is 10.4°C in TR area and 11.2°C in MM
117 area. The mean annual precipitation (MAP) is 353 mm in TR area and 272 mm in MM
118 area, whereas 90% of precipitation occurs during the growing season of larch trees
119 (May to September) [28].

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

126 transects were set on the south-facing slope and southwest-facing slope and sampling
127 was conducted at 10 sites (MM1sw to MM7s) (Figure 1(c)). Among the 21 sites in total,
128 6 sites in TR area (TR1n to TR6n) and 6 sites in MM area (MM1sw to MM3s) were
129 forest sites, and the rest were boundary sites, except for patchy forest (TR1s) and
130 grassland site with no trees (TR2s) in TR area. Both TR and MM areas included
131 forest-grassland gradient, however MM area had more boundary sites with sparser tree
132 distribution on south-facing slope. Observations at those sites in these two areas covered
133 all range of forest-grassland gradient, and also wide range of conditions regarding
134 organic layer accumulation (litter, fermentation, and humus), which was relatively rich
135 in forest sites (mor-type) to poor in boundary sites (mull-type) [32].
136 [Figure 1 and Table 1 near here]

137

138 **2.2. *Sampling***

139 2.2.1. *Foliar samples*

140 Larch needles were collected during the growing season (May to August) from 2004 to
141 2012. Needles from three to four branches at a height of 1 to 5 m were taken from each
142 tree. More than three trees were usually sampled at each site, but only one or two trees
143 in boundary sites due to their limited number. Needles were also collected from several

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

148 2.2.2. *Soil samples*

149 Soil samples were collected at the same sites as needle samples. A small pit (0.6 m × 0.6
150 m × 0.6 m deep) was made, and one to three cores (1.5 cm diameter, 4.5 cm length) of
151 bulk soil were collected from the cross section of the pit every 10 cm from 0 cm (the top
152 of mineral soil) down to 50 cm depth or until a rock appeared. The organic layer was
153 also sampled by collecting the organic matter above the mineral soil. The fresh soil
154 samples were sieved with a 2 mm mesh to remove gravel and living roots, oven-dried at
155 105°C for more than 24 h, and used for isotope analysis. Fresh soil samples collected in
156 2012 were also used for KCl-extractable N (DON, NH₄⁺, and NO₃⁻) analysis.

157

158 2.3. *Analysis*

159 2.3.1. *C/N isotopic ratio and concentrations*

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

162 Environmental Science, Hokkaido University, Japan. The isotope ratio was expressed

163 using the δ notation:

$$164 \quad \delta^{15}\text{N} \text{ (or } \delta^{13}\text{C}) = \left(\frac{R_{\text{sample}}}{R_{\text{std}}} - 1 \right) \times 1000 \text{ (‰)}$$

165 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

166 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

167 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

168 concentration, and 0.1% for the bulk N concentration.

169 2.3.2. *KCl-extractable N*

170 Soil N pools (DON , NH_4^+ , and NO_3^-) were extracted from 4 g of fresh soil with 40 ml of

171 2M KCl after 1 h of shaking and filtration. The extracts were kept in coolers,

172 transported to the laboratory, and stored in a freezer until analysis. The concentration of

173 NO_3^- , NH_4^+ , and total dissolved N (TDN) was analysed colorimetrically using a

174 continuous flow nutrient analyser (QuAatro; BRAN+LUEBBE, Hamburg, Germany).

175 Then, the concentration of dissolved organic N (DON) was calculated by subtracting

176 total inorganic N (NO_3^- and NH_4^+) from TDN. The concentration of nitrite (NO_2^-) was

177 also analysed, but not detected in any samples.

178 2.3.3. *Calculation of average values at each site*

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

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

187 2.3.4. *Isotope mass balance on available N*

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

$$191 \quad F_{\text{input}} + F_{\text{a}} = F_{\text{p}} + F_{\text{m}} + F_{\text{leach}} + F_{\text{gas}} \quad (1)$$

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

$$193 \quad F_{\text{input}} \times \delta_{\text{input}} + F_{\text{a}} \times \delta_{\text{a}} = F_{\text{p}} \times \delta_{\text{p}} + F_{\text{m}} \times \delta_{\text{m}} + F_{\text{leach}} \times \delta_{\text{leach}} + F_{\text{gas}} \times \delta_{\text{gas}} \quad (2)$$

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

198 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,
199 produced N from soil organic matter, plant uptake, immobilization by soil
200 microorganisms, leaching, and gaseous emission of available N, respectively. We
201 assumed that larch was the only plant species (i.e. δ_{p} = needle $\delta^{15}\text{N}$), and that the $\delta^{15}\text{N}$ of
202 available N produced in the soil was the same as that of the soil (i.e. δ_{a} = soil $\delta^{15}\text{N}$).
203 Generally, available N produced in the soil has similar $\delta^{15}\text{N}$ to that of soil organic matter,
204 if all produced N remains in the N pool without any fractionated loss [33]. The
205 schematic representation of each process is shown in Figure S2.

