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High bacterial diversity drives the suppression of a soilborne plant disease

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The rhizosphere microbiome plays a crucial role in the resistance to soilborne plant diseases. However, the principles needed to explain and predict which microbiota will be effective against soilborne pathogens are still lacking due to the complexity of the soil microbial community. We hypothesized that, independent of particular microbial strains, a high diversity is associated with, or increases the probability of, effective suppression. We tested this hypothesis by demonstrating that random combinations of rhizosphere microbial isolates, with the same bacterial diversity, had an equal impact on suppressing root diseases. The incidence of root rot was significantly reduced when soil bacterial diversity was high. We further investigated how high-diversity bacterial communities suppress root rot by constructing synthetic bacterial communities (SynComs). The results suggest that high bacterial diversity suppresses pathogens through mechanisms potentially including nutrient competition and the formation of physical barriers on the root surface. Our study highlights that high bacterial diversity is beneficial for suppressing soilborne plant diseases, offering a nonchemical and sustainable approach for crop disease management.

soilborne disease | bacterial diversity | synthetic microbiota | competition | biofilm formation

Significance

Soilborne diseases pose a significant threat to plant health, often leading to substantial crop losses. Through plant–soil feedback, the preceding crop reshapes the soil microbial community and thereby suppresses soilborne diseases of the following crop. To explore whether the preceding crop can enhance the disease-suppressive potential of community by increasing microbial diversity, we constructed 21 synthetic microbial communities with different bacterial diversities to investigate the relationship between bacterial diversity and soilborne root rot. The results showed that soils with higher bacterial diversity exhibited a reduced incidence of *Astragalus* root rot. Our research provides insights into disease management strategies and highlights the potential of enhancing soil microbial diversity as a sustainable approach to mitigating soilborne pathogens.

Soilborne plant diseases are a major cause for crop losses globally (1). It is thus of pivotal importance to better understand which factors can suppress soilborne disease and contribute to plant health. A range of rhizosphere-associated microorganisms recruited by plants have been shown to play an important role in plant growth and health (2, 3). Plants' immune system and antimicrobial substances produced by beneficial microorganisms can directly alleviate the stress of pathogens on plants (2, 4, 5). Furthermore, after reoccurring outbreaks of severe plant soilborne diseases during continuous cultivation of a susceptible plant, the disease development can sometimes be minimal even in the presence of pathogenic agents and susceptible crops (6–9). This disease-suppressive capacity of soil can be transplanted to conducive soil by microbiota transplantation and can be eliminated by sterilization treatment (10, 11). These results indicate that disease suppression is mainly attributed to a soil microbial community that can combat pathogens. Thus some soil microbial communities can result in plant disease resistance, and hence the plant-associated microbial ecology has been studied intensively (12, 13).

The rhizosphere microbiome is a highly diverse ecological system, encompassing numerous species, some with the potential to contribute to plant resistance to pathogens (14, 15). Furthermore, these species may interact with and influence each other by producing metabolites and by sophisticated signaling and interaction networks that add another layer of complexity (16, 17) which may be critical for plants to resist pathogens. Previous studies have reported that disease suppressive soil, particularly soil fungistasis (18, 19), involves multiple mechanisms including production of antibiotics (20), volatile organic compounds (21), siderophores (22), and chitinases (23), as well as hyperparasitism (24), nutrient and niche space competition (25), microbial composition, and diversity (6, 26). Mono- or multikingdom synthetic communities constructed using strains that were negatively correlated with filamentous pathogenic fungi in roots promoted Arabidopsis survival (27). Wu et al. (26) generated diversity gradients by pasteurizing soil at increasing temperatures and observed a parallel loss of fungistasis as bacterial diversity declined. Yet sterilization simultaneously erases both microbial diversity and total biomass, while high-heat pasteurization also modifies soil physicochemistry and nutrient availability—changes that can themselves impede pathogen hyphal growth and confound any diversity effect. In a parallel system, the gut, a recent study has shown that a high diversity of the gut microbiome can provide protection against two major enteric bacterial pathogens by blocking nutrient availability in vitro and in gnotobiotic mice (28). This study showed that colonization

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resistance is contingent on the presence of key species, yet their inhibitory ability remains modest in isolation. When these bacteria are embedded within a multispecies intestinal community, their collective suppression of pathogens intensifies markedly—underscoring the importance of community-level disease suppression (28). Whether this phenomenon operates in the rhizosphere is unknown, given the difference between the plant rhizosphere and the animal gut. Nonetheless, it remains a significant challenge to predict which microbiota can effectively combat soilborne pathogens, especially in an agricultural setting. This challenge arises due to the complex ecological dynamics of the soil microbial community, which surpasses that of the gut environment.

Numerous studies have examined how plant–soil feedback enhances the disease resistance of subsequent crops and ascribe this "soil legacy" to the enrichment of beneficial microorganisms (6). Yet our previous studies have also shown that *Fusarium oxysporum* infection led to a decrease in the microbial diversity and network complexity of the rhizosphere in *Astragalus* (29). Generally, greater microbial richness typically harbors a broader spectrum of beneficial microbes equipped with pathogen-suppressive traits; we therefore hypothesize that high-diversity microbial community exhibits superior disease prevention. Hence, we constructed plant soil microcosms that varied in microbial diversity and strain composition to evaluate how diversity impacted the disease-suppressive capacity of microbial communities. We added *F. oxysporum* to the microcosms as it can infect a wide range of plant species causing significant damage to the roots (30–33), and explored how highly diverse microbiota resist pathogenic fungi from an ecological perspective.

Results

Ecological Bacterial Diversity Drives Disease Suppression. We first examined whether soil microbial communities with different diversities affect the root rot incidence of the legume *Astragalus* (Fig. 1*A*; Supplementary Methods). *Astragalus* is an important traditional leguminous herb and cash crop, and its growth is severely affected by soilborne root rot after continuous cultivation (34). In order to establish soil microbial communities with different species richness, treatments with sixteen different preceding crops were planted. The incidence of root rot was substantially reduced in *Astragalus* after planting different preceding crops, compared to soils continuously planted with *Astragalus* (Fig. 1*A*). Further, the incidence of root rot was significantly suppressed in soils with higher bacterial diversities, and independent of fungal diversity and in spite of the presence of the fungal pathogen, *F. oxysporum* (Fig. 1 *B* and *C*). This phenomenon is consistent with previous findings that soil fungistasis decreased with the loss of soil bacterial diversity (26). The loss of microbial community diversity is tantamount to the disappearance of some species, including those equipped to restrain pathogens. Disease suppression in the rhizosphere is a multilayered process: antagonistic bacteria synthesize antifungal metabolites (18), microbes emit microbial volatile organic compounds (19), allelochemicals are exuded by roots (35), and beneficial bacteria activate plant induced system resistance (ISR) (36), collectively fortifying the plant against infection. Declining plant disease resistance may be linked to the reduced abundance of specific functional microorganisms. Beyond these mechanisms, it remains unclear whether increasing microbial diversity can also heighten rhizosphere-mediated disease suppression. Thus, we constructed synthetic communities (SynComs) with an experimentally generated gradient of bacterial diversity. In these SynComs, fungi were also added but fungal diversity was unchanged. In total, 974 bacterial and 114 fungal strains were isolated from the rhizosphere and roots of *Astragalus* (Fig. 2*A*). The antagonistic effects of each microbial strain on *F. oxysporum* were examined in *vitro*. Since antagonistic microbes could directly suppress pathogens by producing antimicrobial compounds (2, 4, 5, 37), antagonistic strains were deliberately removed through in *vitro* experiments to reduce this impact on testing for diversity-dependent disease suppression (Fig. 2*A*). To

