Effects of nitrogen addition on leaf nutrient stoichiometry in an old-growth boreal forest

LONGCHAO XU,1,2 AIJUN XING,1,2 ENZAI DU,3 HAIHUA SHEN,1,2† ZHENGBIN YAN,4 LAI JIANG,4 DI TIAN,4 HUIFENG HU,1 AND JINGYUN FANG1

1State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093 China
2University of Chinese Academy of Sciences, Beijing 100049 China
3State Key Laboratory of Earth Surface Processes and Resource Ecology, Faculty of Geographical Science, Beijing Normal University, Beijing 100875 China
4Department of Ecology, College of Urban and Environmental Sciences, and Key Laboratory for Earth Surface Processes of the Ministry of Education, Peking University, Beijing 100871 China

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Abstract. Boreal forests have been evidenced to be highly sensitive to enhanced nitrogen (N) deposition due to prevailing N limitations, and external N inputs from atmospheric deposition are expected to alter plant nutrient stoichiometry. Previous studies have mostly focused on the dominant tree species while neglecting understory plants that often play important role in the nutrient cycles in forest ecosystems. By conducting a six-year N-addition experiment with four treatments of 0 (control), 20 (low N), 50 (medium N), and 100 (high N) kg N ha⁻¹ yr⁻¹ in a boreal forest in Northeast China, we assessed the responses of leaf nutrient stoichiometry (N, phosphorus [P], potassium [K], calcium [Ca], and magnesium [Mg]) for tree, shrubs, and grass. Although the responses of different species to N addition varied, six-year N addition, especially the medium and high N treatments, generally increased the leaf N concentration and decreased the leaf P and Ca concentrations. As a result, the foliar N:P, N:K, N:Ca, and N:Mg ratios increased consistently across plant functional groups under the high N addition compared with the control, and the grass Deyeuxia angustifolia showed a larger increase in its foliar N:P, N:Ca, and N:Mg than the shrubs. The leaf N concentration increased consistently with soil inorganic N in a nonlinear saturating form, while the other leaf nutrients either decreased (P, Ca) or were not affected (Mg, K) by the soil N availability. Consequently, the foliar N:P, N:K, N:Ca, and N:Mg ratios increased linearly with increasing soil N availability. In spite of this alteration of the leaf nutrient stoichiometry with increasing soil N availability, foliar nutrients and their stoichiometry were less affected under the low N treatment in both trees and understory plants, suggesting minor effects of current N deposition rates on the foliar nutrient balance in boreal forests.

Key words: boreal forest; Larix gmelinii; leaf stoichiometry; nitrogen deposition; understory plants.

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† E-mail: shen.haihua@ibcas.ac.cn

INTRODUCTION

Since the industrial revolution, global anthropogenic nitrogen (N) emissions have dramatically enhanced N deposition (Galloway et al. 2004), which is a key driver of plant diversity loss (Bobbink et al. 2010), forest growth (Thomas et al. 2010, Tian et al. 2018), and soil carbon dynamics (Frey et al. 2014, Zak et al. 2017). Since the 1980s, primarily as a result of fossil fuel combustion, traffic, and agricultural activities, large areas of forests in China have received relatively high loads of N deposition, which is a key factor influencing tree growth and forest productivity.
in China (Liu et al. 2011, Du et al. 2014, Jia et al. 2014, Tian et al. 2018). Foliar nutrient concentrations, and especially their stoichiometry ratios, have been shown to correlate with the growth rate of plants and serve as an important indicator of forest health (McNulty et al. 1996, Elser et al. 2010, Penuelas et al. 2013, Wang et al. 2016). For example, the N:phosphorus (P) ratio is regarded as a nutrient limitation indicator for biomass production; in particular, biomass is enhanced by N fertilization when the N:P ratio <10 and by P fertilization when the N:P ratio >20 (Güsewell 2004). Compared with the N:P ratio, the ratios of N to other essential elements (such as potassium [K], calcium [Ca], and magnesium [Mg]) are less understood. Mellert and Göttlein (2012) reported optimal N:K, N:Ca, and N:Mg ratios of major European coniferous tree species in the range of 1.7–4.0, 2.1–9.1, and 10.7–22.9, respectively. These critical ratios can serve as potential references to assess the effects of N deposition on the status of and balance of foliar nutrients in ecosystems dominated by coniferous trees.

