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Co-symbiosis of arbuscular mycorrhizal fungi (AMF) and diazotrophs promote biological nitrogen fixation in mangrove ecosystems

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ABSTRACT

Nitrogen (N) is the most critical nutrient that affects the establishment and stability of mangrove ecosystems. Despite the importance of biological nitrogen fixation (BNF) mediated by diazotrophs in mangrove ecosystems, current studies largely overlook the influence of arbuscular mycorrhizal fungi (AMF) on diazotrophs-driven BNF process in the N-limited ecosystems. Here, we conducted a comprehensive study on root-associated AMF and their interspecific interactions with diazotrophs in mangrove ecosystems using high throughput sequencing methods. Our results showed that the richness/diversity of diazotrophs had significant positive correlations with AMF community in mangrove rhizosphere. BNF rate in the rhizosphere of all mangrove species increased with the increasing of positive co-occurrence between AMF and diazotrophs, suggesting that the positive cooperation may promote N fixation efficiency in mangrove ecosystems. We also identified the potential keystone taxa ($Zi \geq 2.5$ or $Pi \geq 0.62$) and determined their crucial implications for the ecological processes of N fixation in mangrove ecosystems. The random forest analysis further indicated that the AMF keystone taxa were the most important predictors of BNF in mangrove rhizosphere. In addition, path analysis indicated that the α-diversity of AMF and diazotrophs communities, N-related enzymes and sediment nutrition components (e.g., TC, TN, Fe) were the main factors driving BNF process in mangrove ecosystems. This study provides novel insights into the interactions between AMF and diazotrophic communities during BNF process and expands our knowledge of AMF ecological functions of N cycle in mangrove ecosystems.

1. Introduction

Mangrove wetlands are highly prolific and multifunctional ecosystems connecting land and sea, and expanding along tropical and subtropical coastlines worldwide (Friess et al., 2012; Murdiyarso et al., 2015). Mangrove roots are permanently or intermittently submerged, providing precious habitats for coastal biodiversity and form complex food networks for biogeochemical cycling, which are 'hotspots' of microorganisms due to the damp sediments, abundant organic matters and diverse metabolites (Holguin et al., 2001; Schmit and Shearer, 2004).

Arbuscular mycorrhizal fungi (AMF) are an ancient group of symbiotic microorganisms colonizing vascular plant roots, which are closely associated with land colonization in the first appearance of land plants (Bonfante and Genre, 2008; Davison et al., 2015). AMF are obligate biotrophs that require soluble carbohydrates and lipid from their host plants (Brzostek et al., 2015). In return, they also serve as a bridge between soil and plants to support host plant survival by enhancing their nutrient absorbing capacities (Xie et al., 2014; Cheeke et al., 2017). AMF are considered to play key roles in detritus food webs and nutrients cycling in mangrove ecosystems by promoting the degradation of organic matters, especially the abundant lignocellulosic biomass (Hyde and Lee, 1995; Arfi et al., 2013). Also, the extensive mycelium system of AMF, spreading along with plant roots, can provide numerous inhabit spots for plant growth-promoting rhizobacteria to perform their ecological functions such as phosphorus absorption, siderophores production and auxin production (Kothamasi et al., 2005; Veresoglou et al.,

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2012; Gu et al., 2020). Besides, variation in mycorrhizal colonization among different mangrove species plays essential ecological functions in the nitrogen (N) cycle, which is critical for various wetland plant growth and could aid in the maintenance of highly diversified plant community (Cornwell et al., 2001; Montesinos-Navarro et al., 2012). However, current studies of AMF in mangrove wetlands mainly focus on the evaluation of specific AMF groups by traditional purification and isolation methods (Wang et al., 2009; D'Souza and Rodrigues, 2013; Xie et al., 2014; Wang et al., 2015). Very little is known about the rhizophyte-endophyte AMF community structure and species composition, and their ecological contributions among different mangrove habitats.

Mangrove wetlands are N-limited ecosystems, thus N availability constrains the plant productivity and microbial metabolisms, which can further impact the health and stability of mangrove ecosystems (Holguin et al., 2001; Reef et al., 2010). Increasing evidence suggests that AMF could modify the N transformation and storage by coupling the diazotrophic community in the mangrove rhizosphere and hyphosphere (Kaschuk et al., 2010; Veresoglou et al., 2012; Peña et al., 2017; Tsiknia et al., 2021). Furthermore, AMF mostly form hyphae to provide effective transport pathways for chemical compounds, which promotes chemical interaction with the diazotrophic community (Achatz and Rillig, 2014) or allow rhizoplane colonization of non-N-cycling heterotrophic microbes to affect the diazotrophic community (Oliveira et al., 2005; Coskun et al., 2017). However, most studies linking N cycling processes to microbial functions in mangrove ecosystems have overlooked the potential role of interactions between diazotroph and AMF. For example, diazotrophic taxa such as *Frankia*, *Azospirillum* and some species of *Pseudomonas* have been reported to be favored by AMF (Vázquez et al., 2000; Oliveira et al., 2005). Microbial interactions play a fundamental role in the formation and construction of microbial ecological communities and their ecological functions (Barberan et al., 2012). Also, the possible ecological interactions among members within the microbial community could be identified and visualized through co-occurrence networks analysis (Barberan et al., 2012; Ma et al., 2018; Abrego et al., 2020). Therefore, a deeper and comprehensive understanding of co-occurrence networks among AMF and diazotroph communities could contribute to understand their roles in N-cycling in mangrove wetlands.

In mangrove ecosystems, plants and microbial residues are the two primary organic sources, in which the proteins, chitin and peptidoglycan are the most main N source (Cordeiro and Costa, 2010; Arfi et al., 2013). Extracellular depolymerases are required to degrade complex polymers from plant and microbial residues into soluble subunits such as NH_4^+, glutamate and glutamine, which can be taken up by microorganisms (Fitter et al., 2011). In order to meet N demands, some microorganisms can secrete leucine aminopeptidases (LAP) and β-1, 4-N-acetylglucosaminidases (NAG) to degrade protein and chitin, respectively (Jian et al., 2016). Microbial extracellular enzymes, as indicators of microbial nutrient demand and metabolism processes, play an important role in N biogeochemical cycling (Xu et al., 2017). Therefore, studies of the microbial extracellular enzymes responsible for N cycling could provide an insight into the influence of microbial mechanisms on regional N cycling. However, evidence on diazotrophs and AMF regulation of N-related ecological processes via microbial extracellular enzymes is still lacking, thus limited our understanding of the linkages between environmental factors and microbial functional traits in mangrove ecosystems.

In order to fill these knowledge gaps, this study aimed to reveal the composition and structure of rhizophyte-endophyte AMF and their coexistence patterns with diazotrophs in different mangrove species; then further evaluate the ecological contribution of AMF to BNF; and finally determine the main factors driving the BNF process in mangrove ecosystems. We hypothesized that AMF could construct positive cooperative relationships with diazotrophs to increase the N fixation in mangrove ecosystems. To test this hypothesis, we analyzed the rhizosphere and endosphere AMF and diazotrophic communities from three mangrove habitats (*Avicennia marina*, *Laguncularia racemosa*, and *Sonneratia apetala*) by amplicons (18 S rRNA gene of AMF and *nifH* gene of diazotrophs) sequencing. The roles of AMF in driving BNF by increasing the interactions with the diazotroph community and modifying the mangrove rhizosphere microenvironment were clarified. This study largely expanded our understanding of the ecological roles of AMF on N fixation in mangrove ecosystems.

2. Materials and methods

2.1. Site description and sampling

All samples were collected from the Hailing Island National Mangrove Wetland Park (21°38′54″N, 111°58′12″E), which represent the typical subtropical monsoon climate coastal wetland ecosystem located in Guangdong Province, China. The annual average temperature and precipitation are 22.3 °C and 1816 mm, respectively (http://www.hldhsl.com/English/introduction.html). The study area comprises mangrove plantations, sandflat and mudflats. This study investigated three typical habitats which were dominated by *Avicennia marina*, *Laguncularia racemosa* and *Sonneratia apetala*. *A. marina* is natural native species that first appeared in the park in 1988 and it covering over 16.71 ha. *S. apetala* and *L. racemosa* were introduced in 2012, and covers 1.02 ha and 0.39 ha, respectively (Wu et al., 2020). Detailed information about the sampling sites is summarized in Table S1.

Six sediment cores (25-cm deep) adjacent to the mangrove rhizosphere were randomly taken from each type of habitat in May 2018. Sediment cores were packed individually on-site using sealed polythene bags, and kept in a specialized portable cooler before transferred back to the laboratory for further processes. The fine fresh roots were separated and washed with distilled water until there are no visible sediment particles. The roots were further washed twice, 20 min each time, with distilled water in an ultrasonic cleaning machine (Jiemeng, JP-010 S, China) to remove soil residues on the root surface. Then, cleaned root samples were washed in 80 % EtOH and 0.25 % NaClO for 1 min to remove attached microorganisms, and subsequently washed five times in sterilized water to wash out chemical residues. All cleaned roots were stored at −80 °C until subsequent endosphere DNA extraction within 2 days. The sediment adjacent to roots was divided into two subsets: one was stored at 4 °C for sediment physicochemical properties analysis, and the other about 5 g was stored at −80 °C for rhizosphere sediment DNA extraction within 24 h.

2.2. Sediment physicochemical property and nitrogenase activity analysis

In situ temperature of each sediment core was measured on-site with a thermometer (Shunkeda, TR-6, China). The NH_4^+, NO_2^- and NO_3^- were extracted from 2 g sediment with 1 M KCl using a standard protocol (Gómez-Brandón et al., 2016) and measured by a multimode microplate reader (Varioskan LUX, Thermo Scientific, USA). Pre-dried sediments were finely ground and packed into combustion tins for total carbon (TC) and total nitrogen (TN) measurements by an elemental analyzer (Vario TOC, Elemental, Germany). The contents of Fe and Cu in sediment were measured with an inductively coupled plasma optical emission spectrometry (ICP-OES, 5300DV, PerkinElmer, USA) (Yu et al., 2020a). Sediment pH, salinity and moisture were measured using the method as previously described (Liu et al., 2020). Sediment physicochemical properties are summarized in Table S2.

Acetylene reduction assay was used to determine sediment nitrogenase activity. Ambient nitrogenase activity estimates the sediment N fixation rate under field nutrient concentrations without the addition of organic carbon sources. Thus, 10 g fresh sediment was put into a 120 mL serum vial and the vials were sealed with rubber stoppers. Ten mL of acetylene (C_2H_2) was injected into each vial before incubated in the dark at 28 °C (Han et al., 2019). After incubation for 48 h, 200 μL headspace

gas was taken out to determine the concentration of ethylene (C_2H_4) by Agilent gas chromatography (HP7890B, Agilent, USA).

2.3. Sediment N-related enzyme assays

The β-1, 4-N-acetylglucosaminidases (NAG) and leucine aminopeptidase (LAP) can hydrolyze proteins and chitin, respectively (Adlakha et al., 2012; Jian et al., 2016). The activities of NAG and LAP were measured according to the protocol of (German et al., 2011). Briefly, 1 g sediment was homogenized in 20 mL of sodium acetate buffer using a hand blender. The pH value of sodium acetate buffer was set from 4.5 to 5.5 so that it could be close to that of soil samples. Fluorescent substrate proxies specific to each enzyme were added to eight replicate assay wells in optimal concentrations for measuring total potential activity. Assays were run with two standard columns containing sediment homogenate and methylumbelliferone. Each assay microplate also contained substrate blank columns receiving substrate and sodium acetate buffer. Sediment homogenate blanks were also measured simultaneously. Plates were incubated at 37 °C for 1 h, then 0.5 M NaOH was added to each well to terminate enzyme activity. Within 1 min of NaOH addition, fluorescence was measured using a fluorometer set at 450 nm emission (DeForest, 2009). The details of the materials and conditions used are listed in Table S3.

