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Short communication

Contribution of fungi to acetylene-tolerant and high ammonia availability-dependent nitrification potential in tea field soils with relatively neutral pH

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ABSTRACT

This study was conducted to analyze the relative contribution of nitrifying microbial groups in tea field soils with relatively neutral pH where high ammonia availability was expected after heavy fertilization. The nitrification potential in tea field soils supplied with organic fertilizers (OF) or chemical fertilizers (CF) was determined by the chlorate-inhibition method. The response of the potential to the net ammonia concentration, which was employed as an index of ammonia availability, was tested by loading various levels of ammonium sulfate to soil slurries. The potential of tolerance to 10 Pa acetylene was highlighted. The acetylene-tolerant nitrification potential occurred all year at a significant level. It was stimulated by ammonia availability of more than $10^{-5.5}$ mol NH_3 L^{-1}, where the nitrification potential determined under an acetylene-free condition (total potential) was affected negatively. As the test results show, the contribution of the acetylene-tolerant potential ranged from 2 to 38% with OF soils and from 8 to 67% with CF soils. These indicated a significant contribution of heterotrophic microorganisms to the nitrification potential of these neutral tea soils. The addition of cycloheximide rather than streptomycin remarkably suppressed the acetylene-tolerant nitrification potential, suggesting the primary contribution of fungi. A new aspect of heterotrophic nitrification was identified.

1. Introduction

In nitrification, ammonia rather than ammonium ion is the primary substrate for ammonia monooxigenase (AMO), even for intact cells of chemolithotrophic ammonia-oxidizing bacteria (AOB) (Suzuki et al., 1974). Nitrification progresses faster in environments with neutral and slightly alkaline pH than in those with acidic pH because of the larger ammonia availability. AMO is present among ammonia-oxidizing archaea (AOA) as well as AOB (Könneke et al., 2005). Recently, the relative contribution of AOA and AOB to nitrification in soil has been evaluated intensively by means of a quantitative PCR of *amoA*, a gene encoding the active site-containing subunit of AMO, as a functional marker. In many cases, the AOA-*amoA* copy number was hundreds of times higher than that of AOB-*amoA* (see, Tourna et al., 2011; Zhang et al., 2010). Nevertheless, the importance of AOB in agricultural soils was emphasized based on the fact that, compared to AOA, AOB increased in soils with higher ammonium ion concentrations (Di et al., 2010; Höfferle et al., 2010; Matsutani et al., 2011). Yao et al. (2011) reported that the specific AOA and AOB groups were selected in acid tea soils according to both the soil pH and the levels of N fertilizer input. In these studies, the researchers did not attempt to estimate the proportion of ammonia and ammonium ion.

For heterotrophic bacteria, such as *Paracoccus denitrificans* (Crossman et al., 1997; Moir et al., 1996), *Pseudomonas putida* (Daum et al., 1998), and *Bacillus* sp. (Lin et al., 2010), AMO or the corresponding gene was identified. Some other denitrifying bacteria were capable of nitrification (Castignetti and Hollocher, 1984). *Paracoccus pantotrophus* (formerly called *Thiosphaera pantotrophus* and *P. denitrificans* GB 17) has been shown to be a heterotrophic nitrifier/aerobic denitrifier (Robertson and Kuenen, 1984). While several fungal isolates have been demonstrated as nitrifiers (Doxtader and Alexander, 1966; Hatcher and Schmidt, 1971; Hirsch et al., 1961; Marshall and Alexander, 1962; Stroo et al., 1986), the biochemical details of fungal nitrification remain unclear. For *Aspergillus flavus*, nitrate production might be related to aflatoxin biosynthesis (White and Johnson, 1982).

The chlorate-inhibition method (Belser and Mays, 1980; Berg and Rosswall, 1985) gives an estimate of the soil nitrification potential within several hours by minimizing interference caused by

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other microbial nitrogen transformation processes. Acetylene, a suicide substrate for AOB–AMO (Hynes and Knowles, 1982; Hyman and Wood, 1985), is the most specific and effective of the nitrification inhibitors for laboratory use. In combination with the chlorate-inhibition method, acetylene addition has the potential to identify the nitrification potential of heterotrophically nitrifying microorganisms (Daum et al., 1998; Moir et al., 1996) from that of AOB, and presumably of AOA (Gubry-Rangin et al., 2010; Offre et al., 2009).

A large amount of N fertilizer has been applied to tea fields in Japan. A strong nitrification potential developed even in acidic tea soils (Hayatsu and Kosuge, 1993; Yokoyama et al., 2003). In this study, we focused on the nitrification potential of surface tea field soils with relatively neutral pH, although such soil is rare in Japanese tea fields. Nitrifying microorganisms in these soils would be exposed to the accumulation of ammonia after fertilization or manure incorporation and affected in different ways. The relative contribution of each group would shift depending on their sensitivity to high ammonia availability. This study demonstrated that, with either AOB or AOA, heterotrophic microorganisms consistently contributed a significant portion of the nitrification potential in tea field soils with relatively neutral pH.

