



29 **ABSTRACT**

30 Nitrogen (N) resorption is a key strategy for conserving N in forests, and is often affected by soil nutrient  
31 condition and N sink strength within the plant. However, our understanding of the age-related pattern of N  
32 resorption and how increasing N deposition will affect this pattern is limited. Here, we investigated N  
33 resorption along a chronosequence of stands ranging in age from 2 to 100 years old, and conducted a 4-year  
34 exogenous N input experiment in stands at age class 11, 20 and 45 in a *Larix Principis-rupprechtii*  
35 plantation in north China. We found a logarithmic increase in leaf NRE and green leaf N concentration, and  
36 a logarithmic decrease in senesced-leaf N concentration along the stand-age chronosequence. Leaf NRE  
37 was negatively correlated with plant-available N concentration. Stand-level N resorption was positively  
38 correlated with the annual N requirement for tree growth. N resorption contributed to 45%, 62% and 68%  
39 of the annual N supply in the 11-, 20- and 45-year-old stands, respectively. Our exogenous N input  
40 experiment showed that leaf NRE in the 11- and 20-year-old stands decreased 17 and 12% following a 50  
41 kg N ha<sup>-1</sup> yr<sup>-1</sup> input. However, leaf NRE was not affected in the 45-year-old stand. The increases in leaf  
42 NRE and the contribution of N resorption to annual N supply along stand ages suggested that, with stand  
43 development, tree growth depends more on N resorption to supply its N need. Furthermore, the leaf NRE of  
44 mature stand was not decreased under exogenous N input, suggesting that mature stands can be stronger  
45 sinks for N deposition than young stands due to their higher capacity to retain the deposited N within plants  
46 via internal cycle. Ignoring age-related N use strategies can lead to a bias in N cycle models when  
47 evaluating forest net primary production under increasing global N deposition.

48 **Key words:** nitrogen resorption; green leaves; senesced leaves; nitrogen requirement; nitrogen deposition;  
49 stand age; plant-available nitrogen; annual stand biomass production.

## 50 INTRODUCTION

51 Afforestation is one of the most important strategies for mitigating global climate change. In the past two  
52 decades, planted forest area has steadily increased globally by around 4.3 million hectares per year, and  
53 now makes up 6–7% of global forests. Most planted forests are distributed in temperate areas in Asia,  
54 Europe, and North America (Paquette and Messier 2009; FAO 2010). Forest plantations in temperate areas  
55 are often limited by nitrogen (N) availability (Vitousek and Howarth 1991; Magnani and others 2007), but  
56 also encounter rapid increases in anthropogenic N deposition (Galloway and others 2008). As planted  
57 stands develop, the balance between N supply and N requirement for tree growth often changes as well  
58 (Gholz and others 1985; Peri and others 2006). Without understanding how stand development and  
59 exogenous N input interact to affect temperate plantations' internal N cycling and demand, we could not  
60 evaluate the potential role of plantations in alleviating climate warming while supplying wood products.

61 N resorption, the most important aspect of internal plant nutrient cycling (Killingbeck 1996), supplies  
62 about 36–76% of the annual N demand for forest growth (Bond-Lamberty and others 2006).  
63 Downregulation of N resorption efficiency (NRE) has often been observed in trees grown on N-rich soils or  
64 after N fertilization (Small 1972; Yuan and Chen 2015). Meanwhile, NRE can be affected by phloem  
65 transportation rates and loadings (Chapin and Moilanen 1991), and also the sink strength driving by  
66 demand such as producing N-rich reproductive structures (Tully and others 2013). Furthermore, rates of  
67 nutrient translocation may increase linearly with increases in the rates of tree growth (Nambiar and Fife  
68 1991). As young stands begin to mature, tree growth rate tend to get its maximum and soil available N  
69 often decline (Gower and others 1996; Tang and others 2014). To adapt to the increasing severity of N  
70 limitation during stand development, old stands tend to be more economical in their N strategies than  
71 juvenile stands (Gholz and others 1985; Mediavilla and others 2014). To this end, N resorption, an

72 important process of internal N cycling and storage (Wang and others 2013), increased greatly after the  
73 canopy closed (at 8 years old) in a northern Florida pine forest (Gholz and others 1985). A study of several  
74 boreal stands also found that N resorption was significantly higher in older stands (Bond-Lamberty and  
75 others 2006). N resorption as an important component of plant physiological and metabolic processes  
76 (Wang and others 2013) is therefore expected to change with stand aging (Yuan and Chen 2010), although  
77 studies on the topic are scarce.

78 Most forests growth is generally thought to be stimulated by exogenous N input (Thomas and others  
79 2010), which results in increases in annual net primary production (Bown and others 2010; Vicca and  
80 others 2012). However, stands of different ages differ in their growth responses to N input. For example,  
81 exogenous N input stimulated the growth of 1-year-old *Cryptomeria japonica* seedlings in Japan (Nakaji  
82 and others 2001). By contrast, N input stimulated the growth of 18-year-old *Pinus sylvestris* stands in a  
83 boreal forest in Sweden for the first 7 years, but reduced it after that (Högberg and others 2006). Such  
84 age-based responses may occur because the growth rates of dominant tree species, plant community  
85 composition, and N requirements change with stand development (Lehtonen and others 2004; Tang and  
86 others 2014). In addition to vegetation growth and composition, many studies have found that stand age  
87 also regulates the responses of microbial community composition and activity to N deposition (Allison and  
88 others 2010; Ma and others 2013), which could further influence N cycling in the soil. We expect that the N  
89 needed for growth in mature forests depends largely on the internal N cycle, such as greater N withdrawal  
90 from senescing leaves and reduced dependence on external nutrient availability. Thus, the effects of  
91 exogenous N input on N resorption should vary with forest age, we expect older forests to have a higher  
92 NRE and be less responsive to exogenous N inputs, yet few data with which to test this hypothesis are  
93 available.

