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Effects of inorganic nitrogen and phosphorus enrichment on the emission of N_2O from a freshwater marsh soil in Northeast China

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Abstract The freshwater marshes in northern China are heavily impacted by anthropogenic disturbances such as cultivation and fertilization and increased levels of nutrients (especially N and P) through atmospheric deposition and agricultural surface runoff. These disturbances have affected the emission of N₂O from these systems. This laboratory study was conducted to determine the effects of increased inputs of inorganic N and P on N₂O emission from marsh soil in response to different soil moisture conditions. The results showed that the emission of N₂O increased with the enhancement of N inputs when the soil was submerged, but that the highest N treatment suppressed the emission of N₂O when the soil was at 60% water holding capacity (WHC), which may have occurred due to an inadequate amount of available C. Furthermore, the results of this study indicated that a small amount of N fertilizer induced much more N2O evolution from freshwater wetland soil, while P fertilizer inputs appeared to stimulate the emission of N2O only during the first few days of the experiment. Additionally, soil that was treated with P appeared to absorb N₂O when it was at 60% WHC after around 6 weeks of the incubation, which indicates that the input of P fertilizer might serve as a shift of source or N₂O sink in wetland soils under non-flooded conditions. When compared to soil at 60% WHC, submerged soil had significantly higher N₂O emissions, except when

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subjected to the medial N treatment. These findings indicate that the soil moisture condition had a significant effect on N₂O emissions when the same amount of N or P was applied. Therefore, the effects of N and P fertilization in the northern temperate wetlands cannot be neglected from regional or national emissions of N₂O.

Introduction

Nitrous oxide (N₂O) is an important constituent of the global nitrogen cycle. Increases in the atmospheric concentration of N₂O contribute directly to global warming and the destruction of the stratospheric ozone layer. A recently conducted budget of atmospheric N₂O revealed that the cycle is imbalanced, with an increase in the atmospheric N₂O burden of 3.8 Tg N₂O–N year⁻¹ occurring (IPCC 2001). Furthermore, model calculations suggest that the atmospheric N₂O level may increase from the current concentration of 316 ppbv to 354–460 ppbv by 2100 (IPCC 2001). This increase in atmospheric N₂O and the imbalance in its global budget have led to increased interest in the nitrogen budgets of various ecosystems (Baggs et al. 2003; Trujillo-Tapia et al. 2008; Zhang and Han 2008).

The emission of N₂O is closely associated with anthropogenic activities. Human activities such as the combustion of fossil fuels and the application of fertilizer have led to increased levels of N and P in the atmosphere, as well as in most ecosystems on Earth. Galloway et al. (2003) reported that between 1860 and 2000, the anthropogenic N creation rate increased from 15 Tg N to approximately 165 Tg N year⁻¹. In addition, atmospheric P deposition and runoff also increased greatly during this period, especially in areas of intensive agricultural (Ahn and James 2001; Winter et al. 2002). N₂O is produced naturally in soils through the microbial processes of nitrification and denitrification, and is regulated by many factors, such as soil moisture content, temperature, ammonium and nitrate concentrations, the amount of mineralizable carbon, pH and nutrient concentrations (Bouwman 1990; Granli and Bøckman 1994). N fertilizer is considered to be a primary source of N₂O emission from terrestrial and aquatic ecosystems (Mosier et al. 1991; Zhang et al. 2007a). Additionally, P enrichments also influence the microbial biomass and the denitrifiying enzyme activity of some soils (White and Reddy 1999). However, the production of N₂O varies under different soil moisture conditions (Weitz et al. 2001).

Wetlands are considered to be a natural source of N₂O, and many studies have been conducted to evaluate the N2O flux and regulating factors in wetlands (Wang et al. 2006; Song et al. 2005). Due to their unique locations, wetlands receive high levels of nutrient elements (especially N and P) as a result of human activities. The influence of N or P enrichment of wetlands has recently received a great deal of attention. Indeed, many studies have targeted changes in wetland function and structure in response to the influx of N and P, such as shifting plant species compositions (Gaiser et al. 2005; Craft et al. 2007; Tyler et al. 2007), changes in the fluxes of greenhouse gases (Aerts and Toft 1997; Zhang et al. 2007b), or alterations in the chemical and physical parameters involved in biogeochemical cycles in wetlands (Castro et al. 2005; Penton and Newman 2007). However, few studies have been conducted to evaluate the dynamics of N₂O emissions from wetland soils in response to increased levels of N or P. At present, the area of natural wetlands in China is about 36.2 million hm², of which freshwater marsh is 13.7 million hm², covering about 38% of total natural wetlands. Sanjiang Plain mire, which is the largest freshwater marshes in China, has experienced intensive cultivation over the past 50 years (Zhao 1999). As a result, many marshes are being drained for conversion to agricultural production, the average annual N-fertilizer and P-fertilizer were respectively about 5.17 and 2.63 $g m^{-2}$, and showed an increasing trend year by year. As a result of that undrained marshes often receive N or P from agricultural runoff or atmospheric deposition. For example, Sun et al. (2007) found that the total N deposition in this area was 7.57 kg hm⁻² year⁻¹), and that inorganic N accounted for 84.56% of this deposition.

