1 **Nitrogen input ^{15}N-signatures are reflected in plant ^{15}N natural abundances of sub-tropical**

2 **forests in China**

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22 **Abstract**

23 Natural abundance of ^{15}N ($\delta^{15}N$) in plants and soils can provide integrated information on

24 ecosystem nitrogen (N) cycling, but it has not been well tested in warm and humid sub-tropical

25 forests. In this study, we examined the measurement of $\delta^{15}N$ for its ability to assess changes in N

26 cycling due to increased N deposition in an old-growth broadleaved forest and a secondary pine

27 forest in a high N deposition area in southern China. We measured $\delta^{15}N$ of inorganic N in input and

28 output fluxes under ambient N deposition, and N concentration (N%) and $\delta^{15}N$ of major ecosystem

29 compartments under ambient and after decadal N addition at 50 kg N ha^{-1}yr^{-1}. Our results showed

30 that the N deposition was $\delta^{15}N$-depleted (-12 ‰) mainly due to high input of depleted NH_4^+-N.

31 Plant leafs in both forest were also $\delta^{15}N$-depleted (-4 to -6‰). The old-growth forest had higher

32 plant and soil N%, and was more ^{15}N-enriched in most ecosystem compartments relative to the pine

33 forest. Nitrogen addition did not significantly affect N% in both forests, indicating that the

34 ecosystem pools are already N-rich. Soil $\delta^{15}N$ was not changed significantly by the N addition in

35 both forests. However, the N addition significantly increased the $\delta^{15}N$ of plants toward the ^{15}N

36 signature of the added N (~0 ‰), indicating incorporation of added N into plants. Thus, plant $\delta^{15}N$

37 was sensitive to ecosystem N input manipulation although N% was unchanged in these N-rich sub-

38 tropical forests. We interpret the depleted $\delta^{15}N$ values of plants as an imprint from the high and

39 $\delta^{15}N$-depleted N deposition. The signal from the input (deposition or N addition) may override the

40 enrichment effects of fractionation during the steps of N cycling that are observed in most warm

41 and humid forests. Thus, interpretation of ecosystem $\delta^{15}N$ values from high N deposition regions

42 need to include data on the deposition $\delta^{15}N$ signal.

43

44 Key words: Natural ^{15}N abundance, N addition, N deposition, sub-tropical, China

45

1. Introduction

Nitrogen (N) deposition onto terrestrial ecosystems has dramatically increased due to anthropogenic activities (Galloway, 2005), and recently, the increase has been strong in the warm and humid parts of south Eastern Asia, particularly in China (Fang et al., 2011a). Nitrogen deposition onto forests that exceeds plant and microbial demand may increase nutrient leaching and soil acidification (Lu et al., 2014), and potentially causes nutritional imbalances in the forests (Schulze, 1989). Studies of fates and process responses to increased N deposition using coordinated N addition experiments in temperate and boreal forests show that the effects of increased N deposition largely depend on the initial N status of the forests (Gundersen et al., 1998; Hyvönen et al., 2008). Accordingly, N limited forests that often show a growth response to added N, largely retain the deposited N, whereas N saturated forests, with N input in excess of plant and microbial demand, lose N through leaching and denitrification. Although some studies from (sub) tropical regions also suggest that N leaching from tropical forests is related to the initial N status of the forests (Chen and Mulder, 2007; Fang et al., 2009), the observations are far from being conclusive, especially in regions that are subjected to increased anthropogenic N deposition (Townsend et al., 2011).

Natural abundance of ^{15}N (the ratio of $^{15}N:^{14}N$, and given as per mil; $\delta^{15}N$) of ecosystem has been suggested to provide integrated information about effects of both current and past N cycling in ecosystems (Handley and Raven 1992; Robinson, 2001). Using the $\delta^{15}N$ data to interpret N status and changes in N cycling of an ecosystem rely on the fractionation that occurs during N transformation processes and creates differences in $\delta^{15}N$ between substrate and product pools (Högberg, 1997). Accordingly, it was suggested that N-saturated ecosystems are characterized by elevated $\delta^{15}N$ above the atmospheric standard (i.e., 0 ‰) due to increased rate of N cycling (e.g., mineralization and nitrification), and losses of ^{15}N-depleted N-forms by leaching and denitrification. Global data indeed show elevated leaf $\delta^{15}N$ at N rich conditions, i.e. increasing leaf

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70 $\delta^{15}N$ with increasing leaf N concentration and higher leaf $\delta^{15}N$ in warmer climates (Craine et al.

71 2009). Hence, Martinelli et al. (1999) also found tropical forests to have higher leaf $\delta^{15}N$ than

72 temperate forests. However, the influence of increased N deposition on the $\delta^{15}N$ levels is not well

73 known. For example, even though plant $\delta^{15}N$ could increase with N deposition (Emmett et al.,

74 1998), it may not be the case across all regions where not only ecosystem N status but also a region-

75 specific ^{15}N signature of deposited N may influence ecosystem $\delta^{15}N$ (Fang et al., 2011b; Pardo et

76 al., 2006). Moreover, interpretation of ecosystem $\delta^{15}N$ is hampered by the uncertainties in $\delta^{15}N$ of

77 plant N sources, the magnitude of isotopic fractionations during N transformation processes, and the

78 complex behavior of ^{15}N in soils and plants (Robinson 2001). On the other hand, our understanding

79 of ecosystem $\delta^{15}N$ is primarily based on studies from North America, Europe, South America, and

80 Australia (Amundson et al., 2003; Craine et al., 2009; Martinelli et al., 1999). Apparently, there is a

81 data gap over large areas in eastern Asia, particularly sub-tropical China, which is among the hot-

82 spots for N deposition.

