HOKKAIDO UNIVERSITY

Title	Study on nitrogen cycle with special reference to spatial variations in plant and soil nitrogen isotope ratios at forest-grassland boundary in northern Mongolia
Author(s)	藤吉, 麗
Degree Grantor	北海道大学
Degree Name	博士(環境科学)
Dissertation Number	甲第12417号
Issue Date	2016-09-26
DOI	https://doi.org/10.14943/doctoral.k12417
Doc URL	https://hdl.handle.net/2115/63814
Type	doctoral thesis
File Information	Lei_Fujiyoshi.pdf

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Study on nitrogen cycle with special reference to spatial variations in plant and soil nitrogen isotope ratios at forest-grassland boundary in northern Mongolia

（モンゴル北部森林-草原境界域における植物と土壌の窒素同位体比を用いた窒素サイクルの研究）

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Study on nitrogen cycle with special reference to spatial variations in plant and soil nitrogen isotope ratios at forest-grassland boundary in northern Mongolia

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DOCTORAL DISSERTATION

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September 2016

Abstract

Nitrogen isotope ratio ($\delta^{15}N$) of terrestrial plants and soils are recognized as indicators of N cycle, since isotopic fractionations during the processes in N cycle cause variations in these $\delta^{15}N$ values. Recent accumulation of $\delta^{15}N$ data for wide spatial areas has enabled to investigate the characteristics of $\delta^{15}N$ in large spatial scale from region to globe. These studies have revealed that the ecosystem state factors such as climate, soil age, topography, parent material, and cultivation contribute spatial variation of $\delta^{15}N$. However, the contribution of vegetation type, one of the ecosystem state factors, has not been evaluated yet. To interpret the spatial patterns of $\delta^{15}N$ into N cycle, the range of $\delta^{15}N$ variation with associated N process need to be evaluated for each factor. This study aimed to clarify the variations in plant and soil $\delta^{15}N$ and associated processes which arise from vegetation type. Six observation areas were set in the forest-grassland boundary in northern Mongolia, and sampling sites were set along forest to grassland boundary within an area. Each site was classified into forest site or boundary site, and needles of siberian larch (*Larix sibirica* Ledeb.) and soils were investigated. The different type of accumulation of organic layer was observed in this region between forest and boundary sites: “moder type” in forest site and “mull type” boundary site.

Needle and soil $\delta^{15}N$, and the difference in $\delta^{15}N$ between needle and soil ($\Delta\delta^{15}N$)

showed clear spatial trends along forest to grassland boundary in an area, and those trends were also consistent in all areas: needle and soil $\delta^{15}N$ were low in forest sites and high at boundary sites, whereas $\Delta\delta^{15}N$ was large in forest sites and small at boundary sites. The range of $\Delta\delta^{15}N$ observed in this region was 7‰ (-8.4‰ to -1.8‰), which occupied half of the estimated range in globe. The N cycle processes suggested by nitrogen isotope mass balance and observed environmental parameters matched the typical characteristics of moder type in forest sites and and mull type at boundary sites. Small $\Delta\delta^{15}N$ observed at grassland boundary was explained by rapid recycling of N between larch and soil without significant microbial immobilization, whereas large $\Delta\delta^{15}N$ observed in forest was explained by significant microbial immobilization which retained ^{15}N-riched part of available N in soil, and additional N supply from organic layer to larch. This study proposes the new relationship between the type of the accumulation of organic layer and the variation in the difference between plant and soil $\delta^{15}N$ ($\Delta\delta^{15}N$) through the divergence of available N between uptake by larch and immobilization by microbes along forest to grassland boundary.

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Chapter 1 Introduction

1.1. Nitrogen cycle in terrestrial ecosystem

Nitrogen is essential to all life on the earth to make significant component of nucleic acids, protein and other biomolecules that regulate a suite of cell functions. Total quantity of N on the earth is estimated as roughly 1.9 $\times 10^{11}$ Tg N (Hübner 1986), of which 98% are fixed in the lithosphere. The atmosphere contains 2% of the total quantity (3.9×10^{9} Tg N), and the terrestrial biosphere (3.3×10^{5} Tg N) contains less than 0.001% (Table 1.1). More than 99% of this N is not available to more than 99% of living organisms due to N form (Galloway et al. 2003). The most abundant form is N_2, which requires large energy to break the triple bond, hence nonreactive N. Whereas reactive N includes all biologically, photochemically, and radiatively active N compounds in atmosphere and biosphere which composed of reduced forms of N (ammonia [NH_3] and ammonium [NH_4^+]), inorganic oxidized forms (nitrogen oxide [NO_x], nitric acid [HNO_3], nitrous oxide [N_2O], and nitrate [NO_3^-]), and organic compounds (urea, amines, proteins, and nucleic acids).

On land, N cycle in terrestrial plant-soil system consists of internal and external process (Ågren and Andersson 2012) (Figure 1.1). Internal process means the recycling

of N between plant-soil system through litterfall, decomposition and production of biologically available N (NH_4^+, NO_3^-, and amino acids) by microorganisms taken up by plant again. On the other hand, external process is the N input to plant-soil system as N_2-fixation and atmospheric deposition, or N output from plant-soil system as leaching or gas emission. Generally, recycling of N in plant-soil system provides the primary source of N for biological activity in most terrestrial ecosystems (Parton et al. 2007).

Because of its demand for life, N have had their cycles significantly perturbed by human activities to sustain increasing population, and human activities have greatly increased the amount of reactive N that circulates through the land, air and water (Galloway et al. 2003). Once N_2 is converted into reactive N, it can be transported to any part of the system since reactive N is highly mobile, and this cascade through the environment contribute to several environmental impacts such as eutrophication and acidification, or ozone effect and greenhouse effect (Erisman et al. 2011; Bai et al. 2012). Reactive N also influences the climate system indirectly via its role in constraining CO_2 uptake and storage on land (Zaehle et al. 2010; Kroeze and Bouwman 2011). Furthermore, recent global warming will be expected to change the N cycle through change in soil microbial activity via increasing soil temperature or air temperature, especially in high latitude ecosystems (tundra and boreal ecosystems) (Xu

et al. 2011), but its effects is poorly resolved (Bernal et al. 2012). Because of these situation, assessment of N cycle is urgent, however, conventional field observation is still not easy to assess N cycle due to the following reasons (Kroeze et al. 2003); (1) some N fluxes cannot be measured directly and are usually quantified indirectly as the balance in N budgets, (2) direct measurements of N fluxes have inevitable inaccuracies, (3) lack of experimental data and other information (e.g. statistics) needed for upscaling, (4) large spatial and temporal variability of fluxes, and (5) poor understanding of the processes involved.

1.2. Principle of natural abundance of nitrogen isotope ratio ($\delta^{15}N$)

1.2.1. Definition

Isotopes are atoms of the same element that have different numbers of neutrons. Differences in the number of neutrons among the various isotopes of an element mean that the various isotopes have different masses. Nitrogen (N) has seven isotopes with number of neutrons of 5 to 11. Among these isotopes, stable isotopes are ^{14}N and ^{15}N, and the others are radioactive isotopes (Kendall and Caldwell 1998). Stable isotope composition of low-mass element N is normally reported as δ value and calculated by:

$$\delta^{15}N \text{ (in ‰)} = (R_{sa} / R_{std} - 1) \times 1000$$

where R denotes the ratio of the heavy to light isotope (^{15}N / ^{14}N), and R_{sa} and R_{std} are the ratios in the sample and standard, respectively. Standard means international reference standard and for nitrogen atmospheric air is used with $^{15}N/^{14}N$ abundance ratio of 3.677×10^{-3} (Kendall and Caldwell 1998). The $\delta^{15}N$ values in terrestrial materials are widely distributed roughly from -20‰ to +20‰ (Letolle 1988) (Figure 1.2). The $\delta^{15}N$ values of foliage of vascular plants in worldwide have been reported from -11 to +14‰ (Craine et al. 2009), and $\delta^{15}N$ value of surface mineral soils (< 30 cm) has been reported from -4 to +16‰ (Craine et al. 2015).

1.2.2. Isotope fractionation

Variations in $\delta^{15}N$ among materials reflect N isotope fractionation (Robinson 2001). These occur because more energy is needed to break or form chemical bonds involving ^{15}N than ^{14}N. On average, ^{14}N-containing molecules react faster than those containing ^{15}N (Robinson 2001). Biological processes which compose N cycle in plant-soil system are generally unidirectional (e.g. with an enzyme) and are kinetic isotope reactions. This fractionation results in significant difference in $\delta^{15}N$ between the substrate and the biologically mediated product (Table 1.2). Although kinetic isotopic fractionations of biologically-mediated processes change in magnitude, depending on reaction rates, concentrations of products and reactants, environmental conditions, in general, slower

reaction steps show greater isotopic fractionation than faster steps because the organism has time to be more selective (Kendall and Caldwell 1998). In plant-soil system, nitrogen transformations and fractionations associated with all fluxes into and out of plant and soil N pools influence soil and plant $\delta^{15}N$ values through combined effects of N isotope fractionation associated with those processes over time (Pardo and Nadelhoffer 2010).

1.3. Previous studies of plant and soil $\delta^{15}N$

1.3.1. $\delta^{15}N$ of N_2-fixing plant

For a long time from 1950s until now, plant $\delta^{15}N$ have been applied to clarify the relative contribution of different N sources for plant (atmospheric N_2 fixation, soil N, or fertilizer) (Shearer and Kohl 1986; Yoneyama et al. 1990; Schulze et al. 1991). Under the conditions that specific N source (tracer N) has no fractionation as it moves within the system, and other N sources have distinct $\delta^{15}N$ values from $\delta^{15}N$ of tracer N, mixing model can be applied for several N sources to quantify the fraction of tracer N in the plant (Robinson 2001). Recently, most studies applying this method aim to quantify the proportion of N_2 fixation compared to plant N demand in the agriculture field (Mercado et al. 2011; Mokgehle et al. 2014). The $\delta^{15}N$ value of biologically fixed N is 0‰,

whereas that of atmospheric N deposition ranges between -3 to +3‰ globally, although it is known that the $\delta^{15}N$ value of atmospherically derived N exhibits large temporal and spatial variations (Amundson et al. 2003).

1.3.2. Variations in plant and soil $\delta^{15}N$

1.3.2.1. Specific processes which cause variation in $\delta^{15}N$

The variations in non-N_2-fixing plants and soil $\delta^{15}N$ values have been received attention from 1990s, and the characteristics of those variations have been studied to clarify the mechanism of those variation associated with the processes in N cycle. For example, plant $\delta^{15}N$ values show wide variation among the different species (Michelsen et al. 1996; Nadelhoffer et al. 1996) and even within the same species (Garten 1993; Garten and Miegroet 1994). According to a review paper by Hobbie and Högberg (2012), the factors which affect plant $\delta^{15}N$ can be classified into four main factors: (1) physiological processes in plant, (2) rooting depth, (3) difference in N source, and (4) mycorrhizal fungi. First, for (1), difference in physiological processes such as uptake and assimilation of N among species causes variation in $\delta^{15}N$ values in spite of the same N source (Nadelhoffer et al. 1996; Handley et al. 1997; Yoneyama et al. 1997). Furthermore, if the concentrations of N sources are high relative to uptake rates, different degree of fractionation can be observed within the same species (Evans et al.

1996). As for (2), since soil $\delta^{15}N$ commonly shows variation with depth, variation in $\delta^{15}N$ sometimes attributed to differences in rooting depth (in the sense of uptake depth) (Kohzu et al. 2003). For (3), the kinetic fractionations during N decomposition processes from organic N to inorganic N in soil, followed by plant uptake of different forms of N (NH_4^+, NO_3^-, or organic N (amino acids or amino sugar)) affect plant $\delta^{15}N$ values as each form has distinct $\delta^{15}N$ values (Garten and Miegroet 1994; Nadelhoffer et al. 1996; Miller and Bowman 2002; Mayor et al. 2012). And for (4), mycorrhizal fungi influence plant $\delta^{15}N$ in several ways: biochemical reactions within fungi partition N into ^{15}N-enriched and ^{15}N-depleted pools and provide ^{15}N-depleted N into plants; increase plant access to recalcitrant forms of N (organic N) and alter the average $\delta^{15}N$ of the available N sources; increase N access for plant from ^{15}N-depleted surficial litter to ^{15}N-enriched soil at greater depths (Michelsen et al. 1996; Hobbie et al. 1999; Hobbie et al. 2000; Mayor et al. 2012). In addition to the above four factors, N input as atmospheric deposition (Garten 1993; Koopmans et al. 1997; Jung et al. 1997; Korontzi et al. 2000), and N loss as nitrate leaching (Johannison 1994; Hogbom et al. 2002) also affect plant $\delta^{15}N$ values through input or loss of distinct N which has different $\delta^{15}N$ from $\delta^{15}N$ of available N in soil.

Meanwhile, $\delta^{15}N$ values of undisturbed non-cultivated soils typically show increase

with soil depth (Mariotti et al. 1980; Gebauer and Schulze 1991; Högberg et al. 1996). Two processes have been proposed to explain the increase in soil $\delta^{15}N$ (Hobbie and Ouimette 2009): (1) Creation of ^{15}N-depleted N as products of decomposition processes (ammonification, nitrification, and denitrification) followed by leaching, gaseous losses, or uptake of that ^{15}N-depleted N by plants, and (2) Creation of ^{15}N-depleted N by mycorrhizal fungi and transfer of that N into plant. ^{15}N-depleted litter then accumulates at soil surfaces through litterfall, and ^{15}N-enriched N derived from mycorrhizal fungi accumulates at depth. As for (1), through isotope fractionations during the decomposition processes (Table 1.2), ^{15}N-enriched N remains as organic matter and ^{15}N-depleted N is produced and lost through plant uptake or leaching, which cause the increase in soil $\delta^{15}N$ (Mariotti et al. 1980; Gebauer et al. 1994; Nadelhoffer et al. 1996; Marty et al. 2011). For (2), field studies have observed that $\delta^{15}N$ values of mycorrhizal plants depleted relative to co-occurring non-mycorrhizal plants (Nadelhoffer et al. 1996; Michelsen et al. 1998), suggesting the creation and transport of ^{15}N-depleted N to plant by mycorrhizal fungi (Hobbie and Colpaert 2003). In fact, higher $\delta^{15}N$ in mycorrhizal fungal body compared to $\delta^{15}N$ of plant root have been reported, and suggested as the factor to increase soil $\delta^{15}N$ (Högberg et al. 1996). Similarly, N immobilization by soil microorganisms also cause ^{15}N-enriched N in the microorganism body compared to

surrounding soil organic matter (Dijkstra et al. 2008; Coyle et al. 2009), although its contribution to the variation of soil $\delta^{15}N$ has not been reported. In addition to above two, N input as biological N_2 fixation has been reported to affect soil $\delta^{15}N$ (Stock et al. 1995) through input of distinct N which has different $\delta^{15}N$ from $\delta^{15}N$ of N in soil.

In addition to plant $\delta^{15}N$ and soil $\delta^{15}N$, the difference between two values ($\Delta\delta^{15}N$), defined as (plant $\delta^{15}N$ - soil $\delta^{15}N$), has been proposed as a method that compares different sites by normalizing the spatial heterogeneity in soil $\delta^{15}N$ (Pardo et al. 2007). $\Delta\delta^{15}N$ represents the combined isotopic fractionation associated with the processes of production of plant available N from the total soil N pool and fractionation associated with N assimilation by plant, or represents the fractionation associated with plant available N production under the conditions that the fractionation of assimilation can be ignored, and N pools of plant and soil are steady state (Brenner et al. 2001). Four processes have been reported to cause the variation in $\Delta\delta^{15}N$: (1) difference in N source (Garten 1993), (2) N form which plants uptake (Garten and Miegroet 1994; Brenner et al. 2001; Averill and Finzi 2011; Brearley 2013), (3) rate of nitrification (Garten and Miegroet 1994; Schuur and Matson 2001; Kang et al. 2011), and (4) mycorrhizal fungi (Hobbie et al. 1999). For (1), Garten (1993) reported that plants in the more N deficit ridges use atmospheric-derived N and caused large $\Delta\delta^{15}N$, whereas plants in valley

bottom use soil-derived N and caused small $\Delta\delta^{15}N$. For (2), Averill and Finzi (2011) reported that $\Delta\delta^{15}N$ was large at the site in low elevation but large in high elevation, reflecting increasing organic N with uptake with elevation. Similarly, Brearley (2013) compared two distinct type of forest which differed the accumulation of organic layer (mor vs. mull) in Jamaica, and showed that larger $\Delta\delta^{15}N$ in mull-type forest than in mor-type forest, reflecting more fraction of NO_3^- compared to NH_4^+ as N source for the plants in mull-type forest. As for (3), Schuur and Matson (2001) reported that incomplete nitrification (i.e. a part of NH_4^+ is transformed to NO_3^-) caused larger $\Delta\delta^{15}N$ in wet place than in mesic place in montane forest in Hawaii, under assumption that plants uptake only NO_3^- form. As for (4), Hobbie et al. (1999) proposed that isotopic fractionation by mycorrhizal fungi during enzymatic reactions within the fungi produced isotopically depleted amino acids, and these ^{15}N-depleted N were passed on to plant, causing large $\Delta\delta^{15}N$ (Table 1.2).

