Determination of respiration, gross nitrification and denitrification in soil profile using BaPS system

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Abstract: A facility of BaPS (Barometric Process Separation) was used to determine soil respiration, gross nitrification and denitrification in a winter wheat field with depths of 0—7, 7—14 and 14—21 cm. N_2O production was determined by a gas chromatograph. Crop root mass and relevant soil parameters were measured. Results showed that soil respiration and gross nitrification decreased with the increase of soil depth, while denitrification did not change significantly. In comparison with no-plowing plot, soil respiration increased significantly in plowing plot, especially in the surface soil of 0—7 cm, while gross nitrification and denitrification rates were not affected by plowing. Cropping practice in previous season was found to affect soil gross nitrification in the following wheat-growing season. Higher gross nitrification rate occurred in the filed plot with preceding crop of rice compared with that of maize for all the three depths of 0—7, 7—14 and 14—21 cm. A further investigation indicated that the nitrification for all the cases accounted for about 76% of the total nitrogen transformation processes of nitrification and denitrification and the N_2O production correlated with nitrification significantly, suggesting that nitrification is a key process of soil N_2O production in the wheat field. In addition, the variations of soil respiration and gross nitrification were exponentially dependent on root mass ($P < 0.001$).

Keywords: soil respiration; gross nitrification; denitrification; Barometric Process Separation (BaPS)

Introduction

Terrestrial ecosystem carbon cycling plays an important role in the budget of atmospheric CO_2. Second to gross photosynthesis, CO_2 emissions from soils (i.e., soil respiration) exceed all the other terrestrial-atmospheric carbon exchanges (Raich and Schlesinger, 1992). Any increases in soil CO_2 emissions have the potential to exacerbate increasing atmospheric CO_2 levels and to provide a positive feedback to global warming (Raich and Tufekcioglu, 2000). No-plowing has been conceived as one of the conservation tillage methods that mitigate effectively carbon losses from agricultural soils (Reicosky and Lindstrom, 1993). Some studies have addressed the respiration under various plowing methods (Burton and Beauchamp, 1994; Alvarez et al., 2001), however, understanding the outlining respiration at different soil depths will help assess the effect of plowing on soil CO_2 effluxes.

Besides CO_2, N_2O is also an important greenhouse gases that contribute to global warming and ozone depletion. N_2O is produced naturally in the soil microbial processes of nitrification and denitrification (Firestone and Davidson, 1989). Although soil nitrification and denitrification processes have been well documented, however, rare research concentrates simultaneously on nitrification and denitrification rates at different soil depths in various plowing methods and preceding cropping practices. Gross N-turnover rates in soil, on the other hand, are more difficult to measure than soil N mineralization

(Breuer et al., 2002). Nevertheless, gross N-turnover, nitrification and denitrification are essential to comprehensively understand soil nitrogen processes. Current methods for investigation of gross nitrification and denitrification rates involve ^{15}N tracers (Davidson et al., 1992) and acetylene inhibition techniques (Davidson et al., 1986). These methods have the disadvantage of introducing labeled material into soil or changing the composition of soil atmosphere as acetylene was applied (Ingwersen et al., 1999). A facility of BaPS (Barometric Process Separation) was first introduced by Ingwerson et al. (1999) to quantify gross nitrification and denitrification rates by measuring the changes of air pressure in an enclosed gas-tight and isothermal system. Without the involvement of ^{15}N-labelled material or use of acetylene, gross nitrification and denitrification rates of intact soil cores were directly determined in this system. Moreover, soil respiration rate can also be determined during the measurement of nitrification and denitrification (Ingwersen et al., 1999), which was helpful for investigating the three processes of respiration, nitrification and denitrification simultaneously and comparing their variation in different soil depths.

The main objectives of this study were, by using BaPS system, (1) to assess the variation of soil respiration, gross nitrification and denitrification in different soil depths under different cultivation regimes, involving plowing method and preceding cropping practice; (2) to examine the factors influencing the variation of soil respiration,

nitrification and denitrification; and (3) to characterize the relationship between soil N_2O emission and gross nitrification.

