**Tea Tree Cultivation Restructured Soil Microbiota in Mountainous Tea Plantations and Regulated Soil Organic Matter Metabolism and Nitrogen Cycling Functions**

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**Table S1**. Information of primers used in this study.

|  |  |  |  |
| --- | --- | --- | --- |
| Target | Primers | Primer sequence (5’- 3’) | PCR reaction condition |
| 16S | 338F  806R | ACTCCTACGGGAGGCAGCAG  GGACTACHVGGGTWTCTAAT | 95°C for 3 minutes, 27 cycles of (30 s at 95°C, 30 s at 55°C and 45 s at 72°C, 72°C for 10 minutes) |
| 18S | ITS2-2043 R  ITS5-1737F | GCTGCGTTCTTCATCGATGC  GGAAGTAAAAGTCGTAACAAGG | 95°C for 5 minutes, 35 cycles of (30 s at 94°C, 30 s at 52°C, for 30 s at 72°C，72°C for 10 minutes), |

**Table S2**. Soil physicochemical properties and enzyme activities in different locations of tea plantations (Inter-row (IR), Terrace surfaces (TS), and Terrace wall (TW)) under different cultivation years (1Y, 3Y, 5Y).

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| TreaTSent | CK | IR1 | IR3 | IR5 | TS1 | TS3 | TS5 | TW1 | TW3 | TW5 |
| Total N（g·kg-1） | 0.83d | 1.39a | 1.15b | 1.01bc | 1.11b | 0.97c | 0.82d | 0.89d | 0.87d | 0.71e |
| Total P（g·kg-1） | 0.63c | 0.82b | 0.97ab | 1.12a | 0.65c | 0.54d | 0.85b | 0.51d | 0.72c | 0.75c |
| Total K（g·kg-1） | 15.25c | 20.88a | 21.67a | 23.54a | 17.26b | 18.44b | 18.95b | 17.62b | 16.83b | 17.09b |
| Available N（mg·kg-1） | 24.67b | 28.29a | 25.43b | 23.88b | 22.56c | 20.85c | 24.05b | 20.92d | 17.70e | 19.45d |
| Available P（mg·kg-1） | 13.8d | 21b | 24.11a | 23.03a | 15.52c | 17.81c | 20.28b | 11.74e | 13.59d | 16.33c |
| Available K（mg·kg-1） | 84.639e | 106.19b | 115.14ab | 121.54a | 90.88d | 97.55c | 107.18b | 98.36c | 89.14d | 85.93e |
| Organic matter（g·kg-1） | 21.37a | 18.68ab | 17.15bc | 15.6de | 16.56cd | 15.68de | 12.76f | 13.76f | 11.87f | 11.91f |
| pH | 4.54c | 4.6b | 4.53c | 4.45d | 4.58bc | 4.56bc | 4.52c | 4.65a | 4.58bc | 4.54bc |
| Urase[mg/(g·d)] | 0.77bc | 1.04a | 0.89b | 0.82b | 0.62c | 0.65c | 0.88b | 0.52cd | 0.41d | 0.59cd |
| Polyphenoloxidase[mg/(g·2h)] | 0.73e | 1.38c | 1.66b | 1.95a | 0.80de | 0.97e | 1.18cd | 0.62e | 0.58e | 0.76e |
| Acid phosphatase[mg/(g·h)] | 0.98 c | 1.50a | 0.89d | 1.27ab | 1.19ab | 0.85d | 0.74e | 0.99c | 0.87d | 0.79d |
| Protease[mg/(g·d)] | 0.184c | 0.204c | 0.38a | 0.44a | 0.19c | 0.33b | 0.31b | 0.18c | 0.21b | 0.29b |
| Peroxidase[mg/(g·2h)] | 0.97d | 1.33c | 1.68b | 1.94a | 1.3c | 1.1cd | 1.39d | 0.99d | 0.95d | 1.25cd |
| Cellulase[mg/(g·d)] | 0.17e | 0.16e | 0.27c | 0.37a | 0.14f | 0.24c | 0.32b | 0.15ef | 0.21d | 0.22d |

