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Biodiversity of fungal endophyte communities inhabiting switchgrass (*Panicum virgatum* L.) growing in the native tallgrass prairie of northern Oklahoma

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Abstract This study was conducted to explore fungal endophyte communities inhabiting native switchgrass plants from the tallgrass prairie of northern Oklahoma. The primary focus was to isolate these endophytes in pure culture from surface-sterilized plant tissues and provide taxonomic identifications based on comparative analysis of ITS rDNA gene sequences. From these data, we evaluated the biodiversity of these potentially beneficial endosymbionts from this rapidly disappearing habitat of the Great Plains. While important from a strictly conservationist standpoint, this survey further allowed us to identify candidate endophytes for introduction into commercial switchgrass cultivars for biomass enhancement. A total of 210 whole plant samples were collected at early vegetative, full reproductive and senescence stages. Fungal endophytes were isolated, identified to species level when possible, and grouped into communities based on plant part, collection month and part of the prairie from which the plants were collected. Species diversity for each community was estimated by Shannon diversity index, and differences in diversity indices were compared using a *t*-test. The presence of fungal species representing at least 18 taxonomic orders suggests a high level of diversity in switchgrass endophyte communities. The fungal communities from shoot tissue had significantly higher species diversity than communities from the root tissue. The abundance of taxa assigned to the order Hypocreales (to which mutualistic, clavicipitaceous endophytes of cool-season grasses belong) found in shoot (64%) and root tissues (39%) throughout the growing season suggests great potential for utilizing these endophytes for enhancing biomass production and stress resistance of this important bioenergy crop.

Keywords Biodiversity · Bioenergy · Endophyte · Hypocreales · Mutualism · Symbiosis

Introduction

Switchgrass (*Panicum virgatum* L.) has been identified as a bioenergy crop in the United States for cellulosic ethanol production. In addition to a high biomass production potential and a perennial growth habit, this North American prairie grass has broad adaptability and requires minimal nutritional inputs, and thus can be cultivated on marginal lands unsuitable for more input-demanding agronomic crop plants (Bouton 2008; Sanderson et al. 1996). Still, fulfillment of the US Department of Energy's ambitious goal to replace 30% of petroleum-based transportation fuels with biofuel by 2030 will require more acreage dedicated to bioenergy crops and substantial increases in current productivity levels (Bouton 2007). Further, for a renewable biofuel-based economy to fulfill its promise as a path to reduced reliance on fossil fuels, a concerted, multidisciplinary effort is necessary to maximize not only the biomass yield of bioenergy crops, but also the sustainability of acreage dedicated to this purpose for decades and centuries to come. Only then will we be able to supply the continual source of feed-stock to biorefineries necessary for the long-term viability of the biofuel industry.

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Previous research on fertilizer requirements of native warm season grasses, including switchgrass, suggests that nitrogen is the main limiting nutrient required for switchgrass production (Vogel et al. 2002). Arbuscular mycorrhizal fungi (AMF) often enhance phosphorous and nitrogen uptake (Abbott and Robson 1984; George et al. 1995), water uptake and drought tolerance (Safir and Boyer 1971; Sylvia and Williams 1992), and can even provide protection or tolerance to rhizosphere pathogens in a broad variety of plants (Linderman and Hendrix 1982). Similar benefits have been documented for symbiotic associations between host plants and non-mycorrhizal fungal symbionts (Clay et al. 1989; Singh et al. 2000; Waller et al. 2005). These fungi are commonly referred to as fungal endophytes and some, particularly systemic colonizers of aerial plant tissues, can produce secondary metabolites that are toxic to grazing herbivores (Bush et al. 1997; Schardl et al. 2004).

