REGULAR ARTICLE

Diversity of endophytic bacteria and fungi in seeds of *Elymus nutans* growing in four locations of Qinghai Tibet Plateau, China

Jianxiu Guo · Saman Bowatte · Fujiang Hou

Abstract

Aims Seeds are involved in the transmission of micro-organisms from one plant generation to the next, acting as initial inoculum for the plant microbiome, therefore provide a key source of variability in plants. This study aimed to characterize the seed bacteria and fungi communities in *Elymus nutans*, a dominant perennial grass growing in the Qinghai Tibet Plateau (QTP) and explore the effects of plant growth location on the seed microbiome.

Methods Seeds were collected from plants growing in four locations in the QTP. The seed microbial community was examined by Illumina MiSeq sequencing of DNA extracted from the surface sterilized seeds.

J. Guo · S. Bowatte (✉) · F. Hou
State Key Laboratory of Grassland Agro-ecosystems; Key Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture and Rural Affairs; College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730020, China
e-mail: samanbowatte@lzu.edu.cn

J. Guo
e-mail: guojx17@lzu.edu.cn

F. Hou
e-mail: cyhoufj@lzu.edu.cn

Results The seed bacterial community was dominated by the bacteria phylum Proteobacteria (98%) and fungal phyla Ascomycota (83%) and Basidiomycota (15%). At the lower taxonomic level, the bacterial genus *Pseudomonas* dominated in all four locations with an average relative abundance of 83% whereas the fungal genera that dominated the seed microbiome was diverse, the most prominent being *Epichloë*, *Pyrenophora*, *Mycosphaerella* and *Bullera*. Ecologically important bacterial family *Nitrosomonadaceae* (nitrifiers) and fungal phylum Glomeromycota (arbuscular mycorrhizal fungi) were detected in this study for the first time as seed endophytes. The *Elymus nutans* seed bacterial community was not impacted by the plant growth location, in contrast, the seed fungal community varied significantly in four locations.

Conclusions The seeds of *Elymus nutans* host diverse endophytic bacteria and fungi. Unlike the bacteria, the host plant selection of seed fungal endophytes was observed to have been affected by plant growth location. Positive and negative associations in the *Elymus nutans* seed microbiome were observed.

Keywords Seed endophytic bacteria · Endophytic fungi · *Elymus nutans* · Qinghai Tibet Plateau

Introduction

Plants host a diverse community of microbes, both bacteria and fungi, on or inside of various plant components that play important roles in plant biology (Nelson

et al. 2018). How plants acquire their specific microorganisms, whether certain microorganisms are passed down generation to generation or are recruited from the environment, their interactions with external perturbations and the impact of various microbiomes on plant performance remains to be fully understood and has been a hot research topic recently (Shade et al. 2017; Nelson et al. 2018; Shahzad et al. 2018; Verma and White 2019). It was thought that plant microbiomes originated from the soil (Edwards et al. 2015) but recently many studies have demonstrated that seeds are involved in the transmission of microorganisms from one plant generation to the next, acting as initial inoculum for the plant microbiome in a range of plant species (Shade et al. 2017). The seed microbes that inhabit the internal tissues are called seed endophytes. Microbes that inhabit the seed surface are called seed epiphytes. The seed endophytic microbes are more likely to be transmitted vertically than seed epiphytes, however, seed epiphytes can also become endophytes on germination of the seeds (Barret et al. 2015).

Johnston-Monje and Raizada (2011) found a core set of microbes in ancestral wild maize as well as in modern maize cultivars, suggesting core bacterial taxa were conserved across generations even with anthropogenic intervention and cross continental migration. In another recent study by Sánchez-López showed the endophytic bacterial communities across three generations of *Crotalaria pumila* were similar (Sánchez-López et al. 2018). In contrast, Rezki et al. (2018) reported low heritability of seed microbial communities across three generations of *Raphanus sativus*.

The seed microbiome comprises of a diverse range of bacteria (Truyens et al. 2015) and fungi (Shearin et al. 2018), vary according to plant species (Links et al. 2014), genotypes (Adam et al. 2018), the seed maturation process (Mano et al. 2006) and environmental conditions (Klaedtke et al. 2016). The most common seed bacteria across many plant species belong to phyla Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes (Barret et al. 2015; Truyens et al. 2015). The most common seed bacterial genera are *Bacillus*, *Pseudomonas*, *Paenibacillus*, *Micrococcus*, *Staphylococcus*, *Pantoea* and *Acinetobacter* (Truyens et al. 2015). Most seed fungi belong to the phyla Ascomycota and Basidiomycota (Chen et al. 2018).

Several studies have shown the beneficial roles of microbes transmitted through seeds to their host plant (Shahzad et al. 2017; Ab Rahman et al. 2018). Most studies reported their role in plant growth promotion by producing a range of plant growth hormones such as indole acetic acid (IAA) (Faria et al. 2013) and 1-aminocyclopropane-1-carboxylicacid (ACC) deaminase (Johnston-Monje and Raizada 2011). Some seed bacterial endophytes supported plant growth by carrying out nitrogen fixation (Xu et al. 2014) and phosphorus solubilization (Chimwamurombe et al. 2016). Puente et al. (2009) showed that some cactus seed bacteria were able to migrate into the rhizosphere and facilitate the release of mineral nutrients by pulverizing rocks through the production of organic acids, promoting seedling growth. The other major function of seed borne microbes was to provide protection against various pathogens (Cottyn et al. 2009; Faria et al. 2013; Rezki et al. 2016; White et al. 2018). Understanding the contribution made by seed associated microbes to the future plant microbiome has become an emerging area of research, as optimization of this seed microbiome may bring huge benefits to plant breeding and crop improvement (Adam et al. 2018). For example, Mitter et al. (2017) discovered that a specific beneficial endophytic bacterium introduced to the flowers of parent plants could be transferred to the seed microbiome and then passed into offspring generations for expression of beneficial growth traits.

Currently, the seed microbiome of grassland species is poorly defined. Grasslands are a globally important use of land, in terms of feed supply and ecosystem function, covering more than 25% of the earth's landmass. If we are to exploit the plant microbiome for improved grassland productivity, fundamental knowledge of the seed microbiome and understanding of its contribution to the phyllosphere, rhizosphere and endosphere is essential. In this study, we investigated the seed microbiome of *Elymus nutans*, a dominant perennial forage grass, naturally growing in alpine meadow grasslands of the QTP in China. *Elymus nutans* has been promoted for cultivation in alpine areas, due to its high adaptability, good nutrition, high yield and good tolerance to cold, drought and biotic stress (Miao et al. 2011a, b). We collected *Elymus nutans* seeds from four grassland sites located at different geographic locations in the QTP and addressed the following questions:

a) Which bacterial and fungal endophytes colonize the seeds of *Elymus nutans*?

b) Of the bacterial and fungal endophytes that colonize the seeds of *Elymus nutans*, which can be beneficial for the host plant?

c) Are there differences in seed bacterial and fungal endophyte communities among the plant growth locations?
d) Are there interaction patterns of cooperation or competition among the seed endophytic microbial groups?

Materials and methods

Seed collection

The seeds were collected on October 2017 from *Elymus nutans* plants that were growing in alpine meadow grasslands in Maqu County (A), Luqu County (B), Maqin County (C) and Zhuoni County (D) in the QTP. The details of four seed collecting sites are shown in Table 1. Three sub samples (approximately 350 seeds) were separated from the seed collection bag of each location and considered as replicates. The seeds were transported in an ice box to the laboratory and stored at 4 °C until further use.

DNA extraction

Seed surface sterilization was carried out using a method described by Liu et al. (2016). One gram of seeds of each replicate was soaked for 3 min in 70% ethanol, 5 min in 2.5% sodium hypochlorite, 30 s in 70% ethanol, and followed by rinsing 5–7 times with sterilized water. After the seed sterilization, 6 seeds per treatment were immediately placed on sterile R2A agar plates and incubated for 3 days at 25 °C. As there were no colonies on the plates, successful surface sterilization was confirmed. The total genomic DNA of surface sterilized seeds was extracted using the DNeasy Power Soil Kit (QIAGEN, Inc., Netherlands), following the manufacturer's instructions and stored at −20 °C prior to further analysis. Preliminary studies have shown that optimum yield with higher purity can be obtained from the seeds by using soil DNA extraction kit rather than the plant extraction kit (Unpublished observations by Shanghai Personal Biotechnology Co., Ltd). The quality and quantity of extracted DNA was measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively.

