

CONTENTS

The 5th International Symposium on Environmental Economy and Technology (ISEET-2012)

Sensitive voltammetric and amperometric responses of respiratory toxins at hemin-adsorbed carbon-felt Yasushi Hasebe, Yue Wang	1055
Destruction of 4-phenolsulfonic acid in water by anodic contact glow discharge electrolysis Haiming Yang, Baigang An, Shaoyan Wang, Lixiang Li, Wenjie Jin, Lihua Li	1063
Nitrous oxide emissions from black soils with different pH Lianfeng Wang, Huachao Du, Zuoqiang Han, Xilin Zhang	1071
Coulometric determination of dissolved hydrogen with a multielectrolytic modified carbon felt electrode-based sensor Hiroaki Matsuura, Yosuke Yamawaki, Kosuke Sasaki, Shunichi Uchiyama	1077
Palladium-phosphinous acid complexes catalyzed Suzuki cross-coupling reaction of heteroaryl bromides with phenylboronic acid in water/alcoholic solvents Ben Li, Cuiping Wang, Guang Chen, Zhiqiang Zhang	1083

Aquatic environment

Organic matter produced by algae and cyanobacteria: Quantitative and qualitative characterization Maud Leloup, Rudy Nicolau, Virginie Pallier, Claude Yéprémian, Geneviève Feuillade-Cathalifaud	1089
Effects of environmental factors on sulfamethoxazole photodegradation under simulated sunlight irradiation: Kinetics and mechanism Junfeng Niu, Lilan Zhang, Yang Li, Jinbo Zhao, Sidan Lv, Keqing Xiao	1098
Irrigation system and land use effect on surface water quality in river, at lake Dianchi, Yunnan, China Takashi Tanaka, Takahiro Sato, Kazuo Watanabe, Ying Wang, Dan Yang, Hiromo Inoue, Kunzhi Li, Tatsuya Inamura	1107
Temporal and spatial changes in nutrients and chlorophyll- <i>a</i> in a shallow lake, Lake Chaohu, China: An 11-year investigation Libiao Yang, Kun Lei, Wei Meng, Guo Fu, Weijin Yan	1117
Phosphorus speciation in the sediment profile of Lake Erhai, southwestern China: Fractionation and ³¹ P NMR Runyu Zhang, Liying Wang, Fengchang Wu, Baoan Song	1124
Effect of ammonium on nitrous oxide emission during denitrification with different electron donors Guangxue Wu, Xiaofeng Zhai, Chengai Jiang, Yuntao Guan	1131
Adsorption of 2-mercaptobenzothiazole from aqueous solution by organo-bentonite Ping Jing, Meifang Hou, Ping Zhao, Xiaoyan Tang, Hongfu Wan	1139
Differences in rheological and fractal properties of conditioned and raw sewage sludge Hui Jin, Yili Wang, Ting Li, Yujing Dong, Junqing Li	1145
Competitive sorption between 17 α -ethinyl estradiol and bisphenol A/ 4- <i>n</i> -nonylphenol by soils Jianzhong Li, Lu Jiang, Xi Xiang, Shuang Xu, Rou Wen, Xiang Liu	1154
Determination of estrogens and estrogenic activities in water from three rivers in Tianjin, China Kaifeng Rao, Bingli Lei, Na Li, Mei Ma, Zijian Wang	1164

Terrestrial environment

Adsorption and desorption characteristics of diphenylarsenicals in two contrasting soils Anan Wang, Shixin Li, Ying Teng, Wuxin Liu, Longhua Wu, Haibo Zhang, Yujuan Huang, Yongming Luo, Peter Christie	1172
Evaluation of remediation process with soapberry derived saponin for removal of heavy metals from contaminated soils in Hai-Pu, Taiwan Jyoti Prakash Maity, Yuh Ming Huang, Cheng-Wei Fan, Chien-Cheng Chen, Chun-Yi Li, Chun-Mei Hsu, Young-Fo Chang, Ching-I Wu, Chen-Yen Chen, Jiin-Shuh Jean	1180

Environmental biology

Vertical diversity of sediment bacterial communities in two different trophic states of the eutrophic

Lake Taihu, China (**Cover story**)

