Seed-resident microbiomes of leguminous and gramineous grasses are related to host stress tolerance

Ya Dai
chengdu institute of biology, Chinese academy of Sciences
Xin-Yu Li
Chengdu institute of biology,chinese academy of sciences
Yan Wang
chengdu institute of biology, chinese academy of Sciences
Cai-Xia Li
chengdu institute of biology, Chinese academy of Sciences
Yuan He
chengdu institute of biology, Chinese academy of Sciences
Hong-Hui Lin
Sichuan University
Tao Wang
Chengdu institute of biology, Chinese academy of Sciences
Xin-Rong Ma (maxr@cib.ac.cn)
https://orcid.org/0000-0003-2733-9859

Research

Keywords: bacteria, gramineae, fungal, grass, legume, probiotics, seed microbiome, stress tolerance

Posted Date: November 20th, 2019

DOI: https://doi.org/10.21203/rs.2.17468/v1

Abstract

Background: The grasses in adverse environment such as Qinghai-Tibet Plateau are hypothesized to survive the harsh climate in part upon their seed-borne microorganisms, and yet the characteristics of the grass seed microbial communities remain undetermined. Here, we assessed the seed microbial communities of three native gramineous grass species (Avena sativa, Elymus sibiricus and Elymus dahuricus) and four candidate legumes (Vicia villosa, Trifolium repens, Trifolium pretense and Medicago sativa) on the Qinghai-Tibet Plateau by high-throughput sequencing.

Results: A total of 1,013 bacterial operational taxonomic units (OTUs) and 922 fungal OTUs were observed. The OTUs that shared in all the samples were in high abundance but with different relative abundances. The majority of bacterial sequences were assigned to Proteobacteria (54~90%) and Firmicutes (5~41%), and the fungal communities were mainly composed of Ascomycota (23~96%) and Basidiomycota (2~11%). The fungal communities were more affected by host genetic distance than bacteria. The three gramineous grasses were speculated to survive the adversity partly due to their high abundance of beneficial bacteria like Pantoea or Bacillus, and non-pathogenic fungi like Candida or unclassified Helotiaceae. Also enriched with these potential beneficial taxa, the four leguminous grasses may be competent to adapt the Qinghai-Tibet Plateau stress. Furthermore, the higher tolerance grasses (Elymus sibiricus and Elymus dahuricus) possessed a greater number of growth-promoting and tolerance bacterial and non-pathogenic fungi. Conversely, the less tolerance grass Medicago sativa contained lower levels of such microorganisms, and showed higher abundance of pathogenic taxa. Furthermore, the isolated Bacillus subtilis or Pantoea agglomerans could more probably promote seeding growth of hosts with lower abundance of them, while inhibit if the endo-abundance of was high.

Conclusions: Seed-resident microbiome structure of the four cold-tolerance legumes and three Qinghai-Tibet Plateau gramineae is host dependent and related to stress resistance. It also has a strong influence on the response of seedlings to biological seed treatments. This study provides valuable data for studying plant resilience, identifying more biocontrol strains, maximizing microbial functions in ‘smart farming’ practices.

Background

In an era of climate change, maximizing microbial functions in plant growth has become a prerequisite for the sustainability of global agriculture [1].The interiors of plants are usually inhabited by a variety of microbial communities [2]. High diversity of bacteria and fungi have been isolated or sequenced from plant seeds [3], roots [4], shoots [5] and leaves [6]. Some of the microorganisms are typed as commensals with unknown or yet unknown functions in plants, whereas other endophytes confer beneficial effects or latent pathogens to the plants [2]. Whatever, the endophyte communities as a whole have been reported to act important role promote plant growth [7] and improve plant stress resistance to biotic and abiotic stresses [8, 9]. The mechanism mainly involves facilitation of acquisition of essential nutrients including

phosphorous, nitrogen, iron [10] or modulation of plant hormones such as auxin, cytokinin, and ethylene as well as polyamines (Poly-NH2), secondary metabolites (SMs) [2].

The plant seeds are the starting point for the next generation, and also evolved along with diverse microorganisms [3, 11]. Seed endophytes were reported to beneficial the plants starting from seed preservation and germination [12–15] to seedling growth and stress tolerance [16, 17]. In plant microbial assembly, small differences in early colonization processes may lead to large differences in community of later stages of life (that is, priority effects) [18]. Given the importance of priority effects in plant microbiomes, a comprehensive study of seed bacterial and fungal community and managing microbial assembly in seeds and seedlings should be a research priority [1]. However, the study of the seed's microbiota was largely based on culture-dependent investigations in the past [11, 19] and lagged behind the studies of microbiome in leaf and root [20–22]. Seeds from ecologically and geographically diverse were reported to harbor characteristic microbiota [23–25]. The comprehensive study of seed microbiome by means of ribosomal amplicon-based approaches have mostly focused in some field-crop plants like *Maize* [26], *Rice* [27], *Bean [*25] and and *Barley* [28]. However, the comprehensive understand of endophyte microbiome in grass seeds remains enigma.

Some stress tolerant plants like *Agave* and *Quinoa* are reported to have distinguishable microbiome which plays a crucial role in the adverse resilience and productivity of hosts [22, 29]. The Qinghai-Tibet Plateau is the highest altitude plateau in the world, and is called the "Roof of the World" and the "Third Pole". Due to the harsh climate, the pastures in the Plateau are mainly occupied by some gramineous grasses, but the vulnerable legumes are shortage, and only a few leguminous seeds could be harvested. The balance of leguminous and gramineous forges is important to ensure the forage quality and the sustainable development in this area. Thus, it is urgent to introduce high-quality adaptable legumes. We hypothesize that the grasses in Qinghai-Tibet Plateau could survive the harsh climate in part upon their special seed-associated microbial assemblages and some microorganisms that were more enriched in stress-tolerant grasses have the potential to promote plant growth and stress tolerance. The cultivars of *Avena sativa, Elymus sibiricus* and *Elymus dahuricus* used in our study are three economically important gramineous grasses in Qinghai-Tibet Plateau. And the selected four candidate leguminous grass seeds (*Vicia villosa, Trifolium repens, Trifolium pretense* and *Medicago sativa)* are all with high tolerance for the stress environment and prepared to introduce planting in the area. The structure of the bacterial and fungal communities associated with the seven seeds was investigated using 16S rRNA gene and ITS amplicon sequencing.

The plant microorganisms isolated from plants grown in semi-arid environments can promote the growth and improve stress tolerance of a variety of crops [30–32]. Although a number of plant-growth-promoting microorganisms have been reported, they may occasionally outcompete resident microbiomes, potentially having unexpected effects on the hosts [33]. Thus, an understanding of mutual interactions between inoculants and resident microbiome is also required. We were additionally isolated two abundant beneficial microorganisms and their influence on seed germination and seeding growth of

different hosts were also examined, and their interactions with seed-resident microbiomes were discussed.

Material And Methods

Seeds sources

For the microbiome analyses, seven different grass were detected in this study (Table1). The gramineous seeds including *Avena sativa*, *Elymus sibiricus* and *Elymus dahuricus* were collected from Hongyuan County, Sichuan Province, China (elevation 3500~3600 m, 31°50'□33°22'°N, 101°51'□103°23'°W), which was located in the eastern Qinghai-Tibet Plateau. The leguminous seeds including *Vicia villosa*, *Trifolium repens*, *Trifolium pretense* and *Medicago sativa* were purchased from Beijing Baxter Grass Company, and produced from different countries like China, Australia, and USA (Table 1). All of the seeds were collected in the summer of 2017 and stored separately in plastic bags at -20°C until analysis. Host phylogenetic analysis of the seven grass seeds were determined through the analysis of sequence of genes rbcL (Rubisco large subunit) and matK (tRNA-Lys) intron that were downloaded from NCBI [20]. The seeds of *Vicia villosa* and *Avena sativa* have higher seed weight and clustered far from other seeds of same family (Table 1; Additional file 1: Figure S1 and Figure S2a).

Table 1 Seed samples analyzed in this study.

Samples	Family	Geographical origin	Thousand Kernel Weight/g
Vicia villosa	*Leguminosae*	Yunnan, China	62.49
Trifolium repens	*Leguminosae*	Unknown, Australia	0.67
Trifolium pratense	*Leguminosae*	Oregon, USA	1.89
Medicago sativa	*Leguminosae*	Oregon, USA	1.98
Avena sativa	*Poaceae*	Sichuan, China	32.1
Elymus sibiricus	*Poaceae*	Sichuan, China	4.22
Elymus dahuricus	*Poaceae*	Sichuan, China	4.18

Sample preparation

Ten grams of each grass seed sample were Surface-sterilized with 4% Sodium percarbonate ($Na_2CO_3 \cdot 1.5H_2O_2$, aladdin^R) 15 or 30 minutes based on differences in the texture of the seed coat (30 min for *Elymus sibiricus* and *Elymus dahuricus* seeds, and 15 min for other seeds), then the bleach was drained. The surface-sterilization was done at 30°C in an orbital shaker (180 rpm). Then each sort of seeds was rinsed with autoclaved distilled water three times. After draining the water, the seeds were physically Grind into

powder with 75% ethanol-sterilized grinding machine (Royalstar GR150A) for 1-3 minutes. The seed powder was separately packed in a 2 ml centrifuge tube and stored in -20°C for later use.

DNA extraction, PCR and sequencing

Total genomic DNA from 150 mg seed samples described above was extracted according to the descriptions of kit (Quick-DNA Plant/Seed Miniprep Kit of Zymo, Catalog No. D6020). DNA concentration and quality were checked using a NanoDrop Spectrophotometer. All seeds were performed in three biological replicates and three technical replicates. The V4 region of 16S rRNA gene and the ribosomal internal transcribed spacer 2 (ITS2) were PCR-amplified to assess bacterial and fungal diversity, respectively [34, 35]. For 16S rRNA gene amplification, the PLNAM (GTCGAACGTTGTTTTCGGp/ CTTCACCCCAGTCGAAGAp) and PLNAP (GTCGAACGGGAAGTGGTp/ CTTCACTCCAGTCGCAAGCp) primer pairs (phosphorylation at the 3’ end) were used to block amplification of host plant plastid and mitochondrial sequences and increased the proportion of prokaryotic reads for the seed endosphere. Primer: 515F (GTGYCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) were used to amplify the V4 region of 16S rRNA gene and Primer: ITS3_KYO2 (GATGAAGAACGYAGYRAA) and ITS4 (TCCTCCGCTTATTGATATGC) were used to amplify the ITS2 region. Each seed sample was amplified using the specific primer with 12 nt unique barcode.

