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Bidirectional Nitrogen Transfer and Plant Growth in a Mixed Plantation of N_2-Fixing Species and *Eucalyptus urophylla* × *E. grandis* under Different N Applications

Xianyu Yao [1,2], Uromi Manage Goodale [1,3,4], Mei Yang [1], Liangning Liao [1,2], Sufang Yu [1], Shengqiang Wang [1] and Shaoming Ye [1,*]

[1] Guangxi Key Laboratory of Forest Ecology and Conservation, College of Forestry, Guangxi University, Nanning 530004, China; yaoxianyu@scbg.ac.cn (X.Y.); uromi.manage.goodale@outlook.com (U.M.G.); lyangmei@gxu.edu.cn (M.Y.); llning21@scbg.ac.cn (L.L.); yusufang@gxu.edu.cn (S.Y.); shengqiang@gxu.edu.cn (S.W.)

[2] Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

[3] State Key Laboratory of Conservation and Utilization of Subtropical Agro-Bioresources, College of Forestry, Guangxi University, Daxue East Road 100, Nanning 530004, China

[4] Seed Conservation Specialist Group, Species Survival Commission, International Union for Conservation of Nature, 281196 Gland, Switzerland

* Correspondence: yeshaoming@gxu.edu.cn

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Abstract: N_2-fixing species play a crucial role in mixed-plantations as they improve stand productivity. To quantify the N transfer from N_2-fixing species to *Eucalyptus* (*Eucalyptus urophylla* × *E. grandis*) in N_2-fixing species/*Eucalyptus* plantations, we established a pot experiment and confirmed the occurrence of this process under natural conditions. The ^{15}N was traced in labeled species as well as in neighboring tree species after labeling, and the growth was evaluated in short-term natural trials. Our results showed that a bidirectional N transfer occurred. The amount of net N transfer was 21.8–127.0 mg N plant^{-1}, which was equal to 1.5–21.2% of the total nitrogen (TN) that accumulated in *Eucalyptus* plants under pot conditions, was transferred from *Dalbergia odorifera* to *Eucalyptus*. The amount of N transferred significantly decreased with the increasing N application rate but increased with time after labeling. Compared with the results for the *Eucalyptus* monocrop, the soil N concentration (including NO_3^--N and NH_4^+-N) greatly improved when *D. odorifera* was introduced together with *Eucalyptus* under both field and pot conditions. Furthermore, the results under field conditions were consistent with the results of the pot experiment. The dry matter (DM) yield (14.5–16.4%) and the N content (5.1–9.6%) in *Eucalyptus* increased when mixed together with *D. odorifera*, but the N content in and DM yield of *D. odorifera* slightly decreased. It is concluded that the N transfer between *Eucalyptus* and *D. odorifera* is a much more important dynamic process than previously recognized, and *Eucalyptus* and legume intercropping is a successful management practice because N transfer provides a significant amount of N required for *Eucalyptus* productivity.

Keywords: *Eucalyptus urophylla* × *grandis*; *Dalbergia odorifera*; ^{15}N leaf labeling; nitrogen transfer; nitrogen uptake

1. Introduction

Symbiotic nitrogen (N) fixation plays a key role in local and global N cycling [1]. The resulting N is important and may represent a substantial source of N input in legume/non-legume intercropping/systems [2–4]. Through symbiotic N fixation, legumes increase the amount of soil N [5] and subsequently transfer N resources to non-legumes, resulting in biomass increase [6,7]. In intercropping systems, N transfer has the potential to increase the N content and improve the productivity of neighboring non-legumes [8]. However, an

improved understanding of the underlying mechanisms and factors that govern N transfer is needed to determine potential areas for improving yield in intercropping systems.

N transfer from legumes to non-legumes can occur through various pathways. Non-legumes can receive N via (1) the uptake of mineralized N by neighboring plants following leguminous root decomposition, (2) the uptake of soluble N-containing exudates released from legumes and (3) N transfer may occur from legumes directly via common mycorrhizal networks (CMNs) [9,10]. Although the use of different ^{15}N-based techniques has greatly facilitated investigations of N transfer pathways between adjacent leguminous and non-leguminous plants [9,10], reliable quantification of this complex process is difficult [11]. The amount of N transferred from N-fixing leguminous species to non-leguminous species has been found to range from 3.3–72.0% of legume N [6,12–14], such as from faba bean to wheat [15], from peanut to rice [16] or from *Acacia mangium* to *Eucalyptus* [17]. However, the relative importance of N transfer depends to a great extent on the plant species, N management practices, and methods used to determine N resources. While N transfer may occur through interactions between plants of different species or the same species [11], all plants can lose different types of N-containing compounds through their own detritus, leaves, and root secretions [13,18–20]. Some studies have reported that bidirectional N transfer can occur between non-legumes and legumes in intercropping systems [12,21,22], with legumes being the recipient of N from non-legumes. Schimel and Hättenschwiler further confirmed that net N transfer was more strongly dependent on plant N status than on tree species [23]. For example, N transfer between legumes and non-legumes by 9.93%, 5.65% and 4.22% mainly relied on the N status [16]. In addition, for timber species, there is evidence that legume-derived N is transferred to neighboring non-leguminous trees, such as *Eucalyptus*, in plantation systems [3,6,24], resulting in stimulating the nodulation and N_2 fixation of legumes [25].

Species in the *Eucalyptus* genus are among the most widely used in the global commercial timber industry in tropical and subtropical regions worldwide, including China [26]. Compared with *Eucalyptus* monocrop plantations, mixed-species plantations of *Eucalyptus* and N_2-fixing legumes may be an alternative option [27]. Often, the primary objective of using N_2-fixing species in mixed *Eucalyptus* systems is to increase the N available to the *Eucalyptus* species [2]. Such mixed-species plantations are more sustainable, as they have the potential to improve the soil nutrient cycle [28–30] and increase productivity [31–34] while maintaining soil fertility. Although many studies have proposed the use of this N transfer pathway from legumes to neighboring *Eucalyptus* trees as a silvicultural technique for improving *Eucalyptus* productivity [3,6,24], there is little evidence that N transfer occurs from *Eucalyptus* to N_2-fixing species in mixed plantation systems [22]. Therefore, studies quantifying the bidirectional N transfer and the associated effect on the biomass productivity of *Eucalyptus* are highly important for the sustainable development of these valuable plantation systems.

In this study, ^{15}N-labeled urea was applied to the leaves of the N_2-fixing species *Dalbergia odorifera* (*D. odorifera*) or *Eucalyptus urophylla* × *E. grandis* (*Eucalyptus*) (1) to quantify the short-term interspecific N transfer between *D. odorifera* and *Eucalyptus* and determine the net direction of N transfer in an intercropping system and (2) to analyze the effects of the amount of N transferred on *Eucalyptus* growth. However, the evaluation of N transfer with the same method in natural areas may be difficult, and the N transfer cannot be verified with leaf labeling under field conditions. Therefore, a field experiment was conducted in monocultures and mixed plantations of *Eucalyptus* and *D. odorifera* to analyze the trends in the biomass and N content and to evaluate the influence of species mixing on *Eucalyptus* and *D. odorifera* productivity.

2. Materials and Methods

2.1. Study Site and Experimental Layout

2.1.1. Pot Experiment

Three pot experiments (A, B and C) were carried out on 18 May 2017 at the same experimental site used by Yao et al. [24] and were performed in accordance with a completely randomized block (four blocks in total) design with four treatments for N fertilizer at different application rates (0, 3, 6, and 12 g $CO(^{14}NH_2)_2$ pot^{-1}). Seven days after planting, N fertilizer was dissolved in 500 mL water and applied to the soil when we observed that the seedlings surviving and displaying new root growth. Four replicates were included in each treatment, and every replicate corresponds to a block with 15 pots.

The soil used in the three experiments was taken from a *Pinus massoniana* forest at the Liang Fengjiang Experimental Station, Guangxi, China, and the soil chemical properties before the experiment are presented in Table 1. The soil had a clay loam texture, classified as red soil in Chinese soil classification, and the soil pH of soil sample solutions (1:2.5 soil:water (*w*/*v*) ratio) was measured. The total nitrogen (TN), nitrate nitrogen (NO_3^--N) and ammonia nitrogen (NH_4^+-N) in the soil were determined using a continuous-flow chemical analyzer (AA3); the soil organic carbon (SOC), total phosphorus (TP), available phosphorus (AP), available potassium, calcium (Ca) and magnesium (Mg) contents were determined via inductively coupled plasma spectroscopy, as described by Arnesen [35]. Twenty-five kg of air-dried soil was put into each pot and mixed together with perlite at a ratio of 25:1 (*w*/*w*) to maintain water permeability.

Table 1. Soil chemical properties before the planting of *D. odorifera* and *Eucalyptus*.

Soil Chemical Properties	pH	SOC	TN	TP	AN	AP	AK	pH	Ca^{2+}	Mg^{2+}
Mean	4.7	11.46	1.22	0.57	56.17	6.21	104.32	4.65	51.63	12.94

SOC (g kg^{-1}) = soil organic carbon; TN (g kg^{-1}) = total nitrogen; TP (g kg^{-1}) = total phosphorus; AN (mg kg^{-1}) = available nitrogen; AP (mg kg^{-1}) = available phosphorus; AK (mg kg^{-1}) = available potassium; Ca^{2+} (mg kg^{-1}) = exchangeable calcium; Mg^{2+} (mg kg^{-1}) = exchangeable magnesium.

The bidirectional N transfer in the *D. odorifera*/*Eucalyptus* intercropping system was studied in two separate experiments (A and B). In experiments A and B, a ^{15}N-labeled urea solution was applied to the leaves of either *D. odorifera* or *Eucalyptus* one week after N application. Experiment C was identical to the first two experiments except that the plants received no foliar application of the ^{15}N-labeled urea solution (control (CK) experiment; see Supplementary Figure S1).

