Soil acidification of alfisols influenced by nitrate and ammonium nitrogen level in tea plantation

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Abstract: Nitrogen is an important fertilizer in tea production, but it is also an important factor in tea garden soil acidification. The relationship between absorption and transport of different forms of nitrogen in the tea plant and soil acidification is still unknown. In order to explore the different characteristics of absorption, utilization and distribution of nitrogen, stable isotope ^{15}N tracer technique was used to measure the absorption, utilization and allocation of nitrate nitrogen (NO_3-^{15}N) and ammonium nitrogen (NH_4-^{15}N) under the same nitrogen application amount of tea tree seedlings as experimental materials. The results showed that the tea seedlings had the same pattern of nitrogen application: tissue nitrogen content increased after fertilization, remarkable rising at 7 d and the absorption speed increased quickly after 28 d, finally reached its maximum at 56 d. The nitrogen use efficiency of two nitrogen sources in two kinds of soil varied not significantly. The maximum NUE of NO_3-^{15}N reached 12.66%, and at the same time NH_4-^{15}N utilization rose up to 11.54%. According to the absorption of soil nitrogen and nitrogen fertilizer in the two kinds of soil, it is concluded that the soil nitrogen cannot meet the growth needs of tea tree and extra nitrogen supply was required. The declined soil pH indicated that fertilizer should be used in moderation, which can not only satisfy the growth of tea tree but also to restrict soil acidification.

Keywords: tea tree, ^{15}N, nitrate nitrogen, ammonium nitrogen, soil acidification

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1 Introduction

Tea (*Camellia sinensis*) is a major commercial crop in many countries in tropical and subtropical regions, including China, India and Sri Lanka. There are about 2.72 million hm^2 of land used for tea cultivation globally, with 1.02 million hm^2 in China alone[1]. Tea is an unusual crop because the soil after tea planting becomes strongly acidified, and soil pH generally continues to decrease with the increase of stand age[2,3]. The problems of tea garden soil acidification are intensively researched in early 1970-1980s, and many tea producing countries presented the tea garden soil acidification in various degrees. It is reported that 38.64% of Japanese tea gardens had an average soil pH under 3.9, 37.52% of them had an average soil pH from 4.0 to 4.9, and the tea gardens with optimal soil pH for tea tree growth only accounts 15.11%[4]. The pH of tea garden soil in Assam area in South Indian dropped down significantly[5,6].

Fertilization is regarded as an important factor in affecting the tea garden soil acidification. More nitrogen fertilizer and less organic fertilizer can lead to more serious tea garden soil acidification in current China. The tea tree is leaf using crop, it is special fond of nitrogen. Tea plantation generally uses ammonium nitrogen as nitrogen fertilizer. After absorbing ammonium nitrogen, tea tree root release a great amount of H^+, which produced a sustained drop in the soil pH. With the increase application of chemical nitrogen fertilizer and the extension of application time, soil acidification was obviously deepened. In addition to tea tree's metabolism in tea garden, tea garden soil acidification affected by human cultivation process is even more serious. Fertilization is a prime reason which has direct impact on tea garden soil acidification[7-9]. It is reported that China main agricultural soil acidification was significant since 1980s. The survey found that excessive nitrogen fertilization is the main cause of the farmland soil acidification. For tea tree planting, the removal of tea products is the main cause of soil acidification[10]. When nitrogen fertilizer was applied, the yield and biomass of tea tree above ground were increased. The crop harvest led to more alkali removed from soil and further accelerate soil acidification process[11]. Another major reason of nitrogen fertilizer accelerating soil acidification is that the nitrification of ammonium nitrogen in soil and the following NO_3^- leaching[4]. However, the effect of soil nitrogen cycle, the contribution of soil nitrification and soil mineralization on soil acidification is rarely reported.

In order to explore the transferring regularity of different forms of nitrogen in tea tree, the stable isotope ^{15}N tracer technique was used to measure the absorption, utilization and allocation of nitrate nitrogen (NO_3-^{15}N) and ammonium nitrogen (NH_4-^{15}N) under the same nitrogen application rate. Furthermore, this study also demonstrate the effects of soil acidification on soil aluminum chemistry and soil exchangeable base cations, provides theory basis for soil acidification controlling.