PCR amplification

The seed microbial community was examined by Illumina MiSeq sequencing analysis. The Illumina MiSeq sequencing libraries for bacteria were prepared by PCR amplification of V4-V5 hyper variable regions of bacterial 16S rRNA gene by 515F (5'-GTGCCAGC MGCCGCGGTAA-3') and 907R (5'-CCGTCAAT TCMTTTRAGTTT-3') primers (Ren et al. 2015). Sample specific 7-bp barcodes were incorporated into the primers for multiplex sequencing. Thermal cycling consisted of initial denaturation at 98 °C for 2 min followed by 25 cycles including denaturation at 98 °C for 15 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s, and finally at 72 °C for 5 min. The Illumina MiSeq sequencing libraries for fungi were prepared by PCR amplification of the internal transcribed spacer (ITS) region of fungi by ITS1 (5'-TCCGTAGG TGAACCTGCGG-3') and ITS4 (5'-TCCTCCGC TTATTGATATGC-3') primers (Siahmard et al. 2017). The PCR reaction conditions were at 95 °C for 3 min followed by 30 cycles including denaturation at 95 °C for 40 s, annealing at 55 °C for 45 s, and extension at 72 °C for 1 min, and finally at 72 °C for 10 min.

The PCR amplicons were purified with Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN) and quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, amplicons were pooled in equal amounts, and paired-end 2×300 bp sequencing was performed using the Illlumina MiSeq platform with MiSeq Reagent Kit v3 at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China).

Sequence analysis and OTUs generation

The Quantitative Insights Into Microbial Ecology (QIIME, v1.8.0) pipeline was employed to process the sequencing data (Caporaso et al. 2010). Briefly, raw sequencing reads with exact matches to the barcodes were assigned to respective samples and identified as valid sequences. Sequences that had a length of <150 bp, average Phred scores of <20, contained ambiguous bases, and contained mononucleotide repeats of >8 bp were all filtered as low-quality sequences (Gill et al. 2006; Chen and Jiang 2014). Paired-end reads were assembled using FLASH software (Magoč and Salzberg 2011). After detecting chimeras, the remaining high-quality sequences were clustered into operational

Table 1 Details of the seed collection sites

Location	Altitude (m)	UTM coordinates	Annual average rainfall (mm)	Annual average temperature (°C)	Grassland Management
Maqu	3700	753,176.11 N, 3726693.52 E	603	2.0	grazing
Luqu	3500	299,156.05 N, 3871926.84 E	700	2.3	mainly grazing
Maqin	4100	610,232.55 N, 3812705.98 E	490	3.6	grazing
Zhuoni	2541	399,645.49 N, 3875813.85 E	580	4.6	Grazing and cropping

taxonomic units (OTUs) at 97% sequence identity. A representative sequence was selected from each OTU using default parameters. OTU taxonomic classification was conducted by BLAST searching the representative sequences set against the Greengenes Database (DeSantis et al. 2006) using the best hit (Altschul et al. 1997). An OTU table was further generated to record the abundance of each OTU in each sample and the taxonomy of these OTUs. The OTUs containing less than 0.001% of total sequences across all samples were discarded. To minimize the difference of sequencing depth across samples, an averaged, rounded rarefied OTU table was generated by averaging 100 evenly resampled OTU subsets under the 90% of the minimum sequencing depth for further analysis.

Microbial community composition

The rarefied OTUs matrix was used to identify the composition and the relative abundance distribution of phylum, class, order, family and genus in each sample. The shared and unique numbers of OTUs in samples were identified by Venn diagram. The OTUs classified to genus level were used to describe core microbiome (microbes common in all four seed collection locations) and the microbes unique to each seeds collection location.

Microbial community diversity

The microbial community diversity was estimated using rarefied OTUs matrix at a consistent sequencing depth. Alpha diversity of seed bacterial and fungal communities was tested using the Chao 1, ACE, Shannon and Simpson diversity indices using QIIME software. The beta diversity of the communities was assessed by non-metric multidimensional scaling (NMDS) using a weighted UniFrac distance matrix by R software (R Core Team 2014). The differences between microbial

communities between sampling locations were statistically tested by ANOSIM (analysis of similarities) with 999 permutations using QIIME software.

Spearman correlation network analysis

To examine the interaction patterns of cooperation or competition among the microbial groups (bacteria-bacteria, fungi-fungi and bacteria-fungi), Spearman correlation network analysis was carried out (Huang et al. 2018; Tao et al. 2019; Wang et al. 2019). Spearmen rank correlation coefficients between the top 50 dominant genera in terms of abundance were calculated using Mothur software and correlations were established for the groups with rho >0.6 and $p < 0.01$. The networks were visualized using the Cytoscape software.

Sequence acession numbers

The 16S rRNA and ITS gene sequences obtained in this study have been submitted to the NCBI GenBank with the accession numbers SRP211984 and SRP211983 respectively.

Results

The bacterial and fungal community composition

The bacterial communities of *Elymus nutans* seeds were assessed by a total of 21,000 high quality sequencing reads with an average reads of 1750 per replicate sample. The bacterial communities of *Elymus nutans* seeds collected from four locations of QTP were dominated by the bacteria belonging to the phylum Proteobacteria (98%). The rest of the bacterial community belonged to Firmicutes (0.88%), Actinobacteria (0.45%), Bacteroidetes (0.18%), Deinococcus-Thermus (0.05%), Cyanobacteria (0.02%), Verrucomicrobia

(0.02%), Planctomycetes (0.02%) and Acidobacteria (0.01%).

The fungal community was assessed by a total of 14,035 high quality sequencing reads with an average of 1169 reads per replicate sample. The fungal communities of *Elymus nutans* seeds collected from four locations in the QTP were dominated by the fungi belonging to phylum Ascomycota (83%). The rest belonged to Basidiomycota (15%), Glomeromycota (1%), Rozellomycota (0.4%), and Zygomycota (0.3%) phyla.

The relative abundances of the top 5 seed bacterial and fungal phyla, classes and orders, top 10 families and genera, and their distribution, in the seeds of four seed collection locations are shown in Table 2 and in supplementary Table 1S and 2S. Except the *Burkholderiaceae* family, none of the bacterial taxonomic groups relative abundances were significantly different in seeds collected from the four locations while several fungal taxonomic groups were significantly different in seeds collected from the four locations (Table 2, Table 1S and 2S).

Microbial community diversity

The alpha diversity indices of Chao 1, ACE, Shannon and Simpson estimated for both bacterial and fungal communities of *Elymus nutans* seed samples collected from four different locations were not significantly different (Table 3S and 4S). Similarly, the ANOSIM and NMDS analysis showed the beta diversity of the

bacterial community of *Elymus nutans* seeds originated from four locations was also not significantly different (ANOSIM; R statistic = −0.0556, P = 0.662, Fig. 1a). In contrast, the beta diversity of fungal community of *Elymus nutans* seeds originated from the four locations were significantly different (ANOSIM; R statistic = 0.7346, P = 0.002, Fig. 1b).

The *Elymus nutans* core seed microbiome (common microbial community in all four locations)

A total of 663 bacterial OTUs were identified from all four locations, with 130 OTUs in common for all four locations (Fig. 2a). This core seed bacterial community of *Elymus nutans* comprised of 10 known bacterial genera (Table 3a). Bacteria belonging to the *Nitrosomonadaceae* family were also present in seeds of all four locations (Table 3a). The *Elymus nutans* bacterial core seed microbiome comprised of 7 genera from proteobacteria, and one genus from each Actinobacteria and Firmicutes (Table 3a). There were 28 location specific seed bacterial genera with 2, 8, 8 and 10 unique genera at Maqu County, Luqu County, Maqin County, and Zhuoni County, respectively (Table 3a).