N deposition has been shown to be a key driver of global change that alters foliar nutrient concentrations and stoichiometry (Sardans et al. 2012, You et al. 2018, Tian et al. 2019). The responses of tree foliar N and P and their stoichiometric ratios to N deposition have been extensively studied (Tian et al. 2019). N-addition experiments indicate that enhanced N deposition generally increases leaf N concentrations (Bauer et al. 2004, Magill et al. 2004, McNulty et al. 2005, Minocha et al. 2015), which was confirmed by recent meta-analysis (You et al. 2018, Tian et al. 2019). This increase might be nonlinear since leaf N concentration first increased with increasing N availability but followed by a potential slowdown and may eventually become saturated at a threshold level of N availability due to lowered N use efficiency and N demand (Sutton et al. 2004, Du 2017). The responses of leaf P concentrations to N deposition vary among studies, with reported increases (Huang et al. 2012, Lü et al. 2013), decreases (Sardans et al. 2016), or neutral responses (Chen et al. 2015). Consequently, N addition could cause an increase in the N:P ratio (You et al. 2018), thus further intensifying P limitation (Du and Fang 2014) and even shifted from N limitation toward P limitation. In addition to N and P, K, Ca, and Mg are also essential nutrients for plant metabolism (Watanabe et al. 2007, Tian et al. 2019). However, the effects of N deposition on these essential macronutrients are less understood. A study conducted on a red spruce ecosystem found that N addition did not change foliar Ca concentrations over multiple sampling years but decreased Mg concentrations (McNulty et al. 2005), but research in the Harvard Forest found that N addition caused a reduction in foliar Ca concentrations in trees (Minocha et al. 2015). Similarly, Elvir et al. (2010) reported that whole-watershed fertilization of Bear Brook forests resulted in a significant decrease in foliar Ca and K concentrations (Elvir et al. 2010). Overall, an increase in foliar N concentrations and an alteration of other nutrients following N addition might alter leaf nutrient stoichiometry.

Understory plants in forests serve a key role in maintaining ecosystem structure and functioning (Gilliam 2007), though many previous studies have predominately focused on the nutrient stoichiometry responses of dominant trees to N deposition. Different species in the same ecosystem may show diverse responses to increased N deposition due to differences in nutrient use strategies (Mao et al. 2017), and this response may also differ across plant functional groups, such as tree, shrub, and grass; thus, the community nutrient index can serve as a promising indicator of the nutrient status at the community level (Du 2017). With increasing N deposition, a commonly reported phenomenon is an increase in the understory grass abundance (Nordin et al. 2009, Bobbink et al. 2010, Gilliam et al. 2016, Xing et al. 2019), suggesting that the leaf nutrient stoichiometry of grasses might be less affected by increased soil N availability. It is imperative to incorporate both overstory and understory plants and their multiple nutrient responses to better understand the effect of N deposition on forest health.

Boreal forests have been shown to be very sensitive to N deposition (Makipaa 1998, Stengbom et al. 2002), and as such, external N inputs are expected to release plants from N limitations, although they might alter the nutrient stoichiometry by changing the ratios of N to other elements. In an old-growth boreal forest in Northeast China, short-term (three-year) N addition increased the leaf N contents in trees and
understory plants and induced N and P imbalances in canopy trees (Du and Fang 2014, Du 2017). Based on the results of a six-year N-enrichment experiment at the same site, the present study explores the long-term effects of simulated N deposition on leaf nutrient concentrations and the leaf stoichiometry at the community level (including dominant tree and understory plants). Since boreal forests are N-limited, we predict that the foliar N concentration will increase non-linearly with increasing soil N availability and the ratios of N to other elements will increase, thereby altering the leaf nutrient stoichiometry. Moreover, different functional types of understory species may differ in their responses to leaf nutrient stoichiometry.