94 Nitrogen resorption and plant-available N in soil are both vital in whether the supply of N can satisfy  
95 the demand for forest growth. However, to our knowledge, no attempts have been made to assess the  
96 dynamics between N resorption and N demand during stand development under increasing N deposition.  
97 Here, we investigate NRE in larch (*Larix principis-rupprechtii*) plantations of five ages (2–100 years) at  
98 Saihanba National Forest Park in China, the largest plantation (~94,700 ha) in East Asia. We also simulated  
99 N deposition by adding N to Larch plantations of three different ages. We measured N concentration in  
100 both green and senesced leaves of *Larix Principis-rupprechtii* from the various stand-age classes. We  
101 assessed annual production in stand biomass, N required for tree growth, and soil N availability in the  
102 forest under ambient and simulated N deposition treatments. We hypothesized that 1) N resorption and  
103 requirements vary during stand development; 2) the faster growing stands have a higher N requirement and  
104 therefore lead to a greater N resorption; and 3) N deposition alleviates N limitation and therefore reduces  
105 NRE.

106

## 107 **MATERIALS AND METHODS**

### 108 **Site Description and Experimental Design**

109 The study site was located at the Saihanba Ecological Station (42°25′N, 117°15′E, 1505 m a.s.l) of Peking  
110 University in Saihanba National Forest Park, Hebei Province, China. The topography of the study site is  
111 relatively flat, and the soil is predominantly sandy. The mean annual temperature is  $-1.4^{\circ}\text{C}$  ( $-21.8^{\circ}\text{C}$  in  
112 January and  $16.2^{\circ}\text{C}$  in July), and the mean annual precipitation is 450 mm. The site is frost-free for 81 d  
113 each year (Ma and others 2014). Snowfall normally begins in November, and snowmelt occurs in early  
114 April. In winter, the snow depth is typically  $< 30$  cm. The ambient nitrogen deposition is  $13 \text{ kg ha}^{-1} \text{ yr}^{-1}$ .

115 Five stands, aged 2, 11, 20, 45, and 100 years, were selected in August 2009. The dominant tree

116 species was *Larix principis-rupprechtii*, with the 45-year-old stand thinned in 1989. The distance between  
117 any two stands was less than 2 km, and all stands have similar climate and the soils are all classified as  
118 sandy soil. In 2010, the 11-, 20-, and 45-year-old stands were selected for a long-term exogenous N input  
119 experiment. Details of the three stands' properties are listed in Table 1. In each of these three forests, the  
120 experimental area consisted of nine  $20 \times 20\text{-m}^2$  plots, with wide buffer zones ( $> 10$  m) between them. In  
121 each stand, nine plots were randomly assigned as control (no N added, N0), low N input ( $20 \text{ kg N ha}^{-1}$   
122  $\text{year}^{-1}$ , N20), or high N input ( $50 \text{ kg N ha}^{-1} \text{ year}^{-1}$ , N50), with three replicates for each N input level. Urea  
123 was applied to the soil surface six times yearly from early May to early October using backpack sprayers.  
124 The amount of water added to the soil through this N application was equivalent to 0.0625 mm of rainfall,  
125 and the same amount of water was applied to the control plots. Comprehensive investigations of soil and  
126 plant nutrient status were only conducted in N0 and N50, and the results for N20 are therefore not  
127 presented in this study.

128

### 129 Plant and Soil Sampling, and Chemical Analysis

130 To examine leaf NRE, we sampled green and senesced needles from the five age classes in mid-August and  
131 early October, respectively. Appendix table S1 lists the sampling years for each stand age and exogenous N  
132 input treatment. Leaves were collected from upper and lower crown positions. Senescing needles were  
133 collected by hand while still attached to the tree, with the collection time determined by their color and  
134 whether they were ready to flush to the ground, according to Killingbeck and others (1990). We collected  
135 100 needles from each sample tree in mid-August and early October to estimate the leaf mass lost between  
136 the green and senescent stages. Needle samples were placed in plastic bags, transported to the laboratory,  
137 and oven-dried at  $60^\circ\text{C}$  to a constant mass. Leaf litter mass loss was determined by dividing the mass of

138 100 needles sampled in October by the mass of 100 needles sampled in August.

139 We collected litterfall twice per year from 2010 onward, using two traps installed in each plot (for a  
140 total of 18 litter traps in each stand). The traps measured  $1 \times 1 \text{ m}^2$  and were constructed of mesh and metal  
141 frames mounted on 0.4 and 0.8 m high polyvinyl chloride rods in the 11-year-old and 20- and 45-year-old  
142 stands. Samples from the two traps were composited to generate one sample per plot, dried to constant  
143 mass at 60°C, and weighed. Almost no woody or other fraction was found in the traps in our study. Thus  
144 the amount of litterfall was effectively equal to the foliage mass.

145 We sampled branches in the upper and lower crown positions simultaneously with the senesced leaves.  
146 To determine the stem N concentration, three trees were randomly selected in each plot, and the sapwood  
147 and heartwood of each were sampled at a height of 1.3 m using an increment borer. The three samples from  
148 each plot were combined and oven-dried to constant mass at 60°C.

149 To estimate root N concentration, four soil cores (0-40 cm) were obtained using a 10 cm-diameter  
150 metal auger at randomly selected positions in each plot at the end of the growing season. Soil cores were  
151 divided into three depths (0–10, 10–20, and 20–40 cm). Roots were separated by hand from each sample  
152 and oven-dried to constant mass at 60°C. Root N concentration was the average of the four samples.

153 All plant samples were ground in a Wiley mill (2 mm mesh). The C and N concentrations in plant  
154 samples were determined using an elemental analyzer (2400 II CHNS/O Elemental Analyzer, Perkin-Elmer,  
155 Waltham, MA, USA).

156 Soil samples were collected the month before N additions in the growing seasons of 2011 and 2013.  
157 Soil was randomly sampled from three points in each plot using a corer (internal diameter 4 cm) to a depth  
158 of 20 cm, stored in an icebox, and transported to the laboratory immediately after collection. The three  
159 samples from each plot were composited and roots were hand-sorted and soil passed through a 2-mm sieve.

160 We performed a KCl (0.5 M) on soil subsamples, and the extractant was analyzed for  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N  
161 using the Type AA3 Continuous Flow Analytical System (BranLubbe, Germany).