83   Plant leaf and soil $\delta^{15}N$ values are most commonly used to assess N status and changes in N

84 cycling rates, and other ecosystem pools are neglected or rarely measured. The turn-over times of N

85 pools vary among different ecosystem compartments, and thus their $\delta^{15}N$ may respond differently to

86 specific disturbances. For example, within plant compartments, small active N pools such as leaves

87 reflect recent N cycling whereas the larger N pools such as wood might reflect long term-changes in

88 N cycling. Nevertheless, studies that measured $\delta^{15}N$ values of all major ecosystem pools are rare

89 (e.g. Liu, 1995), emphasizing the need for more rigorous studies to provide complete patterns of

90 $\delta^{15}N$ in the leaf-to-soil continuum, and their response to N input manipulation, especially in the

91 tropical forests.

92   We evaluated $\delta^{15}N$ of tropical forests, and its response to increased N deposition using long-

93 term N addition experimental plots established in 2003 in an old-growth broadleaf forest and a pine

94   plantation forest in the Dinghushan Biosphere Reserve (DHSBR) in southern China (Mo et al.,

95   2006). The old-growth forest is more N-rich, and has less N retention capacity than the pine forest

96   (Fang et al., 2006). Nitrogen addition studies in these forests documented that increased N input

97   causes increased N leaching (Fang et al., 2008, 2009), N_2O emission (Zhang et al., 2008) and soil

98   acidification (Lu et al., 2014). Here, our objectives are (1) to determine $\delta^{15}N$ of ecosystem

99   compartments across the leaf-to-soil continuum in the two forest types, (2) to assess response of

100   $\delta^{15}N$ in major ecosystem pools to decadal N addition in the two forests. We hypothesized that i)

101   $\delta^{15}N$ of plants and soil in these forests will be higher (more ^{15}N-enriched) than those reported in

102   temperate forests due to high N status at DHSBR, and subsequent fractionation during leaching and

103   denitrification, ii) decadal N addition would change plant and soil $\delta^{15}N$ towards the ^{15}N signature of

104   the added N due to its incorporation into ecosystem pools, and the changes would differ between

105   the two forests due to their difference N status and N cycling rates.

106

107   **2. Methods**

108   **2.1. Study site**

109   The study was conducted in the Dinghushan Biosphere Reserve, southern China (112°33' E and

110   23°10' N) with typical sub-tropical monsoon climate. Mean annual temperature and mean annual

111   precipitation were 22.2 C^o and 1927 mm, respectively. The reserve DHSBR has been experiencing

112   high rates of atmospheric N deposition (21-38 kg N ha^{-1} yr^{-1} as inorganic N in bulk precipitation)

113   since 1990's (Fang et al., 2008). In 2009 to 2010, total wet N deposition was 34.4 kg N ha^{-1} yr^{-1} (Lu

114   et al., 2013). We used two common forest types that grow on the relatively steep slopes in the

115   reserve; an old-growth broad-leaved forest (hereafter named as BF) and a pine plantation forest

116   (hereafter named as PF) (Mo et al., 2006). The BF is a regional climax mixed broad-leaved forest,

117   which has been protected for at least the last 400 years with minimum human disturbances (Shen et

118   al., 1999). The PF was planted after clear-cut of the original climax forest in the 1930s and has been

119   subjected to human disturbances such as litter and shrub harvesting until the recent past (Mo et al.,

120   2005).

121

122   Table 1. Selected characteristics of the mineral soil (0-10 cm) in the two forest types. Data on soil bulk

123   density, total P and extractable NH_4^+-N are obtained from Fang et al., (2006). Values given in parenthesis

124   indicate SE ($n = 3$).

Parameters	Broad-leaved forest (BF)	Pine forest (PF)
Bulk density (g cm^{-3})	0.9 (0.03)	1.3 (0.03)
pH (H_2O)	3.8 (0.02)	4.0 (0.04)
C concentration (%)	3.8 (0.80)	1.8 (0.03)
N concentration (%)	0.3 (0.04)	0.1 (0.01)
C/N ratio	13.6 (0.9)	13.9 (0.7)
Total P (mg kg^{-1})	59 (3)	43 (3)
Extractable NH_4^+-N (mg kg^{-1})	2.1	3.3
Extractable NO_3^--N (mg kg^{-1})	12.7	2.6

125

126   The major canopy species in the BF were *Castanopsis chinensis*, *Machilus chinensis,*

127   *Schima superba*, *Cryptocarya chinensis*, and *Syzygium rehderianum* and the most common

128   understory species is *Hemigramma decurrins. Pinus massoniana* and *Dicranopteris dichotoma* are

129   the dominant tree and understory species in the PF, respectively. The soil in the reserve is classified

130   as Lateritic Red Earth (Oxisol) formed from Devonian sandstone and shale with a thin layer of

131   forest floor litter (0.5-3.0 cm), but the soil depth is variable ranging from 30 cm in the PF to more

132   than 60 cm in the BF. Probably due to erosion after the clear-cut and the continued human

133   disturbance the PF had lower total soil carbon, N and phosphorus (P) content than the BF (Table 1).

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135

136

2.2. Experimental design

We used an ongoing N addition experiment established in both forests in July 2003 (Mo et al., 2006). The experimental plots consist of control plots and N addition treatments at 50 kg N ha^{-1} yr^{-1} and 100 kg N ha^{-1} yr^{-1} each with three replicates in both forests, and an additional 150 kg N ha^{-1} yr^{-1} in the BF. However, in this study we used only the control plots and the low-N treatment plots at 50 kg N ha^{-1} yr^{-1} (hereafter named as N-plots). Each plot is 10 m x 20 m with at least a 10-m wide buffer strip to the next plot. In the N-plots, NH_4NO_3 is mixed with 20 L of water, and is added monthly below the canopy using a backpack sprayer, whereas the control plots received equivalent 20 L water with no fertilizer. The added N has $\delta^{15}N$ of about -3 ‰ on NH_4^+-N and about 1.8 ‰ on NO_3^--N, with $\delta^{15}N$ of NH_4NO_3 being -0.7 ‰.

