OPEN

Correspondence and
requests for materials
should be addressed to
Z.L. (lizan@scbg.ac.
cn)

* These authors
contributed equally to
this work.

Nitrogen and phosphorus addition impact soil N_2O emission in a secondary tropical forest of South China

Faming Wang[1]*, Jian Li[1]*, Xiaoli Wang[1], Wei Zhang[1], Bi Zou[1], Deborah A. Neher[2] & Zhian Li[1]

[1]Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510160, PR China, [2]Department of Plant and Soil Science, University of Vermont, Burlington, VT 05405, U.S.A.

Nutrient availability greatly regulates ecosystem processes and functions of tropical forests. However, few studies have explored impacts of N addition (*a*N), P addition (*a*P) and N×P interaction on tropical forests N_2O fluxes. We established an N and P addition experiment in a tropical forest to test whether: (1) N addition would increase N_2O emission and nitrification, and (2) P addition would increase N_2O emission and N transformations. Nitrogen and P addition had no effect on N mineralization and nitrification. Soil microbial biomass was increased following P addition in wet seasons. *a*N increased 39% N_2O emission as compared to control (43.3 μgN_2O-N m^{-2}h^{-1}). *a*P did not increase N_2O emission. Overall, N_2O emission was 60% greater for *a*NP relative to the control, but significant difference was observed only in wet seasons, when N_2O emission was 78% greater for *a*NP relative to the control. Our results suggested that increasing N deposition will enhance soil N_2O emission, and there would be N×P interaction on N_2O emission in wet seasons. Given elevated N deposition in future, P addition in this tropical soil will stimulate soil microbial activities in wet seasons, which will further enhance soil N_2O emission.

Nitrous oxide (N_2O) is a long-lived (about 120 years) trace gas that has 310 times the ability of greenhouse effect in the atmosphere compared to carbon dioxide (CO_2)[1,2]. Nitrous oxide accounted for 7.9% of the global anthropogenic greenhouse gas emissions in 2004[2]. It has also been the most dominant ozone-depleting substance since 2009[3,4]. The atmospheric N_2O concentration has increased by about 120% since 1750, from around 270 ppb, to 324 ppb in 2011[4]. Human activities are responsible for 40–50% of the annual increase in N_2O emissions over its pre-industrial levels, with about 57% of the global atmospheric sources of N_2O are estimated to be related to emissions from soils[5]. Thus, there is little doubt that terrestrial soils are the most important sources of atmospheric N_2O.

Nitrous oxide was mainly produced by the microbial processes of nitrification and denitrification in soils[4]. Nitrification and denitrification can occur simultaneously in soils, although the relative rates of the two processes depend on soil aeration and micro-site availability of substrates[6]. Soil N_2O emissions from nitrification and denitrification react sensitively to soil water content, soil temperature, and available N and C in the soils, as well as some other physical and chemical properties of the soil such as texture and pH[7,8]. Increased soil N availability as a result of N inputs by atmospheric deposition has greatly increased N_2O emissions from soils[9]. In a meta-analysis, N addition (mainly NH_4^+ and NO_3^-), ranging from 10 to 562 kg N ha^{-1}yr^{-1}, significantly increased N_2O emission by an average of 216% across various ecosystems[10].

Tropical forests currently represents the largest terrestrial sink for anthropogenic CO_2 emissions, accounting for 59% of the global C pools in forests[11,12]. Recent studies suggested that tropical forests have a key role not only in the C cycle but also in the global atmospheric balance of N_2O[7,13,14]. IPCC[2] in 2007 estimated that N_2O emission in tropical forests was 4.4 Tg N yr^{-1}, accounting for 14 to 23% of all atmospheric N_2O sources. A biogeochemical model estimated 0.9–2.4 Tg N yr^{-1} N_2O emission in tropical rainforests[7]. Recent observations of the N_2O atmospheric column suggested that 50–64% of the atmospheric N_2O was derived from the tropical zone with large temporal variation[15,16]. Furthermore, N addition to a tropical montane forest in Panama increased the N losses via nitrate leaching and N_2O emission[17].

Although our knowledge of sources and sinks of N_2O in tropical forests is increasing, there still remain major knowledge gaps in our understanding of N_2O emission from tropical forests. Differing in soil nutrient status from temperate forests which are usually limited by N supply[18], tropical forests lying on highly weathered soils are

1

Table 1 | Soil physical and chemical characteristics (0–10 cm) in the tropical forest before the start of fertilization (conducted in September 2009)

Variables	aN	aP	aNP	Control
pH(H$_2$O)	3.97 ± 0.05	3.95 ± 0.05	4.02 ± 0.09	3.99 ± 0.06
SOC (%)	2.90 ± 0.12	2.86 ± 0.27	2.90 ± 0.17	2.54 ± 0.16
Total N(g kg^{-1})	2.34 ± 0.21	2.66 ± 0.10	2.68 ± 0.19	2.71 ± 0.15
Total P(g kg^{-1})	0.38 ± 0.02	0.42 ± 0.02	0.43 ± 0.03	0.40 ± 0.03
Available-P(mg kg^{-1})	3.79 ± 0.42	4.06 ± 0.37	3.70 ± 0.60	4.10 ± 0.56
NO$_3^-$-N(mg kg^{-1})	2.72 ± 0.11	2.68 ± 0.31	2.35 ± 0.33	2.88 ± 0.35
NH$_4^+$-N(mg kg^{-1})	1.85 ± 0.13	1.81 ± 0.11	2.03 ± 0.17	2.12 ± 0.12

Note: Data are expressed as means ± SE (n = 5). aN: N addition treatment; aP: P addition treatment; aNP: N and P co-addition treatment.

relatively rich in available N and poor in available P[19,20]. As a result, the productivity is likely limited by P[20,21]. If input of N exceeded the biotic demands, the ecosystem N retention capacity would be exceeded, and gaseous losses of N as N$_2$O would increase[22]. P-limited forests thus could emit more N$_2$O than the N-limited forest after N fertilization[23]. Hall and Matson[23] observed a 54 times higher soil N$_2$O emission in P-limited site than N-limited site after N addition in two tropical forests. Furthermore, recent studies have shown that anthropogenic N deposition is increasing dramatically in tropical regions[24,25] and P deposition may already play an important role as a source of P in tropical regions[26].

The increased nutrient deposition may alleviate nutritional constraints on aboveground plants and soil microbial activities[20,21,27], which would alter soil N$_2$O emissions. Recent work indicated that P application reduced N$_2$O emissions by reducing soil inorganic N due to increased N uptake by plants in a tropical plantation[28] and in a P limited soils[8]. However, Mori et al.[29] found that P addition in a tropical plantation soil increased N$_2$O emission under high soil water content. Some researchers argued that P-enrichment stimulated soil denitrifiers and nitrifiers which would enhance the N$_2$O emission from soils[8,30,31]. White and Reddy[32] found that P addition increased microbial biomass and denitrifying enzyme activity in soil. Moreover, previous study has observed that P addition in tropical forests could alter the composition of soil microbial community[27]. These results suggested that both aboveground plants and belowground microbes would affect the response of soil N$_2$O emission to increased nutrients availability in tropical forests. However, direct evidence of soil N and P availability on soil N$_2$O emission in tropical forests is still rare[33], and most of the studies neglected the interaction of N and P availability on soil N cycling[23,28,29].

