

Distinct spatiotemporal patterns between fungal alpha and beta diversity of soil–plant continuum in rubber tree

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Abstract

Plant-associated microbial communities strongly relate to host health and productivity. Still, our knowledge of microbial community spatiotemporal patterns in soil–plant continuum is largely limited. Here, we explored the spatiotemporal dynamics of fungal communities across multiple compartments (phyllosphere, leaf endosphere, soil, rhizosphere, rhizoplane, root endosphere) of rubber tree in two contrasting seasons collected from Hainan Island and Xishuangbanna. Our results demonstrate that the fungal alpha and beta diversity exhibited distinct pattern, the alpha diversity is highly dependent on seasonal changes, while beta diversity only showed a geographical variation pattern. The season-specific environmental factors (e.g., climatic factors) were the most important factors in shaping fungal alpha diversity across the soil–plant continuum. Physicochemical properties explained some of microbial beta diversity spatiotemporal variation observed, with leaf phosphorus (P) and soil available potassium (AK) likely being the main factors that drove the geographical variation. We further identified the variation of edaphic (e.g., AK) and leaf physicochemical factors (e.g., P) were mainly caused by regional sites ($P < 0.05$). Together, our study provide a solid empirical evidence that the distinct spatiotemporal patterns of alpha and beta diversity of rubber tree fungal diversity and significantly expand our understanding of ecological drivers of plant-associated microbial communities.

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Plant-associated microbial communities strongly relate to host health and productivity. Still, our knowledge of microbial community spatiotemporal patterns in soil–plant continuum is largely limited. Here, we explored the spatiotemporal dynamics of fungal communities across multiple compartments (phyllosphere, leaf endosphere, soil, rhizosphere, rhizoplane, root endosphere) of rubber tree in two contrasting seasons collected from Hainan Island and Xishuangbanna. Our results demonstrate that the fungal alpha and beta diversity exhibited distinct pattern, the alpha diversity is highly dependent on seasonal changes, while beta diversity only showed a geographical variation pattern. The season-specific environmental factors (e.g., climatic factors) were the most important factors in shaping fungal alpha diversity across the soil–plant continuum. Physicochemical properties explained some of microbial beta diversity spatiotemporal variation observed, with leaf phosphorus (P) and soil available potassium (AK) likely being the main factors that drove the geographical variation. We further identified the variation of edaphic (e.g., AK) and leaf physicochemical factors (e.g., P) were mainly caused by regional sites ($P < 0.05$). Together, our study provide a solid empirical evidence that the distinct spatiotemporal patterns of alpha and beta diversity of rubber tree fungal diversity and significantly expand our understanding of ecological drivers of plant-associated microbial communities.

Keywords: Rubber tree; Fungi; Diversity; Spatiotemporal patterns

Introduction

Microbial organisms inhabit all biomes of the Earth (Koskella, 2020), and provide a number of life-support functions for their host (Cordovez et al., 2019). Therefore, we must develop a better understanding of the distribution and ecological drivers of aboveground and belowground microbial communities. Recent

studies have demonstrated the immense role of plant compartment and environmental factors in driving the assembly of microbiomes (Lan et al., 2023; Wei et al., 2022a; Xu et al., 2024). Geographic location and seasonal change have been demonstrated to influence community composition. For example, it was suggested that the assembly of the phyllosphere bacterial and fungal communities is predominantly determined by host compartment (epiphytic and endophytic) and site location (Wei et al., 2022a). As for soil and rhizosphere, microbiomes are influenced by environmental factors (e.g., site, soil properties, and climate) (Grady et al., 2019; Lundberg et al., 2012; Thiergart et al., 2020; Wei et al., 2022b; Xiong et al., 2021; Xu et al., 2024). However, these studies mainly focused on single niche or compartment, and a significant knowledge gap exists on how spatial heterogeneity versus time shape the diversity and structure of microbial communities along the soil–plant continuum. Different scales have varying impact on plant microorganisms (Jumpponen and Jones, 2009; Laforest-Lapointe et al., 2016; Qian et al., 2018; Wang et al., 2023). Moreover, these examples have shown that soil microbial communities are influenced by spatial or temporal change, but, understanding of how seasonal changes (e.g., dry and rainy seasons) affect the compositions and diversity of soil–plant continuum microbial communities at the regional scale is still limited.

Rubber plantation is the most economically important agro-ecosystem in tropical China, particularly in Hainan Island and Xishuanbanna (abbreviated as Banna below) (Lan et al., 2017), where accounting for more than 90% of the total rubber plantation area of China (Xu et al., 2014). It is reported rubber plantation have multiplied quickly throughout Southeast Asia over the last two decades (Chen et al., 2023; Li et al., 2015). As far as we know, rubber forests account for almost 25% and 40% of the total vegetation area in Hainan Island and Banna (Lan et al., 2020), respectively. Previous work in Hainan has shown that seasonal change or site location were the dominant factors resulting in shifts in soil microbial composition at the local and geographic scales, respectively (Lan et al., 2018; Lan et al., 2020; Lan et al., 2019; Wei et al., 2022b). However, these studies were limited, particularly in the sample sizes scales used. Given the importance of microbes in functional roles in tropical forests ecosystem, such as nutrient acquisition, disease resistance, and stress tolerance (Trivedi et al., 2020), and the central part of rubber plantation of terrestrial ecosystems both in Hainan and Banna. Moreover, little attention has been paid to the plant-associated microbial communities in the same scale, as a result, it is difficult to describe the overall pattern of the soil–plant continuum. Therefore, it is necessary to study the spatiotemporal pattern and ecological drivers of the rubber tree soil–plant continuum microbial communities of these two locations.

In this study, we examine fungal communities across multiple compartments (bulk soils, rhizosphere, rhizoplane, root endosphere, phylloplane, and leaf endosphere) based on field samples of rubber tree during both dry and rainy seasons in two major areas of China (Figure S1). We aimed to (1) determine the distribution pattern of fungal communities along the soil–plant continuum of rubber tree; (2) identify the relative importance of spatial heterogeneity versus season and dominant drivers for driving fungal community at the regional scale. We hypothesized that (1) both geographic and seasonal factors will influence the assembly of rubber-associated fungal communities; (2) the spatiotemporal distribution pattern is mediated by the spatiotemporal variation of the driving factors.

Methods

Study site and sampling

The study was conducted in two locations: Hainan Island and Bnna. Hainan Island experiences a tropical maritime monsoon climate, characterized by a rainy season from May to October and a dry season from November to April. Rubber plantations in Hainan are located at low latitudes and altitudes, making them tropical island-type plantations. On the other hand, Bnna has a warm and humid climate throughout the year, with dry (November to April) and rainy seasons (May to October) similar to Hainan. The rubber plantations in Bnna are situated at higher latitudes and altitudes, representing a tropical inland static wind plantation area type with fertile soil (Lan et al., 2020).

