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ORIGINAL PAPER

Diversity and co-occurrence network modularization of bacterial communities determine soil fertility and crop yields in arid fertigation agroecosystems

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Abstract

Irrigation and fertilization practices can improve crop productivity in agroecosystems; however, the role of soil biodiversity in regulating crop production is not well understood. This restricts our ability to understand how changes in soil biodiversity impact soil function and crop productivity. To address this, a 4-year field experiment was conducted in China using three levels of irrigation [high (400 mm), medium (300 mm), and low (200 mm)] and two levels of fertilization [high (600 kg/ha P_2O_5 + 300 kg/ha urea) and low (300 kg/ha P_2O_5 + 150 kg/ha urea)] in arid farmland, to investigate maize production, soil properties, bacterial and fungal communities (diversity, compositions, N-cycling potentials, and co-occurrence networks), and their associations. The results showed that irrigation and fertilization had significant effects on bacterial and fungal community diversity, N-cycling potentials, and co-occurrence network patterns during maize growth. The combined treatment of medium irrigation and low fertilization resulted in higher maize yields, bacterial diversity, nitrification, ammoxidation, N-fixation potentials, and network modularity, compared with the other treatments. Strong and positive associations were observed between the soil N-cycling potentials, maize yields, and bacterial diversity; soils supporting a large number of bacterial taxa with co-occurrence network modularity had high soil nutrient levels (organic C and inorganic N) and maize yields. Structural equation modeling demonstrated that bacteria exhibited higher contribution to soil fertility and maize yields than fungi, and irrigation and fertilization indirectly affected microbial functions by altering bacterial diversity and network modularization, which also affected soil fertility and maize yields. These results highlight the importance of microbial diversity and their co-occurrence networks for maintaining soil fertility and crop production and will improve future irrigation and fertilization practices in arid agroecosystems and provide guidance for sustainable crop production.

Keywords Irrigation · Fertilization · Microbial diversity · Potential functions · Co-occurrence network

Introduction

Microorganisms play an important role in the regulation of multiple ecosystem services, including nutrient cycling and plant growth promotion. For instance, they can increase soil organic matter and total nitrogen (N) content through N fixation and ammonification (Richardson et al. 2009) and enhance plant disease and stress resistance by inducing hormone production (Bharti et al. 2015). However, how soil microbial diversity contributes to crop yield and soil function remains unclear (Fan et al. 2020), especially in arid agroecosystems where soil fertility and water supply are poor. Microorganisms are sensitive to variations in their living environment, including soil nutrients (Sánchez-Cañizares et al. 2017), pH (Wu et al. 2017), soil physical properties (Cui et al. 2018), and plant diversity (Zhang et al.

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2019). Among these, soil nutrients and water content are the two most important factors affecting soil microbial diversity and function. With the enhancement of soil carbon (C) and N contents, the microbial N-fixation ability was improved as the *nifH* gene abundance increased (Levy-Booth and Winder 2010), and the abundance of soil-ammonia oxidizing bacteria was also enhanced (Shen et al. 2011). Additionally, elevated soil moisture may enhance the activities of bacteria involved in ammonia oxidization within limits, but overly high moisture might restrict oxygen transport and thereby inhibit ammonia oxidization (Li et al. 2021). Previous studies have demonstrated that the compositions of soil bacterial and fungal communities were strongly associated with soil C and N (DeForest et al. 2004; Merila et al. 2010), water content (Zhang et al. 2018a), and oxygen levels (Spietz et al. 2015). Consequently, it is expected that regulating the soil nutrient levels and water content via fertilization and irrigation may cause changes in the diversity, composition and functions of soil microbial communities, and this may have potential consequences for crop productivity.

As arid agroecosystems have weak fertility and water supply capacities, fertilization and irrigation are widely used to promote crop yields, resulting in remarkable changes in the composition and functions of soil microorganisms. However, the mechanisms of soil microbial feedback in response to moisture and fertility remain unclear. Microbial responses to increased N inputs are often mixed and lack consistency. For instance, Zeng et al. (2016) conducted a fertilization experiment in the grasslands of Inner Mongolia and found that bacterial diversity of the top 0–10-cm soil layer decreased after N application (> 120 kg N ha^{-1} yr^{-1}). However, Fierer et al. (2012) found that N enrichment had no effect on bacterial diversity in grasslands. Additionally, some studies have suggested that improving soil nutrient levels can lead to improvements in microbial organic matter decomposition and N transformation. Fog (1988) found that addition of N could improve microbial activity and induce microorganisms to produce more enzymes, thereby accelerating the decomposition of organic matter. Jung et al. (2011) indicated that N additions increased the abundance of the *nifH* gene in the soil, thereby improving the ability of microbial N fixation and transformation. However, Li et al. (2015) found that nitrification and denitrification were inhibited when N applications exceeded a certain threshold (> 360 kg·hm^{-2}) in the summer maize fields of the North China Plain. Szukics et al. (2010) assessed the water content by establishing soil microcosms in stainless steel soil cylinders and found that when the soil water content increased from 30 to 70%, the relative abundance of denitrifying bacteria was also enhanced. Nevertheless, excessive water content may form an anaerobic environment, which may inhibit the activities of N fixing microorganisms (Zhou et al. 2020). Consequently, there has been an increase in scientific research into the effects of water and fertilizers on soil microorganisms. Liu et al. (2015) indicated that the diversity of N fixing bacteria decreased as the N applications were enhanced with 263 mm of irrigation, but increased with 526 mm of irrigation. Consequently, it was determined that appropriate fertilization and irrigation applications might retain the balance between soil biodiversity, network structure, and crop yield in arid croplands. Enhancing our understanding of the role and importance of soil biodiversity and network structures in controlling soil processes and crop yields in arid croplands is essential to clarify whether changes to them due to human interference can alter crop yields, which could limit our ability to feed our increasing population.

Soil microbial diversity has a great impact on the stability of underground ecosystems and soil function. He et al. (2009) found that the loss of microbial diversity reduced the number of functions, especially material cycling, thus affecting the ecosystem, which showed a similar pattern in the ecosystems of temperate, temperate monsoon, and subtropical monsoon climates. Bonkowski and Roy (2005) demonstrated that microbial diversity enhanced decomposition, C use efficiency, and N leaching from grassland microcosms. Additionally, functional redundancy caused by high microbial diversity has been reported to improve the resistance of microbial communities to varied surrounding changes and enhance their stability (Wittebolle et al. 2009). Soil multitrophic communities, including bacteria, fungi, and nematodes, can interact with each other to shape complex ecological networks, which are indicative of soil stability and resistance to surrounding changes in the agroecosystem (Fan et al. 2020). These ecological networks provide essential information about the potential links between thousands of soil microbes (Chaffron et al. 2010) and could be applied to reveal microbial co-occurrence patterns. These co-occurrence patterns can help to decipher the composition and assembly of complex microbial communities and predict potential interactions (Barberán et al. 2012). Microbial co-occurrence networks have been used to investigate the stability of microbial communities in response to environmental changes. Wang et al. (2016a) constructed networks of bacterial communities in different hydrocarbon stress conditions and found a complicated network between the bacterial taxa that improved the resistance of the individual bacterial species to environmental stresses. Liu et al. (2020a) demonstrated that a high microbial diversity and complex community interaction networks were conducive to nutrient (e.g., soil organic C, total P, total N, and available N) cycling and accumulation in rice field ecosystems. In agricultural ecosystems, soils with high diversity and stable ecological networks also have higher plant productivity, organic matter decomposition, and pathogen control (Benizri et al. 2005; Fan et al. 2020; Liu et al. 2020c). Despite these recent findings, the contributions of microbial biodiversity

and interaction networks for sustaining soil processes and crop production in agroecosystems are not well understood. Accordingly, a better understanding of the soil microbial communities and ecological network structures that support plant production will be critical to maintaining high crop yields, especially in arid agroecosystems.

To clarify the importance of microbial communities in crop yields, we conducted a 4-year field experiment to investigate crop production, soil properties, bacterial and fungal communities (diversity, compositions, N-cycling potentials, and co-occurrence network), and their associations in different irrigation and fertilization treatments in arid farmlands. The crop plant used for the experiments was maize (*Zea mays* L.), as it is one of the most commercially valuable cereal crops globally. Microbial diversity and their ecological networks are known to play a critical role in nutrient cycling, such as with organic matter decomposition, available N and P transformations, and in the regulation of plant pathogen infections and hormone release. Consequently, we hypothesized that soil microbial diversity and co-occurrence networks would become the essential drivers for soil functions and crop yields in arid agroecosystems.