Experiment A: *D. odorifera* Foliar ^{15}N Labeling

In this experiment, three-month-old *E. urophylla* × *grandis* and 1-year-old *D. odorifera* trees were potted in a pot with a diameter of 50 cm and a depth of 45 cm on 18 May 2017 (Figure 1). The experimental placement of pots followed Yao et al. [24]. On 3 June 2017, a 0.125 mol/L solution of ^{15}N-labeled urea with 10.32 atom% ^{15}N (^{15}N was provided by the Shanghai Stable Isotope Engineering Research Center, Shanghai, China) was first used to label the surface of the *D. odorifera* leaves under different N fertilizer application rates. To prevent contamination of the soil and neighboring *Eucalyptus*, we following steps were taken: first, we used polyvinyl chloride (PVC) cylinders (height of approximately 80 cm) that were open at both ends to enclose the *D. odorifera* canopy; second, the soil surface was covered with two layers of plastic film to prevent ^{15}N contamination of the soil from runoff of the ^{15}N-labeled solution during foliar application, and a 1.5-cm-thick barrier consisting of a sponge with two layers of filter paper above the plastic film was used to absorb leachates, as described by Shen and Chu [16] and Meng et al. [36]; third, 10 mL of ^{15}N-labeled urea was sprayed onto the leaves as described by McNeill et al. [37], and afterward, the leaves were immediately covered with sealable polythene bags until the next day to avoid ^{15}N contamination of the associated *Eucalyptus* plant. All the ^{15}N-labeling processes were strictly controlled as described above to ensure that there was

no ^{15}N contamination of the soil or associated *Eucalyptus* leaves. Two additional labeling applications with 10 mL of ^{15}N-labeled urea were performed on 9 and 15 June 2017. The N transferred from *D. odorifera* to the associated *Eucalyptus* was calculated from the amount of ^{15}N detected and TN accumulated in *Eucalyptus* in the intercropping system.

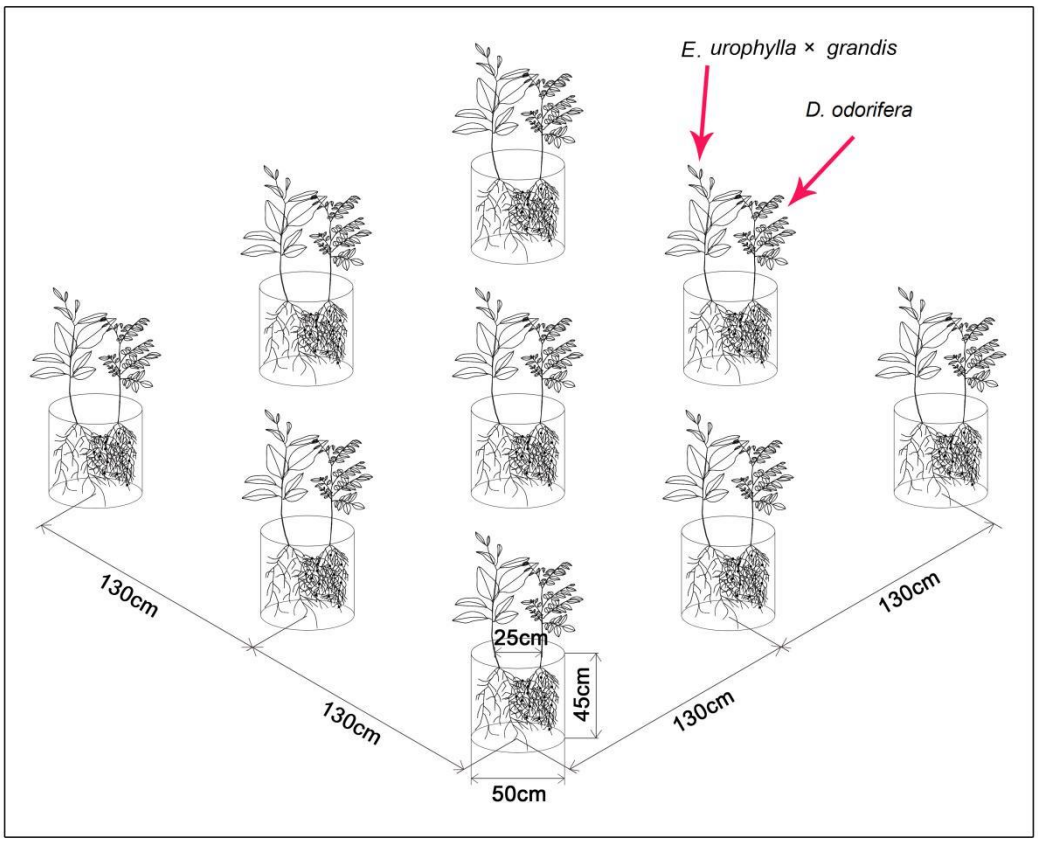

Figure 1. Diagram of *E. urophylla* × *grandis* and *D. odorifera*. Note: In each pot, the plant on the left is *E. urophylla* × *grandis*, and the plant on the right is *D. odorifera*.

Experiment B: *Eucalyptus* Foliar ^{15}N Labeling

The growing conditions in this experiment were similar to those in experiment A; i.e., three N fertilizer application rates (no N and 3, 6, and 12 g pot^{-1}) were added to the soil of the intercropping system. On 3, 9 and 15 June 2017, the leaves of the *Eucalyptus* plants were then treated with 10 mL of 0.75% (m/m) solution of ^{15}N every time in the same manner as the *D. odorifera* leaves were, such that *Eucalyptus* was the donor plant and *D. odorifera* was the receiver plant.

Experiment C: Unlabeling Experiment

Two planting systems, i.e., intercropping and monoculture, were set up here and were used to compare the dry matter (DM) yield of and N accumulation in the two species between the intercropping systems and monocropping systems under different N levels. The growing conditions of this experiment were identical to those of experiments A and B except that the plants received no foliar application of ^{15}N-labeled urea solution.

2.1.2. Field Experiment: DM Yield of and N Content in Plants and Soil N Concentration Analysis

The study site was located at the Experimental Center of Tropical Forestry, Chinese Academy of Forestry (22°07′ N, 106°93′ E), Pingxiang city, Guangxi Zhuang Autonomous Region, China. The mean annual precipitation is approximately 1400 mm; the rainfall occurs mostly from April to September; the mean annual temperature is approximately

21 °C. The soils were formed from granite, classified as red soil in the -Chinese soil classification system, equivalent to oxisol in United States Department of Agriculture (USDA) Soil Taxonomy [38]. The soil has a pH of 4.74 and has the following nutrient contents: TN, 1.13 g kg^{-1}; AN, 54.33 mg kg^{-1}; TP, 0.45 g kg^{-1}; and AP, 5.79 mg kg^{-1}. The determination method was the same as that used for the pot experiment.

Three-month-old *Eucalyptus* and 1-year-old *D. odorifera* seedlings were selected as test materials and planted in February 2015, and the initial establishment of the three treatments was completed in the same year. The treatments (36 plots in total) were 100E (a *Eucalyptus* monocrop), 100D (a *D. odorifera* monocrop) and 50E:50D (a 1:1 ratio of *Eucalyptus* and *D. odorifera*, with the trees of each species planted alternately in each row). The plants of each species were planted in an alternating pattern with a spacing of 2 m in each row and 2.5 m between rows, giving rise to a total planting density of 2000 trees ha^{-1}. In March 2018, an experimental plantation was established in accordance with a completely randomized block design with three replicates. Four N fertilizer treatments and three blocks were established to compare the monospecific and mixed-species stands of *D. odorifera* and *Eucalyptus* in March 2018. The N treatments consisted of no N (N_0) or the addition of 70 kg N ($CO(NH_2)_2$) ha^{-1} (N_{70}), 140 kg urea ha^{-1} (N_{140}), and 210 kg urea ha^{-1} (N_{210}), which was equal to 32.7, 65.4 and 98.1 kg N ha^{-1} to the soil, respectively.

2.2. Sampling, Measurements and Laboratory Procedures

2.2.1. Pot Experiment

Ten trees were harvested 90, 135 and 180 days after the ^{15}N labeling, and trees from the pot experiments (A, B and C) were separated into *Eucalyptus* and *D. odorifera*. The roots of the two species were separated by hand on the basis of their different colors and textures. As many of the roots were collected as possible, with the exception of a few decaying and unidentifiable fine roots. The roots were then washed carefully with a gel tip dropper with a small amount of ultrapure water after soil removal. Soil samples were collected from the middle part of the pots for analysis after planting, and on the same day, the plants were harvested. The harvested materials were dried at 65 °C until a constant dry weight was reached. The dried plant material was ground in a ball mill (<0.1 mm), and the soil samples (experiments A, B and C) were also ground in a separate ball mill (<0.2 mm), avoiding any cross-contamination of samples. The TN and N from ammonium-nitrogen (NH_4^+-N) and nitrate-nitrogen (NO_3^--N) of the plants and soil were determined separately via a continuous-flow chemical analyzer (AA3) [39].

The ^{15}N atom% values were determined (in the samples from experiments A and B) via a mass spectrometer (SN09072D, Homotopic, Thermo Fisher Scientific, Germany). All ^{15}N enrichments were corrected for the background level before the amount of N transferred from the donor to the receiver was calculated. Before labeling was performed, samples were used to measure the atom% ^{15}N background values (^{15}N value before labeling) of the leaves, stems and roots of the two species. The excess amount of ^{15}N of the leaves, stems and roots of the receiver and donor plants were calculated via Equation (1) as follows [9,40,41]:

$$\text{Excess } {}^{15}\text{N amount (mg plant}^{-1}) = \frac{(\text{atom\% } {}^{15}\text{N}_{\text{labeled}} - \text{atom\% } {}^{15}\text{N}_{\text{unlabeled}}) \times \text{ TN (g plant}^{-1}) \times 1000}{100} \quad (1)$$

where atom% $^{15}N_{labeled}$ is the atom% ^{15}N of a labeled sample after 90, 135 and 180 days, and atom% $^{15}N_{unlabeled}$ is the atom% ^{15}N before labeling, and TN is (N concentration) × biomass. The total ^{15}N amount in a whole plant was calculated by summing the amount of ^{15}N of the roots, stems and leaves. The underlying assumption of Equation (1) is that the ^{15}N in the plants at harvest was the same as at "time zero" under unlabeled treatments.

