Agroforestry alters the fluxes of greenhouse gases of Moso bamboo plantation soil

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Abstract

Agroforestry systems are widely applied in China and have both economic and ecological benefits. However, relatively few prior studies have investigated the relative ecological benefits of various agroforestry systems. In the present study, the static chamber method, quantitative polymerase chain reaction, high throughput sequencing were used to establish the differences in greenhouse gases (GHGs) fluxes and explore the bacterial and fungal populations affecting GHGs fluxes under different agroforestry systems, including pure Moso bamboo forest (CK), bamboo + Bletilla striata (BB), bamboo + Dictyophora indusiata (BD), and bamboo + chickens (BC). The highest cumulative CH₄ uptake and N₂O emission in spring occurred in BB while the highest cumulative CO₂ emission and global warming potential (GWP) in spring occurred in BC. The Methylomirabilaceae were the key methanotrophs influencing the comparative differences in NO₃⁻ associated CH₄ uptake among the various agroforestry systems. N₂O emission was associated with pH, and nitrifiers such as the ammonia-oxidizing archaea and bacteria (Nitrospiraceae and Nitrosomonadaceae) rather than denitrifiers may be the key microbes affecting N₂O emission in different agroforestry systems. The bacteria Actinobacteriota and Fibrobacteres and the fungi Ascomycetes and Basidiomycota were the primary microbial taxa influencing CO₂ emission. The lignin-decomposing Basidiomycota played more important roles in CO₂ emission than the cellulose-decomposing fungi and bacteria under the various agroforestry systems. CO₂ emission was positively correlated with NO₃⁻ in the bacterial community and was negatively correlated with NO₃⁻ in the fungal community, implying two C decomposition mechanisms caused by denitrification dominated in bacteria and those caused by microbial nitrogen mining dominated in fungi. The foregoing results suggested that bamboo + B. striata had comparatively higher ecological benefits as it is associated with low GWP and external C fixation. The present study provided valuable information for screening bamboo-based agroforestry systems with high ecological benefits. It also elucidated the microbial mechanism explaining the observed differences in GHGs fluxes between the various agroforestry systems.

1. Introduction

In China, Moso bamboo (Phyllostachys edulis) is widely distributed over an area of ~4.68 Mha and accounts for ~73% of the entire bamboo forest area in China (National Forestry and Grassland Administration 2019). Monoculture is the main type of Moso bamboo plantation (Gai et al 2021). Moso bamboo could serve as a carbon (C) sink and provide considerable ecological benefits. However, the development of the Moso bamboo industry has been hampered by rising labor costs (Zhang et al 2019a). A single-story Moso bamboo forest canopy can provide large areas for herb cultivation or animal husbandry as these managements significantly differ in terms of the ecological niche (Zhang et al 2021). Several bamboo-based agroforestry systems such as the combinations bamboo-medicinal herbs (Zhang et al
2019b, 2020), bamboo-edible fungi, and bamboo-chicken farming (Zhang et al 2019a, Zhang et al 2021) have been established to increase productivity and farmer income. While these managements provide economic benefits, their ecological benefits remain to be determined.

Greenhouse gases (GHGs) such as carbon dioxide (CO$_2$), methane (CH$_4$), and nitrous oxide (N$_2$O) greatly contribute to global warming. As of 2018, their atmospheric concentrations reached 408 ppm, 1869 ppb, and 331 ppb, respectively, and increased by 22%, 46%, and 157%, respectively, above pre-industrial levels (WMO 2019). Moso bamboo forests help regulate the GHGs balance and offset soil CO$_2$ and N$_2$O emissions by sequestering atmospheric CO$_2$ and promoting CH$_4$ uptake by the soil (Song et al 2020). A previous study indicated that soil CO$_2$ and N$_2$O emissions increased and soil CH$_4$ uptake decreased in response to low and medium N deposition (30 and 60 kg N ha$^{-1}$ yr$^{-1}$), respectively, in Moso bamboo plantation (Zhang et al 2021b). This indicates that GHGs fluxes in the soil of Moso bamboo forests depend on N availability. Bamboo-medicinal herb intercropping, bamboo-edible fungus intercropping, and bamboo-chicken farming combination are widely established throughout southern China. Different agroforestry systems may lead to different N input quality and quantity owing to different management types, thus affecting GHGs fluxes. In addition, the interactions between Moso bamboo and herbs, edible fungi, or chickens may differ owing to different competitive relationships. Under low N availability, competition for N between Moso bamboo and herbs may lead to soil organic N mineralization and alterations in soil N$_2$O and CO$_2$ fluxes. Chicken manure input to the Moso bamboo forest floor would enhance N availability and improve plant growth, increasing root exudate and stimulating soil liable C decomposition and CO$_2$ emission. Thus, fertilizer input and the interactions between Moso bamboo and herbs, edible fungi, or chickens affect soil GHGs fluxes.

