The Dark Side Is Not Fastidious – Dark Septate Endophytic Fungi of Native and Invasive Plants of Semiarid Sandy Areas

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Abstract

Dark septate endophytic (DSE) fungi represent a frequent root-colonizing fungal group common in environments with strong abiotic stress, such as (semi)arid ecosystems. This work aimed to study the DSE fungi colonizing the plants of semiarid sandy grasslands with wood steppe patches on the Great Hungarian Plain. As we may assume that fungi colonizing both invasive and native species are generalists, root associated fungi (RAF) were isolated from eight native and three invasive species. The nrDNA sequences of the isolates were used for identification. To confirm that the fungi were endophytes an artificial inoculation system was used to test the isolates; we considered a fungus as DSE if it colonized the roots without causing a negative effect on the plant and formed microsclerotia in the roots. According to the analyses of the ITS sequence of nrDNA the 296 isolates clustered into 41 groups. We found that 14 of these 41 groups were DSE, representing approximately 60% of the isolates. The main DSE groups were generalist and showed no specificity to area or season and colonized both native and invasive species, demonstrating that exotic plants are capable of using the root endophytic fungi of the invaded areas. The DSE community of the region shows high similarity to those found in arid grasslands of North America. Taking into account a previous hypothesis about the common root colonizers of those grasslands and our results reported here, we hypothesize that plants of (semi)arid grasslands share common dominant members of the DSE fungal community on a global scale.

Introduction

Endophytes, which consist of living organisms that colonize plant tissues during some period of their life cycle yet cause no symptoms of tissue damage to their hosts [1,2], are found in all biomes. Among these endophytes, fungi commonly play important roles in ecosystem functioning [3]. Some fungal endophytic interactions have been widely studied due to the general interest in economically important hosts (e.g., tall fescue) or fungi (e.g., clavicipitaceous fungi) [4]. Although there is an increasing interest in fungal endophytes, our knowledge is biased toward grasses and clavicipitaceous fungi) [4]. Although there is an increasing interest in fungal endophytes, our knowledge is biased toward grasses and their above-ground tissues [2].

Dark septate endophytes (DSE) are found worldwide and comprise a group of root-colonizing endophytic fungi that belong to a few orders of the phylum Ascomycota [5]. DSE fungi are septate and generally have melanized hyphae that colonize the cortical cells and intercellular regions of roots and form a densely septated intracellular structure called microsclerotia [5,6]. Historically, there have been several ambiguities in research on DSE fungi regarding the terms, structures or functions of DSE-plant interactions [see [5]]. Although there has been a continual increase in interest in DSE fungi [e.g., [7–11]], our knowledge of DSE fungi diversity and their function in ecosystems is limited and not as well understood as that of the common root colonizer mycorrhizal fungi or the previously mentioned grass endophytes.

Recently, Mandyam and Jumpponen [12] reviewed studies on the distribution and frequency of DSE interactions in plant communities of different ecosystems. Although it seems that DSE fungi are present in all major biome types and climate regions, studies on their presence and diversity are sporadic. One of the pioneering and landmark studies of DSE fungi focusing on alpine biomes [13] hypothesized that the abundance and importance of plant-DSE fungi interactions increase with increasing abiotic stress on the environment. This hypothesis was integrated into the scientific canon and was strengthened by the general view that melanin content is related to abiotic stress, especially drought stress [14]. Fungi with melanized cell walls were used in experimental studies showing increased resistance to heat and drought stress (e.g., [15–17]). These data and the presence of these fungi in certain habitats resulted in the general view that DSE fungi could play important roles in ecosystems with low water availability. Several studies on root-colonizing fungi of such areas – majority of them represents North American biomes – focused on DSE and other root associated fungi (RAF) (e.g., [18–20]).