A model system involving plant-fungal symbioses involves the fungal endophytes of cool season grasses, renowned for imparting a variety of fitness enhancements to their hosts (Clay and Schardl 2002; Schardl et al. 2004). The impact on host survival and persistence can be so substantial that in some cases, their presence is required for sustainable commercial production of cool season grasses in certain parts of the world (Evans 2006; Milne 2006; Pedersen et al. 1990). Conversely, there have been very few studies on the warm season grasses and their associated endophytes (Clay et al. 1989; Ghimire et al. 2009; Kelemu et al. 2001). Previous studies suggest that plants grown in association with certain endophytic fungi produce more tillers, greater biomass, and have enhanced resistance to pathogens when compared to uninfected con-specifics (Ahmad et al. 2001; Clay et al. 1989; Kelemu et al. 2001; Stovall and Clay 1988). Therefore, utilization of fungal endophytes is a feasible strategy to enhance several growth characteristics of warm season grasses, including the important bioenergy crop, switchgrass.

The tallgrass prairie is a unique ecosystem in central North America, originally spanning portions of 14 states from Texas to Minnesota with a total area of 57 million ha (National Park Service 2010). Unchecked urban sprawl and conversion to cropland have left less than 4% of this distinctive landscape intact. Switchgrass is native to these prairies and is the third most dominant species after big bluestem (*Andropogon gerardii*) and Indian grass (*Sorghastum nutans*). However, our understanding of endophytic fungal communities in switchgrass from this magnificent natural habitat is extremely limited. The purpose in this study was to isolate fungal endophytes from surface-sterilized root and shoot tissues of switchgrass plants growing in their native habitat, over the course of an entire growing season with the following specific objectives: (1) to isolate and taxonomically identify shoot and root-associated fungal endophytes of switchgrass; (2) to evaluate these endophyte communities over the course of a growing season to provide insight into symbiont community dynamics; (3) to identify endophyte species that could be introduced into elite switchgrass cultivars for the further enhancement of this promising biofuel crop.

Materials and methods

Study sites

Plant samples were collected from Alfalfa, Grant, Kay and Osage counties of Northern Oklahoma. The sampling points were located between the GPS coordinates of 36°38′38″ to 36° 48′48″ N latitude and 96°10′ 26″ to 98°16′11″ W longitude (Table S1). This region has a sub-humid continental climate, with a mean annual temperature of 15°C and a growing season of 177 to 220 days. Mean annual precipitation ranges from 76 to 112 cm of which more than 70% usually falls in April through October (USDA 1985, 2007a, b, 2008). Although all four counties studied are part of the North American tallgrass prairie, sampling sites from Alfalfa, Grant and Kay counties were from quite diverse habitats (e.g. grassland, pasture, lake shores, salt plains, marshy land). Conversely, those from Osage county were predominantly composed of natural grassland from the Tallgrass Prairie Preserve (TGPP). Since 1989, the Nature Conservancy has owned and managed the TGPP by recreating a semi-natural grazing and disturbance regime with bison herds and a stochastic fire regime (Hamilton 1996). In addition to habitat differences, Alfalfa, Grant and the western part of Kay counties receive significantly lower average annual precipitation than Osage county (82 cm versus 112 cm). Sampling sites from Alfalfa, Grant and Kay counties are henceforth referred to as the west part and those from Osage county are referred to as the east part of the study area.

Field sampling

Plant samples were collected during early vegetative, full reproductive and senescence stages of switchgrass growth in the months of April, July and October of 2009, respectively. Each sampling consisted of 69 to 76 whole plant samples (five to ten tillers per sample) from different parts of the tallgrass prairie that included at least 34 samples each from the east and west. GPS coordinates were recorded for each sampling site in the April sampling, and the same general coordinates (within the same field) were used for subsequent samplings. A total of 214 plant samples were collected and all samples were processed (see below) for shoot and root inhabiting endophytic fungal communities. Twenty-four representative soil samples from these GPS locations, 12 each from east and west

part of prairie were collected and analyzed for pH, organic matter, phosphorus, potassium, calcium, magnesium and sodium content.

Plant sample processing

Collected plants with approximately 25–35 cm of both above and belowground tissues were transported to the laboratory on ice and processed within 24 h of collection. Processing of the root tissues involved thorough rinsing of multiple roots (5–10/plant), with tap water to remove excess soil. The basal portion of shoots was collected for shoot endophyte isolation. Root and shoot samples were cut into 3–4 cm pieces prior to rigorous surface sterilization (95% ethanol for 30 s, 70% ethanol for 5 min followed by 3% sodium hypochlorite for 25–30 min). Surface sterilized tissues were rinsed three times with sterile water, blot dried, cut into small pieces (1–1.5 cm) and plated on PDA plates amended with 100 ppm ampicillin sodium salt, 50 ppm chloramphenicol and 50 ppm streptomycin sulfate. Plates were incubated in the dark for up to 2 months at 24°C and examined regularly for emerging fungal colonies. Emerging fungal colonies were passed through two rounds of subculture prior to preparing agar slants for long-term storage and collecting fungal materials for DNA extraction.

