



Soil nitric oxide emissions after nitrogen and phosphorus additions in two subtropical humid forests

Dejun Li,¹ Xinming Wang,¹ Guoying Sheng,^{1,2} Jiangming Mo,³ and Jiamo Fu^{1,2}

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[1] Soil nitric oxide (NO) emissions after the addition of nitrogen (N) and phosphorus (P) were studied at a broadleaf forest and a pine forest in Dinghushan Biosphere Reserve, south China. N was applied at 5 g N m⁻² (or 50 kg N ha⁻¹) and P was applied at 4 g P m⁻² as NH₄Cl (AN), NaNO₃ (NN), NH₄Cl + Na₂HPO₄ (AP), NaNO₃ + Na₂HPO₄ (NP), and Na₂HPO₄ (P) in water solutions, respectively, in comparison with water and blank controls. Enhancement of NO emission by N addition alone was greater than that by combined addition of N and P in both forests due to the P-limiting soil nutrient status. In both forests, temporal pattern of NO emission after adding NO₃⁻-N was different with that after adding NH₄⁺-N. NO₃⁻-N addition resulted in immediate NO fluxes for the broadleaf and to a lesser extent the pine forest site. In both forests, nitrification contributed more than denitrification to NO production although denitrification also played an important role in the broadleaf forest. N addition induced greater NO emission in the mature broadleaf forest than in the primary pine forest. Over the 50-day experimental period, averaged NO fluxes in plots adding AN, NN, AP, and NP were 3.3, 2.3, 1.9, and 1.8 times that in the water control plots, respectively, for the broadleaf forest and 2.7, 1.6, 1.5, and 1.3 times that in the water control plots, respectively, for the pine forest. Applied N loss as NO-N in AN, NN, AP, and NP plots were 2.8%, 1.6%, 1.1%, and 1.0%, respectively, in the broadleaf forest; and 2.6%, 1.0%, 0.8% and 0.4%, respectively, in the pine forest. On the average, N loss as NO from the forest floors was approximately 2% of the applied N as NH₄⁺ or NO₃⁻ alone, quite similar to that measured by Hall and Matson (1999) from forest floors in the Hawaiian Islands.

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1. Introduction

[2] Over the past century, nitrogen (N) deposition in terrestrial ecosystems has increased more than three times due to anthropogenic activities related to food and energy production [Galloway *et al.*, 2004]. However, until recently, N deposition has been largely a concern in the industrialized world [Hall and Matson, 2003]. In the less industrialized areas, many of which locate in tropics and subtropics, N deposition is expected to increase dramatically with the increase in population and in per-capita energy and food consumption. Due to higher rates of emission and deposition of both oxidized N (NO, NO₂, and other oxidized N compounds) and reduced N (mainly NH₃ and NH₄⁺), some regions in South and East Asia or South and Central

America are projected to be experiencing more than doubled N deposition by year 2050 relative to the early 1990s [Galloway *et al.*, 2004]. Therefore it is essential to improve our understanding of the potential fates for the additional N in these future hotspots.

[3] The human induced increase of N inputs to forests would dramatically alter processes and properties of these systems based on field investigation [Dise and Wright, 1995; Van der Eerden *et al.*, 1998] and several long-term N addition experiments in North America, Europe, Hawaiian Islands and China [Gundersen *et al.*, 1998; Wright and Rasmussen, 1998; Magill *et al.*, 2000; Lohse and Matson, 2005; Mo *et al.*, 2007]. In addition to impacts on forest productivity, soil fertility, species composition, decomposition and nitrate leaching [Venterea *et al.*, 2004], NO emission fluxes have long been proposed to increase after chronic N inputs since soil NO emissions were found to be high in forests experiencing high N deposition [Fenn *et al.*, 1996; Butterbach-Bahl *et al.*, 1997, 2002; Pilegaard *et al.*, 1999; Pilegaard, 2001] or in forests fertilized with N [Kaplan *et al.*, 1988; Bakwin *et al.*, 1990; Hall and Matson, 1999, 2003; Steudler *et al.*, 2002; Vanterea *et al.*, 2003]. Since NO catalyzes the production of ozone and is the precursor of nitric acid (HNO₃) [Crutzen, 1979], soil emissions of NO

¹State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, China.

²School of Environmental and Chemical Engineering, Shanghai University, Shanghai, China.

³South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China.

may also impact regional tropospheric ozone levels and contribute to downwind N deposition. Consequently, studies on soil NO emissions from forests not only help to understand N cycling in these systems, but also help to predict soil NO emission response to increasing N deposition.

[4] N and phosphorus (P) fertilization experiments conducted in tropical rain forests in the Hawaiian Islands indicate that forest N and P status played an important role in soil NO emission [Hall and Matson, 1999, 2003], and NO emissions in response to N addition were much lower at forest sites where primary production was limited by N than those where primary production was limited by P. Northern temperate forests are naturally N-limited, while in tropical/subtropical regions N often is not the limiting nutrient even in relatively undisturbed ecosystems [Martinelli *et al.*, 1999]. This fact, combined with warm, often wet climates in tropical/subtropical regions, can lead to high rates of N loss to atmospheric and aquatic realms, making it likely that even a modest rise in anthropogenic N inputs could lead to rapid increases in N losses to air and water [Matson *et al.*, 1999].

