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Species Differences in Nitrogen Acquisition in Humid Subtropical Forest Inferred From ^{15}N Natural Abundance and Its Response to Tracer Addition

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Abstract: Differences in nitrogen (N) acquisition patterns between plant species are often reflected in the natural ^{15}N isotope ratios ($\delta^{15}N$) of the plant tissues, however, such differences are poorly understood for co-occurring plants in tropical and subtropical forests. To evaluate species variation in N acquisition traits, we measured leaf N concentration (%N) and $\delta^{15}N$ in tree and understory plant species under ambient N deposition (control) and after a decade of N addition at 50 kg N ha^{-1} yr^{-1} (N-plots) in an old-growth subtropical forest in southern China. We also measured changes in leaf $\delta^{15}N$ after one-year of ^{15}N addition in both the control and N-plots. The results show consistent significant species variation in leaf %N in both control and N-plots, but decadal N addition did not significantly affect leaf %N. Leaf $\delta^{15}N$ values were also significantly different among the plant species both in tree and understory layers, and both in control and N-plots, suggesting differences in N acquisition strategies such as variation in N sources and dominant forms of N uptake and dependence on mycorrhizal associations among the co-occurring plant species. Significant differences between the plant species (in both control and N-plots) in changes in leaf $\delta^{15}N$ after ^{15}N addition were observed only in the understory plants, indicating difference in access (or use) of deposited N among the plants. Decadal N addition had species-dependent effects on leaf $\delta^{15}N$, suggesting the N acquisition patterns of these plant species are differently affected by N deposition. These results suggest that co-occurring plants in N-rich and subtropical forests vary in their N acquisition traits; these differences need to be accounted for when evaluating the impact of N deposition on N cycling in these ecosystems.

Keywords: N deposition; ^{15}N natural abundance; subtropical forest; tree species; China

1. Introduction

Enhanced atmospheric nitrogen (N) deposition into forest ecosystems from increased anthropogenic emissions of reactive N [1] has several cascading negative ecological effects on the forest ecosystems including increased N leaching to waters, soil acidification, increased N gas losses from soil and changes in biodiversity [2]. Tropical forests have a disproportionately large effect on the global

carbon (C) and N cycles, and enhanced N deposition is likely to have rapid and deleterious effects on these ecosystems that are naturally regarded as N-rich [3]. The responses of forest ecosystems to N deposition greatly depend on N status of the forests [4]. Plant species can affect N status of forest ecosystems through direct mechanisms including fixing atmospheric N_2, sequestering varying amounts of N in plant biomass, altering the distribution of N to aboveground and belowground plant parts [5,6], and controlling the chemical quality of the litter produced [7]. While study of effects of individual tree species on soil processes using pure stands (plantation forest) of the individual tree species is important to minimize bias due to species interaction [8,9], it may not reveal the species effects that occur when the species co-exist and interact with each other, as in the case of many natural forests. For example, in old-growth forests with multiple co-occurring species in subtropical monsoon climates, the effects of individual species on soil processes are difficult to assess due to the aforementioned species interactions although the functional diversity of these ecosystems has an implication for below- and above-ground processes [10]. On the other hand, there is a need to understand how these ecosystems respond to global change factors such increased N deposition, which is a recognized threat to plant diversity [11] in part through imbalanced nutrition. Ecosystems thought of as not N-limited, such as (sub)tropical systems, may be more vulnerable, and one way to understand and predict how these ecosystems with high taxonomic diversity respond to changes in N deposition is to investigate the effects of N deposition on N cycling traits (e.g., N acquisition) of the co-occurring plant species in the forests. However, to our knowledge, only a few studies have attempted to investigate the effects of enhanced N deposition on N acquisition of (sub)tropical forest plants at the species level [12,13].

Species variation in ^{15}N:^{14}N ratio (natural ^{15}N abundance expressed as $\delta^{15}N$) of plant tissues both within and between sites [14–16] has been explained as representing species differences in N acquisition mechanisms, such as differences in sources and forms of N taken up by the plants [17]. For example, plants that mainly use atmospherically deposited ^{15}N-depleted N may have lower $\delta^{15}N$ values compared to those that mainly use ^{15}N-enriched (relative to atmospheric N) soil N. Similarly, some studies, mostly in predominately N-limited temperate and boreal forests, have shown that leaf $\delta^{15}N$s of mycorrhizal plants are often more ^{15}N-depleted than those of non-mycorrhizal plants [18–20]. Within mycorrhizal groups, leaf $\delta^{15}N$ is more ^{15}N-depleted in ectomycorrhizal plants than in arbuscular mycorrhizal plants due to stronger fractionation in the ectomycorrhizal plants during fungus-to-plant N transfer [18–20]. On the other hand, high within-site species variation in leaf $\delta^{15}N$ (10‰) has been observed in the extremely nutrient-poor tundra ecosystem [21], which was thought to reflect species differences in N acquisition mechanisms (i.e., that they access different N sources with distinct ^{15}N signature) due to competitive partitioning of the overall N pool. Thus, it was suggested [21] that species variation in leaf $\delta^{15}N$ could be minor in N-rich forests as all plant species may display similar N acquisition mechanisms, i.e., rely on common pools of inorganic soil N. However, in some N-rich (sub)tropical forests including those in South America [22,23] and Asia [24], where competition for N sources is not expected due to the abundant N availability in the soil, species leaf $\delta^{15}N$ range has been reported to be comparable to that observed in N-limited ecosystems [21]. The primary underlying mechanisms in these naturally N-rich ecosystems for species variation in leaf $\delta^{15}N$ and its interpretation when evaluating N status and N cycling in the ecosystems are yet to be explored.

