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Thermo Condition Determines the Uptake of Autumn and Winter Applied Nitrogen and Subsequent Utilization in Spring Tea (*Camellia sinensis* L.)

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Abstract: The effect of thermal condition on the uptake of autumn and winter applied N and its subsequent utilization in spring tea (*Camellia sinensis*) was investigated by applying ¹⁵N enriched urea as single or split applications between October and February in two commercial plantations at Xingyang of Henan province and Yongchuan of Chongqing with different thermal conditions. The proportion of N derived from ¹⁵N-labeled urea ($N_{dff\%}$) in fibrous root and mature leaves 15 days after application at Xingyang and the $N_{dff\%}$ of mature leaves on the day of the first spring tea harvest at both sites were the highest in the single October application. The $N_{dff\%}$ of the following spring tea was also the highest in the single October application at both sites. The results showed that application of N fertilizer in October relative to other later months most significantly improves the accumulation of plant N reserves and consequently contributes more significantly to the early spring tea. Such timing effect was related to the thermal condition, i.e., the growing degree days ($^{\circ}\text{C}\cdot\text{d}$, $T > 8^{\circ}\text{C}$) between the dates of fertilization and harvest of young shoots, which represents the combining effect of the temperature and the residence time of N fertilizer in the soil.

Keywords: N reserves; N remobilization; temperature; growing degree days; dormancy; timing of fertilization

Citation: Ma, L.; Jiang, S.; Deng, M.; Lv, L.; Xu, Z.; Ruan, J. Thermo Condition Determines the Uptake of Autumn and Winter Applied Nitrogen and Subsequent Utilization in Spring Tea (*Camellia sinensis* L.). *Horticulturae* **2021**, *7*, 544. <https://doi.org/10.3390/horticulturae7120544>

Academic Editor: Wilfried Rozhon

Received: 12 October 2021

Accepted: 29 November 2021

Published: 2 December 2021

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1. Introduction

Tea is one of the most popular beverages in the world, possessing many health benefits [1]. Between October and February, tea plants are usually in dormant state without active shoot growth because of the inappropriate low temperature. Young shoots of evergreen woody tea plants are harvested during March through the end of September to produce spring (March and April), summer (May, June, and July), and autumn (August and September) teas in subtropical areas of China. The quality of green tea is determined by the season of production and harvest standards. Spring tea has abundant free amino acids, reasonable polyphenol or catechin contents, and, consequently, a low but desirable ratio of polyphenol to total amino acids [2,3]. The high quality (so-called premium) green teas commanding good prices are mostly tip-harvested at the developmental stage of bud with one expanding young leaf in early spring (March and April) [4]. The average price of premium green tea is approximately three times that of common tea produced from later spring and other seasons. Today, the production of premium green teas, which account for over 70% of total tea value with approximately 49% of tea quantity, has become the prevailing pattern for much better economic benefits and marketing prospects [5]. Our previous work showed that the ability of nitrogen (N) storage and remobilization to young shoots is of particular importance to ensure good quality of spring tea [4].

The internal cycling of N reserves contributes a major proportion of N (up to 100%) to support the spring growth of trees, especially when the mineral N availability is low because of the slow mineralization rate under low temperature [6]. In deciduous trees, N reserves are built up by the re-translocation of nutrients in senescing leaves back to branches and roots and/or by absorption in autumn [7–9]. In evergreen trees, the N reserves are contributed from the previous absorption during active vegetative growth or the absorption from the soil during vegetative dormancy [10–13]. The size of N reserves is a determinant factor influencing the amounts subsequently remobilized and its capability to support spring growth [11,13]. Increasing N reserves improves the agronomic performance (e.g., canopy development, flowering, and fruit quality) of horticultural crops [14–18]. External N availability and fertilizer application timing greatly affect the size of N reserves. N supplied in the early phenological stage is absorbed at a high rate and mainly allocated to vegetative and/or fruit growth [19–21]. By contrast, postponing N supply to later stages is preferentially allocated to storage organs; therefore, this effectively increases the size of winter reserves, although the total N uptake is often reduced due to decreasing growth demand [22–25]. However, some works in field-grown peach and orange trees have reported controversial findings that adequate storage does not benefit from fertilization and soil N uptake in the late phenological stages [26,27].

