

## Research Article

# Soil Influences Colonization of Root-Associated Fungal Endophyte Communities of Maize, Wheat, and Their Progenitors

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Root-associated fungal endophytes are vital component of root microbiome as some mitigate their host's abiotic and biotic stress. We characterized root-associated fungal endophytes in cereal grains and their progenitors grown on two different soil-types. We aimed at determining how clay and desert soil affects the colonization of root fungal community. Both culture-dependent and culture-independent methods were employed to identify endophytes that successfully colonized greenhouse-grown host plants. The Internal Transcriber Spacer region of fungal ribosomal DNA was utilized for identification purposes. This study revealed soil as a prominent factor influencing the composition of microfungus communities inhabiting the roots of maize (*Zea mays* subsp. *mays*) and its conspecific progenitor, teosinte (*Zea mays* subsp. *parviglumis*). Similar results were found in wheat (*Triticum aestivum* subsp. *aestivum*) and its progenitor (*Triticum monococcum* subsp. *monococcum*). The multidimensional comparisons of Morisita-Horn similarity values of fungal colonists of various host plant taxa indicated that soil plays a primary role in shaping the root fungal community; a secondary effect was plant host identity, even when the plant host is a conspecific. Future studies focused on characterizing root endophytes in other cereal grains, and studying the effect of edaphic factors on fungal colonization, can ultimately contribute to crop productivity.

## 1. Introduction

Fungal endophytes associated with plants are reported to be ubiquitous in various environments [1–5] and geographical locations [6, 7]. Their relationship with plants varies from being symbiotic, mutualistic, or commensalistic [8], to at times even being pathogenic [9]. Some species of these endophytes are symbiotic to particular host plant species but can be pathogenic to other host species [8, 9]. Symbiotic fungal endophytes are known to enhance plant fitness by conferring abiotic and biotic stress tolerance, and increasing biomass [10]. Most symbiotic root-associated fungal endophytes (RAFE) enhance absorption of water and minerals and in return get direct access to host carbohydrates [11].

The diversity of shoot (especially foliar) fungal endophytes has been well studied in various plants such as turf

grasses [12, 13]; by comparison, RAFE are less understood, especially in cereal crops [14–18]. Similarly, studies examining the soil's effect on RAFE community of cereal grains such as wheat and maize have been rare; most of the published research investigated the identity and abundance of fungal colonists in (or on) the shoots of these crops [14, 16, 18–21]. Therefore, additional information about how soil influences the structure and composition of the RAFE community within wheat and maize would provide the scientific community with much needed information about how RAFE communities are structured and how these economically important and cosmopolitan grasses respond to changes in their soil environment.

The domestication of maize and wheat started approximately 10,000 years ago [22, 23]. During the course of domestication, genotypic and phenotypic changes as a result

of constant artificial selection and demographic bottlenecks could have affected the structure and composition of the root fungal community. In particular, such changes might have decreased the colonization of some symbiotic endophytes associated with nutrient absorption and desiccation tolerance. For example, anthropogenic fertilization to increase levels of nutrients such as nitrogen and phosphorus in the soil might have favored less colonization of root endophytes that facilitate nutrient absorption in domesticated crops [24]. Furthermore, we suspect that additional and adaptive changes in the root physiology of domesticated varieties of cereal grains as a result of constant bottlenecking effects during the course of evolution might have selectively facilitated or impeded colonization or cohabitation by a subset of RAFE, or this change in root physiology might have favored some types of plant-fungal relationship.

Previous studies examining rhizosphere endophyte communities in different cultivars of canola reported a difference in endophyte community structure between the transgenic and nontransgenic cultivars [25]. The modification of the wheat genotype by disomic chromosome substitution had a specific effect that altered the composition of bacterial community [25, 26]. Such evidence supports the plausibility that bottlenecks and continuous artificial selection during domestication might have been associated with favoring/eliminating particular endophytes in domesticated grains. Certain fungal endophytes that are known to mitigate challenges like nutrient deficiencies might have promoted the survival of maize and wheat progenitors in low nutrient environments; the reduction of such environmental deficiencies under domestication might have altered fungal community structure in domesticated grains.

Prior studies also have found that the soil environment plays a critical role in shaping the structure and composition of RAFE in several species of plants [1, 26–28]; however, to our knowledge, none of these studies examined how the soil-type affects the root microfungal community in wheat and maize. This study aims to examine whether different soil-types, desert or clay soil, influence RAFE colonization in maize and wheat. Characterizing and comparing the fungal communities among both the domesticated cereal grasses and their respective progenitors might help provide provisional understanding of domestication's effects on RAFE composition in these crops. RAFE characterization in cereal grains will also help us examine whether they harbor endophytes that are previously known to have a symbiotic relationship with other grass species.