We selected six major plantation sites from Danzhou, Wanning, and Ledong Districts in Hainan Island, as

well as from Jinghong, Menglun, and Mengpeng Districts in Xishuangbanna (Figure S1). At each site, we chose three plots separated by distances of 5-15 km for sampling, resulting in a total of 18 plots. The sample collections were carried out in August to September 2019 and November to December 2020, corresponding to the rainy and dry seasons, respectively. Therefore, we obtained 36 soil samples from each plot, resulting in a total of 216 samples, including those from the phyllosphere, leaf endosphere, soil, rhizosphere, rhizoplane, and root endosphere compartments. For each sample, we analyzed water content (WC), pH, leaf phosphorus (P), potassium (K), nitrogen (N), and organic matter (LOM). Additionally, we quantified soil total nitrogen (TN), total phosphorus (TP), total potassium (TK), organic matter (SOM), available potassium (AK), ammonium nitrogen (AN), nitrate nitrogen (NN), and available phosphorus (AP). The soil, root, and leaf sampling approach, as well as the analysis of physicochemical properties, were conducted based on the methods described in our previous study (Lan et al., 2023; Wei et al., 2022a). We collected latitude, longitude, and elevation data for each site. Additionally, data on average monthly precipitation and average monthly temperature for each month were obtained from the National Meteorological Information Center. (<https://www.data.cma.cn>).

DNA extraction and sequencing

Microbial community DNA was extracted from soil, root, and leaf samples using the FastDNA® Spin Kit for Soil (MP Biomedicals), following the manufacturer's instructions. The hypervariable region ITS of the fungal gene was amplified using the primer pairs ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') (Adams et al., 2013). Sequencing was conducted on the Illumina MiSeq platform, following standard protocols. All samples were pooled in equimolar concentrations and subjected to paired-end sequencing on the Illumina MiSeq platform. The paired-end sequences were merged to generate single sequences of approximately 300 bp. These sequences were then quality-filtered (maximum expected error = 0.2), and singletons were removed using USEARCH v.10. (Edgar, 2010). The raw reads obtained from the sequencing were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP342019).

Data analysis

The raw fastq files were demultiplexed and subjected to quality filtering using QIIME (Caporaso et al., 2010) (version 1.17). Operational Taxonomic Units (OTUs) were clustered at a 97% similarity cutoff using UPARSE version 7.1 (Edgar, 2013). Chimeric sequences were identified and removed using UCHIME. The taxonomy of each representative sequence of the OTUs was determined using the RDP Classifier version 2.2 (Wang et al., 2007) against the ITS database with a confidence threshold of 0.7.

Diversity indices were based on resampled sequences using the MOTHUR program (Schloss et al., 2009). Alpha diversity (observed OTU richness) index was calculated for each sample using the *vegan* package (Oksanen et al., 2022) in the R environment (version 4.3.1). We modeled the alpha diversity as a response variable and site location and seasonal change as fixed effects. The relative importance of the location and season for alpha diversity was evaluated by analysis of variance (ANOVA) (Way and Klasson, 2020), with the p values corrected using the false discovery rate (FDR) method. Principal coordinate analysis (PCoA) was conducted using Bray–Curtis distances for the 216 samples to explore fungal community compositional differences (beta diversity) in different compartments, seasons and locations, and then was visualized by using *ggplot2* (Wickham, 2009). The relative importance of the locations/sites and two seasons for explaining the variation in environmental variables and the alpha diversity of fungi were evaluated by two-way ANOVA. The permutational multivariate analysis of variance (PERMANOVA) in the R *vegan* package was used to test the variations in fungal beta diversity as explained by locations and seasons (Jiao et al., 2022).

Random forest (RF) analysis (*rfPermute* function in *rfPermute* package in R) (Archer, 2013) was used to identify the main environmental drivers for soil fungal alpha diversity (Delgado-Baquerizo et al., 2016; Trivedi et al., 2016). To reveal the relationship between alpha diversity and environmental factors, a linear (linear least-squares regression analysis) or nonlinear regression was used based on RF results. The Mantel test was performed to evaluate the influence of soil properties, climate factors and leaf properties upon fungal

community of different compartments, using the mantel function of the *ecodist* package (Goslee and Urban, 2007) and *vegan* package for R (Oksanen et al., 2022) and visualized by using the *linkET* package (Huang, 2021). We used variation partitioning to quantify the relative importance of seasonal change (dry and rainy season), climatic factors (temperature and precipitation), physicochemical properties (soil including soil pH, WC, SOM, TK, TN, TP, AN, NN, AK, AP; leaf including pH, WC, LOM, K, N, P) and geographic variables (Yeh et al., 2015). Latitudinal and longitudinal data for each site was transferred to rectangular data to represent spatial distance by function *pcnm* of *Vegan* package, and variation partitioning analyses were conducted with function *varpart* in the *vegan* package for R (Oksanen et al., 2022).

Results

Community composition and environmental variables

A total of 9036, 7861, 11001, 10261, 9701, 3846 operational taxonomic units (OTUs) were detected for the six compartments (phyllosphere, leaf endosphere, soil, rhizosphere, rhizoplane, root endosphere, respectively). Among these OTUs, the majority were assigned to the classes *Dothideomycetes* (18.10%) and *Sordariomycetes* (16.51%), *Eurotiomycetes* (10.64%), *Tremellomycetes* (9.46%), and *Agaricomycetes* (8.37%) at the class level (Figure S2).

Among the six measured leaf environmental variables, water content (WC), leaf pH, and organic matter (SOM) showed strong seasonal variations, with higher values during the rainy season compared to the dry season. Leaf phosphorus (P) variables, on the other hand, were more influenced by geographical location or site rather than seasonal changes (Figure 1, S3). In contrast, the ten measured soil physicochemical properties were primarily explained by geographical locations or sites. Variables such as available potassium (AK), soil pH, total nitrogen (TN), total potassium (TK), total phosphorus (TP), water content (WC), organic matter (SOM), nitrate nitrogen (NN), and available phosphorus (AP) were largely influenced by the specific sites where sampling was conducted. In addition, mean seasonal precipitation and mean seasonal temperature showed significant seasonal variations. Approximately 97.6% ($P < 0.001$) of the variation in precipitation and 96.4% ($P < 0.001$) of the variation in temperature were explained by sampling seasons. During the rainy season, leaf water content, precipitation, and temperature were significantly higher compared to the dry season. (Figure 2, S4).