[5] Most subtropical humid forests are distributed in southeastern China in the south of the Yangtze River, the southern tip of the Republic of Korea and the southern half of Japan [FAO, 2001]. Large portions of these regions are experiencing high N deposition ($> 20 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) [Galloway *et al.*, 2004]. Nevertheless, to date few studies have been conducted to measure soil NO emissions from forests in these regions. How would these forests respond to increasing N inputs as projected? How would these forests respond to inputs of different forms of N? What percentage of the input N will emit back to the atmosphere as NO? All these questions need to be answered with extensive studies.

[6] According to Hall and Matson [1999], forests lose equally large amounts of NO after first-time N additions as after chronic, long-term N additions. The results from the long-term N addition experiment at the Harvard Forest in central Massachusetts also confirmed the cumulative responses of ecosystem processes to N addition [Magill *et al.*, 2000]. Thus it is reasonable to postulate that information of NO emission from short-term N addition experiments may help to understand the responses of NO emission to increasing N deposition.

[7] In a previous study, we conducted field measurements of soil NO emission in a broadleaf forest and a pine forest in the Dinghushan Biosphere Reserve, located in Guangdong province, south China [Li *et al.*, 2007]. The present study reports our results of soil NO emission after N and P fertilization in the two forests. The objectives were to (1) explore how soil NO emissions responded to approximately doubled N inputs, and roughly estimate the fraction of applied N loss as NO; (2) determine the role of soil N and P status in the responses of soil NO emission to increasing N inputs; (3) investigate the role of nitrification/denitrification in NO emission at the studied forests.

2. Materials and Methods

2.1. Site Description

[8] The experiment was carried out in a broadleaf forest (BF) in climax successional stage and a pine forest (PF) in primary successional stage in Dinghushan Biosphere

Reserve ($23^{\circ}09'21''$ – $23^{\circ}11'30''\text{N}$, $112^{\circ}30'39''$ – $112^{\circ}33'41''\text{E}$). The reserve is located in the subtropical humid forest life zone with a monsoon climate. Annual mean relative humidity is about 80%. The 6-year-averaged annual rainfall is about 1927 mm with a distinct seasonal pattern. Typically the period from April to September is wet season, and that from October to March is dry season. March and October are transition periods from dry season to wet season and from wet season to dry season, respectively. Annual mean air temperature is about 21°C , with monthly means the lowest in January (13°C) and the highest in July (28°C).

[9] The broadleaf forest, about 250–300 m above sea level, has been protected from direct human interference for more than 400 years. The pine forest, about 50–200 m above sea level, has evolved under human disturbance, like collecting litter, since pine trees were firstly planted in the 1930s. The two forests vary not only in their stages of succession, but also in their species composition. The major species in the broadleaf forest are *Castanopsis chinensis*, *Schima superba*, *Cryptocarya chinensis*, *C. concinna*, *Machilus chinensis* in the tree layer and *Hemigramma decurrens* in the understory layer. Tree heights range from 4 to 30 m and diameters from 5 to 163 cm. The pine forest is dominated by *P. massoniana* with densities of 100–1000 trees ha^{-1} , diameters of 4–32 cm and heights of 3–11 m. Understory species included grasses, ferns, vines and shrubs for a total of 43 species [Mo *et al.*, 2006]. The soil in the two forests is lateritic red earth formed from sandstone. Some important characteristics of the two forests were presented by Li *et al.* [2007].

2.2. Experimental Design

[10] The experiment was arranged in a Randomized Complete Block design. In each forest, three blocks of seven plots (1 m \times 1 m each) were established. In each block, seven treatments, namely, NH_4Cl (AN), NaNO_3 (NN), $\text{NH}_4\text{Cl} + \text{Na}_2\text{HPO}_4$ (AP), $\text{NaNO}_3 + \text{Na}_2\text{HPO}_4$ (NP), Na_2HPO_4 (P), water control (WC), and blank control (BLK), were randomly applied to seven plots, respectively. Treatments with N fertilizer were applied at 5 g N m^{-2} (50 kg N ha^{-1}), and treatments with P fertilizer were applied at 4 g P m^{-2} . The fertilizers were dissolved in 5 L deionized water and sprayed on each plot uniformly with a hand-held sprayer. The water control plots were only sprayed with 5 L water; for the blank control plots nothing was added and the plots were just left under natural conditions. The treatments were completed within 30 min. Water added to the treated plots was equivalent to 5 mm rainfall.

[11] We applied 50 kg N ha^{-1} in order to simulate elevated N deposition that roughly doubles the current N deposition flux, which reached $38.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (wet deposition only) in the year of 1998–1999 [Zhou and Yan, 2001]. The aim of adding P was to investigate the influence of soil P status on NO emission when adding N.