N uptake and distribution in tea plants are determined by the demand imposed from shoot growth and phenological stage [28–30]. Tea yield and quality significantly respond to N nutrition, requiring frequent N fertilization to sustain yield with desirable quality [29,31–33]. Multiple N applications are frequently made in attempts to synchronize the supply and seasonal nutrient demand in accordance with local weather conditions. Fertilizers are applied as base fertilization between October and December and/or top-dressed sometime before the harvests of spring, summer, and autumn teas. Currently, in China, the production of premium green teas of very young tips harvested only in early spring (March until early April) has been the prevailing pattern. Our previous field experiment described a substantial absorption of N fertilizer applied in winter (January and February) by dormant tea plants [30] and contributed to the buildup of plant N reserve which is remobilized and makes a crucial contribution to the quality of spring tea [4,34]. The strategy of proper use of internal N reserves, which has been advocated in the optimization of N nutrition management in fruit trees, should also be considered in tea plantations to increase N use efficiency and avoid environmental contamination [35].

The timing of N fertilization must be finely tuned to focus on spring tea production, of which the N demand dynamics are largely different from summer and autumn teas. Few field experiments have been conducted to evaluate the timing effect of N applied in dormant stage on its absorption by tea plants, limited to just a few pot experiments [28–30]. In the present field experiments, ^{15}N enriched urea was applied on different dates from late October through February at two tea plantations with different heat conditions. The enriched ^{15}N tracing method has been successfully and widely applied to investigate N absorption, storage, remobilization, and cycling by horticultural plants [24,30,36]. We also compared the effect of a single application in late October or split applications in late October and January with identical N rates. The aim of the presented study was to investigate the timing effect of N fertilization on N adsorption and storage of tea plants in vegetative dormancy and subsequent utilization in the following spring tea.

2. Materials and Methods

Two tea plantations were selected for the field experiments. One was located at Yongchuan, Chongqing province (longitude $105^{\circ}53'$, latitude $29^{\circ}23'$, altitude 570 m) belonging to Chongqing Academy of Agricultural Sciences. Another tea plantation was located at Xingyang, Henan province (longitude $114^{\circ}31'$, latitude $32^{\circ}16'$, altitude 57 m) belonging to Xingyang Academy of Agricultural Sciences. The two plantations were established from rooted-cuttings of variety Fudingdaibai and Pingyangtezao, respectively. Both varieties are officially certified clones suitable for green tea. At both sites, bushes,

each consisting of two rooted-cuttings, were cultivated in lines with a distance of 1.2 m. The distance between bushes of the same lines was 0.33 m. Weather data were collected from the local meteorological observatory. The mean annual air temperature and rainfall of the previous 20 years were 17.7 °C and 1015 mm at Yongchuan and 15.5 °C and 1106 mm at Xingyang, respectively. The two plantations were commercially managed before the present experiments to produce nationally known premium green teas, Yongchuan- Xiuya and Xingyang-Maojian, respectively. Prior to the experiment (on October 10), one composite soil sample was taken by auger from about 15 points from each experimental field, thoroughly mixed, and air dried for the analysis of soil chemical properties. Soil pH was measured in a 1:2.5 soil–water solution using a pH meter (Orion 3 Star, Thermo Ltd., Waltham, MA, USA). Contents of soil organic carbon and total nitrogen were measured using a C/N elemental analyzer (Vario Max, Elementar, Langenselbold, Germany). Soil available P, K, and Mg were extracted using the Mehlich 3 method, and then measured using Inductive Coupled Plasma (Thermo Jarrell Ash Ltd., Franklin, MA, USA). The soil properties are presented in Table 1. A total of 48 bushes with visually uniform canopy sizes were selected at each site. Plastic boards (0.4 cm thick) were buried vertically to 30 cm depth to isolate a mini-plot of 0.4 m² (0.66 m in length and 1.2 m in width) containing two bushes [30]. Each plot was separated by one blank bush.

Table 1. Selected soil properties of the experimental tea plantations in Yongchuan, Chongqing, and Xingyang, Henan province.

