1   **Age-related Modulation of the Nitrogen Resorption Efficiency Response to Growth**

2   **Requirements and Soil Nitrogen Availability in a Temperate Pine Plantation**

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ABSTRACT

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

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

INTRODUCTION

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

N resorption, the most important aspect of internal plant nutrient cycling (Killingbeck 1996), supplies about 36–76% of the annual N demand for forest growth (Bond-Lamberty and others 2006). Downregulation of N resorption efficiency (NRE) has often been observed in trees grown on N-rich soils or after N fertilization (Small 1972; Yuan and Chen 2015). Meanwhile, NRE can be affected by phloem transportation rates and loadings (Chapin and Moilanen 1991), and also the sink strength driving by demand such as producing N-rich reproductive structures (Tully and others 2013). Furthermore, rates of nutrient translocation may increase linearly with increases in the rates of tree growth (Nambiar and Fife 1991). As young stands begin to mature, tree growth rate tend to get its maximum and soil available N often decline (Gower and others 1996; Tang and others 2014). To adapt to the increasing severity of N limitation during stand development, old stands tend to be more economical in their N strategies than 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°C (−21.8°C in

112   January and 16.2°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 kg ha^{-1} 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 kg N ha^{-1}

122 year^{-1}, N20), or high N input (50 kg N ha^{-1} 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°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 1×1 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 NH_4^+-N and 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 $$\text{NRE} = \left[\frac{N_{green} - N_{senesced}}{N_{green}}\right] \times 100\% \tag{1}$$

166 where N_{green} and $N_{senesced}$ 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:

$$Ln(biomass) = a \times Ln(D^2H) + b \qquad (2)$$

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:

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

193 where B (Mg ha^{-1}) is the annual biomass production in a plot, B_f (Mg ha^{-1}) is the foliage biomass in a plot

194 in August, B_b', B_s', and B_r' (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 (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 (g m^{-2} 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:

$$Nr = \sum (B_i \times c_i) \qquad (4)$$

204   where Nr (g m^{-2} yr^{-1}) is the annual N requirement for the growth of leaves, branches, stems, and roots in a

205   plot, and B$_i$ (Mg ha^{-1}) and c$_i$ (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 ± 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 ± 0.74 g m^{-2} in the 20-year-old stand and the lowest was 1.8 ± 0.46 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 mg g^{-1} can be considered "complete resorption," and concentrations $>$

290   10 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 decease 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 ± 0.46 to 13.4 ± 1.19 g m^{-2} yr^{-1} (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 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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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 ± 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 ± 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

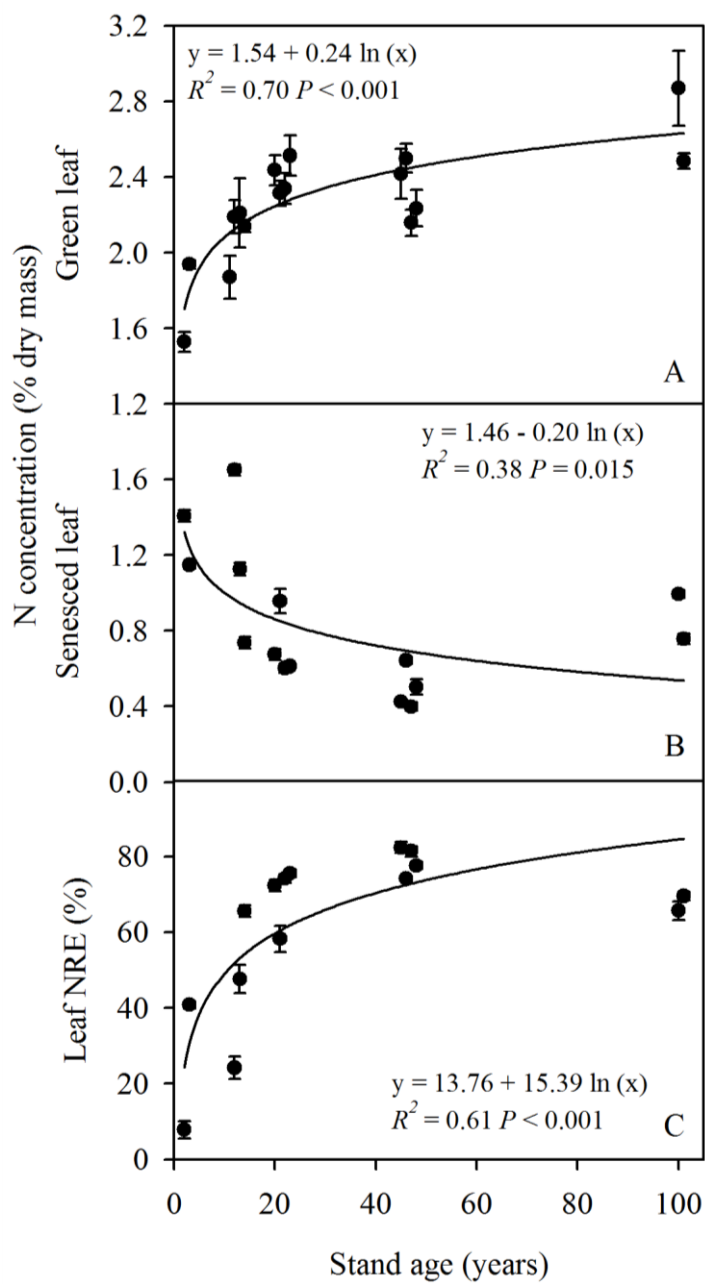523 **Figure 5** The effects of stand age and exogenous N input on the ratio of stand-level N resorption to annual

