The conversion of subtropical forest to tea plantation changes the fungal community and the contribution of fungi to N2O production

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A B S T R A C T
The conversion of natural forests to tea plantations largely affects soil nitrous oxide (N2O) emissions and soil microbial communities. However, the impacts of this conversion on the contribution of fungi to N2O emission and on fungal community structure remain unclear. In this study, we determined the soil N2O emission rate, N2O production by fungi, associated fungal community diversity, and related ecological factors in chronological changes of tea crop systems (3, 36 and 105 years old tea orchards named T3, T36 and T105, respectively), and in an adjacent soil from a natural forest. The results indicate that the tea plantations significantly enhanced soil N2O production compared with the forest soil. Tea plantations significantly decreased soil pH and C/N ratio, but increased soil inorganic nitrogen (N). Furthermore, they increased the fungal contribution to the production of soil N2O, but decreased the bacterial counterpart. We also observed that fungal community and functional composition differed distinctly between tea plantations and forest. Additionally, most of the fungal groups in high N2O emission soils (T36 and T105) were identified as the genus Fusarium, which were positively correlated with soil N2O emissions. The variation in N2O emission response could be well explained by NO3- /C02-N, soil organic carbon (SOC), C/N, and Fusarium, which contributed to up to 97% of the observed variance. Altogether, these findings provide significant direct evidence that the increase of soil N2O emissions and fungal communities are attributed to the conversion of natural forest to tea plantations.

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1. Introduction
Since the 1990s, the conversion of forests to cash crop plantations such as rubber, fruits, palm oil, tree nurseries and tea, has been increasing significantly (Ahrends et al., 2015; Su et al., 2016; Ziegler et al., 2009). Tea plantations (Camellia sinensis) are important for the economy in China. In 2018, one of the most important tea plantations occupied a cultivation area of 2.93 million ha (~61% of the world’s total tea planting area) and was responsible for the production of 2.61 million tons of tea (~45% of the world’s total tea production) in 2018 (International Tea Committee, 2019). However, the conversion of natural ecosystems to plantations generates various problems, such as alteration of local hydrology (Ahrends et al., 2015; Guimberteau et al., 2017), acceleration of soil erosion (Guaillame et al., 2015), biodiversity threats (Wilcove and Koh, 2010), and increase in greenhouse gas (GHG) emissions (Su et al., 2016). Nitrous oxide (N2O), a potent greenhouse gas with long residence time in the atmosphere, can destruct stratospheric ozone and substantially contribute to global warming (Ravishankara et al., 2009; Stein and Klotz, 2016). Soil is the largest source of N2O emissions, and agriculture, forestry and other land uses accounted...
for approximately 82% of N₂O anthropogenic sources (IPCC, 2019). Moreover, forest conversion to croplands has been shown to substantially increase N₂O emission rates, particularly in fertilized systems (van Lent et al., 2015).

Previous studies have reported that tea plantation can decrease soil pH while increasing bulk density, soil organic carbon (SOC) and total N, with increase with plantation age (Alekseeva et al., 2011; Hacisalihoglu et al., 2018; Li et al., 2016; Majaliwa et al., 2010; Wang et al., 2016). Several decades ago, the N₂O-producing metabolic pathway was considered to only exist in bacteria, and eubacteria could not have this ability (Zumft, 1997). However, a fungus (Fusarium oxysporum) was reported to produce N₂O by the denitrification process (Hu et al., 2015; Shoun et al., 1992, 1992). Recently, the existence of fungal denitrification in different soil ecosystems has gradually become an emerging concept and has received much more attention from researchers worldwide (Motheapo et al., 2015; Yu et al., 2019). Moreover, previous studies indicate that denitrification dominates N₂O emissions in acidic soils (Ambus et al., 2006; Zhang et al., 2011; Cai et al., 2012; Chen et al., 2015; Cheng et al., 2015). Paradoxically, fungi might be more important to N₂O production compared to bacteria, given that soil fungi are more tolerant to acidic conditions and can produce N₂O under aerobic or hypoxic conditions (Crenshaw et al., 2008; Fierer and Jackson, 2006; Huang et al., 2017; Laughlin and Stevens, 2002; Yanai et al., 2007; Zhou et al., 2001). Therefore, tea plantations might increase fungal N₂O production compared with natural forest.