206 2.3.5. *Statistical analysis*

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

210

211

212 **3. Results**

213 **3.1. *Temporal variations in $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and N concentration of larch needles***

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

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

223 [Figure 2 near here]

224

225 **3.2. Vertical profile of larch needle, organic layer, and bulk soil $\delta^{15}\text{N}$**

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

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

236

237 **3.3. *Spatial variations along the forest-grassland gradient***

238 Characteristic spatial patterns in $\delta^{15}\text{N}$ values, needle $\delta^{13}\text{C}$, and needle N concentration
239 were observed along the forest-grassland gradient in TR and MM areas (Figure 4). In
240 TR area, needle $\delta^{15}\text{N}$ increased gradually from -3.9‰ at the forest site (TR1n) to +3.3‰
241 at the boundary site (TR8n) on the north-facing slope, whereas on the south-facing
242 slope, needle $\delta^{15}\text{N}$ at TR1s in the patch forest (+1.2‰) was similar to that at TR6n and
243 slightly lower than that at TR8n. In contrast to needle $\delta^{15}\text{N}$, soil $\delta^{15}\text{N}$ slightly varied
244 from +2.9‰ at TR2n to +5.3‰ at TR7n on the north-facing slope, whereas it was
245 higher on the south-facing slope at TR1s (+5.8‰) and TR2s (+7.4‰). The values of
246 $\Delta\delta^{15}\text{N}$ showed the same pattern as needle $\delta^{15}\text{N}$ on the north-facing slope (Figure 4(b));
247 $\Delta\delta^{15}\text{N}$ was larger in the forest (-8‰ at TR1n) and smaller in the boundary (-3‰ at
248 TR7n) on the north-facing slope, whereas $\Delta\delta^{15}\text{N}$ at TR1s on the south-facing slope
249 (-5‰) was similar to that at TR5n. On the north-facing slope, needle $\delta^{13}\text{C}$ increased
250 from -27.8‰ to -26.2‰ from the forest site (TR1n) to the boundary site (TR8n), and
251 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

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

274 [Figure 4 near here]

275

276 **3.4. Relationships between $\delta^{15}\text{N}$ values and other parameters**

277 Significant correlations between different variables of all the sites in TR and MM areas
278 were observed (Figure 5). The correlation was observed between needle $\delta^{13}\text{C}$ and needle
279 $\delta^{15}\text{N}$ ($r_s = 0.877$) (Figure 5(a)), soil $\delta^{15}\text{N}$ and needle $\delta^{15}\text{N}$ ($r_s = 0.718$) (Figure 5(b)),
280 needle N concentration and $\Delta\delta^{15}\text{N}$ ($r_s = 0.591$) (Figure 5(c)), and C/N ratio of bulk soil
281 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
282 -2‰), high needle $\delta^{15}\text{N}$ (+0.34‰ to +4.3‰), high soil $\delta^{15}\text{N}$ (+5.1‰ to +6.8‰), whereas
283 in TR area showed larger variations in those values.

284 [Figure 5 near here]

285

286

287 **4. Discussion**

288 In this study, we observed large spatial variations in needle $\delta^{15}\text{N}$ and the difference
289 between needle and soil $\delta^{15}\text{N}$ ($\Delta\delta^{15}\text{N}$) at each area, whereas relatively small variation in
290 soil $\delta^{15}\text{N}$ (Figure 5(b)). The range of $\Delta\delta^{15}\text{N}$ observed in this study (6‰) (Figure 5(b))
291 accounts for a half of the global latitudinal variation [14], despite the same plant species
292 (*L. sibirica* Ledeb.), soil age, land use history, and climate. The spatial variation in
293 $\Delta\delta^{15}\text{N}$ indicates that the N cycle changes along the forest-grassland gradient. Here, the
294 isotope mass balance of available N in the soil was applied to understand the
295 mechanism of variation in $\Delta\delta^{15}\text{N}$.

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

$$300 \quad F_a = F_p + F_m \quad (3)$$

$$301 \quad \delta_a = \delta_s = f \times \delta_p + (1-f) \times \delta_m \quad (4)$$

302 where δ_s is soil $\delta^{15}\text{N}$, and f is the fraction of available N which is transported to the
303 plants ($f = F_p/F_a$). Namely, under a steady state condition, available N produced in the
304 soil is transported to plants and microorganisms. We assume that larch is the only plant
305 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

