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The conversion of subtropical forest to tea plantation changes the fungal community and the contribution of fungi to N_2O production☆

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ABSTRACT

The conversion of natural forests to tea plantations largely affects soil nitrous oxide (N_2O) emissions and soil microbial communities. However, the impacts of this conversion on the contribution of fungi to N_2O emission and on fungal community structure remain unclear. In this study, we determined the soil N_2O emission rate, N_2O 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 N_2O 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 N_2O, 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 N_2O emission soils (T36 and T105) were identified as the genus *Fusarium*, which were positively correlated with soil N_2O emissions. The variation in N_2O emission response could be well explained by NO_3^--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 N_2O emissions and fungal communities be attributed to the conversion of natural forest to tea plantations.

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 (Guillaume et al., 2015), biodiversity threats (Wilcove and Koh, 2010), and increase in greenhouse gas (GHG) emissions (Su et al., 2016). Nitrous oxide (N_2O), 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 N_2O emissions, and agriculture, forestry and other land uses accounted

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for approximately 82% of N_2O anthropogenic sources (IPCC, 2019). Moreover, forest conversion to croplands has been shown to substantially increase N_2O 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 N_2O-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 N_2O by the denitrification process (Hu et al., 2015; Shoun et al., 1992, 2012). 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 (Mothapo et al., 2015; Yu et al., 2019). Moreover, previous studies indicate that denitrification dominates N_2O 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 N_2O production compared to bacteria, given that soil fungi are more tolerant to acidic conditions and can produce N_2O under either 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 N_2O 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 as 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 N_2O production, we also determined the total soil fungal community structure to investigate the relationship between the dynamics of N_2O emission and N_2O-producing fungi. We hypothesized that (1) the production of N_2O 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 N_2O-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 soil from natural forest was also collected for comparison. The 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 N_2O flux, and the other was stored at −80 °C for DNA extraction.

2.2. Measurement of soil properties

Soil NH_4^+-N and NO_3^--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 $K_2Cr_2O_7$–H_2SO_4 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 N_2O emission rate

To measure the soil N_2O 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 N_2O was measured everyday for three days. Before the measurement of the N_2O 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 N_2O with an electron capture detector.

In addition, soil N_2O 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 N_2O emission

To measure the source of soil N_2O 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 N_2O 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 H_2O 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 KNO_3 (100 μg N g^{-1} dry soil) were added at 60% WFPS. Moreover, the headspace air was replaced by a He/C_2H_2 mixture (90/10 v/v). The soil N_2O flux was measured at 4, 8, 16, and 32 h according to the method described by Laughlin and Stevens (2002). The sources of soil N_2O emissions were calculated by subtracting the N_2O production of the inhibitor treatments from the N_2O production of the control (Herold et al., 2012). We used both inhibitors to distinguish the N_2O 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).

$$IAR = \frac{(a - b) + (a - c)}{a - d} \quad (1)$$

where a, b, c, and d are CO_2 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 TIANgel 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 PRJNA558591.

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 N_2O 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 N_2O-producing fungi, we collected the sequences of N_2O-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 N_2O-producing fungus using the MEGA X 10.1.8 (Kumar et al., 2018). Linear regression and ANOVA analysis were performed to determine the correlation between soil N_2O 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 NO_3^--N and NH_4^+-N, was significantly higher in the soil of tea plantations than in the forest soil ($P < 0.05$); and the NO_3^--N content was significantly higher than that of NH_4^+-N in all soil samples. The NO_3^--N/NH_4^+-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 and forest (3.2 and 2.2, respectively).

3.2. Source of N_2O flux

The soil N_2O flux rate was the highest in T36, followed by T105, T3 and forest (Fig. 1A). In T36 and T105, the N_2O flux rate changed 7 and 10 fold, respectively, compared with that in the forest. However, changes in the N_2O flux rate of soils in the forest and tea plantations were different under aerobic condition (Fig. S1). The highest N_2O flux rate was observed in forest soil (28.6 μg N d^{-1} kg soil), the flux rate in the tea plantation soils increased with planting period.

