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Allelopathic effects of soil pH on nitrogen uptake, its utilization efficiency and soil enzymes in tea bush soil

L. Ding, L. Hong, Y.H. Wang[1], Y.C. Wang[1], S.X. Lin, M.Z. Li, J.B. Yang[2], J.H. Ye[3], X.L. Jia[3] and H.B. Wang*

College of Life Sciences, Longyan University, Longyan 364012, China
E-Mail: dingli8311@163.com; w13599084845@sina.com

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ABSTRACT

We studied the effects of soil pH (3.29, 4.74 and 5.32) on nitrogen uptake and its use efficiency by tea bush. The results showed that with the increase of soil pH value (3.29 ~ 5.32), the nitrogen utilization efficiency of tea bushes increased from 94.28 % to 461.14 %, the nitrogen absorption efficiency increased from 1.05 % to 3.95 % and the nitrogen physiological utilization efficiency increased from 89.79 % to 116.74 %. Besides with the increase of soil pH, the activity of spoil enzymes (Urease, protease, asparaginase, N-acetamide glucose ribosidase) in tea rhizosphere soil was significantly increased, while, that of nitrate reductase and nitrite reductase was decreased significantly. With the increase of soil pH, the expression of *nifH* gene in tea rhizosphere soil increase significantly, while expression of *amoA-AOA*, *amoA-AOB*, *nirK*, *nirS*, *narG* and *nosZ* gene was decreased. Correlation analysis showed that pH value in tea rhizosphere soil was significantly and positively correlated with nitrogen utilization efficiency, nitrogen uptake efficiency, nitrogen physiological utilization efficiency, urease activity, protease activity, asparaginase activity, N-acetamide glucose ribosidase activity and the expression of *nifH* gene. Correlation analysis was negatively correlated with nitrate reductase activity, nitrite reductase activity and the expression of *amoA-AOA*, *amoA-AOB*, *nirK*, *nirS*, *narG* and *nosZ* gene. The results indicated that soil acidity significantly affected the ability of soil nitrogen conversion and nitrogen absorption and utilization ability of tea bushes.

Key word: Allelopathic effects, gene expression, nitrogen uptake, pH value, soil, soil enzyme, tea bush.

INTRODUCTION

Tea bush (*Camellia sinensis*) is a perennial evergreen plant, mainly distributed in tropical and subtropical regions. Tea bushes are harvested for buds and leaves, so there is great demand for nitrogen fertilizer, during the growth phase (14). The annual use of chemical nitrogen fertilizer in tea bush is > twice the amount of nitrogen recommended in tea plantations (1,18). The extensive use of nitrogen fertilizer has resulted in soil acidification. Although tea bush is an acidophilic crop, the optimum soil pH (5.0 and 5.5) and excessive acidity adversely affects the growth of tea bush (19,20). Wang *et al.* (29) studied and analyzed the soil acidity of 363 tea plantations in 9-main tea-planting towns (Longjuan, Huqiu, Xiping, Lutian, Xianghua, Futian, Gande, Changken and Jiandou) in Anxi County and found that 37.67 % of the soils pH was < 4.5. With the decrease in soil pH value, the yield and quality of tea bush decreased. It is known that excessive use of N

*Correspondence author, [1]Fujian Provincial Key Laboratory of Agroecological Processing and Safety Monitoring, College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China. [2]Yizhitai Biotechnology(Longyan) Co., Ltd, Longyan 364012, China. [3]College of Tea and Food, Wuyi University, Nanping 354300, China.

fertilizer results in soil acidification of tea plantation, which inhibited the growth of tea bushes.

Nitrogen is an important nutrient element for the higher yield and quality of tea bushes. For tea bushes growth, the ammonium nitrogen is preferred to avoid leaching losses (32). The lower soil pH decreased the ammonium nitrogen uptake by tea bushes, thus the majority was N was retained, leached or converted into nitrate nitrogen (28). The soil acidification adversely affects the soil nitrogen conversion capacity and thus changed the soil nitrogen from ammonia nitrogen to nitrate nitrogen (3,7,9,12,33). Therefore, it is important to study the effects of acidification on soil nitrogen conversion, nitrogen uptake and utilization efficiency of tea bushes.