We characterized RAFE in domesticated cereal grains (*Triticum aestivum* subsp. *aestivum* [wheat], and *Zea mays* subsp. *mays* [maize]) and their respective progenitors (*Triticum monococcum* subsp. *monococcum* [wild wheat], and *Zea mays* subsp. *parviglumis* [teosinte]), by growing them in desert, or clay soil-type. Although wheat and maize are major world crops, there is a very limited knowledge of their RAFE communities; moreover, most of the studies characterizing root fungal endophytes have been limited to culture-dependent methods [17, 29]. We used 454 pyrosequencing (a more contemporary culture-independent method), in

addition to the more typical culture-dependent method, to characterize the root fungal endophytes.

## 2. Materials and Methods

**2.1. Seed Germination.** Teosinte seeds (Accession PI 566691, Origin: Michoacan, Mexico) and maize seeds (B73; Accession PI 550473, Origin: Iowa, United States) were obtained from the North Central Regional Plant Introduction Station (Iowa State University, Ames, IA). Similarly, seeds of domesticated wheat (GSTR 11562, Origin: Washington, United States) and its progenitor (Accession PI 167526, Origin: Kirklareli, Turkey) were obtained from National Small Grains Germplasm Research Facility (Aberdeen, Idaho).

Based on preliminary germination trials, maize and teosinte seeds were exposed to 20% hydrogen peroxide solution (for two and five minutes, resp.) to help induce seed germination, and to surface-sterilize their seed coats [30]. Similarly, wheat and its progenitor seeds were also exposed to 20% hydrogen peroxide solution (for one minute). The surface sterilized seeds were then wrapped in sterile, wet paper towels and placed in a plastic zip lock bag at room temperature for germination [3]. Once germinated (five days for maize and six for teosinte, and five days for both wheat and its progenitor) seedlings were grown in the Truman State University (TSU) greenhouse.

**2.2. Planting of Seedlings.** Sterile cylindrical plastic pots were filled with either desert soil mixture or clay soil mixture. The desert soil (loamy sand) was collected along a 100 m transect from the Seville National Wildlife Refuge in an area locally known as McKenzie Flats (coordinates: N 32° 21.849 W 106° 41.114) during the month of November 2012 and refrigerated (4°C) until used. The desert soil mixture was a homogenous mixture of autoclaved potting soil (commercial professional growing mix, Berger, BM1) and New Mexican desert soil (in 1:1 ratio by volume). Similarly, the clay soil mixture was a 1:1 combination of autoclaved potting soil and clay soil collected from TSU farm (coordinates: N 40° 10.79334, W 92° 36.10728). More specific information about the characteristics of the desert and clay soils we used can be obtained from the USDA web soil survey [31 April 4th 2015] database.

For simplicity, maize and teosinte plants grown in the desert soil mixture henceforth will be referred to as *Dmaize* and *Dteosinte*, respectively; those grown in the Missouri clay soil as *Cmaize* and *Cteosinte*, respectively. Similarly, wheat and its progenitor grown in desert soil will be referred to as *Daestivum* and *Dmonococcum* and those grown in clay soil mixture will be referred to as *Caestivum* and *Cmonococcum*. Also, the use of terms “wheat plants” and “maize plants” will represent both the domesticated and progenitor plants (e.g., wheat plants = domesticated wheat and its progenitor).

A total of 20 seedlings each of domesticated wheat plants were planted in desert soil mixture as well as clay soil mixture. Similarly, 18 wheat progenitor seedlings were planted in clay soil mixture and 22 in desert soil mixture. A total of 20 seedlings each of maize and teosinte were planted in both soil mixtures. All potted plants (one pot per plant) were kept inside the TSU greenhouse, watered regularly, and pot

placement was changed on alternate days to minimize any positional effects. Weeds growing around the plants were removed periodically. The domesticated wheat was harvested after 56 days of greenhouse growth. Wheat progenitor plants (70 days) and maize and teosinte plants (48 days) were also harvested after the indicated growth periods.

**2.3. Root Sample Preparation.** For each plant, approximately 6 cm healthy roots without visible necrosis or lesions were rinsed with high-pressurized tap water for five minutes with sporadic sprays of 1% Triton X solution [2]. After the rinsing, roots were observed under a dissecting microscope to make sure no soil particles were present on the root surface [31]. The roots were then surface sterilized using the 5% Clorox and 1% Triton X solution for one minute, rinsed with distilled water for an additional minute, and blot-dried with sterile absorbent paper [4]. A portion of these blot-dried roots was immediately used for culture-dependent studies, and the remainder was stored at  $-20^{\circ}\text{C}$  for subsequent DNA extraction and molecular analyses.