In the mycorrhizal status studies of plant communities of the Great Hungarian Plain, the majority of the plant species was found...
to be colonized by DSE fungi [21,22]. One of the areas studied is a 
semiarid open sandy grassland with forest steppe patches, and 
represents a characteristic ecosystem of the plain of the interfluves 
of the two main rivers of the Carpathian basin, the Danube and 
Tisza. From a botanical point of view, this is one of the most 
studied habitats of the region; in addition to the lengthy vegetation 
studies [23,24], one of the three long-term ecological research 
(LTER) projects in Hungary has been performed here [24]. These 
grasslands are the westernmost representation of the Eurasian 
steppe belt [25]. The soil is Danube-origin loose sand with very 
low organic matter content and almost no clay content. Thus, it 
has very limited water-holding capacity that results in a harsh 
environment, especially during the hot summers (Fig. S1). The 
area is considered semiarid, and the open grasslands have semi-
desert characteristics [26]. During the mycorrhizal status studies in 
this semiarid sandy grassland, 89 plant species were studied; 63 of 
these were found to be colonized by melanized, septate hyphae, 
and microsclerotia were detected in 36 species [22]. Due to the 
frequent colonization, we assumed that DSE fungi play an 
important role in this ecosystem.

These results prompted us to study the compositional diversity 
of DSE fungi colonizing the plants of these semiarid sandy areas. 
We aimed to test the area specificity, the seasonality and whether 
the DSE fungi were generalist plant colonizers in the area. To test 
the latter, we sampled invasive plants in addition to the native 
indigenous hosts. The effects of soil microbiota on invasive plants 
are generally established [27–30]. We may assume that a 
successful invasive species either do not have a mutualistic partner 
or can establish functioning interactions not exclusively with 
specific partners. From a myocentric standpoint, we assumed that 
RAF colonizing both the native and invasive plants of the habitat 
are generalists. The fungal isolates were subjected to molecular 
taxonomic characterization and used in an artificial synthesis 
experiment to test whether they could be considered DSE fungi.

**Materials and Methods**

**Sampling**

The samples were collected from three sites on the Great 
Hungarian Plain (Hungary); Bugac (N 46°39’ E 19°36’), 
Fülopháza (N 46°52’ E 19°24’) and Tatárszentgyörgy (N 47°3’ 
E 19°24’) (Fig. S1). The main vegetation types are the open 
grassland at Fülopháza (*Festucetum vaginatae*) and mixed woody 
steppe (*Juniperus-Populetum*) that are characteristic at each site. 
The first two areas are protected and belong to the Kiskunság National 
Park. The three areas are at 100–150 m altitude, the sum of sunny 
hours/year is 2000–2100, the mean annual precipitation is 
between 500 and 600 mm/year and the mean annual temperature is 
10–12°C [31,32]. The soils are sandy, rarely mixed with loess, 
humus contents are less than 1% and the pH is between 7.5 and 
8.2.

The roots of eleven plant species were collected in the area, 
including three invasive species (*tree of heaven, Ailanthus altissima*; 
common ragweed, *Ambrosia artemisiifolia*; and common milkweed, 
*Acelepias syriaca*) and eight native species (*Euphorbia dysctica*, *Festuca 
vaginata*, *Fumana procumbens*, *Helianthemum ovatum*, *Junipers communis*, 
*Medicago minima*, *Populus alba* and *Stipa boryshenica*). These plant 
species were chosen because they represent different life forms and 
they are characteristic components of these plant communities. To 
test season- and area-specificity, samples were collected from 10 
marked *Junipers communis* trees from each of the three areas. In 
three seasons (spring, summer and fall) of 2006 and 2009.

Samples were taken from healthy-looking plants. The complete 
root systems of small plant specimens were removed. Otherwise, 
either the roots were followed from stems and collected or 
10 × 10 × 10 cm soil cubes were sampled under the plants and roots 
were identified according to their characteristics. After transporting 
the roots in soil in plastic bags to the laboratory, the samples 
were stored at 8°C for no longer than three days.

Between 2005 and 2009, samples were taken three to four times 
per year from randomly chosen specimens. Invasive species were 
sampled in the native plant communities or in their close 
proximities. For example, ragweed generally colonizes disturbed 
areas and dirt roads but does not invade stable communities. For 
some species, only a few specimens were sampled (e.g., the strictly 
protected *E. distachia* and *S. boryshenica*).

**Isolation of root associated fungi**

Roots were washed with tap water and cleaned with paint 
brushes. Three or four segments (1–2 cm long) were cut from 
different regions of each root sample. The segments were surface-
sterilized by washing in 2% sodium hypochlorite (90 s), 70% 
ethanol (60 s) and sterile distilled water (180 s). Then, the 
segments were cut into four pieces and placed on MMN media 
[33] (15 g/L agarose, pH 5.8, 10 µg/ml streptomycin) and kept at 
18°C in the dark.