162

### 163 Nitrogen Resorption Calculations

164 The NRE at leaf level was calculated as follows (Aerts 1996; Killingbeck 1996):

$$165 \quad \text{NRE} = \left[ \frac{N_{\text{green}} - N_{\text{senesced}}}{N_{\text{green}}} \right] \times 100\% \quad (1)$$

166 where  $N_{\text{green}}$  and  $N_{\text{senesced}}$  are the nitrogen concentrations (mass of N per unit dry mass) in mature green and  
167 senesced leaves, respectively.

168 Nitrogen resorption at the stand level was determined by the difference in the amount of N between  
169 green and senesced leaves in a plot. The green leaf mass in a plot was assessed by dividing the litterfall  
170 mass by the rate of leaf mass loss.

171

### 172 Annual Stand Biomass Production

173 Annual stand biomass production was used to represent the growth potential of trees in a plot (Chapin and  
174 others 1990). Annual stand biomass production in each plot was determined in the 11-, 20-, and 45-year-old  
175 stands from 2010 to 2013. To examine the increment in tree height and diameter, 15–20 trees were  
176 randomly selected in each plot. We installed metal bands at breast height (1.3 m) on each tree in the spring  
177 of 2010 to measure the diameter at breast height (DBH) of each tree. The change of the window length  
178 between two measurements was the yearly circumference growth, and was recorded at the beginning (early  
179 may) and end (late October) of the growing season. At the same time, tree height was measured using a  
180 hypsometer. The height and DBH of trees in each plot were calculated as the means of 15–20 trees.

181 We estimated the biomass of branches, stems, and roots using an allometric equation relating each

182 biomass component to the DBH and tree height, respectively. This allometric equation was established  
183 using forest inventory data from our study area (personal communication with Chao Yue), and is as  
184 follows:

$$185 \quad \text{Ln}(\text{biomass}) = a \times \text{Ln}(D^2H) + b \quad (2)$$

186 where D and H are the mean DBH and tree height, respectively. Table 2 summarizes the values of a and b  
187 for the biomass of branches, stems, and roots in all study stands.

188

### 189 Measuring the Annual Biomass Production and the N Required for Tree Growth

190 Annual biomass production was defined as the sum of the annual increase in foliage, branch, stem, and root  
191 biomasses. The annual biomass production in a plot was calculated as:

$$192 \quad B = B_f + (B_b - B_b') + (B_s - B_s') + (B_r - B_r') \quad (3)$$

193 where B ( $\text{Mg ha}^{-1}$ ) is the annual biomass production in a plot,  $B_f$  ( $\text{Mg ha}^{-1}$ ) is the foliage biomass in a plot  
194 in August,  $B_b'$ ,  $B_s'$ , and  $B_r'$  ( $\text{Mg ha}^{-1}$ ) are the branch, stem, and root biomasses in a plot at the beginning of  
195 the local growing season, respectively, and  $B_b$ ,  $B_s$ , and  $B_r$  ( $\text{Mg ha}^{-1}$ ) are the branch, stem, and root  
196 biomasses in a plot at the end of the local growing season, respectively.

197 The annual N requirement was defined as the amount of N taken up by vegetation per square meter  
198 per year ( $\text{g m}^{-2} \text{yr}^{-1}$ ), which is difficult to determine directly (Chapin and others 1986). Therefore, we used  
199 total N content in annual accumulated biomass as a surrogate of the annual N requirement for tree growth  
200 (Gholz and others 1985). Thus, the annual N requirement for tree growth was defined as the annual N  
201 accumulation in leaves, branches, stems, and roots in a plot (Berendse and Aerts 1987; Tomaszewski and  
202 others 2003). The annual N requirement in a plot was calculated as:

$$203 \quad \text{Nr} = \sum(B_i \times c_i) \quad (4)$$

204 where  $N_r$  ( $\text{g m}^{-2} \text{yr}^{-1}$ ) is the annual N requirement for the growth of leaves, branches, stems, and roots in a  
205 plot, and  $B_i$  ( $\text{Mg ha}^{-1}$ ) and  $c_i$  ( $\text{mg kg}^{-1}$ ) are the annual production in stand biomass and average N  
206 concentration of foliage, branches, stems, and roots in a plot, respectively.

207

## 208 Statistical Analysis

209 We performed a two-way ANOVA to evaluate the effects of stand age and exogenous N input on all the  
210 variables. Significant effects were determined at  $P < 0.05$  unless otherwise stated. The relationships  
211 between variables were analyzed using exponential or linear models. Analyses were conducted using SPSS  
212 16.0 software (SPSS Inc., Chicago, IL, USA). Data were expressed as mean values  $\pm$  S.E. (standard error).

213

## 214 RESULTS

### 215 Leaf N Concentration and NRE

216 Green leaf N concentration logarithmically increased with increasing stand age (Figure 1A). During stands'  
217 initial development (2–23 years old), green leaf N concentration increased sharply with increasing stand  
218 age from 1.53% to 2.51%. After canopy closure (20–100 years old), green leaf N concentration increased  
219 slowly, ranging from 2.16% to 2.87%.

220 In contrast to green leaf N concentration, senesced-leaf N concentration logarithmically decreased  
221 with increasing stand age (Figure 1B). A sharp decrease (from 1.65% to 0.60%) in senesced-leaf N  
222 concentration occurred from the initial development to canopy closure stages. After canopy closure, the  
223 senesced-leaf N concentration remained relatively low, ranging from 0.40% to 0.99%.

224 Like green leaf N concentration, NRE logarithmically increased with increasing stand age (Figure 1C).

225 In 2–23 years old trees, the NRE increased sharply with increasing stand age from 8% to 76%. After age

226 20, the NRE increased slowly, ranging from 66% to 82%.

227

## 228 Annual Biomass Production and Annual N requirement

229 The annual biomass production was significantly age-related (Figure 2A), and was largest in the  
230 20-year-old stand, followed by the 45-year-old stand, and lowest in the 11-year-old stand. The biomass  
231 production of foliage, branches, stems, and roots displayed the same pattern among stand ages (Figure 2A).  
232 However, biomass allocation to these tissues varied among ages (Figure 2A), with 6.9%, 34.2%, 17.1%,  
233 and 41.9% in the 11-year-old stand, 12.9%, 48.3%, 13.5%, and 25.3% in the 20-year-old stand and 11.6%,  
234 45.1%, 8.6%, and 34.6% in the 45-year-old stand to roots, stems, branches and leaves, respectively (Figure  
235 2A). Exogenous N input significantly increased annual biomass production ( $P = 0.037$ ), and did not alter  
236 the biomass allocation among tissues (Figure 2A).