2.3. NO Flux Measurement

[12] NO fluxes were measured by a dynamic flow-through chamber technique [Pilegaard *et al.*, 1999; Li *et al.*, 2007]. Briefly, the chambers, each covering an area of 30 cm \times 30 cm with a total volume of 9 L, were made of stainless steel and their inner walls were covered with Teflon films. Each chamber has one inlet port, one exhaust

port and one outlet port for sampling. Inside each chamber, a thermosensor was fixed to measure air temperature, and a fan attached to ensure sufficient mixing of air within the chamber. During field measurement, the sampling chambers were put onto the forest floor without soil frames to avoid disturbing the soils. Instead, they were sealed against the outward atmosphere with Teflon foil pinned down by sand bags [Gut *et al.*, 2002]. An additional reference chamber, closed at the bottom with Teflon sheet, was employed for in situ quantification of chemical reactions and chamber wall deposition effects [Kirkman *et al.*, 2002]. Ambient air was pumped into the chambers at a rate of 4 L min⁻¹ through Teflon tubes. The residence time of air in the chambers was about 2.25 min. After about 15 min (over 5 cycles of residence time) when a steady state was reached inside the chambers, NO was analyzed by a model 42C chemiluminescence NO-NO₂-NO_x analyzer (Zero noise 0.2 ppb and detection limit 0.4 ppb, Thermo Electron Corporation, USA). By the contrast of sampling chambers and the reference chamber, net fluxes from the soils could be obtained [Pilegaard *et al.*, 1999].

[13] No corrections were made for the possible removal of NO by chemical reactions taking place in the chambers or by absorption on the chamber wall due to reasons also described by Pilegaard *et al.* [1999]. Firstly, measurements with zero-air and ambient air showed no difference in the calculated fluxes, probably due to low concentrations of O₃ at the forest floor; secondly, very small changes in the concentrations of NO₂ were observed, and on average there was a deposition of NO₂ to the soil, and the NO₂ flux was found to be independent of the NO emission, which indicated that the NO + O₃ reaction did not play a significant role in the chambers.

2.4. Soil Parameter Measurement

[14] In parallel with NO flux measurements, soil temperatures at 5 cm depth, and volumetric water contents of 0–5 cm soil layer were also determined. Soil temperature was measured with soil temperature probes (TES, Ltd., China). Volumetric soil water content was measured with MPA-160 Moisture Probe Meter (ICT international, Australia). In this paper, the volumetric water content was converted to percent water-filled pore space (%WFPS) based on averaged soil bulk density in each forest [Davidson, 1993].

[15] At selected sampling days, soil samples were collected with a soil corer (2.5 cm inner diameter) at each plot for determination of KCl-extractable inorganic nitrogen pools (NH₄⁺-N and NO₃⁻-N (plus NO₂⁻-N)), net rates of N mineralization and nitrification. Briefly, after being taken back to laboratory the same day they were collected, the soil samples were thoroughly mixed and coarse stones and roots in the soils were removed by hand. A ca. 10 g fresh sample was extracted in 50 ml 2 M KCl and filtered through 0.45 μm membrane filter after shaking for 1 hour on an orbital shaker. The extracts were stored in the refrigerator at -18 °C for later analysis. NH₄⁺ was determined by indophenol blue method, and NO₃⁻ (plus NO₂⁻) was analyzed by copper-cadmium reduction method [National Standard Bureau of China, 1987]. Final values of N pools were reported as μg N g⁻¹ dry soil, where dry soils referred to constant weight after drying at 105°C. Net rates of N mineralization and nitrification were determined with a

7-day aerobic incubation method according to Piccolo *et al.* [1994]. A ca. 50 g soil sample was incubated in the dark at room temperature (25°C). After 7 days, the inorganic N pools were determined as described above. Net mineralization rates were determined from the difference between KCl-extractable inorganic N pools before and after the incubation, and the results were expressed on a basis of mean daily inorganic N production. Net nitrification rates were determined from the difference in NO₃⁻-N before and after the incubation, and the results were expressed on a basis of mean daily NO₃⁻-N production.

2.5. Statistical Analyses

[16] Reported data of each treatment were the mean of the triplicate plots on daily basis. ANOVA analyses with post hoc Turkey tests were performed for each sampling date using SPSS 10.0 (SPSS Ltd., USA) to identify differences between treatments within a forest, and independent samples T test was used to compare the fluxes in the same nutrient treatment plots of the two forests. In this paper, analyses with P values < 0.05 were considered significant.