Spatiotemporal pattern of the fungal community

In terms of alpha diversity, the observed OTU richness in the phyllosphere, rhizosphere, rhizoplane, and soil compartments were roughly equal, while the leaf endosphere showed significantly higher alpha diversity in Banna compared to Hainan, while the root endosphere exhibited the opposite trend (Figure S5a, Table S1). When considering the seasonal effect, the observed OTU richness of bacterial communities in the leaf endosphere ($P < 0.01$), root endosphere ($P < 0.05$), and rhizoplane ($P < 0.01$) were significantly higher during the rainy season compared to the dry season (Figure S5b, Table S1). However, when analyzing the seasonal effect separately, distinct patterns were observed. In Banna, the alpha diversity of all compartments was significantly higher during the rainy season compared to the dry season. In Hainan, although the alpha diversity of the root endosphere and rhizoplane were similar between the rainy and dry seasons, and the leaf endosphere showed significantly higher alpha diversity during the rainy season, the phyllosphere, rhizosphere, and soil compartments exhibited significantly higher alpha diversity during the dry season. Overall, the trend in alpha diversity was higher in the dry season compared to the rainy season in Hainan (Figure 3a).

When analyzing the microbial composition based on the Bray-Curtis distance, we observed that geographical location had a significant effect on the beta diversity of fungal communities in all compartments ($P < 0.01$), while seasonal effects were not significant (Figure 3). For instance, geographical location accounted for 6.62%, 7.35%, 9.13%, 8.07%, 17.67%, and 18.78% of the variation in the soil, rhizosphere, rhizoplane, root endosphere, phyllosphere, and leaf endosphere, respectively. Additionally, we observed a much greater effect of site variation compared to seasonal variation in all compartments (Figure S6). In summary, our findings indicate that geographical location/site has a significant impact on fungal composition, while season does not.

Drives of environmental factors in shaping rubber tree fungal community

Among all environmental factors, leaf water content (WC), temperature, and precipitation were identified as the most important predictors of fungal alpha diversity in different compartments (Figure 4, Figure S5b). These findings were further supported by simple linear and nonlinear regression analyses. For instance, significant and positive simple linear regressions were observed between leaf WC, temperature, and fungal alpha diversity in the leaf endosphere and root endosphere compartments. Additionally, a similar mid-peak pattern was observed in four datasets, where richness peaked at mid-temperature in the phyllosphere and rhizosphere compartments, and peaked at mid-precipitation in the soil and rhizoplane compartments. These results indicate that climatic factors play a significant role in driving the alpha diversity of fungi in rubber tree ecosystems.

The major physicochemical and climatic factors to the fungal composition were further identified using the Mantel tests. Of the most important environmental factors contributing to the leaf composition, leaf P had the largest observed effect, followed by **temperature** and precipitation, while soil AK affect the composition most in all root-associated compartments (Figure 5, Table S2). Specifically, temperature and precipitation were significantly correlated with the fungal communities in different compartments to a certain extent (Table S2). The contributions of seasonal, environmental, climatic and geographic variables to the variation in fungal composition were quantified by variation partitioning (VPA). Geographic factors were better predictors of fungal composition than seasonal, environmental and climatic ones (Figure 6), confirming a stronger effect of spatial variation in driving the composition of soil fungi.

Discussion

Revealing the spatiotemporal pattern of microbial communities is a fundamental topic in ecology, which have been explored extensively in microbial ecology over the last two decades (Chu et al., 2020). Furthermore, the significant effect of the seasonal change in regulating the alpha diversity of soil bacteria and fungi was found at the regional scale (Hainan) in our previous study (Wei et al., 2022b). Still very few studies have mapped the temporal and spatial distribution changes of microbial communities of the soil–plant continuum. Here we explored the spatiotemporal of soil–plant continuum fungal community in the rubber tree across different niches and regions, doing so can help to clarify the drivers of space and seasonal change upon microbial community variations. Our findings provide robust empirical evidence that the spatiotemporal variation of fungal diversity in rubber tree was mainly shaped by seasonal change, but only spatial impacts significantly altered microbial beta diversity.

Our results demonstrate that the alpha diversity of soil–plant continuum fungal community is highly dependent on seasonal changes, which is in line with previous studies that have observed significant seasonal variations in microbial diversity (Fournier et al., 2020; Lan et al., 2018; Rasche et al., 2011; Xiang et al., 2021; Zhang et al., 2020). In our study, we provide evidence suggesting that climatic factors play a crucial role in mediating the seasonal variation of fungal alpha diversity. Climatic factors have been identified as the best predictors of soil fungal richness and community composition at a global scale (Bahram et al., 2018; Tedersoo et al., 2014). Importantly, our study further extends this earlier observations at the soil–plant continuum and now provides widespread evidence that climatic factors mediating the alpha diversity of plant microbiome. Among the environmental variables, we found that temperature and precipitation were the dominant predictor for fungal richness, which has also been found in numerous previous studies (Bahram et al., 2018; Delgado-Baquerizo et al., 2017; Fierer, 2017; Hiiesalu et al., 2017; Liu et al., 2020; Tedersoo et al., 2014). Notably, the alpha diversity are sensitive to temperature and precipitation, displaying an unimodal pattern in most compartments, except for the leaf endosphere and root endosphere which showed a linear pattern. Given that temperature and precipitation are generally higher during the rainy season and higher in Hainan compared to Banna, this further supports the observed unimodal pattern in most compartments. Thus, it is not surprising to find that the contrasted seasonal change pattern in Hainan and Banna. Furthermore, we observed a stronger seasonal effect compared to a geographical location effect on some environmental predictors. Specifically, leaf physicochemical factors such as water content (WC) and climatic factors such as temperature and precipitation were primarily influenced by seasonal changes,

contributing to the mechanisms driving microbial seasonal variations.