**2.4. Culture-Dependent Study.** For each plant, cleaned roots were cut into small fragments approximately 1 mm long. Five randomly selected fragments were embedded into each of the three Malt Extract Agar (MEA) and three Potato Dextrose Sugar (PDA) plates with antibiotics (streptomycin sulfate = 0.02 g/mL, and chlortetracycline = 0.01 g/mL) [3]. Then the plates were incubated at  $30^{\circ}\text{C}$  for a minimum of two weeks with regular monitoring [4]. Any fungus observed on the plates was subcultured onto PDA plates with filter paper, scraped, and identified using molecular techniques [3, 32].

**2.5. Culture-Independent Study.** Root DNA was extracted from four randomly selected plants each of species-soil-type combination: *Daestivum*, *Caestivum*, *Dmonococcum*, *Cmonococcum*, *Dteosinte*, *Dmaize*, *Cmaize*, and *Cteosinte* using Qiagen DNeasy Plant Mini Kit (Qiagen Inc., Valencia, USA). The extracted DNA was then standardized to a concentration of  $32\text{ ng}/\mu\text{L}$  utilizing the Nanodrop 2000 spectrophotometer at a wavelength of 260 nm. Loci specific amplicon representing fungal communities was generated for ITS region of fungal rDNA utilizing specific primers (ITS1F and ITS4). Then, the amplicon generated per plant was tagged with unique molecular tags (barcode) using forward and reverse fusion primer sets, specially designed by incorporating the Roche A and B adaptors as specified by the Roche 454 sequencing. The nested PCR amplicon was generated in 25  $\mu\text{L}$  reactions with Qiagen HotStar Taq master mix (Qiagen Inc., Valencia, USA), 1  $\mu\text{L}$  of each 5  $\mu\text{M}$  primer, and 1  $\mu\text{L}$  of template. Reactions were performed on ABI Veriti thermocyclers (Applied Biosystems, Carlsbad, USA) under the following thermal profile:  $95^{\circ}\text{C}$  for 5 min, then 35 cycles of  $94^{\circ}\text{C}$  for 30 sec,  $54^{\circ}\text{C}$  for 40 sec, and  $72^{\circ}\text{C}$  for 1 min, followed by one cycle of  $72^{\circ}\text{C}$  for 10 min and  $4^{\circ}\text{C}$  hold. Products were then pooled equimolar and each pool was cleaned with Diffinity RapidTip (Diffinity Genomics, West Henrietta, USA), and size selected using Agencourt AMPure XP (Beckman Coulter, Indianapolis, USA).

Cleaned amplicon pools were then quantified and sequenced following the Roche Titanium 454 FLX platform with titanium reagents and procedures. Molecular steps after the primary PCR reactions were performed at the Research and Testing Laboratories, LLC (Lubbock, USA), whereas the prior steps were performed at the TSU.

Sequences were clustered into taxonomic groups (species, genus, family, order, and class) at 96.5 percent similarity by Research and Testing Laboratory, LLC. Sequence clustering was based on UPARSE techniques [33]. Additional detailed information on data analysis pipeline involving denoising and chimera checking can be obtained from the data analysis methodology of the Research and Testing laboratory, LLC [34].

**2.6. Analyses.** Shannon diversity indices of RAFF communities were estimated utilizing both fungal species obtained from culture-independent (454 pyrosequencing) data, as well as morphotypes (surrogates for “species”) obtained from culture-dependent data. Likewise, to compare the similarity of RAFF communities among plant groups, Morisita-Horn and Sorensen indices were assessed on both culture-independent and culture-dependent data. The RAFF congruency distances, based on pairwise comparisons of Morisita-Horn or Sorensen values, were plotted utilizing STATISTICA's (version 8.0, Statsoft Inc. [<http://www.statsoft.com/>]) multidimensional scaling (MDS) module [35]. Dimensions one and two represent synthetic variables, generated to maximize the spread of congruency distances between pairs of dependent variable values.

Previous studies conducted throughout much of North America [2, 4, 31] found *Darksidea* spp. (Knapp et al. recently discovered this species as previously mistaken for *Paraphaeosphaeria* spp.) [35] as a prevalent fungal group inhabiting the roots of various grasses (i.e., Poaceae). Thus we assessed the likelihood of occurrence of this cosmopolitan microfungus in the roots of wheat, maize, and their progenitors using SEQUENCHER (version 5.1, Gene Codes [<http://genecodes.com/>]). Around 40 sequences of *Darksidea* spp. previously obtained from other grasses (Genbank Accession nos. GQ923964–GQ923975) were aligned (90% similarity and 40-base-pair overlap) with both the culture-independent and culture-dependent dataset of each plant-type, to calculate its colonization rate.