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.
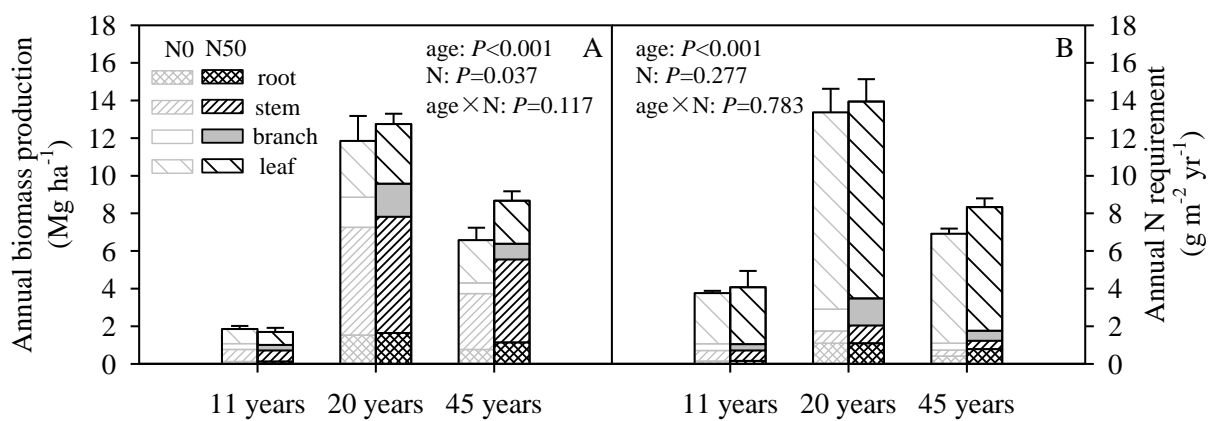
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531   Figure 1
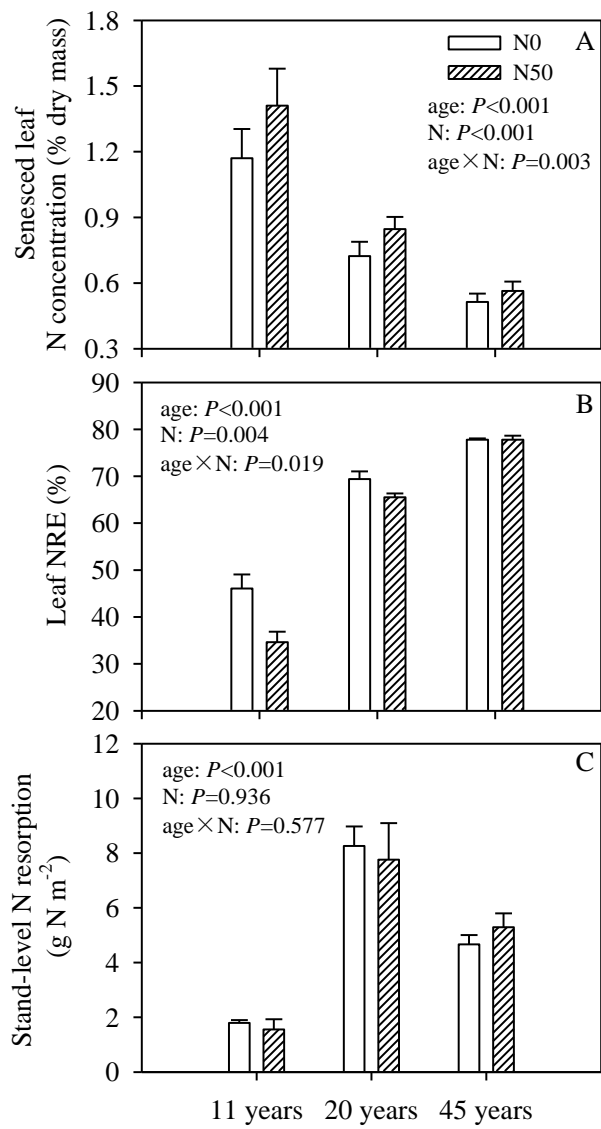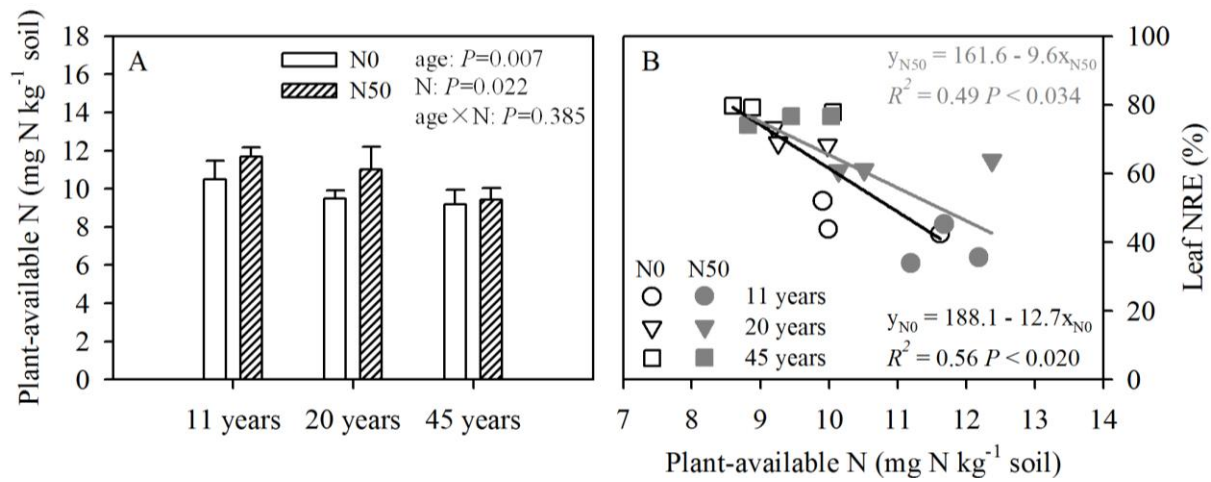