Keqiang Shao, Guang Gao, Yongping Wang, Xiangming Tang, Boqiang Qin 1186

Abundance and diversity of ammonia-oxidizing archaea in response to various habitats

in Pearl River Delta of China, a subtropical maritime zone

Zhixin Li, Wenbiao Jin, Zhaoyun Liang, Yangyang Yue, Junhong Lv 1195

Environmental catalysis and materials

Effect of pretreatment on Pd/Al₂O₃ catalyst for catalytic oxidation of *o*-xylene at low temperature

Shaoyong Huang, Changbin Zhang, Hong He 1206

Efficient visible light photo-Fenton-like degradation of organic pollutants using *in situ* surface-modified

BiFeO₃ as a catalyst

Junjian An, Lihua Zhu, Yingying Zhang, Heqing Tang 1213

Basic properties of sintering dust from iron and steel plant and potassium recovery

Guang Zhan, Zhancheng Guo 1226

Degradation of direct azo dye by *Cucurbita pepo* free and immobilized peroxidase

Nabila Boucherit, Mahmoud Abouseoud, Lydia Adour 1235

Environmental analytical methods

Determination of paraquat in water samples using a sensitive fluorescent probe titration method

Feihu Yao, Hailong Liu, Guangquan Wang, Liming Du, Xiaofen Yin, Yunlong Fu 1245

Chemically modified silica gel with 1-f4-[(2-hydroxy-benzylidene)amino]phenylgethanone:

Synthesis, characterization and application as an efficient and reusable solid phase extractant
for selective removal of Zn(II) from mycorrhizal treated fly-ash samples

R. K. Sharma, Aditi Puri, Anil Kumar, Alok Adholeya 1252

Serial parameter: CN 11-2629/X*1989*m*207*en*P*25*2013-6



Nitrous oxide emissions from black soils with different pH

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Abstract

N₂O fluxes as a function of incubation time from soil with different available N contents and pH were determined. Cumulative carbon dioxide (CO₂) emissions were measured to indicate soil respiration. A 144-hr incubation experiment was conducted in a slightly acidic agricultural soil (pH_{H₂O} 5.33) after the pH was adjusted to four different values (3.65, 5.00, 6.90 and 8.55). The experiments consisted of a control without added N, and with NH₄⁺-N and NO₃⁻-N fertilization. The results showed that soil pH contributed significantly to N₂O flux from the soils. There were higher N₂O emissions in the period 0–12 hr in the four pH treatments, especially those enhanced with N-fertilization. The cumulative N₂O-N emission reached a maximum at pH 8.55 and was stimulated by NO₃⁻-N fertilization (70.4 μg/kg). The minimum emissions appeared at pH 3.65 and were not stimulated by NO₃⁻-N or NH₄⁺-N fertilization. Soil respiration increased significantly due to N-fertilization. Soil respiration increased positively with soil pH ($R^2 = 0.98$, $P < 0.01$). The lowest CO₂-C emission (30.2 mg/kg) was presented in pH 3.65 soils without N-fertilization. The highest CO₂-C emissions appeared in the pH 8.55 soils for NH₄⁺-N fertilization (199 mg/kg). These findings suggested that N₂O emissions and soil respiration were significantly influenced by low pH, which strongly inhibits soil microbial nitrification and denitrification activities. The content of NO₃⁻-N in soil significantly and positively affected the N₂O emissions through denitrification.

Key words: N₂O emission; nitrification; denitrification; pH; soil respiration

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Introduction

Nitrous oxide (N₂O) is of environmental importance because it contributes not only to global warming but also to the depletion of the stratospheric ozone layer. The global warming potential of N₂O is approximately 296 times greater than that of carbon dioxide. Furthermore, the atmospheric concentration of N₂O is increasing linearly at a rate of 0.3% per year (IPCC, 2007).

N₂O is produced by nitrification (Bremner and Blackmer, 1978) and denitrification (Firestone et al., 1980). The contributions of nitrification and denitrification to the formation of N₂O depend on the environmental conditions. Soil pH has a significant influence on biological processes involved in N transformation, e.g., nitrification and denitrification (Barton et al., 2013). The soil pH appears to play a key role in controlling N₂O emissions from soil, partly by affecting the N₂O product ratios of both denitrification (N₂O/N₂) and nitrification (N₂O/(NO₂⁻+NO₃⁻)) (Mørkvad et al., 2007). Autotrophic nitrification is an essential process in N₂O production in arable soil (Tortoso and Hutchinson,