Parameter		Site	
		Yongchuan	Xingyang
pH		3.85	4.34
Organic matter	(mg g ^{−1})	14.1	19.0
Total N	(mg g ^{−1})	1.3	1.1
Available K	(mg kg ^{−1})	62.6	50.0
Available P	(mg kg ^{−1})	24.1	7.2
Available Mg	(mg kg ^{−1})	45.2	205

¹⁵N enriched urea (5.25% abundance, purchased from Shanghai Research Institute of Chemical Industry Co., Ltd., Shanghai, China) was applied to the soil of isolated mini-plot at a rate equivalent to 180 kg N ha^{−1} on five different dates or split into two applications. At Yongchuan, the dates of single N fertilization were October 21, November 25, and December 25, and January 14 and February 1 of the subsequent year. Split applications were implemented on October 21 and January 14 (Figure 1a). At Xingyang, individual N application dates were October 24, November 25, and December 25, and January 14 and February 20 of the subsequent year, or split applications on October 24 and January 14 (Figure 1a). The application in February at Xingyang was intentionally postponed because of the later harvest of spring tea. Other nutrients were applied at identical rates to all plots in case of low availability indicated by soil analysis. There were six treatments (five different dates and split applications) with four replications at each site. At Yongchuan, potash of sulfate (K₂O, 150 kg ha^{−1}) and kieserite (MgO, 30 kg ha^{−1}) were applied on October 21 to compensate for the low availability of K and Mg of the soil. At Xingyang, single superphosphate (P₂O₅, 100 kg ha^{−1}) and potash of sulfate (K₂O, 150 kg ha^{−1}) were applied on October 24 to compensate for its low availability of P and K.

Samples of fibrous roots and mature leaves were taken from the mini-plots in three batches (Figure 1a). The first batch of samples was taken 15 days after each ¹⁵N application (DAA15). Soil cores were taken using an auger (4 cm diameter) from both sides within the application area at a depth of 0–20 cm. Fibrous roots < 2 mm in diameter were carefully sifted through a 2-mm mesh sieve, picked out, and rinsed thoroughly with deionized water, then blotted dry. Mature leaves were taken from the canopy surface at the same time as fibrous roots. The second batch of samples was taken on the day of the first harvest of young spring shoots (referred to as DFH) (March 16 at Yongchuan and April 8 at Xingyang)

(Figure 1a). The third batch of samples was taken after the termination of spring teas (the day post-harvest, referred to as DPH) (May 4 at Yongchuan and May 12 at Xingyang) (Figure 1a). A few days were allowed to the sampling after the last harvest of young shoots to secure the termination of spring tea. Root and leaf samples were dried in an electric oven at 65 °C for constant weight.

Figure 1. The experimental design (a) and daily air temperature (b) at Yongchuan and Xingyang. Fertilization (○); sampling of fibrous roots and mature leaves 15 days after application (DAA15, ▽), on days of the first harvest (DFH, ▲) and post-harvest (DPH, ■); harvest of the following spring tea (X).

Young spring shoots consisting of a bud with one young expanding leaf were harvested by hand, according to the standard for commercial green teas (Yongchuan-Xiuya and Xingyang-Maojian) (Figure 1a). At Yongchuan, the harvest of spring tea started on March 16 and finished on April 29. At Xingyang, the harvest of spring tea started on April 8 and finished on April 26. All harvested young shoots were immediately treated in a microwave oven for 2 min to inactivate the polyphenol oxidase enzyme and finally dried in an electric oven at 65 °C for constant weight. Plant samples were ground to homogeneity with a ball mill (Mixer Mill MM300, Retsch, Haan, Germany) before measurements. Total N concentrations and the abundance of ^{15}N were determined using an elemental analyzer (Thermo NE 1112) coupled (ConFlo III) to an isotope ratio mass spectrometer (ΔPlusAD , Thermo Finnigan, Germany). To reduce analysis costs, samples of March 20 and 28 from the same plot at Yongchuan were pooled and measured. The uptake and utilization of ^{15}N fertilizer were indicated by $N_{\text{dff}\%}$, the proportion of N derived from ^{15}N -labeled urea, which was calculated according to the following Equation (1).

$$N_{\text{dff}\%} (\%) = \frac{\text{Sample } ^{15}\text{N abundance} - \text{natural } ^{15}\text{N abundance}}{\text{Fertilizer } ^{15}\text{N abundance} - \text{natural } ^{15}\text{N abundance}} \times 100\% \quad (1)$$

The growing degree days of heat accumulation (GDD) (the sum of air temperature units above a threshold, T_0) between the date of fertilization and the day of the first harvest (n) was calculated according to the following Equation (2) [37].

$$\text{GDD} (^\circ\text{C}\cdot\text{d}) = \sum_{i=1}^n (T_i - T_0), (T_i > T_0, T_0 = 8^\circ\text{C}) \quad (2)$$

ANOVA was performed on datasets using the analysis module embedded in SigmaPlot 12.5 software (Systat Software, Inc.). Means of parameters from different fertilization dates at the same sampling times and those from different sampling times of the same fertilization dates were further distinguished by the Fisher LSD test in case of initial significance ($p < 0.05$). Statistical analysis was performed separately for each site.

