# Denitrification and $N_2O$ production in subsoil in wheat-maize rotation field in North China Plain

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## Abstract

Excessive application of fertilizer nitrogen (N) in crop production systems in North China Plain has resulted in the accumulation of nitrate ($NO_3^-$) in subsoil and groundwater. Denitrification is a possible pathway for removal of accumulated nitrate in subsoil, but is often neglected and/or regarded as unimportant because of the difficulty of measuring denitrification in the subsoil.

The aim of the study reported here is to quantify the seasonal variations in denitrification activity in a 190cm deep soil profiled under a wheat-maize double cropping system as a function of N fertilizer application. The study was conducted at the Luancheng agro-ecosystem experimental station, CAS, in 2009-2010. The N fertilizer treatments included 0 (CK), 450 (N1) and 750 (N2) kg N $ha^{-1}$ $year^{-1}$, in triplicate. Soil cores were taken by the Geoprobe machine and incubated using acetylene inhibition technique.

Denitrification rates in the 1.9 m deep soil profile showed the commonly observed responses to N fertilizer application, irrigation and rainfall, leading to strong a temporal variability. Nitrogen losses through denitrification were significantly higher (a factor of 2.0 to 2.7) in the maize growing season than in the wheat season, likely because of the more wet and warm weather conditions in the maize growing season. On average, only 26% of total denitrification activity in the 190 cm deep soil profiled occurred in the top soil (0-15 cm); 33% occurred in the 15 to 90 cm soil layer and 41% in the 90 to 190 cm soil layer in CK. The contribution of the top soil (0-15 cm) increased to ~ 45% and that of the subsoil (90-190 cm) decreased to ~ 28%, when the N fertilizer application increased to 750 kg per ha per year. The total amount of N lost via denitrification was 6, 15 and 28 kg N $ha^{-1}yr^{-1}$ in the CK, N1 and N2 treatments, respectively.

In conclusion, the subsoil (15-190 cm) was a large contributor to N losses via denitrification, although the total N losses via denitrification were only in the range of 1 to 6% of total N fertilizer application. Further studies should try to understand the mechanism and controlling factors of denitrification in the low-carbon subsoil.

## Key Words

denitrification, nitrous oxide emission, nitrogen loss, subsoil

## Introduction

Denitrification is an important pathway to remove the nitrate accumulated in subsoil. However, many studies of denitrfication only focus on surface soils, whereas the impacts of management practice and season on denitrification in subsoil and its function to remove nitrate accumulated beneath root zone are largely neglected. Deep soils have a high potential to store nitrate and carbon; therefore any management driven stimulation or repression of microorganisms in subsoil could impact denitrification and its products composition. If the denitrifying process predominantly produces $N_2$ rather than $N_2O$ (one of important greenhouse gases), the denitrification will be benefit to groundwater and atmospheric environment, such as: it will decrease nitrate accumulation in deep soil and repress nitrate leaching to groundwater; meanwhile, it will not induce $N_2O$ accumulation in soil profile and will not cause $N_2O$ emission to atmosphere.

The North China Plain is an important agricultural production area that provides food to hundreds of millions of people. The dominant cropping system is a wheat-maize double-cropping system. The average annual N fertilizer application rate was about 500 kg $ha^{-1}$ $yr^{-1}$ in the last decade. The high N fertilizer application rate contributes to high crop yields, but inevitably also to high $N_2O$ emissions (Qin et al., 2012) and the accumulation of nitrate in subsoil and groundwater (Li et al., 2007). The aim of this study was to understand whether soil management affects (1) denitrfication and $N_2O$ formation in the topsoil (0-15 cm), rooted zone beneath the plough layer (15-90 cm), and in the unrooted zone (90-190 cm); (2) contribution of $N_2O$ produced beneath the plough layer to the $N_2O$ emission.

## Methods

### *Experimental Field and Treatments*

This study was conducted at the Luancheng Agro-ecosystem Experimental station (37.89°N, 114.67°E, at 50 m above sea level) of the Chinese Academy of Sciences. The station is at the piedmont of the Taihang Mountains, in the North China Plain. The area has dry and cold winters and warm semi-humid summers, with a mean annual rainfall of 530 mm. 70% of which is in the period July to September. Annual average temperature is 12.5℃. The soil type of the area is predominantly silt loam (light meadow cinnamon soil). Dominant land use in this area is a continuous wheat-maize crop rotation, with the straw returned to the soil, at least for the last 20 years. Both wheat and maize are irrigated, mainly via flood irrigation using groundwater.