1990). Enrichment of acid heath soils with nitrogen-phosphorus-potassium fertilizer increased the number of autotrophic ammonium- and nitrite-oxidizing bacteria in the organic layer, although the pH of the soil hardly changed with fertilization (de Boer et al., 1988). However, some investigations suggest that a heterotrophic pathway, including bacteria and fungi, may make some contribution to nitrification in acid soils (de Boer and Kowalchuk, 2001; Brierley and Wood, 2001). It is generally thought that fungi are predominant in acid soils and bacteria are more dominant at higher soil pH (Bååth and Anderson, 2003). Raising soil pH increased ammonia-oxidizing bacteria gene copy numbers (Barton et al., 2013). Reduction of N₂O to dinitrogen (N₂) by denitrification is a prevailing natural process in soils. Soil pH significantly regulates the extent to which N₂O is reduced to N₂ via denitrification (Stevens et al., 1998). High pH favors NO₂⁻-N accumulation, and will stimulate N₂O emissions, with NO₂⁻-N as electron acceptor in nitrification. However, low pH will strongly inhibit the activity of N₂O reduction. That results in the increase of the ratio of N₂O to N₂ during denitrification.

In China, the long-term high level of N-fertilization leads to soil acidification and a great number of negative

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environmental effects, including nitrate leaching pollution and nitrous oxide greenhouse gas emission (Ju et al., 2009; Guo et al., 2010). Soil acidification is a major problem in soils of intensive Chinese agricultural systems. Soil pH declined significantly from the 1980s to the 2000s in the major Chinese crop-production areas, with an average pH decline of 0.50 based on 154 agricultural topsoil determinations (Guo et al., 2010). In our previous report, soil $\text{pH}_{\text{H}_2\text{O}}$ in N-fertilized black soil was found to be significantly reduced from 7.22 in 1979 to 6.11 in 2007, with a decrease rate of 0.07 pH units per year (Cai et al., 2010). In recent decades, one of the main forms of black soil degradation has been soil acidification (Cai et al., 2010; Guo et al., 2010). Unsuitable agricultural practices together with adverse environmental conditions have led to the degradation of soil in many areas of China.

The objective of the present study was to investigate the effects of adjusted artificially soil pH on N_2O emissions and soil microbial activities in short-term laboratory incubation. N_2O production processes were analyzed according to N_2O emission amount and available N content.

1 Materials and methods

1.1 Soil descriptions

Soil samples were collected from a long-term experimental field in Harbin State Key Agroecological Station on the campus of the Heilongjiang Academy of Agricultural Sciences, Heilongjiang Province, China (126°35'E, 45°40'N). The soils were taken from 0–20 cm in depth and sieved (2 mm) to remove stones and coarse roots prior to incubation. The soil was derived from alluvial sediments of clay loess, classified as Udic Mollisol according to the US soil taxonomy. The mean annual temperature in the area is 3.5°C, the mean annual precipitation is 533 mm, and the frost-free period is 135 days. Selected soil properties are shown in **Table 1**.

Soil pH was measured in a solution of 10 g of soil (air-dried weight) in 25 mL deionized water that had been shaken at a speed of 300 r/min and then allowed to settle for 15 min, using a glass electrode connected to a pH meter. The pH of the soil slurry was adjusted by adding either 0.1 mol/L NaOH or 0.1 mol/L HCl (Nägele and Conrad, 1990). Then, the slurry was evaporated at 25°C. The slurry pH was adjusted and the above procedure was repeated until the slurry pH values remained stable. The soil pH values were 3.65, 5.00, 6.90 and 8.55.

1.2 Experimental treatments

There were 12 treatments, i.e. 4 pH values (3.65, 5.00, 6.90, and 8.55) multiplied by 3 nitrogen fertilizations: without N (CK), and with ammonium and nitrate addition. $(\text{NH}_4)_2\text{SO}_4\text{-N}$ and $\text{KNO}_3\text{-N}$ solutions were added at the rate of 100 mg/kg soil as ammonium and nitrate resources. Each treatment was performed in triplicate.

