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Short communication

Nitrification results in underestimation of soil urease activity as determined by ammonium production rate

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ABSTRACT

The majority of soil urease activity measurements have been based on the rate of ammonium production under optimal conditions. However, such procedures do not exclude ammonium consumption by the nitrification process. The purpose of this study was to determine the percentage of soil urease activity that is underestimated due to soil nitrification. Six soils with diverse properties were incubated using a standard procedure for measuring soil urease activity. The dynamics of nitrite and nitrate were observed during the incubation. Our results showed that the percentage of underestimation ranged from 7.38% to 15.97%, depending on soil types and whether or not a buffer was used. We recommend that nitrification be taken into account when soil urease activity is assayed by the ammonium production rate method.

Urease is one of the most commonly assayed soil enzymes because it greatly influences the transformation and fate of an important fertilizer – urea (Gianfreda et al. 1992; Schlatte et al. 1998; Sinsabaugh et al. 2000; Caldwell 2005; Andrén et al. 2008). Urease activity has been investigated under a wide range of soil management types or treatments (Aira et al. 2003; Carter et al. 2007; Bastida et al. 2008; Enowashu et al. 2009). Urease activity is generally determined by the rate of ammonium production (Tabatabai and Bremner 1972; Kandeler and Gerber 1988; Kandeler 1996; Sinsabaugh et al. 2000), but occasionally also estimated by measuring the rate of urea loss (Zantua and Bremner 1975) or CO_2 production (Guettes et al. 2002), under alkaline and aerobic condition. However, the alkaline and aerobic conditions, together with the ammonium accumulation during the urease activity assays, tend to enhance the nitrification process in soils (Krave et al. 2002; Warnock et al. 2007). Therefore, it is reasonable to assume that the urease activity determined by the ammonium production rate is more or less underestimated due to the ammonium consumed in nitrification, especially in soils with high nitrification potential, such as the Cambisols in North China (Chu et al. 2007). The aim of the present work was to test if nitrification affects soil assays of urease activity and to quantify this effect, if it exists.

To achieve this goal, we collected 18 surface soil samples (0–20 cm) from six experimental stations (three samples for each station). These stations belong to the Chinese Ecosystem Research Network, which is part of the Chinese Academy of Sciences. The soils have a wide range of nitrification potentials and other properties (Table 1). The urease activities in these soils were assayed by the non-buffer and buffer methods of Kandeler and Gerber (1988). These methods have been widely used in soil urease activity assay. According to ISI Web of Science, the citations of the methods increased yearly and reached 226 by 2009. The major process was described as follows: 5 g of soil (2-mm-sieved and air-dried) were incubated with 0.08 M aqueous or buffered urea solution (pH 10.0) at 37 °C for 2 h. The accumulated ammonium was subsequently extracted with a solution that contained 1 N KCL and 0.01 N HCL. Finally, the ammonium was quantified by spectrophotometry at 690 nm (Kandeler and Gerber 1988).

In order to estimate the ammonium consumption from nitrification in the present study, 10 portions for each soil sample were incubated under the conditions identical to those of the urease activity assay. Subsequently, one portion of each soil sample was selected at 0.5-h intervals to determine nitrite and nitrate contents, respectively. The nitrite and nitrate contents were determined by the methods of Mulvaney (1996) and Norman et al. (1985), respectively. The control samples were treated identically except that distilled water was used instead of a urea solution. The underestimation of urease activity was calculated using the following equation:

$$U = \frac{NA + NI}{NA + NI + UE} 100\% \quad (1)$$

where U, NA, NI, UE represent the percentage of the underestimated urease activity, nitrate-N accumulation, nitrite-N accumulation and urease activity, respectively. Differences in urease activity and its

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Table 1
Major properties of soils collected from field sites.

Soil no.	Site	Soil type [WRB (2006)]	Crop system	pH (H_2O)	SOC ($mg\,g^{-1}$)	TN ($mg\,g^{-1}$)	BD ($g\,cm^{-3}$)	C:N ratio	NP ($\mu g\,g^{-1}\,h^{-1}$)
1	Luangcheng (114°24′47″ E, 37°53′26″ N)	Cambisols	Wheat–maize rotation	8.5	10.3	1.1	1.4	9.4	3.4
2	Hailun (126°55′39″E, 47°27′15″N)	Phaeozems	Maize	7.1	27.5	2.9	0.88	9.2	2.6
3	Fengqiu (114°19′43″, 35°00′40″N)	Fluvisols	Wheat–maize rotation	7.3	9.8	1.0	1.3	9.8	2.4
4	Shenyang (123°22′05″E, 41°31′06″N)	Cambisols	Maize	7.6	9.9	0.9	1.4	11.2	2.1
5	Sanjiang (133°18′03″E, 47°21′07″N)	Fluvisols	Maize	6.5	16.8	1.5	1.6	10.1	1.9
6	Heshan (112°54′01″E, 22°40′47″N)	Acrisols	Peanut	5.5	11.0	0.8	1.4	13.7	0.8

Soil pH, soil organic C (SOC), total N (TN), bulk density (BD) and nitrification potential (NP) were determined by the glass electrode method (Hendershot et al. 1993), the dichromate digestion method (Kalembassa and Jenkinson 1973), the semi-microkjedahl method (Nelson and Sommers 1980), the soil core method (Blake and Hartge 1986) and the shaken slurry method (Hart et al. 1994), respectively.

underestimation were compared using the LSD test at $P < 0.05$ with the SPSS 10.0 software for windows (SPSS 2000).