In contrast, we provide solid evidence that the beta diversity of soil–plant continuum only showed a geographical variation pattern. This aligns with previous studies that have shown spatial factors to be more important in shaping soil microbial communities across large spatial scales (Fierer and Jackson, 2006; Lauber et al., 2013; Wei et al., 2022b; Zhang et al., 2020). The effect of site location was far more influential than the seasonal change in regulating the communities of both soil bacteria and fungi at the regional scale (only in Hainan) in our previous study (Wei et al., 2022b). Given that biogeographic patterns variation of community similarity indicates the influence of historical factors (Ma et al., 2017; Martiny et al., 2006), compare to our previous studies (research in Hainan soil samples) (Wei et al., 2022b), the stronger geographical variation pattern suggests that the pronounced impact of historical event on fungal community structure in soil–plant continuum of rubber tree. For fungal beta diversity, leaf phosphorus (P) and soil available potassium (AK) were the most important factors in leaf and soil samples, respectively. Besides, major taxa belonging to *Dothideomycetes* and *Eurotiomycetes* in leaf samples, according to their life strategies, which have been assigned as copiotrophic fungi, this might explain why leaf samples responded to altered leaf nutrients (e.g., leaf P), which have already been demonstrated (Evans et al., 2014; Ho et al., 2017; Li et al., 2021; Placella et al., 2012). It is worth noting that leaf P and AK which is a highly localized variable, were evidently stronger affected by sampling sites. In all, we found a stronger geographic location effect than seasonal effect upon fungal beta diversity estimates. Furthermore, geographic factors contributed a larger proportion of variation relative to edaphic and climatic factors to the beta diversity of rubber leaf than soils that of soil–plant continuum, indicating a stronger effect of stochastic processes in driving the beta diversity of rubber leaf. These results was also confirmed by the VPA models and Mantel test, and are also consistent with previous observations (Lan et al., 2022).

Conclusion

Our results demonstrate that the alpha diversity is highly dependent on seasonal changes, while beta diversity only showed a geographical variation pattern. Especially, our work showed that spatiotemporal variation in fungal community alpha and beta diversity was mainly driven by climatic factors and soil properties (e.g., AK), leaf properties (e.g., P), respectively. Moreover, the leaf P and AK were mainly explained by the geographic location effect rather than the seasonal effect, and climatic factors showed opposite pattern. Taken together, our study provides empirical evidence for the distinct spatiotemporal patterns and driving factors between alpha and beta diversity in the fungal community of the soil–plant continuum in rubber trees.

Author contributions

Yaqing Wei and Guoyu Lan planned and designed research; Yaqing Wei and Guoyu Lan performed experiments; Yaqing Wei, Guoyu Lan, Zhixiang Wu conducted fieldwork; Yaqing Wei analyzed the data and wrote the manuscript.

Declaration of competing interest

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.

Data Availability

Raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP342019).

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References

- Abdelfattah, A., Wisniewski, M., Schena, L., Tack, A. J. M., 2021. Experimental evidence of microbial inheritance in plants and transmission routes from seed to phyllosphere and root. *Environ Microbiol.* 23, 2199-2214.
- Adams, R. I., Hadly, E. A., 2013. Genetic diversity within vertebrate species is greater at lower latitudes. *Evol Ecol.* 27, 133-143.
- Bahram, M., Hildebrand, F., Forslund, S. K., Anderson, J. L., Soudzilovskaia, N. A., Bodegom, P. M., Bengtsson-Palme, J., et al., 2018. Structure and function of the global topsoil microbiome. *Nature.* 560, 233-237.
- Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., et al., 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods.* 7, 335-6.
- Chen, B., Ma, J., Yang, C., Xiao, X., Kou, W., Wu, Z., Yun, T., et al., 2023. Diversified land conversion deepens understanding of impacts of rapid rubber plantation expansion on plant diversity in the tropics. *Sci Total Environ.* 874, 162505.
- Chu, H., Gao, G. F., Ma, Y., Fan, K., Delgado-Baquerizo, M., 2020. Soil microbial biogeography in a changing world: Recent advances and future perspectives. *mSystems.* 5.
- Cordovez, V., Dini-Andreote, F., Carrion, V. J., Raaijmakers, J. M., 2019. Ecology and evolution of plant microbiomes. *Annu Rev Microbiol.* 73, 69-88.
- Delgado-Baquerizo, M., Maestre, F. T., Reich, P. B., Jeffries, T. C., Gaitan, J. J., Encinar, D., Berdugo, M., et al., 2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nat Commun.* 7, 10541.
- Delgado-Baquerizo, M., Powell, J. R., Hamonts, K., Reith, F., Mele, P., Brown, M. V., Dennis, P. G., et al., 2017. Circular linkages between soil biodiversity, fertility and plant productivity are limited to topsoil at the continental scale. *New Phytol.* 215, 1186-1196.
- Edgar, R. C., 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics.* 26, 2460-1.
- Edgar, R. C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods.* 10, 996-8.
- Evans, S. E., Wallenstein, M. D., Burke, I. C., 2014. Is bacterial moisture niche a good predictor of shifts in community composition under long-term drought? *Ecology.* 95, 110-22.
- Fierer, N., 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. *Nat Rev Microbiol.* 15, 579-590.
- Fierer, N., Jackson, R. B., 2006. The diversity and biogeography of soil bacterial communities. *PNAS.* 103, 626-31.
- Fournier, B., Samaritani, E., Frey, B., Seppey, C. V. W., Lara, E., Heger, T. J., Mitchell, E. A. D., 2020. Higher spatial than seasonal variation in floodplain soil eukaryotic microbial communities. *Soil Bio Biochem.* 147, 107842.
- Goslee, S. C., Urban, D. L., 2007. The ecodist Package for Dissimilarity-based analysis of ecological data. *J Stat Softw.* 22, 1 - 19.
- Grady, K. L., Sorensen, J. W., Stopnisek, N., Guittar, J., Shade, A., 2019. Assembly and seasonality of core phyllosphere microbiota on perennial biofuel crops. *Nat Commun.* 10, 4135.
- Hiiesalu, I., Bahram, M., Tedersoo, L., 2017. Plant species richness and productivity determine the diversity of soil fungal guilds in temperate coniferous forest and bog habitats. *Mol Ecol.* 26, 4846-4858.