1.3 Incubation procedures

Soil samples of 25 g were weighed into 36 150-mL flasks. The nitrogen resource was added as an N solution. Soil samples were incubated at soil moisture of 60% water holding capacity and at 25°C for 144 hr. After flushing the flasks with ambient air for 15 sec, a rubber lid with a septum for gas sampling by syringe was fitted to each flask using HZ-704 silicone adhesive sealant to form a gas-tight seal. Gas samples were taken after 12, 24, 48, 72, 96, 120, and 144 hr. Prior to each sampling, the air in the flasks was mixed by flushing a 60-mL syringe three times, and then a 60-mL gas sample was injected into a sealed gas bag for analysis. After each gas sampling, the headspace air was renewed to compensate for consumed oxygen and to avoid N_2O and CO_2 over build-up in the headspace by evacuating the flasks and adding sufficient pure air again.

1.4 Determination methods

N_2O concentrations were analyzed using a gas chromatograph (Agilent 4890D, Santa Clara, USA) equipped with a ^{63}Ni electron capture detector operated at 330°C. N_2O was separated with a 1-m stainless-steel column with inner diameter 2 mm Porapak Q (80/100 mesh). The column temperature was maintained at 55°C for separation. The carrier gas was argon with 5% methane (CH_4). The nitrous oxide gas standard was provided by the National Standard Material Research Center, China (Cai et al., 2010).

CO_2 was analyzed by a gas chromatograph (Agilent 4890D, Santa Clara, USA) equipped with flame ionization detector (FID). High purity nitrogen (N_2) was used as the carrier gas. The CO_2 was separated with a 2-m stainless steel column (2 mm inner diameter) filled with Porapak Q (60/80 mesh, St. Louis MS, USA). CO_2 was converted to CH_4 prior to analysis with the FID, using a Ni catalyst and H_2 (methanizer) at 375°C. The CO_2 gas standard was provided by the National Standard Material Research Center, China.

At the end of the 144-hr incubation period, soil samples were immediately extracted with a 2 mol/L KCl solution for 1 hr. The extracted solutions were filtered and stored at -18°C until analysis. The contents of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$

Table 1 Selected chemical properties of soils studied

SOM (g/kg)	Total N (g/kg)	Total P (g/kg)	Total K (g/kg)	Avail. N (mg/kg)	Avail. P (mg/kg)	Avail. K (mg/kg)	pH
26.3	1.53	1.02	26.1	156	50.8	202	5.33

N in 2 mol/L KCl soil extracts were determined by the indophenol blue colorimetric method and UV spectrophotometry, respectively.

1.5 Data report and analysis

Data on the N_2O flux, cumulative N_2O and CO_2 were reported as means and standard deviations of three replicates. The increment of NH_4^+-N and $NO_3^- -N$ was calculated from the beginning of incubation minus the end of incubation. Cumulative N_2O and CO_2 emissions were calculated by summing the average of two adjacent fluxes dividing by the time between the two measurements. Statistically significant differences in cumulative N_2O emissions among pH treatments were analyzed by analysis of variance (ANOVA) and least significant difference (LSD) calculations at a 5% significance level.

2 Results

2.1 Nitrous oxide flux

Fertilizers (NH_4^+-N , $NO_3^- -N$) were dissolved in water and applied uniformly in solution when the moisture content of the soil was about 40% water holding capacity prior to fertilization. The results showed that fertilizer application had an immediately positive effect on N_2O flux and reached the maximum in the period of 0–12 hr (Fig. 1). N_2O flux from the treatment pH 8.55 was larger than that from the other three treatments. The highest N_2O-N flux occurred with addition of $NO_3^- -N$ fertilizer at pH 8.55, with peak value of $2.10 \mu\text{g}/(\text{kg}\cdot\text{hr})$. A similar tendency appeared in the treatment at pH 5.90 but with a lower N_2O-N flux ($1.21 \mu\text{g}/(\text{kg}\cdot\text{hr})$). The second highest N_2O-N flux occurred with addition of NH_4^+-N fertilizer at pH 6.90, with the value of $1.67 \mu\text{g}/(\text{kg}\cdot\text{hr})$. The factors such as adequate N and carbon source, and optimal moisture would certainly stimulate N_2O-N emission. However, the N_2O-N flux peaks from all treatments lasted for a short time, and then sharply decreased after 24 hr incubation. Although the N_2O-N fluxes in the treatment at pH 3.65 were very stable, the fluxes were consistently low either with an added N source or without ($< 0.14 \mu\text{g}/(\text{kg}\cdot\text{hr})$). It was worth mentioning that the control treatment nearly had the lowest N_2O fluxes at all times. In general, N-fertilization led to a significant increase in N_2O emission compared to the case without N fertilizer. N_2O emissions were dependent on the soil pH.

