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Effects of soil acidity on soil nitrogen-transforming microorganisms, biochemical intensity and tea quality

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ABSTRACT

To analyze the effects of soil acidity on tea quality and the ability of soil nitrogen transformation, the tea leaves and tea tree rhizosphere soils were collected from 26-tea plantations and determined their contents of theanine and amino acids in tea leaves, the number of soil nitrogen transforming microorganisms and soil biochemical intensity. The results showed that the content of theanine and amino acid in tea leaves, the number of aerobic nitrogen-fixing bacteria and ammonifying bacteria in the soil and the nitrogen fixation intensity and ammonification intensity of the soil increased with the increase of pH value of tea tree rhizosphere soil. However, the number of soil nitrifying bacteria and denitrifying bacteria and soil nitrification intensity and denitrification intensity showed a downward trend. The results of principal component analysis showed that the contribution rate of principal component 1 and principal component 2 was 84.97 % and 7.57 %, respectively. In the soil with different acidity, the main contribution to differentiate the amino acid and theanine content in tea leaves came from the ability of soil for nitrogen fixation and ammonification as well as the ability of soil nitrification and denitrification. The results of correlation analysis showed that soil pH value was significantly positively correlated with theanine, amino acid, aerobic nitrogen-fixing bacteria, ammonifying bacteria, nitrogen fixation intensity and ammoniating intensity, while significantly negatively correlated with nitrifying bacteria, denitrifying bacteria, nitrification intensity and denitrification intensity. The intensification of soil acidity decreased the nitrogen fixation and ammonification activities and boosted nitrification and denitrification activities and decreased the quality of tea leaves.

Key word: Bacteria, denitrification, microbes, nitrification, nitrogen, nitrogen fixation, pH value, quality, rhizosphere soils, soil, tea quality, tea plantation, tea tree.

INTRODUCTION

Although tea tree is an acidophilous plant, the optimal soil pH for its growth is between 5.0 and 5.5 and too acidic or too alkaline soils have an adverse impact on its growth of tea tree (14,16). Fujian Province is one of the major tea-producing provinces in China and it was reported that in 2010, 28 % of 107 tea plantations in Fujian Province had soil pH below 4.0 (28). In 2018, Wang *et al.* (24) studied and analyzed the soil acidity of 363 tea plantations in nine major tea-producing townships in Anxi County and found that 37.67 % soils were acidified and unsuitable for tea plantation. Sun *et al.* (22) found that acidification caused stunted root growth of tea tree seedlings, which inhibited tea tree growth. Secondly, acidification cause soil texture fission, decrease in beneficial microbial population and increase in pathogenic microorganisms, which adversely affected the crop growth and thus

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reduced crop yield and quality (25,26). Thus soil acidification could affect the normal growth of tea trees, hence, it was important to study the effects of acidification on the growth of tea trees.

As a cash crop, tea trees are mainly harvested as shoots and young leaves and therefore have a high demand for fertilizers, especially nitrogen fertilizers, during growth (9). It had been reported that the annual use of chemical nitrogen fertilizers for tea trees exceeds about two times the recommended amount of nitrogen applied to tea plantations and the heavy use of chemical nitrogen fertilizers did not ensure the yield and quality of tea, but rather increases the degree of soil acidification (1,13). Nitrogen in soil often exists in complex forms and usually, nitrate and ammonium nitrogen were the effective forms of nitrogen in soil and could be directly absorbed by plant roots, so the content of effective soil nitrogen (nitrate and ammonium nitrogen) determined the soil nitrogen supply capacity (31). Tea trees preferentially used ammonium nitrogen as the main source of nitrogen during growth (29). As soil pH decreased, a small portion of ammonium N was taken up by tea trees and most of it was sequestered, leached from the soil, or converted to nitrate nitrogen (23). In recent years, numerous studies on the effects of acidification on soil nitrogen transformation and tea tree growth have reported that acidification leads to a decrease in soil nitrogen transformation capacity, which in turn affected the nitrogen uptake and utilization efficiency of tea trees (2,4,6,7,30). However, there were few reports on how acidification changed the soil nitrogen morphology and thus affected the quality of tea and whether there was a link between soil nitrogen transforming microorganisms and biochemical intensity.

Based on the previous studies (4,27), the tea leaves and rhizosphere soil from 26-tea plantations were collected and determined the theanine and amino acid contents of tea leaves, the pH value of tea rhizosphere soil and the number of different types of soil nitrogen transforming bacteria. This study aimed to establish relationship between the soil acidity and tea quality, soil nitrogen transforming bacteria and soil biochemical intensity.