Hyphae growing out of the root segments were isolated to 
separate plates. Endophytes from the same root sample that 
showed similar colony morphology were considered identical. The 
isolates were kept on MMN media in the dark at 18°C, and 
transferred to new plates every three months.

**Molecular identification of the isolates**

Total DNA was extracted from different isolates from each root 
sample using CTAB as described previously [34]. Using *Taq 
polymerase (Fermentas)*, the ITS and LSU regions of rDNA were 
amplified using ITS1F-ITS4 and LR0R-LR5 primer pairs, 
respectively, as described previously [35]. Sequencing of the 
amplified transcriptions was carried out with the primers used for 
the amplification by LGC GmbH (Berlin, Germany).

The sequences were compiled from electropherograms using 
the Pregap4 and Gap4 programs [36] and deposited into 
GenBank (JN859221–JN859491). The obtained sequences were 
compared to sequences in public databases using megablast 
(http://blast.ncbi.nlm.nih.gov/Blast.cgi) [37].

Multiple alignments of our sequences and datasets complemented 
by sequences from GenBank were made using MAFFT 6 
Q-INS-i [38]. The alignments were checked and edited with 
ProSeq 2.9 [39]. The best-fit nucleotide substitution model was 
selected using the program jModelTest [40] considering the 
selection of Akaike Information Criterion (AIC). Maximum 
Likelihood (ML) phylogenetic analyses were carried out with the 
online version of PHYML 3.0 [41]. The GTR nucleotide 
substitution model was used with ML estimation of base 
frequencies. Six substitution-rate categories were set, and the 
gamma distribution parameter was estimated and optimized. 
Bootstrap analysis with 1000 replicates (MLB) was used to test the 
support of the branches. The same substitution model was used in 
Bayesian analyses performed with MrBayes 3.1.2 [42,43]. Four 
Markov chains were run for 5,000,000 generations, sampling 
every 100 steps with a burn-in at 7500 sampled trees. To identify 
the main groups the ITS sequences of our isolates a Minimum 
Evolution tree was inferred using the Maximum Composite 
Likelihood model and pairwise deletion at gaps using MEGA 4.0 
[44]. The phylogenetic trees were visualized and edited using the 
Tree Explorer of the MEGA4.0 program [44] and a text editor.
In vitro tests

In vitro tests were performed with the representatives of each group by the analyses of ITS sequences of the fungal isolates (Fig. S2) using leek (Allium porrum) to test whether an isolate could be categorized as a DSE fungus. Seeds were surface-sterilized with 30% H$_2$O$_2$ for 1 min, then washed twice in sterile tap water for 10 min. Surface-sterilized seeds were germinated and watered on sterile filter paper for approximately seven days. Seedlings were laid to MS medium [45] (Sigma Basal Salt Mixture, 15 g/L agar, pH 5.8, 10 µg/ml Streptomycin) in 9-cm plastic Petri dishes. Two cuts (4–5 mm in diameter) of the representative fungal isolates were placed next to the roots of the leek. The plants were grown in a climatized growth chamber in a 14 h light (24°C):10 h dark (22°C) cycle. A total of five replicates for each fungal isolate and five control plants were incubated in each series. Plants were checked periodically and the roots were harvested 8–9 weeks after inoculation. We considered an isolate to be a DSE fungus if it colonized the root with no visible negative effect to the plant and formed microsclerotia in the roots.

Microscopy

Root samples from the field samples and in vitro experiments were prepared for microscopic analyses similarly to the method described in Kovács and Bagi [21]. The cleared roots were stained using aniline blue and covered in PVLG (polyvinyl-lacto-glycerol). The samples were studied using light microscopes with Nomarski (differential interference contrast, DIC) optics and digital cameras.

Results

Fungal isolates

All the field-collected root samples of the eleven plant species studied were colonized by intraradical melanized septate hyphae and microsclerotia (Fig. 1). In addition to these fungal structures, the roots were generally colonized by arbuscular mycorrhizal fungi.

Nearly 200 samples were collected from the eleven species from the three sampling areas (Table S1). In total, 296 fungal strains were isolated, and the ITS region of 241 isolates were sequenced.
and analyzed (Fig. S2). Based on analyses of the ITS sequences, the 241 isolates were grouped into 7 major and 34 smaller groups, including singletons (Fig. 2). The small groups with one or few isolates were found exclusively on either indigenous or invasive plants, but the groups with greater numbers of isolates colonized both invasive and indigenous plants. All but one of the isolates belonged to the phylum Ascomycota. The ITS of that one isolate showed similarities with sequences from the basidiomycete order Auriculariales (data not shown).