The percentage of the total nitrogen transferred from the donor to the receiver (% NT) was estimated by Equation (3) as follows [16]:

$$\% \text{ NT} = \frac{^{15}\text{N amount}_{\text{receiver}} \text{ (mg plant}^{-1})}{^{15}\text{N amount}_{\text{receiver}} \text{ (mg plant}^{-1}) + ^{15}\text{N amount}_{\text{donor}} \text{(mg plant}^{-1})} \times 100 \quad (2)$$

The amount of N (mg plant^{-1}) transferred from the donor was calculated as follows [42]:

$$\text{N}_{\text{transfer}} \text{ (mg plant}^{-1}) = \frac{\% \text{ NT} \times \text{TN}_{\text{donor (mg plant}^{-1})}}{100} \quad (3)$$

where $\text{N}_{\text{transfer}}$ is the unidirectional net transfer from *D. odorifera* (the ^{15}N donor plant) to *Eucalyptus* (the ^{15}N receiver plant) and vice versa. Notably, the net $\text{N}_{\text{transfer}}$ could be estimated from paired treatments of foliar-labeled legumes and non-legumes according to the different $\text{N}_{\text{transfer}}$ values between *D. odorifera* and *Eucalyptus*.

The proportion of N in the receiver derived from the transfer (% NDFT) was calculated according to the following equation [16]:

$$\% \text{ NDFT} = \frac{\text{NT \%} \times \text{N}_{\text{donor}}}{\text{N}_{\text{reciever}}} \times 100 \quad (4)$$

where N_{donor} is TN of *D. odorifera*, and $\text{N}_{\text{reciever}}$ is TN of *Eucalyptus*.

2.2.2. Field Experiment

Three trees of both *Eucalyptus* and *D. odorifera* were harvested from each plot on 19 March 2019. According to Monsic's stratified clip method [42], the aboveground biomass (AGB) of the stem of trees was divided into 2-meter sections and measured, and each tree was separated into roots, stems, bark, branches and leaves, and their fresh weights were determined. Afterward, 500 g of each component was carefully preserved in the laboratory. The harvested material was dried at 65 °C until a constant weight was reached. The dried material was subsequently ground in a ball mill (<0.1 mm), and the DM of the above- and belowground organs was calculated according to the allometric equations described by Magalhães and Seifert [43]. The TN concentration of each plant was also determined via a continuous-flow chemical analyzer (AA3), and the N content of each plant compartment was calculated as the product of N from the DM yield and TN concentration.

On 19 March 2019, after one year of N application treatment, five soil samples were randomly collected from each plot at a depth of 0–20 cm and uniformly mixed into a composite sample. The corer was wiped clean of obvious soil particles with a paper towel when sampling different plots, and the composite soil samples were divided into two portions. One portion was passed through a 2 millimeter (2 mm) sieve to remove visible stones, soil organisms, roots and other plant material and was stored at 4 °C for analysis of NH_4^+-N and NO_3^--N contents. The other portion was air-dried at room temperature, sieved through a 0.2-mm screen, and then used to analyze the TN content. The TN, NH_4^+-N, and NO_3^--N contents in the soil were also determined via a continuous-flow chemical analyzer (AA3) (the N concentrations of the plants are shown in Supplementary Table S1).

2.3. Statistical Analysis

In both experiments, statistical analyses were performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). For the pot experiment, differences in DM yield of and N content in both species, soil N concentration, atom % ^{15}N and N transfer (%) between the two plantation systems (monoculture vs. intercropping) were assessed by one-way ANOVA with Tukey's post hoc test at $p < 0.05$. Interactions between independent variables were analyzed by two- and three-way ANOVA. Data are reported as means ± standard error ($n = 4$). We also used *t*-tests to evaluate the differences in DM yield or N content of both species between intercropping and monoculture systems, with N level as a fixed

factor. The Pearson correlation between the soil N concentration and the amount of N transferred were assessed via Pearson correlation coefficients.

For the field experiment, differences in N content and DM yield of both species and the soil N concentration under three N treatments were assessed by one-way ANOVA with Tukey's post hoc test at $p < 0.05$. Interactions between independent variables were analyzed by two-way ANOVA. Data are reported as means ± standard error ($n = 3$). The significance level was $\alpha = 0.05$.

3. Results

3.1. Pot Experiment

3.1.1. DM Yields (Experiment C)

The N level, growth duration and planting system, as well as their interactions, significantly affected Eucalyptus and D. odorifera DM yields (see Supplementary Table S2). The DM yield of Intercropped *Eucalyptus* was significantly higher than of the monocropped *Eucalyptus* at 135 and 180 days ($p < 0.05$). In addition, the DM yield of *Eucalyptus* increased with increasing N fertilizer application (Figure 2a–c). In contrast, the DM of *D. odorifera* was lower in the intercropping system than in the monocropping system, which was significant in the later stages (Figure 2d–f). Nevertheless, the DM yield of *D. odorifera* was significantly lower under N_3 than under N_2, except in the intercropping system at 180 days. The DM yield of the stems was greater than that of the leaves and roots of both tree species.

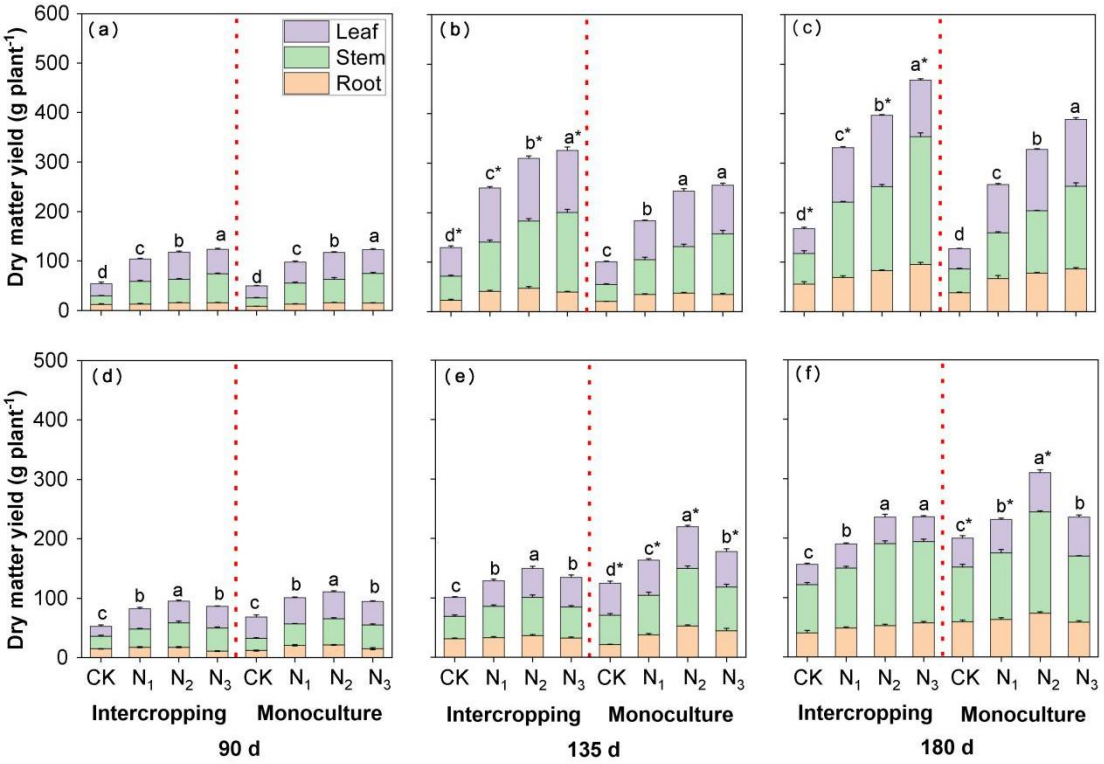

Figure 2. The effects of N application and the planting system on DM accumulation in intercropped and monocropped *E. urophylla* × *grandis* (**a**–**c**) and *D. odorifera* (**d**–**f**) plants. CK indicates plants without N application, and N_1, N_2, and N_3 correspond to N fertilizer application rates of 3, 6, and 12 g urea ($CO(^{14}NH_2)_2$) pot^{-1}, respectively. The different lowercase letters above the bars indicate significant differences ($p < 0.05$) in whole-plant DM accumulation among the N treatments within each planting system. * indicates a significant difference ($p < 0.05$) in *E. urophylla* × *grandis* or *D. odorifera* between intercropped and monocropped plants. Error bars indicate ± SE ($n = 4$).

3.1.2. Plant N (Experiment C)

The N content in *Eucalyptus* and *D. odorifera* was significantly affected by the N level, growth duration and planting system, and the N level × planting system, time × planting system and N level × planting system interactions significantly influenced the N content in both species (Supplementary Table S3). The N content in all the plant parts and in the whole plants of both species increased with increasing N application, the trend of which was the same as that for DM yield (Figure 3). Nevertheless, there were differences in N content between the two species. The N content in *Eucalyptus* was significantly greater (by 7.6–97.3%) under intercropping conditions than under monocropping conditions, but it was 0.5–13.2% lower in *D. odorifera*. Moreover, the N content in the leaves of *Eucalyptus* was always the greatest among all plant parts measured except in the intercropping system at 180 days (Figure 3a–c). The N content in the leaves of *D. odorifera* was the greatest on days 90 and 135 (Figure 3d,e), whereas the N content in stems was greater than that in the leaves and roots on day 180 (Figure 3f).

