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Long-Term Agricultural Management Alters Soil Fungal Communities and Soil Carbon and Nitrogen Contents in Tea Plantations

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Abstract: Soil carbon (C) and nitrogen (N) are vital for enhancing tea production and ensuring the sustainability of tea plantation ecosystems. However, research on the dynamics of soil C and N pools and their associated microbial mechanisms in tea plantations with varying cultivation durations is scarce. We compared soil samples from a forest and two tea plantations—young established (YTP) and century-old (OTP)—to assess changes in soil C and N concentrations and the impact of fungal community structure on these elements. Soil organic carbon (SOC) and total nitrogen (TN) were markedly higher in OTP than in the YTP and forest (65.9% and 30.1%, respectively, relative to YTP). Eurotiomycetes in the YTP group accounted for a relatively higher proportion at 51.6%, surpassing its presence in both the forest (14.3%) and OTP (4.78%) groups and it can be the main microbial factor affecting the C cycle in tea plantation soils and facilitating SOC mineralization. Enhancing planting years or changing land use patterns improves fertilizer and biomass sedimentation and increases the relative abundance of Eurotiomycetes in the soil and the C sink potential of tea plantations. This study provides valuable insights into the role of soil C and N dynamics and fungal communities in tea plantation ecosystems, highlighting the importance of managing these factors for sustainable tea production.

Keywords: tea plantation; carbon and nitrogen pool; fungal community; planting years

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1. Introduction

Dynamic changes in soil carbon (C) and nitrogen (N) pools significantly affect C sequestration and soil fertility in agricultural and forestry ecosystems [1–3]. It has been demonstrated that soil organic carbon (SOC) plays a crucial role in plant growth, and fluctuations in SOC levels can markedly impact soil fertility, consequently influencing plant development [4]. Dynamic changes in the soil C pool significantly affect greenhouse gas emissions [5,6]. Furthermore, N is the main nutrient affecting plant growth, primary productivity, and metabolism [7,8]. High-intensity management measures, such as fertilization and pruning, can significantly affect the content and composition of C and N pools within soil, thereby affecting its C storage stability, N use efficiency, and sustainable development of the agroforestry ecosystem [2,9,10]. Therefore, it is necessary to conduct

thorough investigations of the characteristics and mechanisms of C and N cycling in the soil to promote the sustainable development of agricultural and forestry ecosystems.

Changes in land use patterns and planting duration are the two dominant elements that influence the soil cycling of C and N [11–13]. Management measures, such as fertilization, weeding, plowing, and pruning are also adjusted according to changes in land use patterns [14]; this can influence the microbial community and structure of soils and the availability and content of nutrient sources, which then affect soil C and N cycles [9,15]. In this respect, Wang et al. [13] demonstrated that conversion of forests to tea plantations significantly increased SOC stocks and C sequestration in the soil through enhanced organic C input. Xue et al. [9] found that the nitrification rate in forest soils was lower than that in tea plantations, and this observation suggests that the higher nitrification rate in tea plantations contributes to increased NO_3^--N concentrations, thereby enhancing N utilization efficiency. It has also been observed that with an increase in the number of planting years, alterations in soil physicochemical properties, including the accumulation of nutrients and organic matter, have a substantial impact on the microbial characteristics of soil, consequently influencing C and C cycles in the soil [9,16,17]. Wang et al. [16] and Yang et al. [10] demonstrated that the increase years of tea plantations fostered the accumulation of SOC by mitigating its decomposition and leaching. Moreover, Yang et al. [18] indicated that the SOC and TN contents of young tea plantations were lower than those in ancient tea plantations. In conclusion, the transformation of land use and the duration of cultivation had a significant impact on the dynamics of soil C and N pools, and these effects were mediated by alterations in soil pH and shifts in the structure of microbial communities, among other factors. Therefore, it is essential to investigate the effects of land use changes and different planting durations on soil C and N cycling and to analyze the underlying mechanisms. Such research is crucial for promoting the sustainable development of agricultural and forestry ecosystems.

Previous studies have provided evidence that alterations in the structure and composition of microbial communities, specifically the relative abundance of dominant species, can substantially influence soil C and N cycling [15,17,19]. Changes in land use and an extended duration of planting have been noted to significantly affect the availability of soil C and N, leading to pronounced modifications in the structure and composition of microbial communities. These changes in microbial community characteristics subsequently impact soil C and N cycling processes [15,16,20]. For instance, Wang et al. [21] demonstrated that after the conversion of a coniferous forest to a broad-leaved forest, soil microbial biomass carbon (MBC) had a positive relationship with SOC content and litter C/N, which was the opposite to the trend of litter N. This suggests that the C and N released can significantly affect soil microbial community characteristics and C and N transformation during litter decomposition. In addition, long-term fertilization was found to saturate the soil with nutrients after the forest was converted into tea plantations, and C and N accumulated and significantly affected the characteristics of the soil microbial communities [22–24]. It is known that long-term planting of tea plants can significantly reduce soil pH and affect soil structure and microbial community composition, thus altering soil C and N cycling [14,25,26]. Additionally, alterations in the relative abundance of key species have a significant impact on soil C and N cycling [27]. Research has indicated that Eurotiomycetes are the major microbial predictors of SOC storage [28]. Chen et al. [15] indicated that the relative abundance of Eurotiomycetes produces hydrolases associated with SOC mineralization. Tremellomycetes were found to be positively correlated with SOC and C/N, and the relative abundance of Dothideomycetes was positively correlated with TN content [29]. The aforementioned studies have highlighted the intricate nature of the impacts of land use changes and tea planting duration on soil C and N pools. Consequently, it is imperative to investigate the relationship between C and N cycling and the structure of soil microbial communities. Such research is essential for gaining a deeper understanding of the mechanisms underlying C and N cycling in tea plantations with varying planting durations following land use changes.

Tea is the main cash crop in China, and it has considerable C sink potential [13,30]. As Wang et al. [30] found, China's tea plantations have a SOC reserve of 207.13 Tg, with 124.58 Tg stored in the surface soil (0–20 cm). However, these management measures also alter soil C and N cycles and the sustainable development of tea plantations by changing the structure and composition of microbial communities [16,23,31]. Therefore, studying the relationship between soil C and N cycling and microbial community structure is necessary to further reveal the relevant mechanisms of C and N cycling with different planting years after land use change in tea plantations.

In summary, the aim of this study was to examine the influence of soil C and N pools in different planting years and soil fungal community diversity and composition within two different age tea plantations and forest soils and to investigate the effect of changes in microbial community characteristics on soil C and N pools. We speculated that the accumulation of fertilizer, littering, and the sedimentation of tea biomass would lead to increases in C and N within the surface soil with an increase in tea plantation years and that changes in the C and N contents of deep soil might also be affected by changes in the soil microbial community, such as an increase in the relative abundance of Eurotiomycetes.

To achieve these objectives, we (1) investigated the land utilization type change and planting year increase in tea plantations on soil C and N pools, and (2) elucidated the reciprocities and effects between soil fungal communities and soil C and N cycles in these plantations.