Therefore, a typical soil was selected from freshwater marshes in Sanjiang Plain, Northern China. And a controlled laboratory experiment was then conducted using this soil. The objective of this study was to investigate the dynamics of N_2O emission following increased fertilization (N or P) under different soil moisture conditions, and to evaluate the difference in N_2O emissions in response to application of the same levels of fertilizer under aerobic and aerobic (submerged) conditions.

Materials and methods

Soil sampling

The primary soil types in Sanjiang Plain are typical meadow mire soil and peatland soil, and the corresponding predominant plants are Calamagrostis angustifolia and Carex lasiocarpa, respectively. A typical meadow mire soil was selected for this study because these soils are often found at the margin of the wetland and receive various nutrients before the rest of the system. Fresh soil samples were collected from approximately 0-20 cm, after which the samples were mixed thoroughly in the laboratory and then passed through a 2 mm sieve to remove the visible roots and other impurities. Random aliquots were then used to determine the basic characteristics of the soil (Table 1), while the remaining soil was used in the incubation experiment described below. The total organic carbon was measured by wet oxidation using dichromate in acid medium followed by the FeSO₄ Titration method. The available N was determined by evaluating the diffusion of alkali and extractable P through the extraction of sodium bicarbonate (Lu 2000).

Fertilizers and soil moisture

Three levels of N fertilizer and P fertilizer were evaluated in this study, 1(N1), 2(N2) and 5(N3) mg N g⁻¹ soil, and 0.2(P1), 0.4(P2) and 1(P3) mg P g⁻¹ soil, respectively. In addition, three flasks of soils that did not contain fertilizer were used as controls (CK). N and P fertilizer were added as ammonium nitrate (NH₄NO₃) and sodium dihydrogen phosphate (NaH₂PO₄) solution, respectively. Each N or P fertilizer treatment (including CK) was also subjected to two soil moisture conditions: (A) maintenance at 60% of the water holding capacity (60% WHC) and (B) maintenance of 2 cm of water above the soil layer (submerged). Additionally, three glass flasks that did not contain soil and water were used as blanks. All treatments were conducted in triplicate; therefore, there were 45 incubation flasks used in this study.

Experimental procedure

Twenty gram of soil (on air-dried base) was weighed and placed in each 600 ml glass flask. The corresponding fertilizer treatment was then added and mixed. Next, distilled

Table 1 Selected characteristics of the 0-20 cm soil

Soil type	Total organic carbon (TOC) (g kg ⁻¹)	Available N (AN) (mg kg ⁻¹)	Extractable P (EP) (mg kg ⁻¹)	PH
Meadow soil (0–20 cm)	48.60 (0.199)	316.93 (11.89)	37.45 (3.61)	5.35 (0.08)

Each value represents mean (n = 3). S.E. of means are included in the parenthesis

water was added to bring the soil moisture conditions to the desired level. Each flask was then covered with a rubber stopper containing a needle hole in the middle, which was sealed with silica gel to prevent air from leaking. All flasks were then marked and placed at random in a black chest (approximately 25°C). The air in the headspace was sampled at 1, 3, 6, 10, 15, 21, 27, 33, 39, 45, 55 and 65 days using a 50 ml gas-tight syringe. After sampling, the flasks were flushed with ambient air for 20 min to allow the air to reach equilibrium, after which they were resealed. During incubation, the soil moisture contents were maintained by the weighing method.