[17] Mean gas fluxes over the sampling period were calculated after interpolating rates of fluxes [Stuedler *et al.*, 2002]. Total NO emissions (E_{total}) were calculated by multiplying mean fluxes by sampling days after fertilization, and net NO emission (E_{net}) was obtained by subtracting E_{total} of the water control plot from that of the corresponding N/P treated plots. Fraction of added N loss as NO (F_{NO}) was calculated based on N atoms as the following equation [Tilsner *et al.*, 2003]:

$$\text{emission factor} = \frac{\text{total N emission}_{\text{fertilizer treatment}} - \text{total N emission}_{\text{water control}}}{\text{applied N}} \times 100$$

3. Results and Discussion

3.1. NO Emission After N and N + P Addition

[18] As shown in Table 1, over the experimental period, averaged NO fluxes in AN, NN, AP and NP plots were 3.3, 2.3, 1.9 and 1.8 times that in the water control plots for the broadleaf forest; and 2.7, 1.6, 1.5 and 1.3 times that in the water control plots for the pine forest. In both forests, NO emissions were highest from AN plots, and were significantly greater from AN and NN plots than from AP and NP plots, respectively. This indicated that N addition alone enhanced NO fluxes more significantly than combined addition of N and P. P treatment alone had no significant effects on soil NO emission.

[19] In the broadleaf forest, NO fluxes in the NN plots were significantly higher than those in the NP plots from day 0 to day 2 (Figure 1). NO fluxes in AN plots were significantly higher than those in AP plots from day 7 to day 28. The case in the pine forest was similar (Figure 1). NO fluxes in the NN plots were significantly higher than those in the NP plots from day 14 to day 21, and those in the AN plots were significantly higher than in the AP plots from day 11 to day 40.

[20] Two nutrient addition experiments carried out in tropical rain forests also demonstrated that soil NO emissions responded differently to additions of N and N+P [Stuedler *et al.*, 2002; Hall and Matson, 2003]. At

Table 1. Averaged NO Fluxes (Flux, $\text{ng N m}^{-2} \text{s}^{-1}$), Total NO Emissions (E_{total} , mg), Net NO Emissions (E_{net} , mg), and Fraction of Added N Loss as NO (F_{NO} , %) After Fertilization in the Broadleaf Forest and Pine Forest in Comparison With F_{NO} From Other Forests

	BLK	C	AN	NN	AP	NP	P
<i>Broadleaf Forest</i>							
Flux	13.6	14.3	46.7 ^c	32.4 ^c	27.2 ^c	26.2 ^c	14.7
E_{total}	58.9	61.7	201.8	139.8	117.5	113.1	63.5
E_{net}			140.1	78.1	55.8	51.4	
F_{NO}			2.8	1.6	1.1	1.0	
<i>Pine Forest</i>							
Flux	16.4	18.3	49.0 ^c	29.5 ^c	28.0 ^c	23.3 ^c	18.0
E_{total}	70.9	79.1	211.6	127.2	121.0	100.5	77.8
E_{net}			132.4	48.1	41.8	21.3	
F_{NO}			2.6	1.0	0.8	0.4	
<i>Amazon Forests^a</i>							
F_{NO}			1.05		1.43	0.47	
<i>Hawaiian Tropical Forest (P Limited)^b</i>							
F_{NO}				2			

^a F_{NO} were calculated during a 14-day sampling period [Stuedler et al., 2002].

^b F_{NO} was calculated during a 7-day sampling period after NH_4NO_3 was applied [Hall and Matson, 1999].

^cAveraged NO flux in which treatment is significantly higher than that in the water control.

a P-limited forest in Hawaiian Islands, Hall and Matson [2003] found that NO emissions were greater in N (in the form of NH_4NO_3) addition plots than in N+P addition plots. However, in another study conducted in Brazilian Amazon, NO emissions from AP ($\text{NH}_4^+ + \text{PO}_4^{3-}$ addition) and NP ($\text{NO}_3^- + \text{PO}_4^{3-}$ addition) plots were greater than those from AN (NH_4^+ addition) and NN (NO_3^- addition)

plots, respectively [Stuedler et al., 2002]. The results of the present study were consistent with those reported by Hall and Matson [2003].

[21] The different responses of NO emissions to N and N+P additions are believed to result from the difference in soil nutrient status. Evidence indicates that for many tropical forests N often is not the limiting nutrient [see Martinelli et