Results of the in vitro inoculation tests

For the in vitro tests, 59 representative isolates of the 41 groups were chosen (Fig. S2). The healthy-looking Allium porrum plants generally had well-developed root systems and 3–4 leaves after 8–9 weeks. Nine isolates showed unambiguous negative effects on the hosts; the plants inoculated with these isolates died within 4–5 weeks, after their leaves and roots were shriveled and became yellow. In 21 cases, the leeks did not appear different from the controls. The fungi did not colonize the roots of A. porrum, and neither microsclerotia nor intraradical hyphae were observed. In tests of 29 isolates representing 14 groups, no negative effects were detected, and both septate endogenous hyphae and microsclerotia were observed in the colonized roots (Fig. 1). These 14 groups were considered DSE fungi.

The two most frequently isolated non-DSE groups were the widely distributed plant pathogens Hymenochaetra macrodidyma (group 25) and Fusarium oxysporum (group 27). Both groups were isolated from several native and non-native plants from all sites (Fig. S2, Table S1).

The DSE groups

Of the 41 groups of total isolates, 14 groups (<35%) were considered DSE. These groups contained 142 of the 241 isolates (~60%). Henceforth, these groups will be referred to as DSE groups 1–14 (Fig. 2, Fig. S2, Table S1). The analyses of ITS and LSU sequences showed no difference in the taxonomic position of the isolates. However, different representatives from public databases could be included in the two datasets, which resulted in complementary information about the DSE groups found in our sampling areas (Fig. 3, Table S2). All DSE isolates belonged to five orders of Ascomycota: Pleosporales (seven groups), Helotiales (two groups), Hypocreales (two groups), Eurotiales (one group), Xylariales (one group) and to the family Plectosphaerellaceae (one group) [46]. Some of the DSE groups could be identified at the species or genus level, whereas others could be identified only on higher taxonomic levels (Table S2). Neither the ITS or LSU sequences of some DSE groups (DSE-2, -4, -5, -12) showed similarity with any identified GenBank entry (Fig. 3, Table S2).

The BLAST analysis of the ITS sequences of the DSE isolates showed high similarities with sequences of fungi from roots of different hosts or from soil originating from different geographic and climatic regions (Table S2).

Forty-eight isolates from the marked Juniperus communis trees of the three sampling sites were categorized as DSE fungi. All but one of the isolates belonged to DSE-1, -2 and -3 groups (Table S1). The DSE-1 group contains isolates originating from the three areas and three seasons. Isolates of the DSE-2 group represent two areas (Fülopháza and Tátárszentgyörgy) and three seasons, and isolates from the DSE-3 group were obtained from two areas (Fülopháza and Tátárszentgyörgy) and from two seasons (spring, autumn).

Accordingly, none of these three groups contained an isolate from only one area or one season. The fungi belonging to frequent DSE groups were isolated from both indigenous and invasive plants. In addition to the singleton groups (DSE-11, DSE-12), groups with few isolates (number of isolates indicated by slashes after the groups) originated only from either invasive (DSE-5/2/, DSE-13/3/ and DSE-14/3/) or native (DSE-4/3/ and DSE-8/3/) plant species (Fig. S2, Table S1). The DSE-9 contained more than 3 isolates: these 8 isolates originated solely from native plants from one site (Fülopháza) during different seasons. Apart from the singletons, three DSE groups were from one season/sampling and one site (DSE-4/3/3/ and DSE-8/3/). Finally, one group was from one season/sampling (DSE-10/3/) and two groups were isolated from only one site (DSE-6/5/ and DSE-9/8/).

Discussion

Sandy areas in the Carpathian Basin of Danube-Tiscia interfluve, especially those of the open grasslands, represent semiarid regions with semidesert characteristics [26]. Our previous work on the root colonization of plants of sandy grassland in this region revealed the frequent presence of structures characteristic of DSE fungi [22]. Although our sampling in this study was not designed to address quantitative questions, the rank-order distribution of both the total 41 fungal groups isolated and the 14 groups categorized as DSE fungi showed similar distribution to those generally obtained in fungal endophyte studies [47]. Only a few fungal taxa are represented by the majority of the isolates, thus these groups could be categorized as dominant or at least common members of the RAF community of the area. Twenty-one groups

Figure 2. Frequency rank diagram of the 41 groups formed by the 241 fungal isolates. Filled columns represent the groups considered as DSE fungi.
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were represented by only one isolate. The presence of ‘singletons’ and groups with low numbers of isolates can complicate the test of any specificity. However, a low isolation frequency does not necessarily mean low abundance; a low frequency could be the result of a biased isolation technique.