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

310 From these equations, two processes could explain the small difference in $\delta^{15}\text{N}$
311 between larch needle and soil ($\Delta\delta^{15}\text{N} \cong 0$) observed at boundary sites (Figure 5(b)): (1)
312 available N is transported to larch and/or microorganisms without significant
313 fractionation ($\delta_a = \delta_p = \delta_m$), or (2) almost all available N is transported to larch ($f \cong 1$).
314 However, N immobilization by soil microorganisms (F_m) might not be a suitable
315 explanation, because it is generally accepted that immobilization is associated with
316 significant isotope fractionation, causing higher $\delta^{15}\text{N}$ in microorganisms than that of
317 substrate organic N [34,35]. Therefore, it is reasonable to consider that the observed
318 small $\Delta\delta^{15}\text{N}$ at boundary sites show that available N produced in the soil is mostly
319 transported to larch without significant fractionation. In contrast, large difference in
320 $\delta^{15}\text{N}$ between larch needle and soil ($\Delta\delta^{15}\text{N} < 0$) in forest sites (Figure 5(b)) suggests that
321 the ^{15}N -depleted part of available N is transported to larch, whereas the ^{15}N -enriched
322 part of available N is immobilized in soil microorganisms ($\delta_p < \delta_m$). This process is
323 consistent with previously observed ^{15}N enrichment in soil microorganisms during

324 immobilization [34,35], or with ^{15}N enrichment in the mycorrhizal fungal body and
325 ^{15}N -depletion in the host plant [36,37]. In fact, the observed magnitude of $\Delta\delta^{15}\text{N}$ at
326 TR1n (8‰) was within the range of isotopic fractionation caused by ectomycorrhizal
327 fungi (5‰ to 9‰) [38], although such large enrichment is not always observed [26].
328 Interpretations of $\Delta\delta^{15}\text{N}$ described above are also supported by other observed data. At
329 boundary sites, a high needle N concentration, a low C/N ratio of bulk soil, and a high
330 needle $\delta^{13}\text{C}$ were observed (Figure 5(a), 5(c), and 5(d)). Foliar N concentration and $\delta^{13}\text{C}$
331 have been frequently used as indicators of N availability for trees [39] and light and
332 moisture conditions for plants [40], whereas the soil C/N as an indicator of the
333 decomposition rate of soil organic matter [41]. Therefore, these parameters as well as
334 the high NO_3^- to NH_4^+ ratios at boundary sites in MM area (Table S1) indicate a rapid
335 decomposition of soil organic matter and high N availability for larch under sunny and
336 dry conditions. On the other hand, at forest sites forest, a low needle N concentration, a
337 high C/N ratio of bulk soil, and a low needle $\delta^{13}\text{C}$ were observed (Figure 5(a), 5(c), and
338 5(d)), indicating the slow decomposition of soil organic matter and low N availability
339 for larch under shady and relatively wet conditions. Our results suggest that the
340 quantitative importance of the immobilization of available N, which is in agreement
341 with a previous study that showed the severe competition of available N between plant

342 and soil microorganisms in taiga forest ecosystem [42].

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

346 Generally, recycled N within the plant-soil system provides the primary source of N for
347 biological activities in most terrestrial ecosystems [43]. Although there is not enough
348 data to evaluate the N budget at our study sites, we assume that recycled N is the
349 dominant flux compared with the input and loss fluxes in the plant-soil system. In taiga,
350 the annual N demand of larch has been reported at 1,500 mg N m⁻² yr⁻¹ near at Tura in
351 central Siberia [44], and 850 to 3,100 mg N m⁻² yr⁻¹ at Yakutsk in northeastern Siberia
352 [42]. The estimated N loss as leaching (< 10mg N m⁻² yr⁻¹) at Tura [44] and N input as
353 deposition (48 mg N m⁻² yr⁻¹) at Yakutsk [42] were much smaller than the N demand.

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

356 Furthermore it is unlike that N deposition causes spatial variation in δ¹⁵N observed at
357 such small spatial scale (less than 2 km) in each area, because the amount and isotopic
358 composition of N deposition may be similar at all sites in each area. Although an uptake
359 of atmospheric deposited N by forest canopy has been reported to be a significant N

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

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

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

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

379 Recently, global syntheses of $\delta^{15}\text{N}$ values of plant and soil have been done by
380 comparing the $\delta^{15}\text{N}$ data with climate variables such as mean annual temperature (MAT)
381 and precipitation (MAP). Amundson et al. [14] compiled the $\delta^{15}\text{N}$ values from previous
382 studies, performed regression analysis, and successfully formulated plant and soil $\delta^{15}\text{N}$
383 and their difference ($\Delta\delta^{15}\text{N}$) with MAT and MAP. In addition, Craine et al. [15]
384 compiled foliar $\delta^{15}\text{N}$ and showed that foliar $\delta^{15}\text{N}$ increased with MAT at a rate of
385 $0.23\text{‰ }^{\circ}\text{C}^{-1}$ for ecosystems with $\text{MAT} > -0.5^{\circ}\text{C}$, and decreased by 2.6‰ for every order
386 of magnitude increase of MAP. Similarly, Craine et al. [16] conducted an analysis with
387 compiled soil $\delta^{15}\text{N}$, and reported that soil $\delta^{15}\text{N}$ increased with MAT at a rate of
388 $0.18\text{‰ }^{\circ}\text{C}^{-1}$ for ecosystems with $\text{MAT} > 9.8^{\circ}\text{C}$, showed no with MAT below 9.8°C , and
389 decreased at a rate of 1.78‰ for every order of magnitude increase of MAP. Plant $\delta^{15}\text{N}$
390 values calculated for the MAT and MAP of TR and MM areas with the multi regression
391 equation by Amundson et al. [14] derive -1.1‰ for TR area and -0.8‰ for MM area,
392 and those values are close to the average for all sites in this study ($+1.5 (\pm 2.2)\text{‰}$).
393 Observed range in needle $\delta^{15}\text{N}$ (-3.9‰ to $+4.3\text{‰}$) also drops to the ranges of foliar $\delta^{15}\text{N}$
394 for both MAT and MAP as shown by Craine et al. [15]. Similarly, soil $\delta^{15}\text{N}$ values were
395 calculated to be $+3.2\text{‰}$ and $+3.4\text{‰}$ with MAT and MAP for both TR and MM areas by