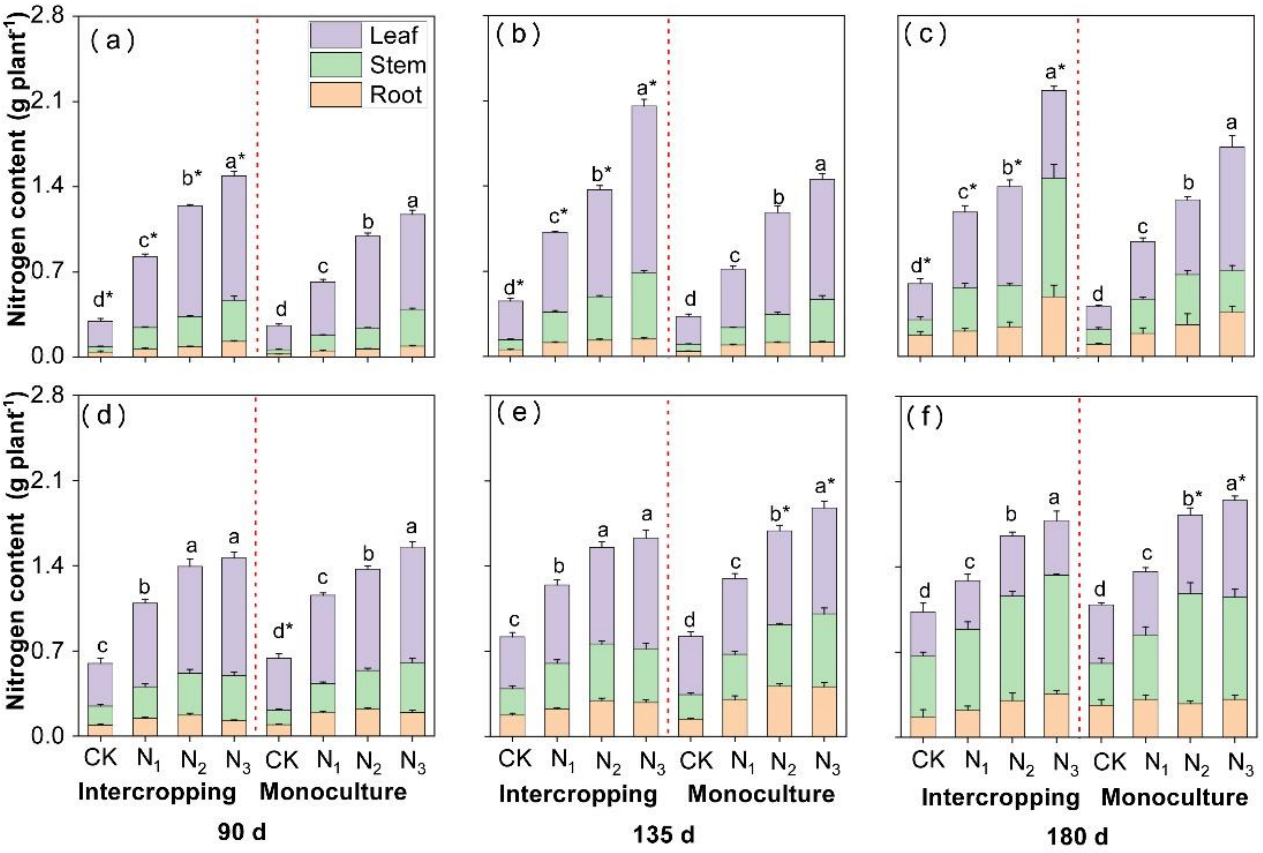

Figure 3. The effects of N application and the planting system on N accumulation in intercropped and monocropped *E. urophylla* × *grandis* (**a**–**c**) and *D. odorifera* (**d**–**f**). CK indicates plants without N application, and N_1, N_2, and N_3 correspond to N fertilizer application rates of 3, 6, and 12 g urea $(CO(^{14}NH_2)_2)$ pot^{-1}, respectively. The different lowercase letters above the bars indicate significant differences ($p < 0.05$) in whole-plant N accumulation among the N treatments within each planting system. * indicates a significant difference ($p < 0.05$) in *E. urophylla* × *grandis* or *D. odorifera* between intercropped and monocropped plants. Error bars indicate ± SE ($n = 4$).

3.1.3. N Transfer between *Eucalyptus* and *D. odorifera* (Experiment A and B)

The excess atom% ^{15}N mean values in *Eucalyptus* prior to labelling were 0.360, 0.352 and 0.388 in the root, stem and leave, respectively, which was slightly higher than those of *D. odorifera* with 0.343, 0.350 and 0.357, respectively (Supplementary Table S4). The atom% ^{15}N values of *Eucalyptus* (with 0.332.82%) were greater than those of *D. odorifera* (with 0.027–0.064%) when the *Eucalyptus* trees were labeled with ^{15}N (Figure 4) and vice-

versa (Figure 5). The excess atom% ^{15}N value of *Eucalyptus* significantly decreased with increasing N fertilizer application ($p < 0.05$) (Figure 4a–c). For *D. odorifera*, the atom% ^{15}N of the stems and roots under N_3 was significantly lower than that under CK and N_1 on day 90, but the excess atom% ^{15}N of the leaves under the N application treatment was greater than that under the CK treatment (Figure 4d). In particular, on days 135 and 180, there was no significant difference between the leaves (Figure 4e,f). The excess atom% ^{15}N of both plant species significantly decreased with increasing N fertilizer application, and the *D. odorifera* leaves under different treatments had a greater excess atom% ^{15}N than did roots (mean values by 54.89–155.29%) and stems (by 11.29–114.18%) when *D. odorifera* was the ^{15}N donor to *Eucalyptus* (Figure 5d–f). In addition, the excess atom% ^{15}N of *Eucalyptus* decreased over time, regardless of whether *Eucalyptus* or *D. odorifera* was the ^{15}N donor. The atom% ^{15}N was greater in *Eucalyptus* donors than in *D. odorifera* donors and was greater in *Eucalyptus* receivers than in *D. odorifera* receivers under all the N treatments.

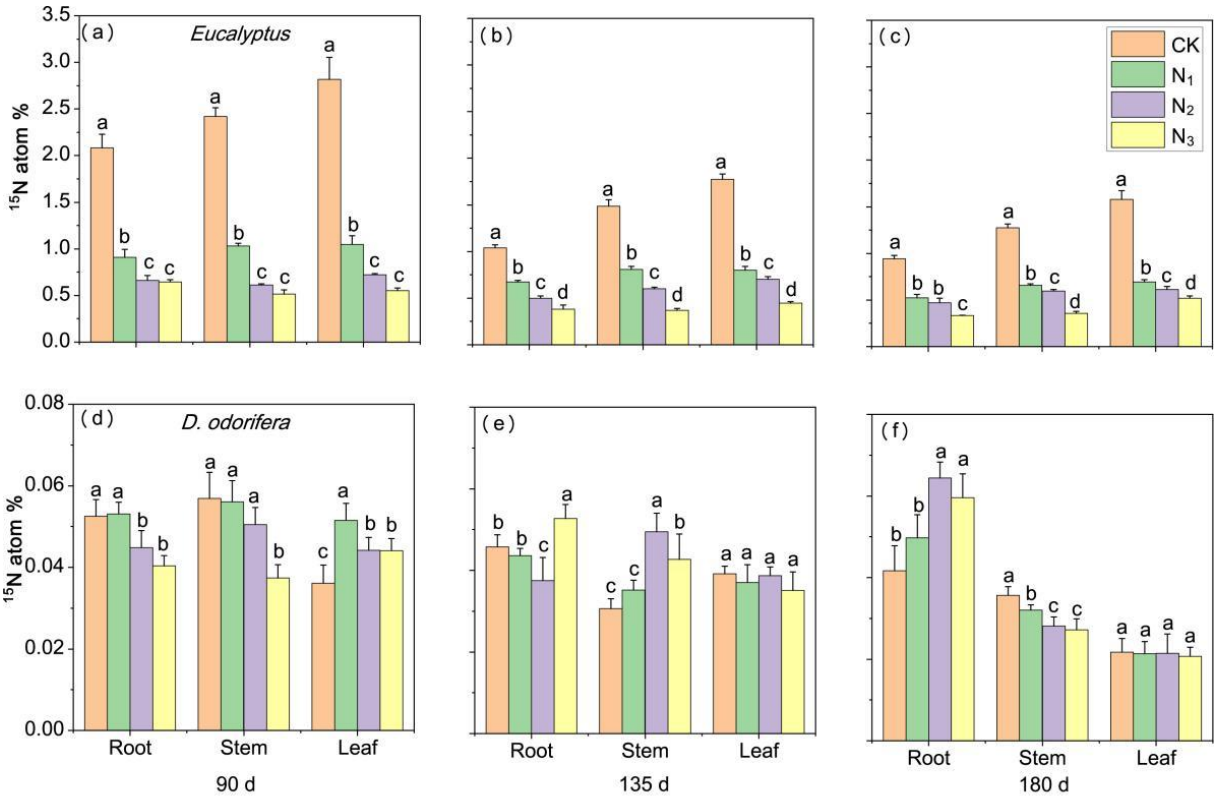

Figure 4. Atom% ^{15}N in excess in plant components of both species in pots where *Eucalyptus* was labeled at different N levels (i.e., *E. urophylla* × *grandis* as the ^{15}N donor and *D. odorifera* as the receiver plant), where atom% ^{15}N is the difference between atom% $^{15}N_{90,\ 135\ 180\ \text{days after labeling}}$ and atom% $^{15}N_{\text{before labeled}}$. Panels (**a**–**c**) show the atom% ^{15}N of *E. urophylla* × *grandis*, and panels (**d**–**f**) show the atom% ^{15}N of *D. odorifera*. CK indicates plants without N application, and N_1, N_2 and N_3 correspond to N fertilizer application rates of 3, 6 and 12 g urea ($CO(^{14}NH_2)_2$) pot^{-1}, respectively. The different lowercase letters above the bars indicate significant differences among N treatments ($p < 0.05$). Error bars indicate ± SE ($n = 4$).

