

Differential Responses of Microbial Network Stability to Post-Tobacco Crop Rotation Driven by Contrasting Soil Nutrient Conditions

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Abstract

Background

Continuous monocropping of flue-cured tobacco disrupts soil nutrient balance and degrades microbial communities, whereas post-tobacco minor crop rotations alleviate these constraints.

Methods

Tobacco fields in Geyi and Xize, Xuanwei City, Yunnan Province, were used to compare six rotation treatments with leguminous and non-leguminous crops to clarify the effects of post-tobacco rotation on soil nutrients and microbial communities in the nutrient-rich Geyi and nutrient-poor Xize regions.

Results

Intercropping with legumes better retained available nutrients in both regions. Bacterial communities showed greater sensitivity to post-tobacco rotation management than fungal communities, as evidenced by greater variation in bacterial α -diversity, higher explanatory rates of bacterial PCoA ordination axes (56.6% and 50.4% in Geyi and Xize, respectively; 31.6% and 36.8% for fungi), and bacterial community assembly being mainly governed by deterministic processes (88.9% and 94.4% in Geyi and Xize, respectively). In contrast, fungal assembly was dominated by stochastic processes (55.6% and 83.3%). Co-occurrence network analysis indicated that network complexity showed no significant differences among treatments at both sites, with positive correlations dominating the networks (> 82%). Two and nine key taxa were identified in Geyi and Xize, respectively, with Chloroflexi identified as a key taxon at both sites. Structural equation modelling indicated that changes in soil nutrients improved network stability directly or indirectly through increased β -diversity, increased contribution of deterministic assembly, and greater network complexity.

Conclusions

Intercropping with legumes better retains available nutrients. Bacterial communities showed greater sensitivity to post-tobacco rotation management. Both increases in β -diversity and greater contributions of deterministic assembly enhanced network stability. This study suggests that broadcasting green manure in Geyi and intercropping pea in Xize represent reasonable locally adapted management strategies for short-term post-tobacco rotation optimization.

1. Introduction

Tobacco (*Nicotiana tabacum* L.) is sensitive to continuous cropping. However, constraints in arable land availability have resulted in persistent monoculture in some tobacco-growing areas [1]. Prolonged

continuous cultivation can induce excessive depletion of soil nutrients, accelerate the proliferation of pathogenic microorganisms, and trigger other soil-related problems [2], thereby constraining flue-cured tobacco growth and reducing yield [1]. This situation poses a serious challenge to both the sustainable utilisation of arable land in tobacco-producing regions and the long-term development of the tobacco industry, highlighting the need to develop improved planting systems capable of sustaining beneficial production cycles.

Conservation tillage includes a range of practices, such as no-tillage, minimum tillage, mulching, or crop rotation [3, 4]. These practices constitute integrated farmland management strategies for improving soil health, maintaining soil productivity, and supporting long-term agricultural sustainability [3, 5]. Among these practices, crop rotation reduces soil disturbance, preserves soil structure, mitigates erosion, and increases soil organic matter, thereby enhancing soil nutrient availability and crop yield [1, 5]. In recent years, post-tobacco intercropping systems, including combinations of tobacco with food or economic crops, have been increasingly promoted in numerous tobacco-growing regions in China. Representative examples include composite planting systems of tobacco-soybean, tobacco-vegetable, tobacco-sweet potato, and tobacco-medicinal herbs, which have been shown to improve land use efficiency and multiple-cropping intensity while also enhancing soil quality and economic returns [6–8]. Conventional crop rotation can also improve tobacco-planting soils by increasing soil organic matter, improving aggregate structure, enhancing microbial diversity and enzyme activity, promoting nutrient cycling and phosphorus activation, and creating a more favourable soil ecological environment [1, 9]. Among different cropping systems, legume-based rotations may provide additional advantages because biological nitrogen fixation and enhanced nutrient activation can further promote soil biodiversity and improve nutrient balance [10, 11]. Leguminous rotations have also been reported to increase soil available phosphorus and significantly improve crop yield by stimulating phosphorus activation and nutrient use efficiency [12]. However, the benefits of conservation tillage are often region-specific or dependent on management context [3]. Consequently, the effects of post-tobacco rotation on tobacco-planting soil remain insufficiently understood.

Moreover, soil microorganisms are fundamental to agricultural ecosystems because they participate in nutrient cycling, improve soil structure, and directly affect crop health and productivity [9]. Microbial diversity is widely regarded as a key indicator of soil quality and fertility [13], and tillage management can regulate both the biodiversity and stability of microbial communities by altering soil nutrient status and physicochemical properties [3, 5]. In parallel, co-occurrence network analysis has been widely used to characterise microbial coexistence and exclusion patterns and identify key taxa closely related to soil functions and crop production [14]. Previous studies have shown that intercropping can increase the complexity and stability of bacterial co-occurrence networks by improving soil nutrient supply and enriching beneficial bacterial groups [15, 16]. In flue-cured tobacco cultivation, rotational cropping is associated with higher complexity and stability of molecular ecological networks, stronger cooperative interactions among microbial taxa, and a more stable community structure [9]. As agricultural intensification continues globally, the stability of soil biological community networks is increasingly viewed as a critical determinant of agricultural sustainability and crop productivity [17]. In addition to

network stability, microbial community stability can also be assessed using indices such as average variation degree (AVD), where lower values indicate smaller shifts in community composition and thus greater stability [18]. Together with network stability, this metric reflects the compositional and functional stability of soil ecosystems. Meanwhile, soil microbial community assembly is jointly governed by deterministic and stochastic processes, and the relative contribution of each is shaped by both biological and abiotic factors [19]. Deterministic processes generally shape community structure through environmental filtering and resource competition. For example, variations in soil pH, nutrient availability, and salinity can intensify environmental filtering and thereby increase the convergence of community assembly [20]. In contrast, in frequently disturbed agricultural ecosystems, stochastic processes, including dispersal limitation and ecological drift, may assume greater importance [21]. Differences in crop root exudates and residue inputs can also alter soil carbon-to-nitrogen ratios and microhabitat conditions, thereby shifting the balance between deterministic and stochastic assembly processes [22]. Evidence suggests that under nutrient regulation or rotational intervention, bacterial communities exhibit stronger environmental filtering, whereas fungal communities are more strongly influenced by stochastic dispersal and drift [19]. Accordingly, clarifying how different post-tobacco crop management strategies affect soil microorganisms is of great significance for optimising agricultural production.

However, the effects of post-tobacco crop rotation and crop type on soil microbial diversity, network structure, and community assembly in tobacco-growing soils remain unclear. Their implications for ecosystem stability, particularly through changes in soil physicochemical properties, also remain poorly understood. This study was conducted within a conservation tillage framework for flue-cured tobacco to examine the effects of post-tobacco crop management on tobacco planting soils. Two crop types were intercropped: leguminous crops (pea and faba bean) and non-leguminous crops (radish and carrot). Additionally, a leguminous crop (smooth vetch) and a non-leguminous crop (ryegrass) were broadcast sown. We hypothesized that post-tobacco crop rotation influences soil microbial community diversity and co-occurrence networks by altering soil available nutrients, and that this effect may vary depending on the specific crop type or species. To test this hypothesis, we: (1) investigated the effects of different post-tobacco crop rotations on soil pH and available nutrients; (2) evaluated the responses of microbial community diversity (α and β diversity), composition, and co-occurrence networks to post-tobacco crop rotation, explored the assembly processes of bacterial and fungal communities under this rotation, and identified keystone taxa alongside their contributions to microbial community stability; and (3) determined the primary factors affecting the stability of the soil microbial community. This study aims to provide a scientific basis for optimizing the cultivation and management of crops in post-flue-cured tobacco rotations, ultimately promoting the sustainable development of agricultural production.

2. Methods

2.1. Field experiment site and soil sampling

The field experiment was conducted at two sites in Xuanwei City, Yunnan Province, China: Lu Village, Geyi Town (26°20' N, 104°25' E; approximately 2060 m above sea level) and Xize Village, Xize Town (26°14' N, 103°51' E; approximately 1800 m above sea level) (Fig. 1-A). Both sites are located within a subtropical monsoon climatic zone, with a mean annual temperature of 13.4°C, annual precipitation of 1098.0 mm, and annual sunshine duration of 2018.5 h. The soils in the study area are predominantly red soils, classified as Ultisols according to the USDA Soil Taxonomy system and as Acrisols in the WRB system. At the Geyi site, the soil pH was 6.83, the soil organic matter content was 49.57 g/kg, and the available nitrogen, phosphorus, and potassium contents were 130.96, 91.68, and 432.73 mg/kg, respectively. At the Xize site, the soil pH was 7.29, soil organic matter content was 27.51 g/kg, and available nitrogen, phosphorus, and potassium contents were 102.23, 31.87, and 299.90 mg/kg, respectively.