### 3. Results

**3.1. Culturing RAFF.** A total of 270 and 210 root segments were assessed from maize and wheat plants, respectively (Tables 1 and 2). In both grasses, *Fusarium* spp. and *Alternaria* spp. were most prevalent. Grasses grown in clay soil had larger Shannon diversity values than those grown in desert soil (Tables 1 and 2). Grasses grown in the same soil-type harbored similar fungal taxa (Figures 1 and 2).

**3.2. Sequence Data.** Altogether, we obtained a total of 148,579 fungal sequences from maize plants (mean = 37,145, SD = 5275,  $n = 4$ ; Table 3). Similarly, 67,927 sequences were obtained from wheat plants (mean = 16,982, SD = 926,

TABLE 1: Culture-dependent data of maize (*Zea mays* subsp. *mays*) and teosinte (*Zea mays* subsp. *parviglumis*).

Plants	# of plants	Total morphotypes	Mean (SD) number of morphotypes/plants	# of species	Shannon diversity indices	Morphotypes represented by the most numerous sequences
Dmaize	7	8	1 (2)	4	0.90	<i>Fusarium</i> spp. (62%) <i>Paraconiothyrium</i> spp. (25%) <i>Alternaria</i> spp. (13%)
Cmaize	7	38	5 (2)	5	0.96	<i>Fusarium</i> spp. (54%) <i>Alternaria</i> spp. (37%) <i>Exserohilum</i> spp. (6%)
Dteosinte	7	5	1 (1)	3	0.50	<i>Fusarium</i> spp. (83%) <i>Darksidea</i> spp. (17%) N/A
Cteosinte	7	25	5 (3)	4	1.11	<i>Alternaria</i> spp. (56%) <i>Fusarium</i> spp. (12%) <i>Chaetomium</i> spp. (12%)
Total	28	44	19 (15)	9	0.87	<i>Fusarium</i> spp. (44%) <i>Alternaria</i> spp. (39%) <i>Chaetomium</i> spp. (4%)

TABLE 2: Culture-dependent data of *Triticum aestivum* subsp. *aestivum* and *Triticum monococcum* subsp. *monococcum*.

Plants	# of plants	Total morphotypes	Mean (SD) number of morphotypes/plants	# of species	Shannon diversity indices	Morphotypes represented by the most numerous sequences
Daestivum	9	48	5 (4)	8	1.71	<i>Fusarium</i> spp. (42%) <i>Darksidea</i> spp. (19%) <i>Cochliobolus</i> spp. (14%)
Caestivum	9	24	3 (3)	7	2.04	<i>Alternaria</i> spp. (32%) <i>Fusarium</i> spp. (23%) <i>Periconia</i> spp. (13%)
Dmonococcum	9	11	1 (1)	4	1.55	<i>Darksidea</i> spp. (50%) <i>Embellisia</i> spp. (30%) <i>Cochliobolus</i> spp. (20%)
Cmonococcum	9	15	2 (2)	9	2.02	<i>Fusarium</i> spp. (27%) <i>Alternaria</i> spp. (20%) <i>Periconia</i> spp. (30%)
Total	36	97	28 (17)	18	1.83	<i>Fusarium</i> spp. (28%) <i>Alternaria</i> spp. (13%) <i>Periconia</i> spp. (12%)

TABLE 3: Culture-independent data of maize (*Zea mays* subsp. *mays*) and teosinte (*Zea mays* subsp. *parviglumis*).

Plants	# of plants	Total sequences	Mean (SD) number of sequences/plants	# of species	Shannon diversity indices	Species represented by the most numerous sequences
Dmaize	4	39971	9993 (3729)	22	1.63	<i>Fusarium</i> spp. (78%) <i>Gibberella</i> spp. (9%) <i>Ceratobasidium</i> spp. (4%)
Cmaize	4	41806	10452 (5166)	22	2.32	<i>Fusarium</i> spp. (38%) <i>Alternaria</i> spp. (32%) <i>Gibberella</i> spp. (24%)
Dteosinte	4	29814	7454 (5276)	22	1.97	<i>Fusarium</i> spp. (53%) <i>Cochliobolus</i> spp. (14%) <i>Podospora</i> spp. (10%)
Cteosinte	4	36988	9247 (3183)	30	2.62	<i>Fusarium</i> spp. (38%) <i>Alternaria</i> spp. (26%) <i>Phoma</i> spp. (11%)
Total	16	148579	37145 (5275)	60	2.14	<i>Fusarium</i> spp. (51%) <i>Alternaria</i> spp. (16%) <i>Gibberella</i> spp. (9%)