2.3. Sampling and analysis of plant and soil pools

Major ecosystem compartments, including leaves, twigs, branches, bark and wood of canopy trees, leaves of understory vegetation, fine roots, and 0-30 cm mineral soil were sampled in January 2013 to determine N concentration (%) and the $\delta^{15}N$ (‰) of the forests. A branch per dominant tree species per plot was cut from the height reached using a pole pruner (c. 7-8 m) taking advantage of the steep slope, and was separated into leaves, twigs and small branches. Bark samples were cutoff the dominate trees at breast height using a knife. After removing the bark, wood cores were sampled using an increment borer and separated visually into sapwood (usually the outer 2-3 cm recent wood) and older wood (heartwood). Dominant understory plant species were cut with a knife and kept separate for each species. A total of seven tree species in the canopy/sub-canopy layer and five plant species in the understory layer (young trees, shrubs, herbs and liana) of the BF were sampled, whereas in the PF the dominant pine tree and five species in the understory layer were sampled. Mineral soil samples were taken using an auger (5.1 cm in diameter) and were divided into three

161　　layers (0-10, 10-20, 20-30 cm). Two soil cores were sampled and pooled together to form one

162　　composite sample for each depth per plot. Living fine roots were hand-sorted from the soil samples

163　　for each depth, but combined to one composite sample for the whole profile (0-30 cm) because the

164　　amount of fine roots in each depth was too small to grind and analyze separately. Litterfall was

165　　collected monthly during July-September 2012 and pooled together to make one composite sample

166　　per plot. The litter was sorted in the laboratory into leaf and others (branches, fruits, flowers, barks),

167　　but only leaf values are reported.

168　　　　　All plant and soil samples were oven-dried at 70°C, and ground to a fine and homogeneous

169　　powder. Mineral soils were sieved (2 mm mesh) to remove non-soil materials, air-dried at room

170　　temperatures and milled to fine powder. Subsamples were dried at 105°C, and all results are

171　　reported on 105°C basis. Based on their approximate N%, about 4-5 mg of the samples were

172　　weighed into tin capsules, and δ^{15}N and N concentration of the samples were determined

173　　simultaneously on an isotope ratio mass spectrometer (Isoprime 100, Isoprime Ltd.) coupled to an

174　　automatic, online elemental analyzer (vario ISOTOPE cube). An internal standard needle sample

175　　from temperate forests, which has been analysed in multiple runs at several laboratories, was used

176　　to check reproducibility of the δ^{15}N determination. We analyzed N % and δ^{15}N separately for each

177　　dominant tree species per plot, but compartment mean values are reported. Natural abundance δ^{15}N

178　　in samples was reported in per mil (‰) relative to atmospheric N_2.

179

180　　**2.4.Sampling and analysis of water samples**

181　　Precipitation, throughfall, surface runoff and soil solution (seepage) from 0-20 cm were sampled

182　　monthly from September 2012 to February 2013 (including a dry period in December and January)

183　　in the control plots to assess the δ^{15}N of N input and output in the two forests under ambient N

184　　deposition. Bulk precipitation was collected at an open area close to the experimental site using an

185   open glass funnel (12 cm in diameter), connected to a 5 L sampling bottle with polypropylene tubes.

186   Throughfall was collected by PVC pipes at five random points within each plot (intercept area 0.8

187   m^2 for each collector) at about 1.3 m above the ground in each forest. Each collector was connected

188   to two 50 L buckets with polypropylene tubes. Soil solution from 20 cm depth (seepage water) was

189   obtained using two zero tension tray lysimeters (755 cm^2 per tray) installed in each plot. Each

190   lysimeter was connected to a 20 L bottle using the steep slope of the sites to facilitate sampling. In

191   addition, since the plots are situated on steep slopes, one plot in both the pine and broad-leaved

192   forests was delimited hydrologically by plastic and concrete barriers to sample and quantify surface

193   runoff.

194   Natural ^{15}N abundance of both NH_4^+-N and NO_3^--N in water samples were analyzed after

195   chemical conversion to nitrous oxide (N_2O). The NH_4^+-N was initially oxidized to nitrite (NO_2^-) by

196   hypobromite (BrO^-) and the NO_2^- is then quantitatively converted into N_2O by hydroxylamine

197   (NH_2OH) under strongly acidic conditions (Liu et al., 2014). Similarly, a series of chemical

198   reactions of vanadium (III) chloride (VCl_3) and sodium azide under acidic conditions was used to

199   convert NO_3^--N into N_2O (Lachouani et al., 2010). The produced N_2O was subsequently analysed

200   for ^{15}N abundance by a purge-and-trap coupled with an isotope ratio mass spectrometer (PT-IRMS)

201   (Liu et al., 2014).

202

203   **2.5. Calculations and Statistics**

204   To evaluate effects of decadal N addition on the whole ecosystem (plant and soil) N% and $\delta^{15}N$, we

205   determined N pool weighted plot means of N% and $\delta^{15}N$ using N pools for each compartment

206   quantified in Gurmesa et al. (2016). We excluded the heartwood and sapwood pools in the plant

207   pool calculations for two reasons; first the low N content in wood samples caused larger

208   uncertainties on the $\delta^{15}N$ determinations, and secondly heartwood and a major part of the sapwood

209   was formed prior to the initiation of the N addition treatment. We expect the later to be the

210   explanation that particular heartwoods showed opposite effects of N addition compared to all other

211   compartments.

212   Differences between the two forests in plot mean N% and $\delta^{15}N$ of the different ecosystem

213   compartments in control plots were analysed using t-test. The effect of treatment on N% and $\delta^{15}N$

214   of each tree compartments and understory leaf in each forest was analyzed using mixed model

215   ANOVA with treatment as explanatory factor and plant species as a random factor, since plant

216   species differed significantly in both parameters (Gurmesa, 2016). Treatment effect on N % and

217    $\delta^{15}N$ of soil in each layer, litter and fine roots in each forest was analyzed using t-test. Effects of

218   treatment on N pool weighted plot means in each forest, and their differences between the control

219   plots of the two forests were also analyzed using t-test.