The experiment was conducted in 2023. Each treatment was replicated three times within the same contiguous field managed by a single farmer. Each replicate plot covered 200 m², resulting in a total treatment area of 600 m² (Fig. 1-B). Tobacco seedlings were transplanted on 8 April at Xize and 12 April at Geyi. The flue-cured tobacco cultivar Yunyan 100 was planted uniformly at a density of 15,000 plants/hm². A basal application of pure nitrogen was supplied at 105 kg/hm², and compound fertilizer (N: P₂O₅: K₂O = 14:10:24) was ring-applied at 70 kg/hm² during transplantation. The first topdressing was applied 20 days after transplanting using a compound fertilizer (N: P₂O₅: K₂O = 14:10:24) at 35 kg/hm². The second topdressing was applied 30 d after transplantation using potassium sulfate (K₂O ≥ 52%) at 225 kg/hm². All other field management practices were kept consistent, and the growth period of flue-cured tobacco was 140 days.

At 108 days after tobacco transplanting, the lower leaves were harvested, and faba bean, pea, carrot, and radish were intercropped separately along the tobacco ridges, with two rows per ridge at a row spacing of 30–35 cm. Plant spacing was 30–35 cm for faba bean, carrot, and radish, and 15–20 cm for pea, with a planting density of 75,000–97,500 plants/hm², 2–4 seeds per hole, and a soil coverage of 1–2 cm. Pea vines were trained onto residual tobacco stalks. All intercropped plots were watered thoroughly after sowing. On the 30th day after tobacco harvest and land preparation, ryegrass and smooth vetch were sown at 3–4 kg/hm² under conventional rotation treatments. No fertilizers or pesticides were applied to the post-tobacco crops. The treatments were designated as tobacco–radish (LB), tobacco–carrot (HLB), tobacco–pea (WD), tobacco–faba bean (CD), ryegrass (MC), and smooth vetch (LF) (Fig. 1-C). For treatments located in the Geyi area, a "G" prefix is used (e.g., GWD, GCD, GLB), whereas an "X" prefix is used for those in the Xize area (e.g., XWD, XCD, XLB). On the 80th day after planting the post-tobacco crops, rhizosphere soil was collected from five points around the crop roots using an S-shaped sampling pattern, and the samples were mixed thoroughly after removing visible impurities. After passing through a 2 mm sieve, each composite sample was divided into two subsamples: one was air-dried for soil nutrient analysis, and the other was stored at – 80 °C for DNA extraction.

2.2. Soil physicochemical property analysis

Soil physicochemical properties were determined according to the methods described by Bao [23]. The soil pH was measured with an Orion Star A211 pH meter at a soil-to-water ratio of 2.5:1. Soil organic matter (SOM) was determined using the external-heating potassium dichromate titration method. Available nitrogen (AN) was measured by converting hydrolyzable nitrogen to ammonium nitrogen using sodium hydroxide. Available phosphorus (AP) was determined using the 0.5 mol/L NaHCO₃ extraction-molybdenum antimony colorimetric method, and available potassium (AK) was measured using the 1 mol/L NH₄OAc extraction–flame photometry method.

2.3. Bioinformatics processing

Details of quality control, clustering, taxonomic annotation, and standardization of amplicon sequencing data are provided in the Supplementary Materials. Raw sequencing data were processed using QIIME 2 (version 2023.5). Briefly, the raw reads were quality-filtered, paired-end assembled, and screened for chimeras, after which operational taxonomic units (OTUs) were generated at 97% sequence similarity. Bacterial OTUs were taxonomically assigned using the SILVA database (release 138), whereas fungal OTUs were annotated using the UNITE database (version 9.0). To minimize the influence of sequencing depth variation on downstream analyses, all samples were rarefied to the same sequencing depth before further analysis.

2.4. Analysis of co-occurrence network construction, community stability, and assembly process

Microbial co-occurrence networks were constructed using bacterial OTUs detected in at least 50% of the samples and with relative abundances > 0.01%, along with all fungal OTUs. After logarithmic transformation of the OTU abundance matrix, pairwise correlations among OTUs were calculated using Spearman's rank correlation. The correlation threshold was determined using Random Matrix Theory (RMT) to distinguish biologically meaningful correlations from random noise. This procedure was implemented in R using the 'stats' and 'RMThreshold' packages. OTU pairs with absolute correlation coefficients exceeding the RMT threshold and with $P < 0.01$ after Benjamini-Hochberg correction were considered significantly correlated [24]. Based on the global network and OTU composition of each sample, OTUs assigned a value of 1 in the binary matrix were selected as nodes, and these nodes, together with their links in the global network, were extracted to generate subnetworks for evaluating treatment-specific changes in network characteristics. For each sample, the OTU abundance table was first converted into a binary matrix, in which OTUs with abundances above the sample mean were assigned a value of 1, and all others were assigned a value of 0. Subsequent analyses were conducted only when the corresponding subnetwork contained more than five nodes [25]. Network topological properties were calculated using the 'igraph' package in R, and network visualization was performed in Gephi v0.9.2 (<https://gephi.org/>) using the Fruchterman-Reingold layout algorithm. The topological features of the networks were calculated to estimate the complexity of the soil network [24], including the total number of nodes, the number of links, network diameter, relative modularity (RM), average clustering coefficient (average CC), and average degree. In the resulting networks, each node represented an OTU, and each edge represented a significant correlation between two OTUs. In this

study, key taxa were defined as follows: (a) nodes identified based on within-module connectivity (Z_i) and among-module connectivity (P_i), with nodes meeting $Z_i > 2.5$ or $P_i > 0.62$ classified as key taxa; (b) OTUs with both specificity and occupancy ≥ 0.7 ; and (c) OTUs shared across all treatments [26].

Microbial community stability can be described in terms of resilience, that is, the recovery of a community towards an alternative stable state, or resistance, that is, the capacity of a community to remain unchanged after disturbance. It can also be evaluated by comparing shifts in the microbiome composition across different environmental conditions [18]. Because no single metric can fully capture microbial stability, two complementary indices, network stability (random node removal) and AVD, were used to provide cross-validation of microbial community stability [18].

The variation degree (a_i) of each OTU was calculated using Eq. (1):

$$|a_i| = |x_i - \bar{x}_i| / \delta_i$$

1

where x_i represents the rarefied abundance of the OTU in an individual sample, and $\bar{x}_i$ and δ_i denote the mean and standard deviation of the rarefied abundances within a specific sample group, respectively.

AVD was calculated using Eq. (2):

$$AVD = \sum_{i=1}^n |a_i| / (k \times n)$$

2

where k is the number of samples (when there is a single sample, $k = 1$, and when there are sample groups, k is the total number of samples in the group), and n is the number of OTUs.

To infer community assembly processes, the 'picante' package in R was first used to calculate the mean nearest taxon distance, after which a previously developed null model framework was applied to estimate βNTI . A value of $\beta NTI > 2$ is interpreted as a phylogenetic turnover significantly greater than expected and thus indicative of variable selection as a deterministic process. A value of $\beta NTI < -2$ indicates a phylogenetic turnover significantly lower than expected and is interpreted as a homogeneous selection as a deterministic process. When $|\beta NTI| < 2$, the observed phylogenetic differences are considered to result from stochastic processes [27].

2.5. Data analysis

All statistical analyses were performed in R (version 4.3.0). Data were first tested for normality and homogeneity of variance using the Shapiro-Wilk test and Levene's test, respectively. When the assumptions for parametric analysis were satisfied, independent-samples t-tests or ANOVA were applied to evaluate the effects of individual factors and their interactions on the measured variables, followed by Tukey's HSD test for post hoc multiple comparisons. When these assumptions were not met, a

logarithmic transformation was performed. The relationships between soil physicochemical factors and microbial community characteristics were examined using Spearman's rank correlation analysis. Structural equation modelling (SEM) was applied to assess the direct and indirect relationships among soil factors, microbial diversity, network properties, and community assembly processes, and all variables were standardized before model fitting. The model fit was evaluated using Fisher's C statistic, with $P > 0.05$ indicating a satisfactory fit. AIC was used to identify the optimal piecewise SEM among comparable models [28].

3. Results

3.1. Effects of post-tobacco rotation on soil properties

Region had a significant effect on all measured soil properties (pH, SOM, AK, AN, and AP). Specifically, AK, AN, AP, and SOM contents were significantly higher in Geyi than in Xize, whereas soil pH was higher in Xize than in Geyi (Fig. 2). Crop type had no significant effect on any soil property, nor was a significant interaction detected between region and crop type. At the regional level, soil pH was significantly higher in LB plots in Geyi, while broadcast-sown smooth vetch plots showed significantly lower soil pH in Xize. In Geyi, AN, AK, and AP were significantly lower under broadcast-sown legumes than under intercropped legumes; the same pattern was observed for AK and AP in Xize.