DNA isolation

Fungal material for DNA extraction was harvested from 1 to 2 week-old cultures grown on potato dextrose agar (PDA) by cutting an agar block of 1.5 cm^3. Colonized agar blocks were placed in a 1.5 ml micro-tube with a single 4.5 mm stainless steel bead. These micro-tubes were arranged in a rack and covered with an AirPore filter, stored at −80°C overnight and lyophilized for 24 h. The DNA was extracted from lyophilized tissue using QIAGEN MagAttract 96 DNA Plant Core Kit according to manufacturers' instructions.

Polymerase chain reaction, sequencing and database search

The internal transcribed spacer (ITS) regions of fungal ribosomal DNA (rDNA) are highly variable in sequence, and thus of great importance in distinguishing fungal species (White et al. 1990). The fungal specific primers ITS1F and ITS4, amplifying the highly variable ITS1 and ITS2 sequences surrounding the 5.8S-coding sequence, were used in this study. These primer sets have been used widely (Gardes and Bruns 1993; Martin and Rygiewicz 2005) and are thus well represented in the NCBI nucleotide database. PCR primers were used to sequence the purified PCR products as described previously (Puckette et al. 2009). Gene sequences were manually inspected, edited and appended into contigs using DNA sequence assembly software Sequencher® version 4.9 (Gene Code Corporation, Ann Arbor, Michigan). These sequences were then subjected to BLASTn searches against the NCBI non-redundant database and the top three hits (with lowest *e*-value) were used to assign identities to test isolates at the deepest possible taxonomic resolution.

Data organization and statistical analysis

Two comprehensive endophyte species lists, one for shoots and one for roots, were generated for each collection date. Comparisons were made between species from the east and west parts of the tallgrass prairie, as well as between sampling dates. In addition, fungal species from shoot and root tissue were grouped according to their higher taxonomic level (ordinal level) to allow ordinal frequencies to be evaluated.

Species diversity in each of these fungal "communities" was estimated using the Shannon diversity index (Bowman et al. 1971) as implemented in the PROC IML program, SAS software version 9.1.3 (SAS 2004). Differences in Shannon diversity indices of any two fungal communities were compared using a Student *t*-test at a 95% confidence level. The mean organic matter, macronutrients, sodium and pH content in soils from the east and west parts of the tallgrass prairie were compared using PROC TTEST in SAS software version 9.1.3 (SAS 2004).

Results

Fungal isolates

The results presented herein are based on ITS sequences of 555 fungal isolates that were isolated on potato dextrose agar from switchgrass plants collected from the tallgrass prairie of northern Oklahoma on three different occasions during the 2009 growing season. Of these 555 isolates, 143 originated from shoots and 412 were from the root tissues.

Endophyte community in shoot tissues

The shoot endophyte community was composed of 143 fungal isolates that belong to at least 51 operational taxonomic units (OTUs) (Fig. 1a). The number of isolates recovered from different collection months varied, with 46, 83 and 14 isolates obtained in April, July and October, respectively. The representation of individual species in the community was variable, as some species were recovered frequently in one or more collections (e.g. *Acremonium strictum*, *Fusarium nygami*, *Fusarium proliferatum*, *Sporisorium everhartii*) and others were recovered infrequently and/or from a single collection (e.g. *Alternaria mali*, *Fusarium subglutinans*, *Phoma glomerata*).