220

221   **3. Results**

222   **3.1.Concentration and $\delta^{15}N$ of NH_4^+-N and NO_3^--N**

223   Dissolved NH_4^+-N in waters samples, including both inputs through precipitation and throughfall

224   and outputs fluxes by surface runoff and seepage at 20 cm depth were ^{15}N-depleted (negative $\delta^{15}N$

225   values) in both forest (Table 2). Nitrate-N was ^{15}N-enriched and had positive $\delta^{15}N$ in the input

226   fluxes, but was slightly ^{15}N-depleted in output fluxes in both forests. Ammonium-N was the

227   dominant N form in precipitation and throughfall, but NO_3^--N appeared to be the dominant N form

228   in the output fluxes through surface runoff and leaching below 20 cm soil (**Fig**. S1). Mean $\delta^{15}N$ of

229   both NH_4^+-N and NO_3^--N in input and output fluxes did not significantly differ between the two

230   forests. The $\delta^{15}N$ of NH_4^+-N in surface runoff and seepage were significantly and positively related

231   to $\delta^{15}N$ of NH_4^+-N in throughfall for combined data in the two forests (Fig 1a, b), but the correlation

232   was not significant for NO_3^--N (Fig 1c, d).

233 Table 2. Mean $\delta^{15}N$ (‰) of NH_4^+-N and NO_3^--N in bulk precipitation, throughfall, surface runoff and seepage

234 at 20 cm depth in control plots from September 2012 to February 2013. Numbers in parenthesis indicate

235 standard error of the mean (SE) ($n = 3$).

Fluxes	Broad-leaved forest (BF)		Pine forest (PF)	
	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N
Precipitation*	-16.6	4.1	-16.6	4.1
Throughfall	-15.2 (2.3)	3.6 (0.2)	-15.5 (1.8)	2.8 (0.3)
Surface runoff	-13.1(1.7)	-1.9 (0.6)	-9.7 (1.0)	-1.5 (0.6)
Seepage	-22.6 (0.9)	-0.9 (1.3)	-21.3 (2.3)	-0.9 (0.2)

236 *Precipitation was collected at open area within the reserve, and was assumed to be the same for both forests

237

238 **3.2.Ecosystem compartment N% and $\delta^{15}N$**

239 In agreement with our expectation based on results from earlier studies in the DHSBR, the BF is

240 more N-rich than PF. Nitrogen concentrations of plant compartments were significantly higher in

241 the BF than in the PF, except in leaves of canopy trees (Table 3). Nitrogen concentration in litter-

242 fall and fine roots in the BF only marginally differed from that of PF. Soil N% was significantly

243 higher in the BF at all depths (Table 3).

244 Natural $\delta^{15}N$ of all plant compartments differ significantly between the two forests with the

245 PF being more ^{15}N-depleted than the BF (Table 4). Soil $\delta^{15}N$ did not show significant difference

246 between the two forests at any depth (test data not shown), though soil still tended to be more ^{15}N-

247 enriched in the BF (Fig. 2).

248

249

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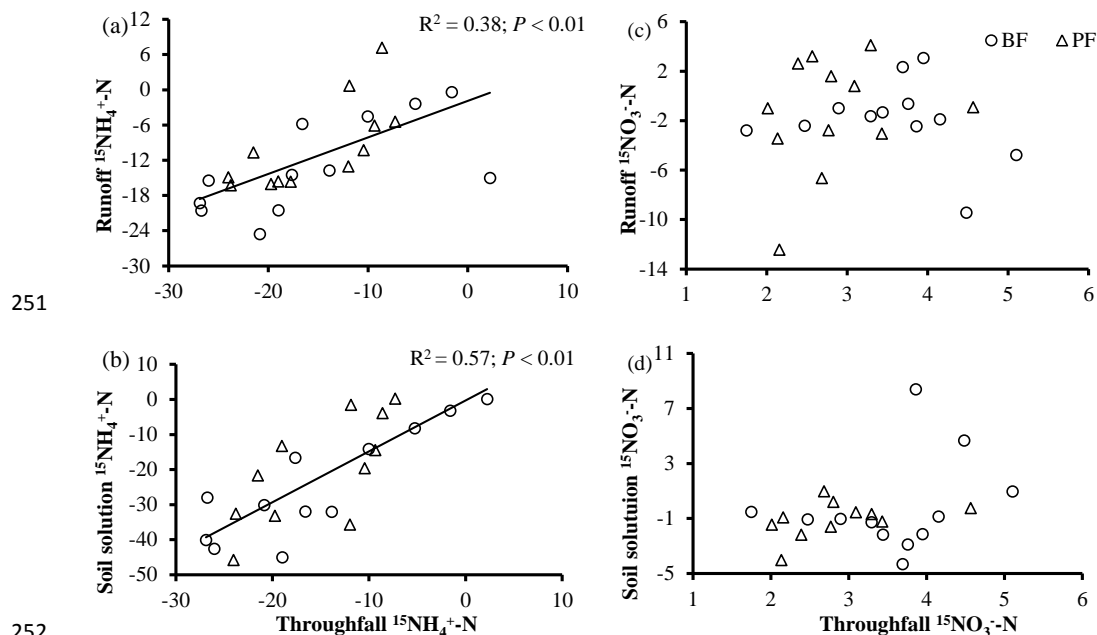
Fig. 1 Correlation between $\delta^{15}N$ (‰) of NH_4^+-N in throughfall and that of NH_4^+-N in surface runoff (a), and soil solution (b), and correlation between $\delta^{15}N$ of NO_3^--N in throughfall and that of NO_3^--N in surface runoff (c), and soil solution (d). No significant effect of forest type was detected; thus the regression line shown was based on data from both forests.

3.3. Effects of N addition on compartment N% and $\delta^{15}N$

Nitrogen concentrations in all measured plant and soil compartments were not significantly affected by N addition in the BF, except in the sapwood (Table 3). In the PF, N% increased in all ecosystem pools, but the change was significant only in leaf of understory plants (Table 3). Nitrogen concentration of N pool weighted average plant pools calculated per plot did not significantly increased by N addition in both forests, but it slightly increased in the PF (Table 3).