Conversion of forests to monoculture plantations such as teak and rubber, can affect the soil microbial communities, which are important for nutrient cycling (Bardgett et al., 2017). This nutrient cycling promoted by microorganisms is vital for soil fertility and plant growth. In this context, the effects of biodiversity loss after tea plantation on microbial communities and underground ecosystem functions are still unclear. A previous study states that the conversion of forest to rubber and palm oil plantations will change the soil bacterial communities in the tropic regions (Berkelmann et al., 2018). In addition to bacteria, fungi are also important for soil functions, which are influenced by changes in nutrient availability and plant types during the conversion of forest to plantation (Ji et al., 2018; Jin et al., 2019). Moreover, soil fungi contribute to plant productivity by enhancing soil nutrient availability and to plant growth through the development of symbiotic relationships and pathogenic infections (Moore et al., 2004; Mortimer et al., 2015; Kernaghan, 2005). Here, we determine how the conversion of forest to tea plantation can induce changes in soil properties. Furthermore, since fungi can be essential for soil N₂O production, we also determined the total soil fungal community structure to investigate the relationship between the dynamics of N₂O emission and N₂O-producing fungi. We hypothesized that (1) the production of N₂O by fungi would increase in the tea plantation area compared with natural forest; (2) conversion of natural forest to tea plantation might change the fungal community and promote soil N₂O-producing fungi.

2. Materials and methods

2.1. Site description and soil sampling

Soil sampling sites were located in Hangzhou, south China (30 11’ N, 120 4’ E), where the annual mean temperature is 17 °C, and the mean annual precipitation is 1438 mm. Soils under tea plantation with different planting ages were selected: 3-years (T3), 36-years (T36), and 105-years (T105) tea plantations. An adjacent forest land was dominated by evergreen broadleaf plants, including Cinnamomum camphora, Castanopsis sclerophylla, and Schima crenata Korthals. The tea plantations had similar management with ~450 kg N ha⁻¹ y⁻¹ ammonium sulfate and urea, whereas the natural forest has no fertilizer input (Yan et al., 2018). The soils were classified as Ultisols, which developed from Anshan quartz-free porphyry (Han et al., 2007). Before the conversion of forest to tea plantation, these areas were categorized as natural forest without artificial field operations such as tillage, fertilization, aeration, and compaction, and were under the same soil erosion and climatic conditions. Based on the particle size distributions of clay (0.36–1.76), silt (31.08–36.45) and sand (63.19–67.16), the soils are classified as sandy loam soils.

All soil samples from four sites were collected from three selected plots. Three cores (0–15 cm) were mixed as a composite sample for one plot. Plant residues and stones were removed, and the samples were placed into an ice box that was transported to the laboratory. The samples were then sieved (2 mm) and divided into two parts. One subset was stored at 4 °C for measurement of soil properties and N₂O flux, and the other was stored at −80 °C for DNA extraction.

2.2. Measurement of soil properties

Soil NH₄-N and NO₃-N were analyzed using a continuous flow colorimeter (SEAL AutoAnalyzer 3, Southampton, UK). Their extraction from soil samples was performed with 1 M KCl solution (1:10 w/v), after shaking for 1 h, and filtering. After conducting soil suspension with water at 1:2.5 of ratio, the pH of soil suspension was measured in the suspension using a pH meter. The SOC was measured by using a K₂Cr₂O₇-H₂SO₄ oil bath pyrolysis method (Walkley and Black, 1934). Finally, the s soil carbon to nitrogen (C/N) ratio was measured using a Vario max CNS analyzer (Elementar, Germany). The soil chemical parameters are listed in Table S1.

2.3. Soil N₂O emission rate

To measure the soil N₂O emission rates from forest and tea plantations, soil samples (10 g dry weight) were placed into a 120 mL serum bottle sealed with a rubber stopper, then incubated aerobically at 60% water-filled pore space (WFPS) at 25 ± 1 °C. Moreover, the concentration of N₂O was measured everyday for three days. Before the measurement of the N₂O emission, the bottles were opened and placed into a fume hood for 30 min to flush the headspace for equilibrium with atmospheric pressure (Yu et al., 2019). The bottles were then closed for one day. Gas sample of 3 mL was extracted from the headspace of bottles by an auto-sampler (CTC analytics AG) equipped with a Gilson Minipuls®3 peristaltic pump. The gas samples were pumped into a gas chromatograph (Agilent 7890 A, Agilent, USA) to measure N₂O with an electron capture detector.