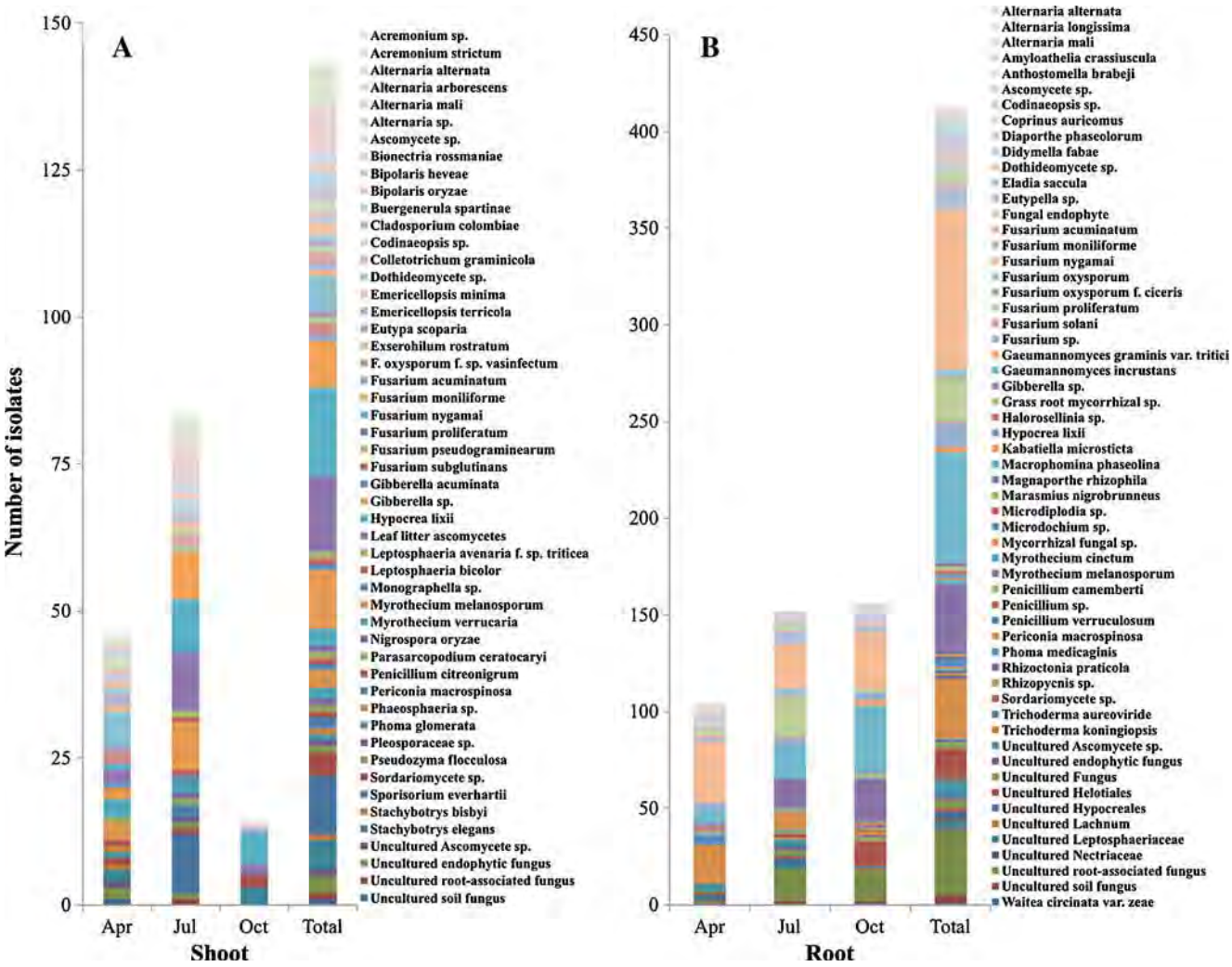

Fig. 1 Temporal distribution of endophytic fungal OTUs in switchgrass (**a**) shoot tissue and (**b**) root tissue in the tallgrass prairie of northern Oklahoma in 2009

Endophyte community in root tissues

The root endophyte community was composed of 412 taxa from at least 58 different OTUs (Fig. 1b). As for the shoot tissues, a number of fungal isolates obtained from root tissues varied between collection months. The highest number of isolates were obtained from the October collection (n=156) and the least from April collection (n=104). A total of 152 isolates were obtained from July collection. As observed for shoot, the representation of individual species in the root endophyte community was variable.