1.3.2.2. Spatial trends in plant and soil $\delta^{15}N$

Recent data accumulation of plant and soil $\delta^{15}N$ in many places has enabled to observe the spatial distribution and characteristics of plant and soil $\delta^{15}N$. For example, Martinelli et al. (1999) showed high $\delta^{15}N$ values of plant and soil in tropical forest than in temperate forest. They attributed this difference to N cycle openness: both inputs and

outputs of N are large relative to N cycling within system in tropical forest than in temperate forest, and differences in both the magnitude and the pathways of N loss cause more ^{15}N enriched in tropical forest. A global scale study has shown that both plant $\delta^{15}N$ and soil $\delta^{15}N$ decrease with increasing MAP (mean annual precipitation) and decreasing MAT (mean annual temperature) along with latitude, with low $\delta^{15}N$ and large $\Delta\delta^{15}N$ in northern high latitude than in low latitude (Amundson et al. 2003) (Figure 1.3). Precipitation (Austin and Vitousek 1998; Schuur and Matson 2001; Ma et al. 2012; Peri et al. 2012; Zhou et al. 2014) or water availability (Handley et al. 1999; Murphy and Bowman 2009) itself has been also reported to cause simultaneous trends in both plant $\delta^{15}N$ and soil $\delta^{15}N$. However, the mechanisms of this trend are not consistent. Taking precipitation as an example, N losses as gas or leaching through nitrification and denitrification processes (Austin and Vitousek 1998; Schuur and Matson 2001; Amundson et al. 2003; Ma et al. 2012), loss as NH_3 volatilization (Ma et al. 2012), water use efficiency of plant (Peri et al. 2012), or soil C and N availability as substrate of microbial activity (Zhou et al. 2014) have been proposed. Furthermore, Amundson et al. (2003) suggested other factors which contribute spatial trend in $\delta^{15}N$: topography, parent material, soil age, and cultivation (Figure 1.3 (c)). Although these factors are also known as ecosystem state factors or soil forming factors (Jenny 1994),

one factor “organism”, especially vegetation type, has not been considered in Amundson et al. (2003).

1.4. Research aim and purpose

To clarify N cycle processes hidden in the spatial variations in plant and soil $\delta^{15}N$ which are reported worldwide, the range of $\delta^{15}N$ and associated N process need to be cleared for each ecosystem state factor. Therefore this study aimed to clarify plant and soil $\delta^{15}N$ variations and associated processes caused by vegetation type. To achieve this aim, forest-grassland boundary in northern Mongolia was focused on. Clear vegetation shift from forest (taiga) to grassland (steppe) in this ecotone and the dominance of single species (*Larix sibirica* Ledeb.) in forest enabled us to investigate $\delta^{15}N$ variation arise only from vegetation type. The purposes of this study were (1) to evaluate the variations of larch and soil $\delta^{15}N$ along forest-grassland gradient, and (2) to clarify the N process controlling $\delta^{15}N$ variations.

Table 1.1. Global inventories of nitrogen (Hübner 1986).

		N (Tg)	N (%)
Terrestrial	total	1.9×10^{11}	98
lithosphere	rocks	1.9×10^{11}	
	sediments	4.0×10^{8}	
	coal	1.2×10^{5}	
biosphere	soil	3.2×10^{5}	
	plant biomass	1.4×10^{4}	
	animal biomass	2.0×10^{2}	
Atmospheric	total	3.9×10^{9}	2.0
	N_2	3.9×10^{9}	
	N_2O	1.3×10^{3}	
	trace gases (NH_3, NOx, etc.)	6.7	
Oceanic	total	2.3×10^{7}	0.012
	organic matter	5.4×10^{5}	
	plant biomass	3.0×10^{2}	
	animal biomass	1.7×10^{2}	
Total		1.9×10^{11}	100

Table 1.2. Nitrogen isotope fractionation for N cycling processes and their effects on soil and plant $\delta^{15}N$ (Hobbie and Ouimette 2009; Pardo and Nadelhoffer 2010), and the difference between soils and plants ($\Delta\delta^{15}N$).

Cycle	flux	Process [form]	Fractionation (‰) ($\delta^{15}N_{substrate}$-$\delta^{15}N_{product}$)	Soil $\delta^{15}N$	Plant $\delta^{15}N$	$\Delta\delta^{15}N$ (plant $\delta^{15}N$-soil $\delta^{15}N$)
External	Input	atmospheric deposition [solute]	n/a	change $\delta^{15}N$ in surface [occupy significant portion of plant N uptake]	change $\delta^{15}N$ in plant tissue [occupy significant portion of plant N uptake]	larger $\Delta\delta^{15}N$ (Garten 1993)
		N fixation [gas→solute]	-2 to 2	move $\delta^{15}N$ toward 0	move $\delta^{15}N$ toward 0	
	Loss	NO_3^- leaching [solute]	n/a	move $\delta^{15}N$ rich in ^{15}N in soil solution and particle [^{15}N-depleted NO_3^- loss]	move $\delta^{15}N$ rich in ^{15}N in plant N pool [^{15}N-depleted NO_3^- loss enrich soil solution ^{15}N]	
		DON leaching [solute]	n/a	change $\delta^{15}N$ depending on $\delta^{15}N$-DON [^{15}N-rich DON loss cause lower soil $\delta^{15}N$]	change $\delta^{15}N$ depending on lossed $\delta^{15}N$-DON from soil [^{15}N-rich DON loss decrease soil solution$\delta^{15}N$ and plant $\delta^{15}N$]	
		NH_3 volatilization [gas]	20 to 40	move $\delta^{15}N$ poor in ^{15}N in surface [^{15}N-depleted NH_4^+ deposition produced from volatilized NH_3]	move $\delta^{15}N$ poor in ^{15}N in plant [deposition of low $\delta^{15}N$-NH_4^+]	
		gaseous loss of denitrification [gas NO, N_2O, N_2]	n/a	move $\delta^{15}N$ rich in ^{15}N in soil solution and particle [^{15}N-depleted compounds loss]	move $\delta^{15}N$ rich in ^{15}N in plant N pool [^{15}N-depleted compounds loss enrich soil solution ^{15}N]	
Internal	in soil	ammonification [DON→NH_4^+]	-1 to 5	may enrich ^{15}N in SOM-N	not alter plant $\delta^{15}N$ regardless of whether uptake NH_4^+, NO_3^- or DON [excluding further transformation of NH_4^+]	
		nitrification [NH_4^+→NO_3^-]	5 to 112	Enrich NH_4^+ pool which lead to enrichment of soil ^{15}N [if NH_4^+ pool is not wholly consumed]	change $\delta^{15}N$ depending on whether NO_3^- loss out of system and plant uptake N form	larger $\Delta\delta^{15}N$ [when plant uptake NO_3^-] [when slow nitrification] (Schmur and Matson 2001)
		denitrification [NO_3^-→gas]	0 to 75	Enrich NO_3^- pool which lead to enrichment of soil ^{15}N [if NO_3^- pool is not wholly consumed]	move $\delta^{15}N$ rich in ^{15}N in plant [if plant uptake NO_3^- and a significant portion of NO_3^- pool is consumed by denitrification]	
		immobilization [DON or NH_4^+→SOM]	-1 to 1.6	depend on the $\delta^{15}N$ of immobilized		
	soil→plant	ion exchange	-8 to -1			
		uptake via mycorrhiza	ectomycorrhizal: 8 to 10 arbuscular mycorrhizal: 0 to 3.5		^{15}N depleted (slight to high)	larger $\Delta\delta^{15}N$ (Hobbie et al. 1999)
	in plant	transport	0 to 2	No direct effect	No effect on total plant $\delta^{15}N$,but root become enrich depleted from shoot	
		assimilation	0	No direct effect	Unknown ,assumed to be negligible	
		retranslocation	0 to 2	No direct effect	No effect on total plant $\delta^{15}N$,but if ^{15}N-depleted N is translocated,the tissue which N moved become enriched	
		litterfall	n/a	Decrease surface soil $\delta^{15}N$ If litter is depleted relative to soil	change $\delta^{15}N$ depending on N retranslocation (if litter is enriched because ^{15}N-depleted N was retranslocated away, litterfall cause net depletion of plant)	

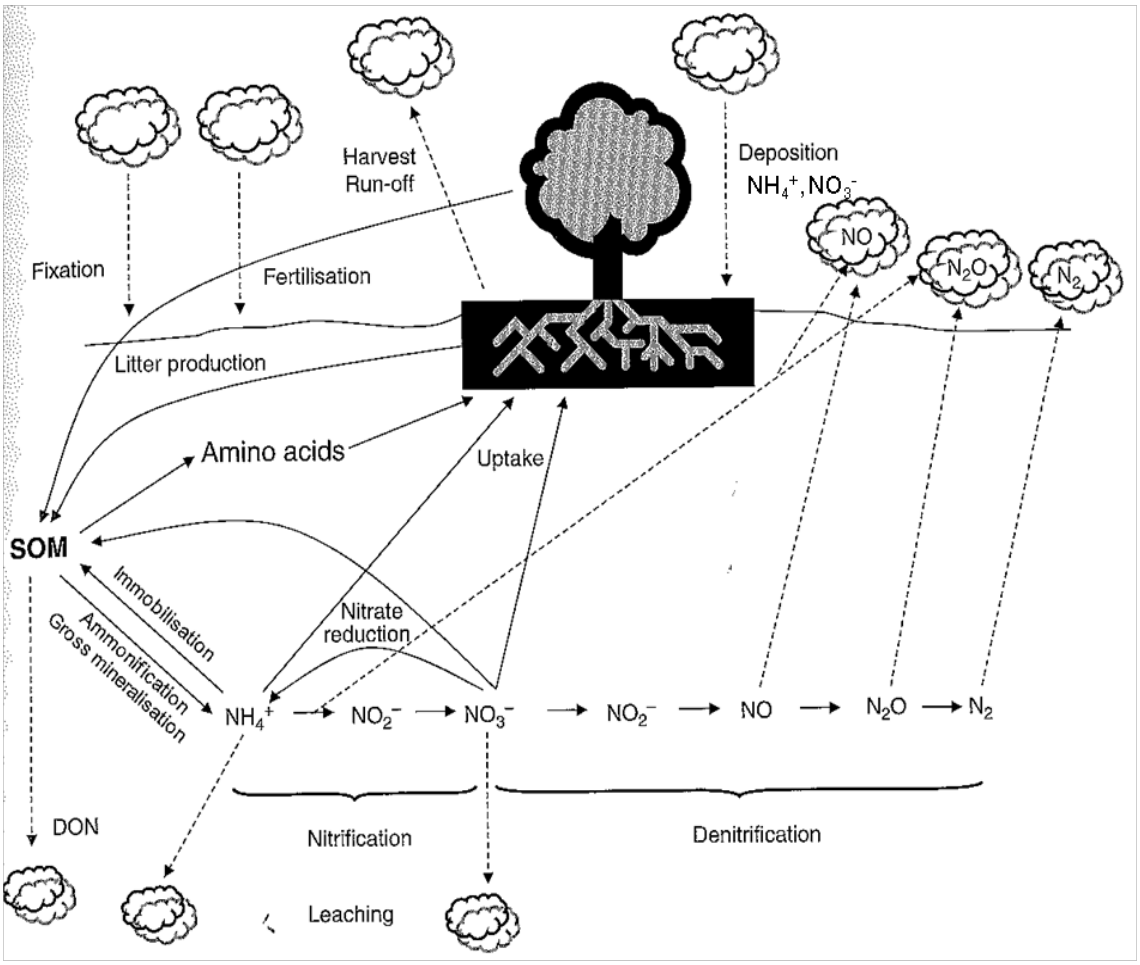


Figure 1.1. Terrestrial nitrogen cycle. Internal fluxes within ecosystm are shown by solid lines and fluxes of exchanges with environment by broken lines (Ågren and Andersson 2012).

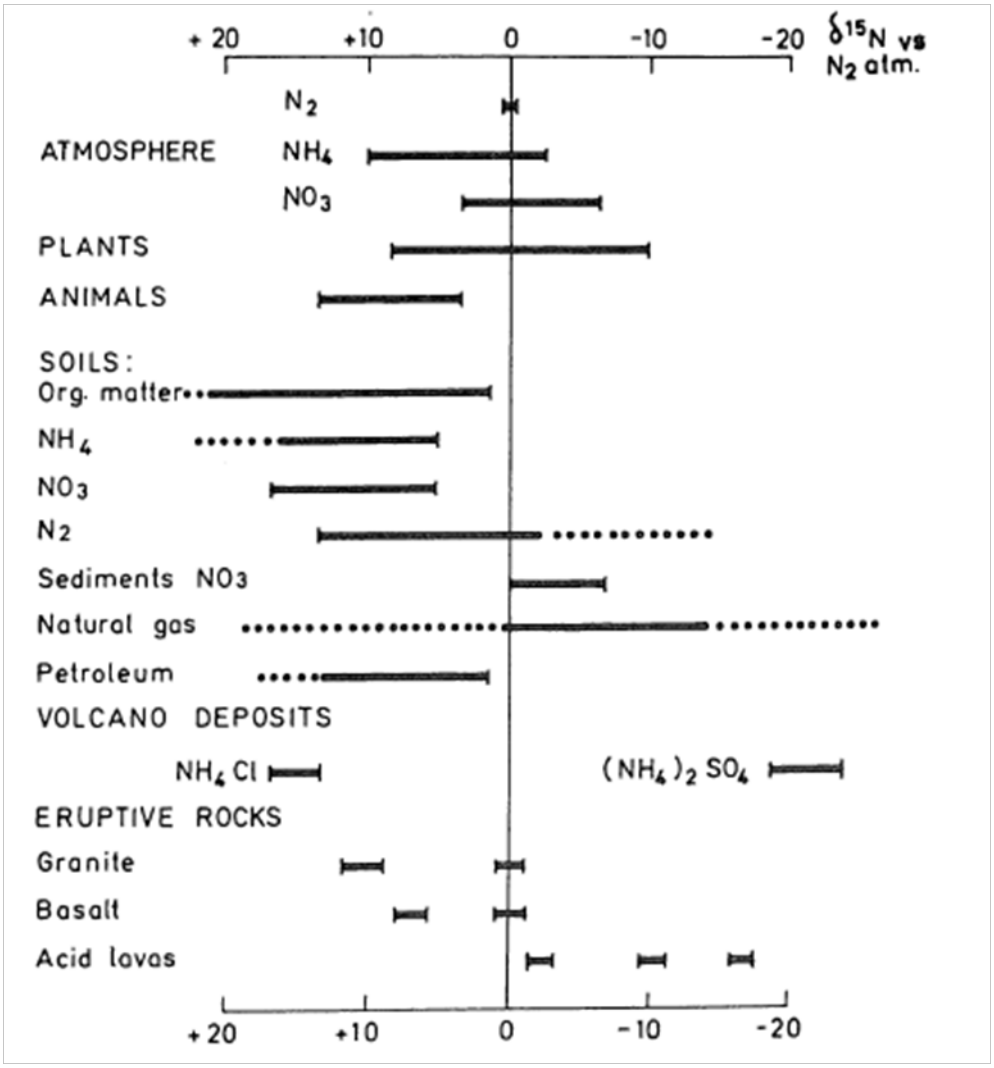
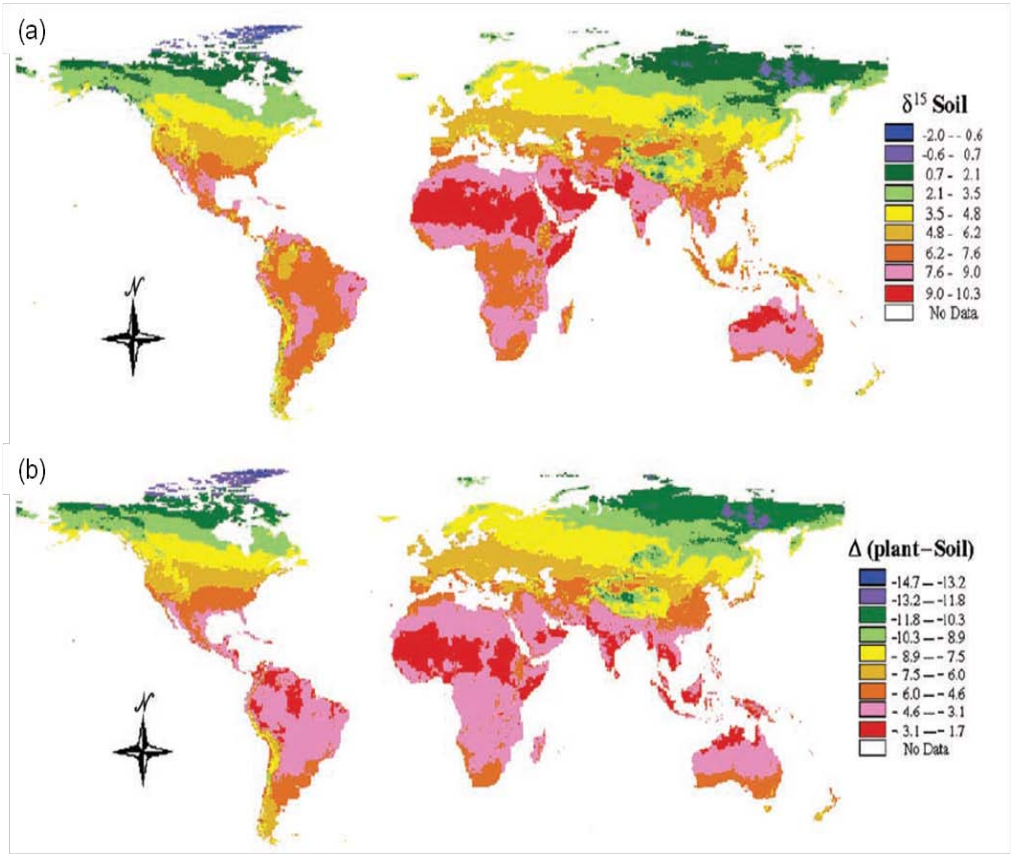


Figure 1.2. The abundance of ^{15}N in terrestrial materials (Letolle 1988).



