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Nitrification potentials of Chinese tea orchard soils and their adjacent wasteland and forest soils

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Abstract

To investigate the nitrifying activities of different soil types, soil samples collected from 8-, 50- and 90-year old tea orchards, the adjacent wasteland, and 90-year old forest were measured for their nitrification potentials using the conventional soil incubation and the liquid incubation method. Among different soil types, the nitrification potential of soil in tea orchards was higher than that of wasteland and forest soils. The slurry shaken liquid incubation method was confirmed to be more accurate and have reliable results than the soil incubation. Interestingly, experimental result revealed that the generally applied pH value of 7.2 for the liquid media was not the optimal pH for these acid soils with a strong buffer capacity. This suggested that tea orchard soils may have nitrifiers requiring pH-neutral condition for the best activity. Our data also showed that treatment with the commonly used nitrogen fertilizer urea significantly improved nitrification potential of the soils; such enhancement effect was stronger on all of three tea orchard soils than on wasteland and forest soils, and also stronger on the younger (8- and 50-year old) tea orchard soils than on the older one (90-year old).

Key words: tea orchard; wasteland; forest; nitrification potential; urea
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Introduction

In the soil ecosystem, nitrification is a crucial nitrogen cycling process of great agricultural and environmental importance. Tea (*Camellia sinensis*) is an important economical crop planted widely on acid red soils in the tropical and subtropical zones of China. To improve the yield and quality of tea leaves, nitrogen fertilizers are commonly used in tea orchards. However, they can acidify tea orchard soil (Han *et al*., 2007), therefore reduce the soil nitrification rate (Chantigny *et al*., 1996; Shi and Norton, 2000; Zhao *et al*., 2007) and the quality of surrounding environment (Tokuda and Hayatsu, 2004).

Nitrification potential is the maximum capacity of nitrifier populations in soil to transform ammonium into nitrate under the optimal conditions. The major influencing factors include the density and diversity of nitrifier populations (Stark and Firestone, 1996), and various environmental factors such as pH (Hayatsu, 1993; De Boer *et al*., 1996; Shen *et al*., 2003), temperature (Emmer and Tietema, 1990; Ellert and Bettany, 1992), soil moisture (Carnol and Ineson, 1999) and structure (Hoffmann *et al*., 2007), substrate (Staley *et al*., 1990) and nutrient availability (Ste-Marie and Paré, 1999), soil organic matter (Sauvé *et al*., 1999), and allelopathic inhibition (White, 1994).

To measure nitrification potentials, soil samples are typically incubated for 24 h under a fixed structure and population size of a nitrifier community. An ideal measurement method should measure nitrification potentials in real time so they can provide the accurate values at different times of sampling, and also reflect human management-related land use changes and the availability of substrates in the soil (Belser, 1979; Hart *et al*., 1994).

For the soil incubation method, the pH value of the incubation medium is an important factor affecting nitrification activity (Sauvé *et al*., 1999; De Boer *et al*., 1996). Generally the nitrification activity is high in a neutral or slightly alkaline environment, so the pH value of the liquid medium is usually set at 7.2. However, since tea orchard soils are severely acidified, a medium of pH 7.2 may not be optimal because the final pH value of the solution may decrease dramatically after the addition of such acid soil samples.

To improve the utilization and management of the tea orchard soil ecosystem, in this study, we determined the nitrification potentials for tea orchard soils of various ages and different land usages, optimized the pH for measuring nitrification potentials of acid soil samples, and examined the effect of urea treatment on soil nitrification potentials.

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1 Materials and methods

1.1 Site description

Soil samples were collected from the West Lake District of Hangzhou, China in three tea orchards, which were constructed on the wasteland in 1914, 1954, and 1996, and were 90-, 50-, and 8-year old, respectively. Each orchard contained many plots separated by footpath, and all the soils received annual applications of N fertilizer two or three times per year averaging approximately 450 kg N/(hm^2·year). To evaluate soil nitrification potential as a function of land use change and human management, a vicinal wasteland and a forest were also chosen as study sites. The wasteland was covered with various grasses. The 90-year old forest was established on the wasteland in 1914 and was a mixed-conifer forest. Both the wasteland and the forest were never exposed to human management. All soils investigated were classified as red soil by the China Classification System and were derived from the same parent material, namely quartzose sandstone interbedded with shales. Some characteristics of soil samples are given in Table 1.

1.2 Sample collection and preparation

Soils were collected from three sampling plots randomly chosen in a 8-year old tea orchard, a 50-year old tea orchard, a 90-year old tea orchard, a wasteland, and a 90-year old forest, in September 2004. Twenty soil columns of 5 cm diameter × 20 cm length were taken from each sampling plot and mixed. Therefore, triplicate soil samples were collected at depth of 0–20 cm at each study site.