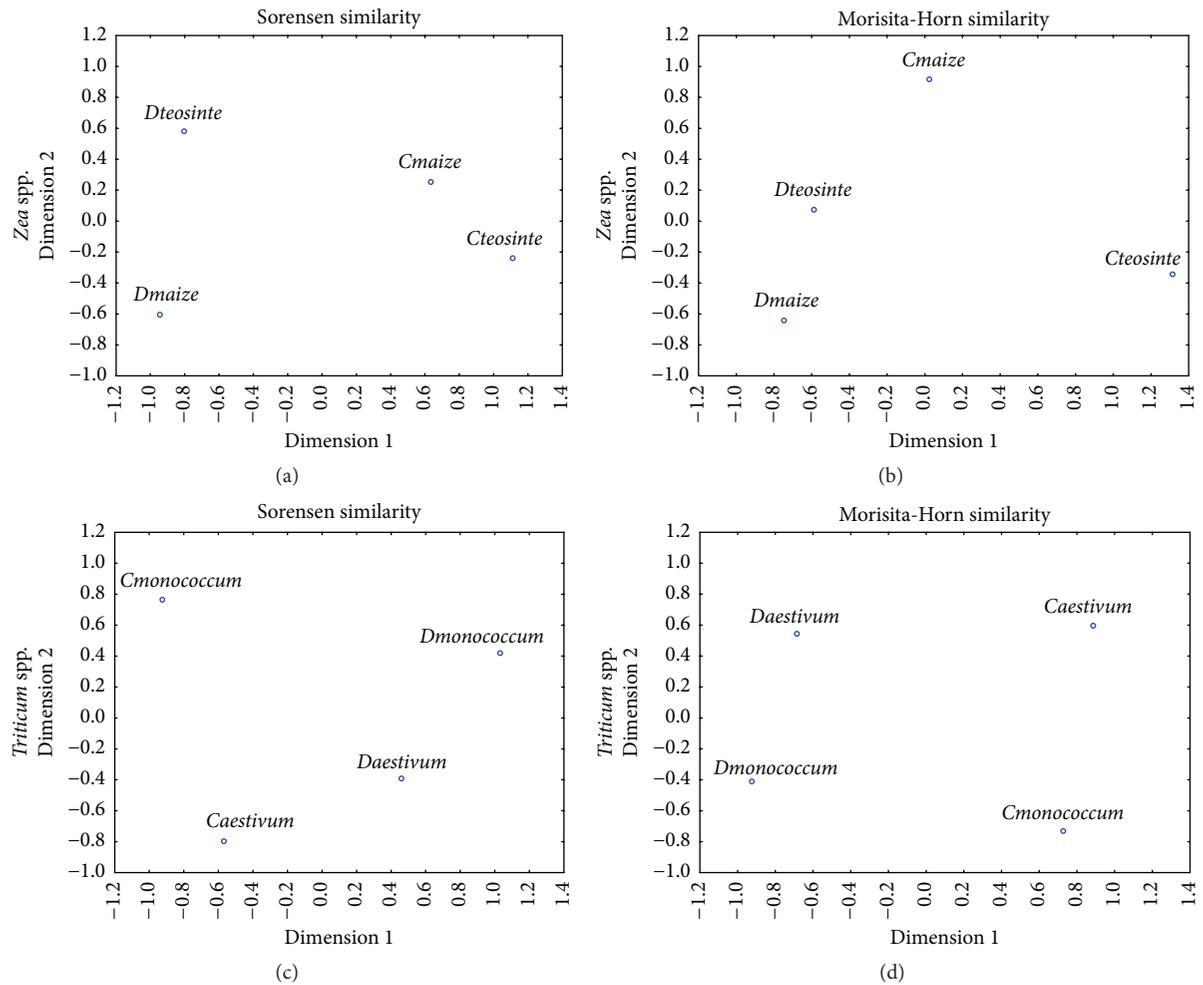


FIGURE 1: Multidimensional scaling diagrams generated using Sorensen ((a) and (c)) and Morisita-Horn ((b) and (d)) similarity values among fungal cultures isolated from root fragments of *Zea* spp. ((a) and (b)) and *Triticum* spp. ((c) and (d)) growing in either desert (DS) or clay (CS) soils. Dimensions one and two represent synthetic variables, generated to maximize the spread of congruency distances between pairs of dependent variable values. Maize (*Zea mays* subsp. *mays*) and teosinte (*Zea mays* subsp. *parviglumis*) plants grown in the desert soil mixture = Dmaize and Dteosinte, respectively; those grown in the Missouri clay soil as Cmaize and Cteosinte, respectively. Similarly, wheat (*Triticum aestivum* subsp. *aestivum*) and its progenitor, wild wheat (*Triticum monococcum* subsp. *monococcum*), grown in desert soil are indicated as Daestivum and Dmonococcum and those grown in clay soil mixture are labeled, Caestivum and Cmonococcum, respectively.