3. Results

3.1. Thermal Condition at Two Sites

The daily air temperature within the experimental period varied between 3.2–27.2 °C and −3.5–28.2 °C from October 1 through May 30 at Yongchuan and Xingyang, respectively (Figure 1b). The air temperature was always above zero at Yongchuan. At Xingyang, it occasionally went down to below zero in November, January, and February. The two sites had the same magnitudes of air temperature in October and in the time beyond late March. In other dates, Yongchuan had higher air temperature than Xingyang. Periodic mean air temperature within 15 days after fertilization (DAA15) was the highest and lowest for applications in October and December at both sites, respectively (Figure 2a,b). The two sites had a similar mean temperature for the application in late October, except for low air temperature in a few days at Xingyang. In other applications, the periodic mean air temperature was lower at Xingyang than Yongchuan. We calculated the growing degree days (GDD, $T_0 > 8$ °C) between the day of fertilization and the first harvest of spring tea (Figure 2c,d). The GDD at Yongchuan was the highest (449 °C•d) for the application in October and decreased in later applications between November and February. At Xingyang, GDD was 330 °C•d for the applications of October and decreased to a constant for later applications. Both sites had relatively low rainfall between October and February and rain increased in March (Figure S1). Yongchuan had higher cumulative rainfall between October and December but Xingyang turned to be higher after December.

Figure 2. Periodic mean air temperature within 15 days after fertilization (a,b) and growing degree days (GDD, ≥ 8 °C) between the fertilization dates and the first harvest of spring tea (c,d) at Yongchuan (a,c) and Xingyang (b,d).

3.2. ^{15}N in Fibrous Roots and Mature Leaves

At Yongchuan, the fertilization dates insignificantly ($p > 0.05$) affected the total N concentrations of fibrous roots and mature leaves on DAA15, DFH (March 16) and DPH (May 5) (Table S1). The N concentrations of root and leaf also changed little in the process from DAA15 to DFH. However, compared to the previous two times, root N concentration on DPH decreased whereas the leaf N concentration increased, as indicated by their means across fertilization dates ($p < 0.001$). The $N_{\text{dff}\%}$ of fibrous roots reached 11.7–21.0% on DAA15 and changed insignificantly in the later times (DFH and DPH) in most treatments (Figure 3a). Only in the November application, the root $N_{\text{dff}\%}$ increased significantly from DAA15 to DFH. The $N_{\text{dff}\%}$ of fibrous roots in all three sampling times was insignificantly affected by fertilization dates. The $N_{\text{dff}\%}$ in mature leaves was generally low (only 0.5–1.6%) on DAA15, which was less than one-tenth of those in fibrous roots (Figure 3b and Table 2). In the duration from DAA15 to DFH, the leaf $N_{\text{dff}\%}$ increased to 1.7–6.2% and the increase was significant only in single and split October applications (Figure 3b). The leaf $N_{\text{dff}\%}$

at DFH was significantly affected by fertilization dates and was the highest in October application and the lowest in January and February applications. On DFH, the $N_{dff\%}$ leaf/root ratio ($LN_{dff\%}/RN_{dff\%}$) was also significantly smaller in the January and February than in other applications (Table 2). There was no significant difference in leaf $N_{dff\%}$ among fertilization dates on DPH. However, the leaf $N_{dff\%}$ increased ($p < 0.05$) by 2.7–9.9 folds to 14–20.4% and resulted in a sharp increase of $LN_{dff\%}/RN_{dff\%}$ ratio during the period from DFH to DPH in all treatments (Figure 3b and Table 2).

Figure 3. $N_{dff\%}$ of fibrous root (a) and mature leaves (b) 15 days after ^{15}N fertilization (DAA15), on the days of first harvest (DFH) and post-harvest (DPH) of spring shoots at Yongchuan, Chongqing province. The data are means and SE ($n = 4$). Single bars without symbols above columns represent LSD values indicating significant differences among DAA15, DFH, and DPH for each of the specified fertilization dates. A single bar without a symbol ticked with (LSD) in (b) represents LSD value indicating significant difference on DFH among fertilization dates.