The 15 bulk samples were transported on ice to the laboratory where they were sieved through a 2-mm mesh to remove plant debris and any small animals. Each of the 15 bulk samples was separated into two portions. The first portion was air dried for chemical analysis except that inorganic N (NH_4^+-N and NO_3^--N) was immediately analysed. The second portion was stored at 4°C for the soil incubation experiment.

1.3 Soil chemical and microbiological analyses

Soil pH was measured by a combination glass electrode (soil:water, 1:2.5). Total organic C was determined by dichromate oxidation, and total nitrogen was determined by Kjeldahl digestion and quantified with a continuous flow analyzer (SA5000, Skalar Inc., the Netherlands). Inorganic N (NH_4^+-N and NO_3^--N) was extracted with 2 mol/L KCl by shaking (1 h, 200 r/min) and filtering through a 0.45-μm polysulfone membrane. The KCl-extractable N was determined colorimetrically by a continuous flow analyzer. Soil microbial biomass C was determined using the chloroform fumigation extraction method.

1.4 Measurement of nitrification potentials by the soil incubation method

Soil samples were pre-incubated at room temperature for one week and were placed in bottles. After 140 mg N/kg $(NH_4)_2SO_4$ was added into the dry soils, all bottles were sealed with caps having holes, and incubated for 24 h under constant temperature of 25°C and a relative humidity of 95%. NO_3^--N was extracted with 1 mol/L KCl at 2, 4, 22 and 24 h of the incubation and analyzed as described above. Nitrification potentials were calculated as daily rates (mg/(kg·d)) from the increases in NO_3^- concentration over time.

1.5 Measurement of nitrification potentials by the liquid incubation method

Nitrification potentials were determined according to the shaken slurry method of Hart *et al*. (1994) with modifications. Soil samples of 15 g portions were pre-incubated at room temperature for one week and individually mixed with 100 mL one of the following solutions (liquid media): S1 solution (pH 7.2) containing 1.5 mmol/L NH_4^+ and 1 mmol/L PO_4^{3-} buffer, S2 solution containing 1.5 mmol/L NH_4^+ with adjustable concentrations of PO_4^{3-} buffer to ensure the pH values within the range of 6.8–7.8 after soil sample addition, and S3 solution containing 1.5 mmol/L NH_4^+ without any buffer. The mixtures in 250-mL Erlenmeyer flasks were made into slurries by incubation under a continuous aerobic condition with shaking at 200 r/min in an orbital shaker. The pH values of the slurries were determined at 2 and 24 h of the incubation. At 2, 4, 22 and 24 h of the incubation, 15 mL sample aliquot was taken from the slurries, and immediately centrifuged; the supernatant was filtered through a 0.45-μm polysulfone membrane, and was immediately analyzed for NO_3^- contents which was similarly to the analysis for KCl-extracted NO_3^--N as described above. Nitrification potential was calculated as the rate of daily increase in NO_3^- concentration in the slurry. To determine the effect of urea treatment on nitrification potential, a portion of the soil sample at each time point incubated with the S2 liquid medium was treated with urea (400 mg urea-N/kg soil) and processed in parallel for the analysis of the nitrification potential.

Table 1 Basic properties of the tested soil samples

Soil No.	pH	Organic C (g/kg)	Total N (g/kg)	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	Microbial biomass (mg C/kg)
1	5.16 ± 0.04 a	7.4 ± 0.2 d	0.85 ± 0.03 e	5.9 ± 0.6 c	6.6 ± 0.9 e	84.4 ± 15.8 d
2	4.22 ± 0.03 b	13.9 ± 0.2 c	1.35 ± 0.04 d	8.0 ± 0.6 b	46.6 ± 2.1 b	165.3 ± 20.5 c
3	4.01 ± 0.04 c	22.2 ± 0.1 b	2.05 ± 0.10 b	7.1 ± 0.5 b	56.1 ± 2.5 a	350.5 ± 16.3 a
4	3.71 ± 0.04 d	26.3 ± 0.5 a	2.29 ± 0.09 a	4.4 ± 0.2 d	40.3 ± 2.0 c	208.9 ± 23.1 bc
5	3.94 ± 0.05 c	27.5 ± 0.5 a	1.75 ± 0.11 c	9.2 ± 0.9 a	13.5 ± 1.1 d	244.2 ± 22.4 b

Different letters within each column indicate significant difference of mean value. Soil No. 1: wasteland; No. 2: 8-year old tea orchard; No. 3: 50-year old tea orchard; No. 4: 90-year old tea orchard; No. 5: 90-year old forest.