In addition, soil N₂O emission rates were determined under anaerobic condition at 60% WFPS at 25 ± 1 °C. The measurement and analyses processes were the same to those in aerobic conditions. To create anaerobic conditions, we selected the Helium (He) gas to replace the headspace air using a He flush vacuum system at the beginning of the incubation and after each sampling time.

2.4. Source of N₂O emission

To measure the source of soil N₂O emission (i.e. fungi and bacteria), four treatments were comprising with (a) Control: soil alone; (b) S: soil treated with 6.0 mg g⁻¹ soil of streptomycin; (c) C: soil treated with 10.0 mg g⁻¹ soil of cycloheximide; and (d) SC: soil treated with 6.0 mg g⁻¹ soil of streptomycin and 10.0 mg g⁻¹ soil of cycloheximide. The streptomycin and cycloheximide were selected...
to inhibit bacterial and fungal activities, respectively (Herold et al., 2012).

The N₂O fluxes were determined following the method of Huang et al. (2017) under different treatments. Briefly, 10 g of soil (dry weight) and 2 mL of distilled H₂O or corresponding antibiotic solutions were pre-incubated overnight at 4 °C. Then, the soils were placed in an incubator (25 ± 1 °C), to which glucose (2 mg C g⁻¹ dry soil) and KNO₃ (100 μg N g⁻¹ dry soil) were added at 60% WFPS. Moreover, the headspace air was replaced by a He/C₂H₂ mixture (90/10 v/v). The soil N₂O flux was measured at 4, 8, 16, and 32 h according to the method described by Laughlin and Stevens (2002).

The sources of soil N₂O emissions were calculated by subtracting the N₂O production of the inhibitor treatments from the N₂O production of the control (Herold et al., 2012). We used both inhibitors to distinguish the N₂O source from bacteria and fungi, and the inhibitor additivity ratio (IAR) was used to evaluate non-target inhibition of cycloheximide and streptomycin based on the microbial respiration response. The IRA was calculated using equation (1).

\[
IAR = \frac{(a - b) + (a - c)}{a - d} \tag{1}
\]

where \(a\), \(b\), \(c\), and \(d\) are CO₂ fluxes from soil: alone, treated with streptomycin, treated with cycloheximide, and treated with both antibiotics, respectively.

According to a previous study, upon use of antibiotics, IAR values close to 1 indicated that there was no non-target inhibition effect after antibiotics used in the study (Chen et al., 2015). Non-target inhibition represents the best concentration to inhibit fungi and bacteria to avoid nonspecific inhibition of non fungal or non bacterial respiration by adding glucose to stimulate the respiration of soil microorganisms. This method was mainly used to determine the appropriate concentration of inhibitors (Anderson and Domsch, 1975).

2.5. DNA extraction, qPCR and Illumina MiSeq sequencing

DNA was extracted from 0.1-g of freeze-dried soil using a Power Soil DNA Isolation kit (MoBio, United States) by using the manufacturer’s instructions. The 18 S rDNA, 16 S rDNA, amoA genes of bacteria and archaea were obtained to quantify the fungi, bacteria, ammonia oxidizing bacteria (AOB), ammonia oxidizing archaea (AOA), respectively. The analyses were conducted on a 96-well plate. The qPCR reaction solution proportion, system and calculation method were used according to our previous study (Zheng et al., 2019). Primers of these genes and thermal conditions have been described in previous studies (Table S2).

The fungal Internal Transcribed Spacer (ITS) region was sequenced on an Illumina MiSeq platform (300 × 2 paired-end). In details, the fungal ITS region was amplified (5 min initial denaturation at 95 °C; followed by 35 cycles at 95 °C for 30 s, 60 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 5 min) using the primer pairs ITS1F/ITS2R, with six-base barcodes attached to the 5’ end (Wang et al., 2018). A total of 50 μL of PCR reaction comprised of 25 μL of 2 × GoTaq Green master mix (Promega, Madison, WI, United States), 2 μL of -10 ng/μL template DNA, 1 μL of 10 mM of each primer, and 21 μL of Milli-Q water. The PCR products were purified using a THANGel Midi Purification Kit (Tiangen Biotech, Beijing, China) and quantified using a NanoDrop™ 2000 spectrophotometer (Thermo Scientific, United States). The purified products were pooled in equimolar and sequenced, and the sequences have been deposited into the NCBI Sequence Read Archive (SRA) under the BioProject PRJNA5558591.