2.4. DNA extraction and amplicon sequencing

Rhizosphere DNA was extracted from 0.5 g sediment samples using a modified sodium dodecyl sulfate extraction method (Zhou et al., 1996; Wang et al., 2020) combined with a PowerSoil DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA). Root samples had been fully ground with liquid nitrogen before DNA extraction. Then, DNA was extracted from 0.2 g root samples by using the same method described above but using a PowerPlant DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA) instead. The DNA was assessed and quantified by NanoDrop ND-2000 Spectrophotometer (Thermo Fisher Scientific, MA, USA), and diluted into 2 ng/μL for subsequent PCR amplification.

The AMF specific primer pair *AMV4*.5NF (5′-GCCTCCCTCGCG CCATCAG-3′) and *AMDGR* (5′-GCCTTGCCAGCCCGCTCAG -3′) were used for amplifying an expected fragment size of 230 bp of 18 S ribosomal RNA gene (Lumini et al., 2010). Both forward and reverse primers were tagged with Illumina adapter sequence, a primer pad, and a linker sequence. Triplicates of PCRs per sample were performed with a reaction volume of 50 μL, which included 25 μL of Phusion High-Fidelity DNA Polymerase (NEB, Inc, USA), 2 μL of both forward and reverse phasing primer, 5 μL of DNA template and 16 μL of RNase free Ultrapure water. The amplification of AMF was performed in a BIO-RAD T100™ thermal cycler (Bio-Rad Laboratory, Hercules, CA, United States) under the following conditions: initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 60 °C for 45 s, extension at 72 °C for 1 min and a final extension at 72 °C for 7 min with a ramp of 3 °C s^{-1}. PCR products from triple reactions were pooled and purified using AMPure XP Beads Kit (NEB, Inc, USA). Purified DNA was quantified by Quant-iT™ dsDNA HS Reagent (Thermo Fisher Scientific, Inc, USA) and diluted to a concentration of 2 nM before sequencing. The *nifH* gene specific primer pair Pol115 F (5′-TGCGAYCCSAARGCBGACTC-3′) and Pol457 R (5′-ATSGCCATCA TYTCRCCGGA-3′), which targets a 320 bp sequence, was used for PCR amplification (Poly et al., 2001). The PCR amplification was performed according to our previous study (Liu et al., 2020). Paired-end sequencing of AMF and diazotrophs were performed using the Illumina HiSeq 2500 sequencer (Illumina, Inc., CA, USA) at Biomarker Biotechnology Co., Ltd (Beijing, China).

2.5. Sequence processing and analysis

The trimming process of AMF gene was conducted by a publicly available pipeline (http://mem.rcees.ac.cn:8080/). Briefly, primer fragments were firstly removed using Cutadapt (Martin, 2011). Poorly overlapped and low-quality sequences such as those with quality score <20 with a window size of 5 were removed by the Btrim program (Kong et al., 2011). Potential chimeric reads were identified and removed from the dataset using UCHIME in reference database mode (Edgar et al., 2011). Operational taxonomic units (OTUs) were identified with a cutoff of 97 % similarity level by using Quantitative Insights into Microbial Ecology (QIIME) implementation of UPARSE (Edgar, 2013). Singletons (OTUs represented by a single observation) were not included in the following analysis. The taxonomic identity of OTUs was determined by BLAST comparison against sequences contained within the MaarjAM database using BLAST (Opik et al., 2010; Martinez-Garcia et al., 2015). Raw AMF amplicon sequences data have been submitted to NCBI Sequence Read Archives under accession number PRJNA692805.

The trimming process of *nifH* gene was applied with the following steps. Primer fragments were firstly removed using Cutadapt (Martin, 2011). Quality control was performed to discard poor quality sequences using Trimmomatic (Bolger et al., 2014), and forward and reverse reads were combined using FLASH (Magoč and Salzberg, 2011). Combined sequences that <285bp and >350bp were eliminated, and sequences that contained one or more ambiguous base(s) ("N") were also removed. Chimeric sequences were identified and removed by using UCHIME (Edgar et al., 2011). Framebot software was used to correct potential frameshifts caused by sequencing errors (Wang et al., 2013), and only DNA sequences that covered >30 % of reference *nifH* protein translations were kept for further analysis. OTUs were identified with a cutoff of 95 % similarity level with protein reference sequences by using QIIME implementation of UPARSE (Edgar, 2013). Taxonomic assignment for *nifH* OTUs was carried out by searching representative sequences against reference *nifH* sequences with known taxonomic information. The raw sequencing data have been made available in the NCBI Sequence Read Archive under accession number PRJNA591108 (Liu et al., 2020).

2.6. Construction of co-occurrence networks of AMF and diazotroph communities

The co-occurrence correlation network was constructed by a phylogenetic Molecular Ecological Network Analysis (MENAP) pipeline through Random Matrix Theory (RMT)-based methods on the publicly available pipeline (http://ieg4.rccc.ou.edu/mena) (Deng et al., 2012). Only the OTUs occurring in 70 % samples were retained for subsequent calculations. According to RMT threshold (from 0.96 to 0.99) and Pearson's correlation coefficient (P value ≤ 0.05), the dominant OTUs and the significant and strong co-occurrence relationships were calculated. Using these dominant OTUs as nodes and significant co-occurrence relationships as edges, a co-occurrence network of metacommunity was established (Newman 2006; Sun et al., 2018). In each network, the size of each node is proportional to the number of connections (i.e., degree). Networks were visualized by the Gephi software (Bastian et al., 2009).

To assess possible topological roles of taxa in the networks, we classified nodes into four categories based on their within-module connectivity (Zi) and among-module connectivity (Pi) values: peripherals ($Zi < 2.5$, $Pi < 0.62$), connectors ($Zi < 2.5$, $Pi \geq 0.62$; connect modules together and important to network coherence), module hubs ($Zi \geq 2.5$, $Pi < 0.62$; critical to its own module coherence) and network hubs ($Zi \geq 2.5$, $Pi \geq 0.62$; vital to both the network and its own module coherence) (Shi et al., 2016). Network hubs, module hubs and connectors have been proposed as potential keystone taxa due to their important roles in network topology.

2.7. Structural equation modeling analysis

The partial least squares path modeling (PLS-PM), a powerful

statistical method to reveal interactive relationships among manifest variables and latent variables, was used to analyze the cascading relationships of biotic and edaphic factors on N fixation function (Barberán et al., 2014). In this study, PLS-PM was conducted using plspm packages in *R* (Sanchez, 2013). We first constructed a priori model with latent variable, manifest variables and path diagram. Latent variables were hypothetical variables that cannot be measured directly and were taken as underlying variables that help explain the association between two or more manifest variables. Several different conceptual structures were tested, and the optimal one was identified with the highest R^2 determination coefficients ($R > 0.60$), the goodness of fit index (GoF) ($GoF > 0.50$) and highest redundancy index (Mean Redundancy > 0.60) after 1000 bootstraps (Trivedi et al., 2016). After assessing the quality of the outer model, the matrix of path coefficients was used to check the inner model quality. Path coefficients represent the direction and strength of the linear relationships between variables. In addition to inspecting the path coefficients, the regression results of each endogenous construct were reviewed by R^2 values of determination of the endogenous latent variables. Finally, we tested the effects that each construct has on the rest of the constructs by taking into consideration the total number of connections in the inner model. The direct effects were given by the path coefficients, while the indirect effects were obtained as the product of the path coefficients by taking an indirect path. The total effects were are the sum of both the direct and indirect effects.

2.8. Statistical analysis

Linear regression analyses and scatter plot were used to assess correlations between any two parameters using GraphPad Prism (version 8.0). The ANOVA and *t*-test were performed to assess the difference of tested parameters (e.g., the sediment physicochemical factors, Shannon and Chao1 index of AMF community) using SPSS version 24.0 (SPSS Inc., USA), and the significant level was examined at $P < 0.05$. AMF community dissimilarity was visualized using principal co-ordinates analysis (PCoA) with Bray–Curtis dissimilarity estimates and we tested the significance among AMF community of three mangrove species by multiple-response permutation procedure (MRPP), analysis of similarities (ANOSIM) and permutational multivariate analysis of variance (PERMANOVA) using the VEGAN package in *R* (version 3.4.4) (Oksanen et al., 2013). Variation partition analysis (VPA) was used to quantify the relative contributions of the sediment physicochemical factors to AMF and diazotrophs communities with the vegan package (Yu et al., 2021). Venn analysis was conducted to identify the number of common and unique OTUs in all samples (Mo et al., 2021). Mantel text was used to determine the correlation between rhizosphere and endosphere microbial communities and sediment physicochemical factors by a publicly available pipeline (http://mem.rcees.ac.cn:8080/). Ternary plots were used to determine distribution of AMF taxa in rhizosphere and endosphere using ggtern packages in R (version 3.4.4) (Yu et al., 2021). We conducted a classification random forest (RF) analysis using the randomForest package to identify keystone taxa (OTUs) predictors for the N fixation (Liaw and Wiener, 2002). The significance of the models was assessed with 2000 permutations of the response variable, by using the "A3" package (Fortmann-Roe, 2015).

3. Results

3.1. The AMF community diversity, structure and composition

We obtained an average of 200,075 high-quality AMF sequences from each sampling plot with minimal sequences of 114,817 from each soil sample collected within the same plot to ensure sufficient sequencing depth. All detected sequences in this study were assigned into 463 unique AMF OTUs after trimming. Our rarefaction curves of observed AMF OTUs numbers showed a good sequencing depth and coverage (Fig. S1). The Shannon and Chao1 index showed that the α-diversity of AMF community in the rhizosphere was significantly ($P < 0.05$) higher than in the endosphere of all three mangrove habitats (Fig. 1). In endosphere, the Shannon index of *A. marina* and *L. racemosa* was significantly ($P < 0.05$) higher than that of *S. apetala* (Fig. 1a). PCoA results showed the structures of rhizosphere and endosphere AMF communities were significantly ($P < 0.05$, adonis test) different among three mangrove species (Fig. 1c and d). Also, three non-parametric tests (MRPP, PERMANOVA, and ANOSIM) using the Bray-Curtis dissimilarity index consistently showed significant ($P < 0.05$) variations of AMF community in the rhizosphere and endosphere among three mangrove species (Table S4), indicating that the mangrove species shifted the structure of the microbial communities.