396 the multi regression equation [14], and these values are also close to the average for all
397 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
398 also within the range reported by Craine et al. [16]. It is worth noting that a large
399 difference in $\delta^{15}\text{N}$ values was observed between forest and boundary sites, but average
400 $\delta^{15}\text{N}$ values observed for all sites, nevertheless, are close to the values obtained from
401 global syntheses.

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

409

410

411 **5. Concluding remarks**

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

414 difference between needle and soil $\delta^{15}\text{N}$ ($\Delta\delta^{15}\text{N}$) showed clear spatial variation along
415 the forest-grassland gradient with large $\Delta\delta^{15}\text{N}$ in the forest (up to -8‰) and small $\Delta\delta^{15}\text{N}$
416 in the grassland boundary (up to -2‰). The difference in $\delta^{15}\text{N}$ between larch and
417 mineral soil reflects the difference in the accumulation patterns of the organic layer. In
418 the grassland boundary, litter decomposes quickly and produced available N is
419 transported to larch without significant fractionation, whereas in the forest, larch trees
420 uptake available N with lower $\delta^{15}\text{N}$ than that produced in the soil. Namely, the
421 ^{15}N -depleted part is taken by larch, whereas the ^{15}N -enriched part remains in the soil,
422 which is balanced by ^{15}N -depleted litter provided by larch. The divergence of available
423 N between larch and microorganisms in the soil, and the accumulation of diverged N in
424 the organic layer control the variation in $\Delta\delta^{15}\text{N}$.

425

426

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646 Table 1. Locations of sampling sites.

Area name	code	Site No. ^a position	Latitude °N	Longitude °E	Elevation 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

647

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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660 Figure 1. A map of the two observation areas (TR and MM) and Ulaanbaatar, the capital
661 of Mongolia (a), and schematic figures of longitudinal cross sections in TR area (b) and
662 MM area (c). Samplings were conducted at Terej (TR) and Mongonmorit (MM) at the
663 forest-grassland boundary in northern Mongolia. In TR area, sampling was conducted at
664 11 sites along a transect line from the north-facing slope to the south-facing slope over a
665 valley. In MM area, two transects were set on the south-facing slope and the
666 southwest-facing slope, and sampling was conducted at 10 sites.

667

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

672

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

676

677 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.

681

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.

