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Interspecific interactions between crops influence soil functional groups and networks in a maize/soybean intercropping system

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ABSTRACT

Despite increasing evidence that intercropping systems may increase crop productivity, little is known about whether and how soil biota change under interspecific competition among plants. A field experiment with maize/soybean intercropping and the corresponding monoculture systems was conducted under four nitrogen fertilization regimes to investigate the effects of interspecific plant interactions on grain yield, soil properties (β-glucosidase and water-soluble carbohydrates), and biotic communities (bacteria, fungi, and nematodes). The soils under maize and soybean in the intercropping system were sampled separately to represent intercropped maize and intercropped soybean, respectively. Compared to monocultures, the complexity and robustness of soil networks comprising bacterial, fungal, and nematode communities increased in intercropped maize soils, but densities of plant parasitic nematodes and β-glucosidase activity were reduced. Intercropped soybean soils had lower C availability due to chronic shading by maize, but a significant increase was found in saprophytic fungi, and plant parasitic nematodes compared to soybean monoculture soils. Although intercropped soybean suffered from interspecific competition with maize, the interactions within the fungal community increased for both crop species in the intercropping system. Our study demonstrates that increased carbon uptake in maize due to increased light capture in the intercropping system can facilitate nutrient cycling by altering the abundance of functional groups of soil organisms, such as saprophytic fungi, and enhancing network complexity and stability. We detected a trade-off between productivity and soil nematode diversity in the intercropping system under nitrogen fertilization, the yield of maize increased but soil nematode richness decreased at low nitrogen level, while the yield of soybean decreased but the nematode richness increased at high nitrogen level. These findings show that both trade-offs and benefits occur in intercropping systems and highlight the role of plant-soil biota interactions in such systems.

1. Introduction

Biodiversity can be essential for the maintenance and functioning of ecosystems (Wright et al., 2021). Higher plant diversity usually results in more productivity in natural ecosystems (Tilman et al., 2001; Huang et al., 2018) and cropland ecosystems (Li et al., 2021). Although mechanisms have been provided by Loreau and Hector (2001) to reveal positive biodiversity effects, the role of plant-plant interactions such as competition and facilitation, and plant-soil interactions remain poorly understood (Barry et al., 2019).

Competition is usually considered as a dominant force in regulating plant diversity (Brown and Cahill, 2022), but interspecific competition can be weaker than intraspecific competition (Adler et al., 2018). Due to niche overlapping, intraspecific competition can speed up resource depletion as conspecific plants use the same resource, while in diverse plant communities, resource partitioning and niche differences enhance

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resource-use efficiency (Tamburini et al., 2020). Previous studies mainly focused on competition for nutrients (Tilman et al., 1997). However, light is also a limiting resource for plant growth (Kothari et al., 2021; Eskelinen et al., 2022). Larger plants may capture more light and shade out their neighbors (Brown and Cahill, 2022). Up to 30% of the total amount of photosynthetically fixed carbon (C) by plants is transferred as root exudates and input into the rhizosphere during plant growth (Kuzyakov and Domanski, 2000). Shading by neighbors which reduces plant growth can also result in a decrease in the allocation of root-derived carbon to the soil of a focal plant (de Vries and Caruso, 2016).

Plants provide the primary resources (C) to the soil and these C are the fuel for the soil food web. Soil biota can take multiple strategies to cope with changes in C availability (Risely, 2020). One of the strategies that soil biota take is to produce specific enzymes to conquer low C availability in soil, such as the C-degrading enzyme β-glucosidase (BG), which is mainly associated with the degradation of organic matter in the soil (Allison et al., 2011). Changes in C availability can also influence the interactions within the entire food web and will lead to changes in trophic interactions (Moore et al., 2003). Carbon limitations in the soil can further change the structure of ecological networks, with a decrease in interactions among species with decreasing C availability (Li et al., 2022).

An increase in soil nutrient availability can also alter carbon availability (Ning et al., 2021). Under nitrogen (N) addition, plants may input fewer root exudates into the soil during plant growth (Rappe-George et al., 2017). This phenomenon can be explained by plant-microbe relationships, as plants are primarily limited by nutrients whereas soil microorganisms are limited by carbon (Soong et al., 2020). Plants can emit root-derived carbon into the soil to facilitate soil microbes to decompose soil organic matter (SOM) and make nutrients available from plant-emitted carbohydrates (Pausch et al., 2016). The stimulation of plant growth via increased N availability through fertilization can result in a reduction in root-derived carbon in the soil, and consequently, a reduction in carbon availability (Soong et al., 2020). In the context of competition, for plants, nutrient addition causes a shift from competition for nutrients belowground to competition for available light aboveground (Hautier et al., 2009). Because the availability of light in the understory will be decreased as productivity increases, and short species will be shaded by taller species. In this case, the amount of root-derived carbon exuded into the soil by plants belonging to the short species will be further limited.

Interspecific facilitation among species and niche partitioning are other mechanisms that can contribute to positive relationships between biodiversity and ecosystem functioning (Loreau and Hector, 2001). Interspecific facilitation among plants can also be via the suppression of species-specific pathogens or the facilitation of nutrient cycling by a particular species (Wright et al., 2021). Such facilitation mechanisms are associated with interactions within the soil food web. Belowground pathogens, for instance, can be regulated by interspecific competition among soil biota for space, nutrients, and niches, and/or via top-down trophic regulation by predators (Thakur and Geisen, 2019). Among coexisting conspecific plants, soil pathogens, and herbivores can have negative density-dependent effects on plants, because these pathogens and herbivores (e.g. plant parasitic nematodes) are usually less mobile and more persistent in soils, resulting in easy infection among conspecific plants and reduction in plant growth (Schnitzer et al., 2011).

Strip intercropping is the simultaneous production of two or more crops on the same field (Vandermeer, 1992). Compared to monocropping, intercropping can increase yield and resource-use efficiency, and when planted with legumes it can increase extra nitrogen input via symbiotic N_2 fixation (Li et al., 2021). Here, we studied how maize (*Zea mays* L.) and soybean (*Glycine max* L.) grown in intercropping and monocultures with different nitrogen addition levels influenced the belowground biotic network and their relations with plant productivity. In the intercropping system, maize which is taller than soybean causes chronic shading for soybean, intercropped maize will increase, whereas intercropped soybean will reduce soil C availability compared to the corresponding monocultures. We hypothesized that to overcome low C availability, in the soil of intercropped soybean, there would be an increase in C-degrading enzymes that are associated with the decomposition of SOM and the functions of its core biota would be more saprotrophic to decompose labile C (Hypothesis 1). Because a reduction in carbon availability would result in reduced interactions among soil biota, we hypothesized that the network in the soil of intercropped soybean would be less complex and robust than the network in the monoculture soybean. On the other hand, the complexity and robustness of the network would be greater under intercropped maize, than in the corresponding monoculture (Hypothesis 2). As nitrogen addition would change the plant's carbon input into the soil as well as belowground community composition, we further hypothesized that nitrogen fertilization would increase C-limitation and change the community composition and associated functions within networks, with more pathogenic species occurring in networks in monoculture soils due to negative density-dependent effects (Hypothesis 3).

2. Materials and methods

2.1. Field experiment description and soil sampling

The field experiment was conducted at the Lishu Experiment Station of the China Agricultural University (43.3°N, 124.4°E), located in Lishu County, Jilin Province, Northeast China. The average air temperature was 6.5 °C, and the average annual precipitation was 577 mm. Basic soil properties were recorded for the 0–20 cm topsoil as 19.1 g kg^{-1} SOM, 1.3 g kg^{-1} total nitrogen, and a pH of 5.8 (H.Yang et al., 2022). The soil is characterized as phaeozem (H. Yang et al., 2022) and consists of 30.9% clay, 45.2% silt, and 23.9% sand.

