ECOLOGY

Nitrogen addition increased CO₂ uptake more than non-CO₂ greenhouse gases emissions in a Moso bamboo forest

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Atmospheric nitrogen (N) deposition affects the greenhouse gas (GHG) balance of ecosystems through the net atmospheric CO₂ exchange and the emission of non-CO₂ GHGs (CH₄ and N₂O). We quantified the effects of N deposition on biomass increment, soil organic carbon (SOC), and N₂O and CH₄ fluxes and, ultimately, the net GHG budget at ecosystem level of a Moso bamboo forest in China. Nitrogen addition significantly increased woody biomass increment and SOC decomposition, increased N₂O emission, and reduced soil CH₄ uptake. Despite higher N₂O and CH₄ fluxes, the ecosystem remained a net GHG sink of 26.8 to 29.4 megagrams of CO₂ equivalent hectare⁻¹ year⁻¹ after 4 years of N addition against 22.7 hectare⁻¹ year⁻¹ without N addition. The total net carbon benefits induced by atmospheric N deposition at current rates of 30 kilograms of N hectare⁻¹ year⁻¹ over Moso bamboo forests across China were estimated to be of 23.8 teragrams of CO₂ equivalent year⁻¹.

INTRODUCTION

Atmospheric nitrogen (N) deposition is considered to be a driver of increasing carbon (C) storage in woody biomass, particularly in N-limited temperate and boreal forests in North America and Europe (1–3). However, there is still debate on the impact of N deposition on the greenhouse gas (GHG) balance of tropical forests, which have abundant available soil N and a much leakier N cycle than temperate forests (4, 5). The tree growth and C storage in woody biomass were not found to significantly respond atmospheric N deposition in temperate forests across Europe and northeastern United States (6, 7). Similar results were also observed in P-limited Hawaiian tropical forests growing on soil of 4.1 million years (8). However, Frey et al. (9) demonstrated that N addition increased soil C accumulation largely due to a suppression of organic matter decomposition rather than enhanced C inputs via litterfall and root production. These results suggest that the responses of forest C sequestration to N addition are variable depending on background limitation, changes in the nutrient use efficiency of plants and decomposers, and limitation of other nutrients such as phosphorus (P) (10, 11). Few studies on N deposition effects have focused on tropical and subtropical regions compared with boreal and temperate regions (12).

Methane (CH₄) and nitrous oxide (N₂O) are the important GHGs that contribute to more than one-quarter of anthropogenic global warming, and their emissions partly offset terrestrial CO₂ uptake at the global scale (13, 14). It has been reported that the emissions of CH₄ and N₂O from terrestrial ecosystems offset 73% of the land CO₂ sink over the North American continent (15). Forest soils are a sink for CH₄, but this sink may have decreased by 77% from 1988 to 2015 (16). Humid tropical forest soils have higher background N₂O losses than temperate forest soils and are the largest source of N₂O across natural ecosystems (12). Nitrogen addition was reported to increase CH₄ and N₂O production and inhibit CH₄ consumption in recent studies, which may shift the net GHG balance toward a source of CO₂ equivalents (CO₂eqs) and could offset 53 to 76% of increased C sequestration induced by N deposition across terrestrial ecosystems (14).

This study focuses on Moso bamboo (Phyllostachys edulis), a widely planted species in East and Southeast Asia, covering 4.43 Mha in subtropical China (17). Moso bamboo has extremely fast growth rates (completing height and diameter growth within 2 months after shoot emergence) and a strong regeneration ability (alternating high and low new bamboo recruitment years). The site studied is intensively managed with one-third of the aboveground biomass being removed by thinning each 2 years, thus leaving tree cohorts younger than 4 years. The residence time of most harvested bamboo products, such as bamboo flooring and bamboo furniture, is larger than 20 years (18, 19). Management practices include fertilization, ploughing, and weeding. Therefore, Moso bamboo forests can always grow and remove CO₂ from the atmosphere; at steady state, their net ecosystem exchange is equal to harvest, and the standing biomass carbon stock remains constant. The net CO₂ sink of the system formed by bamboo forests and their wood products thus depends mainly on the lifetime and accumulation in wood products and waste. Our 5-year observation using eddy covariance measurements found that the mean annual net C sequestration in both trees and soil was 6.03 Mg C ha⁻¹ (17). Over a 30-year time period, the carbon stock of the system including plantation and its products pool is 246 metric tons (t) C ha⁻¹, higher than the 200 t C ha⁻¹ of Chinese fir (Cunninghamia lanceolata), another fast growing tree in China (19).

