Nitrogen Fertilization in Soil Affects Physiological Characteristics and Quality of Green Tea Leaves

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Abstract. To study the effects of soil nitrogen (N) fertilization on tea growth, quality and yield, a controlled experiment with green tea [*Camellia sinensis* (L.) O. Ktze] was conducted. Five N fertilization treatments in soil were designed: 0, 0.97, 1.94, 3.88, and 5.82 g/kg/pot, which were subsequently recorded as N0, N1, N2, N3, and N4. The changes to young shoot biomass, total N and carbon (C), Soil and Plant Analyzer Development (SPAD) value, photosynthetic parameters, senescent characteristics, endogenous hormones, and the quality of green tea leaves were investigated. The results showed that with the increase in N fertilization level, the young shoot biomass, total N and C, SPAD value, net photosynthetic rate (P_N), transpiration rate (T_r), stomatal conductance (g_s), superoxide dismutase activity, indoleacetic acid, gibberellin, zeatin (ZT), caffeine, and amino acids increased at first and then decreased, the maximums appeared at 3.88 g/kg/pot; whereas the intercellular CO_2 concentration (C_i), malondialdehyde contents, abscisic acid (ABA), polyphenol contents, and the ratio of polyphenols (PP) to free amino acid decreased at first and then increased, the minimums appeared at 3.88 g/kg/pot. The immediately significant change in all parameters appeared after 1 month of N treatments. The experiment showed that 3.88 g/kg/pot N fertilization level was the best for growth, quality, and yield of tea, which could provide a theoretical basis for short-term N fertilization management in tea tree.

Tea [*C. sinensis* (L.) O. Ktze] is a kind of traditional economic crop in southern China, and tea industry can quickly promote the local economy development (Zhang et al., 2010). Fertilization is an effective way to supply nutrients for tea tree, and it is also an important factor to improve yield and quality of tea (Ruan et al., 2001).

Nitrogen, either from organic or mineral fertilization, plays an important role in the growth and development of tea tree. Meanwhile, it is an important component of chlorophyll, protein, vitamins, enzymes, alkaloids, amino acids, and hormones in tea leaves, and it can significantly affect the yield and quality of tea leaves (Bethke et al., 2007; Kumar et al., 2004; Zhou et al., 2006). Previous studies have investigated

the effects of N on the growth and physiology of green tea (Mudau et al., 2006; Owour et al., 1991; Owour and Odhiambo, 1994; Ruan et al., 2010). These studies found that N affected CO_2 assimilation and light system function indirectly by influencing chlorophyll content (Berges et al., 1996; Rufty et al., 1988). Moreover, there is a significant positive correlation between N fertilization contents and photosynthetic rate of plant leaves. Some researchers found that N can increase photosynthetic rate (Haukioja et al., 1998), nonstructural carbohydrate accumulation (Wanyoko, 1983), and biosynthesis of C-based secondary metabolites (Haukioja et al., 1998). They also found that the total polyphenol concentrations in green tea leaves were increased by second metabolites (Owour et al., 1991, 2000). Reasonable N fertilization can significantly increase tea yield, free amino acid contents, caffeine, and water-leaching substances, and reduce tea polyphenol contents, whereas excessive use of N fertilization might reduce tea yield (Su et al., 2011). Previous studies mainly

focused on the effects of N fertilization level on tea yield and quality, whereas the effects of N fertilization level on photosynthetic characteristics, senescent characteristics, and endogenous hormones of spring tea have not been reported. In this article, we studied the effects of different N fertilization levels on photosynthetic parameters, chlorophyll content, antioxidant enzyme activity, endogenous hormones, and quality of tea leaves. The aim was to provide a theoretical basis for reasonably using N fertilization and improving yield and quality of tea leaves.