The experiment was established in 2017 and had been planted with monoculture maize prior for decades. This experiment followed a randomized split-plot design replicated in three blocks, with nitrogen fertilization (four levels: N0, N1, N2, and N3) as the whole factor and cropping system as the split factor. Three cropping systems were established for each N level: two monoculture (maize or soybean), and maize/soybean strip intercropping (Fig. S1). Each plot size was 43.2 m^2 (7.2 m × 6 m). The strip intercropping was planted in alternating 2.4-m-wide strips with 0.6 m inter-row distance containing two maize rows and two soybean rows, maize and soybean strips were swapped each year since 2017. Monocultures of maize or soybean were maintained in the same plot since 2017 (i.e. no rotation), with a 0.6 m inter-row distance. The preceding crop residues in the soils of each plot were removed before sowing to minimize the legacy effects of pre-crop on the present crop and rhizosphere microbial communities. N fertilization was split into four levels: N0, N1, N2, and N3, which were 0, 180, 240, and 300 kg N ha^{-1} for maize and 0, 40, 80, and 120 kg N ha^{-1} for soybean, respectively. All the cropping systems received basal nitrogen fertilization of 0, 40, 80, and 120 kg N ha^{-1} in the form of urea to corresponding nitrogen levels. The remaining nitrogen fertilizer was equally fertilized twice at the jointing stage and pre-tasseling stage for both intercropped and monoculture maize. Conventional tillage was applied in each experimental plot following local traditions.

Root zone soil samples of maize and soybean were collected approximately 1–2 cm away from the base of plants during the growing season when maize was at the pre-tasseling stage and soybean was at the flowering stage in early August 2019 and 2020. In each monoculture plot, ten augers of soil from the 0–20 cm topsoil were merged to represent each treatment. In each intercropping plot, the soil samples of intercropped maize and intercropped soybean were collected separately, that is, each intercropped treatment (maize or soybean) was represented by merged soils in ten augers (Fig. S1). Totally, 48 soil samples were collected each year, including monoculture maize (Mono.M), monoculture soybean (Mono.S), intercropped maize (Inter.M), and intercropped soybean (Inter.S) with 4 N levels, all with 3 replicates (Fig. S1). Each soil sample was first sieved through a 2 mm mesh to remove the roots and stones and then divided into three parts: one subsample was stored at -80 °C for soil microbial DNA extraction, one subsample was stored at 4 °C for nematode extraction, soil nitrate, and soil enzyme analysis, and another subsample was air-dried for soil chemical analysis.

2.2. Analysis of chemical properties, grain yield

Total carbon (TC) was determined by combustion (Vario MACRO cube, Germany). Nitrate (NO_3^--N) was extracted with 2 mol L^{-1} KCl and determined with a continuous flow analyzer (FIAstar 5000 Analyzer, Foss Tecator, Hillerød, Denmark). The ratio of C/NO_3^- was calculated as the ratio of TC to nitrate, with a higher C/NO_3^- ratio in soils favoring dissimilatory nitrate reduction (DNR) to ammonia (DNRA) over denitrification (Putz et al., 2018). β-glucosidase (BG) was determined using 4-methylumbelliferone-β-D-glucoside as a substrate linked to a methyl umbelliferyl fluor (Marx et al., 2001). The anthrone-sulfuric acid method was used to quantify water-soluble carbohydrates (WSC) (Grandy et al., 2000), to represent the labile C fraction in the soil (Ning et al., 2021). BG and WSC were only measured in 2019. The grain yield of crops was measured according to H. Yang et al., 2022.

2.3. DNA extraction and amplicon sequencing

For microbial DNA, 0.5 g of fresh soil from each sample was extracted and purified with Fast DNA SPIN Kit for soil (MP Biomedicals, USA) following the manufacturer's protocol. For nematode DNA, 100 g of fresh soil from each sample was used to extract soil nematodes with the cottonwool filter method (Townshend, 1963), and filtered with a Baermann funnel to remove small soil particles and obtain clear nematode suspension, then this nematode suspension was enriched to 0.5 mL for DNA extraction. Nematode DNA was extracted with DNeasy Blood & Tissue Kit (QIAGEN) following the protocol described by Du et al. (2020) and Sun et al. (2023). PCR primers 515 F/907 R (Biddle et al., 2008), primers ITS86F/ITS4R (De Beeck et al., 2014) and NF1F/18Sr2bR (Porazinska et al., 2009) were used to amplify the bacterial 16 S rRNA genes, fungal ITS rRNA genes and nematode 18 S rRNA genes, respectively. The barcoded amplicons were sequenced on an Illumina Miseq platform at Shanghai Majorbio Bio-Pharm Technology Co., Ltd (China).

2.4. Sequence preprocessing

Bacterial, fungal, and nematode amplicon sequences were analyzed with EasyAmplicon v1.14 (Liu et al., 2021). Raw amplicon reads were merged, primers and barcodes were removed, and then quality-control was performed to obtain high-quality amplicon sequences in VSEARCH v2.15 (Rognes et al., 2016). These high-quality sequences were denoised into amplicon sequence variants (ASVs) and representative sequences were picked (Edgar, 2016b) in USEARCH v11.0.667 (Edgar, 2010). The bacterial, fungal, and nematode ASVs were aligned to the RDP v18 database (Cole et al., 2009), UNITE v8.2 database (Kõljalg et al., 2005), and NCBI nucleotide (nt) database (Sun et al., 2023) to remove chimera sequences with the UCHIME algorithm (Edgar et al., 2011), respectively. The taxonomy of bacterial and fungal representative sequences was determined with RDP v18 and UNITE v8.2 using the SINTAX algorithm (Edgar, 2016a). Bacterial ASVs assigned to chloroplasts and mitochondria as well as archaea were removed from the dataset (Xu et al., 2022). Representative sequences of nematodes were blasted against the nt-database to obtain accession IDs (Altschul et al., 1997), then these accession IDs were mapped into taxIDs with the NCBI taxonomy database (Schoch et al., 2020). Finally, nematode taxonomy was done by translating taxID into a lineage with the taxonkit tool (Shen and Ren, 2021). After taxonomic identification, we obtained 14,745 ASVs for the bacteria, 1744 ASVs for the fungi, and 1033 ASVs for the nematodes. All samples were rarefied to a minimum of each sequencing depth of 8644 for bacteria, 19,744 for fungi, and 26,722 for nematodes for downstream analyses. The richness and relative abundance of soil biota was calculated based on rarefied data.

2.5. Effect size of yield, water-soluble carbohydrates, and soil biota richness

Effect sizes (Gong et al., 2022) were calculated as the log ratio of each intercropped plant to its respective monoculture companion for yield, water-soluble carbohydrates (WSC), and soil biota richness, respectively. For example, as an effect size for relative yield in intercropped maize and monoculture maize ($E_{M\text{-}y}$), we used the log ratio of yield in intercropped maize ($Y_{Inter.\mathrm{M}}$) and monoculture maize ($Y_{Mono.\mathrm{M}}$): $E_{M\text{-}y} = \ln\ (Y_{Inter.\mathrm{M}}\ /\ Y_{Mono.\mathrm{M}})$. Similarly, the effect size for the relative WSC ($E_{M\text{-}wsc}$), and relative richness of bacteria ($E_{M\text{-}b}$), fungi ($E_{M\text{-}f}$), and nematodes ($E_{M\text{-}n}$) in intercropped and monoculture maize as well as in intercropped and monoculture soybean (i.e. $E_{S\text{-}y}$, $E_{S\text{-}wsc}$, $E_{S\text{-}b}$, $E_{S\text{-}f}$, and $E_{S\text{-}n}$) were also calculated with the same formula. To compare the strength of the trade-off among yield, WSC, and richness of soil biota, the compatibility index (CI) was used (Gong et al., 2022) by comparing the above effect sizes in maize and soybean separately. For example, the compatibility index of relative yield ($E_{M\text{-}y}$) and relative bacterial richness ($E_{M\text{-}b}$) in maize was defined as $CI_{M:\ y\text{-}b} = E_{M\text{-}y} + E_{M\text{-}b}$. As nitrogen fertilization generally results in an increase in yield and a decrease in soil diversity for intercropped maize and causes the opposite results in the intercropped soybean, the positive value of the compatibility index for maize simply means, for example, that the yield gains exceed the biodiversity losses in the intercropped maize. One sample t-test was used to compare the difference between effect size (or compatibility index) and zero (G. Yang et al., 2022), the significant differences ($P < 0.05$) indicate that variables in intercropped treatment are significantly different from the corresponding monoculture treatment.