The regions where Moso bamboo mainly distributes are today subject to much higher N deposition rates of 30 kg N ha⁻¹ year⁻¹ (20) than Western European (8 to 11 kg) and United States (4 to 5 kg) (21). Moreover, N deposition is predicted to increase by 50 to 100% by 2030 relative to the year 2000 and could reach up to 50 kg N ha⁻¹.
year\(^{-1}\) by 2050 in China (22, 23). It was shown that N deposition significantly increased P uptake (24), photosynthetic capacity (25), decomposition rates of leaf litter (26) and fine roots (27), loss of dissolved organic carbon (DOC) (28), and soil microbial biomass (29) in bamboo forests. However, how N deposition affects ecosystem productivity, soil carbon storage, soil uptake of CH\(_4\) and soil N\(_2\)O emissions, and thus the net GHG balance remains to be quantified. In this study, we performed a 4-year field experiment at three N addition levels of 30, 60, and 90 kg N ha\(^{-1}\) year\(^{-1}\) upon a control (N-free addition with an ambient N deposition rate of 30 kg N ha\(^{-1}\) year\(^{-1}\)) in Moso bamboo forests composed of 1- and 3-year-old bamboo cohorts since January 2013.

RESULTS
Net ecosystem productivity and drivers
Under the N-free treatment, the mean woody biomass increment is 7.28 ± 0.46 t C ha\(^{-1}\) year\(^{-1}\), but there was a net loss of soil organic carbon (SOC) of −0.51 ± 0.32 t C ha\(^{-1}\) year\(^{-1}\), so that net ecosystem productivity (NEP) is 6.77 ± 0.36 t C ha\(^{-1}\) year\(^{-1}\) (Table 1). In response to N addition, woody biomass increment increased by 33.1, 36.8, and 23.9%, respectively (Fig. 1), with significantly increased density and diameter at breast height (DBH) of resprouting bamboos (Fig. S1). SOC further decreased annually compared to the control SOC loss, mainly at depths of 0 to 20 cm and 20 to 40 cm (Fig. 2). The sum of positive biomass increment and SOC losses contributes to a NEP sink increase of 24.5, 29.9, and 18.3%, respectively (Table 1). N addition treatments significantly increased soil available P (AP) concentration in growth season, although this enhancement weakened with time (Fig. S2A).

The response rates of woody biomass increment, SOC change, and NEP to a unit N deposition varied with N addition rate (Table 2). Change in the N response efficiency (ANRE; the difference in response rate per unit N addition between different N levels) of both biomass increment and NEP became negative when N addition exceeded 60 kg N ha\(^{-1}\) year\(^{-1}\), that is, a nonlinear saturating response (Fig. 3) (10).

N\(_2\)O and CH\(_4\) fluxes and drivers
Mean annual soil N\(_2\)O emission was 4.8 kg N\(_2\)O-N ha\(^{-1}\) under the N-free treatment, which is equivalent to CO\(_2\)eq emissions of 2.2 t CO\(_2\)eq ha\(^{-1}\) year\(^{-1}\) (Table 1). The average N\(_2\)O emissions increased by 23.8, 12.8, and 4.8 kg CO\(_2\)eq ha\(^{-1}\) year\(^{-1}\) per 1 kg N ha\(^{-1}\) year\(^{-1}\) input as a response to N additions of 30, 60, and 90 kg N ha\(^{-1}\) year\(^{-1}\), respectively (Table 2 and figs. S3 and S4A). We performed random forest analysis (30) to identify the main predictors of N\(_2\)O fluxes among biotic and abiotic factors and applied structural equation modeling (SEM) (31) to identify direct and indirect relationships between N\(_2\)O emissions and environmental factors. The SEM (fig. S5) explained 60.4% of the variation in N\(_2\)O emissions through positive relationships with NO\(_3\)\(^-\), microbial biomass carbon, pH, and total P.