Diversity of endophyte communities at the ordinal level

Endophytic isolates from the 2009 collections belonged to at least 18 fungal orders (Fig. 2). Isolates from shoot tissues belonged to nine orders and root isolates to 15 orders. Three orders were unique to shoot tissues whereas nine were unique to root tissues. At least six orders were common between shoot and root tissues. Members of the order Hypocreales were the most commonly isolated fungi; constituting approximately 64% and 39% of the fungal communities in shoot and root tissue, respectively.

Diversity of endophyte communities at the species level

The species diversity indices for different fungal communities are presented in Table 1. The shoot fungal community from April had the highest species diversity whereas the least diversity was found in the October community. All three communities from shoot differed in species diversity (P< 0.001); a trend that was also evident between root fungal communities except between April and October communities. The shoot fungal community had significantly higher diversity than the root community in April whereas the opposite was true for the month of October (P<0.001).

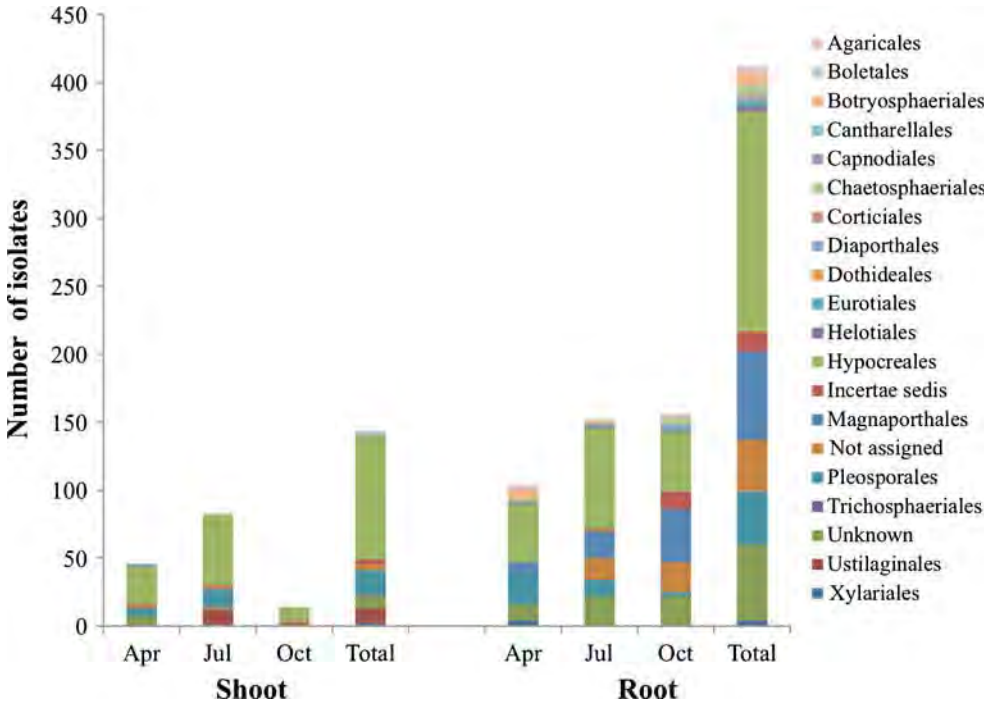

Fig. 2 Temporal distribution of endophytic fungal OTUs in switchgrass at the ordinal level

Overall, fungal communities from shoot tissues had higher species diversity than those from root tissues ($P<0.001$).

Species diversity in the east and west part of the tallgrass prairie

Out of 51 species isolated from shoot tissue, 22 were unique to the east, 18 were unique to the west and the remaining 11 were present in both parts of the prairie. Among 58 species isolated from root tissue, 16 were unique to the east, 20 were unique to the west and 22 species were present in both parts of the prairie (data not shown). The Shannon diversity index revealed that fungal communities from east and west were similar in species diversity ($P>0.05$), irrespective of either plant part or collection month (Table 2).