Materials and methods

Experimental design and sample collection

A 4-year field fertigation experiment was established in 2016 at Shuguang Experimental Station at the Water Conservancy Research Institute of Bayannur City, Inner Mongolia (40°46′N, 107°24′E), China. The soil was derived from alluvial silt sediments and is classified as silt loam according to the American soil classification system. The soil sand content was 29.38%, silt 50.36%, and clay 20.26%. Soil bulk density was 1.34 g cm^{-3}, and the soil texture was the same across all plots. The annual average temperature was 7.8 °C, and the average annual precipitation was 105 mm. As the fields in this arid region have low fertility and water supply capacities, excessive irrigation and high fertilization was commonly adopted to improve soil water and nutrient status. However, the limited use of water by crops causes large water wastage and fertilizer pollution. Based on the local fertilization and irrigation levels, the experiment consisted of an irrigation gradient with three levels, high (400 mm), medium (300 mm), and low (200 mm), and a fertilization gradient with two levels, high (600 kg/ha P_2O_5 + 300 kg/ha urea) and low (300 kg/ha P_2O_5 + 150 kg/ha urea). There were six treatments (each plot area was 36 m^2) with three replicates in a completely randomized block design (Table 1): (1) low irrigation and low fertilization (LI + LF); (2) low irrigation and high fertilization (LI + HF); (3) medium irrigation and low fertilization (MI + LF); (4) medium irrigation and high fertilization (MI + HF); (5) high irrigation and low fertilization (HI + LF); and (6) high irrigation and high fertilization (HI + HF). The HI + HF treatment was treated as a control since this practice was commonly previously used in this region to improve soil water supply capacity and nutrient levels. During the four years of cropping, the treatments on these plots were maintained. Each plot consisted of six rows of spring maize planted on three raised ridges. Each plot was 3.6-m wide and 12-m long, and each ridge-furrow was 1.2-m wide and 12-m long. The ridge and furrow for every group were 50-cm wide (20-cm high) and 70-cm wide (20-cm high), respectively. The furrows were closed at the end of the plot to withhold the irrigation water. The plots were manually covered with transparent plastic film (140-cm wide) on all ridges to mitigate possible spill-over of water. The plastic film was joined at the bottom of the furrow, and a 50-cm space without plastic film was maintained for water infiltration. Two rows of maize (*Zea mays* L.) hybrid "Ximeng No.6" were planted in each ridge, in which row spacing was 40 cm, plant spacing was 30 cm, and artificial hole sowing depth was 5 cm. Before sowing for the high fertilization treatment, 600 kg/ha P_2O_5 (diammonium phosphate) was applied as a base fertilizer, and 150 kg/ha of urea as a base N fertilizer was broadcast. The remaining 150 kg/ha of urea was applied evenly at the tasseling and filling stages of each year. The process for the low fertilization treatment was the same, but half of the fertilizer amount specified for the high treatment was employed. Irrigation occurred four times during the growth period, once a month

Table 1 Experimental treatment and details

Treatment	Irrigation gradients	Fertilization gradients		Details
	mm	P_2O_5 (kg/ha)	Urea (kg/ha)	
LI + LF	200	300	150	Low irrigation and low fertilization
LI + HF	200	600	300	Low irrigation and high fertilization
MI + LF	300	300	150	Medium irrigation and low fertilization
MI + HF	300	600	300	Medium irrigation and high fertilization
HI + LF	400	300	150	High irrigation and low fertilization
HI + HF (the control)	400	600	300	High irrigation and high fertilization

after sowing; each irrigation quota is shown in Table S1. For each year, thinning operations were performed on the 28th day after planting at a spacing of 30 cm, resulting in a population density of 55,000 plants per hectare. The yield data from the most recent year (2019) were used for subsequent analysis.

In 2019, at the seedling, jointing, filling, and harvest stages, soil samples were collected from the top 20 cm of the soil profile in the center of the furrow by using an auger (5 cm in diameter and 20-cm long). Six soil cores were collected from each plot along a sigmoidal transect and then combined to form one sample. Finally, a total of 72 samples were collected. Thereafter, roots, stones, litter, and debris were removed, and each bulked sample was divided into two subsamples. One subsample was immediately stored at -80 °C for DNA extraction, while the other was air dried for physicochemical analysis.

Soil property analysis

Soil moisture content was determined using the oven-drying method, organic C content was determined using the Walkley–Black method (Nelson and Sommers 1982), and soil exchangeable NH_4^+ and NO_3^- were extracted using 2 M KCl for 18 h and then measured by colorimetry (Xu et al. 2010). The molybdenum-antimony resistance colorimetric method was used to determine the total P content (Murphy and Riley 1962).

Microbial DNA extraction and quantitative PCR analyses

The total soil DNA was extracted from 0.5 g of fresh soil collected from the fertigation field by using the E.Z.N.A. Soil DNA Kit (Omega, GA, USA), following the manufacturer's instructions. The concentration and purity of the extracted DNA were determined using a TBS-380 fluorometer (Turner BioSystems, CA, USA) and a NanoDrop ND-1000 spectrophotometer (NanoDrop, Wilmington, DE, USA), respectively. The quality of the extracted DNA was evaluated using 1% agarose gel electrophoresis. The primer 515F (5′-GTGCCAGCMGCCGCGGTAA-3′)/907R (5′-CCGTCAATTCCTTTGAGTTT-3′) was used to amplify the bacterial 16 s rRNA. Primer choice usually biases fungal community studies causing different conclusions (Tedersoo et al. 2015). Although the large subunit (LSU) rRNA gene is suitable for both classification accuracy and resolution to the genus level, here, the primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) were used to amplify the internal transcribed spacer (ITS) region because ITS1 and ITS2 amplicons provide greater taxonomic and functional resolution as well as coverage of the communities compared to LSU amplicons (Xue et al. 2019). A specific peptide barcode, with a length of 8 bp, was added to the 5′ end of the upstream primer for each sample to distinguish it. The PCR products were recovered, and their concentrations were determined. Several samples from the same treatment were mixed to ensure that the DNA concentrations of the samples used for sequencing were the same. The bacterial and fungal sequences were submitted to the NCBI Sequence Read Archive with accession number SRP301343.

Sequence processing

The Quantitative Insight into Microbial Ecology (QIIME 2) pipeline was used to analyze the raw sequence data. Chimeric sequences were observed and removed using USEARCH based on the UCHIME algorithm. Complete-linkage clustering of high-quality sequences into operational taxonomic units (OTUs) was conducted using UCLUST at 97% similarity. Low-abundance OTUs were deleted from the OTU table if they did not show a sum of at least two counts in all samples. The most abundant sequences in each OTU were selected as representative sequences and were compared with PyNAST. Bacteria were identified using the Silva reference database (http://www.arb-silva.de) with the RDP classifier, and fungi were identified using the Unite database (https://unite.ut.ee/) with the BLAST tool Community diversity indicators, including rarefaction curves, observed species, the Shannon–Wiener index, and the Chao1index, which were also computed by QIIME 2.

Functionality prediction and microbial co-occurrence network construction

The functions of the soil bacteria and fungi communities were predicted using the Functional Annotation of Prokaryotic Taxa (FAPROTAX) database 1.1 and FUNGuild database 1.1, respectively. It is worth noting that this investigation only focused on bacterial N reactions and potential fungal plant pathogens, as they are associated with potential risks to plant health and agricultural ecosystems. Microbial co-occurrence networks were constructed using the Molecular Ecological Networks (MEN) analysis (Deng et al. 2012) (http://ieg4.rccc.ou.edu/mena/), where the dominant class acted as nodes and correlations connecting the two classes acted as edges in the network. MEN analysis was performed to obtain all the network indices, with networks visualized using Cytoscape (version 3.7.1, https://cytoscape.org/). The parameters of the subnetwork were extracted using R-3.5.2 and used in the supply table for regression analysis (Table S3). The data of the four growing periods for each treatment were integrated to obtain the overall network parameters. OTUs were divided into four categories: peripheral ($Zi < 2.5$, $Pi < 0.62$), module hub ($Zi > 2.5$, $Pi < 0.62$),

connector (Zi < 2.5, Pi > 0.62), and network hub (Zi > 2.5, Pi > 0.62). Network hubs, module hubs, and connectors are keystone network topological features and are considered to play important roles in the stability and resistance of microbial communities; consequently, OTUs associated with these nodes were defined as keystone species.

Statistical analysis

R-3.5.2 was used for the analysis of variance and multiple comparisons, and all the results were expressed as mean ± standard deviation. Three-way ANOVA, followed by a post hoc least significant difference comparison, were used to examine the differences in soil properties and microbial properties between the six treatments; $P < 0.05$ was considered statistically significant. Analysis of the non-metric multidimensional scaling (NMDS) was applied to examine the differences in the composition of the microbial communities among the different treatments using the "vegan" package in R (V3.5.2), and Adonis was used to test the significance of the difference. The structural equation model (SEM) was used to test the hypothesis of the causal relationship between environmental prediction factors and response variables. The SEM was constructed using Amos (Amos V. 22.0, IBM, USA) to evaluate the direct and indirect effects of the prominent factors: irrigation and fertilization treatments, soil fertility (moisture, OC, TP, exchangeable NO_3^-, and NH_4^+), bacterial communities, and fungal communities on the maize yield. SEM offers the ability to investigate the complex relationships among the biodiversity of microorganisms, microbial co-occurrence network, and crop yields in the fertigation experiment. According to SEM, the relationship between soil microbial diversity, network modularity, and crop yield and soil fertility was further tested by linear regression.