MATERIALS AND METHODS

Experimental

Anxi County, Fujian Province is the origin of Tieguanyin tea tree in China. The county area is 117°36 '-118°17' E, 24°50 '-25°26' N, with mean altitude: 600 m, average annual rainfall: 1,800 mm, average annual relative humidity: 80 % and average annual temperature of 18 °C. In this study, 26-tea plantations were selected to collect tea tree rhizosphere soil and its corresponding tea leaves to determine the soil pH, the number of soil nitrogen converting bacteria, soil biochemical strength and contents of theanine and amino acids in tea tree leaves. Rhizosphere soil of tea trees from 26-tea plantations in Anxi County was collected during March to May 2019 and during the same time mature new leaves of tea trees were also collected to determine their theanine and amino acids. Each of the 26-tea plantations selected had an area of more than 1 hm^2. Five points i.e., 5 replicates were taken for each tea plantation soil sampling using the S-shaped sampling method. For each point, 10 neighbouring tea trees were selected and rhizosphere soil of the tea trees was collected and homogenised and the total amount of soil sample collected was about 1000 g from each site. The soil between the roots of the tea trees was sampled by removing the surface layer of fallen leaves, lightly digging the tea trees, removing the soil attached around the roots of the tea trees and shaking off the soil within 1 cm of the root surface and collecting it separately, that was, rhizosphere soil of tea trees.

Soil pH : It was measured using pH meter (PB-10, Sartorius) with a water-soil ratio of 2.5:1, from 5-replicates for each sample.

Theanine and amino acid content of tea leaves

The theanine and amino acid content of tea leaves was determined using the mature fresh leaves of tea trees, with 5 replicates for each sample. Specifically, 1 g of fresh tea leaves was weighed, ground with 3 mL of ultrapure water, 90 mL of boiling distilled water was added, extracted at a constant temperature of 100 °C for 30 min, filtered and the ultrapure water was fixed to 100 mL, mixed and passed through a 0.45 µm membrane filter for measurement. The theanine content was determined with reference to the national standard GB/T 23193-2017 "Determination of theanine in tea by high performance liquid chromatography" (20). Total amino acid contents were determined as per the method of GB/T 5009.124-2016 "National standard for food safety Determination of amino acids in food" (3).

Number of soil nitrogen transforming bacteria

The number of soil nitrogen transforming bacteria were mainly determined for aerobic free living nitrogen fixing bacteria, ammonifying bacteria, nitrifying bacteria and denitrifying bacteria method of Li *et al*. (8). Among these, soil aerobic auto-nitrogen fixing bacteria were cultured using solid Waxman 77 medium with the formula of 10 g glucose, 0.5 g KH_2PO_4, 0.2 g $MgSO_4$-$7H_2O$, 2 drops of 1 % $MnSO_4$-$4H_2O$ solution, 2 drops of 1 % $FeCl_3$ solution, 20 g agar, 1 L distilled water, pH 7.0-7.2. The determination of aerobic free living nitrogen fixing bacteria was done by plate colony counting method. Soil ammonification bacteria were cultured using liquid peptone ammonification medium with the formula of KH_2PO_4 0.5 g, $MgSO_4$-$7H_2O$ 0.5 g, peptone 5 g, distilled water 1 L, pH 7.0-7.2; the determination of ammonification bacteria was performed by MPN dilution method. Soil nitrifying bacteria were cultured using modified Stephenson's medium with the formula of $(NH_4)_2SO_4$ 2 g, K_2HPO_4 0.75 g, NaH_2PO_4 0.25 g, $MgSO_4$-$7H_2O$ 0.03 g, $CaCO_3$ 5 g, $MnSO_4$-$4H_2O$ 0.01 g, agar 20 g, distilled water 1 L, pH 7.2; and the number was determined plate colony count of nitrifying bacteria. Soil denitrifying bacteria were cultured in liquid denitrifying bacteria medium with the formula of peptone 20 g, glucose 1 g, Na_2HPO_4 2 g, KNO_3 1 g, agar 1 g, distilled water 1 L, pH 7.3; the number of denitrifying bacteria was estimated by MPN dilution method.

Intensity of soil biochemical processes

The intensity of soil biochemical processes was determined by measuring the intensity of nitrogen fixation, ammonification, nitrification and denitrification as per the method of Li *et al*. (8). The intensity of soil nitrogen fixation was determined by using nitrogen-free medium culture and the soil suspension inoculation method. The intensity of soil nitrogen fixation was evaluated by the nitrogen content in 100 mL of culture medium.

Soil ammonification intensity was determined by using ammonification bacteria culture medium inoculated with soil suspension followed by incubation. and soil suspension inoculation method. Soil ammonification intensity was evaluated by the content of NH_4-N in 100 mL culture solution, as per Li *et al*. (8). Determination of soil nitrification intensity, using liquid nitrifying bacteria medium culture and determined by soil suspension inoculation method.