Plant $\delta^{15}N$ was negative in both control and N-plots in both forests, but N addition significantly increased the $\delta^{15}N$ of most plant compartments (Table 4). The changes were more pronounced in the small active plant pools such as leaves of trees and understory plants.

267 Table 3. Mean N% of different ecosystem pools in the broad-leaved (BF) and pine forests (PF). Values in
268 parenthesis indicate SE of plot means ($n = 3$). Within each forest type the effect of N addition is shown by p-
269 values from a mixed model with species as random factor. The last column shows p-values for a difference
270 between the ambient plots of the two forests using t-test. Bolded p-values indicate significant difference.

Compartment	Broad-leaved forest (BF)			Pine forest (PF)			Forest type effect
	Control	N addition	p-values	Control	N addition	p-values	p-values
Plants							
Tree leaf	1.71 (0.19)	1.69 (0.19)	0.48	1.44 (0.11)	1.68 (0.02)	0.10	0.08
Twig	1.28 (0.19)	1.17 (0.05)	0.59	0.99 (0.77)	0.97 (0.08)	0.79	**<0.01**
Branch	0.86 (0.15)	0.81 (0.16)	0.13	0.58 (0.05)	0.60 (0.06)	0.87	**<0.01**
Bark	0.71 (0.16)	0.7 (0.16)	0.55	0.57 (0.02)	0.61 (0.05)	0.52	**<0.01**
Sapwood	0.27 (0.07)	0.3 (0.07)	**<0.01**	0.18 (0.02)	0.11 (0.02)	0.07	**0.02**
Heartwood	0.16 (0.04)	0.16 (0.03)	0.28	0.06 (0.0)	0.09 (0.03)	0.30	**<0.01**
Understory[a]	2.04 (0.02)	1.98 (0.17)	0.09	1.61 (0.41)	1.77 (0.40)	**<0.01**	**<0.01**
Fine root	1.4 (0.16)	1.81 (0.17)	0.14	0.87 (0.13)	0.96 (0.04)	0.43	0.06
Litter-fall	1.56 (0.06)	1.48 (0.08)	0.43	1. 39 (0.03)	1.72 (0.11)	0.56	0.09
Soil							
0-10 cm	0.27 (0.04)	0.28 (0.01)	0.83	0.13 (0.01)	0.12 (0.01)	0.37	**0.03**
10-20 cm	0.18 (0.01)	0.19 (0.01)	0.59	0.07 (0.00)	0.06 (0.00)	0.37	**<0.01**
20-30 cm	0.12 (00)	0.14 (00)	0.10	0.06 (0.00)	0.05 (0.00)	0.12	**<0.01**

271 [a] the values are only for leaf of understory plants

272

273 However, inconsistent effect of N addition on $\delta^{15}N$ was observed in the wood parts (Table

274 4). For heartwoods, the effects were significant, but in different directions than other plant pools for

275 both forests. Due to low N% and challenges in grinding of wood samples it was difficult to get

276 reliable the $\delta^{15}N$. Also much of the sampled wood was formed prior to the treatment and thus, no

277   further evaluation was done for the wood samples. Nitrogen addition did not cause significant effect

278   on δ^{15}N of litter-fall and fine roots. In the BF, there was no correlation between leaf N% and δ^{15}N,

279   but a positive correlation was found for the PF as both N% and δ^{15}N tended to increase in parallel

280   due to N addition (data not shown).

281

282   Table 4. Mean δ^{15}N (‰) of plant pools in the broad-leaved (BF) and pine forests (PF). Values in parenthesis

283   indicate SE of plot means ($n = 3$). Within each forest type the effect of N addition is shown by p-values from

284   a mixed model with species as random factor. The last column shows p-values for a difference between the

285   ambient plots of the two forests using t-test. Bolded p-values indicate significant difference.

Sample type	Broadleaved forest (BF)			Pine forest (PF)			Forest type effect
	Control	N addition	p-values	Control	N addition	p-values	p-values
Tree leaf	-4.0 (0.5)	-3.4 (0.6)	**<0.01**	-5.4 (0.1)	-3.5 (0.3)	**<0.01**	**<0.01**
Twigs	-4.3 (0.8)	-3.8 (0.9)	**0.04**	-5.7 (0.1)	-4.0 (0.3)	**<0.01**	**<0.01**
Branches	-4.6 (0.4)	-4.1 (0.3)	**<0.01**	-5.7 (0.2)	-4.1(0.6)	0.08	**0.03**
Barks	-2.8 (0.8)	-2.4 (0.6)	0.16	-4.0 (0.4)	-2.6 (0.2)	**0.03**	0.03
Sapwood	-1.9 (0.5)	-1.8 (0.3)	0.51	-0.9 (0.4)	1.8 (1.6)	0.18	0.06
Heartwood	**-1.6 (0.9)**	**-2.3 (0.9)**	**<0.01**	**3.2 (0.8)**	**-0.71 (1)**	**0.04**	**< 0.01**
Understory[a]	-3.6 (0.9)	-2.2 (1.1)	**<0.01**	-5.6 (0.5)	-3.54 (0)	**<0.01**	**<0.01**
Fine root	-2.8 (0.6)	-1.7 (0.8)	0.33	-5.1 (0.5)	-3.6 (0.3)	0.06	**0.04**
Litter-fall	-3.9 (0.1)	-3.9 (0.1)	0.98	-4.8 (0.2)	-4.0 (0.3)	0.10	**0.04**

286   [a] the values are only for leaf of understory plants.

287

288   Nitrogen addition tended to decrease soil δ^{15}N values in the BF at all depths, but with no

289   significant change in each layer as well as across the total soil profile ($p = 0.27$, Fig 2a). In the PF,

290   N addition tended to decrease soil δ^{15}N in top 0-10 cm, but the changes were not significant for any

291   layer or in total soil profile ($p = 0.88$, Fig 2b). When compared based on N pool weighted plot

292   mean, the two forests did differ significantly in plant N% and δ^{15}N (Fig. 3a). For the soil, the two

293   forests significantly differ in N pool weighted plot mean N%, with the BF having the higher value,

294   but not in N pool weighted plot mean δ^{15}N (Fig. 3b).