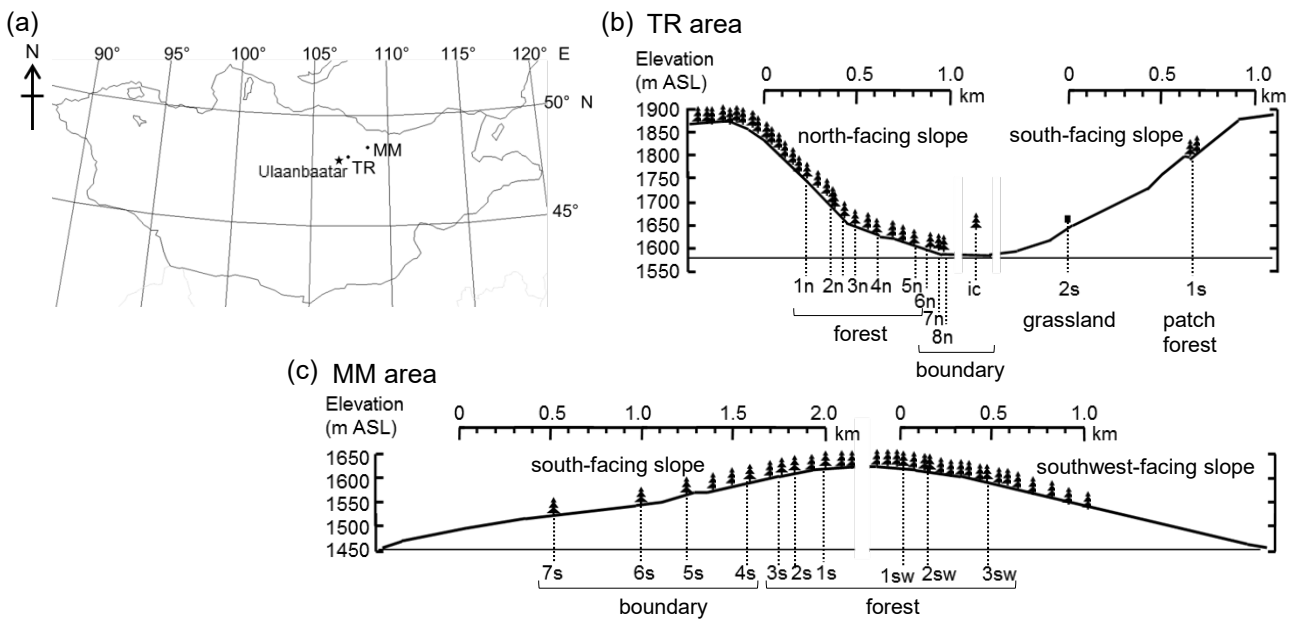


Figure 1.

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.

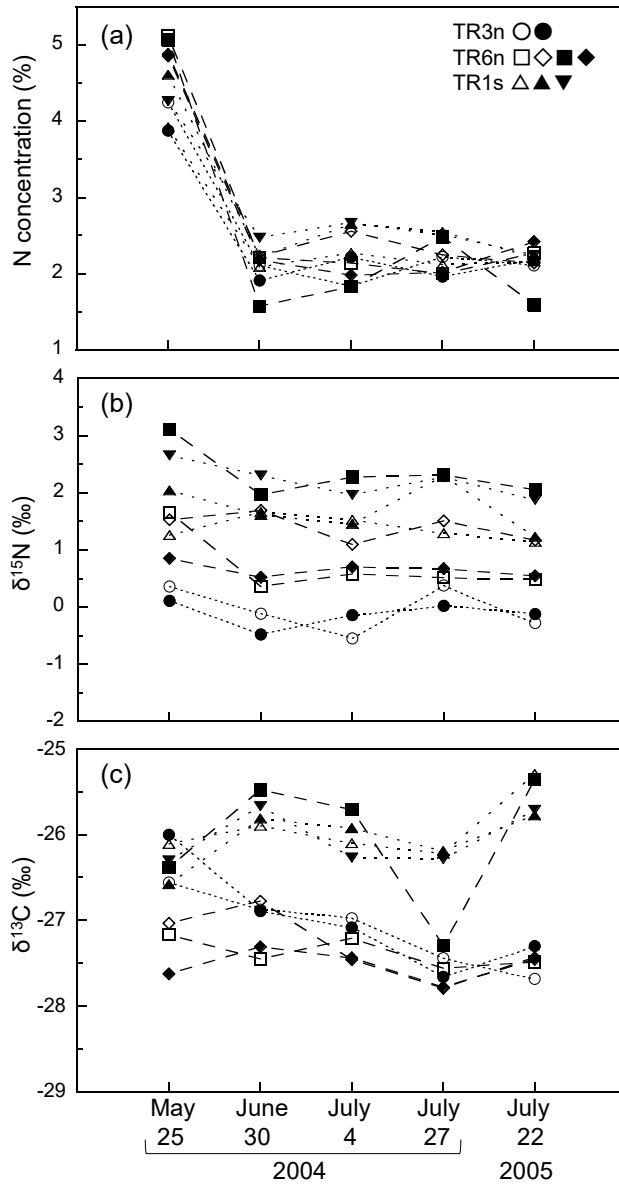


Figure 2.

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.

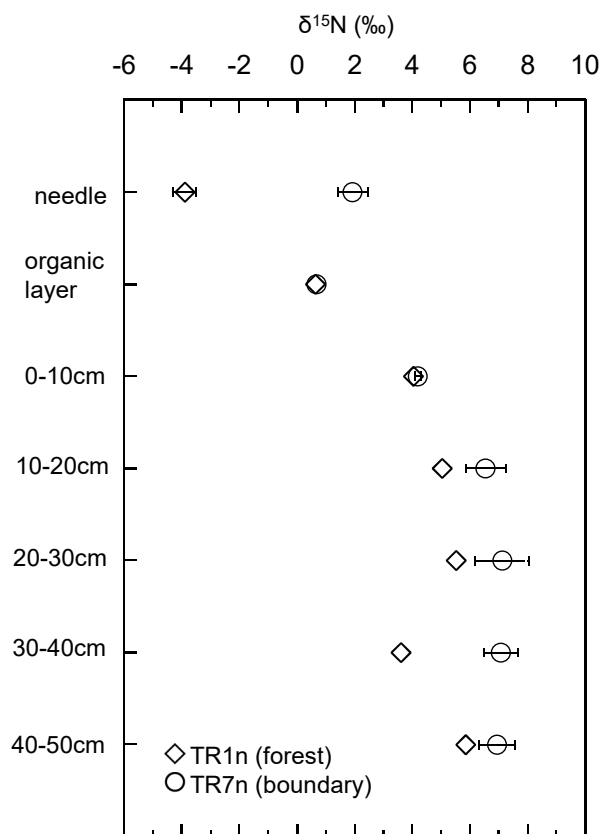


Figure 3.