A total of 450 fungal OTUs were identified from all four locations with 38 OTUs were common to all four locations (Fig. 2b). The core fungal community of *Elymus nutans* comprised of 18 known fungal genera (Table 3b). There were 77 location specific seed fungal

Table 2 Effects of seed collection location on the relative abundance of top five phyla of seed endophytic bacteria (a) and fungi (b)

Phylum	Maqu	Luqu	Maqin	Zhuoni	Mean value	*P* value
a						
Proteobacteria	0.992 ± 0.001	0.988 ± 0.005	0.987 ± 0.001	0.965 ± 0.025	0.983	0.50
Firmicutes	0.003 ± 0.001	0.004 ± 0.003	0.005 ± 0.001	0.022 ± 0.019	0.009	0.48
Actinobacteria	0.004 ± 0.001	0.004 ± 0.003	0.003 ± 0.000	0.008 ± 0.007	0.005	0.78
Bacteroidetes	0.001 ± 0.001	0.003 ± 0.003	0.001 ± 0.001	0.001 ± 0.001	0.002	0.77
Deinococcus-Thermus	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.001	0.001 ± 0.001	0.001	0.34
b						
Ascomycota	0.84 ± 0.03	0.84 ± 0.06	0.70 ± 0.12	0.92 ± 0.03	0.83	0.24
Basidiomycota	0.13 ± 0.03	0.13 ± 0.05	0.27 ± 0.11	0.07 ± 0.03	0.15	0.26
Glomeromycota	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01	0.14
Rozellomycota	0.00 ± 0.00ab	0.01 ± 0.00a	0.01 ± 0.00b	0.00 ± 0.00b	0.00	0.03
Zygomycota	0.00 ± 0.00ab	0.00 ± 0.00b	0.01 ± 0.00a	0.00 ± 0.00b	0.00	0.02

Different lower-case letters in same row indicate statistical significance at 0.05 level
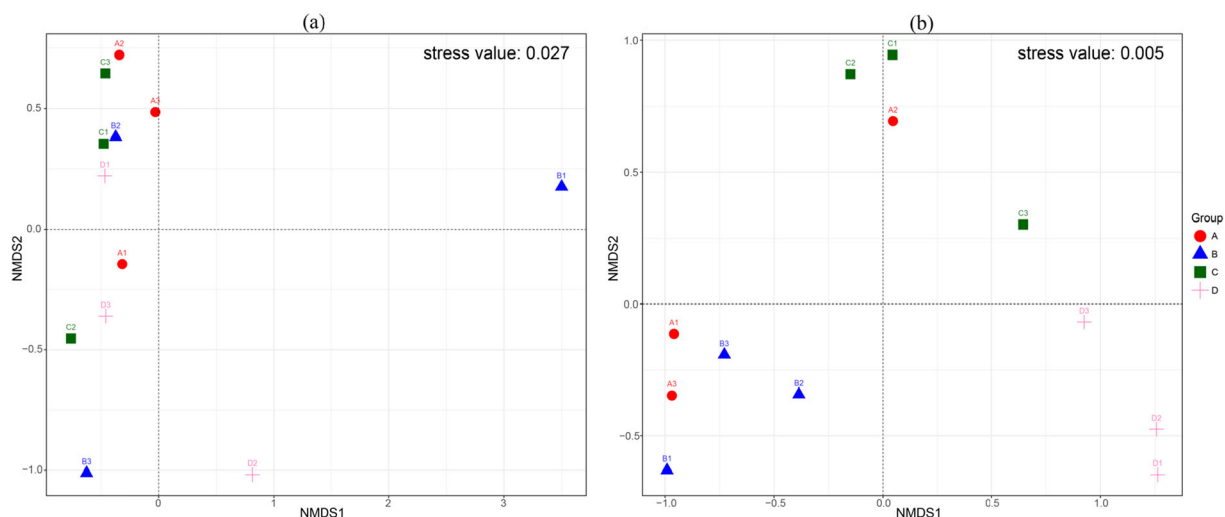
Fig. 1 NMDS analysis of seed bacteria (**a**) and fungi (**b**) community in *Elymus nutans*. Each point represents a replicate, different colors indicate different locations. The closer the distance between the two points, the higher the similarity of microbial community structure. A = Maqu, B = Luqu, C = Maqin, D = Zhuoni

genera with 32, 12, 24 and 9 unique genera at Maqu County, Luqu County, Maqin County, and Zhuoni County, respectively (Table 3b).

Spearman correlation network analysis

The Spearman correlation network analysis of the *Elymus nutans* seed microbiome is illustrated in Fig. 3. The more connections through a node indicate more associations the genus is associated with other members of the community. The correlation network analysis

identified 11 (6 Proteobacteria +3 Actinobacteria +2 Firmicutes) and 16 (10 Ascomycota +6 Basidiomycota) bacterial and fungal genera respectively showing significant correlations within the *Elymus nutans* seed microbiome. There were 8 positive (red) and 2 negative (green) bacteria-bacteria correlations while there were 10 positive and no negative fungi-fungi correlations. There were 2 positive and 2 negative bacteria-fungi correlations. Bacterial genera *Cupriavidus* and *Enterobacter* were involved in most correlations. Bacterial genus *Cupriavidus* showed positive relationships with
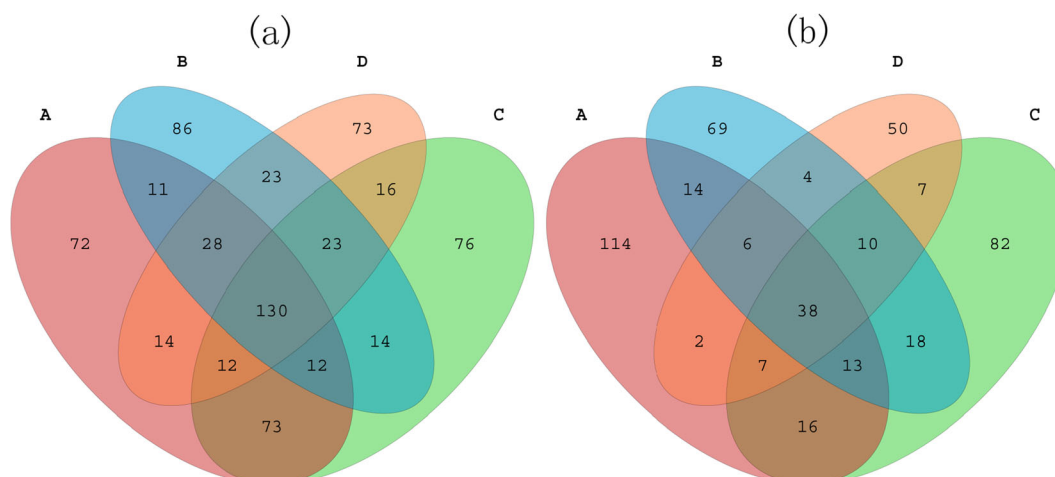

Fig. 2 Venn diagram to indicate number of shared and unique bacterial (**a**) and fungal (**b**) OTUs identified in four seed collecting locations. Each ellipse represents a seed collection location. A = Maqu, B = Luqu, C = Maqin, D = Zhuoni

Table 3 The core and unique bacterial (a) and fungal (b) genera in *Elymus nutans* seeds collected from four locations of QTP in China

a

Core bacterial community	Unique bacteria			
	Maqu	Luqu	Maqin	Zhuoni
Pseudomonas	*Clavibacter*	*Shewanella*	*Veillonella*	*Exiguobacterium*
Halomonas	*Alistipes*	*Vibrionimonas*	*Acidothermus*	*Sanguibacter*
Pantoea	–	*Allobaculum*	*Clostridium*	*Ralstonia*
Ochrobactrum	–	*Ruminococcaceae*	*Enterococcus*	*Lachnospiraceae*
Massilia	–	*Arthrobacter*	*Hamadaea*	*Turicibacter*
Sphingomonas	–	*Faecalibacterium*	*Isosphaera*	*Buttiauxella*
Amycolatopsis	–	*Paeniglutamicibacter*	*Prevotella*	*Parasutterella*
Pelomonas	–	*Propionibacterium*	*Raoultella*	*Caulobacter*
Oceanobacillus	–	–	–	*Citrobacter*
Peptoclostridium	–	–	–	*Staphylococcus*
Uncultured *Nitrosomonadaceae*	–	–	–	–