Ten AMF families were identified from the rhizosphere and endosphere of three mangroves species (Fig. 2a and b). *Glomeraceae*, *Paraglomeraceae* and *Claroideoglomeraceae* were the most abundant families, which cumulatively accounted for more than half of the whole community. Noticeably, some AMF families showed specific symbiosis with different mangrove species. *Glomeraceae* showed the highest relative abundance in the rhizosphere of three mangrove species. *Glomeraceae* was found with a high relative abundance (14.56 %) in the endosphere of *A. marina*. Moreover, the relative abundance of *Paraglomeraceae* was up to 41.85 % in the endosphere of *L. racemosa*, while *Claroideoglomeraceae* (35.08 %) was dominant in the endosphere of *S. apetala*. We also compared the top ten OTUs in three mangrove habitats (Fig. S2). Our results showed that the dominant AMF OTUs varied among three different mangrove species. For instance, OTU_5 (14.15 %), which belongs to *Ambisporaceae*, was the highest in the rhizosphere of *A. marina*, while OTU_3 (14.47 %, *Glomeraceae*) and OTU_8 (10.36 %, *Gigasporaceae*) were the highest in the rhizosphere of *L. racemosa* and *S. apetala*, respectively. The highest relative abundance in the endosphere were OTU_2 (7.69 %, *Claroideoglomeraceae*), OTU_1 (15.30 %, *Paraglomeraceae*) and OTU_2 (27.66 %, *Claroideoglomeraceae*) of *A. marina, L. racemosa and S. apetala*, respectively. *Glomeraceae, Ambisporaceae and Paraglomeraceae* in rhizosphere, and *Paraglomeraceae* in endosphere among three mangrove species showed a significant ($P < 0.05$) difference (Figs. S3a and S3b).

Ternary plots showed a consecutive selection for AMF taxa in the rhizosphere of three different mangrove species and a more specific selection in the endosphere (Fig. 2c and d). Specifically, Glomeraceae (average 6.54 %), Paraglomeraceae (average 6.05 %), Ambisporaceae (average 4.96 %) and Glomeraceae (average 4.94 %) were dominated in the rhizosphere of three different mangrove species, while Claroideoglomeraceae (average 6.54 %), Paraglomeraceae (average 6.05 %), Glomeraceae (average 4.96 %) and Glomeraceae (average 4.94 %) were enriched in the endosphere. Moreover, Venn plots showed that AMF communities in three mangroves shared more common OTUs in the rhizosphere, but had more unique OTUs in the endosphere of each mangrove species (Fig. 2e and f). Specifically, 51.7 % of the AMF community was the same OTUs among three mangroves in the rhizosphere, while only 30.3 % of it was found in the endosphere.

3.2. Assembly processes and coexistence patterns of AMF and diazotroph communities

To explore the topology of interspecies interactions, we constructed the AMF and diazotroph networks in which nodes and links were calculated by the robustness of co-occurrence scores (Fig. 3). Multiple topological properties of the AMF and diazotroph co-occurrence patterns pronouncedly varied in the rhizosphere and endosphere networks. The number of nodes and links, average degree, and average clustering coefficient were higher in rhizosphere, indicating a connected and complicated AMF-diazotroph network in rhizosphere (Fig. 3 and Table S5). Besides, the potential interactions between AMF-diazotroph communities in rhizosphere had higher positive connections, while it has higher negative connections in endosphere. We also found that the rhizosphere had more modules than that in the endosphere, and the

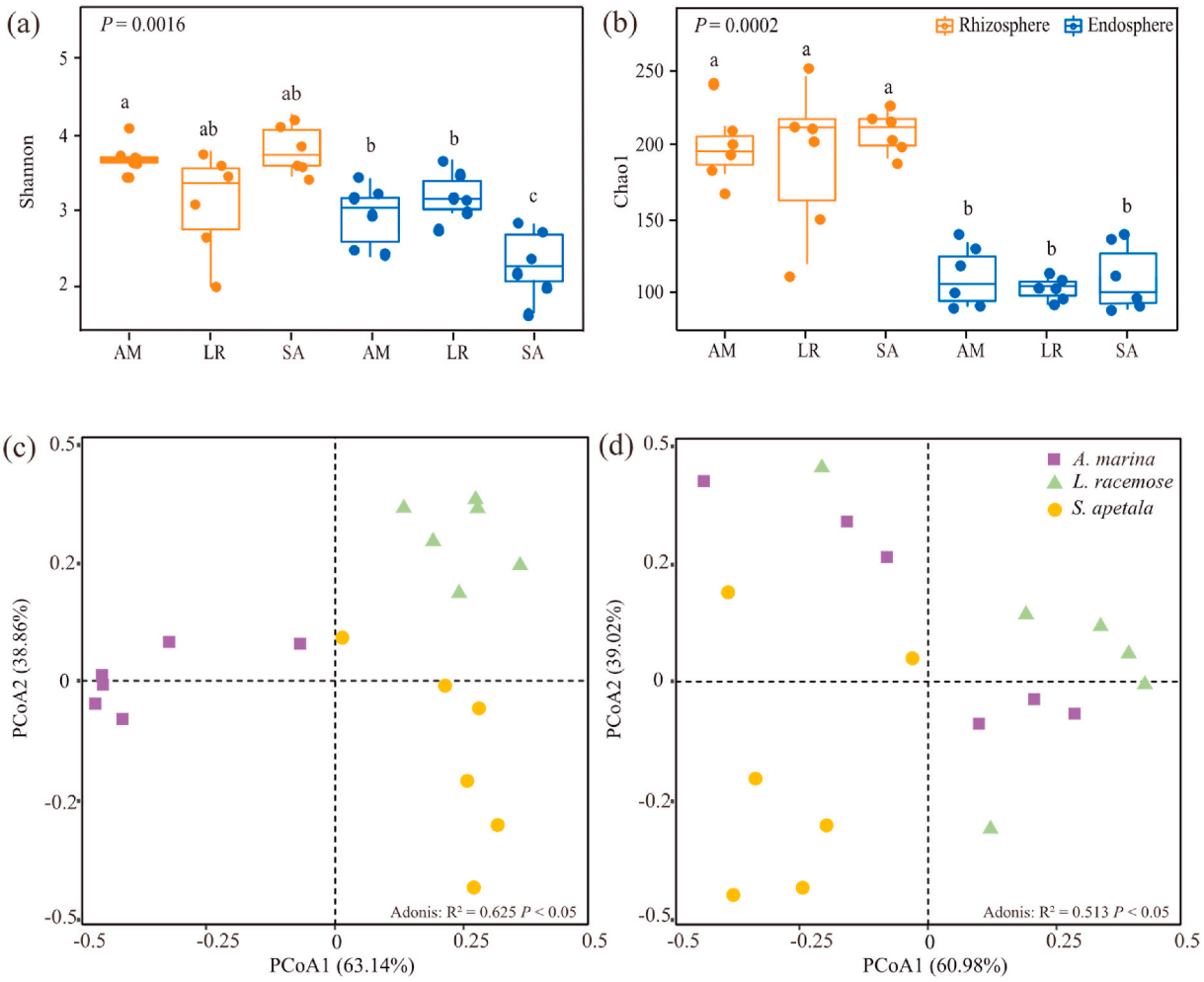

Fig. 1. Successional dynamics of AMF communities in mangroves. The boxplot of Shannon-Wiener (a) and Chao1 index (b) based on OTUs. Principal coordinates analysis (PCoA) of rhizosphere (c) and endosphere (d) AMF communities among three mangroves. The different letters indicated significant difference between mangroves ($P < 0.05$). AM, *A. marina*; LR, *L. racemosa*; SA, *S. apetala*.

proportion of the rhizosphere AMF in AMF-diazotroph co-occurrence networks was higher than that in the endosphere (Fig. 4a and b). The *Zi-Pi* plot showed that rhizosphere had more potential keystone taxa (32 diazotroph OTUs and 7 AMF OTUs) than that in endosphere (26 diazotroph OTUs and 1 AMF OTUs) (Fig. 3e and f). These keystone taxa of AMF played a vital role in module hubs of the rhizosphere co-occurrence network, accounting for about 33 % of the keystone taxa of AMF and diazotroph. The maximum *Pi* value in rhizosphere was AMF OTU_327, suggesting that AMF was also important in the network module. Intriguingly, diazotroph OTU_1132 was found in both rhizosphere and endosphere network hubs, and has a max degree and betweenness, indicating that OTU_1132 may crucial for maintaining the entire community integrity and stabilization.

Given that the proportion of the number of nodes in the rhizosphere is significantly different among three mangroves, we constructed representative networks of three mangroves separately to identify assemblages of potential interaction or share niches in rhizosphere and endosphere. The rhizosphere assemblages in *S. apetala* and *L. racemosa* formed larger and more complex networks with more nodes, links and keystone taxa than that in *A. marina*. Furthermore, the potential interactions between AMF-diazotroph communities in the rhizosphere of *S. apetala* and *L. racemosa* had higher positive connections than those in *A. marina* (Fig. S4). Noteworthy, the higher BNF rates were found in *S. apetala* and *L. racemosa* habitats, suggesting that these positive connections between diazotrophs and AMF could promote nitrogen fixation efficiency (Fig. S5).

3.3. Drivers of AMF and diazotroph communities

Pairwise spearman's correlation matrix of edaphic factors and Mantel tests were used to reveal the environmental factors driving AMF and diazotrophic communities assembly (Fig. 5a and b). Evidently, rhizophyte-endophyte diazotrophs and rhizosphere AMF communities were significantly ($P < 0.05$) correlated with sediment physicochemical factors (e.g., pH, salinity), nutrition components (e.g., TC, TN, Fe), NAG and LAP. Additionally, sediment pH has a strong negative correlation with the content of Fe and the activity of NAG and LAP (Fig. 5a and b). Variation partitioning analysis further indicated that sediment nutrition clearly contributed to a large proportion of variation to AMF (25.9 %) and diazotrophic (30.7 %) communities in the rhizosphere and diazotrophic (41.2 %) community in the endopshere (Fig. S6a, c and d). However, these three factors seem not to be the main driving factors of the endosphere AMF community consturction due to their low interpretations (Fig. S6b). Noteworthy, the combined explanation of sediment nutrition and sediment N-related enzymes to AMF and diazotrophic communities were 16.5 % and 19.5 % respectively, suggesting that the AMF-diazotroph communities in rhizosphere was more likely regulated by a joint effect of the two factors. Our linear regression analysis also showed that LAP and NAG were significantly ($P < 0.05$) positively correlated with TN and have significant ($P < 0.05$) negative