**Materials and Methods**

**Site description**

The study site is located at the National Field Research Station of the Daxing’anling Forest Ecosystem (50°56’N, 121°30’E) in the Great Khingan Mountains, Northeast China. The mean annual temperature is −5.4°C, with long winters (average diurnal temperature <0°C) lasting 9 months and short summers (average diurnal temperature >22°C) of less than one month. The mean annual precipitation is 450–550 mm, and more than 50% of the precipitation occurs in July and August. The boreal forest consists of 3 layers: (1) the canopy layer, with *Larix gmelinii* as the single dominant species; (2) the shrub layer, with mixed species, that is, *Betula fruticosa*, *Ledum palustre*, *Rhododendron parvifolium*, *Vaccinium vitis-idaea*, *Rosa davurica*, and *Vaccinium uliginosum*; and (3) the ground layer, consisting mainly of *Deyeuxia angustifolia* and *Rhytidium rugosum* (Du et al. 2013). The soil is characterized as brown coniferous forest soil with an average depth of 20–40 cm; a permafrost layer occurs below a depth of 60–80 cm. The surface soil pH ranges from 4.5 to 6.5.

**Experimental design**

N has been added in an old-growth larch (*L. gmelinii*) forest since 2010, following the guidelines of the Nutrient Enrichment Experiment in China’s Forests Project (NEECF; Du et al. 2013). Twelve 20 × 20 m plots with a similar stand density (Appendix S1: Fig. S1) were established based on a random block design (4 treatments × 3 replicates) with the following treatments: control (no N added), low N (20 kg N-ha⁻¹-yr⁻¹), moderate N (50 kg N-ha⁻¹-yr⁻¹), and high N (100 kg N-ha⁻¹-yr⁻¹). A 10 m wide buffer zone was established between the plots. From May to September, N additions were applied monthly every year during the period of 2010–2015 to the forest floor as a dilute ammonium nitrate (NH₄NO₃) solution using a backpack sprayer. The control plots received equal amounts of water.

**Sample collection and nutrient analysis**

In July 2015, we collected mature green leaves of the dominant species, including *L. gmelinii* in the canopy layer, *B. fruticosa*, *L. fruticosa*, *V. vitis-idaea*, *R. davurica*, *L. dauricum*, and *V. uliginosum* in the shrub layer, and the herb *D. angustifolia*. Leaf samples were collected according to the protocol proposed by Cornelissen et al. (2003). In each plot, we collected three leaf samples from the middle of the upper canopy from each of three randomly selected larch trees. Five leaf samples from each shrub and herb species were randomly collected in each plot. Three surface soil cores (0–10 cm) were collected randomly in each plot. Part of each fresh soil sample was sieved through a 2-mm sieve to determine the soil inorganic N content, and the rest of the soil samples were air-dried, sieved, and measured to determine the soil pH, available P content, and exchangeable K⁺, Ca²⁺, and Mg²⁺ contents.

Fresh soil was extracted with 2 mol/L KCl and stored at −20°C until the analysis started. The total inorganic N content in the soil layer, defined as the sum of the amounts of ammonium and nitrate, was measured by a continuous flow analyzer (SEAL-AA3, Germany). Ammonium and nitrate contents were calculated based on the concentrations of extracted solutions, the volumes of extracted solutions, and the dry soil mass. The pH was determined by the glass electrode method (decarboxylated water and soil ratio of 5:1, W: V). The soil available P content was measured by the molybdenum blue colorimetric method (Murphy and Riley 1962). Soil exchangeable K⁺, Ca²⁺, and Mg²⁺ contents were measured by the ICP method after extraction with 0.01 mol/L BaCl₂.
Leaf samples were oven-dried (65°C for 48 h), milled, and analyzed to determine the N, P, K, Ca, and Mg contents. Leaf N contents were measured using an elemental analyzer (model PE2400, PerkinElmer, Waltham, Massachusetts, USA). The total P content was determined using the sulfuric acid-perchloric acid digestion/molybdenum-antimony colorimetric method (Liu et al. 1996). We used the HNO₃ digestion-ICP method (iCAP 6300 ICP-OES Spectrometer, Thermo Scientific, Waltham, Massachusetts, USA) to determine the leaf K, Ca, and Mg contents.

