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Soil Nitrification and Denitrification Potentials in a Wheat Field Soil as Affected by Elevated Atmospheric CO_2 and Rice Straw Incorporation

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By the end of this century, atmospheric carbon dioxide (CO_2) concentration is predicted to be up to 549-970 $\mu mol \cdot mol^{-1}$ (IPCC 2001). Such an increase should have a direct effect on soil microbial processes, though the CO_2 concentration in soil is generally higher than that in atmosphere (Baggs *et al.* 2003).

Soil nitrification and denitrification are the microbial processes of great significance in soil N cycling and N_2O production (Carnol *et al.* 2002), and their rates could be modified by elevated atmospheric CO_2 concentration because of the sensitivity of related soil microbes to the changes of soil environmental factors induced by it (Barnard *et al.* 2005). Bagges *et al.* (2003) indicated that the total denitrification in grass swards was increased under elevated atmospheric CO_2, because the increased belowground C allocation provided the energy for denitrification. So far, there is no consistent information about soil microorganisms and their activity involved in nitrification, and little experimental evidence is available on the responses of soil microorganisms involved in denitrification to elevated atmospheric CO_2 (Fromin *et al.* 2005). In this paper, a Chinese Rice/Wheat FACE (free air carbon dioxide enrichment) system was installed on a rice-wheat rotation field of Eastern China to study the effects of elevated CO_2 and rice straw incorporation on soil nitrification and denitrification potentials, the numbers of soil nitrifiers and denitrifiers in a wheat field.

MATERIALS AND METHODS

As a part of the FACE study in China, this study was conducted on a rice-wheat rotation field at the Jiangdu suburb (32 °35' N, 119° 02'E) of Eastern China, consisting three rings for elevated CO_2 (ca. 200μmol CO_2 mol^{-1} over the ambient) and three for ambient CO_2, each with a diameter of 12.5m. The ambient plots were situated at least 90m from the nearest FACE ring (Liu et al. 2002). The test soil type is Vertisols, with organic matter 18.39 g/kg, total nitrogen 1.45g/kg N, total phosphorus 0.63 g/kg P_2O_5, pH 7.9, clay (<0.002mm) fraction 13.6%, and bulk density 1.16 g/cm.

After rice harvest, the field plots were seeded with wheat (*Tritium aestivum L. cv.* Yangmai 14) on Nov. 4th, 2004, and harvested on June 4th, 2005.

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Ammonium-based nitrogen fertilizer was applied in the wheat growing season at a rate of 112.5 kg/ha N, with 50% basally applied on Nov. 2nd, 2004, 10% top-dressed on March 3rd, 2005, and 40% top-dressed on April 5th, 2005. The application rate of phosphorus fertilizer was 75 kg/ha P. All the harvested rice straw from last rice growth season was incorporated at a rate (HR) of ca. 2000 kg/ha C and no rate (NR) of 0 kg/ha C.

Soil sampling was made in wheat growing season (2004-2005). Five fresh soil cores (d=2cm, h=10cm) per treatment were randomly collected at seedling (Dec. 3rd), mid- tillering (March 10th), mid- jointing (April 1st) and heading (April 19th) stages and after harvest (June 2nd). After transport to the laboratory the soil samples were processed immediately and incubated on media within a minimum time period.

Soil potential nitrification (SPN) was measured by the method of suspension incubation (Hart *et al.*, 1994). 15.0g 2mm- sieved field-moist soil sample was placed in a 250ml plasma flask with 100ml culture medium (1.5ml KH_2PO_4, 3.5ml $K_2H_2PO_4$, 15ml $(NH_4)_2SO_4$, pH 7.2). The flask was sealed with a holed rubber stopper, and shaken on an end-to-end shaker for 24h (180r/min). In the course of shaking, soil suspension was extracted 4 times (10ml each time) and centrifuged, and the addition of 10ml soil suspension of same medium to the culture flask after each removal. 1ml supernatant was placed in a 1ml graduated tube with 1ml 2% aminosulfonic acid. After standing for 2 min, 8ml 15% $HClO_4$ was added, and the NO_3^- -N concentration was measured with a spectrophotometer (Specord 50, analytikjenaAG, Germany) at λ=210nm.

Soil denitrification potential (SDP) was measured according to Yeomans *et al.* (1992), with some modifications. 5.0g 2mm- sieved field-moist soil was placed in a 250ml plasma flask with a 5ml solution of 3mg KNO_3-N and 3mg glucose-C. The flask was sealed with a butyl rubber stopper, and its contained atmosphere was replaced by a 90:10 He-C_2H_2 mixture to provide an anaerobic condition and to inhibit N_2O-reductase activity. The flask was placed at 25℃ for 48h, and its N_2O concentration was analyzed on a gas chromatograph equipped with an election capture detector (GC-14B, Shimadzu, Japan). After 10g soil was extracted with 100ml 2M KCl for 1h, its NH_4^+-N and NO_3^--N contents were determined by an automated procedure (Autoanalyzer III, BRAN+LUEBBE, Norderstedt, Germany).