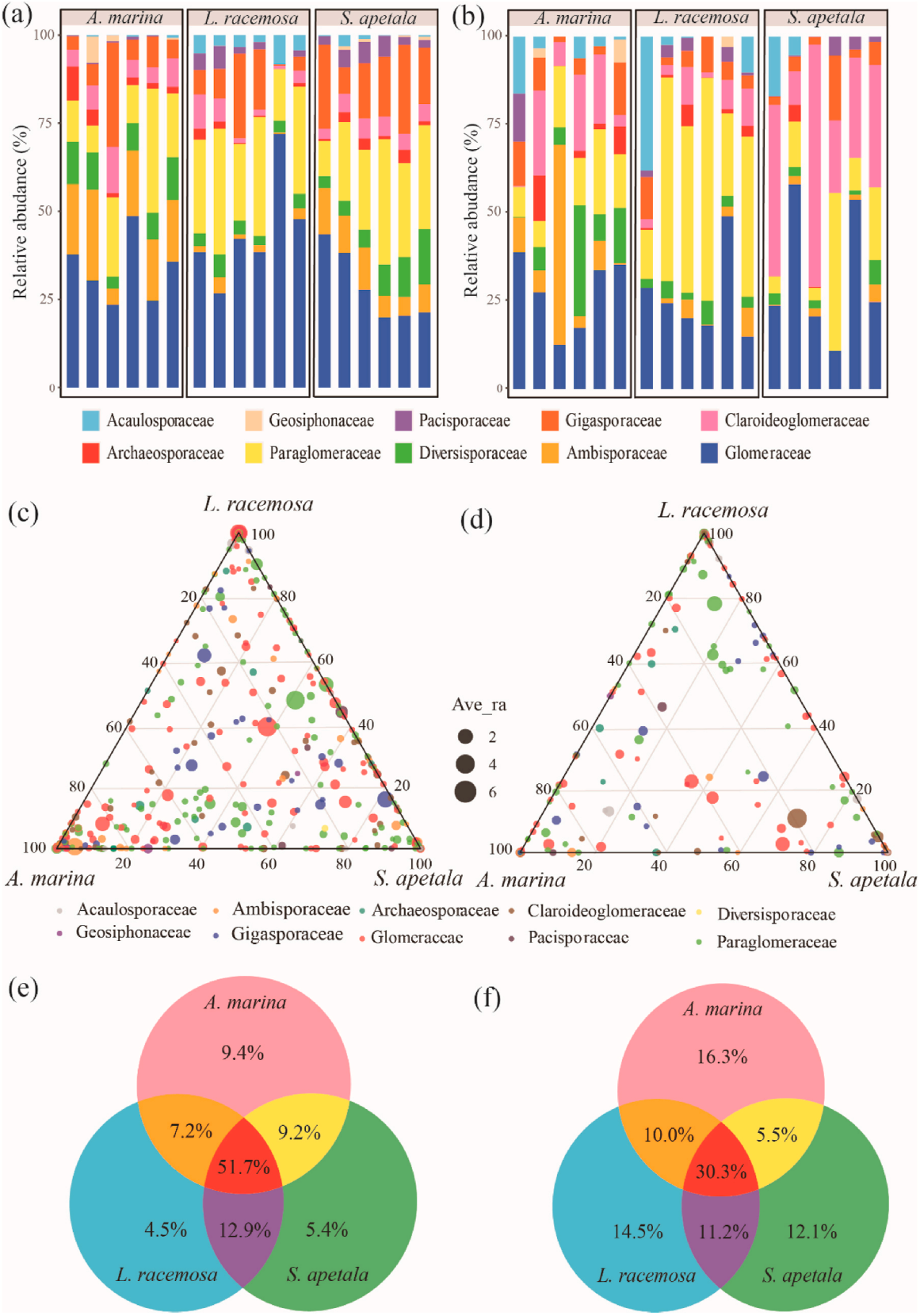

Fig. 2. AMF community composition and distribution traits in three mangroves. AMF community composition of the top ten families in rhizosphere (a) and endosphere (b). Ternary plots of all OTUs detected in rhizosphere (c) and endosphere (d). Each circle represents one OTU, and its size represents the relative abundance (weighted average). The position of each circle is determined by the contribution of the indicated compartments to the total relative abundance. Venn diagrams showing the percentage of shared and unique OTUs among three mangrove sediments in rhizosphere (e) and endosphere (f). AM, *A. marina*; LR, *L. racemosa*; SA, *S. apetala*.

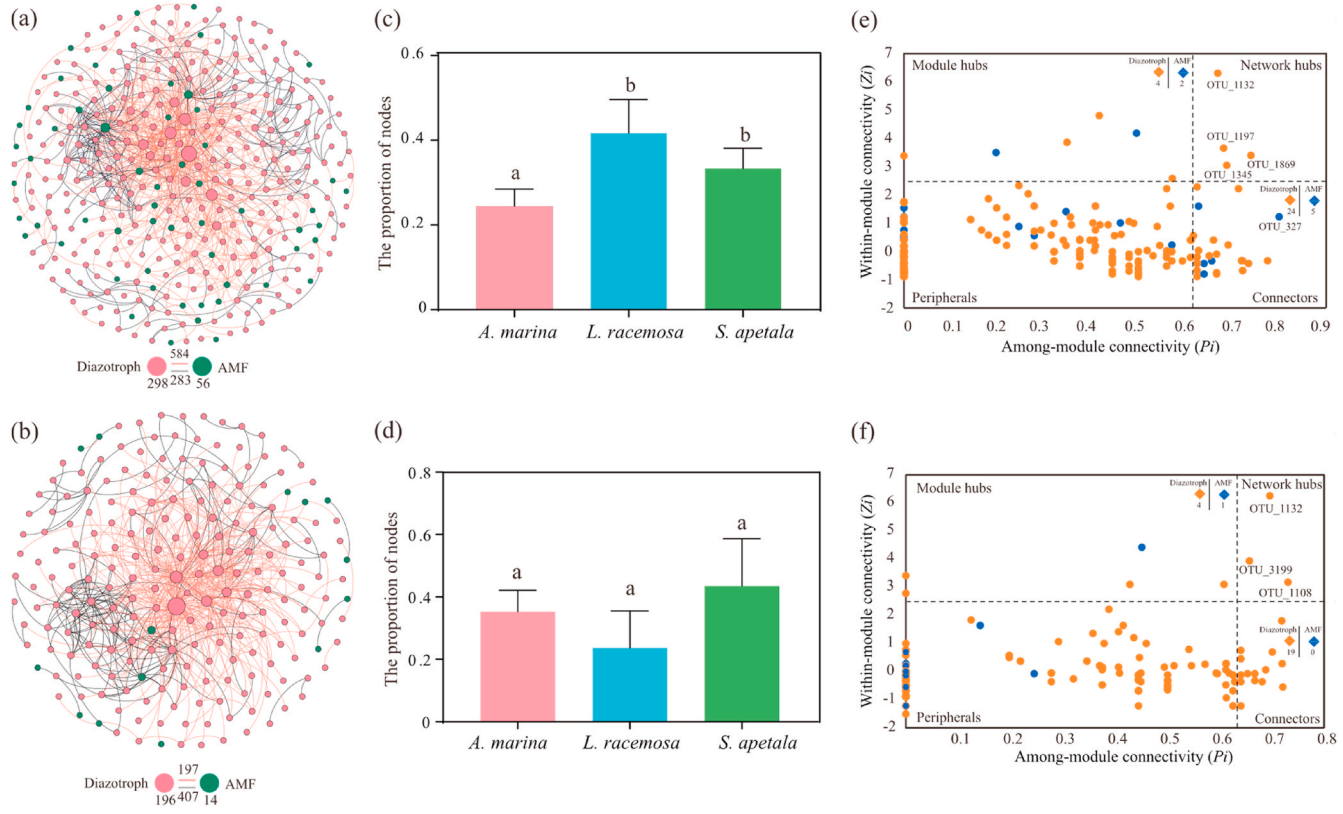

Fig. 3. Co-occurrence network patterns of AMF-diazotroph communities in the rhizosphere (a) and endosphere (b). A connection indicates a strong (Spearman's |r| 0.8 >) and significant ($P < 0.01$) correlation. The green lines represent positive correlations, while black lines represent negative correlations. The node size represents the degree of OTUs. Numbers represent the nodes or edge connections. The nodes proportion of three mangroves in the rhizosphere (c) and endosphere (d). The different letters indicate significant difference between mangroves ($P < 0.05$). *Zi-Pi* plots showed the distribution of OTUs based on their topological roles of networks in the rhizosphere (e) and endosphere (f). Threshold values of *Zi* and *Pi* for categorizing OTUs were 2.5 and 0.62, respectively. A summary of AMF-diazotroph OTUs statistics (only those with $Zi > 2.5$ or $Pi > 0.62$) were also given.

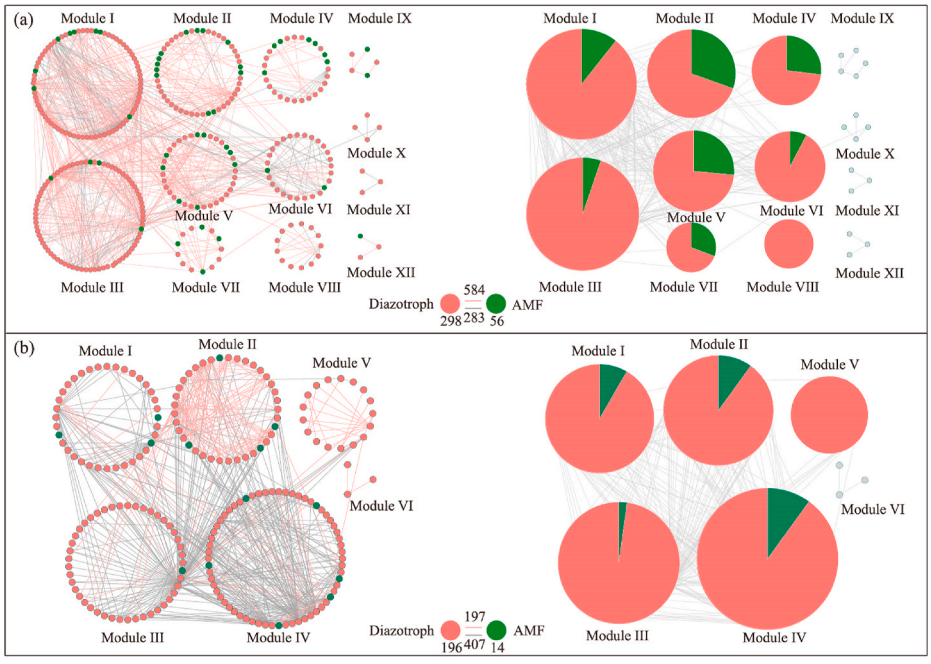

Fig. 4. Highly connected modules (nodes > 2) within rhizosphere (a) and endosphere (b) networks. The red nodes indicate OTUs of diazotroph and green nodes represent OTUs of AMF. The positive links between nodes were indicated in red, whereas negative links were indicated in gray. Numbers represent the nodes or edge connections, and the pie charts represent the composition of modules with >10 nodes.

