

Soil nitrification and foliar $\delta^{15}\text{N}$ declined with stand age in trembling aspen and jack pine forests in northern Alberta, Canada

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Abstract

Background and aims Understanding changes in soil N cycling with stand development is critical for forest management as tree growth is affected by soil N availability. The aim of this study was to evaluate the changes in soil N availability and loss with stand development in trembling aspen (*Populus tremuloides* Michx.) and jack pine (*Pinus banksiana* Lamb.) in northeastern Alberta, Canada.

Methods Soil inorganic N availability (measured as N supply rate) and foliar N chemistry (N concentration and $\delta^{15}\text{N}$) in trembling aspen stands ranged from 52 to 70 years old ($n=7$) and jack pine stands 43 to 78 years

old ($n=8$) were investigated in 2008 and 2009. The relationships among the ratios of NO_3^- -N to total inorganic N (NO_3^- -N/TIN), foliar N concentration, and foliar $\delta^{15}\text{N}$ with stand age were also explored by regression analyses.

Results Total inorganic N supply rates did not systematically change with stand age across stand types, soil layers and measurement periods; whereas NO_3^- -N/TIN showed a decreasing tendency with stand age, suggesting that nitrification and associated N loss potential became smaller in older stands with greater limitation in soil N availability. Foliar $\delta^{15}\text{N}$ decreased with stand age from -1.7 to -4.7‰ for aspen and from -4.1 to

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–7.1‰ for jack pine, and there were positive correlations between foliar $\delta^{15}\text{N}$ and soil NO_3^-/TIN , suggesting that decreased soil N loss led to less ^{15}N -depletion in the inorganic N available for tree uptake in older stands. However, foliar N concentration did not significantly change with stand age, suggesting that there were other N sources such as organic N in the forest floor, in addition to the inorganic N, available for plant uptake.

Conclusions Our results suggest that soil inorganic N availability became more limited as stand age increased. In addition, the ratio of NO_3^-/TIN and its relationship with foliar $\delta^{15}\text{N}$ indicated decreased soil N loss potential and shifted N sources with stand age in boreal forests that are typically N-limited. Our study demonstrated that declining nitrification with increasing stand age might be one of the mechanisms mediating N-limitation in the studied boreal forests.

Keywords Boreal forest · Nitrogen concentration · Nitrogen isotope ratio · Nitrogen loss · Stand development

Introduction

In boreal forests, N availability is often one of the limiting factors for tree growth (Gundersen et al. 2009; Yan et al. 2012); thus, understanding changes in soil N availability with forest development is critical (DeLuca et al. 2002; McLauchlan et al. 2007). In this context, a number of studies have investigated changes in soil N availability with the succession of forest ecosystems and reported that soil N availability may increase or decrease, depending on the stand type and the extent of disturbance imposed on the forests (e.g., Odum 1969; Vitousek and Reiners 1975; Vitousek et al. 1989; Goodale et al. 2003; McLauchlan et al. 2007). Therefore, it is still difficult to assess changes in soil N cycling with the development of forest stand using conventional indicators such as N transformation rates including net N mineralization and nitrification rates and inorganic N concentration that are highly variable both over time and space (Binkley et al. 2000; Prasolova et al. 2000; Aubert et al. 2005; Garten et al. 2011).

The natural ^{15}N abundance ($^{15}\text{N}/^{14}\text{N}$, expressed as $\delta^{15}\text{N}$) of plant samples may serve as a more time- and space-integrating indicator of soil N availability in the rhizosphere where roots assimilate N over the entire growing season (Makarov 2009; Garten et al. 2011;

Hietz et al. 2011; Matsushima et al. 2012). Although plant $\delta^{15}\text{N}$ is affected by many factors and processes such as the sources of N (soil N, atmospheric N, or mycorrhizal-mediated N) and N isotopic fractionation associated with soil N transformations and loss, plant uptake and assimilation of N, and N translocation within plants (Evans 2001; Choi et al. 2003; 2005a; Makarov 2009; Pardo et al. 2013), many studies reported that there is a positive relationship between plant $\delta^{15}\text{N}$ and soil N availability, probably due to the fact that a higher soil N availability induces a greater N loss, resulting in an increase in $\delta^{15}\text{N}$ of plant available N in forest soils (Högberg and Johannisson 1993; Pardo et al. 2006; Garten et al. 2011).

Regarding the use of plant $\delta^{15}\text{N}$ for assessing changes in soil N availability with stand development, McLauchlan et al. (2007) suggested that decreased soil N availability with stand development resulted in a declining patterns of $\delta^{15}\text{N}$ in tree rings over the past 75 years in a northern hardwood forest in the 20th century. In an 8-years free-air CO_2 enrichment experiment, Garten et al. (2011) also found that litterfall $\delta^{15}\text{N}$ of sweetgum (*Liquidambar styraciflua* L.) decreased under limited soil N availability. However, there is still uncertainty on the use of tree ring $\delta^{15}\text{N}$ as an indicator of soil N availability due to N isotopic fractionation associated with inter-tree ring movement of N (Choi et al. 2005c). On the other hand, due to the complexity of soil N processes, plant $\delta^{15}\text{N}$ is often carefully interpreted along with other N indices such as foliar and soil N concentrations that may reflect soil N processes (Evans 2001; Craine et al. 2009). Among many soil N processes affecting soil N availability, nitrification is one of the key processes causing N loss through NO_3^- leaching and N_2O emission through denitrification, unless NH_3 volatilization is severe (Choi et al. 2007). Specifically, if nitrification prevails over other N processes such as immobilization, plant $\delta^{15}\text{N}$ is likely to have a higher $\delta^{15}\text{N}$ while showing a lower N concentration in plant tissues due to N losses through leaching and gaseous emissions (via either nitrification or denitrification) causing ^{15}N -enrichment of the remaining soil N that is available for plant uptake (Högberg and Johannisson 1993). Therefore, the relationship between nitrification potential and plant $\delta^{15}\text{N}$ may serve as a more reliable indicator of changes in soil N availability and N loss with stand development as compared with plant $\delta^{15}\text{N}$ alone (Pardo et al. 2006; LeDuc et al. 2013). In addition, a shift in the N pool available for plant uptake under a N-limited environment such as in older stands, a shift from

inorganic N to mycorrhizal-mediated uptake of small molecule organic N (e.g., amino acid needs), should be considered for the interpretation of foliar $\delta^{15}\text{N}$ (Hobbie et al. 2000; LeDuc and Rothstein 2010; Mayor et al. 2012; LeDuc et al. 2013).

