

1 **Nitrogen input  $^{15}\text{N}$ -signatures are reflected in plant  $^{15}\text{N}$  natural**  
2 **abundances of sub-tropical forests in China**

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18 **Abstract.** Natural abundance of  $^{15}\text{N}$  ( $\delta^{15}\text{N}$ ) in plants and soils can provide time-integrated information related to  
19 nitrogen (N) cycling within ecosystems, but it has not been well tested in warm and humid sub-tropical forests. In this  
20 study, we used ecosystem  $\delta^{15}\text{N}$  to assess effects of increased N deposition on N cycling in an old-growth broad-leaved  
21 forest and a secondary pine forest in a high N deposition area in southern China. We measured  $\delta^{15}\text{N}$  of inorganic N in  
22 input and output fluxes under ambient N deposition, and N concentration (%N) and  $\delta^{15}\text{N}$  of major ecosystem  
23 compartments under ambient deposition and after decadal N addition at  $50 \text{ kg N ha}^{-1}\text{yr}^{-1}$  that has a  $\delta^{15}\text{N}$  of  $-0.7 \text{ ‰}$ . Our  
24 results showed that the total inorganic N in deposition was  $^{15}\text{N}$ -depleted ( $-10 \text{ ‰}$ ) mainly due to high input of strongly  
25  $^{15}\text{N}$ -depleted  $\text{NH}_4^+$ -N. Plant leaves in both forests were also  $^{15}\text{N}$ -depleted ( $-4$  to  $-6 \text{ ‰}$ ). The broad-leaved forest had  
26 higher plant and soil %N, and was more  $^{15}\text{N}$ -enriched in most ecosystem compartments relative to the pine forest.  
27 Nitrogen addition did not significantly affect %N in the broad-leaved forest, indicating that the ecosystem pools are  
28 already N-rich. However, %N was marginally increased in pine leaves and significantly in understory vegetation in the  
29 pine forest. Soil  $\delta^{15}\text{N}$  was not changed significantly by the N addition in either forest. However, the N addition  
30 significantly increased the  $\delta^{15}\text{N}$  of plants toward the  $^{15}\text{N}$  signature of the added N, indicating incorporation of added N  
31 into plants. Thus, plant  $\delta^{15}\text{N}$  was more sensitive to ecosystem N input manipulation than %N in these N-rich sub-  
32 tropical forests. We interpret the depleted  $\delta^{15}\text{N}$  of plants as an imprint from the high and  $^{15}\text{N}$ -depleted N deposition N  
33 that may dominate the effects of fractionation that are observed in most warm and humid forests. Fractionation during  
34 the steps of N cycling could explain the difference between negative  $\delta^{15}\text{N}$  in plants and positive  $\delta^{15}\text{N}$  in soils, and the  
35 increase in soil  $\delta^{15}\text{N}$  with depths. Nevertheless, interpretation of ecosystem  $\delta^{15}\text{N}$  from high N deposition regions needs  
36 to include data on the deposition  $^{15}\text{N}$  signal.

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38 Key words: Natural  $^{15}\text{N}$  abundance, N addition, N deposition, sub-tropical, China

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## 57 1 Introduction

58 Nitrogen (N) deposition onto terrestrial ecosystems has dramatically increased due to anthropogenic activities  
59 (Galloway, 2005) and since the 1980s the increase has been particularly strong in China including in the warm and  
60 humid regions (Liu et al. 2011). Nitrogen deposition that exceeds plant and microbial demand may increase nutrient  
61 leaching and soil acidification (Lu et al., 2014), and potentially causes nutritional imbalances in vegetation (Schulze,  
62 1989). Studies of fates and process responses to increased N deposition using coordinated N addition experiments in  
63 temperate and boreal forests show that the effects of increased N deposition largely depend on the initial N status of the  
64 forests (Gundersen et al., 1998; Hyvönen et al., 2008). Accordingly, N limited forests often show a growth response to  
65 added N and retain most of the deposited N, whereas N saturated forests subjected to N deposition often lose  
66 considerable N through leaching and denitrification. Although some studies from (sub) tropical regions also suggest that  
67 N leaching from tropical forests is related to the initial N status of the forests (Chen and Mulder, 2007; Fang et al.,  
68 2009), observations thus far are not conclusive, especially in regions that are subjected to increased anthropogenic N  
69 deposition (Townsend et al., 2011).

70 The natural abundance of  $^{15}\text{N}$  ( $\delta^{15}\text{N}$ ) in leaves and other ecosystem compartments is relatively easy to measure  
71 and may provide time-integrated information about N cycling in ecosystems (Handley and Raven, 1992; Robinson,  
72 2001). Differences in  $\delta^{15}\text{N}$  between ecosystem compartments and among ecosystems result from isotopic fractionation  
73 during each of the many steps of the N cycle. In particular, N losses through leaching and denitrification lead to  
74 preferential losses of the lighter  $^{14}\text{N}$  forms whereas compounds with isotopically heavier  $^{15}\text{N}$  are retained in the N pools  
75 or further cycled in the ecosystem (Högberg, 1997). Recent advances in the interpretation of  $\delta^{15}\text{N}$  variation among  
76 ecosystems based on the compilation and analysis of global data on foliar and soil  $\delta^{15}\text{N}$  have revealed general global  
77 patterns in relation to climate and N availability (Martinelli et al., 1999; Amundsen et al., 2003; Craine et al., 2009;  
78 2015a, b). Foliar  $\delta^{15}\text{N}$  values are generally elevated under N rich conditions, i.e. increasing leaf  $\delta^{15}\text{N}$  with increasing  
79 leaf N concentration and higher leaf  $\delta^{15}\text{N}$  in warmer climates (Craine et al., 2009). Tropical forests, which are often N-  
80 rich, have higher foliar  $\delta^{15}\text{N}$  than temperate forests (Martinelli et al., 1999). However, global analyses contain almost no  
81 data from eastern Asia, including sub-tropical regions of China now receiving high N deposition (Fang et al., 2011a).

82 The influence of increased N deposition on  $\delta^{15}\text{N}$  levels is not well known. For example, even though plant  
83  $\delta^{15}\text{N}$  could increase with N deposition (Emmett et al., 1998), it may not be the case across all regions where not only  
84 ecosystem N status but also a region-specific  $^{15}\text{N}$  signature of deposited N may influence ecosystem  $\delta^{15}\text{N}$  (Fang et al.,  
85 2011b; Pardo et al., 2006). Moreover, interpretation of ecosystem  $\delta^{15}\text{N}$  is hampered by the uncertainties in  $\delta^{15}\text{N}$  of plant  
86 N sources, the magnitude of isotopic fractionations during N transformation processes, and the complex behavior of  $^{15}\text{N}$   
87 in soils and plants (Robinson, 2001).

88 Plant leaf and soil  $\delta^{15}\text{N}$  are most commonly used to assess N status and changes in N cycling rates, but other  
89 ecosystem pools are neglected or rarely measured. The turnover times of N pools vary among different ecosystem  
90 compartments, and thus their  $\delta^{15}\text{N}$  values may respond differently to specific disturbances. For example, within plant  
91 compartments, small active N pools such as leaves reflect recent N cycling whereas the larger N pools such as wood or  
92 soil might reflect long-term changes in N cycling (Craine et al., 2015a). Nevertheless, reports of  $\delta^{15}\text{N}$  values in all  
93 major ecosystem pools are rare (e.g. Liu, 1995), emphasizing the need for more rigorous studies to provide complete  
94  $\delta^{15}\text{N}$  patterns in the leaf-to-soil continuum, and their response to N input manipulation, especially in the tropical forests.

