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# A soil management strategy for ameliorating soil acidification and reducing nitrification in tea plantations



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# ABSTRACT

The cultivation of tea requires acidic soils which further acidify in plantations due to increased levels of nitrogen fixation. Ameliorating soil acidification by increasing pH is commonly implemented but as consequence, soil microbial nitrification causes losses of nitrate and limits the availability of  $NH_4^+$  for tea, the latter being the preferred nitrogen source. Thus, the aim of this study was to analyze a strategy which can ameliorate soil acidification and simultaneously minimize the stimulation of nitrification in a typical tea soil. Net N transformation rates in different amounts of quicklime (CaO) and rice straw biochar addition treatments were determined through an incubation experiment, to test the effects of quicklime and biochar on acidification and nitrification in a typical tea soil. Our results showed that the addition of quicklime resulted in a significant increase in soil nitrification rate when soil pH increased from 3.77 to 4.10, but it decreased when soil pH increased to > 5.10. Soil nitrification rate with biochar application continued to rise with increasing soil pH (3.77-3.85-4.01-4.38). When nitrification rates from all treatments were plotted against soil pH, we found that nitrification rate increased linearly with increasing soil pH from 3.63 to 4.38, then sharply declined to the values lower than that in the original soil pH when soil pH was increased from 4.38 to 5.10, and finally remained stable. Our results suggest that nitrification may be optimized in tea soil at ca. pH 4.40. Therefore, when we employed pH-raising practices to alleviate soil acidification, the soil pH should be enhanced to more than the optimum pH range for nitrification (approximately 5.10 in this study) to avoid stimulating soil nitrification.

### 1. Introduction

Tea, *Camellia sinensis* L., is an intensively managed broadleaf, evergreen crop in tropical and subtropical regions. While tea plants require acidic soils for successful growth, where the optimum pH range is 5.0–5.6, they can also acidify soil [1,2]. Recently, excessive application of ammonium-based fertilizers and large leave harvests have increased acidification in tea soils [3,4]. Soil acidification invariably increases the toxicity of aluminum to microorganisms and at the same time adversely affects the growth and quality of the tea plants [5], so ameliorating tea soil acidification through addition of an alkaline substance seems logical. At present, the application of lime, quicklime and biochar has been confirmed to be effective in ameliorating soil acidity [6–8]. However, the effect of alkaline substances on the rates of soil nitrification in tea plantations remains poorly understood.

Soil nitrification is highly pH sensitive, and increasing soil pH has

nitrification [9,10], therefore, amelioration of acidification in tea soils may stimulate nitrification. Since tea plants preferably use  $NH_4^+$ -N [11,12], it is likely that  $NO_3^-$ -N produced from ammonium-based fertilizers through nitrification would not be taken up by tea plants. In addition, in tropical and subtropical regions characterized by abundant precipitation and heavy rainfall events where tea plants grow,  $NO_3^-$ -N produced from nitrification would be susceptible to losses through runoff, leaching and denitrification. Therefore, we devoted to find a strategy which can ameliorate soil acidification and simultaneously minimize the stimulation of nitrification. Theoretically, soil nitrifying microorganisms in tea soil may be adapted to the highly acidic tea soil environment [13–15]. Here, we speculated that there is an optimum pH range for nitrification in tea soil, above which it would be suppressed, which has not been previously reported. To verify our speculation, we studied the effects of quicklime and biochar application to tea soil as

been found to stimulate nitrification and decrease the suppression of

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**Fig. 1.** Changes in soil pH over a 21-day incubation period with and without the application of CaO (a) and BC (b). UC, 0.10% CaO, 0.25% CaO and 0.35% CaO represent application rates of 0 g, 0.10 g, 0.25 g and 0.35 g CaO per 100 g soil, respectively, and UC, 1% BC, 2% BC and 5% BC represent application rates of 0 g, 1 g, 2 g and 5 g rice straw biochar per 100 g soil, respectively. Bars represent standard deviation (n = 3). CaO: quicklime; BC: biochar.

acidification and nitrification mitigation strategies.

# 2. Materials and methods

# 2.1. Study samples

Tea soil was sampled from Yixing  $(31^{\circ}07'-31^{\circ}37'N, 119^{\circ}31'-120^{\circ}03'E)$ , in southern Jiangsu Province, China, a region characterized by a subtropical monsoon climate with an annual rainfall of 1177 mm. The brown-red soil (Oxisols, US Soil Taxonomy) was a loam, which comprised 7.3% sand, 41.7% silt, and 51.0% clay, and received N in the form of urea at approximately 600 kg ha<sup>-1</sup> year<sup>-1</sup>. Soil total C, total N, and pH were 9.69 g kg<sup>-1</sup>, 1.18 g kg<sup>-1</sup>, and 3.74, respectively.