b

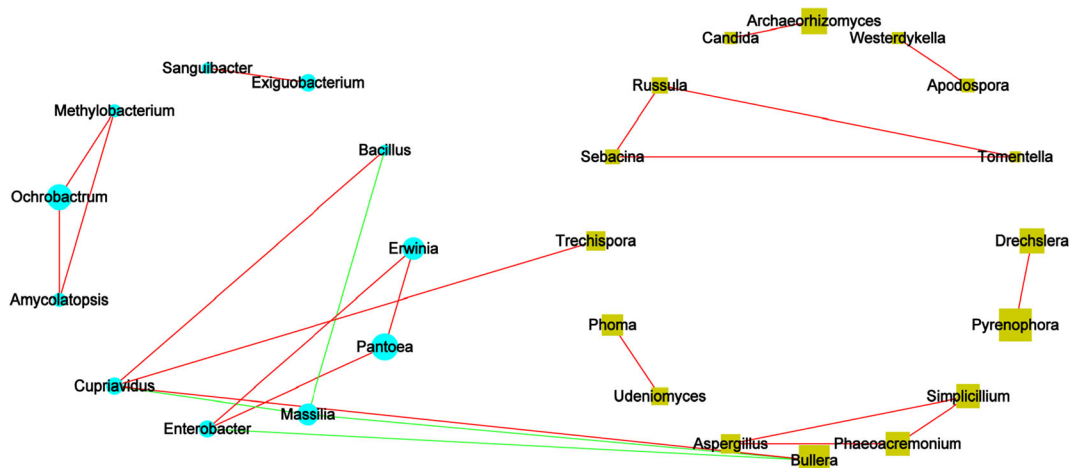| Core fungal community	Unique fungi			
	Maqu	Luqu	Maqin	Zhuoni
Mycosphaerella	*Russula*	*Moniliella*	*Westerdykella*	*Chalastospora*
Bullera	*Sebacina*	*Engyodontium*	*Lanzia*	*Monographella*
Archaeorhizomyces	*Tomentella*	*Cookeina*	*Mrakiella*	*Paecilomyces*
Aureobasidium	*Cercophora*	*Saitoella*	*Mortierella*	*Sclerostagonospora*
Phaeoacremonium	*Cortinarius*	*Rasamsonia*	*Brachyphoris*	*Stachybotrys*
Simplicillium	*Falcocladium*	*Sagenomella*	*Heterobasidion*	*Lulwoana*
Phoma	*Periconia*	*Neurospora*	*Hygrocybe*	*Hypoxylon*
Aspergillus	*Redeckera*	*Piloderma*	*Kondoa*	*Ilyonectria*
Trechispora	*Hysterangium*	*Rhizomucor*	*Bacidia*	*Cladophialophora*
Penicillium	*Capnobotryella*	*Colletotrichum*	*Cylindrocladiella*	–
Cryptococcus	*Savoryella*	*Sphaerulina*	*Myrothecium*	–
Gibberella	*Paratritirachium*	*Tuber*	*Dactylella*	–
Podospora	*Scytalidium*	–	*Ophioceras*	–
Acremonium	*Fistulina*	–	*Scedosporium*	–
Malassezia	*Clavulina*	–	*Lasallia*	–
Staphylotrichum	*Arthrographis*	–	*Pseudogymnoascus*	–
Candida	*Geastrum*	–	*Hypotrachyna*	–
Zopfiella	*Solenopsora*	–	*Sarcosphaera*	–
–	*Inocybe*	–	*Bipolaris*	–
–	*Monascus*	–	*Pleurotus*	–
–	*Mycofalcella*	–	*Knufia*	–
–	*Placopsis*	–	*Phallus*	–
–	*Codinaeopsis*	–	*Pyrenochaetopsis*	–
–	*Darksidea*	–	*Trichosporon*	–
–	*Magnaporthiopsis*	–	–	–
–	*Phialocephala*	–	–	–
–	*Xerocomellus*	–	–	–
–	*Lecanicillium*	–	–	–
–	*Lecythophora*	–	–	–
–	*Ramicandelaber*	–	–	–
–	*Gibberella*	–	–	–
–	*Prosopidicola*	–	–	–

Fig. 3 The Spearman correlation network analysis of the *Elymus nutans* seed microbiome. The nodes denoted by circles (Bacteria) and squares (Fungi) represent the dominant genera associated with significant correlations. The connections between the nodes indicate that there is a correlation between the two genera, the red line indicates positive correlation, and the green lines indicate negative correlation

bacterial genus *Bacillus*, fungal genera *Trechispora* and *Bullera*. Bacterial genus *Enterobacter* showed positive relationships with bacterial genera *Erwinia* and *Pantoea*. Bacterial genus *Enterobacter* and *Massillia* showed negative relationships with fungal genus *Bullera*.

Discussion

The results of our study add vital information on seed microbiome of *Elymus nutans* to the existing limited collection of literature on seed microbiomes of various plant species (Verma and White 2019). We found a variety of bacteria and fungi (66 bacterial and 130 fungal genera) thrive inside the seeds of *Elymus nutans*. The members of the core microbiome identified in this study had been previously recovered in various plant parts of different crops, providing a range of benefits to host plants (Table 4). Therefore, we expect these microbes to become a part of the *Elymus nutans* plant microbiome, potentially providing benefits such as plant growth promotion and plant protection.

Elymus nutans seed bacteria and fungi composition

As previously observed in other plants, the seed microbiome of *Elymus nutans* was also dominated by the bacteria belonging to the phylum Proteobacteria (Johnston-Monje and Raizada 2011; Sánchez-López

et al. 2018) and the fungi belong to phylum Ascomycota (Clay and Holah 1999). Bacteria belonging to Firmicutes, Actinobacteria and Bacteroidetes phyla and fungi belonging to Basidiomycota were also abundantly present in the *Elymus nutans* seeds. These phyla were also observed in seeds of other crops (Barret et al. 2015; Mitter et al. 2017). In our study, the predominance of bacteria phylum Proteobacteria was driven by the high abundance of Proteobacterial genus *Pseudomonas* (Table 1S), with an average relative abundance of 84% in all four locations. A *Pseudomonas* bacterium classified only up to genus level, with an average relative abundance of 18% in all four locations, was the most abundant inside the *Elymus nutans* seeds. The next most abundant was a soil inhabiting *Pseudomonas* bacterium classified only up to genus level. There were another 26-soil inhabiting *Pseudomonas* OTUs were found inside the *Elymus nutans* seeds, perhaps, indicating potential links between soil and the seed microbiome.

We found bacterial phylum Deinococcus-Thermus, one of the most extremophilic phylum of bacteria, resistant to hyperthermal condition, ultraviolet radiation and desiccation (Griffiths and Gupta 2007), was among the top 5 abundant phyla inside *Elymus nutans* seeds. The presence of Deinococcus-Thermus bacteria inside the *Crotalaria pumila* seeds were reported previously by (Sánchez-López et al. 2018) but their exact role for host plant or host plant microbiome is yet to be confirmed.

The members of bacterial family *Nitrosomonadaceae* were detected in this study for the first time as seed

Table 4 Previous reports on the origin and functions of the top 10 core bacteria (a) and fungi (b) identified in this study

a

Core bacterial community	Functions	Origin
Amycolatopsis	Nitrogen metabolism, antibiotics production (Euverink et al. 1992)	–
	–	Seed: *Jatropha curcas* (Miao et al. 2011b)
Cupriavidus	Cadmium tolerance (Punjee et al. 2018)	Root: *Oryza sativa* (Punjee et al. 2018)
Halomonas	Plant growth promotion (Lafi et al. 2016)	Roots: *Cyperus conglomerates* (Lafi et al. 2016)
Massilia	Plant growth promotion, phytohormone and metabolite production (Chimwamurombe et al. 2016)	Seeds: *Tylosema esculentum* (Chimwamurombe et al. 2016)
Oceanobacillus	Salt tolerance (Yang et al. 2016)	Root: *Paris linnaeus* (Yang et al. 2016)
Ochrobactrum	Plant growth promotion (Imran et al. 2014)	Nodules: *Phaseolus vulgaris* (Imran et al. 2014) Nodulation: *Lupinus albus* (Trujillo et al. 2005) Seeds: *Oryza sativa* (Hardoim et al. 2012)
Pelomonas	–	Stems and roots: *Ipomoea batatas* (Terakado-Tonooka et al. 2015)
Pseudomonas	Phosphorus-solubilizing, protease production, plant growth promotion (White et al. 2018); Mitigating metal toxicity, Plant growth promotion (Mastretta et al. 2009)	Seeds: *Phragimates australis* (White et al. 2018) Seeds: *Nicotiana tabacum* (Mastretta et al. 2009)
Pantoea	Plant growth, photosynthates allocations (Feng et al. 2006)	Seeds: *Oryza sativa* (Feng et al. 2006)
	Nitrogen fixing (Loiret et al. 2004)	Plant: *Saccharum officinarum* (Loiret et al. 2004)
	Phosphate solubilizing (Chen et al. 2014)	Root: *Manihot esculenta* (Chen et al. 2014)
Sphingomonas	Plant growth promotion, phytohormone and metabolite production (Chimwamurombe et al. 2016); phosphate-solubilizing (Ruiza et al. 2011); Phytate-solubilizing (López-López et al. 2010);	Seeds: *Tylosema esculentum* (Chimwamurombe et al. 2016) Seeds: *Oryza sativa* (Ruiza et al. 2011) Seeds: *Glycine max* (López-López et al. 2010)