In this study, we conducted a manipulative experiment with a randomized block design to investigate N and P addition and their interaction on soil N$_2$O emission and N transformations in a secondary tropical forest in southern China. We hypothesized that: (1) N addition would increase soil N$_2$O emission and nitrification due to increased substrates for nitrification and denitrification, and (2) P addition would increase soil N$_2$O emission and soil N transformation rates due to stimulated activities of soil microbes from the relief of P limitation in the secondary tropical forest.

Results

Soil available nutrients and N transformations. The general soil properties before fertilization were shown in Table 1. Over the study period, soil NH$_4^+$ concentrations were greatly increased by N addition ($P < 0.001$, Fig. 1A). There was a gradual increase of soil NH$_4^+$ with the time of N addition. In Sep. 2010, one year after the beginning of nutrients addition, the NH$_4^+$ concentration in N-addition plots (aN and aNP) was 2.52 mg/kg, which was about 20% higher than the value in control plots. This percentage in June 2011 and June 2012 became 65% and 67%, respectively (Fig. 1A). Similar to soil NH$_4^+$, soil NO$_3^-$ was significantly increased by N addition since September 2010 ($P < 0.001$. Fig. 1B). Soil NO$_3^-$

concentration under aN and aNP plots increased with the duration of fertilization until June 2011. In June 2011, aN and aNP plots had nearly 3 times higher NO$_3^-$ values than control and aP plots. After that sampling, a decline of soil NO$_3^-$ was observed in aN and aNP plots (Fig. 1B). Soil available P was greatly enhanced by P addition, while N addition and their interactions has no effect on available P. There was also a gradual increase of soil available P with the duration of P addition. In June 2012, soil available P in aP and
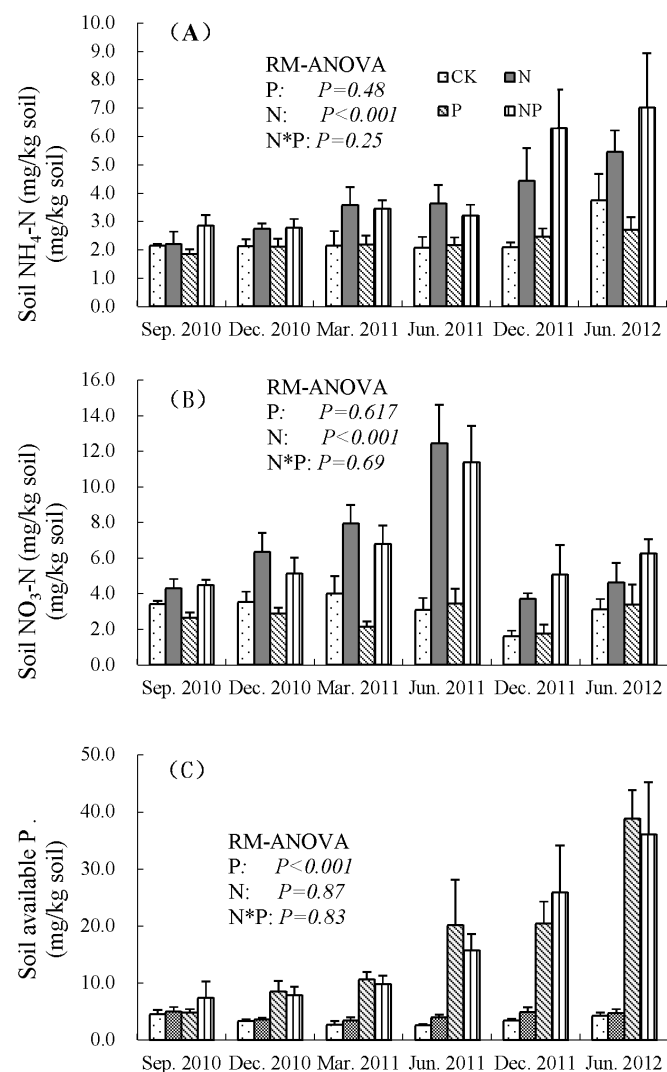

Figure 1 | **Concentrations of soil extractable NH$_4$-N, NO$_3$-N, and available P under different treatments in the secondary tropical forest of Xiaoliang station.** Data are illustrated as means; Error bars represent 1 SE, n = 5.
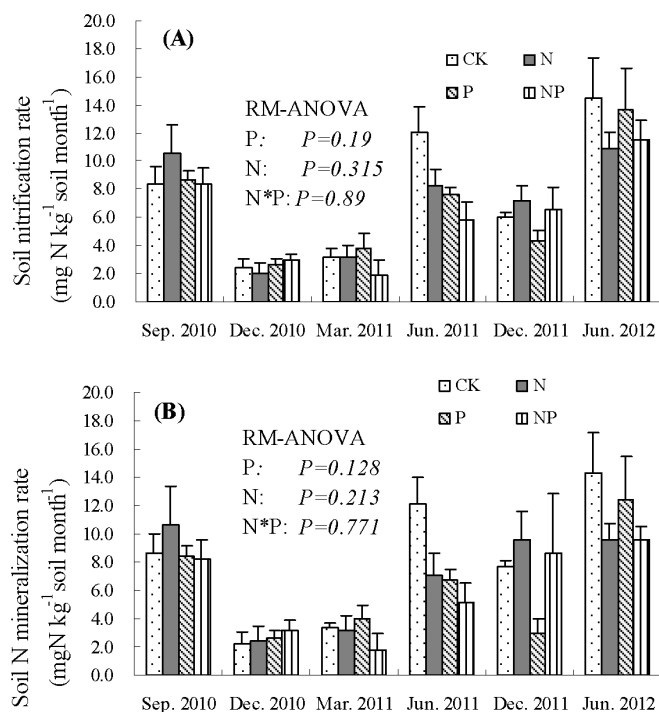
2

Figure 2 | Soil net nitrification and N mineralization rates in the four experiment treatments in the secondary tropical forest of Xiaoliang station. Data are illustrated as means; Error bars represent 1 SE, $n = 5$.

aNP plots were approximately 40 mg/kg, which was nearly ten times as much as the values in control and aN (Fig. 1C).

Neither soil net nitrification nor net N mineralization rates were significantly affected by nutrients addition or their interactions in the 3 yrs experiment (Fig. 2A&B). However, the seasonal pattern of soil

N transformations was obvious. In the dry seasons (Dec. 2010, Mar. 2011, Dec. 2011), soil N transformation rates were always lower than those in the wet season. Furthermore, we observed a high rate of soil nitrification in comparison with N mineralization rate, with the ratio of nitrification to N mineralization was over one. Soil nitrification rate accounted for nearly 88.9% variation of soil N mineralization rate (r = 0.943, $P < 0.001$).