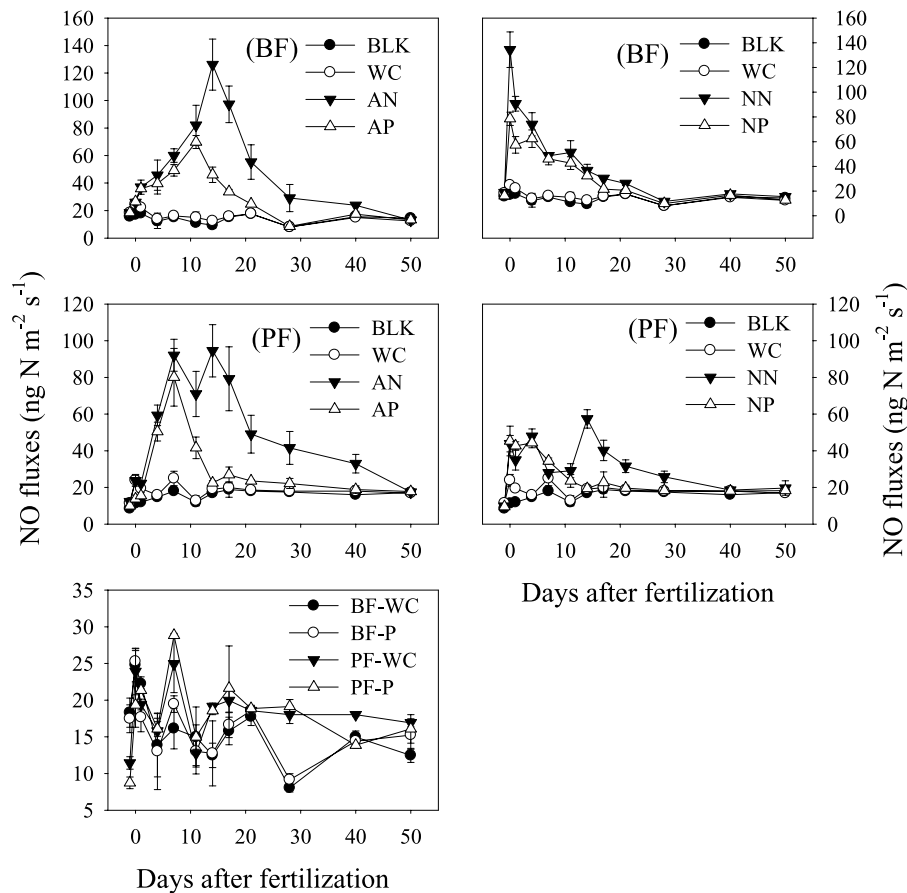


Figure 1. Variation of NO fluxes in the broadleaf forest (BF) and the pine forest (PF) over the sampling period. Error bars represent standard errors.

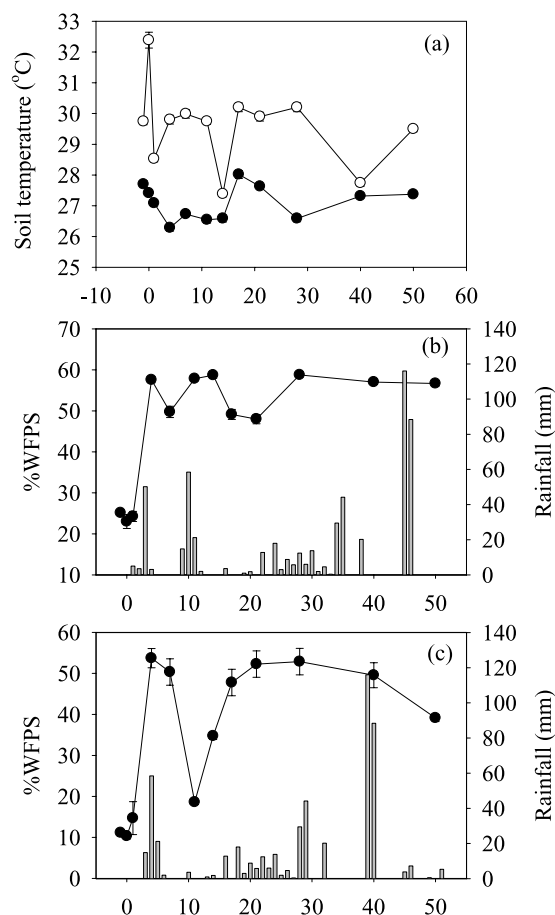


Figure 2. (a) Daily averaged soil temperature (solid circles for the broadleaf forest, open circles for the pine forest). (b) Percent water-filled pore space (% WFPS, solid circles) and rainfall (vertical bars) in the broadleaf forest. (c) Percent water-filled pore space (% WFPS, solid circles) and rainfall (vertical bars) in the pine forest. Values were the means of the seven treatments, except those of -1 , 0 , and 1 , which were the means of the blank control plots (number of days after fertilization, -1 indicates the day before fertilization and 0 indicates the fertilization day). Error bars represent standard errors.

al., 1999, and references therein]. Although no study was conducted with the aim to determine the N or P status in the studied sites, the broadleaf forest was suggested to be saturated with N while limited by P [Mo *et al.*, 2006, 2007]. Considering that the two forests have long been experiencing high N deposition, it is reasonable that soil P instead of N is likely the limiting factor for primary production. This assumption was also supported by the fact that NO fluxes increased dramatically in response to N addition in the two forests, similar to cases in other P-limited forests [Hall and Matson, 1999, 2003]. As explained by Hall and Matson [2003], in these forests that are not N-limited, plants and other microbes consumed much less input N when N was added alone; as a result, nitrifier and denitrifier utilized a much larger portion of the added N. However, when N and P were added in combination, plants and other microbes competed with nitrifier and denitrifier to use the added N because P limitation was relieved. As a

result, the portion of added N utilized by nitrifier and denitrifier decreased, and NO emissions from N + P addition sites were lower than those from N addition alone sites. Hall and Matson [2003] also observed that nitrification, denitrification, and N availability were smaller in the N + P plots compared to N plots, although variability was high. However, in the present study, no significant difference in N pools was found between N and N + P plots.