1 Materials and methods

1.1 Study sites

In 2004—2005, a field experiment was performed in a winter wheat farmland at Jiangsu Academy of Agricultural Sciences (32.0°N, 118.8°E). The site is located in southeast China. Rice/maize-wheat rotation represents the main crop production regime in local area. The annual average temperature of this site is 15.6℃ and annual rainfall averages about 1100 mm. The soil, classified as hydromorphic, has a cultivation horizon (0—20 cm) with an initial sand, silt, and clay content of approximately 29.1%, 16.0% and 54.9%, respectively.

1.2 Experimental design

The experimental design in this study is shown in Table 1, which includes five field treatments. To investigate the effect of plowing, the plots with different plowing depth (0, 12, and 25 cm coded as n, p and dp, respectively) were set up in the winter wheat farmland. And preceding crops of paddy rice and maize are abbreviated as codes of R and M, respectively. A cultivar of winter wheat named Yangmai #158 was sowed on 4 November 2004. The main growth stages of turning green, jointing, heading, and maturing, occurred on 10 February, 17 March, 9 April and 30 May 2005, respectively. Plants were harvested on 31 May. Nitrogen fertilizer as urea was applied at the rates of 54, 81, 45 kgN/hm² on 4 November 2004, 21 February and 3 March, 2005, respectively. Phosphorus as $Ca(H_2PO_4)_2$ and potassium as KCl were applied at the rates of 375 and 150 kg/hm² on 4 November, respectively.

Table 1 Design of field experiment

Treatment	Preceding crop type	Plowing depth, cm
Mp	Maize	12
Mn	Maize	0
Rn	Rice	0
Rdp	Rice	25
Rp	Rice	12

1.3 Taking soil samples

During rapidly growing stages of winter wheat, soil samples were taken on 15 March and 12 April 2005. The soil section planes (each with the depth, length and width of 40 cm × 40 cm × 40 cm) were set up in the field plots (each was 10 m × 10 m), and each treatment had three random section planes.

The BaPS system (Ingwersen *et al.*, 1999) that has a container holding a maximum of 7 soil cores was introduced to determine soil respiration, nitrification and denitrification. Circular stainless

corers (with the diameter of 7 cm) equipped with the BaPS were used for taking soil samples. In each section of plane, intact soil cores in the depth of 0—7 cm, 7—14 cm and 14—21 cm were respectively taken by using the corers. Two soil cores were collected in each depth. Thus the container holds 6 cores (2 cores × 3 section planes) for each depth, representing three random section planes as replicates. The design of sampling depth was due to the fact that CO_2 and N_2O are mainly produced in 0—5 cm (Buchmann and Ehleringer, 1998; Ball *et al.*, 1999; Song *et al.*, 2004) and their concentrations in soil profile decreased dramatically when the depth was below 8 cm (Tang *et al.*, 2003). In addition, the cultivated layer of soils is about 20 cm in this place. Three layers of 0—7 cm, 7—14 cm and 14—21 cm are practical. On each sampling date, a total of ninety soil cores (5 treatments × 3 section planes per treatment × 3 depths per section plane × 2 cores per depth) were sampled, sealed and then transported to laboratory within two hours for analysis.

1.4 Determination of soil respiration, nitrification and denitrification rates using BaPS system

Given a certain soil depth of a treatment on a sampling date, such as in the 0—7 cm of Mn (Table 1) on 15 March, the measurement group including the six replicated soil cores (2 cores × 3 section planes) was put into BaPS system for analysis (Ingwerson *et al.*, 1999; Liu *et al.*, 2005). And measuring samples for other depths and treatments followed such step.

BaPS is based on the measuring of the CO_2, O_2 and total gas balance of soil samples. In such an isothermal and gas tight system, soil respiration, nitrification and denitrification are the main biological processes responsible for gas pressure changes. Based on the gas balance and inverse balance approach, the rates of nitrification, denitrification and respiration can be calculated. The units of gross nitrification, denitrification and respiration rate are $\mu gN/(kg \cdot h)$, $\mu gN/(kg \cdot h)$ and $\mu gC/(kg \cdot h)$, respectively, which represent the amount of N or CO_2-C production per dry weight of soil (kg) per hour. Detailed description about BaPS and relevant measuring processes can be obtained in the reports of Ingwerson *et al.* (1999), Breuer *et al.* (2002), Butterbach-Bahl *et al.* (2002) and Müller *et al.* (2004). A series of previous investigations showed the good agreement in the result of soil respiration measured by BaPS and GC (gas chromatography) (Liu *et al.*, 2005), and in values of gross nitrification and denitrification rates based on the BaPS system and other methods (Ingwerson *et al.*, 1999), such as ¹⁵N-pool dilution technique (Kirkham and Bartholomew, 1954; Barraclough, 1995).