Nitrification intensity = (Nitrite content in the original medium - Nitrite content in the medium after incubation)/nitrite content in the original medium × 100 % (8). Nitrification intensity was calculated as the disappearance rate of NO_2-N. Soil denitrification intensity was determined by using liquid denitrifying bacteria culture medium and soil suspension inoculation method (8). Denitrification intensity = (nitrate content in the original medium - nitrate content in the medium after incubation)/nitrate content in the original medium × 100 % (8). Denitrification intensity was calculated as the disappearance rate of NO_3-N (8).

Statistical analysis

The data were organized, linear equation analysis, principal component analysis and correlation analysis by using Excel 2010 and DPS 9.50 software.

RESULTS AND DISCUSSION

Tea leaf quality index, rhizosphere soil acidity, number of N transforming bacteria

The rhizosphere soil and tea leaves of 26-tea plantations were collected and the pH value of soil, theanine and amino acid content of tea leaves, the number of soil nitrogen transforming bacteria and biochemical strength of soil were determined (Table 1). The results showed that the mean pH value of measured tea plantation soil was 4.23, mean values of theanine and amino acid content of tea leaves were 10.13 mg/g and 21.76 mg/g, respectively. The mean values of soil aerobic free living nitrogen fixing bacteria,

Table 1. Numerical distribution analysis of different soil measurement indexes

Rhizosphere soil parameters	Distribution of measured values	Mean	Standard deviation	Coefficient of variation (%)
pH value	3.15～5.46	4.23	0.81	19.15
Theanine (mg/g)	6.06～13.88	10.13	2.93	28.92
Amino acids (mg/g)	16.43～29.35	21.76	4.05	18.61
Aerobic nitrogen-fixing bacteria (10^4 cfu/g·soil)	5.28～28.45	15.07	7.67	50.90
Ammonifying bacteria (10^7 cfu/g·soil)	4.72～26.87	14.63	7.04	48.12
Nitrifying bacteria (10^5 cfu/g·soil)	7.45～22.38	15.08	4.71	31.23
Denitrifying bacteria (10^3 cfu/g·soil)	2.84～11.26	6.76	2.89	42.75
Nitrogen fixation intensity (mg),	1.02～5.48	2.86	1.39	48.60
Ammoniation intensity (mg/100 mL),	15.26～79.38	41.47	21.34	51.46
Nitrification intensity (%)	14.23～42.36	26.86	8.58	31.94
Denitrification intensity (%)	29.87～48.38	36.93	6.16	16.68

ammonifying bacteria, nitrifying bacteria and denitrifying bacteria were 21.76×10^4 cfu/g, 15.07×10^7 cfu/g, 14.63×10^5 cfu/g and 15.08×10^3 cfu/g of soil, respectively. The mean values of soil nitrogen fixation, ammonification, nitrification and denitrification intensity were 2.86 mg, 41.47 mg/100 mL, 26.86 % and 36.93 %, respectively. Secondly, the analysis revealed that the variable coefficients of different measured indicators ranged from 16.68 % to 51.46 %. The results showed that the measured values of several indexes of the selected samples were widely distributed and typical.

Effects of rhizosphere soil acidity of tea tree on the quality of tea indexes

Soil is the growth medium of tea tree and tea tree that thrives well in acidic environments and changes in soil acidity certainly influenced the formation of tea leaves and yield (24,26). Sun *et al*. (22) used hydroponics to study the effects of acidity on tea tree growth and found that when the pH of culture solution was < 4.5, the number and area of new root system of tea tree decreased significantly, which in turn led to a significant decrease in plant height and biomass of tea tree. In the evaluation of tea quality, the theanine and amino acid content of tea tree leaves were an important basis for evaluating the quality of tea leaves and the theanine and amino acid contents of high-quality tea leaves were significantly higher than those of low-quality tea leaves (11,12,15,18).

In this study, the effects of different acidity of tea tree rhizosphere soil on the theanine and amino acid contents of tea leaves was studied by growing tea tree with rhizosphere soil having different pH values. The results presented in Fig. 1 showed that the theanine and amino acid contents of tea tree leaves have increasing trend with the increase of soil pH in roots of tea trees. It was seen that soil pH significantly affected the quality of tea leaves and lower soil pH resulted in poor quality tea leaves.