295

296
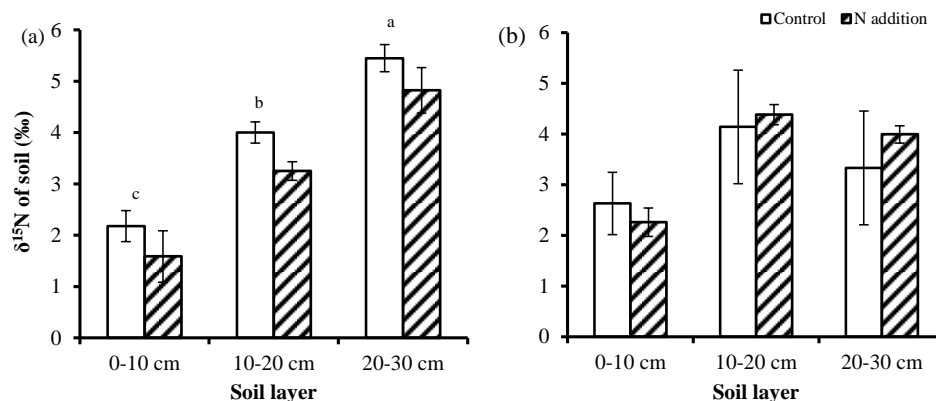
Fig. 2 Mean δ^{15}N (‰) in the soil profiles of broad-leaved forest (a), and pine forest (b). Error bars indicate
SE of plot means ($n = 3$). Different letters indicate significant difference between soil depths. No significant
difference was found between the two forest types at any depth nor was there any effect of N addition

300

301   In summary, the effect of added N on pool weighted plot mean plant N% was neither significant in

302   BF ($p = 0.86$) nor in PF ($p = 0.2$), but the change was more pronounced in the PF (Fig. 3a).

303   However, weighted plot mean plant δ^{15}N values were significantly increased in both forests ($p =$

304   0.03 for BF and 0.01 for PF) from the ^{15}N-enriched (compared to plant tissues) N addition. In the

305   soil, where the N pool is obviously larger than in the plants, the effect of the N addition on weighted

306   average N% was not significant in both forests (Fig. 3b). The direction of change in soil δ^{15}N was a

307   decrease as expected with incorporation of the added N with a δ^{15}N value of -0.7‰, but the change

308   was again not significant (Fig. 3b).

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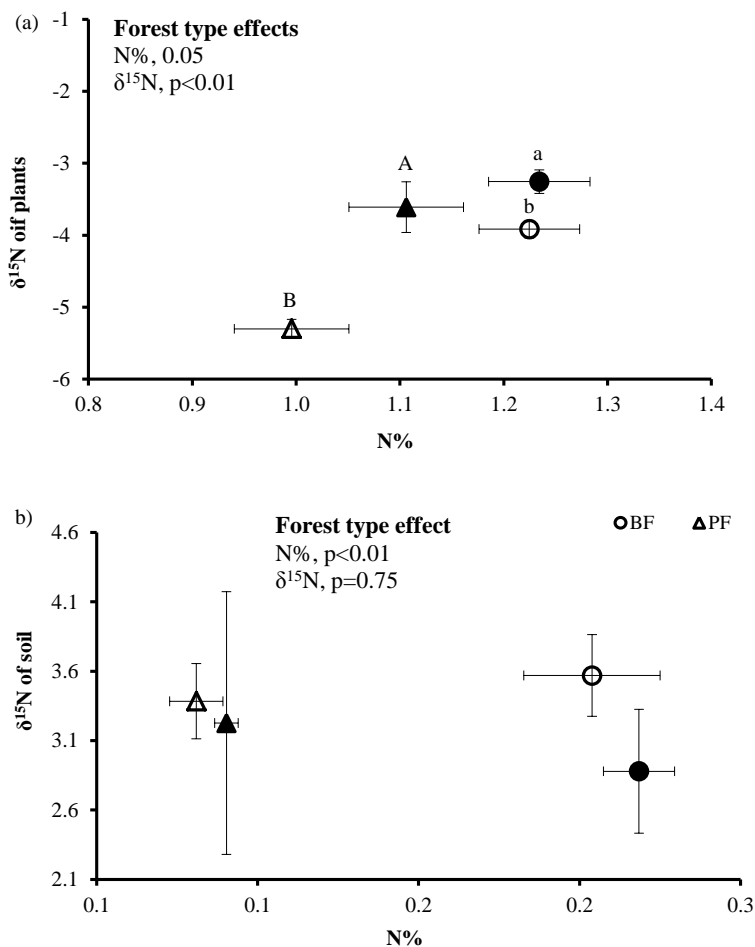
Fig. 3 Overall effect of N addition on plot average weighted N% and δ^{15}N of plants (a), and soil (b) for broad-leaved forest (○) and pine plantation (Δ). Error bars indicate SE of plot means (*n* = 3). Open symbols indicate control plots and closed symbols indicate N-plots. In (a), different lowercase letter for BF and uppercase letter for PF in (a) indicate significant difference. The shown *p*-values are tests for differences in N% and δ^{15}N between the two forests (control plots).

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4. Discussions

4.1. $\delta^{15}N$ of deposition N, plants and soil

The $\delta^{15}N$-depleted deposition N, especially in the NH_4^+-N form both in bulk precipitation and throughfall (Table 2) confirms the widely observed results for forests in southern China (Koba et al., 2012; Zhang et al., 2008) and other regions (Freyer 1978; Russel et al., 1998). The $\delta^{15}N$-depleted NH_4^+-N could indicate effects of NH_3 emissions from agricultural activities, which is usually ^{15}N-depleted (Bauer et al., 2000). Contrary to NH_4^+-N, input NO_3^+-N has positive $\delta^{15}N$ again confirming previous reports (Fang et al., 2011a; Koba et al., 2012), and it has been explained as the contribution of NO_x produced by coal combustion. The very low $\delta^{15}N$ of NH_4^+-N in the soil solution resemble that in precipitation and throughfall (Table 2), and it is likely due to transport of ^{15}N-depleted N throughfall through macrospores as supported by the positive relationship between $\delta^{15}N$ of NH_4^+-N in soil solution and that in throughfall (Fig. 1b).