95 We evaluated  $\delta^{15}\text{N}$  values of sub-tropical forests, and their responses to increased N deposition using long-  
 96 term N addition experimental plots established in 2003 in an old-growth broad-leaved forest and a pine plantation forest  
 97 in the Dinghushan Biosphere Reserve in southern China (Mo et al., 2006). The old-growth forest is more N-rich, and  
 98 has less N retention capacity than the pine forest (Fang et al., 2006). Nitrogen addition studies in these forests  
 99 documented that increased N input causes increased N leaching (Fang et al., 2008, 2009),  $\text{N}_2\text{O}$  emission (Zhang et al.,  
 100 2008) and soil acidification (Lu et al., 2014). Here, our objectives are (1) to compare  $\delta^{15}\text{N}$  values of ecosystem  
 101 compartments across the leaf-to-soil continuum in the two forests, and (2) to assess responses of  $\delta^{15}\text{N}$  in major  
 102 ecosystem pools to decadal N addition in the two forests. We hypothesized that i)  $\delta^{15}\text{N}$  values of plants and soil in these  
 103 forests would follow the global patterns predicted from climate and thus be higher in these sub-tropical forests than in  
 104 those reported for temperate forests, ii) N addition would change plant and soil  $\delta^{15}\text{N}$  towards the  $^{15}\text{N}$  signature of the  
 105 added N due to its incorporation into ecosystem pools, and iii) response of  $\delta^{15}\text{N}$  to N addition would differ between the  
 106 two forests due to differences in their initial N status and N cycling rates.

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## 108 2 Methods

### 109 2.1 Study site

110 The study was conducted in the Dinghushan Biosphere Reserve (DHSBR) in the Guangdong province, southern China  
 111 ( $112^{\circ}33'$  E and  $23^{\circ}10'$  N) with typical sub-tropical monsoon climate. Mean annual temperature (MAT) and mean  
 112 annual precipitation (MAP) are  $22.2\text{ C}^{\circ}$  and  $1927\text{ mm}$ , respectively. The reserve has experienced high rates of  
 113 atmospheric N deposition ( $21\text{-}38\text{ kg N ha}^{-1}\text{ yr}^{-1}$  as inorganic N in bulk precipitation) since 1990's (Fang et al., 2008). In  
 114 2009 to 2010, total wet N deposition was  $34.4\text{ kg N ha}^{-1}\text{ yr}^{-1}$  (Lu et al., 2013). We used two common forest types that  
 115 grow on the relatively steep slopes in the reserve; an old-growth broad-leaved forest (hereafter named as BF) and a pine  
 116 plantation forest (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 al., 1999). The PF  
 118 was planted after a clear-cut of the original climax forest in the 1930s and has been subjected to human disturbances  
 119 such as litter and shrub harvesting until the recent past (Mo et al., 2005).

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121 Table 1. Selected characteristics of the mineral soil (0-10 cm) in the two forest types. Data on soil bulk density, total P and  
 122 extractable  $\text{NH}_4^+\text{-N}$  are obtained from Fang et al. (2006). Values given in parenthesis indicate SE ( $n = 3$ ).

Parameters	Broad-leaved forest (BF)	Pine forest (PF)
Bulk density ( $\text{g cm}^{-3}$ )	0.9 (0.03)	1.3 (0.03)
pH ( $\text{H}_2\text{O}$ )	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 ( $\text{mg kg}^{-1}$ )	59 (3)	43 (3)
Extractable $\text{NH}_4^+\text{-N}$ ( $\text{mg kg}^{-1}$ )	2.1	3.3
Extractable $\text{NO}_3^-\text{-N}$ ( $\text{mg kg}^{-1}$ )	12.7	2.6

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124 The major canopy species in the BF are *Castanopsis chinensis*, *Machilus chinensis*, *Schima superba*,  
 125 *Cryptocarya chinensis*, and *Syzygium rehderianum* and the most common understory species is *Hemigramma decurrins*.  
 126 *Pinus massoniana* and *Dicranopteris dichotoma* are the dominant tree and understory species in the PF, respectively.

127 No N-fixing tree species were found in the plots. The soil in the reserve is classified as Lateritic Red Earth (Oxisol)  
128 formed from Devonian sandstone and shale with a thin layer of forest floor litter (0.5-3.0 cm), but the soil depth is  
129 variable ranging from 30 cm in the PF to more than 60 cm in the BF. Probably due to erosion after the clear-cut and the  
130 continued human disturbance the PF had lower total soil carbon, N and phosphorus (P) content than the BF (Table 1).  
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## 132 **2.2 Experimental design**

133 We used an ongoing N addition experiment established in both forests in July 2003 (Mo et al., 2006). The experimental  
134 plots used for this study consist of control plots and N addition treatment at 50 kg N ha<sup>-1</sup> yr<sup>-1</sup> (hereafter named as N-  
135 plots) each with three replicates in both forests. Each plot is 10 m x 20 m with at least a 10 m wide buffer strip to the  
136 next plot. In the N-plots, NH<sub>4</sub>NO<sub>3</sub> is mixed with 20 L of water, and is added monthly since July 2003 below the canopy  
137 using a backpack sprayer, whereas the control plots received equivalent 20 L water with no fertilizer. The added N has  
138 δ<sup>15</sup>N of about -3 ‰ on NH<sub>4</sub><sup>+</sup>-N and about 1.8 ‰ on NO<sub>3</sub><sup>-</sup>-N, with δ<sup>15</sup>N of NH<sub>4</sub>NO<sub>3</sub> being -0.7 ‰.  
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## 140 **2.3 Sampling and analysis of plant and soil pools**

141 In both forests, major ecosystem compartments including leaves, twigs, branches, bark and wood of canopy trees,  
142 leaves of understory vegetation, fine roots, and 0-30 cm mineral soil were sampled in January 2013 to determine their N  
143 concentration (%) and δ<sup>15</sup>N (‰). A branch per dominant tree species per plot was cut from the height reached using a  
144 pole pruner (c. 7-8 m) taking advantage of the steep slope, and was separated into leaves, twigs and small branches.  
145 Bark samples were cutoff the dominate trees at breast height using a knife. After removing the bark, wood cores were  
146 sampled using an increment borer and separated visually into sapwood (usually the outer 2-3cm recent wood) and older  
147 wood (heartwood). Dominant understory plant species were cut with a knife and kept separate for each species. A total  
148 of seven tree species in the canopy/sub-canopy layer and five plant species in the understory layer (young trees, shrubs,  
149 herbs and liana) of the BF were sampled. In the PF, the dominant pine tree and five species in the understory layer were  
150 sampled. Mineral soil samples were taken using an auger (5.1 cm in diameter) and were divided into three layers (0-10,  
151 10-20, 20-30 cm). Two soil cores were sampled and pooled together to form one composite sample for each depth per  
152 plot. Living fine roots were hand-sorted from the soil samples for each depth, but were combined to one composite  
153 sample for the whole profile (0-30 cm) because the amount of fine roots in each depth were too small to grind and  
154 analyze separately. Litterfall was collected monthly during July-September 2012 and was pooled together to make one  
155 composite sample per plot. The litter was sorted in the laboratory into leaf and others (branches, fruits, flowers, barks),  
156 but only leaf values are reported.