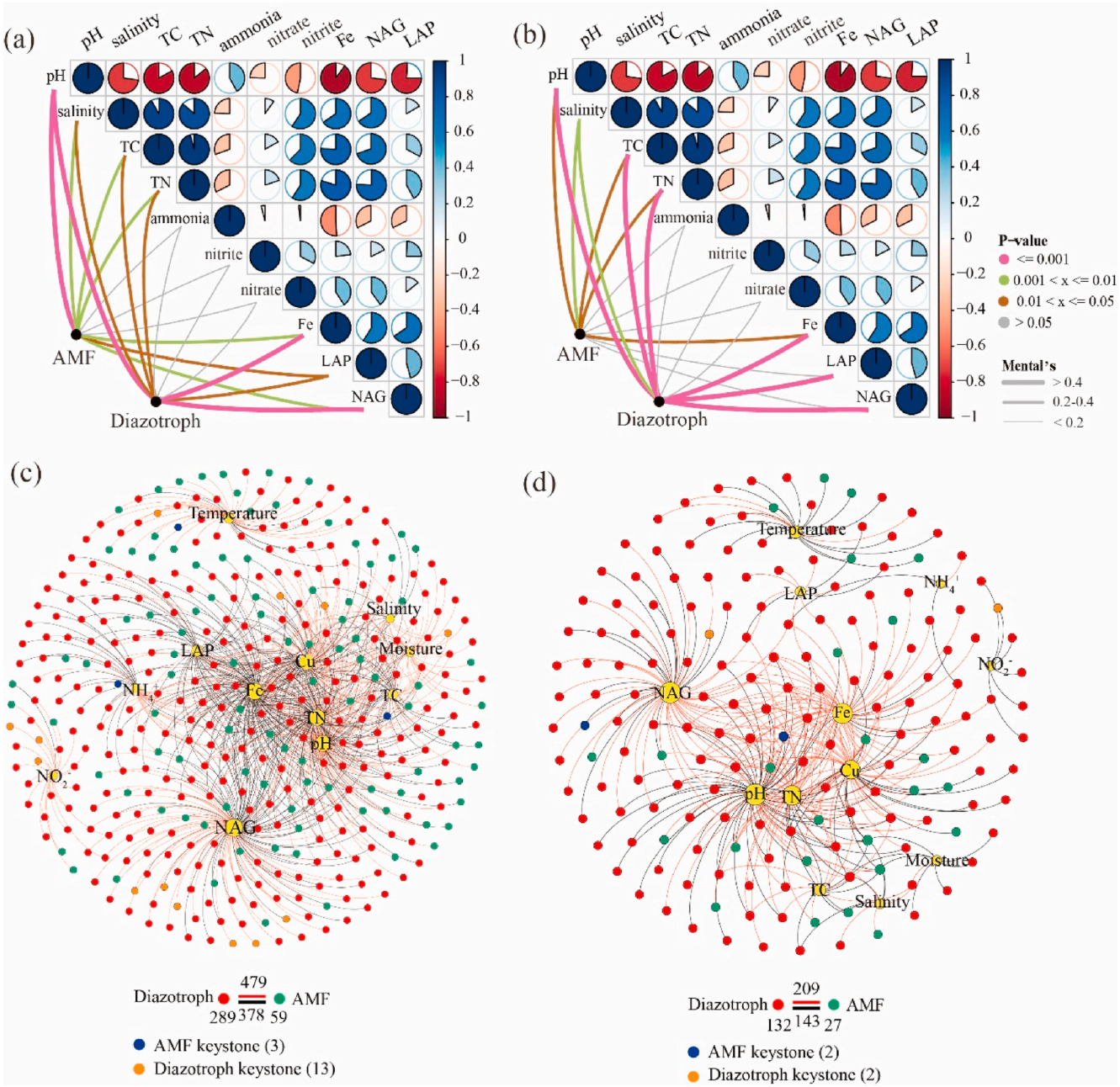

Fig. 5. Factors driving AMF-diazotroph communities in three mangrove species. Pairwise Spearman's correlation matrix of the edaphic factors was shown with pie charts, and diazotrophic community was related to each environmental factor and N-related enzyme by Mantel tests in the rhizosphere (a) and endosphere (b). Edge width means the Mantel's statistic, and edge color means the statistical significance. The AMF and diazotroph co-occurrence networks with the environmental parameters in the rhizosphere (c) and endosphere (d). The node size represents the degree of the OTUs. The red links represent positive correlations, while black links represent negative correlations. A summary of node-edge statistics was also given, and numbers represent the nodes or edges.

correlations with ammonia (Fig. S7). We also constructed the AMF and diazotroph co-occurrence networks with the environmental parameters in rhizosphere and endosphere (Fig. 5c and d). The results showed that pH, NAG, Fe and Cu formed the largest nodes, suggesting that they were the key factors to drive the AMF and diazotroph communities. In addition, the number of diazotroph and AMF keystone taxa linked with the environmental factors in the rhizosphere were more than that in the endosphere.

3.4. Biotic and edaphic factors affected nitrogen fixation function

We conducted a RF modeling to evaluated the biological contribution of keystone taxa to N fixation (Fig. 6). Interestingly, we found that the AMF keystone taxa made a large contribution to predicting N fixation in rhizosphere, accounting for 35 %. For example, AMF OTU_32 was the vital keystone taxa for predicting N fixation in rhizosphere. However, their roles in endosphere seemed weaker. In endosphere, diazotrophs are the main contributors to N fixation, and diazotroph OTU_1119 explained the largest proportion of variation. These results indicated that the potential keystone taxa were important for N fixation in mangrove ecosystems.

To better understand the relative contribution of biotic and edaphic factors to N fixation, we constructed a PLS-PM (Fig. S8). Generally, sediment properties had significant positive effects on sediment nutritions (path coefficients = 0.96, $P < 0.001$) (Fig. 7a), but were negatively related to keystone taxa of AMF and diazotroph (path coefficients =

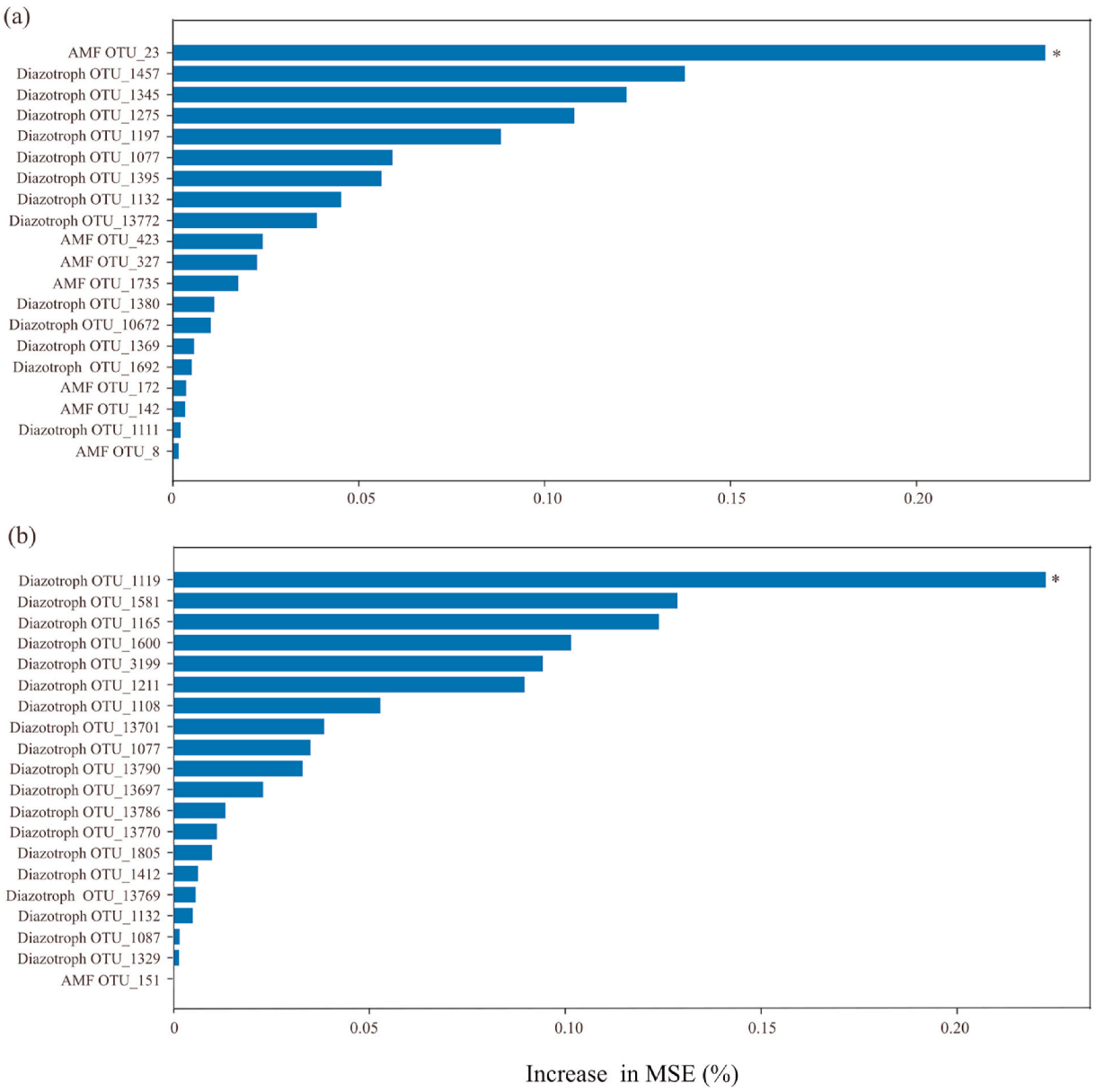

Fig. 6. Random forest analysis identifying the keystone taxa to predict the N fixation in the rhizosphere (a) and endosphere (b). The accuracy importance measure was computed for each tree and averaged over the forest (2000 trees). Percentage increases in the MSE (mean squared error) of variables were used to estimate the importance of these predictors, and higher MSE% values imply more important predictors. *$P < 0.05$.

-0.83, $P < 0.001$). Sediment nutritions showed significant positive relationships with the α-diversity of AMF and diazotroph (path coefficients = 0.55, $P < 0.001$; path coefficients = 0.60, $P < 0.001$). The α-diversity of AMF and diazotroph were positively correlated with sediment N enzymes (path coefficients = 0.78, $P < 0.001$; path coefficients = 0.46, $P < 0.001$) (Fig. 7a). Also, the α-diversity of AMF community showed significant positive relationships with N fixation and diazotroph (path coefficients = 0.48, $P < 0.001$; standardized effects = 0.34) (Fig. 7a), and showed significant positive with the diazotrophic α-diversity in rhizosphere (Fig. S9). Besides, our analysis suggested that the N-related enzymes showed the greatest positive effects on N fixation (standardized effects = 0.75), followed by the keystone taxa of AMF and diazotrophic communities (standardized effects = 0.55) (Fig. 7b). These results indicated that the α-diversity and keystone taxa of AMF and diazotrophic communities, N-related enzymes and sediment nutrition components were the main driving factors of the BNF process in mangrove ecosystems.

4. Discussion

The widespread symbiosis between AMF and mangrove roots implies their potential importance in the biogeochemical cycling of various elements in mangrove ecosystems. However, the contribution of AMF to N-fixing processes remains unclear, causing a knowledge gap in understanding BNF in the N-limited mangrove ecosystem (Wu et al., 2005; Hodge and Storer, 2015). In this study, we found that the rhizosphere and endosphere of different mangrove habitats harbored distinct AMF communities and the dominant AMF taxa. Also, diazotrophs and AMF communities predominantly formed positive co-occurrences, suggesting a facilitative species cooperation. Moreover, path analysis indicated that the α-diversity and keystone taxa of AMF community may affect N fixation by regulating the activities of N-related enzymes and improving the rhizosphere microenvironment (Nuccio et al., 2013).

Niche differentiation in different mangrove habitats is an important factor to govern AMF community (Dickie, 2007; Zobel and Öpik, 2014).

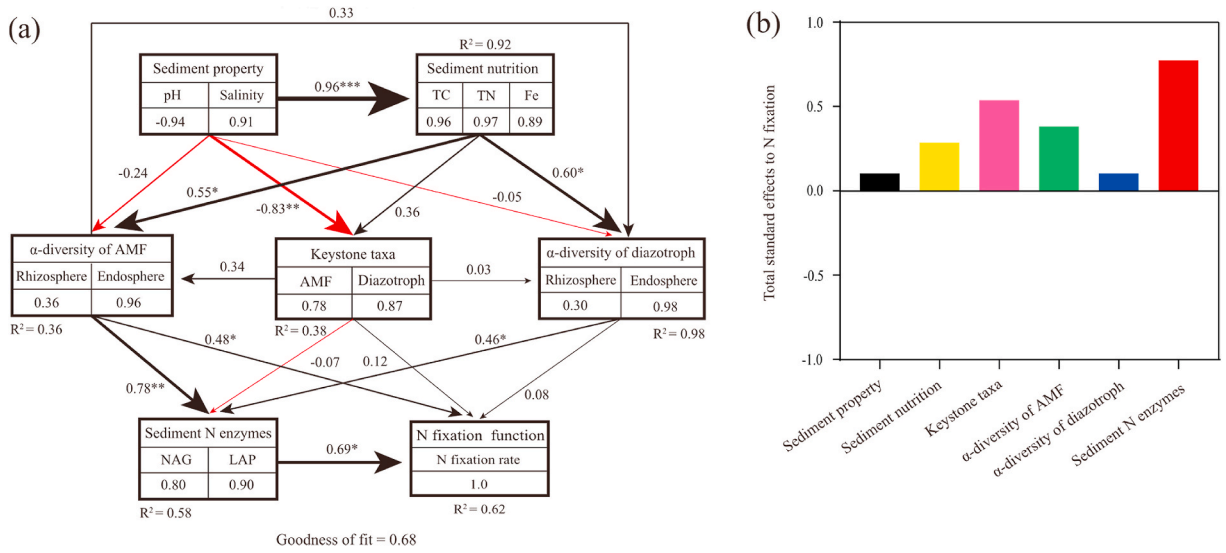

Fig. 7. Cascading relationships of the edaphic factors with N-related enzymes and N fixation in mangroves. Partial least squares path modeling (a) and standardized effects of factors (b) disentangling major pathways of the influences of the latent variables on sediments N fixation in the rhizosphere and endosphere. Each oblong box represents a latent variable and the parameter in each oblong box represents a manifest variable. Path coefficients and coefficients of determination (R^2) were calculated after 1000 bootstraps. Wider arrows indicate greater path coefficients, and black and red lines represent positive and negative effects, respectively. *, **, and *** represent $P < 0.05$, 0.01, and 0.001, respectively.