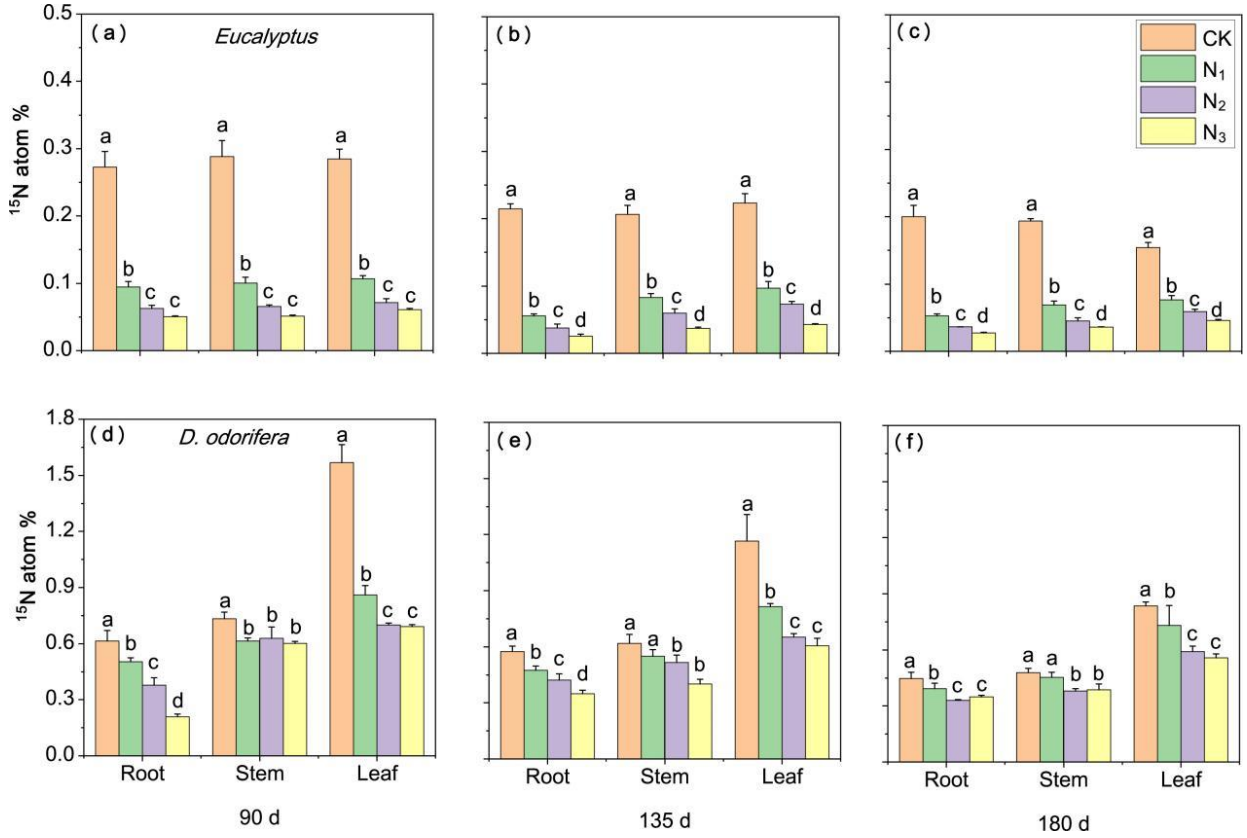

Figure 5. Atom% ^{15}N in excess in plant components of both species in pots where *D. odorifera* was labeled at different N levels, where atom% ^{15}N is the difference between atom% $^{15}N_{90,\ 135\ 180\ \text{days after labeling}}$ and atom% $^{15}N_{\text{before labeled}}$. Panels (**a**–**c**) show the atom% ^{15}N of *E. urophylla* × *grandis*, and panels (**d**–**f**) show the atom% ^{15}N of *D. odorifera*. CK indicates plants without N application, and N_1, N_2 and N_3 correspond to N fertilizer application rates of 3, 6 and 12 g urea ($CO(^{14}NH_2)_2$) pot^{-1}, respectively. The different lowercase letters above the bars indicate significant differences among N treatments ($p < 0.05$). Error bars indicate ± SE ($n = 4$).

Nitrogen transfer was observed in the *Eucalyptus*/*D. odorifera* system at all N application rates. The NT% ranged from 3.2–7.1% when *Eucalyptus* was the ^{15}N donor and significantly increased with N application. In addition, the NT% under N_1 was significantly lower than that under N_2 and N_3 (Figure 6a). In contrast, the NT% ranged from 8.6–14.6% and significantly decreased with increasing N application when *D. odorifera* was the ^{15}N donor (Figure 6b). The N level, growth duration and their interactions had significant effects on the NT% regardless of whether *D. odorifera* or *Eucalyptus* was used as the donor (Supplementary Table S5).

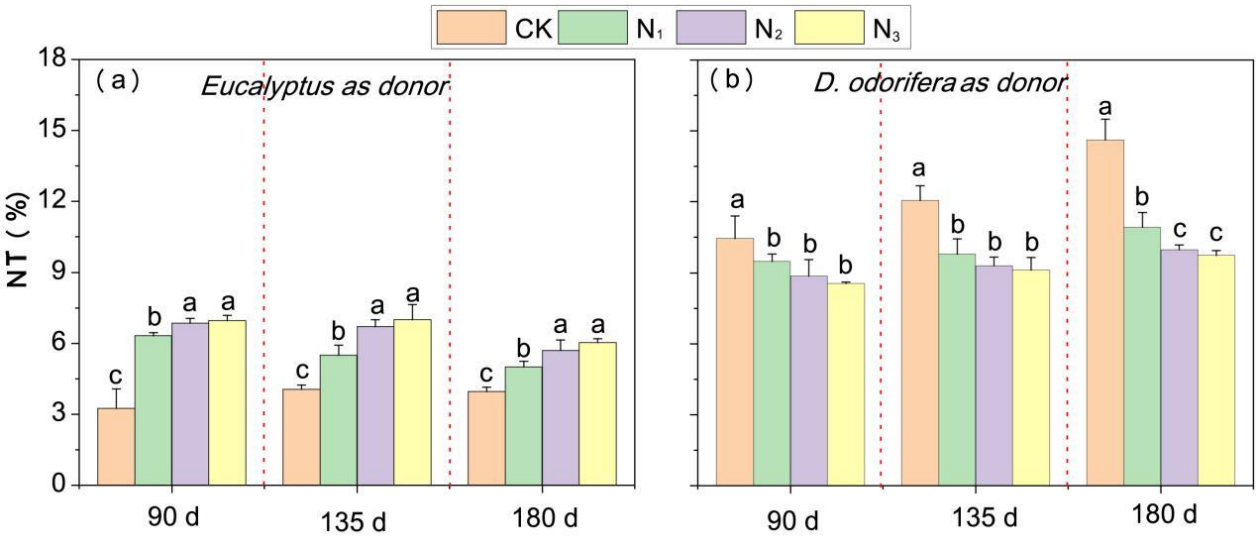

Figure 6. The bidirectional N transfer between *D. odorifera* and *E. urophylla* × *grandis* in an intercropping system with different percentages of N transfer (% NT) from N fertilizer application: (**a**) *E. urophylla* × *grandis* as the ^{15}N donor (i.e., % NT is the unidirectional transfer from *E. urophylla* × *grandis* to *D. odorifera*); (**b**) *D. odorifera* as the ^{15}N donor (i.e., % NT is the unidirectional transfer from *D. odorifera* to *E. urophylla* × *grandis*). CK indicates plants without N application, and N_1, N_2, and N_3 correspond to N fertilizer application rates of 3, 6, and 12 g urea ($CO(^{14}NH_2)_2$) pot^{-1}, respectively. $P_{N\times T}$ indicates the N level × time interaction. The different lowercase letters above the bars indicate significant differences ($p < 0.05$) in N transfer percentage among the N treatments. Error bars indicate ± SE ($n = 4$).

3.1.4. N Transfer Amount (Experiment A and B)

The two-way ANOVA showed that both the N level and the growth duration, as well as the N level × time interaction, significantly affected the NDFT% and the amount of N transferred (Table 2). The amount of N transferred from *D. odorifera* to *Eucalyptus* (62.1–173.5 mg $plant^{-1}$) was greater than that transferred from *Eucalyptus* to *D. odorifera* (9.6–140.4 mg $plant^{-1}$). Therefore, the net N transfer was from *D. odorifera* to *Eucalyptus* and increased over time; the mean values ranged from 20.8–52.5 mg $plant^{-1}$, 23.1–80.3 mg $plant^{-1}$ and 41.8–127.0 mg $plant^{-1}$ under different the N levels at 90, 135 and 180 days, respectively. The net amount of N transferred significantly decreased with increasing N application ($p < 0.05$), except for N_1 at 90 days. The NDFT% in *Eucalyptus* plants ranged from 1.5–21.2% and significantly decreased with the N application rate on days 135 and 180 but tended to slightly increase with time (Table 2).

3.1.5. Soil N Concentration and Its Relationship with N Transfer (Experiment A and B)

The concentrations of soil TN (Figure 7a–c), NH_4^+-N (Figure 7d–f) and NO_3^--N (Figure 7g–i) in the monocrop *D. odorifera* were the greatest (mean values with 0.81–1.34 g kg^{-1} TN, 12.10–26.18 mg kg^{-1} NH_4^+-N and 3.84–10.81 mg kg^{-1} NO_3^--N concentration), while the lowest values were obtained in the monocropped *Eucalyptus* (with 0.61–1.10 g kg^{-1} TN, 11.45–24.56 mg kg^{-1} NH_4^+-N and 3.57–5.24 mg kg^{-1} NO_3^--N concentration). Under the *Eucalyptus* and *D. odorifera* intercropping and monocropping systems, the TN and NH_4^+-N concentrations under the N_3 treatment were significantly greater than those under the CK treatment, except for monocropped *D. odorifera* at 180 days. The NO_3^--N concentrations under the N_3 treatment were significantly greater than those under the other treatments at 90 days ($p < 0.05$). Overall, the concentrations of soil TN, NH_4^+-N and NO_3^--N under the intercropping and monocropping systems increased with increasing N fertilizer rate; particular, those under the N_3 treatment were significantly greater than those under the other treatments at 90 and 135 days. The soil N concentrations were significantly affected by the N level, growth duration and planting system. Moreover, the N level × planting system and time × planting system interactions significantly influenced the NH_4^+-N con-

centration, whereas the N-NO_3^- concentration was significantly affected only by the N level × planting system and time × N level interactions (Supplementary Table S6).