(c)

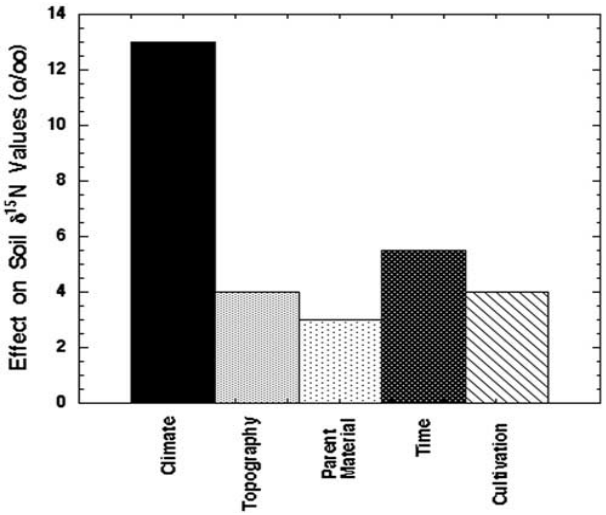


Figure 1.3. Geographical trends in soil $\delta^{15}N$ (a), the difference between plant $\delta^{15}N$ and soil $\delta^{15}N$ (b), and estimated range in the effect of individual ecosystem state factor on the soil $\delta^{15}N$ (c) in Amundson et al. (2003).

Chapter 2 Materials and Methods

2.1. Observation field

2.1.1. Location, vegetation characteristics, and human activities in northern Mongolia

Mongolia is located in the north of the central Asian plateau and has an area of about 1.56 million km^2. It is a landlocked country bordered in the north by the Siberian Russian Federation, and in the east, south and west by China. About half of the land is above 1400 m, and the climate is cold semi-arid and markedly continental (FAO 2006). The vegetation follows a latitudinal zonation with boreal forests in the northern parts and steppe, semidesert, and desert in the center and the south (Figure 2.1). The boreal forest covers about 5% of the Mongolian land area, is distributed primarily in the mountain areas such as Khentii Mountains and the Khangai Mountains of northern Mongolia, whereas steppe covers 83% of the territory (~1.3 million km^2) (Li et al. 2007). The forest is dominated by Siberian larch (*Larix sibirica Ledeb.*) which covers more than 70% of the forested areas (Li et al. 2005). In the northern forest-grassland boundary region, forests are fragmented with dominating the north-facing slopes, whereas temperate grassland dominates the south-facing slopes, dry valleys, and flat plains (Ishikawa et al. 2005; Dulamsuren et al. 2010). This region corresponds to the

southern boundary of permafrost (Ishikawa et al. 2005).

The cold, arid climate is well suited to extensive grazing and transhumance which best makes use of pastures, and the country's pastures have always been heavily stocked, hard grazing is a historical phenomenon (FAO 2006). From 1961 until the early 1990s the number of livestock units remained relatively stable, reflecting the organized management and marketing arrangements by government. However present grazing is lacked of regulation, and this is becoming serious locally, with the abandonment of some areas and over-use of others.

2.1.2. Vegetation change in northern Mongolia from past to present

Many reports have suggested that the vegetation in Mongolia had changed dramatically from past to present (Fowell et al. 2003; Tarasov et al. 2007; Schwanghart et al. 2009; Ma et al. 2013; Sun et al. 2013). These studies have shown that the dominant vegetation changed drastically with time from desert, steppe to taiga in the same place, and also showed that the period of one vegetation continued more than 1000 years. On the other hand, the vegetation was different among studies in the same time. Two studies were held near our study area, the forest-grassland boundary in northern part (Ma et al. 2013, Sun et al. 2013). Ma et al. (2013) observed pollen record in eolian deposit at Shaamar and showed that present landscape of forest-grassland had been developed from past

3200 years by forest expansion to grassland. Sun et al. (2013) observed pollen record in lake sediment at AchitNuur and showed that present landscape of forest-grassland had established from past 1600 years following the forest expansion 6400 years ago. Those vegetation changes were explained to be caused by the intensities of Pacific or Asian monsoons (Fowell et al. 2003; Tarasov et al. 2007; Schwanghart et al. 2009).

2.1.3. Climate and vegetation changes in future

Recently Mongolia is getting warmer and slightly drier. Annual temperature increase by 1.7 °C higher than global average with a slight decrease trend in annual mean precipitation were observed within the last 60 years (1940 to 2001) (Batima et al. 2005). A model of vegetation change across Siberia have shown that Siberian forests are predicted to decrease and shift northwards as far as 600 km, and forest-steppe and steppe ecosystems are predicted to dominate over half of Siberia in the warmer and drier climate by 2080 (Tchebakova et al. 2009). Ecotones are transition zones that may be particularly sensitive to both natural and human-related disturbances to the environment (Pogue and Schnell 2001), and at northern forest-grassland boundary, the decrease in the area covered by Siberian larch is concerned due to increasing aridity (Dulamsuren et al. 2010).

2.2. Site description

Six study areas, Tsagaannuur (TG), Hatgal (HG), Arbulag (AB), and Murun (MR) in western region, Terelj (TR), and Mongonmorit (MM) in eastern region in northern Mongolia were chosen for observation (Figure 2.2). Information on site locations and positions in each area is presented in Table 2.1. Siberian larch (*Larix sibirica* Ledeb.) is the dominant species in this region with scattered white birch (*Betula platyphylla* Sukach.) in TR and MM areas (Ishikawa et al. 2005; Li et al. 2005), and with scattered Siberian spruce (*Picea obovata Ledeb.*) in TG area. This region corresponds to the southern boundary of permafrost (Ishikawa et al. 2005), which coincides with the distribution of the boreal forest. The forest dominates the north-facing slopes, whereas the temperate grassland dominates the south-facing slopes, dry valleys, and flat plains (Ishikawa et al. 2005). The soil is cryosol, and the climate is cold continental climate with dry winters, according to Köppen-Geiger climate classification (Peel et al. 2007). The mean annual temperature ranges from -5.9°C in TG area to 0.1°C in MR area and the average temperatures for five months (May to September) range from 8.9°C in HG area to 14.1 °C in MR area (Figure 2.3). The mean annual precipitation ranges from 201 mm in AB area to 353 mm in TR area, and 90% of precipitation occurs during the growing season of larch trees (May to September) (Li et al. 2005).

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 (1993) (Figure 2.4). 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 (Figure 2.7). 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 2.2b), 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 2.2c). 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. As for the western areas (TG, HG, AB, MR), samplings were conducted at several sites in forest in each area (Figure 2.5 and 2.6). 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 (moder-type) to poor in boundary sites (mull-type) (Ponge 2003) (Figure 2.8).

2.3. Sampling

2.3.1. 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 at 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.

2.3.2. 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 to calculate water content, then used for isotope analysis. In

TR and MM areas, fresh soil samples collected in 2012 were also used for KCl-extractable N (DON, NH_4^+, and NO_3^-) analysis.

2.4. Analysis

2.4.1. C/ N isotopic ratio and concentrations

The $\delta^{13}C$, $\delta^{15}N$, 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 (Figure 2.9). The isotope ratio was expressed using the δ notation:

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

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

2.4.2. 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. Analytical error calculated by repeated analysis of standard solution (1.0 μmol $l^{-1}NH_4^+$, 1.8 μmol l^{-1} for NO_3^-, and 0.2 μmol l^{-1} for NO_2^-) was 0.1 μmol N l^{-1}.

2.4.3. Calculation of average values at each site

To obtain needle N concentration, $\delta^{15}N$, and $\delta^{13}C$ 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 $\delta^{15}N$, $\delta^{13}C$, and N concentrations observed on all sampling dates at each site are shown in Figure S1. For bulk soil $\delta^{15}N$ at each site, the weighted mean of $\delta^{15}N$ 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. $\Delta\delta^{15}N$ was calculated as (needle $\delta^{15}N$ – soil $\delta^{15}N$) at each site.

2.4.4. Isotope mass balance on available N

To interpret the processes reflected in the variation of $\Delta\delta^{15}N$, 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:

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

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

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

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, $\delta_{p,}$ δ_m, δ_{leach}, and δ_{gas} are the $\delta^{15}N$ 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. δ_p = needle $\delta^{15}N$), and that the $\delta^{15}N$ of available N produced in the soil was the same as that of the soil (i.e. δ_a = soil $\delta^{15}N$). Generally, available N produced in the soil has similar $\delta^{15}N$ to that of soil organic matter, if all produced N remains in the N pool without any fractionated loss (Hobbie and Ouimette 2009). The schematic representation of each process is shown in Figure 2.10.

2.4.5. 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).

Table 2.1. Locations of sampling sites.

Area		Site No.[a]	Latitude	Longitude	Elevation
	abbr.	position	°N	°E	m
Tsagaannuur	TG	w1e, w2e, w3e	51.35	99.30	1648-1684
		d1e, d2e	51.34	99.31	1641-1702
Hatgal	HG	1n, 2n	50.42	100.17	1700-1705
Arbulag	AB	1n, 2n	49.87	99.52	2046-2074
Murun	MR	1nw, 2nw	49.65	100.35	1701-1744
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

[a] Each sampling site was expressed by area abbreviation and position on a slope. The position number increases along upper to lower slope. The letter after position number means direction of slope. For example, “n” means north-facing slope and “nw” means northwest-facing slope. TRic is a site in valley

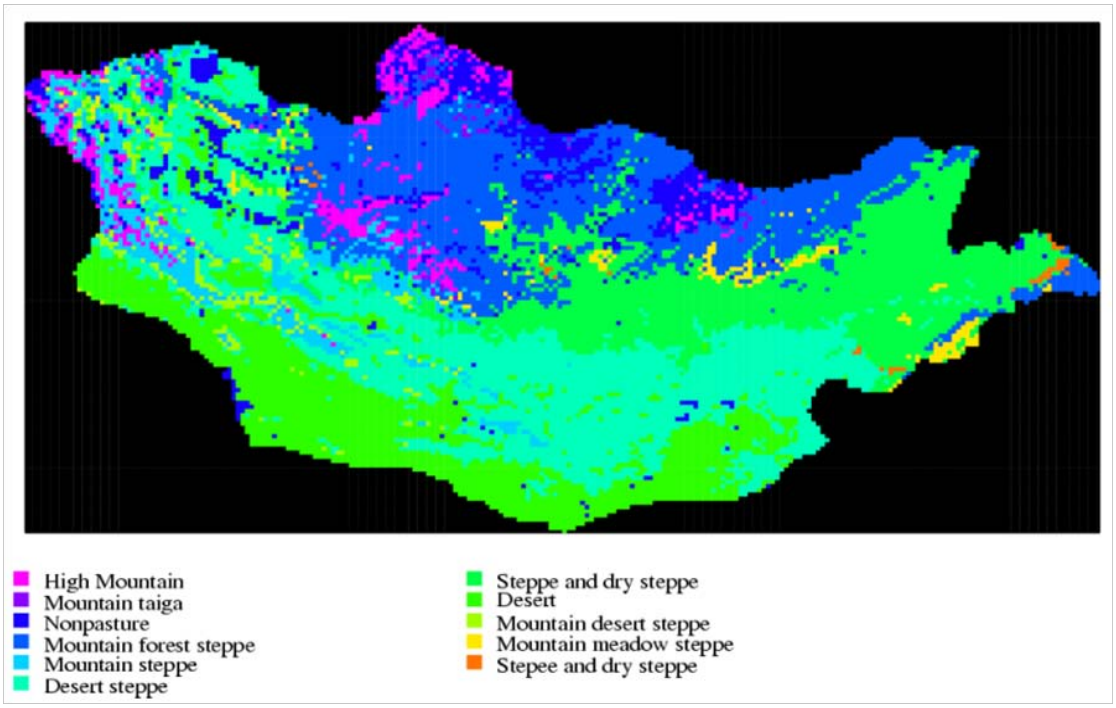


Figure 2.1. Vegetation map of Mongolia with 11 vegetation classes (Saandar and Sugita 2004).

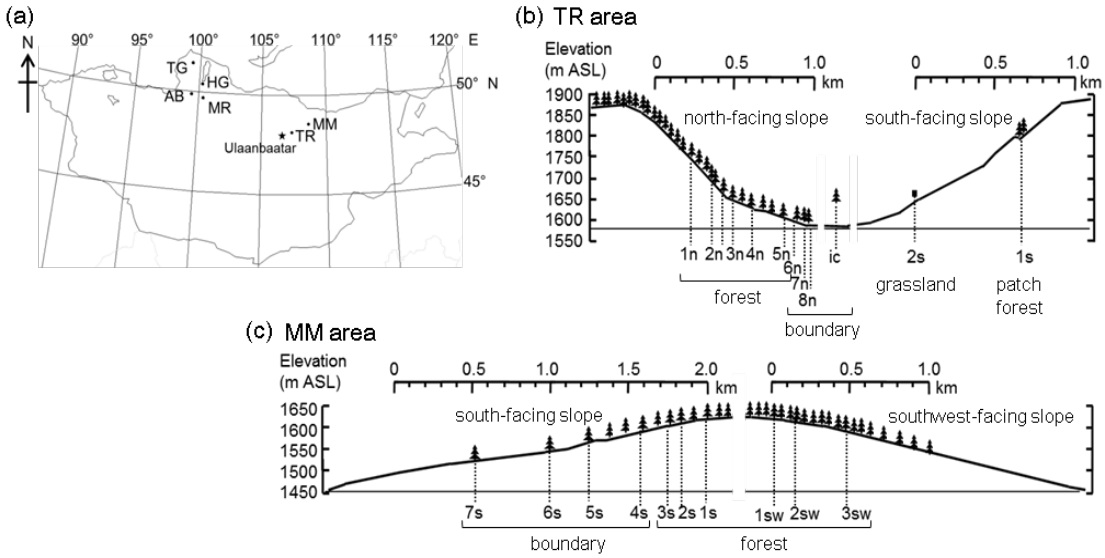


Figure 2.2. A map of the six observation areas (TG, HG, AB, and MR areas in western region and TR, MM areas in eastern region) and Ulaanbaatar, the capital of Mongolia (a), and schematic figures of longitudinal cross sections in TR area (b) and MM area (c). Samplings were conducted at Tsagaanuur (TG), Hatgal (HG), Arbulag (AB), and Murun (MR) in western region, and Terelj (TR), Mongonmorit (MM) in eastern region at 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.

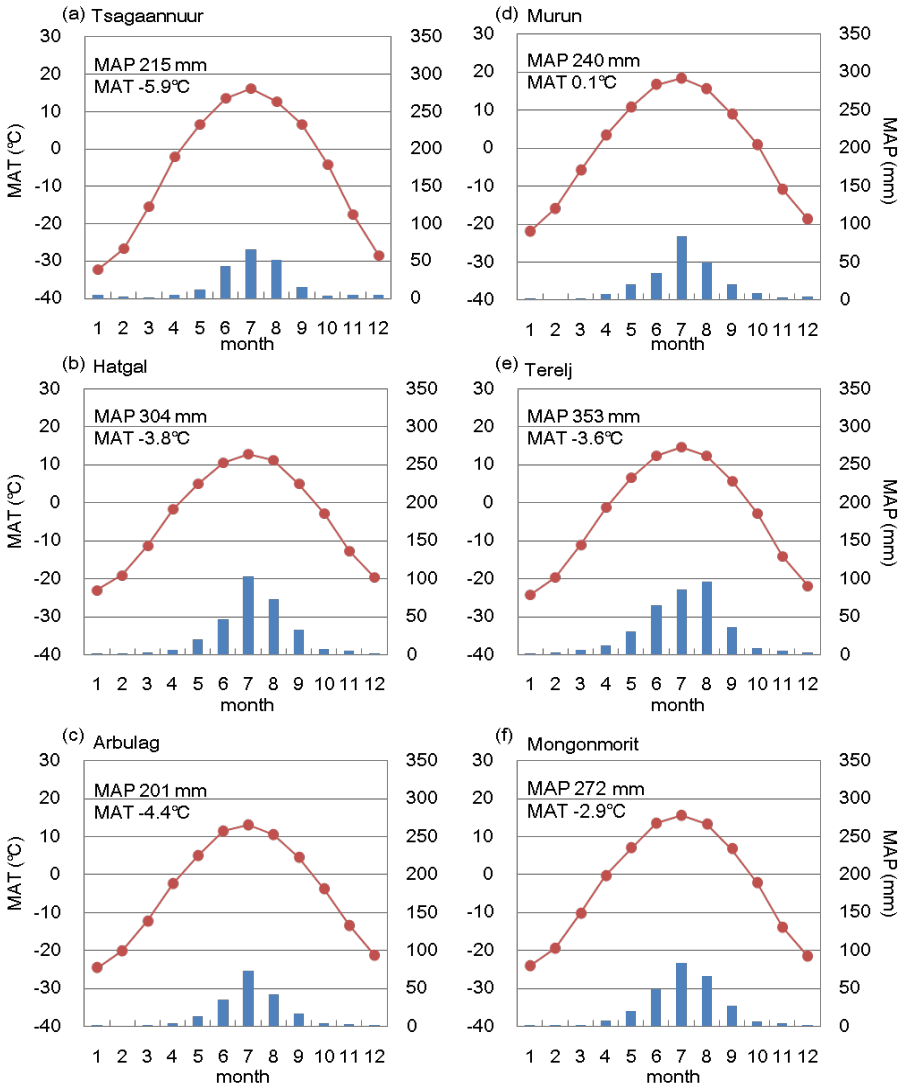


Figure 2.3. Monthly mean temperature (red line) and precipitation (blue bar) observed at meteorological station of study area: TG (a), HG (b), AB (c), MR (d), TR (e), and MM (f) in Mongolia for the period from 1993 to 2006. The latitude, longitude of each station are described above graph, and mean annual precipitation (MAP), mean annual temperature (MAT) are described in graph. The data was provided by meteorological station in institute of Meteorology and Hydrology, Mongolia.