2. Materials and Methods

2.1. Site Description of the Forest, Young (YTP), and Century-Old (OTP) Tea Plantations Groups

The experimental site is characterized by a subtropical monsoon climate featuring four distinct seasons with average annual precipitation and temperature of 1553 mm and 17.0 °C (1.7 °C in January, 33.0 °C in July), respectively. Tea plants thrive from March to September, coinciding with the period when approximately 74% of the total annual rainfall is recorded.

Soil from two tea plantations and adjacent forests of different ages was collected at the Tea Research Institute of the Chinese Academy of Agricultural Sciences to analyze the impacts of tea plantation age on various aspects relating to soil C and N components, contents, and microbial communities. Soil from tea plantations ages 10 years and 100 years old, and the forest were selected for this study, and the associated groups are referred to as YTP, OTP, and Forest groups, respectively. The soil was loamy clay and the tea planting variety was Longjing 43. The fertilization management practices implemented in the experimental setup were consistent with those described in a previous study conducted by Yan et al. [32]. Please see the Supplementary Information for details.

2.2. Soil Sampling and Determination of Related Indicators

The selected tea plantations covered an area of over 300 m^2, and each tea plantation was established with four plots of approximately 75 m^2 for sampling. In October 2017, topsoil was sampled every 10 cm at sampling depths of 0–10 cm and 10–20 cm soil layers (recorded as TS10 and TS20, respectively), and subsoil was sampled every 20 cm at sampling depths of 20–40 and 40–60 cm soil layers (recorded as SS40 and SS60, respectively) using the soil drilling method. Each composite sample comprised soil obtained from 10 points in each block, which had been mixed to form a uniform sample. The fresh soil samples were then sieved through a 5 mm mesh to eliminate any plant residues, roots, and stones, ensuring that the soil used for analysis was free of any extraneous materials that could potentially affect the results. The fresh soil was used to determine the MBC (microbial biomass carbon), MBN (microbial biomass nitrogen), DOC (dissolved organic carbon), and DON (dissolved organic nitrogen) contents and analyze the microbial composition. Soil MBC and MBN were determined by the fumigation–extraction method [33], and soil DOC and DON were determined using the method described by Ghani et al. [34]. These

methods are provided in detail in the Supplemental Information. Air-dried soil specimens were passed through a 2 mm sieve prior to physicochemical analysis [25].

The physical and chemical properties of the soil investigated included pH, total carbon (TC), total nitrogen (TN), NO_3^--N, NH_4^+-N available phosphorus (AP), and available potassium (AK). The soil properties were quantitatively determined at the Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China. The specific determination methods are provided as Supplementary Information in the Supplemental Information.

2.3. DNA Amplicon and Illumina Sequencing of Fungal Communities

A Fast DNA Spin kit was used to extract total DNA (n = 3) from 0.2 g soil samples. The OMEGA Bio-Tek kit was used for purification. The concentration and purity of DNA were determined using a NanoDrop ND-1000 spectrophotometer. Amplification of the first internal transcriptional spacer (ITS1) was conducted using broad-spectrum fungal prims ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) with splicers and barcodes [35]. Polymerase chain reaction (PCR) amplification was performed using the TransStart Fastpfu DNA polymerase (TransGen Biotech, Beijing, China), and five amplicons running parallel PCR (3 × 53 °C) were labeled. Agcourt AMPure XP SPRI magnetic beads were used for purification. Fluorescence (Qubit 2.0) and Qubit dsDNA HS assay kits were used to quantify and normalize the PCR product. The test kits employed and their manufacturers are listed in Table 1.

Table 1. Test kits (and their manufacturers) used in main experimental steps.

Main Steps	Kits and Manufacturers
Extract total DNA of soil samples	Fast DNA Spin kit (MP Biomedicals LLC, Santa Ana, CA, USA)
Purification the total DNA of soil samples	OMEGA Bio-Tek kit (Bio-Tek Instruments, Inc., Charlotte, VT, USA)
Determine the concentration and purity of DNA	NanoDrop ND-1000 spectrophotometer (Thermo Fisher, Waltham, MA, USA).
Purification the PCR products of soil samples	Agcourt AMPure XP SPRI magnetic beads (Beckman Coulter, Indianapolis, IN, USA)
Quantifying and normalizing the PCR product	Qubit 2.0 fluorescence and Qubit dsDNA HS assay kits (Invitrogen, Carlsbad, CA, USA)

Paired-end (29250) sequencing was performed by Personal Biotechnology (Shanghai, China) on the MiSeq platform (Illumina, San Diego, CA, USA) [36]. The analysis included the detection and removal of chimeric sequences using the UCHIME [37]. Centroid sequences of clusters were compared using the USEARCH global alignment algorithm or high-quality sequences from the National Center for Biotechnology Information database [38]. Fungal operational taxonomic units (OTUs) were categorized into functional groups by comparison with the FUNGuild 1.0 database [39,40]. The relative abundance of each functional group was equal to the sum of the relative abundance of all OTUs in that group [41]. The Shannon diversity index of functional groups was determined using the Phyloseq software package in R 4.1.3 [42]. The copy number of the functional genes associated with the C cycle was also considered in the detailed analytical approaches, and these are provided in the Supplemental Information.

2.4. Statistical Analyses

One-way analysis of variance (ANOVA) and Tukey post hoc tests were used to compare the contents of TC, MBC, DOC, TN, MBN, DON, NO_3^--N, and NH_4^+-N, and the microbial diversity and community compositions of the forest, YTP, and OTP groups (IBM SPSS Statistics 26). Normality and homogeneity of variance were tested using ANOVA. Nonlinear regression was employed to describe the relationship between the three groups

at the four depths, the C and N contents and composition, and microbial diversity and richness. Non-metric multidimensional scales (NMDS) was based on the Bray–Curtis distances from the sequencing data, and associations between community composition and environmental factors (such as soil properties, plant richness, and diversity) were assessed. A redundancy analysis (RDA) was used to investigate the correlation between fungal microbial communities and environmental factors in various soil layers. Data processing and visualization were performed using R 4.1.3 and Origin 2021 software (Origin Laboratories Ltd., Northampton City, MA, USA).

3. Results

3.1. Soil Carbon Pool

TC was higher in the OTP group than in the Forest and YTP groups in the TS10, TS20, and SS40 soil layers, with a difference only observed between the Forest and YTP groups in the TS10 layer. The TC content of the YTP and OTP groups increased by 9.29% and 120% in the TS10 soil layer, respectively, compared to that of the Forest group. The TC content of the OTP group rose by 142% and 30.6% compared to that in the Forest group in the TS20 and SS40 soil layers, respectively. Additionally, the TC content was reduced ($p < 0.05$) with increasing soil depth in all groups, whereas no change was observed between the Forest and YTP groups (TS20 and SS40 soil layers) (Figure 1a).

The MBC content of the OTP group was greater than that of the Forest and YTP groups ($p < 0.05$). In the TS10 soil layer, the MBC of the YTP group was lower than that of the Forest group ($p < 0.05$), but more than ($p < 0.05$) that in the TS20 soil layer. There was no distinct change between YTP and Forest groups in other soil layers. The soil MBC content of the OTP group lessened with increasing soil depth (Figure 1b).