In northeastern Alberta, trembling aspen (*Populus tremuloides* Michx.) and jack pine (*Pinus banksiana* Lamb.) are dominant species naturally regenerated following disturbances such as fire and harvesting (Ste-Marie et al. 2007). Although some information on soil N availability and the shift of soil N form with stand development is available (Yermakov and Rothstein 2006; Ste-Marie et al. 2007; LeDuc and Rothstein 2010), the potential mechanisms have rarely been investigated (LeDuc et al. 2013). Therefore, in this study, we aimed to investigate the pattern of long-term changes in soil N availability with development of aspen and jack pine stands using soil (inorganic N supply rates) and plant N indices (foliar N concentration and $\delta^{15}\text{N}$). This study was conducted to test two hypotheses: first, soil N availability may either decrease or increase with stand age depending on stand type and the extent of disturbance; and second, such changes in soil N availability would leave a systematic pattern in foliar $\delta^{15}\text{N}$ with stand age, reflecting an altered magnitude of soil N loss that is dependent on soil N availability and the source of available N. Specifically, we predicted that decreased soil N availability may lead to a lower foliar $\delta^{15}\text{N}$ due both to less N isotopic fractionation associated with lower N loss and a shift to relying on organic N that is ^{15}N -depleted as compared to soil inorganic N.

Materials and methods

Study area and stand characteristics

This study was conducted near Fort McMurray (56°39' N, 111°13' W) in northeastern Alberta within the Boreal Forest Region. This area is characterized by a continental boreal climate, with mean daily temperatures ranging from $-18.8\text{ }^{\circ}\text{C}$ in January to $16.8\text{ }^{\circ}\text{C}$ in July and a mean annual precipitation of about 455 mm, predominantly as rainfall (342 mm) during the summer season (based on Canadian climate normals or averages between 1971 and 2000). Soils in the study area are classified as Eluviated Dystric Brunisols and Orthic Gray Luvisols based on the Canadian System of Soil Classification (Soil Classification Working Group 1998).

In 2008, we selected seven trembling aspen stands ranging from 52 to 70 years old and eight jack pine stands from 43 to 78 years old. The stands established following fire disturbance (Table 1). One $20\times 20\text{ m}$ sampling plot was established within each stand. Tree height and diameter at breast height (DBH) of all living trees were measured in each plot. Aspen foliar samples (sunlit leaves) were randomly collected from the upper-middle part of the canopy in August and jack pine foliar samples in October 2008 and 2009, because deciduous and conifers species have different leaf phenology. At each sampling time, foliar samples were taken from at least 10 trees per plot and combined to form a composite sample.

Soil N supply rates

Soil NH_4^+ and NO_3^- supply rates were determined with Plant Root Simulator (PRSTM, Western Ag Innovations Inc., Saskatoon, SK, Canada) probes consisting of an ion exchange membrane encapsulated in a plastic casing. Four pairs of PRSTM-probes (including four cation exchange probes and four anion exchange probes) were inserted into the forest floor and mineral soil (0 to 20 cm) layers in each plot, and N availability during three periods (Jul. to Sept. 2008, Oct. 2008 to May 2009 and Jun. to Oct. 2009) was determined. At the end of each measurement period, all probes were taken out from the plot and immediately washed with deionized water to remove soil particles. The probes were then placed into clean plastic bags to keep them moist and shipped to Western Ag Innovations Inc. in Saskatoon for analysis.

Foliar N concentration and $\delta^{15}\text{N}$

For foliar $\delta^{15}\text{N}$ and N concentration analysis, the foliar samples were dried at $60\text{ }^{\circ}\text{C}$ for 48 h, then ground to fine powder with a ball mill (MM-200, Retsch GmbH 88 & Co., KG, Haan, Germany). Foliar N concentrations and ^{15}N abundance were determined with an isotope ratio mass spectrometer (VG Optima; Fisons Instruments, Middlewich, Cheshire, U.K.) coupled with an elementary analyzer (Carlo Erba NCS 1500, Milan, Italy). The $\delta^{15}\text{N}$ value was calculated as

$$\delta^{15}\text{N}(\text{‰}) = \left[\left(\frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}}}{(^{15}\text{N}/^{14}\text{N})_{\text{standard}}} \right) - 1 \right] \times 1000$$

where $(^{15}\text{N}/^{14}\text{N})_{\text{sample}}$ is the N isotopic composition of a sample and $(^{15}\text{N}/^{14}\text{N})_{\text{standard}}$ is the N isotopic

Table 1 Location and characteristics of the studied stands

| Stand type | Stand code | Latitude (N) | Longitude (W) | Age (yr) | Density (tree ha ⁻¹) | Height (m) | DBH ^a (cm) | SBA ^b (m ² ha ⁻¹) | % SBA by the dominant species |
|------------|------------|--------------|---------------|----------|----------------------------------|------------|-----------------------|---|-------------------------------|
| Aspen | SV4 | 56.95° | 111.72° | 70 | 2000 | 11.7 | 11.7 | 32.4 | 97 |
| | SV8 | 57.26° | 111.48° | 52 | 1550 | 13.9 | 15.3 | 23.1 | 93 |
| | SV18 | 56.45° | 111.19° | 59 | 1400 | 13.6 | 14.9 | 32.6 | 97 |
| | SV59 | 57.47° | 111.48° | 62 | 2200 | 13.7 | 14.7 | 37.0 | 81 |
| | SV61 | 56.44° | 111.19° | 55 | 1575 | 17.1 | 18.2 | 24.9 | 100 |
| | SV77 | 56.46° | 111.09° | 60 | 2025 | 10.2 | 11.8 | 24.3 | 78 |
| | SV83 | 56.46° | 111.08° | 53 | 1825 | 13.4 | 12.7 | 23.3 | 100 |
| | Jack pine | SV10 | 57.07° | 111.59° | 43 | 1675 | 10.6 | 12.7 | 28.2 |
| SV26 | | 57.51° | 111.43° | 68 | 2075 | 5.0 | 6.9 | 15.1 | 100 |
| SV27 | | 57.51° | 111.44° | 78 | 1075 | 7.8 | 12.6 | 21.6 | 100 |
| SV29 | | 57.10° | 111.64° | 45 | 1650 | 10.9 | 13.0 | 25.5 | 92 |
| SV49 | | 57.10° | 111.64° | 49 | 1325 | 12.6 | 14.9 | 24.4 | 95 |
| SV58 | | 57.47° | 111.47° | 69 | 1375 | 12.0 | 15.5 | 25.8 | 100 |
| SV62 | | 57.50° | 111.52° | 64 | 1100 | 10.0 | 14.6 | 25.1 | 98 |
| SV63 | | 57.50° | 111.52° | 60 | 1150 | 13.4 | 19.1 | 33.8 | 100 |

^a DBH diameter at breast height

^b SBA stem basal area

composition of atmospheric N₂. The accuracy and reproducibility of the $\delta^{15}\text{N}$ measurement were checked with an internal standard material (glycine, $+2.0 \pm 0.1\%$ calibrated against IAEA-N1) and they were better than 0.3 and 0.2‰, respectively.