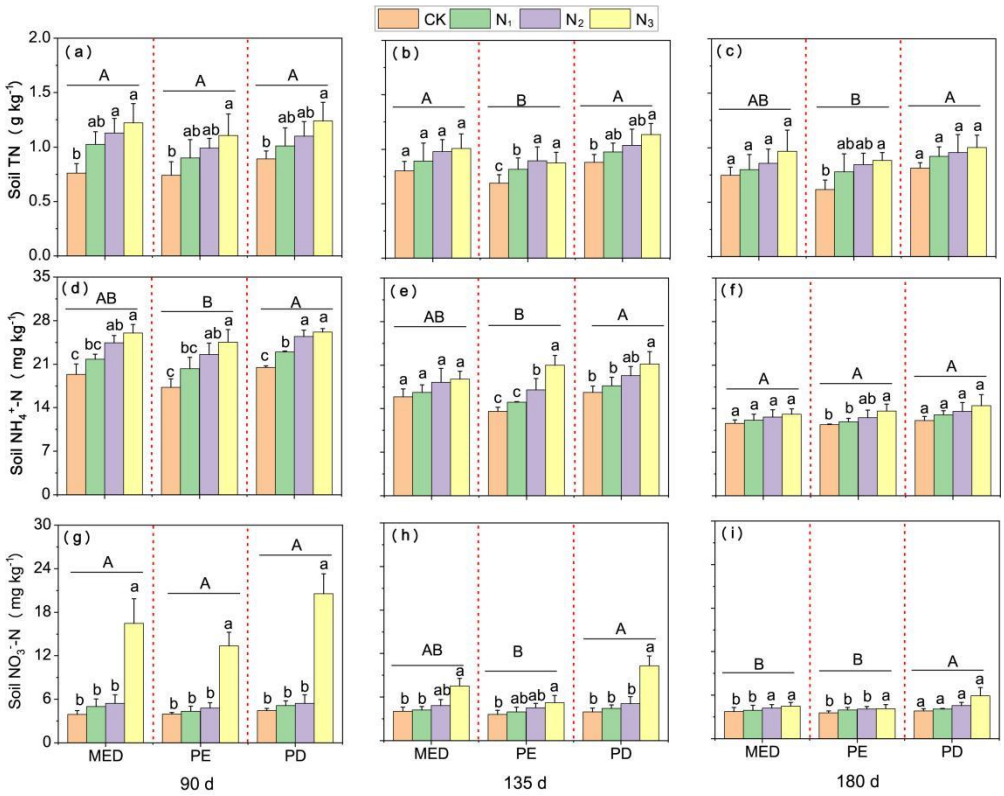

Figure 7. Concentrations of TN (**a**–**c**), N-NH_4^+ (**d**–**f**) and N-NO_3^- (**g**–**i**) in the soil at days 90, 135 and 180. The different lowercase letters above the bars indicate significant differences ($p < 0.05$) in concentrations of TN, NH_4^+-N and NO_3^--N in the soil among the N treatments. MP = *E. urophylla* × *grandis* mixed together with *D. odorifera* plantations; PE = pure *E. urophylla* × *grandis* plantations; PD = pure *D. odorifera* plantations. The different capital letters on the straight lines indicate significant differences in the concentrations of TN, NH_4^+-N and NO_3^--N in the soil among planting systems ($p < 0.05$). CK indicates plants without N application, and N_1, N_2 and N_3 correspond to N fertilizer application rates of 3, 6 and 12 g urea ($CO(^{14}NH_2)_2$) pot^{-1}, respectively. Error bars indicate ± SE ($n = 4$).

The net amount of N transferred was significantly negatively correlated with the soil N concentration. The net amount of N transferred had a weaker correlation with the concentration of N-NO_3^- ($R^2 = 0.17$; Figure 8b) and the concentration of TN ($R^2 = 0.35$; Figure 8a) but was more strongly correlated with the concentration of N-NH_4^+ ($R^2 = 0.43$; Figure 8c).

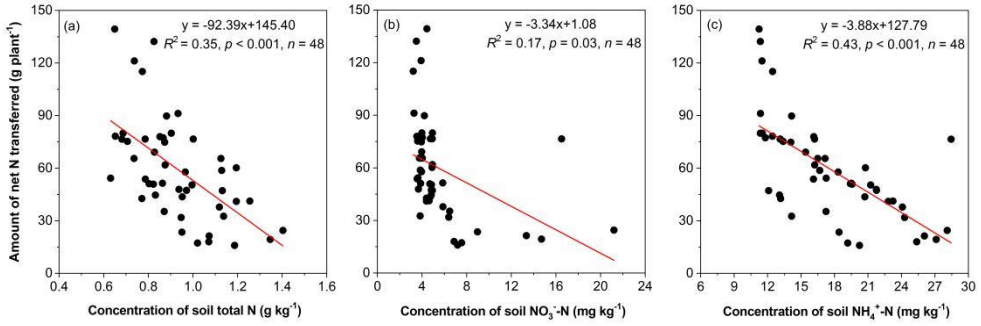

Figure 8. Correlations between the net amount of N transferred and the concentration of total soil N (**a**), nitrate nitrogen (NO_3^--N) (**b**) and ammonium nitrogen (NH_4^+-N) (**c**). The *y*-axis represents the unidirectional transfer per pot, which was from *D. odorifera* to *E. urophylla* × *grandis*.

3.2. Field Experiment

3.2.1. DM Yield and Plant N Content

The DM yield and N content of *Eucalyptus* were significantly greater by 5.1–9.6% and 14.5–16.4% than those in monoculture ($p < 0.05$) (Figure 9a,c). In contrast, the DM yield of and N content in *D. odorifera* were slightly lower in the mixed plantations than in the pure plantations, albeit statistically insignificant (Figure 9b,d). However, the total biomass of monoculture *Eucalyptus* (the equivalent of 79.17–87.50 t ha^{-1}) was greater than that of the mixed plantations (43.99–54.02 t ha^{-1}) and monoculture *D. odorifera* (2.88–4.22 t ha^{-1}). Additionally, the DM yield of and N content in both plant species increased significantly under the N_{140} and N_{210} treatments compared with the CK treatment, and for both plant species in pure plantations, the DM yield under the N_{70} treatment was also significantly greater than that under the CK treatment. Although the N level and planting system significantly affected the DM yield and N content of both species, their interactions had no significant effect (Supplementary Table S7).

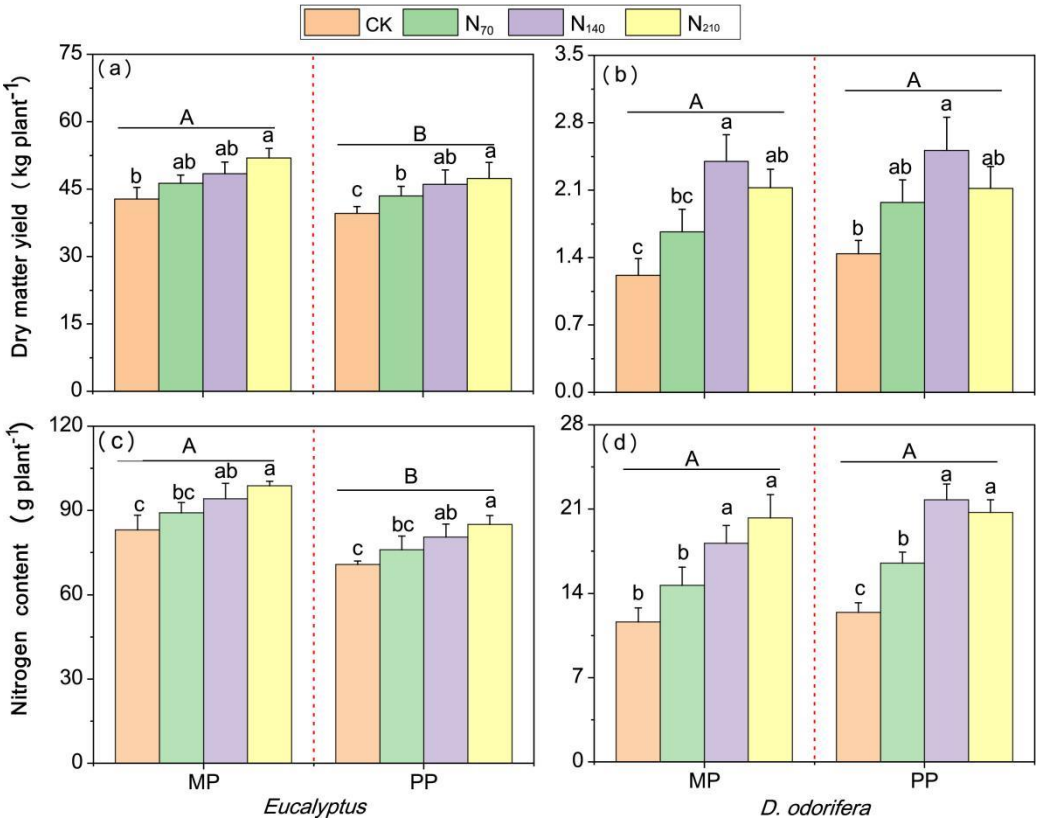

Figure 9. The effects of N application and planting system on the DM yield of and N content in mixed and pure *E. urophylla* × *grandis* and *D. odorifera* plants. CK indicates control plants without N application; N_{70}, N_{140} and N_{210} correspond to N fertilizer application rates of 70, 140 and 210 kg urea ($CO(^{14}NH_2)_2$) ha^{-1}, respectively. (**a**,**b**) represent the DM yield of *E. urophylla* × *grandis* and *D. odorifera*, respectively. (**c**,**d**) represent the N content of *E. urophylla* × *grandis* and *D. odorifera*, respectively MP = *E. urophylla* × *grandis* mixed together with *D. odorifera* plantations; PP = pure plantations. The different lowercase letters above the bars indicate significant differences ($p < 0.05$) in whole-plant DM accumulation among N treatments within each planting system. The different capital letters on the straight lines indicate significant differences in *E. urophylla* × *grandis* or *D. odorifera* between mixed and pure plantations ($p < 0.05$). There was no significant N level × planting system interaction effect on the DM yield of or N content in either species. Error bars indicate ± SE ($n = 3$).