2.2 Cumulative N_2O-N emissions

The cumulative N_2O-N emissions during the 144-hr incubation in different treatments are summarized in Table 2. The highest cumulative N_2O-N emission was $70.4 \mu\text{g}/\text{kg}$ for the treatment at pH 8.55. For the treatments at pH 3.65, cumulative N_2O-N emissions were nearly consistently low with or without N-fertilization ($< 10 \mu\text{g}/\text{kg}$). In the pH 5.00 and pH 6.90 treatments, cumulative N_2O-N emissions were stimulated by both $NO_3^- -N$ and NH_4^+-N fertilizer.

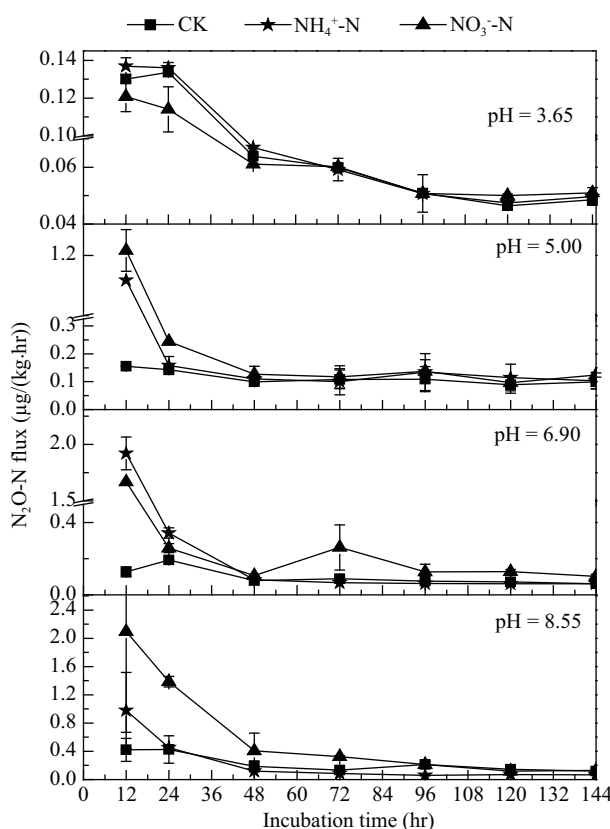


Fig. 1 N_2O fluxes from different treatments during 144-hr incubation. Error bars represent the standard deviation of replicate ($n = 3$) soil samples. CK: without N.

Table 2 A 144-hr cumulative N_2O-N emission from different treatments ($\mu\text{g}/\text{kg}$)

	CK	NH_4^+-N	$NO_3^- -N$
pH = 3.65	$9.63 \pm 0.05a$	$9.85 \pm 0.16a$	$9.37 \pm 0.12a$
pH = 5.00	$12.8 \pm 0.51a$	$29.3 \pm 7.53bc$	$31.9 \pm 2.89ab$
pH = 6.90	$15.7 \pm 4.65a$	$36.9 \pm 1.89c$	$38.9 \pm 0.07b$
pH = 8.55	$29.4 \pm 10.8b$	$26.9 \pm 10.2b$	$70.4 \pm 26.2c$

Different small letters in the same fertilization column indicates significant difference at $p < 0.05$.

The $NO_3^- -N$ fertilization showed a slight increase over NH_4^+-N fertilization. However, in the pH 8.55 treatment, cumulative N_2O-N emissions were dramatically increased with addition of $NO_3^- -N$ fertilization, being 2.62 times higher than those with addition of NH_4^+-N fertilization. The result showed that the N_2O-N emissions in the CK and $NO_3^- -N$ fertilization increased with increasing soil pH (3.65–8.55). The NH_4^+-N fertilization had a dominant role in cumulative N_2O emissions at soil pH 5.00 and pH 6.90.

2.3 Net changes in NH_4^+-N and $NO_3^- -N$

The net changes of the available N (N_{net^-}) were designated as:

$$N_{\text{net}^-} = N_b - N_a$$

where, N_b represents the concentration of available N

before incubation and N_a represents the concentration of available N after incubation. “+” means the concentration was decreased and “-” means increased.