- Ho, A., Di Lonardo, D. P., Bodelier, P. L., 2017. Revisiting life strategy concepts in environmental microbial ecology. *FEMS Microbiol Ecol.* 93.
- Jiao, S., Chu, H., Zhang, B., Wei, X., Chen, W., Wei, G., 2022. Linking soil fungi to bacterial community assembly in arid ecosystems. *Imeta.* 1, e2.
- Jumpponen, A., Jones, K. L., 2009. Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate *Quercus macrocarpa* phyllosphere. *New Phytol.* 184, 438-448.
- Koskella, B., 2020. The phyllosphere. *Curr Biol.* 30, R1143-r1146.
- Laforest-Lapointe, I., Messier, C., Kembel, S. W., 2016. Host species identity, site and time drive temperate tree phyllosphere bacterial community structure. *Microbiome.* 4, 27.
- Lan, G., Li, Y., Lesueur, D., Wu, Z., Xie, G., 2018. Seasonal changes impact soil bacterial communities in a rubber plantation on Hainan Island, China. *Sci Total Environ.* 626, 826-834.
- Lan, G., Quan, F., Yang, C., Sun, R., Chen, B., Zhang, X., Wu, Z., 2022. Driving factors for soil fungal and bacterial community assembly in tropical forest of China. *Appl Soil Ecol.* 177, 104520.
- Lan, G., Wei, Y., Li, Y., Wu, Z., 2023. Diversity and assembly of root-associated microbiomes of rubber trees. *Front Plant Sci.* 14, 1136418.
- Lan, G., Wu, Z., Chen, B., Xie, G., 2017. Species diversity in a naturally managed rubber plantation in Hainan Island, South China. *Trop Conserv Sci.* 10.
- Lan, G., Wu, Z., Yang, C., Sun, R., Chen, B., Zhang, X., 2020. Tropical rainforest conversion into rubber plantations results in changes in soil fungal composition, but underlying mechanisms of community assembly remain unchanged. *Geoderma.* 375, 114505.
- Lan, G., Zhixiang, W., Li, Y., Chen, B., 2019. The drivers of soil bacterial communities in rubber plantation at local and geographic scales. *Arch Agron and Soil Sci.* 66.
- Lauber, C. L., Ramirez, K. S., Aanderud, Z., Lennon, J., Fierer, N., 2013. Temporal variability in soil microbial communities across land-use types. *ISME J.* 7, 1641-50.
- Li, H., Yang, S., Semenov, M. V., Yao, F., Ye, J., Bu, R., Ma, R., et al., 2021. Temperature sensitivity of SOM decomposition is linked with a K-selected microbial community. *Glob Chang Biol.* 27, 2763-2779.
- Li, Y., Xia, Y., Lei, Y.-b., Deng, Y., Chen, H., Sha, L., Cao, M., et al., 2015. Estimating changes in soil organic carbon storage due to land use changes using a modified calculation method. *Iforest.* 8, 45-52.
- Liu, S., Wang, H., Tian, P., Yao, X., Sun, H., Wang, Q., Delgado-Baquerizo, M., 2020. Decoupled diversity patterns in bacteria and fungi across continental forest ecosystems. *Soil Biol Biochem.* 144, 107763.
- Lundberg, D. S., Lebeis, S. L., Paredes, S. H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., et al., 2012. Defining the core *Arabidopsis thaliana* root microbiome. *Nature.* 488, 86-90.
- Ma, B., Dai, Z., Wang, H., Dsouza, M., Liu, X., He, Y., Wu, J., et al., 2017. Distinct biogeographic patterns for Archaea, Bacteria, and Fungi along the vegetation gradient at the continental scale in Eastern China. *mSystems.* 2.
- Martiny, J. B. H., Bohannan, B. J. M., Brown, J. H., Colwell, R. K., Fuhrman, J. A., Green, J. L., Horner-Devine, M. C., et al., 2006. Microbial biogeography: putting microorganisms on the map. *Nat Rev Microbiol.* 4, 102-112.
- Oksanen, J., Simpson, G., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P., Hara, R., et al., 2022. vegan community ecology package version 2.6-2 April 2022.
- Placella, S. A., Brodie, E. L., Firestone, M. K., 2012. Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. *PNAS.* 109, 10931-6.

- Qian, X., Duan, T., Sun, X., Zheng, Y., Wang, Y., Hu, M., Yao, H., et al., 2018. Host genotype strongly influences phyllosphere fungal communities associated with *Mussaenda pubescens* var. *alba* (Rubiaceae). *Fungal Ecol.* 36, 141-151.
- Rasche, F., Knapp, D., Kaiser, C., Koranda, M., Kitzler, B., Zechmeister-Boltenstern, S., Richter, A., et al., 2011. Seasonality and resource availability control bacterial and archaeal communities in soils of a temperate beech forest. *ISME J.* 5, 389-402.
- Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., et al., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol.* 75, 7537-41.
- Tedersoo, L., Bahram, M., Polme, S., Koljalg, U., Yorou, N. S., Wijesundera, R., Villarreal Ruiz, L., et al., 2014. Fungal biogeography. Global diversity and geography of soil fungi. *Science.* 346, 1256688.
- Thiergart, T., Duran, P., Ellis, T., Vannier, N., Garrido-Oter, R., Kemen, E., Roux, F., et al., 2020. Root microbiota assembly and adaptive differentiation among European *Arabidopsis* populations. *Nat Ecol Evol.* 4, 122-131.
- Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hu, H., Anderson, I. C., Jeffries, T. C., Zhou, J., et al., 2016. Microbial regulation of the soil carbon cycle: evidence from gene-enzyme relationships. *ISME J.* 10, 2593-2604.
- Trivedi, P., Leach, J. E., Tringe, S. G., Sa, T., Singh, B. K., 2020. Plant-microbiome interactions: from community assembly to plant health. *Nat Rev Microbiol.* 18, 607-621.
- Wang, Q., Garrity, G. M., Tiedje, J. M., Cole, J. R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microb.* 73, 5261-7.
- Wang, Z., Jiang, Y., Zhang, M., Chu, C., Chen, Y., Fang, S., Jin, G., et al., 2023. Diversity and biogeography of plant phyllosphere bacteria are governed by latitude-dependent mechanisms. *New Phytol.* 240, 1534-1547.
- Way, Klasson, K. T., Two-way ANOVA for Unbalanced Data: The Spreadsheet Way. 2020.
- Wei, Y., Lan, G., Wu, Z., Chen, B., Quan, F., Li, M., Sun, S., et al., 2022a. Phyllosphere fungal communities of rubber trees exhibited biogeographical patterns, but not bacteria. *Environ Microbiol.* 24, 3777-3790.
- Wei, Y., Quan, F., Lan, G., Wu, Z., Yang, C., 2022b. Space rather than seasonal changes explained more of the spatiotemporal variation of tropical soil microbial communities. *Microbiol Spectr.* 10, e0184622.
- Wickham, H., ggplot2 - Elegant Graphics for Data Analysis. Use R!, 2009.
- Xiang, Q., Qiao, M., Zhu, D., Giles, M., Neilson, R., Yang, X. R., Zhu, Y. G., et al., 2021. Seasonal change is a major driver of soil resistomes at a watershed scale. *ISME Communications.* 1, 17.
- Xiong, C., Singh, B. K., He, J. Z., Han, Y. L., Li, P. P., Wan, L. H., Meng, G. Z., et al., 2021. Plant developmental stage drives the differentiation in ecological role of the maize microbiome. *Microbiome.* 9, 171.
- Xu, J., Grumbine, R. E., Beckschafer, P., 2014. Landscape transformation through the use of ecological and socioeconomic indicators in Xishuangbanna, Southwest China, Mekong Region. *Ecol Indic.* 36, 749-756.
- Xu, X., Wei, Y., Lan, G., 2024. Geographical differences weaken the convergence effect of the rhizosphere bacteria of rubber trees. *Forests.* 15, 415.
- Yeh, Y., Peres, P. R., Huang, S., Lai, Y., Tu, C., Shiah, F., Gong, G., et al., 2015. Determinism of bacterial metacommunity dynamics in the southern East China Sea varies depending on hydrography. *Ecography.* 38, 198-212.