The PCR mixture (50 µL) contained 2x PCR buffer, 1.5 mM MgCl2, each deoxynucleoside triphosphate at 0.4 µM, each primer at 1.0 µM, 0.5 U of KOD-Plus-Neo (TOYOBO) and 10 ng template DNA. The PCR amplification program consists of initial denaturation at 94°C for 1 min, followed by 30 cycles of denaturation at 94°C for 20 s, annealing at 55°C (16S rRNA gene) or 48°C (ITS) for 30 s, elongation at 72°C for 30 s, and a final extension at 72°C for 5 min. PCR products from different samples were pooled with equal molar amount and the library was applied to paired-end sequencing (2×250 bp) with the Illumina Hiseq apparatus at Rhonin Biosciences Co., Ltd. All quality sequence files supporting the findings of this article are available in the NCBI Sequence Read Archive (SRA) under the BioProject IDs PRJNA578890 and PRJNA578958.

Data processing and statistical analyses

The sequences were analyzed according to Usearch (http://drive5.com/uparse/) and QIIME5 pipeline. Paired-end reads from the original DNA fragments were merged using FLASH4. Then sequences were assigned to each sample according to the unique barcode. Sequences were clustered into operational taxonomic units (OTUs) at 97% identity threshold using UPARSE algorithms8 [36]. To obtain the taxonomic information of the OTUs, representative sequences of each OTU were generated and aligned against the SILVA and UNITE databases using the uclust classifier in QIIME for 16S rRNA gene and

ITS2, respectively. We picked representative sequences and removed potential chimeras using Uchime algorithm. In case of the influences of sequencing depth on community diversity, the OTUs table was sub-sampled to the lowest number of read counts for further analysis. All data analyses were performed using R7 or Python (https://www.python.org/). Weighted and Unweighted Unifrac distances were calculated in GUniFrac. Calculation of community parameters including Chao1 richness, Simpson's index D and the Shannon-Weiner index H' was performed using mothur. Principal Coordinate Analysis (PCoA) was performed to assess the beta diversity based on Bray-Curtis distance. Possible functions of the bacterial communities were predicted with Tax4Fun [37] and the genes related to plant growth-promoting (PGP) and sporation were selected based on study of grassland soils and Grapevine rootstocks [38, 39]. Kruskal-Wallis rank sum test was performed to show the significance of the difference on alpha diversity and taxa among different groups. To determine if there were significant distincts among different groups, permutational multivariate analysis of variance (PERMANOVA) was performed based on the dissimilarity matrix. Differences in the relative abundances of genera between samples were determined using Student's two-sample t-tests.

Isolation and identification of bacteria from grass seeds

About 0.2g of the seven seeds powder described above was added in a triangular flask, with 20ml of sterile water, 5g of sterile glass beads (3.5mm diameter). The seed microbiota was enriched at 28°C in an orbital shaker (180 rpm) for 4h. Ten-fold serial dilutions of these powder seed suspension (10X and 100X) were streaked on LB solid plates (10 g/L tryptone, 5 g/L, yeast extract and 10 g/L NaCl, pH 7.2, 15g/L agar), followed by incubation for 3 days at 28°C. Morphologically unique bacterial colonies from each plate were selected, and streaked on fresh plates to purify and finally cultured in LB broth for glycerol stocks and DNA extraction. Genomic DNA of each isolates was extracted and PCR was amplified using the primers 27F/1492R of 16s rRNA gene [40](Delong, 1992). Sanger sequencing of the PCR product was carried out in sequencer ABI 3730XL. The obtained 16S rRNA gene sequences were aligned and the affiliations deduced using both BLAST analysis in the EzTaxon server (https://www.ezbiocloud. net/) [41] and NCBI BLAST Resources (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The 16S rRNA genes nucleotide sequences of the two isolates used in our study were deposited to the GenBank® database under accession numbers MN595066 and MN595067.

Plant growth promotion assay

The microbiome structure-dependent effect of the selected bacterial strains on germination of seeds and the growth of grass seedlings were assayed by seed inoculation experiments [42]. The grass seeds of *Vicia villosa*, *Medicago sativa*, *Elymus sibiricus* and *Elymus dahuricus* were Surface-sterilized as above descript. The bacterial strains were grown in

LB medium for 24h at 28°C and the density determined by colony forming unit (CFU) analysis. Bacterial inoculation was performed through soaking seeds in water containing 10^7 bacteria ml^{-1} for 4h at 28°C while control seeds were soaked in sterile water. Incubated seeds were Transferred to Petri dish with two layers of filter paper and 5ml of sterile water, the germination rate of seeds were calculated after 4 days, the seeding of *Medicago sativa*, *Elymus sibiricus* and *Elymus dahuricus* could rooted in the filter paper and growing in the Petri dish without cover, while the seedlings of *Vicia villosa* was larger and cannot be fixed on filter paper, so we transferred the *Vicia villosa* seedings to seed germination pouch (PHYTOTCTM, Size: 140mm*125mm) when it germinated. The fresh weights of the seedlings of the *Vicia villosa* and *Medicago sativa* (7-day-old) or *Elymus dahuricus* and *Elymus sibiricus* (14-day-old) were weighed. The experiment was carried out in 3 replicates for each strain with 100 bio-primed seeds from each grass. Plant growth promoting effects of the microorganisms were statistically analyzed using the SPSS program version 20.0. The significance of the differences in the germination rate and fresh weights between the control and treatment groups was calculated using a pairwise t test with independent samples.

Results

The Sequencing Data Sets

A total of 720,608 and 721,979 clean reads was generated from 16S rRNA gene and ITS sequencing data, respectively. Sequence numbers per sample varied from 30,034 to 39,839 reads (median 34,259) for bacteria and 30,347 to 38,215 reads (median 35,007) for fungi. These reads were assembled into 1,013 distinct bacterial operational taxonomic units (OTUs) (measured at 97% identity) and 922 fungal OTUs. All of the grass seeds had a total 67 common bacterial OTUs, accounting for 74.9% in all sequence reads. OTUs that were unique to each community ranged from 43 to 146, but occupied in low abundance (0.2% to 1.5%) (Fig. 1a). There were 115 fungal OTUs overlapped in the all species seeds, which represented a large fraction (95.5%) of all fungal sequence reads, and the OTUs unique to each grass were also in low abundance (Fig 1b). Thus, the difference of bacterial and fungal community among the seven grass seeds was mainly come from the relative abundance of the shared OTUs rather than the presence or absence of specific OTUs.

(Fig. 1)

Fig. 1 Venn plot of bacterial (a) and fungal (b) communities of seven seed groups. Venn diagrams show absolute number of operational taxonomic units (OTUs) and the relative sequence percentage in the total number of OTUs.

Microbial community diversity of each grass seed

Comparing the bacterial and fungal communities associated with Leguminosae (sample numbers n=12) and Poaceae (n=9), no significant differences were detected through Chao1, Simpson's index and the Shannon index by independent-sample T Test. The bacterial community diversity was significantly higher in seeds of *Vicia villosa* and *Trifolium repens* and lower in *Medicago sativa* and *Avena sativa*, while the fungal community diversity was most highly in seeds of *Avena sativa* and lower in *Elymus sibiricus* and *Elymus dahuricus* (Fig. 2a). Beta diversity analysis based on weighted UniFrac distances (Bray-Curtis) was performed (Fig. 2b). It was shown that the fungal communities of the seven seeds were more distinctively clustered by the host genetic background than bacterial (Additional file 1: Figure S2). Unexpectedly, the bacterial and fungal community of gramineous seeds *Avena sativa* clustered far from *Elymus sibiricus* and *Elymus dahuricus* while closer to leguminous seeds. Especially, the bacterial communities of *Avena sativa* clustered together with the *Trifolium repens* and *Medicago sativa*.

(Fig. 2)

Fig. 2 The Alpha (a) and Beta (b) diversity of the seed bacterial and fungal communities. The four legumes (*Vicia villosa, Trifolium repens, Trifolium pretense Medicago sativa*) and three gramineous grass species (*Avena sativa, Elymus sibiricus* and *Elymus dahuricus*) were abbreviated in the figures. The beta diversity was based on Bray-Curtis distance of OTUs.

Differences in bacterial communities across grass species

After normalization and taxonomic assignment, some highly abundant genera were shared between the seven grass species, but the relative abundance of those showed high variations. (Fig. 3; Table 2). The bacterial communities were mainly composed of *Proteobacteria* (54~90%) and *Firmicutes* (5~41%) in different species. *Firmicutes* was more abundant in seeds of *Vicia villosa* (25%), *Elymus sibiricus* (41%) and *Elymus dahuricus* (24%) than *Trifolium repens*(16%), *Trifolium pretense* (10%), *Avena sativa (10%)* and *Medicago sativa* (5%) (Table 2). *Firmicutes* were mainly composed of *Bacilli* (5~38%). *Proteobacteria* was abundant in all of the grass seeds but had distinct in each species. *Proteobacteria* were represented by the classes of *GammaProteobacteria* (28~80%), *AlphaProteobacteria* (2~31%) and *BetaProteobacteria* (3~10%).

About 73% of the bacterial OTUs could be identified at genus level (Additional file 1: Figure S3a). The relative abundance of the 10 most abundant bacterial genera of seven grass seeds is shown in the Circular visualization picture (Fig. 3). *GammaProteobacteria* was dominated by genera *Pantoea*, *Aeromonas*, *Acinetobacter*, *Salmonella* (Additional file 1: Figure S3a). *AlphaProteobacteria* was represented by genera *Sphingomonas* and *Bacilli* was represented by *Bacillus* and *Lactobacillus*. The seed of *Vicia villosa* and *Trifolium repens* exhibited a high diversity of bacterial taxa and the relative abundance of each taxon

was more balanced than other seeds. *Vicia villosa* had higher abundance of *Acinetobacter* (8%), *Sphingomonas* (5%) *LactoBacillus* (6%), but had lower abundance of *Bacillus* (4%) and *Pantoea* (2%) than other seeds. In the seeds of *Trifolium pratense*, *Medicago sativa* and *Avena sativa*, *GammaProteobacteria* comprised more than half of the seed bacteria microbiota (63~80%). This preponderance was mainly due to the dominance of *Pantoea* (27%) and *Aeromonas* (11%) in *Trifolium pratense*, *Pantoea* (36%) and *Salmonella* (12%) in *Medicago sativa*, and *Pantoea* (42%) in *Avena sativa*. The seeds of *Elymus sibiricus* and *Elymus dahuricus* were more abundant with *Bacillus* (19 and 11%, respectively). And the *Elymus dahuricus* was also enriched with *Sphingomonas* (25%).

(Fig. 3)

Fig. 3 The relative proportion of abundant bacterial taxa on each grass seed. The left side of the figures showed the ten most abundant genus in bacterial communities, and the order level taxonomic groups have been shown above the genus name and bracketed. The right sides represent different seed samples. The different colors of the inner ring represent different taxa or seed samples and the corresponding outer ring shows their proportion.

Differences in fungal communities across grass species

Seed-resident fungal communities of each grass seeds also contained different proportion of shared fungal taxa. However, their relative abundance was more significantly different and more distinctively clustered than bacteria by the host genetic background. The fungal communities were dominated by different taxa even in class and order level (Fig. 4; Table 2). The OTUs of the fungal communities were mainly identified as *Ascomycota* (23~96%), *Basidiomycota* (2~11%) and other unclassified taxa (1~70%) in the seven species seeds. At the class level, it was dominated by *Leotiomycetes* (3~74%), *Dothideomycetes* (9~50%) and *Saccharomycetes* (5~33%) in *Ascomycota*. At order level, *Helotiales* (2~73%) in *Leotiomycetes* was most abundant, followed by *Saccharomycetales* (5~33%) in *Saccharomycetes*, *Pleosporales* (3~43%) and *Capnodiales* (2~23%) in *Dothideomycetes* (Table 2).