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535   Figure 2

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539   Figure 3

542   Figure 4

Panel A: y-axis "Plant-available N (mg N kg^{-1} soil)" ranging 0 to 18. Legend: N0 (open), N50 (hatched). Statistics: age: $P=0.007$; N: $P=0.022$; age×N: $P=0.385$. x-axis categories: 11 years, 20 years, 45 years.

Panel B: x-axis "Plant-available N (mg N kg^{-1} soil)" ranging 7 to 14. y-axis (right) "Leaf NRE (%)" ranging 0 to 100. Equations: $y_{N50} = 161.6 - 9.6x_{N50}$, $R^2 = 0.49\ P < 0.034$; $y_{N0} = 188.1 - 12.7x_{N0}$, $R^2 = 0.56\ P < 0.020$. Legend: N0 / N50 — 11 years, 20 years, 45 years.
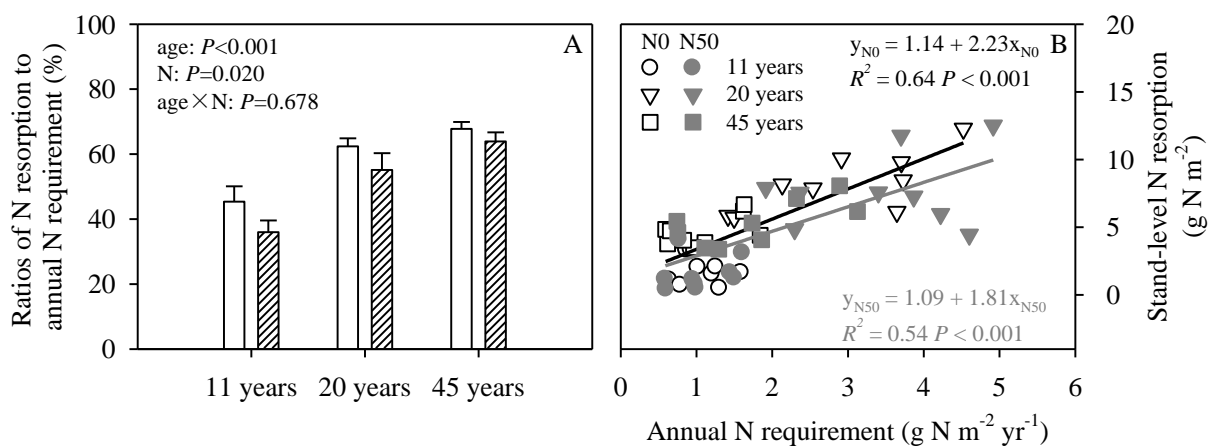
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544   Figure 5

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^{-1})	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)[b]	4.1(0.1)[c]	3.6(0.1)[c]	65.6(2.7)[c]	10.3(0.2)[b]	6.2(0.2)[b]
20	42°23.6´N, 117°14.1´E	3060(132)[a]	10.5(0.1)[b]	8.6(0.1)[b]	79.8(3.1)[b]	11.7(0.3)[a]	6.5(0.0)[a]
45	42°23.9´N, 117°14.8´E	870(48)[c]	20.7(0.2)[a]	16.5(0.2)[a]	102.2(8.0)[a]	13.2(0.9)[a]	6.3(0.2)[ab]

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(biomass) = a × Ln(D²H) + 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^{-2} yr^{-1})			
		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 (P-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	N input treatment		Sampling year			
(years)	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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