2.6. Crop-associated core biota and their potential functions

The abundance-occupancy relationship was used to identify the potential core biota members (Shade and Stopnisek, 2019). As the core biota is plant-specific, we determined the core biota at the ASV level for maize and soybean separately. To detect the enriched and depleted core biota in intercropping plants, a Welch's t-test based on rarefied data (Nearing et al., 2022) was used to compare intercropping to monoculture for maize and soybean. The phylogenetic tree was used to visualize the core biota using the R package ggtree (Yu et al., 2017).

The enriched or depleted core biota under intercropping plants was further analyzed to infer their potential ecological functions at the ASV level. For bacteria, first, the potential ecological functions were assigned by FAPROTAX v1.2.6 (Louca et al., 2016). Secondly, the prediction of gene pathways was determined using the PICRUSt2 pipeline v2.4.2 (Douglas et al., 2020). We mainly focused on carbon and nitrogen cycling based on Enzyme Classification (EC) predictions, and only C-degrading enzymes (Chen et al., 2020) and nitrogen metabolism (https://www.kegg.jp/) were included. The predicted EC number of core ASVs was used to infer bacterial potential functions. The FungalTraits database was used to identify the main fungal functional groups (Põlme et al., 2020). The trophic groups and feeding habits of nematodes were assigned according to the Nemaplex website (http://nemaplex.ucdavis.edu) and Yeates et al. (1993), and the enriched or depleted nematode ASVs were assigned based on their genus level taxonomy.

2.7. Network construction

Co-occurrence network analysis was used to construct a network of potential ecological relationships for the different cropping systems from 2 years' data. For each cropping system, we used 24 samples (4 N levels × 3 replicates × 2 years) to construct the network. To do this, we first combined the bacterial, fungal, and nematode ASV feature tables to represent one community, and ASVs occurring in 50% of the 24 samples were kept to remove rare species. Next, pairwise correlations of combined ASVs were calculated based on the Spearman correlation of their relative abundances, and the correlation cutoffs were determined by the random matrix theory (RMT) method (Luo et al., 2006). The RMT cutoffs were 0.8 for all networks. The correlations above the RMT cutoff for each network were further distinguished to discard the indirect edges using the iDIRECT method (Xiao et al., 2022), and the remaining direct edges were used to construct a network and visualized using the igraph R package (Csárdi and Nepusz, 2006).

The network topological indices were calculated to characterize the structure of networks in igraph and brainGraph packages (Watson, 2020) to reflect the network composition (proportion of nodes represented by each ASV among bacteria, fungi, and nematodes), network interactions (proportion of edges related within and among bacteria, fungi, and nematodes), network complexity (average connectivity and complexity) (Wagg et al., 2019) and small-world property (a network with a small average path length and a high average clustering coefficient is considered as a small-world network) (Watts and Strogatz, 1998). Communications among each species are efficient in a small-world network (Yuan et al., 2021). Natural connectivity was used to assess how quickly network structural robustness degrades after removing nodes, in order of decreasing degree (the number of connected neighbors), decreasing betweenness (the number of paths through a node), or randomly (Peng and Wu, 2016; Ruiz et al., 2017). Furthermore, the species that remained in the network after the random removal of network species or targeted removal of module hubs were also used to determine the network robustness (Yuan et al., 2021). The node degrees were used to fit the power law model, and also to obtain the R^2 of the power model for each network. For each node, its within-module connectivity (Zi) and among-module connectivity (Pi) were calculated to represent its topological roles in the network (Guimerà and Amaral, 2005). We adopted the criteria described in other studies (Olesen et al., 2007; Ling et al., 2016). Network hubs ($Zi \geq 2.5$, $Pi \geq 0.62$), module hubs ($Zi \geq 2.5$, $Pi < 0.62$), and connectors ($Zi < 2.5$, $Pi \geq 0.62$) were identified as keystones, while other nodes were identified as peripherals. Degree centrality, betweenness centrality, and closeness centrality of each node were calculated to assess the importance of nodes in mediating overall species interactions (Girvan and Newman, 2002). To confirm that the empirical network topology represented a nonrandom assembly of microorganisms, 1000 random networks were conducted with the *erdos.renyi.game* functions and the node degrees were calculated to distinguish between empirical and random networks in the topological structure.

To test for nitrogen effects on different cropping systems, the 24 samples from each cropping system were further divided into low nitrogen levels (N0 and N1) and high nitrogen levels (N2 and N3) to prevent biased results yielded by spearman correlation based on small samples (Guseva et al., 2022). ASVs occurring in 6 samples were kept to construct networks. The lowest Spearman's Rho of each network varied between 0.92 and 0.95 after the P-values were corrected with the Benjamini-Hochberg method (Yoav and Daniel, 2001). The RMT method, as well as the iDIRECT method, failed to determine the cutoff of each network and to discard the indirect edges. We, therefore, used the same standards with Spearman's $Rho > 0.95$ and adjusted $P\text{-value} < 0.05$ to conduct all networks. As fungal nodes or edges in all networks increased from low to high nitrogen levels, the functional guilds of common fungal ASVs shared between low and high nitrogen levels, as well as the unique fungal ASVs occurring either in low or high nitrogen levels were identified using FungalTraits to further test the differences in these guilds between low and high nitrogen levels with a Welch's t-test.

2.8. Statistical analyses

All statistical tests were done in R 4.2.1 (R Core Team, 2022). The linear mixed effects model (LMM) was used to estimate the effects of treatments on soil chemistry, richness, and the relative abundance of microbial and nematode groups using lme4 packages (Bates et al., 2015). Maize and soybean plants were analyzed separately, and statistical comparisons were done between each monoculture and its respective intercropped companion. Nitrogen levels, cropping system treatments, and their interactions were considered as fixed effects, whereas block and year were considered as random effects. Type II Wald F tests were used to calculate the P-values from models with the *Anova* functions in the car package (Fox and Weisberg, 2019). Differences in degree, betweenness, and closeness centralities were logarithmically transformed and tested using a Wilcoxon rank-sum test with the ggsignif package (https://github.com/cran/ggsignif).

3. Results

3.1. Crop productivity and soil properties

Intercropping significantly increased grain yields ($P < 0.001$) and soil WSC ($P < 0.05$), but reduced soil BG activity in maize compared with monoculture maize (Fig. 1, A, C, E). In contrast, intercropping significantly reduced the grain yield of soybean ($P < 0.01$), and there was a tendency for reduced soil WSC ($P = 0.044$, one-way ANOVA), and increased soil BG activity compared with monoculture soybean ($P = 0.043$, one-way ANOVA) (Fig. 1, B, D, F; Table S1).

3.2. Enriched and depleted core biota in the intercropping system

According to the abundance-occupancy distributions at the ASV level, most of the core biota were deterministically selected by maize or soybean species (Fig. 2). In total, 28 core (either enriched or depleted, $P < 0.05$) bacterial, 4 fungal, and 36 nematode ASVs were found under intercropped maize (Fig. S2, A-C), and 47 core bacterial, 9 fungal and 24 nematode ASVs were observed under intercropped soybean (Fig. S2, D-F).