Only 23% of the fungal OTUs were identified up to genus and 45% to family level, while 68% could be assigned to order level (Additional file 1: Figure S3b). Thus, we make the circos plot of fungal at the order level (Fig. 4). *Candida* was the representative *genera in Saccharomycetales* and was the most abundant (33%) in *Vicia villosa* seeds. *Trifolium repens* was also enriched with *Candida* (14%), and also distinctively abundant with unclassified *Helotiales*(41%), dominated by *Sclerotiniaceae* (39%). *Trifolium pratense* was only abundant with unidentified fungi (70%). *Medicago sativa* was dominated by *Capnodiales* (23%), represented by unclassified *Mycosphaerellaceae* (21% of all). Moreover, the seed of *Avena sativa* was enriched with both *Candida* (17%) and *Alternaria* (18%), and the latter was assigned to *Pleosporales* (43%). However, *Elymus sibiricus* and

Elymus dahuricus were also in high abundance with *Helotiales*, but it was dominated by unclassified *Helotiaceae* (69 and 59% respectively), which was distincted from the unclassified *Sclerotiniaceae* in *Trifolium repens*. In addition, the unidentified fungi were founded only enriched in the four leguminous grass seeds, and their dominate OTUs in each species were different, such as OTU_2 in *Vicia villosa* (29%), OTU_9 in *Trifolium repens* (16%), OTU_5 in *Trifolium pratense* (55%) and OTU_4 in *Medicago sativa* (38%).

Table 2 The relative abundance of dominated bacterial and fungal taxa (From phylum to order level).

Taxonomic assignment	*Vicia villosa*	*Trifolium repens*	*Trifolium pratense*	*Medicago sativa*	*Avena sativa*	*Elymus sibiricus*	*Elymus dahuricus*
Bacteria							
Proteobacteria [a]	**58%**	**60%**	**77%**	**90%**	**86%**	**54%**	**71%**
GammaProteobacteria [b]	34%	43%	**63%**	**80%**	**74%**	45%	28%
Enterobacteriales [c]	13%	25%	**40%**	**59%**	**52%**	32%	16%
Pseudomonadales [c]	10%	7%	7%	8%	8%	5%	5%
Aeromonadales [c]	3%	4%	**11%**	7%	7%	4%	2%
AlphaProteobacteria [b]	**19%**	7%	3%	3%	5%	2%	**31%**
Sphingomonadales [c]	7%	3%	2%	1%	3%	1%	**25%**
BetaProteobacteria [b]	3%	9%	7%	6%	6%	6%	10%
Burkholderiales [c]	2%	4%	3%	2%	3%	3%	8%
Firmcutes [a]	**25%**	16%	10%	5%	10%	**41%**	**24%**
Bacilli [b]	**19%**	11%	7%	5%	8%	**38%**	**21%**
Bacillales [c]	9%	3%	2%	3%	4%	**25%**	**15%**
Lactobacillales [c]	**10%**	8%	5%	2%	4%	12%	7%
Fungi							
Unidentified Fungi [a]	**39%**	**23%**	**70%**	**49%**	10%	2%	1%
Ascomycota [a]	**56%**	**71%**	**23%**	**46%**	**78%**	**96%**	**94%**
Leotiomycetes [b]	8%	**42%**	8%	3%	7%	**74%**	**63%**
Helotiales [c]	7%	**41%**	7%	2%	6%	**73%**	**62%**
Dothideomycetes [b]	14%	12%	9%	**33%**	**50%**	12%	21%
Pleosporales [c]	10%	8%	3%	9%	**43%**	7%	3%
Capnodiales [c]	2%	3%	5%	**23%**	5%	4%	6%
Saccharomycetes [b]	**33%**	**16%**	5%	9%	**19%**	7%	9%
Saccharomycetales [c]	**33%**	**16%**	5%	9%	**19%**	7%	9%
Basidiomycota [a]	4%	5%	7%	5%	**11%**	2%	5%

[a,b,c]The OTU designation was performed at phylum, class and order level, respectively.

(Fig. 4)

Fig. 4 The relative proportion of abundant fungal taxa on each grass seed. The left side of the figures shows ten most abundant orders in fungal communities, and the genus level taxonomic groups have been shown below the order name and bracketed; The specific OTUs or family name were shown according to the seed samples color when the order dominated by different taxa in different seeds. The right sides represent different seed samples. The different colors of the inner ring represent different taxa or seed samples and the corresponding outer ring shows their proportion.

Predicted bacterial function diversity

To assess how variation in bacterial communities may influence the function diversity of the seven species seeds, Tax4Fun were used to predict the functional properties of taxa units detected by 16S rRNA gene analysis (Fig. 5). The seed bacterial community of *Trifolium repens* and *Trifolium pretense* was predicted to have similar function, and it was also similar between *Medicago sativa* and *Avena sativa*. Functions responsible for metabolism of Energy and Amino Acid were found to be significantly higher in *Vicia villosa* than other seeds, and functions responsible for Nucleotide metabolism were significantly more abundant in seeds of *Trifolium repens* and *Trifolium pretense*. It was predicted that a lower rate of metabolic activity within the bacterial cells of *Elymus* (*Elymus sibiricus* and *Elymus dahuricus*) seeds compared with other seeds (Fig. 5; Additional file 1: Table S1). We also compared the abundance of many KEGG Ortholog (KO) profiles related to plant growth-promoting (PGP) and spore formation traits (Additional file 1: Table S2). The predicted abundances of enzyme-encoding genes involved in general PGP traits and spore formation were more abundant in *Elymus* seeds. It also showed many enrichments of PGP traits in the four leguminous and *Avena sativa* seeds. For example, the function of phosphate solubilization was most abundant in *Medicago sativa*.

(Fig. 5)

Fig. 5 Relative proportions of predicted function of 16S rRNA gene at KEGG level 3. All of the function of gene were with abundance over 1%. The color code refers to Z score of relative abundance, with high predicted abundances (red) and low predicted abundances (Green).

Microbial interaction networks in the grass seed microbiome

A microbial interaction network for the abundant bacterial OTUs (Abundance over 0.2%) in all samples were conducted, and only 75 of 80 OTUs were significant interactions (Pearson Correlation Coefficient > 0.5; $p < 0.05$) and shown in Fig. 6. The bacterial taxa

involved in significant interactions are dominated by *Proteobacteria* (66.7%) followed by *Firmicutes* (24.0%), *Bacteroides* (4.0%) and *Actinobacteria* (2.7%). Among several positive interactions observed, a strong co-occurrence relationship was shown for some members of *Sphingomonas*1/2/3, *Ralstonia*, *Burkholderia*, *Hyphomicrobiaceae_* and *Methylobacterium*. We also found that some of the highly abundant taxa (Rectangle marked) were more likely to co-occurrence with members in same phylum, but co-exclusion with members in different phylum (Fig. 6). The OTU *Pantoea*1 in *Proteobacteria* was only positively correlated with 5 OTUs (*Pantoea*2, 3, *Aeromonas*1, *Haemophilus*1, and *Pseudomonas*1) and all belonged to *Proteobacteria* (5/5, five out of five OTUs), while was more negatively correlated with members in different phylum (6/7), including *Firmicutes* (*LactoBacillus*1, 2*, Enterococcus, RummeliiBacillus and LysiniBacillus*) and *Actinobacteria* (*Rothia*). *Aeromonas*1 was also only positively correlated with same phylum members and negatively correlated with different phylum members. The OTU *Bacillus*1 belonging to *Firmicutes* was also more positively correlated with the members in *Firmicutes* (7/9) than *Proteobacteria* (2/9). The situation was also same for *Sphingomonas*1, among the seven co-occurrence OTUs, six out of seven were same phylum). The other OTUs have same trend but not significant as these abundant taxa. About 39 of 42 Fungal OTUs were significant interactions (Additional file 1: Figure S4), and the fungal taxa are dominated by *Ascomycota* (66.7%), *Basidiomycota* (20.5%) and Unidentified fungi (12.8%). Most of the significant interactions were positive (72/74) and the highly abundant taxa (Rectangle marked) was not more likely to co-occurrence with members of the same phylum and exclusive with members of different phylum. A strong co-occurrence were shown among g__*Alternaria*, OTU_19, o__*Pleosporales*2, f__*Pleosporaceae*2, g__*Phaeosphaeria*, o__*Pleosporales*4.

(Fig. 6)

Fig. 6 Significant co-occurrence and co-exclusion relationships among the grass seeds bacterial microbiome. Each node represents a bacterial OTU (Abundance > 0.2%), describing on genus-level. In cases where the identification of the OTU was not assigned on a genus level, higher-level taxonomic groups have been shown and labeled as "_" after the name. When several OTUs were assigned to the same taxa, the numbers (1–5) were added to the name in order to differentiate between the nodes. The color of nodes corresponds to the phylum, and the rectangle nodes represented the abundant OTUs (Abundance > 4%). Only significant interactions are shown (Pearson Correlation Coefficient > 0.5; $p < 0.05$). Edge width is proportional to the absolute value of the Pearson correlation coefficient, and color indicates the sign of the association (red positive, blue negative).

Different response to the isolated biocontrol strains

From the high-throughput data of 16S rRNA gene, *Pantoea* content was high in all the other seeds except for *Vicia villosa*. Furthermore, *Bacillus* was only significant enriched in the seeds of *Elymus sibiricus* and *Elymus dahuricus*. Reportedly, most of *Pantoea* and *Bacillus* strains were beneficial to plant growth and potential biocontrol strains. We would like to know if the effects of these biological control strains are affected by the inherent abundance of the strain in host seeds. Firstly, we undertook efforts to culture bacteria corresponding to *Pantoea* and *Bacillus* from the seven seeds. By incubating the grass seeds in LB solid medium, about 10 different bacterial colony morphologies were observed. In the isolated plates, the colony similar in morphology to *Bacillus* and *Pantoea* were also in high abundance, a finding in line with results from the sequencing results. Here, *Bacillus* from seeds of *Elymus sibiricus* and *Pantoea* from *Elymus dahuricus* were isolated and chosen as test strains. The 16S rRNA gene full sequencing results showed that the two strains shared highest sequence homology with *Bacillus subtilis* and *Pantoea agglomerans*, respectively.