Soil microbial biomass. The seasonal variation of soil MBC and MBN was very obvious (Fig. 3). In the wet seasons, soil MBC and MBN usually had two times values than the corresponding data in the dry seasons. We also found that the response of soil microbe to N and P addition differed among seasons. P addition significantly increased soil MBC and MBN only in wet seasons ($P = 0.02$, and $P = 0.03$, respectively, Fig. 3A&C). However, neither N addition nor N×P interactions significantly affected soil MBC or MBN in either season.

Soil N_2O emissions, temperature and Water Filled Pore Space (WFPS). Nitrogen addition significantly increased soil N_2O emission in this tropical forest ($P < 0.001$, Fig. 4). The mean soil N_2O emission in aN and aNP were 60.2 and 69.4 μg N_2O-N m^{-2}h^{-1}, which were 39% and 60% higher than that in the control (43.3 μg N_2O-N m^{-2}h^{-1}), respectively (Table 2). The lowest N_2O emission was observed in aP (41.8 μg N_2O-N m^{-2}h^{-1}), which was similar to the control, but significantly lower than aN and aNP (Table 2). Moreover, the temporal variation of soil N_2O emission was obvious. Soil N_2O emission was higher in wet seasons than that in dry seasons (Table 2). In wet seasons, N addition significantly increased N_2O emission ($P < 0.001$, Table 2), and the interaction of N and P addition marginally significantly affected soil N_2O emission ($P = 0.092$, Table 2). aNP had the highest N_2O emission (97.4 μg N_2O-N m^{-2}h^{-1}) in wet seasons, which was significantly higher than the second highest - aN (78.8 μg N_2O-N m^{-2}h^{-1}). However, in dry seasons, we only observed a marginally significant effect of N addition on N_2O emission ($P = 0.06$, Table 2).
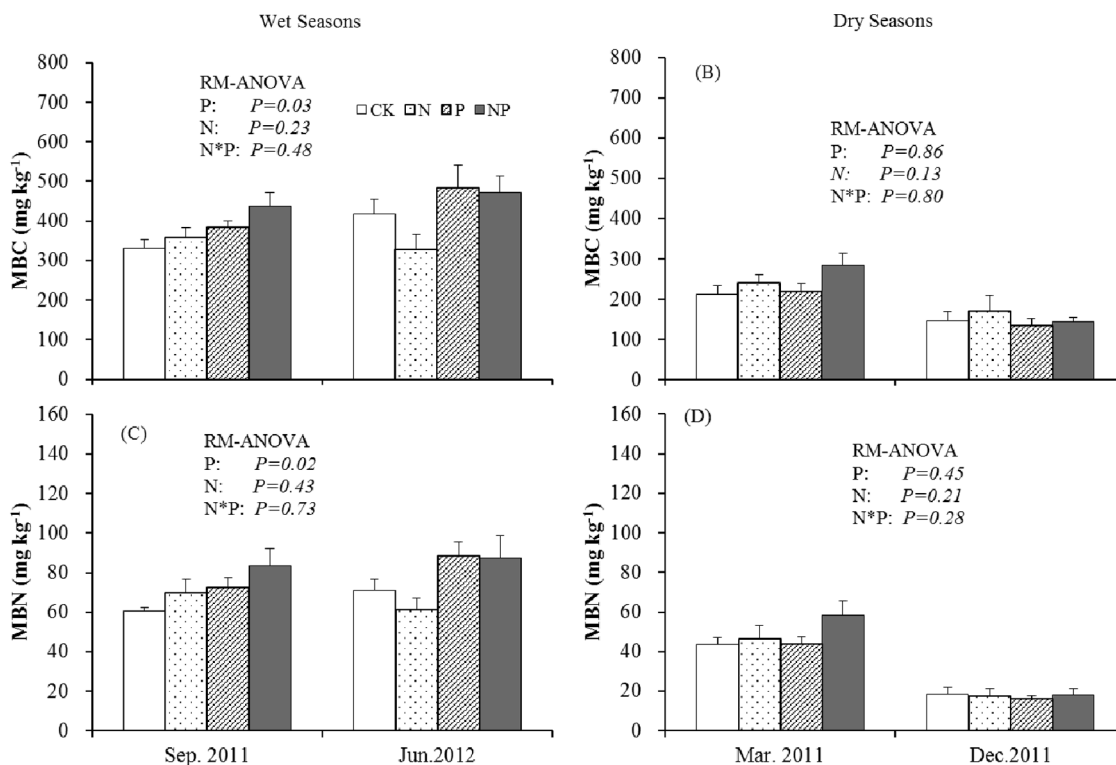

Figure 3 | The wet season and dry season soil microbial biomass C (MBC) and N (MBN) in the four experiment treatments of the secondary tropical forest of Xiaoliang station. Error bars represent 1 SE, $n = 5$.
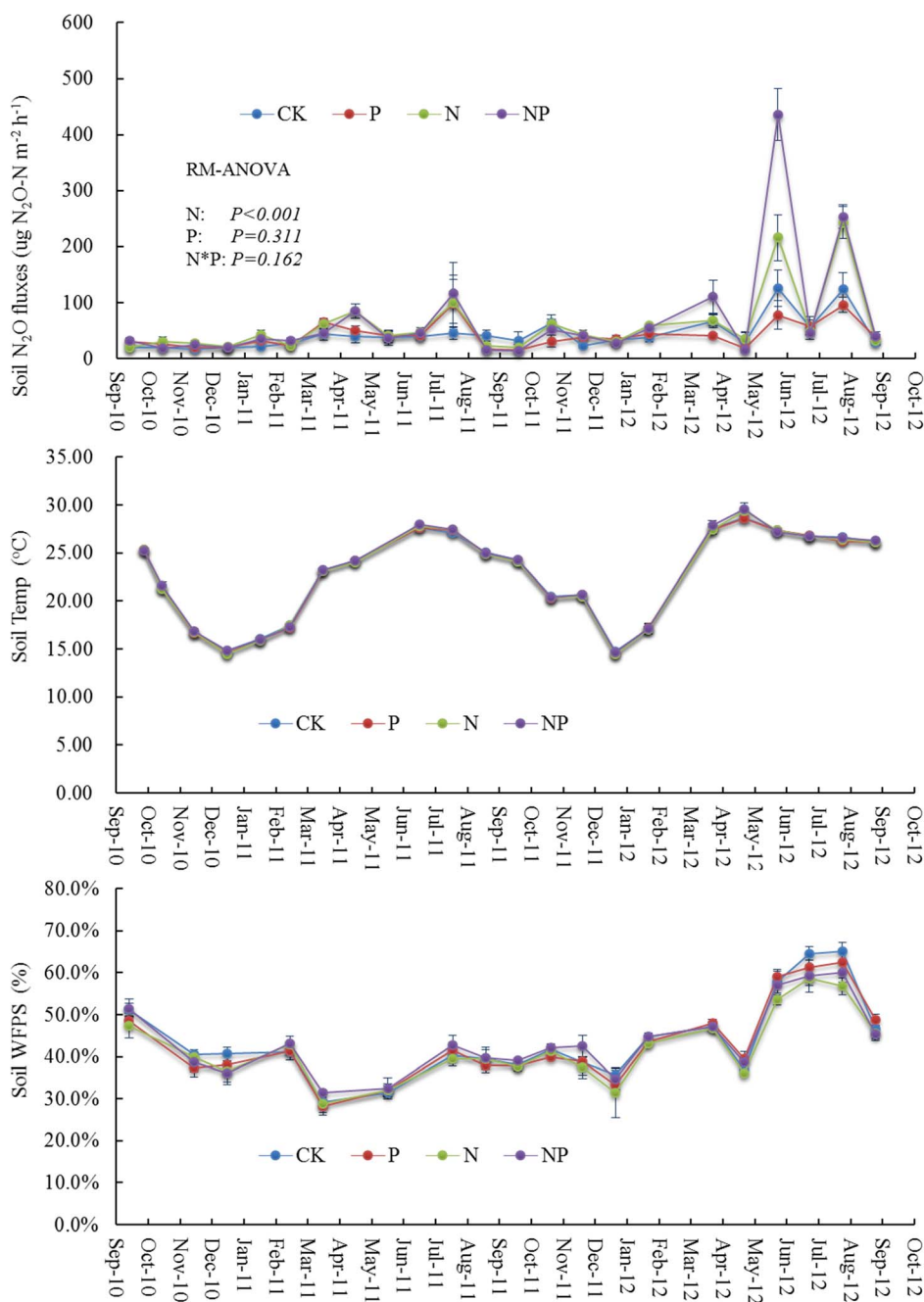
Figure 4 | Monthly soil N₂O emission, temperature and WFPS in the secondary tropical forests of Xiaoliang station from October 2010 to September 2012.