Zhang, K., Delgado-Baquerizo, M., Zhu, Y., Chu, H., 2020. Space is More important than season when shaping soil microbial communities at a large spatial scale. *mSystems*. 5, 10.1128/msystems.00783-19.

Supporting information

Additional supporting information is available in the supplement materials.

Supplementary figures

Figure S1 Study sites on Hainan Island and Xishuangbanna. Red solid circles indicate study sites on the maps. Each site was further divided into 3 plots, totaling 18 sampling plots.

Figure S2 Spatiotemporal changes in fungal community composition (class level) in different compartments of rubber trees.

Figure S3 Environmental variables in dry and rainy seasons among the six sampling sites of rubber tree leaves. The significant differences between seasons and locations were detected by two-way ANOVA. N: Total nitrogen, P: Total phosphorus, K: Total potassium, WC: Water content, LOM: Leaf organic matter.

Figure S4 Environmental variables in dry and rainy seasons among the six sampling sites of rubber tree soils. The significant differences between seasons and locations were detected by two-way ANOVA. WC: Water content, SOM: Soil organic matter, pH: Soil pH, AN: Ammonium nitrogen, NN: Nitrate nitrogen AP: Available phosphorus, AK: Available potassium, TN: Total nitrogen, TP: Total phosphorus, TK: Total potassium.

Figure S5 Boxplots showing the number of OTUs between the two locations (a) and seasons (b). Leaf.WC: Leaf water content.

Figure S6 Principal coordinates analysis (PCoA) of taxonomic similarity based on Bray-Curtis distances (OTU level). Six compartments in Hainan (a) and Banna (b). LD: Ledong, DZ: Danzhou, WN: Wanning; MP: Mengpeng, JH: Jinghong, ML: Menglun.

Supplementary tables

Table S1 The significant effect of location and season on fungal alpha diversity was detected by two-way ANOVA.

Table S2 Mantel test results for the correlation between community similarity and environmental.

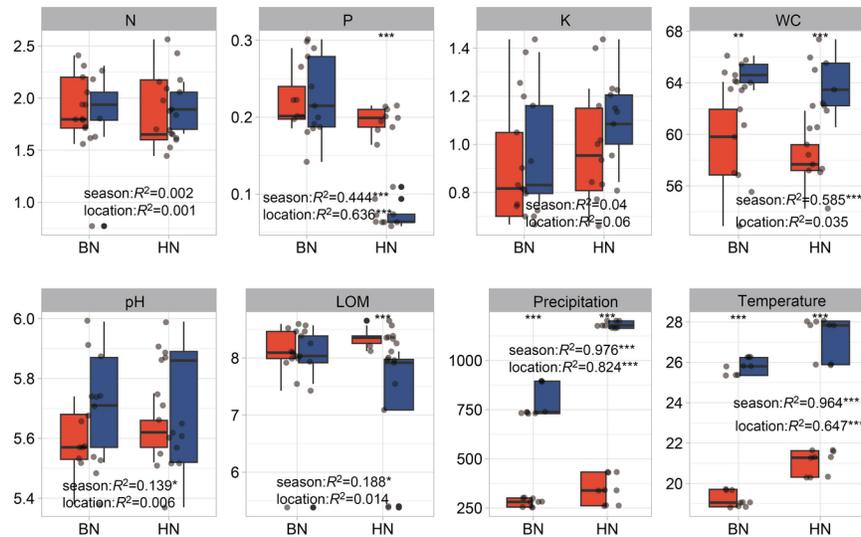


Figure 1 Environmental variables in dry and rainy seasons between two locations of rubber tree leaves. The significant differences between seasons and locations were detected by two-way ANOVA. N: Total nitrogen, P: Total phosphorus, K: Total potassium, WC: Water content, LOM: Leaf organic matter. *, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$

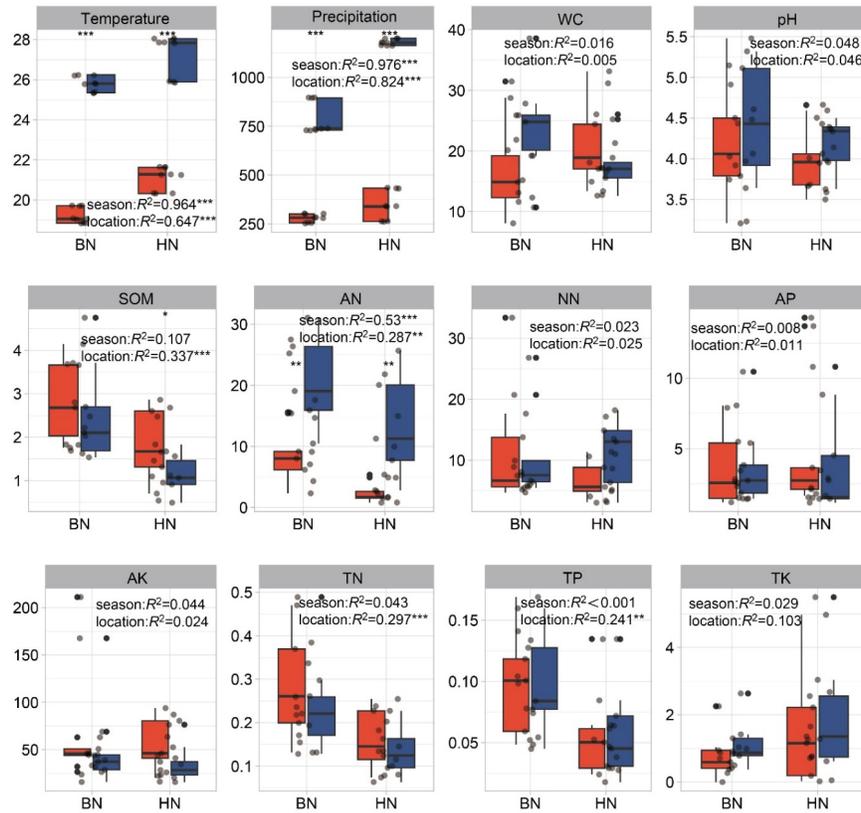


Figure 2 Environmental variables in dry and rainy seasons between two locations of rubber tree soils. The significant differences between seasons and locations were detected by two-way ANOVA. WC: Water content, SOM: Soil organic matter, pH: Soil pH, AN: Ammonium nitrogen, NN: Nitrate nitrogen AP: Available phosphorus, AK: Available potassium, TN: Total nitrogen, TP: Total phosphorus, TK: Total potassium. *, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$