According to the different abundance of *Pantoea* and *Bacillus*, two gramineous grasses (*Elymus sibiricus* and *Elymus dahuricus*) and two leguminous grasses (*Vicia villosa* and *Medicago sativa*) were selected as the test plants. The surface-sterilized seeds were soaked in sterile water (Control) or sterile water containing 10^7 bacteria ml^{-1} of *Bacillus subtilis* or *Pantoea agglomerans* for 4 h at 28°C, separately. The seed germination rates s and the seedling fresh weights were calculated for assessing the effect of growth-promoting. We observed a different influence of the two strains on the growth of the four grasses (Fig. 7). The incubation of *Bacillus subtilis* significantly increased the germination rate ($P<0.001$) and promote the seedling growth of *Vicia villosa* ($P<0.01$). As for the *Medicago sativa*, *Bacillus subtilis* also showed to a stimulating effect on germination and biomass, although this effect was not obvious. Unexpectedly, we detected that *Bacillus subtilis* had a clear detrimental effect on the germination and seedlings growth of *Elymus sibiricus* and *Elymus dahuricus*, and the inhibition was more pronounced in *Elymus sibiricus*. External application of *Pantoea agglomerans* promoted germination and seedling growth of *Vicia villosa*, but the effect was not as obvious as the improvement of germination by *Bacillus subtilis*. While a negative effect of *Pantoea agglomerans* was shown on the growth of *Medicago sativa*, *Elymus sibiricus* and *Elymus dahuricus*, and the inhibition was more significant in *Elymus sibiricus* and *Elymus dahuricus*.

(Fig. 7)

Fig. 7 Effect of the two isolated strains on the germination rate and seedling fresh weights of grasses. The seedling of *Vicia villosa* **(a)** and *Medicago sativa* **(b)** were one week old, and the seedling of *Elymus sibiricus* **(c)** and *Elymus dahuricus* **(d)** were two weeks old. The statistical results of germination rate **(e)**, and seedling fresh weights **(f)** were shown. "*Bacillus*" means treatment with *Bacillus subtilis* and "*Pantoea*" means treatment with *Pantoea agglomerans*. The Fresh weight of *Vicia villosa* were the average weight of 3

plants in one replicate group, and the Fresh weight of *Medicago sativa, Elymus sibiricus* and *Elymus dahuricus* were the average weight 30 plants in one replicate group. Statistical figure was expressed by the geometric mean ± standard deviation.

Discussion

In this study, all the cultivars of the three native gramineous grass species (*Avena sativa, Elymus sibiricus* and *Elymus dahuricus)* from the Qinghai-Tibet Plateau and the four candidate legumes (*Vicia villosa, Trifolium repens, Trifolium pretense* and *Medicago sativa)* have strong cold tolerance, especially the gramineous species. Through the comprehensive analysis of the bacterial and fungal communities, a large fraction of bacterial (74.9%) and fungal (95.5%) OTUs were shared in common, indicating a high commonality among these communities. The bacteria *Proteobacteria* and fungi *Ascomycota* were detected abundant in all of the grass seeds and which were also revealed dominated in many plant seeds [23, 24, 43]. It is inferred that many taxa in the two phyla may be ubiquitous in the endogenous environment of plant seeds. These high abundance bacteria and fungi may act as 'core microbiomes' contributing to seed storage, plant growth and stress resistance [16, 17], and the high commonality may be related to their similar resistance traits.

The previous work showing that *Firmicutes* was more abundant in stress-resistant plants such as *Agave* and *Quinoa [*22, 29]. To our knowledge, *Firmicutes* is commonly considered as monoderm bacteria (lack an outer cell membrane and contain thick cell walls), which might provide an advantage in their capacity to survive in plant seed under harsh conditions such as drought and UV radiation [44](Xu *et al.,* 2018). In our study, we detected higher abundance of *Firmicutes* OTUs in grass species with higher stress resilience like *Vicia villosa* (25%), *Elymus sibiricus* (41%) and *Elymus dahuricus (*24%), and lowest abundance in less tolerance grass *Medicago sativa.* Thus, the abundance of *Firmicutes* may partly explains the different adversity resilience of the seven grasses.

Despite the high commonality, a high species specificity of microbial assemblages was also revealed in this study. And the fungal communities of the seven seeds were clustered more distinctively than bacteria by host genetic background (Fig. 2b; Additional file 1: Figure S2). This was consistent with microbiome study in Brassicaceae [24], sunflower [45] and Apple [46], where host genotypes have a stronger effect on fungal microbiota than bacteria. Unexpectedly, the microbial community of gramineous seeds of *Avena sativa* clustered far from *Elymus sibiricus* and *Elymus dahuricus,* and the bacterial community even clustered together with the *Trifolium repens* and *Medicago sativa.* Indeed, differences in seed bacterial and fungal communities could not only be explained by cultivators or species of host plants, but also some abiotic (geographic location, seed processing and storage) factors [24, 25, 47]. Except genetic background, the seven grass seeds in our study also have different seed weight, morphological structure and geographic location; all of these factors have been reported to influence seed microbiome structure [47]. The gramineous *Avena sativa* seed were significantly differentwith *Elymus sibiricus* and *Elymus dahuricus in* seed weight, morphological structure (Table 1; Additional file 1: Figure S1), which may be the main factors driving the difference of their microbial structures.

Further, we investigated the proportion of potential probiotics in the three gramineous grass seeds. *Avena sativa* was in high abundance with bacteria *Pantoea* (42%) and Fungi like *Candida* (17%) and *Alternaria* (18%). *Pantoea* are featured as IAA producing, salt and osmotic tolerant endophytes [48]. The abundant fungus *Candida* belongs to *Saccharomycetales,* which was mostly reported to have Trophic Mode of Saprotroph and is unlikely to be a pathogen [49]. *Alternaria* is frequently detected with high abundance in healthy plants and some species are considered opportunistic plant pathogens [50], but it may also be beneficial [51]. The species *Alternaria alternate* was reported to enhance fitness of their hosts by stimulating the production of secondary compounds [52]. Although the content of beneficial bacteria *Pantoea* in *Elymus sibiricus (*9%) and *Elymus dahuricus* (12%) was lower than that of *Avena sativa,* it wasalso in high abundant. Besides, the bacteria *Bacillus* (19 and 11%) and fungi unclassified species of *Helotiaceae* (69 and 59%) were only found enriched in seeds of *Elymus sibiricus* and *Elymus dahuricus,* respectively. In addition, *Elymus dahuricus* were also enriched with the bacteria *Sphingomonas* (25%). Both the bacteria *Bacillus* and *Sphingomonas* were considered as beneficial taxa [53]. In addition, the Tax4fun prediction also indicated a lower rate of metabolic activity and a higher ability of spore-forming within the bacterial cells of *Elymus sibiricus* and *Elymus dahuricus* seed. Reduction self-metabolic consumption and dormancy of spore-forming bacteria are thought to provide an opportunity to buffer impacts from changing environment [54, 55]. Reportedly, most members of fungi in *Helotiales* were putative as non-pathogen (Probable Saprotroph-Symbiotroph) [49]. Thus, the high stress tolerance of the three gramineous grasses in Qinghai-Tibet Plateau were speculated to partly due to their high abundance of beneficial bacteria like *Bacillus, Pantoea, Sphingomonas* and Non-pathogenic fungus like *Candida* and classified *Helotiaceae* within seeds.

We further investigated the content of probiotics in the four leguminous grass seeds. The beneficial taxa *Pantoea* was also enriched in *Trifolium repens* (11%), *Trifolium pretense (*27%) and *Medicago sativa (*36%). Although the *Vicia villosa* seeds harbored lower *Pantoea (*2%), it has a wealth of other beneficial taxa like *Acinetobacter* (8%) [53] and *Sphingomonas* (5%). In addition, the Non-pathogenic fungus *Candida* was also enriched in *Trifolium repens* (14%) and *Vicia villosa* (31%). The unclassified *Sclerotiniaceae* in *Helotiales* that also predicted Non-pathogen was abundant in seeds of *Trifolium repens* (39%) [49]. The four legume varieties also enriched with many beneficial taxa, and it indicated that they also have the potential to adapt the harsh environment in Qinghai-Tibet Plateau. Except beneficial taxa, *Medicago sativa* was most abundant with the putative pathogenic fungi (unclassified *Mycosphaerellaceae,* 21%) and bacteria (*Salmonella,* 12%) [49, 56]. Our field observations exhibited that the adaptabilityof *Medicago sativa* to high altitude regions was the worst among the seven species, which was presumably related to its microbial composition. Taken together, the relative abundance of these dominated bacteria and fungi in grass seed may have close relationship to their stress resilience. The higher tolerance grasses (*Elymus sibiricus* and *Elymus dahuricus)* possessed a greater number of growth-promoting bacterial and non-pathogenic fungi. Conversely, the less tolerance grass *Medicago sativa* contained lower levels of such microorganisms, and showed higher abundance of pathogenic taxa.

Furthermore, from Microbial interaction networks, we found that some of the highly abundant bacterial taxa were more likely to co-occurrence with members in same phylum, but co-exclusion with members in different phylum (Fig. 6). It was reported that high abundance bacteria and fungi do not necessarily promote plant growth directly and are more likely to participate in organizing the assembly of plant-associated microbiomes to elevate adverse tolerance or stimulate growth of host plants [1]. One OTU belonging to the putative plant pathogen *Ralstonia* was reported to positively correlated with the beneficial *Burkholderiaceae [*56]. Similarly, *Ralstonia* was also positively correlated with the beneficial taxa like *Sphingomonas*1/2/3 and *Burkholderia* in our results. Thus, these high abundant taxa may also participate in organizing the assembly of plant-associated microbiomes, rather than just promoting host plant growth directly[1].

Rational use of the plant microbiome has the potential to improve the adaption and yield of important agronomical crops [57]. Both *Bacillus* and *Pantoea* were reported to have function of Plant growth promotion, antifungal, IAA production and osmotic stress tolerance [53]. It was also reported that the diversity of communities and the relative abundance of specific taxa influence the effects of biocontrol strains [56]. Rhizobacterial communities were more affected by the plant, environmental stresses and agricultural practices than an exogenous, active PGPR introduced at high levels. The effect of the PGPR on host may largely depends on interactions within and between indigenous populations [33]. Here, both *Bacillus subtilis* and *Pantoea agglomerans* showed positive effects on the germination and seeding growth of *Vicia villosa,* which may be related to the significantly lower abundance of *Bacillus* and *Pantoea* in the seeds. And it is speculated that the overall higher occurrence of *Pantoea* in *Medicago sativa* is responsible for the negative impact of *Pantoea agglomerans* on its growth. Similarly, the negative effect of *Bacillus subtilis* and *Pantoea agglomerans* on *Elymus sibiricus* and *Elymus dahuricus* may also be related to the intrinsic high abundance of host seeds. Microbial dynamic equilibrium with plant systems is vital for the germination, growth in plant life cycle [58]. We deduced that both *Elymus sibiricus* and *Elymus dahuricus,* which come from the Qinghai-Tibet Plateau have co-evolved and adapted to microbes each other, had evolved to most suitable microbial communities that help their host plants nutrient uptakes and stimulate growth to alleviate stresses on the Plateau. Incubation with *Bacillus subtilis* or *Pantoea agglomerans* may disturb the balance within *Elymus sibiricus* and *Elymus dahuricus,* and reduce the germination rate and inhibit seeding growth. Consequently, the structure of the seed microbiome could be an interesting biomarker for breeding strategies and it is better to use the biocontrol strains that are lacking in seeds rather than being enriched.