This study evaluates differences in $\delta^{15}N$ among five co-occurring tree species and seven understory plant species in a subtropical forest using an ongoing N addition experiment that was established in an old-growth forest in Dinghushan Biosphere Reserve (DHSBR), southern China, in July 2003 [25]. We examined species variation in $\delta^{15}N$ in all tree compartments (leaf, twigs, branches, bark and wood) and leaf $\delta^{15}N$ in the understory plants under ambient N deposition (control) and after decadal N addition (N-plots), but we focus primarily on leaf $\delta^{15}N$, as do most other studies [17]. We also examined the variation in leaf $\delta^{15}N$ response to a one-year addition of ^{15}N-enriched N (added as a tracer) in both treatments. With these three data sets (control, N-plots, and after the one-year ^{15}N addition in both treatments), we aim to understand species N acquisition differences in old-growth subtropical forests based on their $\delta^{15}N$ and its response to experimentally manipulated N deposition. Our objectives are:

(1) to evaluate species variation in $\delta^{15}N$ natural abundance among the co-occurring plant species under ambient N deposition (control) as an indicator of long-term differences in N acquisition and cycling traits such as fractionation during uptake and assimilation of soil N and/or deposited N; (2) to evaluate species variation in $\delta^{15}N$ of the plant species in response to a decade of higher N availability (N-plots) with $\delta^{15}N$ of −0.7‰ in added N which, if taken up, is expected to increase (move towards zero) plant $\delta^{15}N$, and (3) to evaluate species variation in leaf $\delta^{15}N$ after a year-long addition of ^{15}N-enriched N fertilizer to both control and N-plots, which could imply short-term species differences in access to and/or uptake of deposited and added N. We hypothesized that: (1) species variation in leaf $\delta^{15}N$ would be significant under both ambient and decadal N addition due to differences in N acquisition mechanism including the primary sources and forms of N used by the plants, and; (2) the response of leaf $\delta^{15}N$ to N and ^{15}N addition would also be different among the plant species, which could imply species differences in access to and/or uptake of deposited and added N.

2. Materials and Methods

2.1. Study Site

The study was conducted in the Dinghushan Biosphere Reserve (DHSBR) in southern China (112°33′ E and 23°10′ N) which occupies an area of approximately 1200 ha. This site has a subtropical monsoon climate with mean annual temperature of 22.2 °C and mean annual precipitation of 1927 mm. Atmospheric N deposition (measured as inorganic N in bulk precipitation) in the reserve since the 1990s has ranged from 21 to 38 kg N ha^{-1} yr^{-1} [26,27]. Recent measurement shows a high total wet N deposition of 51 kg N ha^{-1} yr^{-1} [28]. The soil is lateritic red earth (Oxisol) with a pH value of ~4 and a C:N ratio of 11 in the upper 0–30 cm mineral soil [29]. The forest we used for this study is an old-growth broadleaved forest that covers about 20% of reserve. In terms of vegetation composition, the forest consists of several co-occurring plant species (having the high species diversity typical of humid subtropical forests) in canopy and understory layers that form a complex canopy structure. The most common tree species in the canopy and sub-canopy layers are *Castanopsis chinensis* (Spreng.) Hance, *Cryptocarya chinensis* (Hance) Hemsl., *Memecylon ligustrifolium* Champ. ex Benth., *Syzygium rehderianum* Merr. & L.M.Perry, *Syzygium acuminatissimum* (Blume) DC., *Machilus chinensis*, and *Schima superba*, [30], which represent more than 90% of the total basal area. The understory layer consists of many species (high taxonomic diversity) with the dominant ones including *Alpinia chinensis* (Retz.) Roscoe, *Blastus cochinchinensis* Lour., *Calamus rhabdocladus* Burret, *Cryptocarya concinna* Hance, *Tectaria harlandii* (Hook.) C.M.Kuo, *Maesa salicifolia* E.Walker and *Aidia canthioides* (Champ. ex Benth.) Masam [30], which represent various growth forms including woody plants (mostly >1 m), lianas and herbs (Table 1).

2.2. Experimental Design

We used an ongoing N addition experiment established in the forest in July 2003 [25]. The N addition experiment consisted of four treatments: a control and three N addition treatments at 50, 100 and 150 kg N ha^{-1} yr^{-1}, each with three replicates. In this study, we used only the control plots and the lower N addition plots at 50 kg N ha^{-1} yr^{-1} (hereafter referred to as N-plots). Each experimental plot was 10 m × 20 m, laid out on a mountain slope with at least a 10-m-wide buffer strip to the next adjacent plots. The N-plots have been receiving NH_4NO_3 since July 2003. The fertilizer is dissolved in 20 L of water and added monthly below the canopy using a backpack sprayer. The control plots received the equivalent 20 L of water alone. The NH_4NO_3 fertilizer has a $\delta^{15}N$ value (−0.7‰) close to that of atmospheric N_2 (0‰). From March 2013 to February 2014, ^{15}N-enriched (99.5% atom ^{15}N) $^{15}NH_4{}^{15}NO_3$ was added monthly to both control and N-plots. The monthly ^{15}N tracer dose was mixed with the fertilizer in the N-plots and with the 20 L water in the control plots. The total amount of added ^{15}N (100 mg ^{15}N m^{-2} split in 12 equal monthly doses) was determined so that it would significantly

alter ecosystem $\delta^{15}N$ without significant disturbance of the N pools and fluxes in the control plots or further aggravating effects of the ongoing N addition in the N-plots.

2.3. Sampling and Analysis of Samples

Leaves and other plant compartments were sampled from all the dominant tree species (except *Machilus chinensis*, and *Schima superba*, which were too tall to be sampled) and only leaves were sampled from all the dominant understory plant species (Table 1) in January 2013 and again in June 2014—four months after the last year-long monthly ^{15}N addition. A small branch per tree species per plot was cut from the dense canopy layer using a pole pruner and was separated into leaves (current year and older), twigs (<2 cm) and small branches (>1 cm). For each tree species, a wood core was sampled from the same tree (from which the branch was sampled) using an increment borer and was divided into bark and wood. Leaves of understory plants were cut with a knife from three to four individual plants and were bulked per species. Two mineral soil cores (0–30 cm) per plot were sampled using an auger (5.1 cm in diameter). The two soil cores were analyzed separately but only plot means of $\delta^{15}N$ were used. Living fine roots (<2 mm) were hand-sorted from these soil cores but could not be separated into different species.