The results showed that nitrite-N content increased significantly during the incubation (Fig. 1). The degree of this increase depended on soil types and whether or not the buffer was used. Generally, soils with high nitrification potential, such as Cambisols and Phaeozems, showed greater increases of nitrite-N contents than those with relative lower nitrification potential, such as Acrisols (Fig. 1). Moreover, the buffer method resulted in relatively larger increases than the non-buffer method (Fig. 1). In comparison, the nitrite-N contents in the control samples did not increase during the incubation (Fig. 1). The nitrate-N contents in soils also increased slightly with incubation time, compared to what was observed in the control samples (Fig. 2). These results indicate that a certain percentage of ammonium produced by urease hydrolysis was consumed by nitrification, leading to the potential underestimation of soil urease activity. As can be seen from Table 2, the soil urease activities determined by the ammonium production rate were underestimated by 7.38–15.97%. These percentages were not associated with soil nitrification potential or the use of the buffer. This lack of association could be attributed to both how the level of nitrification and urease activity influence the percentage underestimation, as can be inferred from Eq. (1). Soil nitrification levels are positively related to pH ranging from 6 to 8 (Krave et al. 2002; Kyveryga et al. 2004). Sahrawat (2008) reported that soil nitrification could take place at pH ranging from 6 to 10, with an optimum

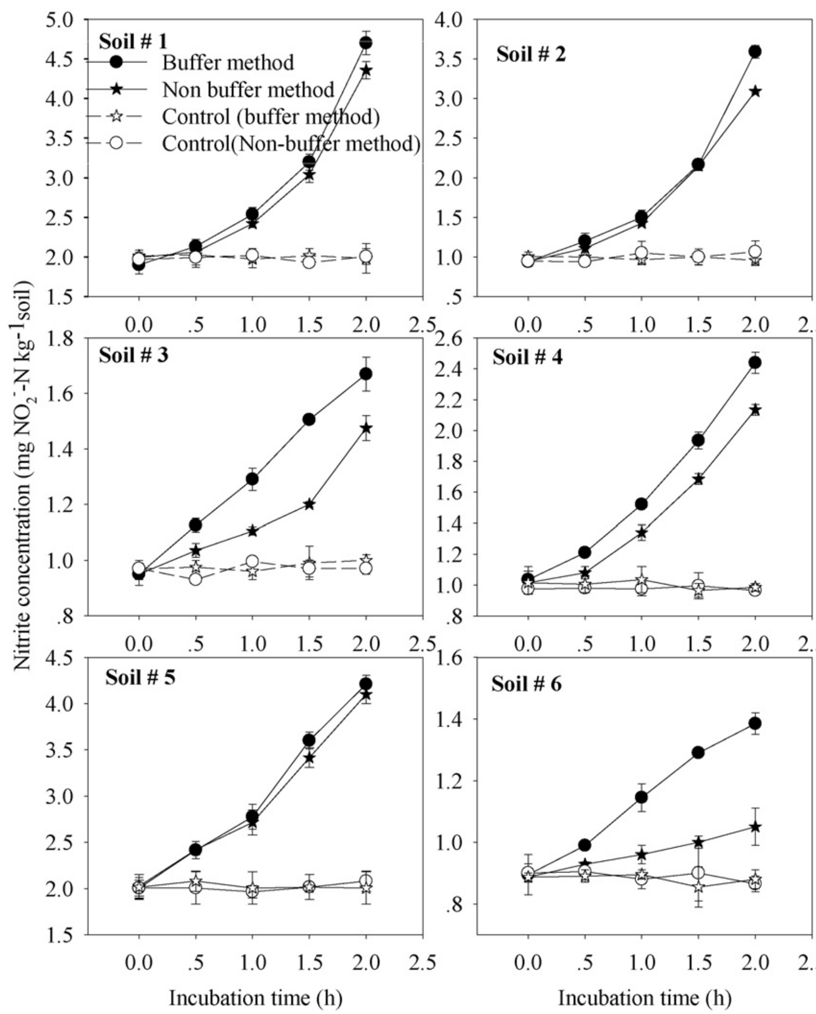

Fig. 1. Nitrite accumulation observed in six soils during the urease activity assay. The incubation conditions were identical to those cited in Kandeler and Gerber (1988).