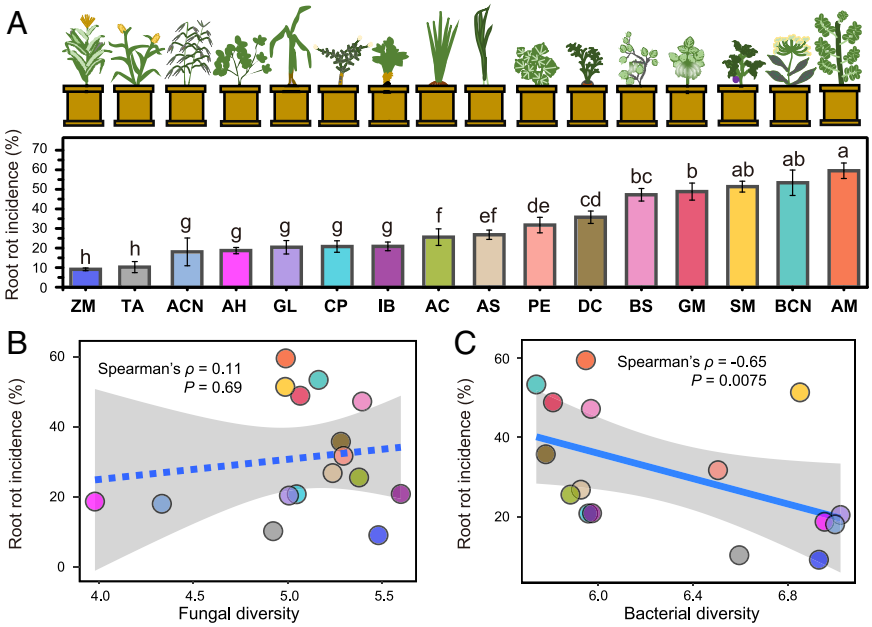

Fig. 1. Effects of microbial diversity on plant disease outcomes. (*A*) The root rot incidence of *Astragalus* in soils pregrown with 16 different crops. 16 crops were as follows: *Zea mays* (ZM), *Triticum aestivum* (TA), *Avena chinensis* (ACN), *Arachis hypogaea* (AH), *Allium sativum* (GL), *Codonopsis pilosula* (CP), *Ipomoea batatas* (IB), *Allium cepa* (AC), *Allium schoenoprasum* (AS), *Pachyrhizus erosus* (PE), *Daucus carota* (DC), *Black soybean* (BS), *Glycine max* (GM), *Solanum melongena* (SM), *Bupleurum chinense* (BCN), *Astragalus* (AM). Different lowercase letters show a significant difference between root rot incidence in *Astragalus* planted in 16 differently cropped soils ($P < 0.05$, according to Kruskal–Wallis tests). (*B*) Correlation between the root rot incidence of *Astragalus* and the fungal diversity was estimated by Spearman's correlation. Dashed lines represent a nonsignificant correlation. (*C*) Correlation between the root rot incidence of *Astragalus* and bacterial diversity was estimated by Spearman's correlation. Solid lines represent the significant correlation.