237 Different from green leaves, the N concentrations in roots, branches, and stems decreased with stand  
238 age (Table 3). The exogenous N input significantly increased N concentration in green leaves, but had no  
239 impact on the N concentrations of branches, stems and roots (Table 3). The annual N requirement,  
240 estimated by summing N content in newly accumulated biomass in different organs, displayed the same  
241 pattern as the annual increase in stand biomass, with the highest value in the 20-year-old stand, followed by  
242 the 45-year-old stand, and the lowest value in the 11-year-old stand (Figure 2B and Table 3). The majority  
243 of annual N supply was used for leaf growth, accounting for 71.6, 78.3 and 84.2% of the total annual N  
244 requirement in 11-, 20- and 45-year-old stands, respectively (Figure 2B). The ratios of annual N  
245 requirement for stems and branches to the total both declined with stand age: 9.5, 8.7 and 5.4% for  
246 branches and 15.1, 4.9 and 4.5% for stems in 11-, 20- and 45-year-old stands, respectively (Figure 2B).  
247 However, the ratios of annual N requirement for roots to the total were higher in the 20- (8.2%) and

248 45-year-old (5.9%) stands than in the 11-year-old stands (3.8%) (Figure 2B). The exogenous N input did  
249 not alter the annual N requirement for leaves, branches and stems, but increased the annual N requirement  
250 for roots, although the increase was significant only in the 45-year-old stands (Table 3).

251

## 252 N Resorption at Leaf and Stand-level

253 Younger stands tended to have higher N concentrations in senesced leaves ( $P < 0.001$ , Figure 3A). The  
254 exogenous N input increased N concentration in senesced leaves ( $P < 0.001$ ), but the degree of the increase  
255 differed among stands. Among the three age classes, the greatest increase in senesced leaf N concentration  
256 occurred in the 11- year-old stands (Figure 3A).

257 The NRE at leaf level ranged from 34.6 to 77.8%, which was increased significantly from the 11- to  
258 45-year-old stands ( $P < 0.001$ , Figure 3B). The exogenous N input decreased NRE in 11- and 20-year-old  
259 stands, while not altered NRE in 45-year-old stand.

260 N resorption at stand-level also differed among the three stands ( $P < 0.001$ , Figure 3C). The highest N  
261 resorption was  $8.3 \pm 0.74 \text{ g m}^{-2}$  in the 20-year-old stand and the lowest was  $1.8 \pm 0.46 \text{ g m}^{-2}$  in the  
262 11-year-old stand. However the exogenous N input did not altered the N resorption at stand level (Figure  
263 3C).

264

## 265 The Effects of Plant-Available N Concentration and N Growth Requirement on N Resorption

266 Plant-available N concentration was different among the three age classes ( $P = 0.007$ ), with the value was  
267 higher in the 11-year-old stand than in the 20- and 45-year-old stands. The exogenous N input significantly  
268 increased the plant-available N concentration ( $P = 0.022$ , Figure 4A). Leaf NRE decreased with increasing  
269 plant-available N concentration, and exogenous N input did not alter this relationship (Figure 4B).

270 The contribution of N resorption to annual N supply, as indicated by the ratio of N resorption at  
271 stand-level to the annual N requirement for stand growth, significantly increased with increasing stand age  
272 ( $P < 0.001$ ), with mean values of  $45 \pm 4.7\%$  for the 11-year-old stand,  $62 \pm 2.5\%$  for the 20-year-old stand,  
273 and  $68 \pm 2.1\%$  for the 45-year-old stand (Figure 5A). The exogenous N input decreased the contribution of  
274 N resorption to annual N supply ( $P = 0.020$ , Figure 5A). The correlation between N requirement and N  
275 resorption at stand level was also analyzed with linearly regression. To avoid autocorrelation, the analysis  
276 was conducted using the total annual N requirement for branches, stems, and roots but not leaves. The  
277 results showed that N resorption at stand level increased with increasing annual N requirement regardless  
278 of with ( $R^2 = 0.54$ ,  $P < 0.001$ ) or without the exogenous N input ( $R^2 = 0.64$ ,  $P < 0.001$ , Figure 5B). The  
279 exogenous N input tended to reduce the slope of the regression line, although the reduction was not  
280 significant (Figure 5B).

281

## 282 **DISCUSSION**

283 We found that along the stand-age chronosequence from 2 to 100 years old, leaf NRE increased  
284 logarithmically from 8% to 82% (Figure 1C). Leaf N is an important component of proteins, which are  
285 abundant in chloroplasts, and minor amounts are found in cytosolic proteins, chlorophyll, and amino acids  
286 (Estiarte and Peñuelas 2015). During leaf senescence, proteins are hydrolyzed into amino acids,  
287 which subsequently retranslocated to woody tissues (Chapin and Kedrowski 1983). 62% of the N is  
288 removed from leaves by this process (Vergutz and others 2012). Killingbeck (1996) suggested that any N  
289 concentration in senesced leaves  $< 7 \text{ mg g}^{-1}$  can be considered “complete resorption,” and concentrations  $>$   
290  $10 \text{ mg g}^{-1}$  “incomplete resorption”. In our study, the N concentration of senesced needles in young stands  
291 ranged from 0.74% to 1.65%. After canopy closure, N in senesced needles decreased to 0.40% to 0.99%

292 (Figure 1B), suggesting that N resorption shifted from incomplete to complete, and indicating that the N  
293 strategy of the larch plantation gradually improved with stand aging. This change in N resorption may be  
294 driven by changes in soil nutrient conditions and forest growth rates during stand development.