3.2. Effects of post-tobacco rotation on soil microbial composition and diversity

A systematic analysis of rhizosphere soil microbial communities was conducted in the two tobacco-growing regions. In total, 12,682 bacterial OTUs and 4,134 fungal OTUs were identified in the 36 samples. The bacterial OTUs were assigned to 39 phyla, 142 classes, 351 orders, 562 families, 1,138 genera, and 2,711 species, whereas the fungal OTUs were assigned to 17 phyla, 62 classes, 138 orders, 322 families, 743 genera, and 1,346 species. Different bacterial and fungal phyla varied in their relative proportions across the two study sites (Fig. S1). Proteobacteria, Actinobacteriota, Acidobacteriota, and Chloroflexi were the dominant bacterial phyla in both Geyi and Xize, collectively accounting for more than 76% of the total relative abundance. Notably, the relative abundances of Actinobacteriota and Acidobacteriota differed significantly between the two sites, and Proteobacteria also showed significant differences, indicating substantial divergence in the proportional composition of dominant bacterial phyla between Geyi and Xize. Among treatments, the relative abundances of Acidobacteriota, Chloroflexi, and Myxococcota exhibited significant variation within the Geyi site. With respect to the fungal community, Ascomycota and Basidiomycota were the overwhelmingly dominant phyla shared across both sites, with their combined relative abundance exceeding 81% across all treatments, followed by Mortierellomycota as a secondary contributor.

Region significantly influenced both bacterial and fungal alpha diversity, with the bacterial Shannon index, bacterial Chao1 index, and fungal Shannon index all being higher in Xize than in Geyi ($P < 0.05$;

Fig. 3). Neither crop type alone nor its interaction with region had a significant effect on microbial diversity. Within Geyi, LB and LF plots exhibited significantly higher bacterial Shannon and Chao1 indices ($P < 0.05$; Fig. 3).

3.3. Differential analysis of soil microbial community composition under post-tobacco rotation

PCoA based on Bray-Curtis distances explained 56.6% and 50.4% of the variation in bacterial community composition in Geyi and Xize, respectively (Fig. 4-A and B), and 31.6% and 36.8% of the variation in fungal community composition (Fig. 4-C and D). Soil bacterial and fungal community compositions differed significantly among the six crop treatments in both regions. In Geyi, LB and LF plots exhibited similar bacterial and fungal community compositions, both of which were clearly distinct from those of the remaining treatments. In Xize, CD and LF plots showed similar bacterial community compositions, while the fungal community under CD was distinguishable from those of the other five treatments.

3.4. Analysis of co-occurrence networks and community assembly of rhizosphere soil microbial communities

The bacterial–fungal co-occurrence network in Geyi comprised 3,596 nodes and 71,818 edges, whereas that in Xize comprised 2,720 nodes and 36,841 edges (Fig. 5A and D; Table S1). The Geyi network exhibited a high clustering coefficient (0.833), high modularity (0.786), and short average path length (5.24), with 82.83% positive edges. In Xize, fungal–fungal associations accounted for 60.78% of all edges, and positive edges comprised 85.31%; both modularity (0.835) and clustering coefficient (0.944) were higher than in Geyi. Two key nodes were identified in Geyi, namely OTU1771 (Chloroflexi) and OTU373 (Ascomycota) (Fig. 5B and C; Table S2), whereas nine key nodes were identified in Xize, belonging to Acidobacteriota, Gemmatimonadota, Proteobacteria, and Chloroflexi (Fig. 5-E and F; Table S2). Region significantly affected network stability, network complexity, and the proportion of positive links (Fig. 6-A, C, and D), but had no significant effect on AVD (Fig. 6-B). Network stability and AVD were higher in Geyi, whereas network complexity and the proportion of positive links were higher in Xize. Within Geyi, LB plots showed higher network stability and AVD, whereas in Xize, LB plots exhibited significantly lower network stability (Fig. 6).

Bacterial community assembly was dominated by deterministic processes (Fig. 6-E and F). Across both sites, 91.7% of samples (33/36) showed $\beta\text{NTI} > 2$, and the contribution of deterministic processes ranged from 66.7% to 100% in Geyi and from 83.3% to 100% in Xize. No samples with $\beta\text{NTI} < -2$ were detected in any treatment, indicating that homogeneous selection did not contribute to bacterial community assembly. Fungal communities showed a contrasting pattern, with 69.4% of all samples across both regions (25/36) satisfying $|\beta\text{NTI}| \leq 2$, indicating an overall dominance of stochastic processes. This stochastic contribution was more pronounced in Xize, where it reached 83.3%, compared with 55.6% in Geyi (Fig. 6-F).

3.5. Response mechanisms of microbial stability

In Geyi, AK directly enhanced network stability and also indirectly influenced stability by reducing soil microbial diversity (Fig. 7-A). Soil pH, AN, and AP affected community assembly, which in turn influenced the co-occurrence network and ultimately network stability; soil pH additionally promoted soil microbial diversity, thereby indirectly affecting stability. In the post-tobacco rotation system, changes in microbial community stability were primarily attributable to increased diversity, elevated soil pH, and increased network complexity (Fig. 7-B).

In Xize, SOM and AK both directly and significantly reduced network stability (Fig. 7-C). AK also indirectly influenced stability by suppressing soil microbial diversity, while increased AK itself and the pH elevation induced by SOM first affected the co-occurrence network and subsequently exerted indirect effects on stability. Among all factors, AK exerted the greatest total effect on network stability (Fig. 7-D).

4. Discussion

4.1. Legume intercropping favors retention of soil available nutrients and affects soil network stability

Cropping systems can regulate soil functional potential by altering the fundamental processes of nutrient cycling and, consequently, the capacity of soil to sustain ecological regulation [3, 5]. Compared with winter fallow, post-tobacco crop rotation without fertilization reduced soil pH, SOM, and available nutrients. Specifically, post-tobacco crop rotation induced a degree of soil acidification, while legume intercropping retained more available nutrients than other rotation crops.

First, post-tobacco crop rotation decreased soil pH compared with winter fallow (Fig. 2). Previous studies have reported lower soil pH under four-year corn-tobacco rotation than under continuous tobacco cultivation. Such acidification may still fall within the favorable range for tobacco growth (5.5–6.5). However, its longer-term consequences under continuous tobacco production or alternative crop rotations remain underexplored. This highlights the need for long-term monitoring and pH regulation through amendments such as lime and biochar [29].

Second, the two broadcast crops (smooth vetch and ryegrass) retained more soil organic matter owing to greater dry matter production and carbon return [30]. Legume intercropping also retained more SOM than non-leguminous cropping (Fig. 2). This may be because the accumulation of available nutrients under legume intercropping reduced the demand of soil microorganisms for SOM decomposition [31].

On the other hand, legume intercropping retained more available nutrients than other treatments in the present study, consistent with previous findings [5]. A plausible explanation is that intercropping enhances the release of root-derived compounds — including organic acids, enzymes, and sugars — which may promote the mobilization of poorly available nutrient fractions such as phosphorus [32].

Moreover, legumes tend to release greater amounts of root exudates [33]. This is supported by the higher AP observed under faba bean and pea intercropping than under non-leguminous intercropping. At the same time, owing to the nitrogen-fixing capacity of leguminous crops, legume intercropping also increased soil AN [5]. The retention of available potassium under legume intercropping may be linked to overall improvements in nutrient cycling and stronger interdependence among nutrients [34]. This is supported by significant positive correlations between available potassium and both AN and AP in this study ($r = 0.72$, $P < 0.001$; $r = 0.34$). Broadcast leguminous cover crops, by contrast, accumulate greater biomass and take up more nitrogen and phosphorus from the soil. At harvest, these nutrients may be removed from the field. This could explain the lower soil available nitrogen and phosphorus observed under broadcast legume management [35, 36].

Changes in soil nutrients resulting from post-tobacco crop rotation can directly affect microbial network stability. Consistent with the findings of Chen et al. [37], AK directly enhanced network stability, particularly in Geyi (Figs. 2, 6, 7). In Xize, lower SOM directly improved network stability. Treatments that retained less SOM – notably faba bean and carrot intercropping – exhibited higher network stability (Figs. 2, 6, 7). Guo et al. [38] conducted a 33-year long-term fertilization experiment and found that soil pH and dissolved organic carbon had significant direct effects on the stability of both bacterial and fungal communities. This suggests that the key soil physicochemical factors governing community network stability may differ across regions. On this basis, future studies could explore in greater depth the mechanisms by which specific nutrients drive improvements in network stability.