Notes: different letters denote significant differences (LSD, P < 0.05)**Table S3**. The study analyzed the topological characteristics of bacterial community co-occurrence networks in three types of soil: Inter-row (IR), Terrace surfaces (TS), and Terrace wall (TW), under different tea cultivation durations (1Y, 3Y, 5Y).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| BACTERIA-Summary Statistics | IR1 | IR3 | IR5 | TS1 | TS3 | TS5 | TW1 | TW3 | TW5 |
| Number of nodes | 193 | 177 | 137 | 176 | 115 | 94 | 137 | 146 | 143 |
| Number of edges | 1549 | 861 | 643 | 1127 | 746 | 545 | 822 | 870 | 607 |
| Avg. number of neighbors | 44 | 16 | 18 | 33 | 30 | 19 | 24 | 19 | 16 |
| Connected components | 32 | 32 | 29 | 24 | 24 | 12 | 25 | 25 | 30 |
| Analysis time (sec） | 0.079 | 0.067 | 0.018 | 0.094 | 0.029 | 0.019 | 0.014 | 0.025 | 0.051 |

**Table S4**. The study analyzed the topological characteristics of fungus community co-occurrence networks in three types of soil: Inter-row (IR), Terrace surfaces (TS), and Terrace wall (TW), under different tea cultivation durations (1Y, 3Y, 5Y).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| FUNGI-Summary Statistics | IR1 | IR3 | IR5 | TS1 | TS3 | TS5 | TW1 | TW3 | TW5 |
| Number of nodes | 139 | 145 | 199 | 160 | 106 | 161 | 86 | 136 | 153 |
| Number of edges | 668 | 977 | 1832 | 1039 | 553 | 1335 | 450 | 1068 | 1335 |
| Avg. number of neighbors | 20 | 26 | 35 | 27 | 20 | 28 | 20 | 28 | 28 |
| Connected components | 22 | 19 | 21 | 21 | 12 | 25 | 12 | 16 | 17 |
| Analysis time (sec） | 0.022 | 0.021 | 0.075 | 0.02 | 0.017 | 0.079 | 0.08 | 0.028 | 0.051 |

**Table S5**. Proportion of variance for PCoA analysis of soil microbial communities.

|  |  |  |
| --- | --- | --- |
| Proportion of Variance | Bacteria | fungi |
| PC1 | 0.3627 | 0.4702 |
| PC2 | 0.245 | 0.1604 |
| PC3 | 0.117 | 0.0669 |
| PC4 | 0.0553 | 0.0575 |
| PC5 | 0.0404 | 0.0533 |
| PC6 | 0.0287 | 0.0454 |
| PC7 | 0.0218 | 0.0315 |
| PC8 | 0.0158 | 0.0305 |
| PC9 | 0.0143 | 0.0277 |
| PC10 | 0.0101 | 0.0105 |
| PC11 | 0.0099 | 0.0098 |
| PC12 | 0.009 | 0.0083 |
| PC13 | 0.0077 | 0.0067 |
| PC14 | 0.0068 | 0.0048 |
| PC15 | 0.0062 | 0.004 |
| PC16 | 0.0059 | 0.0028 |
| PC17 | 0.0056 | 0.0023 |
| PC18 | 0.0052 | 0.002 |
| PC19 | 0.0045 | 0.0017 |
| PC20 | 0.0041 | 0.0009 |
| PC21 | 0.0036 | 0.0008 |
| PC22 | 0.0033 | 0.0006 |
| PC23 | 0.0031 | 0.0005 |
| PC24 | 0.0028 | 0.0004 |
| PC25 | 0.0025 | 0.0002 |
| PC26 | 0.0025 | 0.0002 |
| PC27 | 0.0024 | 0.0001 |
| PC28 | 0.0022 | 0 |
| PC29 | 0.0018 | 0 |
| PC30 | 0 | 0.0001 |