295 The high soil N availability could lead to low efficiency of N use and luxury N consumption of plant  
296 (Vitousek 1982; Yuan & Chen 2015). Numerous studies found that leaf NRE is generally lower in N-rich  
297 conditions than in N-poor conditions (Small 1972; Kobe and others 2005; Vergutz and others 2012; Yuan  
298 and Chen 2015). At our study sites, N availability in the soil decreased with stand age (Figure 4C). The  
299 decline in plant-available N could be due to increased N use for aboveground productivity with stand  
300 development and, simultaneously, soil N availability limited by the return of N from litterfall due to  
301 relatively low litterfall decomposition (Polglase and others 1992; Farley and Kelly 2004). In addition, N  
302 mineralization and nitrification are strongly controlled by litter decomposition, which in turn is controlled  
303 by litter N concentration (Melillo and others 1982; Manzoni and others 2008). At our study site, mature  
304 stands have a relatively low N concentration in senesced leaves, ranging from 0.40% to 1.0% after canopy  
305 closure (Figure 1B). The low N concentration in senesced leaves, coupled with the high C:N ratio of leaf  
306 detritus (Table 1), could slow the litter decomposition rate in mature forest, feeding back on N  
307 mineralization and therefore soil nutrient availability (Aerts 1997). The decrease in plant-available N  
308 during stand development therefore led to an increase in leaf NRE with stands aging (Figure 4B).

309 Temperate forests are facing increasing N deposition, which generally increases plant-available N in  
310 the soils. A recent meta-analysis based on a global dataset found that leaf NRE declined in response to N  
311 fertilization (Yuan and Chen 2015). However, it is largely unknown whether leaf N resorption in stands of  
312 different ages will respond differently to exogenous N input. Our experiments found that exogenous N  
313 input increased N concentration in both green leaves and senesced leaves, however the responses changed

314 with stand development (Table 3, Figure 3A). Compared to older stands, younger stands tend to have a  
315 lower increase in green leaf N concentration, but a greater increase in senesced leaf N concentration under  
316 exogenous N input (Table 3, Figure 3A). Exogenous N input was therefore significantly decreased leaf  
317 NRE in young stands, but such reduction diminished in mature stands (Figure 3C). Our study suggested  
318 that increase in plant-available N could be the main reason leading to the decline in leaf NRE under  
319 exogenous N input (Figure 4B). However, the degree of the responses was regulated by the physiological  
320 stage of the stands (Figure 3B). Increase in N deposition could decrease the efficiency of young stands to  
321 recycle N within plants, whereas mature stands can be a stronger sink for N deposition, with better capacity  
322 to retain the deposited N within plants via internal cycle.

323 Nutrient resorption is a key strategy that plants conserve previously acquired nutrients (Zhang and  
324 others 2015). The N demands of living tissues constitute sinks for resorbed N, as observed in early studies  
325 (Chapin and Kedrowski 1983; Chapin and Moilanen 1991; Tully and others 2013). The potential of forests  
326 to grow living tissues varies with stand ages. We expected that stands with higher growth potential require  
327 more N to support biomass production, therefore should have a higher NRE. This hypothesis was supported  
328 by a study in radiate pine saplings in South Australia. The study found that periods of high N resorption  
329 coincided with periods of high shoot production, indicating that growth demand of N greatly determined  
330 nutrient resorption (Nambiar and Fife 1987, 1991).

331 In the current study, we found that the annual increase in stand biomass and its allocation in tissues  
332 shifted across different stand ages (Figure 2A), in line with many other studies on various tree species  
333 (Gower and others 1996; Ryan and others 2004; He and others 2012; Taylor and others 2014). Due to  
334 changes in the annual accumulated biomass and N concentrations in plant organs, the annual N requirement  
335 showed age-related variation, ranging from  $3.8 \pm 0.46$  to  $13.4 \pm 1.19$  g m<sup>-2</sup> yr<sup>-1</sup> (Figure 2B). Middle-aged

336 stands had the highest N requirements, consistent with the results of Bond-Lamberty and others (2006) for  
337 a boreal black spruce stand. We observed a strong positive relationship between the stand-level N  
338 resorption and the annual N requirement for stand growth (Figure 5B), suggesting that the shift in annual N  
339 requirement could be the main factor responsible for the increase in stand-level N resorption along the age  
340 chronosequence.

341 A recent global analysis suggested that leaf N resorption contributes to 31% of annual N plant demand  
342 (Cleveland and others 2013). In our sites, N derived from senescing leaves can supply 45–68% of the N  
343 required for stand growth (Figure 3A). Both leaf NRE and the contribution of recycled N to annual N  
344 requirement increased with increasing stand age (Figure 3B and Figure 5A). Those evidences indicated that  
345 mature stands are more efficient in recycling N than are younger ones; mature stands are therefore have a  
346 greater capacity to supply growth required N via internal N cycling in plants. Our exogenous N input  
347 experiments further found that N input decreased the contribution of recycled N to the total annual N  
348 requirement by an average of 6.8%, and stand ages did not alter the responses (Figure 5A). With the  
349 continuing increase in N deposition, we expect that the N sources for stand growth would be shifted. Plants  
350 will depend more on root uptake of newly deposited N, but less on recycled N via resorption.

351 In the current study, we use a chronosequence approach to investigate how stand age and its  
352 interaction with N deposition affect N resorption at leaf level and stand level. Our studied stands along the  
353 age chronosequence have similar climate, topography and soil type, we therefore expected that the  
354 difference among the stands is predominately driven by the difference in stand ages. Still the findings  
355 should be interpreted alongside the limitations of the space-for-time approach (Johnson and Miyanishi  
356 2008). Considering factors such as soil nutrient condition and stand successional trajectory could be  
357 changed due to continue receiving N deposition, the chronosequential response of a stand could be different

358 from our prediction using the space-for-time approach. Long-term time series studies are needed to  
359 improve our understanding on this topic.

360

## 361 **CONCLUSIONS**

362 We found a logarithmic shift in the green- and senesced-leaf N concentration and leaf NRE of larch along a  
363 stand-age chronosequence, indicating that NRE can change with stand development. In models, NRE is  
364 often set to a constant value of 50% (Aerts 1996; Van Heerwaarden and others 2003; Vergutz and others  
365 2012). As a key process in biogeochemical models, neglecting age-related changes in N resorption can lead  
366 to bias when evaluating N-derived changes in forest net primary production in N cycle models, especially  
367 with increasing global N deposition. Thus, further experimental and modeling studies are needed to  
368 accurately quantify the age-related pattern of NRE in other tree species and to better address the  
369 implications for N-derived changes in forest growth.