All samples were oven-dried at 70 °C, and ground to a fine and homogeneous powder. Subsamples were dried at 105 °C, and all results are reported on a 105 °C basis. Nitrogen (and C to determine C:N) concentrations (%N) and $\delta^{15}N$ of the samples were determined simultaneously on an isotope ratio mass spectrometer (Isoprime 100, Isoprime Limited, Manchester, UK) coupled to an automatic online elemental analyzer (vario ISOTOPE cube, Elementar, Langenselbold, Germany). We used IAEA-600 and wheat flour with $\delta^{15}N$ of 1.00 and 2.85‰ to correct the measured $\delta^{15}N$ of the samples.

2.4. Calculations and Statistics

The $\delta^{15}N$ data obtained after the one-year of ^{15}N addition (June 2014) were adjusted to express the change in leaf $\delta^{15}N$ by subtracting the $\delta^{15}N$ found prior to the ^{15}N addition in both treatments (January 2013). Species differences in leaf $\delta^{15}N$ among the tree species and understory plant species were separately analyzed using one-way-analysis of variance (ANOVA). Tukey's HSD pairwise comparison test was used to test for significant differences among tree species, and understory plant species. Effects of N addition (control vs. N-plot) on leaf $\delta^{15}N$ of individual species were determined by a simple *t*-test. For ANOVA analyses, all ANOVA assumptions were fulfilled. All analyses were completed using R 3.2.0.

3. Results

3.1. Leaf %N

Leaf %N and C:N ratio in both tree and understory layers differed significantly ($p < 0.001$) among the studied species in both control and N-plots (Table 1). The studied tree species displayed two separate groups with *Syzygium acuminatissimum*, *Castanopsis chinensis*, and *Cryptocarya chinensis* having higher leaf %N (and a lower C:N ratio) than *Memecylon ligustrifolium* and *Syzygium rehderianum* (Table 1). Of the understory plants, *Cryptocarya concinna* had the highest leaf %N, which was significantly different from *Alpinia chinensis*, *Calamus rhabdocladus*, and *Maesa salicifolia*. The shrub *Maesa salicifolia* had significantly lower leaf %N than all the other understory plants. Decadal N addition did not cause significant change in leaf %N of any of the plant species investigated in both canopy and understory layers (Table 1).

Table 1. Mean leaf %N and C:N ratio for dominant tree species and understory plant species in the control and N-plots at Dinghushan Biosphere Reserve (DHSBR) sampled in January 2013. Values in parentheses show SE among plots (n = 3). Different lowercase letters for each variable within each treatment plots and each plant group (tree and understory) indicate significant differences among plant species ($p \leq 0.05$). No significant differences in leaf %N or C:N ratio were observed among control and N-plots for any species.

Species Name	Growth Form	%N		C:N ratio	
		Control	N-plots	Control	N-plots
		Trees			
Syzygium acuminatissimum	Tree	1.95 (0.09)a	2.06 (0.04)a	25.5 (1.0)b	23.7 (0.4)c
Castanopsis chinensis	Tree	1.94 (0.08)a	1.76 (0.02)b	24.9 (1.1)b	27.2 (0.2)bc
Cryptocarya chinensis	Tree	1.97 (0.00)a	1.95 (0.12)ab	25.9 (0.2)b	26.5 (1.7)bc
Memecylon ligustrifolium	Tree	1.34 (0.04)b	1.35 (0.08)c	32.2 (0.9)a	32.4 (1.9)ab
Syzygium rehderianum	Tree	1.37 (0.02)b	1.33 (0.04)c	34.3 (0.6)a	36.0 (1.0)a
		Understory plants			
Alpinia chinensis	Herb	1.40 (0.10)c	1.53 (0.02)c	32.5 (3.3)a	29.2 (0.3)a
Blastus cochinchinensis	Woody shrub	2.09 (0.08)ab	2.15 (0.03)ab	21.3 (0.7)b	20.5 (0.2)c
Calamus rhabdocladus	Liana	1.86 (0.06)b	1.58 (0.03)c	24.3 (0.9)b	28.2 (0.5)a
Cryptocarya concinna	Understory tree	2.50 (0.10)a	2.34 (0.05)a	20.2 (0.8)b	21.5 (0.5)c
Tectaria harlandii	Fern	2.16 (0.04)ab	2.13 (0.00)ab	20.0 (0.3)b	20.7 (0.1)c
Maesa salicifolia	Shrub	2.01(0.02)b	1.96 (0.04)b	23.6 (0.2)b	24.3 (0.6)b
Aidia canthioides	Understory tree	2.23 (0.06)ab	2.18 (0.07)ab	20.6 (0.6)b	21.0 (0.7)c

3.2. $\delta^{15}N$ Values of Tree Compartments

Tree species had a significant ($p < 0.001$) effect on leaf $\delta^{15}N$ in both control and N-plots (Figure 1). In control plots, a significant difference ($p = 0.01$) was observed between *Syzygium rehderianum* and *Castanopsis chinensis*, which had the lowest (−5.2 ± 0.5‰) and highest (−3.0 ± 0.4‰) leaf $\delta^{15}N$s, respectively (Figure 1a, white bar). A similar difference between the two species was observed in the N-plots (Figure 1a, shaded bars). In the N-plots, leaf $\delta^{15}N$ of *Memecylon ligustrifolium* also differed significantly from that of *Castanopsis chinensis* (Figure 1; shaded bars). Nitrogen addition generally increased leaf $\delta^{15}N$, i.e., it moved the $\delta^{15}N$ towards the ^{15}N signature of the added N, with significant change in leaves of *Syzygium acuminatissimum* at $p \leq 0.05$ and *Castanopsis chinensis* at $p \leq 0.1$ (Figure 1a, shaded bars).