2 Materials and methods

2.1 Plant materials

The experimental tea seedlings were collected from Wuxi

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institute of tea science of one-year cutting seedlings. The new tea seedlings' root, stem and leaf were washed by tap water, and the roots were washed three times with deionized water. The uniformly tea seedlings were selected and every three seedlings were collected into one 1 L plastic bucket with 25% nutrient solution, roots were kept away from light. Nutrient solution composition was as follows: $(NH_4)_2SO_4$, 750 μmol/L; $Ca(NO_3)_2{\cdot}4H_2O$, 250 μmol/L; KH_2PO_4, 30 μmol/L; K_2SO_4, 300 μmol/L; $CaCl_2$, 20 μmol/L; $MgSO_4$, 210 μmol/L; $ZnSO_4{\cdot}7H_2O$, 0.51 μmol/L; $CuSO_4{\cdot}5H_2O$, 0.13 μmol/L; $MnSO_4{\cdot}H_2O$, 0.5 μmol/L; H_3BO_3, 3.33 μmol/L; Fe Na EDTA, 2.1 μmol/L; $Na_2MoO_4{\cdot}2H_2O$, 0.17 μmol/L. When large amount of new small white roots grow out, tea seedlings were transferred into greenhouse with 3 h ventilation every morning and afternoon. After 7 d cultivation, the robust tea seedlings under consistent growth condition were chosen for test in September 2014. The pot experiments were conducted in light incubator. The dry weight of each testing basin soil is 0.9 kg.

2.2 Soil sampling and treatment

Four kinds of soil (quaternary red clay, granite and limestone soil parent material development, including yellow brown soil, yellow soil, brown soil and red soil) were choose from north temperate zone and subtropical zone. These soils were not tea planting soil and not polluted wasteland soil. The experimental yellow brown soil was collected from Nanjing, Jiangsu province, the depth of sampling is 0 to 10 cm, soil organic matter content is 30.72 g/kg, cation exchange capacity (CEC) is 14.77 cmol $_{(+)}$/kg, soil pH is 5.86. The experimental yellow soil was collected from Chongqing, depth of sampling is 0 to 10 cm, soil organic matter content is 25.74 g/kg, CEC is 8.72 cmol $_{(+)}$/kg, soil pH is 4.52. The experimental brown soil was collected from Xinyang, Henan province, depth of sampling is 0 to 10 cm, soil organic matter content is 31.65 g/kg, CEC is 15.84 cmol $_{(+)}$/kg, soil pH is 6.71. The experimental red soil was collected from Guilin in Guangxi province, depth of sampling is 0 to 10 cm, soil organic matter content is 28.59 g/kg, CEC is 9.08 cmol $_{(+)}$/kg, soil pH is 5.54. These four kinds of soil samples were natural air dried and crushed to pass 2 mm sieve in cylindrical test POTS (20 cm in diameter, 15 cm high). 8 kg of each kind of soil was added into a barrel, added 2.35 g P_2O_5, 2.89 g K_2O, and blended to mix together. Soil total nitrogen content is 1.0 g/kg.

^{15}N-single marking ammonium nitrate fertilizer with concentration of 10.19% was provided by Shanghai Chemical Industry Research Institute. Treatment 1 applied 0.57142 g NO_3-^{15}N marked NH_4NO_3 in each basin, tagged as NO_3-^{15}N N fertilizer, and applied 0.2 g nitrification inhibitor dicyandiamide at the same time. Treatment 2 applied 0.57142 g NH_4-^{15}N marked NH_4NO_3 in each basin, tagged as NH_4-^{15} N fertilizer. Four replicates were set in each treatment, with a total of 128 pots seedling.