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.

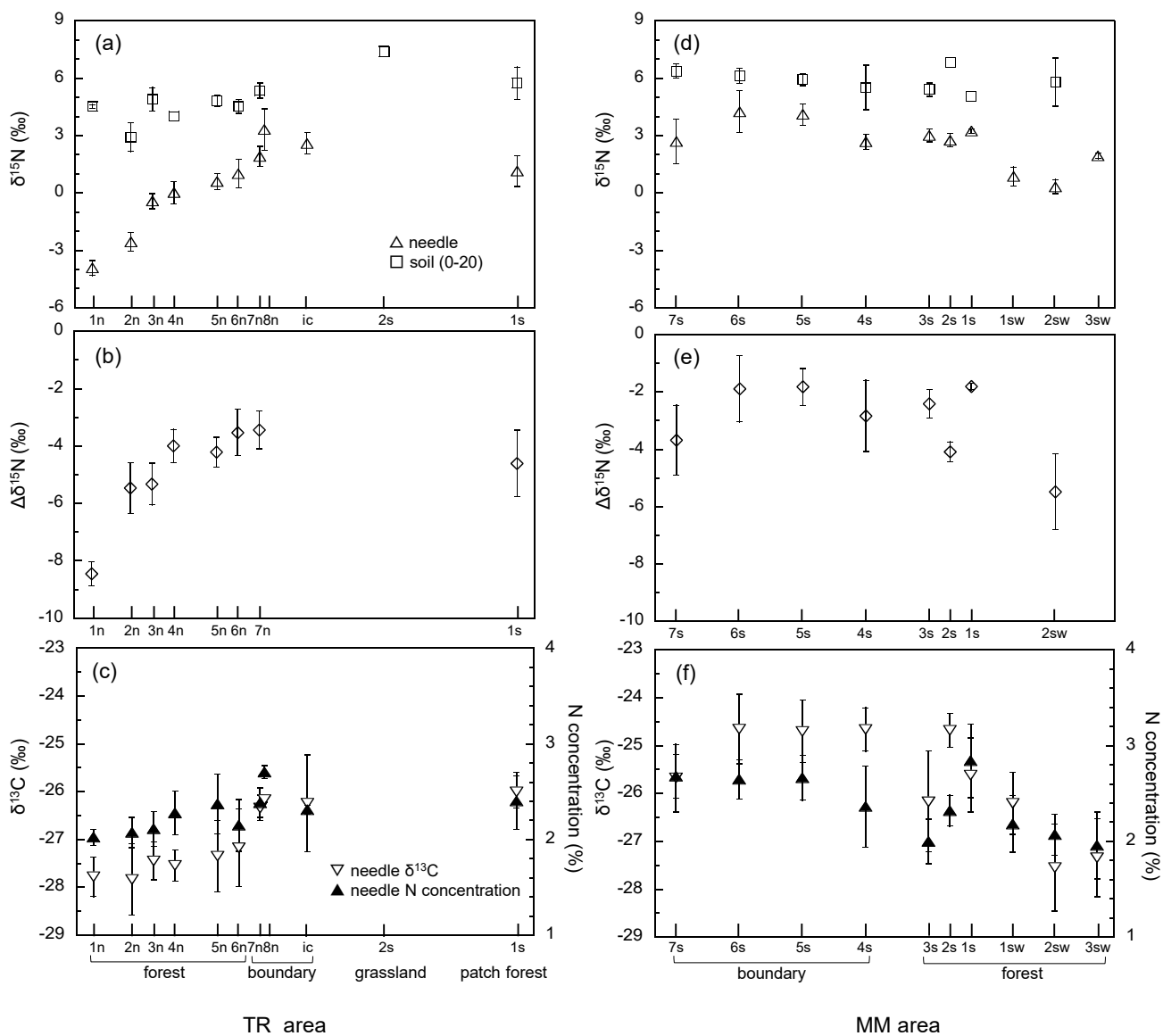


Figure 4.

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.