Soil GHGs fluxes are strongly associated with specific microorganisms implicated in the C and N cycles. CO$_2$ emission is driven by microorganisms involved in C decomposition, including most fungi and certain bacteria and actinomycetes (Brant et al 2006). Bacteria and fungi differ in terms of their abilities to degrade C. Certain bacterial genera such as Cellvibrio, Cellulomonas, and Pseudomonas can only degrade short-chain soluble cellulose via endoglucanase. Meanwhile, the bacterial genera Cellficiacula, Cytaphaga, and Sporocytophaga and the fungal genera Myrothecium and Chaetomium have strong cellulose degradation capacities (Szostk-Kotowa 2004). Furthermore, some Basidiomycota species can degrade lignin. Soil N$_2$O emission is driven by bacteria, archaea, and fungi involved in nitrification, denitrification, nitrifier denitrification, and so on (Butterbach-Bahl et al 2013). N$_2$O is a by-product of nitrification (Law et al 2012), an intermediate product in bacterial denitrification (Guo et al 2013), and an end product in fungal denitrification (Mothapo et al 2015). Its emission is strongly affected by pH and N and O$_2$ availability, etc (Saggar et al 2013). The forest may also act as a CH$_4$ sink (Li et al 2021). CH$_4$ uptake is strongly associated with methanotrophic archaea and bacteria. Methanotrophic bacteria such as the aerobic alpha-, gamma-proteobacteria, Verrucomicrobia, and the anaerobic Phylum Methylmirabilota (NC10) use O$_2$ and NO$_2^-$ as electron acceptors, respectively (Raghoebarsing et al 2006, Shen et al 2019). Hence, CH$_4$ uptake is affected by N form, N availability, and soil aeration. Different agroforestry systems lead to differences in soil physicochemistry, recruit different soil microorganisms, and form microbial communities with stable compositions and functions. N and C inputs quantitatively and qualitatively differ among bamboo-medicinal herb intercropping, bamboo-edible fungus intercropping, and bamboo-chicken farming systems. These differences strongly influence the soil bacteria and fungi implicated in C and N cycling and soil GHGs fluxes. However, the relationships among soil bacterial and fungal populations and GHGs fluxes under various agroforestry systems remain to be elucidated.

Here, the static chamber method, quantitative polymerase chain reaction (qPCR), high throughput sequencing were used to clarify the relationships among GHGs fluxes and the associated soil bacterial and fungal populations under various agroforestry systems, including pure Moso bamboo forest (CK), bamboo + Bletilla striata (BB), bamboo + Dictyophora indusiata (BD), and bamboo + chickens (BC). We hypothesized that (a) GHGs fluxes varied between different agroforestry systems as each management differed in terms of N and C input and soil aeration. (b) The specific bacterial and fungal populations inhabiting the soil under the various agroforestry systems contributed to the relative differences in soil GHGs fluxes. The discoveries of this study could help screen effective methods to improve the ecological benefits of bamboo-based agroforestry and clarify the mechanisms of soil microorganism-mediated GHGs fluxes in these systems.

2. Materials and methods

2.1. Site description

The study site was located in Anji County (30°31’N, 119°36’E), Huzhou City, Zhejiang Province, China. It has a subtropical monsoon climate, mean annual temperature of 15.6 °C, mean annual precipitation of 1380 mm, and average of 226 frost-free days per year. The study was conducted in Moso bamboo forests. The understory vegetation had 10% coverage and included Portulaca oleracea L. and ferns in pure...
Moso bamboo forest. Intensive managements procedures such as annual removal of understory vegetation, annual fertilization (N, 15%; P₂O₅, 6%; K₂O, 20%; 450 kg ha⁻¹) in autumn, biennial thinning of mature bamboo, and agroforestry development were performed. Bamboo-based agroforestry systems were widely distributed at the site and included B. striata, Polygonatum sibiricum, D. indusiata, and chick- ens, among others. Before establishing the systems, understory vegetation was removed.