2.6. Statistics analysis

We used a one-way analysis of variance (ANOVA) to evaluate the difference of chemical and biological properties among the four treatments (Forest, T3, T36, and T105) using SPSS 20.0 (IBM, United States). Raw sequencing reads were demultiplexed, trimmed to the expected size (300–385 bp), and then chimeras were removed using USEARCH v6.1.544. Operational taxonomic units (OTUs) were selected using QiIME 1.9.1 with the UNITE database (12.11) and the blast method with 97% similarity. The alpha and beta diversity were analyzed at a sequencing depth of 30,000 based on the OTU level. Principal coordinate analysis (PCoA) was conducted using the Bray-Curtis distance in PRIMER v7.0.13 (PRIMER-E Ltd, United Kingdom). The significant groups among different soils were determined by using the linear discriminant analysis effect size (LEfSe) method (http://huttenhower.sph.harvard.edu/lefse/), which required a P value lower than 0.05 and a LDA score higher than 2.0. To assign the fungal OTUs to different functional groups, we used an open ecological guilds annotation tool (FUNGuild (Nguyen et al., 2016)), located at https://stbates.org/guilds/app.php. The correlations between soil properties, fungal community and soil N₂O emissions were calculated using Pearson’s correlation. Similarity Percentages analyses (SIMPER) and variance partitioning analysis (VPA) were carried out using the “vegan” package (R 3.5.1). To determine whether the OTUs classified to different taxa were the N₂O-producing fungi, we collected the sequences of N₂O-producing fungus which have been confirmed in previous studies from NCBI GeneBank database. Then a maximum-likelihood tree was generated to analyze the correlation between the OTUs and N₂O-producing fungus using the MEGA X 10.18 (Kumar et al., 2018). Linear regression and ANOVA analysis were performed to determine the correlation between soil N₂O emission and the abundance of different microorganisms.

3. Results

3.1. Soil physicochemical properties

The conversion of forest to tea plantation substantially influenced soil properties (Table S1). The soil pH decreased, but soil organic C increased, especially in T36 and T105. A higher C/N ratio was observed in soils under tea plantation (~12) compared to that under natural forest (~18). Inorganic N, namely NO₃⁻-N and NH₄⁺-N, was significantly higher in the soil of tea plantations than in the forest soil (P < 0.05); and the NO₃⁻-N content was significantly higher than that of NH₄⁺-N in all soil samples. The NO₃⁻-N/NH₄⁺-N ratio ranged from 2.2 to 9.6, and it was significantly higher in T36 and T105 (5.2 and 9.6, respectively) than in T3 forest (3.2 and 2.2, respectively).

3.2. Source of N₂O flux

The soil N₂O flux rate was the highest in T36, followed by T105, T3 and forest (Fig. 1A). In T36 and T105, the N₂O flux rate changed 7 and 10 fold, respectively, compared with that in the forest. However, changes in the N₂O flux rate of soils in the forest and tea plantations were different under aerobic condition (Fig. 1B). The highest N₂O flux rate was observed in forest soil (28.6 μg N d⁻¹ kg⁻¹ soil), the flux rate in the tea plantation soils increased with planting period.

The soils treated with inhibitors showed significantly reduction of N₂O flux rates compared with to soil with no inhibitor treatment (Fig. 1B). Among different soil ecosystems, the IAR results suggest...
the lack of no non-target effect of inhibitors. In the forest soil, the contribution of bacteria to soil \(\text{N}_2\text{O}\) fluxes was much higher than in the soil under tea plantation. In contrast, the contribution of fungi to soil \(\text{N}_2\text{O}\) fluxes was more than 55% higher in tea plantation soils compared with forest soil.

3.3. Quantification of different microorganisms

The quantification results were distinct for each microorganisms and soil ecosystem (Fig. S2). Tea plantations significantly increased the abundance of bacteria and fungi (based on \(16\ S\ r\text{DNA}\) and \(18\ S\ r\text{DNA}\) quantification). The abundance of bacteria decreased in young tea plantation (T3), but the abundance of fungi increased after long tea planting periods (T36 and T105). In addition, the ratio of bacteria to fungi was the highest in T3. In contrast, the \(\text{amoA}\) abundance of archaea and bacteria was elevated in tea plantations. The \(\text{amoA}\) abundance ratio of archaea to bacteria was elevated in T36 and T105 compared with forest and T3.