In order to determine the rates of N_2O emission from soil cores during nitrification and denitrification process, the initial and final concentrations of N_2O in

BaPS system were analyzed by GC. Based on the difference of N_2O concentrations, the weight of soil, temperature, measuring time, etc., the emission rate of N_2O can be calculated. The unit of N_2O emission is ng $N_2O-N/$ (kg·h), which refers to the amount of N_2O-N production per dry weight of soil (kg) per hour.

1.5 Determination of soil and biological characters

During sampling, temperature of all soil depths in each plot was determined by thermometers. Root biomass was gained by repeated sedimentation, decanting, and wet sieving in distilled water from soil cores. Roots were dried at 70℃ for the determination of biomass. Soil samples were dried at 105℃ to measure the volumetric water content. The pH values of soil samples were determined as follows: 10 g soil samples and 25 g distilled water were mixed and stirred for 30 min, and the pH value of the suspension was determined with a pH meter. The soil cores of each plot and depth on 15 March were prepared for organic matter determination.

2 Results

2.1 Variations of some soil and biological characters

Root biomass in surface (0—7 cm) soil was higher than that in deeper soil. An obvious phenomenon that with the increase of soil depth root biomass decreased can be found (Fig.1) on each sampling date. Soil temperature was different between the two sampling dates (15 March and 12 April), with the higher temperature on 12 April. Generally, the pH value and water content of upper layer soil were slightly lower than those of deeper soil. Soil pH ranged from 5.6 to 7.0 on 15 March and from 5.2 to 6.9 on 12 April, respectively. Volumetric soil water content in the treatments of Rn, Rdp and Rp was about 10 percent higher than that in the treatments of Mp and Mn. Soil moisture ranged from 28.0% to 36.6% on 15 March and from 21.2% to 29.7% on 12 April, respectively. Soil organic matter content was in the range of 11.2—19.9 g/kg, higher in the upper layer soil and lower in the deeper soil.
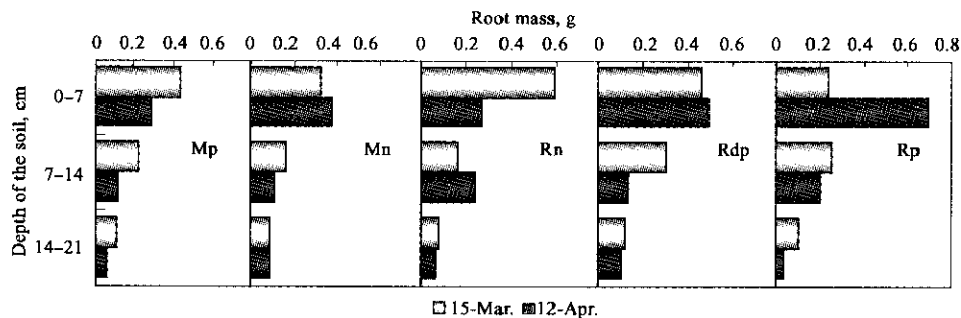

Fig.1 Root biomass in the soil of different depths

2.2 Soil respiration

Soil respiration rates were generally higher on 12 April than those on 15 March in all treatments (Fig.2).

The surface (0—7 cm) soil respiration contributed more than 70% to the summation of respiration in three depths (Fig.2).
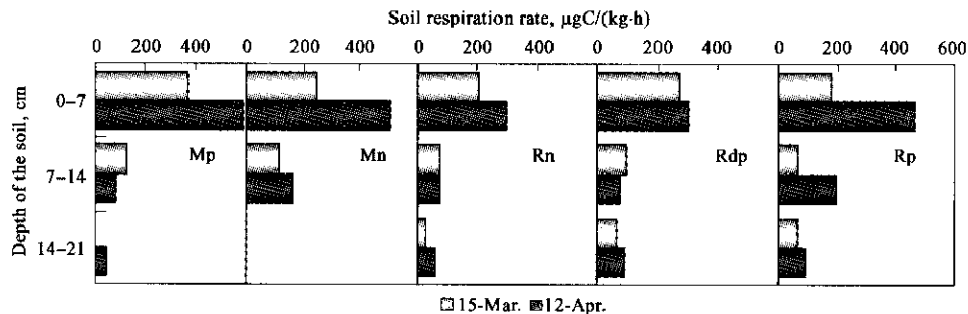