157 All plant and soil samples were oven-dried at 70 °C, and ground to a fine and homogeneous powder. Mineral  
158 soils were sieved (2mm mesh) to remove non-soil materials, air-dried at room temperatures and milled to fine powder.  
159 Subsamples were dried at 105 °C, and all results are reported on 105 °C basis. Based on their approximate %N, about 4-  
160 5 mg of the samples were weighed into tin capsules, and δ<sup>15</sup>N and N concentration of the samples were determined  
161 simultaneously on an isotope ratio mass spectrometer (Isoprime 100, Isoprime Ltd.) coupled to an automatic, online  
162 elemental analyzer (vario ISOTOPE cube). An internal standard needle sample from temperate forests, which has been  
163 analysed in multiple runs at several laboratories, was used to check reproducibility of the δ<sup>15</sup>N determination. We  
164 analyzed %N and δ<sup>15</sup>N separately for each dominant tree species per plot, but compartment mean values are reported.  
165 Natural abundance δ<sup>15</sup>N in samples was reported in per mil (‰) relative to the <sup>15</sup>N content of atmospheric N<sub>2</sub>.

## 166 2.4 Sampling and analysis of water samples

167 Precipitation, throughfall, surface runoff and soil solution were sampled monthly from September 2012 to February  
168 2013 (dry December and January, where there were not enough precipitation to generate water samples) in the control  
169 plots to assess the  $\delta^{15}\text{N}$  of N input and output in the two forests under ambient N deposition. Bulk precipitation was  
170 collected at an open area close to the experimental site using an open glass funnel (12 cm in diameter), connected to a 5  
171 L sampling bottle with polypropylene tubes. Throughfall was collected by PVC pipes at five random points within each  
172 plot (with a total intercept area of  $0.8\text{m}^2$ ) at about 1.3m above the ground in each forest. Each collector was connected  
173 to two 50L buckets with polypropylene tubes. Soil solutions from 20 cm depth (seepage water) were obtained using two  
174 zero tension tray lysimeters ( $755\text{ cm}^2$  per tray) installed in each plot. Each lysimeter was connected to a 20L bottle  
175 using the steep slope of the sites to facilitate sampling. In both forests, one selected plot for each treatment was  
176 delimited hydrologically by placing stable plastic materials and low cement barriers around them. The cement barriers  
177 (covered by the plastic material) on the downslope side of these plots were constructed to enable the sampling of the  
178 surface runoff in three sections, which were then used as pseudo-replicates.

179 Natural  $^{15}\text{N}$  abundances of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  in water samples were analyzed after chemical conversion to  
180 nitrous oxide ( $\text{N}_2\text{O}$ ). The  $\text{NH}_4^+\text{-N}$  was initially oxidized to nitrite ( $\text{NO}_2^-$ ) by hypobromite ( $\text{BrO}^-$ ) and the  $\text{NO}_2^-$  is then  
181 quantitatively converted into  $\text{N}_2\text{O}$  by hydroxylamine ( $\text{NH}_2\text{OH}$ ) under strongly acidic conditions (Liu et al., 2014).  
182 Similarly, a series of chemical reactions of vanadium (III) chloride ( $\text{VCl}_3$ ) and sodium azide under acidic conditions  
183 was used to convert  $\text{NO}_3^-\text{-N}$  into  $\text{N}_2\text{O}$  (Lachouani et al., 2010). The produced  $\text{N}_2\text{O}$  was subsequently analysed for  $^{15}\text{N}$   
184 abundance by a purge-and-trap coupled with an isotope ratio mass spectrometer (PT-IRMS) (Liu et al., 2014).

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## 186 2.5 Calculations and Statistics

187 To evaluate effects of decadal N addition on the whole ecosystem (plant and soil) %N and  $\delta^{15}\text{N}$ , we determined N pool  
188 weighted plot means of %N and  $\delta^{15}\text{N}$  using N pools for each compartment and tree species contribution quantified in  
189 Gurmesa et al. (2016). We excluded the heartwood and sapwood pools in the plant pool calculations for two reasons;  
190 first the low %N in wood samples caused larger uncertainties on the  $\delta^{15}\text{N}$  determinations, and secondly heartwood and a  
191 major part of the sapwood were formed prior to the initiation of the N addition treatment. We expect the later to be the  
192 explanation that particular heartwoods showed opposite effects of N addition compared to all other compartments.

193 Differences between the two forests in plot mean %N and  $\delta^{15}\text{N}$  of the different ecosystem compartments and N  
194 pool weighted plot means in control plots were analysed using *t*-tests. The effect of N addition treatment on %N and  
195  $\delta^{15}\text{N}$  of each tree compartments in the BF and understory leaf in both forests was analyzed using mixed model ANOVA  
196 with treatment as explanatory factor and plant species as a random factor because plant species differed significantly in  
197 both parameters (Gurmesa, 2016). All other tests of treatment effects on %N and  $\delta^{15}\text{N}$  was analysed using simple *t*-test  
198 on plot means.

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## 200 3 Results

### 201 3.1 Concentration and $\delta^{15}\text{N}$ of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in water samples

202 Dissolved  $\text{NH}_4^+\text{-N}$  in water samples in both input (precipitation and throughfall) and output fluxes (surface runoff and  
203 soil solution) were  $^{15}\text{N}$ -depleted (negative  $\delta^{15}\text{N}$ ) in both forests (Table 2). The  $\delta^{15}\text{N}$  of  $\text{NO}_3^-\text{-N}$  was  $^{15}\text{N}$ -enriched in  
204 precipitation and throughfall, and became  $^{15}\text{N}$ -depleted in surface runoff and soil solution. However, for dissolved

205 inorganic N (DIN) the concentration weighted  $\delta^{15}\text{N}$  (calculated based on data in Table 2 and concentration data in Table  
 206 S1) were  $^{15}\text{N}$ -depleted but slightly increased from precipitation input to soil solution. Mean  $\delta^{15}\text{N}$  of both  $\text{NH}_4^+\text{-N}$  and  
 207  $\text{NO}_3^-\text{-N}$  in input and output fluxes did not significantly differ between the two forests. The temporal variation in  $\delta^{15}\text{N}$   
 208 was large (-28 to 2 ‰) for  $\text{NH}_4^+\text{-N}$  but minor (2 to 5 ‰) for  $\text{NO}_3^-\text{-N}$  (Fig. 1b, d, x-axis). The  $\delta^{15}\text{N}$  of  $\text{NH}_4^+\text{-N}$  in surface  
 209 runoff and soil solution were significantly and positively related to the variation in  $\delta^{15}\text{N}$  of  $\text{NH}_4^+\text{-N}$  in throughfall in  
 210 both forests (Fig 1a, b), but the correlation was not significant for  $\text{NO}_3^-\text{-N}$  (Fig 1c, d).

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212 Table 2.  $\delta^{15}\text{N}$  (‰) of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and dissolved inorganic N (DIN) in bulk precipitation, throughfall, surface runoff and soil  
 213 solution at 20cm depth in control plots from September 2012 to February 2013. Numbers in parenthesis for precipitation, throughfall  
 214 and soil solution indicate standard error of the mean (SE) ( $n = 3$ ). For all water fluxes, no significant difference in  $\delta^{15}\text{N}$  of both  $\text{NH}_4^+\text{-N}$   
 215  $\text{N}$  and  $\text{NO}_3^-\text{-N}$  was detected between the two forests.