The factorial field experiment with a continuous wheat-maize crop rotation and with a randomised block design and three replications started in 2002. There were three fertilizer treatments, as follows: no-fertilizer control (CK), 450 (N1) and 750 kg N $ha^{-1}$ $year^{-1}$ (N2) as urea. The annual N application was equally (50% : 50%) split over the wheat and summer maize. The plots (7.5 ×14 m each) were managed under conventional tillage and cultivated in autumn (early in October) to a depth of 20 cm with a rotovator, and seeded to winter wheat with a seeder. Winter wheat was harvested in the beginning of June and summer maize was seeded by a seeder after the winter wheat harvest. Fertilizers were split applied. The basal application was applied in autumn just before the wheat growing season, included 25% of target N rate, 65 kg P $ha^{-1}$ as calcium superphosphate and 75 kg K $ha^{-1}$ as potassium sulfate. Surface applied fertilizers were immediately incorporated with a rotovator to a depth of 20 cm before the winter wheat was sown in rows at 15 cm distance. The supplementary N fertilizer application (25% of target rate) was broadcasted on the surface at the jointing stage in March, just before flood irrigation. 50% of target fertilizer N was applied all at once at the bell stage in July, just before flood irrigation. Denitrification, $N_2O$ formation and emission measurements in the field experiments were conducted from October 2008 to September 2010, including two winter wheat seasons and two summer maize seasons.

### *In-situ measurements of denitrification, $N_2O$ production rates and emissions*

For assessing in-situ denitrification, 8 intact soil cores (5 cm diameter) were taken down to 200 cm depth from each plot per time. The soil cores (100cm length) were collected by Geoprobe machine, which collects intact soil cores in a Teflon tube. The soil cores were cut into 25 cm sections, apart from the uppermost 15 cm layer. The sections of the 8 soil cores were per layer equally divided over two incubation cylinders. One of the cylinders received sufficient $C_2H_2$ to obtain a partial pressure of 10 kPa, whereas the second cylinder remained untreated, as described by Chen et al (1996). After 24 h of incubation, gas samples (12 ml) were taken from the head space with a gas-tight syringe through a three way gas-tight valve and transferred to 10 ml vacuum tubes and then analysed for $N_2O$ concentration by gas chromatography. We assume that the $N_2O$ produced in the presence of $C_2H_2$ is mainly from denitrification, and that $N_2O$ produced in the absence of $C_2H_2$ represents $N_2O$ formation rates from (de)nitrification processes. Cumulative denitrification and $N_2O$-N formation were estimated for each plot by linearly interpolating data points and integrating the curve by Simpson's rule . The sampling schedules were adjusted to focus on periods when denitrification was most probable, that is, after irrigation or rainfall and fertiliser N application. Following N application and irrigation or heavy rainfall, denitrification rate and $N_2O$ production rate were measured once a week for a period of 2 weeks, then once in about 2 week for a month, and thereafter once in a month. Simultaneously, emissions of $N_2O$ were determined using the techniques of static chamber (Horvath et al., 2006). A gas chromatographs (Agilent 6820 series), fitted with an electron capture detector (ECD) and a 30 m × 0.5 mm Poropak Q column, was used for determination of $N_2O$. 99.999% $N_2$ served as the carrier gas (flow rate 20 ml min-1). Oven, injector and detector temperatures were 70, 45, and 300°C, respectively.

### *Measurements of crop yield and data analyses*

To determine grain yield of wheat and maize at maturity, the crops were cut at ground level by hand harvesting fifteen central rows for wheat and seven rows for maize. Grain was air dried and weighed. The yields of grain were calculated from the weights and the corresponding harvest areas. The data were subjected to analysis of variance (ANOVA) by the SPSS procedure (SPSS 13.0 for windows). Least significant difference (LSD) at P≤0.05 was used to determine differences among mean results from treatment.

## Results

### *Maize and wheat yields*

Mean winter wheat and summer maize yields are presented in Table 1. Both wheat and maize yields increased with increasing N fertilizer applications in both years， with the exception of wheat yield in 2010 that wheat yield was slightly higher in N1 than in N2. Significant difference occurred only between control and fertilized treatments. On average, yields were highest in the N2 (750 kg Nha$^{-1}$ y$^{-1}$) treatment, but differences between the N1 (450 kg Nha$^{-1}$ y$^{-1}$) and N2 treatments in grain yield were not always statistical significant. Fertilizer N application increased total annual grain yield from 5900 kg per hectare in the control to almost 10000 kg per hectare.