The average annual soil CH\(_4\) sink rate was 8.0 kg CH\(_4\) ha\(^{-1}\) under the N-free treatment, a CO\(_2\)eq uptake of 0.2 t CO\(_2\)eq ha\(^{-1}\) year\(^{-1}\) (Table 1). Nitrogen addition significantly reduced the CH\(_4\) consumption by soil methanotrophy by 10.3 to 29.7%, and this inhibition was enhanced with increasing N addition rate (Table 1 and figs. S4B and S6). The mean soil CH\(_4\) uptake rate decreased by 0.1, 0.3, and 0.1 kg CO\(_2\)eq ha\(^{-1}\) year\(^{-1}\) per 1 kg N ha\(^{-1}\) year\(^{-1}\) input in response to N additions of 30, 60, and 90 kg N ha\(^{-1}\) year\(^{-1}\), respectively (Table 2). The SEM (fig. S7) explained 63.3% of the variation in CH\(_4\) consumption through positive relationships with total potassium (K) and AP content, as well as pH value, but negative effects of NH\(_4\)\(^+\), total N, and NO\(_3\)\(^-\) content.

Net ecosystem C uptake and offset by N\(_2\)O and CH\(_4\)
Combining N\(_2\)O emission and CH\(_4\) uptake, assuming global warming potential (GWP) at 100-year time horizon gives a net source of 0.56 t C eq ha\(^{-1}\) under the N-free treatment (Table 1) and of 0.70 to 0.79 t C eq ha\(^{-1}\) under the three N addition treatments, an increase of 23.5 to 39.9% compared to the control experiment. The average net sources of these two GHGs increased by 6.7, 3.8, and 1.4 kg C eq ha\(^{-1}\) year\(^{-1}\) per 1 kg N ha\(^{-1}\) year\(^{-1}\) input in response to N additions of 30, 60, and 90 kg N ha\(^{-1}\) year\(^{-1}\), respectively (Table 2). At the ecosystem scale, NEP increased by 1.24 to 2.03 t C ha\(^{-1}\) year\(^{-1}\), while GHG emissions increased by 0.14 to 0.23 t C eq ha\(^{-1}\) year\(^{-1}\) under N additions. That is, the ecosystem remained a net GHG sink, but 10.7 to 12.1% of the increase in C sequestration (NEP) was offset by a higher net GHG source caused by N\(_2\)O emissions and CH\(_4\) sinks (Table 1). After accounting for altered non-CO\(_2\) emissions from added N, the net ecosystem C uptake was 6.2 t C eq ha\(^{-1}\) year\(^{-1}\) under the N-free treatment and 7.3 to 8.0 t C eq ha\(^{-1}\) year\(^{-1}\) under N addition treatments, an increase of 17.8 to 29.0% compared with the N-free treatment (Table 1). The average net ecosystem Ceq sink increased by 48.7, 30.0, and 12.2 kg C ha\(^{-1}\) year\(^{-1}\) per 1 kg N ha\(^{-1}\) year\(^{-1}\) input in response to N addition treatments of 30, 60, and

| Treatment | \(\Delta\)Woody biomass C | \(\Delta\)Soil C | NEP C | GWP | \(\Delta\)Ecosystem C | Offset rate (%) |
|-----------|-------------------------|--------------|-------|-----|----------------|----------------|
| Aboveground | Belowground | Subtotal | Aboveground | Belowground | Subtotal | N\(_2\)O | CH\(_4\) | Subtotal | | |
| Control  | 4.17 ± 0.18c | 3.11 ± 0.29c | 7.28 ± 0.46c | 0.006b | 6.77 ± 0.36c | 0.61 ± 0.10b | 0.050 ± 0.007a | 0.56 ± 0.09b | 6.20 ± 0.28c | 8.32 ± 0.99 |
| N30      | 5.46 ± 0.15ab | 4.34 ± 0.28ab | 9.80 ± 0.43ab | 1.23 ± 0.10b | 8.43 ± 0.54ab | 0.81 ± 0.10a | 0.045 ± 0.006ab | 0.77 ± 0.10a | 7.66 ± 0.64ab | 9.17 ± 1.78 |
| N60      | 5.76 ± 0.05a | 4.33 ± 0.15a | 10.09 ± 0.18a | 1.29 ± 0.08a | 8.80 ± 0.21a | 0.83 ± 0.10a | 0.035 ± 0.006ab | 0.79 ± 0.10a | 8.01 ± 0.11a | 8.97 ± 0.91 |
| N90      | 5.08 ± 0.13b | 4.02 ± 0.22b | 9.10 ± 0.35b | 1.09 ± 0.19a | 8.01 ± 0.28b | 0.73 ± 0.09ab | 0.035 ± 0.006ab | 0.70 ± 0.08ab | 7.31 ± 0.35b | 8.74 ± 1.29 |
90 kg N ha$^{-1}$ year$^{-1}$, respectively (Fig. 4 and Table 2). As for NEP responses to N addition, the responses of the net GHG balance of the ecosystem were found to saturate when N addition rate exceeded 60 kg N ha$^{-1}$ year$^{-1}$ (Fig. 3).