Fluxes	Broad-leaved forest (BF)			Pine forest (PF)		
	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	DIN	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	DIN
Precipitation <sup>a</sup>	-16.6	4.1	-9.9	-16.6	4.1	-9.9
Throughfall	-15.2 (2.3)	3.6 (0.2)	-7.9 (1.2)	-15.5 (1.8)	2.8 (0.3)	-9.9 (0.5)
Surface runoff <sup>b</sup>	-13.1(1.7)	-1.9 (0.6)	-6.2 (1.0)	-9.7 (1.0)	-1.5 (0.6)	-5.4 (0.1)
Soil solution	-22.6 (0.9)	-0.9 (1.3)	-5.7 (0.7)	-21.3 (2.3)	-0.9 (0.2)	-7.3 (1.1)

216 <sup>a</sup> Precipitation was collected at open area within the reserve, and was assumed to be the same for both forests.

217 <sup>b</sup> The indicated SE is for pseudo-replicates within one plot.

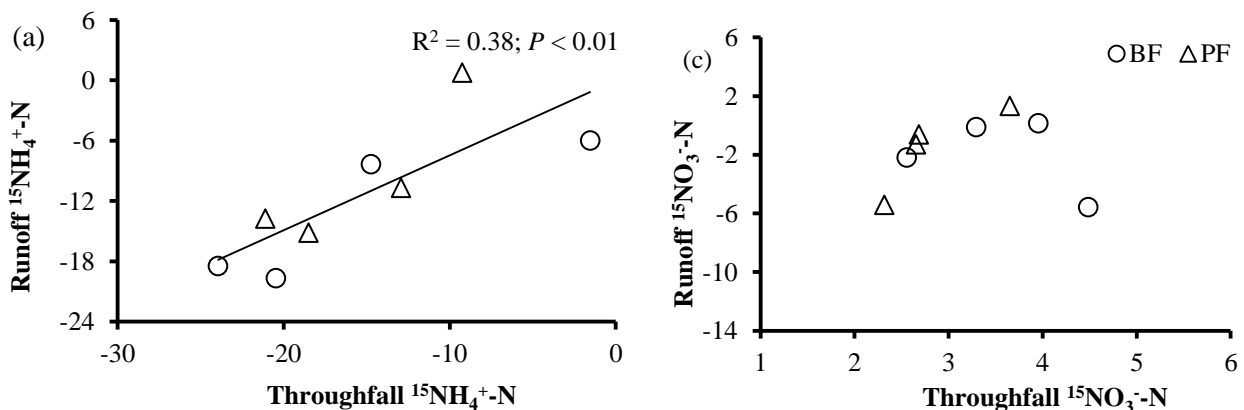
218

### 219 3.2 Effects of forest type

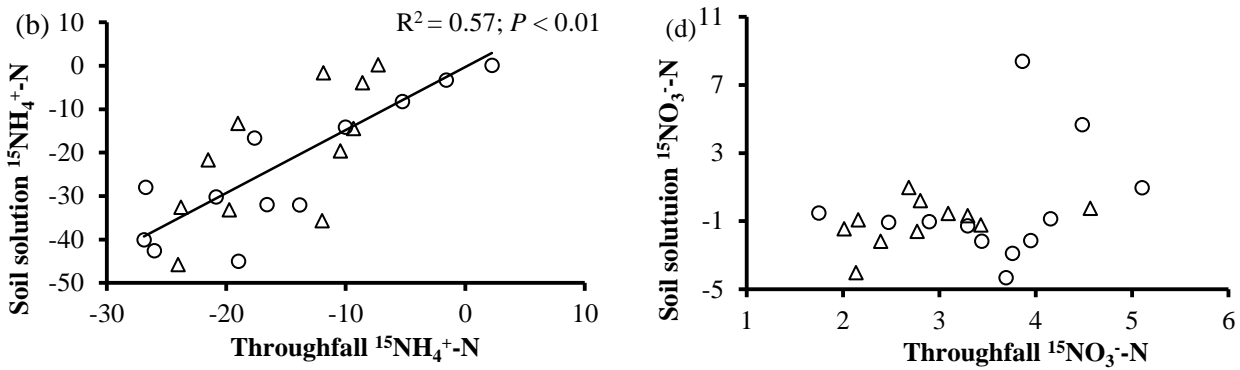
220 As expected based on the differences in disturbance regime, the BF is more N-rich than PF. Nitrogen concentrations of  
 221 plant compartments were significantly higher in the BF than in the PF, except in leaves of canopy trees, litter-fall and  
 222 fine roots for which the difference was marginally significant (Table 3). Soil %N was significantly higher in the BF at  
 223 all depths (Table 3).

224 Most plant compartments are  $^{15}\text{N}$ -depleted with understory and tree leaves, twigs and branches being most  $^{15}\text{N}$ -  
 225 depleted (below -4 ‰) whereas bark and sapwood were less  $^{15}\text{N}$ -depleted within each forest (Table 4). The  $\delta^{15}\text{N}$  of all  
 226 plant compartments differ significantly between the two forests with the PF being more  $^{15}\text{N}$ -depleted than the BF (Table  
 227 4). Soil  $\delta^{15}\text{N}$  did not show significant difference between the two forests at any depth (Table 4).

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231 **Figure 1.** Correlation between  $\delta^{15}\text{N}$  (‰) of  $\text{NH}_4^+\text{-N}$  in throughfall and that of  $\text{NH}_4^+\text{-N}$  in surface runoff (a), and soil solution (b), and  
 232 correlation between  $\delta^{15}\text{N}$  of  $\text{NO}_3^-\text{-N}$  in throughfall and that of  $\text{NO}_3^-\text{-N}$  in surface runoff (c), and soil solution (d). For throughfall and  
 233 soil solution,  $\delta^{15}\text{N}$  were from samples taken monthly between September and February in each of the 3 plots. For surface runoff,  
 234 samples were only from one plot. No significant effect of forest type was detected; thus the regression line shown was based on data  
 235 from both forests.  
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237 When compared based on N pool weighted plot mean, the two forests differed significantly in plant %N and  $\delta^{15}\text{N}$  (Fig.  
 238 2a). For the soil, the two forests also differed significantly in N pool weighted plot mean %N, with the BF having the  
 239 higher value, but not in N pool weighted plot mean  $\delta^{15}\text{N}$  (Fig. 2b).  
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### 241 3.3 Effects of N addition on %N and $\delta^{15}\text{N}$

242 Nitrogen concentrations in all measured plant and soil compartments were not significantly affected by N addition in  
 243 the BF, except in the sapwood (Table 3). In the PF, mean %N values were greater in most plant compartments on  
 244 fertilized plots, but the change was significant only in leaves of understory plants, whereas soil %N was unchanged  
 245 (Table 3).  
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247 Plant  $\delta^{15}\text{N}$  was negative in both control and N-plots in both forests, but N addition significantly increased the  
 248  $\delta^{15}\text{N}$  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.