3.4. Soil fungal diversity and composition

Richness and diversity of fungal communities varied among different soils and significantly increased after tea plantation (Table 1). Additionally, the observed OTUs, Shannon diversity and Chao1 diversity were the highest in T3 and decreased with increasing planting age. Of the 15 fungal phyla detected, Ascomycota accounted for ~69% on average, across the four soils; followed by Basidiomycota (~17%) and Mortierellomycota (~4%) (Fig. 2A). The PCoA coordinates plot shows that the fungal community compositions diverged significantly among the different ecosystems (Fig. 2B) and the ANOSIM indicates a statistically significant divergence among the four soils (\(P < 0.01\)). The distance-based regression analysis identified the relative importance of soil properties related to the variation of fungal communities (Table 2). The results indicate that pH, SOC, and C/N ratio were significantly correlated with the fungal community, with a 67.04% explanation ratio of variation.

There were a total of 40 significantly different fungal groups at the phylum, class, order, family, genus, and species levels (Fig. 3). Only Mucoromycota was higher in forest soil than in soil under tea plantation at the phylum level. Trechisporales, an order of Agaricomycetes, tended to increase with forest to tea plantation conversion, but Cantharellales and Russules decreased (\(P < 0.05\)). Microascales, an order of Sordariomycetes, had a higher relative abundance in soils under tea plantation. Several groups identified as the class of Sordariomycetes (e.g., Fusarium genus, Microascales family) also increased following the land use conversion. Moreover, tea plantation reduced Ceratobasidiaceae, Tricholomataceae and Sporormiaceae at the family level. In particular, tea plantation stimulated the growth of Agaricomycetes (a class of Basidiomycota) and Fusarium but reduced that of Simplicillium, Cordycipitaceae and Russula (Fig. 3).

Table 1

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Observed OTUs</th>
<th>Shannon diversity</th>
<th>Chao1 diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest</td>
<td>1620 ± 89.5</td>
<td>7.42 ± 0.15</td>
<td>2317 ± 143.5</td>
</tr>
<tr>
<td>T3</td>
<td>2045 ± 196.7</td>
<td>8.04 ± 0.21</td>
<td>2795 ± 251.6</td>
</tr>
<tr>
<td>T36</td>
<td>1864 ± 36.8</td>
<td>7.80 ± 0.07</td>
<td>2687 ± 89.0</td>
</tr>
<tr>
<td>T105</td>
<td>1808 ± 87.6</td>
<td>7.68 ± 0.23</td>
<td>2569 ± 95.0</td>
</tr>
</tbody>
</table>

There were approximately 65% of fungal OTUs assigned to the trophic mode according to FUNGuild classification (Table S3). Most of fungi were assigned as saprotroph (~45%), followed by pathogen (~15%) and symbiotroph (~4%). Pathogens were significantly higher in Forest and T3 than in T36 and T105 (\(P < 0.05\)). However, symbiotrophs were the highest in T3 (~8%), followed by Forest (~6%), which presented significantly higher values than in T36 and T105 with an average 3% increase. The guilds assignments in this study are shown in Fig. S3. For the saprotrophic mode, the most abundant guild across four soils was classified under undefined saprotroph (~39%), followed by dung saprotroph (~2.4%). A higher relative abundance of soil saprotroph was found in T3 and T36. However, a significantly higher amount of saprotrophic wood was observed in the tea plantation soils than in the forest soil (\(P < 0.05\)). Nevertheless, the relative abundance of plant pathogen was higher in the tea plantation soils. The amount of ectomycorrhizal was significantly higher in forest and T3 than in T36 and T105, with the highest average relative abundance of symbiotroph guild (~3%).
3.5. Soil chemical and microbial characteristics in relation to the N2O emissions

Some soil properties and fungal groups were correlated with N2O emissions. SOC and NO3 were both positively related to N2O emission (r = 0.70, P < 0.05 and r = 0.89, P < 0.001, respectively). In contrast, a negative correlation was observed between N2O emissions and both pH and C/N ratio (r = 0.71, P < 0.001 and r = 0.79, P < 0.01, respectively; Table S6). Moreover, some fungal genera were correlated with N2O emissions (Table S4). The abundance of Fusarium and Clonostachys were significantly correlated with N2O emissions (r = 0.76, P < 0.01 and r = 0.63, P < 0.05, respectively). In contrast, Neurospora was negatively correlated with N2O emissions (r = -0.65, P < 0.05). The results of the SIMPER analysis indicated that 12 fungal genera contributed above 1% to the overall Bray Curtis dissimilarity between the groups with low (Forest and T3) and high (T36 and T105) N2O flux rate (Table S4), but among them, Fusarium was the only genus correlated with soil N2O emissions (Table S5).