The soils treated with inhibitors showed significantly reduction of N_2O flux rates compared with to soil with no inhibitor treatment (Fig. 1B). Among different soil ecosystems, the IAR results suggest

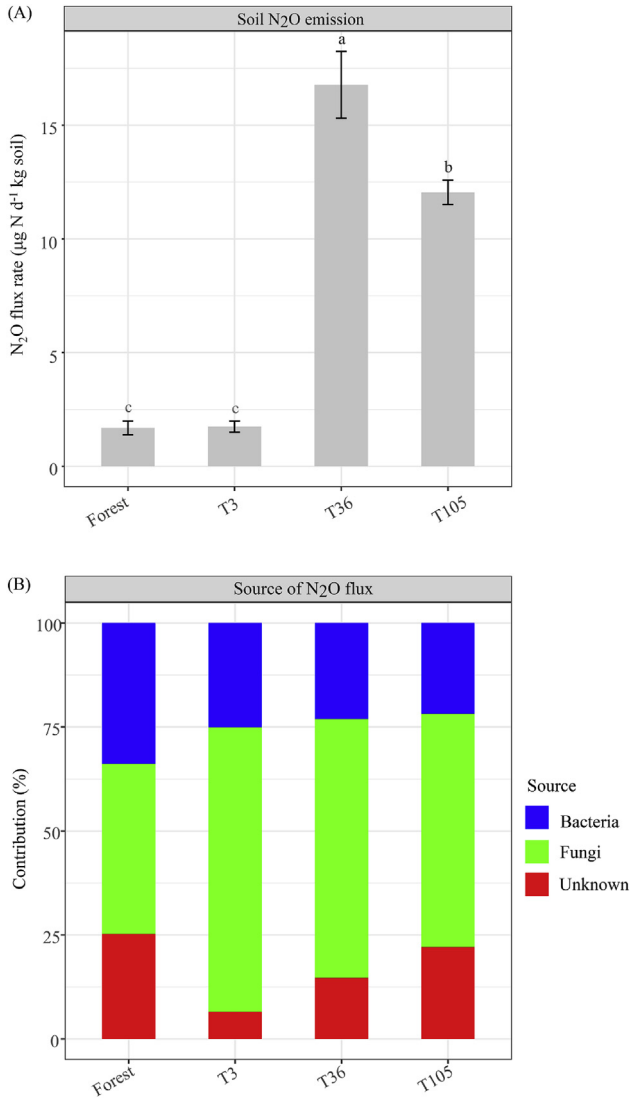

Fig. 1. Soil (A) N_2O flux rates and (B) N_2O flux rates under differential antibiotic treatment. Error bars indicate the standard errors for n = 3. Forest, T3, T36, and T105 represent soil under forest land, and 3-y, 36-y, and 105-y tea plantations. Different letters indicate significant differences, with $P < 0.05$.

the lack of no non-target effect of inhibitors. In the forest soil, the contribution of bacteria to soil N_2O fluxes was much higher than in the soil under tea plantation. In contrast, the contribution of fungi to soil N_2O 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 rDNA and 18 S rDNA 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 *amoA* abundance of archaea and bacteria was elevated in tea plantations. The *amoA* abundance ratio of archaea to bacteria was elevated in T36 and T105 compared with forest and T3.

Table 1
Alpha diversity of fungal communities determined among different soil ecosystems; means ± standard deviation (n = 3). Forest, T3, T36 and T105 represent soils under forest land, and 3-y, 36-y, and 105-y tea plantations. Different letters represent significant differences, with P < 0.05.

	Observed OTUs	Shannon diversity	Chao1 diversity
Forest	1620 ± 89 c	7.42 ± 0.15 c	2317 ± 143 d
T3	2045 ± 196 a	8.04 ± 0.21 a	2795 ± 251 a
T36	1864 ± 36 b	7.80 ± 0.07 a	2687 ± 89.0 b
T105	1808 ± 87 b	7.68 ± 0.23 b	2569 ± 95.0 c

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 *Russulales* 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, *Microascaceae* 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).

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%).

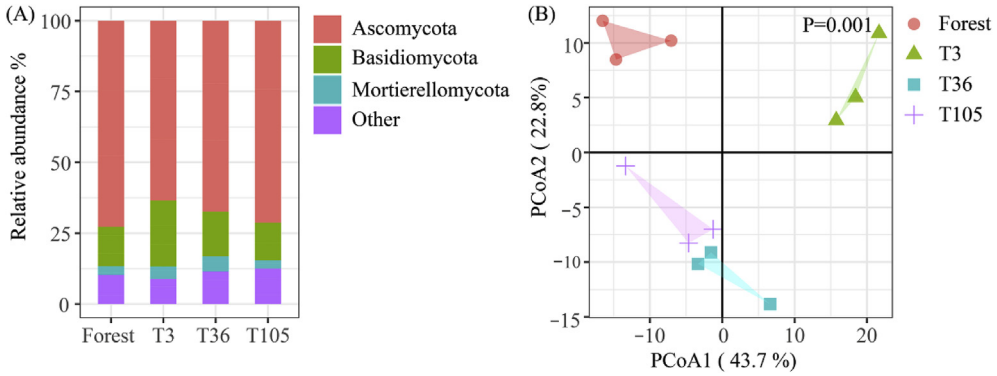

Fig. 2. (A) Relative abundance of fungi at the phylum level and (B) principal coordinate analysis (PCoA) based on Bray-Curtis distance of fungal community in Forest, T3, T36, and T105. See Fig. 1 for abbreviations of treatments.