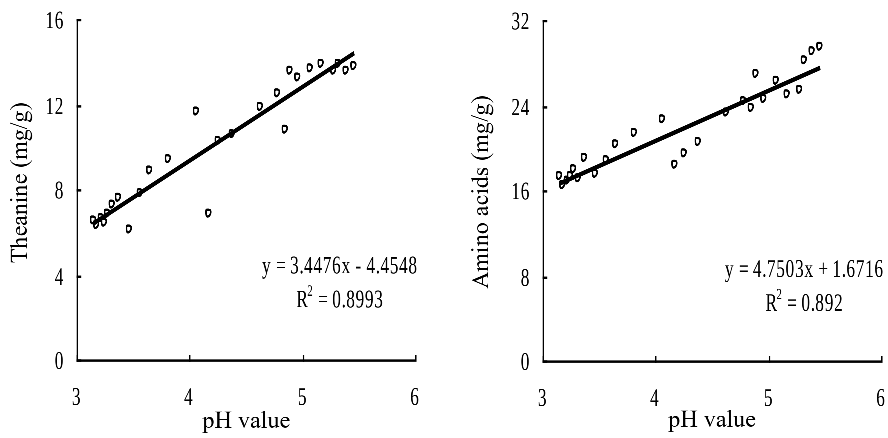

Figure 1. Effects of different acidity soil on theanine and amino acid content in tea leaves

Effects of rhizosphere soil acidity on number of soil N transforming microorganisms

Tea trees were mainly harvested with young shoots and leaves, therefore, the growth of tea trees had a high demand for nitrogen and the formation of tea quality was closely related to nitrogen (9). Tea quality indicators, theanine and amino acids are nitrogenous compounds and the amount of their synthesis in plants was closely related to soil nitrogen conversion capacity and plant nitrogen uptake capacity. Tea plants are ammonium loving plants and ammonium nitrogen facilitates the development of tea roots and improves the accumulation of free amino acids in tea leaves (27). It has been reported that the increase in soil acidity is accompanied with increase in soil nitrification and increase in nitrate-nitrogen content in soil and reduced ammonium-nitrogen content (21). Scarlett *et al*. (19) studied the changes in the content of different nitrogen forms in oak rhizosphere soil and found that the soils with lower pH have lower ammonium-nitrogen content and higher nitrate-nitrogen content in oak inter root soil.

The effects of soil acidity on the number of nitrogen transforming microorganisms in the rhizosphere soil of tea trees was analyzed by taking rhizosphere soil of different pH values as the horizontal coordinates and the number of different nitrogen transforming microorganisms in the soil as the vertical coordinates, respectively. The results (Fig. 2) showed that, with the increase of soil pH, the number of aerobic free living nitrogen fixing bacteria and ammonifying bacteria in the rhizosphere soil of tea tree showed an increasing

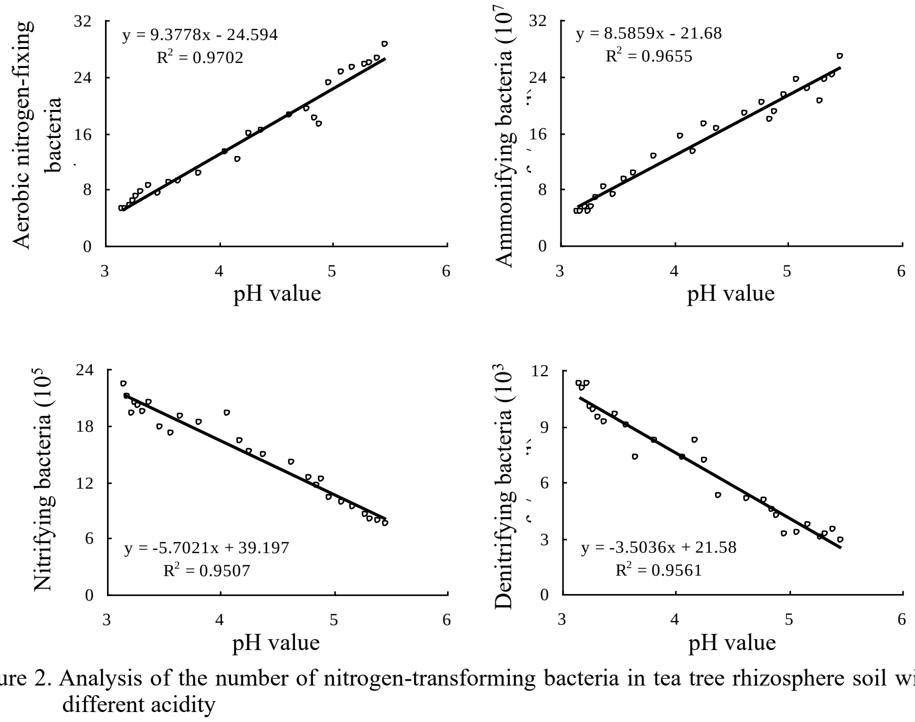

Figure 2. Analysis of the number of nitrogen-transforming bacteria in tea tree rhizosphere soil with different acidity

trend, while the number of nitrifying bacteria and denitrifying bacteria showed a decreasing trend. Furthermore, the effects of soil acidity on the biochemical strength of the soil between the roots of tea trees was analyzed and the results presented in Fig. 3, showed that with the increase in pH of rhizosphere soil of tea tree, the nitrogen fixation intensity and ammonification intensity of inter-root soil of tea tree showed an increasing trend, while the nitrification intensity and denitrification intensity of soil showed a decreasing trend. It is evident that soil acidity could significantly affect the number of microorganisms related to nitrogen transformation in the soil and soil biochemical intensity.