The N₂O levels were analyzed within 6 h of sampling using a modified gas chromatograph (Agilent 4890D) equipped with electron capture detectors (ECD). N₂O was separated using a 1 m stainless steel column with a Porapak Q (80/100 mesh) column with an inner diameter of 2 mm and then measured using an ECD detector at 330°C. The carrier gas was high-purity nitrogen with a flow rate of 30 ml min⁻¹. The column temperatures were maintained at 55°C. The gas concentrations were calculated and compared with that of a standard gas (345 ppb), and the N₂O emission rate was calculated as the average increase in concentration over the experimental period and expressed as $\mu g N_2O$ –N kg⁻¹ soil h⁻¹.

Statistical analysis

Statistical analysis was conducted using SPSS 11.5. Oneway analysis of variance (ANOVA) was used to determine the differences in N_2O emission rates and cumulative amounts in response to various N or P inputs. Differences between the two soil moisture conditions under the same N or P input were analyzed using the Student's *t* test. Differences were considered significant when p < 0.05. All figures were prepared using Origin 7.5.

Results

Dynamics of N_2O emission rate in response to N fertilizer

As shown in Fig. 1, treatment with N fertilizer had a significant effect on the emission of N₂O, especially when higher levels of N were applied. Specifically, the emission rates from CK under 60% WHC condition (Fig. 1a) were very low, with a maximum rate of 0.21 ± 0.01 μ g N kg⁻¹ soil h⁻¹ appearing after 1 day of incubation. N₂O emissions from N1 peaked on the third day after incubation $(3.54 \pm 0.50 \ \mu g \ N \ kg^{-1} \ soil \ h^{-1})$, while the emission rates of N2 and N3 increased continuously until the maximum levels of 59.05 \pm 10.91 and 38.20 \pm 4.77 μ g N kg⁻¹ soil h⁻¹, respectively, were obtained on day 21. The rates then decreased rapidly until day 45, when they increased slightly before decreasing again. It is important to note that the emission rates from the N2 and N3 treatments were significantly higher than those from CK and N1 (p < 0.05), but that the emission rates from N3 were lower than those of N2 after the first 3 days. Under submerged conditions (Fig. 1b), the patterns of N₂O emission were similar among treatments. Specifically, the maximum emissions occurred on day 3 after N application for all treatments except CK, which showed the peak emission on day 6. The maximum emission rates were 21.38 ± 1.26 , $37.86 \pm 2.63, 47.91 \pm 0.85$ and $98.40 \pm 7.07 \ \mu g \ N \ kg^{-1}$

Fig. 1 Variation in N₂O emission rates with time following different N fertilizer application under two soil moisture conditions (mean with SE, n = 3). Note the different Y-axis scales in (**a**) and (**b**). The same blow



soil h^{-1} , for CK, N1, N2 and N3, respectively. After reaching the maximum emission levels, the rates declined rapidly; however, the high emission rates lasted for approximately 4 weeks and remained relatively stable for the remainder of the experiment.

There were significant differences between the two soil moisture conditions in response to the same N treatments. Specifically, the emissions of N₂O from CK, N1 and N3 under submerged conditions were all significantly higher than from the same treatments when the samples were maintained at 60% WHC (*t* test, p < 0.01). However, the emission rate from the N2 treatment was higher under submerged conditions during the first 6 days when compared to the 60% WHC N2 treatment, after which it was lower than that of the N2 treatment that was maintained at 60% WHC.

Dynamics of the N_2O emission rate in response to P fertilizer

Under the 60% WHC condition, the emission rates of N₂O from the treated samples had a similar pattern as that of CK, with maximum emission rates occurring on the first day and then declining rapidly during the first 5 days of the experiment (Fig. 2a). Specifically, the maximum emission rates were 0.21 ± 0.01 (CK), 0.24 ± 0.01 (P1), $0.22 \pm$ 0.00 (P2) and 0.32 ± 0.02 (P3) µg N kg⁻¹ soil h⁻¹. Interestingly, no N₂O was detected from the P treatments after 45 days, which may indicate that the N₂O was absorbed by the soil in these samples. Under submerged conditions (Fig. 2b), the emission rates of N₂O from the P treated soils were elevated on the first day and peaked on day 3, while the emission from CK was very low during the beginning of the experiment and peaked on day 6. Specifically, the maximum emission rates were 21.38 ± 1.26 , 28.82 ± 1.00 , 33.07 ± 0.48 , and $44.93 \pm 2.72 \ \mu g \ N$ kg^{-1} soil h^{-1} for CK, P1, P2 and P3, respectively. The rates from all treatments decreased rapidly after the maximum emission rates were attained, and a steady state was attained after approximately 3 weeks. The emission rates of N_2O increased as the P fertilizer input increased during the first 3 days, after which there were no significant differences among groups that received different levels of P fertilizer.