Results

Crop yield and soil physicochemical properties

The irrigation and fertilization treatments had a significant effect on maize yield (Table S2, $p < 0.01$). Compared to HI + HF (control), MI + LF, and MI + HF treatments showed significantly enhanced maize yield, while HI + LF, LI + HF, and LI + LF treatments showed significantly decreased maize yield, but no significant difference was detected between MI + LF and MI + HF treatments (Fig. 1). The irrigation and fertilization treatments also caused significant changes in the soil physicochemical properties (Table 2). MI + LF and MI + HF treatments showed significantly enhanced levels of OC, exchangeable NO_3^-, and NH_4^+ ($p < 0.05$); however, there was no difference between them, and they were followed by the control, LI + HF, HI + LF, and LI + LF

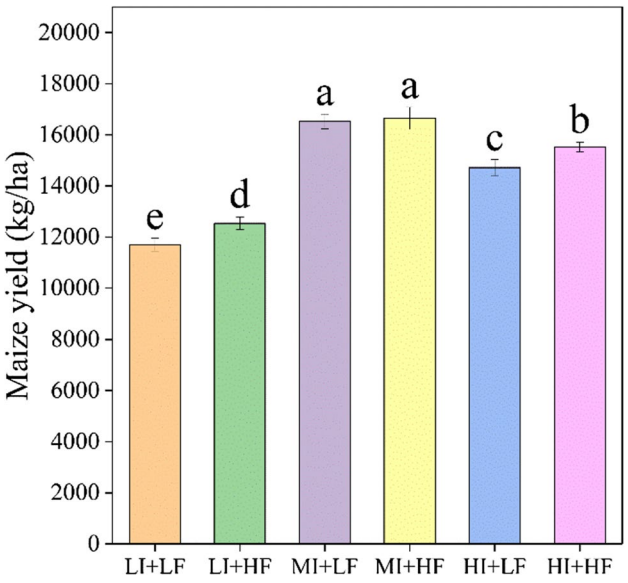

Fig. 1 Maize yields for the different treatments. Different letters indicate significant differences between treatments at the 0.05 level of the least significant difference (LSD) test. *LI + LF* low irrigation and low fertilization, *LI + HF* low irrigation and high fertilization, *MI + LF* medium irrigation and low fertilization, *MI + HF* medium irrigation and high fertilization, *HI + LF*, high irrigation and low fertilization, *HI + HF* high irrigation and high fertilization (the control)

treatments successively. Except for the seedling stage, no significant difference was detected in the soil moisture content among the control, HI + LF, MI + HF, and MI + LF treatments, which were significantly higher than that of LI + HF and LI + LF treatments. The lowest soil moisture content was observed at the jointing stage and increased gradually in the next two stages. Soil pH did not differ significantly among the six treatments. Irrigation, fertilization, and stage had significant effects ($p < 0.05$; Table S2) on soil physicochemical properties. The interaction of fertilization and stage had a significant effect on TP, OC, exchangeable NO_3^-, and NH_4^+ ($p < 0.05$; Table S2), while the interaction of irrigation and stage had a significant effect on SM, TP, OC, and exchangeable NO_3^- ($p < 0.01$; Table S2).

Microbial community diversity and composition

Based on Chao1 and Shannon index (Table 3), the α-diversity of bacteria and fungi in each sample was assessed. Compared to control, MI + LF and MI + HF treatments showed enhanced bacterial diversity, while HI + LF, LI + LF, and LI + HF treatments showed decreased bacterial diversity. No significant difference was detected between MI + LF and MI + HF treatments ($p > 0.05$). However, fungal diversity showed a different trend in that MI + LF treatment showed the lowest diversity. Stage, irrigation, and fertilization had significant effects on the diversity of bacteria and

Table 2 Soil physicochemical properties for the different treatments. All the data are presented as mean ± SE ($n = 3$)

Stage	Treatment	Moisture %	Total P g/kg	Organic C g/kg	NO_3^- -N mg/kg	NH_4^+ -N mg/kg	pH
Seeding	LI + LF	15.1 ± 1.2cA	0.09 ± 0.00bA	1.19 ± 0.02aAB	1.50 ± 0.02cB	0.46 ± 0.03 dB	8.26
	LI + HF	16.0 ± 1.2cA	0.12 ± 0.01aA	1.20 ± 0.04aA	1.65 ± 0.05bB	0.55 ± 0.02cB	8.25
	MI + LF	19.5 ± 1.4bA	0.07 ± 0.00cB	1.22 ± 0.03aB	1.81 ± 0.02aB	0.83 ± 0.02aB	8.27
	MI + HF	21.1 ± 1.3bA	0.09 ± 0.00bB	1.22 ± 0.02aB	1.85 ± 0.04aB	0.85 ± 0.03aB	8.26
	HI + LF	25.6 ± 1.4aA	0.07 ± 0.01cB	1.20 ± 0.03aAB	1.48 ± 0.05cB	0.57 ± 0.04cB	8.27
	HI + HF	25.2 ± 0.9aA	0.08 ± 0.00bAB	1.20 ± 0.03aB	1.66 ± 0.05bB	0.68 ± 0.02bB	8.27
Jointing	LI + LF	10.8 ± 0.9bA	0.09 ± 0.00dA	1.22 ± 0.00cA	21.75 ± 2.05cdA	0.84 ± 0.06dA	8.27
	LI + HF	12.8 ± 0.8bA	0.12 ± 0.02cA	1.29 ± 0.02bA	25.47 ± 2.72bA	1.02 ± 0.15cA	8.26
	MI + LF	19.1 ± 0.4aA	0.16 ± 0.02aA	1.56 ± 0.03aA	34.74 ± 2.37aA	1.35 ± 0.03abA	8.25
	MI + HF	17.6 ± 0.8aA	0.14 ± 0.03bA	1.59 ± 0.04aA	37.00 ± 2.01aA	1.39 ± 0.04aA	8.25
	HI + LF	19.1 ± 1.1aB	0.08 ± 0.00eA	1.22 ± 0.04cA	20.36 ± 1.55dA	0.95 ± 0.04cdA	8.27
	HI + HF	18.9 ± 0.9aA	0.09 ± 0.00deA	1.35 ± 0.05bA	24.21 ± 1.07bcA	1.25 ± 0.04bA	8.26
Filling	LI + LF	13.3 ± 1.2bA	0.08 ± 0.00cdA	1.05 ± 0.13cBC	15.63 ± 1.61cA	0.73 ± 0.06dAB	8.26
	LI + HF	14.9 ± 0.9bA	0.09 ± 0.01cB	1.21 ± 0.03bA	18.87 ± 1.75bcA	0.85 ± 0.06cAB	8.28
	MI + LF	20.6 ± 0.7aA	0.13 ± 0.03aAB	1.38 ± 0.02aAB	28.11 ± 2.03aA	1.13 ± 0.04aAB	8.25
	MI + HF	21.5 ± 2.0aA	0.11 ± 0.01bAB	1.38 ± 0.01aAB	29.75 ± 2.24aA	1.12 ± 0.07aAB	8.24
	HI + LF	19.6 ± 1.0aAB	0.08 ± 0.00dAB	1.18 ± 0.02bAB	17.11 ± 1.71cA	0.8 ± 0.03cdAB	8.28
	HI + HF	22.3 ± 1.1aA	0.08 ± 0.01cdAB	1.22 ± 0.05bAB	22.22 ± 2.72bA	0.97 ± 0.03bAB	8.26
Harvest	LI + LF	15.1 ± 0.9bA	0.07 ± 0.01cB	0.91 ± 0.06dC	13.14 ± 1.77bAB	0.56 ± 0.04dAB	8.25
	LI + HF	15.6 ± 1.5bA	0.09 ± 0.00bB	1.20 ± 0.04bcA	16.17 ± 1.92bAB	0.68 ± 0.03cAB	8.27
	MI + LF	20.5 ± 1.2aA	0.10 ± 0.01aAB	1.30 ± 0.02aB	23.28 ± 1.33aA	0.95 ± 0.02aB	8.27
	MI + HF	20.3 ± 0.7aA	0.10 ± 0.01aB	1.29 ± 0.02aB	22.76 ± 2.71aAB	0.93 ± 0.04aB	8.21
	HI + LF	20.7 ± 1.5aAB	0.07 ± 0.00cAB	1.15 ± 0.03cB	16.23 ± 2.07bA	0.62 ± 0.03cB	8.25
	HI + HF	21.7 ± 1.4aA	0.08 ± 0.00bcB	1.22 ± 0.03bAB	20.67 ± 2.72aA	0.78 ± 0.04bB	8.25