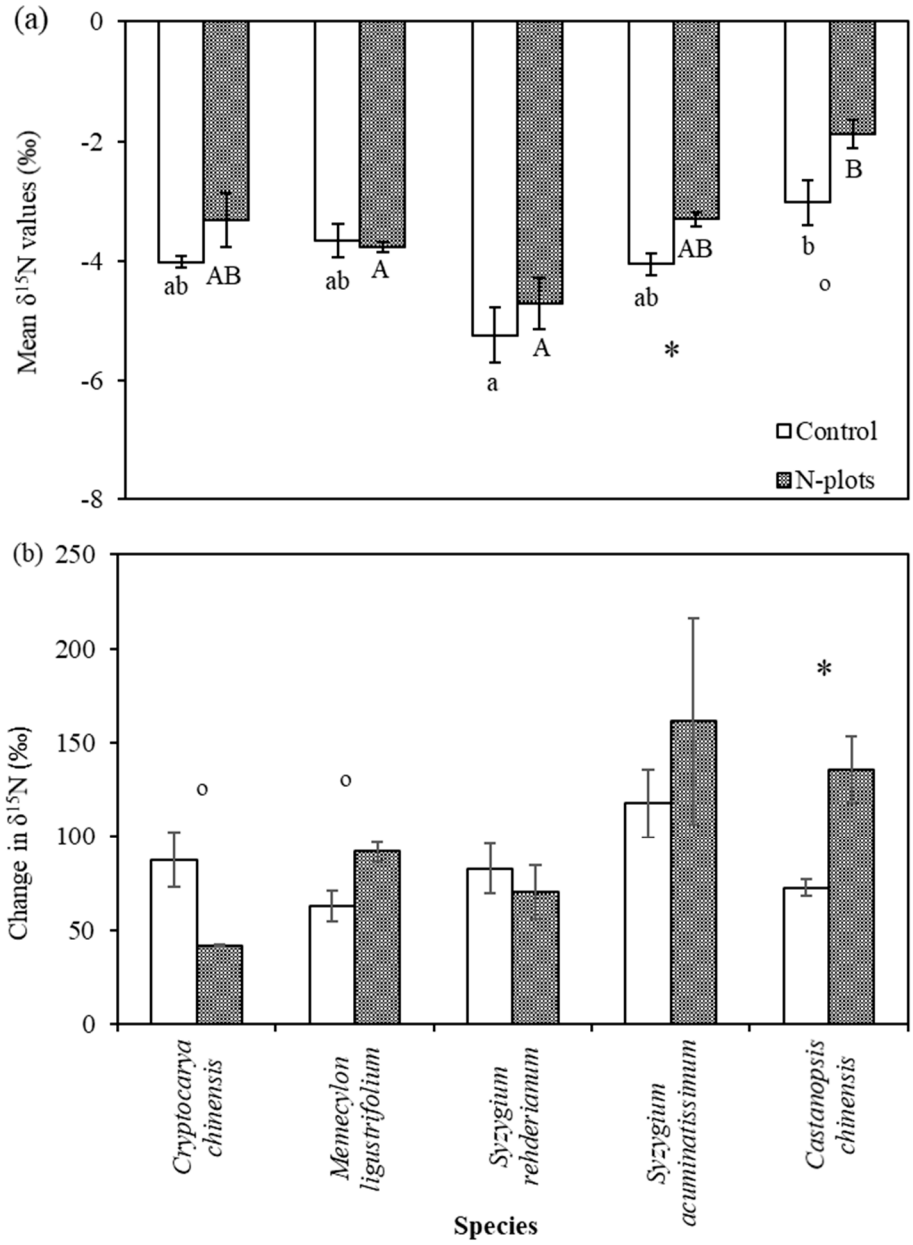

Figure 1. Leaf $\delta^{15}N$ values of co-occurring dominant tree species in Dinghushan Biosphere Reserve (DHSBR): (**a**) natural $\delta^{15}N$ abundance in control and N-plots; and (**b**) changes in leaf $\delta^{15}N$ values after one year of ^{15}N tracer addition in both treatments. The change in leaf $\delta^{15}N$ values in (**b**) was determined by subtracting the $\delta^{15}N$ found prior to the ^{15}N addition presented in (**a**) from the leaf $\delta^{15}N$ values obtained after the one-year ^{15}N tracer addition in each treatment. Error bars indicate SE among plots ($n = 3$). In graph (**a**), tree species means with different letters indicate significant species differences ($p < 0.05$) in the control plots (lowercase letters) and in the N-plots (uppercase letters). In graph (**b**), species differences are not significant. In both (**a**) and (**b**), significant effects of N addition on leaf $\delta^{15}N$ of a given species are indicated by * ($p < 0.05$) and ^{o} ($p < 0.1$).

Leaf ^{15}N values of all studied tree species were significantly increased above their natural abundance ($\delta^{15}N$) values after ^{15}N addition, as indicated by the changes in leaf $\delta^{15}N$ of the tree species (Figure 1b). Leaf $\delta^{15}N$ of the tree species obtained after the one-year ^{15}N addition ranged from 40‰ to 160‰ (Table S1). There was no apparent significant species difference in the changes in leaf $\delta^{15}N$ in either control and N-plots although leaf $\delta^{15}N$ of the two tree species (*Syzygium acuminatissimum* and

Castanopsis chinensis) that were more affected by the decadal N addition (Figure 1a) also showed larger changes in leaf $\delta^{15}N$ after the ^{15}N addition, particularly in the N-plots (Figure 1b, shaded bars).