The observed leaf $\delta^{15}N$ values ranging from -4 ‰ in BF to -6 ‰ in the PF in DHSBR (Table 4) are within the range (-2 ‰ to -5 ‰) found in eastern Asia (Fang et al., 2011a; Wang et al., 2014; Kitayama and Iwamoto, 2001). However, they are more depleted than the average (3.7 ‰) given for tropical forests in a global synthesis (Martinelli et al., 1999), and those reported from a major survey across Amazonas (3.1 ± 2.3 ‰) by Nardoto et al. (2014). Surprisingly, the average leaf $\delta^{15}N$ are closer to, but even more ^{15}N-depleted than that of temperate forests (-2.8 ‰) (Martinelli et al., 1999). Several studies (Martinelli et al., 1999; Craine et al., 2009; Craine at al., 2015) suggested that leaf $\delta^{15}N$ increases with N availability. The suggestion was based on the hypothesis that N losses by fractionation pathways at sites with high N availability cause plant and soil to become relatively more ^{15}N-enriched. Supporting the hypothesis, Craine et al (2009) observed positively correlation between foliar $\delta^{15}N$ and the enrichment factor (difference in $\delta^{15}N$ between leaf and soil), and suggested that it reinforce the idea that the general patterns of N availability can be assessed

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345 with foliar δ^{15}N alone. Our result rejects this hypothesis for the high N deposition systems that we

346 study because the expected ecosystem enrichment from fractionation occurring during N

347 transformations under the high N availability at DHSBR is overridden by other depleting factors.

348 We believe that the main depleting factor at DHSBR (and in other Chinese forests with high N

349 deposition) is a direct uptake of ^{15}N-depleted deposition N, particularly NH_4^+-N (Table 2) by leaf

350 from throughfall or by roots from the soil solution (Wang et al., 2014). Sun et al. (2010) found that

351 tree ring δ^{15}N of *Pinus massoniana* at DHSBR (PF) decreased from 2 ‰ in the 1960s to -1 ‰ in the

352 late 1990s, and that the decrease was found to coincide with the increasing deposition of ^{15}N-

353 depleted N over the last 50 years. This finding also supports our conclusion that the δ^{15}N of the

354 deposition N sources is more important in explaining patterns of ecosystem δ^{15}N data in highly

355 polluted regions. Thus, we highlight the importance of region-specific interpretations for patterns of

356 observed foliar signatures that may not be explained by increased N availability.

357 Soil δ^{15}N at DHSBR are also below the average for tropical forests, but are closer to that

358 observed for temperate forests (Martinelli et al., 1999). In agreement with the global trends, plants

359 at DHSBR are more δ^{15}N-depleted than the soils (Table 4, Fig 2). This trend has been explained by

360 isotopic discrimination against ^{15}N during mineralization and nitrification that makes the N

361 available for plants more ^{15}N-depleted than the source soil organic matter pools (Amundson et al.,

362 2003). The increase of soil δ^{15}N with soil depth in the BF is also a well-documented phenomenon in

363 undisturbed soils (Boeckx et al., 2005; Emmet et al., 1998; Koba et al., 2010). It is caused by

364 continuous input of ^{15}N-depleted litter that keeps δ^{15}N of the top soil low, whereas ^{15}N-enriched soil

365 fractions are transported and accumulated at deeper soil profile (Nadelhoffer et al., 1988) when the

366 lighter ^{14}N is removed by plants, microbes, or through leaching following decomposition (Boeckx

367 et al., 2005). Enrichment of deeper soil layers could also occur due to fractionation when

368 mycorrhiza fungi transfer ^{15}N-depleted N to their host plants ^{15}N (Högberg et al., 2011; Hobbie and

369   Högberg, 2012) leading to accumulation of ^{15}N-enriched N of fungal origin in deeper soil layers.

370   However, the role of mycorrhiza in N uptake is less in N saturated ecosystems and hence the

371   increase in δ^{15}N with soil depth may be less steep (Högberg et al., 1996, 2011; Emmett et al., 1998;

372   Hobbie and Ouimette, 2009). This might explain the minor observed increase in soil δ^{15}N (~3 ‰)

373   with depth at DHBSR in BF compared to that found for several temperate sites (Vervaet et al.,

374   2002; Emmet et al., 1998; Bauer et al., 2000), and the 3 to 9 ‰ increase with soil depth reported in

375   global data from temperate forests (Martinelli et al., 1999).

376

377   **4.2. Effects of N addition on N% and δ^{15}N**

378   The increase in plant δ^{15}N caused by N addition (Table 4, Fig 3a) is consistent with previous

379   measurements on leaves of the two forests at DHSBR (Fang et al., 2011a) and in several temperate

380   forests (Högberg et al., 2011; Högberg et al., 2014; Korontzi et al., 2000; McNulty et al., 2005;

381   Näsholm et al., 1997). It was interpreted mainly as effects of fractionation during N uptake and

382   cycling as discussed above. For our experiment, we found another explanation more likely: inspite

383   of the higher N deposition with more negative δ^{15}N than that of plants, plant δ^{15}N moved toward the

384   more positive ^{15}N signature of the added N fertilizer (-0.7 ‰), indicating acquisition of the added N

385   by the plants (Table 4; Fig. 3). However, the changes in δ^{15}N slightly varied among compartments

386   because they differ in terms of N turnover time. Thus, the most pronounced changes in δ^{15}N

387   occurred in small but more active pools (e.g. leaves, roots) compared to the large and relatively

388   inactive/stable pools (e.g. woods and soil pools). Added N is more likely incorporated into the

389   active pools that are responsive to contemporary N input manipulation (Fang et al., 2006;

390   Johannisson and Hogberg, 1994; Pardo et al., 2002). Response of weighted plot mean δ^{15}N of the

391   plants also showed that δ^{15}N of the whole plant pools were significantly increased in both forests