b

Core fungi community	Functions	Origin
Mycosphaerella	Plant growth inhibition (Shaw and Royle 1989)	Plant: *Triticum aestivum* (Shaw and Royle 1989)
	–	Leaves: *Citrus limon* (Douanla-Meli et al. 2013)
	–	Plant: *Phaeophyta* (Fries 1979)
Bullera	Production of galacto-oligosaccharides from lactose (Shin et al. 1998)	–
Aureobasidium	Cadmium uptake (Mowll and Gadd 1984)	–
	Galactooligosaccharide production (Shin et al. 1998)	–
Phaeoacremonium	Plant cell activity inhibition (Luini et al. 2010)	Plant: *Vitis vinifera* (Luini et al. 2010)
Simplicillium	Nematodes control (Dong et al. 2018)	–
Phoma	Germination and seedling growth improvement (Shearin et al. 2018)	seeds: *Invasive Phragmites* (Shearin et al. 2018)
Aspergillus	Antifungal and antibacterial activity (Xiao et al. 2014)	Plant: *Melia azedarach* (Xiao et al. 2014)
	Deoxy podophyllotoxin production (Kusari et al. 2009)	Twigs: *Juniperus communis* (Kusari et al. 2009)
Trechispora	Metal phytoremediation (Hur et al. 2012)	Rhizosphere: poplars (Hur et al. 2012)
Penicillium	Germination and seedling growth improvement (Shearin et al. 2018)	Seeds: *Phragmites australis* (Shearin et al. 2018)
Cryptococcus	Germination and seedling growth improvement (Shearin et al. 2018)	Seeds: *Phragmites australis* (Shearin et al. 2018)
	–	Leaves: *Oryza sativa* (Tantirungkij et al. 2015)

endophytes. All the *Nitrosomonadaceae* OTUs identified in this study were classified only up to the family level. All cultivated representatives of the *Nitrosomonadaceae* family are ammonia oxidizers (Prosser et al. 2014), mostly found in soil. They control the rate limiting step of soil nitrification by oxidizing ammonia to nitrite, which is subsequently oxidized by bacterial nitrite oxidizers to nitrate (Kowalchuk et al. 2000). They therefore play major roles in mediating the nitrogen cycle. They are also of significant economic and environmental importance, as nitrification is a key determinant of nitrous oxide production; a strong greenhouse gas and nitrate pollution. Therefore, we emphasize the need for future research to investigate the role of seed born ammonia oxidizing bacteria in nitrogen cycle in agricultural systems.

The Glomeromycota was the third highest fungal phylum observed in this study (Table 2b) and were found in *Elymus nutans* seeds collected from all four locations. The members of Glomeromycota include widely distributed, ecologically important fungal group arbuscular mycorrhizal fungi (AMF), those form intimate associations with plants (Schüßler et al. 2001), in which the plant acquires inorganic nutrients through the fungus, whilst the fungus obtains carbohydrates from the plant. In addition, mycorrhizal fungi play an important role in soil aggregation through hyphae networking and producing soil particle binding substances such as glomalin and maintaining the physical properties of soil (Miller and Jastrow 2000). To the best of our knowledge, seed endophytic mycorrhizal fungi have not been detected in previous seed microbiome studies. Further studies warrant investigating host seed endophytic mycorrhiza of plant species, their establishment in next generation plants and contribution to the host plant and ecosystems.

In contrast to the bacteria, there was greater diversity in dominant fungal genera inside *Elymus nutans* seeds. The fungal genera *Epichloë*, *Pyrenophora* and *Mycosphaerella* were the most dominant inside the *Elymus nutans* seeds. Although *Epichloë* accounted for the highest average relative abundance of fungal genera in this study, they were only found in seeds collected from Maqu County and Luqu County. The seed born *Epichloë* fungi are known to develop systematic associations with aboveground tissues of grasses. This plant-fungal association has been utilized for its economic importance in grass seed industries in countries such as New Zealand, Australia and the USA, providing both economic and sustainable agriculture

solutions (Easton et al. 2001). Song et al. (2015) reported that in their study materials, the *Epichloë bromicola* species was the *Epichloë* endophyte in all the *Elymus* species including *Elymus nutans* seeds collected from China while *Epichloë amarillas, Epichloë clarkii,* and *Epichloë elymi* were infected with other *Elymus* grass species collected from North America. Interestingly, in our study, all the *Elymus nutans* seeds, which were collected from the QTP of China, were infected by *Epichloë glyceriae*. The *Epichloë glyceriae* had been previously reported as an endophyte in *Glyceria striata*, a wetland perennial grass, whose infection had significant effects on carbon translocation in the host plant (Pan and Clay 2004).

Among the fungal genera the second highest relative abundance was observed from the genus *Pyrenophora*, in which all members identified in this study were *Pyrenophora tritici-repentis*, a major pathogen of wheat, behind the tan spot disease in most wheat growing areas in worldwide (Aghamiri et al. 2015). Interestingly, in our study, this genus was only observed in the seeds collected from Zhuoni (D).

Microbial community diversity

The alpha diversity indices of both bacterial and fungal communities of *Elymus nutans* seed samples collected from four different locations were not significantly different, suggesting *Elymus nutans* seed microbial community species richness and evenness were similar, irrespective of geographic origin of the seeds. The beta diversity indicated by the seed bacterial community composition among the four locations (Fig. 1a) was also not significantly different but the seed fungal community composition was significantly different (Fig. 1b). These results suggest that the *Elymus nutans* seed bacterial community is conserved across the four seed collection sites indicating little effect of environment on host plant selection of seed bacterial endophytes. This result is somewhat consistent with Johnston-Monje's study that demonstrated the conservation of seed bacterial endophytes in maize across wider geographic regions. In contrast, the *Elymus nutans* seed fungal composition significantly varied in four locations (Fig. 1b) suggesting a strong environmental effect on host plant selection on seed fungal endophytes. A greater effect of geographic location on bean seed fungal community than seed bacterial community was observed by Klaedtke et al. (2016). They found that seed

collection farm sites explained 12% and 40% of variation in bacterial and fungal beta diversity.

The core seed microbiome of four locations

In our study, the seed microbiome consisted 130 and 30 shared bacterial and fungal OTUs respectively among the four seed collecting sites, indicating a potential core seed microbiome of *Elymus nutans* (Fig. 2). Bacterial core microbiome included 8, 2 and 1 genera belong to Proteobacteria, Actinobacteria and Firmicutes phyla respectively while fungal core microbiome included 14 and 4 genera of Ascomycota and Basidiomycota phyla (Table 3). These bacteria and fungi can potentially contribute range of benefits to the host plants by promoting various biological functions for plant growth and plant protection (Table 4). The bacterial core seed microbiome was dominated by the *Pseudomonas* genus (106 OTUs). The *Pseudomonas* species were previously found in numerous plant species in various plant parts such as roots, stems, leaves, xylem sap, root nodules and seeds, and known for their contribution to plant growth promotion and plant protection to host plants (Mercado-Blanco and Bakker 2007). White et al. (2018) demonstrated that *Pseudomonas* species isolated from the seeds of *Phragmites australis* were capable of plant growth promotion, disease resistance, and release bacteria into the soil to create favorable environment to host seedlings and less favorable to competitor plants.

The core microbiome included 18 fungal genera, Ascomycota and Basidiomycota claiming 14 and 4 of those, respectively (Table 3b). The *Mycosphaerella tassiana*, a pathogenic fungus of several host plants (Petrie and Vanterpool 1978; Aghamiri et al. 2015) was the most dominant in the core microbiome. The fungal core microbiome was diverse and included plant pathogenic fungi (e.g. *Phaeoacremonium*), soil fungi (e.g. *Archaeorhizomyces*, *Simplicillium*), antibiotic producing fungi (e.g. *Penicillium*), mycotoxin producing fungi (e.g. *Aspergillus*), plant growth hormone producing fungi (e.g. *Gibberella*) and human infectious fungi (e.g. *Malassezia*).

In order to test the consistency of our findings with other crops, we compared seed associated bacteria of maize, bean, rice and Salvia, as reported in Chen et al. (2018). The bacterial genera *Pseudomonas*, *Pantoea* and *Sphingomonas* were found in all species, as well as in *Elymus nutans* seeds in our study. Chen et al. (2018) also looked at the seed associated fungal genera among Salvia, Brassicaceae and bean, finding

Alternaria in all three plant species. However, we did not observe *Alternaria* in *Elymus nutans* seeds in our study.