Fig. 2 Respiration rate of the soil in different depths
No data sets were accepted for the soil samples of 14—21 cm (Mp and Mn, 15 March), and 14—21 cm (Mn, 12 April), because the response signals were weaker than the detecting limitation of the BaPS

For plowing and deep plowing treatments of Rp and Rdp, the respiration rates for surface (0—7 cm) soil samples were about 20% higher than that from no-plowing treatment of Rn on average of the values of two sampling dates (Fig.2). And for plowing treatment of Mp, the respiration rate for surface soil

sample was also about 20% higher than that from no-plowing treatment of Mn. However, for the soil depth of 7—14 cm and 14—21 cm, the tendency that plowing practice enhanced respiration of deeper soil was not clear.

Both on 15 March and 12 April, for the

treatments under the same plowing practice, the soil respiration rates from Mp and Mn (preceding crop was maize) were a little higher than those from Rp and Rn (preceding crop was rice) in 0—7 cm, respectively (Fig.2).

Q_{10}, that represents the factor of respiration rate increased with an increment of $10^{\circ}C$ in soil temperature, varies from 1.4 to 2.9 (Raich and Schlesinger, 1992; Raich and Potter, 1995) with a mean value of 2 (Koizumi *et al.*, 1999). We accept the Q_{10} value of 2 that is very close to the previous report at this site (Zou *et al.*, 2004) to calibrate the respiration rates under the temperature on 15 March to those under the temperature on 12 April. This calibration was made to avoid the effect of temperature and characterize the effect of other presumed factors (e.g. root biomass) on respiration. Respiration rates in soil cores taken from the five treatments both on 15 March and 12 April exhibited a strong dependency on changes in root biomass. Exponential regression analysis (Fig.3) confirmed the close relationship between soil respiration rate and root biomass, with $P<0.001$. It could be observed that when root biomass extrapolated to zero, respiration rate was about 70.6 µgC/(kg·h) (Fig.3), which was very low as compared with the respiration rate in soil cores involving root biomass.

No statistical significant dependence of soil respiration rate was observed on pH, organic matter content or volumetric water content from our available
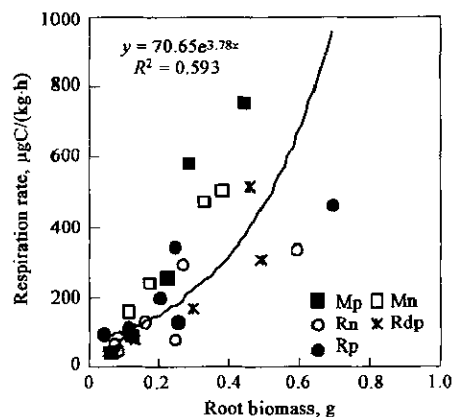

Fig.3 Relationship between soil respiration rate and root biomass

data. A possible explanation would be attributed to the fact that the values of pH, volumetric water content and organic matter content varied in a relative narrow range among all measurements, although the difference among three depths was significant for some treatments. Moreover, organic matter content controls large-scale variation of soil respiration (Raich and Potter, 1995) rather than its site-specific variation.

2.3 Soil nitrification and denitrification

Gross nitrification rates for the treatments of Rn, Rdp and Rp, where rice was planted in previous season, were generally higher on 12 April than on 15 March. For the treatments of Mp and Mn, where the preceding crop was maize, no such clear tendencies could be observed (Fig. 4).
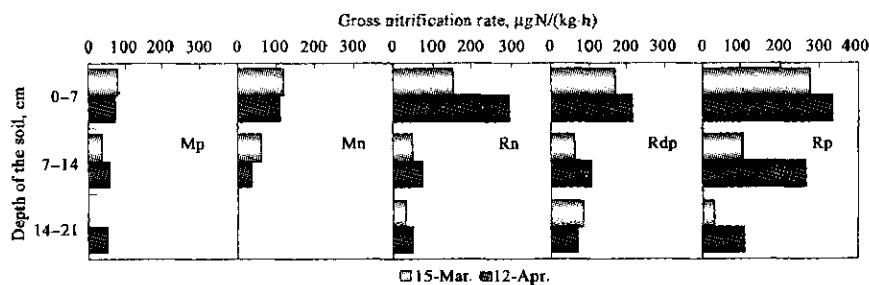