Variance partitioning was used to determine the relative contribution of soil variables to the variation in N2O emissions. Four variables among the soil properties i.e., NO3/N, SOC, C/N, and the fungal genera Fusarium, could explain most of the basal N2O emissions (Fig. 4). Fusarium was found to contribute the most to the variations in N2O emissions (16%), while soil physico-chemical properties contributed less (<2%). The joint effect of C/N, SOC, and NO3/N was found to be the most important factor in determining N2O emissions.

Table 2
Results of DistLM analyses for the fitting environmental variables, namely pH, soil organic carbon (SOC), C/N, NH4+, NO3-, and fungal community.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted R²</th>
<th>SS(trace)</th>
<th>Pseudo-F</th>
<th>P</th>
<th>Prop.%</th>
<th>Cumul. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>0.12744</td>
<td>877.30</td>
<td>2.6066</td>
<td>0.027</td>
<td>20.68</td>
<td>20.68</td>
</tr>
<tr>
<td>SOC</td>
<td>0.33897</td>
<td>1070.90</td>
<td>4.2001</td>
<td>0.006</td>
<td>25.24</td>
<td>45.92</td>
</tr>
<tr>
<td>C/N</td>
<td>0.54679</td>
<td>896.27</td>
<td>5.1268</td>
<td>0.001</td>
<td>21.12</td>
<td>67.04</td>
</tr>
<tr>
<td>NH4+</td>
<td>0.55384</td>
<td>193.85</td>
<td>1.1264</td>
<td>0.373</td>
<td>4.57</td>
<td>71.61</td>
</tr>
<tr>
<td>NO3-</td>
<td>0.60767</td>
<td>296.69</td>
<td>1.9604</td>
<td>0.097</td>
<td>6.99</td>
<td>78.60</td>
</tr>
</tbody>
</table>

Prop.: percentage variance explained by specific variable; Cumul., cumulative total proportion of explained variation.

Fig. 2. (A) Relative abundance of fungi at the phylum level and (B) principal coordinate analysis (PCoA) based on Bray-Curtis distance of fungal community in Forest, T3, T36, and T105. See Fig. 1 for abbreviations of treatments.

Fig. 3. Cladogram of LDA scores computed for differentially abundant fungal taxa across four soils. Significantly different taxonomic distribution of fungal groups presents LDA score >2.0. See Fig. 1 for abbreviations of treatment.
and *Fusarium* was 25%, followed by SOC × NO$_3$ (23%), C/N × NO$_3$ (13%) and all these variables (18%). Other joint effects were less than 5.0%. Meanwhile, a substantial amount of the variation in N$_2$O emission (97%) could not be explained by these variables. Moreover, the R square was indicates that these four variables were significantly correlated with N$_2$O emissions, and the R square values for NO$_3$–N, SOC, C/N and *Fusarium* were 0.82, 0.63, 0.48 and 0.57, respectively.

The phylogenetic analysis indicates that more than 36% of OTUs classified in the *Fusarium* genus were close to the N$_2$O-producing *Fusarium* species (Fig. 5a). The most abundant OTU under this genus (>80%) was clustered to one branch with the N$_2$O-producing *Fusarium oxysporum*, and this OTU contributed the most to the most variation of soil N$_2$O emissions from forest and tea plantation within this genus. The results of the linear regression analysis indicates that N$_2$O emissions were significantly correlated with the abundance of fungi and archaea but not of bacteria ($P < 0.05$) (Table S7).

### 4. Discussion

Previous literature indicates that deforestation for crop plantation can decrease soil pH while increasing bulk density, SOC and total N, following the increase in planting age (Alekseeva et al., 2011; Li et al., 2012; Li et al., 2016; Wang et al., 2016; Hacisalihoglu et al., 2018; Majaliwa et al., 2010; Yüksek et al., 2009). However, Guo and Gifford (2002) shows that the effects of land use change on soil carbon stocks decline after this type of land-use conversion (approximately ~13%), and the trend in soil carbon varies between tree types (i.e., broadleaf vs conifer), planting age and precipitation. In the present study, the conversion of forest to tea plantation increased SOC stocks and N$_2$O emissions in the long term but not in the short term (Chiti et al., 2018). Moreover, we observed that SOC was positively correlated with N$_2$O emissions. In addition, there is a lower C/N ratio in the tea plantation soils than in the forest soil, which favors NO$_3$ reduction by denitrifiers with N$_2$O as the end product (Huang et al., 2004; Kiese and Butterbach-Bahl, 2002). Furthermore, the tea plantations in this study often receive a high rate of N fertilization (~450 kg N ha$^{-1}$), which likely contributes to the significantly higher soil inorganic N (van Lent et al., 2015; Zhu et al., 2014). The soil NO$_3$ content was much higher than that of NH$_3$, which could trigger significantly higher N$_2$O emissions by the denitrification process rather than by the nitrification process; and soil NO$_3$ was significantly higher in T36 and T105 than in forest, which supports this speculation. However, compared with longer-term tea plantation, T3 presented a lower N$_2$O emission rate, which occurred probably due to the pH value, which was similar to the forest.