3.2. Controls on NO Emissions

3.2.1. Soil Temperature and Soil Moisture

[22] Soil temperature and soil moisture are among the major factors controlling soil NO emissions [Ludwig *et al.*, 2001]. As shown in Figure 2, in the present study, soil temperature over the experimental period varied in a narrow range in both forests (26.3–28.0°C for the broadleaf forest and 27.4–32.4°C for the pine forest). No significant relationship was found between NO fluxes and soil temperature in both forests probably due to the narrow temperature variation. %WFPS in the BLK plots in the first three sampling days was relatively low ($24.1 \pm 0.6\%$ for the broadleaf forest and $12.1 \pm 1.3\%$ for the pine forest) because of several days without rains, but increased after rainfalls in both forests in the following sampling days ($54.8 \pm 1.5\%$ for the broadleaf forest and $44.3 \pm 3.9\%$ for the pine forest). For the broadleaf forest, significant correlation ($P < 0.05$) between NO fluxes and % WFPS only existed in the blank control, water control and P addition plots; for the pine forest, significant correlation only existed in blank and water controls. The reason for the absence of correlation between NO emission and soil moisture in the N addition plots was probably that the effects of additional N masked those of soil moisture. However, for NO fluxes from AN and NN plots, the decrease at day 11 and the increase at day 14 were consistent with variation of % WFPS in the pine forest, probably indicating that soil moisture did play a role even in the N fertilization plots.

3.2.2. Nitrogen Availability

[23] Since pools of NO_3^- and NH_4^+ , net rates of nitrification and mineralization were all higher in the broadleaf forest than in the pine forest, soil N status is better in the broadleaf forest than in the pine forest [Figure 3, also see Li *et al.*, 2007]. This was largely because the pine forest was under human disturbance, such as collecting litter, since it was firstly planted in 1930s, while the broadleaf forest has been protected without direct human interference for more than 400 years. The difference of soil N status between the two forests was reflected by NO emissions. Due to greater available N status, more applied N would be left for nitrifier and denitrifier producing NO in the broadleaf, although averaged background NO flux (as reflected in the control plots) over the experimental period was significantly greater in the pine forest than in the broadleaf forest, which was mainly a result of soil moisture effect [Li *et al.*, 2007]. As indicated in Table 1, N addition-induced NO emissions (as reflected by E_{Net} and F_{NO}) in the broadleaf forest were higher than in the pine forest, especially for the NN, AP, and NP treatments, where significant difference in N addition-induced NO emission existed between the two forests.

[24] As shown in Figure 3, significant correlation existed between NO fluxes and soil NO_3^- pools in both forests. Since NO_3^- was a product of nitrification and a source