Fig.4 Gross nitrification rate of the soil in different depths
No data sets were accepted for the soil samples of 14—21 cm (Mp and Mn, 15 March), and 14—21 cm (Mn, 12 April),
because the response signals were weaker than the detecting limitation of the BaPS

Gross nitrification rates of surface (0—7 cm) soil samples were higher than those in deeper soil (Fig.4). Surface (0—7 cm) soil contributed more than 60% to the summation of nitrification rates in three depths. This tendency was similar with the vertical variation of respiration.

Generally, plowing practice in Rdp and Rp increased the gross nitrification rates by about 15 and 70 µgN/(kg·h), respectively, compared with the no-plowing treatment of Rn on average of the values in three depths and on two sampling dates. However, the gross nitrification rate was about 12 µgN/(kg·h) higher in no-plowing treatment of Mn than that in Mp. It can also be observed that nitrification rates in Rn,

Rdp and Rp were about two times of that in Mp and Mn on average of the values in three soil depths and on two sampling dates.

We analyzed the possible soil and biological factors affecting the variation of gross nitrification rate. As no clear temperature dependency of nitrification was reported so far, unlike soil respiration, we plotted the raw nitrification rates for regression. When three datasets that are far scattered from all the samples were excluded, the nitrification rate correlates the root mass well (Fig.5). The relationship between the variables could be described by an exponential function ($P<0.001$). A base nitrification rate with approximate 41.5 µgN/(kg·h)

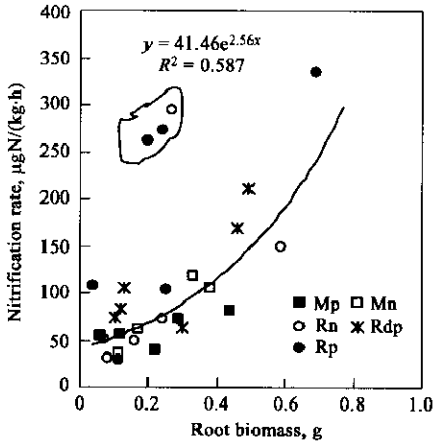can be obtained when the root biomass was extrapolated to zero.

Fig.5 Relationship between gross nitrification rate and root biomass

Basing on our available data, the phenomenon that denitrification were generally higher on 12 April than that on 15 March can be found (Fig.6). Unlike soil respiration and nitrification, the tendency that the denitrification of surface (0—7 cm) soil was higher than that in deeper soil tended to be not clear. It can also be observed that denitrification was higher in the treatments of Rn and Rp, than that in the treatments of Mp and Mn, respectively, especially for the depth of 0—7 cm (Fig.6).

2.4 The role of nitrification in N_2O emission

Previous investigation reported the main process for N_2O production was nitrification and denitrification. In order to investigate the relationship between nitrification and denitrification and address the percentage of these two processes in nitrogen transformation, a regression analysis was performed.
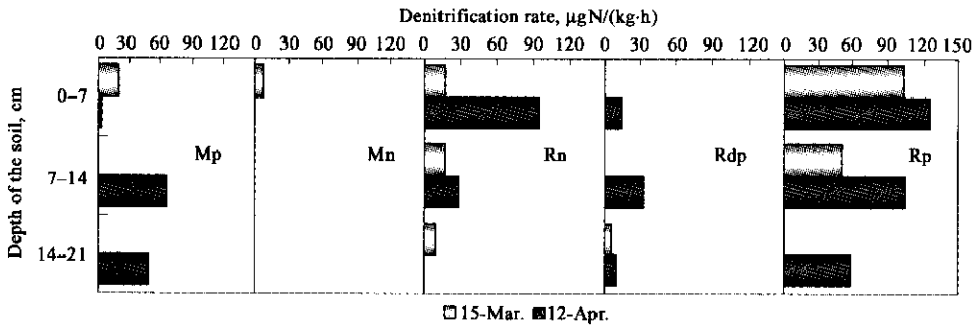