The emission rates were very low ($<0.32 \pm 0.02 \mu gN kg^{-1} soil h^{-1}$) under the 60% WHC condition, although a rapid increase in emissions from the P3 treated soil was observed in the beginning of the experiment. However, the emission rates of N₂O under the submerged condition were significantly higher than those under 60% WHC (*t* test, *p* < 0.01) when the same levels of fertilizer were used.

Cumulative emissions of N_2O with time as affected by N fertilizer

Figure 3 shows the accumulation of N₂O emissions as a function of time following the application of N fertilizer. During the 65 days incubation period, the cumulative emissions of N₂O from the N treatments were all higher than those from the CK under both the 60% WHC and the submerged condition. These findings indicate that the application of N fertilizer could promote the emission of N₂O from marsh soils. The accumulation of N₂O at 60% WHC increased with N application, except for the emissions from the N3 treatment, which were lower than those from the N2 treatment. Nevertheless, N2 and N3 had significantly higher emissions than CK and N1 (p < 0.01). Additionally, the highest percentage of N₂O was emitted from the N2 treatment (Table 2). The accumulation of N_2O under the submerged conditions increased as the level of N applied increased and was greatest during the first 3 weeks of the experiment, after which it increased much slower. Under submerged conditions, the highest percentage of



Fig. 2 Variation in N₂O emission rates with time following different P fertilizer application under two soil moisture conditions (mean with SE, n = 3)



Table 2 Cumulative emission of N₂O-N and total losses N as N₂O with the application of N fertilization after the 65-day incubation

Treatments	Nutrient addition (mg g^{-1} soil)	60%WHC		Submerged	
		Cumulated N ₂ O–N (mg N kg ^{-1} soil)	Percentage of applied N as N ₂ O	Cumulated N ₂ O–N (mg N kg ^{-1} soil)	Percentage of applied N as N ₂ O
СК	0	0.10 (0.01)a	-	5.53 (0.34)a	-
N1	1	2.11 (0.22)a	0.21	19.02 (1.42)b	1.90
N2	2	36.98 (4.07)c	1.85	23.94 (1.22)b	1.20
N3	5	26.59 (2.35)b	0.53	41.86 (5.96)c	0.84
P1	0.2	0.091a	-	5.42 (0.63)a	-
P2	0.4	0.089a	-	5.47 (0.21)a	-
P3	1	0.090a	-	6.08 (0.20)a	-

Each value represents mean (n = 3). Different letters within each column indicate significant difference at p < 0.01. SE of means are included in the parenthesis

emitted N₂O–N from the added N occurred in N1 treatment and declined as the increase of N inputs. Moreover, the accumulation of N₂O emissions from CK, N1 and N3 under submerged conditions was greater than under 60% WHC when the same level of N was applied.

Cumulative N_2O emissions with time as affected by P fertilizer

Figure 4 shows the accumulation of N_2O emissions as a function of time following the application of P fertilizer. The accumulation of N_2O including CK at 60% WHC were quite smaller, as mentioned previously (Fig. 2). Specifically, there was no N_2O observed after 45 days of incubation, which implies that the soil may absorb N_2O from the atmosphere after the application of P. The P treatments had a larger accumulation of N_2O emissions than control within 45 days, but this difference was not significant. In the earlier period of incubation, more N_2O was emitted from the P1 and P2 treated soil than from CK under submerged conditions, while the accumulation of N_2O emissions from the P3 treatment was greater than that of CK throughout the experiment.

Discussion

Soil moisture condition

Production of N₂O is primarily related to nitrification, nitrifier denitrification and denitrification (Wrage et al. 2001). Water is the most important factor in nitrification and denitrification and thus the production of N₂O (Bandibas et al. 1994). It is well known that the production of N₂O increases with increased water content. Ponce-Mendoza et al. (2006) reported that the emission of N_2O increased significantly (32 times) when soil was incubated at 100% WHC when compared to 40% WHC. When the soil water content increases, nitrifier denitrification and denitrification is induced (Wrage et al. 2001). Indeed, in this study, soil moisture was found to have a significant effect on N₂O emission. The higher N₂O emission observed under submerged conditions was likely due to increased denitrification anaerobic microbes. by Conversely, nitrification may be responsible for the N₂O emission that was observed at 60% WHC. The occurrence of a few rapid emission peaks and the higher emission rates observed under submerged conditions imply that

Fig. 4 Cumulated N₂O emission over time during 65 days incubation with different P fertilizer application under two soil moisture conditions (mean with SE, n = 3)



significant N₂O production could occur immediately after dry soil becomes submerged.