**DISCUSSION**

The NEP of Moso bamboo forest under the N-free treatment of 6.77 ± 0.36 t C ha$^{-1}$ year$^{-1}$ (Table 1) is close to NEP of 6.03 ± 0.6 t C ha$^{-1}$ year$^{-1}$ obtained from eddy covariance data (17). Biomass increment response to N addition (increasing 23.9 to 36.8%) (Fig. 1) was far higher than previously measured effects of added N to the growth of temperate forest (<20%) (6, 7), which likely reflects the always-young structure of the bamboo plantation with very high trees growth rate causing a high N demand. Soil P availability could limit biomass increment in humid tropical forests and Moso bamboo forests (24) and was generally expected to decline with declining pH (fig. S2B). In this study, soil AP significantly increased with N addition rates (fig. S2A). The AP were most likely derived from applied fertilizer (N, 15%; P$_2$O$_5$, 6%; K$_2$O, 20%) (26), larger litterfall and P return (32), and faster decomposition of leaf litter (26) and fine roots (27) induced by N addition, which may swamp the effect of declining pH. The increase in soil AP and N addition could sustain enhanced P uptake by plants (24), as well as higher carboxylation efficiency and photosynthetic rates (especially the youngest plants) (25), and a higher density and DBH of resprouting new bamboos cohorts (fig. S1), which, together, contributed to the increases in woody biomass increment and NEP.

Our results showed that the ratio of carbon sequestration per unit N incorporated in the aboveground woody biomass was 27:1 to 43:1 (Table 2) for N addition rates below the saturation threshold. This ratio is slightly higher than in European forests (20:1 to 40:1) (4) or (21:1 to 26:1) (33) and in a global meta-analysis (20:1 to 30:1) (34), but only half of the ratios reported for northeastern and north-central United States (51:1 to 82:1) (3). The ratios of woody biomass carbon increment per unit N were 84:1 and 47:1 under N addition treatments of 30 and 60 kg N ha$^{-1}$ year$^{-1}$, respectively (Table 2), which is close to the reported ratio for tree carbon (73:1) across the northeastern and north-central United States (3). The response rates of NEP sink to per unit N were 55:1 and 34:1 under N addition treatments of 30 and 60 kg N ha$^{-1}$ year$^{-1}$, respectively (Table 2), which is close to the values for European forests (35:1 to 47:1) (35) and higher than a mean value for global forests (25:1) (14).

Our previous studies at this site indicated that N deposition increased soil available N (NO$_3^-$-N and NH$_4^+$-N) (28) and that N released from the larger litterfall and N return (32) and faster decomposition of leaf litter and fine roots (26, 27) produced more reaction substrates for nitrification and denitrification and thus increase N$_2$O emissions. In the present study, N addition treatments significantly decreased the diversity of ammonia oxidizing archaea and denitrobacteria (norB)
(fig. S8, A and B), but the N30 treatment significantly increased the amount of norB ($P < 0.05$) (fig. S9B), indicating that the $N_2O$ emission may be mainly driven by denitrification process in the Moso bamboo forest.

Previous studies in temperate woodlands found that $N$ addition decreased soil $CH_4$ consumption by 14 to 51% (36, 37), consistent with our results. The oxidation of both $CH_4$ and ammonium ($NH_4^+$) relies on the same methane monooxygenase enzyme (MMO), and

| Aboveground biomass C | $\Delta$BC C | SOC | NEP C | GWP $N_2O$ | GWP $CH_4$ | GWP Subtotal | Ecosystem C |
|-----------------------|-------------|-----|-------|------------|------------|--------------|-------------|
| N30                   | 43.1 ± 7.6a | 84.1 ± 14.0a | −28.7 ± 9.6a | 55.4 ± 12.5a | 6.5 ± 0.3a | −0.17 ± 0.03b | 6.7 ± 0.3a | 48.7 ± 12.6a |
| N60                   | 26.5 ± 3.5b | 46.8 ± 7.4b | −13.0 ± 6.2b | 33.8 ± 2.6b | 3.5 ± 0.5b | −0.25 ± 0.03a | 3.8 ± 0.6b | 30.0 ± 2.9b |
| N90                   | 10.1 ± 2.4c | 19.1 ± 5.9c | −5.5 ± 1.7b | 13.6 ± 4.9c | 1.2 ± 0.4c | −0.18 ± 0.02b | 1.4 ± 0.4c | 12.2 ± 5.0c |

