

Supplementary Information:

Analysis of changes in soil carbon and nitrogen content by soil microbial communities in tea plantations (*Camellia sinensis* L.) with different planting years

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Site description of the Forest, YTP and OTP treatments

Fertilization management

fertilization management was consistent across the tea plantations of different ages. In this respect, 2250 kg ha⁻¹ organic fertilizer was applied at 1500 kg ha⁻¹ at the end of September or in early October. Rapeseed cake contained 45% organic C, 4.6% N, 0.9% P, 1.2% potassium (K), and the compound fertilizer contained 8.0% N, 6.0% P, 3.4% K. In mid-February and May, 250 kg/ha of N fertilizer was applied before and after spring tea-picking, respectively. Other agronomic management techniques applied in the tea plantations, including pruning, tillage, and weeding, were consistent. The forest is an evergreen broad-leaved forest, and the main trees are *Schima crenata* korthals, *Cinnamomum camphora*, and *Castanopsis sclerophylla*. No additional fertilizer was applied and the litter layer was returned to the soil at a rate of approximately 10 Mg ha⁻¹ year⁻¹ [1].

The soil properties

Soil NO₃⁻-N and NH₄⁺-N were extracted with 0.1 mol L⁻¹ KCl and analyzed by continuous flow analysis (TRAACS 2000; Seal Analysis, Mequon, WI, USA). Soil pH was measured in a paste of 1:1 (w/v) soil: distilled water mixed with an ORION 3 STAR pH meter (Thermo Ltd., Waltham, MA, USA). The SOC and soil TN concentrations were measured using a Vario Max CN analyzer (Element Analysensystem GmbH, Langenselbold, Germany). Available P (AP) and available K (AK) were extracted using the Mehlich 3 method and determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) [2].

Soil microbial biomass carbon (MBC) and nitrogen (MBN) were determined by fumigation–extraction method [3]. Within each plot, three of the six subsamples (each weighing 10.0 g of fresh soil) were subjected to fumigation with ethanol-free chloroform for a period of 24 hours at 25°C within an evacuated extractor. The remaining samples served as controls. Both fumigated and non-fumigated samples were then extracted with 40 ml of 0.5 mol/l K₂SO₄, at a ratio of soil to extractant of 1:4, and agitated for 1 hour on a reciprocal shaker. Following extraction, the samples were filtered through 7 cm diameter Whatman No.42 filter paper and stored at -15°C until analysis. The concentrations in the extracts were subsequently determined utilizing a Multi N/C 3000 analyzer (Elementar Analysensysteme GmbH).

MBC was calculated as:

$$MBC = E_C / K_{EC}$$

where E_C = (organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils) and $K_{EC} = 0.45$.

MBN was calculated as:

$$MBN = E_N / K_{EN}$$

where E_N = (total N extracted from fumigated soils) - (total N extracted from non-fumigated soils) and $K_{EN} = 0.54$.

Field-moist soil samples were extracted for DON and DOC using 0.5 M K₂SO₄ at a soil-to-extractant ratio of 1:5 at 20°C. Duplicate subsamples, equivalent to 5 g of oven-dried soil weight, were aliquoted into 50-ml polypropylene centrifuge tubes. Each tube received 25 ml of the extractant solution, after which the tubes were sealed and agitated at 15 revolutions per minute for a duration of 2 hours. Post-shaking, the samples were centrifuged at 750 g for 10 minutes and subsequently filtered through Whatman 42 filter paper. DON concentration was ascertained by subtracting NH₄⁺-N from Kjeldahl-N. Concurrently, the DOC content within the soil extracts was quantified using a Shimadzu TOC Analyzer 5000 A (Shimadzu Corporation, Melbourne, Australia) [4].

DNA Amplicon and Illumina Sequencing of Fungal Communities

Amplification of the first internal transcriptional spacer (ITS1) was conducted using broad-spectrum fungal primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') with spacers and barcodes [5]. Polymerase chain reaction (PCR) amplification was performed using TransStartFastpfu DNA polymerase, with a reaction volume of 20 µL. Amplification was first denatured at 95°C for 5 min, followed by 35 cycles of 30 s at 95°C, 30 s at 53°C, and 50 s at 72°C. Five amplicons running parallel PCR (3 × 53°C) were labeled [3]. The noise reduction using a noise reduction device was 0.851 [6]. The chimeric sequence was detected using UCHIME [7] and deleted. The sequence was shortened to 300 bp and any sequence shorter than 300 bp was deleted. The sequences were independently clustered using USEARCH, and with similarity of 97%. To determine the identity of each remaining sequence, the sequence quality was determined and demultiplexed using denoised data, and the sequences were clustered into operational taxonomic units (OTU) using the UPARSE algorithm. The centroid sequence of each cluster was compared using the USEARCH global alignment algorithm or high-quality sequences

from the NCBI database [8]. The output was analyzed using an internally developed Python program that assigns classification information to each sequence, and then computes and writes the final analysis file. Fungal OTUs were divided into functional groups based on comparisons with the FUNGuild 1.0 database [9,10]. Assignments to functional guilds were made at the generic level, and only assignments with a confidence level of "highly likely" or "probable" were reserved for analysis. Approximately 60% of OTUs are matched to the functional guilds. The relative abundance of each functional group was equal to the sum of the relative abundances of all OTUs in that group [11]. The Shannon diversity index of the functional groups was calculated using the Phyloseq software package [12].