Moreover, the correlation analysis showed that pH was negatively correlated with soil N$_2$O emissions, which suggests that soil pH is essential to regulate soil N$_2$O emission in T3 and forest. The variance partitioning results indicate that SOC and C/N were the most important soil properties affecting N$_2$O emissions. However, SOC and C/N were significantly higher in T3 due to the most recent forest clearing compared to T36 and T105. The nutrient content and physical characteristics of soil, such as soil particle size, SOC, N availability and soil pH can be influenced by the climate and ground vegetation (Agus et al., 2009). The vertical altitude of all analyzed soils were close (below 100 m) and ranged from 50–100 m, thereby presenting limited effects on the soil properties and types of ground vegetation. Therefore, the analysis of adjacent soil can reflect the real effects of the conversion from forest to tea plantation on N$_2$O emissions and microbial communities. In addition, compared with land use change, effects of different original organic C, N and P in the chronosequence of the forest would be limited and/or would only influence N$_2$O emissions in the first few years (Chen et al., 2019). The present study incorporates three years of consistent N application rates, which could minimize the total N application effects of different tea planting ages on N$_2$O emissions. Varying inorganic N content in tea plantations might be caused by greater organic N mineralization than N application, the latter of which was higher in T36 and T105 than in T3 (Chen et al., 2019). Furthermore, soil organic matter accumulation combined with fertilization produced higher N$_2$O emission in T36 and T105 than in T3.

Low soil pH can enhance the primary N$_2$O-producing process by denitrification process under acidic conditions in temperate and subtropical forest ecosystems (Ambus et al., 2006; Cai et al., 2012; Zhang et al., 2011). However, the soil pH decreased 0.1–0.4 units upon conversion to tea plantation (Table S1). This pH decrease might have inhibited the bacterial N$_2$O reductase, which catalyzes the N$_2$O to N$_2$ step, thus leading to a higher soil N$_2$O/(N$_2$O + N$_2$) ratio (Rösch et al., 2002). Tea plantations can decrease the soil pH and change the soil nutrient contents (e.g., SOC and C/N ratio),

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**Fig. 4.** Variable partitioning used to analyze the effects of NO$_3$–N, C/N, SOC, and abundance of *Fusarium* on variance of N$_2$O emissions. Variance partitioning of (a) outline and (b) N$_2$O emissions; all numbers indicate the proportion of explained variation.
which can affect the fungal community (Aleksieva et al., 2011; Chiti et al., 2018; Yang et al., 2017).

In the present study, the tea plantations significantly changed the soil properties. The DistLM analysis indicates that the soil physiochemical factors (SOC, C/N ratio, and soil pH) can explain the alteration in the soil fungal community well. Ascomycota and Basidiomycota were the two main fungal communities, accounting for more than 85% of the total fungal phyla in this study. These two phyla are the main soil fungal decomposers breaking down soil organic matter, and thereby regulating the balance of soil carbon and nutrients (Zifčáková et al., 2016). Plant litter and decaying wood input would enhance these groups in the forest and tea plantation, thus impacting soil carbon and nitrogen balance according to differences in soil aggregates (Wang et al., 2019). This is shown in Table 1, where a stable system (natural forest) is dominated by fewer species, and presents low alpha diversity. Upon disturbances from conversion to tea plantation, a more diverse cohort of species emerges until stability is reestablished, thereby reducing diversity once again in the long-term (e.g., fungal community in T105).

Fungal groups (including Mucoromycota, and Russulales), which can form associations with the plants were significantly decreased in the tea plantation soil compared to the forest soil. Mucoromycota includes a variety of mycorrhizal fungi with a broad host to form intimate symbioses, e.g., Glomeromycotina and Mucoromycotina, which is consistent with the FUNGuild results (Hoysted et al., 2018). This might have been caused by the land use change from natural forest to monoculture tea plantation.

