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Soil Enzyme Activities and N_2O Emissions under Different Land Management Conditions

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Nitrous oxide (N_2O) is a main greenhouse gas, which is not only a strong absorber of infrared radiation, but also contributes to the destruction of stratospheric ozone by opto-chemical reaction (Rodhe, 1990). Farmland ecosystems are a main source for the emissions of N_2O, amounting to 60-70% of the anthropological N_2O emissions (IPCC, 1992). N_2O emissions from soil are mainly a consequence of microbiological processes, i.e. nitrification and de-nitrification. These processes are largely controlled by a number of soil factors, such as soil moisture, nitrate content, temperature and carbon availability, which irregularly and substantially vary in temporal and space and also interact with each other (Tiedje, 1988). Changes of single factor are hard to reflect variance of soil N_2O emissions. Soil enzyme activities have been reported as critical indicators of soil N_2O emissions, for examples, Bai Hong-ying et al. (2002) thought that soil urease activities could reflect N_2O emission. Shi Yi (1999) reported that soil denitrifying enzyme activities could distinguish the pathway of N_2O emission in dry land. However, at present, there are no ideal models of soil N_2O emissions that can be able to predict N_2O emissions under different land management conditions (Mosier et al, 1996; Henault et al, 1998). Thus, the measurement of in-situ N_2O emissions and further investigation on the relationship between N_2O emissions and soil enzyme activities under different land management conditions are still very necessary.

The objectives of this study are to find out the effects of different land management conditions on N_2O emissions and soil nitrate reductase (NR), nitrite reductase (NiR) and acid phosphatase activities, and further to investigate the relationships between soil N_2O emissions and the activities of these enzymes under different land management conditions in the dry land of northwest China.

MATERIALS AND METHODS

Experiments were carried out in the 1st farm station of Northwestern Sci-tech University of Agriculture & Forestry (NWSUAF) in the south of the Loess Plateau in China, located at 108°38'N, 35°42'E. The site is at an elevation of 320m to 600m,

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with an annual mean temperature of 11.0 to 14.0℃ and an annual mean precipitation of 415mm to 630mm. The accumulated temperature above 10℃ in the zone ranges from 3500 to 4000℃ with an annual mean evaporation of 800 to 900mm. Based on Chinese classification system, the dominant soil is classified as Earth-Cumuli-Qrthic Anthrosols (B2.4) having 490 g kg^{-1} physical clay, 210 g kg^{-1} clay analyzed by the pipette method. The soil has 11.5 g kg^{-1} organic matter, 92.9 g kg^{-1} $CaCO_3$ and a pH (1:1) of 7.9. Water Holding Capacity (WHC) was determined on 50g soil dry weight after soaking and draining for 24 hours. The water-filled pore space (WFPS) was 65.8%. WFPS was calculated using the following equations.

$$WFPS = \frac{\theta_v}{\phi} \qquad (1)$$

$$\theta_v = \theta_g \cdot \frac{D_b}{D_w} \qquad (2)$$

$$\phi = 1 - \frac{D_b}{D_p} \qquad (3)$$

Where: θ_v is soil volumetric moisture (%), ϕ is soil total porosity, θ_g is soil gravimetric moisture (%), D_b is soil bulk density (g·cm^{-3}), D_w is water density (g·cm^{-3}), and D_p is soil particle density (g cm^{-3}). $D_p = 2.65$ g·cm^{-3}.

Experiments treatments consisted of: 1) Fallow (F), 2) Fallow with mulching (FM), 3) Wheat-planted (W), and 4) Wheat-planted with mulching (WM). These land management conditions are very common in this area except the FM treatment. They were randomized for each of 3 blocks. Every treatment had an area of 24 m^2. The amount of chemical fertilizer inputs was 150 kg N ha^{-1} and 40 kg P ha^{-1} as the base fertilizer. Peasants in this area largely used the amount and method. The two kinds of fertilizers were carefully sprayed on the plots and ploughed into the topsoil. The clear plastic film was applied after harrowing and leveling the soil, and winter wheat was dibbed. The trial variety of winter wheat was Xiaoyan 503. No artificial irrigation was applied during the whole growth period. Plastic films were not removed before harvest in accordance to the practice of farmers in these areas.