Conclusions

Seed-resident microbiomes of the four cold-tolerance legumes and three Qinghai-Tibet Plateau gramineous grasses contain different proportion of shared taxa, and most of these taxa are beneficial and non-pathogenic. These high abundance of shared bacteria and fungi probably act as ‘core microbiomes’ for the seven host plants to stimulate growth and help the hosts live in the harsh climate. Importantly, they are more likely to participate in organizing the assembly of plant-associated microbiomes, rather than just promoting host plant growth directly. The four legume grasses as

candidates are speculated to survive on the Qinghai-Tibet Plateau. Compared with bacteria, the fungal communities in all the seven grass seeds were more affected by host genetic distance, seed traits and geographical location. Additionally, the host plants with higher specific beneficial taxa (*Bacillus* or *Pantoea)* tended to counteract the effect of the same probiotics (*Bacillus subtilis* or *Pantoea agglomerans)* and even reflect negative function for overuse of the biocontrol strains. This study provides valuable data for investigating plant resilience, identifying more biocontrol strains, maximizing microbial functions in 'smart farming' practices and has important reference value for the evaluation of the introduction of exotic high quality legumes and other grass species.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in this

published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the National Key Research and Development Program of China (Grant No. 2017YFD0201301).

Authors' contributions

XRM, YD and TW conceived and designed research. YD, YH and CXL conducted experiments. YD, XYL, and YW analyzed the data. YD and XRM wrote the manuscript with assistance from TW and HHL. All authors read and approved the manuscript.

Acknowledgements

We thank the anonymous reviewers and the editors for their constructive comments and suggestions to improve the manuscript.

Author details

[1] Chengdu Institute of Biology, Chinese Academy of Sciences, No. 9, Section 4, Renmin South Road, Chengdu 610041, Sichuan, China

[2] Key Laboratory of Bio-Resources and Eco-Environment of the Ministry of Education, College of Life Science, Sichuan University, Chengdu, 610065, Sichuan, China

[3] University of Chinese Academy of Sciences, 100049, Beijing, China

References

1.Toju H, Peay KG, Yamamichi M, Narisawa K, Hiruma K, Naito K, Fukuda S, Ushio M, Nakaoka S, Onoda Y *et al: Core microbiomes for sustainable agroecosystems. Nature Plants* 2018, *4(*5):247–257.

2.Hardoim PR, van Overbeek LS, Berg G, Pirttila AM, Compant S, Campisano A, Doring M, Sessitsch A: *The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. Microbiol Mol Biol R* 2015, *79(*3):293–320.

3.Truyens S, Weyens N, Cuypers A, Vangronsveld J: *Bacterial seed endophytes: genera, vertical transmission and interaction with plants. Environmental Microbiology Reports* 2015, *7(*1):40–50.

4.Murphy BR, Doohan FM, Hodkinson TR: *Fungal root endophytes of a wild barley species increase yield in a nutrient-stressed barley cultivar. Symbiosis* 2015, *65(*1):1–7.

5.Rojas X, Guo J, Leff JW, Mcnear DH, Fierer N, Mcculley RL: *Infection with a Shoot-Specific Fungal Endophyte (Epichloë) Alters Tall Fescue Soil Microbial Communities. Microbial Ecology* 2016, *72(*1):197–206.

6.Yumlembam R, Borkar SG: *Assessment of antibacterial properties of medicinal plants having bacterial leaf endophytes against plant pathogenic Xanthomonads.* 2014, *67:*353–357.

7.Compant S, Clément C, Sessitsch A: *Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. Soil Biology & Biochemistry* 2009, *42(*5):669–678.

8.Kuldau G, Bacon C: *Clavicipitaceous endophytes: Their ability to enhance resistance of grasses to multiple stresses. Biological Control* 2008, *46(*1):0–71.

9.Gond SK, Bergen MS, Torres MS, Jr JFW: *Endophytic Bacillus spp. produce antifungal lipopeptides and induce host defence gene expression in maize. Microbiological Research* 2015, *172:*79–87.

10.Luo S, Liang C, Chen J, Chan R, Xiao X, Yong W, Zeng G, Fei L, Liu C, Liu Y: *Endophyte-assisted promotion of biomass production and metal-uptake of energy crop sweet sorghum by plant-growth-*

promoting endophyte Bacillus sp. SLS18. Applied Microbiology & Biotechnology 2012, *93(*4):1745–1753.

11.Shahzad R, Khan AL, Bilal S, Asaf S, Lee IJ: *What Is There in Seeds? Vertically Transmitted Endophytic Resources for Sustainable Improvement in Plant Growth. Frontiers in Plant Science* 2018, *9:*24-.

12.Cheesanford JC, li MMW, Davis AS, Sims GK: *Do microorganisms influence seed-bank dynamics? Weed Science* 2006, *54(*3):575–587.

13.Goggin DE, Emery RJN, Kurepin LV, Powles SB: *A potential role for endogenous microflora in dormancy release, cytokinin metabolism and the response to fluridone in Lolium rigidum seeds. Annals of Botany* 2015, *115(*2):293.

14.Rodríguez CE, Mitter B, Barret M, Sessitsch A, Compant S: *Commentary: seed bacterial inhabitants and their routes of colonization. Plant & Soil* 2018(1–2):1–6.

15.Shearin ZRC, Filipek M, Desai R, Bickford WA, Kowalski KP, Clay K: *Fungal endophytes from seeds of invasive, non-native Phragmites australis and their potential role in germination and seedling growth. Plant & Soil* 2018, *422(*1–2):1–12.

16.Santoyo G, Moreno-Hagelsieb G, Orozco-Mosqueda MDC, Glick BR: *Plant growth-promoting bacterial endophytes. Microbiological Research* 2016, *183(*5):92–99.

17.Shahzad R, Khan AL, Bilal S, Waqas M, Kang SM, Lee IJ: *Inoculation of abscisic acid-producing endophytic bacteria enhances salinity stress tolerance in Oryza sativa. Environmental & Experimental Botany* 2017, *136:*68–77.

18.Fukami, Tadashi: *Historical Contingency in Community Assembly: Integrating Niches, Species Pools, and Priority Effects. Annual Review of Ecology Evolution & Systematics, 46(*1):150805153913004.

19.Chimwamurombe PM, Gronemeyer JL, Reinhold-Hurek B: *Isolation and characterization of culturable seed-associated bacterial endophytes from gnotobiotically grown Marama bean seedlings. Fems Microbiol Ecol* 2016, *92(*6).

20.Naylor D, Degraaf S, Purdom E, Coleman-Derr D: *Drought and host selection influence bacterial community dynamics in the grass root microbiome. Isme Journal* 2017, *11(*12):2691–2704.

21.Horton MW, Bodenhausen N, Beilsmith K, Meng D, Muegge BD, Subramanian S, Vetter MM, Vilhjálmsson BJ, Nordborg M, Gordon JI: *Genome-wide association study of Arabidopsis thaliana leaf microbial community. Nature Communications* 2014, *5(*5):5320.

22.Coleman-Derr D, Desgarennes D, Fonseca-Garcia C, Gross S, Clingenpeel S, Woyke T, North G, Visel A, Partida-Martinez LP, Tringe SG: *Plant compartment and biogeography affect microbiome composition in cultivated and native Agave species. New Phytologist* 2016, *209(*2):798–811.

23.Links MG, Tigst D, Tom GF, Hill JE, Hemmingsen SM, Dumonceaux TJ: *Simultaneous profiling of seed-associated bacteria and fungi reveals antagonistic interactions between microorganisms within a shared epiphytic microbiome on Triticum and Brassica seeds. New Phytologist* 2014, *202(*2):542–553.

24.Barret M, Briand M, Bonneau S, Préveaux A, Valière S, Bouchez O, Hunault G, Simoneau P, Jacques MA: *Emergence shapes the structure of the seed-microbiota. Appl Environ Microbiol* 2015, *81(*4):1257.

25.Klaedtke S, Jacques MA, Raggi L, Préveaux A, Bonneau S, Negri V, Chable V, Barret M: *Terroir is a key driver of seed-associated microbial assemblages. Environmental Microbiology* 2016, *18(*6):1792–1804.

26.Yang L, Shan Z, Zou Y, Wang J, Wei S: *Investigation on diversity and population succession dynamics of endophytic bacteria from seeds of maize (Zea mays L., Nongda108) at different growth stages. Annals of Microbiology* 2013, *63(*1):71–79.

27.Midha S, Bansal K, Sharma S, Kumar N, Patil PP, Chaudhry V, Patil PB: *Genomic Resource of Rice Seed Associated Bacteria. Front Microbiol* 2015, *6.*

28.Yang L, Danzberger J, Schöler A, Schröder P, Schloter M, Radl V: *Dominant Groups of Potentially Active Bacteria Shared by Barley Seeds become Less Abundant in Root Associated Microbiome. Frontiers in Plant Science* 2017, *8:*1005-.

29.Pitzschke A: *Developmental Peculiarities and Seed-Borne Endophytes in Quinoa: Omnipresent, Robust Bacilli Contribute to Plant Fitness. Frontiers in Microbiology* 2016, *7(*482):2.

30.Marasco R, Rolli E, Ettoumi B, Vigani G, Mapelli F, Borin S, Abou-Hadid AF, El-Behairy UA, Sorlini C, Cherif A: *A drought resistance-promoting microbiome is selected by root system under desert farming. Plos One* 2012, *7(*10):e48479.

31.Mengual C, Schoebitz M, Azcón R, Roldán A: *Microbial inoculants and organic amendment improves plant establishment and soil rehabilitation under semiarid conditions. Journal of Environmental Management* 2014, *134(*2):1–7.

32.Rolli E, Marasco R, Vigani G, Ettoumi B, Mapelli F, Deangelis ML, Gandolfi C, Casati E, Previtali F, Gerbino R: *Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. Environmental Microbiology* 2014, *17(*2):316–331.

33.Castro-Sowinski S, Herschkovitz Y, Okon Y, Jurkevitch E: *Effects of inoculation with plant growth-promoting rhizobacteria on resident rhizosphere microorganisms. Fems Microbiology Letters, 276(*1):1–11.

34.Caporaso JG, Gordon JI: *Global patterns of 16s rrna diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci U S A* 2011, *108(*Supplement_1):4516–4522.

35.Tedersoo L, Anslan S, Bahram M, Põlme S, Riit T, Liiv I, Kõljalg U, Kisand V, Nilsson H, Hildebrand F: *Shotgun metagenomes and multiple primer pair-barcode combinations of amplicons reveal biases in metabarcoding analyses of fungi. MycoKeys* 2015, *10:*1–43.

36.Edgar RC: *UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods* 2013, *10(*10):996.

37.Asshauer KP, Wemheuer B, Daniel R, Meinicke P: *Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data. Bioinformatics:*btv287.