Soil temperature had a clear seasonal pattern with the highest temperature in wet seasons and the lowest in dry seasons (Fig. 4). However, N and P addition neither affected soil temperature nor soil WFPS. Soil WFPS also shows a seasonal variation with higher soil WFPS in wet seasons and lower in dry seasons (Fig. 4). Both soil temperature and WFPS was positively correlated with soil N₂O emission in the control plots along the two years duration (Soil temperature vs N₂O: $R^2 = 0.18$, $P < 0.01$; Soil WFPS vs N₂O: $R^2 = 0.26$, $P < 0.01$).

Pathways determining soil N₂O emission by N and P addition. Due to the large seasonal difference of the soil microbial and N₂O emission variables, we conducted SEM (structural equation model) analyses separately for wet season and dry season data (Fig. 5). Consistent

with the ANOVA results (Fig. 1), both of wet and dry season SEM showed that N addition significantly increased soil inorganic N concentrations, and P addition significantly increased soil available P ($P < 0.05$ for all, Fig. 5A&B). In wet seasons, the final SEM adequately fitted the data describing interaction pathways analysis of N and P addition on soil N₂O emission and nitrification (Probability level = 0.416, Fig. 5A). The final wet season SEM explained 58% of the variation in soil N₂O emission and 21% of the variation in soil nitrification. The increased soil NO_3^- by N addition significantly increased N₂O emission ($P < 0.05$, Fig. 5A), while soil NH_4^+ had no direct effect on N₂O emission. The increased soil available P after P addition marginally significantly increased soil MBC ($P = 0.06$, Table S1), which further increased soil N₂O emission ($P < 0.05$, Fig. 5A). In the dry season, P addition did not have clear

Table 2 | Mean soil N_2O emission from 2010 to 2012 after N and P additions in the secondary tropical forest of South China (Mean ± SE, n = 5)

Treatments	Soil N_2O emission (ug N_2O-N $m^{-2}h^{-1}$)		
	Total	Wet Seasons	Dry Seasons
Control	43.3 ± 3.6[b]	54.6 ± 5.6[c]	28.6 ± 3.0[b]
aP	41.8 ± 3.3[b]	50.5 ± 5.1[c]	30.2 ± 2.5[b]
aN	60.2 ± 6.0[a]	78.8 ± 9.6[b]	36.1 ± 2.9[a]
aNP	69.4 ± 9.2[a]	97.4 ± 15.0[a]	33.7 ± 2.3[ab]
P-values of RM-ANOVA			
P	0.311	0.201	0.548
N	**<0.001**	**<0.001**	0.063
N*P	0.162	0.092	0.547

Note: Different letters denote significant difference (P < 0.05) between treatments by LSD. *aN*: N addition treatment; *aP*: P addition treatment; *aNP*: N and P co-addition treatment.

Figure 5 | **The final structural equation model of N and P addition effects on soil N transformations and N_2O emission in the secondary tropical forest in wet seasons ($X^2 = 16.53$; df = 16, Probability level = 0.417; RMSEA = 0.04; AIC = 56.5) and dry seasons ($X^2 = 11.51$; df = 13, Probability level = 0.568; RMSEA = 0.00; AIC = 57.5).** Numbers on arrows are standardized path coefficients (equivalent to correlation coefficients). Width of the arrows indicates the strength of the causal influence. Green arrows indicate significant positive relationships and red arrows indicate significant negative relationships ($P < 0.05$). Black arrows indicate nonsignificant positive relationships and grey arrows indicate nonsignificant negative relationships ($P > 0.05$). Grey dashed arrows indicate paths removed to improve model fits (see Methods). Percentages close to Nitrification and N_2O flux indicate the variance explained by the model (R^2).

pathway to affect soil N transformations, and the final SEM only explained 7% of the variation in soil N_2O emission and 23% of the variation in soil nitrification. Soil NO_3^- was negatively correlated with nitrification rate in dry season ($P < 0.05$, Fig. 5B). The relationships between the remaining exogenous and endogenous variables were not significant, but improved the model fit (Table S1).
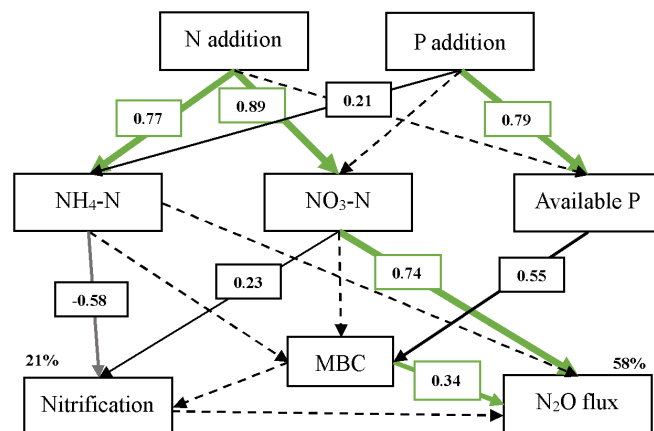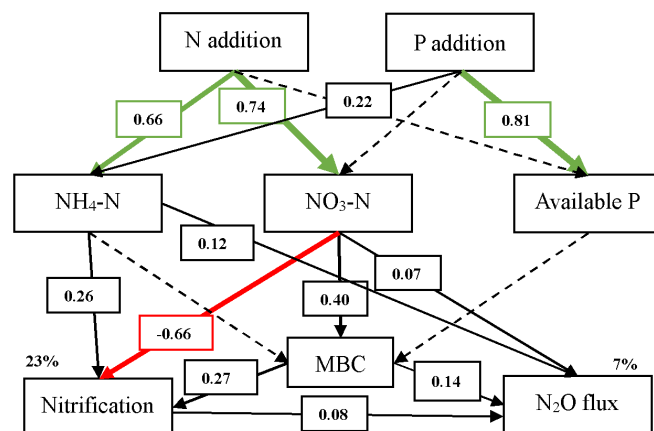
Discussion