Statistical analyses

The effects of forest type (aspen vs. jack pine), soil layer (forest floor vs. mineral soil) and measurement period (Jul. to Sept. 2008, Oct. 2008 to May 2009, and Jun. to Oct. 2009) on soil NH₄⁺-N, NO₃⁻-N, TIN and NO₃⁻-N/TIN ratio were evaluated with a three-way analysis of variance (ANOVA), and the effect of forest type and sampling time (2008 vs. 2009) on foliar N concentrations and $\delta^{15}\text{N}$ were analyzed with a two-way ANOVA using the general linear model as described in the SPSS PASW Statistics (ver. 18.0, SPSS Inc.). When the effects were significant, Tukey's test was further conducted to make multiple comparisons among measurement periods. The relationships between stand age and N supply rate, foliar $\delta^{15}\text{N}$ and foliar N concentrations were explored with polynomial regressions analysis to arrive at the best fit. Pearson's correlation analysis between foliar $\delta^{15}\text{N}$ and soil NO₃⁻-N/TIN were conducted. In this study, correlation between foliar $\delta^{15}\text{N}$ was tested not

only with the soil NO₃⁻-N/TIN ratio in the same year but also with the NO₃⁻-N/TIN ratio in the previous year, to account for part of N that was assimilated from soils in the previous year and then re-translocated to foliage of next year via re-sorption of foliar N before senescence, because such reuse of foliar N may affect foliar $\delta^{15}\text{N}$ in the next year (Choi et al. 2005b). All statistical significance was set at $\alpha=0.05$.

Results

Changes in soil N availability and NO₃⁻-N/TIN with stand age

Mean soil inorganic N availability measured as N supply rates across the stand ages were quite variable and not affected by forest type and soil layer. Soil NH₄⁺-N, NO₃⁻-N and NO₃⁻-N/TIN ratio were different among the measurement periods (Table 2). Specifically, soil NH₄⁺-N supply rates had an increasing trend while soil NO₃⁻-N supply rates, NO₃⁻-N/TIN ratio tended to decrease with measurement time regardless of the stand type and soil layer (Table 2).

The analysis on the relationship between TIN concentration and stand age revealed no systematic trend of

Table 2 Availability of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, total inorganic N (TIN, $\text{NH}_4^+\text{-N}+\text{NO}_3^-\text{-N}$) measured as N supply rate, and the $\text{NO}_3^-\text{-N}/\text{TIN}$ ratio in soils under aspen and jack pine stands across stand ages. Values are the means of 7 stands for aspen and 8 for pine with standard errors in the parentheses

| Tree species | Soil layer | $\text{NH}_4^+\text{-N}$ ($\mu\text{g N (10 cm}^{-2}\text{) per month}$) | | | $\text{NO}_3^-\text{-N}$ ($\mu\text{g N (10 cm}^{-2}\text{) per month}$) | | | TIN ($\mu\text{g N (10 cm}^{-2}\text{) per month}$) | | | $\text{NO}_3^-\text{-N}/\text{TIN}$ ratio | | |
|------------------|--------------|--|---------------------|----------------|--|---------------------|----------------|---|---------------------|----------------|---|---------------------|----------------|
| | | Jul-Sept (2008) | Oct-May (2008–2009) | Jun-Oct (2009) | Jul-Sept (2008) | Oct-May (2008–2009) | Jun-Oct (2009) | Jul-Sept (2008) | Oct-May (2008–2009) | Jun-Oct (2009) | Jul-Sept (2008) | Oct-May (2008–2009) | Jun-Oct (2009) |
| Aspen | Forest floor | 0.48b ^d (0.25) | 0.36ab (0.10) | 1.01a (0.33) | 1.02a (0.22) | 0.49b (0.08) | 0.20b (0.11) | 1.50 (0.43) | 0.85 (0.15) | 1.21 (0.32) | 0.8a (0.1) | 0.6b (0.1) | 0.2c (0.1) |
| | Mineral soil | 0.52 (0.15) | 0.89 (0.34) | 1.02 (0.26) | 1.07 (0.20) | 0.46 (0.08) | 0.33 (0.11) | 1.59 (0.24) | 1.36 (0.39) | 1.35 (0.35) | 0.7 (0.10) | 0.4 (0.10) | 0.3 (0.1) |
| Jack pine | Forest floor | 0.73 (0.19) | 1.58 (0.49) | 1.65 (0.64) | 1.28 (0.26) | 0.59 (0.16) | 0.38 (0.12) | 2.02 (0.26) | 2.16 (0.59) | 2.02 (0.58) | 0.6 (0.1) | 0.3 (0.1) | 0.3 (0.1) |
| | Mineral soil | 0.28 (0.0.9) | 0.68 (0.22) | 0.81 (0.08) | 0.98 (0.17) | 0.48 (0.16) | 0.99 (0.57) | 1.26 (0.21) | 1.16 (0.35) | 1.79 (0.60) | 0.8 (0.1) | 0.4 (0.1) | 0.4 (0.1) |
| Effect | | <i>Probability > F</i> | | | | | | | | | | | |
| Tree species (S) | | ns ^b | | | | | | | | | | | |
| Soil layer (L) | | ns | | | | | | | | | | | |
| Time (T) | | * | | | | | | | | | | | |
| S × L | | * | | | | | | | | | | | |
| S × T | | ns | | | | | | | | | | | |
| L × T | | ns | | | | | | | | | | | |
| S × L × T | | ns | | | | | | | | | | | |

^a Values followed by different lowercase letters in the same row indicate significant difference among measurement periods at $\alpha=0.05$

^b ns, non-significant; *, $P<0.05$; ***, $P<0.001$

change in soil N availability with stand age regardless of stand type and soil layer, except for N availability in mineral soil in jack pine stands (Fig. 1). However, there was a systematic pattern in terms of the change in NO_3^-/TIN ratio (an indicator of the intensity of nitrification) with stand age for both species during the active growing seasons, in that the NO_3^-/TIN ratio decreased with stand age in the mineral soil in aspen stands but in both the forest floor and the mineral soil in jack pine stands (Fig. 2).