370 Our data also suggested that the age-related pattern of stand-level N resorption was controlled by the  
371 changes in soil N availability and annual N requirement for forest growth during stand development. In  
372 addition, the ratios of stand-level N resorption to the annual N requirement for stand growth increased with  
373 stand age. These results not only imply that the growth potential of trees can influence their NRE with  
374 stand development, but also show that mature stands are probably stronger sinks for N from atmospheric  
375 deposition. Ignoring these results could lead to a bias in N-cycling models when evaluating N-derived  
376 changes in forest net primary production under increasing global N deposition.

377

378

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385

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502 **Figure legends**

503

504 **Figure 1** Variations in green (A) and senesced (B) leaf N concentrations and leaf NRE (C) along a  
505 stand-age chronosequence in a Larix plantation in north China. The regression equations represent green-  
506 and senesced-leaf N concentrations and leaf NRE vs. stand age. The vertical bars represent the mean  $\pm$  1  
507 SE.

508

509 **Figure 2** Annual stand biomass productions (A), N requirement for stand growth (B) in different stand-age  
510 classes. Annual stand biomass production and N requirement for stand growth include the contributions of  
511 leaves, branches, stems, and roots in each plot. The error bars represent the standard deviations of the  
512 annual stand biomass increase or the N requirement for stand growth.

513

514 **Figure 3** The effects of stand age and exogenous N input on senesced-leaf N concentrations (A), leaf NRE  
515 (B) and the amount of stand-level N resorption (C). Vertical bars represent the mean  $\pm$  1 SE.

516

517 **Figure 4** The effects of stand age and exogenous N input on the plant-available N concentration (A) and  
518 the relationship between plant-available N concentration (0–20-cm soil depth) and leaf NRE under two N  
519 input levels (B). Data in figure B are the mean values from the three stands in 2011 and 2013. The circle,  
520 triangle and squares symbols represent the stands aged 11-, 20- and 45-year-old, respectively. Black and  
521 gray lines are regressions of the plant-available N and NRE under N0 and N50 treatments, respectively.

522

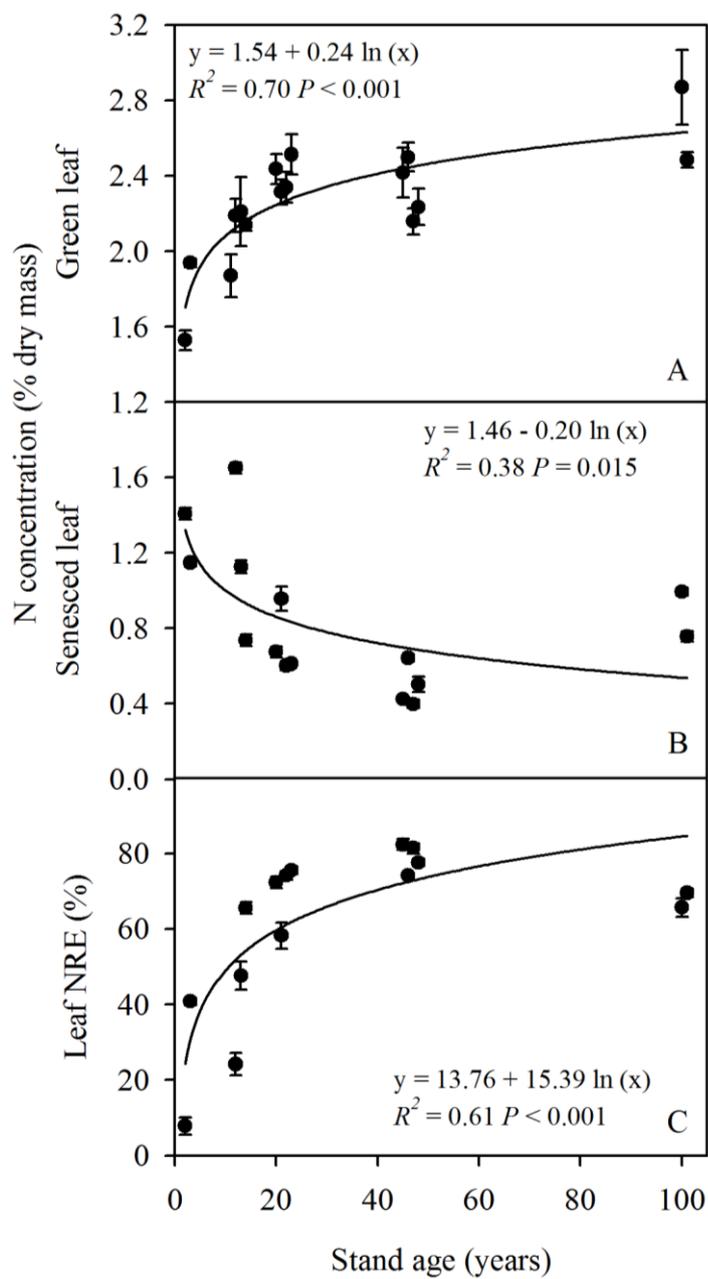
523 **Figure 5** The effects of stand age and exogenous N input on the ratio of stand-level N resorption to annual

524 N requirement for stand growth (A), and the relationship between the total annual N requirement for  
525 branches, stems, and roots and the amount of stand-level N resorption for stands aged 11 (circles), 20  
526 (triangles), and 45 (squares) years (B). The data are mean values. Black and gray lines are regressions of  
527 the amount of stand-level N resorption and the annual N requirement for tree growth under N0 and N50  
528 treatments, respectively.