The soil N_2O emission in the secondary tropical forest ranged from 28.6 μg N_2O-N $m^{-2}h^{-1}$ in dry season to 54.6 μg N_2O-N m^{-2} h^{-1} in wet season, with an average N_2O emission of 43.3 μg N_2O-N $m^{-2}h^{-1}$. This value is lower than the N_2O emission reported by Tang et al.[34] for a nearby old-growth tropical forest (84 μg N_2O-N m^{-2} h^{-1}), and by Konda et al.[35] for an *Acacia mangium* plantation in Indonesia (77.01 μg N_2O-N m^{-2} h^{-1} in wet season), but is higher than the data reported in many African, South American, and Australian tropical forests. Castaldi et al.[14] reported an average 26.6 μg N_2O-N m^{-2} h^{-1} emission for a rainforest in Africa. Werner et al.[36] found that the N_2O emission in a Kenya rainforest was 42.9 μg N_2O-N m^{-2} h^{-1}, which was comparable to our data. Two other studies estimated lower N_2O emission than our study, i.e., 24.2 μg N_2O-N m^{-2} h^{-1} in a moist tropical forest in the Amazon Basin[37] and 16.3–24.3 μg N_2O-N m^{-2} h^{-1} in a lowland tropical rainforest in Australia during wet season[38].

The high atmospheric N deposition might explain the relatively high N_2O emission data in this secondary tropical forest. South China is a region suffering from serious N deposition due to the rapid industrial growth. Bulk N deposition (NH_4^+ + NO_3^-) ranged from 16.2 to 38.2 kg N $ha^{-1}yr^{-1}$ in 14 tropical and subtropical forests of South China[39]. There was also an approximately 40 kg N $ha^{-1}yr^{-1}$ atmospheric wet N deposition (DON+ NH_4^++ NO_3^-) in this forest (Wang et al. unpublished). In a nearby old-growth forests with 84 μg N_2O-N m^{-2} h^{-1} emission[34], the bulk N deposition was measured at 38 kg N $ha^{-1}yr^{-1}$in 1999[40]. Moreover, we have found that N addition have greatly enhanced N_2O emissions from soils in this tropical forest (See below).

In the present study, N addition enhanced soil N_2O emission by 39–60% in comparison to control. The increased N_2O emission following NH_4^+ or NO_3^- addition was observed in many N-saturated ecosystem[6,33,41,42]. In this study, the ratio of net nitrification to net N mineralization was >1, which was a common phenomenon in many P-limited forests[33]. The value of this ratio suggested that the majority of N that is mineralized is also nitrified, and the soil of this tropical forest is not a strong consumer of N (N saturated)[33]. In N saturated forests, if inputs of N exceeded the biotic demands for N, the ecosystem N retention capacity would be exceeded, with the potential for increased leaching losses of N as NO_3^- and DON and elevated gaseous losses of N as N_2O and N_2[22]. Liu and Greaver[10] reported that

tropical forest emitted more N_2O (on average +739%) under N enrichment compared with other ecosystem types. Hall and Matson[33] suggested that N_2O emissions from P-limited tropical forests would be especially sensitive to N inputs, since they might respond to even small initial N additions with larger losses than predicted by models developed for N-limited temperate forests. In a P-limited Hawaiian forest, N_2O emissions increased rapidly and by much larger amounts than in a nearby N-limited forest after N additions[23].

Nitrous oxide was mainly produced by the microbial processes of nitrification and denitrification in soils[4]. The increased soil NH_4^+ and NO_3^- after N addition would increase N substrates to nitrification and denitrification processes. However, N addition did not

increased soil net nitrification in the present study, which is contrary to our hypothesis. In the final SEM models, no significant relationships were observed between soil net nitrification and soil NH_4^+, and the final model only explained 21%–23% variation of soil net nitrification in wet and dry seasons. This finding supports Hall and Matson[33] who also found that N addition did not change soil net nitrification in a P limited tropical forest. One explanation was that the method we used to quantity nitrification here was net nitrification rate, which was basically calculated from the change of the soil NO_3^- pool across time by incubation, thereby disregarding consumption of nitrate by assimilation and denitrification. Direct comparisons of net and gross nitrification rates reveal that net nitrification rates can be an order of magnitude lower than gross nitrification rates and, thus, changes in the NO_3^- pool do not necessarily reflect the total N turnover from NH_4^+ to NO_3^-[38].

Besides nitrification, denitrification process is also an important source of N_2O flux. From a kinetic perspective, denitrification is assumed to be most efficient in soils with prevailing warm and moist conditions[7,24]. This secondary tropical forest in wet seasons thus has favorable conditions for denitrification to emit considerable amounts of N_2O, i.e., low oxygen content due to high water-filled pore-space (WFPS) and temperature high enough to allow microbial activity to occur. In some tropical and subtropical forests, denitrification contributed more than 70% of soil N_2O emission[43]. In the present study, the SEM shows that soil NO_3^- was significantly associated with soil N_2O emission, and the model totally explained 58% of the variation of soil N_2O emission in wet seasons. It is thus highly possible that in this tropical forest, denitrification greatly contributed to the increased N_2O emission in wet seasons.

Although P addition did not greatly affected N_2O emission, the interaction of N and P marginally significantly increased N_2O fluxes during wet seasons. The result indicated a stimulation of P addition on soil N_2O after N addition. Consistent with our results, Mori et al.[29] also observed increased N_2O emissions after P addition under high soil water content in an N-fixing *Acacia mangium* plantation[6]. In *a*NP plots, substrate N was high even though the increased plant uptake of N (Fig. 1). The stimulated nitrifying and/or denitrifying bacteria by P thus could enhance N_2O emission without substrate N limitation. The wet season SEM model also suggested that soil available P increased soil MBC, and MBC significantly increased N_2O emission. Furthermore, White and Reddy[32] found that P enrichments increased soil microbial biomass and the denitrifiying enzyme activity. Since major part of soil N_2O production originated from denitrification process under high soil WFPS[43], we assumed that increased soil N_2O emission as a result of P addition under elevated N soils in wet seasons was mainly through stimulated soil microbial denitrification process in this forest. The increased N_2O emission in wet season of 2011 also coincided with the decline of NO_3^- between June 2011 and December 2011 (Fig. 1B), which could support above assumption.