2.3 Soil and plant analysis

After fertilization, plant and soil samples were collected every 7 d, 14 d, 28 d and 56 d. 2 plant strains were selected into samples each treatment every time, the total is 8 plant strains for each treatment. Samples were separated into root, stem and leaf, washed with tap water and distilled water three times, oven-dried under 105°C-110°C for 30 min, then dried in 65°C with 48 h. The plant samples were grinded to pass 0.125 mm sieve and the dry weight of root, stem and leaf was weighted. Soil samples were also air-dried and ground to pass 2 mm sieve, and stored in plastic bags[12,13].

Total nitrogen content is analyzed by carbon and nitrogen analyzer. ^{15}N abundance of each plant part was determined by MAT-251 mass spectrometer in Institute of Atomic Energy, Chinese Academy of Agricultural Sciences.

The soil pH was determined using a combination glass electrode in 1:2.5 (w/v) ratio of soil-deionized water mixture[14]. Exchangeable Ca, Mg, K, and Na were extracted by 1.0 mol/L NH_4Ac at pH 7.0, and the cations were measured using atomic adsorption spectrometry[15]. The CEC was determined by the Kjeldahl Nitrogen Determination method[16].

Exchangeable acidity and exchangeable Al of soils were extracted with 1.0 mol/L KCl followed by titrating with standard NaOH solution[17].

2.4 Data analysis

The NUE was calculated according to the method of Dong et al.[12,13]

SPSS 15.0 (SPSS Inc., Chicago, IL, USA) was used for data processing and statistical analysis.

3 Results and discussion

3.1 Soil pH

The pH of the soil samples in different treatments are shown in Table 1. The incubation of tea seedlings caused decreases of pH in all types of soil with most of the pH decreases occurred within 56 d. The four types of soil have different pH values. With the increase of cultivation time, soils pH value of four treatment samples were both declined. In 56 d, soil pH of brown soil under NO_3-^{15}N treatment dropped from 6.71 to 6.48, soil pH of brown soil under NH_4-^{15}N treatment dropped from 6.62 to 6.38; soil pH of red soil under NO_3-^{15}N treatment dropped from 5.54 to 5.29, and soil pH of red soil under NH_4-^{15}N treatment dropped from 5.42 to 5.18. Yellow soil and yellow brown soil follows the same pattern, while pH of yellow soil is significantly higher than yellow brown soil. It is also noticed that in all kinds of soil, NH_4-^{15}N treatment leads to lower soil pH than NO_3-^{15}N treatment.

Table 1 Different tea root soil pH value

Soil	Treatment	0	7 d	14 d	28 d	56 d
Brown soil	NO_3-^{15}N	6.71±0.15	6.65±0.24	6.59±0.24	6.56±0.18	6.48±0.37
	NH_4-^{15}N	6.62±0.28	6.57±0.21	6.52±0.15	6.43±0.23	6.38±0.32
Red soil	NO_3-^{15}N	5.54±0.13	5.48±0.19	5.43±0.24	5.36±0.12	5.29±0.25
	NH_4-^{15}N	5.42±0.16	5.39±0.21	5.32±0.16	5.25±0.25	5.18±0.15
Yellow brown soil	NO_3-^{15}N	5.21±0.15	5.16±0.25	5.07±0.24	4.95±0.18	4.89±0.30
	NH_4-^{15}N	5.22±0.28	5.2±0.21	5.15±0.15	4.93±0.23	4.85±0.32
Yellow soil	NO_3-^{15}N	6.62±0.16	6.59±0.24	6.54±0.19	5.95±0.25	5.48±0.15
	NH_4-^{15}N	6.65±0.13	6.6±0.19	6.43±0.24	5.83±0.18	5.45±0.25

It is reported that tea tree absorbed ammonium nitrogen greatly higher than nitrate nitrogen. After absorption of ammonium nitrogen, tea tree roots released H^+ to decreased soil pH[18-20]. Compare to ammonium nitrogen, the adsorption of nitrate nitrogen releases more OH^- and relatively increase soil pH. However, this experiment applied same amount of ammonium nitrate and nitrate nitrogen with only differences on ^{15}N labeling, so soil pH change trend of the different treatments in same soil roughly belonged to normal phenomenon.