Studies using molecular diversity screening methods generally detect basidiomycetes, mostly Agaricales, as common members of RAF communities (e.g., [10,18,20]). We found only a single basidiomycete isolate with ITS showing similarities to the genus *Auricularia* (Auriculariales). Unfortunately, the isolate was lost before we could perform any experiments with the fungus.

Although there is an increasing interest in DSE fungi, there are no clearly defined criteria to determine whether a fungus is DSE. Such criteria would be useful in avoiding confusion caused by inconsequent terminology as has occurred in some mycorrhizal examples [48]. Several studies use a simple assumption: a fungus is ‘endophytic’ if it has been isolated from a healthy plant after surface sterilization [3]. In addition to the cultivation-based techniques, data arising from molecular diversity studies using fungal-specific primers are now used to characterize RAF and other endogenous fungal communities. In our study, we isolated fungi from surface-sterilized roots and used an artificial synthesis system to decide whether they may be categorized as DSE fungi. Based on our results, the majority of the most frequently isolates was DSE fungi. The DSE interactions, similarly to any plant-fungal interaction, depend on hosts and environmental factors [9].

Based on our artificial test, we may assume that each isolate that was categorized as DSE could be endophytic fungi. However, we cannot exclude the possibility that the isolates that did not fit our assumptions are DSE.

**The main DSE groups**

The DSE isolates identified in this study belong to those ascomycete groups that are the most frequent root-colonizing endophytes, such as Pleosporales, Hypocreales and Helotiales.

Some of our isolates represented well-known root colonizing taxa or lineages. *Cadophora* formed the largest (44 isolates) DSE group (DSE-1); *Cadophora* is a widely distributed, common root colonizer, especially *C. finlandia* (syn: *Phialophora finlandia*) [49]. However, the species identity of our *Cadophora* group is dubious and it may represent more than one taxon (Fig. S3).

The group DSE-3 comprises *Rhizopycnis vagum* isolates. Several fungal root endophytes grouped together with our isolates from different geographic and host origin: such as the Mediterranean plants, *Pinus halepensis* and *Rosmarinus officinalis* [50], and *Dioscorea zingiberensis* from China [51] (Fig. S4, Table S2). Some studies characterize *R. vagum* as a pathogen. Armengol et al. [52] studied the pathogenicity of eight *R. vagum* isolates with different geographic and host origins. All isolates were pathogenic to muskmelon roots, although the disease reaction was not severe. *R. vagum* was also described as a weak pathogen and presumably a putative contributor to mature watermelon vine decline [53,54]. There is a hypothesis stating that endophytic fungi might be latent pathogens [3]. The different effects of conspecific isolates reported in previous works might be the result of this, or there could be an unrevealed heterogeneity of the group masked by morphological and ITS sequence similarities.

Our DSE-8 group is the known DSE fungus *Periconia macrospinosa* (Fig. S5, Table S2). This species is widely distributed and is generally associated with grasses. The hyaline hyphae of the

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**Figure 3.** The maximum likelihood (ML) tree of the partial LSU sequences of representative isolates of DSE groups and similar sequences from GenBank. *Saccharomyces cerevisiae* (FJ972212) was used as outgroup. Sequences obtained in this study are shown in bold. Name of the DSE groups and accession number were shown in brackets. NJ bootstrap (not shown below 70%) values are above and the Bayesian posterior probabilities as percentage (not shown below 90%) are below the branches. Abbreviations: un culturred (u.), clone (c.).

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fungal fungus are barely visible in the roots [55], except their microsclerotia, which are melanized. The colonization by this DSE fungus could remain hidden when roots are studied using routine methods. Three different P. macrospinosa isolates have been used in experimental studies of the effect of DSE fungal inoculation with several hosts [11,19]. The results suggest a trend; namely, the grasses show stronger reactions to the inoculation with the species. We isolated this fungus only from grasses. This, together with the results of the aforementioned study, suggests that the fungus might be mainly associated with grasses.