For intercropped soybean, functional annotations by FAPROTAX revealed that most of the enriched ($P < 0.05$) core bacteria were related to chemoheterotrophy, and methane decomposition functional groups (Fig. 3A). The result of PICRUSt2 (Fig. 3A) showed that enriched bacteria under intercropped soybean were related to C-degrading enzymes, mainly β-glucosidase (EC3.2.1.21), following β-xylosidase (EC3.2.1.37), β-cellobiohydrolase (EC3.2.1.91) and polyphenol oxidase (EC1.14.18.1). The enriched or depleted bacteria under intercropped maize were similar in quantity (13 vs 15), but distinct in functions, as some of the depleted bacteria were only related to β-glucosidase. Besides, enriched bacteria, both under intercropped maize and intercropped soybean, were related to nitrogen metabolisms (Fig. 3A), such as nitrogen fixation (Nfix), denitrification (Deni), and dissimilatory nitrate reduction (DNR) to ammonia. In particular, the relative abundance of DNR was significantly greater than Nfix and Deni under Inter.S (Fig. S3B), and the C/NO_3^- ratio was also significantly greater under Inter.S than Inter.M (Fig. S3A). According to FungalTraits assignments, enriched fungi under Inter.S were assigned as saprotrophs (Fig. 3B), and enriched nematodes belonged to bacterivores, fungivores, and plant parasites (Fig. 3C). For Inter.M, most of the enriched nematodes were bacterivores, whereas the depleted nematodes were plant parasites (Fig. 3C). Inter.M both enriched and depleted some saprotrophic fungi (Fig. 3B).

3.3. Co-occurrence networks of different cropping systems

The networks for different cropping systems showed scale-free patterns, with the degree of distributions following the power law distributions with R^2 values from 0.85 to 0.91 (Fig. S4). Although the numbers of nodes under Inter.M and Inter.S were lower than those under Mono.M and Mono.S (Fig. 4, A-D), the proportion of fungal and

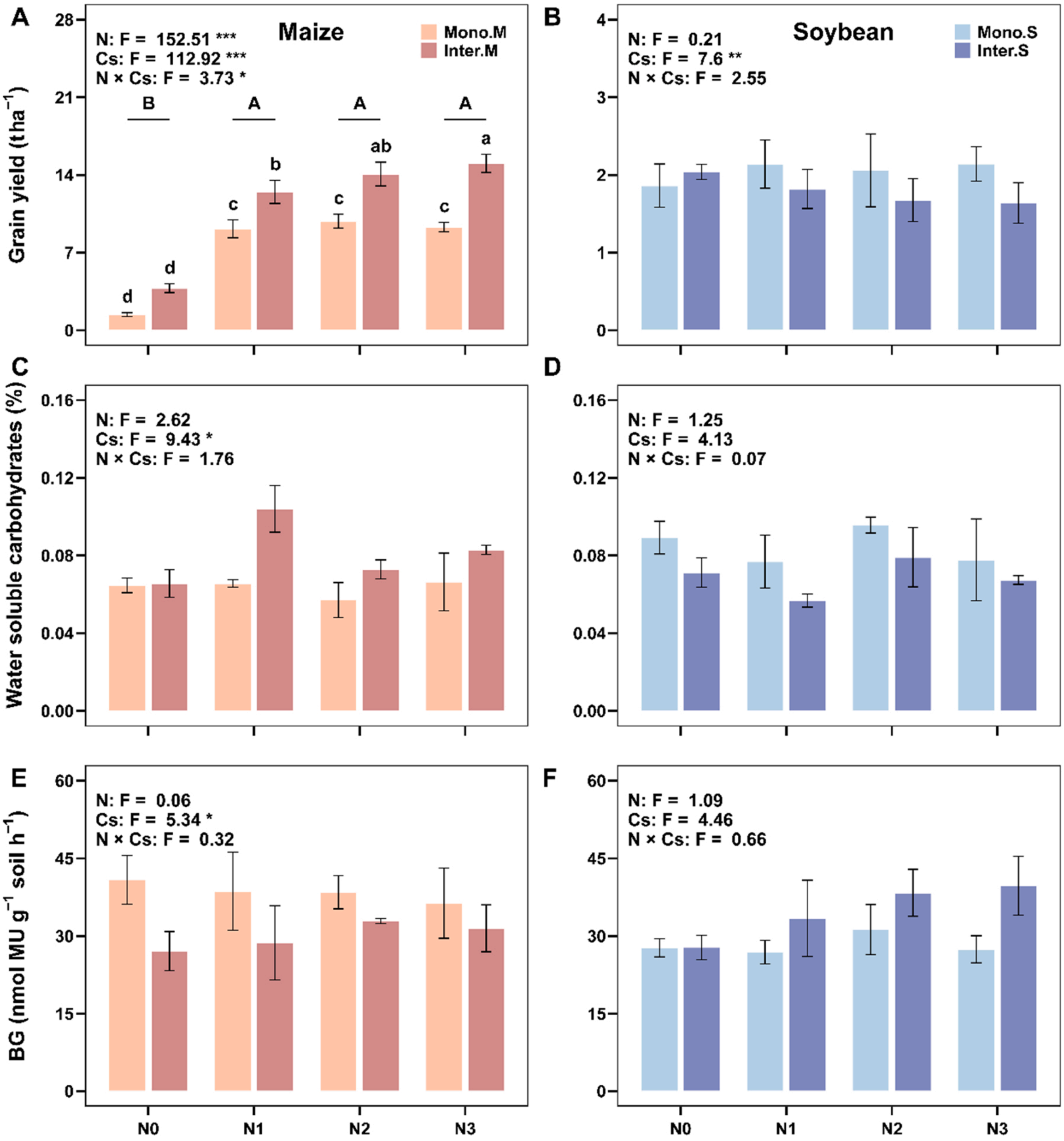

Fig. 1. The grain yield of two years (2019–2020) (A, B), water-soluble carbohydrates (WSC) (C, D), and β-glucosidase (BG) (E, F) for monoculture and intercropped maize cropping system (Cs) and soybean at different N addition levels. *P $<$ 0.05; * *P $<$ 0.01; * **P $<$ 0.001.

nematode nodes were enriched with more arbuscular mycorrhizal fungi, fungivores, predators and omnivores under Inter.M compared with Mono.M (Fig. S5). More saprotrophic fungi and fewer herbivores were found under Inter.S than under Mono.S (Fig. S5). The edges linked within the fungi community dominated among network interactions under Inter.M (56%), Inter.S (46.1%), and Mono.S (42.3%), whereas network interactions under Mono.M were dominated by links between bacteria (57.7%) (Fig. 4F). Furthermore, keystones consisting of bacteria, fungi, and nematodes were identified under Inter.M and Inter.S, compared to only bacteria under Mono.M and Mono.S (Fig. 4G). Additionally, the degree and betweenness centralities were significantly greater for fungi than for bacteria and nematodes under Inter.M and Inter.S (Fig. S6). The complexity and robustness (natural connectivity) of the networks were greater under Inter.M than under Mono.M, while in Inter.S, network complexity and robustness were reduced relative to Mono.S (Fig. 4, H, I; Fig. S7). The natural connectivity of all networks linearly decreased with random species removal (Fig. 4H) but dropped quickly after degree-based and betweenness-based species removal (Fig. S7). Furthermore, the remaining species in networks after the random removal of network species and targeted removal of module hubs also followed the network robustness pattern but with the lowest species remaining in networks under Mono.M after random species or targeted module hubs removal (Fig. S7), and the differences between intercropping and monoculture were greater for maize than for soybean associated species (Fig. S7). Both Inter.M and Inter.S had small-world properties with greater average clustering coefficients and smaller average path lengths than the corresponding monocultures (Fig. 4J).