Figure 2 shows the net changes of NO_3^- -N concentration. The NO_3^- -N concentrations were generally decreased at all soil pH values (5.00, 6.90, 8.55) except for the soil with pH 3.65 under CK and NH_4^+ -N fertilization. The greatest net changes in concentration of NO_3^- -N all appeared without N-fertilization and the values were about +44.5 $\mu\text{g/g}$ soil. However, under the NH_4^+ -N fertilization, the NO_3^- -N concentration hardly changed and there was no accumulation of NO_3^- -N. These results indicated that the depletion of NO_3^- -N had an important role in N_2O -N fluxes. Nitrate is not only a product of nitrification, but the substrate for denitrification. Under the CK condition, the soil NH_4^+ -N concentrations increased significantly at low pH values (pH 3.65 and pH 5.00), but were decreased at higher pH value (pH 6.90). Compared with the treatments at low soil pH, the NH_4^+ -N concentration in soil at pH 8.55 had a slight increase; with NH_4^+ -N fertilization, the soil NH_4^+ -N concentration represented a decreasing tendency, especially at low pH (pH 3.65). Under the NO_3^- -N fertilization, the NH_4^+ -N concentration had slight changes at low soil pH but not at pH 8.55 (< -18 $\mu\text{g/g}$). These results indicated the heterotrophic nitrification could not be ignored, especially at low soil pH.

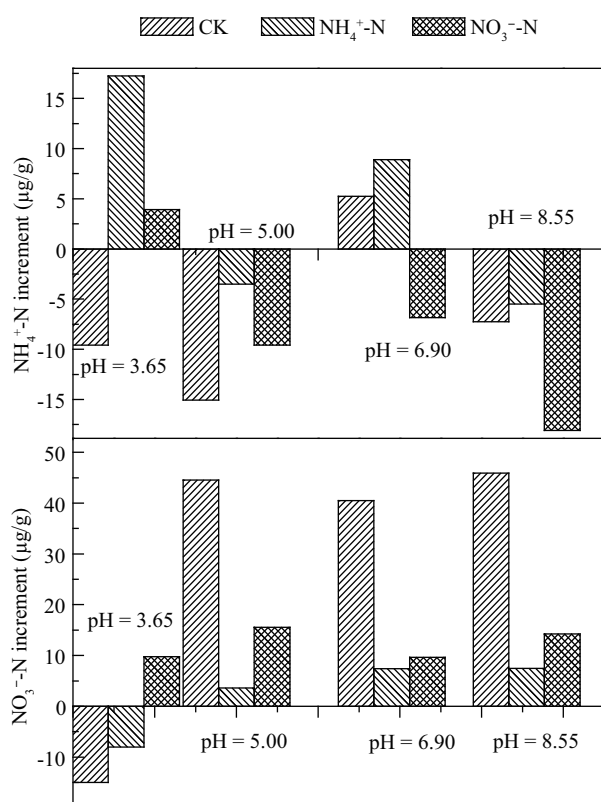


Fig. 2 Net changes of NH_4^+ -N and NO_3^- -N in different treatments during 144-hr incubation.

2.4 Soil respiration

The biogenic sources of CO_2 -C efflux from soils can be grouped into root respiration, rhizosphere microbial respiration, decomposition of plant residues, the priming effect induced by root exudation or by addition of plant residues, and basal respiration by microbial decomposition of soil organic matter (Kuzyakov, 2006). In the present incubation study, CO_2 -C was produced by soil microbial respiration. N-fertilization significantly increased cumulative CO_2 -C emissions from acidic soil to alkaline soil (**Fig. 3**). In the absence of N-fertilization (CK), cumulative CO_2 -C emission increased positively with soil pH ($R^2 = 0.98$, $P < 0.01$), and cumulative CO_2 -C emission from pH 8.55 soil was as 3.07 times that at pH 3.65. For NH_4^+ -N fertilization, the lowest accumulative CO_2 -C emission (132 mg/kg) was presented in the soil with pH 5.00, which was very near to the soil background pH of 5.33. This indicated that traditional cultivation with soil background pH and urea fertilization had the lowest CO_2 -C emission, and alkaline soil increased CO_2 -C emission. Cumulative CO_2 -C emission from pH 3.65 soils was significantly lower than that from the other three pH soils with nitrate fertilization. There were insignificant differences in cumulative CO_2 -C emissions among pH 5.00, 6.90 and 8.55.