Root-related rhizophytes and endophytes represent a variety of plant-associated fungi with different evolutionary and trophic origins, which complicate the separation of factors driving fungal compositional shifts in ecosystems (Rodriguez et al., 2009; Neuenkamp et al., 2018). Current research believe that the microbial community succession is due to the comprehensive action of habitat and host plants and it is determined by the driver/passenger hypothesis and habitat hypothesis (Zobel and Öpik, 2014; Martinez-Garcia et al., 2015; Neuenkamp et al., 2018). We found that a consecutive and homogeneous selection govern the rhizosphere AMF and diazotrophs, suggesting that the selection of habitats effect on AMF partners in the rhizosphere of different mangrove may be converged. The rates of energy transformation and resources cycling in mangrove ecosystems are 3–4 times higher than those of other ecosystems (Van Groenigen et al., 2014; Yu et al., 2020b). Rapid nutrient cycling in the mangrove rhizosphere would result in a small difference between the AMF communities and other symbionts, thus reducing the limitation of mangrove species against AMF partners (Kiers et al., 2011). Additionally, the endophytic AMF taxa have a relatively strict host specificity (Wang et al., 2016). For example, *Paraglomeraceae* and *Claroideoglomeraceae* showed distinct host preferences in the endosphere of *L. racemosa* and *S. apetala*. The increased unique AMF OTUs were found in the endosphere of mangrove. One possible explanation is that the mangrove root surface constitutes a stronger filter (Sikes et al., 2014; Jiao et al., 2019). Moreover, different mangroves have unique root architecture, lifespan and rooting depth, all of which will contribute to a particular endosphere environment. Therefore, AMF purposefully selects the internal parasitic environment of the appropriate host (Krauss et al., 2003; Naylor et al., 2017).

Species co-occurrences can reveal the roles of interspecific interactions in determining the community structure and ecological functions (Abrego et al., 2020). In this study, diazotrophs and AMF predominantly formed positive co-occurrence networks, suggesting that the pattern reflects facilitative interactions. Besides, the BNF rate in the rhizosphere of three mangrove species increased with increasing of the positive connections between AMF and diazotrophs, suggesting that this pattern could promote nitrogen fixation efficiency in mangrove ecosystems. Although the associations between plant growth-promoting rhizobacteria and mycorrhizal fungi were confirmed, our findings further extend this knowledge to the root-associated functional bacteria and AMF (Frey-Klett et al., 2007; Abrego et al., 2020). AMF could be benefit for root-associated diazotrophs through improving nutrition and reducing adversities (Frey-Klett et al., 2007). The positive interspecific interactions reflected a positive synergistic response of root-associated AMF and diazotrophs to harsh mangrove environments (Welsh et al., 2009; Acuña-Rodríguez et al., 2020). Also, a significant positive correlation between the diversity/richness of AMF and diazotrophs community supports the conclusion. Therefore, cooperations rather than competitions between AMF and diazotrophs were the main interspecies relationship in mangrove ecosystems.

AMF-diazotrophs co-occurrences patterns may be mediated by their keystone taxa. The keystone taxa connected highly in networks can individually account for microbiome interactional turnover and highlights the crucial roles of keystone taxa in maintaining the integrity and the function of entire microbial community (Agler et al., 2016; Chen et al., 2019). We found that the keystone taxa of AMF and diazotrophs displayed intense interactions with their contacted members, and these interactions were mostly positive. Given the positive effects of AMF (e.g., *Frankiaceae*, *Pseudomonadaceae* and *Rhodospirillaceae*) on the abundance and α-diversity of diazotrophic community, AMF keystone taxa could promote the N-fixing ability of diazotrophs (Vázquez et al., 2000; Oliveira et al., 2005; Acuña-Rodríguez et al., 2020). For example, *Paraglomeraceae,* the AMF keystone taxa, were observed in AMF-diazotrophs co-occurrence networks in the rhizosphere. They could break down plant and microbial residues to obtain limited resources (Wang et al., 2015). Besides, they could alleviate soil aggregation to reduce the toxicity of metal ions (Faggioli et al., 2019). Thus, those keystone taxa play a crucial role to regulate N fixation process in whole AMF-diazotroph communities. Collectively, the facilitative interactions between the root-associated AMF and diazotrophs could promote their respective diversity and ecological functions.

Although our understanding of the roles of AMF in BNF has been improved recently, their potential impacts may be still grossly underestimated (Welsh et al., 2009; Veresoglou et al., 2012; Tsiknia et al., 2021). Our results found that AMF keystone taxa were the most important predictor to N fixation in mangrove rhizosphere, indicating that AMF may be an important facilitator of N fixers. Some AMF species can directly mediate the structure and composition of root-related diazotrophic communities to improve the N-fixing capacity of diazotrophs (Kaschuk et al., 2010; Tian et al., 2010). In addition, AMF can help diazotrophs gain soluble carbohydrates, mine nutrients, phosphate,

and inorganic N from the host plant (Talbot et al., 2008; Brzostek et al., 2015; Cheeke et al., 2017). Thus, the increased performance of N fixers in our study is predictable. Besides, additional N fixation may occur in AMF extraradical hyphae as its extraradical hyphae have a potential ability to fix atmospheric nitrogen (Minerdi et al., 2001). This may partially explain the direct effect of rhizospheric AMF on N fixation rate. Therefore, the ecological role of AMF is critical in BNF process in mangrove ecosystems.

AMF could mediate shifts of N fixation by N-related enzymes and edaphic factors (Xie et al., 2014; Coskun et al., 2017). AMF may serve as a connecting channel to facilitate the release of various enzymes to accelerate the transformation of nutrition in rhizosphere environments (Tian et al., 2010). Previous studies indicated that extra-radical hyphae of AMF can assimilate N into the form of NH_4^+ and NO_3^- through the glutamine synthetases and the nitrate reductases (Fitter et al., 2011; Kiers et al., 2011; Jian et al., 2016). We observed that AMF could affect N fixation via mediating sediment N-related enzyme activities. A reasonable speculation is that AMF can improve availability of sediment nutrition, which will contribute to the expression of genes encoding for N-related enzyme (Trivedi et al., 2016). However, the impacts of N-related enzymes on N fixation are not simply single-factor pathway effects. The synergistic effects of soil nutrients and N-related enzymes on N fixation could partly explain the variation of AMF and diazotrophic communities (Jian et al., 2016). Besides, pH and salinity could affect the mobility and availability of sediment nutrient. For example, a slightly acidic environment contributes to the decomposition of mangrove litters and high salinity inhibited it, both of which could affect N and C pools in mangrove ecosystems (Wang et al., 2018; Yu et al., 2020b). In turn, the nutrient levels and types of nutrient resources in sediments could regulate the structure of endophyte rhizophyte AMF and diazotrophic communities (Liu et al., 2020). Also, pH could directly affect diazotrophic community diversity and the keystone taxa. The optimal pH value for diazotrophs growth is between 7.0 and 8.0 and a low pH (5.02–7.00) could inhibit their nitrogenase activities (Wang et al., 2017). Taken together, N-related enzymes, sediment nutrition components and sediment properties may be critical for BNF in mangrove ecosystems.

5. Conclusions

This study expands our understanding of AMF community and their relationships with diazotrophs and their potential impacts on BNF in mangrove ecosystems (Fig. 8). The results indicated that the effects of mangrove habitats and host effects were the major forces driven the succession of AMF community in the rhizosphere and endosphere, respectively. We also found that the AMF community could affect the BNF process in mangrove ecosystems by regulating the microbial extracellular enzyme activity and modifying the mangrove rhizosphere microenvironment. Moreover, AMF and diazotrophs predominantly formed positive co-occurrences, which could promote N fixation efficiency in mangrove ecosystems. However, future studies are needed to evaluate their significance for plant growth. This study highlights the ecological significance of functional bacterial-fungal facilitative interactions in mangroves restoration.

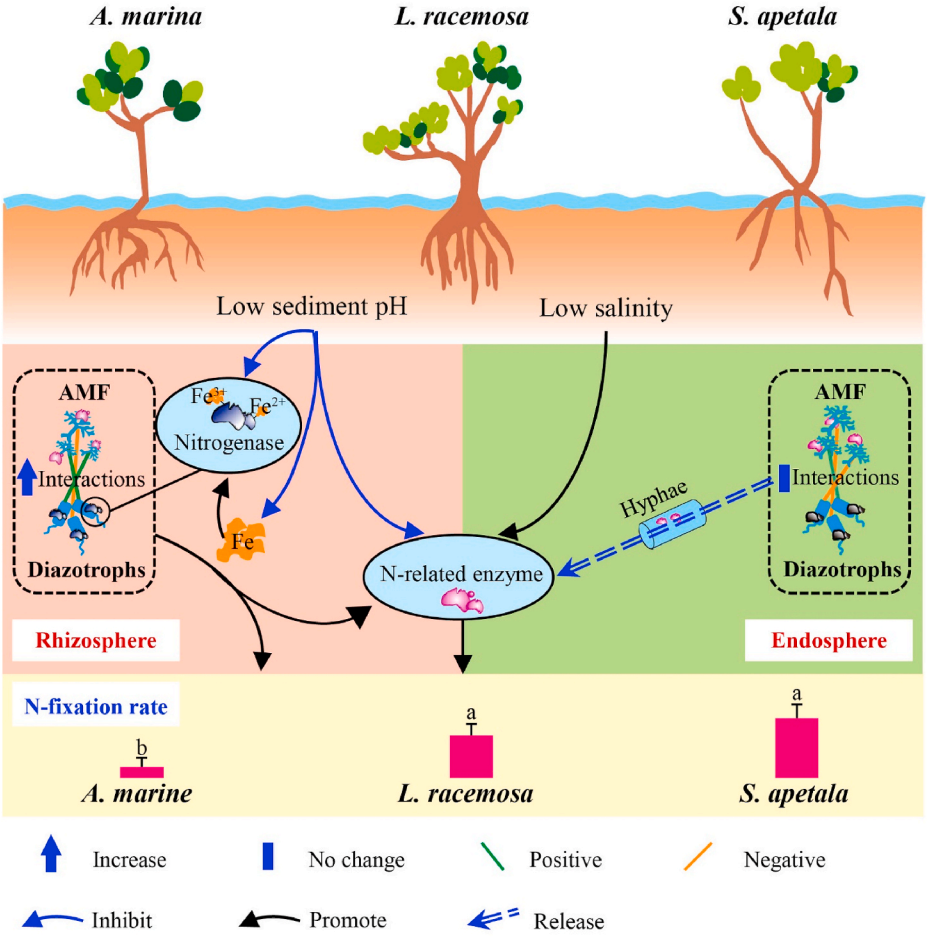

Fig. 8. A conceptual model of N fixation processes and their influence path in the rhizosphere of three mangroves. Solid lines indicate that such linkages are supported by experimental data in this study or literatures.