The lowercase letters indicate significant differences between the different treatments at the same stage at the 0.05 level of the least significant difference (LSD) test. The uppercase letters indicate significant differences between the same treatments at different stages at the 0.05 level. *LI + LF* low irrigation and low fertilization, *LI + HF* low irrigation and high fertilization, *MI + LF* medium irrigation and low fertilization, *MI + HF* medium irrigation and high fertilization, *HI + LF* high irrigation and low fertilization, *HI + HF* high irrigation and high fertilization (the control)

fungi ($p < 0.01$), while the interaction of irrigation and stage only had significant effects on bacterial diversity ($p < 0.05$; Table S2). Gammaproteobacteria was the dominant bacterial class (16.42%) across all samples, followed by Bacteroidia (12.35%), Gemmatimonadetes (10.24%), and Alphaproteobacteria (7.79%), all of which together accounted for more than 46.80% (Fig. S1). In the critical periods of crop growth, the jointing stage and filling stage, MI + LF treatment showed enhanced relative abundance of Phycisphaerae, but that of Anaerolineae, Bacteroidia, and Gammaproteobacteria were relatively low. Sordariomycetes was the most abundant fungal class across the treatments, accounting for 58.84% of all the taxa on average, followed by Pezizomycetes (12.44%) and Mortierellomycetes (7.79%) (Fig. S2). MI + LF treatment had a lower relative abundance of Sordariomycetes, an increased relative abundance of Mortierellomycetes at the jointing stage, and an enhanced Pezizomycetes abundance during the filling stage. Stage had significant effects on the relative abundance of bacteria and fungi, while fertilization only influenced the relative abundance of bacteria (Table S2, $p < 0.01$). The NMDS clearly identified variations in the compositions of bacterial and fungal communities, respectively, among the six treatments (Fig. S3 and Fig. S4). The compositions of bacterial and fungal communities from MI + LF and MI + HF treatments appeared to be more similar and more closely clustered together than those detected in the other treatments. In contrast, the compositions of the microbial communities among control, HI + LF, LI + HF, and LI + LF treatments showed obvious differences.

Microbial co-occurrence networks and microbial predictive functional profiles

Compared with the control and other treatments, MI + LF treatment showed the highest bacterial subnetwork modularity and the lowest connectivity, and there were significant differences between MI + LF treatment and the other treatments ($p < 0.05$, Table 4). For the fungal subnetworks,

Table 3 Soil microbial diversity for the different treatments

Stage	Treatment	B-Shannon	B-Chao1	F-Shannon	F-Chao1
Seeding	LI + LF	6.17 ± 0.11cA	3044 ± 349cA	2.64 ± 0.31abA	344 ± 17bcB
	LI + HF	6.6 ± 0.49abcAB	3374 ± 208bcA	2.81 ± 0.13aB	377 ± 18aAB
	MI + LF	7.07 ± 0.61abB	3679 ± 195bAB	2.03 ± 0.22cA	306 ± 12 dB
	MI + HF	7.23 ± 0.63aB	4180 ± 283aAB	2.24 ± 0.08bcA	320 ± 21cdA
	HI + LF	6.27 ± 0.1bcB	3377 ± 257bcA	2.69 ± 0.31abA	376 ± 22abB
	HI + HF	6.81 ± 0.66abcB	3610 ± 260bA	3.11 ± 0.42aA	390 ± 19aA
Jointing	LI + LF	6.46 ± 0.97dA	3356 ± 198cA	2.84 ± 0.28bcA	389 ± 14bA
	LI + HF	7.74 ± 0.71cdA	3669 ± 252bcA	3.30 ± 0.35abA	403 ± 28bA
	MI + LF	12.65 ± 0.96aA	4493 ± 151aA	2.35 ± 0.18dA	347 ± 19cA
	MI + HF	11.03 ± 1.30bAB	4602 ± 314aA	2.53 ± 0.30cdA	342 ± 20cA
	HI + LF	8.00 ± 0.18cA	3494 ± 309cA	3.24 ± 0.17abA	453 ± 21aA
	HI + HF	8.39 ± 0.18cA	4013 ± 225bA	3.59 ± 0.30aA	441 ± 10aA
Filling	LI + LF	5.85 ± 0.31cAB	3116 ± 260cA	2.75 ± 0.56bcA	360 ± 16cdAB
	LI + HF	6.64 ± 0.29cAB	3456 ± 223bcA	3.31 ± 0.15aA	377 ± 11bcAB
	MI + LF	8.52 ± 0.54abAB	4158 ± 258aAB	2.41 ± 0.20cA	324 ± 4eAB
	MI + HF	9.21 ± 0.51aAB	4225 ± 350aAB	2.37 ± 0.22cA	336 ± 11deA
	HI + LF	6.53 ± 0.24cB	3317 ± 264bcA	2.97 ± 0.20abA	398 ± 10bAB
	HI + HF	7.84 ± 0.72bAB	3761 ± 214abA	3.28 ± 0.25aA	429 ± 30aA
Harvest	LI + LF	4.98 ± 0.49cB	2908 ± 223cA	2.37 ± 0.19bcA	340 ± 9bcB
	LI + HF	6.27 ± 0.19bB	3409 ± 226abA	3.26 ± 0.13aA	351 ± 14bB
	MI + LF	7.61 ± 0.35aB	3508 ± 298abB	2.06 ± 0.30cA	317 ± 11cAB
	MI + HF	8.10 ± 0.51aAB	3732 ± 223aB	2.18 ± 0.18cA	334 ± 10bcA
	HI + LF	6.38 ± 0.14bB	3348 ± 150bA	2.64 ± 0.42bA	396 ± 20aAB
	HI + HF	6.91 ± 0.32bAB	3655 ± 90abA	3.23 ± 0.13aA	410 ± 23aA

All the data are presented as mean ± SE ($n = 3$). The lowercase letters indicate significant differences between the different treatments at the same stage at the 0.05 level of the least significant difference (LSD) test. The uppercase letters indicate significant differences between the same treatments at different stages at the 0.05 level. *LI + LF* low irrigation and low fertilization, *LI + HF* low irrigation and high fertilization, *MI + LF* medium irrigation and low fertilization, *MI + HF* medium irrigation and high fertilization, *HI + LF* high irrigation and low fertilization, *HI + HF* high irrigation and high fertilization (the control). *B-Shannon* bacterial Shannon index, *B-Chao1* bacterial Chao1 estimator, *F-Shannon* fungal Shannon index, *F-Chao1* fungal Chao1 estimator

LI + LF and control treatments had the highest connectivity and modularity, respectively, and the connectivity of control and HI + LF treatments was lower than that of LI + HF and LI + LF treatments (Table 4). Overall, for both fungi and bacteria, MI + LF treatment showed the highest modularity and the lowest connectivity (Table 5), and the bacterial network modularity for MI + HF and MI + LF treatments was higher than that for LI + HF and LI + LF treatments. Except for MI + HF and MI + LF treatments, the microbial overall network modularity of the control and LI + HF treatments was higher than that of HI + LF and LI + LF treatments, respectively (Table 5). Irrigation had significant effects on the connectivity and modularity of microbial networks, while stage only significantly influenced the modularity of fungal networks ($p < 0.001$; Table S2). As shown in Figs. 2 and 3, for both fungi and bacteria, the co-occurrence network under MI + LF treatment had the smallest number of links and the largest number of modules. The proportion of hubs and connectors for MI + HF and MI + LF treatments was higher than that of the control and HI + LF treatments, indicating a more hub-based and connected network structure in MI + LF and MI + HF treatments (Table S5). For bacteria, there were no module hub OTUs in the treatments, and the network hubs and connectors mainly belonged to the class Gemmatimonadetes and the phyla Bacteroidetes, Proteobacteria, and Acidobacteria. The number of network nodes and links for the OTUs within bacterial communities was higher than that within fungal communities.