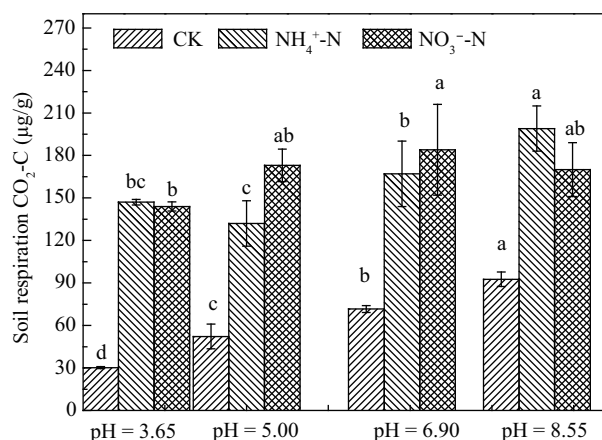


Fig. 3 Soil respiration (cumulative CO_2 emission) in different treatments during 144-hr incubation. Vertical bars denote the standard deviations of the means ($n = 3$). Different letters (a,b,c,d) denote significant difference among soil pHs at $P < 0.05$.

3 Discussion

The results showed that the cumulative N_2O -N emissions increased when the pH of slightly acidic black soil was increased from 3.65 to 8.55 (**Table 2**). It is likely that the microorganisms had inadequate capacity to adapt to the strong changes of soil pH and a certain portion of the community was inhibited (e.g. nitrifiers) (Allison and Prosser, 1993; Hayatsu et al., 2008). The findings also demonstrated that NH_4^+ -N and NO_3^- -N fertilizer significantly stimulated the emission of N_2O -N, especially for

the addition of NO_3^- -N fertilizer (Granli and Bøckman, 1994). The stimulation of N_2O -N emission by NH_4^+ -N fertilizer reached the maximum at soil pH 6.90, which has been widely accepted as the most favorable pH for nitrifier population and/or nitrification activity (Hayatsu et al., 2008; Ward, 2008; Barton et al., 2013). However, the stimulation of N_2O -N emission by NO_3^- -N fertilizer increased with the increase of soil pH (Šimek and Cooper, 2002).

In both cases, the net changes in the concentration of NO_3^- -N did not indicate accumulation for NH_4^+ -N and NO_3^- -N fertilizer (**Fig. 2**). This observation demonstrated that the rate of NO_3^- -N reduction strictly controlled the N_2O -N fluxes. Many studies have investigated the effect of soil pH on the emission of N_2O -N. This may be a result of different susceptibilities of the denitrification enzymes (NO_3^- reductase, NO_2^- reductase and N_2O reductase) to different soil pH (Šimek and Cooper, 2002). Low pH inhibits the N_2O reductase activity of N_2O reduction to N_2 (Kesik et al., 2006). The highest N_2O -N emission and largest net changes in concentration of NO_3^- -N all appeared with the addition of NO_3^- -N fertilizer at high soil pH (**Table 2** and **Fig. 2**), in which denitrification dominates the N_2O -N emission (Skiba, 2008). Meanwhile, raising soil pH decreased N_2O emissions from nitrification (Barton et al., 2013). Changes of NO_3^- reduction by the microbial community dramatically contributed to N cycling. The relationship between soil pH and denitrification as determined by various incubation methods remains unclear, because results were influenced both by the original conditions in soil samples and by unknown changes during incubation (Šimek and Cooper, 2002).

Soil pH affects all chemical, physical and biological soil properties and inevitably disturbs the carbon flux. At lower pH, cumulative CO_2 -C emission decreased (**Fig. 3**). Soil acidification inhibits the soil microbial respiration. The higher pH soils had significantly higher CO_2 -to-soil organic carbon (SOC) ratios, and consequently SOC in the alkaline soils did not seem as chemically stable against mineralization (Nambu et al., 2008). Soil respiration decreased markedly in samples from acidified soil plots (Popovic, 1984). Impacts of anthropogenic activity on increased CO_2 -C emission have been investigated for many years. However, there have been few reports on how acidification affects CO_2 -C emission from black arable soil. The intensity of soil respiration is closely related to the decomposition of SOC, and even small changes of organic matter content and soil pH will dramatically affect the dynamics of carbon (Silva et al., 2008). Increasing pH with N-fertilization enhanced CO_2 -C emission (**Fig. 3**). The microorganisms seemed to be more adapted to high pH than to low pH (Cookson et al., 2007). N-fertilization improves soil microbial activity, leading to more CO_2 -C emission.

Acknowledgments

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