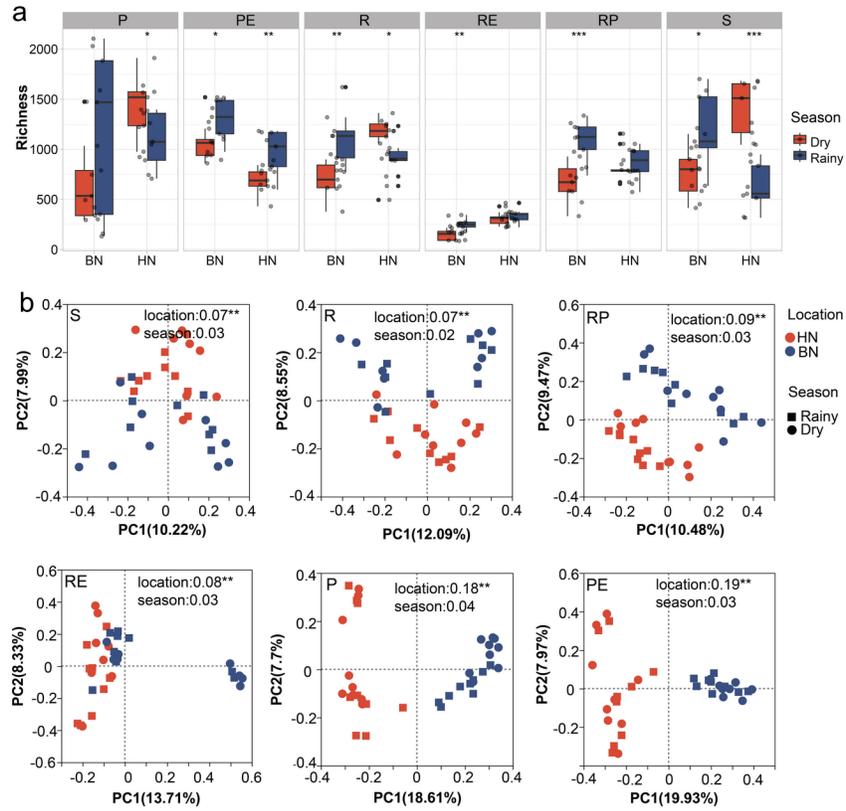


Figure 3 Alpha diversity of rubber tree fungal community (a). Significant differences between seasons in each sampling area are marked by stars. (b) Principal coordinates analysis (PCoA) of taxonomic similarity based on Bray-Curtis distances (OTU level). P: phyllosphere, PE: leaf endosphere, S: soil, R: rhizosphere, RP: rhizoplane, RE: root endosphere, *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

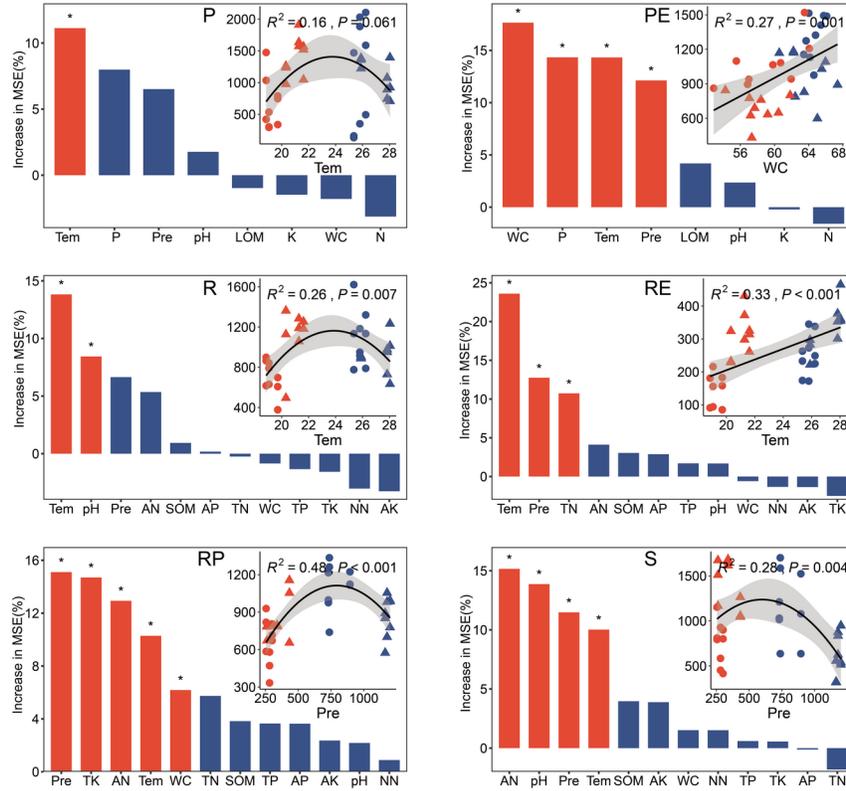


Figure 4 Drivers of soil bacterial α -diversity across different compartments. Random forest (RF) analyses identifying the importance of potential predictors of fungal richness. RF Importance = Increase in % mean square error. Red and blue columns represent $P < 0.05$ and $P > 0.05$, respectively. Relationships between environmental factors and the bacterial α -diversity was estimated via linear least-squares regression analysis. Solid circle (red: dry season; blue: rainy season) indicate samples from Banna; Solid triangle (red: dry season; blue: rainy season) indicate samples from Hainan. P: phyllosphere, PE: leaf endosphere, S: soil, R: rhizosphere, RP: rhizoplane, RE: root endosphere, WC: Water content, N: Leaf nitrogen, P: Leaf phosphorus, K: Leaf potassium, LOM: Leaf organic matter, SOM: Soil organic matter, pH: Soil pH, AN: Ammonium nitrogen, NN: Nitrate nitrogen AP: Available phosphorus, AK: Available potassium, TN: Total nitrogen, TP: Total phosphorus, TK: Total potassium, Pre: Precipitation, Tem: Temperature.