The DOC content of the OTP group was more than that of the Forest and YTP groups ($p < 0.05$), with an average increase of 28.9% and 38.2% compared to those in the Forest and YTP groups, respectively. The DOC content of the YTP group was lower than that of the Forest group only in the TS20 soil layer ($p < 0.05$). The DOC content attenuated ($p < 0.05$) with deepened soil depths; it was more abundant in TS10 and TS20 than in SS40 and SS60, but there was no obvious difference between the contents of TS20 and SS40 soil layers (Figure 1c).

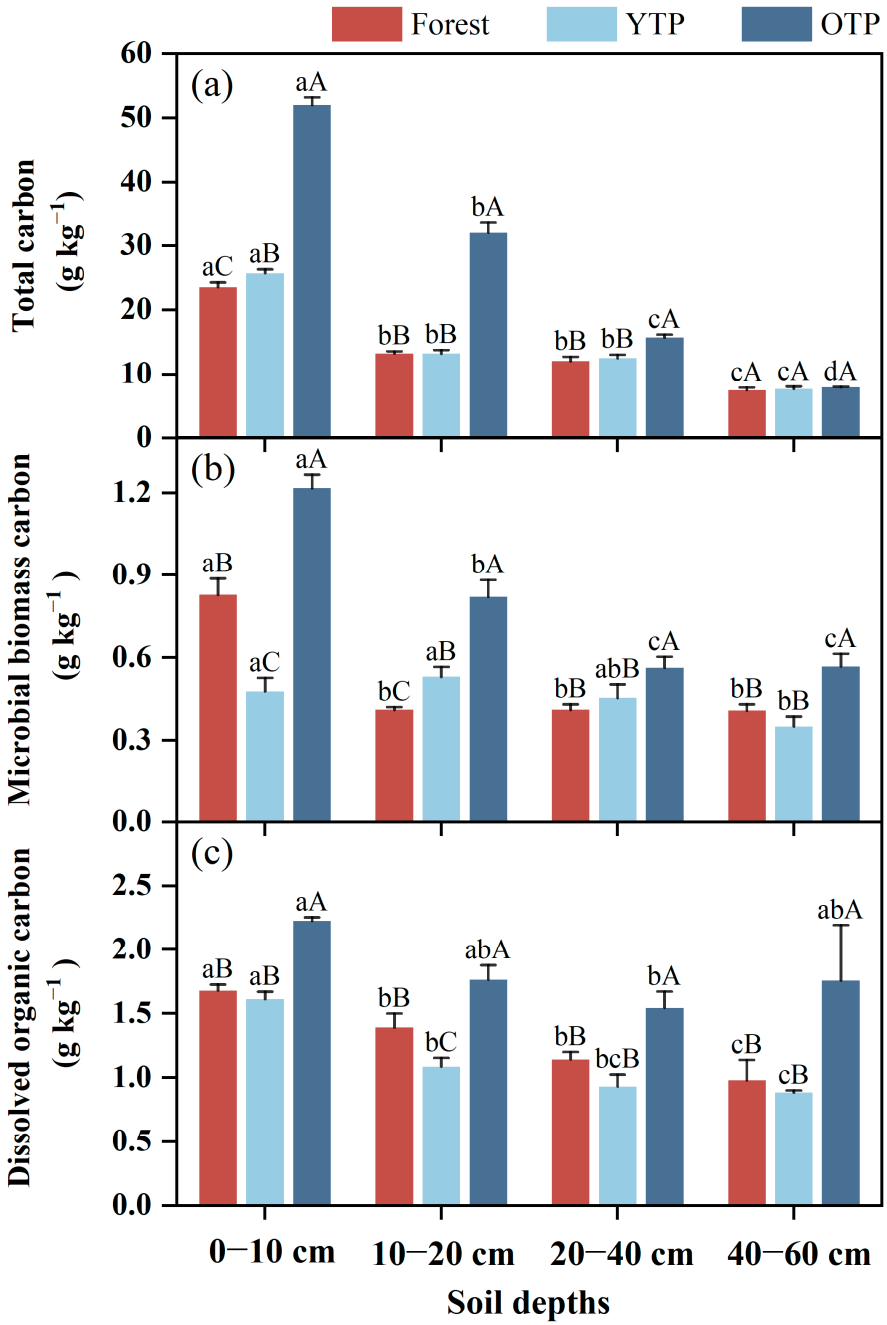

Figure 1. Effects of the Forest, YTP, and OTP groups on soil C contents at different soil depths. (**a**) Total carbon. (**b**) Microbial biomass carbon. (**c**) Dissolved organic carbon. Soil depths were recorded as 0–10 cm (TS10), 10–20 cm (TS20), 20–40 cm (SS40), and 40–60 cm (SS60). Different capital letters indicate significant differences among the three treatments ($p < 0.05$); different lowercase letters indicate significant differences between soil depths ($p < 0.05$). Error bars represent the standard deviations (n = 3).

3.2. Soil N Pool

The TN content of the soil reduced ($p < 0.05$) with increasing soil depths, regardless of the group, except in the Forest and YTP group within TS20 and SS40 soil layers. The TN content of the Forest group was lower than that of the YTP and OTP groups in all soil layers ($p < 0.05$). The mean TN content of the YTP and OTP groups increased by 58.5% and 61.7%, respectively, compared with that of the Forest group. The soil TN content of the OTP group was higher ($p < 0.05$) than that of the YTP group under the TS10 and TS20, and lower ($p < 0.05$) than YTP under the SS40 and SS60 (Figure 2a).

The MBN content in the OTP group was higher than that of the Forest and YTP groups in all soil layers ($p < 0.05$), except for within TS20. The average MBN content of the YTP and OTP groups increased by 34.9% and 51.5%, respectively, compared to those in the Forest group. There was no distinction between the MBN content of the Forest and YTP groups in the TS10 and SS60 cm soil layers. The MBN content of the Forest group was reduced ($p < 0.05$) in the TS20 and SS40 soil layers (Figure 2b).

The OTP and YTP groups had higher DON content than the Forest group, except for the SS60 soil layer ($p < 0.05$). The average DON content in the YTP and OTP groups increased by 48.0% and 65.7%, respectively, compared to that in the Forest group. The DON content of the OTP group was more than that of the YTP group in the TS10 and SS40 soil layers ($p < 0.05$), but no change was observed under the OTP group. Additionally, the DON content decreased with increasing soil depth in the Forest and YTP groups ($p < 0.05$), (Figure 2c).

The inorganic N content of the OTP group was greater than that of the Forest and YTP groups (Figure 2d,e). The average NO_3^--N and NH_4^+-N contents of the OTP group increased by 78.9% and 91.3%, respectively, whereas those of the YTP group increased by 61.6% and 73.0%, respectively, compared to those of the Forest group. The NO_3^--N content was higher ($p < 0.05$) in the YTP group than in the Forest group. Additionally, the NO_3^--N content decreased ($p < 0.05$) in the SS40 and SS60 soil layers under the Forest group, increased in the TS20 and SS40 soil layers under the YTP group, and increased in the SS60 soil layer under the OTP group in comparison to that under the TS10 soil depth (Figure 2d).