Changes in foliar N concentrations and $\delta^{15}\text{N}$ with stand age

Both foliar N concentrations and $\delta^{15}\text{N}$ were higher ($P<0.001$) in aspen than in jack pine stands (Table 3). Although foliar N concentrations were different with the measurement period ($P=0.013$), the seasonal variation was much smaller than the variation with stand type. Foliar N concentration was not related with stand age for both stand types; however, foliar $\delta^{15}\text{N}$ was negatively related with stand age (Fig. 3). For aspen, $\delta^{15}\text{N}$ of foliage sampled in 2009 ($\delta^{15}\text{N}_{2009}$) and averaged foliar $\delta^{15}\text{N}$ across 2008 and 2009 ($\delta^{15}\text{N}_{\text{AVE}}$) linearly decreased with stand age ($R=0.87$, $P<0.01$ and $R=0.80$, $P<0.05$, respectively). For jack pine, there was a non-

linear (quadratic) relationship ($R=0.89$, $P<0.05$) between the $\delta^{15}\text{N}_{\text{AVE}}$ and stand age (Fig. 3). However, for both species, $\delta^{15}\text{N}$ of foliage sampled in 2008 ($\delta^{15}\text{N}_{2008}$) was not related with stand age.

Relationships between foliar $\delta^{15}\text{N}$ and soil NO_3^-/TIN

A positive correlation was frequently found between foliar $\delta^{15}\text{N}$ and soil NO_3^-/TIN ratio in the period Jul. to Sept. 2008 in this study. In aspen stands, NO_3^-/TIN ratio in the forest floor for the period from Jul. to Sept. 2008 was positively correlated ($R=0.83$, $P<0.05$) with foliar $\delta^{15}\text{N}_{2008}$, and the NO_3^-/TIN ratio in the mineral layer for the period from Jul. to Sept. 2008 was positively correlated with $\delta^{15}\text{N}_{2008}$ ($R=0.89$, $P<0.01$), $\delta^{15}\text{N}_{2009}$ ($R=0.89$, $P<0.01$), and $\delta^{15}\text{N}_{\text{AVE}}$ ($R=0.95$, $P<0.01$) (Table 4). In jack pine stands, there were positive correlations between NO_3^-/TIN ratio in the forest floor for the period Jul. to Sept. 2008 and foliar $\delta^{15}\text{N}_{2009}$ ($R=0.73$, $P<0.05$) and $\delta^{15}\text{N}_{\text{AVE}}$ ($R=0.80$, $P<0.05$); the NO_3^-/TIN ratio in the forest floor from Jun. to Oct. 2009 and from Jul. 2008 to Oct. 2009 were also positively correlated with $\delta^{15}\text{N}_{\text{AVE}}$ ($R=0.90$, $P<0.01$) and $\delta^{15}\text{N}_{2008}$ ($R=0.71$, $P<0.05$), respectively. However, there was no correlation between foliar $\delta^{15}\text{N}$ and NO_3^-/TIN ratio in mineral soil layer.

Fig. 1 Relationships between stand age and soil total inorganic N supply rates measured at different periods in forest floor and mineral soil layers in aspen and jack pine stands: **a** forest floor of aspen, **b** mineral soil of aspen, **c** forest floor of jack pine, and **d** mineral soil of jack pine

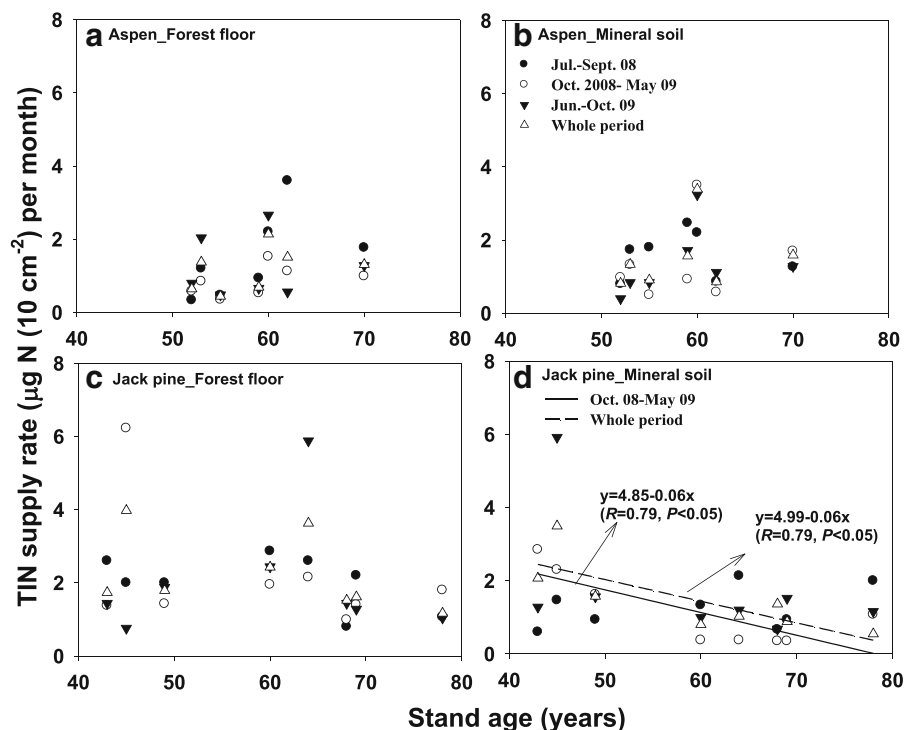
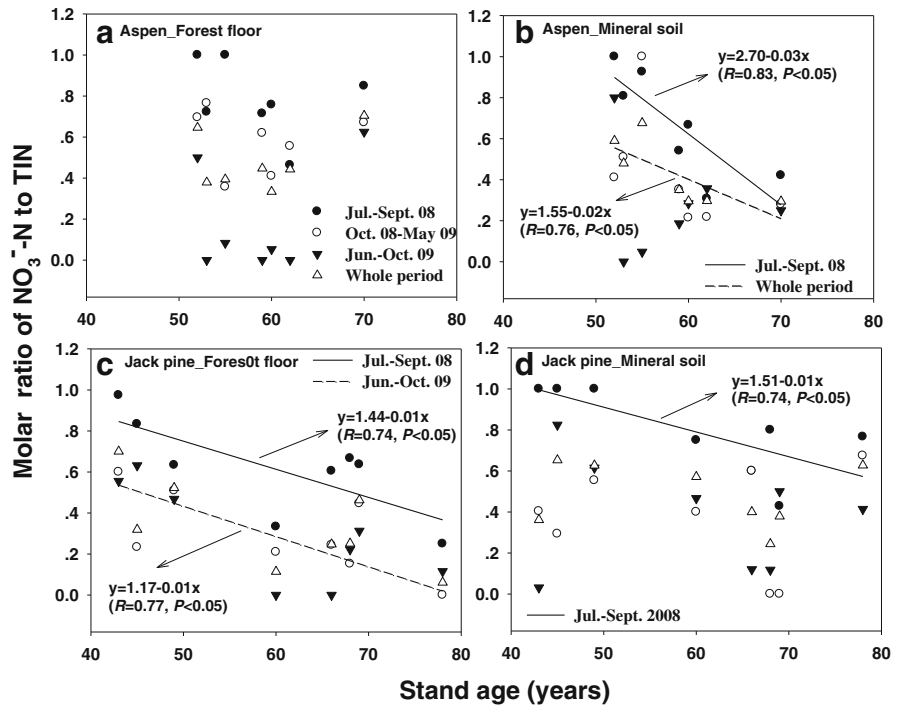