Soil enzymes and microorganisms are important for the soil nitrogen cycle transformation and are closely related to soil pH (24,31). Soil microbial mineralization and nitrogen fixation maintains the soil fertility and played an important role in nitrogen conversion (34). Soil nitrogen transformation is done by microbial populations with specific functional genes, for example, the microbes contain *nifH* gene could convert N_2 to NH_3 in the air, the microbes contains *amoA-AOA* and *amoA-AOB* genes would oxidize NH_4^+ to NO_3^- and the microbes contain *nirK* and *nirS* gene for reduction of NO_2 to NO, these microbes interact with each other to maintain the balance of nitrogen in the soil (2,17,21). The inorganic soil nitrogen uptake by plant roots was mainly due to enzymatic degradation products of soil microorganisms (6). For example, nitrate reductase and nitrite reductase could be directly involved in the denitrification process in soil, urease causes urea hydrolysis and help the plant roots to absorbs NH_4^+, N-acetamide glucose ribosidase and asparaginase catalyze the metabolism of proteins and peptides and these enzymes activities are fully responsible for soil nitrogen conversion and supply status (4,5,23). Therefore, soil microbial specific functional gene expression and soil enzyme activity are often used to evaluate soil nitrogen cycle and conversion capacity. However, there are few reports on the effects of acidification on specific functional gene expression, soil nitrogen-related invertase activity and the relationship between nitrogen uptake and utilization efficiency of tea bushes.

This study aimed to determine the effects of soil acidity (i). on nitrogen utilization efficiency, nitrogen absorption efficiency, nitrogen physiological utilization efficiency, (ii). the activity of soil nitrogen inverter-related enzyme and (iii). the expression of soil nitrogen transformation genes to provide basis for scientific and rational fertilization of tea plantations.

MATERIALS AND METHODS

Experimental materials

The Longjuan Township (117°93' E, 24°97' N), Anxi County, Quanzhou City, Fujian Province, China was choosen as the research site. Based on previous studies (29), soil samples (50 Kg each) of 3-tea plantations with different acidity (3.29, 4.74 and 5.32) were collected in March 2020. The collected soil was air-dried and ground, screened through 40 mesh and the basic physicochemical properties of the soil were determined.

The physicochemical properties were shown in Table 1. Further, tea plantation soils with different pH values were used to replant the tea bush seedlings. After 1 year of planting, tea bush seedlings were collected to measure the nitrogen uptake and utilization efficiency of tea bush. Tea bush rhizosphere soils were also collected to measure the activity of soil nitrogen conversion related enzymes and the expression intensity of nitrogen conversion related genes.

Table 1. Basic physico-chemical properties of soils with different pH values

pH value	Total nitrogen (g/kg)	Total phosphorus (g/kg)	Total potassium (g/kg)	Available nitrogen (mg/kg)	Available phosphorus (mg/kg)	Available potassium (mg/kg)
3.29±0.11c	2.63±0.14a	1.35±0.12a	1.76±0.05a	27.64±0.15a	77.45±1.23a	305.26±4.35a
4.74±0.07b	2.61±0.16a	1.38±0.03a	1.72±0.08a	27.17±0.31a	78.04±1.05a	301.37±3.72a
5.32±0.09a	2.58±0.19a	1.34±0.08a	1.73±0.12a	27.16±0.23a	77.17±1.16a	303.26±2.38a

Note: Different lowercase letters indicate the significant difference at $P < 0.05$ levels among soil of different sample.