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

Variable	Adjusted R^2	SS(trace)	Pseudo-F	*P*	Prop.%	Cumul. %
pH	0.12744	877.30	2.6066	0.027	20.68	20.68
SOC	0.33897	1070.90	4.2001	0.006	25.24	45.92
C/N	0.54679	896.27	5.1268	0.001	21.12	67.04
NH_4^+	0.55384	193.85	1.1264	0.373	4.57	71.61
NO_3^-	0.60767	296.69	1.9604	0.097	6.99	78.60

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

3.5. Soil chemical and microbial characteristics in relation to the N_2O emissions

Some soil properties and fungal groups were correlated with N_2O emissions. SOC and NO_3^- were both positively related to N_2O emission ($r = 0.70$, $P < 0.05$ and $r = 0.89$, $P < 0.001$, respectively). In contrast, a negative correlation was observed between N_2O 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 N_2O emissions (Table S4). The abundance of *Fusarium* and *Clonostachys* were significantly correlated with N_2O emissions ($r = 0.76$, $P < 0.01$ and $r = 0.63$, $P < 0.05$, respectively). In contrast, *Neurospora* was negatively correlated with N_2O 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) N_2O flux rate (Table S4), but among them, *Fusarium* was the only genus correlated with soil N_2O emissions (Table S5).

Variance partitioning was used to determine the relative contribution of soil variables to the variation in N_2O emissions. Four variables among the soil properties i.e., NO_3^--N, SOC, C/N, and the fungal genera *Fusarium*, could explain most of the basal N_2O emissions (Fig. 4). *Fusarium* was found to contribute the most to the variations in N_2O emissions (16%), while soil physic-chemical properties contributed less (≤2%). The joint effect of C/N, SOC,

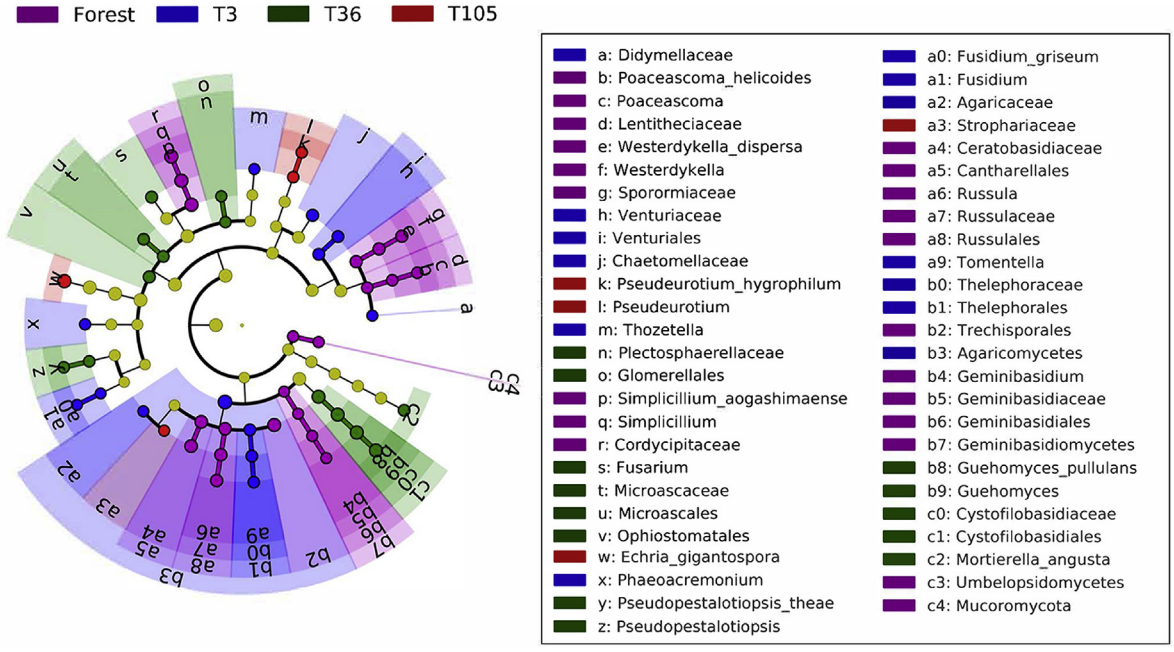

Fig. 3. Cladogram of LDA scores computed for differentially abundant fungal taxa across four soils. Significantly different taxonomic distribution of fungal groups presents LDA score >2.0. See Fig. 1 for abbreviations of treatment.