Fig. 2 Relationships between stand age and NO_3^- -N/TIN (total inorganic N) ratio measured at different periods in forest floor and mineral soil layers in aspen and jack pine stands: **a** forest floor of aspen, **b** mineral soil of aspen, **c** forest floor of jack pine, and **d** mineral soil of jack pine



Discussion

Soil NO_3^- -N/TIN as an indicator of N cycling

Generally speaking, in N-limited boreal forests, soil N loss is likely to decrease with stand development and with decreased rates of nitrification, a process that is linked to soil N loss via NO_3^- leaching and N_2O emission through denitrification (Driscoll et al. 1999; DeLuca et al. 2002). Soil nitrification and net N mineralization decreased with stand age in Scots pine (*Pinus sylvestris* L.) forests in northern Sweden (DeLuca et al. 2002), and soil NO_3^-

concentrations were found to decrease with stand age in hybrid white spruce (*Picea glauca* × *engelmannii*) forests in central interior British Columbia, Canada (Driscoll et al. 1999). In this study, the availability of inorganic N measured as soil N supply rates were quite variable (Table 2) and thus a consistent decreasing pattern of soil N availability with stand age was not found (Fig. 1). However, a decreasing pattern of soil NO_3^- -N/TIN ratio (particularly during the active growing season) with stand age (Fig. 2) suggests that the N loss potential through NO_3^- leaching and N_2O emission might decrease with stand development in trembling aspen and jack pine forests. Therefore, we suggest that soil NO_3^- -N/TIN ratio can serve as a better indicator of changed soil N cycling with stand age than soil N availability data in the studied stands. Considering that a negative relationship was more likely between stand age and NO_3^- -N/TIN ratio in the growing season (from Jul. to Sept. 2008 and from Jun. to Oct. 2009) rather than in the non-growing season due to a more active N cycling during the growing season when substrates for microbes are abundant under warm conditions (Vance and Chapin 2001), determination of NO_3^- -N/TIN ratio in the growing season would be better for assessing nitrification potential in forest stands.

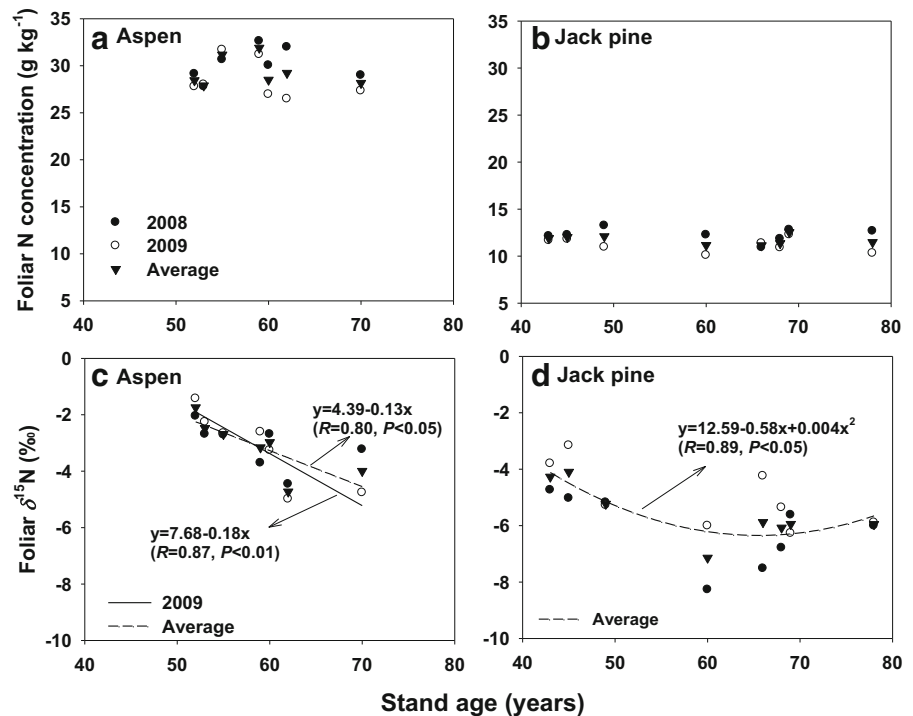
A few mechanisms have been proposed to explain why nitrification potential and thus N loss becomes lower with stand development. The first is the increased

Table 3 Mean foliar N concentrations and $\delta^{15}\text{N}$ in 2008 and 2009 in aspen and jack pine stands (with standard errors in the parentheses) across stand ages

| Stand type | Foliar N (g N kg^{-1}) | | Foliar $\delta^{15}\text{N}$ (‰) | |
|----------------|-----------------------------------|------------|----------------------------------|------------|
| | 2008 | 2009 | 2008 | 2009 |
| Aspen | 30.2 (0.6) | 28.5 (0.8) | -3.1 (0.3) | -3.1 (0.5) |
| Jack pine | 12.2 (0.2) | 11.2 (0.3) | -6.1 (0.4) | -5.0 (0.4) |
| Effect | <i>Probability > F</i> | | | |
| Stand type (S) | <0.001 ^a | | <0.001 | |
| Year (Y) | 0.01 | | 0.11 | |
| S × Y | 0.57 | | 0.16 | |

^a Bold values indicate significant *P* values

Fig. 3 Relationships of foliar N and $\delta^{15}\text{N}$ in 2008, 2009, and average values with stand age in aspen and jack pine stands: **a** foliar N concentration of aspen, **b** foliar N concentration of jack pine, **c** foliar $\delta^{15}\text{N}$ of aspen, and **d** $\delta^{15}\text{N}$ of jack pine



presence of phenolic compounds that could depress nitrification rates and thus reduce N loss (Rice and Pancholy 1972; Olson and Reiners 1983). Another plausible explanation is that the accumulation of the recalcitrant N pool or the increased thickness of the forest floor with stand age may decrease soil N mineralization rate (DeLuca et al. 2002; Kielland et al. 2006). An increase in moss coverage on the ground with stand development is common in the field and may result in increased uptake of soil NO_3^- and reduced soil NO_3^- concentration. Increased N immobilization by well-established soil microbes with stand development may result in a low availability of NH_4^+ , the substrate for nitrification in older stands (Stark and Hart 1997). In addition, gradual shifts of N fraction available for plant uptake from NO_3^- to NH_4^+ and from inorganic N to organic N under N-limited conditions could also contribute to a more conservative N cycling in old stands as uptake of NH_4^+ and organic N affords less opportunity for N loss (Kahmen et al. 2008; LeDuc and Rothstein 2010) through leaching of NO_3^- or denitrification.