For all tree species, $\delta^{15}N$ increased from leaves to wood with visible variation among the five tree species in both control and N-plots and across tree compartments (Figure 2). $\delta^{15}N$ values of all tree compartments lay between $\delta^{15}N$ values of N in precipitation and soil N. Species variation in the twig $\delta^{15}N$ was similar to the variation observed for leaf $\delta^{15}N$ (Figure 1a). *Syzygium rehderianum* and *Memecylon ligustrifolium* had higher leaf C:N; *Syzygium rehderianum* tended to have the lowest $\delta^{15}N$ across all compartments while *Memecylon ligustrifolium* also tended to be separated from the three other species with lower C:N ratios of 24 to 27 (Table 1).

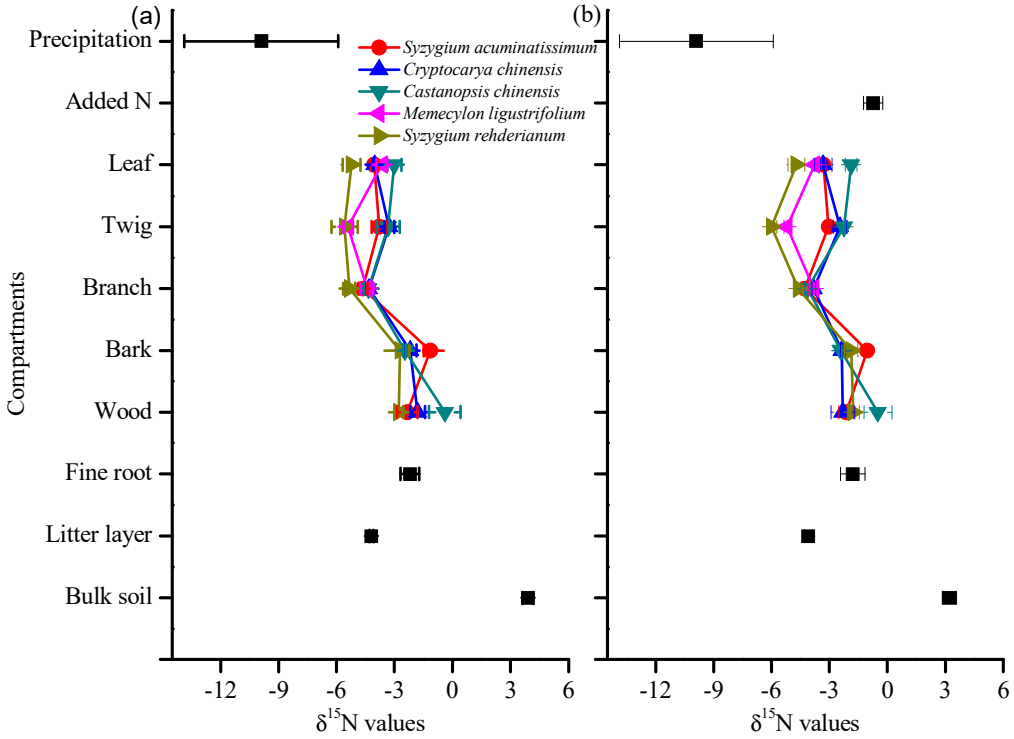

Figure 2. $\delta^{15}N$ values of tree compartments of dominant co-occurring tree species in Dinghushan Biosphere Reserve (DHSBR) sampled prior to ^{15}N addition: (**a**) in the control plots; and (**b**) in the N-plots including the $\delta^{15}N$ values of the N source end points (precipitation, added N and bulk soil). $\delta^{15}N$ of N in precipitation are from Gurmesa (2016) [28]. Error bars indicate SE (n = 3).

The five tree species constitute different proportions of tree biomass in the experimental plots (Table S2). Thus, we calculated the N pool weighted plant $\delta^{15}N$ for the trees and the results showed significant species variation in N pool weighted plant $\delta^{15}N$ in both control (p = 0.01) and N-plots ($p < 0.01$) (Figure S1), which is similar to the pattern observed in leaf $\delta^{15}N$ (Figure 1a).

3.3. Leaf $\delta^{15}N$ Values of Understory Plants

As in the tree layer, understory leaf $\delta^{15}N$ values differed significantly ($p < 0.001$) among the plant species in both control (Figure 3a, white bars) and N-plots (Figure 3a, shaded bars). In the control plots, *Blastus cochinchinensis* had the lowest leaf $\delta^{15}N$ value (−6.6 ± 0.2‰), which was significantly lower than that of all the other species. *Maesa salicifolia* had the highest leaf $\delta^{15}N$ value (−1.6 ± 0.4‰), which was significantly different from that of the other species, except *Alpinia chinensis*. Nitrogen addition significantly increased leaf $\delta^{15}N$ of *Alpinia chinensis*, *Tectaria harlandii* and *Maesa salicifolia* (Figure 3a, white bars vs. shaded bars). Leaf $\delta^{15}N$ of the understory species after the year-long ^{15}N tracer addition ranged from 170‰ to 1070‰ (Table S1). Thus, the ^{15}N addition significantly increased the ^{15}N abundance of all sampled understory plant species with *Blastus cochinchinensis*, *Alpinia chinensis* and

Tectaria harlandii showing a stronger increase in leaf ^{15}N in the control plots (Figure 3b, white bars). Similar species differences in the change in $\delta^{15}N$ were apparent in the N-plots (Figure 3b, shaded bars). Three species, *Tectaria harlandii*, *Maesa salicifolia*, and *Cryptocarya concinna*, showed significantly higher changes in leaf $\delta^{15}N$ in the control plots than in the N-plots (Figure 3b, shaded bars). *Aidia canthioides* had the lowest change in leaf $\delta^{15}N$ compared to the other species in both the control and N-plots (Figure 3b).