38.Griffith JC, Lee WG, Orlovich DA, Summerfield TC: *Contrasting bacterial communities in two indigenous Chionochloa (Poaceae) grassland soils in New Zealand. PLoS ONE* 2017, *12(*6):e0179652.

39.Marasco R, Rolli E, Fusi M, Michoud G, Daffonchio D: *Grapevine rootstocks shape underground bacterial microbiome and networking but not potential functionality. Microbiome, 6(*1):3.

40.Delong EF: *Archaea in coastal marine environments. Proceedings of the National Academy of Sciences of the United States of America* 1992, *89(*12):5685–5689.

41.Ok-Sun K, Yong-Joon C, Kihyun L, Seok-Hwan Y, Mincheol K, Hyunsoo N, Sang-Cheol P, Yoon Seong J, Jae-Hak L, Hana Y: *Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol* 2012, *62(*Pt 3):716–721.

42.R S, M N, MA J, A S, T R, B M: *The genomes of closely related Pantoea ananatis maize seed endophytes having different effects on the host plant differ in secretion system genes and mobile genetic elements.* 2015, *6(*440):440.

43.Rezki S, Campion C, Simoneau P, Jacques MA, Shade A, Barret M: *Assembly of seed-associated microbial communities within and across successive plant generations. Plant & Soil* 2018:1–13.

44.Xu L, Naylor D, Dong Z, Simmons T, Pierroz G, Hixson KK, Kim YM, Zink EM, Engbrecht KM, Wang Y: *Drought delays development of the sorghum root microbiome and enriches for monoderm bacteria. Proc Natl Acad Sci U S A* 2018, *115(*18):201717308.

45.Leff JW, Lynch RC, Kane NC, Fierer N: *Plant domestication and the assembly of bacterial and fungal communities associated with strains of the common sunflower, Helianthus annuus. New Phytologist* 2016, *214(*1).

46.Liu J, Abdelfattah A, Norelli J, Burchard E, Schena L, Droby S, Wisniewski M: *Apple endophytic microbiota of different rootstock/scion combinations suggests a genotype-specific influence. Microbiome, 6(*1):18.

47.Khalaf EM, Raizada MN: *Taxonomic and functional diversity of cultured seed associated microbes of the cucurbit family. Bmc Microbiology* 2016, *16(*1):131.

48.Walitang DI, Kim K, Madhaiyan M, Kim YK, Kang Y, Sa T: *Characterizing endophytic competence and plant growth promotion of bacterial endophytes inhabiting the seed endosphere of Rice. Bmc Microbiology, 17(*1):209.

49.Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG: *FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology* 2016, *20(*1):241–248.

50.Thomma BPHJ: *<I>Alternaria</I> spp.: from general saprophyte to specific parasite. Molecular Plant Pathology* 2003, *4(*4):225–236.

51.Pan JJ, Baumgarten AM, May G: *Effects of host plant environment and Ustilago maydis on the fungal endophyte community of maize (Zea mays). New Phytologist* 2008, *178(*1):147–156.

52.Aschehoug E, Callaway R, Newcombe G, Tharayil N, Chen S: *Fungal endophyte increases the allelopathic effects of an invasive forb. OECOLOGIA* 2014, *175(*1):285–291.

53.Martínez-Rodríguez A, Macedo-Raygoza G, Huerta A, Reyes-Sepulveda I, Lozano-Lopez J, García-Ochoa E, Fierro-Kong L, Medeiros M, Di Mascio P, White J *et al: Agave Seed Endophytes: Ecology and Impacts on Root Architecture, Nutrient Acquisition, and Cold Stress Tolerance.* In*.;* 2019: 139–170.

54.Jones SE, Lennon JT: *Dormancy contributes to the maintenance of microbial diversity. Proc Natl Acad Sci U S A* 2010, *107(*13):5881–5886.

55.Lennon JT, Jones SE: *Microbial seed banks: the ecological and evolutionary implications of dormancy. Nature Reviews Microbiology* 2011, *9(*2):119–130.

56.Rybakova D, Mancinelli R, Wikström M, Birch-Jensen AS, Postma J, Ehlers RU, Goertz S, Berg G: *The structure of the Brassica napus seed microbiome is cultivar-dependent and affects the interactions of symbionts and pathogens. Microbiome* 2017, *5(*1):104.

57.Turner TR, James EK, Poole PS: *The plant microbiome. Genome Biology, 14(*6).

58.Li H-Y, Parmar S, Sharma VK, White JF: *Seed Endophytes and Their Potential Applications;* 2019.

Figures

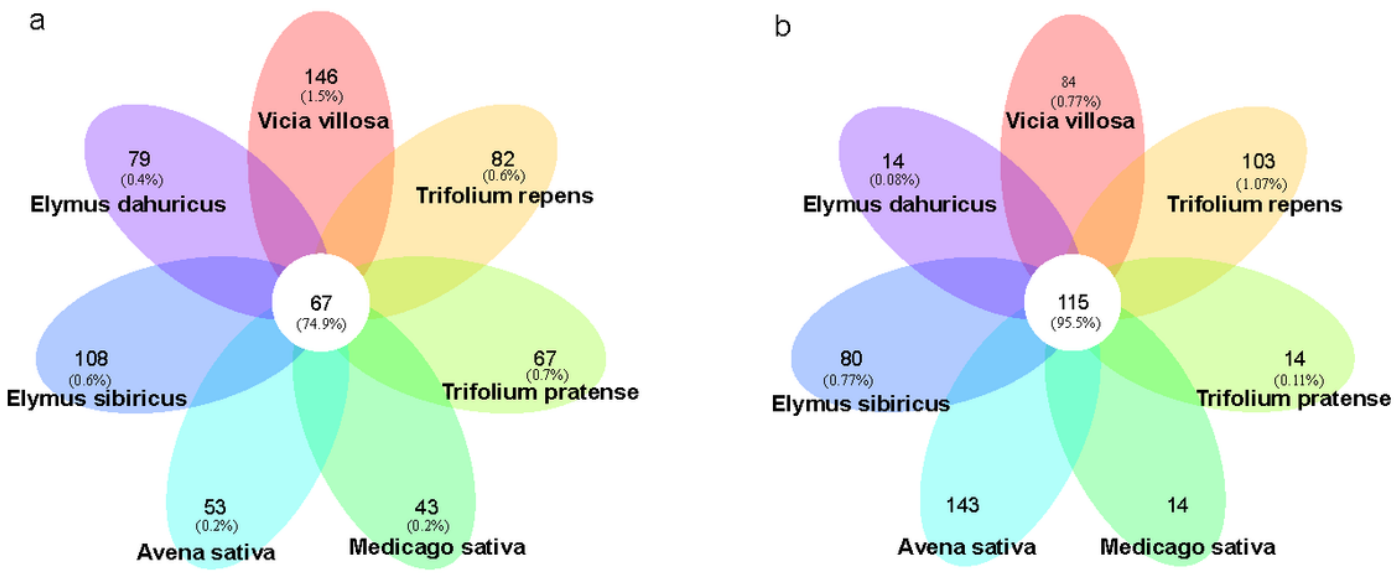

Figure 1

Venn plot of bacterial (a) and fungal (b) communities of seven seed groups. Venn diagrams show absolute number of operational taxonomic units (OTUs) and the relative sequence percentage in the total number of OTUs.

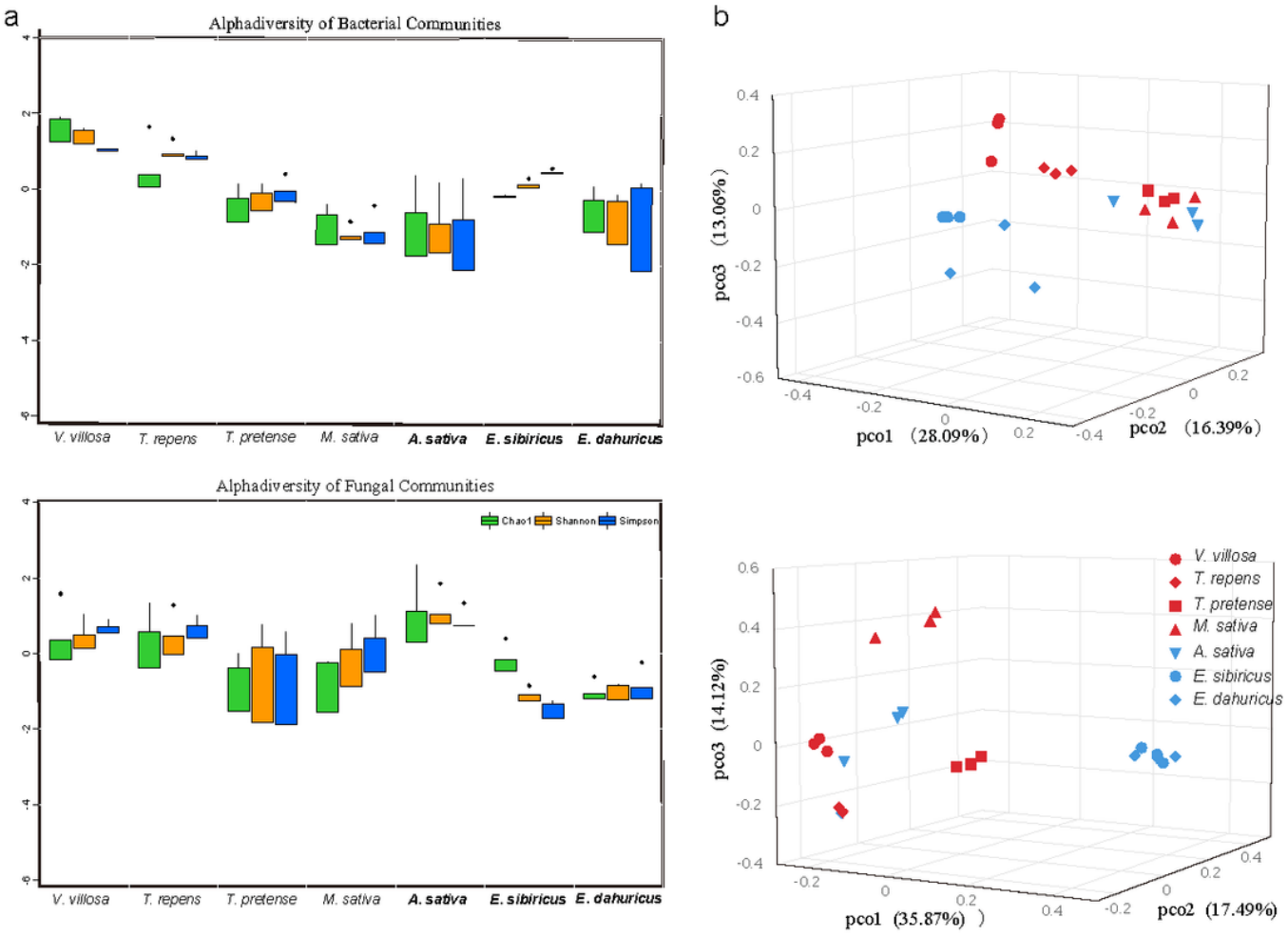

Figure 2

The Alpha (a) and Beta (b) diversity of the seed bacterial and fungal communities. The four legumes (Vicia villosa, Trifolium repens, Trifolium pretense Medicago sativa) and three gramineous grass species (Avena sativa, Elymus sibiricus and Elymus dahuricus) were abbreviated in the figures. The beta diversity was based on Bray-Curtis distance of OTUs.