4.2. Bacterial communities are more sensitive than fungal communities to post-tobacco rotation, and diversity and community assembly affect improvements in network stability

We propose that bacterial communities were more sensitive than fungal communities to post-tobacco rotation in the present study. First, bacterial α -diversity showed differential responses to post-tobacco rotation, whereas fungal α -diversity did not. This may be related to the different soil pH preferences of bacteria and fungi [37]. Bacteria have a narrower optimal pH range for growth, whereas fungi tolerate a broader range [39, 40], making fungi less responsive to the pH decline induced by post-tobacco crop rotation. As shown in the present study, carrot intercropping and broadcast smooth vetch in Geyi maintained significantly higher soil pH ($P < 0.05$), which increased bacterial α -diversity but had no effect on fungal α -diversity. Second, the explanatory power of PCoA ordination axes was higher for bacteria than for fungi (56.6% and 50.4% for bacteria; 31.6% and 36.8% for fungi). In Geyi, the alleviation of acidification under carrot intercropping and broadcast smooth vetch may have driven shifts in both bacterial and fungal community composition, causing their separation from other crop treatments in terms of β -diversity [41]. However, the explanatory power remained lower for fungi than for bacteria (Fig. 4). In Xize, faba bean rotation also helped improve water-use efficiency and, by enhancing microbial activity and nutrient mineralization potential, strengthened microbial interactions, resulting in β -diversity that differed from other legume rotations [4]. Finally, consistent with Huo et al. [19], bacterial community assembly in the present study was dominated by deterministic processes, indicating a strong responsiveness of bacterial communities to environmental change. Fungal community assembly, in

contrast, was primarily governed by stochastic processes and was not significantly influenced by crop type.

α -Diversity is widely recognized as an indicator of species richness and evenness and is closely related to soil multifunctionality, ecological maintenance, and disturbance resistance [13]. Existing evidence has indicated that the contribution of microbial diversity to community stability becomes stronger in more fertile soils. Radujković et al. [42] similarly demonstrated a clear soil background dependency in the effects of soil biodiversity on community stability. A comparable pattern was observed in the present study, where the total effect of diversity on network stability was greater in Geyi than in Xize (Fig. 7-B and D). At the same time, increases in β -diversity can directly enhance network stability [43] and can also improve network complexity, ultimately contributing to higher network stability (Fig. 7) [9, 44].

Previous studies have indicated that increases in the stochasticity of soil microbial community assembly are associated with improvements in network stability [45, 46]. However, in the present study, improved network stability was associated with more deterministic community assembly (Fig. 7). This suggests that bacterial communities – dominated by deterministic processes – played a stronger role in network stability under post-tobacco rotation than fungal communities, which were primarily governed by stochastic processes (Fig. 6). This is consistent with the observation that high salinity increases the contribution of deterministic processes while strengthening microbial interactions, thereby enhancing network stability [47]. In the present study, the overall low nutrient content in Xize created a nutrient-deficient environment. Post-tobacco crop rotation in Geyi also caused a substantial decline in AP (from 91.68 mg/kg to 33.39 mg/kg), potentially inducing a degree of phosphorus limitation. Both conditions may have increased the dependence of network stability on deterministic processes. In addition, compared with Geyi, network stability in the nutrient-poor Xize depended more strongly on the regulation of community assembly under soil environmental conditions (Fig. 7-B and D) [48].

4.3. Co-occurrence network characteristics affect network stability

Microbial co-occurrence networks can partially represent soil microbial interactions and thereby influence network stability. First, increases in network complexity led to higher network stability (Fig. 7). As Yuan et al. [24] argued, complexity promotes stability, and complex microbial networks strengthen community cooperation through integrated resource utilization and metabolic coordination, thereby improving co-occurrence network stability [49]. However, no significant differences in network complexity indices were detected among treatments in the present study. Second, positive correlative connections dominated microbial interactions at both sites (Fig. 6, > 82%), thereby influencing network stability. This can be explained by two mechanisms: inter-species interactions ("true cooperation") or shared environmental preferences ("spurious cooperation") [14, 50]. Environmental stress imposes strong selective pressure on microbial communities, driving members toward "true cooperation" (mutualism) rather than competition for survival and functional maintenance [26, 51]. This resulted in high positive correlative connections in Xize, where greater cooperation enhanced network resistance. In

contrast, under favorable environmental conditions, many species co-occur because of shared habitat preferences rather than genuine mutualism [50]— a form of "spurious cooperation." In Geyi, carrot intercropping and broadcast smooth vetch significantly reduced positive correlative connections (Fig. 6-D) and thereby significantly improved network stability (Fig. 6-A). This is consistent with previous findings that reduced positive correlative connections are associated with improved network stability [47].

Finally, key taxa in the co-occurrence networks provided additional evidence for understanding the regulatory mechanisms of network stability. Only 2 key nodes were identified in the nutrient-rich Geyi, whereas 9 were identified in the environmentally stressed Xize. Xiao et al. [52] similarly found that a greater number of key nodes occurred in areas with higher pollutant concentrations, maintaining microbial metabolic functions. In both regions, the key taxa in the microbial co-occurrence networks were Chloroflexi and Ascomycota in Geyi, and Chloroflexi, Gemmatimonadota, Proteobacteria, and Acidobacteriota in Xize. They exhibited high connectivity within the network (Figs. 5, 6) and were also dominant taxa (Fig. S1). The enrichment of oligotrophic bacterial groups (Acidobacteria and Chloroflexi) and the nitrogen-cycling-related Gemmatimonadota may have partially enhanced soil nutrient turnover capacity [53, 54]. Copiotrophic Proteobacteria typically respond rapidly to nutrient inputs and rhizosphere environmental changes, whereas copiotrophic Ascomycota, as the dominant fungal group in soil, are commonly involved in plant residue decomposition and soil organic matter breakdown [55]. Notably, Chloroflexi was identified as a key taxon in both regions, and increases in its abundance have been shown to improve crop disease resistance [56, 57]. Overall, microbial interactions and the formation of key taxa under post-tobacco rotation enhanced microbial functional collaboration. Future studies could specifically validate the role of Chloroflexi strains in improving plant disease resistance in tobacco-cultivated soils.

It should be noted that key taxa may create a compositionally more stable rhizosphere environment. Their aggregation may primarily serve to maintain existing community composition or function (AVD) rather than to immediately enhance network stability [58]. In Geyi, the key groups included both copiotrophic fungi (Ascomycota) and oligotrophic bacteria (Chloroflexi), indicating functional complementarity that may stabilize nutrient transformation processes [59]. Yu et al. [60] found that under nutrient-rich conditions, key bacterial and fungal groups can jointly enhance soil multifunctionality. Under nutrient-limited conditions, however, this role depends more strongly on key bacterial groups. This distinction likely reflects the fact that fungal ecological functions are more nutrient-dependent, whereas bacterial functions are more broadly adaptable [61]. Building on these findings, we infer that a similar pattern may apply to compositional stability in the present study. In the nutrient-rich Geyi, stability is maintained through the complementary functions of bacteria and fungi. In the nutrient-poor Xize, bacteria serve as the primary contributors. As shown in Fig. 6, increases in the abundance of key taxa improved compositional stability. Specifically, the abundance of all key groups in Geyi and two-thirds of key groups in Xize directly contributed to this improvement. In line with this, a previous study also demonstrated that increases in key taxa abundance over eight years of intercropping led to

improvements in network stability [15]. The role of key taxa in maintaining microbial community stability may therefore be time-dependent, and this mechanism warrants further long-term investigation.

In the present study, changes in network stability and compositional stability were not always synchronized. More specifically, improvements in network stability were often accompanied by a reduction in compositional stability. A similar decoupling between compositional and functional stability has been reported by Li et al. [62], particularly in Xize (Fig. 6). The preceding sections used network stability to clarify the links among soil properties, microbial assembly processes, diversity, and network characteristics with respect to microbial community stability. In Geyi, soil pH and diversity showed relatively high effect values on network stability (Fig. 7). Carrot intercropping and broadcast smooth vetch significantly alleviated soil acidification and maintained high α -diversity (Figs. 2, 3). However, broadcast smooth vetch achieved more favorable compositional stability and network stability (Fig. 6-B). Broadcast smooth vetch is therefore recommended as the preferred post-tobacco rotation crop in Geyi. In Xize, faba bean, pea, and ryegrass intercropping produced higher network stability. However, given the generally low soil nutrient content in Xize, pea intercropping can more effectively reduce soil pH to an appropriate range in the short term and retain more soil nutrients for the subsequent tobacco-growing season. Pea intercropping is therefore recommended for Xize.