N application effect

The input of inorganic N could provide available substrates to nitrifiers and denitrifiers for nitrification and denitrification and thus the production of N₂O. In this study, the addition of N led to increased N2O emission under both soil moisture conditions (Figs. 1, 3), which indicates that there is a limit to the level of N that can be converted to N₂O in this wetland soil. The input of N fertilizer has been regarded as a primary controlling factor of the emission of N₂O from many soils (Hou et al. 2000; Merbach et al. 2001; Trujillo-Tapia et al. 2008). In addition, boreal freshwater marsh systems are normally limited by nitrogen (Koerselman and Meuleman 1996; Sun et al. 2006). Therefore, the addition of exogenous nitrogen likely induces a series of changes in biogeochemical processes, including the N cycle. In this laboratory experiment, N fertilizer was added in the form of NH₄NO₃, which resulted in there being large concentrations of substrates available for nitrification and denitrification. We concluded that the production of N₂O was primarily due to nitrification of NH_4^+ -N under 60% WHC, while N₂O production was likely a result of denitrification under submerged conditions. Zhang et al. (2007a) found that exogenous nitrogen had a significant impact on N₂O fluxes in this area. White and Reddy (1999) also reported significant increases in denitrification rates in response to high N treatments in a study conducted using soil from the Everglades.

In the present study, the emission rates and accumulation of N₂O increased as the level of N added increased (Table 2), and there was a much higher emission of N₂O immediately after the application of N (Maljanen et al. 2003). However, the highest N application suppressed the emission of N₂O at 60% WHC, which was likely due to a lack of readily available organic C (Stapleton et al. 2005) and/or a possible adverse effect on microbial activity in response to the high NH_4^+ –N concentration. Hadi et al. (2000) reported that N fertilization may suppress N₂O emissions by inhibiting nitrification in some tropical peatlands. It was also found that the mineralization of organic carbon was suppressed after the first few days of stimulation under the highest N application (Liu et al. 2008). Therefore, the activities of soil microbes may have been limited by the availability of C in this study due to the abundance of nitrogen. Various studies have shown that when sufficient NO_3^- is available to induce the denitrifying activity, an increase in soil organic matter (SOM) increases soil denitrification rate (Bijay-Singh et al. 1988; Gale et al. 1993).

P application effect

The addition of P had no significant effects on the cumulative emission of N₂O-N after 65 days of incubation (Table 2). Specifically, only a relatively small priming effect was observed during the first few days, and lower emissions and N₂O absorbance was observed after 45 days of incubation under the 60% WHC condition (Fig. 2). These findings may have been due to nitrogen limitation in the boreal wetland. Additionally, only the highest P treatment had larger emission rates than the non-fertilized treatment under submerged conditions during the 65-day incubation period, and there were no significant differences among P treatments. Moreover, the emission rates and accumulation of N2O were much higher under the submerged conditions when compared to the 60% WHC conditions for all P treatments (Figs. 2, 4), which supports the view that soil water content plays an important role in N₂O emissions. White and Reddy (2003) reported that denitrifiers are more sensitive to P limitations than to nitrifiers, and that the effect of P enrichment was much greater on denitrifiers. Furthermore, the level of dissolved organic carbon was reportedly higher under submerged conditions than at 60% WHC in response to the same P treatment (Liu and song 2008). Taken together, these findings indicate that the high denitrification rates were due to a combination of high P availability and an adequate supply of available labile organic C. Therefore, the emission

rates and accumulation of N_2O were much higher under submerged conditions than under 60% WHC when the same levels of P were applied. However, nitrification was more dependent on inorganic substrates such as NH_4^+ and high alkalinity, while P availability only had minimal effects on nitrification. As a result, the emission rates decreased rapidly and the accumulation of N_2O increased slowly as the amount of mineral nitrogen was reduced.