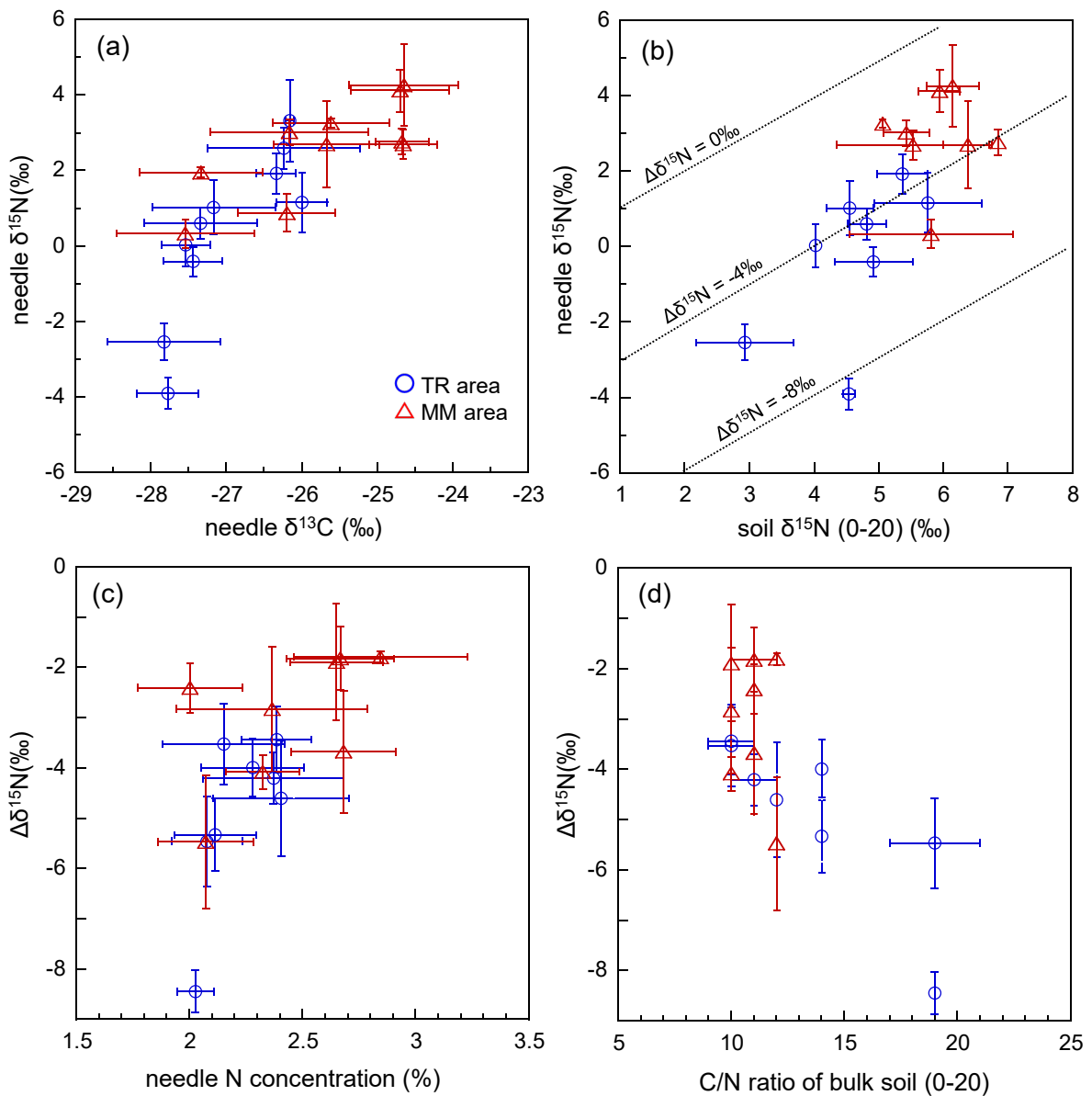


Figure 5.

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.