Foliar $\delta^{15}\text{N}$ and N concentration

Plant $\delta^{15}\text{N}$ has served as an indicator of plant N acquisition pathways (i.e., source of N) and ecosystem N

cycling (Högberg and Johansson 1993; Pardo et al. 2006; Garten et al. 2011); however, plant $\delta^{15}\text{N}$ should be carefully interpreted since it is affected by many factors that are associated with N source such as mycorrhizal fungal associations (Chang and Handley 2000; Compton et al. 2007; Craine et al. 2009), root distribution (Compton et al. 2007), N deposition (Hietz et al. 2011), N isotopic fractionation associated with soil N processes causing N loss (Evans 2001), and plant N assimilation and intra-plant translocation (Pardo et al. 2013). Therefore, plant $\delta^{15}\text{N}$ can better serve as an indicator of soil N processes when it is used together with other N indices (Evans 2001). For both aspen and jack pine in this study, the decreasing pattern of $\text{NO}_3^-/\text{N/TIN}$ ratio (Fig. 2) and foliar $\delta^{15}\text{N}$ (Fig. 3) with stand age in combination with the positive relationship between $\text{NO}_3^-/\text{N/TIN}$ ratio and foliar $\delta^{15}\text{N}$ (Table 4) suggest that decreased nitrification in an older stand under more N-limited conditions might lead to less N loss and thus allow aspen trees to assimilate less ^{15}N -enriched N from the soil (Chang and Handley 2000; Pardo et al. 2006; Compton et al. 2007). Under N-limited conditions, the availability of NH_4^+ that is the substrate of nitrification decreases (Stark and Hart 1997) and thus the $\delta^{15}\text{N}$ of N available for plant uptake is likely to be lower, leading to less ^{15}N -enriched foliar

Table 4 Correlation coefficients between foliar $\delta^{15}\text{N}$ and the $\text{NO}_3^-/\text{N}/\text{TIN}$ ratio of forest floor and mineral soil layers in aspen and jack pine stands

| Species | Soil N measurement period | | | | |
|------------------------------------|--------------------------------------|--------------------|-----------------------|-------------------|-------------------------|
| | | Jul.-Sept. 2008 | Oct.2008- May 2009 | Jun.-Oct. 2009 | Jul. 2008- Oct. 2009 |
| Aspen | | Forest floor | | | |
| | $\delta^{15}\text{N}_{2008}^a$ | 0.83* | NA ^b | NA | 0.13 |
| | $\delta^{15}\text{N}_{2009}^a$ | 0.59 | 0.16 | -0.06 | -0.11 |
| | $\delta^{15}\text{N}_{\text{AVE}}^a$ | 0.73 | 0.13 | 0.1 | -0.02 |
| | | Mineral soil | | | |
| | $\delta^{15}\text{N}_{2008}$ | 0.89** | NA | NA | 0.63 |
| | $\delta^{15}\text{N}_{2009}$ | 0.89** | 0.43 | 0.19 | 0.71 |
| | $\delta^{15}\text{N}_{\text{AVE}}$ | 0.95** | 0.44 | 0.22 | 0.72 |
| | Jack pine | | Forest floor | | |
| $\delta^{15}\text{N}_{2008}$ | | 0.62 | NA | NA | 0.71* |
| $\delta^{15}\text{N}_{2009}$ | | 0.73* | 0.22 | 0.57 | 0.37 |
| $\delta^{15}\text{N}_{\text{AVE}}$ | | 0.80* | 0.46 | 0.90** | 0.66 |
| | | Mineral soil | | | |
| $\delta^{15}\text{N}_{2008}$ | | 0.48 | NA | NA | 0.15 |
| $\delta^{15}\text{N}_{2009}$ | | 0.58 | 0.16 | -0.02 | 0.04 |
| $\delta^{15}\text{N}_{\text{AVE}}$ | | 0.63 | 0.04 | 0.15 | 0.12 |

^a $\delta^{15}\text{N}_{2008}$, $\delta^{15}\text{N}$ of foliage sampled in 2008; $\delta^{15}\text{N}_{2009}$, $\delta^{15}\text{N}$ of foliage sampled in 2009; $\delta^{15}\text{N}_{\text{AVE}}$, average foliar $\delta^{15}\text{N}$ across 2008 and 2009

^b NA, not applicable

* $P < 0.05$; ** $P < 0.01$

$\delta^{15}\text{N}$ than those under N abundant conditions (Högberg and Johannisson 1993). The lower foliar $\delta^{15}\text{N}$ usually indicates more N-limited conditions in forests, with high dissolved organic N (DON) usage and a high dependence on ectomycorrhizal fungi (Mayor et al. 2012). The depletion of plant ^{15}N in older stands were also found in jack pine forests ranging from 1 to 72 years in northern Lower Michigan, USA (Leduc et al. 2013) and in the other species (Chang and Handley 2000; Compton et al. 2007; McLauchlan et al. 2007).

In spite of the changed soil N cycling with stand age, there is not a decreasing pattern of foliar N concentration with stand age (Fig. 3a and b) and this suggests that there were other N sources such as organic N beside the inorganic soil N (Hobbie et al. 2000; LeDuc and Rothstein 2010; Mayor et al. 2012; LeDuc et al. 2013). For example, in N limited forests, trees show a high reliance on ectomycorrhizal fungi which have a lower $\delta^{15}\text{N}$ than soil inorganic N and thus leads to a lower foliar $\delta^{15}\text{N}$ (Hobbie et al. 2000). Trees can take up

organic N such as simple amino acids as well as inorganic N, particularly when inorganic N availability is low such as in old stands (LeDuc and Rothstein 2010), or when the majority of the root system is acquiring N from the forest floor, which is a larger organic N pool than the mineral soil (Yermakov and Rothstein 2006). In addition, Yan et al. (2012) found that fine root biomass in the forest floor increases with stand development in boreal forests. Because $\delta^{15}\text{N}$ of N in the forest floor is lower than that in the mineral layer due to N isotope fractionation associated with mineralization of organic N (Koba et al. 1998; Kwak et al. 2009), increases in the contribution of N derived from the forest floor to the available soil N pool with stand age is likely to lower foliar $\delta^{15}\text{N}$. Therefore, this study in combination with data from previous studies suggest that not only changed soil N dynamics (i.e., decreases in nitrification rates in this study) but also shift in soil N availability from inorganic N to organic N resulted in a decreasing pattern of foliar $\delta^{15}\text{N}$ with stand age.