Materials and Methods

Plant materials and treatments. The experiment was conducted on 15 Jan. 2014 at Nanjing University of Information Science and Technology, China (lat. 32°2'N, long. 118°7'E, and altitude 22 m). Three 2-year-old rooted cuttings (cultivar Wuniuzao, plant height 30 ± 1 cm) were transferred to each pot (39 cm × 36 cm, height × diameter) filled with 8 kg air-dried soil that passed through a 5-mm sieve. The physicochemical properties of the growing media were determined by Hanlon (1994): organic matter 17.6 g·kg⁻¹, total N 0.78 g·kg⁻¹, available N 21.57 mg·kg⁻¹, available phosphorus 37.21 mg·kg⁻¹, available potassium 80.34 mg·kg⁻¹, and pH 5.7.

Five N fertilization levels were established at urea concentrations of 0, 0.44, 0.88, 1.76, and 2.64 g/kg/pot, which is equivalent to 0, 0.97, 1.94, 3.88, and 5.82 g/kg/pot N fertilization levels, respectively; these levels are subsequently recorded as N0, N1, N2, N3, and N4 according to Ruan et al. (2001). Each treatment had 15 pots. The fertilization was dissolved in water and applied to each treatment on 15 Feb. 2014. Plants were placed in the greenhouse at 28/18 °C day/night air temperature with natural light. The relative humidity ranged from 60% to 70%. The plants were watered as needed.

Collection of samples for chemical analyses. Samples of young shoots (plant height 40–50 cm and crown diameter 30–40 cm) consisting of one bud with two leaves were collected and frozen quickly in liquid N, and the samples were stored at –20 °C until freeze-dried. Collection of the young shoot samples began at the N supplements (15 Feb.) and continued at intervals of 15 Mar., 1 Apr., 16 Apr., and 1 May 2014.

Chlorophyll measurements. The relative content of chlorophyll in tea leaves was measured by the SPAD chlorophyll meter. The first mature and undamaged leave was randomly taken from tea trees in each treatment, and the SPAD was read with SPAD-502 (Minolta Co., Ltd., Osaka, Japan) (Yang et al., 2008). Three replications were measured for each leaf and 20 leaves for each treatment.

Photosynthetic parameters measurements. The fifth to eighth functional leaves from the top of plants were selected, and P_N , g_s , C_i , T_r were measured between 09:00 and 11:00 AM

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Table 1. Effects of nitrogen fertilization treatments on young shoot biomass (g DW/plant) of green tea on different dates.

Treatments	Date				
	2/15	3/15	4/1	4/16	5/1
N0	0.34 ± 0.01 Da	0.46 ± 0.02 Cd	0.55 ± 0.04 Bc	0.77 ± 0.05 Ad	0.82 ± 0.05 Ad
N1	0.33 ± 0.02 Ea	0.49 ± 0.03 Dcd	0.62 ± 0.04 Cc	0.89 ± 0.05 Bcd	1.14 ± 0.06 Ac
N2	0.35 ± 0.02 Ea	0.55 ± 0.04 Dbc	0.73 ± 0.05 Cb	0.95 ± 0.06 Bc	1.26 ± 0.06 Abc
N3	0.32 ± 0.02 Ea	0.68 ± 0.04 Da	0.89 ± 0.05 Ca	1.22 ± 0.07 Ba	1.73 ± 0.08 Aa
N4	0.35 ± 0.02 Ea	0.62 ± 0.03 Dab	0.76 ± 0.05 Cb	1.08 ± 0.05 Bb	1.35 ± 0.05 Ab

Results are presented as mean ± SD ($n = 3$). Uppercase and lowercase letters indicate significance of $P < 0.05$ by Duncan's test within each row and column, respectively. N0, N1, N2, N3, and N4 refer to 0, 0.97, 1.94, 3.88, and 5.82 g/kg/pot N fertilization, respectively.

Table 2. Effects of nitrogen fertilization treatments on total N contents (mg·g⁻¹) of green tea on different dates.