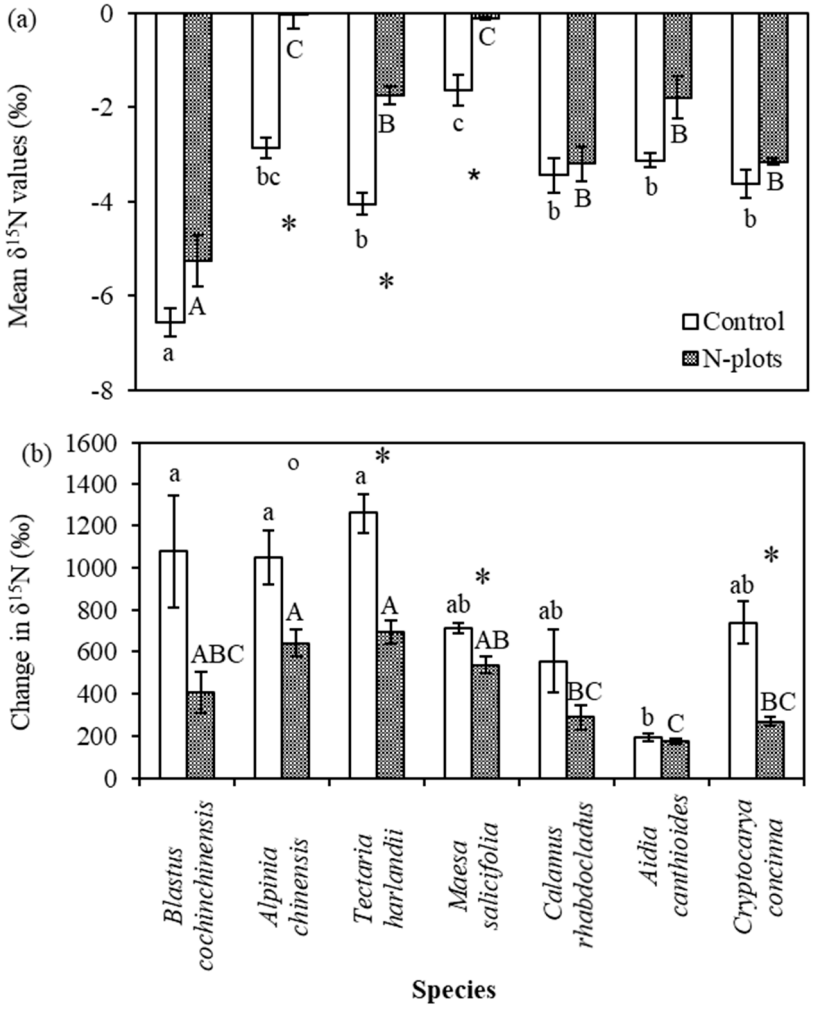

Figure 3. Leaf $\delta^{15}N$ values of dominant co-occurring understory plant species in Dinghushan Biosphere Reserve (DHSBR): (**a**) natural $\delta^{15}N$ abundance in control and in N-plots; and (**b**) changes in leaf $\delta^{15}N$ values after one year of ^{15}N tracer addition in both treatments. The change in leaf $\delta^{15}N$ values in (**b**) was determined by subtracting the $\delta^{15}N$ found prior to the ^{15}N addition presented in (**a**) from the leaf $\delta^{15}N$ values obtained after the one-year ^{15}N tracer addition in each treatment. Error bars indicate SE (n = 3). For each graph, species means with different letters indicate significant species differences ($p < 0.05$) in control plots (lowercase letters) and in N-plots (uppercase letters). Significant effects of N addition within species are indicated by * ($p < 0.05$) and º ($p < 0.1$).

4. Discussion

4.1. Species Variation in %N and Effects of N Addition

In N-limited ecosystems, variation in leaf %N among co-occurring plant species has been reported to reflect differences in N use efficiency [31] and interspecific competition for N [32]. Since the forest at DHSBR is regarded as N saturated, the cause of species differences in leaf %N observed at different levels of high N availability (control and N-plots) both in the tree and understory layers (Table 1)

is unlikely to be interspecific competition. Instead, the species variation in leaf %N may simply indicate variation in composition of organic N compounds in their tissues as a result of high taxonomic diversity that creates significant chemical and structural variation in the canopy [33], which is often reflected in plant leaf chemistry [34,35]. For example, Phillips et al. [35] showed that variation in leaf %N, phosphorus (P) and the N:P ratio of species within individual tropical forest canopies (trees and large lianas) or plant families exceed that of a biome-wide database on all temperate trees. Lack of significant effects of decadal N addition on leaf %N of any of the plant species we sampled (Table 1) could indicate that the plants are already N-rich, hence N addition may not affect N availability in the forest. A previous study conducted in the old-growth forest at DHSBR showed that plant (tree and understory) growth generally did not show a positive growth response to N addition [36,37], indicating that N is a non-limiting nutrient at the study site. Observed high leaching loss of N, under both ambient and N addition conditions [26,38,39], and soil acidification due to N addition in the forest [40] also confirm that the forest is N saturated as a result of long-term N accumulation and high ambient N deposition >30 kg N ha^{-1} yr^{-1} over the past 15–20 years [26,39,41]. Another reason for the lack of an N addition effect (increase) on leaf %N could be because of P-limitation in the forest, which is evident from high average foliar N:P ratios of leaves (28.3) and forest floors (37) [42], suggesting strong P-limitation at our study site. Thus, in addition to the increased N deposition in the region, P-limitation in the study forest may make the forest sensitive to increased N input with additional external N input only leading to negative consequences.