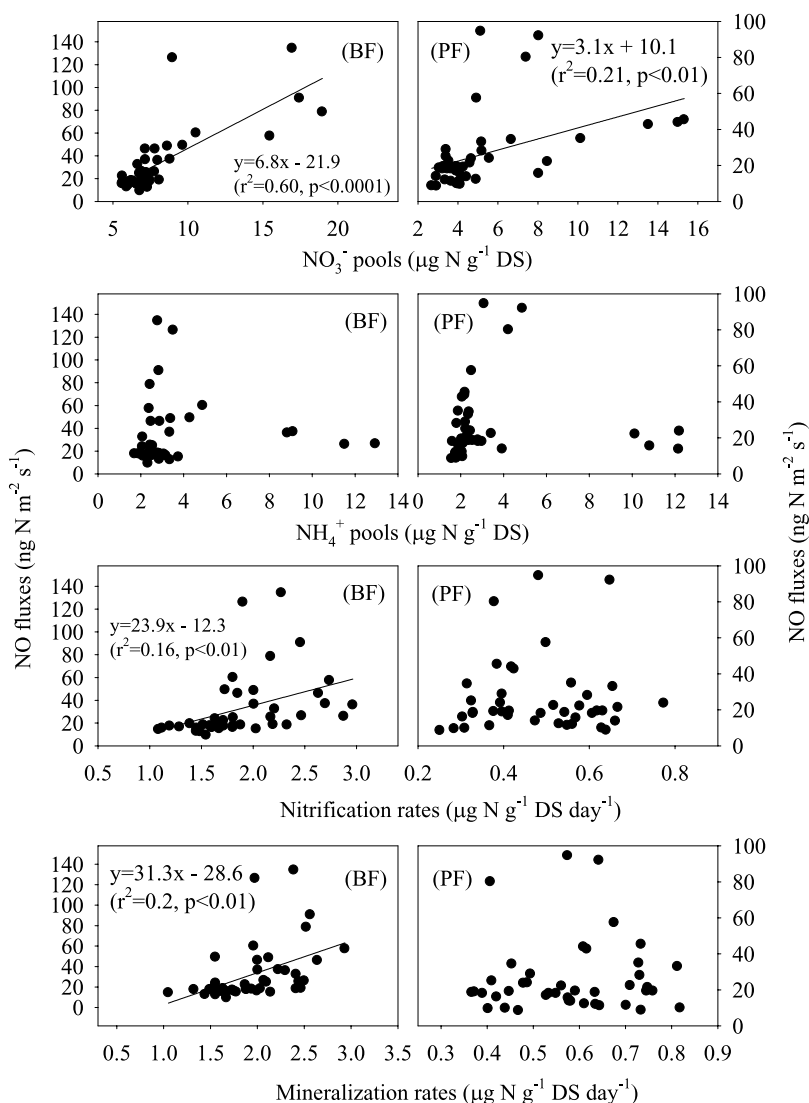


Figure 3. NO fluxes as a function of indices of N availability in the broadleaf forest (BF) and the pine forest (PF), $n = 42$.

material for denitrification, this strong correlation did not imply that nitrification dominated over denitrification in NO production. However, the significant correlation between NO fluxes and net rates of nitrification and mineralization did indicate that nitrification was a source of NO fluxes in the broadleaf forest.

[25] Since soil N availability is one of the most important factors controlling NO production, addition of NO_3^- or NH_4^+ was often found to increase soil NO emission [Ludwig *et al.*, 2001]. In some studies, addition of NO_3^- stimulated NO emissions greatly, while addition of NH_4^+ had little or even no effect [Kaplan *et al.*, 1988; Bakwin *et al.*, 1990; Sanhueza *et al.*, 1990; Cárdenas *et al.*, 1993]. In a study by Kaplan *et al.* [1988], addition of $200 \text{ kg NO}_3^- \text{ N ha}^{-1}$ to soils at a tropical forest resulted in 3- to 4-fold enhancement of soil NO fluxes over nontreated sites. In another experiment manipulated in a nearby forest, addition of NO_3^- caused 5- to 150-fold increase of NO fluxes just 30 min after treatment; meanwhile, one NH_4^+ treated plot showed a response similar to that of the NO_3^- treated plots, but other NH_4^+ treated plots showed no

pronounced increase in NO emissions [Bakwin *et al.*, 1990]. Sanhueza *et al.* [1990] also observed a 40-fold increase of NO emissions immediately after NaNO_3 application. Other studies, however, found NH_4^+ -based fertilizers elevated soil NO emissions the most [Serca *et al.*, 1994; Parsons and Keller, 1995; Steudler *et al.*, 2002]. For example, in the study by Steudler *et al.* [2002], NH_4^+ addition increased NO fluxes by 4- to 9-fold in an Amazon forest.

[26] NO emission also showed different patterns in the two forests after addition of NH_4^+ -N and NO_3^- -N. As shown in Figure 1, in the present study, NO fluxes increased sharply in the treatment day for the NN and NP treated plots in both forests, especially in the broadleaf forest, where NO fluxes for the NN and NP treated plots reached their peak values of 5.4 and 3.2 times the water controls, respectively. For the NH_4^+ -N treated plots, after AP fertilization NO fluxes peaked 7–11 days for both the pine forest (~ 3.2 times the water control) and the broadleaf forest (4.7 times the water control), while NO fluxes after AN fertilization peaked 14 days after fertilization for the pine forest (4.9 times the water control) and the broadleaf forest

(10.1 times the water control). Except for the AP plots, peak fluxes of the other N treatments in the broadleaf forest were higher than those in the pine forest. Increase of NO fluxes after NO_3^- addition indicated denitrification was responsible for NO production in both forests, especially in the broadleaf forest where denitrification played a stronger role than in the pine forest; while increase of NO fluxes after NH_4^+ addition indicated nitrification existed in both forests. However, nitrification should be more important in both forests especially in the pine forest, since more NO was lost from the NH_4^+ -N addition plots.

[27] The different responses of the two forests to different N forms might result from their different soil properties. Due to its higher content of soil organic matter and clay, the soil in the broadleaf forest had a better water holding capacity [Li *et al.*, 2007], which is defined as the ability of a soil to retain water against the pull of gravity and is positively correlated with content of soil organic matter and clay [Khaleel *et al.*, 1981; Zhang and Zhuo, 1985]; on the contrary, water drains more easily from the coarser textured soil under the pine forest. For this reason and other properties like much more developed root systems in the broadleaf forest, even in wet season WFPS in the pine forest would not reach levels as high as those in the broadleaf forest owing to its poorer water holding capacity (Figure 2). As WFPS is negatively correlated with gas diffusibility, the soil in the broadleaf forest would be more anoxic. Due to this more anoxic environment for soil micro-organisms in the broadleaf forest, denitrification would be stronger in the broadleaf forest than in the pine forest. That is, most likely why NO fluxes peaked earlier and were much higher in the broadleaf forest than in the pine forest when NO_3^- -N was added. Accordingly the peak fluxes in NH_4^+ added plots occurred earlier in the pine forest where nitrification was favored more than in the broadleaf forest due to lower %WFPS and higher porosity.