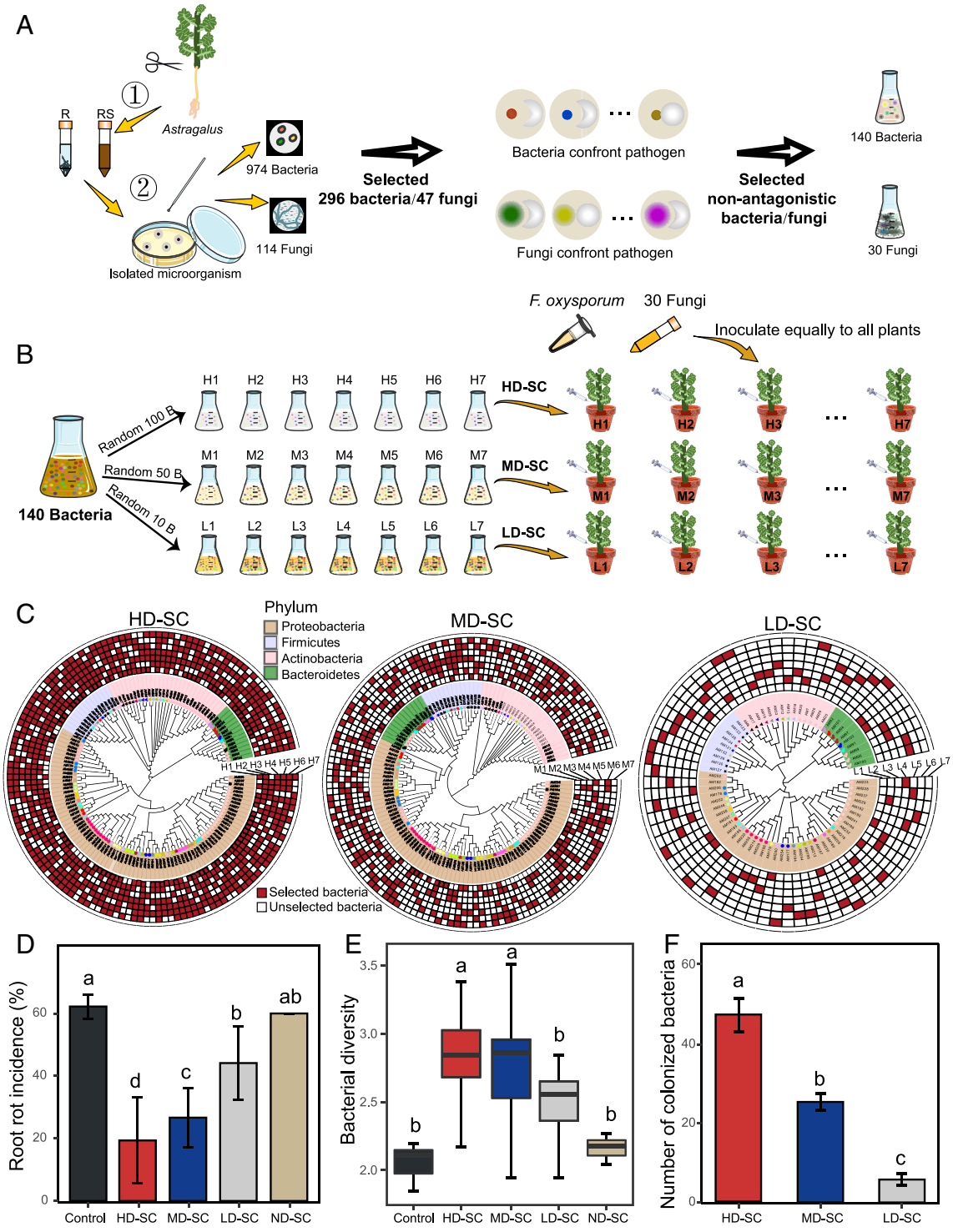

Fig. 2. Experimentally increasing bacterial diversity enhanced disease resistance. (*A*) 974 bacterial and 114 fungal strains were isolated from the rhizosphere (RS) and roots (R) of healthy and diseased *Astragalus*. All bacteria and fungi that are antagonistic to *Fusarium oxysporum* in *vitro* were removed, a total of 140 bacterial and 30 fungal strains were used to construct cross-kingdom synthetic microbiota. R: *Astragalus* root; RS: rhizosphere soil of *Astragalus*. (*B*) 10, 50, and 100 bacterial strains were randomly selected from 140 bacterial strains to form distinct cross-kingdom synthetic communities with high, medium, and low bacterial diversity, respectively, and 30 fungal strains remain unchanged. Synthetic communities were inoculated into the soil to form different bacterial diversity level soil for planting *Astragalus*. At the same time, pathogens were also inoculated into all soils. (*C*) Phylogenetic trees of all bacteria in high (HD-SC), medium (MD-SC), and low (LD-SC) bacterial diversity synthetic communities were constructed respectively. Colors for the innermost shape represent different genera, and colors for the second ring represent different Phyla. The colors of the third to ninth rings represent the composition of bacteria in different synthetic communities, the red square represents that the synthetic community contains this strain, and the white represents that it does not contain this strain. (*D*) Root-rot incidence of *Astragalus* inoculated with HD-SC, MD-SC, LD-SC, and community contains only 30 fungi (ND-SC), and 0.3% sterile saline water (control). Lowercase letters suggest a significant difference in the root rot incidence of *Astragalus* grew in soils with different bacterial diversity ($P < 0.05$; according to Kruskal–Wallis tests). (*E*) Rhizosphere soil bacterial diversity (Shannon Index) at the end of the experiments when inoculated synthetic communities with high (HD-SC), medium (MD-SC), and low (LD-SC) bacterial diversity. (*F*) Number of species detected in the rhizosphere of SynComs with high (HD-SC), medium (MD-SC), and low (LD-SC) bacterial diversity. Different letters show significant differences among different bacterial diversity synthetic communities by multiple comparisons with Kruskal–Wallis tests ($P < 0.05$).

capture a diverse array of phylogenetic clades, 140 bacterial and 30 fungal strains were retained for constructing SynComs, whose members were evenly distributed among 56 bacterial genera and 15 fungal genera (Fig. 2*A* and *SI Appendix*, Fig. S1).

Then 100, 50, and 10 bacterial strains were randomly chosen from these 140 bacterial strains to form synthetic communities with high (HD-SynComs), medium (MD-SynComs), and low (LD-SynComs) bacterial diversity, respectively (Fig. 2*B*). Specifically, we conducted repetitive sampling from the species pool of 140 bacterial strains to generate seven replicates for each diversity level, i.e., each replicated synthetic community had completely distinct bacterial compositions (Fig. 2*C*). The species number of 100 was set as the diversity of HD-SynComs to generate more variable compositions among replicates. In building the SynComs, we deliberately included fungal strains to approach the natural rhizosphere community. To ensure consistent fungal diversity across all synthetic communities, 30 fungal were integrated strains into each community. This was based on the typical ratio of bacterial to fungal diversity in the rhizosphere, which was approximately 3:1. Thus, a total of 21 cross-kingdom SynComs were generated. Three replicates for each SynComs were also established when assessing their impact on disease suppression. To ensure that effects were related to differences in bacterial diversity and not due to differences in microbial biomass, the SynComs were incubated in soil for 4 wk so that the quantity of bacteria in the soils was similar (*SI Appendix*, Fig. S2). The results showed that the root-rot incidence was significantly decreased with increasing diversity of the experimental SynComs (Fig. 2*D* and *SI Appendix*, Fig. S3). Among SynComs of the same diversity level, there was no significant difference in their ability to suppress root rot, despite their distinct bacterial compositions (*SI Appendix*, Fig. S4). This suggests that bacterial diversity is also related to the suppression of root rot. Species-rich microbiota form denser, more intricate networks of interaction, potentially expanding the spectrum of antifungal metabolites produced or amplifying the antagonistic traits of beneficial microbes with suppressive abilities (38–40). We also evaluated the rhizosphere colonization of the members of the SynComs at the end of the experiments. The number of species detected in the rhizosphere corresponded to our design (Fig. 2*F*), although a few strains were not detected. Note that sequences of several noninoculated taxa were observed and these probably reflect dead taxa or their DNA that survived sterilization. The inclusion of these taxa did not influence the generation of the diversity gradient (Fig. 2*E* and *SI Appendix*, Fig. S5). This indicates the successful generation of a diversity gradient. In addition, we observed that *Pseudomonas* accounted for a large proportion of HD-SynComs and MD-SynComs, and many of this taxon can provide biocontrol, thus the synthetic communities after removing *Pseudomonas* were also reconstructed. The results were consistent with previous results showing that these HD-SynComs were also more effective in suppressing disease than LD-SynComs (*SI Appendix*, Fig. S6), though other bacterial taxa, besides *Pseudomonas*, may contribute to disease suppression.

In order to investigate whether the rule of increased bacterial diversity benefiting plant disease control extends to other plant species, we implemented the above 21 cross-kingdom synthetic communities in a soybean cropping system, one of the most valuable commercial crops. The results showed that HD- and MD-SynComs significantly reduced the disease index of soybean root rot, while LD-SC did not alleviate soybean root rot (*SI Appendix*, Fig. S7). This result verifies that high bacterial diversity can contribute more generally to the control of plant diseases.