**Table S6**. Parameters related to Neutral community mode (NCM) for different soil microbial communities.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Neutral community model (NCM) data | | | | | |
|
| GROUP | | R2 | N | m | Nm |
| Bacteria | IR | 0.7767 | 47501 | 0.6411 | 30452.1894 |
| TS | 0.7953 | 47501 | 0.6392 | 30360.6524 |
| TW | 0.799 | 47501 | 0.6268 | 29771.7668 |
| Fungi | IR | 0.5064 | 63214 | 0.186 | 11755.5085 |
| TS | 0.4785 | 63214 | 0.1574 | 9952.4611 |
| TW | 0.4311 | 63214 | 0.1319 | 8338.0233 |

Notes: The R2 value represents the overall goodness of fit of the Neutral Community Model; The migration rate (m) quantifies the community-level dispersal and is consistent for all community members; N describes the metacommunity size, which is the total abundance of all OTUs in each sample; Nm, which is the product of the community size (N) and mobility (m) (Nm = N\*m), quantifies estimates of dispersal between communities.

**Table S7**. Proportion of variance for RDA analysis of soil microbial communities.

|  |  |  |
| --- | --- | --- |
| **Proportion of Variance** | **Bacteria** | **Fungi** |
| RDA1 | 0.7375 | 0.4448 |
| RDA2 | 0.1083 | 0.1789 |
| RDA3 | 0.0585 | 0.132 |
| RDA4 | 0.0361 | 0.1003 |
| RDA5 | 0.0082 | 0.0448 |
| RDA6 | 0.0063 | 0.0328 |
| RDA7 | 0.005 | 0.0155 |
| RDA8 | 0.0044 | 0.0104 |
| RDA9 | 0.0024 | 0.0059 |
| RDA10 | 0.002 | 0.0016 |
| RDA11 | 0.0015 | 0.0003 |
| RDA12 | 0.0008 | 0.0002 |
| RDA13 | 0.0006 | 0.0001 |
| RDA14 | 0.0005 | 0 |
| RDA15 | 0.0003 | 0.0183 |
| PC1 | 0.0102 | 0.0048 |
| PC2 | 0.0074 | 0.0032 |
| PC3 | 0.0034 | 0.0027 |
| PC4 | 0.0019 | 0.0013 |
| PC5 | 0.0012 | 0.0009 |
| PC6 | 0.0012 | 0.0005 |
| PC7 | 0.0008 | 0.0003 |
| PC8 | 0.0006 | 0.0002 |
| PC9 | 0.0005 | 0.0001 |
| PC10 | 0.0002 | 0 |
| PC11 | 0.0002 | 0 |

**Table S8**. The significant relationships between soil physicochemical properties and enzyme activity with soil microbial community in the RDA analysis.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | RDA1 | RDA2 | ef.vectors.r | ef.vectors.pvals |
| N | -0.728432024 | 0.685118082 | 0.337864069 | 0.007 |
| P | -0.737882219 | 0.674929501 | 0.251034834 | 0.032 |
| K | -0.868337774 | -0.495973296 | 0.117854547 | 0.202 |
| AN | -0.386297049 | -0.92237443 | 0.302249955 | 0.016 |
| AP | -0.595903079 | 0.803056362 | 0.556214234 | 0.001 |
| AK | -0.459006714 | 0.888432798 | 0.134942857 | 0.176 |
| SOM | 0.871358788 | -0.490646372 | 0.500794614 | 0.002 |
| MC | 0.484883836 | -0.874578565 | 0.749063285 | 0.001 |
| pH | 0.736842608 | -0.676064325 | 0.666486575 | 0.001 |
| UE | -0.819457583 | -0.573139835 | 0.333174052 | 0.007 |
| PPO | -0.980200395 | 0.198008046 | 0.373871002 | 0.001 |
| ACP | -0.086092111 | -0.996287182 | 0.328573436 | 0.01 |
| POD | -0.426002887 | 0.904721802 | 0.507809024 | 0.001 |
| PT | -0.686977174 | 0.726678995 | 0.724757134 | 0.001 |
| CE | -0.508040508 | 0.861333177 | 0.759204456 | 0.001 |