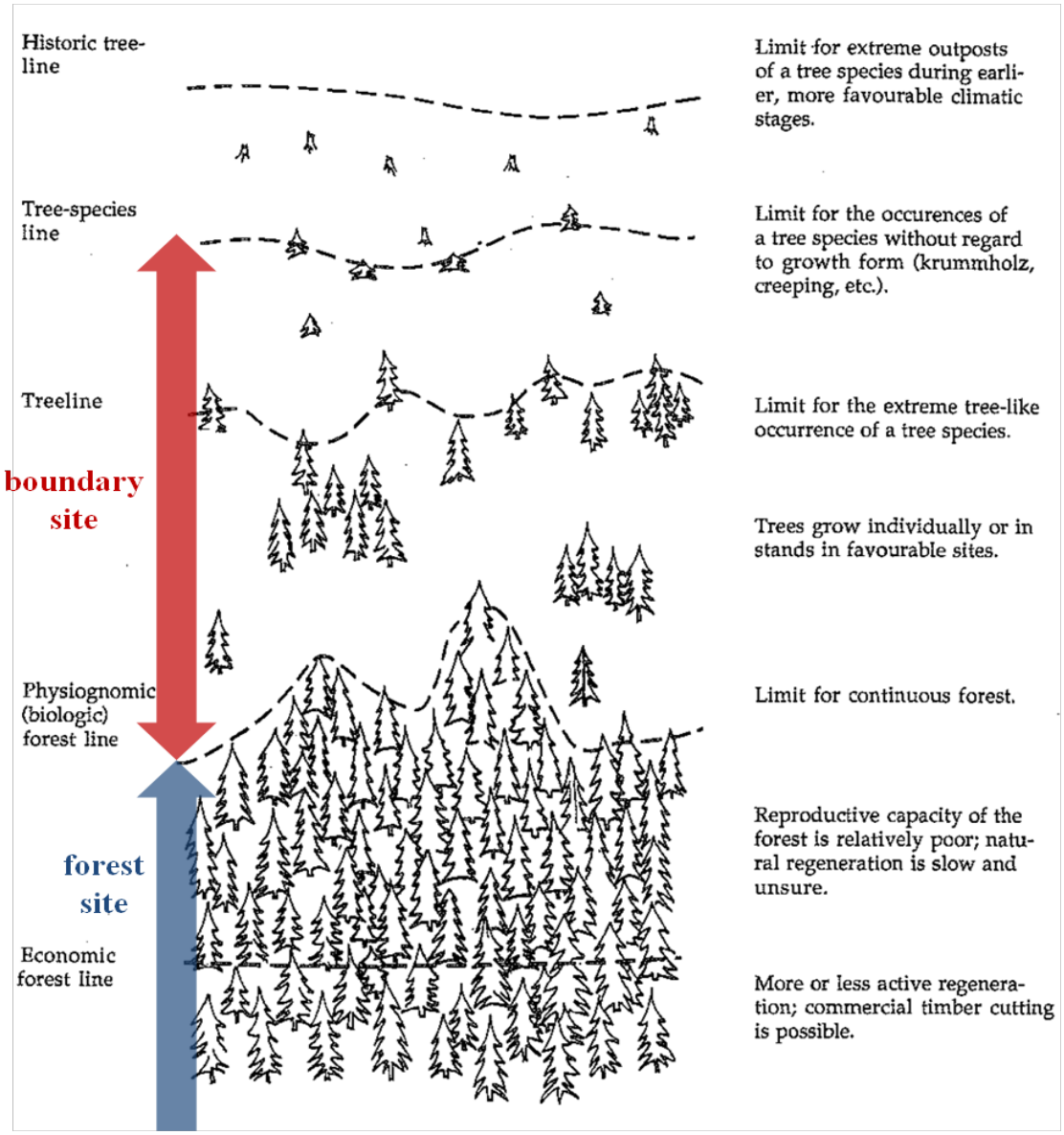


Figure 2.4. Definition of forest/boundary sites in this study, according to Tuhkanen (1993). In this study, the sampling sites were classified as forest or boundary as described by Tuhkanen (1993). Forest site was defined as the site in the continuous forest (zone with blue arrow), whereas boundary site was defined as the site between the edge of continuous forest and grassland (zone with red arrow).

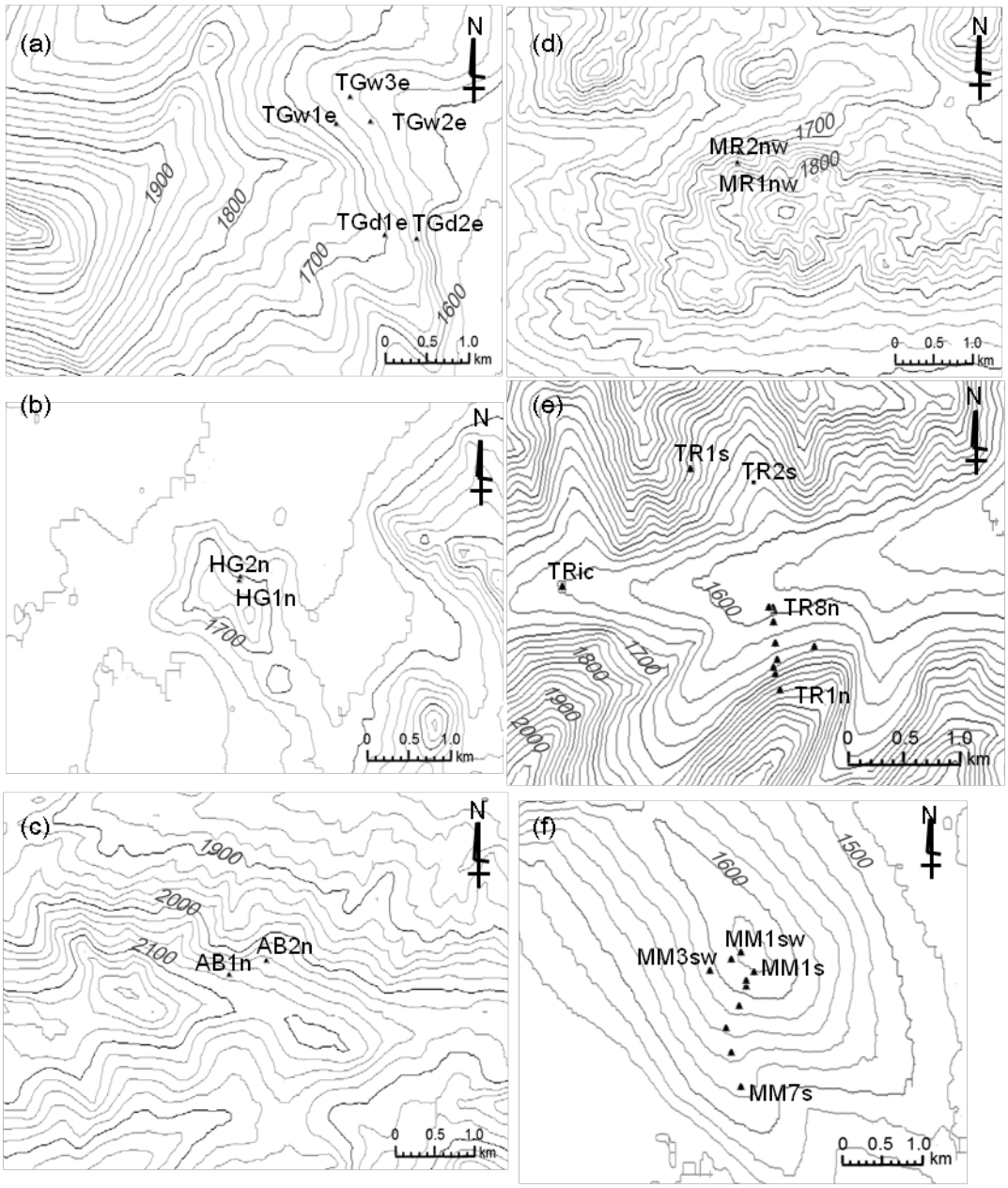


Figure 2.5. Contour maps of observation areas in Tsagaanuur (TG) (a), Hatgal (HG) (b), Arbulag (AB) (c), Murun (MR) (d) in western region, and Terelj (TR) (e), Mongonmorit (MM) (f) in eastern region.

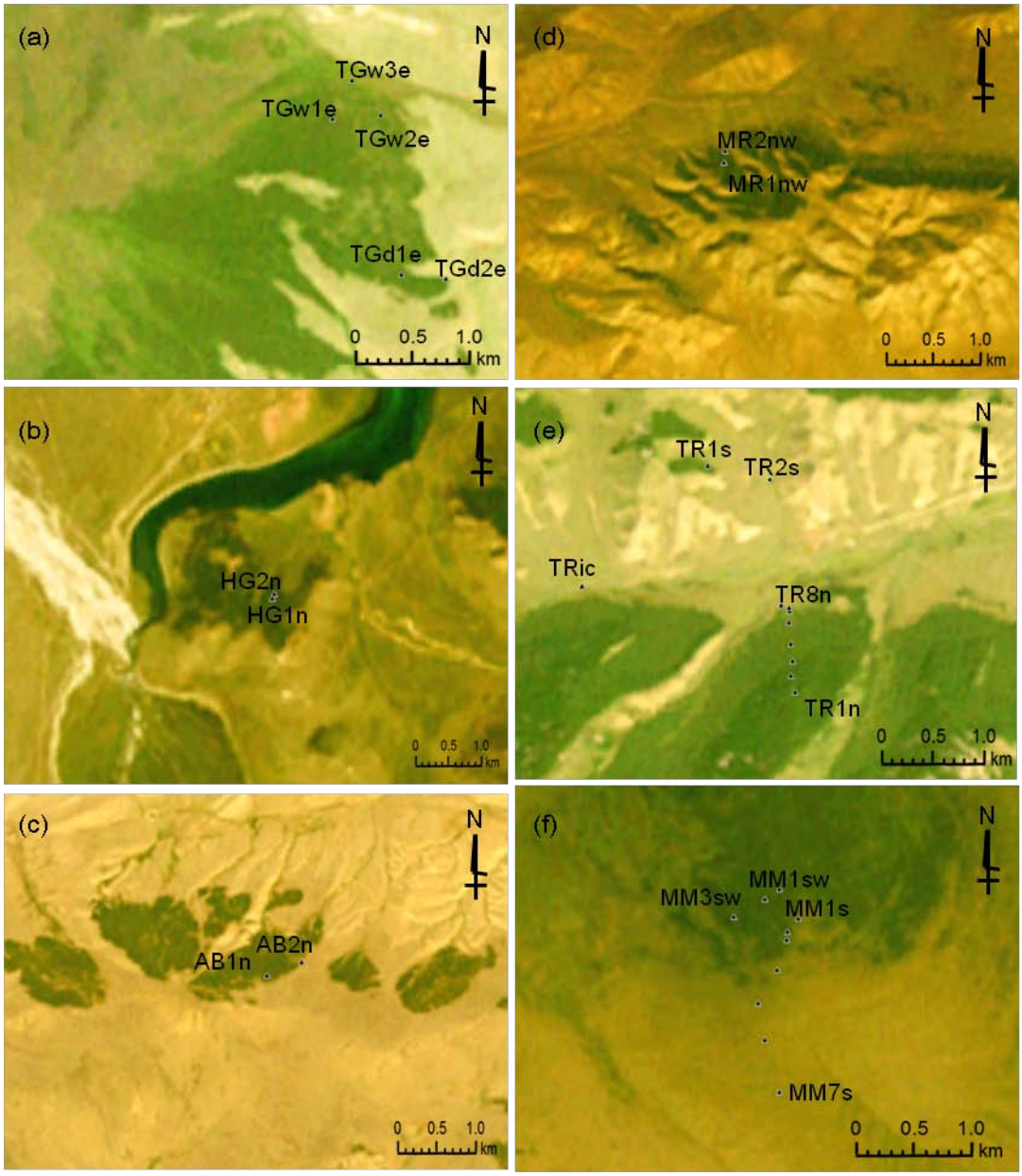


Figure 2.6. Satellite images of observation areas in Tsagaanuur (TG) (**a**), Hatgal (HG) (**b**), Arbulag (AB) (**c**), Murun (MR) (**d**) in western region, and Terelj (TR) (**e**), Mongonmorit (MM) (**f**) in eastern region (Landsat ETM+, combined images of August2001 and June-July 2002).

(a)

(b)

Figure 2.7. Typical forest site (TGw3e) in TG area (a) and typical boundary site (MM4s) in MM area (b) in August 2012. Trees were dense at forest sites, whereas trees were sparse and grasses were dominant at boundary sites.

Figure 2.8. Soil profiles at typical forest site in TR area (TR2n) (a) and typical grassland boundary site in MM area (b) which were observed in August 2012. At forest site, accumulation of organic layer (moder-type) was observed at soil surface, whereas at boundary site, the accumulation was poor (mull-type).

Figure 2.9. Conflo system with DELTA V Plus and FlashEA 1112 (Thermo Fisher Scientific) at the Graduate School of Environmental Science, Hokkaido University, Japan. Photo by R. Shingubara.

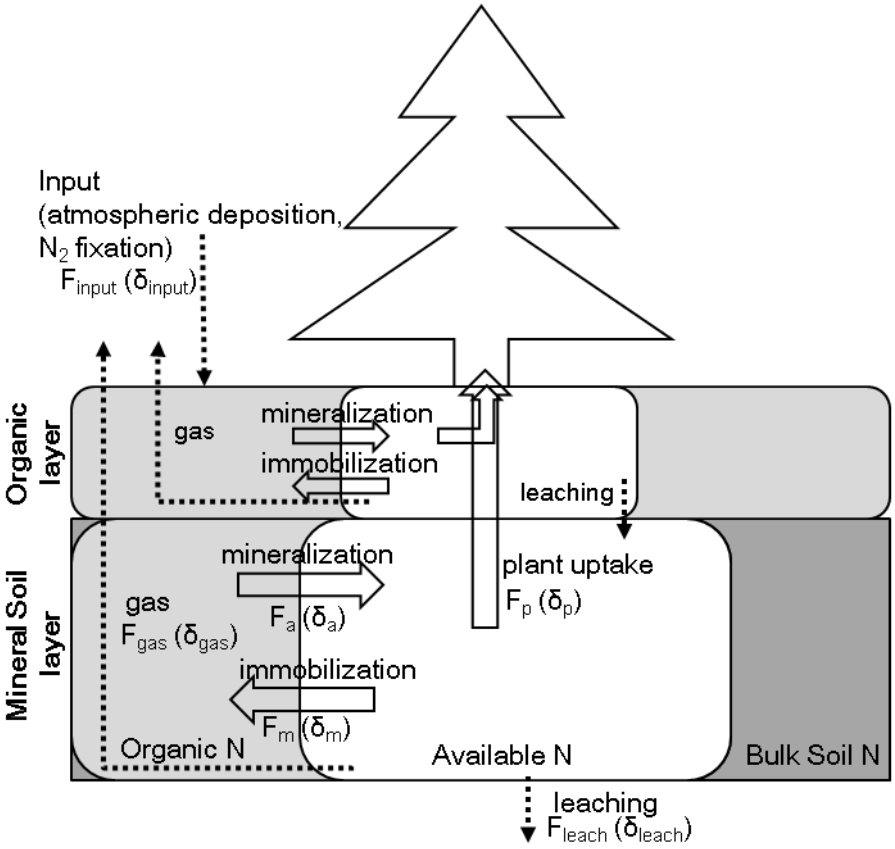


Figure 2.10. Schematic representation of the N cycle in the plant-soil system. Arrows with its flux and $\delta^{15}N$ in parentheses are the processes that included in the mass balance. White arrows are the processes within the system, whereas black dotted arrows are the processes of N input and loss. Under the rapid decomposition of organic matter in grassland boundary, most of the available N produced in the soil (F_a) is transported to larch (F_p) without significant N isotope fractionation ($\delta_a = \delta_p = \delta_m$), leading to small $\Delta\delta^{15}N$. Under the significant accumulation of organic layer in forest, the ^{15}N-depleted part of available N is transported to larch (F_p), whereas the ^{15}N-enriched part remains in the soil immobilized by microorganisms (F_m) ($\delta_p < \delta_m$), leading to large $\Delta\delta^{15}N$.

Chapter 3 Temporal and spatial variations in larch needle and soil components at a forest-grassland boundary in northern Mongolia

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

Temporal variations in needle N concentration, $\delta^{15}N$, and $\delta^{13}C$ of nine individual tree were observed during the growing season of 2004 and in July 2005 at three sites in TR area (TR3n, TR6n, and TR1s), and two sites in MM area (MM1sw and MM2s) (Figure 3.1). In both areas, needle N concentrations were significantly higher in May 2004 than the following months in 2004 and July in 2005 ($p < 0.05$) (Figure 3.1(a) and (d)). Similarly, high needle $\delta^{15}N$ in May 2004 was observed in MM area (Figure 3.1(e)), whereas temporal variations were not significant for needle $\delta^{15}N$ in TR area and needle $\delta^{13}C$ in TR and MM areas (Figure 3.1(b), (c), and (f)). When the data collected in May were excluded, the standard deviations of individual tree during the observed period in TR and MM areas were 0.5%, 0.5‰, and 0.9‰ for N concentration, $\delta^{15}N$, and $\delta^{13}C$, respectively.

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

The $\delta^{15}N$ 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 3.2). At the forest site (TR1n) and the boundary site (TR7n), soil $\delta^{15}N$ increased up to 20-30 cm soil depth. This pattern in soil $\delta^{15}N$ was common at all sites in this study (Figure 3.3). However, changes in the $\delta^{15}N$ of needle, organic layer, and soil were different between these two; a gradual increase in $\delta^{15}N$ was observed at forest site (TR1n), whereas a slight increase was observed at boundary site (TR7n) (Figure 3.2). Similarly, in MM area, a gradual increase in the $\delta^{15}N$ 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 (Figure 3.3).