Table 2. Leaf/root ratio of $N_{dff\%}$ ($LN_{dff\%}/RN_{dff\%}$) measured 15 days after ^{15}N application (DAA15), on the days of the first harvest (DFH) and post-harvest (DPH) of young spring shoots at Youngchuan (March 16 and May 4) and Xingyang (April 8 and May 12), respectively.

Site	Fertilization Date	DAA15	DFH	DPH
Yongchuan	October 21	0.030	0.25 b ¹	0.91
	November 25	0.045	0.19 ab	1.01
	December 25	0.045	0.25 b	0.92
	January 14	0.105	0.09 a	0.71
	February 1	0.041	0.09 a	1.03
	October 21 + January 14	0.049	0.29 b	1.19
Xingyang	October 24	0.042 bc	0.19	0.27
	November 25	0.015 ab	0.23	0.22
	December 25	0.003 a	0.14	0.26
	January 14	0.015 ab	0.22	0.27
	February 20	0.053 c	0.22	0.37
	October 24 + January 14	0.049 c	0.17	0.33

¹ Different letters following data of the same column indicate significant difference at each site. Data are means of four replications.

At Xingyang, total N concentration of fibrous roots was significantly affected by fertilization dates on DFH, but not on DAA15 and DPH (Table S1). Root N concentration was the highest in October application and lower in the applications of December, January, and February. The root N concentration changed little from DAA15 to DFH, but decreased significantly from DFH to DPH. The total N concentration of mature leaves was also insignificantly affected by fertilization dates. The leaf N concentration across fertilization

dates declined at DPH in comparison with the previous two sampling times on DAA15 and DFH (Table S1). The root $N_{dff\%}$ reached 10.5–31.1% on DAA15 and continued to increase ($p < 0.05$) on DFH in all other applications except the October one (Figure 4a). On both DAA15 and DFH, root $N_{dff\%}$ was significantly affected by application dates and was the largest in single or split October application. From DFH to DPH, the root $N_{dff\%}$ had little change. On DAA15 the $N_{dff\%}$ in mature leaves was generally low (0.01–1.6%), but they were significantly affected by fertilization dates. Both the leaf $N_{dff\%}$ and $LN_{dff\%}/RN_{dff\%}$ were the highest in October applications but very low in November, December, and January applications (Figure 4b and Table 2). The $N_{dff\%}$ of mature leaves increased to 4.0–6.7% on DFH ($p < 0.05$), and the increase continued until DPH (Figure 4b). Nevertheless, on both DFH and DPH, the leaf $N_{dff\%}$ and $LN_{dff\%}/RN_{dff\%}$ were insignificantly affected by different fertilization dates.

Figure 4. $N_{dff\%}$ of fibrous root and mature leaves 15 days after ^{15}N fertilization (DAA15), on the days of first harvest (DFH) and post-harvest (DPH) of spring shoots at Xingyang, Henan province. The data are means and SE ($n = 4$). Single bars without symbols above columns represent LSD values indicating significant differences among DAA15, DFH, and DPH for each of the specified fertilization dates. A single bar without a symbol ticked with (LSD) represent LSD values indicating significant difference on DAA15, DFH (a), and DAA15 (b) among fertilization dates.

3.3. ^{15}N in Young Spring Shoots

At Yongchuan, the harvest of young shoots started on March 16. Shoot N concentrations were the highest in the first harvest (March 16, mean 57.7 mg g^{-1}) and decreased afterward to the lowest in the last harvest (April 29, mean 46.3 mg g^{-1}) (Table 3). The young shoot $N_{dff\%}$ of the first harvest reached 4.8–16.9%, and they were significantly different among fertilization dates (Figure 5a). In the first harvest, young shoot $N_{dff\%}$ was the highest in the single October application, which was 3.6-fold of the lowest in February application. Split applications (October and January) had a slightly lower shoot $N_{dff\%}$ than the single October application. This tendency was continued in the following harvests on March 20–28 and April 1. Their difference became smaller ($p > 0.05$) in the last harvest (April 29). The mean $N_{dff\%}$ of young shoots increased from 10.9% of the first to 15.3% of the last harvest round (Table 3). The increases were particularly remarkable in the February and January applications (Figure 5a). Split applications had a significantly lower $N_{dff\%}$ than the single application.

Table 3. Mean N concentration and $N_{dff\%}$ of young shoots across fertilization dates.