Variability of foliar $\delta^{15}\text{N}$ (-3.1 to -6.1‰, Table 3) reported in this study is similar to values observed in similar natural stands, e.g., Kwak et al. (2009) reported that foliar $\delta^{15}\text{N}$ of *Pinus densiflora* in a natural park in Korea ranged from -3 to -6‰. Meanwhile, foliar $\delta^{15}\text{N}$ of trees grown with N fertilization showed a relatively higher value; for example, -0.8 to -0.2‰ for *Picea glauca* (Moench) Voss in Alberta, Canada (Matsushima et al. 2012) and -0.8 to +0.5‰ for *Pinus taeda* in North Carolina, USA (Choi et al. 2005a). Comparing between the two stand types, mean foliar $\delta^{15}\text{N}$ of aspen (-3.1‰) across stand ages and sampling years was higher than that (-5.6‰) of jack pine (Table 3), and this corroborates with other studies where hardwoods tend to have higher foliar $\delta^{15}\text{N}$ than conifers grown under similar environments (Hobbie et al. 2000; Fang et al. 2011). In general, litter from broadleaf trees is known to be a better substrate for microbial decomposition due to its high N and Ca concentrations and low lignin content than that from conifer trees (Moore et al. 1999), which might result in the higher foliar $\delta^{15}\text{N}$ in stands dominated by deciduous tree species due to increased N isotopic fractionation as a result of faster N mineralization that stimulates N loss (Gundersen et al. 2009). However, such difference in the magnitude of N isotopic fractionation between aspen and jack pine species was not supported by our data of soil $\text{NO}_3^-/\text{N}/\text{TIN}$ ratio (Table 2), suggesting again that soil inorganic N is a very active pool and thus it may not be a reliable

indicator of soil N availability in boreal forests. Instead, many other factors associated with N sources such as mycorrhizal associations and rooting depth, as well as plant N uptake, assimilation and transport within the plant could have contributed to the different foliar $\delta^{15}\text{N}$ signals between the species (Nadelhoffer et al. 1996; Pardo et al. 2013). In this study, the lower foliar N concentration in jack pine implied that the increase in the intensity of mycorrhizal infection could lead to a difference of 1.3 to 10‰ in foliar $\delta^{15}\text{N}$ among tree species (Hobbie et al. 2000).

Conclusions

Although the patterns of change in soil inorganic N availability with stand age were not consistent across soil layers, stand types, and soil N measurement periods, decreasing patterns of NO_3^- -N/TIN ratio with stand age indicated that nitrification potential and thus the subsequent N loss decreased with stand development in the N-limited boreal forests studied in this project. The lower foliar $\delta^{15}\text{N}$ in older stands and the positive relationship between foliar $\delta^{15}\text{N}$ and NO_3^- -N/TIN suggested that a decrease in foliar $\delta^{15}\text{N}$ with stand age was associated in part with reduced N loss that leaves less ^{15}N -enriched inorganic N in older stands. On the other hand, foliar N was not related to stand age in spite of soil N limitation in the older stand. This implies the presence of other N sources such as organic N from the forest floor and organic N uptake likely through mycorrhizal associations that are ^{15}N -depleted relative to inorganic N. The latter might have further contributed to a lower foliar $\delta^{15}\text{N}$ in older stands. Our results in combination with other published data suggested that a decreasing pattern of foliar $\delta^{15}\text{N}$ of aspen and jack pine with stand age could be an indicator of both reduced N loss and a shift of N source from inorganic N to organic N, likely due to greater reliance on organic N from the forest floor and organic N uptake through mycorrhizal associations in older stands.

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References

- Aubert M, Bureau F, Vincelas-Akpa M (2005) Sources of spatial and temporal variability of inorganic nitrogen in pure and mixed deciduous temperate forests. *Soil Biol Biochem* 37: 67–79
- Binkley D, Son Y, Valentine DW (2000) Do forests receive occult inputs of nitrogen? *Ecosystems* 3:321–331
- Chang SX, Handley LL (2000) Site history affects soil and plant ^{15}N natural abundances ($\delta^{15}\text{N}$) in forests of northern Vancouver Island, British Columbia. *Funct Ecol* 14:273–280
- Choi WJ, Ro HM, Lee SM (2003) Natural ^{15}N abundances of inorganic nitrogen in soil treated with fertilizer and compost under changing soil moisture regimes. *Soil Biol Biochem* 35: 1289–1298
- Choi WJ, Chang SX, Allen HL, Kelting DL, Ro HM (2005a) Irrigation and fertilization effects on foliar and soil carbon and nitrogen isotope ratios in a loblolly pine stand. *For Ecol Manage* 213:90–101
- Choi WJ, Chang SX, Hao X (2005b) Soil retention, tree uptake, and tree resorption of $^{15}\text{NH}_4\text{NO}_3$ and $\text{NH}_4^{15}\text{NO}_3$ applied to trembling and hybrid aspens at planting. *Can J For Res* 35: 823–831
- Choi WJ, Lee SM, Chang SX, Ro HM (2005c) Variations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in *Pinus densiflora* tree-rings and their relationship to environmental changes in eastern Korea. *Water, Air, Soil Pollut* 164:173–187
- Choi WJ, Chang SX, Bhatti JS (2007) Drainage affects tree growth and C and N dynamics in a minerotrophic peatland. *Ecology* 88:443–453
- Compton JE, Hooker TD, Perakis SS (2007) Ecosystem N distribution and during a century of forest regrowth after agricultural abandonment. *Ecosystems* 10:1197–1208
- Craine JM, Elmore AJ, Aidar MP, Bustamante M, Dawson TE, Hobbie EA, Kahmen A, Mack MC, McLaughlan KK, Michelsen A, Nardoto GB, Pardo LH, Peñuelas J, Reich PB, Schuur EA, Stock WD, Templer PH, Virginia RA, Welker JM, Wright IJ (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytol* 183:980–992
- DeLuca TH, Nilsson MC, Zackrisson O (2002) Nitrogen mineralization and phenol accumulation along a fire chronosequence in northern Sweden. *Oecologia* 133:206–214
- Driscoll KG, Arocena JM, Massicotte HB (1999) Post-fire soil nitrogen content and vegetation composition in Sub-Boreal spruce forests of British Columbia's central interior, Canada. *For Ecol Manage* 121:227–237
- Evans RD (2001) Physiological mechanisms influencing plant nitrogen isotope composition. *Trends Plant Sci* 6:121–126
- Fang HJ, Yu GR, Cheng SL, Zhu TH, Zheng JJ, Mo JM, Yan JH, Luo YQ (2011) Nitrogen-15 signals of leaf-litter-soil continuum as a possible indicator of ecosystem nitrogen saturation by forest succession and N loads. *Biogeochemistry* 102:251–263