The MPN of nitrifiers and denitrifiers were quantified by following the procedures described by Xu (1986). Soil extracts were used instead of fermentation solutions. Soil extracts were prepared as follows: 1000 ml distilled water was added to 1 kg fresh soil. The mixture was mixed thoroughly for 30 min, and then was left overnight. Clear liquor was obtained by filtering repeatedly. The liquor was sterilized at 121°C for 30 min and stored at 4°C (Hou 2000). A half ml of the suspension at 10^{-2} to 10^{-7} dilution was inoculated to the tubes containing 5 ml medium to measure nitrifiers. The tubes were incubated at 30°C for 14d. Parameter measured for determining nitrifiers was NO_2^-, and the method applied

was Griess reaction. The culture media for nitrifiers were: $(NH_4)_2SO_4$ 2.0 g; NaH_2PO_4 0.25 g; $MnSO_4 \cdot 4H_2O$ 0.01 g; K_2HPO_4 0.75 g; $MgSO_4 \cdot 7H_2O$ 0.03 g; $CaCO_3$ 5.0 g; distilled water 1000 ml. For enumeration of denitrifiers, a sample of 10 g fresh soil was transferred into a conical flask containing 90 ml distilled water. Then 1 ml suspension at 10^{-2} to 10^{-7} dilution was inoculated to the tubes containing 10 ml medium. The tubes were incubated at 30°C for 14d. Parameters measured for determining denitrifiers were NO_2^- and N_2O+N_2 by using Griess reaction and Durham tube as determination methods, respectively. The culture media for denitrifiers were: $C_4H_4KNaO_6 \cdot 4H_2O$ 20 g; KNO_3 2 g; K_2HPO_4 0.5 g; $MgSO_4 \cdot 7H_2O$ 0.2 g; distilled water 1000 ml.

All data were subjected to statistical analysis of variance (ANOVA) in the SPSS statistical package.

RESULTS AND DISCUSSION

Some researchers reported a decreased soil nitrate concentration in their long-term elevated CO_2 experiments. Barnard *et al.* (2005) observed a sharp decrease (-50%) of soil NO_3^--N under elevated CO_2. Our results also showed an obvious decrease (10.5-54.8%) of soil NO_3^--N in elevated CO_2 treatments at wheat heading stages. Hungate *et al.* (1997) indicated that the enhanced root production and soil C:N ratio under elevated CO_2 decreased the availability of soil NH_4^+ and NO_3^- to soil nitrifier and denitrifier, and thereby, decreased soil potential nitrification and denitrification potential. Our determinations (Table 1) showed that the soil denitrification potential in FACE was decreased by 26.5-42.1%, compared with ambient. Similarly, soil NH_4^+-N and potential nitrification under elevated CO_2 was decreased by15.0-7.0% and 37.6-12.0%, respectively, compared with ambient, and the decrements were larger in the treatment of high rate rice straw incorporation, which suggested that elevated CO_2 limited the N utilization of soil nitrifier, the incorporation of high rate rice straw exacerbated this limitation.

Table 1. Soil potential nitrification (SPN), denitrification potential (SDP), and extractable soil mineral N contents in treatments FACE and ambient at wheat heading stages.

Treatments	SPN mg/kg h	SDP mg/kg 48h	NH_4^+-N mg/kg	NO_3^--N mg/kg
FACE HR	16.31(±3.65)	26.16(±3.58)	1.02(±0.61)	9.53(±5.37)
Ambient HR	26.12(±13.74)	35.60(±2.51)	1.20(±0.12)	10.65(±2.01)
FACE NR	22.61(±4.47)	26.84(±5.18)	2.13(±0.42)	8.47(±2.13)
Ambient NR	25.71(±7.17)	46.38(±5.80)	2.29(±0.03)	18.74(±8.91)

Data shown are the means ± standard error

At two straw levels, the nitrifier declined with crop growth but ascended at maturing stage, and its seasonal dynamics could be well described by $Y = 0.054X^3-0.2239X^2-0.4479X+5.641$ ($R^2 = 0.99$, $p<0.01$) and $Y=0.0585X^3-0.1104X^2-1.0699X+5.7692$ ($R^2=0.999$, $p<0.01$) for treatments HR and NR, respectively, where Y is the logarithm of MPN of soil nitrifier, and X is the sampling date in Fig. 1a.