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270 Table 3. Mean %N of different ecosystem pools in the broad-leaved (BF) and pine forests (PF). Values in parenthesis indicate SE of  
 271 plot means ( $n = 3$ ). Within each forest type  $p$ -values for the effect of N addition are shown. The last column shows  $p$ -values for a  
 272 difference 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 $p$ -values
	Control	N addition	$p$ -values	Control	N addition	$p$ -values	
<b><i>Plants</i></b>							
Tree leaf	1.71 (0.19)	1.69 (0.19)	0.48 <sup>§</sup>	1.44 (0.11)	1.68 (0.28)	0.16	0.12
Twig	1.28 (0.19)	1.17 (0.23)	0.59 <sup>§</sup>	0.99 (0.05)	0.97 (0.08)	0.79	<b>0.01</b>
Branch	0.86 (0.15)	0.81 (0.16)	0.13 <sup>§</sup>	0.58 (0.05)	0.60 (0.06)	0.85	<b>0.03</b>
Bark	0.71 (0.16)	0.7 (0.16)	0.55 <sup>§</sup>	0.57 (0.02)	0.61 (0.05)	0.53	<b>0.01</b>
Sapwood	0.27 (0.07)	0.3 (0.07)	<b>&lt;0.01</b> <sup>§</sup>	0.18 (0.02)	0.11 (0.02)	0.07	<b>0.03</b>
Heartwood	0.16 (0.04)	0.16 (0.03)	0.28 <sup>§</sup>	0.06 (0.00)	0.09 (0.03)	0.35	<b>&lt;0.01</b>
Understory leaves	2.04 (0.02)	1.98 (0.17)	0.09 <sup>§</sup>	1.61 (0.41)	1.77 (0.40)	<b>&lt;0.01</b> <sup>§</sup>	<b>&lt;0.01</b>
Fine root	1.4 (0.16)	1.81 (0.17)	0.15	0.87 (0.13)	0.96 (0.04)	0.58	0.06
Litter-fall	1.56 (0.05)	1.48 (0.06)	0.45	1.39 (0.04)	1.72 (0.09)	0.06	0.09
<b><i>Soil</i></b>							
0-10 cm	0.27 (0.04)	0.28 (0.01)	0.83	0.13 (0.01)	0.12 (0.01)	0.39	<b>0.03</b>
10-20 cm	0.18 (0.01)	0.19 (0.01)	0.59	0.07 (0.00)	0.06 (0.00)	0.37	<b>&lt;0.01</b>
20-30 cm	0.12 (0.00)	0.14 (0.00)	0.14	0.06 (0.00)	0.05 (0.00)	0.18	<b>&lt;0.01</b>

273 <sup>§</sup> Due to significant differences between the sampled tree or understory plant species the effect of N addition was tested in a mixed  
 274 model ANOVA with species as random factor.

275

276 However, effect of N addition on  $\delta^{15}\text{N}$  was inconsistent in the wood parts (Table 4). For heartwood, the effects  
 277 were significant, but in different directions than in other plant pools for both forests. Due to low %N and challenges in  
 278 grinding of wood samples it was difficult to get reliable  $\delta^{15}\text{N}$  results for these samples. Also much of the sampled wood  
 279 was formed prior to the treatment and thus, no further evaluation was done for the wood samples. Nitrogen addition did  
 280 not cause significant effects on  $\delta^{15}\text{N}$  of litter-fall and fine roots. In the BF, there was no correlation between leaf %N  
 281 and  $\delta^{15}\text{N}$ , but a positive correlation was found for the PF as both %N and  $\delta^{15}\text{N}$  tended to increase in parallel due to N  
 282 addition (data not shown).

283 Nitrogen addition tended to decrease soil  $\delta^{15}\text{N}$  in the BF at all depths, but with no significant changes in any  
 284 layer (Table 4). In the PF, soil  $\delta^{15}\text{N}$  was unchanged by N addition (Table 4).

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288

289 Table 4. Mean  $\delta^{15}\text{N}$  (‰) of plant pools in the broad-leaved (BF) and pine forests (PF). Values in parenthesis indicate SE of plot  
 290 means ( $n = 3$ ). Within each forest type  $p$ -values for the effect of N addition is shown. The last column shows  $p$ -values for differences  
 291 between the ambient plots of the two forests using  $t$ -test. Bolded  $p$ -values indicate significant differences.

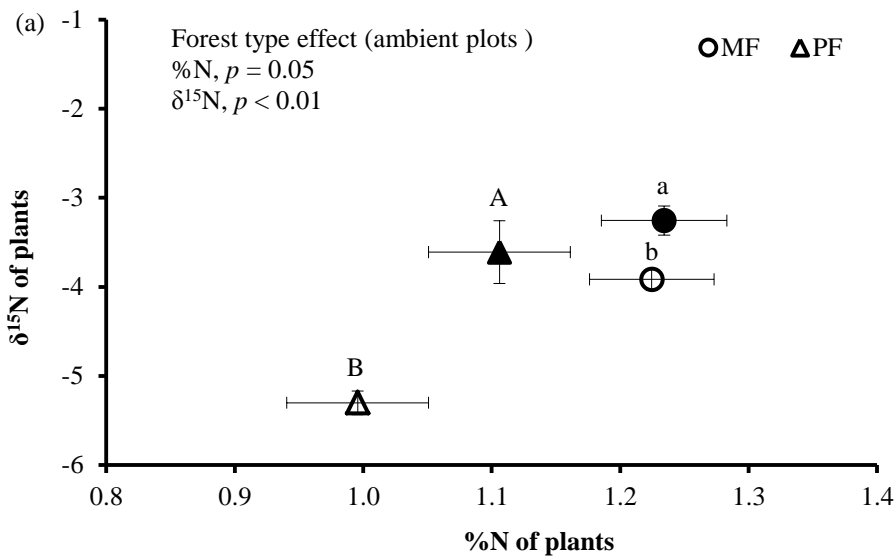
Sample type	Broad-leaved forest (BF)			Pine forest (PF)			Forest type effect
	Control	N addition	$p$ -values	Control	N addition	$p$ -values	$p$ -values
Tree leaf	-4.0 (0.5)	-3.4 (0.6)	<b>0.02</b> <sup>§</sup>	-5.4 (0.1)	-3.5 (0.3)	<b>0.01</b>	<b>&lt;0.01</b>
Twigs	-4.3 (0.8)	-3.8 (0.9)	0.09 <sup>§</sup>	-5.7 (0.1)	-4.0 (0.3)	<b>0.02</b>	<b>&lt;0.01</b>
Branches	-4.6 (0.4)	-4.1 (0.3)	<b>&lt;0.01</b> <sup>§</sup>	-5.7 (0.2)	-4.1(0.6)	0.12	<b>0.03</b>
Bark	-2.8 (0.8)	-2.4 (0.6)	0.05 <sup>§</sup>	-4.0 (0.4)	-2.6 (0.2)	<b>0.03</b>	0.06
Sapwood	-1.9 (0.5)	-1.8 (0.3)	0.51 <sup>§</sup>	-0.9 (0.4)	1.8 (1.6)	0.23	0.09
Heartwood	-1.6 (0.9)	-2.3 (0.9)	<b>0.05</b> <sup>§</sup>	3.2 (0.8)	-0.71 (1)	<b>0.04</b>	<b>0.03</b>
Understory leaves	-3.6 (0.9)	-2.2 (1.1)	<b>&lt;0.01</b> <sup>§</sup>	-5.6 (0.5)	-3.54 (0)	<b>&lt;0.01</b> <sup>§</sup>	<b>0.01</b>
Fine root	-2.8 (0.6)	-1.7 (0.8)	0.33	-5.1 (0.5)	-3.6 (0.3)	0.08	<b>0.04</b>
Litter-fall	-3.9 (0.1)	-3.9 (0.1)	0.98	-4.8 (0.2)	-4.0 (0.3)	0.11	<b>0.04</b>
<b><i>Soil</i></b>							
0-10 cm	2.2 (0.4)	1.6 (0.6)	0.46	2.6 (0.8)	2.3 (0.4)	0.69	0.63
10-20 cm	4.0 (0.3)	3.2 (0.2)	0.09	4.1 (1.4)	4.4 (0.3)	0.88	0.93
20-30 cm	5.4 (0.3)	4.8 (0.5)	0.39	3.3 (1.4)	4.0 (0.2)	0.68	0.26

292 <sup>§</sup> Due to significant differences between the sampled tree or understory plant species the effect of N addition was tested in a mixed  
 293 model ANOVA with species as random factor.