- Garten CT, Iversen CM, Norby RJ (2011) Litterfall ^{15}N abundance indicates declining soil nitrogen availability in a free-air CO_2 enrichment experiment. *Ecology* 92:133–139
- Goodale CL, Aber JD, Vitousek PM, McDowell WH (2003) Long-term decreases in stream nitrate: successional causes unlikely; possible links to DOC? *Ecosystems* 8:334–337
- Gundersen P, Sevel L, Christiansen JR, Vesterdal L, Hansen K, Bastrup-Birk A (2009) Do indicators of nitrogen retention and leaching differ between coniferous and broadleaved forests in Denmark? *For Ecol Manage* 258:1137–1146
- Hietz P, Turner BL, Wanek W, Richter A, Nock CA, Wright SJ (2011) Long-term change in the nitrogen cycle of tropical forests. *Science* 334:664–666
- Hobbie EA, Macko SA, Williams M (2000) Correlations between foliar $\delta^{15}\text{N}$ and nitrogen concentrations may indicate plant-mycorrhizal interactions. *Oecologia* 122:273–283
- Högberg P, Johannisson C (1993) ^{15}N abundance of forests is correlated with losses of nitrogen. *Plant Soil* 157:147–150
- Kahmen A, Wanek W, Buchmann N (2008) Foliar values characterize soil N cycling and reflect nitrate or ammonium preference of plants along a temperate grassland gradient. *Oecologia* 156:861–870
- Kielland K, Olson K, Ruess RW, Boone RD (2006) Contribution of winter processes to soil nitrogen flux in taiga forest ecosystems. *Biogeochemistry* 81:349–360
- Koba K, Tokuchi N, Yoshioka T, Hobbie EA, Iwatsubo G (1998) Natural abundance of ^{15}N in a forest soil. *Soil Sci Soc Am J* 62:778–781
- Kwak JH, Choi WJ, Lim SS, Arshad MA (2009) $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, N concentration, and Ca-to-Al ratios of forest samples from *Pinus densiflora* stands in rural and industrial areas. *Chem Geol* 264:385–393
- LeDuc SD, Rothstein DE (2010) Plant-available organic and mineral nitrogen shift in dominance with forest stand age. *Ecology* 91:708–720
- LeDuc SD, Rothstein DE, Yermakow Z, Spaulding SE (2013) Jack pine foliar $\delta^{15}\text{N}$ indicates shifts in plant nitrogen acquisition after severe wildfire and through forest stand development. *Plant Soil*. doi:10.1007/s11104-013-1856-0
- Makarov MI (2009) The nitrogen isotopic composition in soils and plants: its use in environmental studies (a review). *Eurasian Soil Sci* 42:1335–1347
- Matsushima M, Choi WJ, Chang SX (2012) White spruce foliar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ indicate changed soil N availability by understory removal and N fertilization in a 13-year-old boreal plantation. *Plant Soil* 361:375–384
- Mayor JR, Schuur EAG, Mack MC, Hollingsworth TN, Bååth E (2012) Nitrogen isotope patterns in Alaskan black spruce reflect organic nitrogen sources and the activity of ectomycorrhizal fungi. *Ecosystems* 15:819–831
- McLaughlan KK, Craine JM, Oswald WW, Leavitt PR, Likens GE (2007) Changes in nitrogen cycling during the past century in a northern hardwood forest. *Proc Natl Acad Sci U S A* 104:7466–7470
- Moore TR, Trofymow JA, Taylor B, Prescott C, Camiré C, Duschene L, Fyles J, Kozak L, Kranabetter M, Morrison I, Sitanen M, Smith S, Titus B, Visser S, Wein R, Zoltai S (1999) Litter decomposition rates in Canadian forests. *Global Change Biol* 5:75–82
- Nadelhoffer K, Shaver G, Fry B, Giblin A, Johnson L, McKane R (1996) ^{15}N natural abundances and N use by tundra plants. *Oecologia* 107:386–394
- Odum EP (1969) The strategy of ecosystem development. *Science* 164:262–270
- Olson R, Reiners W (1983) Nitrification in subalpine balsam fir soils: tests for inhibitory factors. *Soil Biol Biochem* 15:413–418
- Pardo LH, Templer PH, Goodale CL, Duke S, Groffman PM, Adams MB, Boeckx P, Boggs J, Campbell J, Colman B, Compton J, Emmett B, Gundersen P, Kjønaas J, Lovett G, Mack M, Magill A, Mbila M, Mitchell MG, McGee G, McNulty S, Nadelhoffer K, Ollinger S, Ross D, Schleiippi P, Spoelstra J, Wessel W (2006) Regional assessment of N saturation using foliar and root $\delta^{15}\text{N}$. *Biogeochemistry* 80:143–171
- Pardo LH, Semaoune P, Schaberg PG, Eagar C, Sebilo M (2013) Patterns in $\delta^{15}\text{N}$ in roots, stems, and leaves of sugar maple and American beech seedlings, saplings, and mature trees. *Biogeochemistry* 112:275–291
- Prasolova NV, Xu ZH, Saffigna PG, Dieters MJ (2000) Spatial-temporal variability of soil moisture, nitrogen availability indices and other chemical properties in hoop pine (*Araucaria cunninghamii*) plantations of subtropical Australia. *For Ecol Manage* 136:1–10
- Rice EL, Pancholy SK (1972) Inhibition of nitrification by climax ecosystems. *Am J Bot* 59:1033–1040
- Soil Classification Working Group (1998) The Canadian system of soil classification, 3rd edn. NRC Research Press, Ottawa
- Stark JM, Hart SC (1997) High rates of nitrification and nitrate turnover in undisturbed coniferous forests. *Nature* 385:61–64
- Ste-Marie C, Paré D, Gagnon D (2007) The contrasting effects of aspen and jack pine on soil nutritional properties depend on parent material. *Ecosystems* 10:1299–1310
- Vance ED, Chapin FS III (2001) Substrate limitations to microbial activity in taiga forest floors. *Soil Biol Biochem* 33:173–188
- Vitousek PM, Reiners WA (1975) Ecosystem succession and nutrient retention: a hypothesis. *BioScience* 25:376–381
- Vitousek PM, Matson PA, Van Cleve K (1989) Nitrogen availability and nitrification during succession: primary, secondary, and old-field seres. *Plant Soil* 115:229–239
- Yan ER, Hu YL, Salifu F, Tan X, Chen ZC, Chang SX (2012) Effectiveness of soil N availability indices in predicting site productivity in the oil sands region of Alberta. *Plant Soil* 359:215–231
- Yermakov Z, Rothstein DE (2006) Changes in soil carbon and nitrogen cycling along a 72-year wildfire chronosequence in Michigan jack pine forests. *Oecologia* 149:690–700