Tea bush planting

In May 2020, 10 kg soil of different pH values were put into the pots (diameter 17.5 cm, depth 22 cm). The one year old 6- tea bush seedlings (about 35 cms tall) were transplanted per pot. There were 3 replicates of pots of each pH value soil. The management and fertilization (phosphorus 75 kg/ha, potassium 90 kg/ha) were done as per the traditional methods of tea planting, but no nitrogen fertilizer was applied. In May 2021, one year old tea bush seedlings were collected to determine the nitrogen uptake and utilization efficiency of tea bushes and tea rhizosphere soil samples were collected to determine the activity of enzymes involved in soil nitrogen transformation and the expression intensity of genes of nitrogen cycle.

Nitrogen efficiency and nitrogen absorption efficiency of tea bush

(i). Before planting : Before being replanted (in May 2020), 6-tea bush seedlings with 3 replicates were randomly selected as a sample to determine the biomass and nitrogen content. The whole tea plant was dried at 150 °C for 5 min and then at 80 °C till constant weight, its biomass was obtained and grinded to powder form by Mill. Nitrogen of tea bush was determined by Kjeldahl method (30).

(ii). After planting : After one year of growth (May, 2021), 6- tea bushes with 3 replicates from each pot were collected to determine the biomass and nitrogen content of tea bush and each sample. The whole plant of tea bushes was dug out and its roots were washed. The biomass and nitrogen of tea bush was determined as above. The rhizosphere soil of tea bushes was collected to further analyze the activity of nitrogen transformation related enzymes and the expression of nitrogen transformation related genes in soil.

The nitrogen utilization efficiency, nitrogen absorption efficiency, nitrogen physiological utilization efficiency by tea bushes were calculated as under (30):

(i) Nitrogen utilization efficiency (NE) = [(B1 - B2) / Ns)] × 100 %
(ii) Nitrogen absorption efficiency (NAE) = [(N1 - N2) / Ns)] × 100 %
(iii) Nitrogen physiological utilization efficiency (NPE) = [(B1 - B2) / (N1 - N2))] × 100 %

Where, B1 : Total biomass and N1: Nitrogen content of whole one year old tea bush; while B2: Total biomass and N2 : Nitrogen content of the whole plant at planting; while Ns : Soil total nitrogen content.

Soil enzymes activity

We determined 6-enzymes [urease (EC 3.5.1.5), protease (EC 3.4.2.21), nitrate reductase (EC 1.7.1.3), nitrite reductase (EC 1.7.2.1), asparaginase (EC 3.5.1.1) and N-acetamide glucose ribosidase (EC 3.2.1.30)], involved in soil nitrogen transformation by Enzyme Linked Immunosorbent Assay Kit. The determination method was as under: 1 g fresh soil was extracted with PBS buffer solution (The soil to water ratio was 1:10), thereafter, the Elisa enzyme-linked immunoassay kit (Beijing Huadeboyi biological technology co., LTD.,) was used to extract and the OD value at 450 nm of extracting solution was detected by multifunctional enzyme mark (BioTek Synergy2 Gene 5, American). The results of enzyme activity were expressed as the molar mass (μmoL) of the enzyme produced per unit volume (L^{-1}) and per unit time (min^{-1}). The kit principle used double antibody clamp method to determine the level of enzyme activity in the soil samples. As an example of nitrite reductase determination, the purified nitrite reductase antibody was coated with microporous plates to produce solid phase antibodies; the extracted test solution was added to the micropores coated with monoclonal antibody, further, the horseradish peroxidase (HRP)-labeled nitrite reductase antibody was added to form the antibody-antigen-enzyme-labeled antibody complex. After that, thoroughly washed the compound and added tetramethyl benzidine (TMB) for colour development. TMB was converted to blue under the catalysis of HRP enzyme and finally to yellow under the action of acid. The colour intensity was positively correlated with the nitrite reductase in the sample. Other enzymes were determined using similar method.

Gene expression analysis

Expressions of 7-genes (*nifH*, *amoA-AOA*, *amoA-AOB*, *nirK*, *nirS*, *narG* and *nosZ*) related to soil nitrogen transformation were determined by fluorescence quantitative PCR as under:

Total DNA of soil microbial population was extracted using PowerSoil^{TM} Total DNA Isolation Kit. Briefly, the DNA was extracted as per instructions of the kit by taking 0.5 g of fresh soil sample. DNA extraction was detected by 1 % agarose electrophoresis and DNA concentration and purity were determined by microspectrophotometer. The extracted DNA was stored at -20 °C.