Since the initial cultivate soil nitrogen mineralization absorb protons and urea hydrolysis, which increases in line with Table 1, the beginning of the cultivation of soil ammonium nitrogen content; As the nitrification, 1 mol NH_4-^{15}N oxidized to NO_3-^{15}N, then 2 mol H^+ release into the soil, so the soil pH decreases. Soil pH changes only four different amplitudes, the number of produced mainly with soil buffering capacity, urea hydrolysis degree and nitrogen transformation process related to proton[21-24].

3.2 Nitrogen absorption of tea tree and soil

3.2.1 Nitrogen absorption in brown soil and yellow soil

As shown in Figure 1, the different processing of tea tree nitrogen absorption markers presented a certain dynamic change. 7 d after fertilization, tea tree absorbed low nitrogen, NO_3-^{15}N nitrogen treatment absorption of brown soil of tea tree is 0.0047 g/kg, NH_4-^{15}N absorption treatment of brown soil of tea tree nitrogen is 0.0051 g/kg; Yellow soil uptake in 7 d is 0.0004 g/kg, but the uptake of 28 d is 0.0103 g/kg.

After 28 d, nitrogen absorption of tea tree was getting slowed, at 56 d is in the max, NO_3-^{15}N treatment of brown soil of tea tree reached the maximum, the absorption amount is 0.0163 g/kg, NH_4-^{15}N treatment of brown soil reached maximum ,the amount is 0.0176 g/kg, and nitrogen uptake in yellow soil is 0.0134 g/kg. In generally, for two kinds of soils, tag nitrogen absorption of NH_4-^{15}N treatment in tea tree is higher than NO_3-^{15}N treatment of tea tree.

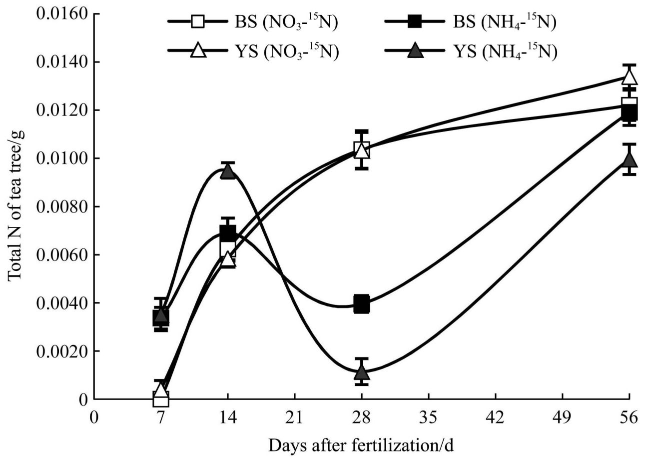

Figure 1 Nitrogen fertilizer uptake in different treatment and different types of soil (brown soil and yellow soil)

3.2.2 Nitrogen absorption in yellow brown soil and red soil

The nitrogen fertilizer absorption in yellow brown soil and red soil can be seen from the Figure 2. The absorption of different nitrogen species of tea tree presented certain regularity.

Nitrogen absorption was kept at a high ratio in the first 14 d after fertilization, the absorption amount in red soil NH_4-^{15}N treatment is 0.0055 g/kg, and the absorption amount in red soil NO_3-^{15}N treatment t is 0.0049 g/kg. From 14 d to 28 d after fertilization, nitrogen absorption in tea tree was slowly increased. The soil applied ^{15}N- NO_3 fertilizer, the absorption rate of 0 to 28 d is faster, yellow brown soil uptake is 0.0039 g/kg in 7 d, but the uptake in 28 d increased to 0.0104 g/kg. After 28 d, the absorption rate declined, uptake of nitrogen in yellow brown soil in 56 d is 0.0122 g/kg NO_3-^{15}N nitrogen uptake in red soil in 56 d is 0.0169 g/kg, while NH_4-^{15}N nitrogen uptake in read soil is 0.0182 g/kg. To exert NH_4-^{15}N for two kinds of soil nitrogen volatile, absorption rate in 0 to 7 d rose rapidly, but declined after 28 d. After 56 d the absorption presented the same level of NO_3-^{15}N. These results suggest that urea hydrolysis in yellow brown soil in tea garden is faster than in red soil in tea garden, while the absorption and utilization of NH_4-^{15}N in these two soils takes longer time, which is conducive to tea for NH_4-^{15}N absorption and utilization. At the same time, two kinds of tea garden soil nitrification lag existed, in favor of tea to ammonium nitrogen[25-27].