3.4. Effects of N additions on co-occurrence networks of different cropping systems

To test whether the stability of co-occurrence networks changes with the addition of nitrogen (N) and how this differs between cropping systems, eight networks were conducted (Fig. S8). More fungal nodes

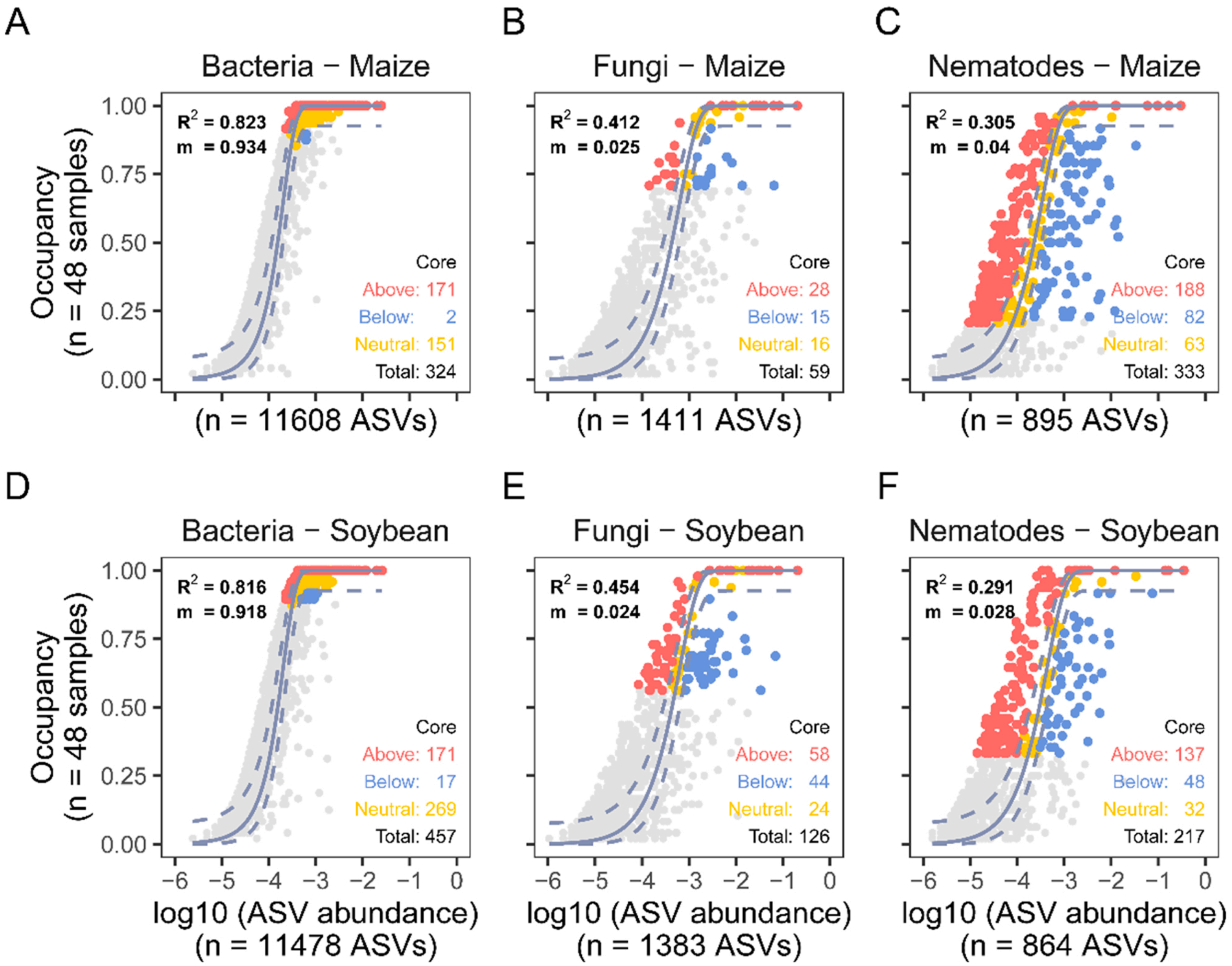

Fig. 2. Abundance-occupancy distributions were used to identify core ASVs of maize/soybean for bacteria, fungi, and nematodes. The solid lines indicate the best fit of Sloan's neutral model and the dashed lines are 95% confidence intervals around the model fit. The point within the 95% confidence intervals (points in yellow) are inferred to be neutrally selected. The points above (points in red) and below (points in blue) the 95% confidence are inferred to be deterministically selected.

were observed in high N than in low N across all networks (Fig. 5A). Edges linked within the nematode community and edges linked between bacterial and nematode communities were all reduced in networks under Inter.M, Inter.S, and Mono.S, but edges linked within fungal community increased (Fig. 5B; Fig. S8). Besides, increasing nitrogen addition enhanced the complexity of all networks (Fig. 5C). There were many more unique species for low and high nitrogen levels than common species shared between two nitrogen levels in all networks (Fig. S9). For the unique fungi, pathogenic fungi (Path) were significantly greater under monocultures, whereas mycoparasite fungi (Myco) were significantly greater under intercropping systems in high N than in low N, and AMF was more abundant in high than in low N but only in the Inter.M system (Fig. 5, H-K).

3.5. *Trade-offs between plant productivity and soil biodiversity*

Overall, the majority of compatibility indices for bacteria, fungi, and nematodes in the soil were significantly larger than 0 for intercropped maize, but smaller than 0 for intercropped soybean (Fig. 6; Table S3). For intercropped maize, the relationship between bacteria richness and grain yield was close to a win-win situation in the N3 treatment, and there were trade-offs with yield gain and nematode richness loss in the N1 treatment (Fig. 6; Table S2). For intercropped soybean, there were trade-offs with yield loss and nematode richness gain in the N3 treatment (Fig. 6). Intercropping significantly reduced nematode richness for maize and increased nematode richness for soybean compared with monoculture treatments (Fig. S10, E, F).

4. Discussion

4.1. *Variations in carbon allocation result in a shift in soil enzymes and functional groups in the intercropping system*

In line with our first hypothesis, compared with the monoculture, intercropping did significantly increase soil water-soluble carbohydrates (WSC) under maize plants, while it was reduced under soybean plants. A previous study in the same field showed that intercropped maize significantly increased root length, and root weight, compared with monoculture maize (H. Yang et al., 2022). Together, these results indicate that growing maize in intercropping will increase plant-derived carbon in the soil. However, soybean in intercropping suffers from severe shading stress from maize during the inter-growth period (Cheng et al., 2022), and this leads to lower photosynthetic rates and less C input into the soil (de Vries and Caruso, 2016). Variations in carbon allocation in intercropping can result in a shift in enzyme activity and potential functions of soil biota. For instance, there was a tendency for increased C-degrading enzyme β-glucosidase (BG) in intercropped soybean to cope with relatively low WSC (Allison et al., 2011), while intercropped maize reduced BG activity because of higher root-derived carbon input (WSC), relative to monocultures. To further overcome low WSC, the soils of