CRediT authorship contribution statement

Huang Yu: designed and conducted the experiments, Formal analysis. **Xingyu Liu:** designed and conducted the experiments, Formal analysis. **Chao Yang:** Writing – review & editing. **Yisheng Peng:** Writing – review & editing. **Xiaoli Yu:** Writing – review & editing. **Hang Gu:** Writing – review & editing. **Xiafei Zheng:** Writing – review & editing. **Cheng Wang:** Writing – review & editing. **Fanshu Xiao:** Writing – review & editing. **Longfei Shu:** Writing – review & editing, All authors read and approved the final manuscript. **Zhili He:** Writing – review & editing. **Bo Wu:** Writing – review & editing. **Qingyun Yan:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2021.108382.

References

Abrego, N., Roslin, T., Huotari, T., Tack, A.J., Lindahl, B.D., Tikhonov, G., Somervuo, P., Schmidt, N.M., Ovaskainen, O., 2020. Accounting for environmental variation in co-occurrence modelling reveals the importance of positive interactions in root-associated fungal communities. Molecular Ecology 29, 2736–2746.
Achatz, M., Rillig, M.C., 2014. Arbuscular mycorrhizal fungal hyphae enhance transport of the allelochemical juglone in the field. Soil Biology and Biochemistry 78, 76–82.
Acuña-Rodríguez, I.S., Newsham, K.K., Gundel, P.E., Torres-Diaz, C., Molina-Montenegro, M.A., 2020. Functional roles of microbial symbionts in plant cold tolerance. Ecology Letters 23, 1034–1048.
Adlakha, N., Sawant, S., Anil, A., Lali, A., Yazdani, S.S., 2012. Specific fusion of β-1, 4-endoglucanase and β-1, 4-glucosidase enhances cellulolytic activity and helps in channeling of intermediates. Applied and Environmental Microbiology 78, 7447–7454.
Agler, M.T., Ruhe, J., Kroll, S., Morhenn, C., Kim, S.T., Weigel, D., Kemen, E.M., 2016. Microbial hub taxa link host and abiotic factors to plant microbiome variation. PLoS Biology 14, e1002352.
Arfi, Y., Chevret, D., Henrissat, B., Berrin, J.G., Levasseur, A., Record, E., 2013. Characterization of salt-adapted secreted lignocellulolytic enzymes from the mangrove fungus *Pestalotiopsis* sp. Nature Communications 4, 1–9.
Barberan, A., Bates, S.T., Casamayor, E.O., Fierer, N., 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. The ISME Journal 6, 343–351.
Barberán, A., Ramirez, K.S., Leff, J.W., Bradford, M.A., Wall, D.H., Fierer, N., 2014. Why are some microbes more ubiquitous than others? Predicting the habitat breadth of soil bacteria. Ecology Letters 17, 794–802.
Bastian, M., Heymann, S., Jacomy, M., 2009. Gephi: an Open Source Software for Exploring and Manipulating Networks. AAAI Press, Menlo Park, California.
Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120.
Bonfante, P., Genre, A., 2008. Plants and arbuscular mycorrhizal fungi: an evolutionary-developmental perspective. Trends in Plant Science 13, 492–498.
Brzostek, E.R., Dragoni, D., Brown, Z.A., Phillips, R.P., 2015. Mycorrhizal type determines the magnitude and direction of root-induced changes in decomposition in a temperate forest. New Phytologist 206, 1274–1282.
Cheeke, T.E., Phillips, R.P., Brzostek, E.R., Rosling, A., Bever, J.D., Fransson, P., 2017. Dominant mycorrhizal association of trees alters carbon and nutrient cycling by selecting for microbial groups with distinct enzyme function. New Phytologist 214, 432–442.
Chen, L., Jiang, Y., Liang, C., Luo, Y., Xu, Q., Han, C., Zhao, Q.G., Sun, B., 2019. Competitive interaction with keystone taxa induced negative priming under biochar amendments. Microbiome 7, 1–18.
Cordeiro, C., Costa, T.M., 2010. Evaluation of solid residues removed from a mangrove swamp in the São Vicente Estuary, SP, Brazil. Marine Pollution Bulletin 60, 1762–1767.
Coskun, D., Britto, D.T., Shi, W., Kronzucker, H.J., 2017. How plant root exudates shape the nitrogen cycle. Trends in Plant Science 22, 661–673.
Cornwell, W.K., Bedford, B.L., Chapin, C.T., 2001. Occurrence of arbuscular mycorrhizal fungi in a phosphorus-poor wetland and mycorrhizal response to phosphorus fertilization. American Journal of Botany 88, 1824–1829.
D'Souza, J., Rodrigues, B.F., 2013. Biodiversity of arbuscular mycorrhizal (AM) fungi in mangroves of Goa in West India. Journal of Forestry Research 24, 515–523.
Davison, J., Moora, M., Öpik, M., Adholeya, A., Ainsaar, L., Bâ, A., Burla, S., Diedhiou, A. G., Hiiesalu, I., Jairus, T., Johnson, N.C., Kane, A., Koorem, K., Kochar, M., Ndiaye, C., Pärtel, M., Reier, Ü., Saks, Ü., Singh, R., Vasar, M., Zobel, M., 2015. Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. Science 349, 970–973.
DeForest, J.L., 2009. The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and l-DOPA. Soil Biology and Biochemistry 41, 1180–1186.
Deng, Y., Jiang, Y.H., Yang, Y., He, Z., Luo, F., Zhou, J., 2012. Molecular ecological network analyses. BMC Bioinformatics 13, 1–20.
Dickie, I.A., 2007. Host preference, niches and fungal diversity. New Phytologist 174, 230–233.
Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200.
Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods 10, 996–998.
Faggioli, V., Menoyo, E., Geml, J., Kemppainen, M., Pardo, A., Julieta Salazar, M., Becerra, A.G., 2019. Soil lead pollution modifies the structure of arbuscular mycorrhizal fungal communities. Mycorrhiza 29, 363–373.
Fitter, A.H., Helgason, T., Hodge, A., 2011. Nutritional exchanges in the arbuscular mycorrhizal symbiosis: implications for sustainable agriculture. Fungal.Biol.Rev. 25, 68–72.
Fortmann-Roe, S., 2015. Consistent and clear reporting of results from diverse modeling techniques: the A3 method. Journal of Statistical Software 66, 1–23.
Frey-Klett, P., Garbaye, J., Tarkka, M., 2007. The mycorrhiza helper bacteria revisited. New Phytologist 176, 22–36.
Friess, D.A., Krauss, K.W., Horstman, E.M., Balke, T., Bouma, T.J., Galli, D., Webb, E.L., 2012. Are all intertidal wetlands naturally created equal? Bottlenecks, thresholds and knowledge gaps to mangrove and saltmarsh ecosystems. Biological Reviews 87, 346–366.
German, D.P., Weintraub, M.N., Grandy, A.S., Lauber, C.L., Rinkes, Z.L., Allison, S.D., 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biology and Biochemistry 43, 1387–1397.
Gómez-Brandón, M., Juárez, M.F.D., Zangerle, M., Insam, H., 2016. Effects of digestate on soil chemical and microbiological properties: a comparative study with compost and vermicompost. Journal of Hazardous Materials 302, 267–274.
Gu, T.Y., Yu, H., Li, F., Zeng, W.M., Wu, X.L., Shen, L., Liu, Y.D., Yu, R.L., Liu, Y.D., Li, J. K., 2020. Antimony-oxidizing bacteria alleviate Sb stress in Arabidopsis by attenuating Sb toxicity and reducing Sb uptake. Plant and Soil 452, 397–412.
Han, L.L., Wang, Q., Shen, J.P., Di, H.J., Wang, J.T., Wei, W.X., He, J.Z., 2019. Multiple factors drive the abundance and diversity of the diazotrophic community in typical farmland soils of China. FEMS Microbiology Ecology 95 fiz113.
Hodge, A., Storer, K., 2015. Arbuscular mycorrhiza and nitrogen: implications for individual plants through to ecosystems. Plant and Soil 386, 1–19.
Holguin, G., Vazquez, P., Bashan, Y., 2001. The role of sediment microorganisms in the productivity, conservation, and rehabilitation of mangrove ecosystems: an overview. Biology and Fertility of Soils 33, 265–278.
Hyde, K.D., Lee, S.Y., 1995. Ecology of mangrove fungi and their role in nutrient cycling: what gaps occur in our knowledge? Hydrobiologia 295, 107–118.
Jian, S., Li, J., Chen, J., Wang, G., Mayes, M.A., Dzantor, K.E., Luo, Y., 2016. Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: a meta-analysis. Soil Biology and Biochemistry 101, 32–43.
Jiao, S., Xu, Y., Zhang, J., Lu, Y., 2019. Environmental filtering drives distinct continental atlases of soil archaea between dryland and wetland agricultural ecosystems. Microbiome 7, 1–13.
Kaschuk, G., Leffelaar, P.A., Giller, K.E., Alberton, O., Hungria, M., Kuyper, T.W., 2010. Responses of legumes to rhizobia and arbuscular mycorrhizal fungi: a meta-analysis of potential photosynthate limitation of symbioses. Soil Biology and Biochemistry 42, 125–127.
Kiers, E.T., Duhamel, M., Beesetty, Y., Mensah, J.A., Franken, O., Verbruggen, E., Fellbaum, C.R., Kowalchuk, G.A., Hart, M.M., Bago, A., Palmer, T.M., West, S.A., Vandenkoornhuyse, P., Jansa, J., ücking, H., 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science 333, 880–882.
Kong, Y., 2011. Btrim: a fast, lightweight adapter and quality trimming program for next-generation sequencing technologies. Genomics 98, 152–153.
Kothamasi, D., Kothamasi, S., Bhattacharyya, A., Kuhad, R.C., Babu, C.R., 2005. Arbuscular mycorrhizae and phosphate solubilising bacteria of the rhizosphere of the mangrove ecosystem of Great Nicobar island, India. Biology and Fertility of Soils 42, 358–361.
Krauss, K.W., Allen, J.A., Cahoon, D.R., 2003. Differential rates of vertical accretion and elevation change among aerial root types in Micronesian mangrove forests. Estuarine, Coastal and Shelf Science 56, 251–259.