Fig. 3. Responses of Moso bamboo forest to $N$ addition gradient. NRE, the response per unit $N$ addition, is indicated by the slopes in the straight lines. $\Delta$NRE is the difference in NRE between different $N$ levels, indicating the difference between the slopes. Under $N$ limited conditions, the indices increase linearly with excess $N$; thus, NRE is highest and constant with changing $N$ input, while $\Delta$NRE equals 0. Under high $N$ enrichment, the indices show a slower than linear response; thus, NRE decreases with $N$, while $\Delta$NRE is smaller than 0. The dash lines indicate the $N$ saturation threshold for these indices nonlinearity.
thus, more NH$_4^+$ likely suppressed CH$_4$ consumption through competition for MMO (38). Furthermore, NO$_2^-$ resulting from NH$_4^+$ oxidation and NO$_3^-$ can poison methanotrophic bacteria (39), increased soil available NO$_3^-$ induced by N deposition in this study site (28), and thus also inhibit CH$_4$ oxidation. The optimum pH range for most methanotrophic bacteria is from 4 to 7.5. The significantly lower pH value induced by N input in the current study site (fig. S2B) could inhibit the activity of methanotrophic bacteria, thus suppress CH$_4$ consumption (40), and contributed to cumulative effect of years of N addition on steadily declining CH$_4$ uptake rates (fig. S4B). These effects contributed to the significantly negative correlation between soil CH$_4$ uptake and soil NH$_4^+$ total N content, as well as the significantly positive correlation between CH$_4$ uptake and pH (fig. S7). Moreover, our results show that N deposition significantly decreased the diversity of methanotrophs (fig. S8D) but significantly increased the amount of methanogen (fig. S9C), indicating that N deposition decreased CH$_4$ uptake, likely through two paths: a large decrease in CH$_4$ oxidation and slight increase in CH$_4$ emission. The mechanism by which total K and AP positively affect CH$_4$ uptake remains unclear and needs further study.

The contribution of current atmospheric N deposition input (30 kg N ha$^{-1}$ year$^{-1}$) (20) across southern China to the C uptake of the Moso bamboo forest ecosystem (biomass and soil) was estimated at 1.66 Mg C ha$^{-1}$ year$^{-1}$, accounting for 19.3 $\pm$ 5.4% of the total C sequestration (Table 1). Note that terrestrial plants may emit CH$_4$ under aerobic conditions when soils are saturated. To date, it is still unclear whether Moso bamboo can emit CH$_4$ and how N deposition may influence plant CH$_4$ emissions, which contributes to uncertainty of our results. Our previous study in the same site also found that N deposition significantly increased the loss of soil DOC and dissolved organic nitrogen (28) and soil respiration rate (41), which might contribute to soil C variation and N$_2$O emission. In addition, continued N fertilization and atmospheric N deposition can result in continuous declines in soil pH, which likely induce aluminum toxicity or other problems, and eventually maybe suppress plant growth and C uptake capacity.

A meta-analysis across terrestrial ecosystems suggested that 53 to 76% of the increased CO$_2$ sequestration induced by N deposition may be offset by N-induced stimulation of N$_2$O and CH$_4$ emissions from multiple ecosystems at a global scale (14). In the present study, the proportion of CO$_2$ uptake offset by N$_2$O and CH$_4$ emissions was only 10.7 to 12.1%, indicating a lower effect than the global analysis (14). The present study reports the net N$_2$O and CH$_4$ fluxes changes induced by N deposition coincident with CO$_2$ fluxes in the same forest. Previous meta-analysis lacked collocated data for the three GHGs from the same ecosystem (14) and thus might overestimate the offset effect of N$_2$O and CH$_4$ fluxes.

The global forest C sequestration benefits induced by N deposition were estimated as 0.24 to 2.0 Pg C year$^{-1}$ using stoichiometric scaling approaches that multiply a C/N response by an estimate of the N deposition rate, assuming that the patterns of N assimilation in wood and soil in temperate forests are representative and the tree and soil C/N ratios are constant along N deposition gradients (3, 6). Using this simple approach, we estimated that N deposition could account for a 11.18 Tg C year$^{-1}$ sink in Moso bamboo forest biomass and a soil C loss of 3.81 Tg C year$^{-1}$, thus a net combined of 7.37 Tg C year$^{-1}$ sink (both trees and soil) in China over 4.43 million ha of plantations. These estimates were calculated by multiplying the C/N response measured in this study (84.1:1 for biomass and 55.4:1 for ecosystem) by an average regional N deposition rate of 30 kg N ha$^{-1}$ year$^{-1}$ (20). This estimation assumed that all Moso bamboo forests assimilated deposited N with the similar way in which the present Moso bamboo forest did (42), although the proportion of overall N allocation fraction between bamboo tissues and soil layers remains unknown, which will contribute to the uncertainty of our estimation to some extent.