Treatments	Date				
	2/15	3/15	4/1	4/16	5/1
N0	7.6 ± 0.51 Eb	10.8 ± 0.62 Dd	16.5 ± 0.99 Ce	23.8 ± 1.00 Be	26.4 ± 1.02 Ae
N1	8.1 ± 0.67 Eab	13.5 ± 0.85 Dc	19.8 ± 1.02 Cd	27.6 ± 1.09 Bd	31.7 ± 1.10 Ad
N2	8.5 ± 0.57 Eab	19.7 ± 0.94 Db	29.3 ± 1.07 Cc	34.5 ± 1.10 Bc	38.3 ± 1.22 Ac
N3	9.1 ± 0.62 Ea	25.1 ± 1.04 Da	39.6 ± 1.14 Ca	48.4 ± 1.56 Ba	52.6 ± 01.64 Aa
N4	8.8 ± 0.59 Eab	20.6 ± 0.93 Db	34.5 ± 0.99 Cb	42.7 ± 1.31 Bb	46.9 ± 1.28 Ab

Results are presented as mean ± SD ($n = 3$). Uppercase and lowercase letters indicate significance of $P < 0.05$ by Duncan's test within each row and column, respectively. N0, N1, N2, N3, and N4 refer to 0, 0.97, 1.94, 3.88, and 5.82 g/kg/pot N fertilization, respectively.

Table 3. Effects of nitrogen fertilization treatments on total C contents (mg·g⁻¹) of green tea in different dates.

Treatments	Date				
	2/15	3/15	4/1	4/16	5/1
N0	429.4 ± 4.98 Ca	442.4 ± 5.10 Bc	463.6 ± 5.67 Bc	475.6 ± 6.07 Ac	483.5 ± 5.53 Ac
N1	432.5 ± 4.87 Ca	451.4 ± 4.94 Bbc	472.4 ± 6.21 Bc	482.5 ± 5.75 Abc	490.6 ± 5.69 Abc
N2	440.6 ± 6.31 Ba	455.6 ± 6.87 Ab	476.5 ± 6.45 Abc	488.3 ± 6.02 Aabc	495.8 ± 6.15 Aabc
N3	453.7 ± 5.76 Ca	464.5 ± 5.88 Ba	483.7 ± 5.68 Ba	490.1 ± 6.12 Aa	497.6 ± 6.65 Aa
N4	442.8 ± 5.08 Da	452.9 ± 4.90 Cab	476.8 ± 5.83 Bab	480.8 ± 5.47 Aab	488.4 ± 5.96 Aab

Results are presented as mean ± SD ($n = 3$). Uppercase and lowercase letters indicate significance of $P < 0.05$ by Duncan's test within each row and column, respectively. N0, N1, N2, N3, and N4 referred to 0, 0.97, 1.94, 3.88, and 5.82 g/kg/pot N fertilization, respectively.

using a portable photosynthesis measurement system (LI-6400; LI-COR Bioscience, Lincoln, NE). For each measurement, the leaf was exposed to 1000 μmol (photon)·m⁻²·s⁻¹ photosynthetic photon flux density, the temperature in the leaf chamber was set to 25 °C, the CO₂ concentration was 380 ± 10 μmol (CO₂)·mol⁻¹, and relative humidity was 60% to 70%.

Antioxidant enzyme measurements. The same functional leaves as those used in the photosynthesis measurements were sampled and immediately frozen in liquid N and stored at -40 °C for further enzyme analyses. Superoxide dismutase (SOD) (EC 1.15.1.1) activity was measured with extracts obtained from 300 mg frozen leaf tissue followed by Beauchamp and Fridovich (1973). The frozen leaves were homogenized in an extraction buffer containing 50 mM phosphate buffer (pH 7.8), 0.1% (w/v) ascorbate, and 0.05% (w/v) β -mercaptoethanol. The 3-mL assay mixture contained 50 mM phosphate buffer (pH 7.8), 9.9 mM·L⁻¹ methionine, 0.025% (w/v) nitroblue tetrazolium chloride (NBT), and 0.0044% (w/v) riboflavin. SOD activity was measured using the fact that the enzyme inhibits the photoreduction of NBT.