Using ITS clone libraries of RAFs of Bouteloua gracilis, a dominant grass in a North American semiarid grassland, Porras-Alfaro et al. [18] found a dominant, unidentified fungal lineage belonging to Pleosporales. The lineage was detected in the RAF community of different grasses of semiarid grasslands (e.g., [10,20,56,57]). In this study, we also found this particular fungal group: our group DSE-7 belongs to this clade. The group remains unidentified [18], and the analyses of our LSU sequences could not help identify the lineage either. At this time, this group has been detected in grass species. However, the previous studies detecting the lineage did not study non-grass plants. The majority of our isolates originated also from dominant grasses of our grasslands, such as Festuca vaginata and Stylo borythenico, but the fungus was isolated from the roots of the dwarf-shrub Festuca procumbens and the invasive tree Ailanthus altissima. The isolates of the DSE-7 group could help us study the function and taxonomy of this DSE fungus that is distributed worldwide and seems to be associated with plants, especially with grasses, of (semi)arid habitats.

A mutual increase of heat tolerance was demonstrated when an endophytic Curcularia species was used in experiments with its natural host Dichanthium lanuginosum originating from an area with strong geothermal activity [15]. The study showed not only the beneficial effect of the melanized fungus on the plant but also that the fungus needs the plant to survive the conditions of its natural habitat. In a later study, the same fungus was used with different host crops and yielded similar results [16]. Our DSE-10 group also belongs to the genus Curcularia. Based on the available sequence-similarity information given in the original article, our isolates are likely different from the fungus isolated by Redman et al. [15], on which a US patent on increasing heat tolerance using endophytic fungi is based (US Patent # 7906313).

Microdochium strains were isolated in prairie ecosystems of Kansas, United States, and the fungus was used in experimental studies about effects on plants and enzyme activities [11,19]. The effect of the Microdochium varied according to different hosts, but the fungus is considered endophytic. The singleton group DSE-11 is a Microdochium showing high similarity with the isolate used in the aforementioned experiments [11,19].

The DSE-14 group is composed of isolates of the widely distributed plant pathogen genus Fusarium that includes endophytic lineages [58-60]. RAF sequences represent fungi from grasslands of North America, and they also can be found in this group [10,20], suggesting that the lineage could be widely spread.

**Specificity, seasonality, invasive plants**

There are many results suggesting that DSE fungi are generalists and colonize several hosts [5]. However, only a few studies have addressed the area-, host- and organ-specificity and seasonality of these fungi. Herrera et al. [10] found only exceptional cases in which RAF could be detected in organs other than root. In the same study, in addition to some lineages showing geographically related specificity, a core of root-colonizing fungi was identified as constant members of a community of grass root endophytes. A similar pattern was found by Khidir et al. [20], who concluded that the dominant colonizers are similar in the American semiarid grasslands that they studied. We isolated specimens not only from grasses but also from other native and invasive plants of the area. Among the frequent groups there was only one, the group DSE-9 representing an Embellisia sp., that was isolated solely from native plants. The puzzling question of host specificity could be addressed on several levels [61]. Although we can conclude that the most frequent DSE fungi are generalists, more studies are necessary to obtain data from the individual to the ecosystem level. Mandiyam et al. [11,19] studied several Periconia isolates and concluded that DSE fungi have ‘broad host range’, but there was a considerable difference in the characteristics and effects of the conspecific isolates. Mandiyam et al. [11] suggested a kind of ‘greater compatibility’ of the DSE isolates they studied with grasses than with other plants used in the experiments. It is relatively difficult to address whether there is a certain DSE fungus with specificity for grasses, as studies characterizing RAF and DSE communities are dominated with works focusing on grasses.

The seasonality of DSE fungi has been addressed in previous works [62] and references therein). However, these works studied the seasonality of colonization by DSE fungi. Based on the results of our sampling in different seasons, the dominant members of the DSE community were not restricted to a certain period of the year. Nevertheless, understanding the seasonal dynamics, both functional and compositional, of DSE communities requires further studies.