Soils in the east and west parts of the tallgrass prairie

The soils from the east and west parts of tallgrass prairie differed significantly in organic matter, phosphorus, calcium and sodium contents ($P\leq 0.049$; Table 3). The organic matter and calcium content was high in soils from east whereas phosphorus and sodium content was high in the soils from the west.

Table 1 Comparison of species diversity between switchgrass fungal endophyte communities from the tallgrass prairie of northern Oklahoma in 2009

Parameter	Comparisons	Number of		Shannon index (H_S)	*t*-value	Degree of freedom	*P* value
		Isolate	Species				
Shoot	April vs. July	46 vs. 83	30 vs. 28	3.241 vs. 2.898	2.775	127	<0.001
	April vs. October	46 vs. 14	30 vs. 7	3.241 vs. 1.730	7.158	58	<0.001
	July vs. October	83 vs. 14	28 vs. 7	2.898 vs. 1.730	6.167	95	<0.001
Root	April vs. July	104 vs. 152	30 vs. 34	2.610 vs. 2.865	4.476	254	<0.001
	April vs. October	104 vs. 156	30 vs. 28	2.610 vs. 2.512	1.631	258	>0.100
	July vs. October	152 vs. 156	34 vs. 28	2.865 vs. 2.512	6.920	306	<0.001
April	Shoot vs. Root	46 vs. 104	30 vs. 30	3.241 vs. 2.610	5.311	148	<0.001
July	Shoot vs. Root	83 vs. 152	28 vs. 34	2.898 vs. 2.865	0.497	233	>0.500
October	Shoot vs. Root	14 vs. 156	7 vs. 28	1.730 vs. 2.512	4.237	168	<0.001
All three months	Shoot vs. Root	143 vs. 412	51 vs. 58	3.456 vs. 3.006	9.857	553	<0.001

Table 2 Comparison of species diversity between switchgrass fungal endophyte communities from the east and west part of tallgrass prairie in 2009

Month	Plant part	Part of prairie	Number of		Shannon index (H_S)	*t*-value	Degree of freedom	*P* value
			Taxa	Species				
April	Shoot	East vs. West	18 vs. 28	14 vs. 19	2.553 vs. 2.818	1.060	44	>0.200
	Root	East vs. West	67 vs. 37	20 vs. 17	2.292 vs. 2.358	0.555	102	>0.500
July	Shoot	East vs. West	44 vs. 39	21 vs. 15	2.752 vs. 2.486	1.965	81	>0.050
	Root	East vs. West	82 vs. 70	23 vs. 25	2.647 vs. 2.770	1.477	150	>0.100
October	Shoot	East vs. West	7 vs. 7	5 vs. 3	1.550 vs. 1.004	1.491	12	>0.100
	Root	East vs. West	81 vs. 75	19 vs. 19	2.319 vs. 2.420	1.407	154	>0.100

Discussion

Endophytic fungi are ubiquitous in nature, infecting virtually all plants in both natural and agronomic ecosystems (Arnold et al. 2003; Hyde and Soytong 2008; Petrini 1986). Some of these likely have no beneficial effects on host fitness, and may be either latent pathogens or saprophytes that remain quiescent until environmental cues trigger a developmental shift for the fungus to continue its lifecycle. However, at least some endophytic fungi are consistently associated with enhanced host plant growth, persistence and stress tolerances in one or more challenging environments. It is these endophytes we hope to utilize to create associations with improved switchgrass cultivars that will maximize their utility as a bioenergy crop.

This study documents fungal community diversity in switchgrass plants from the native tall grass prairies of northern Oklahoma. We demonstrated that a wide range of fungal species from at least 18 different taxonomic orders exist as endophytes in switchgrass plants. The number of fungal isolates obtained from root tissues (n=412) was almost three times higher than that from shoot tissues (n=143). This is not surprising, as soil-borne fungi are typically much more prevalent and diversified than those that infect aerial plant tissues, via air-borne spores for example. The shoot and root endophytes were composed of at least nine and 16 orders, respectively. Despite the low diversity of the shoot endophyte community at the ordinal level, the species diversity exceeded that of the root community ($H_{S\ SHOOT}$= 3.456, $H_{S\ ROOT}$=3.006, P<0.001; Table 1). Such a discrepancy in diversity can be explained by the fact that Shannon diversity index incorporates both evenness and richness aspects of the species present in the community/population (Groth and Roelfs 1987), and suggests that the orders that are represented in the shoot community were populated with a greater number of distinct species.