In summary, these results preliminarily demonstrate that increased β -diversity, increased contribution of deterministic assembly, more complex networks, and decreased abundance of key taxa all enhance network stability. However, this study was confined to a specific region, and single-season sampling data are insufficient to comprehensively reflect the long-term dynamics of microbial community stability. The research scope could be expanded, and a long-term dynamic monitoring framework is still needed to capture sustained responses over time and refine the associated evaluation indicators. Furthermore, although this study identified the potential roles of multiple key taxa, future research should integrate multi-omics approaches, including metagenomics, metatranscriptomics, and metabolomics, to clarify the functional gene composition and metabolic activity of these key taxa. Such work can help verify their functional roles, reveal the molecular mechanisms underlying stability maintenance, and provide a stronger basis for identifying optimal management strategies.

5. Conclusions

Based on co-located field experiments conducted at two sites, this study revealed region-specific regulatory mechanisms by which post-tobacco rotation shaped microbial communities in tobacco-growing soils of nutrient-rich Geyi and nutrient-poor Xize. Intercropping with leguminous crops consistently retained more available nutrients, and soil SOM and AK directly influenced network stability. Bacterial communities showed greater sensitivity than fungal communities in terms of α -diversity, β -diversity, and community assembly. Both increases in β -diversity and greater contributions of deterministic assembly enhanced network stability. A decrease in the proportion of positive correlations in Geyi and an increase in that proportion in Xize both contributed to improved network stability. Xize required more keystone bacterial taxa to maintain microbial interactions, whereas fungi were identified

as keystone taxa only in the nutrient-rich Geyi region. These findings suggest that broadcasting green manure in Geyi and intercropping peas in Xize represent ideal crop choices for post-tobacco rotation, effectively improving soil environmental conditions and achieving higher soil microbial network stability. These findings not only provide a novel perspective for optimizing post-tobacco rotation patterns but also offer a scientific basis for elucidating the regulatory mechanisms of soil microbial stability under different nutrient backgrounds.

Abbreviations

AK	available potassium
AN	available nitrogen
ANOVA	analysis of variance
AP	available phosphorus
AVD	average variation degree
Bac-Bac	bacteria–bacteria interactions
Bac-Fun	bacteria–fungi interactions
β NTI	beta nearest taxon index
C	crop type
CD	tobacco–faba bean rotation
df	degrees of freedom
Fun-Fun	fungi–fungi interactions
G	Geyi
HLB	tobacco–carrot rotation
Leg	leguminous crops
LB	

tobacco–radish rotation
LF
smooth vetch monoculture
MC
ryegrass monoculture
NoLeg
non-leguminous crops
OTU
operational taxonomic unit
PC1
first principal component
PCA
principal component analysis
PCoA
principal coordinates analysis
PERMANOVA
permutational multivariate analysis of variance
pH
soil pH
 P_i
among-module connectivity (participation coefficient)
S
sampling site
SE
standard error
SEM
structural equation modelling
SOM
soil organic matter
WD
tobacco–pea rotation
X
Xize
 Z_i
within-module connectivity (z-score).

Declarations

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

Not applicable.

Declaration of competing interest:

Chengguang Shi, Yingying Qian, and Quanzhen Wang were employed by China Tobacco Hebei Industrial Co., Ltd, China Tobacco Yunnan Industrial Co., Ltd, and the Qujing Branch of Yunnan Tobacco Company, respectively. All authors declare that these affiliations did not influence the design, execution, or interpretation of the study, and that no other competing interests exist.

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Author Contribution

ZJ wrote the original draft of the manuscript and was responsible for data curation. TX contributed to funding acquisition and designed the experimental methodology. CS provided resources and contributed to methodological development. YQ provided resources and conducted the field investigation. YP carried out the investigation and acquired funding. YZ developed and applied the software tools and performed the formal analysis. SY carried out the investigation and prepared the data visualizations. YW acquired funding and contributed to the visualization. ZT reviewed and edited the manuscript, supervised the overall study, and acquired funding. QW reviewed and edited the manuscript and administered the project. All authors read and approved the final manuscript.

Data Availability

Data will be made available on request. The raw sequence data have been deposited in NCBI SRA under the BioProject ID of (PRJNA1453413).

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Figures

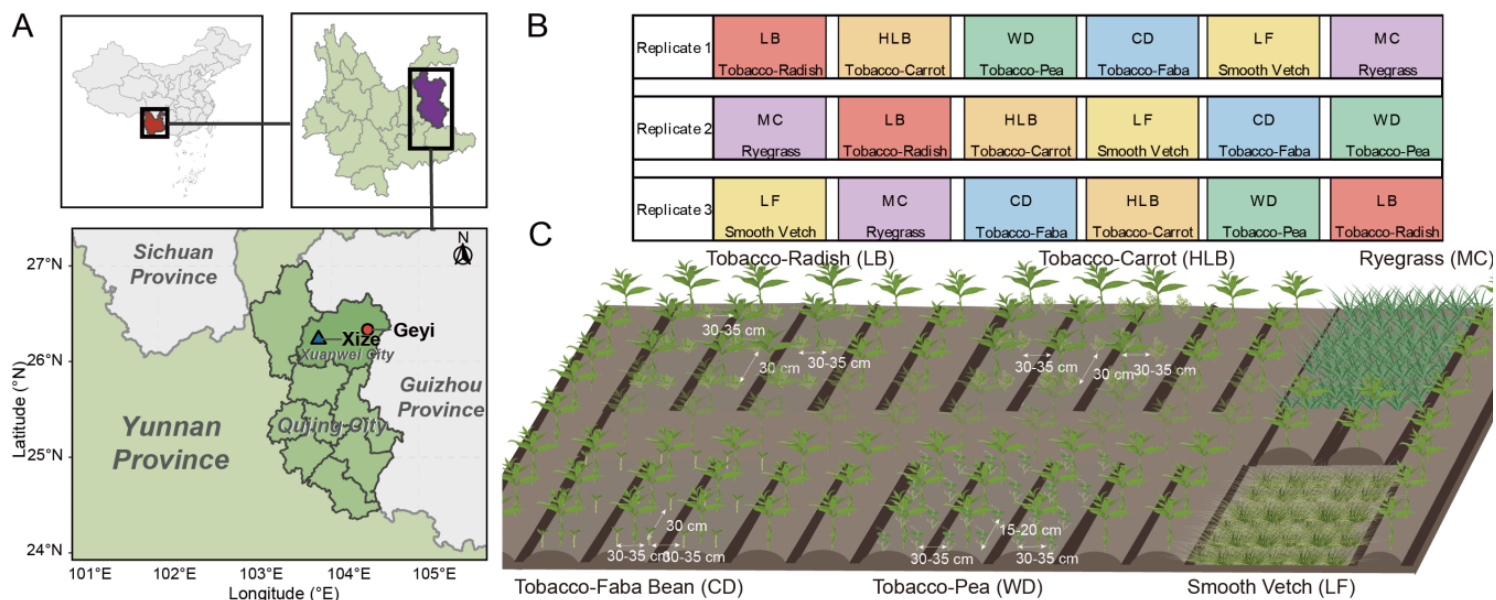


Figure 1

Geographical location of sampling sites (A), random block design of the field experiment (B), and plant spatial structures under the six planting patterns (C). (A) Geographical distribution of the two sampling sites (Geyi and Xize) in Yunnan Province. (B) The random block design in the field experiment demonstrated three repeated setups for six different treatment methods. (C) Schematic diagrams of the plant spatial structures under the six planting patterns. LB, tobacco–radish; HLB, tobacco–carrot; WD, tobacco–pea; CD, tobacco–faba bean; LF, smooth vetch; MC, ryegrass.