Site	Harvest Date	N Concentration (mg g ⁻¹)	$N_{dff\%}$ (%)
Yongchuan	March 16	57.7 c ¹	10.9 a
	March 20–28	53.3 b	12.6 b
	April 1	47.7 a	11.8 ab
	April 29	46.3 a	15.3 c
Xingyang	April 8	64.4 b	18.2 ab
	April 13	61.0 a	17.4 a
	April 26	61.1 a	19.6 b

¹ Different letters following data of the same column indicate significant differences at each site. Fertilization dates were taken as replications in one-way repeated ANOVA.

Figure 5. $N_{dff\%}$ of spring young shoots harvested on different dates at Yongchuan (a) and Xingyang (b) from tea plants receiving ¹⁵N enriched urea individually in October, November, December, January, and February and split in October and January. Data are means and SE ($n = 4$). Bars without data points above columns represent LSD values indicating significant ($p < 0.05$) difference among fertilization dates on each of the specified harvest dates.

At Xingyang, the harvest of young shoots started on April 8, a time much later than Yongchuan for lower winter and spring temperature. Again, the mean shoot N concentration was significantly higher in the first than in later harvests (Table 3). The young shoot $N_{dff\%}$ of the first harvest reached 16.8–22.2% and was significantly different among fertilization dates (Figure 5b). $N_{dff\%}$ was the highest in the single October application, which was 1.2–1.3 fold of those applied in other months. The split application had significantly lower $N_{dff\%}$ than that of the single October application. This tendency was continued in the following harvest on April 13, with smaller but significant differences (Figure 5b). The young shoot $N_{dff\%}$ of the last harvest (on April 26) was insignificantly different among fertilization dates. The mean $N_{dff\%}$ across fertilization dates of the last harvest was higher than two tier harvests (Table 3).

The $N_{dff\%}$ of root, mature leaves, and young shoots on the DFH was closely associated with the change of growing degree days at both sites (Figure 6). At Yongchuan, their relationship could be well described by two regression lines and $N_{dff\%}$ reached the maximum at the GDD of about 220 °C•d. At Xingyang, their relationship was described by one regression line as there was little difference among the last four fertilization dates. At this site, $N_{dff\%}$ reached the maximum at the GDD of about 330 °C•d.

Figure 6. Relationship of $N_{diff}\%$ of root, mature leaves, and young shoots on the day of the first harvest (DFH) with growing degree days ($T > 8^\circ\text{C}$, GDD) between fertilization dates and DFH at Yongchuan (a) and Xingyang (b). Black, green and blue colors of symbols and lines indicate root, mature leaves, and young shoots, respectively. Symbols ○, ▽, □, ◇, and △ indicate fertilization dates in October, November, December, January, and February, respectively.

4. Discussion

In the present study, we applied ^{15}N enriched urea on different dates from late October through February at two tea plantations. These two sites were selected for their different thermo conditions during November and March, but very similar conditions in other months (Figure 1b). We measured ^{15}N uptake and utilization after a short (DAA15) and two longer times (DFH and DPH) to investigate the interacting effect of fertilization date and thermo conditions on fertilizer N uptake and utilization in the following spring.

4.1. Temperature and the Short-Term Autumn ^{15}N Absorption and Upward Transport

Temperature is a key factor affecting the rate of nutrient uptake. Low temperature reduces nutrient uptake by decreasing plant growth rate and thereby nutrient demand and root respiration then less energy supply [38]. With decreasing temperature in October, tea plants in subtropical area stop vegetative growth and turn into vegetative dormancy. Demand of nutrients therefore largely decreased, but persisted as indicated by the present experiment that fertilizer N applied in the months between October and February was taken up by fibrous roots at both sites (Figures 3–5). This finding coincides with previous experiments showing N uptake in the late autumn and winter dormancy in the absence of active shoot growth in subtropical ever-green woody plants such as tea and citrus [18,30]. A significant difference between application dates was observed at Xingyang (Figure 4), where $N_{diff}\%$ in root and $\text{LN}_{diff}\%/\text{RN}_{diff}\%$ within DAA15 were much lower in November, December, and January than other dates. This is likely linked to different thermo conditions. At this site, periodic air temperatures within DDA15 occasionally fell below zero and were conspicuously lower in application dates other than October (Figure 2c), possibly leading to little N uptake. Previous work showed that N uptake by cool-season turfgrasses is greatly decreased when the temperatures approach 0°C [39]. However, at Yongchuan, we observed little difference in ^{15}N uptake and transport with DAA15 among fertilization dates (Figure 3). At this site, the periodic air temperature never fell below zero ($3.7\text{--}8.5^\circ\text{C}$), even in the coldest December and January (Figures 1b and 2a). There is no information about the lowest temperature below which N uptake by tea plant root ceases. However, it is assumed that the air/soil temperature at Yongchuan within DAA15 of all applications met the minimum requirement for the nutrient uptake as a result of weak demand at vegetative dormancy.