The primer design of genes related to soil nitrogen transformation is shown in Table 2 (13). The PCR system was 50 μL, including 2×*Fine Taq*^{TM} PCR supermix 25 μL, DNA template 1 μL, forward primer 1 μL (10 μmol/L), reverse primer 1 μL (10 μmol/L) and 22 μL of ddH_2O. The amplification procedure was 98 °C for 10 s, 52 °C for 30 s, 72 °C for 30 s, 35 cycles, finally, it was kept at 72 °C for 5 min. During the operation of this experiment, each sample has 3 replicates and gene copy number was determined and converted by ABI7500 real-time fluorescence quantitative PCR system.

Table 2. Soil nitrogen conversion gene primers

Gene	Forward primer	Reverse primer
nifH	AAAGGYGGWATCGGYAARTCCACCAC	TTGTTSGCSGCRTACATSGCCATCAT
amoA-AOA	CCCCTCKGSAAAGCCTTCTTC	GCCATCCATCTGTATGTCCA
amoA-AOB	GGGGTTTCTACTGGTGGT	CCCCTCKGSAAAGCCTTCTTC
narG	TAYGTSGGGCAGGARAAACTG	CGTAGAAGAAGCTGGTGCTGTT
nirK	ATYGGCGGVCAYGGCGA	GCCTCGATCAGRTTRTGGTT
nirS	GTSAACGYSAAGGARACSGG	GASTTCGGRTGSGTCTTSAYGAA
nosZ	AGAACGACCAGCTGATCGACA	TCCATGGTGACGCCGTGGTTG

Statistical analysis

Data were classified by EXCEL, variance analysis, significance analysis and correlation analysis were carried by SPSS software package 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Tea bush nitrogen uptake and use efficiency

The nitrogen uptake and use efficiency of tea bushes planted in different acidic soils showed that as soil pH value (3.29 ~ 5.32) increased, the nitrogen utilization efficiency of tea bushes increased from 94.28 % to 461.14 %, the nitrogen absorption efficiency from 1.05 % to 3.95 % and the nitrogen physiological utilization efficiency increased from 89.79 % to 116.74 % (Figure 1). The soil acidity significantly influenced the nitrogen utilization efficiency, nitrogen absorption efficiency and nitrogen physiological utilization efficiency of tea bushes. From tea bush we harvested young buds and leaves, which have large demand for nitrogen and tea yield was closely related to nitrogen absorption and utilization efficiency (15). The low pH inhibits the nitrogen uptake by plants, thereby inhibiting the plant growth (8). Liu *et al*. (16) reported that the soil pH value was significantly correlated with plant nitrogen uptake and utilization efficiency and increasing the soil pH improved the nitrogen uptake and utilization efficiency of plant. Pan *et al*. (22) found that the increased soil pH increased the nitrogen sources of plant in the soil, improved the nitrogen physiological and nitrogen utilization efficiency of plant, increased the nitrogen accumulation of plant and increased the yield of plant.

These results showed that with the decrease in soil pH, the nitrogen utilization efficiency, nitrogen absorption efficiency and nitrogen physiological utilization efficiency of tea bushes decreased. The soil acidity adversely affects the nitrogen absorption and utilization efficiency of tea bushes, thereby, reducing the yield of tea bushes.