Ten measurements were carried out over two successive growing seasons of winter wheat. The first sampling were taken on Mar 10^{th}, Apr 16^{th}, May 12^{th} and Jun 1^{st} between March 2001 and June 2001 in the order of tiller stage, elongation stage, flowering stage and ripening stage. The second sampling took place on Oct 23^{rd}, Mar 10^{th}, Mar 30^{th}, Apr 25^{th}, May 27^{th} and Jun17^{th} between October 2001 and June 2002 in the order of seeding stage, tiller stage, ante- and post-elongation stages, flowering stage and ripening stage. All sampling positions were treated the same. Sampling of N_2O was done using closed chamber technique (diam.31.0 cm, height 54.0 cm) fitted with rubber septa for gas sampling. Chambers were inserted 4-8 cm depth into the soil depending on the structure of the soil. For mulching treatments, plastic film was uncovered for about 3 to 5 minutes before sampling based on the air temperature. That was expected to remove the antecedent N_2O accumulated between mulch and topsoil due to the long time covering. Gas was sampled within 10 and 60 minutes after the chambers were sealed. On each treatment, 5 parallel chambers were sampled using injectors. Every measurement took place in a fixed time (between 9 a.m. and 11 a.m.) and at a similar site, with the removal of above ground crop parts to reduce the influence of N_2O emissions from the wheat. Nitrous oxide was measured immediately after sampling on a gas chromatograph (Varian GC-3800) equipped with a Porapak Q column (60℃). EC detector (^{63}Ni) run at 350℃.The injector temperature was 100℃. High purity Nitrogen with a flow velocity of 60 ml min^{-1} was used as a carrier gas. The N_2O standard gas coming from Beijing was 9.6μl l^{-1}, and was calibrated with 0.3282μl l^{-1} gas produced in Sweden. Samples were diluted with 99.999% nitrogen. Computer program had been set up to push out the vapor by a valve device before entering ECD. Variance is less than 5%.

Soil samples for analysis of soil enzymes activities were collected from 0-20 cm depth, air dried, and sieved (1 mm mesh size) with removal of all large roots. Then, samples were analyzed for soil enzymes activities. NR and NiR activities were determined as reported by Han jian-gang (2002b), Acid phosphatase activities was determined as described by Guan Song men (1986).

Variables were analyzed by two-way ANOVA (analysis of variance), and a PLSD's (protected least significant difference) test was used to compare means. Simple linear regressions were performed to check the relationship between soil N_2O emissions and soil enzymes.

RESULTS AND DISCUSSION

Coefficients of variation of N_2O emissions rates often exceed 100%. Temporal variations of N_2O emissions rates have been demonstrated on diurnal and seasonal time scales. Diurnal variation is primarily associated with temperature variations (Slemr et al, 1984), whereas seasonal variations also include other climatic factors and the abundances of different substrates in soil (Robertson, K. 1994). Results of ten measurements in the two successive growing seasons of winter wheat, 2000 to 2001 and 2001 to 2002 were shown in figure 1. It can be seen that clear plastic film mulching practice (FM and WM treatments) and the planting of winter wheat (W and WM treatments) both obviously increased soils N_2O emissions as compared to F and W treatments, and to F and FM treatments, respectively. Further, regarding the growing stages of winter wheat as recessive replications (10 times), two-way ANOVA was used to analyze the variance of variables (4 treatments and 10 growing stages of winter wheat), and different letters following the means presented the significant level of differences between treatments (PLSD's test) (Table 1). Significant differences (at 0.01 levels) can be found for N_2O emissions. Mulching practice increased soil N_2O fluxes by 69.80% and 60.20% as compared to the F and W treatments, respectively.

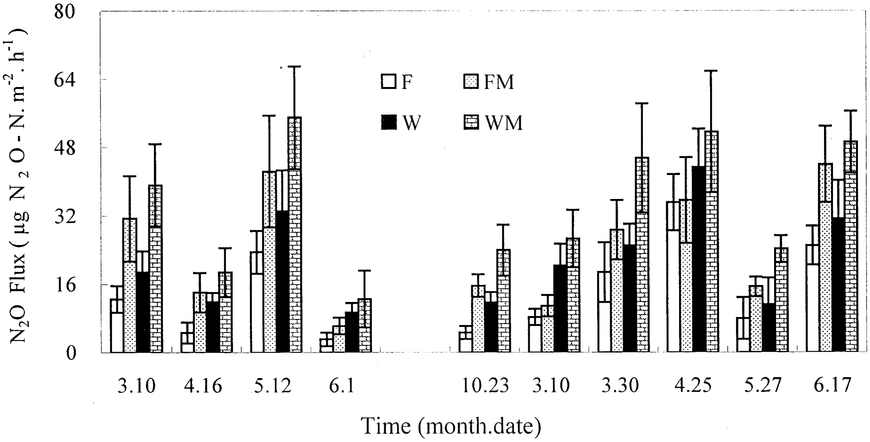

Figure 1. N_2O emissions under different land management conditions.