**Statistical analysis**

We calculated the community nutrient (N, P, K, Ca, and Mg) indicator (CNI) for the shrub layer by integrating the shrub species composition and the leaf nutrient concentrations according to the following equation (Du 2017):

\[
\text{CNI} = \frac{\sum \text{LNC}_i \times \text{SC}_i}{\sum \text{SC}_i}
\]

where \(\text{LNC}_i\) indicates the leaf nutrient content (g/kg) of each shrub species, and \(\text{SC}_i\) indicates the coverage (%) of each shrub species. CNI indicates the community nutrient (N, P, K, Ca, and Mg) indicators in the shrub layer. To compare the sensitivity of the nutrient stoichiometry responses to addition of N, we calculated the normalized response ratio of the treatment effects by taking the natural logarithm of the ratio of the low, medium, and high treatments against the control at the species and plant functional group levels.

One-way analysis of variance (ANOVA) with a least significant difference (LSD) post hoc test was used to identify the effects of N additions on the soil pH, soil inorganic N content, soil available P content, soil exchangeable K, Ca²⁺, and Mg²⁺ contents, and leaf nutrient contents for each species. Two-way ANOVA was used to determine the effects of the plant species/plant functional groups, N addition, and their interaction on the leaf nutrients and stoichiometry and their response ratios. Regression analysis was conducted to explore the relationships between the leaf N, P, K, Ca, and Mg concentrations and their stoichiometry and the soil available N content. Specifically, the response of foliar N to

![Fig. 1. Effect of N addition on soil properties.](image)

Figure a, b, c, d, e, f, g and h represent soil pH, soil ammonium, soil nitrate, soil inorganic N, soil available P, soil exchangeable K, soil exchangeable Ca, and soil exchangeable Mg, respectively. Different letters in the same graph indicate a significant difference at \(P < 0.05\) as revealed by one-way ANOVA followed by multiple comparisons.
increased N deposition was fitted with a nonlinear saturation curve based on previous studies (Sutton et al. 2004, Du 2017), while the response of other foliar nutrients to increasing N availability was first visually observed and further explored with linear regression or nonlinear regression with an exponential curve fitting. The model fit was assessed with the AIC criteria, and a nonlinear model was selected when the AIC difference between the nonlinear and linear models was >2; otherwise, a linear regression was used (Appendix S1: Table S1). Values are reported as the mean ± standard error unless noted otherwise. All statistical analyses were conducted using R 3.5.1 (R Core Team 2018).

**RESULTS**

**Changes in soil inorganic N and available P contents and pH**

One-way ANOVA found that N addition had no significant effect on the soil pH (Fig. 1a). A

![Fig. 2. Response of foliar nutrients (N, K, Ca, Mg, and P) to N addition across different plant species. In each subplot, different letters indicate a significant difference at P < 0.05 among treatment as revealed by one-way ANOVA followed by multiple comparisons. Inserted plot was to better illustrate the effect of N addition on leaf Mg and P, which were otherwise difficult to observe due to low concentration.](image-url)
significant effect of N addition on soil ammonium, soil nitrate, soil inorganic N, and soil exchangeable K was revealed by one-way ANOVA. Multiple comparisons indicated that the soil ammonium, soil nitrate, and soil inorganic N contents under medium and high N treatments were significantly higher than those of the control, while no significant difference was found between the low N treatment and the control (Fig. 1b–d). The soil K content under the low N treatment was significantly higher than that of the high N treatment (Fig. 1f). In contrast, N addition showed no significant effect on the soil available P, Ca\(^{2+}\), and Mg\(^{2+}\) contents (Fig. 1e, g, h).

Changes in N, P, K, Ca, and Mg contents in leaves

Two-way ANOVA indicated a significant effect of N addition, plant species, and their interaction on the leaf N contents (Appendix S1: Table S2). The leaf N contents generally increased with N additions, but the response varied among the species (Fig. 2). One-way ANOVA revealed that only the leaf N contents under the medium and high N treatments were significantly higher than those of the control for *L. gmelinii*, shrub *R. parrifolium*, and *V. uliginosum*, while the leaf N contents were significantly increased by the low, medium, and high N treatments for *V. vitis-idaea* and *D. angustifolia*.