Soil NH_4^+-N was more ($p < 0.05$) in the YTP group than in the Forest group within the TS10 and TS20 layers. The NH_4^+-N content decreased ($p < 0.05$) in the SS40 and SS60 soil layers under the YTP group, and decreased ($p < 0.05$) in the SS60 soil layer under the OTP group. Additionally, it decreased ($p < 0.05$) in other soil layers under the Forest group compared to that in the TS10 soil layer, where the NH_4^+-N content was lower in the TS20 than in the SS40 and SS60 layers (Figure 2e).

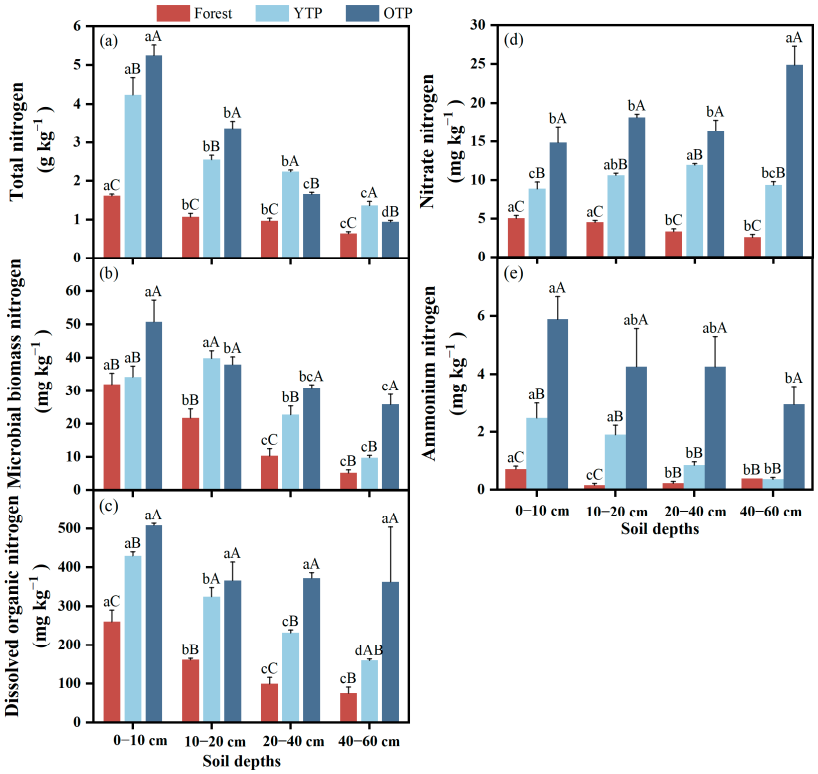

Figure 2. The effects of the Forest, YTP, and OTP groups on soil N contents at different soil depths. (**a**) Total nitrogen. (**b**) Microbial biomass nitrogen. (**c**) Dissolved organic nitrogen. (**d**) Nitrate

nitrogen. (**e**) Ammonium nitrogen. The soil depths were recorded at 0–10 cm (TS10), 10–20 cm (TS20), 20–40 cm (SS40), and 40–60 cm (SS60). Different capital letters indicate significant differences among the three treatments ($p < 0.05$); different lowercase letters indicate significant differences soil depths ($p < 0.05$). Error bars represent the standard deviations (n = 3).

3.3. Soil Microbial Community

Fungal richness (Chao1 index) increased at the TS20 soil depth in the OTP group but decreased at the SS60 soil layer in the YTP group compared to that in the Forest group. Moreover, fungal richness was higher in the OTP group than in the YTP group in TS20 soil layer (Figure 3a). In contrast, fungal diversity (Shannon index) in the OTP group ranked as follows for the various soil layers: SS40 > TS10 = TS20 > SS60 (Figure 3b).

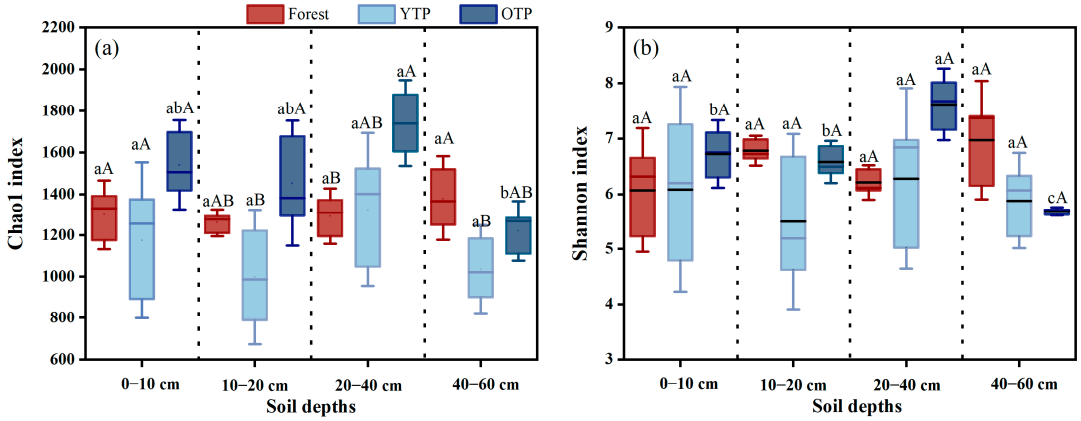

Figure 3. Richness (Chao1 index) and microbial diversity (Shannon index) of the fungal community in a *Camellia sinensis* (L.) soil for the different groups (Forest, YTP, and OTP). The soil depths were recorded at 0–10 cm (TS10), 10–20 cm (TS20), 20–40 cm (SS40), and 40–60 cm (SS60). (**a**,**b**) Different capital letters indicate significant differences among the three treatments ($p < 0.05$); different lowercase letters indicate significant differences soil depths ($p < 0.05$). Error bars represent the standard deviations (n = 3).

With respect to the Bray–Curtis distances, the non-metric multidimensional analysis results showed that fungal communities were clearly distinguishable between the OTP and YTP groups in all soil layers (Figure 4). With respect to the relative abundance of the fungal community (Top 10), as shown in Figure 5, Dothideomycetes was the main microbial population in the Forest group (21.5%) within TS10, and its component percentage was higher than that in the same layer of the YTP and OTP groups (2.38% and 8.70%, respectively). The relative abundance of Eurotiomycetes in the YTP group (51.6%) was higher than that in the Forest (14.3%) and OTP (4.78%) groups, whereas the relative abundance of Tremellomycetes in the YTP group (6.65%) was lower than that in the Forest (13.2%) and OTP (17.3%) groups in the TS20 soil layer. The main microbial community component of the OTP groups was Tremellomycetes (16.8%), whereas the main microbial community component of the YTP groups was Eurotiomycetes (33.3%) in the SS40 soil layer. Furthermore, the relative abundance of Eurotiomycetes was higher in the YTP group than in the OTP (4.75%) and Forest (20.7%) groups. The relative abundance of Leotiomycetes in the YTP groups (23.1%) was higher than that in the Forest (6.19%) and OTP (13.0%) groups within the SS60 soil layer, whereas the relative abundance of Eurotiomycetes in the Forest (17.8%) soil layers was higher than that in the YTP (7.97%) and OTP (4.36%) groups. The OTP group mainly contained Dothideomycetes (15.7%) and Tremellomycetes (16.0%) in the SS60 soil layers, whereas in the Forest group, the relative abundances of Tremellomycetes and Dothideomycetes was only 9.50% and 3.88% in the SS60, respectively.