**Table S9.** Structural equation modeling parameters and Confidence index (Fig 6C).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Summary table of model regression coefficients | | | | |
| X | → | Y | p | Regression coefficient |
| soil location | → | N nutrient | 0.04 | -0.52 |
| soil location | → | Bacteria diversity | 0.14 | 0.29 |
| soil location | → | Nitrification function | 0.01 | -0.44 |
| soil location | → | Denitrification function | 0.00 | -0.76 |
| soil location | → | organic matter | 0.01 | -0.72 |
| soil location | → | Fungi diversity | 0.68 | 0.09 |
| soil location | → | saprotroph function | 0.45 | 0.20 |
| soil location | → | Plant pathogen function | 0.02 | -0.43 |
| Bacteria diversity | → | N nutrient | 0.00 | 0.65 |
| Bacteria diversity | → | Nitrification function | 0.00 | 0.67 |
| Bacteria diversity | → | Denitrification function | 0.01 | -0.71 |
| Fungi diversity | → | Plant pathogen function | 0.02 | 0.42 |
| Fungi diversity | → | saprotroph function | 0.04 | 0.30 |
| Fungi diversity | → | organic matter | 0.02 | -0.42 |
| Nitrification function | → | N nutrient | 0.05 | 0.35 |
| Denitrification function | → | N nutrient | 0.01 | 0.45 |
| Plant pathogen function | → | organic matter | 0.66 | 0.15 |
| saprotroph function | → | organic matter | 0.02 | 0.45 |

Note：→Representing the regression relationships

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Confidence index | χ2/df | p | GFI | RMSEA | RMR | CFI | NFI | NNFI |
| value | 1.247 | 0.085 | 0.541 | 0.017 | 0.037 | 0.932 | 0.918 | 0.619 |
| Confidence index | TLI | AGFI | IFI | PGFI | PNFI | PCFI | SRMR |  |
| value | 0.9 | 0.85 | 0.907 | 0.479 | 0.568 | 0.466 | 0.097 |  |

Note: Confidence index

**Table S10.** Structural equation modeling parameters and Confidence index (Fig 6D).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Summary table of model regression coefficients | | | | |
| X | → | Y | p | Regression coefficient |
| Planting Years | → | N nutrient | 0.08 | -0.42 |
| Planting Years | → | Bacteria diversity | 0.00 | -0.88 |
| Planting Years | → | Nitrification function | 0.01 | -0.61 |
| Planting Years | → | Denitrification function | 0.00 | 0.81 |
| Planting Years | → | organic matter | 0.00 | -0.87 |
| Planting Years | → | Fungi diversity | 0.44 | 0.18 |
| Planting Years | → | saprotroph function | 0.01 | 0.67 |
| Planting Years | → | Plant pathogen function | 0.02 | 0.39 |
| Bacteria diversity | → | N nutrient | 0.03 | 0.39 |
| Bacteria diversity | → | Nitrification function | 0.00 | 0.90 |
| Bacteria diversity | → | Denitrification function | 0.01 | -0.57 |
| Fungi diversity | → | Plant pathogen function | 0.02 | 0.39 |
| Fungi diversity | → | saprotroph function | 0.03 | -0.23 |
| Fungi diversity | → | organic matter | 0.03 | 0.27 |
| Nitrification function | → | N nutrient | 0.01 | 0.75 |
| Denitrification function | → | N nutrient | 0.01 | -0.48 |
| Plant pathogen function | → | organic matter | 0.00 | -0.81 |
| saprotroph function | → | organic matter | 0.01 | -0.57 |