References

1. Yan, P. Shen, C. Fan, L.C.; Li, X.; Zhang, L.P. Zhang, L.; Han W.Y. Tea planting affects soil acidification and nitrogen and phosphorus distribution in soil. *Agr Ecosyst Environ.* **2018**, 254:20-25.
2. Yan, P.; Shen, C.; Zou, Z.; Fan, L.; Li, X.; Zhang, L.P.; Zhang, L.; Dong, C.; Fu, J.; Han, W.; Shi L.L. Increased Soil Fertility in Tea Gardens Leads to Declines in Fungal Diversity and Complexity in Subsoils. *Agronomy.* **2022**, 12, 1751.
3. Yang, K.; Zhu, J.J.; Zhang, M.; Yan, Q.L.; Sun, O.J.X. Soil microbial biomass carbon and nitrogen in forest ecosystems of Northeast China: a comparison between natural secondary forest and larch plantation. *J. Plant Ecol.* 2010,3,3,175-182.
4. Ghani, A.; Dexter, M.; Carran, R.; A. Theobald, P. W. Dissolved organic nitrogen and carbon in pastoral soils:the New Zealand experience. *Eur. J. Soil Sci.* 2007,58,832-843.
5. White, T.J.; Bruns, T.; Lee, S.; Taylor, J.; Innis, M.A.; Gelfand, D.H. Sninsky, J. Amplification and Direct Sequencing of Fungal Ribosomal Rna Genes for Phylogenetics. In PCR Protocols; Academic Press: Cambridge, MA, USA, **1990**; pp. 315–322.
6. Reeder, J.; Knight, R. Rapidly denoising pyrosequencing amplicon reads by exploiting rank-abundance distributions. *Nat. Methods.* **2010**, 7, 668–669.
7. Edgar, R.C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics.* **2010**, 26, 2460–2461.

8. Pruitt, K.D.; Brown, G.R.; Hiatt, S.M.; Thibaud-Nissen, F.; Astashyn, A.; Ermolaeva, O.; Farrell, C.M.; Hart, J.; Landrum, M.J.; McGarvey, K.M.; Murphy, M.R.; O’Leary, N.A.; Pujar, S.; Rajput, B.; Rangwala, S.H.; Riddick, L.D.; Shkeda, A.; Sun, H. Z.; Tamez, P.; Tully, R.E.; Wallin, C. Webb, D.; Weber, J.; Wu, W.; DiCuccio, M.; Kitts, P.; Maglott, D.R.; Murphy, T.D.; Ostell, J.M. RefSeq: An update on mammalian reference sequences. *Nucleic Acids Res.* **2013**, *42*, D756–D763.
9. Nguyen, N.H.; Williams, L.J.; Vincent, J.B.; Stefanski, A.; Cavender-Bares, J.; Messier, C.; Paquette, A.; Gravel, D.; Reich, P.; Kennedy, P.G. Ectomycorrhizal fungal diversity and saprotrophic fungal diversity are linked to different tree community attributes in a field-based tree experiment. *Mol. Ecol.* **2016**, *25*, 4032–4046
10. Yang, X.; Yu, H.; Zhang, T.; Guo, J.; Zhang, X. Arbuscular Mycorrhizal Fungi Improve the Antioxidative Response and the Seed Production of Suaedoideae Species Suaeda physophora Pall Under Salt Stress. *Not. Bot. Horti Agrobotanici. Cluj-Napoca.* **2016**, *44*, 533–540.
11. Delgado-Baquerizo, M.; Fry, E.L.; Eldridge, D.J.; de Vries, F.T.; Manning, P.; Hamonts, K.; Kattge, J.; Boenisch, G.; Singh, B.K.; Bardgett, R.D. Plant attributes explain the distribution of soil microbial communities in two contrasting regions of the globe. *New Phytol.* **2018**, *219*, 574–587.
12. McMurdie, P.J.; Holmes, S. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* **2013**, *8*, e61217.

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.

Depth (cm)	pH			Available phosphorus (mg kg ⁻¹)			Available potassium (mg kg ⁻¹)		
	Forest	Young tea plantation	Century-old tea plantation	Forest	Young tea plantation	Century-old tea plantation	Forest	Young tea plantation	Century-old tea plantation
0-10	4.12 ± 0.02dA	3.97±0.01cB	3.73±0.09aC	17.8±0.7aB	11.1±0.6aC	79.8±3.6aA	51.3±1.1dC	181±2.1aA	135±16.9aB
10-20	4.26±0.02cA	4.02±0.02bB	3.52±0.06bC	11.0±0.7bB	12.6±1.0aB	36.6±3.4bA	58.1±0.8bC	181±4.6aA	118±1.8aB
20-40	4.31±0.01bA	4.25±0.01aB	3.54±0.03abC	9.1±0.3bB	8.2±0.2bB	23.5±3.5cA	54.6±0.6cC	101±1.9bB	147±16.6aA
40-60	4.35±0.01aA	4.23±0.02aA	3.4±0.12bB	2.6±1.4cB	4.0±0.5cAB	8.7±3.5dA	70.2±1.7aC	82±2.4cB	129±2.6aA

Different lowercase letters indicate significant differences among the three groups ($P < 0.05$) and different capital letters indicate significant differences in soil depth ($P < 0.05$). The mean values after “±” are the standard deviation. Different lowercase letters indicate significant differences among the three treatments ($P < 0.05$); different capital letters indicate significant differences soil depths ($P < 0.05$). Error bars represent the standard deviations ($n = 3$).