Few studies have been conducted to evaluate the influence of P fertilizer on the emission of N2O. Minami and Fukushi (1983) reported enhanced N₂O emissions in response to the addition of P to aerobic soils and Keller et al. (1988) found the same phenomenon in a tropical forest. Furthermore, Bauhus et al. (1993) and Falkiner et al. (1993) found that P application promoted nitrification in several forest soils. Conversely, Sahrawat et al. (1985) observed no significant changes in nitrification in six acid forest soils in response to the addition of P, while Steudler et al. (2002) found no changes in nitrification in soil from tropical forests and pastures. Zhang and Han (2008) reported increased N₂O emission in abandoned cropland, but no significant changes in nitrification in grasslands following the application of P. Furthermore, the effects of P on N₂O emission were found to be site specific in a review conducted by Zhang and Han (2008). Indeed, the mechanisms of N_2O emission in response to P are still not clear. Nevertheless, these positive and negative responses suggest that extensive application of P fertilizer plays an important role in the emission of N₂O. Further investigation is required to identify the mechanisms involved in the effects of P application on the emission of N₂O from various soils.

The potential effects of increased N and P fertilization on N_2O emission from freshwater marsh soil

White and Reddy (1999) evaluated the potential denitrification rates and denitrifying enzyme activity (DEA) in response to the addition of different nutrients in a P-limited everglade and only observed significant differences in response to treatment with the two highest levels of N. Their findings demonstrated that denitrification and DEA values in Everglades soils were controlled by the NO₃-N input, not P. Accordingly, although they found a significant positive correlation (r = 0.93, p < 0.001) between total P and DEA for detritus and the 0-10 and 10-30 cm soil depths, they concluded that this was incidental rather than causal because NO3⁻ was determined to be the limiting factor for DEA in the field. After several years, White and Reddy (2003) reported that the total P was correlated with potential denitrification (r = 0.85). They went on to state that P was the limiting nutrient with respect to potential denitrification rates and that it had the potential to affect the N balance in the northern Everglades.

The results of the present study indicated that N affected N₂O emission in a N-limited freshwater wetland within 30-40 days of the application of N fertilizer, while the effects of P fertilizer were only observed during the first few days of the experiment. This study also demonstrated that, at the present background of N and P concentrations, significantly higher rates of N₂O evolution can be produced when only a small amount of nitrogen is added to the freshwater wetland soil (Table 2). For example, if the input amount increased from 1 to 2 mg N g^{-1} soil, the emission of N₂O would increase by approximately 17.53 times at 60% WHC. These findings indicate that N fertilizer enrichment of freshwater wetland soils in northern China can result in a substantial increase in regional and national N2O emission; therefore, their roles in the N₂O budget cannot be neglected. Which were consistent with researches in cropland (Xiang et al. 2007; Wu et al. 2008). There were fewer N₂O emissions following large inputs of P at 60% WHC, and no significant differences were observed among treatments under submerged conditions. Huang et al. (2005) reported input of P fertilizer stimulated the emission of N₂O in rice field, but the intensity were smaller than N fertilizer. These findings may have been due to the low amount of available N in the soils evaluated in this study. It is possible that, if N were loaded at levels sufficient to prevent it from being a limiting factor, P inputs would induce greater emissions of N₂O. In addition, the simultaneous application of N and P fertilizer would likely have resulted in a much more complex effect on N2O emissions. Meanwhile, N2O is considered to be an important greenhouse gas with CO₂ and CH₄ for the warming effects and longer resort time in atmosphere. The global warming potential of N₂O is 275, 296 and 156 times as compared to CO₂, with a span of 20, 100 and 500 years, respectively (IPCC 2001). The N and P fertilizer input would affect the greenhouse affect either in short time scale or long time scale, whose future global warming forcing effects is not neglected.

Conclusions

The results of the present study suggest that increased loading of N nutrients would stimulate the emission of N_2O from the boreal freshwater marsh soil, especially under submerged conditions. However, the highest N application evaluated in this study suppressed the emission of N_2O , which might indicate that there was not an adequate organic carbon source for the microbes in the soil. The results of this study also suggest that the wetland soil had a strong capacity to remove N through nitrification and

denitrification. However, the removal fractions in response to additional N were not constant.

Phosphorus loading appeared to enhance N_2O emission only during the first several days, and no N_2O was detected at the end of the incubation period under 60% WHC. Furthermore, P loading had no significant effects on the emission of N_2O under submerged conditions. These results indicate that the input of P fertilization may cause a shift in the source or reduction in N_2O under non-flooded conditions due to the lack of available N. Further investigations suggested that, at present background N and P concentrations, significantly higher rates of N_2O evolution would be produced when there is little nitrogen input into the freshwater soil, and that the effects of P loading would be smaller than those of N fertilization. Moreover, the effects of N + P loading in this wetland ecosystem should be evaluated.

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