529

530

531 Figure 1

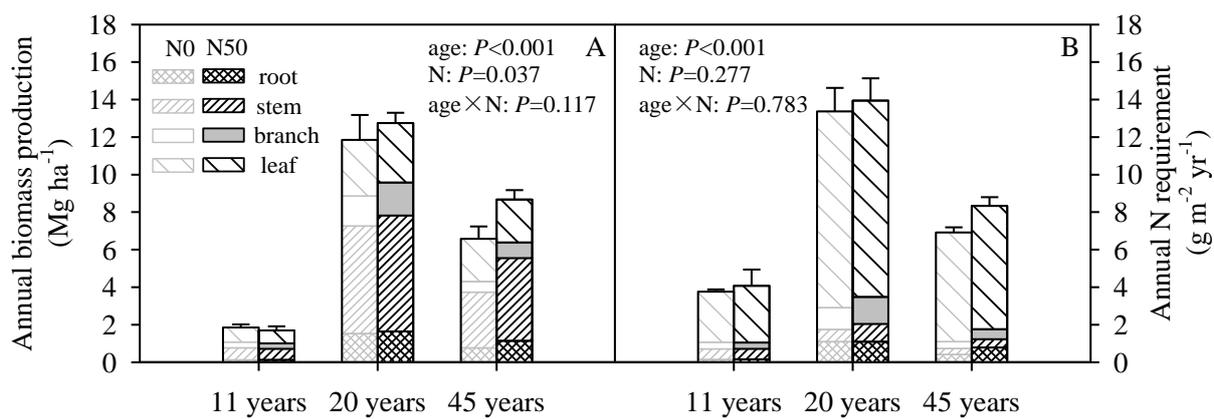


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535 Figure 2

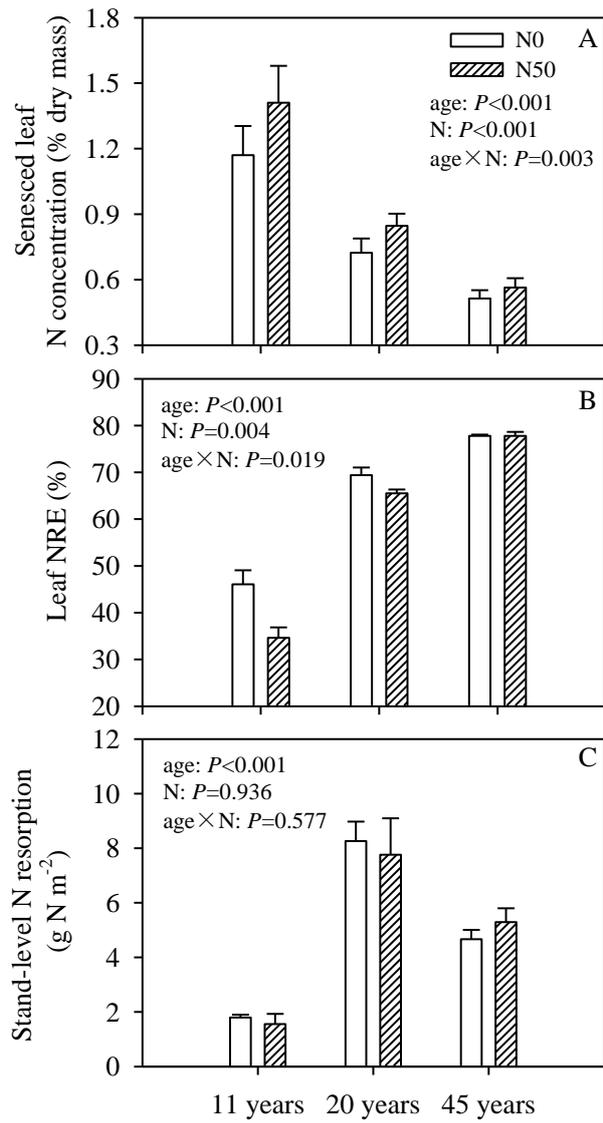


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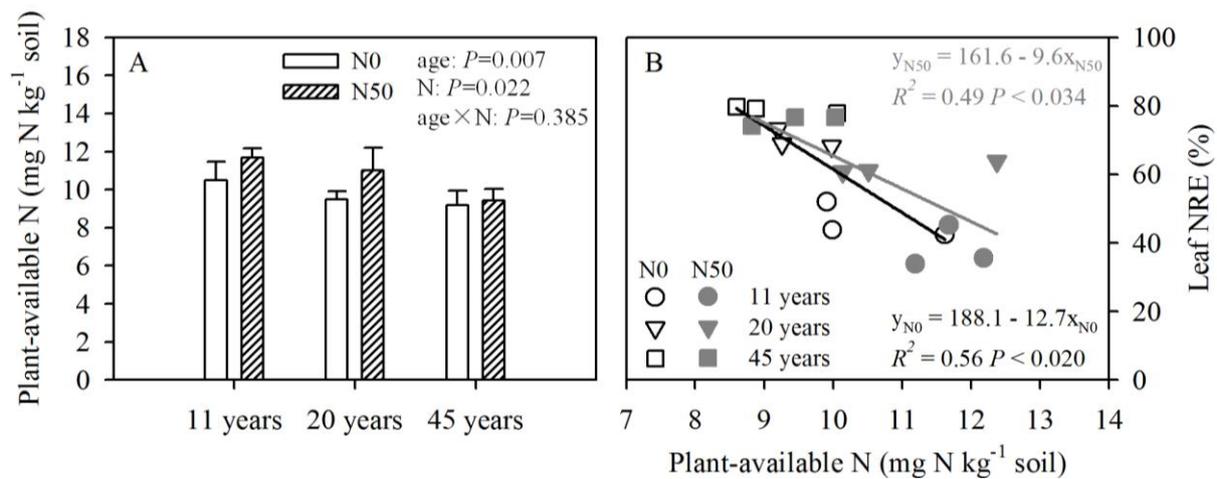
539 Figure 3