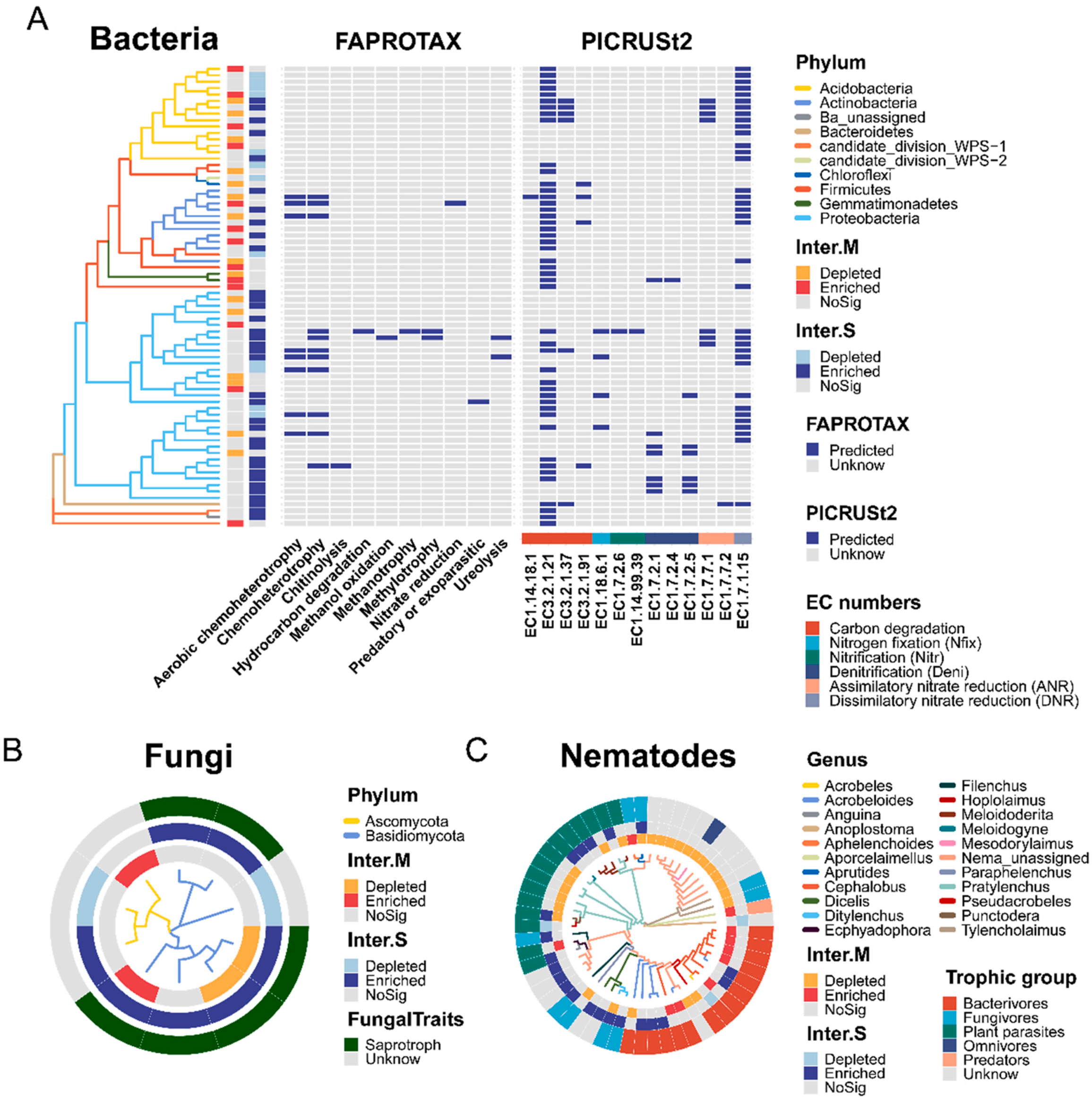

Fig. 3. Functional groups of core biota members under intercropping plants were shown by phylogenetic trees. Functional groups of bacterial ASVs colored at the phylum level were predicted by FAPROTAX and PICRUSt2 (A); functional traits of fungal ASVs colored at the phylum level were assigned by FungalTraits (B); trophic groups of nematode ASVs colored at genus level were assigned according to Nemaplex website (C). Color in red/orange represents core biota that significantly enriched/depleted in intercropped maize (Inter.M), respectively; color in purple/blue represents core biota that significantly enriched/depleted in intercropped soybean (Inter.S), respectively.

intercropped soybean were enriched with core bacteria with C-degrading functions and saprotrophic fungi. In terms of the number of taxa, the enriched fungal taxa belonging to the Basidiomycota phylum outnumbered those belonging to the Ascomycota phylum in the intercropped soybean soils, suggesting that fungal succession may exist during the decomposition, as Basidiomycota phylum is distinctively dominated during the later stages of decomposition because they are more capable to use resistant carbon than the Ascomycota phylum (Voříšková and Baldrian, 2013). In addition, the functional prediction from PICRUSt2 indicated that nitrogen-fixing bacteria were only enriched under intercropped soybean, and intercropped maize and intercropped soybean together increased the relative abundance of bacteria that are associated with dissimilatory nitrate reduction to ammonia (DNRA). Besides, DNRA was stronger than nitrogen fixation under intercropped soybean, indicating that nitrogen fixation of soybean may not be the only way to enhance soil nitrogen availability in the intercropping system, and DNRA may also play an important role in nutrient retention.

4.2. Interspecific competition between crop species shifts soil networks

In line with our second hypothesis, networks under intercropped maize were more complex and robust relative to monoculture. While an opposite pattern was found under intercropped soybean. This suggests that WSC is different in two crops when used in intercropping, and acts as the driver of bottom-up regulation (Moore et al., 2003). As C is the primary energy source of soil biota, C-limitation results in limited microbial activities and thus reduces interactions among soil biota (Pausch et al., 2016). Interestingly, the natural connectivity in networks under intercropped soybean was also lower than in those under monocultures, which suggests that interspecific competition might have been stronger than the intraspecific competition in our study. According to coexistence theory, a core tenet of species coexistence is that interspecific competition must be weaker than intraspecific competition (Adler et al., 2018),