Lipid peroxidation measurements. Lipid peroxidation was estimated in terms of malondialdehyde (MDA) content. The MDA content was determined according to Zhao et al. (1994). Fresh leaves (1.0 g) were ground in 10% trichloroacetic acid and then centrifuged at 3000 rpm for 10 min. Two milliliters of the supernatant was mixed with 2 mL of 0.6% thiobarbituric acid (TBA) and incubated

Fig. 1. Changes in leaf chlorophyll content (Soil and Plant Analyzer Development) in 'Wuniuzao' after N fertilization. Uppercase and lowercase letters indicate significance of $P < 0.05$ by Duncan's test under the same N level and date, respectively. Error bars represent SD, $n = 3$. N0, N1, N2, N3, and N4 refer to 0, 0.97, 1.94, 3.88, and 5.82 g/kg/pot N fertilization, respectively. IAA = indole-3-acetic acid; GA₃ = gibberellin A₃; ZT = zeatin, and ABA = abscisic acid.

for 30 min at 100 °C to form an MDA-TBA2 adduct. The mixture was cooled rapidly in an ice bath. After centrifugation at 5000 rpm for

10 min, the absorbance was measured at 450 nm, 532 nm, and 600 nm. Lipid peroxidation was expressed as $\mu\text{mol}\cdot\text{g}^{-1}$ (FM) using the

following formula: $\text{MDA} [\mu\text{mol}\cdot\text{g}^{-1} (\text{FM})] = 6.45 (A_{532}-A_{600})-0.56A_{450}$, where A_{532} , A_{600} , and A_{450} are absorbance measured at 532, 600, and 450 nm, respectively.

Endogenous hormones measurements. The youngest leaf buds consisting of one bud with two leaves were collected, immediately frozen in liquid N, and then stored in a low-temperature freezer (-40°C). The frozen samples were used for analyses of indole-3-acetic acid (IAA), gibberellin A_3 (GA_3), ZT, and ABA contents with a high-performance liquid chromatography (Agilent 1200 series HPLC-ultraviolet) according to Pan and Qian (2006). Finely milled samples were extracted in 10 mL cold methanol (80%) overnight. After the samples had been filtered, the residue was extracted twice in 10 mL cold methanol (80%) and combined with the supernatant. The samples were then extracted and bleached with an equal volume of light petroleum. The ether phase was discarded and the water phase was retained. This process was repeated three times. The samples were then vacuumed and evaporated to 1/4 of their original volume at 37°C . The pH of the solution was adjusted to 2.8 and the required sample was extracted with an equal volume of acetic ether. The water phase was discarded and the ester phase was extracted three times. The samples were then

vacuumed and evaporated to 1 mL at 37°C , 2 mL of methanol was added, and then the samples were measured by LC 600 chromatography workstation (LC 600 supporting software) over a $0.45\text{-}\mu\text{m}$ microporous filter membrane. A chromatographic column [Agilent 5 HC-C18 ($150\text{ mm} \times 4.6\text{ mm}$, $5\text{ }\mu\text{m}$)] was used to separate the compounds. The mobile phase was methanol–0.075% glacial acetic acid aqueous solution (45:55), the flow velocity was $0.7\text{ mL}\cdot\text{min}^{-1}$, the column temperature was 35°C , the sample volume was $20\text{ }\mu\text{L}$, and the detection wavelength was 210 nm .

Elemental concentrations and inherent quality determination. Finely milled, dried plant samples of 0.5 g from each treatment were used for analysis of total N and C with an elemental analyser (Carlo Erba, Milano, Italy). Polyphenols and caffeine were extracted twice with 70% aqueous methanol (v/v) at 70°C for 10 min at a ratio of 1:25 (w/v). The concentration of total PP was measured with the Folin–Ciocalteu method (Astill et al., 2001). Caffeine was analyzed by HPLC on a column packed with ODS-5 ST ($5\text{ }\mu\text{m}$, $150 \times 4.6\text{ mm}$; Grom, Rottentburg-Hailfingen, Germany) (Yang, 2011). The elution solutions and gradients were essentially the same as previously described. Standards of caffeine were purchased from Sigma.