3.3. Spatial variations along the forest-grassland gradient

Characteristic spatial patterns in $\delta^{15}N$ values, needle $\delta^{13}C$, and needle N concentration were observed along the forest-grassland gradient in TR and MM areas (Figure 3.4). In TR area, needle $\delta^{15}N$ 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 $\delta^{15}N$ at TR1s in the patch forest (+1.2‰) was similar to that at TR6n and slightly lower than that at TR8n (Figure 3.4a). In contrast to needle $\delta^{15}N$, soil $\delta^{15}N$ 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 $\Delta\delta^{15}N$ showed the same pattern as needle $\delta^{15}N$ on the north-facing slope; $\Delta\delta^{15}N$ was larger in the forest (-8‰ at TR1n) and smaller in the boundary (-3‰ at TR7n) on the north-facing slope, whereas $\Delta\delta^{15}N$ at TR1s on the south-facing slope (-5‰) was similar to that at TR5n (Figure 3.4(b)). On the north-facing slope, needle $\delta^{13}C$ 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 south-facing slope, needle $\delta^{13}C$ at TR1s (-26.0‰) was as high as that at TR8n, and needle N concentration at TR1s (2.4%) was similar to that at TR7n (Figure 3.4(c)). KCl-extractable N (DON, NH_4^+, and NO_3^-) pools in the 0-20 cm soil layer were observed at two forest sites on the north-facing slope (TR2n and TR5n) in August 2012 (Table 3.1). At both sites, DON was more than one-order of magnitude larger than NH_4^+ and NO_3^- pools. Additionally, fractions of NO_3^- relative to inorganic N were low (< 0.2).

In MM area, needle $\delta^{15}N$ values were higher on the south-facing slope (+2.7‰ to +4.3‰) where most sites were located in the boundary, than those on the southwest-facing slope (+0.34‰ to +2.0‰) where all sites were located in the forest (Figure 3.4d). In contrast, soil $\delta^{15}N$ slightly varied (+5.1 to +6.8‰) in MM area. The $\Delta\delta^{15}N$ slightly changed from -4‰ to -2‰ at the sites on the south-facing slope, which

were smaller than those on the southwest-facing slope (-6‰) (Figure 3.4(e)). Needle $\delta^{13}C$ and needle N concentration were higher at the sites on the south-facing slope (-26.2‰ to -24.6‰) than those on the southwest-facing slope (-27.5‰ to -26.2‰) (Figure 3.4(f)). KCl-extractable N (DON, NH_4^+, and NO_3^-) pools in the 0-20 cm soil layer were observed at one forest site (MM3s) and three boundary sites (MM4s, MM5s, and MM6s) on the south-facing slope in August 2012 (Table 3.1). 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 fractions of NH_4^+ nitrified in the soil (NO_3^-/inorganic) (close to 1).

In western areas (TG, HG, AB, MR), needle $\delta^{15}N$, soil $\delta^{15}N$, $\Delta\delta^{15}N$, needle $\delta^{13}C$, and needle N concentration were mostly intermediate between those in MM and TR areas, whereas low values were observed for several variables (Figure 3.5). The lowest needle $\delta^{15}N$ (-5.4‰), soil $\delta^{15}N$ (+2.0‰), and needle N concentration (1.7%) were observed at TGw3e, a forest site where thick accumulation of organic layer was observed.

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

Significant correlations between different variables of all the sites in TR area and MM

area were observed (Figure 3.6). The correlation was observed between needle $\delta^{13}C$ and $\Delta\delta^{15}N$ ($r_s = 0.753$), soil $\delta^{15}N$ and needle $\delta^{15}N$ ($r_s = 0.718$), needle N concentration and $\Delta\delta^{15}N$ ($r_s = 0.591$), and C/N ratio of bulk soil and $\Delta\delta^{15}N$ ($r_s = -0.541$) ($p < 0.05$). Data in MM area showed small $\Delta\delta^{15}N$ (-5‰ to -2‰), high needle $\delta^{15}N$ (+0.34‰ to +4.3‰), high soil $\delta^{15}N$ (+5.1‰ to +6.8‰), whereas those in TR area showed large variations in those values. When the western areas were included, all the above relationships were still significant (Figure 3.7). The correlation was observed between needle $\delta^{15}C$ and $\Delta\delta^{15}N$ ($r_s = 0.678$), soil $\delta^{15}N$ and needle $\delta^{15}N$ ($r_s = 0.795$), needle N concentration and $\Delta\delta^{15}N$ ($r_s = 0.499$), and C/N ratio of bulk soil and $\Delta\delta^{15}N$ ($r_s = -0.793$) ($p < 0.05$).

Table 3.1. KCl-extractable N[a] in the 0-20 cm soil layer observed in August 2012 in TR and MM area.

Area	Site	DON (sd) g N m^{-2}	NH_4^+ (sd) g N m^{-2}	NO_3^- (sd) g N m^{-2}	total[b] (sd) g N m^{-2}	inorganic N[c] (sd) g N m^{-2}	NO_3^- to inorganic N -
TR	TR2n	2.7 (0.6)	0.32 (0.17)	not detected	3.1 (0.6)	0.32 (0.17)	0
	TR5n	4.0 (0.1)	0.25 (0.01)	0.056 (0.064)	4.3 (0.2)	0.31 (0.07)	0.2
MM	MM3s	3.2 (1.4)	0.58 (0.17)	0.010 (0.018)	3.8 (1.4)	0.59 (0.18)	0.02
	MM4s	3.5 (0.3)	0.38 (0.10)	0.39 (0.45)	4.2 (0.5)	0.77 (0.46)	0.5
	MM5s	3.7 (0.4)	0.090 (0.095)	0.060 (0.052)	3.8 (0.4)	0.15 (0.11)	0.4
	MM6s	3.0 (0.3)	0.10 (0.12)	0.15 (0.11)	3.3 (0.3)	0.25 (0.16)	0.6

[a] KCl-extractable NO_2^- was analyzed but not detected.

[b] total is the sum of DON, NH_4^+, and NO_3^- concentrations.

[c] inorganic N is the sum of NH_4^+ and NO_3^- concentrations.

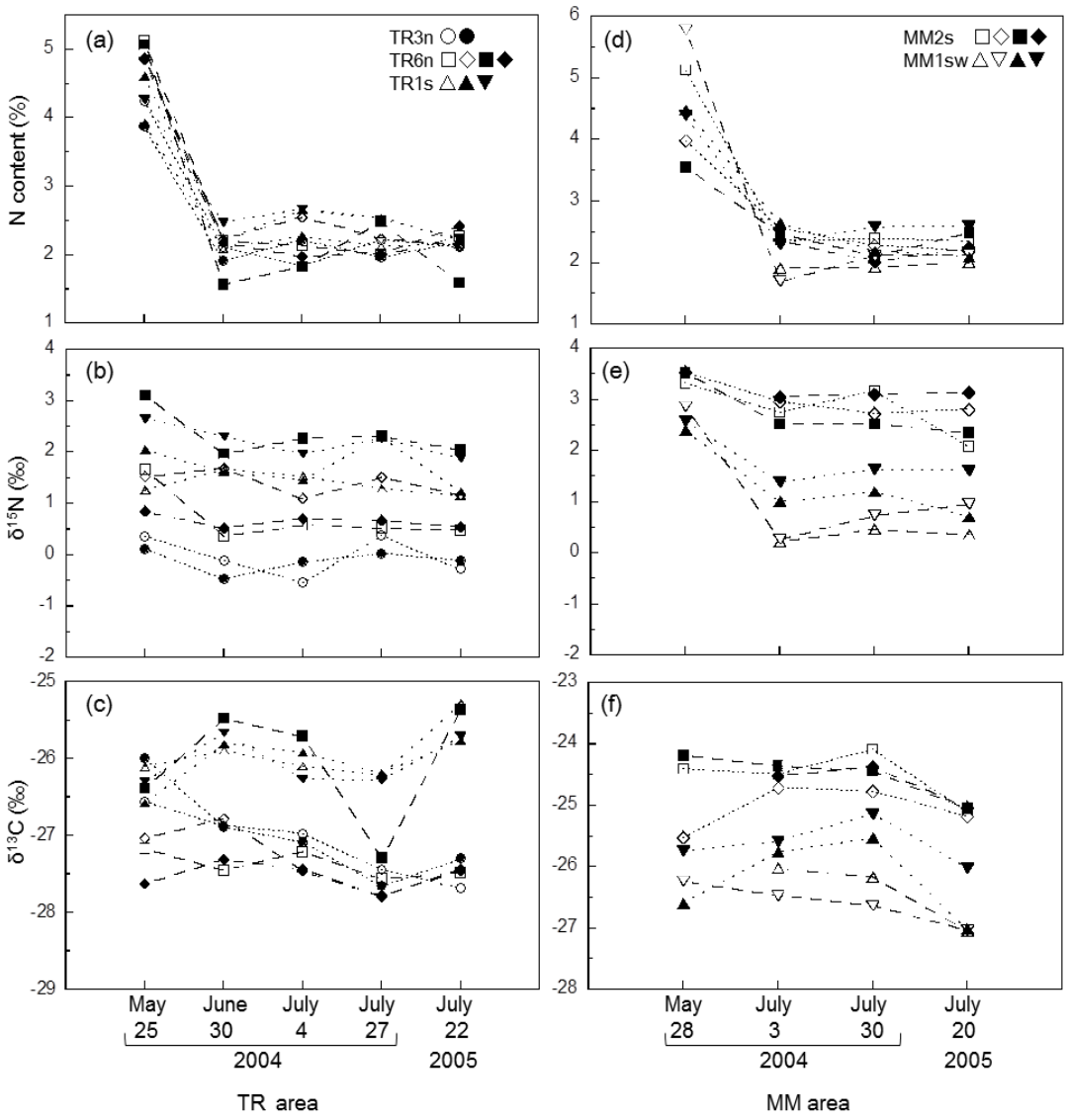


Figure 3.1. Temporal variations in larch needle N concentration (a), $\delta^{15}N$ (b), and $\delta^{13}C$ (c) in TR area, and the same as (d), (e), and (f) in MM area. Data from two trees at TR3n, four trees at TR6n, and three trees at TR1s in TR area, as well as four trees at MM1sw and four trees at MM2s in MM area were shown. Each tree was expressed by a different shape (circle, rectangle, or triangle) and colour (black or white). Trees within the same site share the same shape.

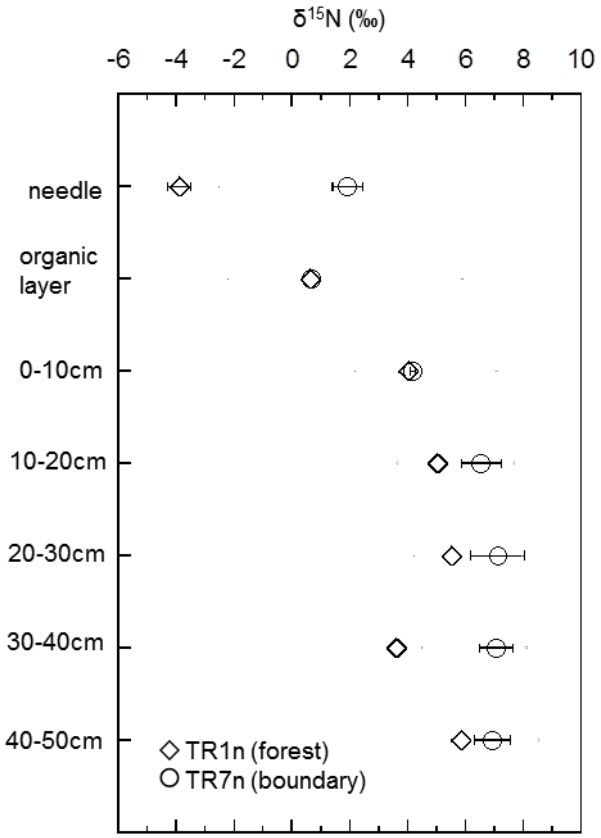


Figure 3.2. Vertical profiles of larch needle, organic layer, and bulk soil $\delta^{15}N$ values at the forest site (TR1n) and the boundary site (TR7n) in TR area. Bars represent standard deviation of the mean.

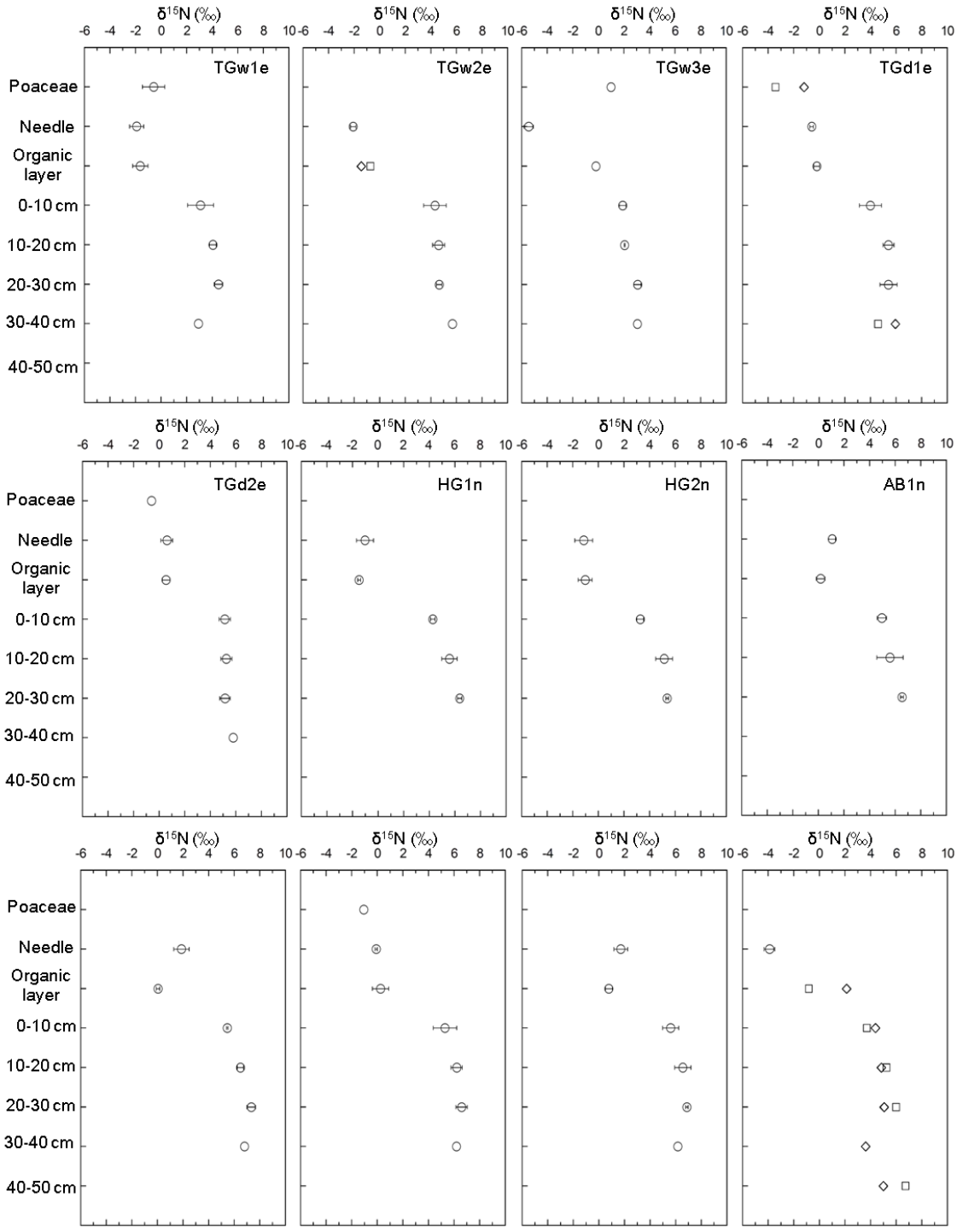


Figure 3.3. Vertical profiles of Poaceae, larch needle, organic layer, and bulk soil $\delta^{15}N$ values at all sites in this study. Bars represent standard deviation of the mean.

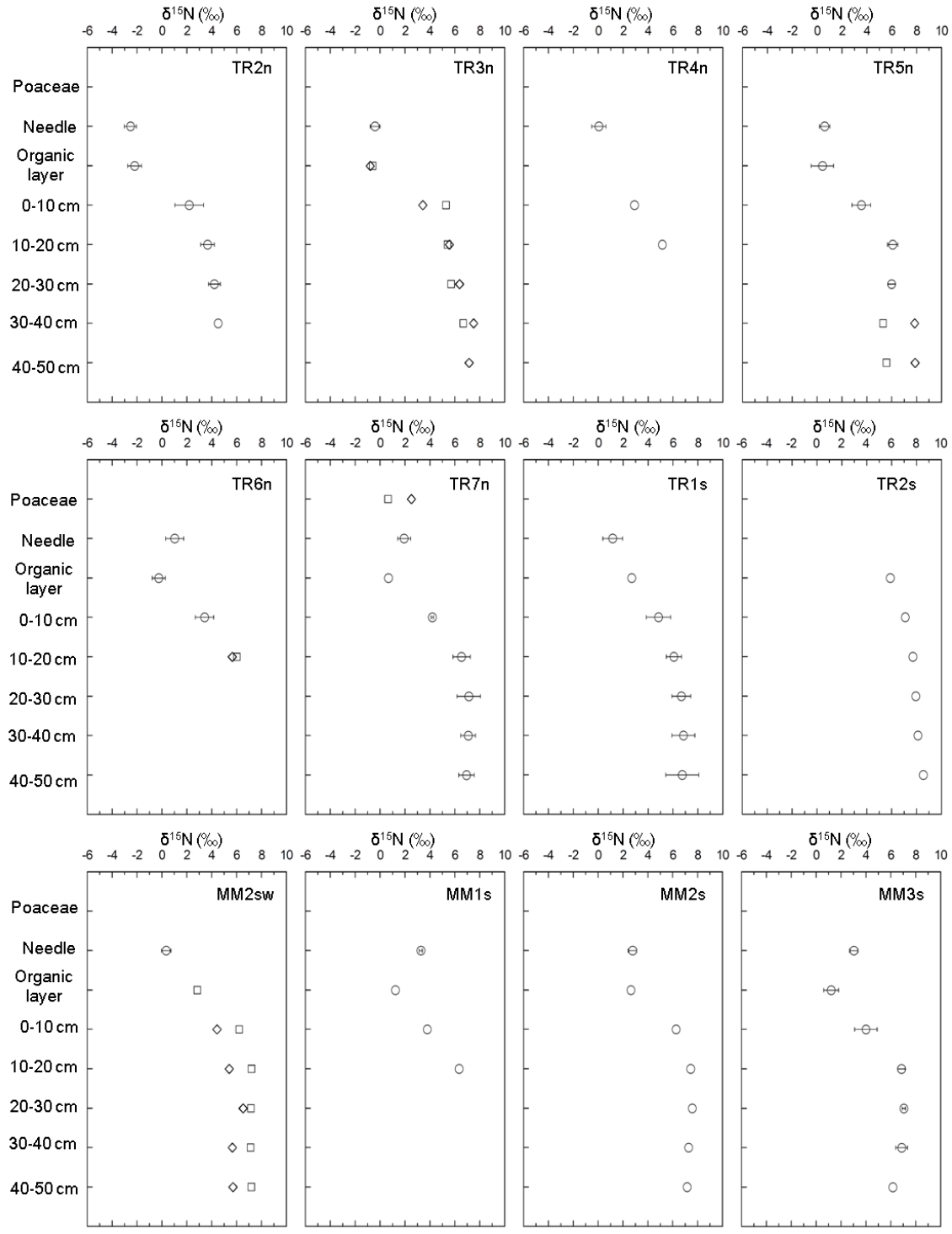


Figure 3.3. Continued.