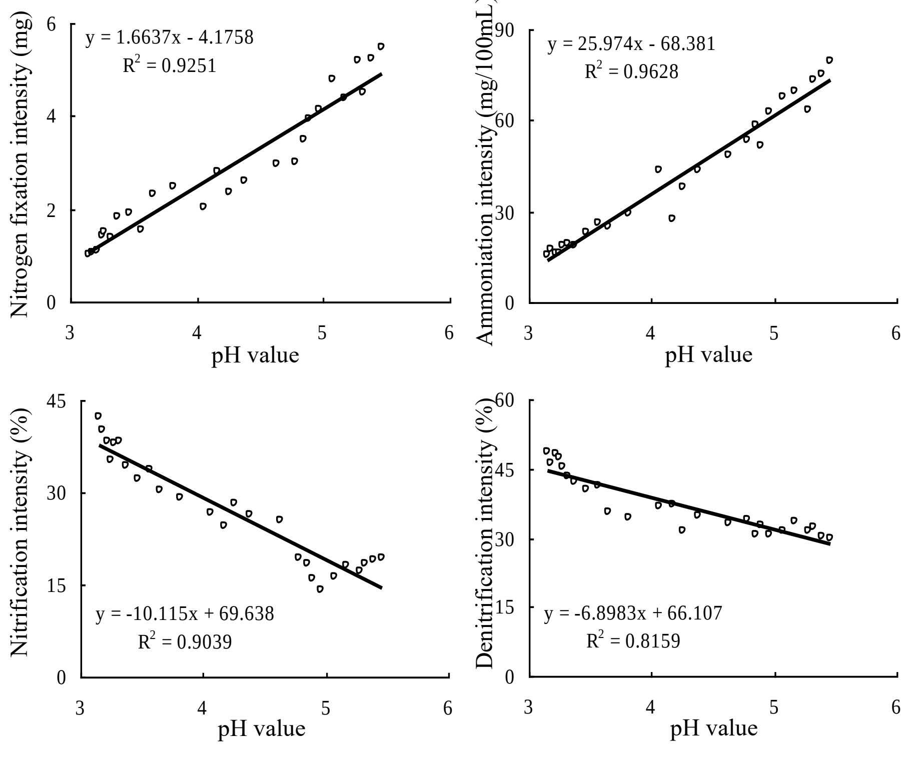

Figure 3. Analysis of the biochemical intensity of tea tree rhizosphere soil with different acidity

PCA of tea quality indicators, soil N transforming bacteria and biochemical intensity in different acidity soils

PCA (Principal Component Analysis) is a statistical method. Through orthogonal transformation, a group of variables that may be correlated is transformed into a group of linearly unrelated variables, which are called principal components. At the same time, it can divide the indexes with strong correlation into the same area. In this study, the principal component analysis (Fig. 4) showed that the two main components could effectively distinguish different indicators, where the contribution of principal component 1 was

84.97 % and the contribution of principal component 2 was 7.57 %. Among these amino acids and theanine content, ammonification bacteria and ammonification intensity were distributed at the positive end of principal component 1 and principal component 2; aerobic free living nitrogen fixing bacteria and nitrogen fixing intensity were distributed at the positive end of principal component 1 and the negative end of principal component 2; nitrifying bacteria, denitrifying bacteria, nitrification intensity and denitrification intensity were distributed at the negative end of principal component 1 and the positive end of principal component 2. It could be seen that principal component 1 and principal component 2 could effectively distinguish different indicators and the main contribution to distinguish the amino acid and theanine content of tea leaves in soils with different acidity comes from the nitrogen fixation and ammonification capacity of the soil and the nitrification and denitrification capacity of the soil.