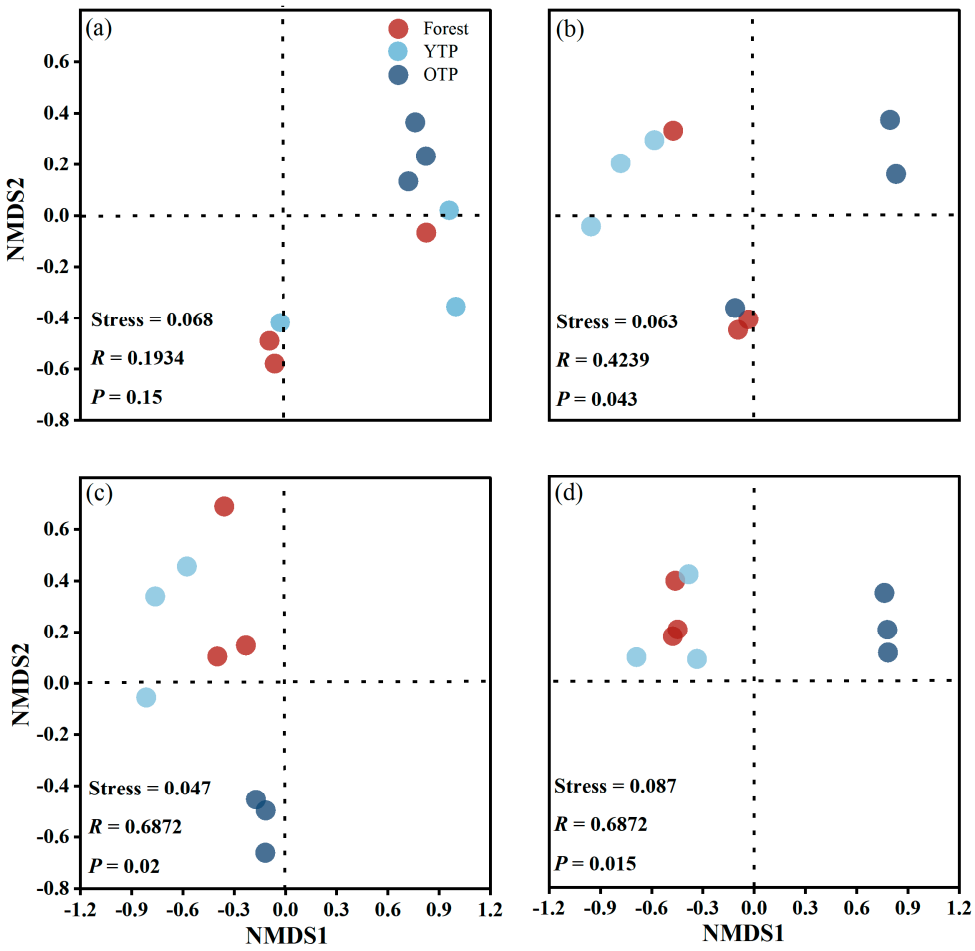

Figure 4. Results of beta diversity analysis used to estimate the effect of the Forest, YTP, and OTP different groups on soil microbial community composition. Non-metric multidimensional scaling (NMDS) analysis comparing differences in the fungal community variance of the Forest, YTP, and OTP groups at different soil layers: (**a**) 0–10 cm (TS10); (**b**) 10–20 cm (TS20); (**c**) 20–40 cm (SS40); and (**d**) 40–60 cm (SS60).

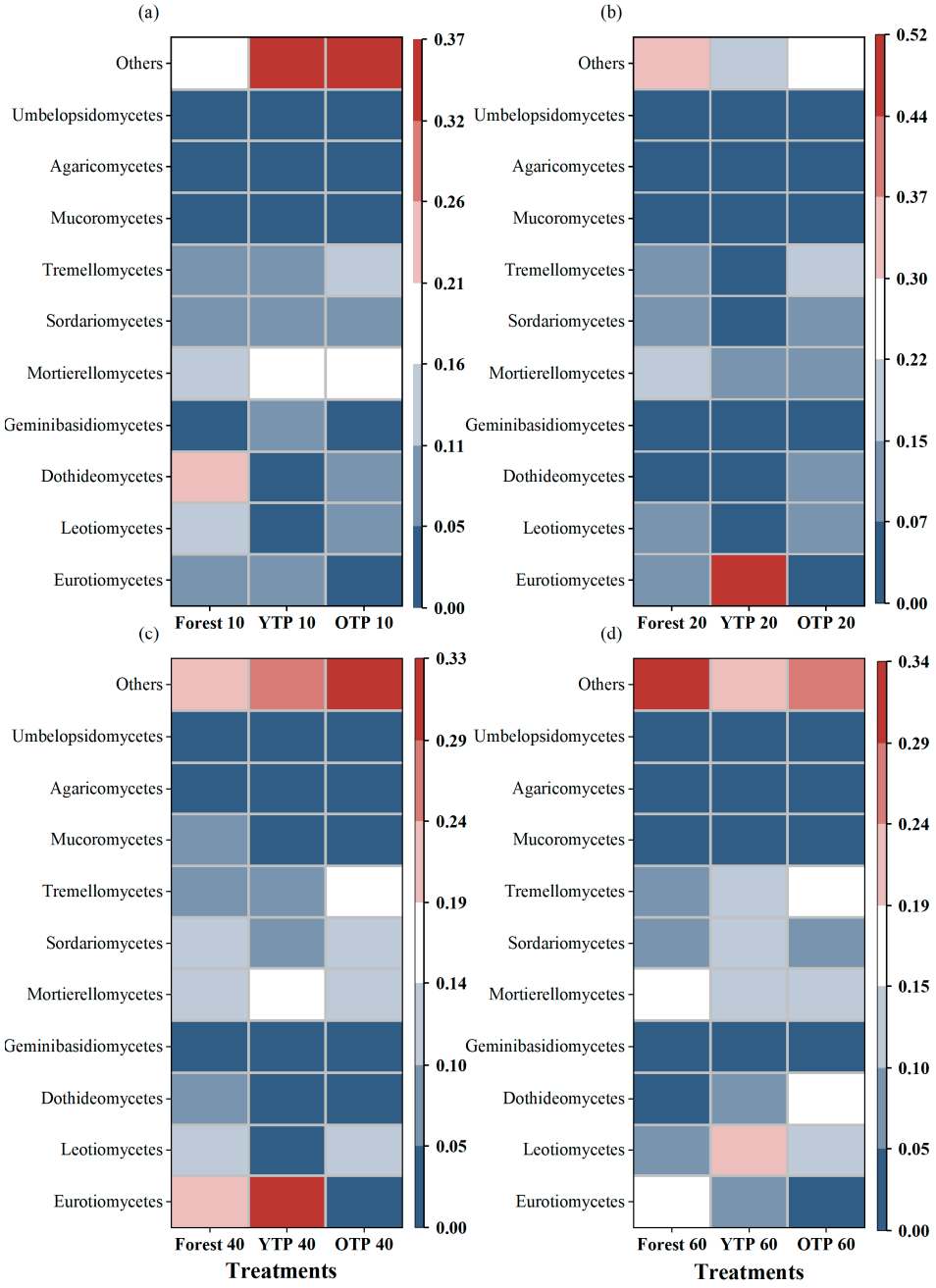

Figure 5. Differences in soil microbial composition of Forest, YTP, and OTP groups. Heatmaps depicting the relative abundance of fungal classes in different groups (Forest, YTP, and OTP groups) within four soil layers: (**a**) 0–10 cm (TS10); (**b**) 10–20 cm (TS20); (**c**) 20–40 cm (SS40); and (**d**) 40–60 cm (SS60). Heatmap showing the fungal classes with the Top 10 average relative abundance. Minor classes (fungi < 1%) are grouped into "Others". The color scale represents the square root of relative abundance.