Note：→Representing the regression relationships

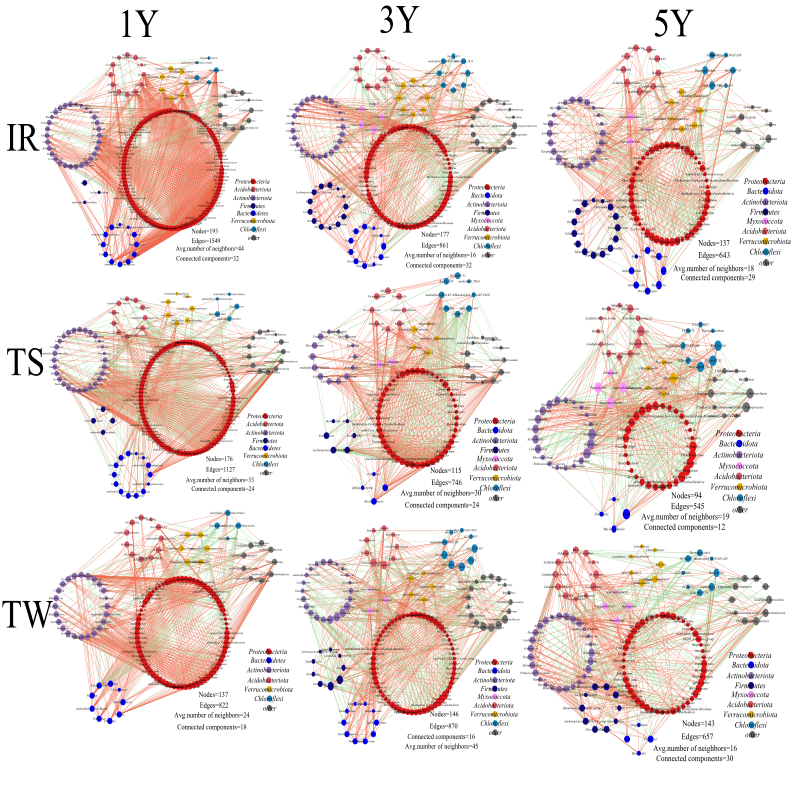
|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Confidence index | χ2/df | p | GFI | RMSEA | RMR | CFI | NFI | NNFI |
| value | 1.351 | 0.058 | 0.5 | 0.074 | 0.04 | 0.918 | 0.686 | 0.871 |
| Confidence index | TLI | AGFI | IFI | PGFI | PNFI | PCFI | SRMR |  |
| value | 0.871 | 0.247 | 0.918 | 0.332 | 0.52 | 0.66 | 0.036 |  |