Within DAA15, $N_{diff}\%$ of mature leaves was below a tenth of those in roots, showing only weakly upward transport from root to above-ground. The low demand of above-

ground at dormancy, reduced ability of roots to conduct water, and weak transpiration flow might all have contributed to the slow upward ^{15}N translocation under low temperature [40,41]. On DAA15, the root $N_{\text{dff}\%}$ did not differ significantly between the two sites for each specific application date, whereas leaf $N_{\text{dff}\%}$ was greater ($p < 0.05$) at Yongchuan than Xingyang in the applications of November, December, and January. The $\text{LN}_{\text{dff}\%}/\text{RN}_{\text{dff}\%}$ ratios in these applications were disproportionately low at Xingyang, possibly suggesting that ^{15}N transport from the root to above-ground had been particularly inhibited by the lower temperature at this site (Figure 2 and Table 2).

4.2. Thermo Condition and the Accumulation of ^{15}N Reserves during Winter Dormancy

For ever-green trees nitrogen absorbed in winter, dormancy is stored in plants as N reserves [18]. In the present work, we were unable to precisely evaluate the effect of fertilization timing on the size of ^{15}N reserves in tea plants at the time when young spring shoots started to grow. On DFH, ^{15}N reserves might have already been utilized in young shoots. However, the build-up process of N reserves and the contribution of fertilizer ^{15}N could be partly reflected by the change of ^{15}N in fibrous roots and mature leaves between DAA15 and DFH. Root $N_{\text{dff}\%}$ changed only slightly and insignificantly between DAA15 and DFH at Yongchuan in all applications, except November's (Figure 3a). There was also little change in the root $N_{\text{dff}\%}$ of October applications at Xingyang during DAA15 and DFH. It therefore appeared that the root $N_{\text{dff}\%}$ reached their maximum within DAA15 in these applications. In contrast, at Xingyang in all other applications except October's and at Yongchuan in November's application the $N_{\text{dff}\%}$ of fibrous roots had not reached their maximum on DAA15, thereafter, continued to increase until DFH (Figures 3a and 4a). In all applications at both sites, $N_{\text{dff}\%}$ of mature leaves increased by 5–8 fold, and $\text{LN}_{\text{dff}\%}/\text{RN}_{\text{dff}\%}$ ratio increased by 3.6–6 fold during the period from DAA15 to DFH (Figures 3b and 4b and Table 2). These increases likely were the result of an accumulating effect of ^{15}N upward transport from roots, a process which also became stronger in response to rising temperature from DAA15 to DFH (Figure 1b) [30]. Taking fibrous roots and mature leaves together, it was concluded that N reserves were greater in the October application than those of other months at both sites (Figures 3b and 4b).

4.3. Thermo Condition and ^{15}N Utilization in the following Young Spring Shoot

From DFH to DPH, N concentrations of fibrous roots at both sites decreased significantly (Table S1). Such a decrease of N concentration during spring growth was frequently observed in other trees and might be the result of remobilization to young shoots as the new sink [42]. On the other hand, N concentrations of mature leaves showed the opposite changes at the two sites (Table S1): it significantly increased at Yongchuan but decreased at Xingyang. The $N_{\text{dff}\%}$ of mature leaves and their ratios to fibrous roots corresponded to these changes, with a more remarkable increase at Yongchuan than at Xingyang (Figures 3b and 4b and Table 2). The changes of leaf total N and ^{15}N amounts were estimated based on $N_{\text{dff}\%}$ values assuming constant biomass between DFH and DPH. We found that about 65% (60–81%) of the increase of leaf N content could be accounted for by the change of ^{15}N amount at Yongchuan. Thus, the concomitant root ^{15}N uptake had contributed mainly to the increase of leaf N from DFH to DPH, indicating that ^{15}N had been allocated to both young shoots and mature leaves. However, at Xingyang, the N content of mature leaves decreased, suggesting a net N export to young shoots during this period. Meanwhile, leaf ^{15}N content increased only minimum (by 0.24-fold), suggesting that ^{15}N had been predominantly allocated to young shoots. The differing ^{15}N allocation to mature leaves and young shoots between the two sites might be attributed to different inherent properties of the two varieties which deserves future work.

