

## Vertical distribution of fungal communities in tallgrass prairie soil

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**Abstract:** We used 454 sequencing of the internal transcribed spacer region to characterize fungal communities in tallgrass prairie soils subdivided into strata 0–10, 10–20, 30–40 and 50–60 cm deep. The dataset included more than 14 000 fungal sequences distributed across Basidiomycota, Ascomycota, basal fungal lineages and Glomeromycota in order of decreasing frequency. As expected the community richness and diversity estimators tended to decrease with increasing depth. Although species richness was significantly reduced for samples from the deeper profiles, even the deepest stratum sampled contained richness of more than a third of that in the topmost stratum. More importantly, nonparametric multidimensional scaling (NMS) ordination analyses indicated that the fungal communities differed across vertical profiles, although only the topmost and deepest strata were significantly different when the NMS axis scores were compared by ANOVA. These results emphasize the importance of considering the fungal communities across the vertical strata because the deeper soil horizons might maintain a distinct community composition and thus contribute greatly to overall richness. The majority of operational taxonomic units (OTUs) declined in frequency with increasing depth, although a linear regression analysis indicated that some increased with increasing depth. The OTUs and BLAST-assigned taxa that showed increasing frequencies were mainly unculturable fungi, but some showed likely affinities to families Nectriaceae and Venturiaceae or to genus *Pachnocybe*. Although the ecological roles of the fungi in the deeper strata remain uncertain, we hypothesize

that the fungi with preferences for deeper soil have adequate access to substrates and possess environmental tolerances that enable their persistence in those environments.

**Key words:** 454 sequencing, pyrosequencing, soil depth, soil fungi, tallgrass prairie

### INTRODUCTION

The soil is the host of diverse fungal communities, which derive energy from mutualistic associations with plants (Smith and Read 2008) and from consuming the abundant organic substrates embedded in the soil matrix (Cooke and Rayner 1984). The fungal community in soil has been assessed traditionally by isolating fungi in pure culture media. These studies have provided much of the fundamental understanding on the structure of the fungal communities in soil (e.g. Domsch et al. 1980) as well as necessary isolates for physiological studies and experimental manipulations of typical, culturable soil fungi. However molecular microbial community analyses indicate that culture-dependent methods are incomplete for describing soil mycobiota because a large proportion of fungi can escape detection in such studies (Amann et al. 1995, Borneman and Hartin 2000, Jumpponen and Johnson 2005, Porter et al. 2008, Schadt et al. 2003).

Soil microbial diversity has been considered nearly innumerable, even in studies using so-called high throughput molecular tools (see Buée et al. 2009, Roesch et al. 2007). The recent development of such high throughput tools, including massively parallel 454 sequencing approaches (Margulies et al. 2005), has great promise for a comprehensive community analysis. Depending on the chosen technology, a single sequencing run can produce several million base pairs (bp) of sequence reads (Cardenas and Tiedje 2008) thus allowing an unprecedented saturation of organism richness in complex environmental samples (see Roesch et al. 2007). Similarly combination of these tools with DNA-tagging allows simultaneous examination of a large number of samples without an additional sequencing cost (Hamady et al. 2008, Hoffmann et al. 2007, Meyer et al. 2008). Combined with DNA tagging, the application of these tools opens the microbial ecologist's toolbox to adequate replication of experimental units and also enables more complex and revealing experimental designs.

Because most detritus inputs and most microbial activity and biomass occur in the topmost soil profile

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TABLE I. Selected physical and chemical properties of soil cores by depth stratum

Depth	Bulk density (g/cm <sup>3</sup> ) <sup>a</sup>	% Sand <sup>a,b</sup>	% Silt <sup>a,b</sup>	% Clay <sup>a,b</sup>	% C <sup>a,c</sup>	% N <sup>a,c</sup>
0–10 cm	0.95 ± 0.01	19 ± 2	59 ± 2	21 ± 1	4.29 ± 0.28	0.34 ± 0.02
10–20 cm	1.15 ± 0.02	21 ± 5	53 ± 3	26 ± 3	2.80 ± 0.07	0.24 ± 0.01
30–40 cm	1.24 ± 0.05	14 ± 1	46 ± 1	40 ± 1	1.94 ± 0.07	0.17 ± 0.01
50–60 cm	1.32 ± 0.04	19 ± 2	44 ± 1	37 ± 1	1.21 ± 0.10	0.12 ± 0.01

<sup>a</sup>Values are means (± 1 SE) based on six soil cores, except for texture, which was based on three samples formed by compositing soil samples from 2 cores.