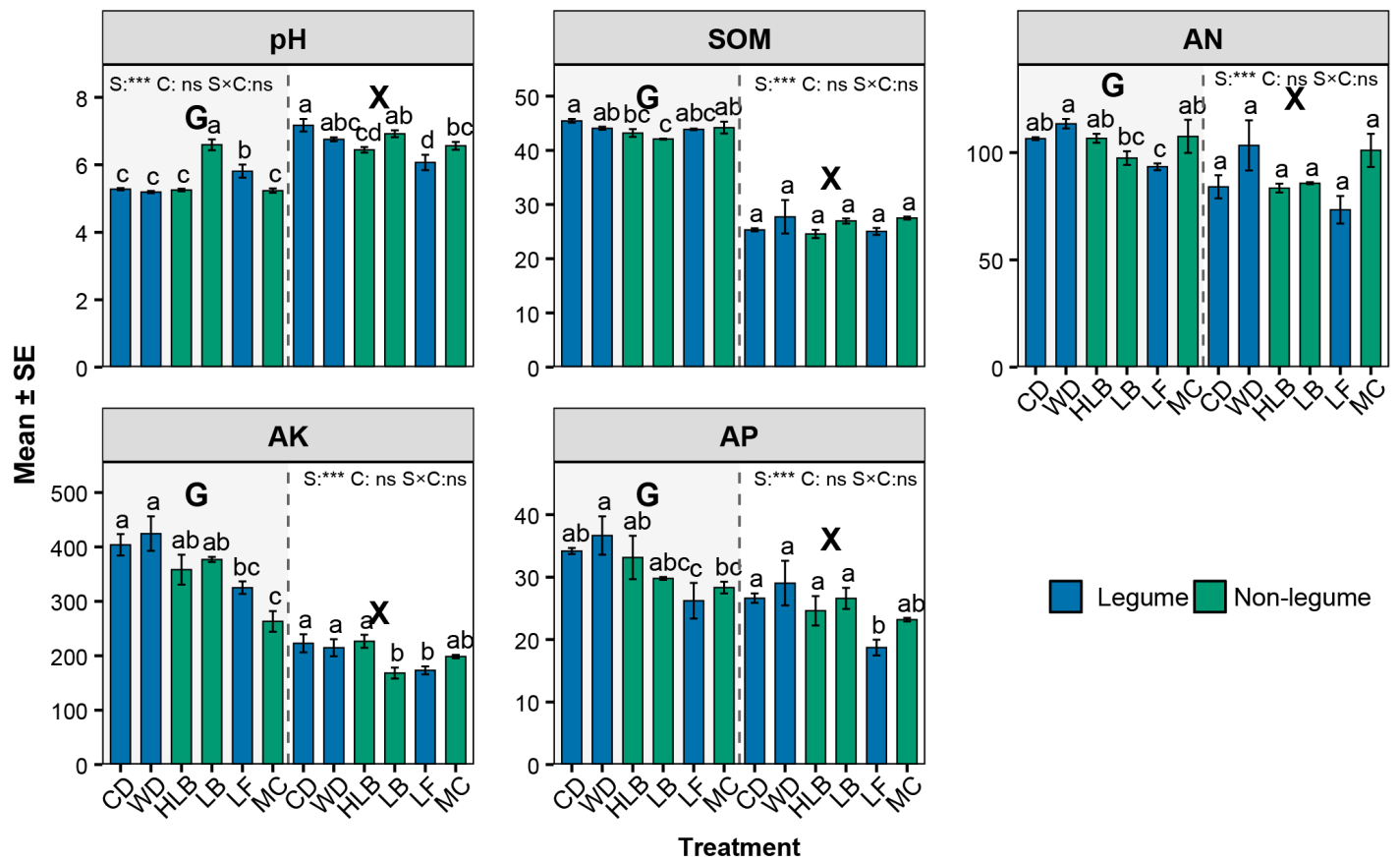


Figure 2

Soil physicochemical properties (pH, SOM, AN, AP, and AK) under six planting patterns across two regions (Geyi and Xize). S, region; C, crop type. Region codes: G, Geyi; X, Xize. Crop categories: Leg, leguminous crops; NoLeg, non-leguminous crops. Individual treatments: WD, tobacco–pea; CD, tobacco–faba bean; LB, tobacco–radish; HLB, tobacco–carrot; LF, smooth vetch; MC, ryegrass. pH, soil pH; SOM, soil organic matter; AN, available nitrogen; AP, available phosphorus; AK, available potassium. Data are presented as mean ± SE (n = 3). Statistical significance derived from ANOVA is indicated by asterisks: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Different lowercase letters indicate significant differences between treatments within the same region ($P < 0.05$). The relative positions of the letters G and X represent the comparison results of the mean values between the two locations.

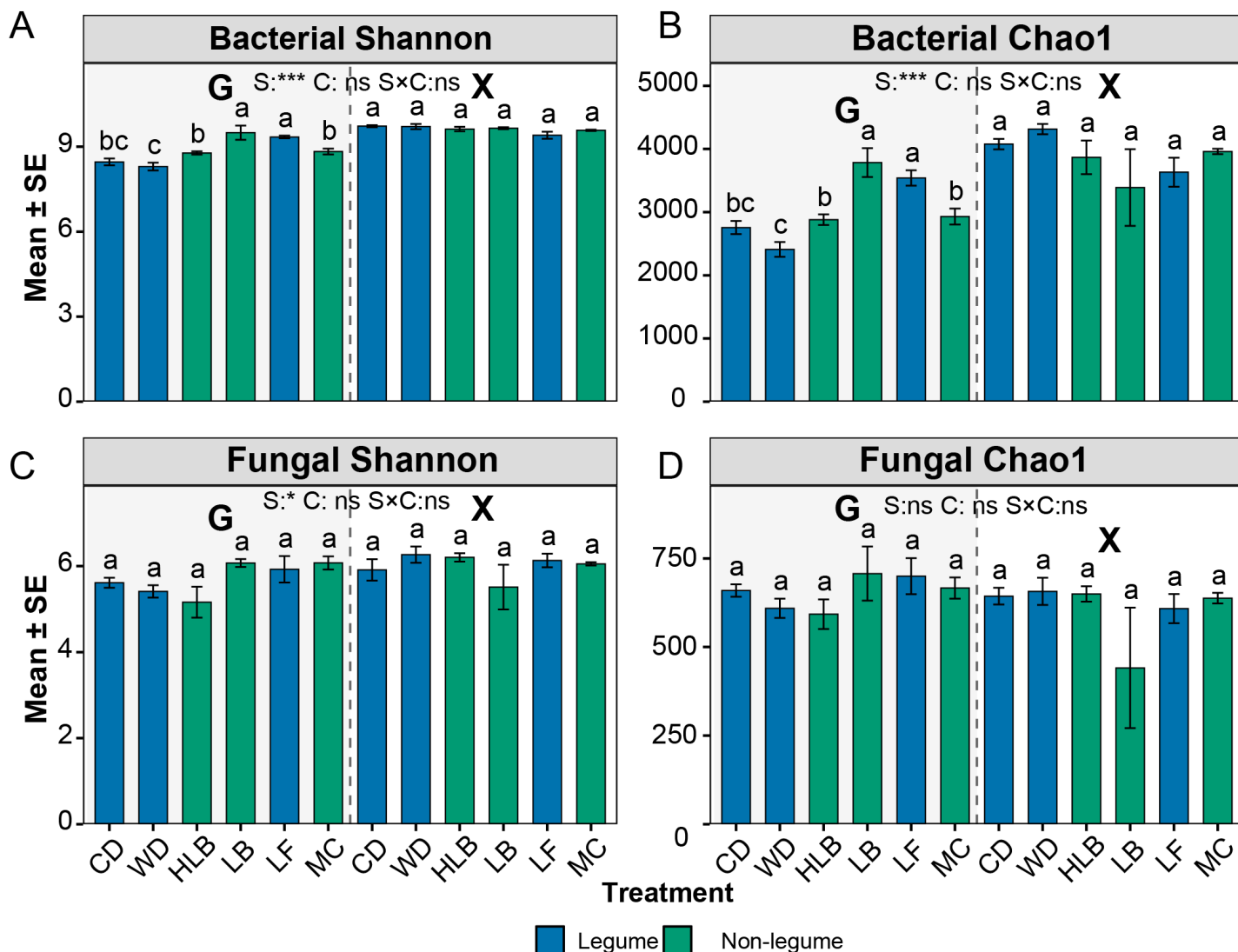


Figure 3

Shannon and Chao1 indices of α -diversity for soil bacterial communities (A and B) and fungal communities (C and D) across two regions and six planting patterns. G, Geyi; X, Xize. Blue and green bars represent leguminous and non-leguminous treatments, respectively. Letters G and X denote comparisons of mean values between the two sites. Different lowercase letters indicate significant differences among treatments within the same region at $P < 0.05$ (mean $\pm$ SE, $n = 3$).