FAPROTAX analysis was adopted to predict ecological and biological functions from bacterial OTUs, although poor taxonomic identification was noted in this database. As shown in Fig. 4, the relative abundance of nitrification was the highest, accounting for 6.61%, followed by bacteria involved in aerobic ammonia oxidation (5.50%), nitrite respiration (1.98%), denitrification (0.28%), and N fixation (0.20%). In general, compared with the control and other treatments, MI + LF and MI + HF treatments had more abundant groups capable of nitrification, ammoxidation, and N fixation, but fewer groups

Table 4 Microbial subnetwork connectivity and modularity for the different treatments

Stage	Treatment	B-connectivity	B-modularity	F-connectivity	F-modularity
Seeding	LI + LF	0.020 ± 0.00c	0.174 ± 0.008d	0.072 ± 0.003a	0.323 ± 0.010c
	LI + HF	0.010 ± 0.00 cd	0.411 ± 0.019c	0.045 ± 0.001b	0.454 ± 0.021b
	MI + LF	0.006 ± 0.000d	0.700 ± 0.008a	0.034 ± 0.004c	0.181 ± 0.058d
	MI + HF	0.042 ± 0.009b	0.444 ± 0.025bc	0.038 ± 0.002c	0.494 ± 0.045b
	HI + LF	0.056 ± 0.012a	0.514 ± 0.088b	0.039 ± 0.003c	0.485 ± 0.021b
	HI + HF	0.058 ± 0.008a	0.461 ± 0.047bc	0.027 ± 0.003d	0.608 ± 0.041a
Jointing	LI + LF	0.019 ± 0.002bc	0.197 ± 0.061c	0.083 ± 0.005a	0.315 ± 0.019d
	LI + HF	0.009 ± 0.001c	0.450 ± 0.052b	0.047 ± 0.003b	0.486 ± 0.013c
	MI + LF	0.006 ± 0.000c	0.719 ± 0.016a	0.027 ± 0.003d	0.657 ± 0.020b
	MI + HF	0.046 ± 0.008a	0.415 ± 0.005bc	0.033 ± 0.003c	0.608 ± 0.054b
	HI + LF	0.033 ± 0.028ab	0.301 ± 0.265bc	0.033 ± 0.003c	0.626 ± 0.053b
	HI + HF	0.047 ± 0.011a	0.272 ± 0.152bc	0.021 ± 0.001e	0.759 ± 0.012a
Filling	LI + LF	0.019 ± 0.002b	0.180 ± 0.029c	0.077 ± 0.008a	0.338 ± 0.020c
	LI + HF	0.009 ± 0.001b	0.421 ± 0.0504b	0.045 ± 0.005b	0.501 ± 0.004bc
	MI + LF	0.006 ± 0.000b	0.691 ± 0.007a	0.028 ± 0.004d	0.485 ± 0.216bc
	MI + HF	0.051 ± 0.012a	0.417 ± 0.186b	0.042 ± 0.004bc	0.527 ± 0.046b
	HI + LF	0.051 ± 0.012a	0.513 ± 0.08b	0.037 ± 0.001c	0.528 ± 0.016b
	HI + HF	0.061 ± 0.017a	0.376 ± 0.089b	0.025 ± 0.003d	0.701 ± 0.048a
Harvest	LI + LF	0.019 ± 0.001b	0.185 ± 0.047c	0.074 ± 0.005a	0.339 ± 0.010d
	LI + HF	0.009 ± 0.000bc	0.440 ± 0.039b	0.044 ± 0.001b	0.482 ± 0.037c
	MI + LF	0.006 ± 0.000c	0.709 ± 0.001a	0.03 ± 0.002d	0.600 ± 0.027ab
	MI + HF	0.048 ± 0.004a	0.492 ± 0.059b	0.037 ± 0.000c	0.534 ± 0.034bc
	HI + LF	0.051 ± 0.012a	0.401 ± 0.083b	0.039 ± 0.002c	0.485 ± 0.056c
	HI + HF	0.048 ± 0.006a	0.461 ± 0.122b	0.025 ± 0.002e	0.656 ± 0.055a

All the data are presented as mean ± SE ($n = 3$). The lowercase letters indicate significant differences between the different treatments at the same stage at the 0.05 level of the least significant difference (LSD) test. *LI + LF* low irrigation and low fertilization, *LI + HF* low irrigation and high fertilization, *MI + LF* medium irrigation and low fertilization, *MI + HF* medium irrigation and high fertilization, *HI + LF* high irrigation and low fertilization, *HI + HF* high irrigation and high fertilization (the control). *B* bacterial subnetwork. *F* fungal subnetwork

Table 5 Microbial overall network connectivity and modularity for the different treatments

Treatment	B-connectivity	B-modularity	F-connectivity	F-modularity
LI + LF	7.457	0.364	4.367	0.591
LI + HF	4.335	0.622	3.309	0.699
MI + LF	2.512	0.836	2.672	0.715
MI + HF	3.648	0.665	3.783	0.659
HI + LF	3.985	0.626	4.44	0.626
HI + HF	3.982	0.677	3.203	0.665

LI + LF low irrigation and low fertilization, *LI + HF* low irrigation and high fertilization, *MI + LF* medium irrigation and low fertilization, *MI + HF* medium irrigation and high fertilization, *HI + LF* high irrigation and low fertilization, *HI + HF* high irrigation and high fertilization (the control). *B* bacterial overall network. *F* fungal overall network

with the ability for nitrite respiration or denitrification. No significant differences were detected in the relative abundance of these functional groups between MI + LF and MI + HF groups ($p > 0.05$). For fungi, the relative abundance of the plant pathogens was 11.28% according to FUNGuild. Compared to the control, MI + LF and MI + HF treatments had lower relative abundance for plant pathogens (Fig. S5). Irrigation, fertilization, and stage had significant effects on the abundance of bacterial N functions and plant pathogens ($p < 0.001$), and the interaction of irrigation and stage also had significant effects on the same variables ($p < 0.001$; Table S2).

Possible drivers of soil fertility and maize yield

To explore the potential mechanisms for the yield differences in spring maize, a SEM was constructed (Fig. S6). The fitting model (CFI = 0.994, RMSEA = 0.067, $\chi^2/f = 1.076$) complies with the fitness and significance standards of the SEM. The final model explained 99.0% of the variance in maize yield. Clearly, changes in irrigation and fertilization had a direct effect on the diversity and network modularity of bacteria and fungi, which resulted in significant changes in the bacterial N-cycling functions, soil fertility, and maize yield. Bacterial diversity had a direct and considerable

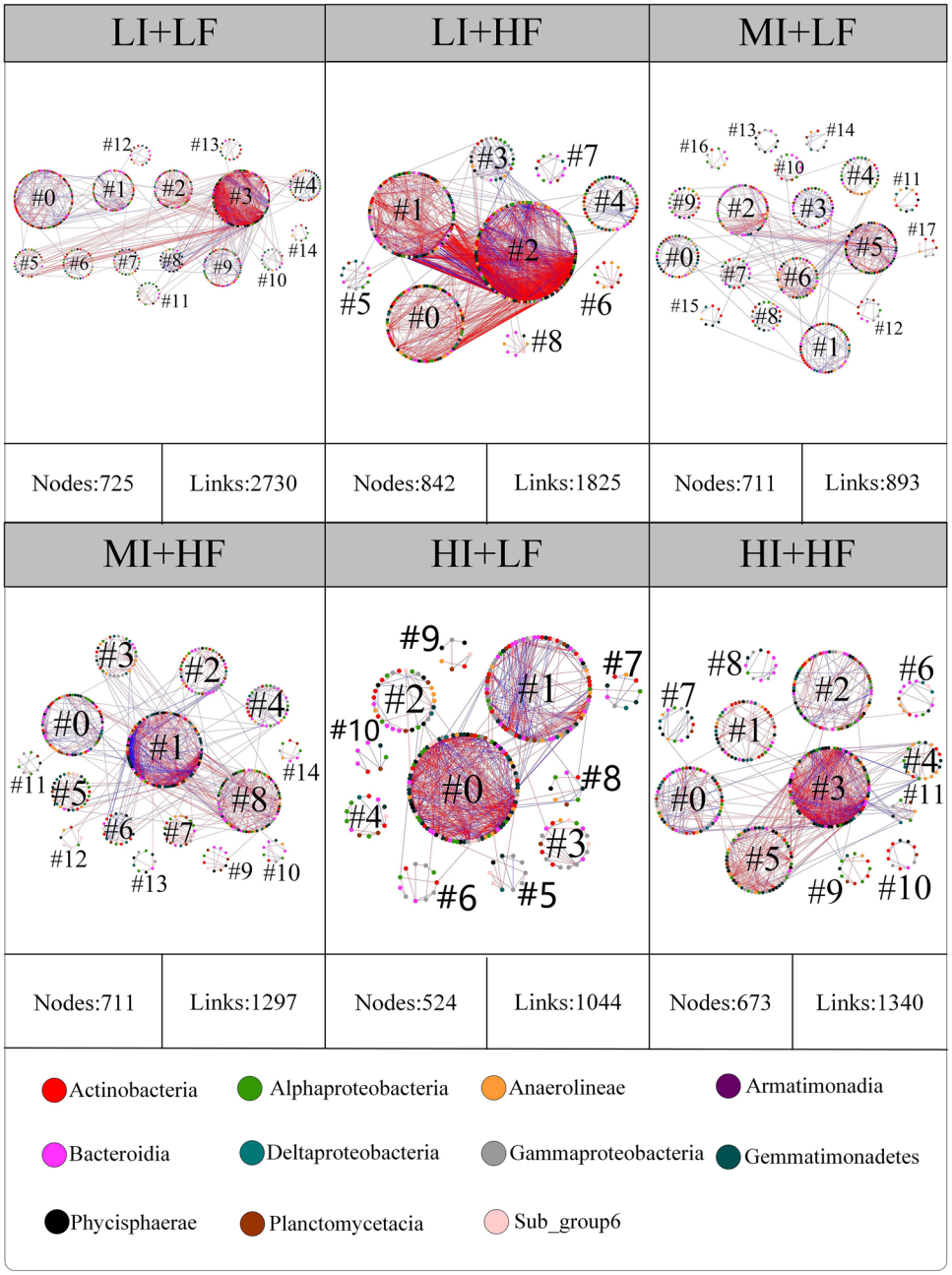

Fig. 2 The bacterial co-occurrence networks for the different treatments [*LI* + *LF* low irrigation and low fertilization, *LI* + *HF* low irrigation and high fertilization, *MI* + *LF* medium irrigation and low fertilization, *MI* + *HF* medium irrigation and high fertilization, *HI* + *LF* high irrigation and low fertilization, *HI* + *HF* high irrigation and high fertilization (the control)]. Each node corresponds to an OTU, and edges between nodes correspond to either positive (red) or negative (blue) correlations. OTUs belonging to different microbial class have different color codes