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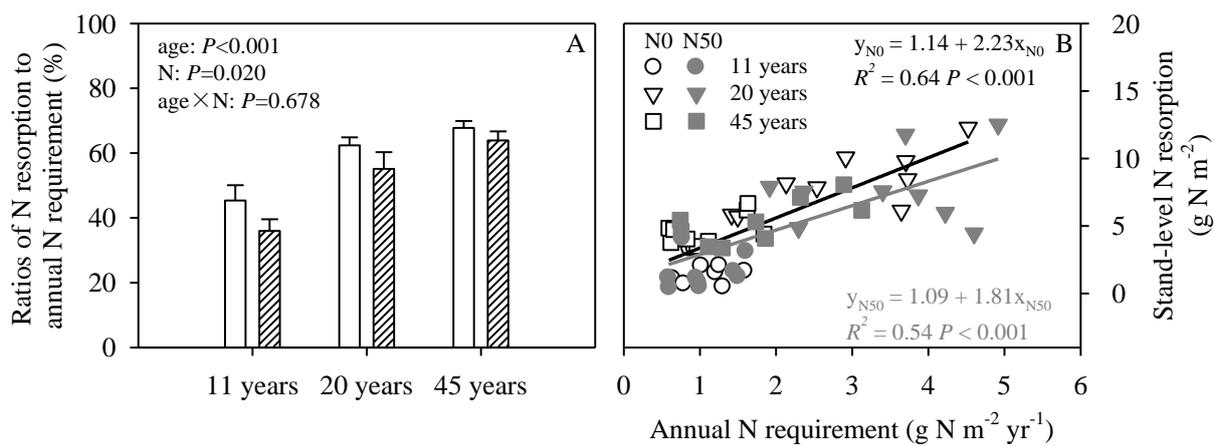
541

542 Figure 4



543

544 Figure 5



545

546

547 **Table 1** Forest structure and litter (organic horizon) and soil (0–10-cm depth) properties of the three stand ages of *Larix principis-rupprechtii*  
 548 plantations in 2013.

Stand age (years)	Location (latitude, longitude)	Community			Litter and soil physiochemical properties (0–10 cm)		
		Density (Trees ha <sup>-1</sup> )	DBH (cm)	Height (m)	Litter C:N	Soil C:N	Soil pH (soil:water = 1:2.5)
11	42°23.3'N, 117°14.0'E	2640(157) <sup>b</sup>	4.1(0.1) <sup>c</sup>	3.6(0.1) <sup>c</sup>	65.6(2.7) <sup>c</sup>	10.3(0.2) <sup>b</sup>	6.2(0.2) <sup>b</sup>
20	42°23.6'N, 117°14.1'E	3060(132) <sup>a</sup>	10.5(0.1) <sup>b</sup>	8.6(0.1) <sup>b</sup>	79.8(3.1) <sup>b</sup>	11.7(0.3) <sup>a</sup>	6.5(0.0) <sup>a</sup>
45	42°23.9'N, 117°14.8'E	870(48) <sup>c</sup>	20.7(0.2) <sup>a</sup>	16.5(0.2) <sup>a</sup>	102.2(8.0) <sup>a</sup>	13.2(0.9) <sup>a</sup>	6.3(0.2) <sup>ab</sup>

549 Values in parentheses are SDs of the mean (n = 3). Different superscript letters within the same column indicate significant differences between stands (one-way  
 550 ANOVA, post hoc LSD test,  $P < 0.05$ ). DBH = diameter at breast height.

551 **Table 2** Allometric regression parameters used to predict biomass of branches, stems, and  
 552 roots in forest stands of different ages.

Items	a	b	$R^2$	$P$
11-year-old stand				
Stems	0.5360	4.7647	0.89	< 0.01
Branches	0.3481	5.2004	0.52	< 0.01
Roots	0.3896	4.0268	0.62	< 0.01
20- and 45-year-old stands				
Stems	0.8882	3.6574	0.99	< 0.01
Branches	0.7051	3.8495	0.90	< 0.01
Roots	0.8725	2.4581	0.95	< 0.01

553 *The models were developed from Larix principis-rupprechtii plantations in Saihanba National Forest.*

554 *The equation is :  $\ln(\text{biomass}) = a \times \ln(D^2H) + b$ , where  $D$  is the mean DBH, and  $H$  is the mean tree*

555 *height.*

556 **Table 3** N concentrations and annual N requirement for newly accumulated biomass in different organs.

Stand age (years)	N treatment	N concentration (%)				Annual N requirement (g m <sup>-2</sup> yr <sup>-1</sup> )			
		Green leaves	Branches	Stems	Roots	Leaves	Branches	Stems	Roots
11	N0	2.18(0.10)	1.14(0.01)	0.90(0.03)	1.14(0.05)	2.70(0.11)	0.36(0.01)	0.57(0.04)	0.14(0.01)
	N50	2.15(0.05)	1.12(0.04)	0.93(0.02)	1.34(0.08)	3.02(0.88)	0.33(0.07)	0.55(0.13)	0.16(0.04)
20	N0	2.39(0.03)	0.76(0.06)	0.11(0.01)	0.72(0.09)	10.47(0.98)	1.16(0.12)	0.65(0.07)	1.09(0.14)
	N50	2.48(0.02)	0.84(0.05)	0.16(0.02)	0.67(0.03)	10.47(1.31)	1.43(0.14)	0.96(0.10)	1.09(0.04)
45	N0	2.30(0.06)	0.62(0.02)	0.11(0.02)	0.53(0.02)	5.82(0.32)	0.37(0.02)	0.31(0.07)	0.41(0.02)
	N50	2.54(0.08)	0.60(0.05)	0.10(0.01)	0.69(0.05)	6.57(0.48)	0.53(0.07)	0.45(0.07)	0.78(0.08)
Source ( <i>P</i> -values)									
	age	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	N	0.014	0.669	0.272	0.058	0.590	0.081	0.065	0.043
	age × N	0.024	0.467	0.585	0.112	0.892	0.265	0.22	0.036

557 *Data are the means of 3 years from 2011 to 2013. P-values were results of the LSD test which followed the Two-way ANOVA on the effects of stand age and*  
 558 *exogenous N input on the concentrations of branches, stems and roots and N requirement of foliage, branches, stems and roots.*

559 **Appendix**

560

561 **Table S1** The arrangement of exogenous N input treatments and leaf sampling for each stand  
562 of the five age classes.

563

Stand age (years)	N input treatment		Sampling year			
	N0	N50	2010	2011	2012	2013
2	√			√	√	
11	√	√		√	√	√
20	√	√	√	√	√	√
45	√	√	√	√	√	√
100	√			√	√	

564 “√” indicates that the exogenous N input treatment was carried out in this stand, or that green and senesced  
565 leaves were sampled in this year.

566 The English in this document has been checked by at least two professional editors, both native  
567 speakers of English. For a certificate, please see:

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