3.3. Loss of Added N as NO

[28] As shown in Table 1, in both forests AN plots had the greatest fraction of applied N lost as NO (F_{NO}). For the broadleaf forest and the pine forest, F_{NO} in the AN plots were 2.8% and 2.6%, respectively. For all N treated plots F_{NO} were higher in the broadleaf forest than their counterparts in the pine forest; and in each forest; F_{NO} in plots treated with N alone were greater than those in plots treated with N and P.

[29] In an Amazon forest [Stuedler *et al.*, 2002], NO emissions accounted for 0.47%, 1.05%, and 1.43% of the applied N in NP, AN and AP treated plots, respectively. Our study observed comparatively greater F_{NO} at AN treated plots in the two forests (Table 1). In another study conducted in a Hawaiian tropical forest (P limited), about 2% of the applied N (as NH_4NO_3) was lost as NO over a 7-day sampling period [Hall and Matson, 1999]. This fraction was higher than those at plots applying AN or NN in the present study over the same period. Unlike our results, Stuedler *et al.* [2002] found plots treated with NP or AP had greater F_{NO} than those treated with NN or AN, respectively, in a mature moist tropical forest in the Brazilian Amazon.

3.4. Implications of the Current Study

[30] As observation in the present study, N addition alone enhanced NO emission greater than combined addition of N and P, indicating that NO emissions from more P-limited

sites were higher than from more N-limited (N+P addition) sites in response to N addition. This was consistent with that reported by Hall and Matson [1999], who found that N addition to P-limited forests induced NO and N_2O fluxes 10–100 times higher than those from the nearby N-limited forests treated with the same fertilizer. As most temperate forests in northern hemisphere were N-limited, while most tropical forests were P-limited, increase of N deposition would likely induce more NO emissions from tropical forests than from temperate forests.

[31] As noted above, forests lost equally large amounts of NO after first-time N additions as after chronic, long-term N additions [Hall and Matson, 1999]. In the present study, E_{net} also represented the emission increment potential after N deposition doubled in this region since the amount of applied N in the present study was roughly twice the annual wet N deposition to the two forests.

[32] Annual NO emissions in the broadleaf forest and the pine forest were estimated to be 6.5 and 4.2 kg N $\text{ha}^{-1} \text{yr}^{-1}$, respectively, based on the observed NO flux data throughout the year 2005 [Li *et al.*, 2007]. Since N deposited to forests are mainly in the form of NH_4^+ and NO_3^- , NO emission increment for additional N deposited in the forest would likely lie between those in AN and NN treated plots. Thus according to our experiments NO emissions will likely increase by 12.0%–21.5% in the broadleaf forest and by 11.5%–31.5% in the pine forest for 5 g N m^{-2} more N deposition, which almost doubles the present deposition rates in the two forests.

[33] With development in less developed regions, over 60% of the N fertilizers will be used in the tropics and subtropics by 2020; at the same time fossil fuel use is expected to increase by several times in many regions of the tropics over the coming decades [Galloway *et al.*, 1994]. Assuming ca. 22 Tg N yr^{-1} will be deposited on tropical forests by year 2050, Hall and Matson [1999] estimated an enhanced NO emission of 0.4 Tg N yr^{-1} from tropical forest floor, and 0.2 Tg N yr^{-1} into the atmosphere if 50% of the NO will be taken up by the forest canopy, which is about 18% of current NO emission from soils of tropical evergreen forests as estimated by Davidson and Kinglerlee [1997]. In the present study, if the fraction of the input N loss as NO was calculated as the average of those from the AN and NN treated plots since ambient N deposition includes both NH_4^+ and NO_3^- , then the fraction of input N loss as NO would be 2.2% in the broadleaf forest, and 1.8% in the pine forest. These percentages are quite similar to that (2%) measured by Hall and Matson [1999] in Hawaiian Islands. It seems that for additional N deposition 2% would be an appropriate percentage of NO loss from tropical forest floors.

4. Conclusions

[34] By applying N and/or P to forest floors, we found that N addition alone enhanced NO emission more than combined addition of N and P in both a mature and a primary humid subtropical forest in south China. This indicated that soil nutrient status played an important role in soil NO emission response to additional N deposition. N deposition would likely induce more NO emissions in the P-limited tropical forests than in most temperate N-limited forests. NO

emission responded differently to the addition of different N forms, and NO emission pattern also varied between the two forests due to difference in soil properties. On average, N loss as NO from the forest floors was approximately 2% of the applied N, quite similar to that measured by Hall and Matson [1999] in the Hawaiian Islands.

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J. Fu, D. Li, G. Sheng, and X. Wang, State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, P.O. Box 1131, Wushan Guangzhou, Guangdong 510640, China. (wangxm@gig.ac.cn)

J. Mo, South China Botanical Garden, Chinese Academy of Sciences, Xingke Road 723, Tianhe District, Guangzhou 510650, China.