3.4. Relationships Between Soil Carbon, Nitrogen, and Microbial Community

The redundancy analysis (RDA) demonstrated that variations in soil environmental factors significantly influenced the community structure of soil microbial communities (Figure 6). Under the TS10 layer, the first axis accounted for 40.3% of the variation in the microbial community structure and was primarily related to DOC, DON, TC, AK, and AN. The second axis was mainly associated with DOC, explaining 10.8% of the variation (Figure 6a). In the TS20 layer, the first axis illustrated that 58.5% of the variation in the soil

microbial community structure was mainly related to DOC and TN. The second axis revealed that 5.56% of the variation was primarily associated with AN. The DOC and TN contents were identified as the dominant factors responsible for the differentiation of microbial communities in the TS20 soil layer compared to those in other soil layers (Figure 6b). In the SS40 layer, the first axis illustrated that 41.0% of the variation in the soil microbial community structure was mainly related to DOC, DON, MBC, TN, and MBN. The second axis accounted for 10.2% of the variation and was primarily associated with DOC (Figure 6c). In the SS60 soil layer, the first axis demonstrated that 42.3% of the variation in the soil microbial community structure was mainly related to AP, TN, AK, AN, NN, and MBN, and the second axis indicated that 25.0% of the variation was primarily associated with TN and AP (Figure 6d).

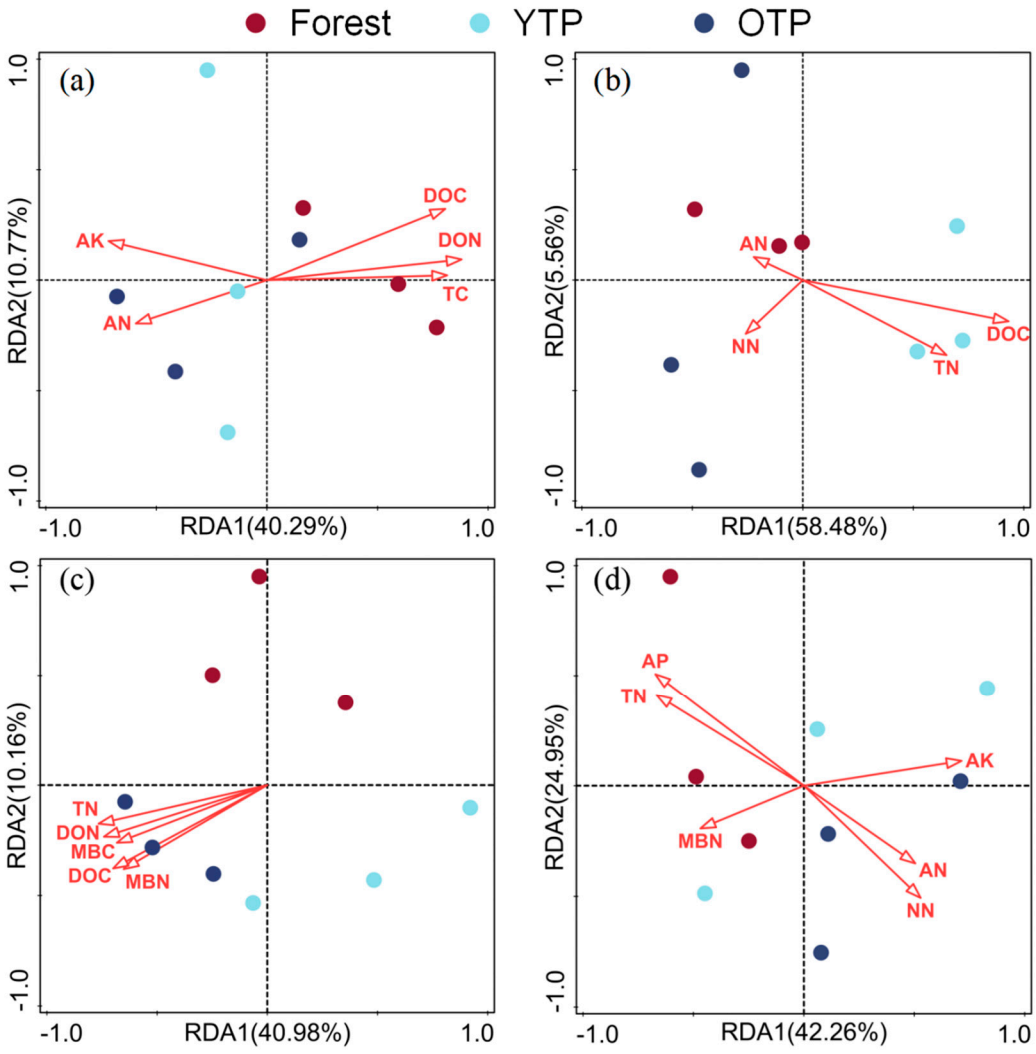

Figure 6. Redundancy analysis (RDA) of soil fungal community composition with soil physicochemical parameters in Forest, YTP, and OTP groups within four soil layers: (**a**) 0–10 cm (TS10); (**b**) 10–20 cm (TS20); (**c**) 20–40 cm (SS40); and (**d**) 40–60 cm (SS60). The meaning of the abbreviation is the same as above.

The correlation analysis (Figure 7) revealed that Eurotiomycetes exhibited a negative correlation with the DOC content, richness, and diversity, as measured by the Chao1 and Shannon indices while demonstrating a positive correlation with the TC content ($p < 0.05$). Geminibasidiomycetes showed a positive correlation with the AP, DOC, DON, and AN contents ($p < 0.05$), but a negative correlation with pH ($p < 0.05$). A positive correlation was

also observed between Mucoromycetes and TN and TC contents ($p < 0.05$). Umbelopsidomycetes were positively correlated with the contents of DOC, DON, NN, and AN contents, but negatively correlated with the pH, TC, and TN contents ($p < 0.05$).