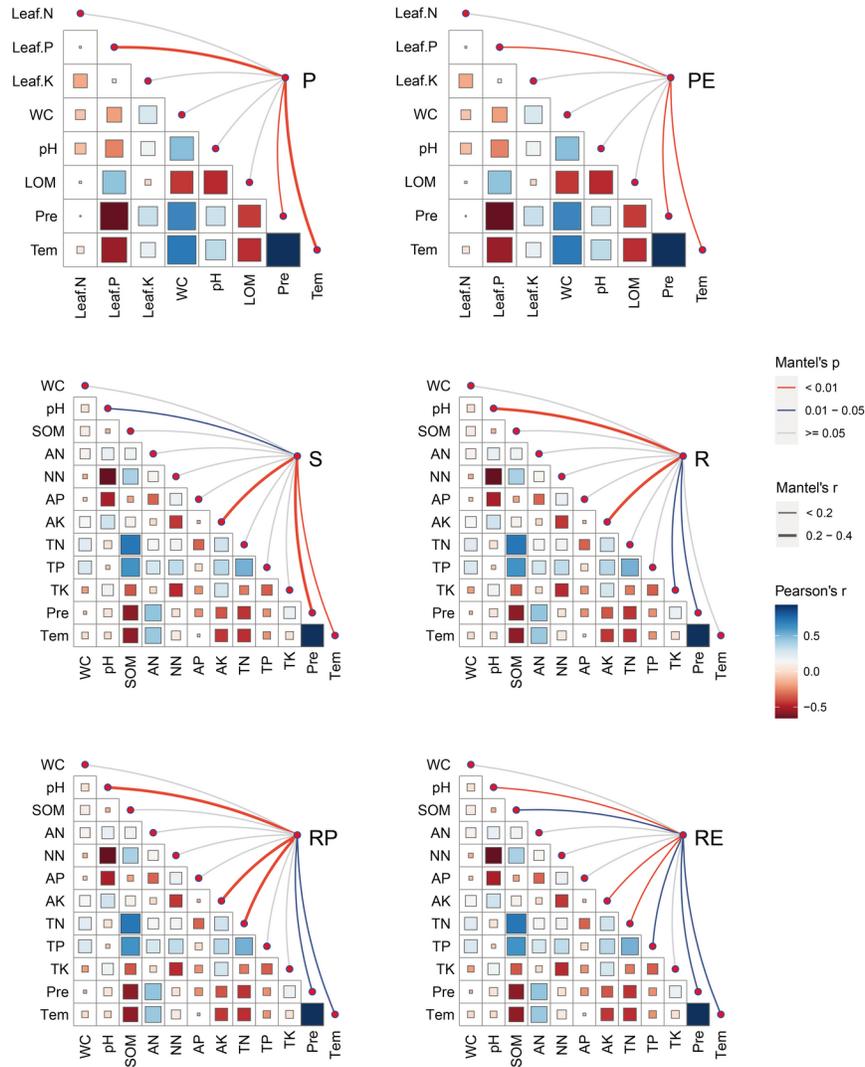


Figure 5 Correlation analysis among edaphic properties, climatic characteristics and microbial communities in the rubber tree microbial community determined using the Mantel test. The color of the line represents the significance of the correlations (P-values). The width of the line represents the size of the correlation coefficients (Mantel's r). The size and color of the squares represents the values of the correlation coefficients (Pearson's r). WC: Water content, Leaf.N: Leaf nitrogen, Leaf.P: Leaf phosphorus, Leaf.K: Leaf potassium LOM: Leaf organic matter; SOM: Soil organic matter, pH: Soil pH, AN: Ammonium nitrogen, NN: Nitrate nitrogen AP: Available phosphorus, AK: Available potassium, TN: Total nitrogen, TP: Total phosphorus, TK: Total potassium, Pre: Precipitation, Tem: Temperature.

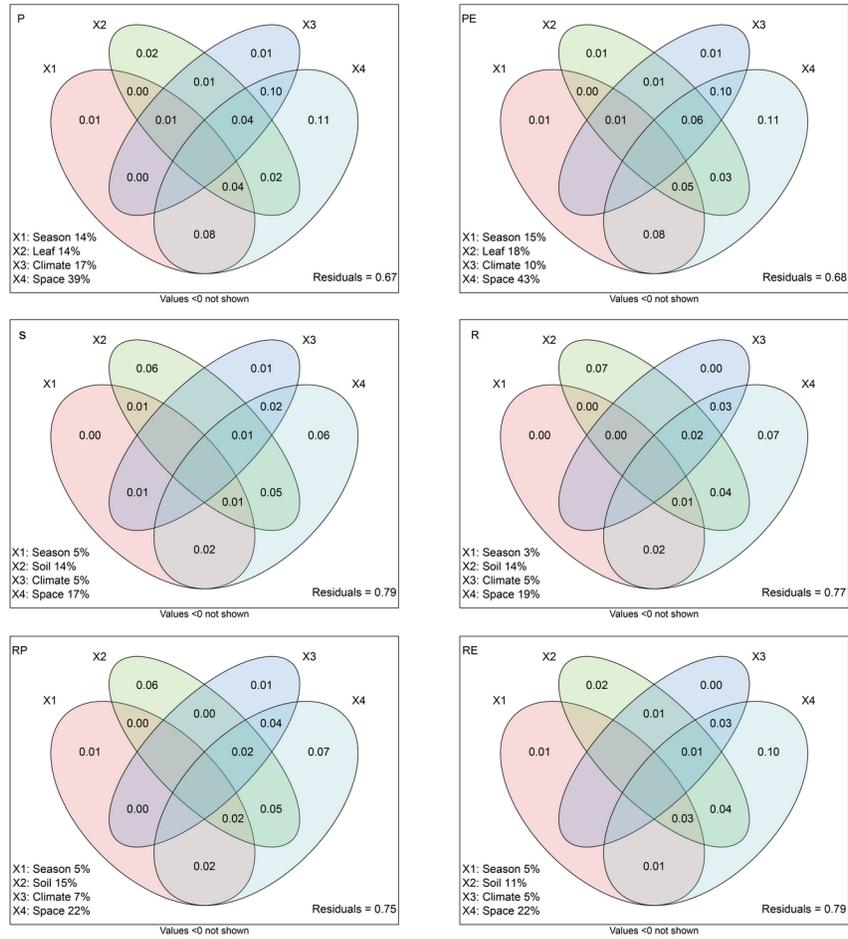


Figure 6 Variation partitioning analysis (VPA) showing the effects of leaf, soil properties, climatic factors and geographic factors, and season, on the microbial community composition in the microbial beta diversity. The values indicate the percentage of variation significantly explained by each section.