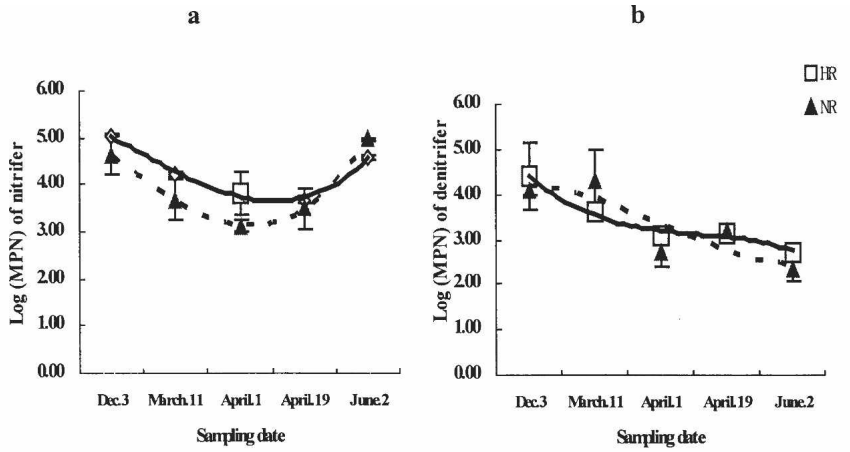

Figure 1. Seasonal dynamics of the logarithm of nitrifier and denitrifer population in the soil during the wheat season. Data shown are the means of the logarithm of MPN under both CO_2 levels. Error bars are standard errors. (a) nitrifier; (b) denitrifier

The denitrifier was the largest at seeding stage, and the smallest at harvesting stage. From tillering to heading stage, the denitrifier remained relatively stable. Its seasonal dynamics during the growth period could be described by $Y=-0.0591X^3+0.6294X^2-2.3615X+6.2112$ ($R^2=0.98$, $p<0.01$), and $Y=0.0443X^3-0.402X^2+5.997X+3.9598$ ($R^2=0.75$, $p>0.05$) for treatments HR and NR, respectively, where Y is the logarithm of MPN of soil denitrifier, and X is the sampling date in Fig. 1b.

Across the wheat growth stages, elevated atmospheric CO_2 decreased the numbers of soil nitrifier at joining and heading stages, with the mean differences in the logarithm of most probable number being significant at $p<0.05$ or $p<0.01$ between treatments FACE and ambient, and more decrement was found in the treatment incorporated with high rate rice straw. Soil denitrifier was also lowered significantly by elevated CO_2 during the whole growth period of wheat (Fig.2).

Torber *et al.* (2000) reviewed that elevated atmospheric CO_2 could promote root growth, making an increase of root biomass (the root biomass in this study was increased by37.85%, $p<0.001$) and root exudates, and thus, an increase of carbon substrate for soil microbes. But, in another hand, the elevated CO_2 could also stimulate plant N uptake (+9%, $p<0.01$, Yang and Wang's unpublished data in this study) and soil biological N fixation (Hungate *et al.* 1997), further reduce mineral N availability for nitrification and denitrification as showed by lower available soil ammonia and nitrate under elevated CO_2 (Table 1).

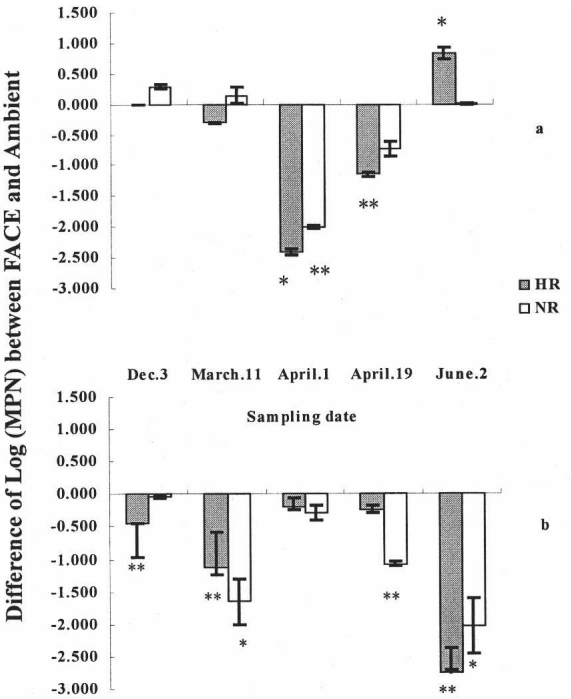

Figure 2. Increment of soil nitrifer and denitrifier in wheat field soil under elevated CO_2, as compared to the ambient. Columns are the mean differences of logarithm of the most probable number (MPN) of nitrifer and denitrifiers between FACE and ambient. Errors are standard errors. * is significant at $p < 0.05$, and ** at $p < 0.01$ in ANOVA analysis. (a) nitrifier; (b) denitrifier, (HR) incorporated with high rate rice straw of ca. 2000 kg/ha C, (NR) incorporated with no rate of 0 kg/ha C

Additionally, high rate rice straw incorporation increases N immobilization and also limits ammonia availability for nitrification, and cause lower numbers of nitrifers at joining and heading stages (Figure 2). Soil potential nitrification (Table 1) reflects the number/activity of organism at heading stages. Thereby, all of these suggested that elevated CO_2 caused lower soil nitrification and denitrification potentials in a wheat field soil and incorporation of high rate rice straw exacerbated the lower potentials.

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