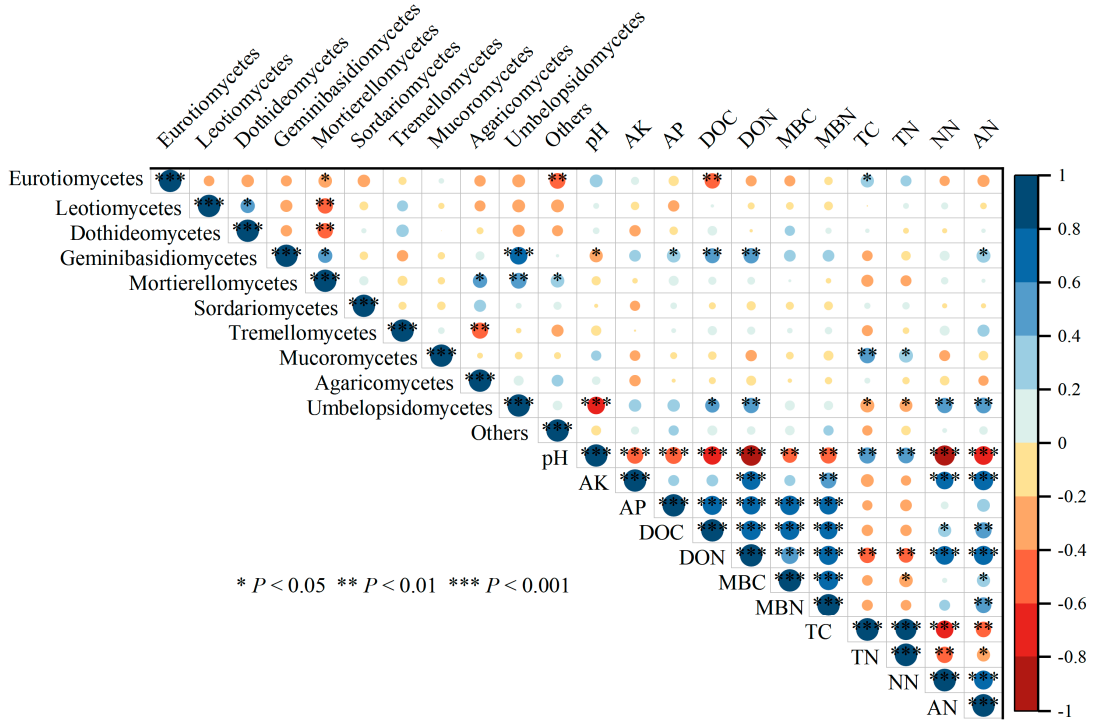

Figure 7. Results of correlation analysis between soil fungi and environmental factors in the Forest, YTP, and OTP groups. Asterisks indicate the significance of the ANOVA results (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). The "blue to red" color gradient represents the value of the Pearson correlation index. Deepening blue represents a greater positive Pearson correlation value, while deepening red represents a greater negative correlation value. TC, Total carbon; TN, Total nitrogen; DOC, Dissolved organic carbon; DON, Dissolved organic nitrogen; NN, Nitrate nitrogen; AN, Ammonium nitrogen; MBC, Microbial biomass carbon; MBN, Microbial biomass nitrogen; AP, Available phosphorus; AK, Available potassium.

4. Discussion

4.1. Effects of Planting Year on the Soil Carbon Pool at Different Depths

Numerous studies have identified significant differences in SOC content associated with the replacement of forests by tea plantations [11,43]. In this study, we observed an increase in the SOC content following such conversions (Figure 1a). This finding aligns with the results reported by Chiti et al. [44], who noted an increase in SOC content during the transition from forest to cypress and tea plantations. Additionally, the year of planting was found to affect the SOC pool in tea plantations [13,21]. In the current study, the SOC content was observed to rise with increasing planting years and was consistently higher compared to the OTP group than in the YTP group across all soil layers (Figure 1a). These results align with previous research that compared the SOC content of a 19-year-old plantation to that of a 43-year-old tea plantation, demonstrating a significant increase in the SOC content in the latter [44].

This phenomenon can be attributed to several factors. First, the conversion of forests to tea plantations can lower soil pH, a trend that intensifies with the number of years following planting. This alteration in pH can lead to changes in the microbial composition (Table S1). Some dominant microbial populations are replaced due to reduced pH, particularly those associated with C and N metabolism, which significantly impacts soil C and N [25,45]. Second, the above-ground biomass of the tea plant increases with the number

of years since planting, resulting in a greater accumulation of SOC. For instance, the tea-pruned litter is returned to the soil, which plays a crucial role in regulating C and nutrient cycling in forest and agroforestry ecosystems. This process leads to a higher input of C than its consumption, thereby enhancing SOC [10,16,46]. The results of previous studies on changes in SOC content align with those observed in our study (Figure 1). These findings can be attributed to an increase in the total vegetation C stock derived from shade trees, tea bushes, and litter biomass, particularly at soil depths of 0–50 cm [11]. Furthermore, nutrients in the soil are saturated owing to long-term fertilizer application following forest conversion to a tea plantation, and sufficient nutrients can maintain the growth of microorganisms. However, reverting to the field of tea pruning, litter can weaken the decomposition ability of microorganisms and lead to an increase in SOC storage with increasing planting years [47].

MBC is also known to change with alteration in land use and an increase in planting years [48,49]. This study found that the MBC content increased following the conversion of forest to tea plantations and was higher in the OTP group than in the YTP group (Figure 1b). This indicates that microbial activity rises with increasing planting years. A meta-analysis revealed that in subtropical forest mineral soils, MBC decreased by 39% after the removal of aboveground litter and increased by 26% following the addition of litter [50]. In addition, Li et al. [14] observed a decreasing trend in MBC concentration, followed by an increase in both pure and mixed stands of *Pinus massoniana* and *Cinnamomum camphora* from ages 10 to 24, which contrasts with the results of our study. This discrepancy may be attributed to the fact that tea plantations are more intensively managed, which results in a greater supply of nutrients, and has a higher amount of pruning litter returned compared to that in these forest ecosystems. Furthermore, the MBC content indicates a trend of decreasing rapidly with increasing soil depth [51], which is consistent with the findings of our research.

Changes in the DOC contents are significantly related to land use patterns and management measures [11–13]. Yang et al. [52] demonstrated that the DOC is involved in soil microbial metabolic activity and plays a crucial role in the C cycle. Our study discovered that the DOC content in the OTP group was higher than that of the YTP group across all the soil layers (Figure 1c). Likewise, Clarke et al. [53] showed that pruning litter increased the DOC content of a Norway spruce forest, with the accumulation of pruning litter also rising with the number of planting years. Our results align with this trend, particularly in the YTP and Forest groups. These findings suggest that converting forest land to tea plantations enhances DOC content, and this pattern intensifies with longer planting durations. This phenomenon can be attributed to two main factors: (1) the replacement of forests with tea plantations increases pruning and fertilizer inputs, which have a cumulative effect over time [16,18,25]; and (2) the application of pruned litter and fertilizer application can affect soil pH and soil structure, thereby affecting the stoichiometry of N, P, and so on, as well as the living environment of microorganisms, thus leading to changes in activated C, like DOC [25,50].

4.2. Effects of Planting Year on Soil N Pool at Different Depths

Planting years and land use changes significantly affect the soil N pool [12,20]. A higher TN content was observed in the OTP group compared to the YTP group in the TS10 and TS20 layers (Figure 2a). These findings align with previous research indicating that TN content increases as planting years progress [12]. This effect is associated with fertilizer application, which enhances TN content and leads to accumulation over time. Consequently, there was a marked difference between the TN contents of the OTP and YTP groups under management, with the former showing higher levels. Additionally, our results demonstrated a significant decrease in TN content with increasing soil depth (Figure 2a), which may be attributed to the impact of soil N retention [54].