2. Materials and methods

The fields of Humic Hapludult (Cultivated Soil Classification Committee, 1995) were located on a gentle slope (7°) facing south in Ono, Ube City, Yamaguchi Prefecture, Western Japan (+34°4′ latitude, +131°19′ longitude). A tea field with an area of 20 (width) m × 40 (length) m planted with *Camellia sinensis* (L) O. Kuntze (Yabukita) was studied. Organic fertilizers had been applied to the tea bushes planted in the upper half of the area (OF soil), while the plants grown in the lower half had received chemical fertilizers (CF soil). Nitrogen fertilizers were supplied at 640 kg N ha^{-1} annually. Bean cakes and blended organic fertilizers were incorporated into the OF soil. The CF soil received ammonium sulfate or compound fertilizers as N sources. For each soil, a surface layer of furrow (0–15 cm depth) was collected after litter removal. Soil samples were collected 7 times in 2003 and twice in 2004 for a year-round study.

Table 1
Some properties of OF and CF soils.

Soil	Texture	pH		Total C	Total N
		H_2O	KCl	(g kg^{-1})	(g kg^{-1})
OF	SCL	6.2	6.0	36.3	3.1
CF	CL	5.9	5.6	41.7	4.9

The data were determined for soil samples collected in November 2003.

Each soil was collected from at least four different positions along furrows, and more than 10 kg in total was composited. The soil temperature at 5 cm depth ranged from 10 °C in winter to 25 °C in summer. Soil passed through a 2 mm sieve was stored at 4 °C. The pH of these soils was almost 6, probably due to liming with dolomite and oyster shell powder (Table 1). This would be a rare case among Japanese tea field soils, which generally have strong acidity of pH 4 or less (Hayatsu, 1993; Tokuda and Hayatsu, 2000; Yokoyama et al., 2003). The total carbon and nitrogen contents were slightly larger for CF (41.7 and 4.9 g kg^{-1}) than OF (36.3 and 3.1 g kg^{-1}), respectively (Table 1). This difference could be attributed to the incorporation of a large quantity of litter fragments into the surface layer rather than fertilization.

Within 4 days of sampling, the nitrification potential was determined according to the chlorate-inhibition method (Belser and Mays, 1980; Berg and Rosswall, 1985) with some modifications (Yokoyama et al., 2003). Ten grams of fresh soil was suspended in a 25 mL potassium chlorate solution (15 mmol L^{-1}) in each of three flasks per treatment. To test the effect of ammonia availability on the nitrification potential, an ammonium sulfate solution was loaded into the soil suspensions at 25, 50, 100, or 200 mmol NH_4^+ L^{-1} in addition to indigenous ammonium ion in soil. The suspension pH was allowed to equilibrate under the given conditions unless adjusted by an extraneous acid or alkaline addition. Before shaking, 1 mL of suspension was pipetted out as a 0 h sample. The flasks were closed with a double-layer stopper of butyl gum. The required amount of acetylene gas was injected into a headspace. Routinely, 10 Pa as partial pressures was employed. The inhibition efficiency of acetylene was tested at 10, 500, or 1000 Pa with CF soil suspensions loaded with 200 mmol NH_4^+ L^{-1} and nitrifying at 38.2 nmol NO_2^- g^{-1} h^{-1}. For the determination

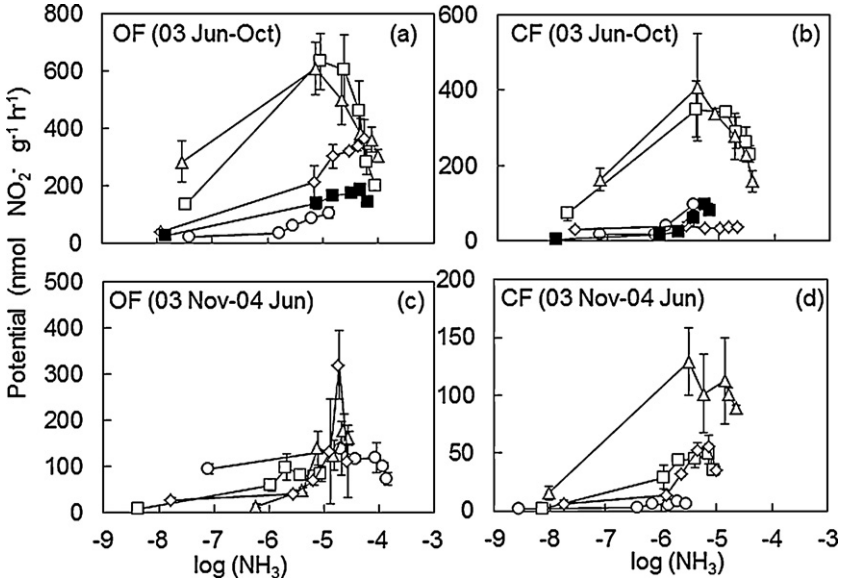

Fig. 1. Changes in the total nitrification potentials of OF and CF soils as the function of ammonia availability. Bars indicate the standard deviations of replicate experiments (n = 3). Panels (a) and (b) show potentials determined from June 2003 to October 2003. Panels (c) and (d) show those from November 2003 to June 2004. The horizontal axis indicates the logarithmic values of the net NH_3 concentration (mol L^{-1}). Symbols in (a) and (b) are (○) June 2003, (□) July 2003, (△) August 2003, (◇) September 2003, and (■) October 2003. Symbols in (c) and (d) are (○) November 2003, (□) December 2003, (△) April 2004, and (◇) June 2004.