Our work supports the general hypothesis that a more diverse microbiome can carry soil and plant health benefits (14, 41, 42). Although much discussed, evidence for this hypothesis is typically based on observations of the existing rhizosphere microbiomes and the correlations drawn between the diversity and plant health outcomes are usually impacted by multiple confounding factors (14, 42). Here, we provide direct experimental evidence that bacterial diversity can indeed provide plant health benefits through an increased ability to protect against pathogens. While previous studies have found that a few antagonistic bacteria can significantly reduce plant disease incidence in a targeted treatment, but pathogen-specific antagonism lacks the generalized utility to combat a wide variety of diseases seen in soil (2, 5, 14, 15).

Beneficial microbes can suppress pathogens by triggering the plant's immune system (43). In our study, we did not observe a significant difference in gene expression related to the plant's immune system among different diversity SynComs (*SI Appendix*, Fig. S8), indicating that the inhibition by synthetic communities to root-rot incidence was not through more efficient expression of plant resistance genes. The extent to which these SynComs may modulate the expression of defense-related genes in *Astragalus* when the pathogen is present, i.e., their priming potential (44), was not examined. Hence, we further examined how diversity confers disease resistance.

Nutrient Competition Suppresses the Growth of Pathogen. When inoculating HD-SynComs on an agar plate, the colony diameter of *F. oxysporum* was significantly reduced, and most of the hyphae were hyaline and lie flat against the agar surface (Fig. 3 *A* and *B* and *SI Appendix*, Fig. S10). Additionally, curling and wrinkling of the hyphae were observed when *F. oxysporum* interacted with both HD-SynComs and MD-SynComs (Fig. 3*C* and *SI Appendix*, Fig. S11). These findings indicate that synthetic microbiota with high diversity exhibit more pronounced antagonism, resulting in the inhibition of hyphae formation. Although we assessed the antagonistic potential of individual strains in vitro, nonantagonistic isolates may still produce antifungal compounds in vivo. Consequently, our SynComs cannot entirely exclude antagonists; isolates exhibiting antagonism in *vitro* were excluded and the other microbes were used to assemble the SynComs in an effort to minimize antagonism among community members.

Further results showed that the root-rot incidence was the lowest when the synthetic community was inoculated first, followed by simultaneous inoculation of the synthetic community and *F. oxysporum*, and root rot incidence was highest when first inoculating *F. oxysporum* (Fig. 4*A*), indicating that priority effect, the order of species occupancy, can play an important role in suppressing pathogen invasion (45, 46). This may be that plants have a finite amount of energy for root exudation hence a limited resource for the rhizosphere microbiome and nutrients in the rhizosphere are a key limiting resource that is critical to microbial colonization of root (47, 48). Previous studies have indicated that beneficial microbes can inhibit pathogens by out-competing them for key nutrients (22, 49). We therefore investigated how carbon source competition between the pathogen and the SynComs as microbial diversity increases. Based on a competition experiment with 32 carbon sources, we found that the number of carbon sources overlapping between *F. oxysporum* and HD-SynComs was the highest, whereas it was lowest in LD-SynComs (Fig. 4*B* and *SI Appendix*, Fig. S13). Considering that glucose could be consumed by all SynComs and *F. oxysporum*, we further measured the efficiency of glucose consumption among SynComs with different diversities. The glucose degradation efficiency of HD-SynComs and MD-SynComs was significantly higher than that of LD-SynComs after 12- and 24 h of SynComs growth (Fig. 4 *C* and *D*).

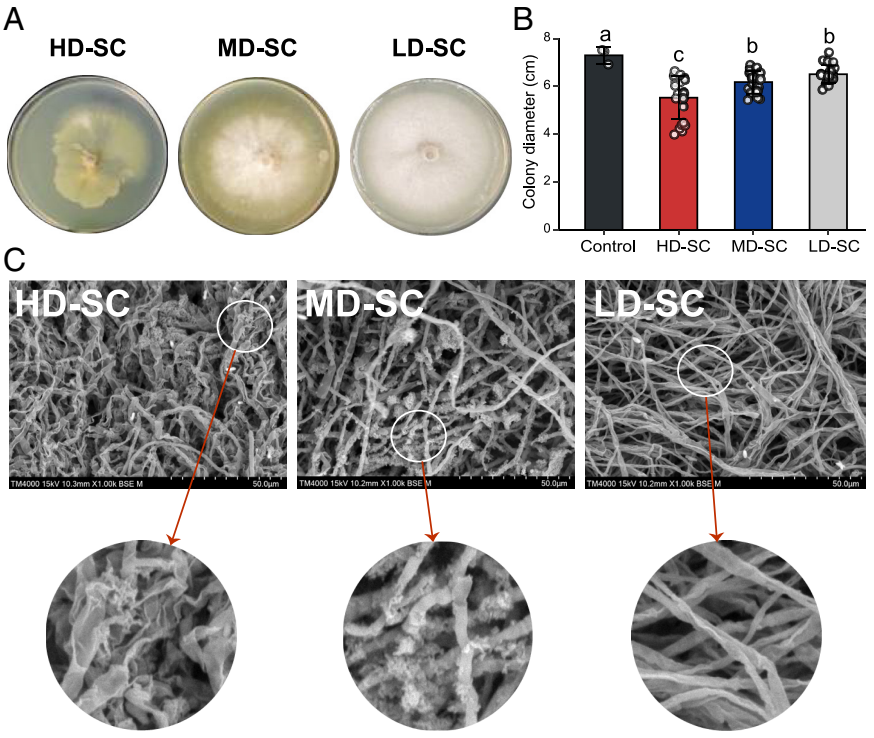

Fig. 3. High diversity communities antagonize *F. oxysporum*. (*A*) *F. oxysporum* colony structure on plates inoculated with high, medium, and low diversity synthetic communities. (*B*) Variation in the colony diameter of *F. oxysporum* when inoculated with high-, medium-, and low-diversity synthetic communities on the plate. The colony diameter of *F. oxysporum* without synthetic communities was taken as a control. (*C*) Change in the *F. oxysporum* hyphal morphology was observed through scanning electron microscopy when *F. oxysporum* interacted with high-, medium-, and low-diversity synthetic communities.

To further investigate the potential impact of microbiota glucose consumption on the biomass of *F. oxysporum* hyphae, we collected and sterilized the glucose fermentation fluid from different SynComs at 12 and 24 h. This fluid was then used to feed *F. oxysporum* cultures. Interestingly, we observed a significant decrease in mycelial biomass of *F. oxysporum* when cultivated in the fermentation fluid of HD-SynComs and MD-SynComs, compared to LD-SynComs (Fig. 4*E*). These findings suggest that bacterial communities with higher diversity effectively compete for the available nutrients, such as glucose, thereby suppressing the growth of *F. oxysporum*.