In addition to the native plants, we studied the DSE fungi that colonized alien, invasive plants of the area. Ailanthus altissima, Asclepias syriaca and Ambrosia artensissifolia are important invaders [63-65] causing not only ecological but economic problems as well. Each of the three species was found to be colonized by DSE fungi in the present work and in previous studies carried out in the region [21,22]. The possible effects of soil microbiota on invasive plants and on the success of invasion is generally known, and the effects of mycorrhizal colonization have been frequently studied [29]. However, we are not aware of any study on the DSE fungi that colonize invasive plants. The DSE colonization of Asclepias syriaca and Ambrosia artensissifolia were studied in their native environments in North America, and both were found to be colonized by DSE; the former was also used in experiments with Periconia isolates [11]. A successful invasive plant cannot depend exclusively on specific symbiotic partners, and we assume that the generalist root-colonizing fungi will colonize alien and invasive plants. In our study, the dominant, frequent DSE groups were isolated from the roots of the invasive plants as well as the native plants, which supports the hypothesis that those fungi are generalists with a wide host range. As the DSE fungi are frequent and their role in plant survival in a stressful environment could be important, we may assume that those fungi could also help invasive plants in these environments. Subsequent experiments are needed to study the effects of DSE fungi on the success of invasive plants.

**Conclusions**

Based on the results of several studies, Khidir et al. [20] concluded that ‘grasses of the arid regions of North America share a general community of RAF’. Based on our own results, we may extend this statement by hypothesizing that plants of (semi)arid grasslands share common dominant DSE fungal community. Global, inter-continental comparative studies could test this hypothesis further. DSE fungi are frequently found in arid and semiarid environments, and these communities share some
important global key-players. The need for a better understanding of the role of DSE fungi has been stressed by many authors (e.g., [3,12,19]). The appropriate studies would require diversity screening, experimental studies and systems biology approaches [3]. All the dominant DSE groups identified in our study were generalists that colonized different native and invasive plants and showed no specificity to any area. Furthermore, they showed high similarity, even identity, with root colonizers from different continents; this strengthens the evidence that DSE fungi are generalists. Thus, our isolates from the semiarid sandy grasslands could be used in experiments to help understand the ‘elusive function’ [12] of DSE fungi in semiarid environments on a broader scale.

Supporting Information

Figure S1 The three sampling site on the Great Hungarian Plain; Bugac (a), Fülpöháza (b) and Tatařszentgyörgy (c).
(PDF)

Figure S2 Minimum Evolution tree of the ITS sequences of 241 isolates originating from roots of indigenous (O) and invasive (A) plants. Name of the groups is shown in brackets. Isolates used in the artificial inoculation experiments are marked (X). Tree was inferred using the Maximum Composite Likelihood model and pairwise deletion at gaps using MEGA 4.0. Bootstrap values obtained from 1000 replicates are shown as percentages and not shown below 70%. Bar = 2 characters.
(PDF)

Figure S3 The maximum likelihood (ML) tree of the ITS sequences of representatives of group DSE-1 and similar sequences from GenBank. Sequences obtained in this study are shown in bold. Pyrenopeziza recta (AJ430224) was used as outgroup. Accession number, isolation source and geographic origin of sequences from public databases are shown. NJ bootstrap (not shown below 70%) values are above and the Bayesian posterior probabilities as percentage (not shown below 90%) are below the branches. Abbreviations: uncultured (u.), clone (c.). Bar = 0.05 expected change on one nucleotide.
(PDF)

Figure S4 The maximum likelihood (ML) tree of the ITS sequences of representatives of group DSE-3 and similar sequences from GenBank. Sequences obtained in this study are shown in bold. Massarina tortuosa (FR668004) was used as outgroup. Accession number, isolation source and geographic origin of sequences from public databases are shown. NJ bootstrap (not shown below 70%) values are above and the Bayesian posterior probabilities as percentage (not shown below 90%) are below the branches. Abbreviations: uncultured (u.), clone (c.). Bar = 0.1 expected change on one nucleotide.
(PDF)

Table S1 The list of the 241 isolates with their name, group, accession number of ITS sequences, host plant, sampling site and season of the collection.
(PDF)

Table S2 Closest BLAST matches of representative isolates of DSE groups. Name, source, provenance country, query coverage and max ident of sequences from GenBank are indicated. Abbreviations: uncultured (u.), clone (c.), strain (st), Host (H), Isolation Source (IS), Tissue Type (TT), Environmental Sample (ES).
(PDF)

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Author Contributions

Conceived and designed the experiments: GMK. Performed the experiments: DGK AP GMK. Analyzed the data: DGK GMK. Contributed reagents/materials/analysis tools: GMK. Wrote the paper: DGK GMK.

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