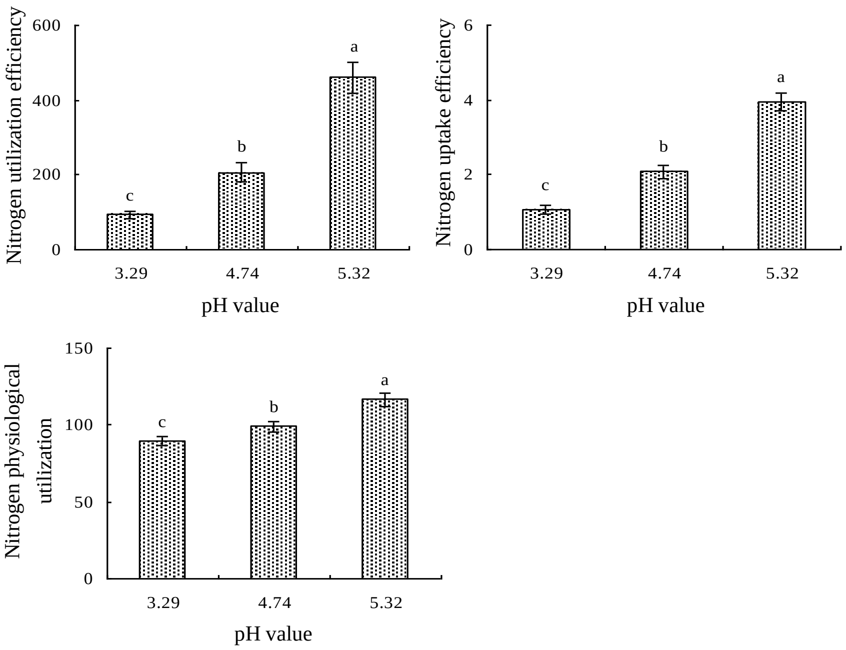

Figure 1. Effects of soil pH value on nitrogen uptake and utilization efficiency of tea tree
Note: Different lowercase letters indicate the significant difference at $P < 0.05$ levels among the different soil pH value.

Nitrogen conversion activities

The soil pH influenced the activities of enzymes involved in nitrogen transformation in the rhizosphere soil of tea bushes grown in soils of different pH (3.29~5.32) (Figure 2). With the increase in pH the activities of enzymes (urease, protease, asparaginase and N-acetamide glucose ribosidase) increased significantly. The activity of urease increased from 148.99 μmol/min·L to 203.53 μmol/min·L, protease from 8.92 μmol/min·L to 26.83 μmol/min·L, asparaginase from 251.59 μmol/min·L to 336.31 μmol/min·L and N-acetamide glucose ribosidase from 0.11 μmol/·L to 0.23 μmol/min·L. On the contrary, the increase in rhizosphere soil pH decreased the activities of the enzymes (nitrate reductase and nitrite reductase) involved in nitrate reduction. The activities of enzymes nitrate reductase and nitrite reductase were reduced from 6.25 mol/min·L to 3.10 mol/min·L and from 167.72 mol/min·L to 86.89 mol/min·L. respectively.

The nitrogen uptake and utilization ability of tea bush was closely related to the nitrogen form and its conversion in soil. The soil nitrogen pools consist of organic and inorganic nitrogen pools according to their forms of existence. The plants mainly absorb inorganic forms of nitrogen [ammonium nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-)] from the soil (34). The results of this study showed that with the decrease in soil pH, the activity of soil urease, protease, asparaginase, N-acetamide glucose ribosidase decreased. The soil urease enzyme hydrolyse the urea to produce ammonia, which is converted into ammonium nitrogen for direct absorption and use by plants or oxidized to NO_3^- as the main nitrogen form absorbed by the plants for their growth (5). Soil protease, hydrolyses the complex organic nitrogen (proteins) into amino acids, as one of the sources of nitrogen in higher plants (25). Asparaginase and N-acetamide glucose ribosidase catalyzed soil organic nitrogen mineralization, improves the available soil nitrogen content (4,23). With the decrease in soil pH, the nitrogen cycling capacity of soil decreases and the nitrogen absorption and utilization capacity of tea bush decreases.