2.2. Experimental design

The experiment consisted of three bamboo-based agroforestry systems, namely bamboo + B. striata (BB), bamboo + D. indusiata (BD), and bamboo + chickens (BC) and there were three replicates per system. Pure Moso bamboo forests (CK) served as the control. In May 2020, 12 plots (20 m × 20 m) with similar slope direction (south), degree (20°–30°), and position were established to minimize the influences of topographic factors. In the BB system, B. striata was grown at a density of 120,000 ± 30,000 ha⁻¹ and ~300 kg ha⁻¹ compound fertilizer (15:6:20 N:P₂O₅:K₂O) was applied in autumn. In the BD system, D. indusiata was grown at a density of 15,000 ± 3000 ha⁻¹ and 750 kg ha⁻¹ urea and gypsum, 375 kg ha⁻¹ calcium phosphate, and 60,000 kg ha⁻¹ bamboo sawdust were applied in autumn. In the BC system, chickens were bred at a density of 480 ± 25 ha⁻¹ and approximately 2000 kg ha⁻¹ (fresh manure) excrement (the equivalent of 32.6 kg ha⁻¹ N, 30.8 kg ha⁻¹ P, and 16.5 kg ha⁻¹ K) were applied into soils each year. Details of the study site are listed in Table 1.

2.3. Sample collection and index determination

The closed static chamber method was used to collect the gas samples using three chambers in each plot. The static chamber consisted of a base (40 cm × 40 cm × 10 cm) with a U-shaped groove (50 mm length × 50 mm depth) and a box (40 cm × 40 cm × 40 cm). Gas sampling was performed according to Zhang’s method (Zhang et al 2021b). In short, during gas sampling, the cover boxes were placed onto the base boxes and the grooves filled with water to serve as an air seal. A small fan was installed in each chamber to mix the air. Four samples were collected using a 60-ml plastic syringe attached to a three-way stopcock every 10 min for 30 min (i.e. at 0, 10, 20, and 30 min) in each chamber. Gases were sampled between 9:00 am and 11:00 am daily, twice monthly from March to May 2021. The gas samples were analyzed by gas chromatography (GC-2014, Shimadzu Corp., Kyoto, Japan), with flame ionization detector (FID) for CH₄ and CO₂ detection and electron capture detector (ECD) for N₂O detection.

Soil samples were collected at 0–20 cm depth with a soil sampler (i.d. = 3.5 cm) on April 29, 2021. One part of each sample was stored at −20 °C before microbial DNA extraction while the other was air-dried and its physicochemical properties were analyzed. The pH of 1:2.5 soil-water mixtures was measured with a pH meter. Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined using the chloroform fumigation–extraction method (Cui et al 2018). Total N (TN), NH₄⁺—N, and NO₃⁻—N were determined using the Kjeldahl method (Bremner 1996), the indophenol blue colorimetry method (Dorich and Nelson 1983), and ultraviolet spectrophotometry (Norman and Stucki 1981), respectively. Soil organic carbon (SOC) was determined using wet oxidation with K₂Cr₂O₇ (Nóbrega et al 2015). Alkali-hydrolyzable nitrogen (AHN) was determined according to the methodology of Roberts et al (2011).

2.4. Calculation

The CO₂, CH₄, and N₂O fluxes were calculated using the following equation (Zhang et al 2021b):

\[
F = \frac{dc}{dt} \times \frac{M}{V_0} \times \frac{273}{273 + T} \times \frac{V}{A},
\]

where \( F \) is the gas flux (mg CO₂ m⁻² h⁻¹ for CO₂, mg CH₄ m⁻² h⁻¹ for CH₄, and mg N₂O m⁻² h⁻¹ for N₂O), \( M \) is the molar mass of the gas (g mol⁻¹), \( V_0 \) is the molar volume under standard conditions (1 mol⁻¹), \( V \) is the volume of the static chamber (m³), \( A \) is the area covered by the static chamber, dc/dt is the change in gas concentration (dc) over a certain time (dt), and \( T \) is the air temperature (°C).

The cumulative soil CO₂, CH₄, and N₂O fluxes in spring were calculated with the following equation:

\[
F_\text{g} = \sum_{i=6}^{(F_{i+1} + F_i)/2 \times (t_{i+1} - t_i) \times 24 ÷ 100,
\]

where \( F_\text{g} \) is the cumulative soil flux in spring [CO₂ (kg CO₂ ha⁻¹), CH₄ (kg CH₄ ha⁻¹), or N₂O (kg N₂O ha⁻¹)], \( F \) is the soil CO₂ (mg CO₂ m⁻² h⁻¹), CH₄ (mg CH₄ m⁻² h⁻¹), or N₂O (mg N₂O m⁻² h⁻¹) flux determined at each sampling time, \( t \) is the sampling number, and \( t \) is the sampling time.