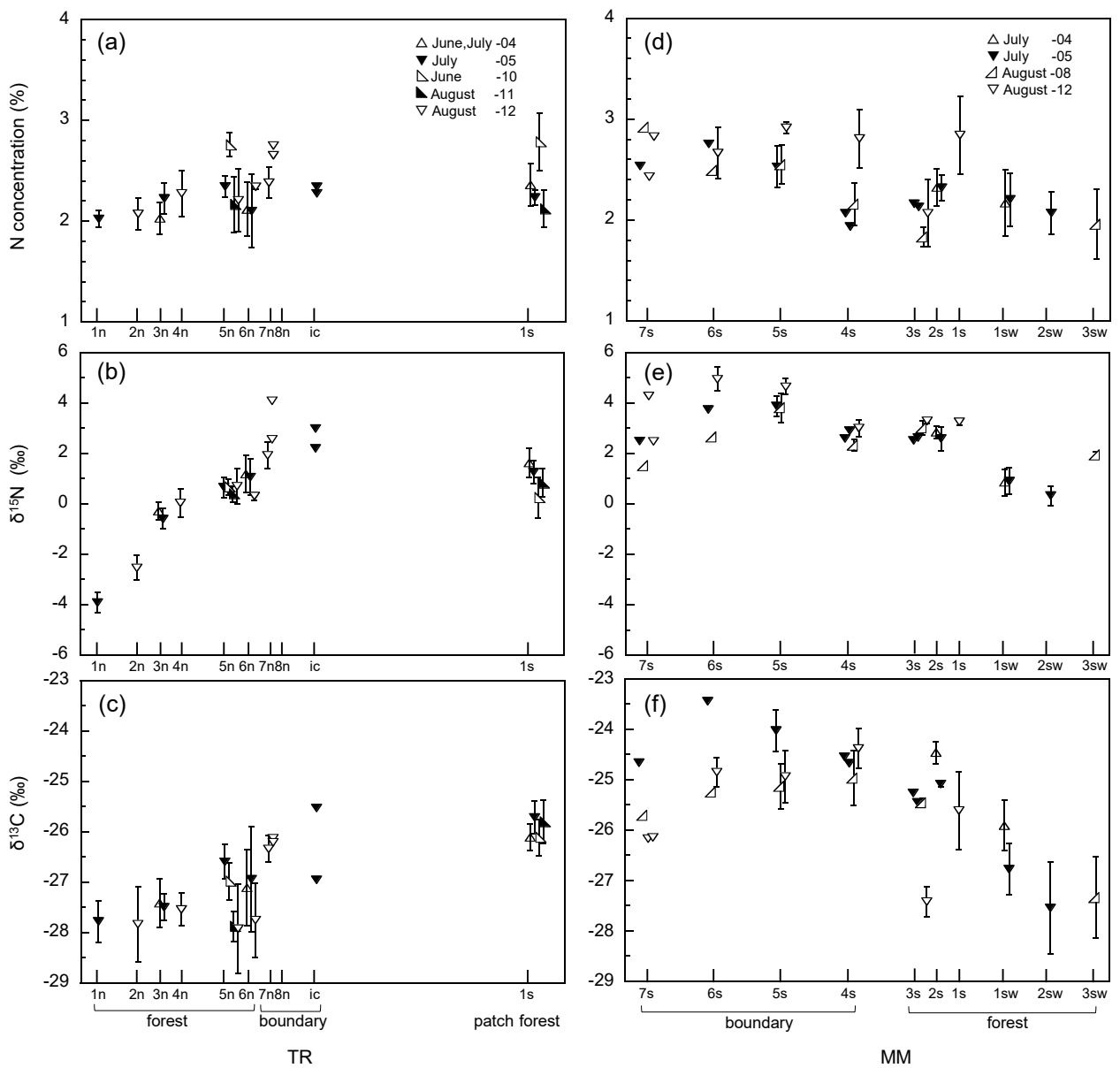


Figure S1.

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.

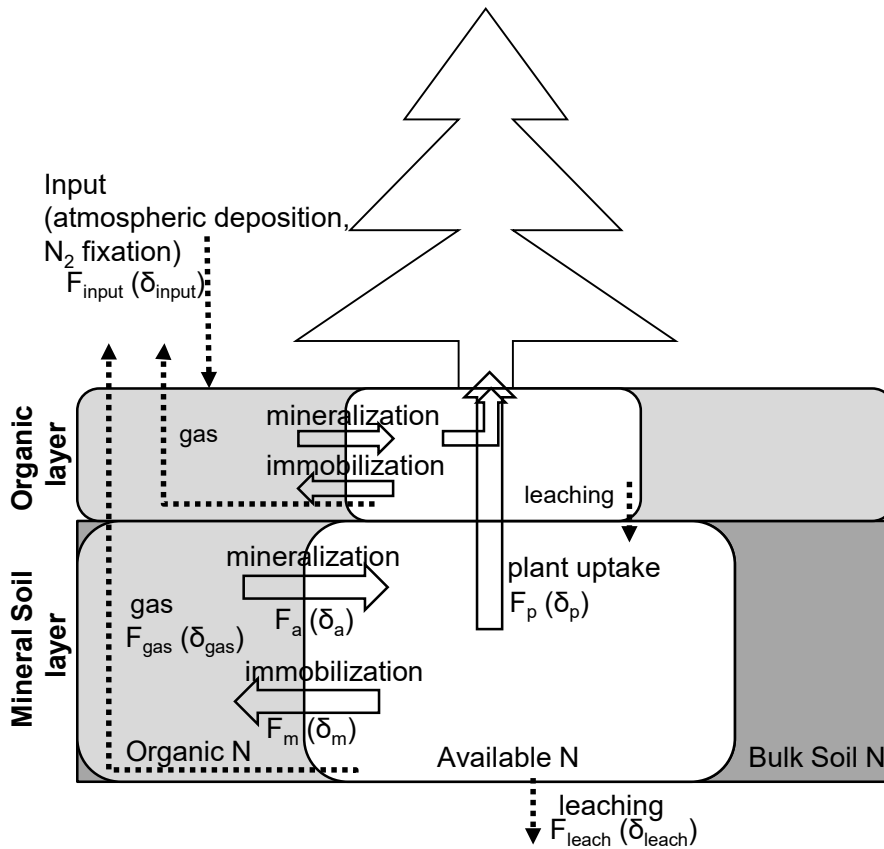


Figure S2.

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:

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

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. $\delta_{\text{p}} = \text{needle } \delta^{15}\text{N}$), and that the $\delta^{15}\text{N}$ of available N produced in the soil was the same as that of the soil (i.e. $\delta_{\text{a}} = \text{soil } \delta^{15}\text{N}$).