impact on bacterial function but was negatively correlated with the relative abundance of plant pathogens. The bacterial Shannon diversity, N functions, and network modularity and fungal network modularity exerted a direct effect on soil fertility and maize yield. As shown in Figs. 5 and 6, we further selected a few examples to illustrate the link between microbial diversity, co-occurrence networks, and maize yield. Specifically, we found that the bacterial Shannon diversity was positively correlated with maize yield, N cycle functional groups, OC, NO_3^- and exchangeable NH_4^+ content, and bacterial network modularity but negatively correlated with plant pathogen abundance; the fungal Shannon diversity index was also positively correlated with fungal network modularity. In addition, microbial network modularity had a significant positive correlation with maize yield and soil fertility.

Discussion

Effects of irrigation and fertilization on crop production, microbial diversity, functions, and co-occurrence networks

Irrigation and fertilization, when appropriately used, can be beneficial for soil fertility and crop production, but excessive

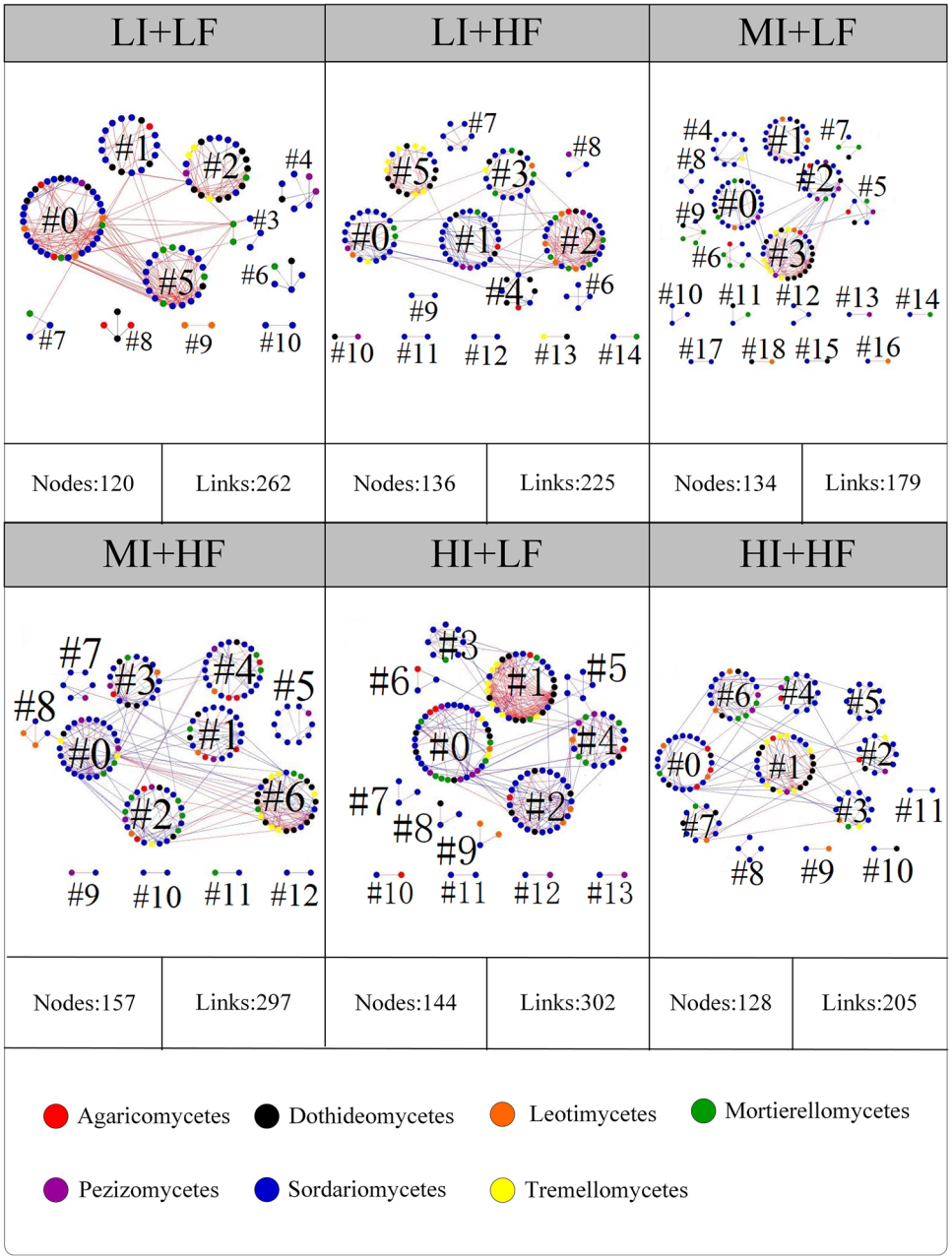

Fig. 3 The fungal co-occurrence networks for the different treatments [*LI + LF* low irrigation and low fertilization, *LI + HF* low irrigation and high fertilization, *MI + LF* medium irrigation and low fertilization, *MI + HF* medium irrigation and high fertilization, *HI + LF* high irrigation and low fertilization, *HI + HF* high irrigation and high fertilization (the control)]. Each node corresponds to an OTU, and edges between nodes correspond to either positive (red) or negative (blue) correlations. OTUs belonging to different microbial class have different color codes

water and soil nutrient levels could have negative effects. Our results showed that MI + LF and MI + HF treatments resulted in higher maize yields, OC and inorganic N contents, bacterial diversity, nitrification, ammoxidation, N-fixation potentials, and network modularity, compared with the other treatments. This suggests that limited or excessive water conditions could have negative impacts on the maintenance of microbial diversity and functions, even under sufficient fertilization. Our study found that both OC and inorganic N in the low irrigation treatments were lower than those in the medium irrigation treatment, and their changes were significantly positively related to bacterial diversity. This is expected as the lack of water reduces soil OC and N availability and thus decreases microbial diversity. Consistent with our results, Liu et al. (2014) reported that when the content of active OC decreased by 18.4% with drought stress, the Shannon diversity index for the soil microorganisms was reduced significantly from 1.13 to 0.52. An alternative explanation for the reduction in diversity with low irrigation was that water deficiency led to the reduction of soluble nutrients, which resulted in competition among the microbial species. Previous studies have observed that increases in these competitive relationships resulted in a reduction of bacterial network complexity and a decrease in bacterial diversity in high altitude areas (Li et al. 2020a). In the present study, excessive water was also not conducive