Nitrogen deposition of 30 kg N ha$^{-1}$ year$^{-1}$ was scaled up to increase N$_2$O emission by 0.86 Tg Ceq year$^{-1}$ and decrease CH$_4$
uptake by 0.02 Tg Ceq year⁻¹ in the region considered, leaving a net GHG sink of 6.49 Tg Ceq every year. Considering a scenario where N deposition increase by 50 to 100% in 2030 relative to 2000 (23), it can be inferred that the bamboo forests remain net GHG sinks but weakening over time due to the saturating C uptake enhancement (Fig. 3 and Table 2). Our results provide new evidence of the effects of N deposition on net ecosystem C uptake of Moso bamboo forests and also new data to test the accuracy of terrestrial ecosystem models for quantifying the effects of N deposition on net ecosystem C uptake of tropical forests in China.

**METHODS**

**Study site**

The study site was located at Qingshan Town, Lin’an City (30°14’N and 119°42’E), Zhejiang Province, China. The area has a monsoonal subtropical climate with four distinct seasons. The mean annual precipitation is 1420 mm, and the mean annual temperature is 15.6°C, which ranges from 24°C in July to 3°C in January. The area receives an average of approximately 1847 hours of sunshine per year and sees an average of 230 frost-free days per year. The monthly air temperature and rainfall for the observation period (2013–2016) are shown in fig. S10.

The Moso bamboo forest was originally established in the late 1970s from native evergreen broadleaf forests in sites of similar topography (southwest slopes of approximately 6°) and soil type (Ferrisols derived from granite). The bamboo forest was managed with annual fertilization (N, 15%; P₂O₅, 6%; K₂O, 20%; 450 kg ha⁻¹), ploughing, weeding with herbicide (26), digging bamboo shoots, and biennial mature bamboo thinning. There were several understory species with a forest floor coverage of 5% and a total herbal biomass of 14.6 kg ha⁻¹. The initial stand and soil characteristics can be found in (41).

Moso bamboo exhibits a different growth pattern than arborous species. Shoots usually begin to emerge from the ground at the end of March and complete their height and diameter growth within the following 2 months. Afterward, the diameter, height, and volume of the young bamboos remain unchanged, owing to scarce secondary cambium, and the bamboos begin to accumulate biomass (17). Moso bamboo forests are characterized by alternating high and low recruitment years, as a result of long-term management activities. The recruitment of Moso bamboo shoots at the study site happened only during even-numbered years (e.g., 2012, 2014, and 2016) (17). Furthermore, since the biomass accumulating capacity of Moso bamboo tends to decrease rapidly 4 years after shoot emergence (17), Moso bamboos more than 4 years old are usually harvested in November during even-numbered years to maximize the economic benefits. Thus, the Moso bamboo forests are uneven aged forests with a 2-year interval (24). The fast growth pattern, biennial new bamboo recruitment and mature bamboo thinning, and long durable time of bamboo products make Moso bamboo forest to always keep a high and continuous productivity and C sequestration capacity.

**Experimental design and measurement**

In November 2012, 12 experimental plots of 20 m by 20 m were established with a 20-m-wide buffer zone to avoid disturbing the nearby plots. Three N addition treatments (low N, medium N, and high N) and a control (N-free addition) with three replicate plots per treatment were established randomly in Moso bamboo forests. Nitrogen deposition over most parts of southern China, the main distribution area of Moso bamboo forests, has reached 30 kg N ha⁻¹ year⁻¹ with an average NH₄⁺/NO₃⁻ ratio of 1.28 (20, 27) and was predicted to reach 50 kg N ha⁻¹ year⁻¹ by 2050 (22). Referring to the similar methods simulating atmospheric N deposition across global forests (7, 9), NH₄NO₃ was used as N source and the N addition rate equaling, doubling, and tripling the local N deposition rate with a low N treatment of 30 kg N ha⁻¹ year⁻¹ (N30), a medium N treatment of 60 kg N ha⁻¹ year⁻¹ (N60), and a high N treatment of 90 kg N ha⁻¹ year⁻¹ (N90) (26, 41). Beginning in January 2013, quantitative NH₄NO₃ was weighed, mixed with 10 liters of water, and sprayed evenly onto the forest floor of each plot using an electric sprayer at the beginning of every month for a total of 12 equal applications over the entire year throughout the experimental period. These water-based additions were equivalent to an increase of 0.3 mm of rainfall per year (26, 41). Each control treatment plot received 10 liters of N-free water to control for the effects of the added water.