#### 2.2. Incubation experiment

A preliminary experiment was carried out to determine the exact amounts of quicklime (CaO) and rice straw-derived biochar (BC; pH: 9.16, 62% C, and 1.3% N) addition required for obtaining the desired final pH range (Fig. 1). The treatments of the actual incubation experiment comprised untreated control (UC); 0.10% (20 mg) CaO; 0.25% (50 mg) CaO; 0.35% (70 mg) CaO; 1% (0.2 g) BC; 2% (0.4 g) BC; and 5% (1.0 g) BC. Fresh soil (equivalent to 20 g dry weight) were mixed with quicklime or biochar in 250 mL flasks, and the moisture content of each mixed sample was adjusted to 40% water holding capacity (WHC). The flasks were sealed with rubber stoppers and pre-incubated at 25 °C in the dark for 7 days. Following pre-incubation, net N transformation rates were determined by incubating the soil samples, which had been treated with urea (100 mg N kg<sup>-1</sup>), for 21 days at 25 °C and 60% WHC. During incubation, the flasks were opened for 30 min each day to renew the atmosphere inside each flask. The moisture content of the incubated soil samples was maintained by adding water every 3 days to compensate for water lost through evaporation. After 2, 5, 9, 15 and 21 days of incubation, gas samples were taken during 6-h sealed incubation from the headspace of the flasks to analyze NO concentration using a NOx analyzer (ThermoFisher 42i, chemiluminescence detector, USA) and N<sub>2</sub>O and CO<sub>2</sub> concentrations using gas chromatography (Agilent 7890 A, USA). Soil concentration of  $NH_4^+$  and  $NO_3^-$  in 100 mL 2 M KCl solution was determined using a San++ Continuous Flow Analyzer (Skalar, Netherlands) and net N mineralization rates were calculated as the difference between final and initial mineral N concentrations divided by 21 days. Net nitrification rates were calculated in the same manner as the daily mean accumulation of  $NO_3^{-1}$ .

#### 2.3. Statistical analyses

One-way ANOVA was used to compare the differences in net N mineralization and nitrification rates and cumulative  $N_2O$ , NO, and  $CO_2$  emissions.

# 3. Results

After 7 days of pre-incubation, soil pH of the UC, 0.10% CaO, 0.25% CaO and 0.35% CaO treatments was 3.63, 4.04, 4.91, and 6.40, respectively (Fig. 1a); and 3.84, 3.95, and 4.27 in the 1% BC, 2% BC and 5% BC treatments, respectively (Fig. 1b). Although soil pH in all treatments following urea application was more or less maintained (Fig. 1), the ameliorating effect of quicklime was superior to biochar.

For all treatments,  $NH_4^+$ -N concentrations increased during the first 5 days of incubation, due to hydrolysis of the urea; subsequently it tended to decrease or remain stable (Figs. S1a and b);  $NO_3^-$ -N concentrations gradually increased over the incubation period (Figs. S1c and d). Rates of net mineralization over the 21 day incubation period in the control were not significantly different from those in the 0.10% CaO and 0.25% CaO treatments, but were significant lower than that in the 0.35% CaO treatment (p < 0.05) (Fig. 2a). In contrast, the addition of biochar had no effect on net mineralization rates, regardless of application rate (Fig. 2b). Rates of net nitrification in the control were significantly higher than in the 0.25% and 0.35% CaO treatments (p < 0.05) (Fig. 2c). In contrast, the net nitrification rates gradually increased with increasing application rate of biochar (p < 0.05) (Fig. 2d).

Cumulative N<sub>2</sub>O emission over the 21 day incubation period was largely enhanced by quicklime and the application of 2% and 5% BC, compared with the untreated control (Fig. 4a and b). In contrast, cumulative NO emission declined due to the quicklime application, but increased in the 1% BC and 2% BC treatments (Fig. 4c and d). Cumulative CO<sub>2</sub> emission, as an indicator of soil microbial activity, was enhanced by the quicklime application, but unaffected by biochar application (Fig. 4e and f).

#### 4. Discussion

Generally, soil pH is the main factor affecting nitrification, where increasing and decreasing pH stimulates and depresses net nitrification, respectively [9,10]. In our study, the nitrification rate may have been constrained by low soil pH (3.77) and thus the increase in soil pH as a result of biochar application increased the rate of nitrification (Fig. 3a). Biochar has been shown to optimize conditions for nitrification in creased the rates of nitrification [7,16]. We suggest that nitrification in our study was driven by pH-sensitive nitrifying microorganisms [17,18]. In contrast, biochar can also decrease nitrification by limiting the NH<sub>3</sub> or NH<sub>4</sub><sup>+</sup> availability for oxidation due to either the surface adsorption [19,20], or increased emissions of NH<sub>3</sub> because of enhanced soil pH.

In comparison with biochar application, we found that quicklime increased soil pH but decreased net nitrification rate (Fig. 3b), which was inconsistent with previous studies that report that quicklime and liming promote nitrification in acidic soils [6,8,21]. Previous studies found that when soil pH increased from 3.60 to 4.50 to 6.30–6.88 by lime application, net nitrification rate was significantly increased in soils dominated by a single species (pine, rhododendron or tea), but was significantly inhibited in the mixed species forest soil [22]. There is a possibility, therefore, that acid-tolerant or even acidophilic nitrifying microorganisms were responsible for nitrification in this study, since nitrification was not stimulated or was even suppressed by an increase



**Fig. 2.** Net mineralization rate (a, b) and net nitrification rate (c, d) of treatments applied with CaO and BC over the 21-day incubation, respectively. UC, 0.10% CaO, 0.25% CaO and 0.35% CaO represent application rates of 0 g, 0.10 g, 0.25 g and 0.35 g CaO per 100 g soil, respectively, and UC, 1% BC, 2% BC and 5% BC represent application rates of 0 g, 1 g, 2 g and 5 g rice straw biochar per 100 g soil, respectively. Bars represent standard deviation (n = 3). CaO: quicklime; BC: biochar.