Fig.6 Denitrification rate of the soil in different depths

No data sets were accepted for the soil samples of 7—14 cm (Mp, 15 March), 14—21 cm (Mp and Mn, 15 March), and 14—21 cm (Mn, 12 April), because the response signals were weaker than the detecting limitation of the BaPS. The values of denitrification for the soil samples of 0—7 cm (Mn, 12 April), 7—14 cm (Mn, 15 March and 12 April), 14—21 cm (Rn, 12 April), 0—7 cm (Rdp, 15 March), 7—14 cm (Rdp, 15 March) and 14—21 cm (Rp, 15 March) were 0

Fig.7 reveals that nitrification acted as a key role in this nitrogen transformation process. The linear regression function showed the significant relationship ($P<0.0001$) between nitrification and the summation
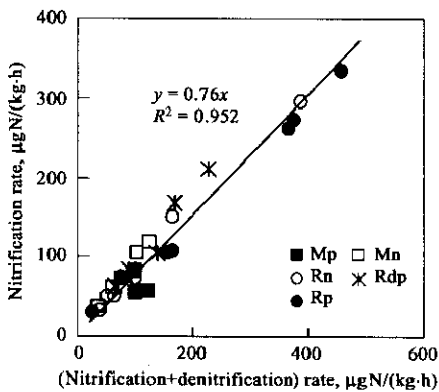

Fig.7 Relationship between nitrification rate and (nitrification+denitrification) rate

of nitrification and denitrification. Nitrification rate averaged about four times of denitrification rate.

A correlation analysis was also conducted to determine whether a link between the rates of N_2O

production as measured by GC (gas chromatography) and gross nitrification rates as measured by BaPS system existed. Before conducting regression analysis, all data were natural-logarithmically transformed to make the data normalized. Fig.8 showed a significant positive correlation between normalized N_2O emission and normalized nitrification rate ($P=0.001$), when pooling all available data for one regression analysis. It is obvious that the adjusted squared multiple R is 0.421, which indicates that more than 40 percent in
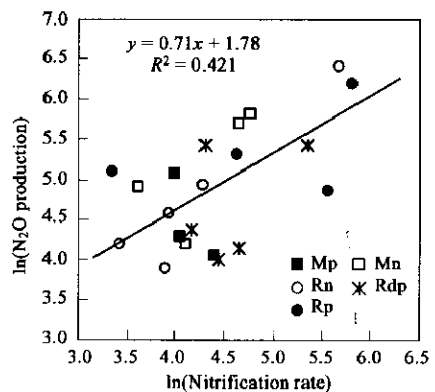

Fig.8 Relationship between N_2O production and gross nitrification rate

the variation of N_2O emission can be explained by nitrification process.

3 Discussion

3.1 Effect of plowing practice on respiration, nitrification and denitrification in soil profile

Plowing practice, compared with reduced tillage method, often decreases soil organic matter (Gebhart *et al.*, 1994) and increases the flux of CO_2 from soil (Reicosky and Lindstrom, 1993; Alvarez *et al.*, 1995) through enhanced biological oxidation of soil carbon. Short-term measured losses of soil carbon due to plowing are considerable (Alvarez *et al.*, 2001). And long-term no-tillage has been proposed to attenuate negative trends in soil organic matter contents and to reduce soil respiration (Lal and Kimble, 1997).

Our data confirmed the assumption that reducing plowing intensity may lead to decrease in respiration of soil profile (Fig.2) and increase in soil carbon (data were not shown), given the same cropping practice in preceding crop season before planting wheat. This phenomenon was more evident in surface soil (0—7 cm). Plowing accelerates organic carbon oxidation to CO_2 by improving soil aeration, by increasing contact between soil and crop residues, and by exposing aggregate-protected organic matter to microbial attack (Beare *et al.*, 1994; Curtin *et al.*, 2000). Given the amount of amended crop residues unchanged in the future, widespread adoption of conservation tillage (e. g. no-plowing) in winter wheat farmland may result in net increase in carbon sequestration in agricultural lands, reversing the decline caused by intensive tillage practices used for decades (Kern and Johnson, 1993).

Nitrification was a little higher in Mn than that in Mp. However, the generally higher nitrification rate was found in the plowing treatments of Rp and Rdp than that in the no-plowing treatment of Rn. The reason was not clear so far and necessary to be further investigated. Plowing practice (Mp and Rp) increased soil denitrification rate, compared with the treatments of Mn and Rn (Fig.6). However, deep plowing maybe significantly increased the oxidation of soil and decreased the rate of denitrification in Rdp treatment, compared with Rp and Rn treatments.