1.6 Statistics

All measurements were repeated for three times and their arithmetic means were reported and expressed on an oven-dried soil basis (105°C). Statistical analyses was performed with the software packages SPSS10.0 for Windows; means and least significant differences (LSD) at the 5% significance level were calculated by the one-way ANOVA.

2 Results

2.1 Nitrification potentials measured with the soil incubation method

The soil incubation experiment with adequate NH_4^+-N showed that the nitrification potential of 50-year old tea orchard soil was higher than those of 8-year and 90-year old tea orchard soils (Fig. 1); and the potential of soil for all tea orchards were also significantly higher than those of wasteland and forest soils.

2.2 Effect of liquid medium pH on soil nitrification potential

After adding of different soil samples into the liquid media S1, S2 or S3, pH values were measured at 2 h. The result showed that liquid media S1 and S3 became acidic while the S2 liquid medium remained neutral or slightly alkaline with a pH around 7.0 (Table 2).

The nitrification potentials measured in S1 and S3 acid liquid media showed similar trends as those obtained from the soil incubation method among different soil samples (Fig. 2). The soil nitrification potential of 8-year old tea orchard soil was lower than those of 50-year and 90-year old tea orchard soils, and there was a significant difference between 8-year old tea orchard and 90-year forest soil, while the wasteland soil had the lowest nitrification potential. Soil nitrification potential obtained by liquid incubation with S2 medium was much higher than those with S1 and S3 media (Fig. 2). Soil nitrification potentials of all soil samples increased in different solutions by the order of S3, S1 and S2. The nitrification potentials of different soil samples (three tea orchard soils, wasteland and forest soils) incubated with S2 neutral medium were 1.23, 1.85, 2.34, 1.65 and 1.32 times, respectively, higher than those of the corresponding soil samples incubated with S1 acid medium. The nitrification potential of all soil samples measured by the liquid incubation method were significantly higher than those by the soil incubation method.

Table 2 pH values of the liquid media S1, S2 or S3 at 2 h after incubation with soil samples

Soil No.	S1	S2	S3
1	5.08 ± 0.12 a	7.20 ± 0.13 a	4.87 ± 0.13 a
2	4.24 ± 0.10 b	6.99 ± 0.12 b	4.06 ± 0.09 b
3	4.04 ± 0.08 c	6.89 ± 0.11 b	3.86 ± 0.05 c
4	4.03 ± 0.09 c	6.91 ± 0.08 b	3.82 ± 0.08 c
5	4.10 ± 0.06 c	6.93 ± 0.07 b	3.91 ± 0.10 bc

S1: solution (pH 7.2) containing 1.5 mmol/L NH_4^+ and 1 mmol/L PO_4^{3-}; S2: solution containing 1.5 mmol/L NH_4^+ with adjustable PO_4^{3-} concentrations to ensure the slurry pH within the range of 6.8–7.8 after addition of each sample; S3: solution containing 1.5 mmol/L NH_4^+ without any buffer. Soil No is the same as described in Table 1. Different letters within each column indicate significant difference of mean value.

2.3 Effect of urea treatment on soil nitrification potential

The slurry shaken liquid incubation method with S2 liquid medium was determined to be the best, therefore, was used to further assess the effect of nitrogen fertilizer application on soil nitrification potential. Urea treatment (400 mg urea-N/kg soil) significantly improved the soil

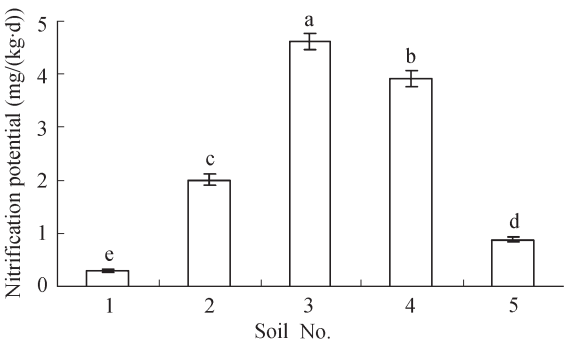

Fig. 1 Soil nitrification potential measured by soil incubation. Soil No.1–5 are the same as that in Table 1. Different letters indicate significant difference of mean value.

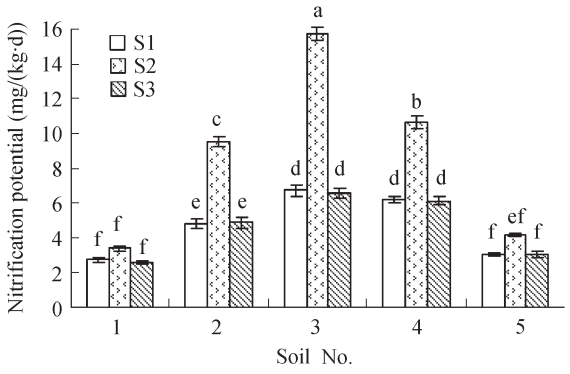

Fig. 2 Soil nitrification potential determined by using different pH in liquid medium. S1–3 are the same as that in Table 2. Soil No. is the same as in Fig. 1. Different letters indicate significant difference of mean value.