Two-way ANOVA found a significant effect of N addition, species, and their interaction on the leaf K and P contents, while a significant effect was found only for N addition and species on the leaf Ca and Mg contents (Appendix S1: Table S2). One-way ANOVA revealed that the leaf K contents under the high N treatment were significantly lower than those of the control for *L. gmelinii*, shrub *R. parrifolium*, *B. fruticosa*, and *V. uliginosum*, while the leaf N contents were significantly increased by the low, medium, and high N treatments for *V. vitis-idaea* and *D. angustifolia*.

Table 1. Two-way ANOVA results showing the effects of N addition, plant functional group, and their interaction on foliar nutrient.

| Foliar nutrient | Source                  | df  | F      | P     |
|-----------------|-------------------------|-----|--------|-------|
| N               | Treatment               | 3   | 73.92  | <0.001|
|                 | Functional group        | 2   | 41.56  | <0.001|
|                 | Treatment × functional group | 6   | 4.55   | 0.003 |
| K               | Treatment               | 3   | 2.5    | 0.08  |
|                 | Functional group        | 2   | 3.34   | <0.001|
|                 | Treatment × functional group | 6   | 2.1    | 0.09  |
| Ca              | Treatment               | 3   | 5.22   | 0.006 |
|                 | Functional group        | 2   | 3.9    | <0.001|
|                 | Treatment × functional group | 6   | 2.48   | 0.05  |
| Mg              | Treatment               | 3   | 1.03   | 0.39  |
|                 | Functional group        | 2   | 2.65   | <0.001|
|                 | Treatment × functional group | 6   | 0.27   | 0.95  |
| P               | Treatment               | 3   | 13.92  | <0.001|
|                 | Functional group        | 2   | 1.41   | 0.26  |
|                 | Treatment × functional group | 6   | 1.67   | 0.17  |

Note: Bold numbers indicate a significant effect at P < 0.05.
tree and shrubs decreased linearly, while those of the grass decreased exponentially with increasing soil N availability (Fig. 3). Only the leaf K contents of the trees decreased linearly as the soil inorganic N increased, while the leaf Mg contents were not affected by soil N availability (Fig. 3).

**Changes in stoichiometric ratios of multiple nutrients in leaves**

A significant effect of N addition on the leaf N: K, N: Ca, N: Mg, and N: P ratios of the trees, shrubs, and grasses was revealed by one-way ANOVA (Fig. 4). Most leaf stoichiometry for the
trees and shrubs was significantly increased only by the medium and high N treatments compared with the control, while that for the grasses was significantly increased even under the low N treatment, except for the leaf N:Mg ratio (Fig. 4). Two-way ANOVA showed a consistent effect of N addition and functional group on the response ratio of leaf stoichiometry without a significant interaction between them (Table 2, Fig. 5). Multiple comparisons of the plant functional group revealed that the magnitude of change in the leaf N:Ca and N:Mg ratios of the grasses was significantly higher than that of the shrubs, and the magnitude of the changes in the leaf N:P ratio of the grasses was significantly higher than that of both the trees and shrubs (Fig. 5). The leaf N:K, N:Ca, N:Mg, and N:P ratios increased linearly with increasing soil N availability (Fig. 6).

**DISCUSSION**

*Varied effects of N deposition on foliar nutrients*

Our results indicate that the effects of N deposition on different elements varied greatly among
the species (Fig. 2, Appendix S1: Table S1). Foliar N generally increased with N addition, which was in accordance with previous studies (Bauer et al. 2004, Magill et al. 2004, McNulty et al. 2005, Minocha et al. 2015). Consistent with our first hypothesis, the leaf N contents increased nonlinearly with soil N availability (Fig. 3). The most significant change in the leaf N contents occurred in the grass *D. angustifolia*, and the largest extent of the leaf N response ratio increase was revealed by two-way ANOVA followed by multiple comparisons (Appendix S1: Fig. S2, Table S2). This was because of the nitrophilic character of graminoids (Bobbink et al. 2010) and was consistent with the substantial increase in abundance previously reported at our site (Du 2017, Xing et al. 2019).