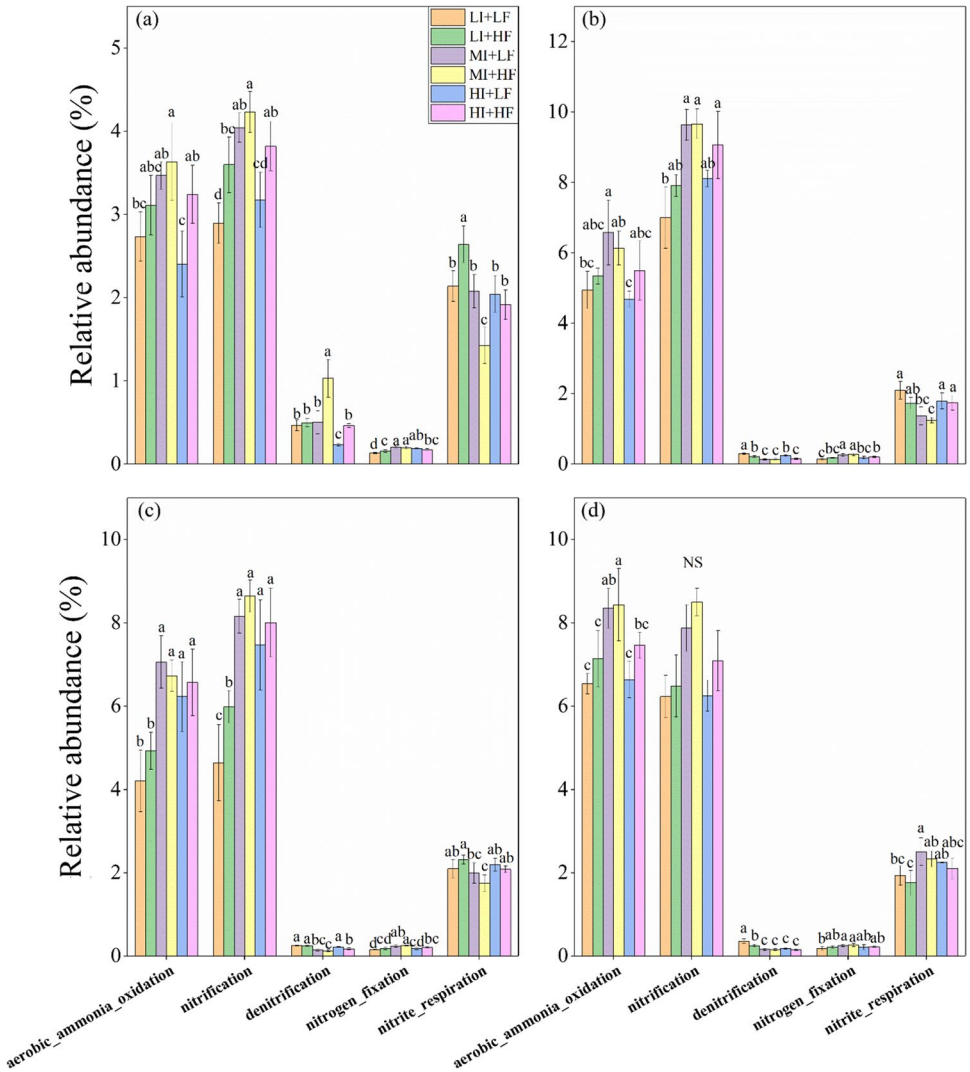

Fig. 4 Putative functional profiles of soil bacterial for the different treatments [*LI* + *LF* low irrigation and low fertilization, *LI* + *HF* low irrigation and high fertilization, *MI* + *LF* medium irrigation and low fertilization, *MI* + *HF* medium irrigation and high fertilization, *HI* + *LF* high irrigation and low fertilization, *HI* + *HF* high irrigation and high fertilization (the control)] at the **a** seeding stage, **b** jointing stage, **c** filling stage, and **d** harvest stage. All the data are present as mean ± SE ($n = 3$). Different letters indicate the significance difference at the 0.05 level of the least significant difference (LSD) test

to the maintenance of microbial diversity. Our result was in agreement with a report by Li et al. (2020b), who found that the abundance of aerobic microorganisms (e.g., Actinobacteria) decreased significantly, while that of anaerobic microorganisms (e.g., Chloroflexi and Firmicutes) increased significantly when dryland was converted into paddy, and the diversity of the soil microbial community was quickly reduced by this conversion. This suggested that soil aeration plays an important role in this process. Excessive irrigation reduced soil aeration and oxygen content, which had a positive impact on the abundance of anaerobic microbial species and decreased the abundance of aerobic microbial species, resulting in the reduction of bacterial diversity.

MEN analysis reveals the potential interactions between soil microorganisms and reflects the stability and complexity of ecological processes and ecosystem functions (Deng et al. 2016). Our results showed that the network connectivity within the fungal community with high levels of irrigation was lower than that within the fungal community with low levels of irrigation, potentially due to higher accessibility to resources because of the enhanced moisture. These additional nutrients reduced competition; thus, they may enable more fungal species to maintain free-living populations, resulting in fewer dependencies between individual microorganisms and consortia (Upton et al. 2020). The medium irrigation treatment had higher modularity of bacteria in the overall network when compared with the low irrigation treatment, regardless of fertilization. Our results are in agreement with those of Li et al. (2020b), who found flood irrigation enhanced the modularity of bacterial networks in paddies from 0.694 to 0.822, compared with drylands. This suggested that increased irrigation may enhance the soluble OC content and thus drive the change in bacterial community composition. However, excessive soil moisture could decrease the modularity, possibly due to the reduction of bacterial diversity. We found that the increased bacterial Shannon diversity index was accompanied by increased bacterial subnetwork modularity. Reduced diversity caused by excessive soil

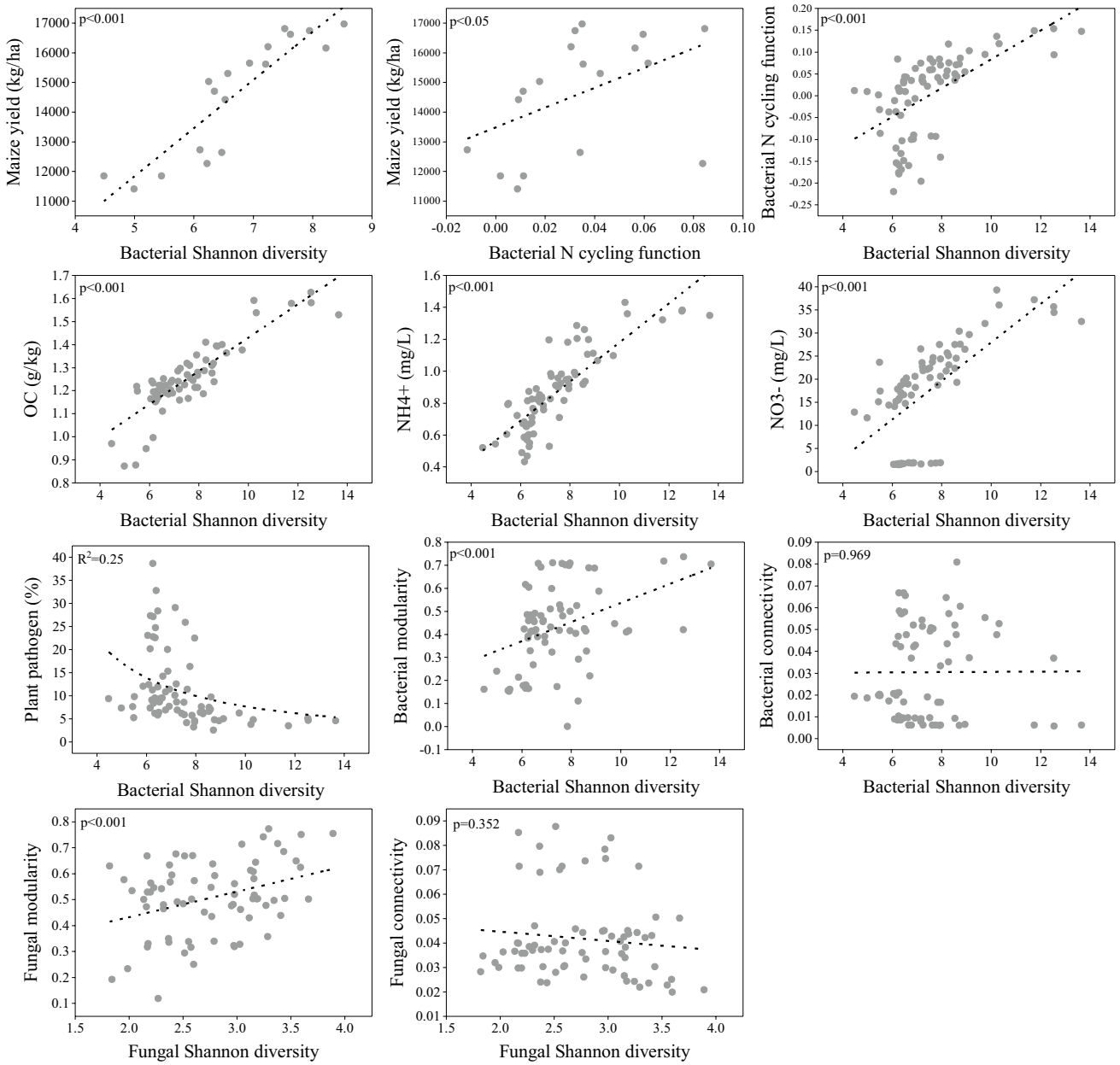

Fig. 5 Ecological relationships between biodiversity of bacteria, N cycling function, and crop production and fertility. The first principal component (PC1) from the principal component analysis (PCA) was selected as an integrative variable for representing bacterial N cycling function

moisture may decrease the number of keystone species (hubs and connectors, Table S5) which played an essential role in the network structure. The network analysis results demonstrated that the increase in fertilizer application enhanced the modularity of the overall microbial network, except for in the MI + HF and MI + LF treatments (Table 5). Our results are consistent with those of Yao et al. (2020), who reported that the modularity of the microbial network was improved by the application of chemical fertilizers for wheat and corn rotations. Additionally, regardless of irrigation, the higher subnetwork modularizations for fungi were observed with high level fertilization treatments, while for bacteria, they were observed with low level fertilization treatments, which indicated that they had different responses to soil fertility. Compared to fungi, bacteria were not necessarily as dependent on pre-existing resources, such as N and organic compounds (Zhang et al. 2018b). Certain bacteria can fix N and C in the atmosphere, but most fungi cannot fix N (Duc et al. 2009). Therefore, bacteria still showed strong cooperation and had greater network modularity with lower fertility conditions. Our results demonstrated that bacterial communities exhibited more network nodes and links than fungal communities