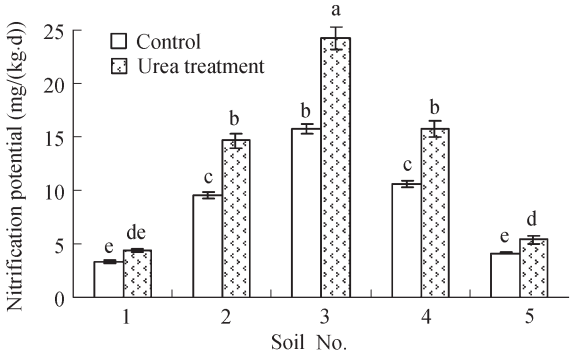

Fig. 3 Effect of urea treatment on nitrification potential. Soil No. is the same as in Table 1. Different letters indicate significant difference of mean value.

nitrification potential (Fig. 3). Before and after urea treatment, the soil samples from tea orchards of 8-, 50-, 90-year old, wasteland and 90-year forest showed increases in the net nitrification rates of 53.8%, 55.0%, 48.4%, 28.8%, and 29.6%, respectively. From these data, urea treatment enhanced the soil nitrification potential of the younger (8-year and 50-year old) tea orchard soils to a greater extend than of the older one (90-year old), and such enhancement was also more prominent on all the three tea orchard soils than on wasteland and 90-year forest soils.

3 Discussion

Our survey of different soil types show that tea orchard soils displayed significantly higher nitrification potential than wasteland and forest soils. This may be explained by the fact that tea orchards are always fertilized by N fertilizers but wasteland and forest are not subjected to such human management. Previous studies (Martikainen, 1985; Hayastu and Kosuge; 1993; Aarnio and Martikainen, 1996; Mendum *et al*., 1999; Chu *et al*., 2008) showed that the fertilized soils have a higher nitrifying activity due to the increased release of ammonium from fertilizers and the accelerated growth of nitrifying population. Our results also show that N fertilizer has stimulating effect on soil nitrification. Since the vegetation type, the quantity and chemical composition also largely determine the quantity and quality of the soil microbial community (Waid, 1999), the difference in soil microbial communities between all sampling sites may also account for the difference in the net nitrification rate and the nitrification potential among different types of soils.

Among various methods of measuring soil nitrification potentials, the shaken slurry method of Hart *et al*. (1994) is a well-established classical method where a liquid medium of pH 7.2 is typically used as the optimal pH to measure the activity of nitrifiers. However, our results show that the pH value of slurry made with the mixed soil sample in the medium of pH 7.2 can be significantly reduced to below 5.0 due to the strong buffer capacity of tested acid soils. Therefore, using such a liquid medium at pH 7.2 was not the best strategy to measure acid soil samples as analyzed in this study. Instead, nitrification potentials measured in S2 liquid medium was significantly higher, suggesting that a liquid medium with a stronger buffering capacity is more suitable or readjustment of the pH to a neutral level after soil sample addition may be necessary. The nitrification potential determination in traditional way by using the pH 7.2 medium can not reflect the maximum nitrification capacity of the soil nitrifier population in acid soils. Moreover, the data suggest that some nitrifiers present in tea orchard soils may have higher activities in the pH-neutral conditions.

The nitrification potentials determined by the soil incubation method were significantly lower than by the liquid incubation method. Liquid incubation can make soil particles evenly dispersed so that all nitrifiers can be exposed to N sources. However, this is harder for soil incubation method, therefore, some nitrifiers may not be directly exposed to N sources during the measurement. On the other hand, liquid incubation can ensure the pH environment homogeneous to all nitrifiers while soil incubation can hardly achieve this. Therefore, the liquid incubation method was preferable to soil incubation for measuring soil nitrification potentials.

4 Conclusions

Among different soil types surveyed in this study, the tea orchard soils display higher nitrification potentials. To measure soil nitrification potential, the liquid incubation method yields more accurate and reliable results than the soil incubation method; with the liquid incubation method, a pH 7.2 traditional applied to the liquid medium is not optimal for acid soil samples with strong buffer capacities. In addition, the decreased nitrification potential obtained under a sub-optimal acidic condition indicated the presence of nitrifiers in the tea orchard soils requiring a pH-neutral condition for optimal activity. Our data also show that urea treatment significantly improves nitrification potentials of the soils, whch is more prominent to tea orchard soils than to wasteland and forest soils, and also more significant to the younger tea orchard soils than to the older one.

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