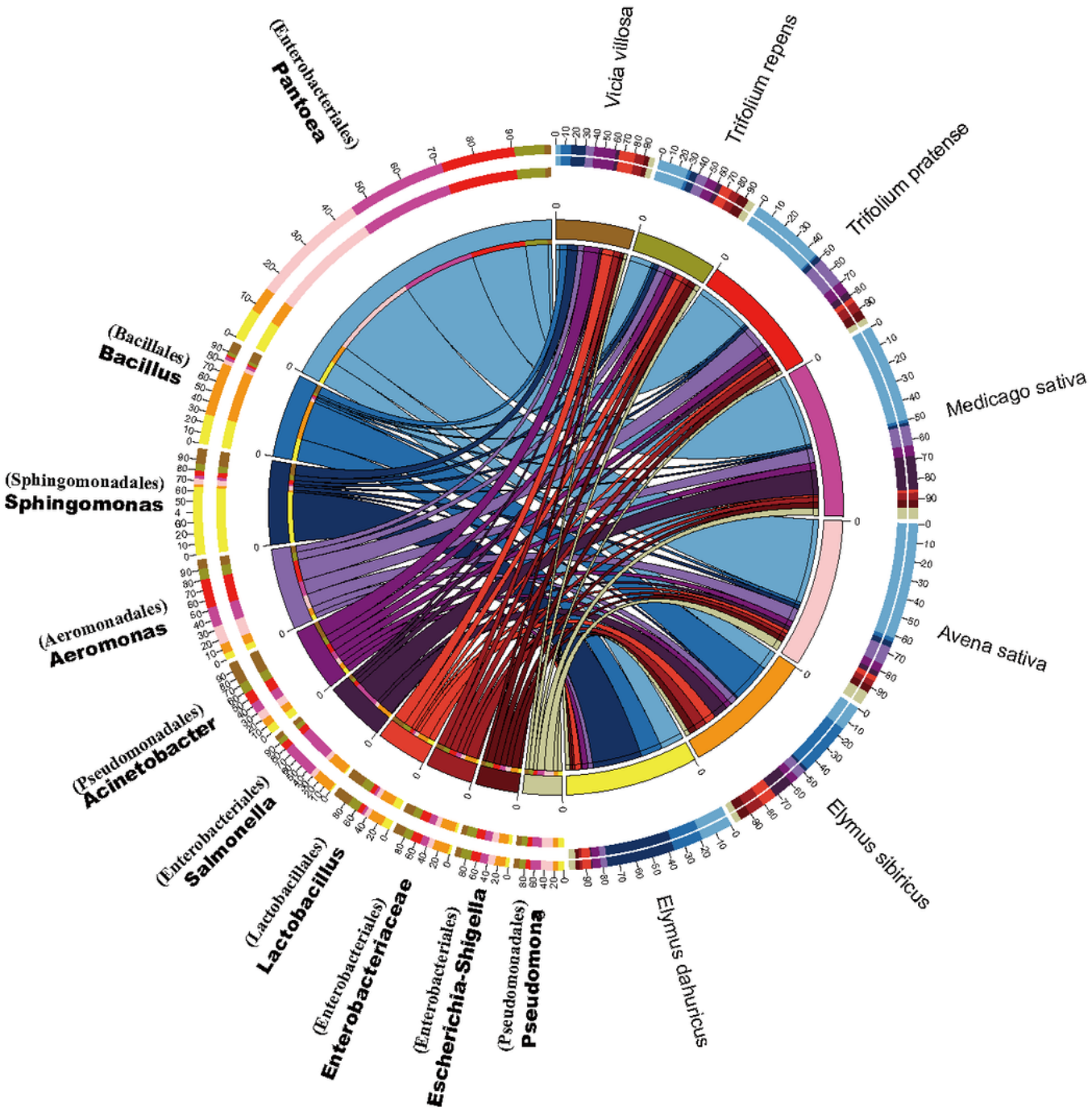

Figure 3

The relative proportion of abundant bacterial taxa on each grass seed. The left side of the figures showed the ten most abundant genus in bacterial communities, and the order level taxonomic groups have been shown above the genus name and bracketed. The right sides represent different seed samples. The different colors of the inner ring represent different taxa or seed samples and the corresponding outer ring shows their proportion.

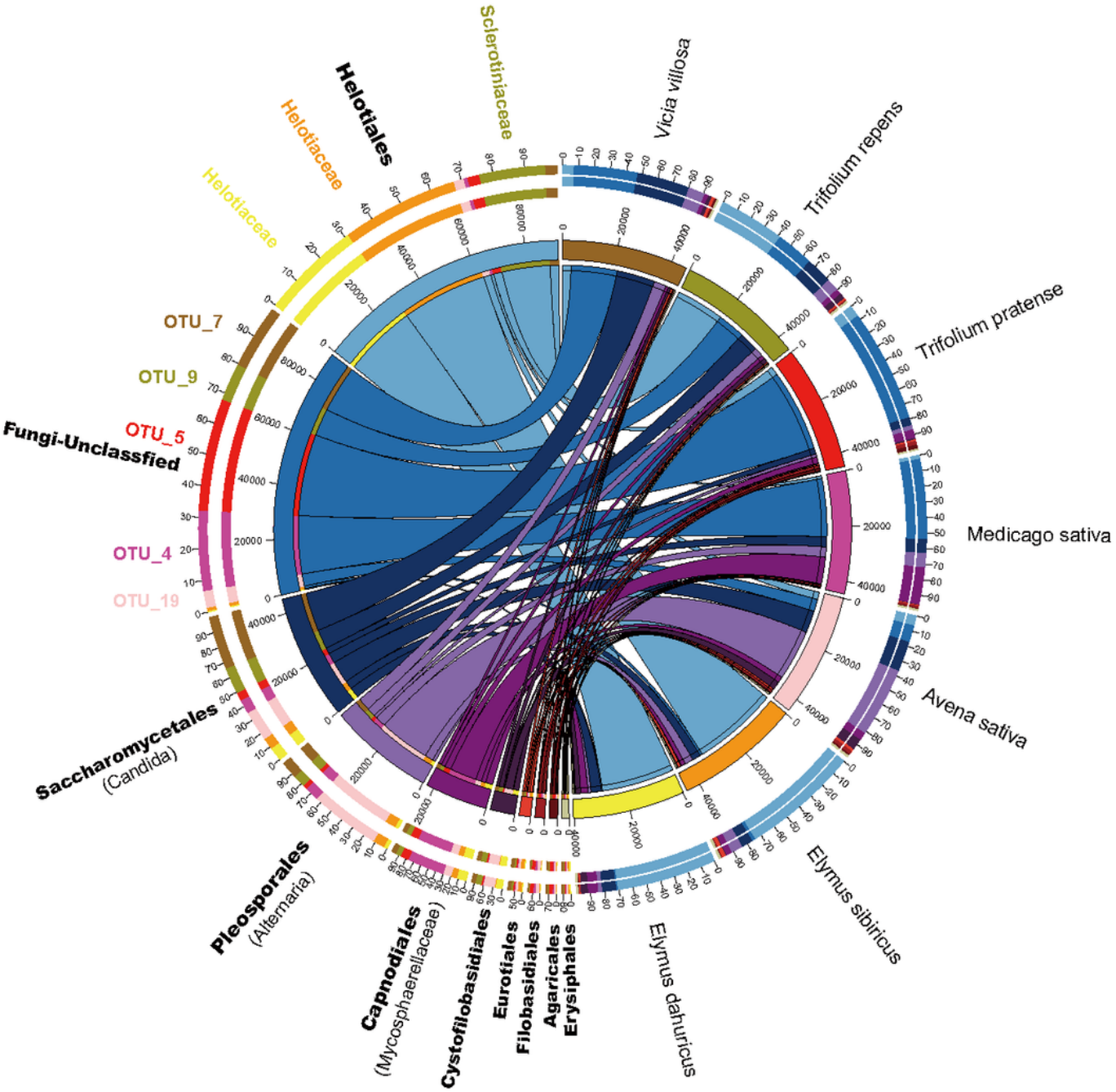

Figure 4

The relative proportion of abundant fungal taxa on each grass seed. The left side of the figures shows ten most abundant orders in fungal communities, and the genus level taxonomic groups have been shown below the order name and bracketed; The specific OTUs or family name were shown according to the seed samples color when the order dominated by different taxa in different seeds. The right sides

represent different seed samples. The different colors of the inner ring represent different taxa or seed samples and the corresponding outer ring shows their proportion.