The OTP group exhibited a higher MBN content than that the YTP and Forest groups (Figure 2b). This finding aligns with the results of Gui et al. [55], who observed a

significant increase in the MBN content in long-term monoculture tea plantations. Similarly, Zhang et al. [56] demonstrated that the MBN content increased with the progression of planting years, ranging from 3 to 43 years. The main reason for the increase in the MBN content over the years of cultivation may be as follows: (1) Long-term fertilizer application provides sufficient nutrient sources for microorganisms in the soil, which benefits microbial growth and promotes an increase in MBN content [55–57]; (2) The SOC content increases significantly with increasing planting years and provides a C source for the survival of microorganisms, thus promoting an increase in the MBN content [47,53].

We also found that the DON content of the OTP group was greater than that of the YTP group. In addition, the DON content in both the Forest and YTP groups decreased with increasing soil depth (Figure 2c). These results correlate with those of Gong et al. [58], who found that the DON content of shrubland that had been established for 25, 35, 46, and 56 years increased remarkably with increasing planting age. Fertilizer application is one of the main reasons for the increased DON content following forest conversion to tea plantations [57,59], and the DON content also increases significantly owing to the accumulative effect of fertilizer application over the years [57]. Thus, the increased MBN and DON contents are attributed to the continuous application of fertilizer and the return of pruning litter to the field after forest conversion to tea plantations.

Inorganic N is a crucial component of N in the soil, and its fluctuations have a significant impact on N availability and plant growth [59,60]. We found that the NH_4^+-N and NO_3^--N contents of the OTP group were higher than those of the YTP and Forest group soils (Figure 2d,e). Numerous studies have shown that after forests are converted to tea plantations or other crops, there is considerable uncertainty in the inorganic N content occurs with an increase in planting years [9,16,26]. For instance, Gong et al. [58] showed that with increased forest age (5, 35, 45, 55, and 65 years), the inorganic N content first increased and then decreased. Nevertheless, the inorganic N content in a mature monsoon evergreen broad-leaved forest (older than 400 years) was shown to be higher than that in a mixed pine and broad-leaved forest planted in the 1930s [61]. In addition, the conversion of tropical forests to rubber and tea plantations resulted in an increase in NH_4^+-N concentration and a decrease in NO_3^--N concentration. This effect was particularly pronounced in plantations with a high frequency of fertilization application [16]. Our study found that the increased inorganic N content may be related to the high-intensity ecosystem management practices applied in tea plantations with increasing planting years, and the long-term application of fertilizers provides a stable N source to the soil of tea plantations.

4.3. Association Between the Soil C and N Pool and Microbial Communities

The conversion of land from forest to tea plantation significantly impacts the physicochemical properties and structure of the soil, consequently influencing the composition and structure of the soil microbial community, and these alterations evolve over the years [11,17,31]. In this study, the fungal community diversity was greater in the OTP group than in the Forest and YTP groups, specifically in the TS20 and SS40 soil layers (Figure 3a). Additionally, fungal community richness increased in the SS40 soil layers with increasing planting years (Figure 3b). Similarly, the NMDS analysis showed clear distinctions in the fungal communities between the OTP and YTP groups across all soil layers (Figure 4). These findings align with those of Liao et al. [62], who observed that transition from cropland to orchards led to changes in the soil microbial community composition. Our RDA results also showed that variations in DON, DOC, and other contents significantly altered the structure and composition of the fungal community (Figure 6). These changes primarily affected the composition and function of the soil microbial community by modifying soil C and N input [9,11,53]. The change from forest to tea plantation resulted in a significant increase in SOC content, with notable cumulative effects from fertilizer application and the return of pruning litter to the field. Additionally, the TC and TN contents of the OTP group were higher than those of the YTP group. Soil acidification is known to intensify in tea plantations as planting years increase [32], and such changes in

pH affect the living environment of microorganisms, thereby affecting their community compositions [25].

Furthermore, the soil C and N contents are known to be significantly interrelated with the soil microbial structure and composition [15,31,63]. Our study showed that land use change and an increase in the planting year have significantly altered the fungal community structure and composition in the tea plantations (Figure 5). The conversion of forest to tea plantations outlined a decrease in the relative abundance of Dothideomycetes in the TS10 soil layer, while the relative abundance of Eurotiomycetes increased significantly in the TS20 and SS40 soil layers (Figure 6a–c). Chen et al. [15] showed that Eurotiomycetes are the main decomposers of many refractory substances, and they consume DOC to produce hydrolase enzymes associated with organic C mineralization to break down SOM [15,17]. The Eurotiomycetes have been confirmed to secrete decomposition enzymes that promote the mineralization of SOC, thereby accelerating the conversion of SOC to CO_2. These results can explain why the relative abundance of soil Eurotiomycetes within the TS20 and SS40 soil layers of YTP in our study is significantly higher than that of the OTP group, and that of the Forest group is greater than that in the OTP group. Also, the SOC content in YTP is significantly lower than that of OTP (Figure 1a).

Meanwhile, with an increasing number of planting years, the relative abundance of Geminibasidiomycetes decreased in the TS10 soil layer, while the abundance of Dothideomycetes increased in SS40 and SS60. Additionally, Tremellomycetes increased across all soil layers in the OTP group compared to the YTP group (Figure 5). The correlation analysis conducted in this study revealed that the relative abundance of Eurotiomycetes was negatively correlated with the DOC content. Conversely, the relative abundance of Umbelopsidomycetes exhibited a positive correlation with both the DOC and DON content (Figure 7). This shows that two key fungal taxa, Umbelopsidomycetes and Tremellomycetes maybe could enhance the activated C and N contents, while Eurotiomycetes may utilize DOC and stimulate SOC mineralization. These taxa play important roles in C and N cycling. Furthermore, Eurotiomycetes may be the primary microbial factor in the YTP group, where the SOC content was lower than that in the OTP group.

5. Conclusions

Our results indicated that the transition from forest to tea plantations augments the C and N pools in the soil. Specifically, the SOC, TN, MBC, MBN, DOC, DON, and inorganic N contents all increased with the number of planting years, particularly in the topsoil layers (TS10 and TS20). The accumulation of litter and the application of fertilizers were the primary reasons for the higher concentrations of C and N in the topsoil layers. Furthermore, our findings revealed that the conversion of forests to tea plantations, along with an increase in planting years, significantly affects the microbial composition of fungal communities. Tea is the main cash crop in China, and it has considerable C sink potential. Eurotiomycetes is the main microbial difference that leads to soil C and N contents and composition variations between forests and tea plantations, and these are the key contributors to the C cycle in tea plantation soils, as they utilize DOC to produce hydrolase enzymes that facilitate the mineralization of organic C, thereby breaking down SOC. In summary, tea plantations that employ high-intensity management practices, including fertilization and long-term return of litter, provide rich nutrients for the microbial community, which significantly increases the soil C and N contents. In addition, long-term cultivation and high-intensity management practices alter the soil microbial community composition and increase the abundance of fungal populations associated with C and N cycling in the soil.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14122779/s1, Table S1: Soil chemical properties under Forest, YTP (10-year-old tea plantation) and OTP (100-year-old tea plantation) groups at different soil depths.

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