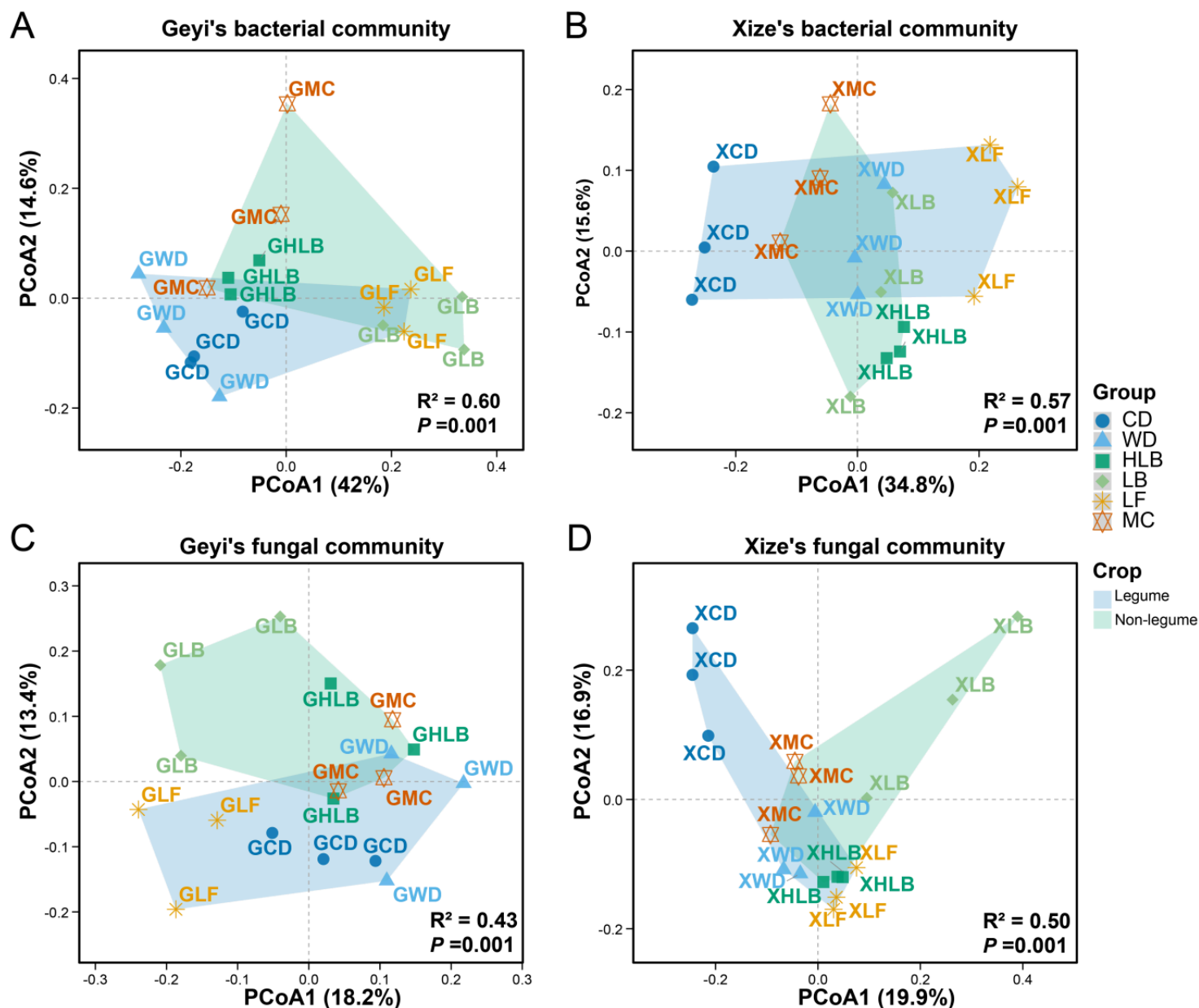


Figure 4

PCoA of soil bacterial (A and B) and fungal (C and D) community structures in the Geyi (A and C) and Xize (B and D) regions based on Bray–Curtis dissimilarity. Distinct geometric shapes denote specific planting treatments. Blue and green shading delineate the clustering ranges of leguminous and non-leguminous communities, respectively. PERMANOVA R^2 values for panels A–D were 0.60, 0.57, 0.43, and 0.50, respectively (all $P = 0.001$).

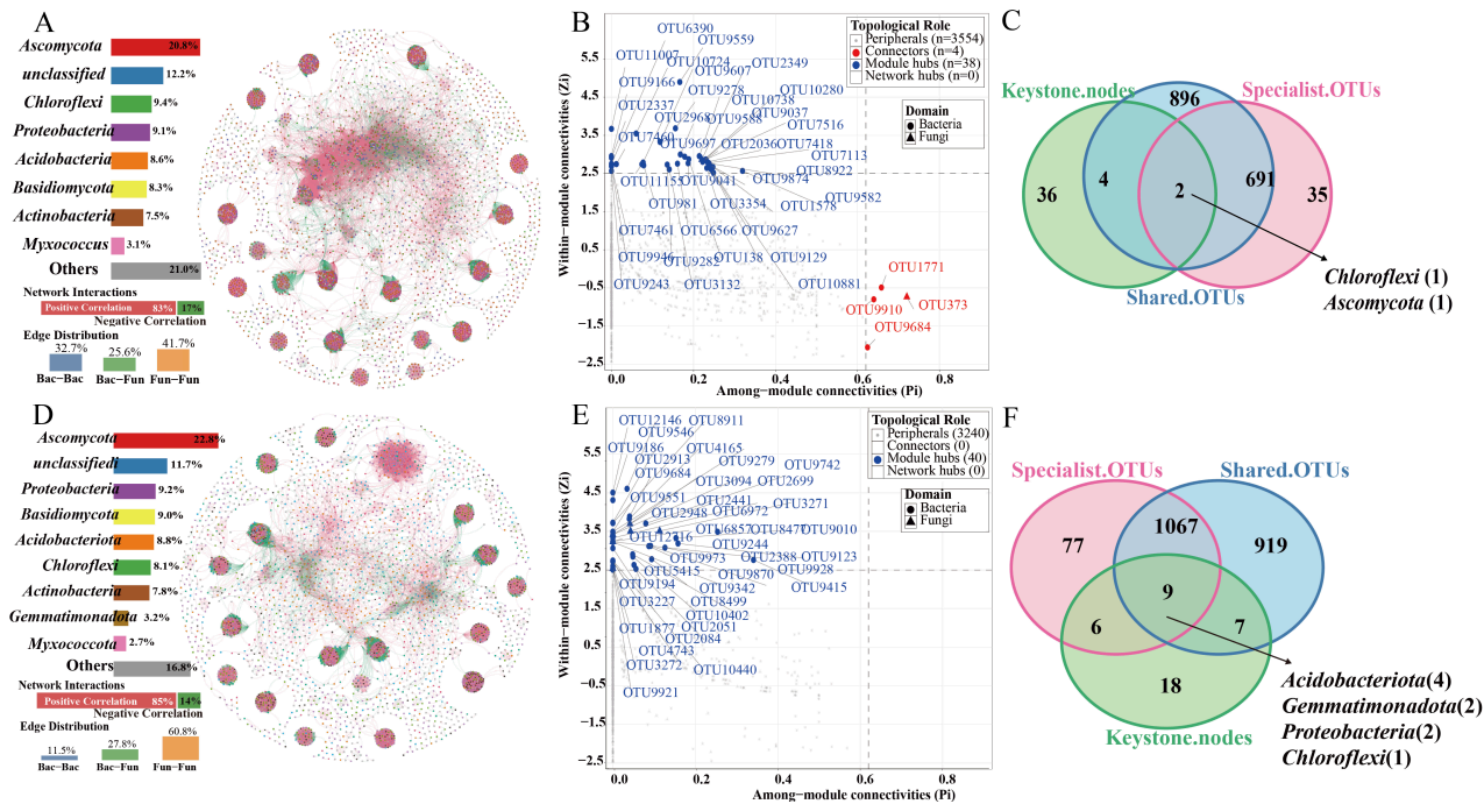


Figure 5

Inter-domain co-occurrence networks (A and D), topological role classification of nodes (B and E), and Venn diagrams of key node and OTU distributions (C and F) in soil microbial communities of the Geyi (A–C) and Xize (D–F) regions. Node size is proportional to OTU relative abundance; node color indicates phylum-level taxonomic affiliation; red and green edges represent significant positive and negative correlations, respectively ($P < 0.05$). Bar charts quantify taxonomic composition and interaction types (Bac-Bac, Bac-Fun, and Fun-Fun). Nodes are classified as peripherals, connectors, module hubs, or network hubs based on within-module (Z_i) and among-module (P_i) connectivity, with key nodes explicitly labelled. Venn diagrams illustrate the distribution and overlap of key nodes, specialist OTUs, and shared OTUs within each regional network.