The global warming potential (GWP) was calculated with the following equation (Song et al 2020):

\[
\text{GWP (t C eq ha⁻¹)} = (25 \times F_{\text{CH}_4} + 298 \times F_{\text{N}_2\text{O}} + F_{\text{CO}_2}) \times 12/44,
\]

where \( F_{\text{CO}_2}, F_{\text{CH}_4}, \) and \( F_{\text{N}_2\text{O}} \) are cumulative emissions of CO₂, CH₄, and N₂O in spring. The fractions of 12/44 convert the mass of CO₂ into C mass. 25 and 298 indicate the radiative forcing of CH₄ and N₂O, respectively, in terms of a CO₂ eq unit at a 100 year time horizon.

2.5. DNA extraction and fluorescent qPCR

Microbial DNA was extracted from each 0.25 g fresh soil sample with a Qiagen DNA Easy Kit for Soil
Qiagen, Hilden, Germany). The functional genes related to nitrification and denitrification included archaeal amoA and bacterial amoA, nirK, nirS, and nosZ. The functional genes related to C decomposition included cbhI, GH48, and chIA targeting fungal cellobiohydrolase I, bacterial glycoside hydrolase, and family 18 group A chitinases, respectively. The pmoA served as a bacterial methanotroph biomarker. The foregoing genes were quantified with MoA qPCR Kit (Takara Bio, Dalian, China). Details of the primers and thermal protocols are shown in Supplementary Table S2. The V3–V4 hypervariable region of the bacterial 16S rRNA gene and the fungal ITS1 fragment were amplified with the primer pairs 338F (5′-GTGACCTAGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) and the primer pairs ITS1F (5′-CTTGGTCAATTAGAGGAA GTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGA GC-3′), respectively, using an ABI GeneAmp® 9700 PCR Thermocycler (Applied Biosystems Inc., Waltham, MA, USA). Equimolar quantities of purified amplicons were pooled and paired-end sequenced on an Illumina MiSeq PE300 platform (Illumina, San Diego, CA, USA) by Majorbio BioPharm Technology Co. Ltd (Shanghai, China) according to standard protocols.

Table 1. Details of the study site.

| Agroforestry system | Bamboo density (hm⁻²) | Diameter at breast height (DBH; cm) | NH₄⁺ (mg kg⁻¹) | NO₃⁻ (mg kg⁻¹) | TN (g kg⁻¹) | SOC (g kg⁻¹) | pH |
|---------------------|-----------------------|-------------------------------------|----------------|----------------|-----------|------------|----|
| Pure bamboo forest (CK) | 3057 ± 184 | 10.3 ± 0.3 | 9.9 ± 0.2 | 2.9 ± 0.2 | 1.9 ± 0.1 | 21.9 ± 1.0 | 5.9 ± 0.4 |
| Bamboo + B. stratiata (BB) | 3022 ± 285 | 10.1 ± 0.4 | 14.7 ± 0.6 | 10.1 ± 0.7 | 2.5 ± 0.2 | 31.7 ± 1.4 | 6.1 ± 0.3 |
| Bamboo + D. indica (BD) | 3084 ± 302 | 9.8 ± 0.4 | 17.6 ± 0.2 | 6.9 ± 0.5 | 3.8 ± 0.2 | 48.7 ± 4.9 | 6.0 ± 0.3 |
| Bamboo + chickens (BC) | 2810 ± 203 | 9.6 ± 0.2 | 11.8 ± 0.8 | 5.9 ± 0.5 | 2.2 ± 0.2 | 24.9 ± 3.0 | 5.3 ± 0.3 |

2.7. Statistical analyses

Bioinformatic analysis of the soil was conducted using the Majorbio Cloud platform (https://cloud.majorbio.com). Based on the ASVs information, rarefaction curves (figure S1) and Shannon and Simpson indices were calculated using Mothur v. 1.30.0 (https://mothur.org/wiki/mothur_v.1.30.0/) (Schloss et al 2009). Similarities among microbial communities under various agroforestry systems were determined by non-metric multidimensional scaling (NMDS) based on the Bray-Curtis dissimilarities. Anosim tests were used to assess the significance of the bacterial and fungal communities among the various agroforestry systems. Linear discriminant analysis (LDA) effect size (LEfSe) (Segata et al 2011) was run to identify significantly abundant bacterial and fungal taxa (phylum–family) under the different agroforestry systems (LDA score > 3.5; p < 0.05). Redundancy analyses (RDA) were performed in the vegan package of R v. 2.5-3 (R Core Team, Vienna, Austria) to investigate the effects of soil physicochemistry on soil bacterial and fungal community structures. Forward selection was based on 9999 Monte Carlo permutations. Variance partial analyses (VPA) were conducted to quantify species distributions according to N nutrients and C sources. Bacterial and fungal functions were predicted using FAPROTAX (www.loucalab.com/archive/FAPROTAX) and FUNGuild (https://github.com/UMNFuN/FUNGuild), respectively, based on the representative ASVs sequences.
One-way analysis of variance and Duncan’s multiple comparison tests were performed in Statistical Product and Service Solutions software (SPSS 20.0, IBM Corp., Armonk, NY, USA) to compare differences in GHGs fluxes, GWP, Shannon and Simpson indices, and functional genes among the various agroforestry systems. GHGs fluxes and functional genes were plotted using SigmaPlot 12.5. Heatmaps depicting specific bacterial and fungal populations were plotted using TBtools (https://github.com/CJ-Chen/TBtools).