A recent meta-analysis indicated that N addition led to P reduction in N-limited ecosystems (You et al. 2018), and this was the case in this boreal forest (Fig. 2). The leaf P contents of the trees and shrubs decreased linearly, while those of the grasses decreased exponentially with increasing soil N availability (Fig. 3). This might be explained by the fact that graminoids develop arbuscular mycorrhizal fungi (AMF) associations, and AMF associations are efficient in acquiring inorganic P when N availability increases (Aerts 2003). Different shrub species showed varied responses in the leaf P contents with N addition. The foliar P concentrations of the shrub *B. fruticosa* were significantly decreased, while those of the ericaceous species (*L. palustris, V. vitis-idaea, and V. uliginosum*) were less affected by N addition (Fig. 2). This divergent response among shrub species might be the result of different mycorrhizal associations. Ericaceae plants develop ericoid mycorrhizal associations, which play a crucial role in the utilization of both inorganic and organic P forms (Myers and Leake 1996), and an increase in N availability, especially ammonium (Fig. 1), might have negative effects on ecto-mycorrhizal fungi and to a lesser extent on ericoid mycorrhizae (Aerts 2003, Read and Perez-Moreno 2003).

Leaf Ca reduction following N addition has been reported previously (Elvir et al. 2010, Minocha et al. 2015, Tian et al. 2019) since increased N availability might reduce the Ca uptake capacity of plants due to reduced carbon allocation to roots and detrimental effects on mycorrhiza (van Diepen et al. 2010, Kjoller et al. 2012). Again, the leaf Ca contents of the trees and shrubs decreased linearly, while those of the grasses decreased nonlinearly with increasing soil N availability. This nonlinear decrease in P and Ca implied that the grasses showed a larger resistance to increasing N availability than the trees and shrubs and were thus able to maintain their demand for P and Ca under high N availability. Consequently, the grasses were able to adapt to elevated N deposition. In spite of the decrease in P and Ca with increasing soil N availability, most of the significant reduction in the leaf P and Ca contents occurred under the medium and high N treatments (Fig. 2). Considering the low N deposition rates in boreal regions (Gundale et al. 2011) and the lack of response under the low N

### Table 2. Two-way ANOVA results showing the effects of N addition, plant functional group, and their interaction on foliar nutrient response ratios.

| Foliar nutrient ratio | Source                        | df | F     | P     |
|----------------------|-------------------------------|----|-------|-------|
|                      | Treatment                     | 2  | 17.99 | <0.001|
|                      | Functional group              | 2  | 6.31  | 0.01  |
|                      | Treatment × functional group  | 4  | 1.87  | 0.16  |
| N:Ca                 | Treatment                     | 2  | 11.99 | <0.001|
|                      | Functional group              | 2  | 21.26 | <0.001|
|                      | Treatment × functional group  | 4  | 0.48  | 0.75  |
| N:Mg                 | Treatment                     | 2  | 7.07  | 0.01  |
|                      | Functional group              | 2  | 4.92  | 0.02  |
|                      | Treatment × functional group  | 4  | 0.68  | 0.62  |
| N:P                  | Treatment                     | 2  | 19.93 | <0.001|
|                      | Functional group              | 2  | 11.88 | <0.001|
|                      | Treatment × functional group  | 4  | 1.41  | 0.27  |

**Note:** Bold numbers indicate a significant effect at $P < 0.05$. 

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treatment, N deposition might not induce a severe reduction in the foliar Ca and P concentrations in boreal forests.