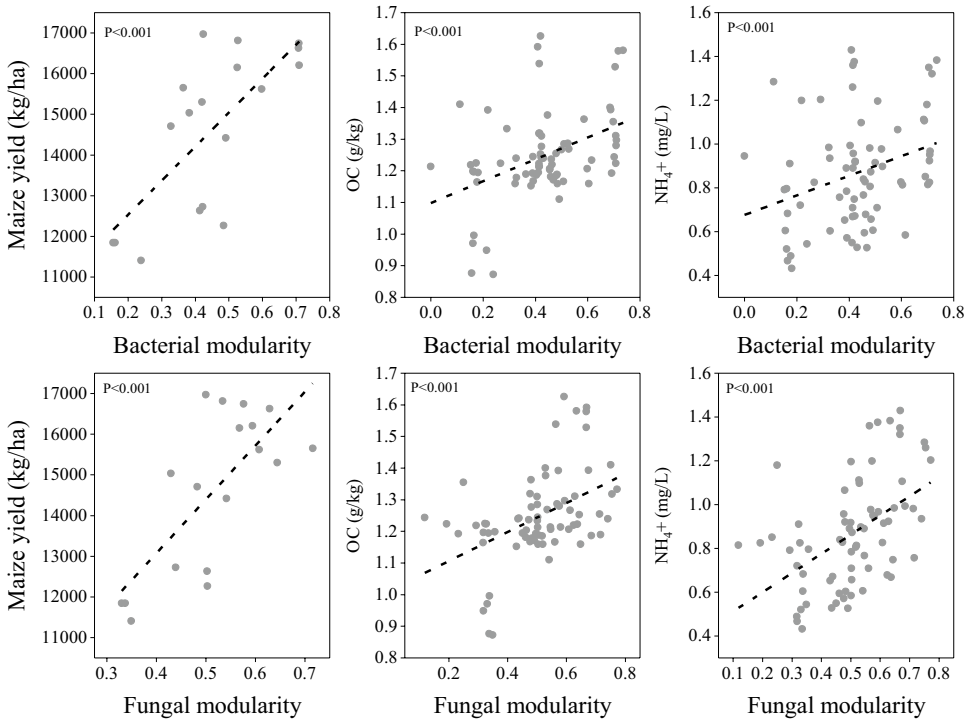

Fig. 6 Ecological relationships between microbial network modularity and crop production and fertility

under arid agroecosystem conditions, potentially due to the differences in the predicted metabolism for these organisms. Most bacteria responded much more rapidly to changes in environmental conditions compared to fungi, which can acquire nutrient from plant through mycorrhizae (Chen and Ma 2016), thus reducing their obligate dependency on bacterial taxa.

The growth stage of plants affected microbial diversity and functions, which could be due to the differences in nutrient requirements at different phenological stages (Bardgett and van der Putten 2014). Interestingly, the growth stage had no significant effect on the connectivity and modularity of bacterial networks but did have an influence on the fungal network structure in our study. This may have been because the fungi were the first to degrade soil inert organic matter and release nutrients when plants need a lot of nutrients to grow, especially in poor soil (Schneider et al. 2012), which may depend on the interactions among fungi. Additionally, soil moisture was significantly affected by the stage and was the lowest at the jointing stage, which may be related to the enhancement of the water demand of crops at the jointing stage.

Irrigation and fertilization increased soil fertility and maize yield via changes in bacterial diversity and network modularity

The application of MI + LF and MI + HF treatments led to higher bacterial community diversity, which was associated with higher levels of maize production, nutrient availability, and a lower relative abundance of potential fungal plant pathogens after 4 years of treatment. These findings suggested that the diversity of bacterial communities potentially affected maize production in an arid fertigation agroecosystem. Several mechanisms may explain this phenomenon. First, soils with high bacterial community diversity usually had higher availability of nutrients (Franklin and Mills 2006), as supported by our findings of direct and positive correlations between bacterial diversity and available nutrients (NO_3^--N, NH_4^+-N, and OC), which indicated that bacterial community diversity facilitated more nutrients for plants and that there was less competition from microbes. Second, high bacterial community diversity may lead to a higher relative abundance of keystone species. In our study, the bacterial networks of MI + LF contained multiple keystone species, such as those of Flavobacterium, which can degrade complex organic matter (Krakat et al. 2011), and play an important role in the C cycle; other keystone species that belonged to genera of Steroidobacter and Gillisia also play essential roles as soil utilizers of C substrates and in nutrient turnover (Werner and Newton 2005; Philippot et al. 2013). The increase in bacterial diversity may drive these keystone species to proliferate and promote the accumulation of available N and OC in the soil and consequently promote crop yield. Similarly, Huang et al. (2019) reported that the keystone taxa in the central module were enriched in the rhizosphere soil of healthy plants. All of these results indicated that keystone taxa are essential for the healthy growth of plants. Additionally, high bacterial diversity may

lead to a lower relative abundance of plant pathogens; this was confirmed by the linear regression analysis (Fig. 5). Irrigation and fertilization resulted in significant variations in microbial community composition and functions (Van Der Heijden et al. 2008), and high bacterial diversity may suppress the competition for the limited resources by plant pathogens (Fargione and Tilman 2005) and control the relative abundance of potential plant pathogens. We found that the HI + HF, HI + LF, LI + HF, and LI + LF treatments resulted in increases in the relative abundance of potential plant pathogens, which might trigger plant diseases and have negative effects on crop production. Although the interactions between legacy effects from fertigation on fungal pathogens and other soil microbial community effects were difficult to control, further investigations in the future are required to address this important aspect.

In line with our hypothesis, microbial co-occurrence networks were essential in sustaining soil fertility and crop yield. Generally, communities with higher network modularity are regarded as better organized or better operational communities as they have more functionally interrelated members (Chen et al. 2020). However, it should be noted that network analyses do not distinguish whether niche sharing is casual or whether it is due to functional interactions between different taxa (Nannipieri et al. 2020). These members comprise the functional units to perform specific functions, such as N cycling. In addition, the high modularity of the microbial community may result in a more complex ecosystem (Chen et al. 2020), and the ambient disturbance was unlikely to spread to other modules, resulting in a more stable network structure (Wang et al. 2016b). Taken together, our results indicate that soil fertility and yield enhancements were due to the increased specific functional units and stable network structures caused by modularity. Interestingly, Liu et al. (2020b) reported a positive correlation between bacterial network complexity and aboveground biomass in a glass greenhouse in which soil moisture was maintained at 20% and with abundant N fertilizer legacy. The reason for the different conclusions may lie in the different soil moisture and nutrient supply conditions between the two experimental plots. Drought and barren conditions may drive the formation of different specific functional units (e.g., N-cycling functions) in microbial communities and thus increase the microbial network modularity to resist external disturbance in arid agroecosystems, indicating that the stronger stability of the microbial community was primarily driven by ecological network modularity rather than connectivity in the arid agroecosystems.

In this study, bacteria contributed more to soil fertility and maize yield, compared to fungi. A possible explanation for this was that the dominant bacteria, such as Bacteroidia, Gemmatimonadetes, Alphaproteobacteria, and Gammaproteobacteria, contributed more to nutrient turnover in the soils, while the dominant fungi, such as Dothideomycetes (Schoch et al. 2009) and Leotiomycetes (Wang et al. 2006), combated the pathogenic taxa in the soil. Furthermore, bacteria but not fungi can fix C and atmospheric N (Feng et al. 2018). Therefore, bacteria played a vital role in the accumulation of nutrients in arid cropland.

Conclusions

This study demonstrated the essential roles of microbial diversity in maintaining soil fertility and crop production. The contribution of bacteria to soil fertility and maize production was higher than that of fungi, and irrigation and fertilization indirectly affected microbial functions by altering bacterial diversity and co-occurrence network modularization, which in turn affected soil fertility and maize yields. The combined application of the medium irrigation treatment and low fertilization could improve the soil nitrification, ammoxidation, N-fixation potentials, bacterial diversity and their network modularity, and maize yields. It is worth noting that the exact nature of bacterial and fungal interactions remains unknown through co-occurrence networks and detection of gene is not its expression. Additionally, given the important roles of rare taxa in soil functions and interactions with plants, their contributions to microbial co-occurrence should be considered in future studies, especially in the rhizosphere zones where plants and microbes more commonly interact.

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