$n = 4$ ; Table 4). In all grasses, fungal endophytes of the orders Hypocreales and Pleosporales were most common. *Fusarium* spp. (order Hypocreales) were the most prevalent microfungal taxa, constituting more than 25% of the fungal sequences. *Alternaria* spp., *Gibberella* spp., *Cochliobolus* spp., *Periconia* spp., *Phoma* spp., and *Cercophora* spp. were other commonly encountered fungal taxa (Tables 3 and 4).

Shannon diversity indices describing the diversity and abundance of fungal species were calculated from maize and wheat plants grown in desert and clay soil. Plants grown on the clay soil had greater fungal diversity values than those grown on the desert soil (Tables 3 and 4). However, one-way ANOVA showed no significant difference in mean Shannon diversity indices of maize and teosinte plants grown on each soil-type at level  $P < 0.05$  [ $F(3,12) = 1.94$ ,  $P = 0.177$ , ANOVA]. Similarly, wheat plants also indicated no significant difference between domesticated and progenitor

plants grown on each soil-type at level  $P < 0.05$  [ $F(3, 12) = 0.091$ ,  $P = 0.963$ , ANOVA]. The Morisita-Horn and Sorensen similarity index values were generated to compare fungal species similarity among plants. These values indicated that plants grown on the same soil-type harbored similar fungal communities. The MDS diagrams then generated by pairwise comparisons of similarity values of culture-independent data clarified that plants grown on the same soil-type were colonized by similar microfungal community (Figures 1 and 2). This finding is consistent with culture-based data, where plants grown in the same soil harbored similar microfungal communities (Figures 1 and 2).

Furthermore, while assessing the sequences of *Darksidea* spp. (a genus frequently found in semiarid and arid grassland) [2, 31, 35], we found that only desert-soil-grown plants, *Dmaize* and *Dteosinte*, harbored *Darksidea* spp. (0.0009% and 0.0027% of their sequences, resp.). This soil-type-specific