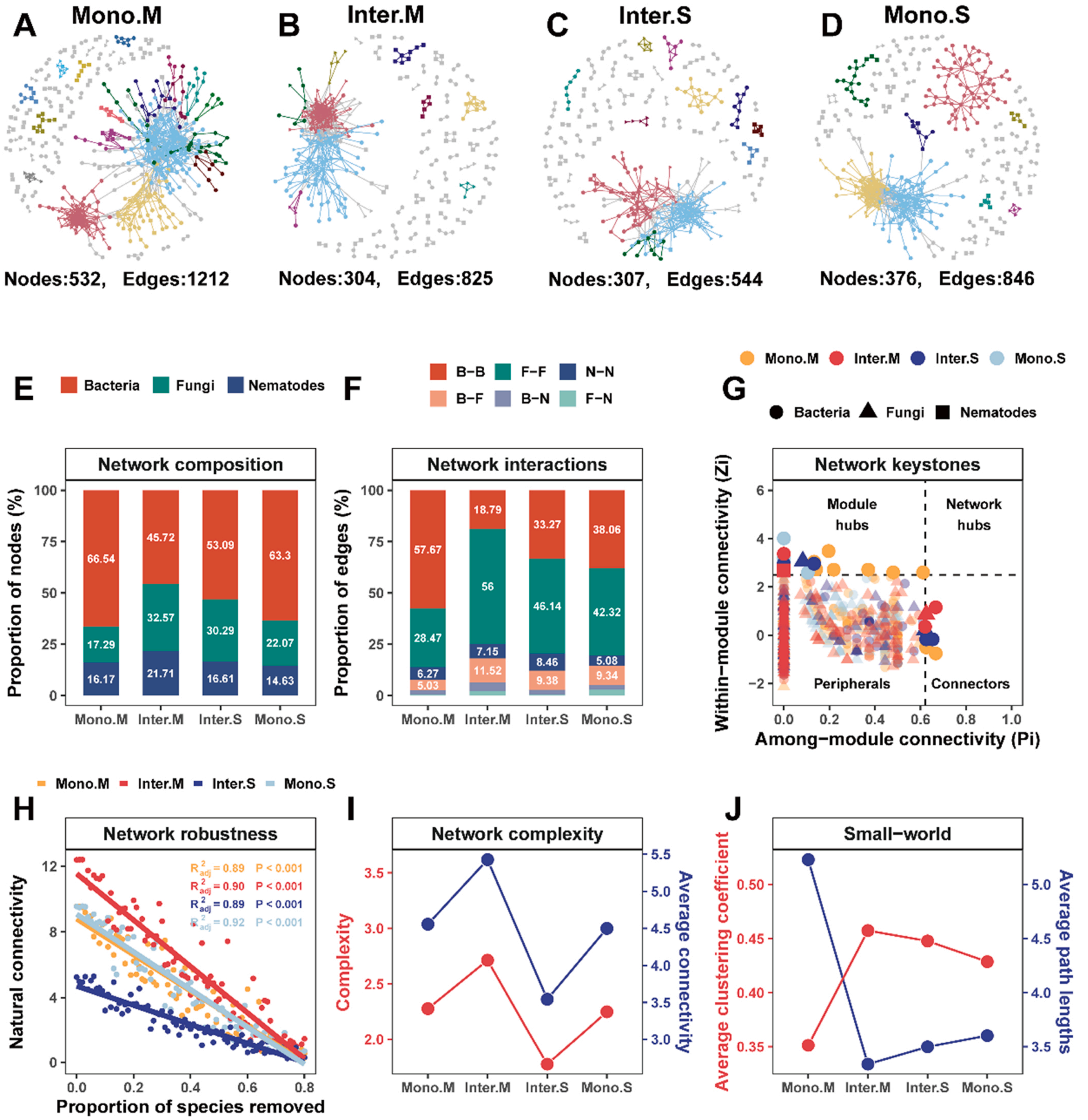

Fig. 4. Network analysis (A-D) shows interkingdom co-occurrence patterns in monoculture maize (Mono.M), intercropped maize (Inter.M), intercropped soybean (Inter.S), and monoculture soybean (Mono.S), respectively. The proportion of nodes (E), and edges (F) were used to represent network composition and network interactions, respectively. Network keystones (G) were determined based on within/among-module connectivity in networks, and module hubs, network hubs, and connectors were selected as keystones. Network robustness (H) is measured as the proportion of species randomly removed from each network. Complexity and average connectivity were used to represent network complexity (I). High average clustering coefficient and small average path lengths were characteristic as small-world properties (J).

thus intercropped maize may outcompete intercropped soybean to acquire more resources, resulting in more complex soil food web interactions in belowground. However, both the proportion of fungal nodes and links within fungal species increased indicating that fungi might play a crucial role in the intercropping system. Furthermore, the network core taxa of intercropped maize and soybean included both fungi and nematodes rather than pure bacteria under monocultures, although the role of these keystone species needs to be tested by further experiments (Röttjers and Faust, 2019). Previous work has shown that fungal communities are more responsive than bacteria to vegetation changes (Dassen et al., 2017). In the intercropping system, not only does the composition of aboveground vegetation change, but also the quality and quantity of plant-derived C input. Furthermore, evidence is accumulating that fungi may be the first consumers of root-derived C (Ballhausen and de Boer, 2016). Although intercropped soybean reduced the complexity and robustness of the networks, their small-world properties

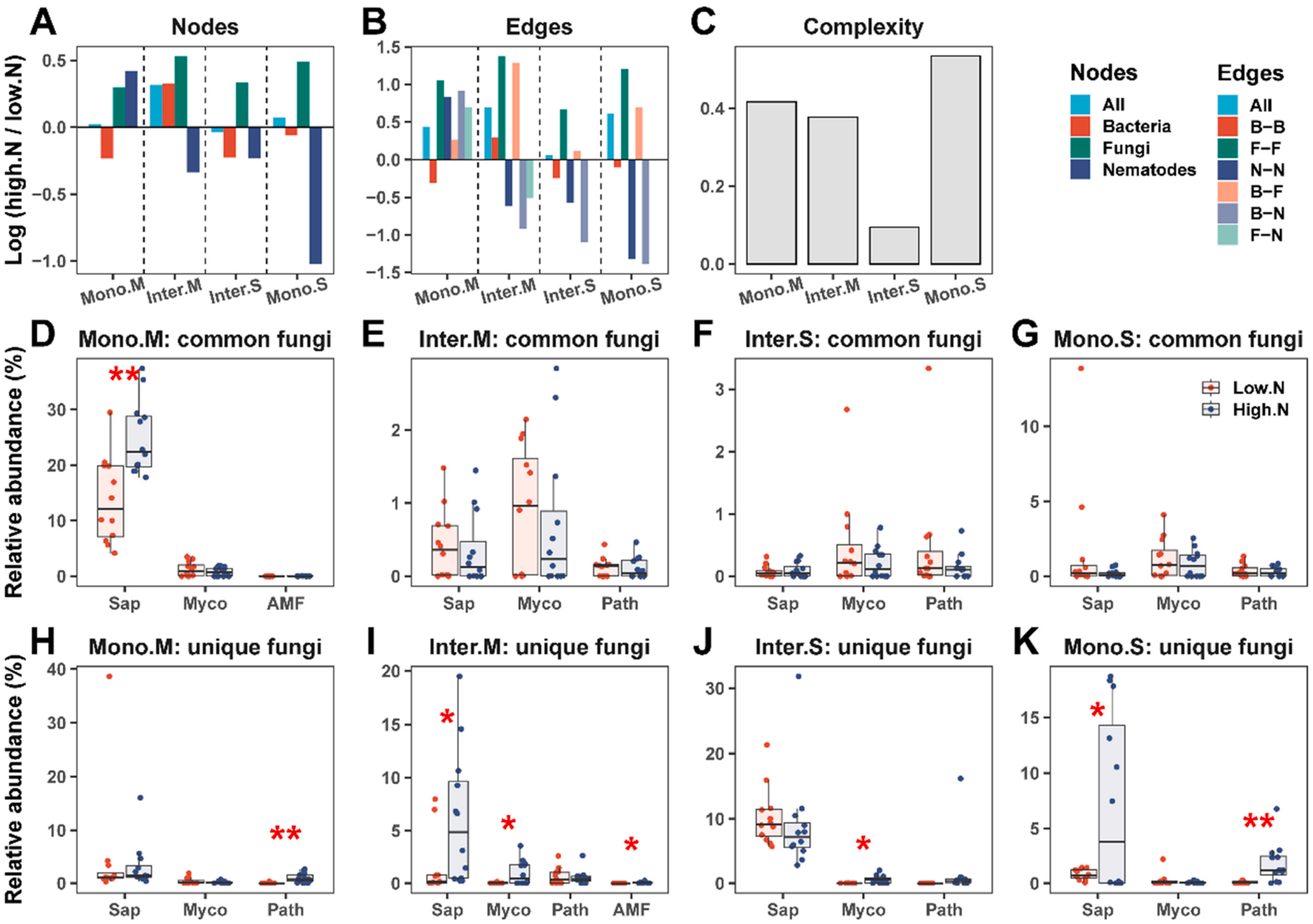

Fig. 5. Log response ratio of high nitrogen (high.N) to low nitrogen (low.N) for network nodes (A), edges (B), and complexity (C) under monoculture maize (Mono.M), intercropped maize (Inter.M), intercropped soybean (Inter.S) and monoculture soybean (Mono.S). The difference of common (D-G) and unique (H-K) fungal guilds between and within low and high nitrogen for each cropping system, respectively. B, F, and N represent bacterial, fungal, and nematode nodes, respectively. B-B, F-F, and N-N represent edges linked within bacterial, fungal, and nematode communities, respectively. B-F, B-N, and F-N represent edges linked between bacterial-fungal, bacterial-nematode, and fungal and nematode communities, respectively. Sap, Myco, Path, and AMF represent saprotrophic fungi, mycoparasite fungi, plant pathogenic fungi, and arbuscular mycorrhizal fungi, respectively. $^{*}P < 0.05$; $^{**}P < 0.01$.

were greater, suggesting that communications among species in the intercropping networks are efficient (Yuan et al., 2021). In the longer term, this may lead to interspecific facilitation between intercropped species through intensified biotic interactions in the intercropping system.