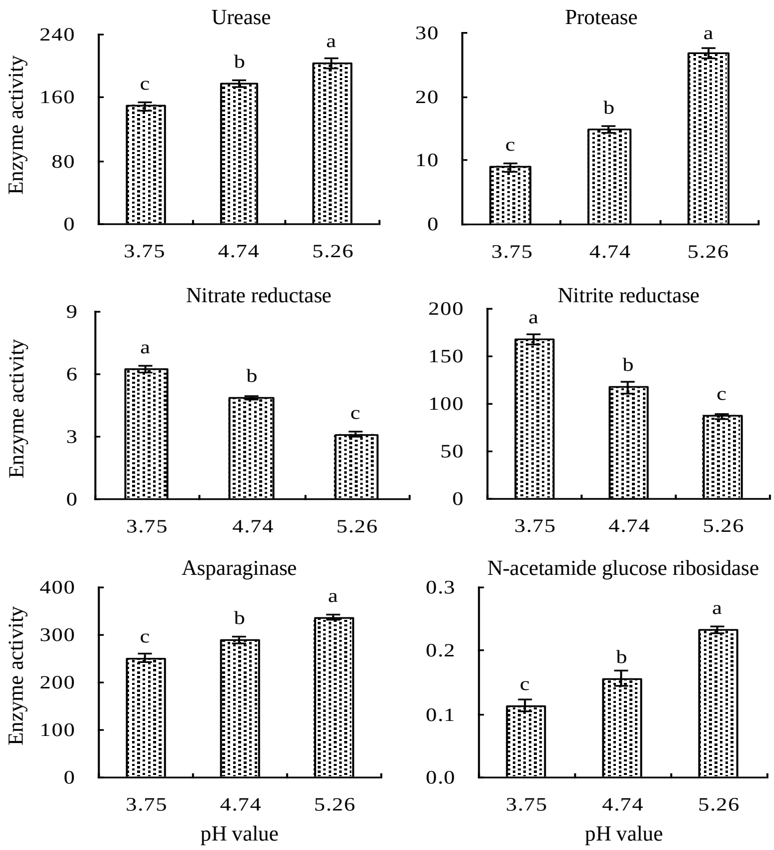

Figure 2. Effects of pH value on the activity of soil nitrogen transformation-related enzymes
Note: Different lowercase letters indicate the significant difference at $P < 0.05$ levels among the different soil pH value.

Gene expression related to nitrogen transformation

The results (Fig. 3) of expression of nitrogen transformation genes in tea bush rhizosphere soil with different pH values showed that with the increase in soil pH (3.29 ~

5.32), the expression of *nifH* gene in tea bush rhizosphere soil significantly increased from 1.57×10^6 Copy number/g·soil to 3.27×10^6 Copy number/g·soil. However, with the increase in soil pH (3.29 ~ 5.32), genes such as *amoA-AOA*, *amoA-AOB*, *nirK*, *nirS*, *narG* and *nosZ* showed significant downward trend. Among them, *amoA-AOA* gene decreased from 858.69×10^6 Copy number/g·soil to 618.91×10^6 Copy number/g·soil, *amoA-AOB* gene decreased from 11.45×10^6 Copy number/g·soil to 5.14×10^6 Copy number/g·soil, *nirK* gene decreased from 37.89×10^6 Copy number/g·soil to 27.85×10^6 Copy number/g·soil, *nirS* gene decreased from 34.98×10^6 Copy number/g·soil to 24.67×10^6 Copy number/g·soil, *narG* gene decreased from 1.62×10^6 Copy number/g·soil to 0.88×10^6 Copy number/g·soil and *nosZ* gene decreased from 34.33×10^6 Copy number/g·soil to 23.69×10^6 Copy number/g·soil.