Isolation of 92 distinct fungal species from asymptomatic plant tissues demonstrates that switchgrass harbors a diverse group of cultivable endophytic mycobiota. A total of 51 species were isolated from shoot tissues whereas 58 species were isolated from roots, and there were 17 species in common between these two plant parts. These common species were mainly from the genera *Alternaria*, *Codinaeopsis*, *Fusarium*, *Gibberella*, *Hypocrea* and *Periconia*, and constituted 50% and 58% of the shoot and root fungal communities, respectively. The number of endophytic fungal

Table 3 Organic matter, macronutrients, sodium and pH content of soils from the east and west parts of the tallgrass prairie in 2009

Parameter	Part of Prairie	Mean	Standard error	*t*-value (at 22 df)	*P* value
Organic matter (%)	East	4.90	0.34	4.460	0.0002
	West	2.48	0.42		
Phosphorus (ppm)	East	9	0.99	2.370	0.0267
	West	35	11		
Potassium (ppm)	East	354	63	0.350	0.7310
	West	395	99		
Calcium (ppm)	East	7684	654	3.540	0.0018
	West	4154	751		
Magnesium (ppm)	East	412	41	1.540	0.1380
	West	599	114		
Sodium (ppm)	East	40	8	2.080	0.0491
	West	96	26		
pH (1 to 14)	East	7.5	0.16	0.090	0.9310
	West	7.5	0.24		

species in switchgrass was 23% and 70% higher than those found in two perennial C_3 grasses *Ammophila arenaria* and *Elymus farctus*, respectively (Sanchez Marquez et al. 2008). However, *Holcus lanatus* and *Dactylis glomerata* both harbored more fungal endophytes than switchgrass, with 46% and 18% greater number of species recovered, respectively (Sanchez Marquez et al. 2007, 2010).

We observed an unequal distribution of isolate richness among fungal species in switchgrass. For example, 17 species that were common between shoot and root tissues constituted 56% of total fungal isolates in this study. Similarly, *Fusarium nygami* alone accounted for 18% of the study isolates. Such unequal distributions of endophytic fungal species have been reported in other grasses (Neubert et al. 2006; Sanchez Marquez et al. 2007, 2008, 2010; Wirsel et al. 2001). At the ordinal level, switchgrass fungal community was dominated by the members of Hypocreales. This order consists of at least seven families and 2,647 species that are parasitic or pathogenic to plants, other fungi and insects (Bisby et al. 2010; Kirk et al. 2008). The majority of Hypocreales in switchgrass were from the family Nectriaceae. It is somewhat surprising that none of the switchgrass isolates belonged to family Clavicipitaceae (also order Hypocreales) because these fungi are very common endophytes in cool-season grasses and *Balansia henningsiana*, a member from this family is reported to cause systemic infection in switchgrass and caespitose grass (*Panicum agrostoides*) in North America (Clay et al. 1989; Diehl 1950).

Several seasonal trends were distinguishable from our data. From a broad phylogenetic perspective, there are examples wherein fungi in certain orders appear to accumulate throughout the growing season (e. g. Magnaporthales in root), decline throughout the growing season (e.g. Pleosporales in root), or are represented fairly consistently throughout the growing season (e. g. Hypocreales). A similar trend is evident at the species or genus level of resolution, wherein some OTUs were present in all three collections representing the entire growing season (e. g. *Acremonium sp*., *Fusarium nygami*, *Gaeumannomyces instructans*, *Periconia macrospinosa*), some were present in two of three collections (e. g. *Gibberella sp*., *Hypocrea lixii*, *Microdochium sp.*, *Stachybotrys elegans*) and others were present in one of three collections only (e. g. *Microdiplodia sp*., *Myrothecium melanosporum*, *Sporisorium everhartii*). The number of isolates obtained belonging to a given OUT also varies, with some represented quite prominently in the community whereas others were only rarely recorded. Thus, examples of endophyte gain, loss and maintenance are evident at all levels of taxonomic resolution. The significance of these seasonal trends is not clear at present, but it is obvious that a population model of OTU abundance and/or diversity increasing linearly over time does not fit the data and that other factors, such as antagonism among fungi and/or abiotic effects on the host plant may be shaping endophyte community dynamics.