4.3. Shifts in soil networks of different cropping systems in response to nitrogen fertilization

In line with our third hypothesis, nitrogen addition greatly enhanced the complexity of the networks except under intercropped soybean, which suggests that the shading effect caused by intercropped maize may still override the nitrogen effect. Recent studies have shown that increasing nitrogen addition can reduce photosynthetic C input to microbes (Ning et al., 2021) when nitrogen addition leads to increased plant growth, and the plants decrease the exudation of carbon via the roots. Nitrogen addition also changed the community composition of the networks. Interestingly, the relative abundance of unique fungi rather than common fungi increased with nitrogen addition, suggesting that nitrogen addition filters out most of the fungal species that are adapted to low nitrogen levels and enriches N-favored fungi instead. High nitrogen addition also increased the relative abundance of pathogenic fungi in comparison with the low nitrogen treatment in both monocultures. Mycoparasites belonging to the genus *Trichoderma* were found to increase in the intercropping system in the high nitrogen compared with the low nitrogen. Interestingly, mycoparasites are used for the biological control of fungal pathogens and are supposed to control plant pathogens as well (Kim and Vujanovic, 2016). Arbuscular mycorrhizal fungi (AMF) were only enriched in networks under intercropped maize in high nitrogen addition. AMF can provide more nutrients to the host plant. Therefore, AMF under intercropped maize might have alleviated effects on intercropped soybean, as they can uptake and transfer nitrogen from intercropped soybean to intercropped maize (Powell and Rillig, 2018). Taken together, increased nutrients in the intercropping system may further increase C limitation in soils of soybean species, and plant pathogens could increase in abundance with increasing nutrients when crop species are grown alone.

4.4. Trade-offs between plant productivity and soil biodiversity

The trade-off relationship between plant productivity and soil biodiversity may exist in our study. The yield of intercropped maize increased but the soil nematode richness decreased in the N1 treatment, while the yield of intercropped soybean reduced but the soil nematode richness increased in the N3 treatment. Nitrogen fertilization changed the intercropping effects on both plant productivity and soil nematodes, with intercropping effects being much close to a double-win situation under no fertilization, while nitrogen fertilization shifted these positive

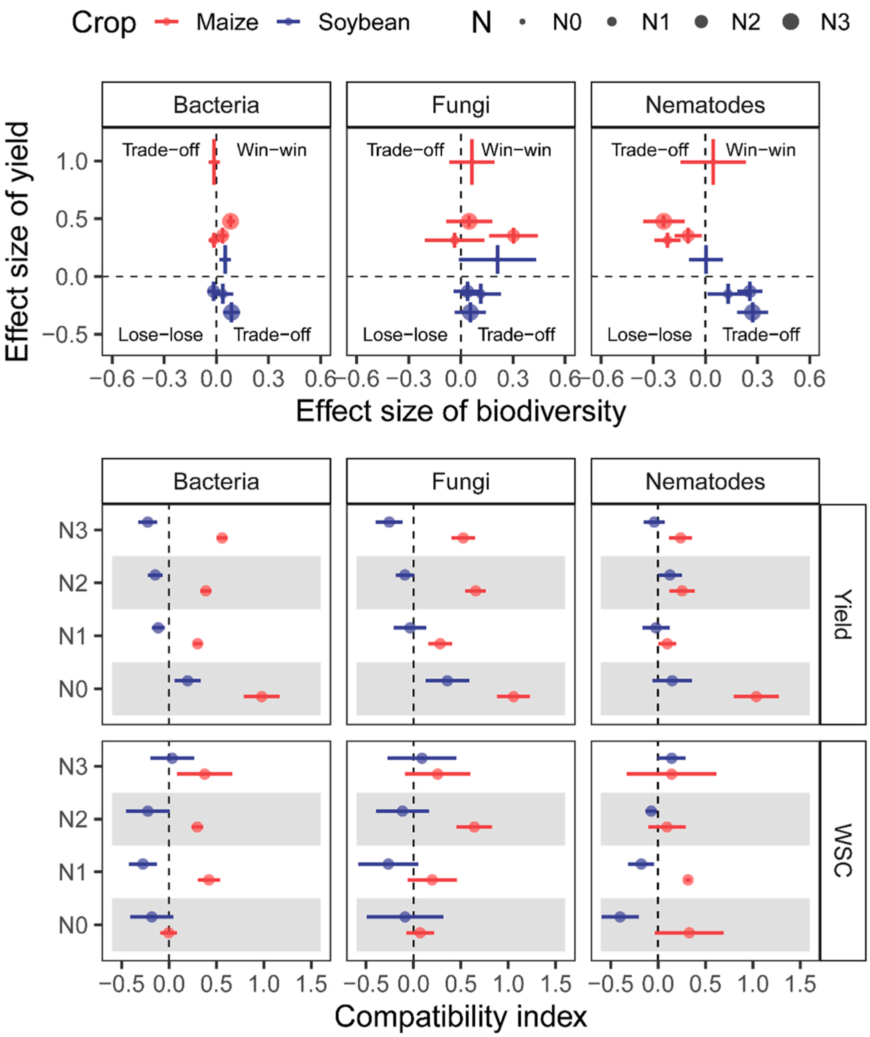

Fig. 6. The log ratio in yield and biodiversity between intercropping and monoculture systems for bacteria, fungi, and nematodes in maize or soybean. The compatibility index (the contrast in log biodiversity and yield, or water-soluble carbohydrates (WSC)) for bacteria, fungi, and nematodes. Dots and error bars show the mean values and standard error.

effects into negative effects, either in increases or losses in plant productivity and soil nematode richness. Resource competition theory predicts that nutrient enrichment usually results in increased plant productivity but in decreased diversity (Tilman, 1982). This is because nutrient addition eliminates limited nutrients, resulting in fast-growing species outcompeting other species, and thus reducing the number of nematodes that coexist in soils of intercropped maize. However, C-limitation under intercropped soybean would allow more nematodes to coexist in soils. A global meta-analysis also confirmed that high nutrient input, such as N fertilization, weakens intercrop overyielding (Zhao et al., 2022), and these are similar to our results, as the effect size in the N0 treatment was greater than those in the nutrient addition treatments. Previous studies demonstrated that annual non-legume crops are primarily limited by nutrients (Soong et al., 2020), thus increasing nitrogen is expected to benefit intercropped maize productivity in the short term. However, in the longer term, reduced soil biodiversity caused by long-term nitrogen fertilization could consequently result in a decrease in productivity due to the loss of positive diversity effects (Seabloom et al., 2021). Therefore, increased crop diversity with appropriate nutrient fertilization is needed to produce food for the increasing population worldwide.

Previous studies showed that soil and rhizosphere microbial communities can be strongly influenced by the preceding crop in the crop rotation (Benitez et al., 2021). In our study, we switched the maize and soybean strips each year in the intercropping system according to traditional farmer practices in China (Cong et al., 2015) to avoid problems associated with repeatedly growing the same crop in the soils, which could lead to an increase of soil-borne diseases (Jing et al., 2022). Simultaneously, the crop residues, primarily dead roots or crop litter, were removed from the soils before sowing in our study, which could help to minimize the legacy effects of previous crops and their associated microorganisms. The previous crop legacies can persist in the soils during an entire season or even for several years (Jing et al., 2022), or can be transient as the present crop also influences the rhizosphere (Benitez et al., 2021). Our study investigated the short-term effects of maize/soybean intercropping on the belowground soil communities, and further research on the long-term effects of the preceding crop is required to better understand this knowledge gap.

5. Conclusions

Intercropped maize resulted in higher C availability in belowground communities, which further increased the complexity and stability of soil networks, as well as pest control of plant parasitic nematodes compared with the corresponding monoculture. In contrast, under intercropped soybean, the activity of BG and the population of saprophytic fungi increased to overcome relatively low water-soluble carbohydrates, and this resulted in reduced trophic interactions in the networks. In addition, the trade-offs between crop productivity gain and soil biodiversity loss caused by nitrogen fertilization under intercropped maize clearly showed the pros and cons of nitrogen addition effects and we propose that these negative effects should be avoided by increasing crop diversity and reducing nitrogen fertilization. Our study demonstrates how interspecific interactions among soil biota change in response to intercropping. A better understanding of these belowground interactions is essential to better manipulate interspecific interactions among plants and soil biota in intercropping systems.

Declaration of Competing Interest

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

Data Availability

Data will be made available on request.

Acknowledgements

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2023.108595.

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