The leaf K contents of the trees decreased linearly with soil inorganic N, while those of the shrubs and grasses did not (Fig. 3). A decrease in the leaf K contents might lead to tree growth and productivity declines (Aber et al. 1995, McNulty et al. 1996, Hoosbeek et al. 2002, Wright et al. 2011) and thus constrain carbon accumulation in tree biomass under high N availability. Foliar Mg was not affected by N addition, which has been reported previously (Minocha et al. 2015, Tian et al. 2019). A study along an N deposition gradient also demonstrated that Ca was far more susceptible to depletion than Mg with increasing N availability because the Mg demand in plants can be supplied by atmospheric deposition of Mg, while plants rely largely on soil reserves to maintain their Ca demand (Perakis et al. 2006).

Consistent N addition induced changes in foliar nutrient stoichiometry

Despite the varied effects of N addition on plant foliar nutrients, we found a consistent alteration of the foliar nutrient stoichiometry; further,

Fig. 5. Response ratio of leaf nutrient stoichiometry and their response to N addition and plant functional groups. In each subplot, different letters indicate a significant difference in leaf stoichiometry change magnitude among plant functional groups as revealed by two-way ANOVA followed by multiple comparisons.
the addition of N induced an increase in the N versus other elements ratios (Fig. 4). Nutrient stoichiometry might be a better measure of forest health (McNulty et al. 1996), and N:P ratios have been used widely to indicate nutrient limitation (Vitousek et al. 2010). Because the grass *D. angustifolia* experienced a substantial increase in abundance under the high N treatment (Du 2017, Xing et al. 2019), we expected that its leaf stoichiometry might be less affected by N addition than that of the shrubs. In contrary, the N:P ratio of the grass *D. angustifolia* showed the largest magnitude of changes (Fig. 5). N:P ratio thresholds of <10 and >20 have been proposed to detect plant N and P limitations, respectively (Güsewell 2004). The N:P ratio of the grass *D. angustifolia* was 9.7 under the control, which implied N limitation according to Güsewell (2004). However, 6 yr of N addition increased the N:P ratio up to 22.0, which was higher than 20, and thus, *D. angustifolia* should be P-limited and its growth under the high N treatment should be less than or equal to the control at best. The grass abundance under the high N treatment was higher than that of the control (Du 2017, Xing et al. 2019), which implied that P limitation for the grasses might not be the case. In fact, *D. angustifolia* was able to maintain its leaf P content

**Fig. 6.** Relationship between foliar nutrient stoichiometry and soil inorganic N.
under high soil N availability (Fig. 3). This suggested that the actual critical ratio should be used with caution and can only provide insight into plant nutrient limitations by considering the growth response simultaneously.

It has been reported that plants are K-limited when N:K $>1.4$ (Hoosbeek et al. 2002) or N:K $>3$ (Bragazza et al. 2004). Huttl (1990) suggested that a balanced nutrient status exists for an N:K range of 1–3, and Olde Venterink et al. (2003) found that the critical ratio of N:K was 2.1. Furthermore, the increase in the leaf N:Mg ratio is considered to be an important cause of forest degradation (Schulze 1989). In our current study, N addition induced changes in the leaf nutrient stoichiometry between N and base cations (K, Ca, Mg) with higher ratios of N versus other elements with increasing soil N availability; however, these alterations were mostly significant under the medium and high N treatments, as revealed by one-way ANOVA (Fig. 5). The foliar N:K ratios of the trees, shrubs, and grasses were significantly increased under the high N addition, and the magnitude of change was higher for the grasses (Fig. 5). The N:Ca ratio was increased by high N addition as well, but to a lesser extent for the shrubs (Fig. 2). Despite this alteration of the leaf nutrient stoichiometry with increasing soil N availability, foliar nutrients and their stoichiometry were less affected under the low N treatment for both the trees and understory plants, which suggested that N deposition had a minor effect on the foliar nutrient balance in boreal forests.

**Conclusions**

Six-year N-addition experiment indicated that N fertilization affected foliar nutrients differentially among species, with grass species being more sensitive. With the increase in the soil N availability, the leaf N content increased nonlinearly in the tree and grass species, while it increased linearly in the shrub species. N addition increased the N to other nutrient ratios, which increased with increasing soil N availability. Foliar nutrients and their stoichiometry were not affected under the low N treatment for both trees and understory plants, suggesting a minor effect of current N deposition rates on the foliar nutrient balance in boreal forests.

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Supporting Information

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