Note: Confidence index

**Table S11.** Microbial genera functions and corresponding references.

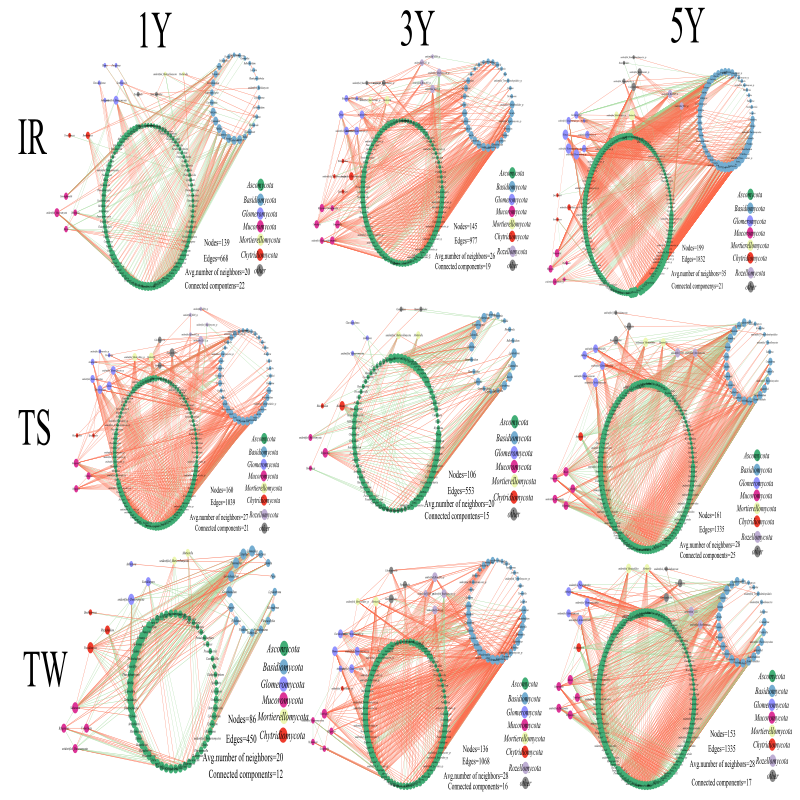
|  |  |  |
| --- | --- | --- |
| Genus | Funtions | References |
| *Burkholderia* | Nitrogen fixation | (Lin et al., 2012; Zhong et al., 2022) |
| *Bradyrhizobium* | Nitrogen fixation，Nitrogen transformation | (Sharma et al., 2020; Ormeño-Orrillo and Martínez-Romero, 2019) |
| *Gemmatimonas* | Nitrogen fixation，Nitrogen transformation | (Park et al., 2017; Oshiki et al., 2022) |
| *Xanthobacter* | Nitrogen fixation，Conversion of plant hormones | (Juan et al., 2022) |
| *Chujaibacter* | Degradation, Organic metabolism | (Feng et al., 2023) |
| *Candidatus\_Solibacter* | Degradation, Organic metabolism | (Wang et al., 2017; Wang et al., 2022) |
| *Sphingomonas* | Soil remediation, Organic metabolism | (Luo et al., 2019; Asaf et al., 2020) |
| *Kitasatospora* | Organic metabolism | (Rahmani et al., 2019; Klaysubun et al., 2022) |
| *Sinomonas* | Amylolytic, Organic metabolism | (Lee et al., 2015; Narsing Rao et al., 2018) |
| *Bryobacter* | Soil remediation, Organic metabolism | (Li et al., 2021; Hu et al., 2022) |
| *Granulicella* | Organic metabolism | (Costa et al., 2020) |
| *Cladosporium* | Mildew | (Simão and Gomes, 2001; Prasannath et al., 2021) |
| *Curvularia* | Cuvularia leaf spot | (Zhang et al., 2019) |
| *Sarocladium* | Cause Brown Spot, Rice Sheath Rot | (Hou et al., 2019; Peeters et al., 2020) |
| *Fusarium* | Leaf blight, Mildew | (Rai et al., 2020) |
| *Geminibasidium* | Organic metabolism, Disease resistance | (Pulido-Chavez et al., 2021; Ren et al., 2021) |
| *Gongronella* | Solubilizing capacity, Organic metabolism | (Doilom et al., 2020) |
| *Penicillium* | Cellulose degradation | (Vaishnav et al., 2018) |
| *Talaromyces* | Cellulose degradation, Lignin degradation | (Houbraken et al., 2020; Méndez-Líter et al., 2021) |
| *Trichoderma* | Cellulose Degradation, Disease resistance | (Sood et al., 2020; Zin and Badaluddin, 2020) |

**Fig. S1**. The co-occurrence networks of bacterial communities in different soils under varying tea cultivation durations.



Notes: Different circle colors represent different Phylums(Spearman,P<0.01,r> 0.70).

**Fig. S2**. The co-occurrence networks of fungus communities in different soils under varying tea cultivation durations.



Notes: Different circle colors represent different Phylum (Spearman<0.01,r> 0.70).

**Fig. S3.** LEfSe analysis of distinctive bacteria in different soil types (Figure4A.B.C).

s3

Notes: A: Inter-row (IR), B: Terrace surfaces (TS), C: Terrace wall (TW). Different column colors represent different years of the same sample. (LDA > 3.5). D: LEfSe multilevel species hierarchy tree. Different colored nodes indicate the microbial taxa that were significantly enriched in the corresponding groups and had a significant impact on the differences between groups.

**Fig. S4.** LEfSe analysis of distinctive fungi in different soil types (Figure5A.B.C)

S4

Notes: A: Inter-row (IR), B:Terrace surfaces (TS), C: Terrace wall (TW).Different column colors represent different years of the same sample. (LDA > 3.5). D:LEfSe multilevel species hierarchy tree.Different colored nodes indicate the microbial taxa that were significantly enriched in the corresponding groups and had a significant impact on the differences between group

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