3.2.2. Soil N Concentration

The soil N concentration results were also consistent with the results of the pot experiments; i.e., the concentrations of soil TN, N-NH_4^+ and N-NO_3^- in the *D. odorifera* monocrop were greatest, while the lowest values were observed in the *Eucalyptus* monocrop (Table 3). Compared with the values in the *Eucalyptus* monocropping system, the concentrations of soil TN, N-NH_4^+ and N-NO_3^- in the intercropping system were 3.5–9.1%, 14.6–33.5% and 6.5–50.1% greater, respectively, but the values were 19.3–26.5%, 2.2–23.1% and 17.5–30.7% lower, respectively, than those in the *D. odorifera* monocropping system (Table 3). Moreover, the soil N-NH_4^+ concentration in the *D. odorifera* monocrop was significantly greater than that in other plantations except under the N_{210} treatment in the mixed plantation. Although two-way ANOVA showed that both the N level and the planting system significantly affected all the tested soil N concentrations, the N level × planting system interaction did not significantly affect the soil N concentrations (Table 3).

Table 2. The mean values of the net amount of N transferred and the percentage of TN content (NDFT (%)) in *Eucalyptus* plants as a result of N level, time, and their interaction.

Source of Variation	Amount of N Transferred (mg plant^{-1})									NDFT (%)		
	From *Eucalyptus* to *D. odorifera*			From *D. odorifera* to *Eucalyptus*			Net Transfer					
	90 Days	135 Days	180 Days	90 Days	135 Days	180 Days	90 Days	135 Days	180 Days	90 Days	135 Days	180 Days
CK	9.6 ± 1.4 d	18.4. ± 0.8 d	23.6 ± 1.1 d	62.1 ± 2.3 c	98.6± 6.5 d	150.6 ± 11.8 c	52.5 ± 1.7 a	80.3. ± 6.4 a	127.0 ± 10.9 a	17.8 ± 0.9 a	17.7 ± 1.3 a	21.2 ± 1.0 a
N_1	52.1 ± 1.4 c	56.2 ± 4.9 c	59.4 ± 4.7 c	103.6 ± 5.3 b	121.7 ± 6.3 c	140.5 ± 5.6 b	51.4 ± 6.0 a	65.5 ± 2.9 b	81.2 ± 7.0 b	6.20± 0.8 b	6.4 ± 0.3 b	6.8 ± 0.7 b
N_2	85.3 ± 1.6 b	91.6 ± 2.1 b	87.2 ± 4.3 b	123.3 ± 4.8 a	144.5 ± 7.9 b	164.7 ±5.9 a	38.0 ± 4.4 b	52.9 ± 6.9 c	77.2 ± 2.1 b	3.1 ± 0.4 c	3.9 ± 0.5 c	5.0 ± 0.2 c
N_3	103.3 ±3.0 a	140.4 ± 10.9 a	131.0 ± 6.7 a	124.1 ± 4.8 a	163.3 ± 6.2 a	173.5 ± 3.2 a	20.8 ± 2.7 c	23.1 ± 8.4 d	41.8 ± 6.4 c	1.5 ± 0.1 c	1.5 ± 0.5 d	1.9 ± 0.4 d
N		35.93 ***			265.95 ***			254.84 ***			55.86 ***	
T		880.02 ***			120.32 ***			6.16 ***			860.30 ***	
N × T		11.15 ***			13.17 ***			72.11 ***			5.28 ***	

The different lowercase letters (a, b, c, d) indicate significant differences among N levels ($p < 0.05$) in each column. 'N', 'T' and'N × T' indicate the N level, time and N level × time interaction. *** = *F*-value significant at $p \leq 0.001$.

Table 3. The mean values of TN, NH_4^+-N and NO_3^--N concentration in the soil as a result of planting system (P-S), N level (N) and their interaction after 4 years under field conditions.

Source of Variation	TN (g kg^{-1})			NH_4^+-N (mg kg^{-1})			NO_3^--N (mg kg^{-1})		
	MED	PE	PD	MED	PE	PD	MED	PE	PD
CK	1.03 ± 0.2 b	0.95 ± 0.18 b	1.41 ± 0.06 a	11.20 ± 2.16 c	8.39 ± 1.73 b	14.56 ± 0.87 a	3.38 ± 0.43 b	2.26 ± 0.37 b	4.89 ± 0.21 a
N_{70}	1.14 ± 0.1 b	1.11 ± 0.20 ab	1.50 ± 0.28 a	12.79 ± 1.69 bc	10.28 ± 1.21 ab	15.87 ± 3.48 a	4.03 ± 0.62 a	2.95 ± 0.35 b	4.88 ± 0.23 a
N_{140}	1.22 ± 0.1 b	1.15 ± 0.07 ab	1.57 ± 0.31 a	14.81 ± 2.06 b	12.92 ± 2.03 ab	16.54 ± 1.22 a	4.17 ± 0.17 ba	3.59 ± 0.17 ab	5.12 ± 0.21a
N_{210}	1.42 ± 0.1 a	1.33 ± 0.27 a	1.76 ± 0.15 a	16.99 ± 3.17 a	13.55 ± 2.01 a	17.36 ± 0.52 a	4.44 ± 0.51 a	4.17 ± 0.44 a	5.66 ± 0.66 a
P-S		16.77 ***			16.93 ***			68.03 ***	
N		5.91 **			8.88 ***			15.74 ***	
P-S × N		0.02 ^{ns}			0.48 ^{ns}			1.68 ^{ns}	

The different lowercase letters (a, b and c) in each column indicate significant differences ($p < 0.05$) in the concentrations of TN, NH_4^+-N and NO_3^--N in the soil among different N treatments. MED = mixed plantation; PE = pure *Eucalyptus* plantation; PD = pure *D. odorifera* plantation. ns, ** and *** = *F*-value not significant, significant at $p > 0.05$ and $p < 0.01$ and significant at $p < 0.001$, respectively.

4. Discussion

4.1. Bidirectional N Transfer Occurs in Mixed Systems, and Net N Transfer Occurs from D. odorifera to Eucalyptus

The N transfer process may provide a significant amount of the N requirements of trees close to legumes, and investigating this process could lead to an improved understanding of N cycling driven by interactions between plants [6,10]. Significant ^{15}N enrichment was already detectable in unlabeled species in our experiment after leaf labeling of neighboring plants, which showed that N transfer in the intercropping system between *Eucalyptus* and *D. odorifera* occurred in the short term. Direct and indirect routes can account for the belowground N transfer observed between trees [11]. First, short-term belowground N transfer may occur directly via CMNs [44], and these CMNs can be formed by arbuscular mycorrhizal fungi (AMF) [45] or ectomycorrhizal fungi [22]. The roots of both species can be colonized by ectomycorrhizal fungi and may potentially form CMNs [46], promoting the occurrence of N transfer. Second, excreted N from roots, which is rapidly produced after ^{15}N is applied to leaves [20,47], can be rapidly taken up by neighboring plants. It should be emphasized that the percentage of ^{15}N that was transferred from *D. odorifera* to *Eucalyptus* trees in our study ranged from 8.6–14.6% depending on N supply, which is higher than the N transfer range of 6.5–9.6% from *D. odorifera* to *Eucalyptus* observed with ^{15}N dilutions in the soil [24]. The explanation for it could be our calculation method of %NT (Equation (2)) in the study; the result may be an overestimation of NT % over the course of the experiment due to part of exuded N remaining in soil even over time periods of several months and not being fully absorbed by neighboring plants [9,47]. Although we have not determined the N exudation in this study, previous studies have frequently found that non-leguminous plants absorption of N exuded by leguminous plants was relatively low [47].

Our results show that the net transferred N was from *D. odorifera* to *Eucalyptus* was consistent with previous studies. There are two possible reasons for it, and one is that the N transfer via CMNs and flow from the more N-sufficient plant to the less N-sufficient one [20]. Indeed, our results showed that the N concentration was greater in *D. odorifera* plants than in *Eucalyptus* plants (Table S1), resulting in the N transferred being greater from *D. odorifera* to *Eucalyptus* than from *Eucalyptus* to *D. odorifera*, which is consistent with findings of previous reports [6,23]. The other potential reason could be that leguminous plants with a low C:N ratio can release more N via root decay than those with a high C:N ratio, which suggests that legume root exudates potentially are a good source of N to adjacent plants, either directly absorbed or after rapid recycling via soil microbial organisms [47], thus increasing the amount of N transferred from leguminous species to non-leguminous species [14]. Overall, although bidirectional N transfer was verified between *Eucalyptus* and *D. odorifera*, the net transferred N was still from legumes plants to *Eucalyptus*.