After the measurement of PP and caffeine, free amino acids were extracted with chloroform:methanol (3:7, v/v) on ice for 30 min. Homogenates were then extracted twice in 3 mL of distilled water, evaporated to dryness in a rotatory evaporator, and redissolved in 2 mL ultrapure H_2O . Amino acids were analyzed as *o*-phthalaldehyde derivatives on a reverse C_{18} column (Hypersil ODS, $3\text{ }\mu\text{m}$, $250 \times 4.6\text{ mm}$) (Knauer GmbH, Berlin, Germany) using an automated HPLC system (Gerendás et al., 1998). Standards were prepared from authentic compounds, and norvaline was used as internal standard.

The ratio of tea polyphenol to amino acids can be used as a basis for determining tea quality.

Statistical analysis. Differences and correlation analysis between N fertilization and date, with regard to the growth, total N and C, photosynthetic parameters, SPAD value, SOD, MDA, endogenous hormones, and inherent quality were tested by one-way analysis of variance using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL). The reported data were the mean $\pm$ SD of three biological replications.

Results

Plant growth and elemental concentrations. The level of N fertilization had a profound

Fig. 2. Changes in leaf photosynthetic rate (P_N) (A), transpiration rate (T_r) (B), stomatal conductance (g_s) (C), and intercellular CO_2 concentration (C_i) (D) in 'Wuniuzao' after N fertilization. Uppercase and lowercase letters indicate significance of $P < 0.05$ by Duncan's test under the same N level and date, respectively. Error bars represent SD, $n = 3$. N0, N1, N2, N3, and N4 refer to 0, 0.97, 1.94, 3.88, and 5.82 g/kg/pot N fertilization, respectively.

effect on tea growth and mineral nutrition. The biomass of young shoots increased at first and then decreased by the increasing N level except for the start of N treatments, and it peaked under N3 (Table 1). Meanwhile, after 1 month of N treatments (3/15), the young shoot biomass increased significantly compared with that on 2/15. The changing trend of total N contents was consistent with that of young shoot biomass, which also increased to the maximum at N3, and there was a significant difference between N3 and N4 except for 2/15 (Table 2). Compared with the start of N treatments (2/15), there was a significant difference after 1 month of treatments. The changes in total C contents were the same as that of total N, and we also found the immediate change after 1 month of N treatments (Table 3).

Chlorophyll (SPAD value). The chlorophyll contents in 'Wuniuzao' leaves after N treatments are shown in Fig. 1. The N levels affected SPAD values of 'Wuniuzao' leaves, and the SPAD values were higher than that of N0 after all N treatments. At the beginning of N treatments (2/15), the SPAD value changed slowly with an increase in N level, and there was no significant difference with the increase in N level. After 1 month of growth, the SPAD value under all N treatments increased significantly compared with that of date 2/15. The SPAD value under N3 reached the maximum with the increase in treatment dates.

Photosynthetic parameters. The P_N , T_r , g_s , and C_i of tea leaves after N treatments were determined (Fig. 2). The P_N under different N levels was higher than that of N0. The P_N increased at first and then decreased with the increase in N level, it reached the maximum under N3, and there was a significant difference between N3 and other N treatments. After 1 month of N treatments, the P_N significantly increased compared with that of the beginning N treatments (Fig. 2A). The T_r under all N treatments increased with the increase in N level and it peaked under N3. There was a significant difference between date 2/15 and all other dates at the same N level (Fig. 2B). After N fertilization treatments, the g_s in tea leaves was significantly higher than that of N0 at the same date (Fig. 2C). At the end of N treatments, the maximum of g_s also appeared at N3; this was consistent with that of P_N and T_r . The C_i of tea leaves significantly decreased after N fertilization treatments (Fig. 2D). Among the four N treatments, the C_i of N3 decreased the most compared with that of N0.