3. Results

3.1. Effects of bamboo-based agroforestry systems on soil GHGs fluxes in spring

The dynamic changes of GHGs fluxes and accumulation of GHGs had different responses to different bamboo-based agroforestry systems in spring (figures 1 and S2). Soil CO$_2$ flux gradually increased over time in CK, while showing the opposite trend in BB. In addition, soil CO$_2$ flux showed first falling and then rising levels in BD and BC. The cumulative CO$_2$ flux was the highest in BC, followed by BD and BB, which were 36.36%, 29.49%, and 16.16% higher than that in CK ($p < 0.05$), respectively. CH$_4$ uptake showed a downward, then upward trend in CK, BB, and BC over time whereas it increased in BD. Meanwhile, cumulative CH$_4$ uptake in BB was significantly higher than that in BD, CK, and BC ($p < 0.05$). N$_2$O emission showed first a rising and then a falling trend in CK and BB; in contrast, N$_2$O emission in BD and BC decreased first and then increased. The highest N$_2$O emission was observed in BB, followed by BD and CK, while the lowest N$_2$O emission occurred in BC. In addition, GWP was the highest in BC, followed by BD and BB, which was 35.91%, 29.55%, and 16.10% higher than that in CK. CO$_2$ and N$_2$O emissions contributed 100.31%–100.68% and 1.22%–2.40% to GWP, respectively; in contrast, CH$_4$ uptake offset 1.90%–2.81% to GWP.

3.2. Effects of bamboo-based agroforestry systems on bacterial and fungal community diversity

The Shannon and Simpson indices indicated that the various bamboo-based agroforestry systems had no significant effect on the bacterial alpha diversity but altered the fungal alpha diversity. The latter was significantly lower in BC than in CK (table 2).

3.3. Effects of bamboo-based agroforestry systems on soil microbial community composition

The NMDS plot and Anosim indicated that bacterial and fungal community composition significantly differed among agroforestry systems ($p < 0.05$) (figures 2(A) and (B)). The dominant bacterial phyla were Acidobacteria, Proteobacteria, Chloroflexi, Actinobacteriota, Methyloirabilota, and Bacteroidota. Collectively, they accounted for ~80% of all ASVs (figure 3(A)). The LEfSe analysis revealed that the Vicinamibacteria class, Planctomycyctota and Nitrospirota phyla were significantly abundant in BB; the Chloroflexia and Patescibacteria phyla,
and Acidobacteria, Ktedonobacteria and Planctomycetes classes were significantly abundant in BC; the Proteobacteria phylum, Cytophagales order, and Microscillaceae family were significantly abundant in BD; and the Alphaproteobacteria and Actinobacteria classes, Rhizobiales, Sphingomonadales, and Micromonosporales orders, and Xanthobacteraceae family were significantly abundant in CK (figures 4(A) and S3(A)).

The dominant fungal phyla were Basidiomycota, Ascomycota, Mortierellomycota, Glomeromycota, and Rozellomycota. Collectively, they accounted for ~58.63%–79.51% of all ASVs (figure 3(B)). The LEfSe analysis revealed that the Glomeromycota phylum, Mortierellomycetes class, and Sordariales and Tubeufiales orders were significantly abundant in BB; the Basidiomycota phylum, Agaricomycetes and Saccharomycetes classes, and Trechisporales, Agaricales, Botryosphaeriales, Cantharellales, Tremellales, and Leucosporidiales orders were significantly abundant in BC; the Ascomycota and Rozellomycota phyla, Archaeorhizomycetes and Spiizellomycetes classes, Tremellodendropsidales and Trichosporonales orders were significantly abundant
in BD; the Mortierellomycota and Entorrhizomycota phyla, and Polyporales order were significantly abundant in CK (figures 4(B) and S3(B)).