The interaction patterns of the *Elymus nutans* seed microbial genera

In our study, the Spearman correlation network analysis of the *Elymus nutans* seed microbiome revealed a greater number of positive bacteria-bacteria and fungi-fungi associations than negative associations. In fact, there was only one bacteria-bacteria negative association and negative fungi-fungi associations were absent altogether. There were two bacteria-fungi negative associations, both involving fungal genus *Bullera* (Fig. 3). Some species of the *Bullera* genus show antifungal activity by producing killer toxins (Golubev et al. 1997), which may be the reason for its absence in any positive associations with any other fungal genera. Its negative relationships with two bacterial genera, *Massilla* and *Enterobacter* (Fig. 3), may suggest potentially deleterious effects of the killer toxins on some bacteria, or vise vasa. In nature, a microbial community is established in a specific niche through the mutualistic (positive), competitive (negative) and commensalism (neutral) networks among different microbial groups (Faust and Raes 2012), and such associations of plant-bacteria-fungi play important roles in plant biology (Jambon et al. 2018). We speculate that existence of such microbial networks in the seed microbiome may play a role in setting the keystone microbial network for the next generation's plant microbiome. Agler et al. (2016) found the microbial community network in the *Arabidopsis thaliana* plant microbiome was controlled by a small number of taxa that strongly interconnected to have a severe effect on ultimate community. A comparison of seed microbial network and the microbial network of next generation plant would be helpful to test the above hypothesis.

Implications

The results of our study add vital information on *Elymus nutans* seed microbiome to the existing literature regarding the seed microbiomes of various plant species. Contributing such information on various plant species is required to fully understand seed microbial evolution and the functions for host plants. In addition to characterizing the *Elymus nutans* seed microbiome, we have

presented here a number of new findings that may lead to future scientific advancements in ecology. For example:

a) The environmental impact on genotype is an important component of plant phenotypic variation. Plant breeding has traditionally focused on plant trait variation under different environments. New knowledge suggests that the plant phenotype not only depends on plant traits, but is also associated with microbial traits (Gopal and Gupta 2016). The seed microbiome is a key source for the plant microbiome (White et al. 2018), and is thus important for plant phenotypic variation. If the future of plant breeding involves consideration of seed microbiomes, it is important to question whether abiotic filtering (environmental effects) of seed microbial structure should occur. Our results suggest that such environmental effects are important in *Elymus nutans* seed fungal communities, but not for seed bacterial communities. The fungal seed endophyte industry has already been used for wider agronomic benefits. For future development of novel endophyte cultivars, our findings would be advantageous for the selection of specific cultivars that correspond to respective environments.

b) For the first time, our study has shown that ecologically important soil nitrifiers and arbuscular mycorrhizal fungi can be seed endophytes. Our results support the notion that the plant microbiome not only contributes to host plant biology, but that it may also interact with the host plant environment. This concept has not previously been examined thoroughly in ecology. Our study lays a foundation for future studies to explore manipulation of the seed microbiome for soil biological functions that could improve plant productivity while minimizing environmental pollution. This might include, for example, exploring seed borne ammonia oxidizing bacteria for improvement of plant nitrogen use efficiency, in which the environmental benefits would include a reduction in soil nitrous oxide emissions.

c) We reported evidence of the existence of seed microbe community networks that could potentially lead to keystone networks in the plant microbiome. Although this idea is speculative, if proven, biotechnological advances could be explored by the selecting beneficial plant-microbe associations through seed microbiome manipulations.

d) Over the past few decades, traditional plant breeding that considered only plant genetic variability may have resulted in significant losses of beneficial indigenous seed microbes (Berg and Raaijmakers 2018). Having information on seed microbiomes of natural ecosystems is beneficial when investigating the impact of domestication or plant breeding on the seed microbiome. The seed microbiome of *Elymus nutans,* which was growing in the natural grasslands of the QTP, would be a valuable data set for such explorations. Comparing native grassland species with modern grass cultivars, that have been developed for intensive grassland framing, gives scientists the opportunity to develop strategies for reinstating beneficial microbes found in wild cultivars into the seeds of their modern relatives (Wassermann et al. 2019).

Conclusions

Diverse consortia of bacteria and fungi thrive inside the seeds of *Elymus nutans*. The seed microbiome of *Elymus nutans* was dominated by the bacterial genera *Pseudomonas* and fungal genera *Epichloë*, *Pyrenophora* and *Mycosphaerella*. The *Elymus nutans* seed bacterial community was not impacted by the plant growing environment. In contrast, the *Elymus nutans* seed fungal composition significantly varied in four plant growing locations, suggesting a strong environmental impact for host plant selection on seed fungal endophytes. Positive and negative associations of *Elymus nutans* seed microbiome were observed and those associations may play a role in initiating keystone plant bacteria fungi associations in next generation plants.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Ab Rahman SFS, Singh E, Pieterse CM, Schenk PM (2018) Emerging microbial biocontrol strategies for plant pathogens. Plant Sci 267:102–111. https://doi.org/10.1016/j.plantsci.2017.11.012

Adam E, Bernhart M, Müller H, Winkler J, Berg G (2018) The *Cucurbita pepo* seed microbiome: genotype-specific composition and implications for breeding. Plant Soil 422:35–49. https://doi.org/10.1007/s11104-016-3113-9

Aghamiri A, Mehrabi R, Talebi R (2015) Genetic diversity of *Pyrenophera tritici-repentis* isolates, the causal agent of wheat tan spot disease from northern Iran. Iran J Biotechnol 13:39–44. https://doi.org/10.15171/ijb.1118

Agler MT, Ruhe J, Kroll S, Morhenn C, Kim S, Weigel D, Kemen EM (2016) Microbial hub taxa link host and abiotic factors to plant microbiome variation. PLoS Biol 14:e1002352. https://doi.org/10.1371/journal.pbio.1002352

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402. https://doi.org/10.1093/nar/25.17.3389

Barret M, Briand M, Bonneau S, Préveaux A, Valière S, Bouchez O, Hunault G, Simoneau P, Jacques MA (2015) Emergence shapes the structure of the seed microbiota. Appl Environ Microbiol 81:1257–1266. https://doi.org/10.1128/AEM.03722-14

Berg G, Raaijmakers JM (2018) Saving seed microbiomes. ISME J 12:1167–1170

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336. https://doi.org/10.1038/nmeth.f.303

Chen H, Jiang W (2014) Application of high-throughput sequencing in understanding human oral microbiome related with health and disease. Front Microbiol 5:508. https://doi.org/10.3389/fmicb.2014.00508

Chen Y, Fan J, Du L, Xu H, Zhang Q, He Y (2014) The application of phosphate solubilizing endophyte *Pantoeadispersa* triggers the microbial community in red acidic soil. Appl Soil Ecol 84:235–244. https://doi.org/10.1016/j.apsoil.2014.05.014

Chen H, Wu H, Yan B, Zhao H, Liu F, Zhang H, Sheng Q, Miao F, Liang Z (2018) Core microbiome of medicinal plant *Salvia miltiorrhiza* seed: a rich reservoir of beneficial microbes for secondary metabolism? Int J Mol Sci 19:672. https://doi.org/10.3390/ijms19030672

Chimwamurombe PM, Grönemeyer JL, Reinhold-Hurek B (2016) Isolation and characterization of culturable seed-associated bacterial endophytes from gnotobiotically grown Marama bean seedlings. FEMS Microbiol Ecol 92. https://doi.org/10.1093/femsec/fiw083

Clay K, Holah J (1999) Fungal endophyte symbiosis and plant diversity in successional fields. Science 285:1742–1744. https://doi.org/10.1126/science.285.5434.1742

Cottyn B, Debode J, Regalado E, Mew TW, Swings J (2009) Phenotypic and genetic diversity of rice seed-associated bacteria and their role in pathogenicity and biological control. J Appl Microbiol 107:885–897. https://doi.org/10.1111/j.1365-2672.2009.04268.x

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72:5069–5072. https://doi.org/10.1128/AEM.03006-05

Dong Q, Dong R, Xing X, Li Y (2018) A new antibiotic produced by the cyanobacterium-symbiotic fungus *Simplicillium lanosoniveum*. Nat Prod Res 32:1348–1352. https://doi.org/10.1080/14786419.2017.1343320

Douanla-Meli C, Langer E, Mouafo FT (2013) Fungal endophyte diversity and community patterns in healthy and yellowing leaves of *Citrus limon*. Fungal Ecol 6:212–222. https://doi.org/10.1016/j.funeco.2013.01.004

Easton HS, Christensen MJ, Eerens J, Fletcher LR, Hume DE, Keogh RG, Lane GA, Latch G, Pennell C, Popay AJ (2001) Ryegrass endophyte: a New Zealand grassland success story. Proc N Z Grassl Assoc 63:37–46