**Table 1. Mean grain yields of winter wheat and summer maize from Oct. 2008 to Sept. 2010, in kg ha$^{-1}$**

| Treatments | Winter wheat | | Summer maize | |
|---|---|---|---|---|
| | 2009 | 2010 | 2009 | 2010 |
| CK | 2186±214b | 1612±277b | 3035±201c | 5076±313b |
| N1 | 5798±490a | 3764±60a | 5679±284b | 7137±210a |
| N2 | 6382±381a | 3691±213a | 6682±338a | 7389±710a |

*: yield±SE (standard error). Values within the same column followed by the same letter are not significantly different as determined by ANOVA and Fisher protected LSD (*P<0.05*)

### *Denitrification and $N_2O$ formation rates in soil profile*

Strong spatial and temporal variability of denitrification and $N_2O$ formation rates in the 1.9 m deep soil profile were observed and showed the commonly observed responses to N fertilizer application, irrigation and rainfall. Denitrification rates and $N_2O$ formation rates at various soil depths exhibited the same seasonal pattern whatever the treatments, respectively. Both rates of denitrification and $N_2O$ formation observed in maize season were significantly higher than those in wheat season, likely because of the more wet and warm weather conditions in the maize growing season. The supplementary fertilizer N application in April and July both of 2009 and 2010 induced short bursts of denitrification and $N_2O$ formation throughout the soil profile. The peak height increased with N fertilizer application rate. There were 2-3days lags between peaks occurrence and fertilization or irrigation events. In the same N application treatment, maximum peaks were recorded at 0-15cm (Fig. 1) and the rates of denitrification and $N_2O$ formation decreased with depth downward (Fig.2).

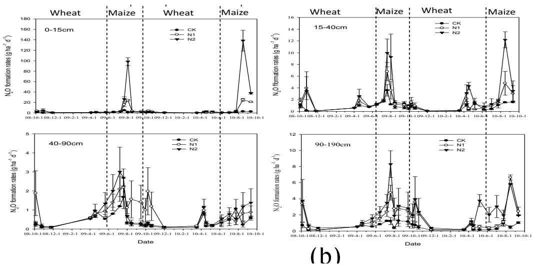

Figure 1. The seasonal variation of (a) denitrification rates (n = 3) and (b) $N_2O$ formation at various soil depths in wheat-maize double cropping rotation receiving 0(CK), 450(N1), 750(N2) kg of N per hectare per year from Oct. 2008 to Sept. 2010. Bars in figures indicate 1 standard deviation.

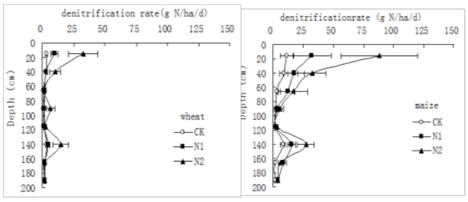

Fig 2. Mean (± standard error, n=13 for two wheat seasons, n=12 for two maize seasons) of denitrification rates in the soil profile during the wheat and maize growing seasons from Oct. 2008 to Sept. 2010, as function of N application rate. Bars in figures indicate 1 standard deviation.

### *Total denitrification and $N_2O$ production in soil profile*

Nitrogen application is a main trigger for denitrification and $N_2O$ production. The total amount of N lost via denitrification was 6.3, 14.6 and 28.0 kg N ha$^{-1}$yr$^{-1}$ in the CK, N1 and N2 treatments (Table 1), respectively, while the total $N_2O$ production was 1.7, 4 .0 and 6.5 kg N ha$^{-1}$yr$^{-1}$ corresponding to the treatments of CK, N1 and N2 (Table 2), respectively. Nitrogen losses through denitrification were significantly higher (a factor of

2.0 to 2.7) in the maize growing season than in the wheat season, likely because of the more wet and warm weather conditions in the maize growing season. The seasonal patterns of $N_2O$ production in 1.9 m soil profile were similar to those of denitrification. The total production of $N_2O$ in 0-190cm soil was 2~8 kg N ha$^{-1}$ in wheat and 4~21 kg N ha$^{-1}$ in maize (Table 2). The effects of N application on denitrification /$N_2O$ production were different between different soil layers. Under no N application, annual denitrification in the top soil (0-15 cm) was 1.6 kg N ha$^{-1}$, only 26% of total denitrification activity in the 190 cm deep soil profiled; 2.1 kg N ha$^{-1}$ observed in the 15 to 90 cm soil layer and 2.6 kg N ha$^{-1}$ observed in the 90 to 190 cm soil layer, accounting for 33% and 41% of total denitrification activity in the 190 cm deep soil profiled, respectively. When the N fertilizer application increased to 750 kg per ha per year, the annual denitrification increased to 12.7, 7.6 and 7.8 kg N ha$^{-1}$ in 0-15, 15-90, 90-190 cm soil layers, respectively. The contribution of the top soil (0-15 cm) increased to ~ 45% and that of the subsoil (90-190 cm) decreased to ~ 28% (Table 1) in N2 treatment.