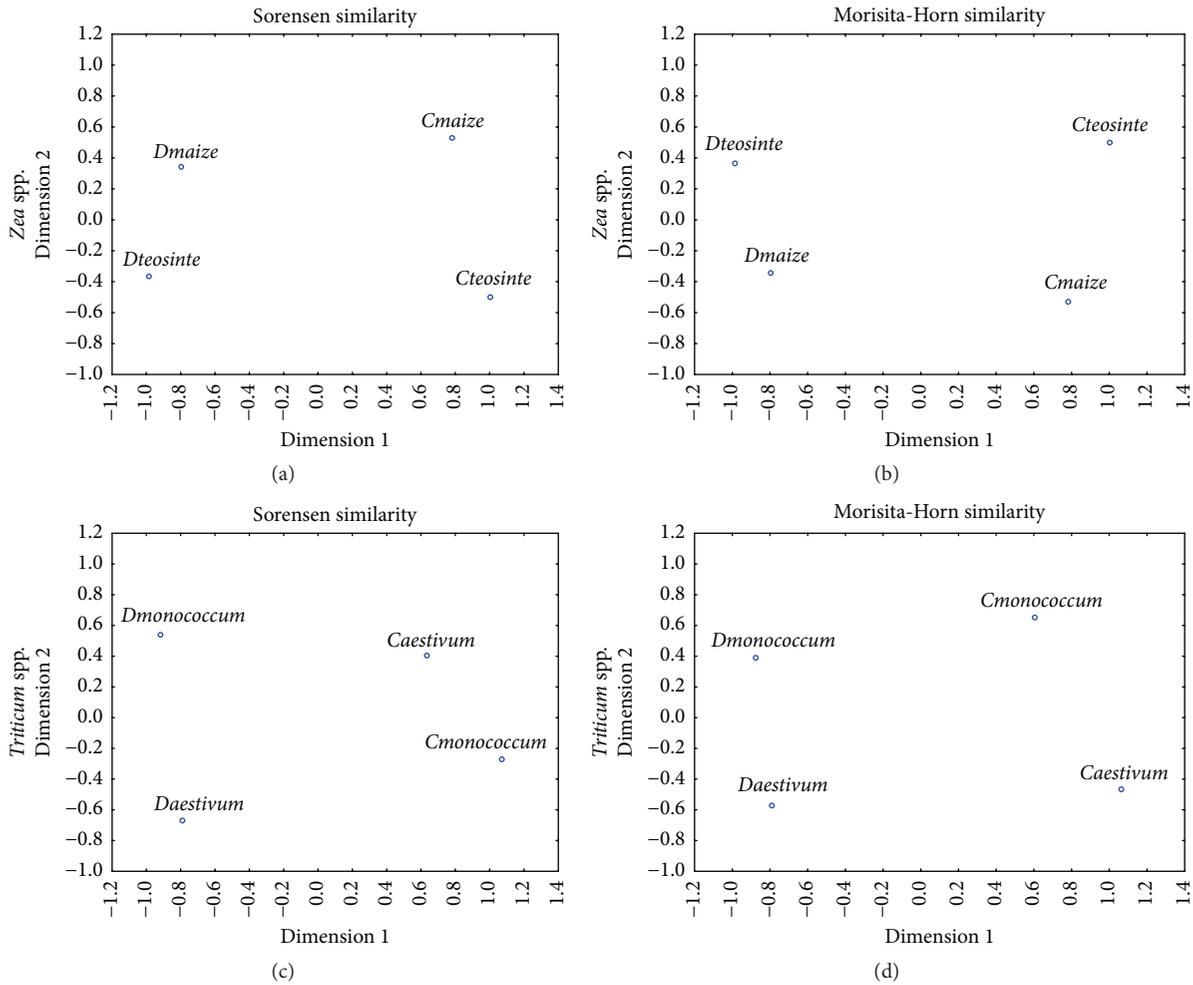


FIGURE 2: Multidimensional scaling diagrams generated using Sorensen ((a) and (c)) and Morisita-Horn ((b) and (d)) similarity values of sequence data, showing relative similarity in root-associated fungal communities colonizing *Zea* spp. ((a) and (b)) and *Triticum* spp. ((c) and (d)) in either desert (DS) or clay (CS) soils. Dimensions one and two represent synthetic variables, generated to maximize the spread of congruency distances between pairs of dependent variable values. Maize (*Zea mays* subsp. *mays*) and teosinte (*Zea mays* subsp. *parviglumis*) plants grown in the desert soil mixture = Dmaize and Dteosinte, respectively; those grown in the Missouri clay soil as Cmaize and Cteosinte, respectively. Similarly, wheat (*Triticum aestivum* subsp. *aestivum*) and its progenitor, wild wheat (*Triticum monococcum* subsp. *monococcum*), grown in desert soil will be referred to as Daestivum and Dmonococcum and those grown in clay soil mixture are labeled, Caestivum and Cmonococcum, respectively.

observation of *Darksidea* spp. also held true for wheat and its progenitor (*Daestivum* and *Dmonococcum*, 0.63 and 0.56% of their sequences, resp.).

#### 4. Discussion

This study documented soil as the prominent factor determining the composition and structure of microfungus communities inhabiting the roots of all grasses; that is, plants grown in the same soil-type harbored similar microfungus communities. Similar to our findings, research studying the effects of sandy soil and salt marshes on RAFF communities of 24 plant species in different habitats, coastal and inland, found soil-type influences on RAFF colonization [1]. Maciá-Vicente et al. [1] investigated the influence of the geomorphology and the physicochemical characteristics of the soil

on microfungus colonization. Prominently, these researchers found that, as opposed to the geographic origin of the soil, the physicochemical characteristics of the soil such as the percentage of clay, silt, sand, and soil conductivity tend to play the most prominent role in fungal colonization [1]. Future studies focused on learning how these various individual physicochemical characteristics of soil exert influence on the root endophyte community will help us understand how each factor contributes to overall fungal colonization.

Fungal colonization of host plants at the early developmental stage may provide a crucial competitive advantage to these plants, potentially improving their ability to cope with stress and/or utilize limited resources. The effectiveness of microfungus communities to influence host plant fitness might not depend solely on the level of colonization or fungal diversity, but also on the timing of colonization [29].

TABLE 4: Culture-independent data of *Triticum aestivum* subsp. *aestivum* and *Triticum monococcum* subsp. *monococcum*.