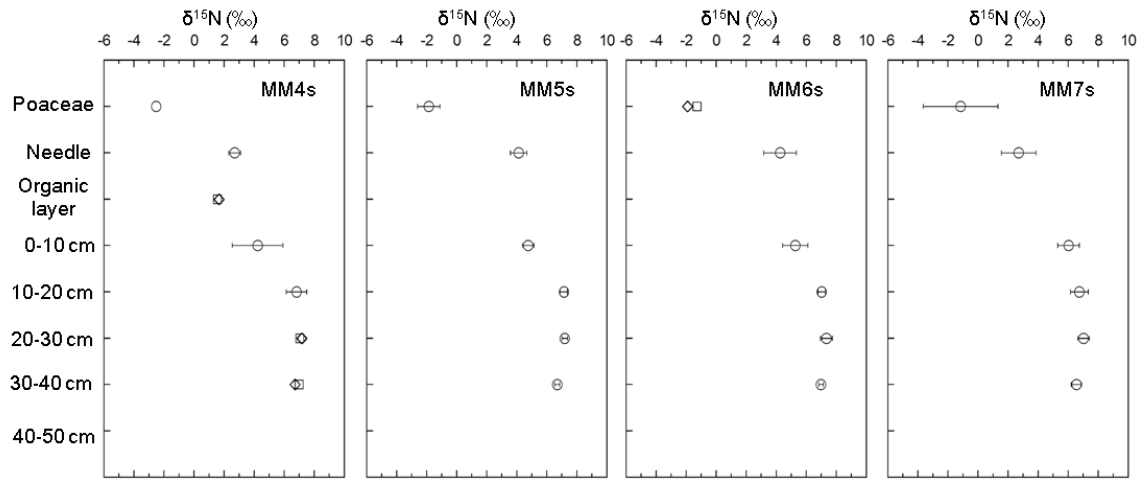


Figure 3.3. Continued.

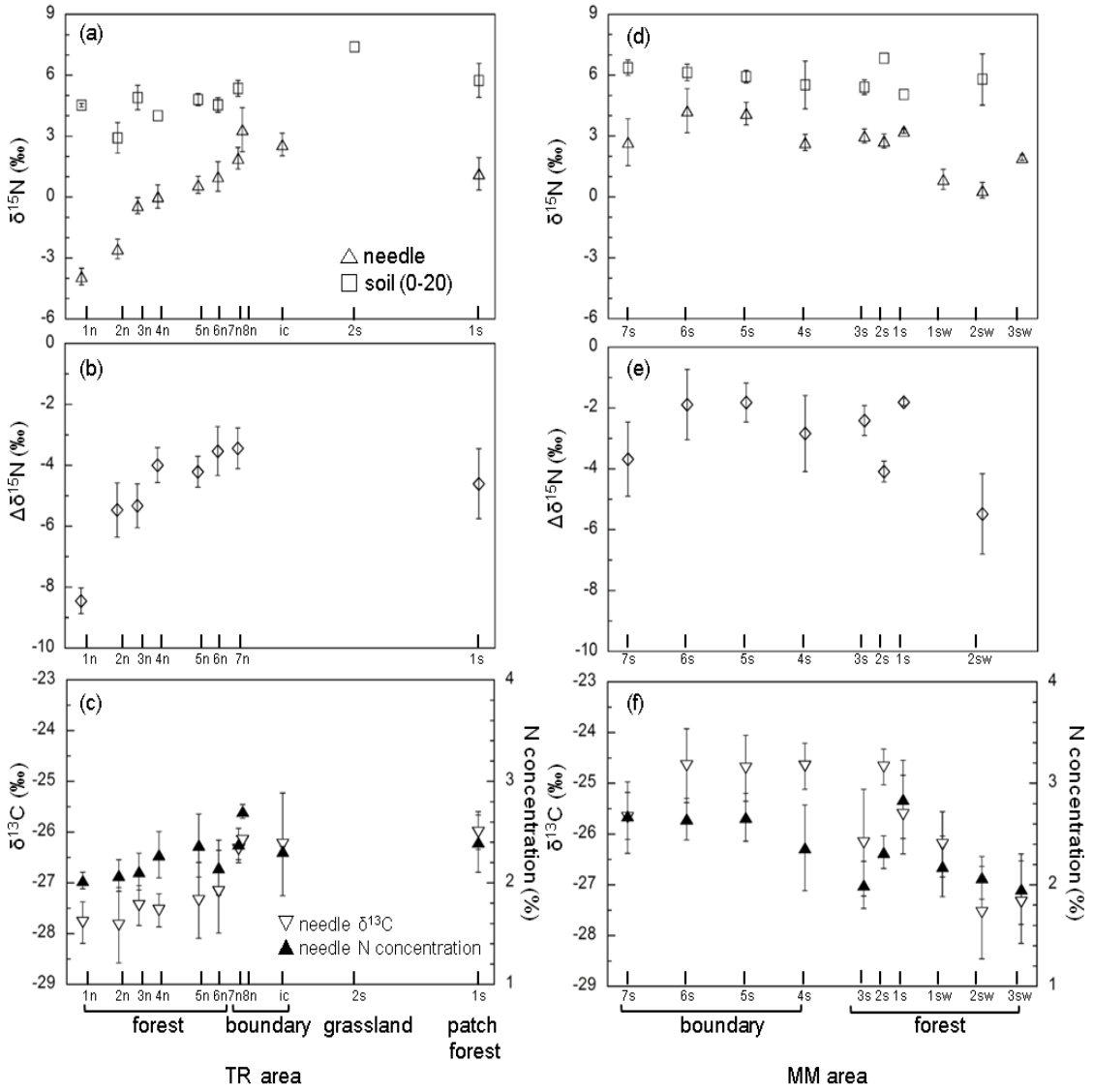


Figure 3.4. Spatial variations in larch needle (triangle) and soil (square) $\delta^{15}N$ (a), differences in $\delta^{15}N$ between needle and soil ($\Delta\delta^{15}N$) (b), and needle $\delta^{13}C$ (open-triangle) and N concentration (filled-triangle) (c) in TR area, and the same as (d), (e), and (f) in MM area. Bars represent standard deviation of the mean.

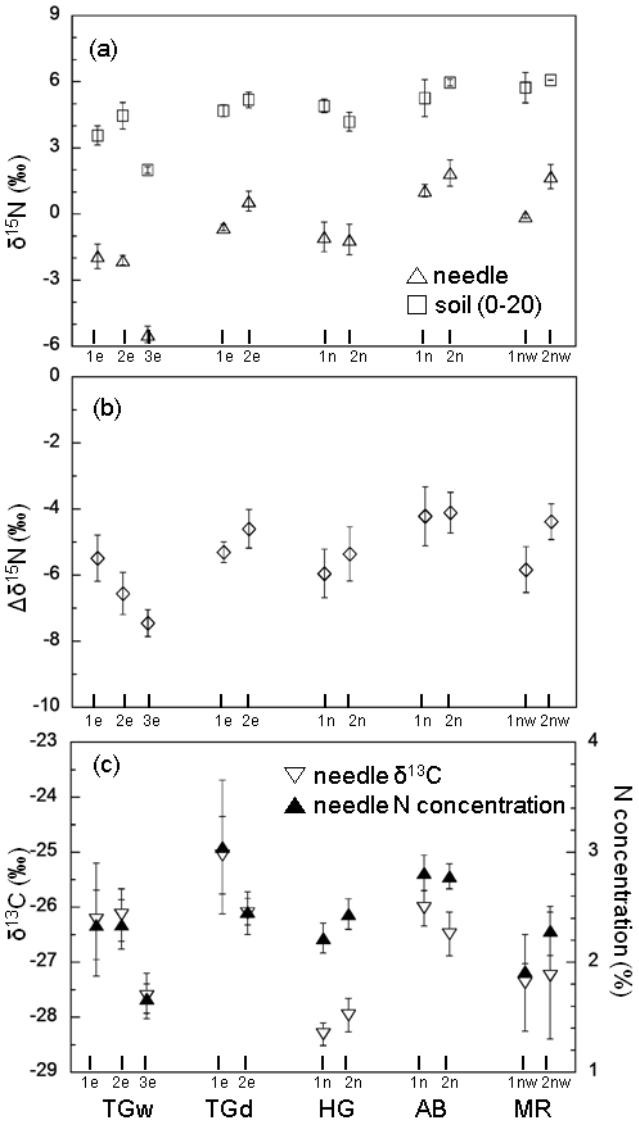


Figure 3.5. Spatial variations in larch needle (triangle) and soil (square) $\delta^{15}N$ (a), difference in $\delta^{15}N$ between needle and soil ($\Delta\delta^{15}N$) (b), and needle $\delta^{13}C$ (open-triangle) and N concentration (filled-triangle) (c) in all areas in western region. Bars represent standard deviation of the mean.

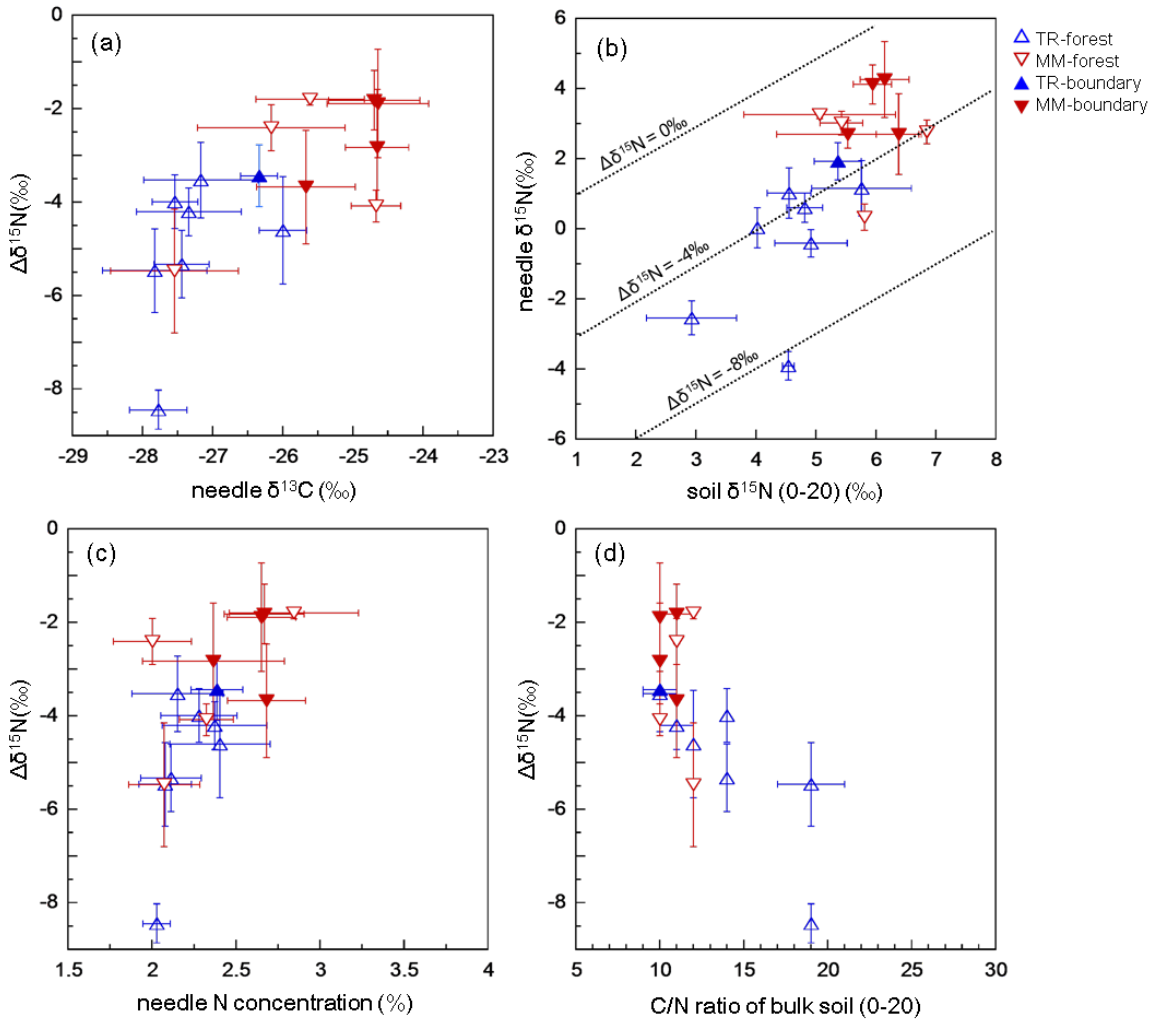


Figure 3.6. Correlations between larch needle $\delta^{13}C$ and $\Delta\delta^{15}N$ (r_s = 0.753) (a), needle and soil $\delta^{15}N$ (r_s = 0.718) (b), needle N concentration and $\Delta\delta^{15}N$ (r_s = 0.591) (c), and C/N ratio of bulk soil and $\Delta\delta^{15}N$ (r_s = -0.541) (d) at all sites in TR area and MM area. Dotted lines in (b) indicate $\Delta\delta^{15}N$ values at 0‰, -4‰ and -8‰. Correlation coefficients are significant at $p < 0.05$. Bars represent standard deviation of the mean.

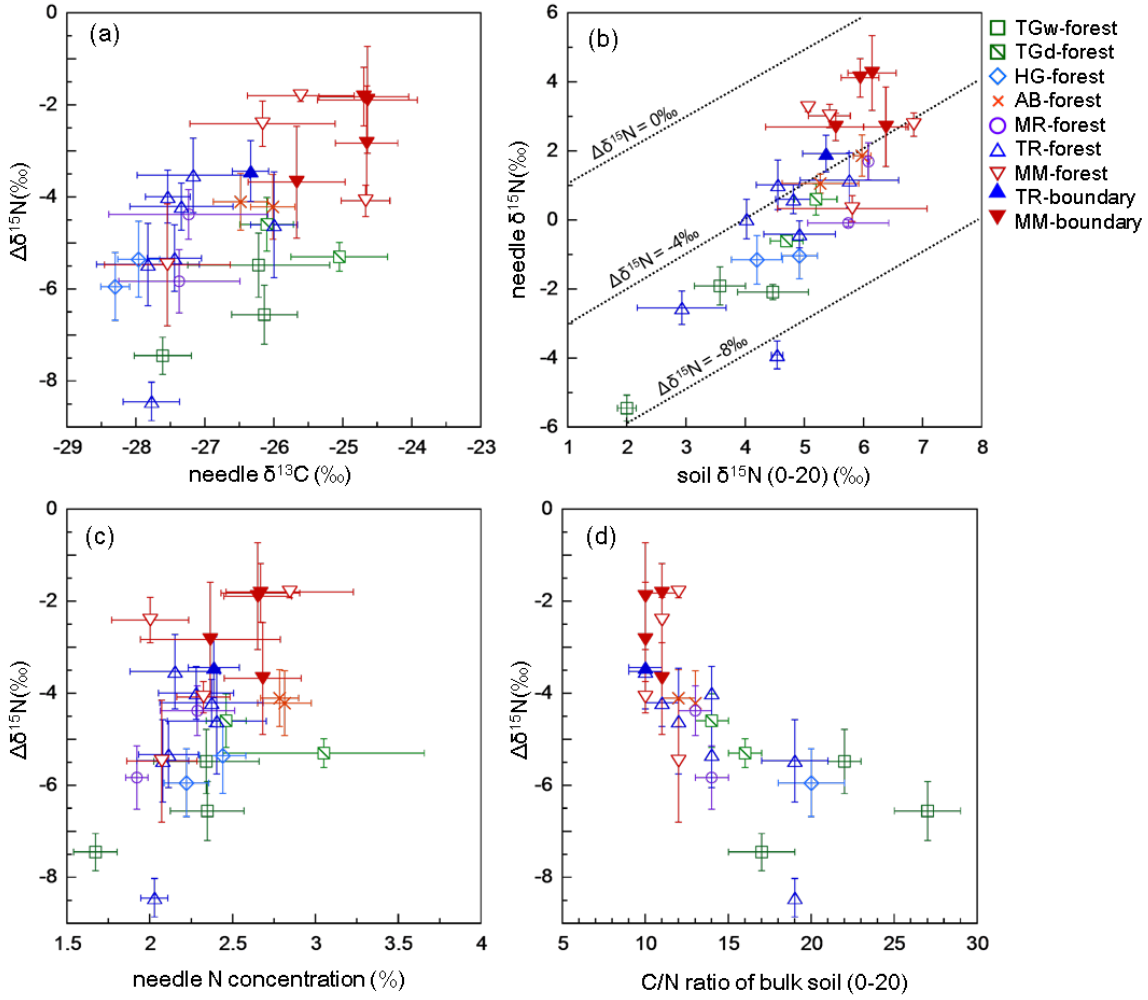


Figure 3.7. Correlations between larch needle $\delta^{13}C$ and $\Delta\delta^{15}N$ (r_s = 0.648) (a), needle and soil $\delta^{15}N$ (r_s = 0.795) (b), needle N concentration and $\Delta\delta^{15}N$ (r_s = 0.499) (c), and C/N ratio of bulk soil and $\Delta\delta^{15}N$ (r_s = -0.793) (d) at all sites in all areas. Dotted lines in (b) indicate $\Delta\delta^{15}N$ values at 0‰, -4‰ and -8‰. Correlation coefficients are significant at $p < 0.05$. Bars represent standard deviation of the mean.