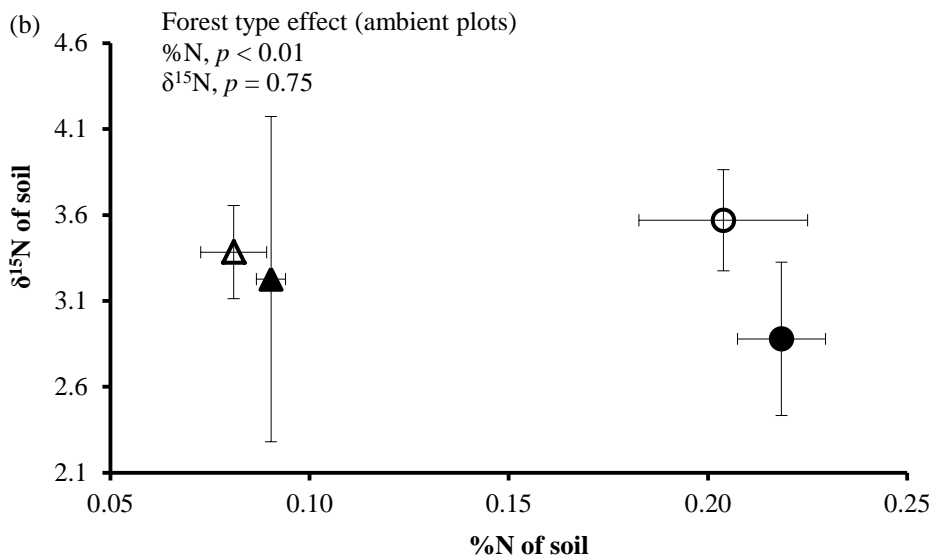
294

295 In summary, the effect of added N on pool weighted plot mean plant %N was not significant in either BF ( $p = 0.86$ ) or  
 296 in PF ( $p = 0.25$ ) more pronounced in the PF (Fig. 2a). However, weighted plot mean plant  $\delta^{15}\text{N}$  were significantly  
 297 increased in both forests ( $p = 0.04$  for BF and  $p = 0.03$  for PF) by the N addition. In the soil, where the N pool is  
 298 obviously larger than in the plants, the effect of the N addition on weighted average %N was not significant in both  
 299 forests (Fig. 2b). The direction of change in soil  $\delta^{15}\text{N}$  was a decrease as expected with incorporation of the added N  
 300 ( $\delta^{15}\text{N} = -0.7$  ‰), but the change was again not significant (Fig. 2b).

301



302



303

304 Figure 2. Overall effect of N addition on plot average weighted %N and  $\delta^{15}\text{N}$  of plants (a), and soil (b) for broad-leaved forest (○)  
 305 and pine plantation (△). Error bars indicate SE of plot means ( $n = 3$ ). Open symbols indicate control plots and closed symbols  
 306 indicate N-plots. In (a), significant effects of N addition within forest type is indicated by different letters; lowercase for BF and  
 307 uppercase for PF. The  $p$ -values shown in the upper right corners are tests for differences in %N and  $\delta^{15}\text{N}$  between the two forests  
 308 (ambient plots).  
 309

## 310 4 Discussions

### 311 4.1 $\delta^{15}\text{N}$ of N in deposition and soil solution

312 Deposition N (bulk precipitation and throughfall) was  $^{15}\text{N}$ -depleted in  $\text{NH}_4^+\text{-N}$  and  $^{15}\text{N}$ -enriched in  $\text{NO}_3^-\text{-N}$  (Table 2),  
 313 but since  $\text{NH}_4^+\text{-N}$  is the dominating N form (Table S1) DIN deposition is  $^{15}\text{N}$ -depleted (-10 to -8 ‰) as also previously  
 314 reported in the region (Zhang et al., 2008; Koba et al., 2012). The source of the  $\text{NH}_4^+\text{-N}$  is likely  $\text{NH}_3$  emissions from  
 315 activities in the intensively used agricultural land surrounding DHSBR. Agricultural  $\text{NH}_3$  emissions are usually  $^{15}\text{N}$ -  
 316 depleted (Bauer et al., 2000). The source of the  $\text{NO}_3^-\text{-N}$  contribution may originate from  $\text{NO}_x$  produced by coal  
 317 combustion in mega-cities in the Guangdong province.

318 The low  $\delta^{15}\text{N}$  of  $\text{NH}_4^+\text{-N}$  in the soil solution of both forest resemble that in precipitation and throughfall (Table  
319 2), and it is likely due to transport of  $^{15}\text{N}$ -depleted throughfall N through macrospores as supported by the positive  
320 relationship between  $\delta^{15}\text{N}$  of  $\text{NH}_4^+\text{-N}$  in soil solution and that in throughfall (Fig. 1b). The further  $^{15}\text{N}$ -depletion of  
321  $\text{NH}_4^+\text{-N}$  (6 to 7 ‰) from throughfall to soil solution may occur by preferential retention of the heavier  $^{15}\text{N}$  isotope by  
322 cation exchange on soil surfaces (e.g. Karamanos and Rennie, 1978), although preferential nitrification of the lighter  
323 isotope could work in the opposite direction. This fractionation effect of nitrification (leaving the substrate  $\text{NH}_4^+\text{-N}$   $^{15}\text{N}$ -  
324 enriched and the product  $\text{NO}_3^-\text{-N}$   $^{15}\text{N}$ -depleted (Högberg, 1997)) may explain the relative  $^{15}\text{N}$ -enrichment of  $\text{NH}_4^+\text{-N}$  (2  
325 to 6 ‰) from throughfall to surface runoff in both forests (Table 2). A contribution of  $\text{NO}_3^-\text{-N}$  from nitrification of  $^{15}\text{N}$ -  
326 depleted throughfall  $\text{NH}_4^+\text{-N}$  during surface runoff passing through the biological active litter layer may also explain the  
327 4 to 6 ‰  $^{15}\text{N}$ -depletion of  $\text{NO}_3^-\text{-N}$  from throughfall to surface runoff (Table 2).

328 While  $\text{NO}_3^-\text{-N}$  is the dominant N-form in soil solution (Table S1) and the N leaching fluxes are almost as large  
329 as the N inputs by deposition in both forests (Fang et al., 2009), nitrification is an important process in the soils at  
330 DHSBR. However, as soil solution  $\text{NO}_3^-\text{-N}$  was  $^{15}\text{N}$ -enriched (-1 ‰) relative to the  $^{15}\text{N}$ -depleted throughfall  $\text{NH}_4^+\text{-N}$  (-  
331 15 ‰) this cannot be the main substrate for nitrification in the soil. Also the relative narrow temporal variation of  $\delta^{15}\text{N}$   
332 for soil solution  $\text{NO}_3^-\text{-N}$  (Fig. 1d) indicate dominance of a substrate for nitrification with stable  $\delta^{15}\text{N}$  content such as soil  
333 organic N and/or adsorbed  $\text{NH}_4^+$ . On the other hand, gaseous losses of  $^{15}\text{N}$ -depleted N by denitrification would  $^{15}\text{N}$ -  
334 enrich soil N as well as soil solution  $\text{NO}_3^-\text{-N}$  (Houlton et al., 2006). For the BF, denitrification N losses have been  
335 estimated to be as high as  $2.6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  as  $\text{N}_2\text{O}$  (Zhang et al., 2008) and  $30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  as  $\text{N}_2$  (Fang et al., 2015).  
336 This may explain why DIN in soil solution is slightly  $^{15}\text{N}$ -enriched relative to the DIN input (bulk precipitation or  
337 throughfall), despite the apparent importance of fractionation via nitrification in the soils of both forest.