Plants	# of plants	Total sequences	Mean (SD) number of sequences/plants	# of species	Shannon diversity indices	Species represented by the most numerous sequences
Daestivum	4	15978	3995 (1840)	118	2.42	<i>Fusarium</i> spp. (28%) <i>Gibberella</i> spp. (37%) <i>Phoma</i> spp. (9%)
Caestivum	4	17890	4473 (1970)	112	2.48	<i>Periconia</i> spp. (38%) <i>Fusarium</i> spp. (17%) <i>Alternaria</i> spp. (11%)
Dmonococcum	4	17637	4410 (2866)	89	2.42	<i>Fusarium</i> spp. (26%) <i>Alternaria</i> spp. (18%) <i>Embellisia</i> spp. (23%)
Cmonococcum	4	16422	4106 (1258)	118	2.46	<i>Fusarium</i> spp. (57%) <i>Alternaria</i> spp. (8%) <i>Cercophora</i> spp. (7%)
Total	16	67927	16982 (926)	248	2.45	<i>Fusarium</i> spp. (31%) <i>Alternaria</i> spp. (12%) <i>Gibberella</i> spp. (12%)

Early colonization of symbiotic endophytes might play a vital role in enhancing absorption of nutrients and water and mitigating desiccation tolerance, thus conferring accelerated development. Maize seedlings grown in desert soil reached the 4-leaf stage (10th day, average value) earlier, compared to plants grown in clay soil (18th day) (J. Herrera and D. Bokati, unpublished data from greenhouse-grown maize and wheat grasses). This might have resulted from earlier colonization of symbiotic microfungal communities present in desert soil. However, comparing the shoot dry mass of the plants grown in different soils upon harvesting indicated no significant difference at level  $P < 0.05$  [ $F(3, 20) = 0.88$ ,  $P = 0.468$ , ANOVA] (J. Herrera and D. Bokati, unpublished data from greenhouse-grown maize and wheat grasses). Any advantage to host plants provided by earlier fungal colonization could be relatively unimportant when host plants are in favorable conditions, as was the case in our greenhouse study. Future studies could assess whether endophytes can boost early growth in crops in stressful environments.

Pleosporales taxa made up the greatest proportion of the microfungal community in Chihuahuan Desert soil [30], and it is highly prevalent in native grasses of the Chihuahuan Desert, such as *Bouteloua gracilis* [2, 30]. A particular group of fungal species known as Dark Septate Endophytes (DSE) of order Pleosporales are prevalent microfungal taxa of Chihuahuan Desert grasses [2, 30]. DSE, a conidial or sterile fungus, are reported to colonize around 600 plant species representing 320 genera and about 300 families [36]. Symbiotic DSE seems to gain access to carbohydrates from hosts' cells, and, in return, hosts benefit from improved nutrient and water acquisition [11]. Some of these DSE are known to mitigate desiccation stress [30, 32]. In this study, the decrease in Pleosporales colonization in *Dmaize* (2%) relative to its progenitor *Dteosinte* (26%) may mean that genotypic and phenotypic changes during maize domestication could have decreased its dependence on Pleosporales.

Cosmopolitan Pleosporalean fungi, *Darksidea* spp., colonize grass species inhabiting different environments and geographic regions [2–6, 30, 31, 35] (J. Herrera, and R. Poudel,

unpublished data from grasses in Nepal and South Africa). All of the plants grown in the desert soil were colonized by these particular fungi, and only these plants supported these fungi. Some taxa of DSE fungi, especially of order Pleosporales, are known to provide desiccation tolerance [4]. The uniquely biased colonization of desert-soil-grown cereal grains by *Darksidea* spp. signals its potential for symbiotic relationships with these host plant taxa, particularly in arid environments. Also, this ability of *Darksidea* spp. to colonize roots of a wide variety of grasses may be evidence of a fairly ancient relationship between these particular fungal taxa and host plants in the Poaceae [2] (J. Herrera, and R. Poudel, unpublished data from grasses in Nepal and South Africa). Additional studies on a more diverse set of grasses can test this assumption more rigorously.

Even though plants grown in the same soil harbored similar fungal taxa, they were typically in different concentrations. For example, based on culture-independent data, a greater number of *Fusarium* spp. were observed in *Dmaize* (78%) than in *Dteosinte* (53%) (Table 3); this difference might have been the result of the host's role in microfungal colonization, as found in other studies [10, 11, 37]. Since this was a greenhouse experiment, plants were provided with optimal growth conditions. However in farms or natural ecosystems those plants might not have such moderate growing conditions, a situation that could alter fungal colonization and fungal community development. It would be especially interesting to investigate root fungal community in arid lands because many studies have found that some symbiotic fungal endophytes tend to selectively colonize in stressful environmental conditions [38]. Thus, additional studies in more variable and realistic environmental conditions are needed to determine some of the nuances of the effect soil-type has on fungal colonization of grass roots.

## Conflict of Interests

There is no conflict of interests to declare.

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