Chapter 4 Discussion

4.1. Seasonal variation in needle N concentration of larch tree

The N concentration of larch needle showed seasonal variation in which significantly high in May compared to the following months (Figure 3.1). The observed decrease in N concentration from May to July can be explained by a dilution of N with addition of carbon as the needles mature from May to July, as generally observed (Chapin and Kedrowski1983; Schlesinger and Bernhardt 2013).

4.2. The depth of available N production in soil

In our study, soil $\delta^{15}N$ at all sites showed the maximal of $\delta^{15}N$ value above 20-30 cm layer (Figure 3.2 and 3.3). This suggests that soil $\delta^{15}N$ in the 0-20 cm soil layer seem to be representative of the layer which provides nutrient to plants, as decomposition processes in the soil cause increase in soil $\delta^{15}N$ (Hobbie and Ouimette 2009). The KCl-extractable N showed higher concentration in 0-20 cm layer than the deeper layers (Figure 4.1), which supports this idea.

4.3. Interpretation of observed $\Delta\delta^{15}N$ in forest and grassland boundary

4.3.1. Isotope mass balance

The range of $\Delta\delta^{15}N$ observed in this study (6‰) (Figure 3.7) accounts for a half of the range reported in globe (13‰) (Amundson et al. 2003). This large variation in $\Delta\delta^{15}N$ indicates that the N cycle changes along the vegetation gradient between forest and grassland. Here the isotope mass balance of available N in the soil was applied to understand the mechanism of the variation in $\Delta\delta^{15}N$.

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 (Brenner et al. 2001), the flux of N input in the system equals to that of N loss from the system, the equation ($F_{input} = F_{leach} + F_{gas}$) can be established, and then the equations (1) and (2) can be simplified as follows:

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

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

where δ_s is soil $\delta^{15}N$, 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. δ_p = needle $\delta^{15}N$), and also $\delta^{15}N$ 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 $\delta^{15}N$ at 0-20 cm layer (Figure 3.2 and 3.3), in which creation of ^{15}N-depleted N as products of N processes such as mineralization, nitrification is suggested (Hobbie and Ouimette 2009).

From these equations, two processes could explain the small difference in $\delta^{15}N$ between larch needle and soil ($\Delta\delta^{15}N \cong 0$) observed at boundary sites (Figure 3.7(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 $\delta^{15}N$ in microorganisms than that of substrate organic N (Dijkstra et al. 2008; Coyle et al. 2009). Therefore, it is reasonable to consider that the observed small $\Delta\delta^{15}N$ at boundary sites show that available N produced in the soil is mostly transported to larch without significant fractionation. In contrast, large difference in $\delta^{15}N$ between larch needle and soil ($\Delta\delta^{15}N < 0$) in forest sites (Figure 3.7(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 (Dijkstra et al. 2008; Coyle et al. 2009), or consistent with ^{15}N enrichment in mycorrhizal fungal body and ^{15}N-depletion in host plant (Gebauer et al. 1993; Michelsen et al. 1998). In fact, the observed magnitude of $\Delta\delta^{15}N$ at TR1n (8‰) was within the range of isotopic fractionation caused by ectomycorrhizal fungi (5‰ to 9‰) (Hobbie and Hogberg 2012), though such large enrichment is not always observed (Gebauer et al. 2003).

Interpretations of $\Delta\delta^{15}N$ 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 $\delta^{13}C$ were observed (Figure 3.7(a), (c), and (d)). Foliar N concentration and $\delta^{13}C$ have been frequently used as indicators of N availability for trees (Liang et al. 2014) and of light and moisture conditions for plants (Farquhar 1989), and the soil C/N as the decomposition rate of soil organic matter (Finzi et al. 1998). Therefore, these parameters as well as the high fraction of NH_4^+ nitrified in the soil in MM area (Table 3.1) indicate rapid decomposition of soil organic matter and high N availability for larch under sunny and dry conditions. On the other hand, at forest sites, a low needle N concentration, a high C/N ratio of bulk soil, and a low needle $\delta^{13}C$ were observed (Figure 3.7(a), (c), and (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 (Popova et al. 2013). The only study which compared the immobilization between moder and mull type of organic layer in Czech Republic (Frouz and Novakova 2005) has shown the decrease in bacterial immobilization along the shift from moder to mull along succession stage from shrub (*Salix caprea*) to forest (*Populus tremuloides* and *Betula spp*). They attributed the decrease of immobilization to the decrease in organic carbon content in topsoil layer caused by earthworm-mediated soil mixing and decrease in the availability of soil organic matter for bacteria from moder to mull. In our study, as the boundary sites were influenced by grasses dominant in steppe, the decrease in carbon availability of litter from forest to grassland boundary might have caused the decrease in bacterial immobilization at boundary sites.

In the above mass balance, we assumed that only the 0-20 cm soil layer provided available N to larch. However, in reality, the organic layer may also provide available N (Tietema et al. 1992). The organic layer showed lower $\delta^{15}N$ than the bulk soil (Figure 3.2), suggesting that the $\delta^{15}N$ of available N produced in the organic layer might be lower than that in the 0-20 cm soil layer (Koba et al. 2003). In 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 $\delta^{15}N$ of available N from the organic layer might contribute to large $\Delta\delta^{15}N$.

The physiological processes in plant such as N uptake and assimilation have been reported to cause in variations in plant $\delta^{15}N$ among species or among parts in a plant (Evans 2001). However, this study can be ignored these variations since the single species (*Larix sibirica* Ledeb.) and the same part (needles on previous year shoot) were treated.

4.3.2. Validity of isotope mass balance in yearly timescale

In the mass balance equations from (1) to (4), we assumed that the available N pool was theoretically at a steady state condition. Also to obtain (3) and (4), we assumed that input and loss fluxes could be ignored. Strictly speaking, these assumptions are not always true. However in this study, here we show our assumption is plausible in yearly timescale.

Generally, recycled N within the plant-soil system provides the primary source of N for biological activities in most terrestrial ecosystems (Parton et al. 2007). Although there is no enough data to evaluate the N budget at our study sites, we assume that

recycled N is the dominant flux compared to the input and loss fluxes in the plant-soil system. In taiga, the annual N demand of larch (F_p) has been reported at 1,500 mg N m^{-2} yr^{-1} near at Tura in central Siberia (Tokuchi et al. 2010), and 850 to 3,100 mg N m^{-2} yr^{-1} at Yakutsk in northeastern Siberia (Popova et al. 2013). For other plant than larch, annual N demand by perennial grass in steppe is estimated more than 2000 mg N m^{-2} $year^{-1}$ (He et al. 2008; Bai et al. 2008). Microbial immobilization (F_m) with plant absence has been reported in taiga forest (~14000 mg N m^{-2} $year^{-1}$) (Popova et al. 2013) and in steppe (~28000 mg N m^{-2} $year^{-1}$) (Wu et al. 2012). Compared to these N fluxes within soil-plant system, N input as N deposition in TR area has been observed to be 96 to 289 mg N m^{-2} yr^{-1} (EANET 2011), biological N_2 fixation is estimated up to 200 mg N m^{-2} yr^{-1} in boreal forest (DeLuca et al. 2002) and 100 mg N m^{-2} yr^{-1} in grassland in inner Mongolia (Yang et al. 2011). In sum up, total annual N input (F_{input}) is calculated as up to 500 mg N m^{-2} yr^{-1}, and this supply is much smaller than annual N demand by larch trees (F_p) or microorganisms (F_m). As for N loss from system, gaseous N flux (N_2O and NO_x) (F_{gas}) has been reported ~1 mg N m^{-2} $year^{-1}$ in taiga forest (Koide et al. 2010) and ~32 mg N m^{-2} $year^{-1}$ at steppe in Inner Mongolia (Xu et al. 2003; Holst et al. 2007). N loss as leaching (F_{leach}) is negligible both in taiga forest (Shugalei and Vedrova 2004) and at steppe in this region (Iijima et al. 2012). In sum up, total N loss (< 40 mg N m^{-2}

year^{-1}) is much smaller than annual N demand by larch trees (F_p) or microorganisms (F_m). Therefore, in yearly timescale, our assumption in mass balance (1) which neglected input and loss fluxes are plausible, and we assume that internal processes (F_p, F_m ,and F_a) are the factors which control quality and quantity of available N pool.

In yearly timescale, change in available N pool size might be ignored in our study region. In taiga forest of northeastern Siberia, Popova et al. (2013) observed seasonality of soil inorganic N pool: in the beginning of summer the pool was small and increased rapidly in August due to mineralization, then by the next June it was small again due to microbial immobilization. In another study, Robinson (2002) reviewed that a major part of N mineralized in ecosystems at high latitudes may be immobilized very quickly in microbial biomass. These suggest that at our forest sites, available N pool size in soil can be regarded as stable (i.e. not increase or decrease) in yearly timescale, although it varies seasonally. Meanwhile, at steppe in Inner Mongolia, Wu et al. (2012) estimated annual net ammonification of -0.9 gN m^{-2} year^{-1}, net nitrification of 3 gN m^{-2} year^{-1} , hence total 2 gN m^{-2} of available N is accumulated in 0-10cm soil with plants absence in one year. However this available N might be used up by dominant perennial grass in at steppe in Inner Mongolia (*Laymus chinensis*), since this grass contains 2 gN m^{-2} in 0-10 cm soil layer (He et al. 2008) due to rebirth of roots which has 81 days of lifespan

(Bai et al. 2008). These results suggest that the net annual accumulation of available N is used up by grass for new production of roots at our boundary sites. From all above, the steady state condition for available N pool in yearly timescale might be plausible in this study.

Furthermore it is unlike that N input and loss causes spatial variation in $\delta^{15}N$ observed in such small spatial scale (less than 2 km) in each area. As for N input (N_2 fixation and N deposition), the amount and isotopic composition of N input 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 (Harrison et al. 2000), it may not affect much our results because of the similar reasons described above. 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 (Martinelli et al. 1999). This process is opposite to our interpretation to bear large $\Delta\delta^{15}N$ in the forest (i.e. larch uptake ^{15}N-depleted N which exists in soil). Hence, also from viewpoint of $\delta^{15}N$, both N input and loss processes may not suitable to explain the variation in $\Delta\delta^{15}N$ observed in this study.

4.4. Long timescale change in $\delta^{15}N$

In above discussion, we have proposed that the difference between larch needle and soil $\delta^{15}N$ ($\Delta\delta^{15}N$) reflects internal process which occurs in plant-soil system yearly timescale: the divergence of available N between larch and microorganisms in the soil, and the accumulation of diverged N in the organic layer. On the other hand, we also observed spatial trends in needle and soil $\delta^{15}N$ values: in this study, the forest sites showed lower needle $\delta^{15}N$ and soil $\delta^{15}N$ than those in the boundary sites (Figure 3.2 and Figure 3.7(b)). It is well known that $\delta^{15}N$ values of terrestrial ecosystem N pools are influenced by not only internal processes but also temporal change of input-output balances (Pardo and Nadelhoffer 2010), so here we calculate ecosystem $\delta^{15}N$ (Compton et al. 2007), the averaged $\delta^{15}N$ weighted by the size of each N pool (larch, organic layer, and soil) for forest site (TR1n), boundary site (TR7n), and grassland site (TR2s) in TR area (Table 4.1). We hypothesize that if variations in needle $\delta^{15}N$ and soil $\delta^{15}N$ along forest-grassland gradient are caused by only internal processes, ecosystem $\delta^{15}N$ should be the same among forest, boundary, and grassland. However, the calculated ecosystem $\delta^{15}N$ values are not same but lower in the order of forest (+1.3 to +1.5‰), boundary (+4.8 to +5.0‰), and grassland (+7.4‰) in TR area (Table 4.1). This indicates that needle $\delta^{15}N$ and soil $\delta^{15}N$ values cannot be explained only by internal process, but N input-output effect significantly contributes those variations. In northern Mongolia,

present landscape of forest-grassland boundary has been reported to be established from past 3200 to 6400 years ago followed by forest expansion to grassland (Ma et al. 2013; Sun et al. 2013). This means that present forest sites was once grassland and have changed from grassland to forest with time. Here we propose that the present difference in the type of the accumulation of organic layer (moder in forest and mull in grassland boundary) was caused by the change in dominant plant species (larch or Poaceae) along forest to grassland boundary. The change in the dominant plant species from grass (Poaceae) to tree (larch) along forest expansion might have caused the change in litter quality (e.g. more recalcitrant litter in larch) and caused the difference in decomposition rates or microbial activities in soil (Chapman et al. 2005), then the difference in the type of accumulation of organic layer “moder” and “mull” might have been formed. Furthermore, we suggest that the lower $\delta^{15}N$ values in the forest sites might be explained by the accumulation of input N for a long timescale (~millennial) with forest development. Long-term decrease in soil $\delta^{15}N$ during forestation can be explained by the accumulation of organic layer of which $\delta^{15}N$ is much lower than that in the soil, and the source of N accumulated in the forest is expected to be N input such as N fixation and/or atmospheric N deposition with low $\delta^{15}N$ (Handley et al. 1999; Houlton et al. 2006). In fact, for example, decrease in ecosystem $\delta^{15}N$ over the century due to

accumulation of N input has been reported at mixed forests in Rhode Island, USA (Compton et al. 2007). Also it has been reported that in boreal forest, a significant amount of N_2 is fixed by cyanobacteria living in association with mosses, contributing up to 50% to the total N input (Rousk et al. 2014). Meanwhile, soil $\delta^{15}N$ in grassland site was higher than those in boundary and forest. This suggests that soil $\delta^{15}N$ in grassland might have increased from 0‰ (typical $\delta^{15}N$ of input N) during grassland establishment, possibly through the losses of ^{15}N-depleted gases as N_2O (Holst et al. 2007; Wolf et al. 2010) since hydrological loss is unlikely in our sites (Iijima et al. 2012). As for needle $\delta^{15}N$, its value might have been affected by $\delta^{15}N$ of N source for larch (soil $\delta^{15}N$) and fractionation between soil and larch ($\Delta\delta^{15}N$). The significant correlations between needle $\delta^{15}N$ and soil $\delta^{15}N$ or $\Delta\delta^{15}N$ observed in this study support this idea (Figure 3.7(b)).

The mechanisms of the observed variations in larch needle $\delta^{15}N$, soil $\delta^{15}N$, and $\Delta\delta^{15}N$ in forest-grassland boundary in northern Mongolia is summarized as follows and in schematic figure, taking $\delta^{15}N$ values in TR area as an example (Figure 4.2).

(1) More than 6400 years ago, grassland was dominant in this region and the soil $\delta^{15}N$ (8‰) was higher than $\delta^{15}N$ of input N (around 0‰) by gaseous N loss (mainly as N_2O).

(2) Around 6400 to 3200 years ago, forest expansion to grassland started, and soil $\delta^{15}N$ in the tree expanded areas started to decrease through accumulation of input N such as N_2 fixation.

(3) In association with forest development, soil $\delta^{15}N$ in the forest have become lower than in grassland due to accumulation of input N in the forest, and at the same time, the divergence of available N between larch and microorganisms in the soil with accumulation of diverged N in the organic layer have caused large $\Delta\delta^{15}N$ in the forest.

4.5. Comparison of$\delta^{15}N$ with global data-set

Recently, global syntheses of $\delta^{15}N$ values of plant and soil have been done by comparing the $\delta^{15}N$ data with climate variables such as mean annual temperature (MAT) and precipitation (MAP). Amundson et al. (2003) compiled the $\delta^{15}N$ values from previous studies, performed regression analysis using MAT and MAP assuming that climate exerts a first-order control on ecosystem $\delta^{15}N$, and successfully formulated plant and soil $\delta^{15}N$ and their difference ($\Delta\delta^{15}N$) with MAT and MAP. In addition, Craine et al. (2009) compiled foliar $\delta^{15}N$ and showed that foliar $\delta^{15}N$ increased with MAT at a rate of 0.23‰ $°C^{-1}$ for ecosystems with MAT > −0.5°C, and decreased by 2.6‰ for every order

of magnitude increase of MAP. Similarly, Craine et al. (2015) conducted an analysis with compiled soil $\delta^{15}N$, and reported that soil $\delta^{15}N$ increased with MAT at a rate of 0.18‰ $°C^{-1}$ for ecosystems with MAT > 9.8°C but invariant with MAT below 9.8°C. As for MAP, soil $\delta^{15}N$ decreased at a rate of 1.78‰ for every order of magnitude increase of MAP. Plant $\delta^{15}N$ values calculated for the MAT and MAP of all areas with the multi-regression equation by Amundson et al. (2003) derive from -1.2‰ for TG area to -0.3‰ for MR area, and those values are close to the average for all sites in this study (0.0 ±2.2‰). Observed range in needle $\delta^{15}N$ (-5.4‰ to +4.3‰) also drops to the ranges of foliar $\delta^{15}N$ for both MAT and MAP as shown by Craine et al. (2009). Similarly, soil $\delta^{15}N$ values are calculated to be from +2.8‰ for TG area to +4.0‰ for MR area with MAT and MAP by the multi-regression equation (Amundson et al. 2003), and these values are slightly lower but also close to the average for all sites in this study (+5.1 ±1.1‰). The observed range of soil $\delta^{15}N$ (+2.0‰ to +6.8‰) is also within the range reported by Craine et al. (2015). It is worth noting that a large difference in $\delta^{15}N$ values was observed between forest and boundary sites, but average $\delta^{15}N$ values observed for all sites, nevertheless, are close to the values obtained from global syntheses. Furthermore, observed $\Delta\delta^{15}N$ data are consistent with the global trend of $\Delta\delta^{15}N$ (Craine et al. 2015) (Figure 4.3). All our data were plotted below the solid line (0‰), which was

consistent with the data shown by Craine et al. (2015) under each MAT in each area.