4.2. Soil N Concentration and the Growth Duration of Plants Are the Main Factors Affecting N Transfer

Chalk et al. pointed out that N transfer depended on the soil N status and the number of labeled urea applications, and transfer from leguminous to non-leguminous plants improved when the soil available N content was low [9]. Oliveira et al. suggested that higher rates of belowground transfer of N from leguminous plants to *Eucalyptus* trees in non-fertilized than in fertilized mixed-species stands [17]. Our results were also consistent with this finding. The net amount of N transferred in the N application treatments was lower than that under the CK treatment suggested that N transfer decreased with increasing soil N application. In addition, soil ammonium and nitrate are responsible for driving changes in the ^{15}N content of plants in space and time [10], thereby affecting N transfer in legume/non-legume intercropping systems. *Eucalyptus* seems to be more sensitive to N in the form of ammonium than other N forms [4]. This finding is consistent with the observation that the growth of many forest plant species is greatest when ammonium is supplied [48]. He et al. suggested that N transfer was greater from *Casuarina* to *Eucalyptus*

when N was supplied as $^{15}NH_4^+$-N rather than $^{15}NO_3^-$-N [22]. In agreement with previous studies, our results suggest the relationship between ammonia N concentrations and N transfer is greater than that between nitrate N concentrations (Figure 8b,c). N transfer observed in this study was affected by the soil N concentration, especially the N-NH_4^+ concentration, confirming the findings on N transfer by He et al. [22].

The growth advantage associated with mixed cropping systems increases over time for non-leguminous plants [49], and the amount of N transferred is affected by time [6]. The proportion of transferred N in *Eucalyptus* (% Ndft) increased throughout this experiment, probably as a result of an increase in N exudation with tree growth (Table 2). Previous studies have found that the amount of N transferred between leguminous and non-leguminous plants was shown to be greater at 79 days than at 50 days [21]. A similar time-dependent growth advantage was also confirmed in a study by Paula et al. [6]. Our results were consistent with these previous studies: the net amount of N transferred increased from 20.8 to 127.0 mg plant^{-1} with time, probably due to the continuous absorption of N exudation by *D. odorifera* over time periods of several months [24]. This relationship may explain the increasing growth advantage of *Eucalyptus*, i.e., that caused by enhanced N transfer. Additionally, the amount of N transferred from leguminous plants increased with time, as outlined by Chalk et al. [9], increasing the N content in non-leguminous plants. Importantly, two-way ANOVAs showed that the NT % was significantly affected by N application, growth time and their interactions regardless of whether *Eucalyptus* or *D. odorifera* was the ^{15}N donor (Table S5). Therefore, we suggested that soil N concentration and growth duration were key factors affecting N transfer in our study.

4.3. Competition Increasing the Growth of Eucalyptus but Limiting That of D. odorifera

Previous studies have shown that establishing commercial *Eucalyptus* plantations on poor soils leads to a high loss of N after wood harvesting, and the introduction of leguminous species appears to be a promising strategy to increase the N content of *Eucalyptus* [49]. In our study, compared with that in *Eucalyptus* in monocrops, the biomass and N content in *Eucalyptus* in mixed plantations significantly increased under the field and pot conditions suggesting that the improvement in productivity may be due to N transfer from the legume [6,50], which were consistent with other studies [6,8,49]. Another crucial reason is that leguminous plants can rely heavily on fixed N, which can represent 10% to nearly 100% of the N used by the leguminous plant [51]. Thus, more soil N may be available to non-leguminous plants before the fixed N is cycled and transferred to the non-leguminous plants. *D. odorifera* can obtain N through symbiotic N_2 fixation, while *Eucalyptus* may obtain a more than the proportional share of soil inorganic N due to a high competitive ability to take up this N, which was proved in our preliminary study [24]. Namely, *Eucalyptus* was the dominant species taking up more amounts of N from soil in the intercropping system, which decreased the soil N concentration compared with the *D. odorifera* monoculture [52]. Therefore, biological N fixation by *D. odorifera* in the intercropping system was improved, and N sparing was increased, suggesting that increased *Eucalyptus* growth is not only the result of N transfer but also N sparing.

Studies have shown that positive and negative effects between species in terms of N absorption coexist in various systems [53] or promote the growth of both N-fixing species and *Eucalyptus* in mixed-species plantations [54]. Our results from both experiments are consistent with those of Hong et al. [52]; i.e., the positive effects improved the growth of *Eucalyptus*, but the growth of and N content in *D. odorifera* were limited in the mixed system. One explanation for these findings is the competition of *Eucalyptus* was greater than that of *D. odorifera* [24], and increased competition for light, soil water and nutrition contributed to the decreased growth of *D. odorifera* in the mixture. Another reason is related to N transfer; *Eucalyptus* benefits from transferred N and has a positive effect in mixed systems. In addition, root symbiotic relationships and root exudate allelochemicals are commonly involved in root interactions [55,56]. Some species contain unique allelochemicals in their exudates to inhibit neighboring plants [57]. Xia et al. showed that broadleaf species

chemically mediate the growth of neighboring plants (*Chinese fir*) through root exudates [58]. *Eucalyptus* has been widely studied for its high allelochemical content, such as phenolic acids [59]. For example, Liu et al. suggested that three phenolic acids were isolated from the roots of *Eucalyptus*, and they would decrease the *Albizia jjulibrissin* plant growth [60]. Therefore, they also may inhibit biomass growth of *D. odorifera* in our study, but further studies are needed to test whether *D. odorifera* biomass would be inhibited by phenolic acids of *Eucalyptus*. Importantly, the total biomass of the *Eucalyptus* monoculture plantations was greater than that of the mixed plantations and *D. odorifera* monoculture plantations under different N levels. Compared with pure *Eucalyptus* plantations, although the soil N concentration was increased (Table 3), the total productivity in the mixture decreased in a short time. Therefore, suggesting that the mixed plantations of *D. odorifera* and *Eucalyptus* should be maintained as long-term silvicultural systems with sustainable management. Additionally, to solve the problem of wood shortage, we suggested that the proportion of *Eucalyptus* and *D. odorifera* with 67% and 33% probably more scientific and reasonable reflect the current management in commercial plantations than previously recognized.

4.4. N Transfer Probably Occurs but Differs between Natural Areas and Commercial Areas

N transfer was studied via seedlings in a greenhouse, which might yield substantially different findings compared with those of field experiments because of differences in growing conditions. The main reason for this was to avoid loss of N due to rain; our pot experiment was conducted in the greenhouse. However, we confirmed the substantial bidirectional transfer of N occurred between the litters using experiments under field conditions, which showed the net transfer of N from high-N to low-N litter [13]. This is consistent with results obtained by Godoi et al., in which it was found that the N leaf concentration correlated positively with the N in the *Eucalyptus* litter under field conditions [61]. This result suggested that transferred N in *Eucalyptus* (% Ndft) would be increased in the field due to the increase the absorption N from the leguminous plants' litter, while litter and microbes had smaller effects on N transfer in our experiment compared with those in natural and commercial areas [13,62]. In addition, more N may be absorbed from the soil during the growth process in natural and commercial areas than in pot experiments because of differences in growth duration, as demonstrated by experiments on *Eucalyptus* and casuarina (*Casuarina cunninghamiana*), where the % NT after one year was double that at six months [22].

Although the increasing N content and biomass of *Eucalyptus* benefited from N transfer under field conditions, the NT % was probably lower than in the pots because N was mainly released belowground close to the N_2-fixing trees [6]. First, different trees can exert effects through belowground chemical interactions, where the root exudates of neighbors influence root placement patterns [58], and there may be different root placement patterns and distances between the pot and field experiments. For example, the ^{15}N values in young *Eucalyptus* leaves and roots were shown to decrease with increasing distance from labeled *Acacia* trees [6]. Second, for the plantations, the moderate reduction in stand density of forests was beneficial to the growth of trees and maintaining soil nutrients and soil water, which helps to achieve sustainable management in the long run [63]. Therefore, the distance between different trees under the field conditions was greater than that under the pot conditions, resulting in a lower NT % in the natural areas than that in the pot experiment. For example, the % NT values in the pot conditions for *D. odorifera* transferring N to its hemiparasite (*Santalum album*) (68–72%) with nodulation treatment [14] or *D. odorifera* transferring N to *Eucalyptus* (6.5–9.6%) [24] were greater than those determined for *Acacia* trees transferring N to *Eucalyptus* (3.33%) within approximately 6.2 m of each other [6].

5. Conclusions

Under pot conditions, a foliar ^{15}N labeling approach was used for the first time to detect the bidirectional N transfer in seedlings of *Eucalyptus* and an N_2-fixing species, *D. odorifera*, and our study demonstrated the feasibility of detecting the bidirectional N transfer

between woody plant species in pot experiments via the foliar ^{15}N labeling approach. The findings suggested that the net N transfer occurred from *D. odorifera* to *Eucalyptus* and was affected by the N application rate and growth duration. Transferred N is an important N source and increases the productivity of *Eucalyptus*, particularly when the soil already has low N concentrations (at most, 127.0 mg plant^{-1} N, which was 21.2% of the TN accumulated in *Eucalyptus* plants). Further studies on the possible benefits of the N transfer from *D. odorifera* to *Eucalyptus* in the field are warranted to evaluate the long-term influence on *Eucalyptus* productivity and to provide evidence for the sustainable development of *Eucalyptus* and N-fixing species in mixed forests.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f12091171/s1, Figure S1: Flow diagram of ^{15}N-labeled experiment, Table S1: The N concentration of *Eucalyptus* and *D. odorifera* under different N application rate and planting system at 90, 135 and 180 days. Table S2: The F statistic and *p* values for nitrogen levels (N), time (T) and planting systems (P-S) ANOVA effects on the dry matter yield of *Eucalyputs urophylla* × *grandis* and *D. odorifera*. Table S3: The F statistic and *p* Values for nitrogen levels (N), time (T) and planting systems (P-S) ANOVA effects on the N content of *Eucalyputs urophylla* × *grandis* and *D. odorifera*. Table S4: The mean of atom % ^{15}N (standard error) in plants compartments of *D. odorifera* and *Eucalyptus* before ^{15}N labeling (^{15}N abundance values). Table S5: The F statistic and *p* values for nitrogen levels (N) and time (T) ANOVA effects on N transfer. Table S6: The F statistic and *p* values for nitrogen levels (N), time (T) and planting systems (P-S) ANOVA effects on soil TN, NH_4^+-N and NO_3^--N concentration under the pot conditions. Table S7: The F statistic and *p* values for Nitrogen levels (N) and planting system (P-S) ANOVA effects on dry matter yield under field conditions.

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