Table 1. Effects of different land management conditions on soil enzymes activities and N_2O emissions (average±sd).

Treatment	N_2O Flux (μg N_2O-N·m^{-2}·h^{-1})	NR activities (mgNO_3^--N·10g $soil^{-1}$·$24h^{-1}$)	NiR activities (mgNO_2^--N·10 g $soil^{-1}$·$24h^{-1}$)	Acid phosphatase activities (mgP_2O_5·100g $soil^{-1}$·$2h^{-1}$)
F	14.43±10.78 C	18.23±3.47 b	14.42±3.92 a	71.95±3.99 b
FM	24.49±13.65 B	18.91±2.86 b	13.90±4.01 a	70.51±3.90 b
W	21.68±11.39 B	18.43±3.80 b	12.70±3.98 a	71.97±6.02 b
WM	34.73±15.20 A	20.99±3.75 a	13.34±1.98 a	76.81±4.86 a

Note: Within column, means followed by different letter show significant difference using PLSD's test, and the lower case and capital letter show the significance at 0.05 and 0.01, respectively. Values followed by the same letter are not significantly difference at the 0.05 or 0.01 levels.

WM treatment had the highest soil N_2O flux with a mean of 34.73±15.20 μg N_2O-N·m^{-2}·h^{-1}. In addition, the planting of winter wheat increased soil N_2O fluxes by 50.27% and 41.77% as compared to F and FM treatment, respectively. This observation supported the findings of Huang Guo-hong (1998), who reported that N_2O

emission flux from the soil with crop (maize) is evidently higher than from fallow soil. Table 1 also showed that soil NR, NiR and acid phosphatase activities were influenced by the different land management practices as compared to the FW and W treatments, NR and acid phosphatase activities of the WM treatment were significantly increased because of mulch and planting. No significance differences were found between the F, W and FM treatments for NR and acid phosphatase. In contrast, NiR activities between the 4 treatments had no significant differences. As a result of long-term evolution, soil enzymes activities were initially in a state of dynamic equilibrium. Although soils enzymes were influenced by soil physical and chemical properties and environmental factors, they could not change sharply unless some limiting factors changes occurred. This observation supported the findings of Bai Hong-ying (2002), who reported that soil urease activities could be regarded as an effective indicator to reflect N_2O emissions that is not largely influenced by other environmental factors. It can also be referred to table 1 that soil NR activities changed synchronously with acid phosphatase activities. That implicated that there might exist some relationships between the two enzymes. However, that is poorly reported for the moment. These observations will motivate further study in this field. Therefore, we concluded that the practice of clear plastic film mulching in the winter wheat-soil eco-system significantly increased N_2O emissions in the dry lands of northwest China. Moreover, NR or acid phosphatase activities in the whole top 20cm of the soil could be regarded as a bio-indicator of N_2O emissions because of their close correlations with soil N_2O fluxes and their synchronous changes under these different soil management conditions.

Different land management conditions could lead to a very heterogeneous soil environment, and thus, soil N_2O emissions and enzymes activities would have great variance. Han Jian-gang (2002a) presented the schematic pathways of N_2O emissions in soil nitrification and denitrification processes (figures 2, 3). It can be seen that nitric oxide (NO), N_2O and dinitrogen (N_2) are the gaseous compounds in the two microbiological processes, and NR and NiR involved in the sequential reduction pathway of nitrate. Specific enzymes control each step of denitrification. However, only a few denitrifies are able to bring this reaction to completion. Shi Yi (1999) reported that soil NR and NiR activities could distinguish the pathway of N_2O emission. Therefore, simple linear regressions between soil enzyme activities and N_2O fluxes were performed (table 2). Results showed that there existed a significant positive correlation between soil N_2O emissions and NR and NiR activities. The relationship between them can be expressed well by the equation $y=ax^b$. All the determinant coefficients (R^2) are significant at 0.05 levels. In these equations, when soil NR or NiR activities are very small (near to 1 mg·NO_3^--N (NO_2^--N) ·10g soil^{-1}·24h^{-1}), the amount of N_2O emissions ranged from 0.001 to 0.417 μg N_2O-N·m^{-2}·h^{-1}, which can be neglected. In other words, the lower NR or NiR activities indicate the smaller soil N_2O fluxes. Thus, we think that denitrification process dominated soil N_2O emissions in the dry land of northwest China, and N_2O emissions