4.2. ^{15}N-Depleted Plants at DHSBR

Nitrogen-rich forests, like the one we used in this study, are expected to be more ^{15}N-enriched compared to predominately N-limited temperate and boreal forests [43]. This is thought to be due to increased fractionation during soil N transformation processes in N-rich soil that cause gaseous and leaching loss of the isotopically lighter ^{14}N and plant uptake of ^{15}N-enriched residual soil N [44]. Indeed, ^{15}N-enriched leaf δ^{15}Ns (>3‰) have been observed in tropical plants in the Amazonian basins [22,45,46]. In contrast to this expectation for tropical forests, leaf δ^{15}N values of all the studied plant species at DHSBR are ^{15}N-depleted (−3‰ to −7‰) (Figures 1a and 3a), even compared to the average leaf δ^{15}N values (~ −3‰) reported for global temperate forests [43]. A previous study in the same old-growth forest at DHSBR reported similar ^{15}N-depleted (−3‰ to −7‰) leaf δ^{15}N [47]. Similarly, the observed leaf δ^{15}N range at our study site is within the range (−7‰ to 1.3‰) observed in different tropical forests located across southern China [24,48]. Similar ranges indicating low leaf δ^{15}N values have also been reported in eastern Asia, including −4.8% to 0.02% for tropical forests in Malaysia [49] and −6% to −2.2% for a subtropical forest in Taiwan [50]. However, comparison of leaf δ^{15}N among sites and interpretation of any observed difference and/or similarity is not straight forward due to many confounding factors related to site and plant (species-specific) traits [17]. For example, leaf δ^{15}N values of the same plant species appear to vary across forest successional stages [24]. Nevertheless, the consistent ^{15}N-depletion of plants at DHSBR and other subtropical forests in southeastern Asia regardless of their differences in vegetation (species) composition could be caused by ^{15}N-depleted high N deposition, particularly NH_4-N [51]. Our observation of increased δ^{15}N values of the studied plant species following addition of N and ^{15}N confirm the previous observation that δ^{15}N of input N can directly affect plant δ^{15}N [17]. Moreover, increased fractionation during plant uptake under the high N availability in the region [17,52] could contribute to the ^{15}N-depletion of leaf δ^{15}N in these forests.

4.3. Species Variation in δ^{15}N and Its Response to N and ^{15}N Addition

Differences often exist among plant parts (leaves, branches, stems and roots) [53,54] due to multiple assimilation events, organ-specific loss of N, different patterns of N assimilation and reallocation of N that leads to the intra-plant variation in δ^{15}N [52]. Although, the magnitude of the differences may depend on species type [55] and plant functional type [56], any average differences are generally small, and foliar δ^{15}N values are often used as an index of whole plant δ^{15}N. We also observed a

similar species variation pattern between N pool weighted plant $\delta^{15}N$ in trees (Figure S1) and leaf $\delta^{15}N$ (Figure 1a). Thus, we focus on species variation in leaf $\delta^{15}N$, which is more significant compared to the small variation in $\delta^{15}N$ of the other tree compartments both within and among the tree species (Figure 2).

The significant variation in leaf $\delta^{15}N$ of up to 3‰ among understory and tree species in both control and N-plots (Figures 1a and 3a) confirmed our first and second hypotheses that leaf $\delta^{15}N$ would significantly vary among the co-occurring plant species under both ambient and decadal N addition. The species variation in leaf $\delta^{15}N$ observed at our study site is within the range (0–10‰) often observed among co-occurring plant species [57], and it is consistent with results reported from some forests in southeastern Asia [49,50,58]. Given that the range of variation in plant $\delta^{15}N$ observed at a particular site increases with the number of plant species sampled [59], the narrow species variation observed for few plant species (dominant ones that are found in all replicate plots) at our site may not indicate a general pattern in tropical forests. A wider leaf $\delta^{15}N$ range (>10‰) reported in N-rich tropical forests such as those in the Amazon where higher number of plant species were sampled [22,45] evidently indicates biogeochemical heterogeneity of tropical plant species both in single sites and biome-wide. Interestingly, at a larger scale, a considerably larger range of $\delta^{15}N$ has been found in the tropical forests than in temperate forests [22,43]. Thus, different mechanisms may result in high leaf $\delta^{15}N$ ranges in N-limited and N-rich systems. Interpreting variation among species within a site is complicated due to the multiple processes influencing $\delta^{15}N$ values. Various mechanisms including differences in N sources (biological N_2 fixation, depositional N, soil N) and forms of N (e.g., NH_4^+, NO_3^-) utilized [60,61], mycorrhizal dependence [20] and discrimination against ^{15}N during root N uptake and assimilation [55,62] have been proposed to explain species differences in leaf $\delta^{15}N$ within an ecosystem. Here, we discuss some of the plausible mechanisms that could underlie the observed species variation in leaf $\delta^{15}N$ in our study forest.

None of the plant species included in this study are N_2-fixing (i.e., they do not belong to the Fabaceae family), indicating that the plants predominately acquire N from inorganic soil N and/or depositional N, which have different $\delta^{15}N$ values (Table S3). The observed variation in leaf $\delta^{15}N$ among the plants indicates that the studied co-occurring plants uptake different proportions of N sources (deposition or soil) and N forms (NH_4^+, NO_3^-) with different ^{15}N signatures due to species-specific preference and/or due to differences in their ability to access these N forms (e.g., different rooting depth). Moreover, previous studies in the same forest observed that $\delta^{15}N$ of bulk soil N increased with soil depth [41] and the $\delta^{15}N$ values of NH_4^+ and NO_3^- are different within the soil profile [47]. The studied plant species may access soil N from different soil layers (with distinct $\delta^{15}N$ values) because of differences in their rooting patterns that were previously observed for the pioneer plant species in DHSBR [63]. ^{15}N signature of deposited N can alter plant $\delta^{15}N$ when directly acquired by plants on leaf surfaces or by altering the ^{15}N signature of available N in the soil [17,64]. Difference in direct uptake of the deposited N (with multiple N compounds with variable ^{15}N signature) among the plants can also contribute to the observed species variation in leaf $\delta^{15}N$ [64]. We observed that leaf $\delta^{15}N$ of all the studied species increased after decadal N addition in both tree (Figure 1a, shaded bars) and understory layers (Figure 3a, shaded bars), but the magnitude of the changes were different. This confirms part of our second hypothesis that the response of leaf $\delta^{15}N$ to N would be different among the plant species, and it suggests differences among the plant species in access to (use of or dependence on) external N input (deposited or added N). Our second hypothesis was further confirmed by the observed species difference in leaf $\delta^{15}N$ in response to the year-long ^{15}N tracer addition, especially in the understory plants, where the species difference was significant in both control and N-plots (Figure 3b). The change in leaf $\delta^{15}N$ after ^{15}N addition was higher in the understory plants than in the tree (Figures 1b and 3b), indicating understory plants have more access to the added ^{15}N. Within understory plants, smaller understory species that were directly spayed on (*Blastus cochinchinensis, Calamus rhabdocladus and Cryptocarya concinna*) showed larger changes in leaf $\delta^{15}N$ compared to the other relatively taller plants, indicating likely foliar uptake of the added ^{15}N in the smaller plants (Figure 3b). In the dominant