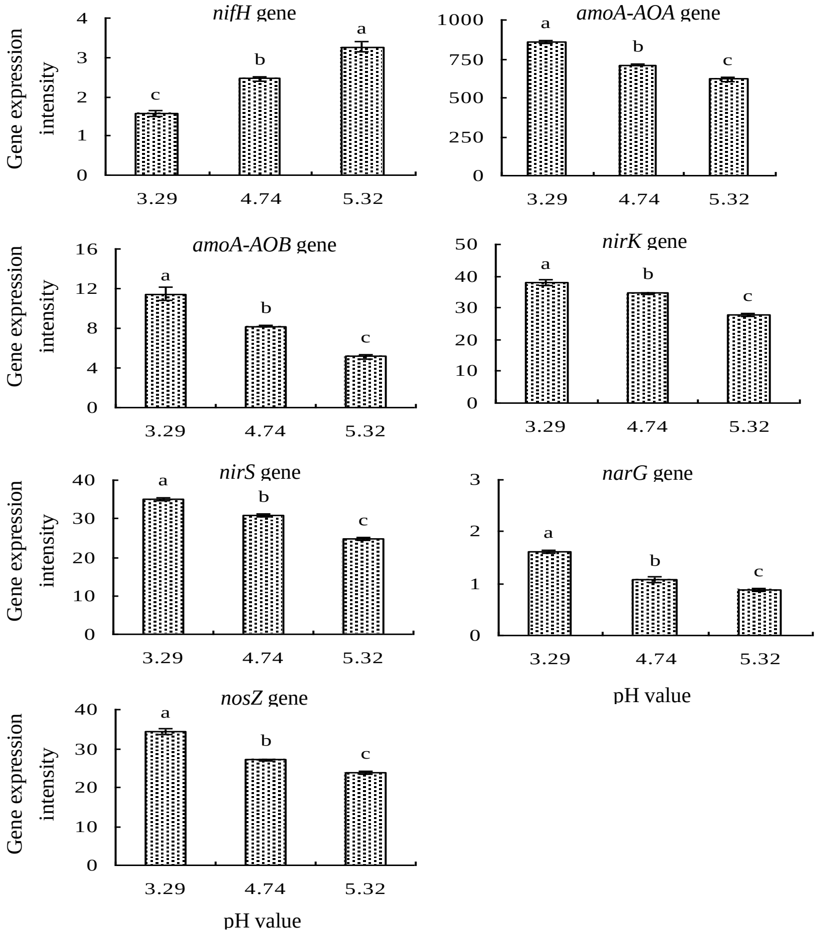

Figure 3. Effects of pH value on the expression intensity of genes related to soil nitrogen transformation
Note: Different lowercase letters indicate the significant difference at $P < 0.05$ levels among the different soil pH value.

The soil ammonia monooxygenase oxidize the NH_4^+ into NO_3^- and the abundance of gene *amoA-AOA* and *amoA-AOB* gene encoding ammonia monooxygenase could accelerate the soil nitration process (10). The expression abundance of *narG* and *nosZ* gene reflects soil nitrate reductase activity, while *nirK* and nir*S* gene expression abundance reflects soil nitrite reductase activity, thereby accelrating the soil denitrification (2,17). These results showed that with the increase of soil pH, the expression of *amoA-AOA*, *amoA-AOB*, *nirK*, *nirS*, *narG* and *nosZ* gene in tea bush rhizosphere soils showed a significant downward trend.

Tea bushes are ammonia-loving plants and ammonium nitrogen is conducive to the development of tea bush roots and the accumulation of free amino acids in tea leaves (11). Scarlett e*t al*. (26) studied the changes of different nitrogen forms in plant rhizosphere soil and found that lower the soil pH, the ammonium nitrogen content was lower and at higher pH the nitrate nitrogen content was higher in rhizosphere soil. Stopnisek *et al*. (27) reported that lower the soil acidity, the stronger the nitrification and higher the nitrate nitrogen content and lower ammonium nitrogen content in the soil. With the decrease in soil pH, the nitrification and denitrification capacity of soil increased and the quantity of ammonium nitrogen absorbed and utilized by tea bushes decreased.