Edwards J, Johnson C, Santos-Medellín C, Lurie E, Podishetty NK, Bhatnagar S, Eisen JA, Sundaresan V (2015) Structure, variation, and assembly of the root-associated microbiomes of rice. Proc Natl Acad Sci 112:E911–E920. https://doi.org/10.1073/pnas.1414592112

Euverink GJ, Hessels GI, Vrijbloed JW, Coggins JR, Dijkhuizen L (1992) Purification and characterization of a dual function 3-dehydroquinate dehydratase from *Amycolatopsis methanolica*. Microbiology 138:2449–2457. https://doi.org/10.1099/00221287-138-11-2449

Faria DC, Dias ACF, Melo IS, de Carvalho CFE (2013) Endophytic bacteria isolated from orchid and their potential to promote plant growth. World J Microbiol Biotechnol 29:217–221. https://doi.org/10.1007/s11274-012-1173-4

Faust K, Raes J (2012) Microbial interactions: from networks to models. Nat Rev Microbiol 10:538–550. https://doi.org/10.1038/nrmicro2832

Feng Y, Shen D, Song W (2006) Rice endophyte *Pantoea agglomerans* YS19 promotes host plant growth and affects allocations of host photosynthates. Appl Environ Microbiol 5:938–945. https://doi.org/10.1111/j.1365-2672.2006.02843.x

Fries N (1979) Physiological characteristics of *Mycosphaerella ascophylli*, a fungal Endophyte of the marine Brown alga *Ascophyllum nodosum*. Physiol Plant 45:117–121. https://doi.org/10.1111/j.1399-3054.1979.tb01674.x

Gill SR, Pop M, DeBoy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE (2006) Metagenomic analysis of the human distal gut microbiome. Science 312:1355–1359. https://doi.org/10.1126/science.1124234

Golubev W, Ikeda R, Shinoda T, Nakase T (1997) Antifungal activity of *Bullera alba* (Hanna) Derx. Mycoscience 38:25–29

Gopal M, Gupta A (2016) Microbiome selection could spur next-generation plant breeding strategies. Front Microbiol 7. https://doi.org/10.3389/fmicb.2016.01971

Griffiths E, Gupta RS (2007) Identification of signature proteins that are distinctive of the Deinococcus-Thermus phylum. Int Microbiol 10:201

Hardoim PR, Hardoim CC, van Overbeek LS, van Elsas JD (2012) Dynamics of seed-borne rice endophytes on early plant

growth stages. PLoS One 7:e30438. https://doi.org/10.1371/journal.pone.0030438

Huang X, Li C, Li F, Zhao J, Wan X, Wang K (2018) Cervicovaginal microbiota composition correlates with the acquisition of high-risk human papillomavirus types. Int J Cancer 143(3):621–634. https://doi.org/10.1002/ijc.31342

Hur M, Lim YW, Yu JJ, Cheon SU, Choi YI, Yoon S, Park S, Kim D, Yi H (2012) Fungal community associated with genetically modified poplar during metal phytoremediation. Chin J Microbiol 50:910–915. https://doi.org/10.1007/s12275-012-2491-9

Imran A, Saadalla MJA, Khan SU, Mirza MS, Malik KA, Hafeez FY (2014) Ochrobactrum sp. Pv2Z2 exhibits multiple traits of plant growth promotion, biodegradation and N-acyl-homoserine-lactone quorum sensing. Ann Microbiol 64: 1797–1806. https://doi.org/10.1007/s13213-014-0824-0

Jambon I, Thijs S, Weyens N, Vangronsveld J (2018) Harnessing plant-bacteria-fungi interactions to improve plant growth and degradation of organic pollutants. J Plant Interact 13:119–130. https://doi.org/10.1080/17429145.2018.1441450

Johnston-Monje D, Raizada MN (2011) Conservation and diversity of seed associated Endophytes in Zea across boundaries of evolution, ethnography and ecology. PLoS ONE 6. https://doi.org/10.1371/journal.pone.0020396

Klaedtke S, Jacques MA, Raggi L, Préveaux A, Bonneau S, Negri V, Chable V, Barret M (2016) Terroir is a key driver of seed-associated microbial assemblages. Environ Microbiol 18: 1792–1804. https://doi.org/10.1111/1462-2920.12977

Kowalchuk GA, Stienstra AW, Stephen JR, Woldendorp JW (2000) Changes in the community structure of ammonia-oxidizing bacteria during secondary succession of calcareous grasslands. Environ Microbiol 2:99–110. https://doi.org/10.1046/j.1462-2920.2000.00080.x

Kusari S, Lamshöft M, Spiteller M (2009) Aspergillus fumigatus Fresenius, an endophytic fungus from Juniperuscommunis L. Horstmann as a novel source of the anticancer pro-drug deoxypodophyllotoxin. Appl Environ Microbiol 107:1019–1030. https://doi.org/10.1111/j.1365-2672.2009.04285.x

Lafi FF, Ramirez-Prado JS, Alam I, Bajic VB, Hirt H, Saad MM (2016) Draft genome sequence of Halomonas elongata strain K4, an Endophytic growth-promoting bacterium enhancing salinity tolerance in Planta. Genome Announc 4:1214–1216. https://doi.org/10.1128/genomeA.01214-16

Links MG, Demeke T, Grafenhan T, Hill JE, Hemmingsen SM, Dumonceaux TJ (2014) Simultaneous profiling of seed-associated bacteria and fungi reveals antagonistic interactions between microorganisms within a shared epiphytic microbiome on Triticum and Brassica seeds. New Phytol 202:542–553. https://doi.org/10.1111/nph.12693

Liu Y, Zhao R, Ni L I, Cao Y H, Zhang C, Bai F R, Zhang X, Yuan L P, Wang W P, Cheng C (2016) Diversity of endophytic bacterial communities in seeds of super hybrid rice (Oryza staiva L). Food Ferment Ind 42(1):31–36

Loiret FG, Ortega E, Kleiner D, Ortega-Rodés P, Rodés R, Dong Z (2004) A putative new endophytic nitrogen-fixing bacterium Pantoea sp. from sugarcane. J Appl Microbiol 97:504–511

López-López A, Rogel MA, Ormeño-Orrillo E, Martínez-Romero J, Martínez-Romero E (2010) Phaseolus vulgaris seed-borne endophytic community with novel bacterial species such as Rhizobium endophyticum sp. nov. Syst Appl Microbiol 33: 322–327. https://doi.org/10.1016/j.syapm.2010.07.005

Luini E, Fleurat-Lessard P, Rousseau L, Roblin G, Berjeaud J (2010) Inhibitory effects of polypeptides secreted by the grapevine pathogens Phaeomoniella chlamydospora and Phaeoacremonium aleophilum on plant cell activities. Physiol Mol Plant Pathol 74:403–411. https://doi.org/10.1016/j.pmpp.2010.06.007

Magoč T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27: 2957–2963. https://doi.org/10.1093/bioinformatics/btr507

Mano H, Tanaka F, Watanabe A, Kaga H, Okunishi S, Morisaki H (2006) Culturable surface and Endophytic bacterial Flora of the maturing seeds of Rice plants (Oryza sativa) cultivated in a Paddy field. Microbes Environ 21:86–100. https://doi.org/10.1264/jsme2.21.86

Mastretta C, Taghavi S, Van Der Lelie D, Mengoni A, Galardi F, Gonnelli C, Barac T, Boulet J, Weyens N, Vangronsveld J (2009) Endophytic bacteria from seeds of Nicotiana tabacum can reduce cadmium phytotoxicity. Int J Phytoremediation 11:251–267. https://doi.org/10.1080/15226510802432678

Mercado-Blanco J, Bakker PAHM (2007) Interactions between plants and beneficial Pseudomonas spp.: exploiting bacterial traits for crop protection. Antonie Van Leeuwenhoek 92: 367–389. https://doi.org/10.1007/s10482-007-9167-1

Miao Q, Qin S, Bian G, Yuan B, Xing K, Zhang Y, Li Q, Tang S, Li W, Jiang J (2011a) Amycolatopsis endophytica sp. nov., a novel endophytic actinomycete isolated from oil-seed plant Jatropha curcas L. Antonie Van Leeuwenhoek 3:333–339. https://doi.org/10.1007/s10482-011-9588-8

Miao J, Zhang X, Chen S, Ma X, Chen Z, Zhong J, Bai S (2011b) Gliadin analysis of Elymus nutans Griseb. from the Qinghai-Tibetan plateau and Xinjiang, China. Grassl Sci 57:127–134. https://doi.org/10.1111/j.1744-697X.2011.00219.x