Physical Barrier Formation Hinders Pathogen Invasion. Root cell surface contains key structures for bacterial attachment and expansion (50). Colonization of beneficial bacteria on the root surface via biofilm matrix formation contributes to plant tolerance against biotic stress (51, 52). Here, we observed that the root surface was densely covered with bacteria when incubated with HD-SynComs and MD-SynComs but were only discontinuously covered with the LD-SynComs when plants were grown hydroponically and inoculated with similar bacterial densities at each diversity level (Fig. 3*E*, and *SI Appendix*, Fig. S12). The diversity of probiotic consortia has been shown to enhance rhizoplane colonization to suppress disease (52), and microbiota exhibiting *Fusarium* wilt suppression were enriched in genes involved in biofilm formation (5). Root exudates trigger conidial germination, and the subsequent growth of hyphae to the root surface, where they weave into an infective network, constitutes the first step in *F. oxysporum* pathogenesis (53). By gathering at the root surface, bacteria may simultaneously deplete these exudates (54), potentially curbing spore germination, and form a barrier to prevent the already formed hyphae reaching the root (55). Thus, these results suggest that high bacterial diversity may suppress pathogen colonization by creating a physical barrier on the root surface.

Our results show that under hydroponic conditions, high levels of bacterial diversity can increase the total number of resources that bacteria can collectively utilize as a community to compete with *F. oxysporum* for nutrients. This notion is supported by a recent study demonstrating that gut microbiome diversity protects against enteric pathogens' colonization by nutrient blocking (28). In addition, high bacterial diversity formed physical barriers on root surface to prevent *F. oxysporum* from colonizing on the roots.

While our study provides important insights into the effects of bacterial diversity on plant disease resistance, we also acknowledge that other factors such as protists or viruses could also play significant roles in soil disease suppression, important avenues for future investigation. By considering the broader microbial community and potential interactions among various microorganisms, a more comprehensive understanding of the role of microbial diversity in soil disease suppression can be achieved, thereby enhancing soil health and disease management.

Overall, our study demonstrates that a high-diversity synthetic bacterial community effectively reduces the incidence of soilborne disease. This indicates that pathogen suppression can be achieved through diversity manipulation at the community level, underscoring the potential of leveraging microbial diversity as a promising, nonchemical, and sustainable strategy for crop disease management. Although we cannot directly inoculate SynComs in soil to obtain a desired level of bacterial richness, diversified cropping, organic amendments, and the retention of crop residues can substantially raise microbial diversity (56–58). Crop rotation, for instance, markedly decreases the copy number of pathogenic fungi in the soil of apple orchards and suppresses peanut root rot (59, 60), and our result offers a mechanistic rationale for this protection. Importantly, we provide evidence of the diversity-driven disease resistance which is vital for the development of sustainable agricultural systems. Moreover, as agricultural intensification decreases cropland microbial diversity (61), our findings highlight the urgency of actively protecting microbial diversity to maintain agroecosystem services.