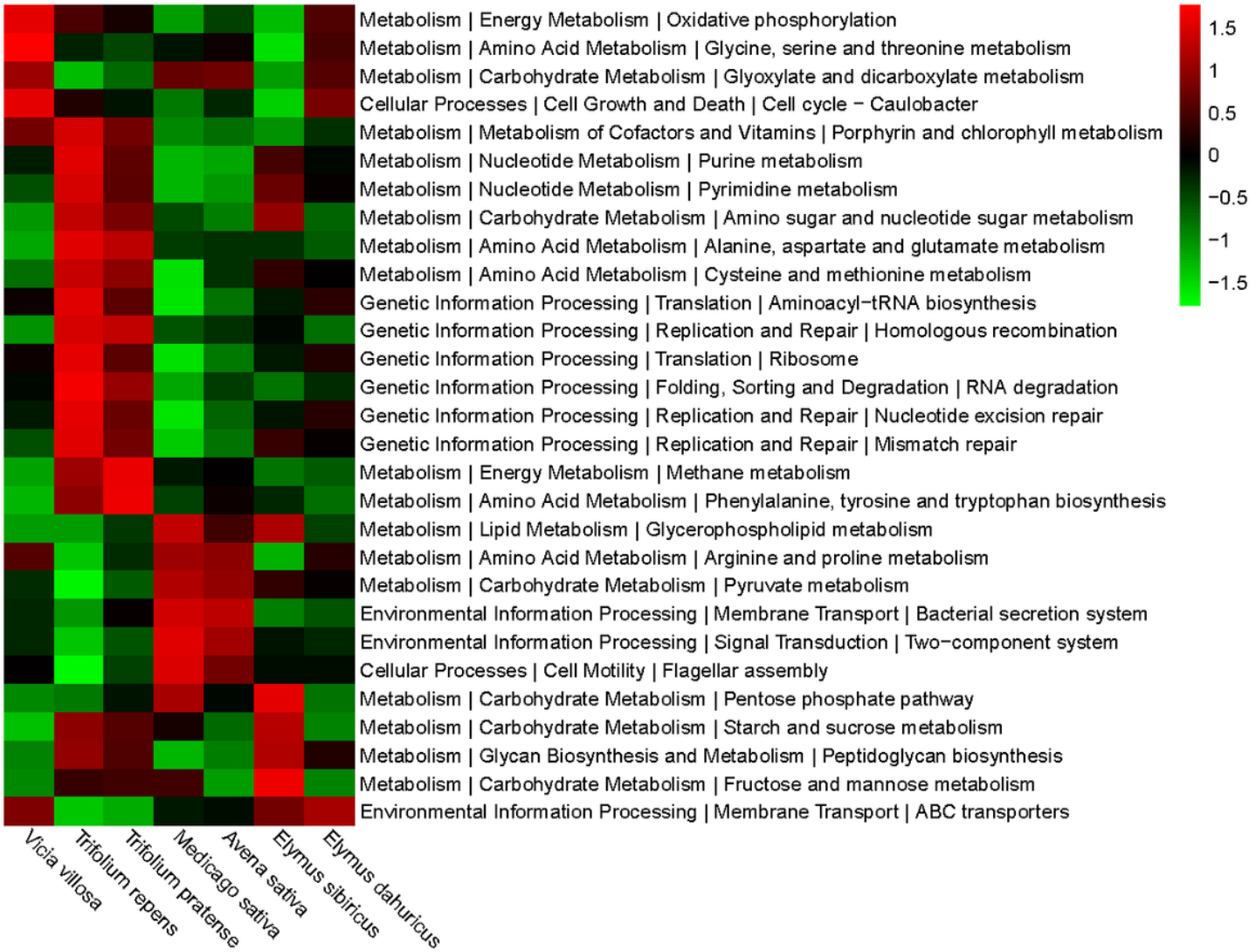

Figure 5

Relative proportions of predicted function of 16S rRNA gene at KEGG level 3. All of the function of gene were with abundance over 1%. The color code refers to Z score of relative abundance, with high predicted abundances (red) and low predicted abundances (Green).

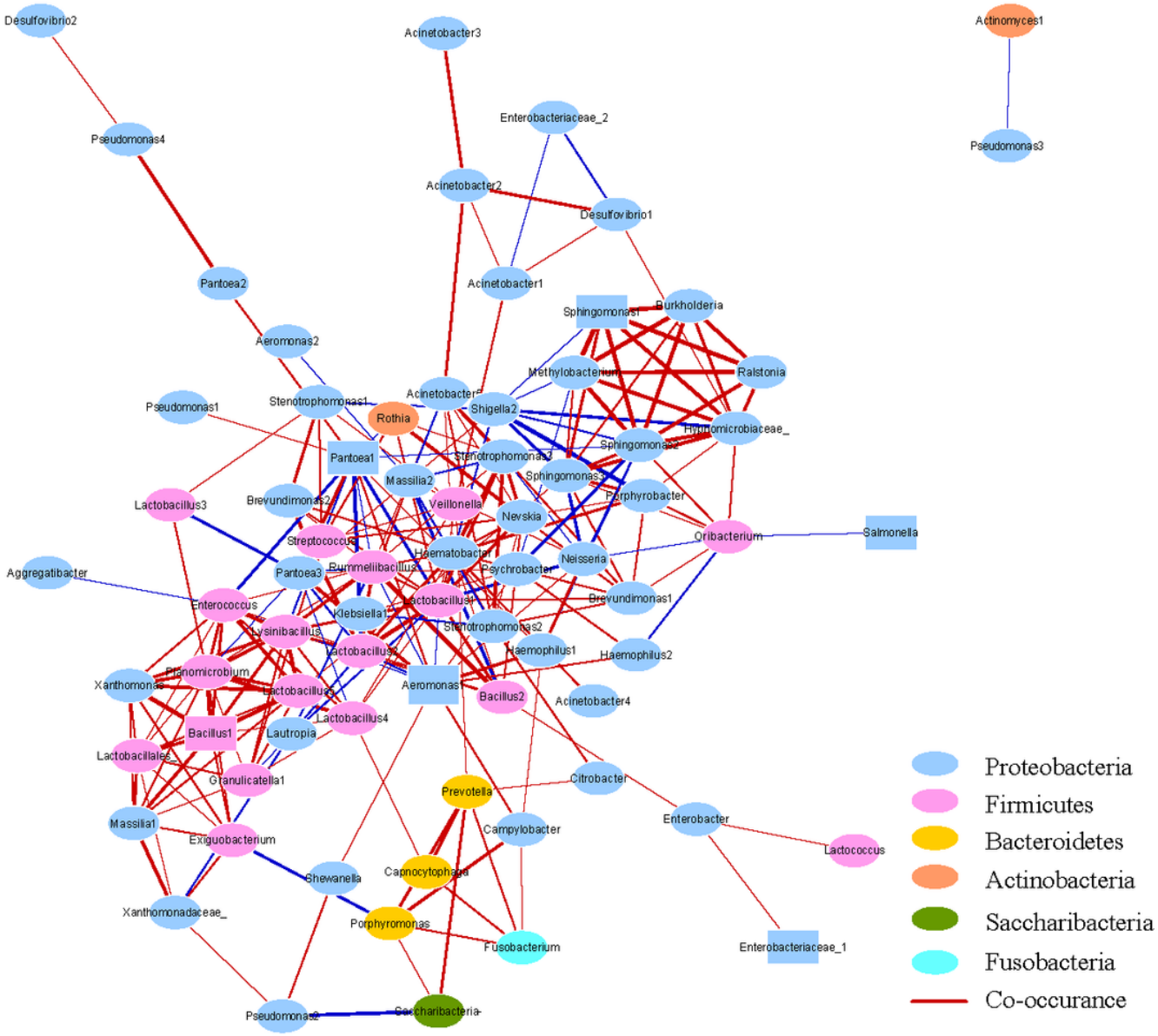

Figure 6

Significant co-occurrence and co-exclusion relationships among the grass seeds bacterial microbiome. Each node represents a bacterial OTU (Abundance > 0.2%), describing on genus-level. In cases where the identification of the OTU was not assigned on a genus level, higher-level taxonomic groups have been shown and labeled as "_" after the name. When several OTUs were assigned to the same taxa, the numbers (1–5) were added to the name in order to differentiate between the nodes. The color of nodes corresponds to the phylum, and the rectangle nodes represented the abundant OTUs (Abundance > 4%).

Only significant interactions are shown (Pearson Correlation Coefficient > 0.5; p < 0.05). Edge width is proportional to the absolute value of the Pearson correlation coefficient, and color indicates the sign of the association (red positive, blue negative).

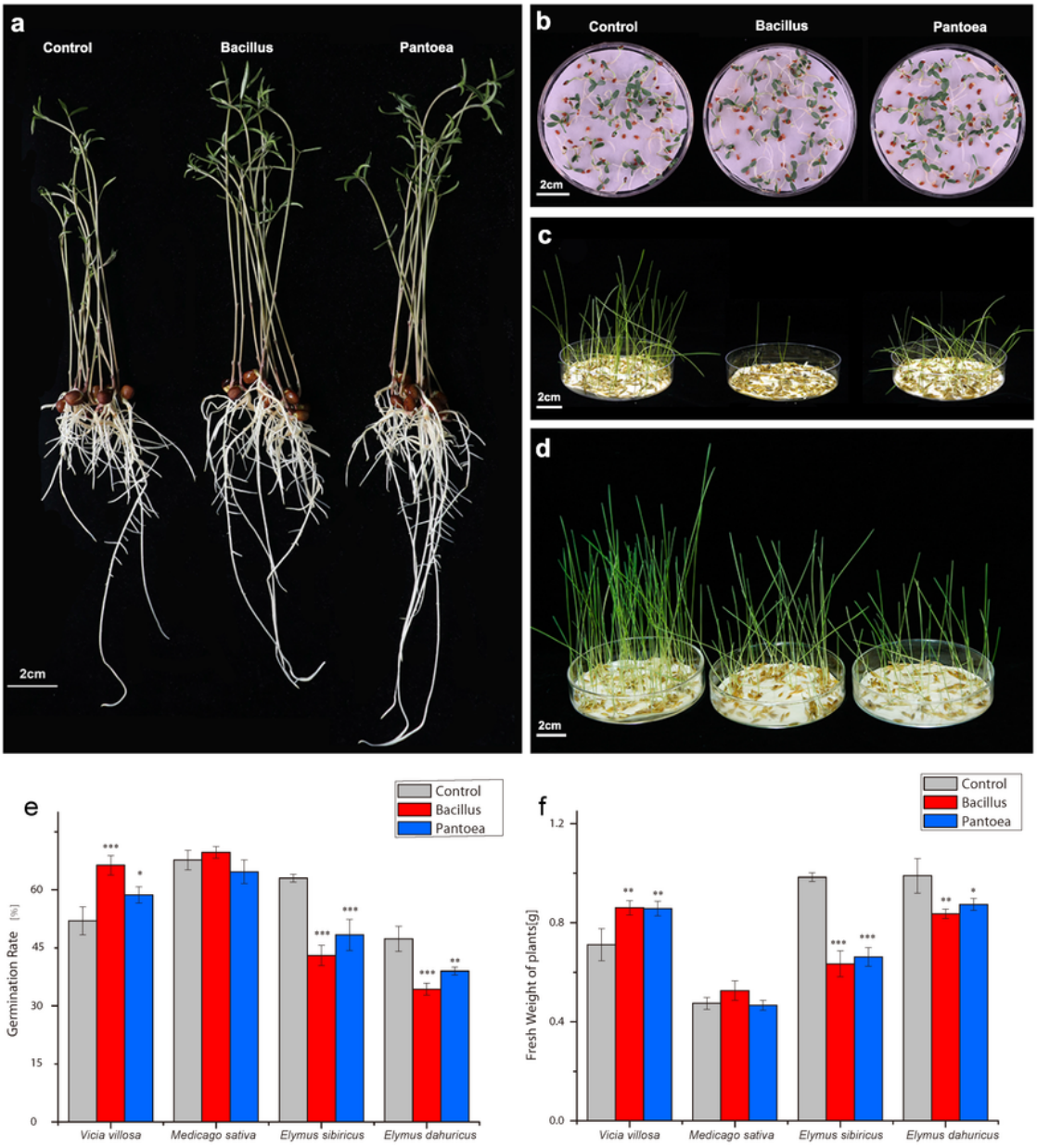

Figure 7

Effect of the two isolated strains on the germination rate and seedling fresh weights of grasses. The seedling of Vicia villosa (a) and Medicago sativa (b) were one week old, and the seedling of Elymus

sibiricus (c) and Elymus dahuricus (d) were two weeks old. The statistical results of germination rate (e), and seedling fresh weights (f) were shown. “Bacillus” means treatment with Bacillus subtilis and “Pantoea” means treatment with Pantoea agglomerans. The Fresh weight of Vicia villosa were the average weight of 3 plants in one replicate group, and the Fresh weight of Medicago sativa, Elymus sibiricus and Elymus dahuricus were the average weight 30 plants in one replicate group. Statistical figure was expressed by the geometric mean ± standard deviation.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.docx