392   (Fig 3a). The increase in plant δ^{15}N after decadal N addition (Fig 3a), i.e., toward the ^{15}N signature

393   of the added N is in line with our second hypothesis, and provides evidence for the incorporation of

394   the decadal N addition even though the forest is considered as N-rich. A substantial incorporation of

395   newly added N into the ecosystem N pools was confirmed by a recent tracer study in the BF

396   (Gurmesa et al., 2016). More importantly, it again showed the importance of ^{15}N signature of input

397   N in controlling ecosystem δ^{15}N.

398   Our result showed an insignificant decrease of soil δ^{15}N by N addition moving the ^{15}N

399   signature towards that of the added N (Fig. 3b), again pointing to an imprint of the added N, as also

400   discussed by (Högberg et al., 2014). This contradicts results in similar long-term experimental N

401   addition (Högberg, 1991; Högberg et al., 1996, 2011), where an increase in δ^{15}N in N-fertilized

402   plots also after addition of ^{15}N-depleted N was observed, and interpreted as fertilizer-induced

403   fractionation due to increased N transformation rates.

404

405   **4.3. Difference between BF and PF**

406   We observed differences in N% and δ^{15}N that can be interpreted as a difference in N status and N

407   cycling difference between the two forests. As expected from previous studies, the BF is more N-

408   rich than the PF as indicated by higher N% in major ecosystem pools in BF (Table 3). Trees leaves

409   in BF are more ^{15}N-enriched than the PF (Table 4) as also previously observed by Fang et al.,

410   (2011c) for a few tree species in the same forests. The two forests also differ in plant species and

411   successional age, and (Wang et al., 2014) reported that these are important factors that affect δ^{15}N

412   in different tropical forests in southern China. The difference could be partly related to higher N

413   cycling rates and subsequent losses of the lighter ^{14}N in the BF through fractionating processes, and

414   subsequent plant uptake of ^{15}N-enriched soil N (Magill et al., 2000; Zhang et al., 2008; Nadelhoffer

415   and Fry, 1994). On the other hand, leave δ^{15}N in PF can be more affected by δ^{15}N-depleted

416   deposition as the forest is still expanding in biomass and has lower N availability, thus it might

417   depend more on the ^{15}N-depleted atmospheric N input than the BF does. Another explanation could

418   be that the PF is dominated by *Pinus massoniana*, which has ectomycorrhizal fungi whereas

419   majority of the plants in the BF have arbuscular mycorrhizal association (Gurmesa, 2016), and

420   ectomycorrhizal plants are found to be more ^{15}N-depleted than arbuscular mycorrhizal plants

421   (Craine et al., 2009; 2015).

422   No apparent significant difference in soil δ^{15}N was observed between the BF and PF, except

423   the clear increase in soil δ^{15}N with soil depth only in the BF (Fig 2a). Absence of a clear profile

424   pattern in soil δ^{15}N with depth in the PF could be due to effects of erosion and soil mixing caused

425   by human disturbances until recent years. Soil δ^{15}N are reported to increase with organic matter age

426   (Bauer et al., 2000), and we expect soil organic matter of the top soil to be older in the BF, because

427   this layer might have been lost by erosion in the PF as it could be noted from the lower C, N and P

428   concentration (Table 1), and lack of depth pattern of soil δ^{15}N in the PF (Fig 2b).

429   The differences in plant N% and δ^{15}N between the two forests are also confirmed by their

430   response to the decadal N addition. In the BF, N addition did not significantly change plant N% in

431   each plant compartment and in the pool weighted plant pools because the plant tissues was already

432   saturated with N as also suggested by Fang et a1. (2011c). In contrast, plant N% of tree

433   compartments (and pool weighed N%) in the relatively N-poor PF tended to increase, and

434   significantly increased in understory plants (Table 3, Fig. 3a), indicating more dependence of plant

435   on input N in the PF, although the pool weighed N% was neither significantly increased in the PF.

436   Although the plant δ^{15}N after decadal N addition was not significantly different between the two

437   forests, the more pronounced effect of N addition in PF could still hint an ecological interpretation

438   of a difference in N status. In line with our expectation, the larger changes in plant δ^{15}N in the PF

439   could indicate larger incorporation of added N in to plants in the PF compared to that in the BF. In

440   support of this, calculations based on an isotope mixing model (Dawson et al., 2002) estimated the

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441   fraction of added N that was incorporated into the plant N pools to be larger (~30 % of the total 500

442   kg N ha^{-1} over a decade) in PF compared to ~20 % in the BF.

443

444   **5. Conclusion**

445   Our findings show that plants and soil in humid tropical forests of southern China are ^{15}N-depleted

446   likely due to an imprint from ^{15}N-depleted N atmospheric deposition. This effect of the input N

447   (deposition) ^{15}N signature was further confirmed by our observation that δ^{15}N of both plants and

448   soil moved toward the ^{15}N signature of added fertilizer N, which also shows that the added fertilizer

449   is incorporated into the forest N pools. We found that broad-leaved forests and early successional

450   forests differ in their N% and δ^{15}N, and accordingly differ in their response to increased N input.

451   Significant changes in the overall ecosystem δ^{15}N after decadal N addition in both forests indicate

452   that the ^{15}N signature of incoming N is more important for ecosystem δ^{15}N than fractionation during

453   the steps of N cycling. Thus, the use of ecosystem δ^{15}N as a tool to interpret changes in ecosystem

454   N cycling suggested in the literature is hampered by the ^{15}N imprint of increased N deposition,

455   particularly in regions with high N emissions.

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465 **Authors contribution:** Gundersen P. and Mo J. conceived and designed the experiments. Gurmesa

466 A.G., Lu X., Mao Q. and Zhou K. performed the data acquisition. Gurmesa A.G. analyzed the data.

467 Gurmesa A.G. and Gundersen P. wrote the manuscript. Lu X. and Mo J. commented and edited the

468 article.

469 **Conflicts of interest**: The authors declare that they have no conflict of interest.

470

471

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