3.4. Specific bacterial and fungal populations associated with GHGs fluxes under various agroforestry systems

Soil bacterial and fungal populations associated with GHGs fluxes differed across the four agroforestry systems. The anaerobic phylum Methylomirabilota was enriched in CK. In contrast, the Methylomirabilaceae family, belonging to the Methylomirabilota phylum, was significantly more enriched in BB than in the other agroforestry systems. The anaerobic WX65 and aerobic Methylphilaceae families were enriched in BB and BC. The aerobic Methylolgellaceae family was enriched in BD (figure 5(A)).

The relative abundances of nitrifying bacteria (phylum Nitrospirota and families Nitrospiraecae and Nitrosomonadaceae) were significantly higher in BB than in the other agroforestry systems. The relative abundances of the typical denitrifying bacteria Sphingobacteriaceae, Flavobacteriaceae, Comamonadaceae, and Pseudomonadaceae were reduced in BB. The relative abundance of Sphingobacteriaceae increased in BC. The relative abundances of Flavobacteriaceae and Comamonadaceae increased in BD (figure 5(B)). The fungal denitrifier genera Bionectria, Clonostachys, Mortierella, and Acremonium were enriched in BB; Botrytis, Trichoderma, Penicillium, Phoma, and Talaromyces were enriched in BD; and Shiraia, Fusarium, Oidiodendron, Gibberella, Candida, Cladosporium, and Calonectria were enriched in BC (figure S4).
Typical C-degrading bacteria were enriched in CK and BD. However, only the Acidobacteriota phylum and the Cellvibrio genus were enriched in BB and BC, respectively (figure 6(A)). The relative abundances of typical C-degrading fungi were low in CK. The cellulose- and lignin-degrading fungi were specifically distributed in other agroforestry systems. The Ascomycota phylum and the Chaetomium, Xylaria, and Pleurotus genera were enriched in BB. The Ascomycota, Chytridiomycota, and Basidiomycota phyla and the Penicillium, Trichoderma, Agaricus, and Thielaria genera were enriched in BD. The Basidiomycota phylum and the Cladosporium, Aspergillus, Arrhenia, and Myrothecium genera were enriched in BC (figure 6(B)).

3.5. Effects of bamboo-based agroforestry systems on functions of bacterial and fungal community related to GHGs fluxes

The abundance of pmoA related to CH₄ uptake was the highest in BB and its level in the latter was significantly higher than that in CK (figure 7(A)). The functional gene related to N₂O emission had different responses to different agroforestry systems. The abundance of the archaeal ammA was the highest in BB, 4–5 times higher than those in BD and BC, and 75 times higher than that in CK (figure 7(B)). The abundance of bacterial ammA was the highest in BD and BC and was significantly higher than those in BB and CK (figure 7(C)). In contrast, the abundances of nirS, nirK, and nosZ involved in denitrification did not significantly differ between agroforestry systems (figures 7(D)–(F)). In addition, the abundance of cbhI was the highest in BD followed by BC. The levels of both were significantly higher than that in CK (figure 7(G)). By contrast, the abundance of GH48 was enriched in CK and higher than those in the other agroforestry systems (figure 7(H)). The abundance of chiA was the highest in BD and its level in the latter was significantly higher than that in BB (figure 7(I)).

Based on the predictions of 16S rRNA sequencing, bacterial functions related to N₂O emission (nitrite, nitrate, and nitrous oxide denitrification) were enriched in CK and BD but reduced in BB and BC. Bacterial functions related to CO₂ emission (cellulolysis, chemoheterotrophy, and aerobic chemoheterotrophy) were enriched in CK and BC but reduced in BB and BD, while another function related to CO₂ emission (chitinolysis) was enriched in CK and BB. Bacterial functions related to CH₄ oxidation (methanol oxidation) were enriched in BB and BC (figure 8(A)). Based on the predictions of ITS sequencing, Undefined Saprotroph and Arbuscular Mycorrhizal were enriched in BB, Soil Saprotroph was enriched in BD, Wood Saprotroph and Leaf Saprotroph were enriched in BC, and unknown was enriched in CK (figure 8(B)).

3.6. Relationships among environmental factors and bacterial and fungal community composition

The RDA revealed that F_N₂O, pH, TN, MBC, and SOC were the key environmental factors affecting bacterial community composition (p < 0.05), whereas F_N₂O, pH, NO₃⁻, NH₄⁺, MBC, and MBN were the key environmental factors affecting fungal community composition (p < 0.05) (table S2). The pH controlled
bacterial and fungal community composition in BB, while MBC significantly affected bacterial and fungal community composition in BD. The N₂O and CH₄ fluxes were greatly influenced by the bacterial and fungal communities in BB and positively correlated with the pH. The CH₄ flux was positively correlated with NO₃⁻ in the bacterial community and with NO₃⁻ and NH₄⁺ in the fungal community. The CO₂ flux was markedly influenced by the bacterial community in BC and by the fungal communities in BC and BD. The CO₂ flux was positively correlated with NO₃⁻ in the bacterial community and negatively correlated with NO₃⁻ in the fungal community (figures 9(A) and (B)).