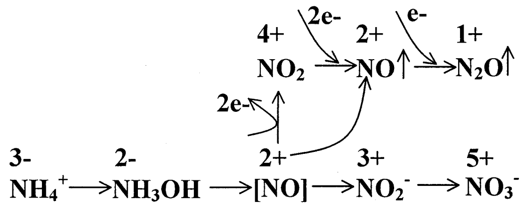

Figure 2. Schematic pathway of N_2O emission in nitrification process.

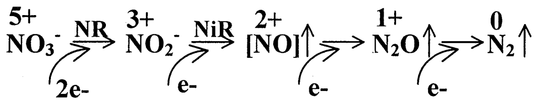

Figure 3. Schematic pathway of N_2O emission in denitrification process.

Table 2. Regression equations and determinant coefficients (R^2) between soil enzyme activities (x) and N_2O fluxes (y).

Treatment	NR activities	NiR activities	Acid phosphatase activities
F	$y = 0.001x^{3.2267}$ $R^2 = 0.5282$	$y = 0.0139x^{2.5256}$ $R^2 = 0.6388$	$y = -2.4969x + 194.09$ $R^2 = 0.8531$
FM	$y = 0.0007x^{3.5026}$ $R^2 = 0.6655$	$y = 0.3896x^{1.5304}$ $R^2= 0.4523$	$y = -3.2536x + 253.9$ $R^2 = 0.8626$
W	$y = 0.0468x^{2.0758}$ $R^2 = 0.578$	$y = 0.417x^{1.5267}$ $R^2 = 0.6015$	$y = -1.6829x + 142.8$ $R^2 = 0.7919$
WM	$y = 0.0893x^{1.9354}$ $R^2 = 0.5496$	$y = 0.0859x^{2.2862}$ $R^2 = 0.4853$	$y = -2.3556x + 215.66$ $R^2 = 0.5673$

Note: n=10, the critical R^2 value at 0.05 and 0.01 levels are 0.3994 and 0.5852, respectively.

from nitrification are very low. Moreover, we advised that nitrification inhibitors for reducing N_2O emissions from soils in the dry lands of northwest China should be applied cautiously. Soil acid phosphatase activity is an effective indicator of phosphorous availability. Table 2 shows that there existed a significant negative correlation between soils N_2Oemissions and acid phosphatase activities, and it could be expressed well by the equation $y=a+bx$. All of R^2 are significant at 0.05 levels. In these equations, when x (acid phosphatase activities) is near to 0, the values of y ranged from 142.9 to 253.9μg N_2O-N·m^{-2}·h^{-1}. The lower acid phosphatase activities are, the higher soil N_2O fluxes would be. That is to say, the deficiency of soil available phosphorous would lead to the increase of N_2O emissions.

In this study, the relationship between N_2O emissions and soil enzymes activities in the all treatments could be expressed well by the equations $y=ax^b$ and $y=a+bx$ in the two years. Thus, we thought equations $y=ax^b$ and $y=a+bx$ could be used to predict N_2O emissions under different land management conditions in the dry land of northwest China. Additional innovative finding is that the values of a in all equations varied broadly, while b values were much steady. The averages of b values for the F, FM, W and WM treatments are 2.7497±0.4133, 2.7622±1.0740, 1.7618±0.2829 and 2.1924±0.2253, respectively. According to these observations, we speculated that b might be involved with some soil properties such as soil bulk density under the different land management conditions. Based on this study, we think the practice should be evaluated impartially for the sake of environment quality, although the application of much practice in a large scale in northwest China increased significantly crop yields. Some good retrieval or substitute practices should be encouraged, for example, a timing removal of mulch may be effective for the N_2O reduction and for steady and high yields production. Our next activities are to investigate the relationships between soil NR and acid phosphatase, and the implications of the parameter b. Hopefully this study will lead to further research in the field in the future.

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