On the other hand, observed variations $\delta^{15}N$ in this study cannot be explained by climate effect proposed by Amundson et al. (2003), suggesting that vegetation type is another factor controlling $\delta^{15}N$. The observed range of soil $\delta^{15}N$ (5‰) was comparable to other ecosystem state factors (Figure 1.3(c)). Furthermore, the observed range of $\Delta\delta^{15}N$ (7‰) occupies the half of the range estimated by climate in Amundson et al. (2003). Since the calculation of $\Delta\delta^{15}N$ by Amundson et al. (2003) is based on a first-order control of climate on N cycle, the calculated values and range might reflect only direct effect of climate on N cycle (i.e. precipitation and/or temperature affect the form of loss N from soil and cause variation in $\Delta\delta^{15}N$). Therefore, here we propose that this study has clarified the new factor and new process controlling $\Delta\delta^{15}N$: vegetation type, which was equivalent to the type of accumulation of organic layer, causes significant variation in $\Delta\delta^{15}N$ through divergence of available N between larch uptake and microbial immobilization.

4.6. Implication of this study

This study has shown that the variation in $\Delta\delta^{15}N$ can be attributed to the type of accumulation of organic layer from moder to mull type, which was corresponded to

vegetation type from forest to grassland. On the other hand, vegetation type is not always equivalent to the type of accumulation of organic layer. For example, Brearley (2013) observed both the mor and mull type within the same Jamaican montane forest. This means that the relationship between vegetation type and the type of accumulation of organic layer is not always established. However, we suggest that the relationship between the type of accumulation of organic layer and the variation in $\Delta\delta^{15}N$ is permanent under climax ecosystem where plant and soil N pools are steady state, since the process which is typical in each moder and mull type is consistent with the process proposed by isotope mass balance. To prove this, further studies in other plant species and other places are needed. In the same way, further studies to clarify the relationship between another ecosystem state factor and $\delta^{15}N$ of plant and soil with underlying N process, and to clarify the relative importance of each factors in various spatial scales will promote the interpretation of spatial trend of $\delta^{15}N$.

Table 4.1. Calculation of ecosystem $\delta^{15}N$ value at forest site (TR2n), boundary site (TR7n), and grassland site (TR2s) in TR area.

Site			$\delta^{15}N$ (‰)				N content (gN m^{-2})			Ecosystem $\delta^{15}N$ (‰)[e]
		dominance	Larch	Poaceae	Organic layer	Soil	Plant	Organic layer	Soil (0-20 cm)	
TR2n	forest	Larch	-2.5 (0.5)	n/a	-2.2 (0.6)	+2.9 (0.8)	2.6 to 8.8[b]	167 (20)	391 (82)	+1.3 to +1.5
TR7n	boundary	Poaceae	+1.9 (0.5)	+1.6	n/a	+5.4 (0.4)	1.0 to 5.2[c]	n/a	1365 (140)[d]	+4.8 to +5.0
TR2s	grassland	Poaceae	n/a	+1.6[a]	n/a	+7.4	1.0 to 5.2[c]	n/a	1220[d]	+7.4

[a]$\delta^{15}N$ of Poaceae at TR2s was not measured, so that $\delta^{15}N$ of Poaceae at TR7n was applied.

[b]Larch N content was calculated by needle N content obtained in this study and needle biomass (tonnes ha^{-1}) of taiga forest in Mongolia from Tsolmon et al. (2002) (International Journal of Remote Sensing, 23(22), 4971-4978) and Battulga et al. (2013) (Journal of Forestry Research, 24(3), 431-437).

[c]Poaceae N content was calculated by whole N content obtained in this study and aboveground biomass (g m^{-2}) of steppe in Inner Mongolia from Zhou et al. (2009) (Soil Biology and Biochemistry, 41(4), 796-803) and Bai et al. (2004) (Nature, 431(7005),

181-184).

[d]Soil N content was calculated by bulk N content (gN g^{-1}) obtained in this study and assumed bulk soil density of 1.5 g cm^{-3}.

[e]Ecosystem $\delta^{15}N$ value was calculated as weighted average of $\delta^{15}N$ value by N content of each N pool (i.e. plant, organic layer, or soil).

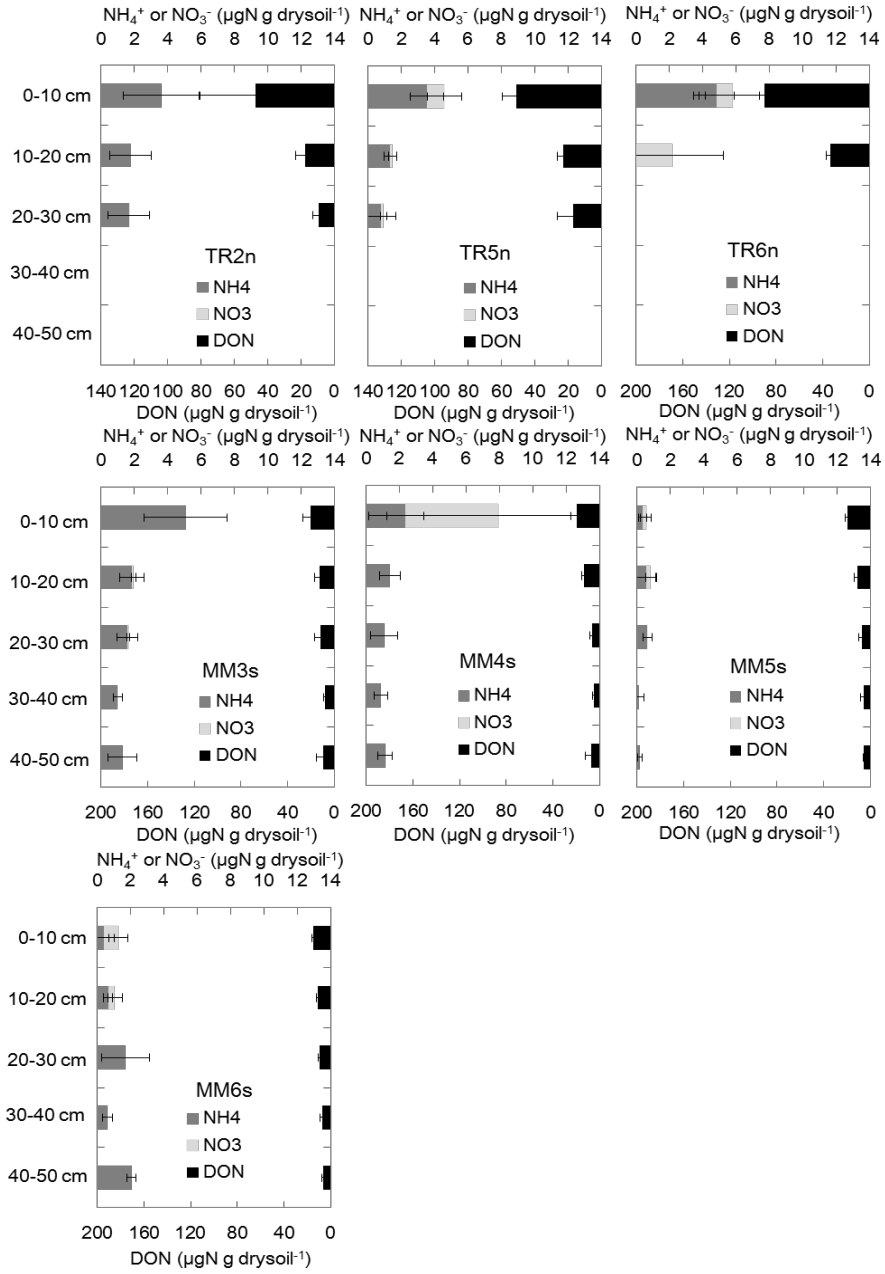


Figure 4.1. Profiles of KCl-extractable N pools in each soil layer in TR and MM area. Three forest sites (TR2n, TR5n, and TR6n) in TR area, and one forest site (MM3s) and three boundary sites (MM4s, MM5s, and MM6s) in MM area were observed in August 2012.

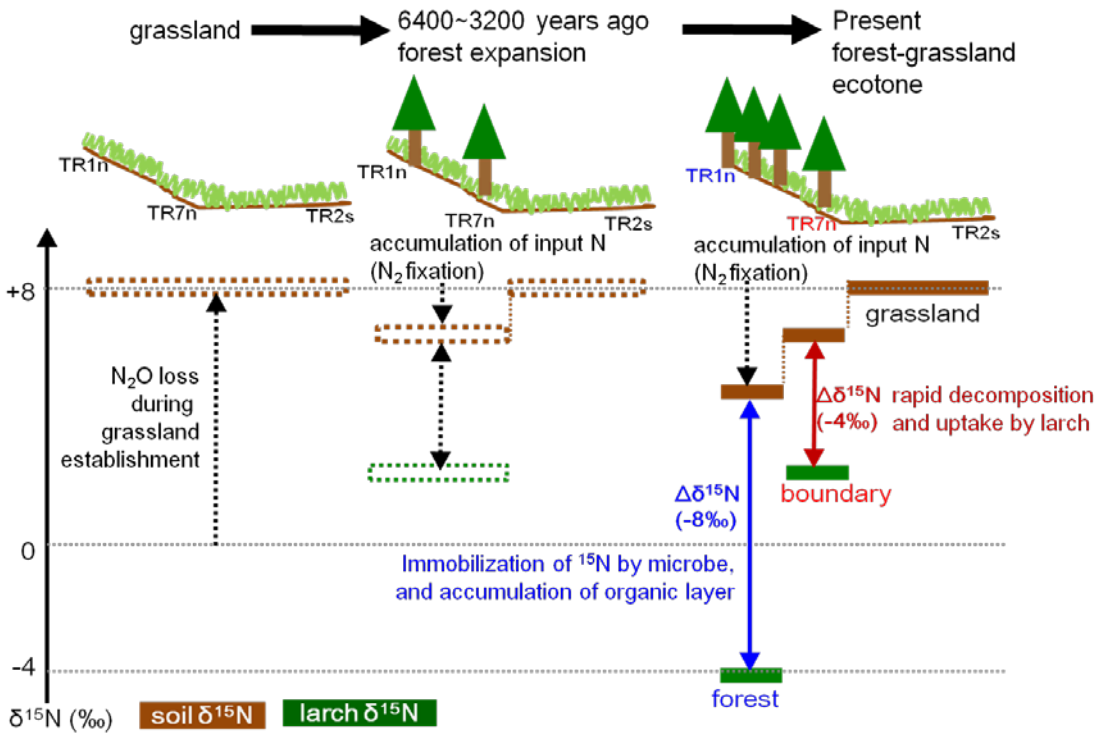


Figure 4.2. Schematic representation of the mechanism of spatial variations in $\delta^{15}N$ values along forest-grassland gradient in northern Mongolia, taking TR area as an example. The brown square is soil $\delta^{15}N$ and green square is larch needle $\delta^{15}N$. The observed $\delta^{15}N$ values are expressed as filled square, whereas assumed $\delta^{15}N$ values are expressed as dashed square. More than 6400 years ago, grassland was dominant in this region and the soil $\delta^{15}N$ (8‰) was higher than $\delta^{15}N$ of input N (around 0‰) by gaseous N loss (mainly as N_2O). Around 6400 to 3200 years ago, tree expansion to grassland started, and soil $\delta^{15}N$ in the tree expanded areas started to decrease through accumulation of input N such as N_2 fixation. In association with forest development,

soil $\delta^{15}N$ in the forest have become lower than in grassland due to accumulation of input N in the forest, and at the same time, the divergence of available N between larch and microorganisms in the soil with accumulation of diverged N in the organic layer have caused large $\Delta\delta^{15}N$ in the forest.

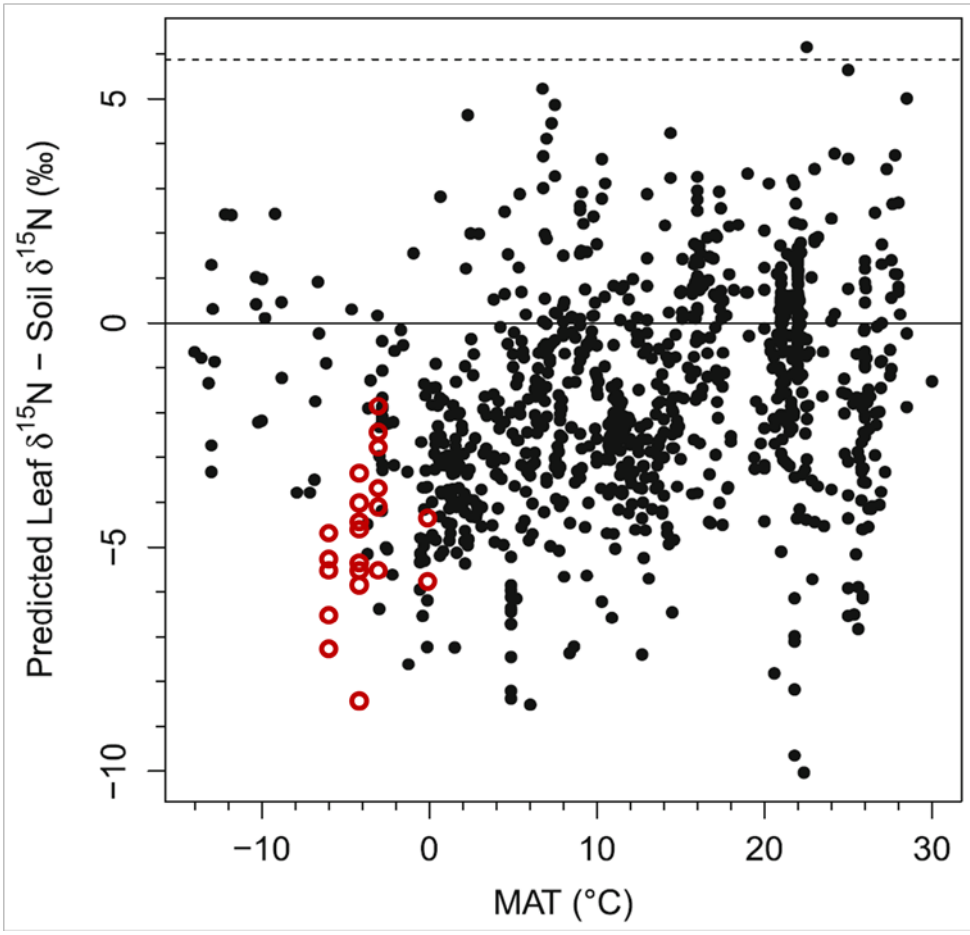


Figure 4.3. Relationship between mean annual temperature (MAT) and the difference between $\delta^{15}N$ of leaves and soil in Craine et al. (2015). In this figure, the data obtained in this study were plotted as red circle. MAT was -6°C in TG area, -4°C in HG, AB, TR area, -3°C in MM area, and 0°C in MR area.

Chapter 5 Conclusion

To clarify the variations of plant and soil $\delta^{15}N$ and associated N processes which arise from vegetation type, larch needle and soil $\delta^{15}N$ were investigated at forest-grassland boundary in northern Mongolia. Along forest to grassland boundary, clear variations of $\delta^{15}N$ were observed: larch needle and soil $\delta^{15}N$ increased, whereas the difference of needle and soil $\delta^{15}N$ ($\Delta\delta^{15}N$) became smaller from forest to boundary. The observed range was 9‰ (-5.4‰ ~ +4.3‰) for needle $\delta^{15}N$, 5‰ (+2.0‰ ~ +6.8‰) for soil $\delta^{15}N$, and 7‰ (-8.4‰ ~ -1.8‰) for $\Delta\delta^{15}N$ in this region. The observed range of $\Delta\delta^{15}N$ was large enough to occupy half of the global range estimated by climate effect. The vegetation type corresponded to the type of accumulation of organic layer: mull type at boundary and moder type in forest. Isotope mass balance suggested that small $\Delta\delta^{15}N$ at boundary could be explained by rapid recycling of N between larch and soil without significant microbial immobilization, whereas large $\Delta\delta^{15}N$ in forest could be explained by the significant microbial immobilization as well as uptake by larch. The isotope mass balance and observed environmental parameters matched to the characteristics reported in mull and moder type, which means that observed variation of $\Delta\delta^{15}N$ along forest-grassland boundary can be explained by the processes representative of the type

of the accumulation of organic layer (moder-mull). We suggest the relationship between the type of accumulation of organic layer and variation of $\Delta\delta^{15}N$ is permanent under climax ecosystem where plant and soil N pools are steady state.

Acknowledgments

I am deeply grateful to Professor Atsuko Sugimoto for the opportunity of this study and the patient support throughout my study for seven years. I also appreciate Associate Professor M. Yamamoto (Hokkaido University), Associate Professor Y. Yamashita (Hokkaido University), and Assistant Professor T. Irino (Hokkaido University) for many critical advice on my thesis and kind encouragement, and all teachers in the course in geochemistry for many suggestions for my PhD defense.

I express special thanks to Associate Professor Lopez C. M. Larry (Yamagata University) for the big support during the fieldwork in Mongolia, staying in Yamagata, and processes of writing article. I also thank all members of the fieldwork in Mongolia for their supports.

I would like to express my gratitude to Associate Professor Isao Kudo (Hokkaido University) and the members in his laboratory for their support on the measurement of dissolved N by autoanalyzer (QuAAtro).

I appreciate Ms. Y. Hoshino, Ms. H. Kudo and all the secretaries in Sugimoto laboratory for their kind encouragement, and I would like to thank A. Ueta, A. Popova, S. Tei, M. Liang, X. Li, F. Li, R. Shingubara, S. Takano, A. Kitayama, T. Morozumi, R. Fan, R. Shakhmatov, H. Shimada, D. Zhou, S. Saito, and all graduated members in

Sugimoto laboratory, and all members in our hensen-group for their supports.

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