In our study, the ratio between fungi and bacteria varied among the different soils, thus potentially affecting the soil N2O emission rate. The bacterial biomass can increase after conventional tillage in the tea plantation, especially in young tea planting period (Frey et al., 1999). In contrast, tea plantation increases soil litter, which would increase fungal biomass and the fungal decomposers. In this study, as the tea plantations aged, the fungal contribution to N2O production increased and the contribution by bacteria decreased, particularly compared to that of forest land. Therefore, there is a significant correlation between soil N2O emissions and fungal abundance due to the absence of N2O reductase enzyme in most N2O-producing fungi. Consequently, it can be implied that fungi are essential in acidic tea plantation soils (Chen et al., 2015; Huang et al., 2017). Moreover, most of fungal groups enriched in the soil under tea plantation were identified as Sordariomycetes, a class of Ascomycota, which includes numerous members with N2O-producing capability (Huang et al., 2017; Mothapo et al., 2015). In particular, the genus Fusarium, known for its considerable N2O production among fungi, significantly increased land-use conversion. Based on the correlation analysis and the variance partitioning, it can be concluded that Fusarium is likely the most important genus contributing to soil N2O emission following the conversion of forest to tea plantation. In addition, Fusarium was the fungal genus contributing the most (>1% contribution) to the differences between soils with low and high N2O flux level, i.e., Forest and T3 at low N2O flux levels and T36 and T105 were at high levels.

Further, the most abundant genus of Fusarium (OTU1) was close to N2O-producing fungus, which might indicate this group contributes to the deviation of N2O emissions from different soils. According to previous studies, archaea produce minimum N2O gas compared with fungi, thereby providing a small contribution to soil N2O emissions (Hink et al., 2017; Huang et al., 2017). Finally, the difference between N2O emissions from different soils under aerobic and rigorously anaerobic conditions indicates that there might be other interpretative mechanisms. That is, bacterial denitrification could be higher in the forest under anaerobic condition, and the byproducts of denitrification (N2O and N2) would also be higher in the forest compared to tea plantations. Compared with T3, a higher abundance of the Fusarium genus was found in T36 and T105, and was highest in T36, which could explain the N2O emission trends (T36 > T105 > T3). Furthermore, the ratio of AOA and AOB (T36 > T105 > T3) had good correlation with N2O emissions according to different planting ages of tea plantations. AOA commonly contributes much more than AOB to ammonia oxidation in acidic soils, which can produce N2O as a byproduct (Li et al., 2018). Finally, the combination of these soil abiotic and biotic soil properties contributed to N2O emission following the conversion from natural forest to tea plantation. Overall, these observations provide effective evidence that the conversion of forest to tea plantation affects the fungal community and their functional composition. Additionally, the differential fungal community’s response to the conversion of forest to tea plantation reflects the changes in underground conditions.

5. Conclusion

Our results demonstrate that the sources of soil N2O production (fungi and bacteria) and soil properties can be used to simulate soil N2O emissions. Tea plantation increased the fungal contribution to soil N2O flux but decreased the bacterial counterpart compared to that in forest soil. Moreover, the conversion of forest to tea plantation affected the fungal community and their functional composition. The soil pH, SOC and C/N ratio were determining in shaping the soil fungal community following the conversions. In particular, tea plantations promoted some fungal groups capable of N2O production. Moreover, NO3-N, SOC and C/N ratio were essential for the high N2O emissions. Altogether, these findings provide significant direct evidence of increased soil N2O emissions, and the involvement of the fungal community in this increase following the conversion of natural forest to tea plantation. Moreover, further research is needed to investigate the contributions of soil microbiota under different soil depths and climate zone to the N2O emissions of tea plantation soil.

CRediT authorship contribution statement

Ningguo Zheng: Conceptualization, Methodology, Software, Data curation, Validation, Writing - original draft, Visualization, Investigation, Writing - review & editing. Yongxiang Yu: Conceptualization, Data curation, Validation, Visualization, Investigation, Writing - review & editing. Juan Wang: Conceptualization, Data curation, Validation, Visualization, Investigation, Writing - review & editing. Stephen J. Chapman: Conceptualization, Visualization, Investigation, Writing - review & editing. Huaiying Yao: Conceptualization, Methodology, Software, Data curation, Validation, Visualization, Investigation, Writing - review & editing. Yingying Zhang: Conceptualization, Data curation, Validation, Visualization, Investigation, Writing - review & editing.

Declaration of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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