**Sample collection**

In November 2016, the DBH and age of each Moso bamboo were recorded in each plot, which included three age stages (bamboo shoots emerging in 2012, 2014, and 2016). Nine bamboos (three at each age stage) that were close to the mean DBH were cut in each plot, and the leaf, branch, trunk, and underground trunk were collected, then oven-dried at 65°C to a constant weight, and weighed to calculate the biomass proportion of the different organs. Three subplots of 1 m by 1 m were set up in each plot, and the rhizomes and roots at a depth of 0 to 60 cm were collected and mixed into a sample. The samples were cleaned, then oven-dried at 65°C to a constant weight, and weighed. Oven-dried samples were ground with a grinder (DFT-50A, Wenling LINDA Machinery Co., Ltd., Wenling City, China) for C content analysis. Five soil cores at three depths of 0 to 20 cm, 20 to 40 cm, and 40 to 60 cm were randomly collected from each plot and mixed together, respectively. The cutting ring method was used to measure the bulk density of soil at the three depths. Soil samples were sieved through a 2-mm mesh to remove roots, plant residues, and stones for the analysis of C content. The total carbon value was determined using a Sumigraph NC-80 high-sensitivity CN analyzer (Shimadzu, Japan). The methods for analyzing soil physicochemical properties and microbe biomass were the same as those described by Li et al. (29). The soil samples at a depth of 0 to 20 cm were also collected from each plot in spring and summer every year to measure soil physicochemical properties and microbe biomass (29).

Fluxes in N₂O and CH₄ were measured using widely applicable static chamber and gas chromatography techniques (43). The static chambers were made of opaque polyvinyl chloride panels consisting of a square base box (0.4 m by 0.4 m by 0.1 m) with a U-shaped groove (50 mm wide and 50 mm deep) at the top of the edge to hold a removable top (0.4 m by 0.4 m by 0.4 m). In each plot, three boxes were inserted directly into the soil to a depth of 10 cm a month before the first sampling and remained fixed during the entire observation period. The chamber tops were placed onto the base boxes during gas sampling with the grooves being filled with water to act as an air seal. A small fan was installed inside at the top of each chamber to generate turbulence in the chamber during sampling. A 60-ml plastic syringe attached to a three-way stopcock was used to collect gas samples at 0, 10, 20, and 30 min following chamber closure. The gas samples were injected into vacuum bags made of inert aluminum-coated plastic (Desen Inc., Dalian, China), and the
concentrations of N₂O and CH₄ were analyzed within 2 days using gas chromatography (GC-2014, Shimadzu Corporation, Kyoto, Japan). The chamber tops were immediately removed after sampling. Gas samples were collected once in the middle of each month. Sampling time was between 9:00 and 10:00 a.m., as during this period, GHG fluxes have been demonstrated to be close to daily means (43). Soil temperature was monitored with button thermometers (iButton DS1923, Wdsen Electronic Technology Co. Ltd., China), which automatically recorded soil temperature at 1-hour intervals. A button thermometer was buried at 5-cm depth in the middle of each base box, and the recorded data were collected once every 6 months; the recorded temperature in the sampling time was used for further analysis. Soil volumetric water content at 5-cm depth was monitored in each chamber with TDR100 (Spectrum Technologies Inc., USA) immediately after the gas samples were collected.

**Statistical analysis**

The aboveground biomass of a Moso bamboo was calculated using a widely applied existing model, which was developed on the basis of biomass data from whole Moso bamboo in Zhejiang province with a correlation coefficient (R² = 0.937) and accuracy (96.43%) with 95% confidence intervals (44)

\[
f(D,A) = 747.78 D^{2.771} \left( \frac{0.1484 A}{0.28 + A} \right)^{5.555} + 3.772
\]

where \( f \) is the aboveground biomass of a Moso bamboo including the leaf, branch, trunk, and underground trunk and \( D \) and \( A \) are the DBH (centimeters) and age of Moso bamboo, respectively.