To date, most work has focused on the role of N status in N_2O emission[23], with very little attention on how P availability may interact with plant and soil microbes on N_2O emission[8]. The potential influence of P availability and N×P interaction on the tropical N_2O emission remains largely ignored. Increased P availability would increase NPP (net primary productivity) and biomass C sequestration in tropical forests[44], which could mitigate the global warming effects[28]. In the secondary tropical forest, our vegetation investigation data also support this assumption: from 2010 to 2013, *a*P had the highest tree growth rate, followed by *a*NP, *a*N and control (Wang et al. unpublished data). In this study, P addition thus could increase biomass C sequestration and has no significant effect on soil N_2O emission under ambient atmosphere N deposition. However, under expectedly elevated N deposition conditions in the future, P addition will enhance the emission of N_2O in wet seasons. Some of the potential mechanisms behind our results remain unclear, but the

implications are not. Given the dramatically increasing of anthropogenic N deposition in tropical regions[24,25], P addition in this tropical soils will stimulate soil microbial activities in wet seasons, which will further enhance soil N_2O emission, and would offset the plant biomass C sequestration.

Methods

Site description. This study was conducted at the Xiaoliang Tropical Coastal Ecosystem Research Station of the Chinese Academy of Science (CAS) (21°27′N, 110°54′E), southwest of Guangdong Province, China. This region experiences a tropical monsoon climate, with mean annual temperature of 23°C. The annual rainfall ranges from 1400 to 1700 mm with a distinct variation of dry and wet seasons. The wet season is from April to October and the dry season is from November to March. The soil is a latosol developed from granite[45].

Our experimental site was a secondary mixed forest. The forest started as *Eucalyptus exserta* plantation in 1959, then 312 native tress species were introduced in 1960s[45,46]. Now, the most common tree species are: *Castanopsis fissa, Cinnamomum camphora, Carallia brachiata, Aphanamixis polystachya, Ternstroemia pseudoverticillata, Acacia auriculaiformis, Cassia siamea, Albizia procera, Albizia odoratissima, Leucaena leucocephala, Aquilaria sinensis* and *Chakrasia tabularis.* The forest is considered a typical tropical secondary forest with regards to biodiversity and structure complexity of the forest community[47].

Experimental design. An N and P addition experiment was designed as a randomized complete block (n = 5) and established in September 2009. Each block was located in a site more than 50 meters apart in the forest. Within each block, four 10×10 m plots were established and each plot was surrounded by a 2-m-wide buffer strip in each site. The treatments, N addition (*a*N), P addition (*a*P), N and P co-addition (*a*NP), and control (no addition of mineral nutrients) were assigned randomly to the four plots within each block. Both N and P were applied at 100 kg ha^{-1}yr^{-1}. Briefly, 476.6 g NH_4NO_3 (equal to 166.6 g N) and/or 808 g NaH_2PO_4 (equal to 166.6 g P) were dissolved in 30 L groundwater and, then, applied to the corresponding plots near soil surface using a backpack sprayer in each two months since September 2009 for three years. Thirty liters of groundwater was applied to control plots in each treatment event.

Soil N transformations. An *in situ* soil-core technique[48,49] was used to estimate soil net nitrogen mineralization in September 2010 (12 months), December 2010 (15 months), March 2011 (18 months), September 2011 (24 months), and July 2012 (34 months). Briefly, in each replicate plot, 4 points were randomly located. In each of these points, two PVC (polyvinyl chloride) tubes of 4.6 cm in diameter and 15 cm in height were hammered into the soil to a depth of 10 cm. Before sampling, forest floor litter was removed. One of the two tubes from each subplot was retrieved and sent to the lab. The other tube, with a lid on the top and some holes on the sidewall for aeration, was retained *in situ* for one month, 30 days, before being retrieved.

All soil cores were transported to the lab immediately and stored at 4°C, and extracted for mineral N within 48 hrs. Before extraction, each of the four cores from the same plot was manually mixed thoroughly. Visible roots and stones were removed manually. Twenty grams of fresh soil from each layer were extracted with 100 ml of 2M KCl solution (1 : 5 ratio) and filtered (Shuangquan quantitative filter paper 202#). Concentrations of ammonium and nitrate in the extraction solution were determined by a flow injection autoanalyzer (FIA) (Lachat Instruments, USA), ammonium by the salicylate-nitroprusside method and nitrate by sulfanilamide colorimetry after the Cd-core reduction to nitrite. Soil moisture was determined by weight loss after oven drying at 105°C for 24 hrs. Bulk density (dry soil) was calculated based on soil weight in all tubes and soil moisture. Net N mineralization was calculated as the increase in ammonium plus nitrate N between the initial soil sample and the incubated sample, while net nitrification was the increase in nitrate and net ammonification was the increase in ammonia.

Soil general properties. Soil chemical properties (i.e. soil pH, organic matter, total N, total P,) were determined using the soil samples obtained in September 2009 (Table 1), before the fertilization started. All soil samples were air-dried and passed through a 2 mm sieve. Soil pH was measured in a 1 : 2.5 mixture of soil:deionized water. Soil available P was extracted with Bray-2 solution[48] and determined by the molybdate blue colorimetric method. Soils for analyses of total N (TN) and organic matter were grounded to pass through a sieve of 60 mesh. Total N concentration was determined by micro-Kjeldahl digestion followed by salicylate-nitroprusside colorimetric determination on the Lachat FIA. The measured total N thus only included organic N and ammonium. Soil organic C (SOC) was determined by the wet combustion method[50], with SOM calculated as with Van Bemmelen's factor, following the guidelines of Liu, et al.[51].

Soil microbial biomass C and N (MBC and MBN) were determined by the chloroform fumigation extraction method[52] in the March, September, December 2011 and June 2012.

N_2O flux measurement. Nitrous oxide fluxes were measured monthly, from October 2010, one year after nutrients addition started. Gas fluxes were monitored once every month using the static chamber and a gas chromatograph (Agilent 4890D). The static chamber was a 25 cm diameter by 16 cm tall PVC pipe permanently anchored 8 cm

into the soil. During gas collection, a 30 cm tall removable cover chamber was attached tightly to the anchor ring with a rubber band. Gas samples were collected from each chamber from 9:00–10:00 local time. Gas samples were taken with a 60 ml plastic syringe at 0 min, 15 min and 30 min after the chamber closure. Before each sampling, syringes were flushed three times with chamber gas to mix the headspace. Laboratory tests showed that chambers and syringes were inert to N_2O[53,54]. Gas samples were analyzed within 12 h in a gas chromatograph (Agilent 4890D) fitted with an electron capture detector (ECD) for N_2O. Calibration gases (N_2O at 321ppbv, bottle's No. 070811) were obtained from the Institute of Atmospheric Physics, Chinese Academy of Sciences.