3.2 Effect of preceding cropping practice on respiration, nitrification and denitrification in soil profile

As depicted above, the difference of preceding crop-planting regimes resulted in the difference of some soil variables, soil water content etc. Volumetric soil water content in the treatments of Rn, Rdp and Rp was about 10 percent higher than that in the treatments of Mp and Mn, which may influence the variation of soil respiration, nitrification and denitrification.

The preceding cropping practice influenced soil respiration in the wheat-growing season, given the same plowing method. Soil respiration in the treatments of Mp (preceding crop was maize) was a little higher than those in the treatments of Rp (preceding crop was rice) (Fig.2), especially in surface soil of 0—7 cm. And the similar phenomenon was found in Mn and Rn. Higher water content in Rp and Rn may restrain the activity of soil microorganisms and thereby result in the decrease of soil respiration. Moreover, C_4 crop of maize will input more carbon (root) into soil (Monson *et al.*, 1986), which facilitates the soil respiration in the following wheat season.

The effect of preceding cropping practice on soil nitrification in the wheat-growing season was pronounced. It is obvious that the gross nitrification rates in the treatments of Rn, Rdp and Rp were higher than those in Mn and Mp for all soil depths. The possible reason is as follows. Firstly, higher water content enhanced soil nitrification rate (Breuer *et al.*, 2002). Secondly, the values of pH in Mp and Mn were lower than those in Rn, Rdp and Rp, with about 7 for the latter. The neutral condition may be favorable for nitrification. A further investigation indicates that the denitrification rate in Rn and Rp was higher than that in Mn and Mp, respectively, which tends to be also attributed to the facilitation of higher water content on denitrification (Firestone and Davidson, 1989).

3.3 The role of root in soil respiration and nitrification

Soil respiration is derived from root respiration, rhizosphere respiration and heterotrophic respiration of soil organic matter by microorganisms. Root plays a key role in soil respiration. About 10%—15% of belowground allocated carbon is respired by roots, and about 15%—25% of belowground allocated carbon is exuded from the roots into the soil (Kuzyakov *et al.*, 2000, 2002). Rhizosphere microorganisms utilize these substances as easily available C and energy sources for fast growth and reproduction. Roots represent a dynamic portion of belowground carbon, nutrient capital, and a significant part of net primary production in ecosystems (Tufekcioglu *et al.*, 1999). For our study, Fig.4 also showed the significant effect of wheat root on soil respiration, which was consistent with previous investigations.

Nitrification is a nitrogen transformation process, in which microorganisms consume carbon and nitrogen nutrition. Our result showed that the nitrification rate correlates the root mass well (Fig.5). It suggests that the process of nitrification is affected by rhizodeposition such as root secretion. Root is an important factor driving soil nitrification, and root growth maybe enhanced gross nitrification.

3.4 Nitrification and N_2O emission

Nitrification dominated the nitrogen transformation of nitrification and denitrification (Fig.7), which was the main process of producing N_2O. A

close correlation between N_2O emission and gross nitrification rate existed (Fig.8). Our data strongly suggest that, based on the BaPS result, nitrification influences the N_2O emission from soil significantly. There are grounds for the assumption that nitrification is a key process of N_2O production in wheat farmland soil, where urea was applied as nitrogen fertilizer. Firstly, ammonium is transformed to nitrate through nitrification, and N_2O was produced simultaneously through this process. Secondly, nitrification leads to the accumulation of substrate (nitrate) utilized for denitrification, which is the other important process of producing N_2O (Firestone and Davidson, 1989).

4 Conclusions

Soil respiration and gross nitrification in wheat field generally decreased with the increase of soil depth, while such tendency was not pronounced for denitrification. Plowing practice enhanced soil respiration in surface soil of 0—7 cm but had no significant effect on gross nitrification and denitrification. Cropping system affected soil gross nitrification. Gross nitrification in the plot with preceding crop of paddy rice was higher than that with maize. Nitrification rather than denitrification played a key role in nitrogen transformation and N_2O production in wheat field where urea was used as nitrogen fertilizer. The variations of soil respiration and gross nitrification rates can be quantitatively described by root mass.

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