Young shoot $N_{\text{dff}\%}$ was markedly affected by fertilization dates (Figure 5). Young shoot ^{15}N was contributed from the remobilization of N reserves before bud breaking and the current uptake during the spring growth. Application in October improved N absorption throughout winter and early spring, which was stored in the tea plants and

contributed to greater accumulation of N reserves in roots and mature leaves than other applications (Figures 3 and 4). The largest difference of $N_{dff\%}$ among dates of applications occurred in the first harvest (March 16 and April 8) at both sites, showing that N fertilizer applied in late October makes a relatively greater contribution to the early spring tea (Figure 5). This finding is in line with previous works demonstrating that the N taken up in the late phenological stages (i.e., in autumn before winter dormancy or after the harvest of fruits) is preferentially remobilized to support early spring growth [22–25,43]. The decreasing total N concentrations of young shoots from the first to the latest harvest, in contrast with the increasing $N_{dff\%}$ at both sites reflected weakening support of N reserves, which progressively depleted (Table 3). The difference of shoot $N_{dff\%}$ between application dates became smaller (Figure 5 and Table 3), likely suggesting the increasing ability of concurrent uptake in the late spring, and contribution to new spring growth with rising temperature [7,44]. The difference of young shoot $N_{dff\%}$ among fertilization dates was closely associated with the GDD values between dates of fertilization and the first harvest (Figures 2 and 6). At Yongchuan where there was large difference in GDD, there were significant different $N_{dff\%}$ of young shoots among different fertilization dates. At Xingyang where there was different GDD and shoot $N_{dff\%}$ only among October and other applications. Our finding confirms a few earlier works showing that air or soil GDD depicts the combining effect of temperature and residence time and thereby well predicted ^{15}N uptake by citrus, apple and tea plants [30,45,46].

There were clear differences of $N_{dff\%}$ in fibrous roots, mature leaves, and young spring shoots between the single October application and split applications in October and January. The split application supplied only half of the ^{15}N in October, likely weakened the N reserves and subsequently, the amount remobilized to young spring shoots. On the other hand, splitting another half to January application has shortened the residence time of this N part in the root zone and cumulative heat condition estimated from GDD [30,45,46]. Our work suggests that a decrease or withdrawal of autumn fertilization cannot be compensated by an later application, an observation has been previously shown in young peach trees [17].

5. Conclusions

In summary, application of N fertilizer in October relative to other later months most significantly improves the N absorption and accumulation of plant N reserves throughout the winter dormancy, and consequently contributes more significantly to the early spring tea. Such timing effect was closely associated with the growing degree days between the dates of fertilization and harvest of young shoots, which represents the combining effect of the temperature and the residence time of N fertilizer in the soil. The result showed the determining effect of thermal condition on the efficiency of fertilizer N applied in the autumn–winter period and provides practical information to assist the optimization of N nutrition management in green tea production. Due to the relatively short experimental duration, future works at more sites and for a longer period would further deepen our understanding about the interacting effect of thermal condition and N utilization by tea plants.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/horticulturae7120544/s1>, Figure S1: Cumulative rainfall from October 1 to May 30 at Yongchuan, Chongqing (CQ) and Xingyang, Henan (HN) province, China, Table S1: Total N concentration of fibrous roots and mature leaves measured 15 days after ^{15}N application (DAA15), on the days of the first harvest (DFH) and post-harvest (DPH) of young spring shoots at Yongchuan (March 16 and May 4) and Xingyang (April 8 and May 12), respectively.

Author Contributions: Conceptualization and experimental design, J.R. and L.M.; field investigation, sample collection and preparation, S.J., M.D., L.L. and Z.X. sample analysis, L.M., data curation, J.R., L.M., S.J. and M.D. writing of manuscript, J.R., L.M, Z.X. and L.L.; funding acquisition, J.R., L.L. and Z.X. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Science Foundation of China under Grant 32172634, the Earmarked Fund for China Agriculture Research System under Grant CARS 19, the Agricultural Science and Technology Innovation Program under Grant CAAS-ASTIP-TRICAAS, and Yunnan provincial special fund for construction of science and technology innovation base (202102AE090038).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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