The total aboveground biomass in each plot was the sum of the biomass of each bamboo (\( f \)). The total aboveground biomass carbon in each plot was the sum of the biomass carbon for each bamboo, which was calculated as the sum of the carbon from the different organs using carbon concentration multiplied by organ weight at each age stage. The annual aboveground biomass carbon increment was calculated as one-sixth of the current total biomass carbon due to biennial thinning of the Moso bamboo and the harvest of one-third of the biomass (i.e., 4-year-old bamboos) (45). Considering the leaf biomass contributing small fractions of 7.0 to 8.3% of total aboveground biomass increment and high decomposition rates of 0.34 to 0.47 year⁻¹ of leaf litter under the control and N addition treatments (26), leaf increment was excluded when calculating annual aboveground biomass carbon increment and subsequent net ecosystem carbon uptake.

The total belowground biomass carbon in each plot was calculated by extrapolating the biomass carbon of the rhizomes and roots via carbon concentration multiplied by the weight of the rhizomes or roots from three subplots. The ratio of belowground biomass to aboveground biomass was calculated, and this was then used to estimate the annual belowground biomass carbon increment according to the annual aboveground biomass carbon increment. The biomass carbon increment is the sum of the annual increments of the above- and belowground biomass carbon.

SOC storage was calculated using the following formula

\[
SOC = \sum_i d_i \times b_i \times 0.2
\]

where SOC is SOC storage at a depth of 0 to 60 cm, \( i \) is the soil layer (0 to 20 cm, 20 to 40 cm, and 40 to 60 cm), and \( d_i \) and \( b_i \) are carbon concentration and bulk density at depth \( i \), respectively.

NEP was calculated as the sum of annual woody biomass carbon increment and soil carbon increment. The soil N₂O and CH₄ fluxes were calculated using the following formula (41)

\[
F = \left( \frac{dc}{dt} \right) \times (M/V_0) \times (273.15/T) \times (V/A)
\]

where \( F \) (milligrams meter⁻² hour⁻¹) is the soil N₂O and CH₄ flux and positive and negative values that indicate emissions and uptake of N₂O and CH₄, respectively; \( dc/dt \) is the slope of the linear regression between the change in N₂O and CH₄ concentrations (dc) and change in time (dt) in the chamber; \( M \) and \( V_0 \) are the molar mass and molar volume of N₂O and CH₄ under standard conditions, respectively; \( T \) is the absolute air temperature during sampling; \( V \) (cubic meters) and \( A \) (square meters) are the effective volume and bottom area of the chamber, respectively. The daily soil N₂O and CH₄ fluxes were accumulated to monthly and annual fluxes.

The GWP was used to define the cumulative impacts of the emission of 1 g of CH₄ or N₂O on the planetary energy budget relative to 1 g of reference CO₂ gas over a period of 100 years. The GWP of fluxes of soil N₂O and CH₄ was calculated using the formula (15)

\[
GWP = 25 \times F_{\text{CH}_4} + 298 \times F_{\text{N}_2\text{O}}
\]

Index refers to aboveground biomass carbon, biomass carbon increment, soil carbon, NEP, GWP Ceq of CH₄ and N₂O fluxes, and net carbon sequestration of Moso bamboo forests. ΔNRE is the difference in NRE between different N levels, indicated by the difference between the slopes.

A classification random forest analysis was performed to identify the main predictors of N₂O and CH₄ fluxes among biotic and abiotic factors using the randomForest package (30) in R statistical software, version 3.0.2 (http://cran.r-project.org/). SEM (31) analysis was conducted to evaluate the direct and indirect relationships between N₂O and CH₄ fluxes and environmental factors using Amos 21.0 (IBM SPSS Amos, USA). Some goodness-of-fit indices, such as the \( \chi^2 \) test, root mean square error of approximation and their \( P \) values, degrees of freedom, normed fit index, and comparative fit index, were used to identify the best-fit model. Our observation number of 72 is demonstrated to be acceptable.

Pearson’s correlations between N₂O and CH₄ fluxes and soil physicochemical properties and microbe biomass were analyzed. One-way analysis of variance (ANOVA) and least significant difference tests were used to determine the statistical significance of the differences in biomass C, soil C, and N₂O and CH₄ fluxes among N addition treatments. All data were tested for homogeneity of variance and distribution normality before conducting the ANOVA. Data analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA) for Windows.
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