understory species, the change in leaf $\delta^{15}N$ was generally higher in the control plots than in the N-plots for all species (Figure 3b). Since the added ^{15}N was more diluted in the N-plots (added with fertilizer) than in the control plots, this contrast (higher changes in leaf $\delta^{15}N$ in control plots) was expected in the change in leaf $\delta^{15}N$ assuming similar uptake rates of the added ^{15}N in the two treatments.

Another potential source of the species variation in $\delta^{15}N$ could be the difference in mycorrhizal association [21]. In N-limited ecosystems, ectomycorrhizal plants (ECM) are found to be more ^{15}N-depleted compared to co-occurring arbuscular mycorrhizal plants (AM) [65]. Emerging evidence from N-rich (sub)tropical ecosystems [66,67] and temperate forests subjected to high N deposition [18,68], however, indicate more ^{15}N-enrichment of ECM plants compared with co-occurring AM plants. We found that the two ECM trees (*Syzygium acuminatissimum* and *Castanopsis chinensis*), on average, have significantly lower leaf C:N ratio in both control and N-plots (Table S4) and are more ^{15}N-enriched than the three AM trees (*Cryptocarya chinensis*, *Memecylon ligustrifolium* and *Syzygium rehderianum*) in both control and N-plots (Figure S2). The ^{15}N-enrichment of ECM in N-rich systems is reported to indicate either direct uptake of soil N by the trees without mediation by ectomycorrhiza, or absence of strong ^{15}N-fractionation during the fungal transfer of N to the host trees [65,66,69]. We also found that the change in leaf $\delta^{15}N$ was more pronounced in the ECM trees than in the AM trees (Figure S2), as also reported by a recent tracer study [70], indicating that the ECM trees may have more access to deposited N than AM trees, which in turn suggests that N acquisition of ECM plants may be more sensitive to N deposition than that of AM trees. In the understory layer, only *Blastus cochinchinensis*, the most ^{15}N-depleted with low $\delta^{15}N$ values in both control (−6.6‰) and N-plots (−5.3‰), and *Calamus rhabdocladus* (also with lower $\delta^{15}N$ value) (Figure 3a) were identified as AM plants. Information about mycorrhizal association of the other understory species is not available, thus the pattern between mycorrhizal type and leaf $\delta^{15}N$ in the understory plants could not be discussed. Nevertheless, our findings support previous remarks that plants with different mycorrhizal groups differ in their N cycling, and information about the contrasting N cycling in the mycorrhizal groups could be useful in predicting how these plants will respond to environmental change factors [71]; this could be an important area of research in these ecosystems.

5. Conclusions

Our study found significant species differences in leaf %N and $\delta^{15}N$ among the studied plant species both in the tree and understory layers. The observed species variation in leaf $\delta^{15}N$ was attributed to differences in N acquisition, mainly the primary sources and forms of N used by the different plant species. Leaf %N of all plant species was not significantly affected by decadal N addition, likely because the plant tissues are N-rich due to N saturation of the forest from long-term N accumulation and high ambient N deposition. The species-dependent responses of leaf $\delta^{15}N$ to N and ^{15}N additions were interpreted as representing species differences in accessing or in dependence on N sources and N forms (with different ^{15}N signatures), which are partly related to their differences in mycorrhizal association. The results suggest that N acquisition traits or strategies of these co-occurring plants may differ, and they may also be differently affected by atmospheric N deposition. Our study also highlights that information about the mycorrhizal type of tropical plants (particularly at our study site) could be important to fully understand N acquisition of the plants and its response to changes in N input. However, such information is very scant, and it merits further investigation. Overall, our results highlight the importance of considering plant species variation in subtropical forests characterized by high taxonomic diversity when studying N cycling and its response to N deposition.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/10/11/991/s1, Figure S1: N pool-weighted plant $\delta^{15}N$ of five dominant tree species in Dinghushan Biosphere Reserve (DHSBR) in control plots and N addition plots, Figure S2: Mean leaf $\delta^{15}N$ of arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) trees in Dinghushan Biosphere Reserve (DHSBR); Table S1: Foliar $\delta^{15}N$ values of dominant co-occurring tree and understory plant species in Dinghushan Biosphere Reserve (DHSBR) in control and N-plots before (sampled in January 2013) and after one-year ^{15}N addition (sampled in June 2014) in the two treatments, Table S2:

Estimated leaf biomass (kg ha^{-1}) of dominant tree species in the experimental plots of the old-growth broad-leaved forest at Dinghushan Biosphere Reserve (DHSBR), southern China, Table S3: Mean concentration (mg N L^{-1}) and $\delta^{15}N$ values of NH_4-N, NO_3-N and DON in precipitation, throughfall and soil solution in control plots (September 2012 to February 2013). Table S4: Leaf C:N ratio of the studied tree species grouped as ectomycorrhizal (ECM) and arbuscular mycorrhizal plants (AM).

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