Antioxidant enzyme and MDA content. SOD activity of tea leaves gradually declined with the increase in N treatments time (Fig. 3A). The SOD activity was the highest under N3, and there was a significant difference between N3 and N0 at the same date (except for 2/15). Meanwhile, the SOD activity of all N treatments significantly declined compared with that of date 2/15. The MDA content of tea leaves decreased significantly after N fertilization treatments, except for the beginning treatments (Fig. 3B). The MDA of N3 was the lowest among all the N

treatments; at the end of N treatments (5/1), it was 75.3% of N0. There was a significant change after 1 month of N treatments.

Endogenous hormones. The peak time of IAA, GA₃, ZT, and ABA for a standard sample is shown in Fig. 4. The peak time of

Fig. 3. Changes in leaf superoxide dismutase (SOD, **A**) and malondialdehyde (MDA, **B**) content in 'Wuniuzao' after nitrogen fertilization. Uppercase and lowercase letters indicate significance of $P < 0.05$ by Duncan's test under the same N level and date, respectively. Error bars represent sd, $n = 3$. N0, N1, N2, N3, and N4 refer to 0, 0.97, 1.94, 3.88, and 5.82 g/kg/pot N fertilization, respectively.

Fig. 4. The peak time diagram of indoleacetic acid, gibberellin, zeatin, and abscisic acid standard samples.

ZT for the standard sample was 2.98 min, GA₃ was 4.27 min, IAA was 6.53 min, and ABA was 8.45. The recovery of ZT for the standard-spiked sample was 89.53%, GA₃ was 93.76%, IAA was 101.45%, and ABA was 110.88%.

The changes in IAA, GA₃, ZT, and ABA in tea leaves were shown after N fertilization treatments (Fig. 5). The results showed that IAA could be significantly promoted by N fertilization from N0 to N3, and the IAA peaked under N3 among all treatments dates (Fig. 5A). After 1 month of N treatments, the IAA increased obviously. Except for N1 on 3/15, GA₃ contents in other N treatments were higher than that of N0. The highest GA₃ content appeared in N3 during the whole treatment date (Fig. 5B). N fertilization treatments could also increase ZT contents of tea tender buds, with the maximum in N3; it indicated that N3 had a significant effect on the increase in ZT contents (Fig. 5C). One month later, ZT contents were significantly higher than that at the beginning of N treatments. The changes in ABA contents were contrary to those of IAA, GA₃, and ZT; it declined at first and then increased, the minimums appeared under N3 (Fig. 5D). There was a significant

difference after 1 month of N treatments (except for N4 at 5/1).

Inherent quality. The experiment showed that N fertilization increased caffeine contents (Fig. 6A). At the end of N treatments (5/1), caffeine contents increased at first and then decreased with the increase in N level, and the peak appeared under N3. There was a significant difference between N3 and N0 under all N treatments, and caffeine contents significantly increased after 1 month of N treatments. N fertilization treatments decreased PP contents from N0 to N3, and PP was the lowest under N3 (Fig. 6B). The difference between the start of N treatments and 1 month was significant. The N fertilization treatments increased AA contents from N0 to N3; the highest value appeared in N3 (Fig. 6C). After 1 month of N treatments, AA contents changed significantly compared with those of the beginning (2/15). The changes in phenol ammonia were consistent with those of PP, decreased at first and then increased and the lowest value appeared at N3 (Fig. 6D). The less the phenol ammonia, the better the inherent quality.

Correlation analysis. Table 4 shows that there was a significant negative correlation between PP and P_N, SOD, and ABA, and

there was a significant positive correlation between PP and young shoot biomass, total N, SPAD, C_i, MDA, and IAA. The correlation between AA and total N, total C, SPAD, T_r, g_s, IAA, GA₃, and ZT; there was a significant positive correlation between caffeine and young shoot biomass, total N, SPAD value, T_r, g_s, IAA, GA₃, and ZT; there was a significant negative correlation between caffeine and ABA. The phenol ammonia and P_N, g_s, SOD, and ZT negatively correlated, whereas positively correlated with C_i and MDA. The results indicated that there was a close correlation between the inherent quality and young shoot biomass, total N and C, photosynthesis, antioxidant enzyme activity, and endogenous hormones after N fertilization treatments. The changes in these parameters after N fertilization treatments improved the 'Wuniuzao' green tea quality.