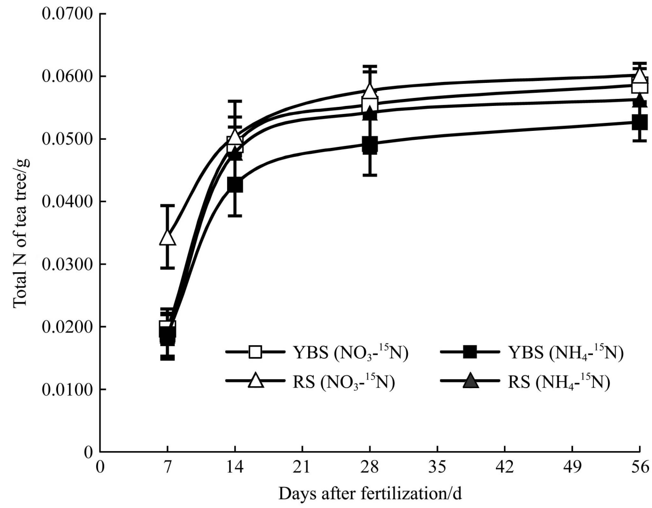

Figure 2 Nitrogen fertilizer uptake in different treatment and different types of soil (yellow brown soil and red soil)

3.3 Soil nitrogen use efficiency

Nitrogen use efficiency is the percentage of nitrogen absorption in plant utilization of the total nitrogen in soil. Nitrogen use efficiency is an important indicator to measure the rationality of nitrogen fertilizer. As seen from Table 2, a similar pattern of utilization rate of nitrogen fertilizer with nitrogen uptake was discovered. After the four kinds of soil are applied with NO_3-^{15}N fertilizer, utilization rate of 0 to 56 d is continuously increased, utilization rate in brown soil at 7 d was 4.40%, and utilization rate at 28 d was 10.93%, at the end of 56 d utilization rate reached 11.68%. The utilization rate in red soil at 7 d was 4.83%, utilization rate at 28 d was 8.54%, at 56 d it reached 11.98%. The utilization rate of NH_4-^{15}N in four kinds of soil are also increased consistently, at 7 d utilization rate in brown soil was 5.37%, at 28 d it was 12.38%, and at last it reached 14.41% at 56 d. The utilization rate in red soil also showed the same trend. Comparison showed that nitrogen fertilizer utilization rate is on the rise, but utilization rate of NH_4-^{15}N fertilizer was obviously higher than that of NO_3-^{15}N fertilizer. The results show that the cultivation of tea can cause soil acidification, strongly acidic soils in tea plantation area can still occur even in the absence of additional nitrogen source. With the increase of cultivation d the soil produced high concentrations of ammonia, create toxic circumstance for nitrification microorganisms to inhibit nitrification. In comparison, yellow soil has stronger nitrification than other soil, because N_2O in yellow soil mainly came from aerobic nitrification process[28]. In addition, organic carbon, total nitrogen, inorganic nitrogen, porosity and other parameters in yellow soil were significantly higher than the red soil, which can provide sufficient substrate for the soil microbial activity and a higher rate of N_2O emissions[29]. Increase amount of nitrogen leads to higher N_2O emission, in which microbial growth may have primary effect than red soil property, but the exact reason need to be further studied[30].