The VPA (figures 10(A) and (B)) showed that N nutrients (TN, AHN, MBN, NH₄⁺, and NO₃⁻) and C sources (SOC and MBC) explained 19.53% and 33.32% of the variation in the bacterial community composition, respectively. The N nutrients and C sources explained 63.52% and 87.83% of the variation in the fungal community composition, respectively. The N nutrient indices had stronger influences than the C source indices on the bacterial and fungal community composition.

4. Discussion

4.1. Relationship among GHGs fluxes and environmental factors under various agroforestry systems

The highest cumulative CH₄ uptake and N₂O emission in spring were determined for BB. The highest CO₂ emission occurred in BC. Previous studies indicated that decreased soil disturbance and increased soil macroporosity could increase CH₄ uptake (Nair et al 2010, Christiansen and Gundersen 2011). Moreover, decreased soil moisture levels (drought) could increase CH₄ uptake rates in forest soils (Liu et al 2019). Here, NO₃⁻ also influenced CH₄ uptake. The relative abundance of Methylomirabilaceae, which carry out nitrite-dependent anaerobic methane oxidation using CH₄ as an external C source and NO₃⁻ or NO₂⁻ as electron acceptors (Song et al 2022), was significantly higher in BB than in the other agroforestry systems. Previous studies indicated that N₂O emission increased under agroforestry systems and was associated with soil pH and N availability (Hall et al 2006, Kim et al 2016). This result was somewhat different from our study. In the present study, the N₂O emission was lower in BC than in BB, BD, and CK. A previous study reported that seasonal total N₂O emissions decreased to a greater extent in response to cattle manure-derived organic fertilizer than to chemical fertilizer (Geng et al 2021), indicating the different effects of fertilizer types on N₂O emission.

In this study, CO₂ emissions from the bacterial and fungal communities were positively and negatively correlated with NO₃⁻, respectively. This implies different C decomposition mechanisms in bacterial
Figure 7. Variations in the functional genes related to GHGs fluxes among bamboo-based agroforestry systems. (A): gene related to CH$_4$ uptake (pmoA); (B)–(F): genes related to N$_2$O emission (archaeal amoA, and bacterial amoA, nirS, nirK, and nosZ); (G)–(I): genes related to CO$_2$ emission (ehl, GH48, and chiA). CK: pure bamboo forest; BB: bamboo + B. striata; BD: bamboo + D. indusiata; and BC: bamboo + chickens. Each bar represents the average of three replicates. Error bars indicate standard deviation (SD). Different lowercase letters indicate significant differences between different agroforestry systems ($p < 0.05$).

Figure 8. Functional profiles predicted by FAPROTAX for bacteria (A) and by FUNGuild for fungi (B). CK: pure bamboo forest; BB: bamboo + B. striata; BD: bamboo + D. indusiata; BC: bamboo + chickens.

The bacterial community denitrified using organic carbon as the C source and NO$_3^-$ as the electron acceptor when NO$_3^-$ was abundant. Under NO$_3^-$ depletion, microbial N mining led to organic C decomposition and CO$_2$ emission. The CO$_2$ flux was positively correlated with MBN and MBC in the fungal community, which provides strong evidence for a C degrading mechanism in the fungal community. Therefore, we linked the CO$_2$ flux and NO$_3^-$ concentration in different agroforestry
systems and speculated that organic C decomposition by fungi was the primary contributor to CO$_2$ emission.

4.2. Specific bacterial and fungal populations associated with GHGs fluxes

Functional prediction for bacteria revealed that methanol oxidation was enriched in BB. Methanol is an intermediate metabolite during methane oxidation. Methane is first oxidized to methanol by methane mono-oxygenase. Methanol is then oxidized to formaldehyde and the latter is oxidized to CO$_2$ (Mateos-Rivera et al. 2018). This indicates that methane oxidation was enriched in BB. Linking the pmoA gene and typical methanotrophs and CH$_4$ uptake, we believe Methylomirabilaceae were the key methanotrophs explaining the differences in the pmoA gene and CH$_4$ uptake between the various agroforestry systems.