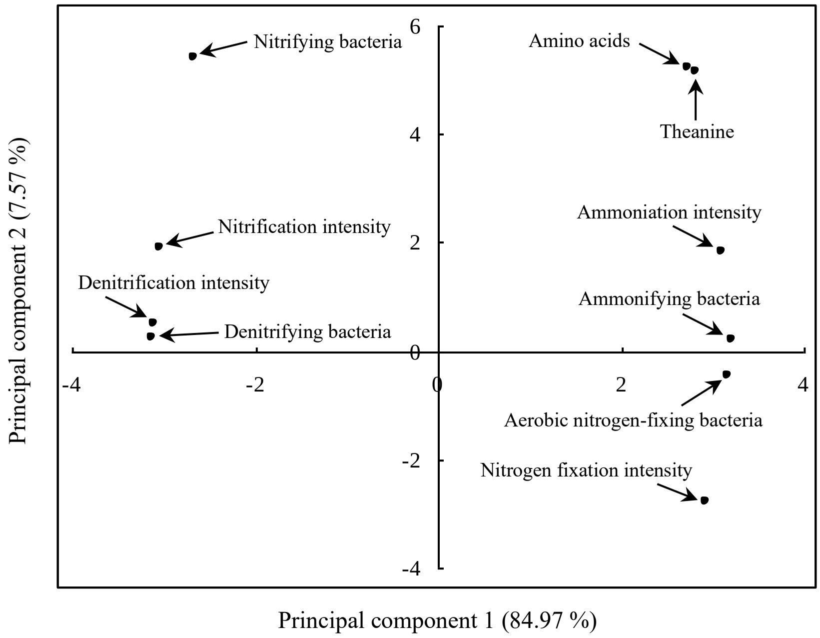

Figure 4. Principal component analysis of tea quality index, soil nitrogen-transforming bacteria and biochemical intensity of different acidity soil

Low pH inhibits the plant nitrogen uptake and thus plant growth (5). Liu *et al*. (10) found that soil pH was significantly correlated with plant nitrogen uptake and utilization efficiency and increasing soil pH could improve nitrogen uptake and utilization efficiency of rice. Pan *et al*. (17) studied the effects of different pH soils on maize yield, nitrogen uptake and utilization efficiency and found that increasing pH was beneficial to increase the source of nitrogen for maize in soil, improve the nitrogen physiological utilization, increase the nitrogen accumulation of maize and improve the yield and quality of maize. It could be seen that with the decrease of soil pH, the nitrogen fixation and ammonification capacity of rhizosphere soil of tea tree decreased, nitrification and denitrification capacity increased, the nitrogen content in the soil available for tea tree uptake and utilization decreased and consequently the theanine and amino acid content in the leaves of tea tree decreased (Figure 4).

Correlation analysis between soil pH and tea quality, soil N transforming bacteria and biochemical intensity

The correlation analysis of soil pH with different indicators showed (Table 2) that soil pH has highly significant positive correlation with theanine, amino acid, aerobic free living nitrogen fixing bacteria, ammonifying bacteria, nitrogen fixing intensity and ammonification intensity, while it showed highly significant negative correlation with nitrifying bacteria, denitrifying bacteria, nitrifying intensity and denitrifying intensity.

Table 2. Correlation analysis of soil pH with tea leave quality index, number of soil nitrogen-transforming bacteria and biochemical intensity

	A	B	C	D	E	F	G	H	I	J
B	0.95**									
C	0.94**	0.95**								
D	0.98**	0.95**	0.94**							
E	0.98**	0.96**	0.94**	0.98**						
F	-0.98**	-0.90**	-0.91**	-0.97**	-0.94**					
G	-0.98**	-0.96**	-0.94**	-0.97**	-0.97**	0.95**				
H	0.96**	0.90**	0.94**	0.96**	0.93**	-0.96**	-0.95**			
I	0.98**	0.95**	0.96**	0.99**	0.97**	-0.97**	-0.96**	0.95**		
J	-0.95**	-0.91**	-0.89**	-0.91**	-0.93**	0.91**	0.95**	-0.91**	-0.91**	
K	-0.90**	-0.87**	-0.84**	-0.87**	-0.93**	0.85**	0.92**	-0.86**	-0.87**	0.91**

Note: A: pH value; B: Theanine; C: Amino acids; D: Aerobic nitrogen-fixing bacteria; E: Ammonifying bacteria; F: Nitrifying bacteria; G: Denitrifying bacteria; H: Nitrogen fixation intensity; I: Ammoniation intensity; J: Nitrification intensity; K: Denitrification intensity. ** indicate the significant difference at $P < 0.01$ levels between different indicators.

Theanine and amino acid content was highly significant and positively correlated with aerobic free living nitrogen fixing bacteria, ammonifying bacteria, nitrogen fixing intensity and ammonification intensity, while highly and negatively correlated with nitrifying bacteria, denitrifying bacteria, nitrification intensity and denitrification intensity. It could be seen that soil acidity could significantly affect the content of theanine and amino acids, the number of soil nitrogen transforming microorganisms and soil biochemical intensity in tea leaves. In turn, the number of soil nitrogen transforming microorganisms and biochemical intensity could affect the content of theanine and amino acids in tea leaves.