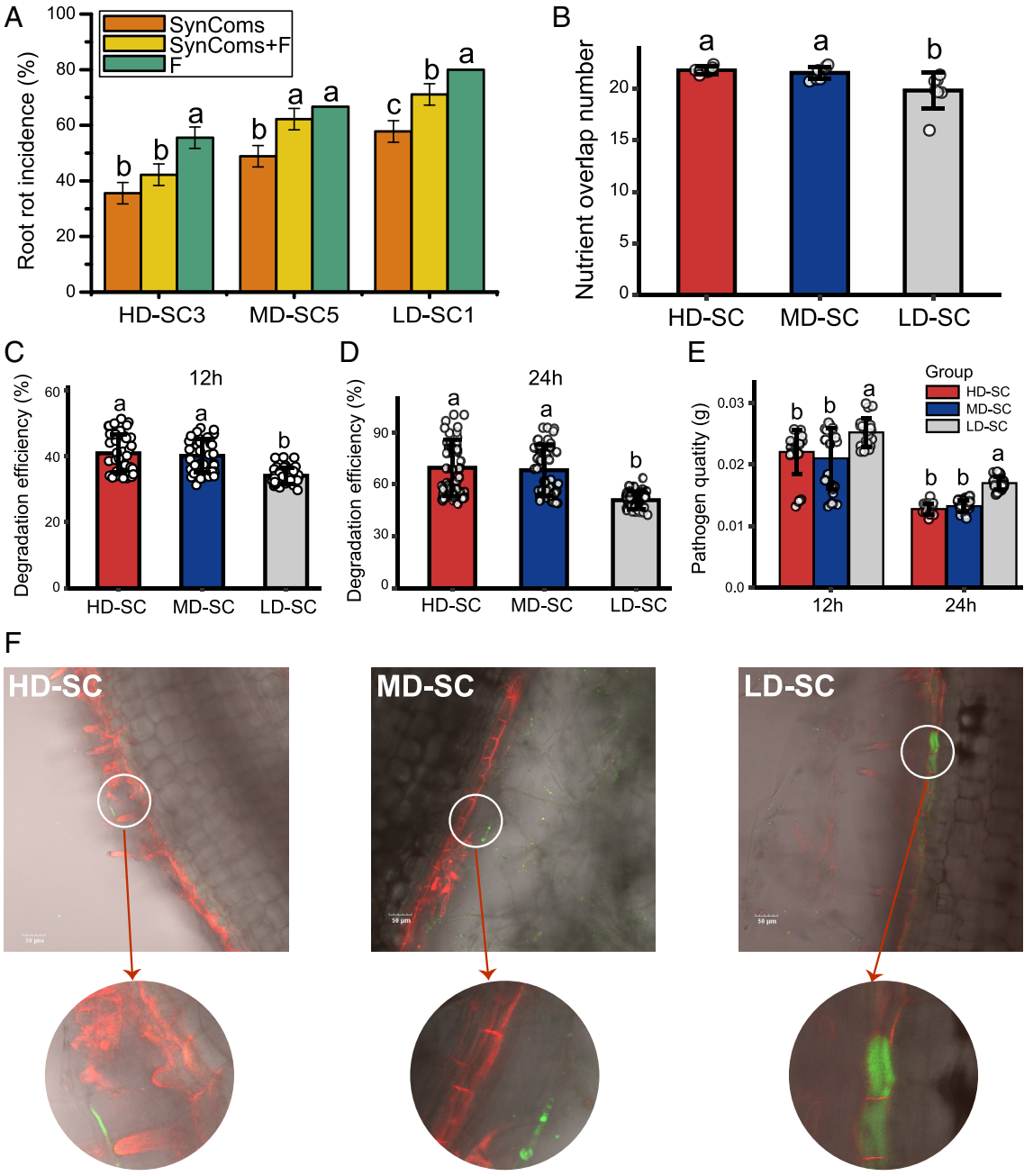

Fig. 4. Nutrient competition and Physical barrier prevents pathogen infection. (*A*) Root-rot incidence in *Astragalus* examined when first inoculating the synthetic communities and then *F. oxysporum* (SynComs), or when first inoculating *F. oxysporum* (F) and then the synthetic community, or when simultaneous inoculating the synthetic community and *F. oxysporum* (SynComs+F). (*B*) The number of carbon sources overlapping between *F. oxysporum* and synthetic communities was examined. The glucose degradation efficiency of the synthetic communities with high-, medium-, and low-diversity was measured at 12 h (*C*) and 24 h (*D*), respectively. (*E*) The quantity of fungal hyphae was measured when *F. oxysporum* were cultured in the sterile spent medium, which originated from glucose medium consumed by SynComs for 12- and 24-hours, respectively. Different letters show significant differences among different treatments by multiple comparisons with Kruskal–Wallis tests ($P < 0.05$). (*F*) The colonization of synthetic communities and *F. oxysporum* on the root surface of *Astragalus* was observed by a laser scanning confocal microscope when first inoculating synthetic communities then *F. oxysporum*. The bacteria in biofilm on the root surface of *Astragalus* was stained with Nile blue oxazone, and *F. oxysporum* was labeled with GFP (green regions). Different letters show significant differences among different treatments by multiple comparisons with Kruskal–Wallis tests ($P < 0.05$).

Materials and Methods

Experimental Design and Soil Sample Collection. To examine the effect of soil microbial diversity on the resistance of *Astragalus* to root rot, 16 crops were planted in soil to modify the soil microbial composition and diversity. After the plants grew for 5 mo, the preceding crops were harvested. *Astragalus* was planted in the corresponding soil and grew for 21 d, then the soil microbial community composition and the root rot incidence of *Astragalus* was determined.

Impacts of Different Diversity Level SynComs On Disease Resistance. Bacterial and fungal strains were isolated from *Astragalus* rhizosphere soil. After removing redundant and antagonistic strains, 100, 50, and 10 bacterial strains were randomly selected from nonantagonistic bacteria for SynComs construction, respectively. Each diversity level bacterial communities contained 7 different SynComs each with three replicates. Thus 21 synthetic bacterial communities (7 in each of high, medium, and low diversity SynComs) were obtained. The same 30 fungi was added to each bacterial SynComs. Then the

21 cross-kingdom synthetic communities were inoculated into sterile planting bags and incubated 4 wk, ten germinated seedlings were sown in a planting bag. After 7 d, five *Astragalus* seedlings were kept in each planting bag. Each treatment contains three replicated bags, and the root rot incidence was counted after 21 d growth.

Quantifying the Expression of Disease Resistance Related Genes in *Astragalus*. To investigate the response of the *Astragalus* defense system to the SynComs, the *Astragalus* seedlings inoculated with different SynComs were harvested at 21 dpt (day post treatment) and frozen in liquid nitrogen, respectively. Then the plant RNA was extracted and reverse transcribed to cDNA. The expression of nine genes related to plant's immune system, encoding Polyphenol oxidase (PPO), Phenylalanine ammonia lyase (PAL), Endoglucanase (EGase), Superoxide dismutase (SOD), pathogenesis-related protein-4 (PR-4), pathogenesis-related protein-10 (PR-10), pathogenesis-related protein-3 (PR-3), pathogenesis-related protein-2 (PR-2), and pathogenesis-related protein-1 (PR-1), respectively, were determined by fluorescence quantitative PCR.

Synthetic Communities Compete With *F. oxysporum* for Niche on the Plate. Twenty-one SynComs were respectively coated on LB plate to obtain bacterial cakes, and the *F. oxysporum* was inoculated into PDA plate to obtain pathogenic cake. Subsequently, an agar column (d = 8 mm, h = 2 mm) was put in the center of a sterile PDA plate, and a bacterial cake and a pathogenic cake were positioned at the top of the agar column, allowing the SynComs to compete with the pathogen (*SI Appendix*, Fig. S9). The control group consisted solely of pathogens at the top of the agar column without any bacterial cake. On the 6th day, the *F. oxysporum* colony radius was measured with the vernier caliper. In addition, scanning electron microscopy (SEM) was performed to observe the hyphal morphology of *F. oxysporum* on plates treated with different SynComs.

Synthetic Communities Form Biofilms on the Root Surface of *Astragalus*. Five *Astragalus* seedlings were placed on a sterile plate consisting of half sterile agar and half sterile nutrient solution, and the *Astragalus* roots were immersed in the nutrient solution. These plates were incubated at 25 °C under a 16-h light/8-h darkness cycle. After five days, the bacterial SynComs were inoculated into nutrient solution to final concentration of 10^7 cfu /mL. Two days later, the green fluorescent protein (GFP)-tagged *F. oxysporum* was inoculated into the nutrient solution at final concentration of 10^5 spores/mL. Three days after inoculation with pathogens, the root sample of *Astragalus* was dyed in Nile red solution at 37 °C for 3 min, and then the sample was washed with water for 2 to 3 times, put on a microscope slide and covered for further confocal microscopy analysis. The bacterial cells in biofilm matrix and *F. oxysporum* on root surface of *Astragalus* were examined using a Digital Laser scanning system (Olympus Corporation, Japan).

Synthetic Communities Competition With Pathogens for Carbon Sources. The Biolog EcoPlate was used to assess the overlap in carbon substrate utilization between SynComs and pathogen. Biolog EcoPlate contained three replicate wells of 32 carbon sources and a control without substrate. An OD_{600} of 21 SynComs and spore suspension concentration of *F. oxysporum* was adjusted to 0.1 and 10^5 spore/mL, respectively. SynComs or spore suspensions of 150 μL were inoculated into each well in Biolog EcoPlate. The EcoPlates were incubated at 25 °C for 7 d and the absorbance was determined at 590 nm daily. The OD values of SynComs in each well were normalized by subtracting OD values of the control and the initial OD values. To evaluate the effect of the spent medium on the growth of the pathogen, the 21 SynComs (OD_{600} = 0.1) were inoculated in inorganic salt medium with glucose as the sole carbon source, and the control was inoculated in sterile water. The SynComs were incubated under 250 rpm at 28 °C. The spent medium was collected at 12 h and 24 h after inoculation, and the glucose content in spent medium was determined with Glucose Assay Kit (Sinobestbio, Shanghai, China). Then 100 μL spore suspension (10^5 spores/mL) was inoculated into 10 mL of sterile spent medium, and cultured in a shaker at 28 °C for 3 d. The mycelium of *F. oxysporum* in the culture solution was collected, then dried in an oven and weighed. Additional details of experimental methods are provided in *SI Appendix*.