**Table 1. Total denitrification at various soil depths in wheat-maize double cropping rotation from Oct. 2008 to Sept. 2010, averages of two wheat seasons and two maize seasons, respectively.**

| Depth (cm) | Wheat season （kg N/ha） | | | Maize season （kg N/ha） | | | Annual （kg N/ha） | | |
|---|---|---|---|---|---|---|---|---|---|
| | CK | N1 | N2 | CK | N1 | N2 | CK | N1 | N2 |
| 0-15cm | 0.5 | 1.5 | 3.5 | 1.1 | 3.3 | 9.1 | 1.6 | 4.8 | 12.7 |
| 15-90cm | 0.6 | 0.7 | 2.0 | 1.5 | 3.6 | 5.6 | 2.1 | 4.4 | 7.6 |
| 90-190cm | 1.0 | 1.8 | 2.5 | 1.6 | 3.7 | 5.3 | 2.6 | 5.4 | 7.8 |
| 0-190cm | 2.1$^c$ | 4.0$^b$ | 7.9 $^a$ | 4.2$^c$ | 10.6$^b$ | 20.1$^a$ | 6.3 $^c$ | 14.6 $^b$ | 28.0 $^a$ |
| Percentage of applied N % | | 0.8 | 1.6 | | 2.8 | 4.2 | | 3.7 | 5.8 |

*:Values within the same line followed by the same letter are not significantly different as determined by ANOVA and Fisher protected LSD (*$P<0.05$*)

**Table 2. Total amount of $N_2O$ formation at various soil depths and the contribution of subsoil (15cm-190cm) to the total $N_2O$ emission in wheat-maize double cropping rotation from Oct. 2008 to Sept. 2010, averages of two wheat seasons and two maize seasons, respectively.**

| | Wheat (kg N/ha） | | | Maize (kg N/ha） | | | Annual (kg N/ha） | | |
|---|---|---|---|---|---|---|---|---|---|
| | CK | N450 | N750 | CK | N450 | N750 | CK | N450 | N750 |
| $N_2O$ emissions (static chamber) | 0.3 | 0.9 | 1.5 | 0.6 | 1.4 | 4 | 0.9 | 2.3 | 5.5 |
| $N_2O$ production in 0-15cm | 0.1 | 0.2 | 0.4 | 0.2 | 1.1 | 2.7 | 0.3 | 1.3 | 3.1 |
| $N_2O$ production in 15-90cm | 0.3 | 0.4 | 0.4 | 0.3 | 0.5 | 0.7 | 0.6 | 0.9 | 1.1 |
| $N_2O$ production in 90-190cm | 0.4 | 0.6 | 0.8 | 0.4 | 1.2 | 1.5 | 0.7 | 1.7 | 2.3 |
| $N_2O$ production in 0-190cm | 0.8 $^c$ | 1.2 $^b$ | 1.6 $^a$ | 0.9 $^c$ | 2.8 $^b$ | 4.9 $^a$ | 1.7 $^c$ | 4.0 $^b$ | 6.5 $^a$ |
| $N_2O$ emissions from subsoils beneath 15cm | 0.2 | 0.7 | 1.1 | 0.4 | 0.3 | 1.3 | 0.6 | 1.0 | 2.4 |

*:Values within the same line followed by the same letter are not significantly different as determined by ANOVA and Fisher protected LSD (*$P<0.05$*)

### *Contribution of subsoil to the total $N_2O$ emission*

We assume that all of the $N_2O$ produced in top soil (0-15cm) can emit to the atmosphere and the difference value between $N_2O$ emission measured with static chamber method and $N_2O$ produced in 0-15cm soil measured with intact soil core incubation method represents the contribution of subsoil to the total $N_2O$ emission. The result showed that 44%~67% of the annual $N_2O$ emission came from the $N_2O$ diffusion beneath the plough layer in wheat-maize double cropping rotation in North China Plain (Table 2).

## Conclusion

Denitrification rates in the 1.9 m deep soil profile were on average rather low and showed the commonly observed responses to N fertilizer application, irrigation and rainfall, leading to strong a temporal variability. Nitrogen losses through denitrification were significantly higher (a factor of 2.0 to 2.7) in the maize growing season than in the wheat season, likely because of the more wet and warm weather conditions in the maize growing season.

Denitrification rates and $N_2O$ production rates were higher in the surface soil layers than in the subsoil. However, denitrification under root zone cannot be neglected. On average, about 41%, 37% and 28% of total denitrification activity in the 190 cm deep soil profiled occurred in the unrooted zone (90-190 cm) corresponding to CK, N1 and N2 treatments, respectively. Hence, the subsoil (90-190 cm) was a large contributor to N losses via denitrification, although the total N losses via denitrification were only in the range of 1 to 6% of total N fertilizer application. Our results do not exclude the possibility that denitrification activity occurs below a depth of 190 cm. Further studies should try to understand the mechanism and controlling factors of denitrification in the subsoil.

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