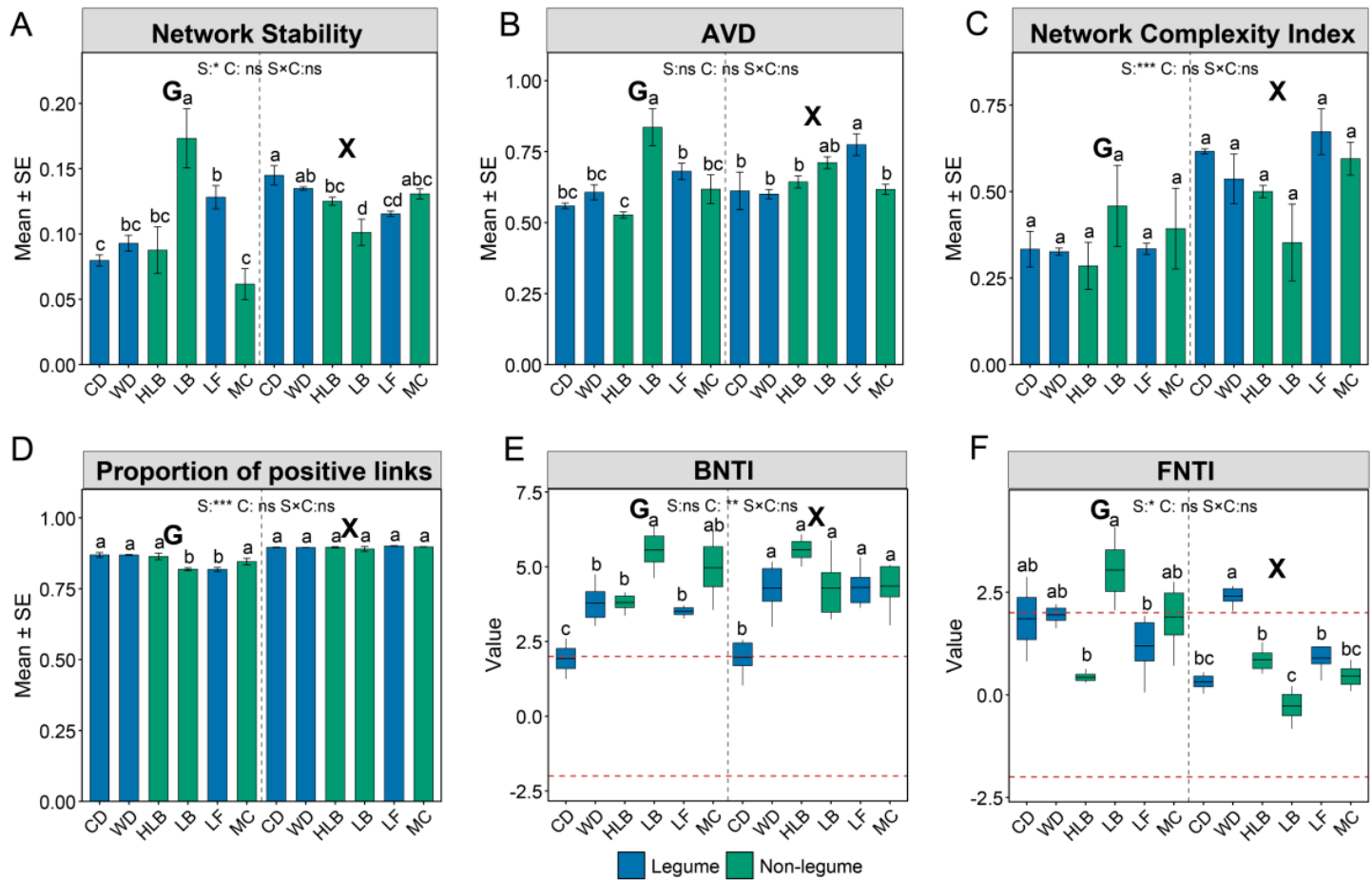


Figure 6

Topological features of soil microbial co-occurrence networks (A–D) and community assembly mechanisms indicated by β NTI for bacterial (E) and fungal (F) communities across the Geyi (G) and Xize (X) sites. Panels A–D compare network stability (A), average variation degree (AVD, B), network complexity index (C), and proportion of positive links (D) across crop treatments. Red dashed lines in panels E and F ($y = \pm 2$) indicate thresholds distinguishing deterministic ($|\beta\text{NTI}| > 2$) from stochastic ($|\beta\text{NTI}| < 2$) assembly processes. Different lowercase letters indicate significant differences among treatments within the same region ($P < 0.05$). Letters G and X denote comparisons of mean values between the two sites.

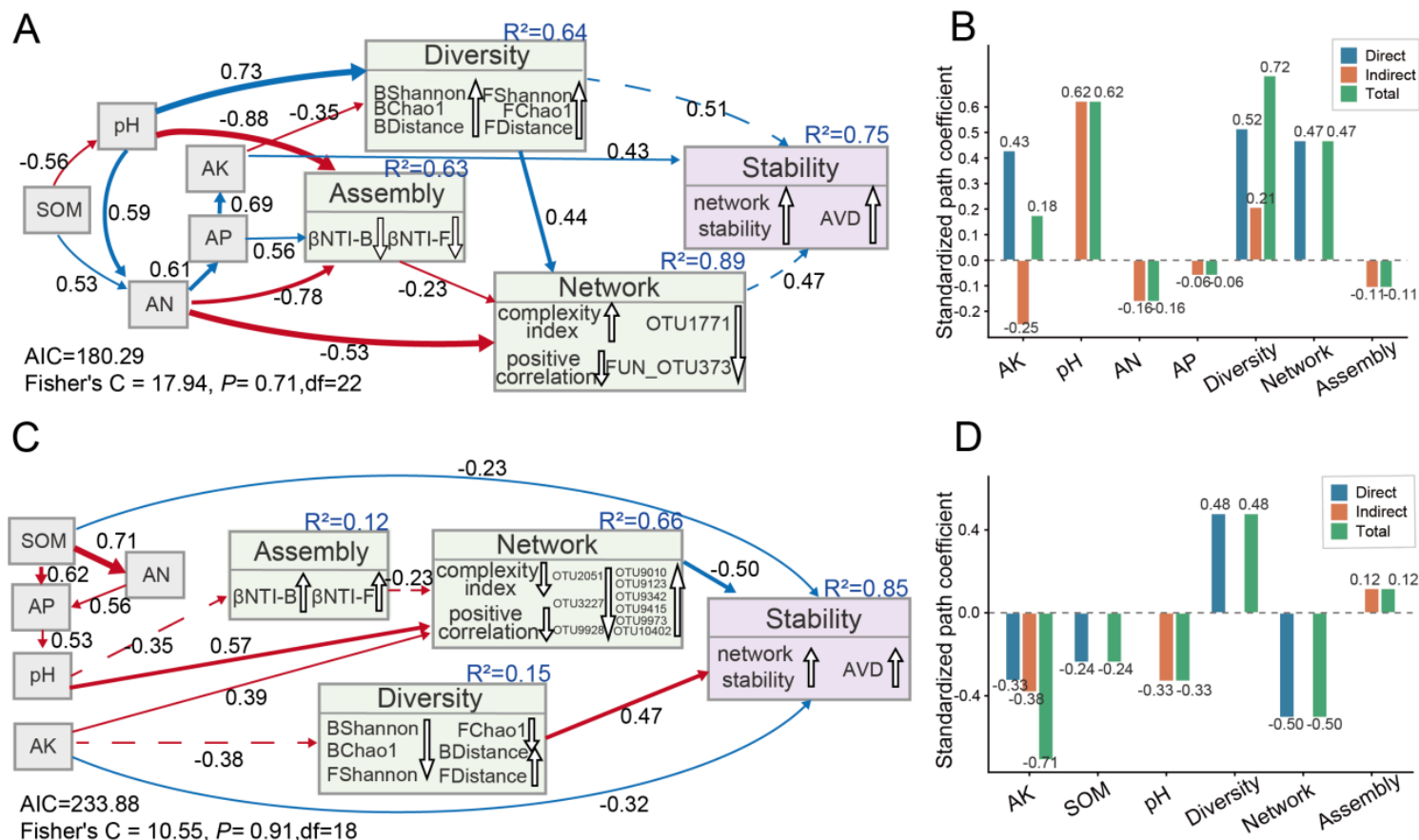


Figure 7

Piecewise SEM path diagrams (A and C) and standardised direct, indirect, and total effects on community stability (B and D) for the Geyi (A and B) and Xize (C and D) sites, illustrating pathways linking soil properties, microbial attributes, and community stability. Model fit indices: AIC=180.29 Fisher's C = 17.94, $P = 0.71$, $df = 22$ (Geyi); AIC=233.88 Fisher's C = 10.55, $P = 0.91$, $df = 18$ (Xize). Community diversity, assembly, co-occurrence network structure, and stability are each represented by PC1 of their respective PCAs; arrows within boxes indicate the loading direction of each indicator relative to PC1. Solid and dashed arrows denote significant and non-significant paths, respectively; red and blue arrows indicate negative and positive correlations, respectively. Numbers alongside arrows are standardised path coefficients; line thickness corresponds to significance level. Blue R^2 values (upper right of each box) indicate the proportion of variance explained. pH, soil pH; SOM, soil organic matter; AN, available nitrogen; AP, available phosphorus; AK, available potassium.

Supplementary Files

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