Data, Materials, and Software Availability. The soil microbiome dataset of 16 crops has been deposited in the NCBI Sequence Read Archive under accession number PRJNA1082258 (62). And the datasets of soil bacterial community at the end of the experiment of inoculating 21 synthetic communities to *Astragalus* have been deposited in SRA under BioProject PRJNA1086885 (63). All code is available at GitHub (https://github.com/BaiXL111/Manipulating-bacterial-diversity-suppresses-soil-borne-plant-disease) (64).

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1. J. R. Lamichhane, M. J. Barbetti, M. I. Chilvers, A. K. Pandey, C. Steinberg, Exploiting root exudates to manage soil-borne disease complexes in a changing climate. *Trends Microbiol.* **32**, 27–37 (2024).
2. Z. Li *et al.*, A simplified synthetic community rescues *Astragalus mongholicus* from root rot disease by activating plant-induced systemic resistance. *Microbiome* **9**, 217 (2021).
3. D. L. McRose, J. Li, D. K. Newman, The chemical ecology of coumarins and phenazines affects iron acquisition by pseudomonads. *Proc. Natl. Acad. Sci. U.S.A.* **120**, e2217951120 (2023).
4. P. Trivedi, J. E. Leach, S. G. Tringe, T. Sa, B. K. Singh, Plant–microbiome interactions: From community assembly to plant health. *Nat. Rev. Microbiol.* **18**, 607–621 (2020).
5. X. Zhou *et al.*, Cross-kingdom synthetic microbiota supports tomato suppression of Fusarium wilt disease. *Nat. Commun.* **13**, 7890 (2022).
6. D. Schlatter, L. Kinkel, L. Thomashow, D. Weller, T. Paulitz, Disease suppressive soils: New insights from the soil microbiome. *Phytopathology* **107**, 1284–1297 (2017).
7. J. Spooren *et al.*, Plant-driven assembly of disease-suppressive soil microbiomes. *Annu. Rev. Phytopathol.* **62**, 1–30 (2024).
8. H. L. Hayden, K. W. Savin, J. Wadeson, V. V. S. R. Gupta, P. M. Mele, Comparative metatranscriptomics of wheat rhizosphere microbiomes in disease suppressive and non-suppressive soils for *Rhizoctonia solani* AG8. *Front. Microbiol.* **9**, 859 (2018).
9. M. Mazzola, Mechanisms of natural soil suppressiveness to soilborne diseases. *Antonie Van Leeuwenhoek.* **81**, 557–564 (2002).
10. R. Mendes *et al.*, Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science.* **332**, 1097–1100 (2011).
11. M. van der Voort, M. Kempenaar, M. van Driel, J. M. Raaijmakers, R. Mendes, Impact of soil heat on reassembly of bacterial communities in the rhizosphere microbiome and plant disease suppression. *Ecol. Lett.* **19**, 375–382 (2016).
12. W. de Boer, P. Verheggen, P. J. Klein Gunnewiek, G. A. Kowalchuk, J. A. van Veen, Microbial community composition affects soil fungistasis. *Appl. Environ. Microbiol.* **69**, 835–844 (2003).
13. V. J. Carrión *et al.*, Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome. *Science* **366**, 606–612 (2019).
14. J. D. van Elsas *et al.*, Microbial diversity determines the invasion of soil by a bacterial pathogen. *P. Natl. Acad. Sci. U.S.A.* **109**, 1159–1164 (2012).
15. C. Yin *et al.*, Rhizosphere community selection reveals bacteria associated with reduced root disease. *Microbiome* **9**, 86 (2021).
16. Z. Wei *et al.*, Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health. *Nat. Commun.* **6**, 8413 (2015).
17. M. Li *et al.*, Facilitation promotes invasions in plant-associated microbial communities. *Ecol. Lett.* **22**, 149–158 (2019).
18. J. L. Lockwood, Soil fungistasis. *Annu. Rev. Phytopathol.* **2**, 341–362 (1964).
19. S. Liu *et al.*, Plant pathogen resistance is mediated by recruitment of specific rhizosphere fungi. *ISME J.* **17**, 931–942 (2023).
20. E. M. Perkins, C. C. Mundt, Associations among cultivar cropping sequence, 2, 4-diacetylphloroglucinol-producing pseudomonad populations, and take-all disease of winter wheat in Oregon. *Plant Dis.* **108**, 3604–3613 (2024).
21. T.-P. Nguyen *et al.*, Antifungal mechanism of volatile compounds emitted by Actinomycetota Paenarthrobacter ureafaciens from a disease-suppressive soil on Saccharomyces cerevisiae. *mSphere* **8**, e0032423 (2023).
22. S. Gu *et al.*, Competition for iron drives phytopathogen control by natural rhizosphere microbiomes. *Nat. Microbiol.* **5**, 1002–1010 (2020).
23. E. Morales-Ruiz, R. Priego-Rivera, A. M. Figueroa-López, J. E. Cazares-Álvarez, I. E. Maldonado-Mendoza, Biochemical characterization of two chitinases from *Bacillus cereussensu lato* B25 with antifungal activity against *Fusarium verticillioides* P03. *FEMS Microbiol. Lett.* **368**, fnaa218 (2021).
24. S. Parratt, A. L. Laine, The role of hyperparasitism in microbial pathogen ecology and evolution. *ISME J.* **10**, 1815–1822 (2016).
25. E. Bakkeren, V. Piskovsky, K. R. Foste, Metabolic ecology of microbiomes: Nutrient competition, host benefits, and community engineering. *Cell Host Microbe.* **33**, 790–807 (2025).
26. M. Wu *et al.*, Soil fungistasis and its relations to soil microbial composition and diversity: A case study of a series of soils with different fungistasis. *J. Environ. Sci (China)* **20**, 871–877 (2008).