CONCLUSIONS

The theanine and amino acid content of tea leaves was decreased with decrease of rhizosphere soil pH. Likewise, with decrease of rhizosphere soil pH, there was significant decrease in number of aerobic free living nitrogen fixing bacteria and ammonifying bacteria as well as the nitrogen fixing intensity and ammonification intensity of inter-root soil of tea tree. While the number of nitrifying bacteria and denitrifying bacteria as well as the nitrification and denitrification intensity were significantly increased. Therefore, with the decrease of rhizosphere soil pH of tea tree, (i). Nitrogen fixation capacity and ammonification capacity of rhizosphere soil of tea tree was also decreased, (ii). Nitrification and denitrification capacity increased, (iii). Total soil nitrogen decreased, thereby, the nitrogen content available for tea tree uptake and utilization decreased, (iv). which decreased the nitrogen accumulation capacity of tea tree leaves and (v). Theanine and amino acid synthesis capacity of tea tree leaves and their contents decreased. This study provided a certain research basis for rational use of nitrogen fertilizers to regulate pH of acidified tea plantations

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CONFLICT OF INTEREST

The authors declare no conflict of interest. All authors agree to publish it.

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.

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.

REFERENCES

1. Akiyama, H., Yan, X. and Yagi, K. (2006). Estimations of emission factors for fertilizer-induced direct N_2O emissions from agricultural soils in Japan: summary of available data. *Soil Science and Plant Nutrition* **52:** 774-787.
2. Chen, Y., Wang, J., Zhang, J.B., Müller, C. and Wang, S.Q. (2015). Mechanistic insights into the effects of N fertilizer application on N_2O-emission pathways in acidic soil of a tea plantation. *Plant and Soil* **389**: 45-57.

3. General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China. (2017). GB/T5009.124-2016 *Determination of Amino Acids in Foods.* China Standards Press, Beijing. pp 1-12.
4. Gogoi, S., Mishra, G. and Deka, A.K. (2016). Soil nutrients dynamics in tea agroforestry ecosystem of Golaghat district of Assam, India. *Agricultural Science Digest* **36**: 185-190.
5. Gu, X.Y., Li, K.Q., Pang, K., Ma, Y.P. and Wang, X.L. (2017). Effects of pH on the growth and NH_4-N uptake of Skeletonema costatum and Nitzschia closterium. *Marine Pollution Bulletin* **124**(2): 946-952.
6. Huang, Y., Long, X.E., Chapman, S.J. and Yao, H. (2015). Acidophilic denitrifiers dominate the N_2O production in a 100-year-old tea orchard soil. *Environmental Science and Pollution Research* **22**: 4173-4182.
7. Li, S.Y., Li, H.X., Yang, C.L., Wang, Y.D., Xue, H. and Niu, Y.F. (2016). Rates of soil acidification in tea plantations and possible causes. *Agriculture Ecosystems Environment* **233**: 60-66.
8. Li, Z.G., Luo, Y.M. and Teng, Y. (2008). *Soil and Environmental Microbiology Research Method*. Science Press, Beijing. pp 435-480. (Chinese)
9. Liu, J.W., Fang, H.H., Yuan, X.Y., Luo, D.L., Yang, Q., Yu, X., Dang, H.E. and Xia, Z.J. (2018). Research progress on effect of nitrogen on physiological metabolism and major quality-related constituents in tea plants. *Acta Tea Sinica* **59**: 155-161. (Chinese)
10. Liu, R., Hafeez, A., Li E.L., Meng, J.L., Tian, J.H. and Cai, K.Z. (2020). Effects of nitrogen fertilizer reduction and biochar application on paddy soil nutrient and nitrogen uptake of rice. *Chinese Journal of Applied Ecology* **31**(7): 2381-2389. (Chinese)
11. Nakagawa, M. (1975). Contribution of green tea constituents to the intensity of taste element of brew. *International Journal of Food Sciences and Nutrition* **22**: 59-64.
12. Nakagawa, M. (1970). Constituents in tea leaf and their contribution to the taste of green tea liquor. *Jarq-Japan Agricultural Research Quarterly* **5**: 43-47.
13. Ni, K., Liao, W.Y., Yi, X.Y., Niu, S.Y., Ma, L.F., Shi, Y.Z., Zhang, Q.F., Liu, M.Y. and Ruan, J.Y. (2019). Fertilization status and reduction potential in tea gardens of China. *Journal of Plant Nutrition and Fertilizers* **25**(3): 421-432. (Chinese)
14. Mehra, A. and Baker, C.L. (2007). Leaching and bioavailability of aluminium, copper and manganese from tea (*Camellia sinensis*). *Food Chemistry* **100**: 1456-1463.
15. Miyauchi, S., Yuki, T., Fuji, H., Kojima, K., Yonetani, T., Tomio, A., Bamba, T. and Fukusaki, E. (2014). High-quality green tea leaf production by artificial cultivation under growth chamber conditions considering amino acids profile. *Journal of Bioscience and Bioengineering* **118**: 710-715.
16. Mohammad, N., Samar, M. and Alireza, I. (2014). Levels of Cu, Zn, Pb and Cd in the leaves of the tea plant (*Camellia sinensis*) and in the soil of Gilan and Mazandaran farms of Iran. *Food Measure* **8**: 277-282.
17. Pan, X., Baquy, A.A., Guan, P., Yan, J. and Xie, L. (2020). Effect of soil acidification on the growth and nitrogen use efficiency of maize in Ultisols. *Journal of Soils and Sediments* **20**(3): 1435-1445.
18. Ruan, L., Wei, K., Wang, L.Y., Cheng, H., Wu, L.Y. and Li, H.L. (2019). Characteristics of free amino acids (the quality chemical components of tea) under spatial heterogeneity of different nitrogen forms in tea (*Camellia sinensis*) plants. *Molecules* **24**: 415-443.
19. Scarlett, K., Denman, S., Clark, D.R., Forster, J., Vanguelova, E., Brown, N. and Whitby, C. (2020). Relationships between nitrogen cycling microbial community abundance and composition reveal the indirect effect of soil pH on oak decline. *ISME Journal* **15**: 623-635.
20. Standardization administration of the People's Republic of China. (2018). GB/T23193-2017 *Determination of Theanine in Tea-Using High Performance Liquid Chromatography.* China Standards Press, Beijing. pp 1-6.
21. Stopnisek, N., Gubry, R.C., Hofferle, S., Nicol, G.W., Mandic, M.I. and Prosser, J.I. (2010). Thaumarchaeal ammonia oxidation in an acidic forest peat soil is not influenced by ammonium amendment. *Applied and Environmental Microbiology* **76**: 7626-7634.
22. Sun, L.L., Zhang, M.S., Liu, X.M., Mao, Q.Z., Shi, C., Kochian, L. and Liao, H. (2020). Aluminium is essential for root growth and development of tea plants (*Camellia sinensis*). *Journal of Integrative Plant Biology* **62**(7): 984-997.
23. Wan, X.C. and Xia, T. (2015). *Secondary Metabolism of Tea Plant*. Science Press, Beijing. pp 41-100.
24. Wang, H.B., Chen, X.T., Ding, L., Ye, J.H., Jia, X.L., Kong, X.H. and He, H.B. (2018). Effects of soil acidification on yield and quality of tea tree in tea plantations from Anxi county, Fujian Province. *Chinese Journal of Applied and Environmental* **24**: 1398-1403. (Chinese).