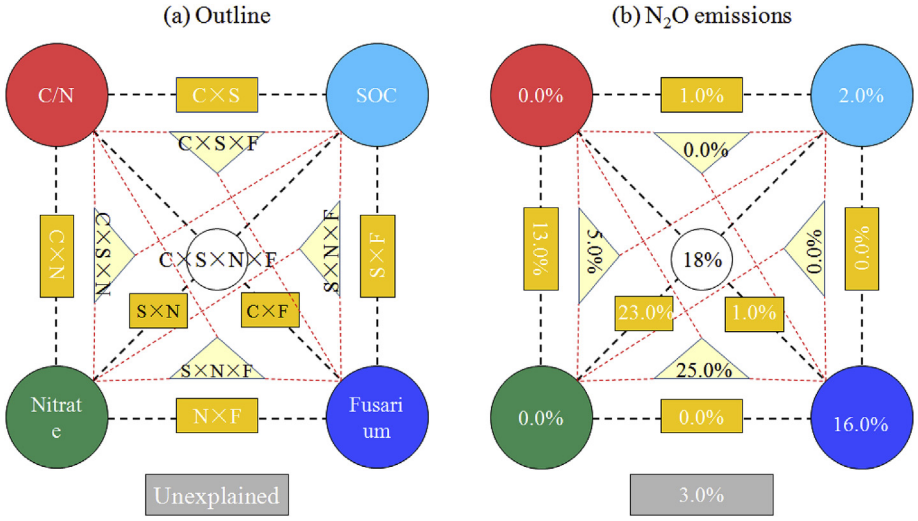

Fig. 4. Variable partitioning used to analyze the effects of NO_3^--N, C/N, SOC, and abundance of *Fusarium* on variance of N_2O emissions. Variance partitioning of (a) outline and (b) N_2O emissions; all numbers indicate the proportion of explained variation.

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_2O emission (97%) could not be explained by these variables. Moreover, the R square was indicates that these four variables were significant correlated with N_2O 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_2O-producing *Fusarium* species (Fig. S4). The most abundant OTU under this genus (>80%) was clustered to one branch with the N_2O-producing *Fusarium oxysporum*, and this OTU contributed the most to the most variation of soil N_2O emissions from forest and tea plantation within this genus. The results of the linear regression analysis indicates that N_2O 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_2O 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_2O 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_2O 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_4^+, which could trigger significantly higher N_2O 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_2O 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_2O emissions, which suggests that soil pH is essential to regulate soil N_2O emission in T3 and forest. The variance partitioning results indicate that SOC and C/N were the most important soil properties affecting N_2O 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_2O 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_2O 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_2O 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_2O emission in T36 and T105 than in T3.

Low soil pH can enhance the primary N_2O-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_2O reductase, which catalyzes the N_2O to N_2step, thus leading to a higher soil $N_2O/(N_2O + N_2)$ 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),

which can affect the fungal community (Alekseeva 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 (Žifcáková 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 N_2O 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 fungi biomass and the fungal decomposers. In this study, as the tea plantations aged, the fungal contribution to N_2O production increased and the contribution by bacteria decreased, particularly compared to that of forest land. Therefore, there is a significant correlation between soil N_2O emissions and fungal abundance due to the absence of N_2O reductase enzyme inmost N_2O-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 N_2O-producing capability (Huang et al., 2017; Mothapo et al., 2015). In particular, the genus *Fusarium*, known for its considerable N_2O 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 N_2O 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 N_2O flux level, i.e., Forest and T3 at low N_2O flux levels and T36 and T105 were at high levels.

Further, the most abundant genus of *Fusarium* (OTU1) was close to N_2O-producing fungus, which might indicate this group contributes to the deviation of N_2O emissions from different soils. According to previous studies, archaea produce minimum N_2O gas compared with fungi, thereby providing a small contribution to soil N_2O emissions (Hink et al., 2017; Huang et al., 2017). Finally, the difference between N_2O 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 (N_2O and N_2) 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 N_2O emission trends (T36 > T105 > T3). Furthermore, the ratio of AOA and AOB (T36 > T105 > T3) had good correlation with N_2O 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 N_2O as a byproduct (Li et al., 2018). Finally, the combination of these soil abiotic and biotic soil properties contributed to N_2O 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 N_2O production (fungi and bacteria) and soil properties can be used to simulate soil N_2O emissions. Tea plantation increased the fungal contribution to soil N_2O 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 N_2O production. Moreover, NO_3^--N, SOC and C/N ratio were essential for the high N_2O emissions. Altogether, these findings provide significant direct evidence of increased soil N_2O 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 N_2O 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, Supervision, Funding acquisition. **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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