The calculation of N_2O flux followed that described in Zhang et al.[55], based on a linear regression of chamber gas concentration versus time. Atmospheric pressure was measured at the sampling site using an air pressure gauge (Model THOMMEN 2000, Switzerland). Air temperature (enclosure), soil temperature (at 5 cm depth) and moisture (0–10 cm depth) were measured during each sampling. Soil moisture content was quantified using a TDR-probe (Model Top TZS-I, China). Soil moisture (0–10 cm depth) values were converted to WFPS (Water Filled Pore Space) according to the following formula:

$$WFPS[\%] = Vol\ [\%]/\left(1 - SBD\left[g\ cm^{-3}\right]/2.65\left[g\ cm^{-3}\right]\right)$$

Where SBD is soil bulk density, Vol is volumetric water moisture and 2.65 is the density of quartz.

Statistical analyses. Repeated measures ANOVA was used to examine the effect of N and P additions on soil N_2O fluxes, soil temperature and WFPS from October 2010 to September 2012, Soil NH_4^+, NO_3^-, available P and soil N transformations were also analyzed by the repeated measures ANOVA. Soil microbial biomass C and N were separately analyzed in wet seasons and dry seasons.

Structural equation modelling (SEM) was performed to analyze different hypothetical pathways that may explain soil N_2O emission and N transformations in wet seasons and dry seasons (Fig. S1). Because of a positive correlation between net N mineralization and nitrification, we only used net nitrification rate in the SEM analysis. We used soil NH_4^+, NO_3^-, available P as the soil abiotic variables, and soil MBC as soil microbial variables. In the SEM analysis, data were fit to the models using the maximum likelihood estimation method. Adequacy of the models was determined using X^2 tests, Akaike Information Criteria (AIC), and root square mean errors of approximation (RMSEA). Adequate model fits are indicated by a nonsignificant X^2 test (P > 0.05), low AIC, and low RMSEA (<0.05). We improved the adequacy of the model by removing relationships between observed variables in the prior models based on Modification Indices. Repeated-measures ANOVA were performed in SPSS 18.0 (SPSS Inc., USA), and SEM was performed in Amos 21.0 (SPSS Inc., USA).