338

#### 339 4.2 $\delta^{15}\text{N}$ of plants and soil under ambient condition

340 Climate is important in regulating global patterns of  $\delta^{15}\text{N}$  in plants and soils (Amundson et al., 2003; Craine et  
341 al., 2009; 2015b). Based on the relationships between plant and soil  $\delta^{15}\text{N}$  and climate parameters (MAT and MAP)  
342 established by Amundson et al. (2003), the expected  $\delta^{15}\text{N}$  at DHSBR are 0.4 ‰ for plants and 5.2 ‰ for the top 10cm  
343 soil. In a global synthesis for forests Martinelli et al. (1999) reported an average leaf  $\delta^{15}\text{N}$  at  $3.7 \pm 3.5$  ‰ for tropical  
344 forests and a major recent survey across Amazonas observed similar  $^{15}\text{N}$ -enriched leaf  $\delta^{15}\text{N}$  levels ( $3.1 \pm 2.3$  ‰)  
345 (Nardoto et al., 2014). For tropical forest soils Martinelli et al. (1999) reported  $9.3 \pm 1.8$  ‰ for the top 10cm. However,  
346 the observed leaf  $\delta^{15}\text{N}$  at DHSBR were much lower, between -4 ‰ and -6 ‰ for the two forests (Table 4). Similar low  
347 leaf  $\delta^{15}\text{N}$  (-2 to -5 ‰) were found in other (sub) tropical forest in eastern Asia (Fang et al., 2011a; Wang et al., 2014;  
348 Kitayama and Iwamoto, 2001). The top 10 cm soil  $\delta^{15}\text{N}$  at DHSBR (2.2 to 2.6 ‰, Table 4) were again lower than  
349 expected from local climate or observed in tropical forest. Apparently, the ecosystem  $\delta^{15}\text{N}$  values at DHSBR are more  
350 close to the values reported for temperate forests by Martinelli et al. (1999) for leaves ( $-2.8 \pm 1.8$  ‰) and for soil (1.6  $\pm$   
351 3.6 ‰) as well as those reported from N-saturated temperate forests (Koopmans et al. 1997; Sah & Brumme, 2003).

352 Thus, our results reject our first hypothesis that ecosystem  $\delta^{15}\text{N}$  at DHSBR would compare with other  
353 observations from warm and humid climates; also DHSBR forests were not more  $^{15}\text{N}$ -enriched than temperate forests.  
354 Martinelli et al. (1999) discussed reasons for the  $^{15}\text{N}$ -enrichment of tropical ecosystems (relative to temperate forest)  
355 and concluded it could result from open N cycles in tropical forests, with fractionation during microbial activities  
356 resulting in losses of isotopically light  $^{14}\text{N}$  forms which leave isotopically heavy N to cycle internally within tropical

357 ecosystems. Despite noticeable fractionation processes in the soil at DHSBR (section 4.1) and high N availability  
358 leading to considerable N losses, there is no evident ecosystem  $^{15}\text{N}$ -enrichment at DHSBR or in other Chinese forests  
359 with high N deposition (Fang et al., 2011a; Wang et al., 2014).

360 We suspect this phenomenon to be an imprint from the high and  $^{15}\text{N}$ -depleted N deposition (Table 2). The  $^{15}\text{N}$   
361 signature of deposition N can alter plant  $\delta^{15}\text{N}$  by direct uptake in the canopy and by altering the signature of available N  
362 in the soil (Craine et al., 2015a) (as it is noticeable for  $\text{NH}_4^+$ -N in soil solution; Fig 1b). A similar mechanism involving  
363 preferential uptake of particularly  $^{15}\text{N}$  depleted  $\text{NH}_4^+$ -N could also explain the occurrence of  $^{15}\text{N}$ -depleted plants in  
364 tropical rainforests in southern China (Wang et al., 2014). Such influence of deposition N can be region-specific as  
365 shown for some forests in Europe that appear to follow a different trajectory for increasing leaf  $\delta^{15}\text{N}$  with N deposition  
366 than forests in USA (Pardo et al., 2006).

367 The conclusion that plant  $\delta^{15}\text{N}$  is influenced by the  $^{15}\text{N}$ -depleted N deposition is further supported by the result  
368 that tree ring  $\delta^{15}\text{N}$  of *Pinus massoniana* at DHSBR (sampled nearby the PF plots) decreased from 2 ‰ in the 1960s to -  
369 1 ‰ in the late 1990s, and that the decrease was found to coincide with the increasing deposition of  $^{15}\text{N}$ -depleted N  
370 over the last 50 years (Sun et al., 2010). In line with that long-lived plant compartments (bark and wood) were less  $^{15}\text{N}$ -  
371 depleted than short-lived compartments (leaves, twigs and branches) in both forests (Table 4).

372 The lower soil  $\delta^{15}\text{N}$  in DHSBR relative to the global average for tropical forest soils may in part also be an imprint from  
373 the  $^{15}\text{N}$ -depleted N deposition. However, with an N-pool at  $\sim 2400 \text{ kg N ha}^{-1}$  (equal to more than 60 years of N  
374 deposition) alone in the top 10 cm (Gurmesa et al., 2016), the influence should be minor compared to that in short-lived  
375 plant compartments that holds an N-pool an order of magnitude less.

376 The steep slopes at DHSBR may contribute slightly to lower the soil  $\delta^{15}\text{N}$ , because steeper slopes promote  
377 non-fractionating erosional losses of soil organic matter and decrease the residence time of soil N compared to forests  
378 on more gentle slopes, that on the other hand may have more fractionation from denitrification due to greater soil  
379 moisture (Amundson et al., 2003; Hilton et al., 2013; Perakis et al., 2015).

380

#### 381 **4.3 Effects of N addition on $\delta^{15}\text{N}$**

382 Nitrogen addition increases N availability and is thus expected to increase plant  $\delta^{15}\text{N}$  as a result of  
383 fractionation during N uptake and cycling, as discussed above. Several N addition experiments in temperate forests  
384 indeed observed this effect (Högberg et al., 2011; Högberg et al., 2014; Korontzi et al., 2000; McNulty et al., 2005;  
385 Näsholm et al., 1997). Accordingly, plant  $\delta^{15}\text{N}$  in both forests at DHSBR were increased by N addition (Table 4, Fig  
386 2a). The changes in  $\delta^{15}\text{N}$  occurred in small and short-lived plant compartments (e.g. leaves, roots) that are responsive to  
387 contemporary N input manipulation (Fang et al., 2006; Johannisson and Hogberg, 1994; Pardo et al., 2002) compared to  
388 the large, long-lived and less responsive compartments (e.g. bark and wood). Such changes in plant  $\delta^{15}\text{N}$  could be a  
389 result of fractionation processes, but alternatively it may originate from uptake and incorporation of the added N  
390 fertilizer, that had an enriched  $^{15}\text{N}$  signature (-0.7 ‰) relative to  $\delta^{15}\text{N}$  of the plants (e.g. -4 to -6 ‰ in leaves).