Miller RM, Jastrow JD (2000) Mycorrhizal fungi influence soil structure. In: Kapulnik Y, Douds DD (eds) Arbuscular mycorrhizas: physiology and function. Springer, Dordrecht, pp 3–18

Mitter B, Pfaffenbichler N, Flavell R, Compant S, Antonielli L, Petric A, Naveed M, Sheibani-Tezerji R, von Maltzahn G, Sessitsch A (2017) A new approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds. Front Microbiol 8:11. https://doi.org/10.3389/fmicb.2017.00011

Mowll J L, Gadd G M (1984) Cadmium uptake by Aureobasidium pulluians. Microbiol 130:279–284

Nelson EB, Simoneau P, Barret M, Mitter B, Compant S (2018) Editorial special issue: the soil, the seed, the microbes and the plant. Plant Soil 422:1–5. https://doi.org/10.1007/s11104-018-3576-y

Pan JJ, Clay K (2004) Epichloë glyceriae infection affects carbon translocation in the clonal grass Glyceriastriata. New Phytol 164:467–475. https://doi.org/10.1111/j.1469-8137.2004.01203.x

Petrie GA, Vanterpool TC (1978) Mycosphaerella tassiana on Cruciferae in Western Canada. Can Plant Dis Surv 58:77–79

Prosser JI, Head IM, Stein LY (2014) The family Nitrosomonadaceae. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (eds) The prokaryotes. Springer, Berlin, Heidelberg, pp 901–918

Puente ME, Li CY, Bashan Y (2009) Endophytic bacteria in cacti seeds can improve the development of cactus seedlings. Environ Exp Bot 66:402–408. https://doi.org/10.1016/j.envexpbot.2009.04.007

R Core Team (2014) R: a language and environment for statistical computing. R foundation for statistical computing, Vienna

Ren W, Ren G, Teng Y, Li ZG, Li L (2015) Time-dependent effect of graphene on the structure, abundance, and function of the soil bacterial community. J Hazard Mater 297:286–294. https://doi.org/10.1016/j.jhazmat.2015.05.017

Rezki S, Campion C, Iacomi-Vasilescu B, Preveaux A, Toualbia Y, Bonneau S, Briand M, Laurent E, Hunault G, Simoneau P (2016) Differences in stability of seed-associated microbial assemblages in response to invasion by phytopathogenic microorganisms. PeerJ4. https://doi.org/10.7717/peerj.1923

Rezki S, Campion C, Simoneau P, Jacques M, Shade A, Barret M (2018) Assembly of seed-associated microbial communities within and across successive plant generations. Plant Soil 422:67–79. https://doi.org/10.1007/s11104-017-3451-2

Ruiza D, Agaras B, de Werrab P, Wall LG, Valverde C (2011) Characterization and screening of plant probiotic traits of bacteria isolated from rice seeds cultivated in Argentina. J Microbiol 49:902–912. https://doi.org/10.1007/s12275-011-1073-6

Sánchez-López AS, Thijs S, Beckers B, González-Chávez MC, Weyens N, Carrillo-González R, Vangronsveld J (2018) Community structure and diversity of endophytic bacteria in seeds of three consecutive generations of *Crotalaria pumila* growing on metal mine residues. Plant Soil 422:51–66. https://doi.org/10.1007/s11104-017-3176-2

Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. Mycol Res 105:1413–1421. https://doi.org/10.1017/S0953756201005196

Shade A, Jacques MS, Barret M (2017) Ecological patterns of seed microbiome diversity, transmission, and assembly. Curr Opin Microbiol 37:15–22. https://doi.org/10.1016/j.mib.2017.03.010

Shahzad R, Waqas M, Khan AL, Alhosni K, Kang SM, Seo CW, Lee IJ (2017) Indoleacetic acid production and plant growth promoting potential of bacterial endophytes isolated from rice (*Oryza sativa* L.) seeds. Acta Biol Hung 68:175–186. https://doi.org/10.1556/018.68.2017.2.5

Shahzad R, Khan AL, Bilal S, Asaf S, Lee I (2018) What is there in seeds? Vertically transmitted endophytic resources for sustainable improvement in plant growth. Front Plant Sci 9:24. https://doi.org/10.3389/fpls.2018.00024

Shaw MW, Royle DJ (1989) Estimation and validation of a function describing the rate at which *Mycosphaerella graminicola* causes yield loss in winter wheat. Ann Appl Biol 115:425–442. https://doi.org/10.1111/j.1744-7348.1989.tb06562.x

Shearin ZRC, Filipek M, Desai R, Bickford WA, Kowalski KP, Clay K (2018) Fungal endophytes from seeds of invasive, non-native *Phragmites australis* and their potential role in germination and seedling growth. Plant Soil 422:183–194. https://doi.org/10.1007/s11104-017-3241-x

Shin H, Park J, Yang J (1998) Continuous production of galacto-oligosaccharides from lactose by *Bullera singularis* β-galactosidase immobilized in chitosan beads. Process Biochem 8:787–792. https://doi.org/10.1016/S0032-9592(98)00045-4

Siahmard OJ, Pableo RMB, Novero AU (2017) Molecular identification of Rhizospheric fungi associated with 'Saba' Banana via the amplification of internal transcribed spacer sequence of 5.8S ribosomal DNA. Asian J Plant Sci 16:78–86. https://doi.org/10.3923/ajps.2017.78.86

Song H, Song QY, Li XZ, Nan ZB (2015) Are *Epichloë* endophytes specific to *Elymus* grass hosts? Genet Mol Res 14:17463–17471. https://doi.org/10.4238/2015.December.21.17

Tantirungkij M, Nasanit R, Limtong S (2015) Assessment of endophytic yeast diversity in rice leaves by a culture-independent approach. Antonie Van Leeuwenhoek 108:633–647. https://doi.org/10.1007/s10482-015-0519-y

Tao K, Zhang X, Chen X, Liu X, Hu X, Yuan X (2019) Response of soil bacterial community to bioaugmentation with a plant residue-immobilized bacterial consortium for crude oil removal. Chemosphere 222:831–838. https://doi.org/10.1016/j.chemosphere.2019.01.133

Trujillo ME, Willems A, Abril A, Planchuelo A, Rivas R, Ludeña D, Mateos PF, Martínez-Molina E, Velázquez E (2005) Nodulation of *Lupinus albus* by strains of *Ochrobactrum lupini sp. nov.* Appl Environ Microbiol 3:1318–1327

Truyens S, Weyens N, Cuypers A, Vangronsveld J (2015) Bacterial seed endophytes: genera, vertical transmission and interaction with plants. Environ Microbiol Rep 7:40–50. https://doi.org/10.1111/1758-2229.12181

Verma SK, White JF Jr (2019) Seed endophytes. Springer, Cham

Wang J, Tao D, Wang S, Li C, Li Y, Zheng F, Wu Z (2019) Disinfection of lettuce using organic acids: an ecological analysis using 16S rRNA sequencing. RSC Adv 9(30):17514–17520. https://doi.org/10.1039/C9RA03290H

Wassermann B, Adam E, Cernava T, Berg G (2019) Understanding the indigenous seed microbiota to design bacterial seed treatments. In: Verma S, White J Jr (eds) Seed endophytes. Springer, Cham, pp 83–99

White JF, Kingsley KI, Kowalski KP, Irizarry I, Micci A, Soares MA, Bergen MS (2018) Disease protection and allelopathic interactions of seed-transmitted endophytic *pseudomonads* of invasive reed grass (*Phragmites australis*). Plant Soil 422:195–208. https://doi.org/10.1007/s11104-016-3169-6

Xiao J, Zhang Q, Gao YQ, Shi XW, Gao JM (2014) Antifungal and antibacterial metabolites from an endophytic *Aspergillus sp.* associated with *Melia azedarach*. Nat Prod Res 28:1388–1392. https://doi.org/10.1080/14786419.2014.904308

Xu M, Sheng J, Chen L, Men Y, Gan L, Guo S, Shen L (2014) Bacterial community compositions of tomato (*Lycopersicum esculentum* Mill.) seeds and plant growth promoting activity of ACC deaminase producing *Bacillus subtilis* (HYT-12-1) on tomato seedlings. World J Microbiol 30:835–845. https://doi.org/10.1007/s11274-013-1486-y

Yang L, Tang S, Chu X, Jiang Z, Xu L, Zhi X (2016) *Oceanobacillus endoradicis sp. nov.*, an endophytic bacterial species isolated from the root of *Paris polyphylla* Smith var. *yunnanensis*. Antonie Van Leeuwenhoek 109:957–964. https://doi.org/10.1007/s10482-016-0695-4