Liaw, A., Wiener, M., 2002. Classification and regression by randomForest. R News 2, 8–22.
Liu, X.Y., Yang, C., Yu, X., Yu, H., Zhuang, W., Gu, H., Xu, K., Zheng, X.F., Wang, C., Xiao, F.S., Wu, B., He, Z., Yan, Q.Y., 2020. Revealing structure and assembly for rhizophyte-endophyte diazotrophic community in mangrove ecosystem after introduced *Sonneratia apetala* and *Laguncularia racemosa*. The Science of the Total Environment 721, 137807.
Lumini, E., Orgiazzi, A., Borriello, R., Bonfante, P., Bianciotto, V., 2010. Disclosing arbuscular mycorrhizal fungal biodiversity in soil through a land-use gradient using a pyrosequencing approach. Environmental Microbiology 12, 2165–2179.
Ma, B., Zhao, K., Lv, X., Su, W., Dai, Z., Gilbert, J.A., Brookes, P.C., Faust, K., Xu, J.M., 2018. Genetic correlation network prediction of forest soil microbial functional organization. The ISME Journal 12, 2492–2505.
Mo, Y., Peng, F., Gao, X., Gao, X.F., Logares, R., Jeppesen, E., Ren, K.X., Xue, Y.Y., Yang, J., 2021. Low shifts in salinity determined assembly processes and network stability of microeukaryotic plankton communities in a subtropical urban reservoir. Microbiome 9, 1–17.
Montesinos-Navarro, A., Segarra-Moragues, J.G., Valiente-Banuet, A., Verdu, M., 2012. Plant facilitation occurs between species differing in their associated arbuscular mycorrhizal fungi. New Phytologist 196, 835–844.
Magoč, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27, 2957–2963.
Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.J. 17, 10–12.
Martinez-Garcia, L.B., Richardson, S.J., Tylianakis, J.M., Peltzer, D.A., Dickie, I.A., 2015. Host identity is a dominant driver of mycorrhizal fungal community composition during ecosystem development. New Phytologist 205, 1565–1576.
Minerdi, D., Fani, R., Gallo, R., Boarino, A., Bonfante, P., 2001. Nitrogen fixation genes in an endosymbiotic *Burkholderia* strain. Applied and Environmental Microbiology 67, 725–732.
Murdiyarso, D., Purbopuspito, J., Kauffman, J.B., Warren, M.W., Sasmito, S.D., Donato, D.C., Manuri, S., Krisnawati, Haruni, Taberima, Sartji, Kurnianto, Sofyan, 2015. The potential of Indonesian mangrove forests for global climate change mitigation. Nature Climate Change 5, 1089–1092.
Naylor, D., DeGraaf, S., Purdom, E., Coleman-Derr, D., 2017. Drought and host selection influence bacterial community dynamics in the grass root microbiome. The ISME Journal 11, 2691–2704.
Neuenkamp, L., Moora, M., Opik, M., Davison, J., Gerz, M., Mannisto, M., Jairus, T., Vasar, M., Zobel, M., 2018. The role of plant mycorrhizal type and status in modulating the relationship between plant and arbuscular mycorrhizal fungal communities. New Phytologist 220, 1236–1247.
Newman, M.E.J., 2006. Modularity and community structure in networks. Proceedings of the National Academy of Sciences of the United States of America 103, 8577–8582.
Nuccio, E.E., Hodge, A., Pett-Ridge, J., Herman, D.J., Weber, P.K., Firestone, M.K., 2013. An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. Environmental Microbiology 15, 1870–1881.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. Package 'vegan'. Community Ecology Package. Version 2.
Oliveira, R.S., Castro, P.M.L., Dodd, J.C., Vosátka, M., 2005. Synergistic effect of Glomus intraradices and *Frankia spp.* on the growth and stress recovery of *Alnus glutinosa* in an alkaline anthropogenic sediment. Chemosphere 60, 1462–1470.
Opik, M., Vanatoa, A., Vanatoa, E., Moora, M., Davison, J., Kalwij, J.M., Reier, U., Zobel, M., 2010. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (*Glomeromycota*). New Phytologist 188, 223–241.
Peña, T.C.D.T., Fedorova, E., Pueyo, J.J., Lucas, M.M., 2018. The Symbiosome: legume and rhizobia co-evolution toward a nitrogen-fixing organelle? Frontiers of Plant Science 8, 2229.
Poly, F., Monrozier, L.J., Bally, R., 2001. Improvement in the RFLP procedure for studying the diversity of nifH genes in communities of nitrogen fixers in soil. Research in Microbiology 152, 95–103.
Reef, R., Feller, I.C., Lovelock, C.E., 2010. Nutrition of mangroves. Tree Physiology 30, 1148–1160.
Rodriguez, R.J., White, J.F., Arnold, A.E., Redman, R.S., 2009. Fungal endophytes: diversity and functional roles. New Phytologist 182, 314–330.
Sanchez, G., 2013. PLS Path Modeling with R, 383. Trowchez Editions, Berkeley.
Schmit, J.P., Shearer, C.A., 2004. Geographic and host distribution of lignicolous mangrove microfungi. Botanica Marina 47, 496–500.
Shi, S., Nuccio, E.E., Shi, Z.J., He, Z., Zhou, J., Firestone, M.K., 2016. The interconnected rhizosphere: high network complexity dominates rhizosphere assemblages. Ecology Letters 19, 926–936.
Sikes, B.A., Maherali, H., Klironomos, J.N., 2014. Mycorrhizal fungal growth responds to soil characteristics, but not host plant identity, during a primary lacustrine dune succession. Mycorrhiza 24, 219–226.
Sun, W., Xiao, E., Krumins, V., Häggblom, M.M., Dong, Y.R., Pu, Z.L., Li, B.Q., Wang, Q., Xiao, T.F., Li, F.B., 2018. Rhizosphere microbial response to multiple metal(loid) s in different contaminated arable soils indicates crop-specific metal-microbe interactions. Applied and Environmental Microbiology 84, 701–718.
Talbot, J.M., Allison, S.D., Treseder, K.K., 2008. Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. Functional Ecology 22, 955–963.
Tian, C., Kasiborski, B., Koul, R., Lammers, P.J., Bücking, H., Shachar-Hill, Y., 2010. Regulation of the nitrogen transfer pathway in the arbuscular mycorrhizal symbiosis: gene characterization and the coordination of expression with nitrogen flux. Plant Physiology 153, 1175–1187.
Tsiknia, M., Tsikou, D., Papadopoulou, K.K., Ehaliotis, C., 2021. Multi-species relationships in legume roots: from pairwise legume-symbiont interactions to the plant–microbiome–soil continuum. FEMS Microbiology Ecology 97 fiaa222.
Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hu, H.W., Anderson, I.C., Jeffries, T.C., Zhou, J.Z., Singh, B., 2016. Microbial regulation of the soil carbon cycle: evidence from gene-enzyme relationships. The ISME Journal 10, 2593–2604.
Van Groenigen, K.J., Qi, X., Osenberg, C.W., Luo, Y., Hungate, B.A., 2014. Faster decomposition under increased atmospheric CO_2 limits soil carbon storage. Science 344, 508–509.
Vázquez, M.M., César, S., Azcón, R., Barea, J.M., 2000. Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum, Pseudomonas, Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. Applied Soil Ecology 15, 261–272.
Veresoglou, S.D., Chen, B., Rillig, M.C., 2012. Arbuscular mycorrhiza and soil nitrogen cycling. Soil Biology and Biochemistry 46, 53–62.
Wang, B.H., Zheng, X.F., Zhang, H.J., Xiao, F.S., Gu, H., Zhang, K.K., He, Z., Liu, X., Yan, Q.Y., 2020. Bacterial community responses to tourism development in the xixi national wetland park, China. The Science of the Total Environment 720, 137570.
Wang, H., Gilbert, J.A., Zhu, Y., Yang, X., 2018. Salinity is a key factor driving the nitrogen cycling in the mangrove sediment. The Science of the Total Environment 631, 1342–1349.
Wang, Q., Quensen, J.F., Fish, J.A., Lee, T.K., Sun, Y., Tiedje, J.M., Cole, J.R., 2013. Ecological patterns of *nifH* genes in four terrestrial climatic zones explored with targeted metagenomics using FrameBot, a new informatics tool. mBio 4 e00592-00513.
Wang, Y., Qiu, Q., Yang, Z., Hu, Z., Tam, N.F.Y., Xin, G., 2009. Arbuscular mycorrhizal fungi in two mangroves in South China. Plant and Soil 331, 181–191.
Wang, Y., Li, T., Li, Y., Qiu, Q., Li, S., Xin, G., 2015. Distribution of arbuscular mycorrhizal fungi in four semi-mangrove plant communities. Annals of Microbiology 65, 603–610.
Wang, Y., Li, C., Kou, Y., Wang, J., Tu, B., Li, H., Li, X.Z., Wang, C.T., Yao, M.J., 2017. Soil pH is a major driver of soil diazotrophic community assembly in Qinghai-Tibet alpine meadows. Soil Biology and Biochemistry 115, 547–555.
Wang, Y., Li, Y., Bao, X., Björn, L., Li, S., Olsson, P., 2016. Response differences of arbuscular mycorrhizal fungi communities in the roots of an aquatic and a semiaquatic species to various flooding regimes. Plant and Soil 403, 361–373.
Welsh, A.K., Burke, D.J., Hamerlynck, E.P., Hahn, D., 2009. Seasonal analyses of arbuscular mycorrhizae, nitrogen-fixing bacteria and growth performance of the salt marsh grass *Spartina patens*. Plant and Soil 330, 251–266.
Wu, S.C., Cao, Z.H., Li, Z.G., Cheung, K.C., Wong, M.H., 2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma 125, 155–166.
Wu, M.X., He, Z.Y., Fung, S.T., Cao, Y.J., Guan, D.S., Peng, Y.S., Lee, S.Y., 2020. Species choice in mangrove reforestation may influence the quantity and quality of long-term carbon sequestration and storage. The Science of the Total Environment 714, 136742.
Xie, X.Y., Weng, B.S., Cai, B.P., Dong, Y.R., Yan, C.L., 2014. Effects of arbuscular mycorrhizal inoculation and phosphorus supply on the growth and nutrient uptake of *Kandelia obovata* (Sheue, Liu & Yong) seedlings in autoclaved soil. Applied Soil Ecology 75, 162–171.
Xu, Z., Yu, G., Zhang, X., He, N., Wang, Q., Wang, S., Wang, R., Zhao, N., Jia, Y., Wang, C., 2017. Soil enzyme activity and stoichiometry in forest ecosystems along the North-South Transect in eastern China (NSTEC). Soil Biology and Biochemistry 104, 152–163.
Yu, H., Yan, X.Z., Zheng, X.F., Xu, K., Zhong, Q.P., Yang, T., Liu, F.F., Wang, C., Shu, L.F., He, Z., Xiao, F.S., Yan, Q.Y., 2020a. Differential distribution of and similar biochemical responses to different species of arsenic and antimony in *Vetiveria zizanioides*. Environmental Geochemistry and Health 42, 3995–4010.
Yu, H., Zheng, X.F., Weng, W.L., Yan, X.Z., Chen, P.B., Liu, X.Y., Peng, T., Zhong, Q.P., Xu, K., Wang, C., Shu, L.F., Yang, T., Xiao, F.S., He, Z., Yan, Q.Y., 2021. Synergistic effects of antimony and arsenic contaminations on bacterial, archaeal and fungal communities in the rhizosphere of Miscanthus sinensis: insights for nitrification and carbon mineralization. Journal of Hazardous Materials 411, 125094.
Yu, X.L., Yang, X.Q., Wu, Y.J., Peng, Y.S., Yang, T., Xiao, F.S., Zhong, Q.P., Xu, K., Shu, L. F., He, Q., Tian, Y., Yan, Q.Y., Wang, C., Wu, B., He, Z., 2020b. *Sonneratia apetala* introduction alters methane cycling microbial communities and increases methane emissions. Soil Biology and Biochemistry 144, 107775.
Zhou, J., Bruns, M.A., Tiedje, J.M., 1996. DNA recovery from soils of diverse composition. Applied and Environmental Microbiology 62, 316–322.
Zobel, M., Öpik, M., 2014. Plant and arbuscular mycorrhizal fungal (AMF) communities–which drives which? Journal of Vegetation Science 25, 1133–1140.