391 Assuming fractionation effects are minor, the decadal N addition with  $\delta^{15}\text{N}$  value of -0.7 ‰ can be viewed as a  
392 tracer addition, since it differs from the abundance in the major ecosystem pools. Based on a  $^{15}\text{N}$  mass balance  
393 calculation (Nadelhoffer and Fry, 1994), and using the control plots as reference, the fraction of added N that was  
394 incorporated into plants could be estimated (Table S2). Since the calculation relies on the difference in  $\delta^{15}\text{N}$  between the  
395 control and the N-plots in the target pool, it is only meaningful when this difference is significant. Thus, the fraction of

396 added N incorporated could only be estimated for the total plant N pool, but not for the soil (Fig. 2). The results showed  
397 that ~15 % of the total 500 kg N ha<sup>-1</sup> added over a decade was incorporated into plant pools in both forests. For BF this  
398 was less than the estimated fate (24 % to plants) of a stronger tracer (Gurmesa et al., 2016). Nevertheless, it indicates  
399 substantial incorporation of input N into plants in BF even though the N addition did not increase the net uptake in the  
400 forest, i.e. no change in %N in plant compartments at BF.

401 For soils, N addition tended to decrease  $\delta^{15}\text{N}$ , opposite to results in other long-term experimental N addition  
402 (Högberg, 1991; Högberg et al., 1996, 2011) where soil  $\delta^{15}\text{N}$  increased after addition of N. The authors explained that  
403 the increase was the result of fertilizer-induced fractionation due to increased N transformation rates. In our study,  
404 fractionation may also occur, but with the decreasing tendency of soil  $\delta^{15}\text{N}$  indicates incorporation of the isotopically  
405 lighter added N (relative to the soil) is likely as discussed by Högberg et al. (2014).

406 The result supports our second hypothesis that the added N is incorporated into the ecosystem N pools with  
407 plant (and soil)  $\delta^{15}\text{N}$  changing slowly toward the  $^{15}\text{N}$  signature of the decadal N addition. This again highlights the  
408 importance of the  $^{15}\text{N}$  signature of input N in controlling ecosystem  $\delta^{15}\text{N}$ .

409

#### 410 4.4 Effects of forest type

411 As expected from previous studies, the BF is more N-rich than the PF as indicated by higher %N in major ecosystem  
412 pools in BF (Table 3). Accordingly, plant %N in short-lived compartments (and in the pool weighted plant pools) did  
413 not respond to the decadal N addition in BF, whereas plant %N in PF tended to increase, though only significantly in  
414 understory plants (Table 3, Fig. 2a). In the BF, the plant tissues were apparently saturated with N, while the PF still  
415 could retain part of the addition (Fang et al., 2009). Most plant compartments in BF are more  $^{15}\text{N}$ -enriched than the PF  
416 (Table 4) and the change in plant  $\delta^{15}\text{N}$  after decadal N addition was most pronounced in PF (Fig. 2a). This again could  
417 hint a difference in N status, where the larger changes in plant  $\delta^{15}\text{N}$  in the PF indicate larger incorporation of added N  
418 into plants in PF than in BF in agreement with our third hypothesis.

419 The difference under ambient conditions may in part be related to higher N cycling rates and subsequent  
420 losses of the lighter  $^{14}\text{N}$  in the BF through fractionating processes, and subsequent plant uptake of  $^{15}\text{N}$ -enriched soil N  
421 (Magill et al., 2000; Zhang et al., 2008; Nadelhoffer and Fry, 1994). On the other hand, leaf  $\delta^{15}\text{N}$  in PF can be more  
422 affected by  $^{15}\text{N}$ -depleted deposition as the forest is still expanding in biomass and has lower N availability, thus it might  
423 depend more on the  $^{15}\text{N}$ -depleted atmospheric N input than the BF does. An additional explanation could be that the PF  
424 is dominated by *Pinus massoniana*, which has ectomycorrhizal fungi whereas majority of the plants in the BF have  
425 arbuscular mycorrhizal association (Gurmesa, 2016), and ectomycorrhizal plants are found to be more  $^{15}\text{N}$ -depleted than  
426 arbuscular mycorrhizal plants (Craine et al., 2009; 2015a).

427 Soil  $\delta^{15}\text{N}$  did not significantly differ between the BF and PF (Table 4; Fig. 2b), although we expected soil to be  
428 more  $^{15}\text{N}$ -enriched in the BF than in the PF. Soil  $\delta^{15}\text{N}$  are reported to increase with organic matter age (Bauer et al.,  
429 2000), and we expect soil organic matter of the top soil to be older in the BF, because this layer might have been lost by  
430 erosion in the PF as it could be noted from the lower C, N and P concentration (Table 1), and lack of depth pattern of  
431 soil  $\delta^{15}\text{N}$  in the PF (Fig 2b). A common feature in soil profiles is  $^{15}\text{N}$ -enrichment with soil depth (Bauer et al., 2000;  
432 Emmet et al., 1998; Koba et al., 2010; Boeckx et al., 2005) as observed in the undisturbed BF, but not in the disturbed  
433 PF (Table 4). The absence of a  $^{15}\text{N}$ -enrichment profile may again be an effect of erosion and soil mixing from human  
434 disturbances that may shape soil N and  $\delta^{15}\text{N}$  patterns over ecosystem succession (Perakis et al., 2015). The  $^{15}\text{N}$ -

435 enrichment with depth is known to occur as a result of fractionation followed by removal of lighter  $^{14}\text{N}$  by plants,  
436 microbes, or through leaching following decomposition, whilst the  $^{15}\text{N}$ -enriched N fraction is transported and  
437 accumulated at deeper soil profile (Högberg et al., 2011; Hobbie and Högberg, 2012; Nadelhoffer et al., 1988).

438

## 439 **5 Conclusion**

440 We show that forests at DHSBR (and other humid tropical forests of southern China) are likely  $^{15}\text{N}$ -depleted due to  
441 imprints from  $^{15}\text{N}$ -depleted N deposition, particularly  $\text{NH}_4^+$ -N in the region. This effect of the input N (deposition)  $^{15}\text{N}$   
442 signature was further supported by our observation that  $\delta^{15}\text{N}$  of plants (and soil) were changed toward the  $^{15}\text{N}$  signature  
443 of added fertilizer N, which also shows that fertilizer additions are incorporated into forest N pools even at high N  
444 availability. We found that broad-leaved forests and early successional forests differ in their %N and  $\delta^{15}\text{N}$ , and  
445 accordingly differ in their response to increased N input. The significant changes in plant  $\delta^{15}\text{N}$  toward the  $\delta^{15}\text{N}$  value of  
446 the added N observed in both forests indicate that the  $^{15}\text{N}$  signature of incoming N could dominate the effects from  
447 fractionation during the steps of N cycling. Thus, the  $^{15}\text{N}$  imprint of increased N deposition should be considered in  
448 using ecosystem  $\delta^{15}\text{N}$  to interpret ecosystem N cycling characteristics, particularly in regions with high N emissions.

449

450 **Authors' contribution:** Gundersen P. and Mo J. conceived and designed the experiments. Gurmesa A.G., Lu X., Mao  
451 Q. and Zhou K. performed the data acquisition. Gurmesa A.G. analyzed the data. Gurmesa A.G. and Gundersen P.  
452 wrote the manuscript. Lu X., Fang Y. and Mo J. commented and edited the article.

453

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

455

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