27. P. Durán *et al.*, Microbial interkingdom interactions in roots promote *Arabidopsis* survival. *Cell.* **175**, 973–983 (2018).
28. F. Spragge *et al.*, Microbiome diversity protects against pathogens by nutrient blocking. *Science.* **382**, eadj3502 (2023).
29. J. Ai *et al.*, Silica nanoparticles suppress fungal pathogenic allies to alleviate *Astragalus* root rot. *Microbiome* **13**, 209 (2025).
30. X. Li *et al.*, Acidification suppresses the natural capacity of soil microbiome to fight pathogenic *Fusarium* infections. *Nat. Commun.* **14**, 5090 (2023).
31. L. E. Johns, D. P. Bebber, S. J. Gurr, N. A. Brown, Emerging health threat and cost of *Fusarium* mycotoxins in European wheat. *Nat. Food.* **3**, 1014–1019 (2022).
32. S. Vitale, A. Di Pietro, D. Turrà, Autocrine pheromone signalling regulates community behaviour in the fungal pathogen *Fusarium oxysporum*. *Nat. Microbiol.* **4**, 1443–1449 (2019).
33. L. J. Jia *et al.*, A linear nonribosomal octapeptide from Fusarium graminearum facilitates cell-to-cell invasion of wheat. *Nat. Commun.* **10**, 922 (2019).
34. M. Yang *et al.*, Ecological niche modeling of *Astragalus membranaceus* var. *mongholicus* medicinal plants in Inner Mongolia, China. *Sci. Rep.* **10**, 12482 (2020).
35. S. Cadot *et al.*, Specific and conserved patterns of microbiota-structuring by maize benzoxazinoids in the field. *Microbiome* **9**, 103 (2021).
36. Y. Yu *et al.*, Induced systemic resistance for improving plant immunity by beneficial microbes. *Plants.* **11**, 386 (2022).
37. Z. Pang *et al.*, Microbiota-mediated nitrogen fixation and microhabitat homeostasis in aerial root-mucilage. *Microbiome* **11**, 85 (2023).
38. T. Netzker *et al.*, Microbial interactions trigger the production of antibiotics. *Curr. Opin. Microbiol.* **45**, 117–123 (2018).
39. T. Sun *et al.*, Interactions with native microbial keystone taxa enhance the biocontrol efficiency of *Streptomyces*. *Microbiome* **13**, 126 (2025).
40. M. E. E. Franco *et al.*, Hyperdiverse, bioactive, and interaction-specific metabolites produced only in co-culture suggest diverse competitors may fuel secondary metabolism of xylarialean fungi. *mSystems* **10**, e0046825 (2025).
41. S. Banerjee, M. G. A. van der Heijden, Soil microbiomes and one health. *Nat. Rev. Microbiol.* **21**, 6–20 (2023).
42. C. Wagg, K. Schlaeppi, S. Banerjee, E. E. Kuramae, M. G. A. van der Heijden, Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. *Nat. Commun.* **10**, 4841 (2019).
43. C. M. Pieterse *et al.*, Induced systemic resistance by beneficial microbes. *Annu. Rev. Phytopathol.* **52**, 347–375 (2014).
44. M. Hönig, V. M. Roeber, T. Schmülling, A. Cortleven, Chemical priming of plant defense responses to pathogen attacks. *Front. Plant Sci.* **14**, 1146577 (2023).
45. R. Debray, A. Conover, X. Zhang, E. A. Dewald-Wang, B. Koskella, Within-host adaptation alters priority effects within the tomato phyllosphere microbiome. *Nat. Ecol. Evol.* **7**, 725–731 (2023).
46. M. C. Urban, L. De Meester, Community monopolization: Local adaptation enhances priority effects in an evolving metacommunity. *Proc. R. Soc. B Biol. Sci.* **276**, 4129–4138 (2009).
47. S. McLaughlin, K. Zhalnina, S. Kosina, T. R. Northen, J. Sasse, The core metabolome and root exudation dynamics of three phylogenetically distinct plant species. *Nat. Commun.* **14**, 1649 (2023).
48. A. Essarioui, N. LeBlanc, H. C. Kistler, L. L. Kinkel, Plant community richness mediates inhibitory interactions and resource competition between *Streptomyces* and *Fusarium* populations in the rhizosphere. *Microb. Ecol.* **74**, 157–167 (2017).
49. E. Bakkeren, V. Piskovsky, K. R. Foster, Metabolic ecology of microbiomes: Nutrient competition, host benefits, and community engineering. *Cell Host Microbe.* **33**, 790–807 (2025).
50. R. M. Wheatley, P. S. Poole, Mechanisms of bacterial attachment to roots. *FEMS Microbiol. Rev.* **42**, 448–461 (2018).
51. H. C. Flemming, J. Wingender, The biofilm matrix. *Nat. Rev. Microbiol.* **8**, 623–633 (2010).
52. J. Hu, Z. Wei, V. P. Friman, Probiotic diversity enhances rhizosphere microbiome function and plant disease suppression. *MBio* **7**, 01790–01816 (2016).
53. C. Olivain *et al.*, Colonization of tomato root by pathogenic and nonpathogenic *Fusarium oxysporum* strains inoculated together and separately into the soil. *Appl. Environ. Microbiol.* **72**, 1523–1531 (2006).
54. K. Wu *et al.*, Competitive use of root exudates by *Bacillus amyloliquefaciens* with *Ralstonia solanacearum* decreases the pathogenic population density and effectively controls tomato bacterial wilt. *Sci. Hortic.* **218**, 132–138 (2017).
55. J. Li, C. Wang, W. Liang, S. Liu, Rhizosphere microbiome: The emerging barrier in plant-pathogen interactions. *Front. Microbiol.* **12**, 772420 (2021).
56. A. Williams, H. W. G. Birt, A. Raghavendra, P. G. Dennis, Cropping system diversification influences soil microbial diversity in subtropical dryland farming systems. *Microb. Ecol.* **85**, 1473–1484 (2023).
57. Y. Xiang *et al.*, Grass cover increases soil microbial abundance and diversity and extracellular enzyme activities in orchards: A synthesis across China. *Appl. Soil Ecol.* **182**, 104720 (2023).
58. G. Guan, S. Zhang, T. He, F. Guo, J. Zhu, Sod culture treatments positively affect soil fungal diversity, soil enzyme activities, and nutrient uptake in navel orange orchards. *J. Soil Sci. Plant. Nutr.* **24**, 5130–5143 (2024).
59. Y. Zhou *et al.*, Crop rotation and native microbiome inoculation restore soil capacity to suppress a root disease. *Nat. Commun.* **14**, 8126 (2023).
60. H. Wang *et al.*, The effects of crop rotation combinations on the soil quality of old apple orchard. *Hortic. Plant J.* **8**, 1–10 (2022).
61. M. R. Felipe-Lucia *et al.*, Land-use intensity alters networks between biodiversity, ecosystem functions, and services. *P. Natl. Acad. Sci. U.S.A.* **117**, 28140–28149 (2020).
62. X. Bai, Correlation between microbial diversity and plant disease. NCBI. https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1082258. Deposited 29 February 2024.
63. X. Bai, Correlation between microbial diversity and plant disease. NCBI. https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1086885. Deposited 12 March 2024.
64. X. Bai, Manipulating-bacterial-diversity-suppresses-soil-borne-plant-disease. GitHub. https://github.com/BaiXL111/Manipulating-bacterial-diversity-suppresses-soil-borne-plant-disease. Accessed 2 March 2024.