Fig. 3. The relationship between pH and net nitrification rate in treatments treated with CaO (a), BC (b) and both (c). Bars represent standard deviation (n = 3). CaO: quicklime; BC: biochar.



**Fig. 4.** Cumulative N<sub>2</sub>O emission (a, b), cumulative NO emission (c, d) and cumulative CO<sub>2</sub> emission (e, f) of treatments treated with CaO and BC over the 21-day incubation, respectively. UC, 0.10% CaO, 0.25% CaO and 0.35% CaO represent application rates of 0 g, 0.10 g, 0.25 g and 0.35 g CaO per 100 g soil, respectively, and UC, 1% BC, 2% BC and 5% BC represent application rates of 0 g, 1 g, 2 g and 5 g rice straw biochar per 100 g soil, respectively. Bars represent standard deviation (n = 3). CaO: quicklime; BC: biochar.

in soil pH [13-15].

The contrasting effects of biochar and quicklime application on soil nitrification in our study were surprising given that the main role of quicklime and biochar addition was the same (amelioration soil acidification). It is unlikely that acid-sensitive nitrifying microorganisms were responsible for nitrification following biochar application, but acid-tolerant or acidophilic nitrifying microorganisms may have accounted for nitrification following quicklime application. When the nitrification rates from all the UC, biochar and quicklime application treatments were plotted against soil pH, we found that they increased linearly with increasing soil pH from 3.77 to 4.38, but then sharply dropped to values lower than that in the original soil pH when soil pH was increased from 4.38 to 5.10, and finally remained stable (Fig. 3c). Such results may suggest that soil nitrifying microorganisms may have been well adapted to growth in the highly acidic soil, and that nitrification was optimized with the addition of 5% BC that resulted in a soil pH of c. 4.40. In fact, ammonia-oxidizing archaea probably played a

more important role than ammonia-oxidizing bacteria in autotrophic ammonia oxidation in strongly acidic soils especially in the acidic tea soils [23,24]. Alternately, the decreased nitrification rates to do lime application might be attributed to the lag effect of lime in raising the pH and nitrification rate in the acidic mixed forest soil [22] and in a degraded acid soil [21]. However, this explanation was not practicable in our studied tea soil as low application rate of quicklime significantly stimulated nitrification rate.

Nitrification and denitrification are two main processes involved in  $N_2O$  production [25] and as soil pH increased due to the application of quicklime, nitrification rate declined and  $N_2O$  emission increased. These results suggest that nitrification was probably not the dominant process involved in  $N_2O$  production and the increased  $N_2O$  emission that followed the application of quicklime could be due to enhanced denitrification activity as indicated by an increase in  $CO_2$  emission (Fig. 4e). The relatively higher  $N_2O$  emission in the 2% BC and 5% BC treatments than the control could be attributed to enhanced

nitrification rates, as also reported before [26]. Our results conflict with previous studies that biochar addition decreased emissions of N2O, due to enhanced N<sub>2</sub>O reductase enzyme activity associated with increasing pH during denitrification [27-29]. Furthermore, the effects of biochar application on soil N availability and associated N<sub>2</sub>O emission could be time dependent. Biochar greatly reduced NH<sub>4</sub><sup>+</sup>-N one month after application whereas no significant change of NH4<sup>+</sup>-N was observed for longer residence times of biochar in soil up to 1 year, while biochar significantly reduced NO<sub>3</sub><sup>-</sup>-N at all time intervals except  $\leq 1$  year [16]. Adecrease in N<sub>2</sub>O emissions were observed in soil amended with Douglas fir wood derived biochar (pyrolyzed at 510 °C) for 3 days out of the 42-day growing season, which suggests that effects of biochar on decreasing  $N_2O$  emissions may be transient [30]. It was therefore likely that the results from short-term laboratory incubation experiment in this study may not reflect the actual conditions in situ and thus future studies using longer term field experiments should be performed.

# 5. Conclusions

In conclusion, our results suggest that the optimum pH for nitrification in tea soil was ca. pH 4.40, achieved by the addition of 5% BC. Tea plants prefer  $NH_4^+$ -N rather than  $NO_3^-$ -N, thus nitrification would be detrimental to the N uptake of tea, while  $NO_3^-$ -N produced from nitrification would be vulnerable to losses via runoff, leaching and denitrification. Therefore, when we employed the quicklime, biochar and other pH-raising treatments to alleviate soil acidification, the soil pH should be enhanced to more than the optimum pH range for nitrification (approximately 5.10 in this study) to avoid stimulating soil nitrification activity.

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#### Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx. doi.org/10.1016/j.ejsobi.2018.06.001.

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