25. Wang, H.B., Wang, Y.H., Zhang, Q., Lin, S.X., Zhang, Q.X., Ye, J.H., Ding, L., Lin, S. and He, H.B. (2020). Construction of the microbial protein metabolism map of tea rhizosphere soil in acidified plantations. *Scientia Sinica (Vitae)* **50**(08): 849-865. (Chinese)
26. Wang, H.B., Zhang, Q.X., Chen, X.T., Wang, Y.H. and He, H.B. (2020). Analysis of microbial diversity of tea tree (*Camellia sinensis* (L.) O. Ktze.) sick rhizospheric soil using soil metaproteomic technology. *Allelopathy Journal* **51**(2): 147-156.
27. Wang, Y.H., Hong, L., Wang, Y.C., Yang, Y.W., Lin, L.W., Ye, J.H., Jia, X.L. and Wang, H.B. (2022). Effects of soil nitrogen and pH in tea plantation soil on yield and quality of tea leaves. *Allelopathy Journal* **55**(1): 51-60.
28. Yang, D.X., Zhong, Z.M., Chen, J.X., Jiang, N.Z., Lin, X.M., Wang, Y.X., Guo, W. and Li, C.P. (2016). Evaluation of soil nutrients in tea gardens in Fujian Province. *Straits Science* **42**: 129-131. (Chinese)
29. Yang, Y.Y., Li, X.H., Ratcliffe, R.G. and Ruan, J.Y. (2013). Characterization of ammonium and nitrate uptake and assimilation in roots of tea plants. *Russian Journal of Plant Physiology* **60**: 91-99.
30. Yamamoto, A., Akiyama, H., Naokawa, T., Miyazaki, Y., Honda, Y., Sano, Y., Nakajima, Y. and Yagi, K. (2014). Lime-nitrogen application affects nitrification, denitrification and N_2O emission in an acidic tea soil. *Biology and Fertility of Soils* **50**: 53-62.
31. Zhang, C., Song, Z.L., Zhuang, D.H., Wang, J., Xie, S. and Liu, G. (2019). Urea fertilization decreases soil bacterial diversity, but improves microbial biomass, respiration and N-cycling potential in a semiarid grassland. *Biology and Fertility of Soils* **55**: 229-242.

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