Table 2 Different soils of different forms of nitrogen utilization rate

Soil	Treatment	Nitrogen fertilizer use efficiency/%			
		7 d	14 d	28 d	56 d
Brown soil	NO_3-^{15}N	4.40±0.07	8.20±0.45	10.93±0.68	11.68±0.02
	NH_4-^{15}N	5.37±0.09	11.29±0.36	12.38±0.19	14.41±0.05
Red soil	NO_3-^{15}N	4.83±0.09	6.51±0.24	8.54±0.47	11.98±0.21
	NH_4-^{15}N	7.56±0.05	10.83±0.15	11.52±0.28	13.61±0.72
Yellow brown soil	NO_3-^{15}N	3.66±0.05	5.90±0.08	9.80±0.10	11.54±0.06
	NH_4-^{15}N	3.18±0.09	6.52±0.12	3.74±0.04	11.27±0.013
Yellow soil	NO_3-^{15}N	0.38±0.07	5.53±0.15	9.76±0.11	12.66±0.18
	NH_4-^{15}N	3.33±0.10	8.98±0.09	1.08±0.03	9.42±0.08

3.4 Soil exchange properties

The amount of exchangeable base cations, exchangeable acidity and CEC of the soils are shown in Table 3. After the cultivation of tea plants, the amount of soil exchangeable base cations decreases, hence the soil exchangeable acidity increases, and the soil base cation saturation decreases substantially. After the cultivation of tea plants for 56 d the exchangeable acidity of yellow brown soil increased to 5.25 cmol/kg, and the total base cations decreased to 5.60 cmol/kg. Similarly, the base cation saturation decreased to 50.82%. These results are consistent with the trends of the change of the corresponding soil pH. In comparison for the data from different soils, the total base cations and base cation saturation of the soils generally increased with the increase of fertilization, while the soil exchangeable acidity changed in the opposite trend (Table 3). Soil acidification also changes soil CEC. Therefore, soil acidification decreases not only the content of soil base cations and base cation saturation of the soil, but also the soil CEC.

Table 3 Chemical properties of the soils with different treatments

Soil	Treatment	Exchangeable base cations/(cmol·kg^{-1})					Exchangeable acidity /(cmol·kg^{-1})	CEC	Base cation saturation/%
		Ca^{2+}	Mg^{2+}	K^+	Na^+	Total			
Brown soil	NO_3-^{15}N	7.80	4.67	0.25	0.52	13.24	0.62	13.36	99.10
	NH_4-^{15}N	7.45	4.77	0.16	0.26	12.64	0.58	13.26	95.32
Red soil	NO_3-^{15}N	5.95	2.82	0.19	0.27	9.23	3.05	10.61	86.99
	NH_4-^{15}N	5.09	2.70	0.18	0.15	8.12	3.13	11.39	71.29
Yellow brown soil	NO_3-^{15}N	4.38	1.66	0.18	0.17	6.39	5.14	9.98	64.03
	NH_4-^{15}N	3.79	1.29	0.21	0.31	5.60	5.25	11.02	50.82
Yellow soil	NO_3-^{15}N	7.07	4.30	0.12	0.35	11.84	3.06	13.10	90.38
	NH_4-^{15}N	6.96	3.67	0.21	0.28	11.12	3.23	12.17	91.37

4 Conclusions

This study showed that with the increase of cultivation time, the soil pH decreased obviously, and the growth of tea tree is the cause of soil acidification. For the two types of nitrogen fertilizer, soil applied with NO_3-^{15}N have slower pH drop than soil applied with NH_4-^{15}N in the four types of soil. Nitrogen cycle accompanied by H^+ uptake and release. Ammonification each release 1 mol NH_4^+, can absorb 1 mol H^+; nitrification oxide 1 mol of NH_4^+ can change into 1 mol NO_3^-, release 2 mol H^+ into the environment. It can be concluded that ammonium nitrogen nitrification process released H^+ leading to the emergence of this phenomenon, so the ammonium nitrogen fertilizer applied exacerbate tea garden soil acidification. The absorption of nitrogen and nitrogen fertilizer utilization efficiency of tea tree showed that the growth of tea tree is closely related with the nitrogen fertilizer. The demand of nitrogen for tea tree is particularly large, and application of nitrogen fertilizer benefits tea tree growth and production. At the same time ammonium nitrogen uptake and utilization of tea tree were significantly higher than nitrate nitrogen, showed that tea tree is fond of ammonium nitrogen.

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