Table 3. Correlation analysis of tea tree rhizosphere soil pH value, tea tree nitrogen uptake and utilization efficiency, soil nitrogen invertase activity and soil nitrogen conversion gene expression intensity

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
B	0.98*															
C	0.97*	1.00**														
D	0.95*	1.00**	1.00**													
E	0.98*	0.97*	0.98*	0.98*												
F	0.95*	1.00**	1.00**	1.00**	0.98*											
G	-0.95*	-0.99*	-1.00**	-0.99**	-0.99**	-0.99**										
H	-1.00**	-0.95*	-0.95*	-0.95*	-0.99**	-0.95*	0.98*									
I	0.95*	0.99*	1.00**	0.99**	0.99**	0.99**	-1.00**	-0.98*								
J	0.95*	0.99**	1.00**	1.00**	0.99**	1.00**	-1.00**	-0.97*	1.00**							
K	0.98*	0.97*	0.98*	0.98*	1.00**	0.98*	-1.00**	-0.99**	1.00**	0.99**						
L	-1.00**	-0.93	-0.95*	-0.95*	-0.99**	-0.95*	0.98*	1.00**	-0.98*	-0.97*	-0.99**					
M	-0.98*	-0.97*	-0.98*	-0.98*	-1.00**	-0.98*	1.00**	0.99**	-1.00**	-0.99**	-1.00**	0.99**				
N	-0.95*	-1.00**	-1.00**	-1.00**	-0.97*	-1.00**	0.99**	0.95*	-0.99**	-0.99**	-0.98*	0.95*	0.98*			
O	-0.95*	-0.99**	-1.00**	-1.00**	-0.99**	-1.00**	1.00**	0.97*	-1.00**	-1.00**	-0.99**	0.97*	0.99**	1.00**		
P	-1.00**	-0.95*	-0.97*	-0.97*	-0.97*	-0.95*	0.95*	0.99**	-0.97*	-0.95*	-0.97*	0.99**	0.97*	0.95*	0.95*	
Q	-1.00**	-0.97*	-0.98*	-0.98*	-0.99*	-0.95*	0.96*	1.00**	-0.96*	-0.95*	-0.98*	1.00**	0.98*	0.97*	0.95*	1.00**

Note: A: pH value; B: Nitrogen utilization efficiency; C: Nitrogen uptake efficiency; D: Nitrogen physiological utilization efficiency; E: Urease; F: Protease; G: Nitrate reductase; H: Nitrite reductase; I: Asparaginase; J: N-acetamide glucose ribosidase; K: *nifH* gene; L: *amoA-AOA* gene; M: *amoA-AOB* gene; N: *nirK* gene; O: *nirS* gene; P: *narG* gene; Q: *nosZ* gene. *: Indicate the significant difference at $P < 0.05$ levels between different indicators. **: Indicate the significant difference at $P < 0.01$ levels between different indicators.

Correlation analysis

The correlation analysis between soil pH and nitrogen absorption and utilization efficiency of tea bush, activity of soil nitrogen invertase and expression intensity of soil nitrogen transformation genes showed that soil pH was significantly positively related to nitrogen utilization efficiency, nitrogen uptake efficiency, nitrogen physiological utilization efficiency, activities of enzymes urease, protease, asparaginase, N-acetamide glucose ribosidase and expression of *nifH* gene (Table 3). However, there was significant and negative correlation with nitrate reductase activity, nitrite reductase activity and the expression of genes *amoA-AOA*, *amoA-AOB*, *nirK*, *nirS*, *narG* and *nosZ*. Thus soil acidity affected the nitrogen conversion in rhizosphere soil of tea bush and thereby affected the nitrogen absorption and utilization efficiency of tea bush.

CONCLUSIONS

This study analyzed effects of soils with different acidity on nitrogen uptake and utilization efficiency of tea bush, soil nitrogen transformation activity and the expression of soil nitrogen invertase related gene. The results showed that as the soil pH value decreased, soil nitrogen conversion capacity and the nitrogen absorption and utilization capacity of tea bushes were decreased, while nitrification and denitrification capacity increased. The soil acidity significantly affected soil nitrogen conversion, nitrogen absorption and utilization capacity of tea bushes. This study provided a research basis for soil regulation and rational use of nitrogen fertilizer in acidified tea plantations.

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DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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