Intriguingly, while the total number of fungal isolates obtained from shoot tissues increased steadily from April to July and declined sharply in the October collection, the number of isolates obtained from root tissues in different collections was unaffected (Fig. 1). One possibility for this difference may be that, while root tissues remain more or less persistent throughout the growing season, foliar tissues of senescing or pre-senescent switchgrass plants have substantially lower nutrient content (Yang et al. 2009), which could be less suitable for endophytic growth. Further, the declining ambient temperatures towards the end of the growing season might have a negative impact on growth and/or maintenance of several endophytic fungi in foliar tissues.

What characteristics or criteria do we use to mine these fungi for promising candidates for incorporation into elite switchgrass cultivars? First, those endophytes that appear to be prevalent in switchgrass and present during the entire growing season may indicate significant compatibility and stability of the infection. Those strains found in our first collection trip may be exceptionally promising candidates as we would most likely inoculate seedlings with endophytes at the initiation of the growing season and they obviously can persist in switchgrass at this early stage of crop development. Taxonomic identity is also a major consideration. Members of Hypocreales were the most commonly isolated from switchgrass shoot and root tissues. The seed-borne *Neotyphodium* endophytes that very commonly form endophytic, and often mutualistic, associations with grasses also belong to the order Hypocreales (Christensen et al. 2000; de Jong et al. 2008). As mentioned previously, *Neotyphodium* endophytes are not known to infect warm-season grasses like switchgrass, but several of their relatives in Hypocreales (e.g. *Fusarium* spp. and *Acremonium* spp.) do infect these grasses, and have even been used as bio-control agents in both food crops and forage C4 grasses (Dongyi and Kelemu 2004; Horinouchi et al. 2007; Kaur et al. 2010; Kelemu et al. 2001). *Acremonium strictum*, represented in our collection at least eight times, is an effective mycoparasite, preventing the colonization and subsequent disease of the host plant caused by at least five different plant pathogenic fungi (Choi et al. 2009). A natural endophyte of maize, *Acremonium zeae*, is used to effectively eliminate mycotoxin accumulation in the kernels caused by *Fusarium verticillioides* and *Aspergillus flavus* (Wicklow et al. 2005). Further, *Acremonium zeae* produces a full complement of hemicellulolytic enzymes capable of hydrolyzing arabinoxylans from several industrially important feedstocks, demonstrating its potential for application in the bioconversion of lignocellulosic biomass into fermentable sugars

(Bischoff et al. 2009). The abundance of isolates in our collection belonging to these and related genera bodes well for our efforts.

Finally, knowledge of the soil parameters we documented from each collection site can be useful, particularly if one or more of our target planting sites are depleted or have accumulated unusually high levels of macro- or micronutrients that could inhibit switchgrass establishment and/or development. For example, one of the collection sites in the western part of the Plains borders a great salt flat with soils that are laden with sodium (286 ppm). Thus, it is reasonable to assume that some of the endophytic microbes collected from these areas may be useful in imparting some level of halotolerance to the cultivars into which we incorporate these isolates.

To our knowledge, the collection of endophytic fungi we have accumulated from switchgrass represents the largest repository of such symbionts from this important bioenergy crop. Given the constraints involved in producing a bioenergy crop, these beneficial endophytes have the potential to play an important role in maximizing the sustainability and minimizing the economic cost of growing such crops. Our approach can be likened to probing a genetically diverse natural plant population for novel genes that could be either introgressed or genetically transformed into related food crops. In this case, we are simply evaluating the natural symbiont populations of a wild grass for incorporation into elite, bred cultivars of the same species. We reasoned that the best place to look for such beneficial microbes would be in a center of switchgrass biodiversity, such as the tallgrass prairie of northern Oklahoma, where switchgrass dominates as a part of one of the last remaining native habitats for this plant.

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