Discussion

Photosynthesis is an important physiological process in plants, which can synthesize organic matter and generate energy (Caesar, 1989; Richardson et al., 2002). The

Fig. 5. Changes in leaf indoleacetic acid (IAA, **A**), gibberellin (GA₃, **B**), zeatin (ZT, **C**), and abscisic acid (ABA, **D**) contents in 'Wuniuzao' after nitrogen fertilization. Uppercase and lowercase letters indicate significance of $P < 0.05$ by Duncan's test under the same N level and date, respectively. Error bars represent SD, $n = 3$. N0, N1, N2, N3, and N4 refer to 0, 0.97, 1.94, 3.88, and 5.82 g/kg/pot N fertilization, respectively.

Fig. 6. Changes in leaf caffeine (A), tea polyphenols (B), amino acids (C), and phenol ammonia (D) in 'Wuniuzao' after nitrogen fertilization. Uppercase and lowercase letters indicate significance of $P < 0.05$ by Duncan's test under the same N level and date, respectively. Error bars represent SD, $n = 3$. N0, N1, N2, N3, and N4 refer to 0, 0.97, 1.94, 3.88, and 5.82 g/kg/pot N fertilization, respectively.

Table 4. Correlation between inherent qualities and biomass, total N and C, photosynthetic parameters, enzyme activities, and endogenous hormones of Wuniuzao.

Factors	PP	AA	Caffeine	Phenol ammonia
Biomass	0.518**	0.275	0.826**	0.224
Total N	0.505**	0.482*	0.857**	0.102
Total C	0.351	0.834**	0.394	0.294
SPAD value	0.407*	0.626**	0.828**	-0.038
P_N	-0.894**	0.268	-0.302	-0.835**
T_r	0.174	0.925**	0.637**	-0.387
g_s	-0.128	0.911**	0.608***	-0.628**
C_i	0.871**	-0.371	0.240	0.867**
SOD	-0.843**	0.351	-0.229	-0.847**
MDA	0.879**	-0.266	0.274	0.819**
IAA	0.551**	0.559**	0.815**	0.105
GA ₃	0.280	0.467*	0.691**	-0.040
ZT	-0.031	0.881**	0.516**	-0.534**
ABA	-0.554**	-0.615**	-0.804**	-0.081

*Mean significantly correlated at 0.05 level; **mean significantly correlated at 0.01 level.

P_N , net photosynthetic rate; T_r , transpiration rate; g_s , stomatal conductance; C_i , intercellular CO₂ concentration; SOD, superoxide dismutase; MDA, malondialdehyde; IAA, indole-3-acetic acid; GA₃, gibberellin A₃; ZT, zeatin; ABA, abscisic acid; PP, polyphenol; AA, amino acid.

chlorophyll content determines the intensity of photosynthesis; SPAD value is a dimensionless ratio value, and there was a positive correlation between SPAD value and chlorophyll content (Li et al., 2006). The application of N fertilization increased SPAD value, P_N , T_r , and g_s of 'Wuniuzao' leaves, which

were consistent with those of Haukioja (Haukioja et al., 1998), where N3 was superior to other treatments, indicating that increased N fertilization could improve the photosynthesis of tea tree. Increasing N level can increase the chlorophyll content in tea leaves. This may be due to two factors: N is

an element of chlorophyll composition and N is an important element of enzymes, proteins, nucleic acids, and other macromolecules, which were related to chlorophyll synthesis in a plant. This was consistent with the results of Gu (Gu et al., 2013). Nitrogen regulates plant photosynthesis by affecting leaf stomatal and nonstomatal factors. Nitrogen may regulate the opening and closing of stomata, and it is also an element of RuBP carboxylase composition, which comprehensively affects photosynthetic characteristics of leaves (Gao, 2013). N3 treatment is superior to N4 treatment; the excess N exceeds the tolerance of plants, for too high concentration of soil solution and plant water loss will result in "burn seedlings," and the excessive N fertilization will also cause resource waste and environmental pollution.