The functional genes related to nitrification and denitrification indicated that N$_2$O emission was associated mainly with ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB). The Nitrospirotia phylum and the Nitrospiraceae and Nitrosomonadaceae families were the primary bacteria responsible for N$_2$O emission. AOA might play a comparatively more important role than AOB in N$_2$O emission. In BB alone, AOA abundance was one order of magnitude higher than that of AOB. Previous studies disclosed that fungi may contribute >50% of the total soil N$_2$O emissions (Crenshaw et al. 2008, Chen et al. 2014). The various agroforestry systems in the present study contained different soil fungal denitrifiers. As there were only minor relative differences among denitrification-associated functional genes, fungal denitrifiers may not be the key microbes explaining the differences in N$_2$O emission among the various agroforestry systems.

C-decomposing bacteria and fungi play vital roles in CO$_2$ emission. The comparatively enriched bacterial C decomposition but lower cumulative CO$_2$ emission in CK suggested the weak roles of bacteria in soil C decomposition. The cbh1 and GH48 genes are biomarkers in fungal and bacterial cellulose degradation, respectively. They had different

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Figure 9: RDA plot of relationships among environmental variables and phylum-level bacterial (A) and fungal (B) community composition. Correlations among environmental variables and RDA axes are represented by arrow length and angle. CK: pure bamboo forest; BB: bamboo + _B. striata_; BD: bamboo + _D. indusiata_; BC: bamboo + chickens.

Figure 10: Variance partitioning analysis on bacterial (A) and fungal (B) community composition. C: SOC and MBC; N: TN, AHN, MBN, NH$_4^+$, and NO$_3^−$.

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responses in each agroforestry system. The results indicated that fungi were greater contributors to cellulose degradation than bacteria. Unlike bacteria, fungi can degrade lignin, which is a recalcitrant structural compound unique to terrestrial vascular plants (Boer *et al* 2005). Therefore, enriched wood saprotroph fungi in BC and enriched soil saprotroph fungi in BD indicated that BC had greater C decomposition capacity and higher CO$_2$ emission rates than BD. Moreover, bacteria and fungi differ in their C degradation abilities. Acidobacteriota can degrade short-chain C compounds (*Su* *et al* 2020) whereas Actinobacteria and Fibrobacteres can degrade cellulose (Ransom-Jones *et al* 2012). Meanwhile, many Ascomycota degrade cellulose, whereas most Basidiomycota degrade lignin (*Dashtban* *et al* 2010). Therefore, the cumulative CO$_2$ emissions were comparatively greater in BC and BD than in other agroforestry systems. The foregoing findings suggest that an elevated microbial C decomposition ability may lead to increased CO$_2$ emissions.

4.3. Ecological benefits of various bamboo-based agroforestry systems

GWP was significantly higher under bamboo-based agroforestry systems than under pure bamboo forests, and CO$_2$ was the primary contributor to GWP. However, *B. striata* in BB could fix atmospheric CO$_2$ via photosynthesis and partially offset the CO$_2$ and N$_2$O emissions. In addition, SOC increased by 17.32% in BB compared with those at the beginning of the experiment. For these reasons, bamboo + *B. striata* was suitable for plantation in Zhejiang Province. *Kim et al* (2016) found that tree-crop agroforestry stands (average age 14 yr) sequestered 7.2 ± 2.8 t C ha$^{-1}$ yr$^{-1}$, of which biomass and soil C sequestration contributed ~70% and ~30%, respectively. Soils under agroforestry oxidized 1.6 ± 1.0 kg CH$_4$ ha$^{-1}$ yr$^{-1}$ and emitted 7.7 ± 3.3 kg N$_2$O ha$^{-1}$ yr$^{-1}$. This discovery demonstrated the ecological benefits of agroforestry characterized by plant growth under trees.

5. Conclusion

Bamboo-based agroforestry systems altered soil GHGs fluxes and increased the GWP, to which CO$_2$ was the primary contributor. Anaerobic methanotrophs such as Methylocirrabilaceae and WX65 family members played relatively more important roles in CH$_4$ uptake among the various agroforestry systems. Nitrifying bacteria, such as AOA and AOB (Nitrospirae and Nitrosomonadaceae), primarily accounted for the observed differences in N$_2$O emission between the various agroforestry systems. Fungi played comparatively more important roles in CO$_2$ emission than bacteria among the different agroforestry systems. The pH was a key factor affecting N$_2$O emission, and NO$_3^-$ was a key factor affecting CH$_4$ uptake and CO$_2$ emission. Under different agroforestry systems, the NO$_3^-$ concentration may be associated with two C decomposition pathways carried out by bacteria and fungi, leading to the difference in CO$_2$ emission. The foregoing results indicate that bamboo + *B. striata* provided the greatest benefit in terms of carbon emission reduction. The present study clarified the relationships among soil microorganisms and GHGs fluxes and provided novel insights into improving the ecological benefits of various agroforestry systems.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

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