1. Cicerone, R. J. Changes in stratospheric ozone. *Science* **237**, 35–42 (1987).
2. IPCC. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. (Cambridge, UK, 2007).
3. Portmann, R. W., Daniel, J. S. & Ravishankara, A. R. Stratospheric ozone depletion due to nitrous oxide: influences of other gases. *Philos. Trans. R. Soc. B: Biol. Sci.* **367**, 1256–1264 (2012).
4. Bouwman, A. F. et al. Global trends and uncertainties in terrestrial denitrification and N_2O emissions. *Philos. Trans. R. Soc. B: Biol. Sci.* **368**, 20130112 (2013).
5. Mosier, A. et al. Closing the global N_2O budget: nitrous oxide emissions through the agricultural nitrogen cycle. *Nutr. Cycl. Agroecosys.* **52**, 225–248 (1998).
6. Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R. & Zechmeister-Boltenstern, S. Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philos. Trans. R. Soc. B: Biol. Sci.* **368**, 20130122 (2013).
7. Werner, C., Butterbach-Bahl, K., Haas, E., Hickler, T. & Kiese, R. A global inventory of N_2O emissions from tropical rainforest soils using a detailed biogeochemical model. *Global Biogeochem. Cycles* **21**, GB3010 (2007).
8. Baral, B. R., Kuyper, T. W. & Van Groenigen, J. W. Liebig's law of the minimum applied to a greenhouse gas: alleviation of P-limitation reduces soil N_2O emission. *Plant Soil* **374**, 539–548 (2014).
9. Erisman, J. W. et al. Consequences of human modification of the global nitrogen cycle. *Philos. Trans. R. Soc. B: Biol. Sci.* **368**, 20130116 (2013).
10. Liu, L. & Greaver, T. L. A review of nitrogen enrichment effects on three biogenic GHGs: the CO_2 sink may be largely offset by stimulated N_2O and CH_4 emission. *Ecol. Lett.* **12**, 1103–1117 (2009).
11. Dixon, R. K. et al. Carbon pools and flux of global forest ecosystems. *Science* **263**, 185–90 (1994).
12. Sayer, E. J. et al. Variable Responses of Lowland Tropical Forest Nutrient Status to Fertilization and Litter Manipulation. *Ecosystems* **15**, 387–400 (2012).
13. Hietz, P., Dunisch, O. & Wanek, W. Long-Term Trends in Nitrogen Isotope Composition and Nitrogen Concentration in Brazilian Rainforest Trees Suggest Changes in Nitrogen Cycle. *Environ. Sci. Technol.* **44**, 1191–1196 (2010).
14. Castaldi, S., Bertolini, T., Valente, A., Chiti, T. & Valentini, R. Nitrous oxide emissions from soil of an African rain forest in Ghana. *Biogeosciences* **10**, 4179–4187 (2013).
15. D'Amelio, M. T. S., Gatti, L. V., Mille, J. B. & Tans, P. Regional N_2O fluxes in Amazonia derived from aircraft vertical profile. *Atmos. Chem. Phys.* **9**, 8785–8797 (2009).
16. Kort, E. A. et al. Tropospheric distribution and variability of N2O: Evidence for strong tropical emissions. *Geophys. Res. Lett.* **38**, L15806 (2011).
17. Corre, M. D., Veldkamp, E., Arnold, J. & Wright, S. J. Impact of elevated N input on soil N cycling and losses in old-growth lowland and montane forests in Panama. *Ecology* **91**, 1715–29 (2010).
18. Vitousek, P. M. & Sanford, R. L. Nutrient cycling in moist tropical forest. *Annu. Rev. Ecol. Syst.* **17**, 137–167 (1986).
19. Vitousek, P. M. & Matson, P. A. Nitrogen transformations in a range of tropical forest soils. *Soil Biol. Biochem.* **20**, 361–367 (1988).
20. Vitousek, P. M., Porder, S., Houlton, B. Z. & Chadwick, O. A. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. *Ecol. Appl.* **20**, 5–15 (2010).
21. Cleveland, C. C., Townsend, A. R. & Schmidt, S. K. Phosphorus limitation of microbial processes in moist tropical forests: Evidence from short-term laboratory incubations and field studies. *Ecosystems* **5**, 680–691 (2002).
22. Galloway, J. N., Leach, A. M., Bleeker, A. & Erisman, J. W. A chronology of human understanding of the nitrogen cycle. *Philos. Trans. R. Soc. B: Biol. Sci.* **368**, 20130120 (2013).
23. Hall, S. J. & Matson, P. A. Nitrogen oxide emissions after nitrogen additions in tropical forests. *Nature* **400**, 152–155 (1999).
24. Galloway, J. N. et al. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science* **320**, 889–892 (2008).
25. Hietz, P. et al. Long-Term Change in the Nitrogen Cycle of Tropical Forests. *Science* **334**, 664–666 (2011).
26. Okin, G. S., Mahowald, N., Chadwick, O. A. & Artaxo, P. Impact of desert dust on the biogeochemistry of phosphorus in terrestrial ecosystems. *Global Biogeochem. Cycles* **18**, GB2005 (2004).
27. Liu, L., Gundersen, P., Zhang, T. & Mo, J. Effects of phosphorus addition on soil microbial biomass and community composition in three forest types in tropical China. *Soil Biol. Biochem.* **44**, 31–38 (2012).
28. Mori, T. et al. Soil greenhouse gas fluxes and C stocks as affected by phosphorus addition in a newly established Acacia mangium plantation in Indonesia. *Forest Ecol. Manag.* **310**, 643–651 (2013).
29. Mori, T. et al. Effects of phosphorus addition on N_2O and NO emissions from soils of an Acacia mangium plantation. *Soil Sci. Plant Nutri.* **56**, 782–788 (2010).
30. Falkiner, R., Khanna, P. & Raison, R. Effect of superphosphate addition on N mineralization in some Australian forest soils. *Soil Res.* **31**, 285–296 (1993).
31. Minami, K. & Fukushi, S. Effecfs of phosphate and calcium carbonate application on emission of N20 from soils under aerobic conditions. *Soil Sci. Plant Nutri.* **29**, 517–524 (1983).
32. White, J. R. & Reddy, K. R. Influence of nitrate and phosphorus loading on denitrifying enzyme activity in Everglades wetland soils. *Soil Sci. Soc. Am. J.* **63**, 1945–1954 (1999).
33. Hall, S. J. & Matson, P. A. Nutrient status of tropical rain forests influences soil N dynamics after N additions. *Ecol. Monogr.* **73**, 107–129 (2003).
34. Tang, X., Liu, S., Zhou, G., Zhang, D. & Zhou, C. Soil-atmospheric exchange of CO_2, CH_4, and N_2O in three subtropical forest ecosystems in southern China. *Global Change Biol.* **12**, 546–560 (2006).
35. Konda, R., Ohta, S., Ishizuka, S., Heriyanto, J. & Wicaksono, A. Seasonal changes in the spatial structures of N_2O, CO_2, and CH_4 fluxes from *Acacia mangium* plantation soils in Indonesia. *Soil Biol. Biochem.* **42**, 1512–1522 (2010).
36. Werner, C., Kiese, R. & Butterbach-Bahl, K. Soil-atmosphere exchange of N_2O, CH_4, and CO_2 and controlling environmental factors for tropical rain forest sites in western Kenya. *J. Geophys. Res.-Atmospheres* **112**, D03308 (2007).
37. Davidson, E. A., Nepstad, D. C., Ishida, F. Y. & Brando, P. M. Effects of an experimental drought and recovery on soil emissions of carbon dioxide, methane, nitrous oxide, and nitric oxide in a moist tropical forest. *Global Change Biol.* **14**, 2582–2590 (2008).
38. Kiese, R., Hewett, B. & Butterbach-Bahl, K. Seasonal dynamic of gross nitrification and N_2O emission at two tropical rainforest sites in Queensland, Australia. *Plant Soil* **309**, 105–117 (2008).
39. Fang, Y. et al. Nitrogen deposition and forest nitrogen cycling along an urban–rural transect in southern China. *Global Change Biol.* **17**, 872–885 (2011).
40. Mo, J. et al. Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. *Global Change Biol.* **14**, 403–412 (2008).
41. Venterea, R. T. et al. Nitrogen oxide gas emissions from temperate forest soils receiving long term nitrogen inputs. *Global Change Biol.* **9**, 346–357 (2003).
42. Koehler, B., Corre, M. D., Veldkamp, E., Wullaert, H. & Wright, S. J. Immediate and long-term nitrogen oxide emissions from tropical forest soils exposed to elevated nitrogen input. *Global Change Biol.* **15**, 2049–2066 (2009).
43. Zhu, J. et al. Spatial and temporal variability of N_2O emissions in a subtropical forest catchment in China. *Biogeosciences* **10**, 1309–1321 (2013).
44. Cleveland, C. C. & Townsend, A. R. Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere. *Proc. Natl. Acad. Sci. U S A* **103**, 10316–10321 (2006).
45. Ren, H. et al. Changes in biodiversity and ecosystem function during the restoration of a tropical forest in south China. *Sci. China Ser. C* **50**, 277–284 (2007).

46. Ding, M., Yi, W., Liao, L., Martens, R. & Insam, H. Effect of afforestation on microbial biomass and activity in soils of tropical China. *Soil Biol. Biochem.* **24**, 865–872 (1992).

47. Yu, Z. & Peng, S. *Ecological studies on vegetation rehabilitation of tropical and subtropical degraded ecosystems* (Guangdong Science and Technology Press, Guangzhou, 1996).

48. Wang, F. *et al.* Effects of nitrogen-fixing and non-nitrogen-fixing tree species on soil properties and nitrogen transformation during forest restoration in southern China. *Soil Sci. Plant Nutri.* **56**, 297–306 (2010).

49. Wang, F., Zhu, W., Xia, H., Fu, S. & Li, Z. Nitrogen Mineralization and Leaching in the Early Stages of a Subtropical Reforestation in Southern China. *Restor. Ecol.* **18**, 313–322 (2010).

50. Walkley, A. & Black, I. A. An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. *Soil Sci.* **63**, 251–263 (1934).

51. Liu, G. S., Jiang, N. H., Zhang, L. D. & Liu, Z. L. *Soil physical and chemical analysis & description of soil profiles* 266 (Standards press of China, Beijing, 1996).

52. Vance, E., Brookes, P. & Jenkinson, D. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* **19**, 703–707 (1987).

53. Steudler, P. A., Bowden, R. D., Melillo, J. M. & Aber, J. D. Influence of nitrogen fertilization on methane uptake in temperate forest soils. *Nature* **341**, 314–316 (1989).

54. Bowden, R. D., Steudler, P. A., Melillo, J. M. & Aber, J. D. Annual Nitrous-Oxide Fluxes from Temperate Forest Soils in the Northeastern United-States. *J. Geophys. Res.-Atmospheres* **95**, 13997–14005 (1990).

55. Zhang, W. *et al.* Emissions of nitrous oxide from three tropical forests in Southern China in response to simulated nitrogen deposition. *Plant Soil* **306**, 221–236 (2008).

Author contributions

F.M.W. and Z.A.L. designed the experiments. F.M.W., J.L. and B.Z. carried out the experiments and performed the analyses. F.M.W., J.L., X.L.W., W.Z. and D.A.N. substantially contributed to interpreting the results and writing the paper.

Additional information