In addition to the degradation of chlorophyll and photosynthetic capacity, the imbalance of reactive oxygen metabolism is also a main characteristic of plant senescence. Some studies suggested that leaf senescence was due to the balance destruction between production and clearance of intracellular reactive oxygen and the accumulation of reactive oxygen caused cell damage (Jiang et al., 2007). This study showed that SOD

activity in 'Wuniuzao' leaves significantly increased after N treatments, and SOD activity increased at first and then declined with the increase in N content. SOD is an important antioxidant enzyme in plants, and it can remove excessive reactive oxygen produced by internal stress; its activity level reflects the ability of plant resistance to senescence (Liu et al., 2007). MDA is a product of scavenging cellular reactive oxygen, and there is a positive correlation between MDA content and the peroxidation degree of the cell membrane. The results showed that MDA content of 'Wuniuzao' leaves decreased after N fertilization treatments, the MDA content increased at first and then decreased with the increase in N fertilization level. A possible reason is that physiological process of plants is the interaction of various organs and excess N will affect the balance of plant C and N metabolism, and it also inhibits the enzyme activity (Li, 2012). The increase in SOD activity and decrease in MDA content showed that N fertilization could delay the senescence of 'Wuniuzao' leaves, and this result was consistent with that of Zhang and Wang (2010). It indicates that N fertilization can increase SOD activity and further enhance their capacity for scavenging reactive oxygen and, thus, delay the senescence of plants.

Plant hormones play an important role in cell division and elongation, differentiation of tissue, organ, flowering, seeding, ripening, senescence, dormancy, germination, plant morphological building, and in vitro tissue culture (Hung et al., 2008; Takei et al., 2001). This study showed that IAA, ZT, and GA₃ contents of 'Wuniuzao' leaves after N treatments were higher, whereas ABA content was lower than that of CK. Nitrogen is an element of plant hormone composition and significantly affects the endogenous hormones of plants. The ZT, GA₃, and IAA are growth-promoting hormones, N can promote their accumulation, and ABA is a growth-inhibiting hormone, which starts and promotes plant senescence (Wang et al., 1994). This study showed that the contents of ZT and GA₃ were the highest in N3, the content of ABA was the minimum, and the IAA content was the highest in N4. The reason may be that high auxin concentration inhibits growth, whereas low IAA concentration promotes growth. The effects of ABA on plant growth are relatively complex; it not only promotes senescence but also can regulate the switch of stoma and change in *T_r* (Yue et al., 2012). The tea polyphenol and amino acids are not only the metabolite materials but also are the main components that constitute tea quality, so that the phenol/ammonia ratio directly affects the quality of tea leaves (Deng et al., 2012).

This study suggested that N fertilization may improve contents of free amino acids and caffeine, and the conclusions were consistent with those of Yang (2011). This study confirmed that the content of tea polyphenol decreased with the increase in N fertilization level, the possible reason is that too much N

makes most photosynthesis products to be used for protein synthesis, limiting the transformation from sugar to polyphenol (Zhao, 2007). Caffeine is the most abundant alkaloids in tea leaves, and it is an important taste substance (Yang, 2011). Nitrogen is a component of caffeine; previous studies showed that the caffeine content increased with the increase in N level, which is a result of the N nutrition situation being improved in tea tree.

Conclusion

In brief, we carried out this research in pot trials, and the results showed that the supply of N fertilization can affect the growth and quality of green tea in a short time. From the results, we can see that an optimal N level can promote growth and development, delay senescence, and improve the taste of green tea, which showed N3 (3.88 g/kg/pot) was the appropriate N level for 'Wuniuzao' tea tree. We will conduct field trials in the future work.

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