<sup>b</sup>Soil texture was determined with the hydrometer method following dispersal of soils with sodium hexametaphosphate.

<sup>c</sup>Soil C and N content were determined by coupled combustion and gas chromatography with a Flash EA1112 analyzer.

(Fritze et al. 2000, Lenz and Eisenbeis 1998, Snajdr et al. 2008), soil microbial communities, their diversity and richness have rarely been evaluated in the deeper soil (Fierer et al. 2003). In addition most studies on vertical distribution of fungal communities in soil focus on mycorrhizal associates of plants (Dickie et al. 2002, Egerton-Warburton et al. 2003, Oehl et al. 2005, Rillig and Field 2003). Studies on ectomycorrhizal or soil fungal community composition show vertical separation of the fungal communities in boreal forest ecosystems (Baier et al. 2006, Dickie et al. 2002, Rosling et al. 2003). From a functional standpoint Lindahl et al. (2007) suggested that saprobic fungi are confined largely to recent plant litter on the forest floor whereas ectomycorrhizal fungi tend to dominate the underlying litter and humus. Studies in arable or grassland ecosystems similarly show vertical patterning in their fungal communities. Arbuscular mycorrhizal (AM) root colonization declines with depth (Jakobsen and Nielsen 1983, Rillig and Field 2003) as do the number of AM propagules (An et al. 1990), the number of AM spores (Oehl et al. 2005, Zajicek et al. 1986), the amount of AM hyphae (Kabir et al. 1998) or the number of observed species (Oehl et al. 2005, Zajicek et al. 1986). Although AM spore densities tend to decline with soil depth, Oehl et al. (2005) observed that soil up to 70 cm deep can maintain considerable AM species richness thus supporting other findings that deeper soil can contribute substantially to the microbial diversity in soil (Fierer et al. 2003).

Our primary goals in this study were to apply 454 sequencing and DNA tagging to explore the vertical distribution of the fungal communities in a native tallgrass prairie soil and to test whether they differ in richness, diversity or composition along the vertical gradient. In addition we also aimed to pinpoint taxa that show a clear preference for deeper soil; we expected that a majority of fungi would be limited to the topmost profile. Oehl et al. (2005) identified AM species that increased in relative abundance with

increasing depth (e.g. *Scutellospora calospora* and *S. castanea*), one of which (*S. castanea*) was detected only below 20 cm. Our data show that, while fungal richness and diversity do decrease with increasing depth, the profiles below 50 cm maintain considerable diversity and some taxa seem near exclusive to these deeper soils.

#### MATERIALS AND METHODS

*Site.*—The study was conducted at the Konza Prairie Biological Station (KPBS, 39°05'N, 96°35'W), a long-term ecological research (LTER) site representative of native tallgrass prairie in the Flint Hills of eastern Kansas. KPBS spans 3487 ha, and the majority of the site remains undisturbed by agriculture. The vegetation is dominated by native grasses: big bluestem (*Andropogon gerardii* Vitman), indian grass (*Sorghastrum nutans* (L.) Nash.), little bluestem (*Schizachyrium scoparium* (Michx.) Nash.) and switch grass (*Panicum virgatum* L.) (see Towne 2002 for a complete list of vascular plants at the KPBS). The Flint Hills are generally characterized by shallow soils overlying chert-bearing limestone and shale (Ransom et al. 1998). Topographic relief divides the landscape into upland plateaus with shallow soils, slopes with outcrops of limestone and lowlands with deeper alluvial and co-alluvial soils. January mean temperature is –3 C (range –9 to –3 C) and the July mean is 27 C (range –20 to –33 C). Annual precipitation averages 835 mm, 75% of which falls in the growing season. In the present study we sampled soil profiles in the unsheltered control plots of an ongoing climate change study (Fay et al. 2000). These plots are located on relatively deep Irwin silt-like clay loams (fine, mixed, mesic Pachic Argiustolls) with slopes less than 4%. Depth to limestone varies approximately 75 cm–1.2 m. Soil texture and bulk density vary with depth, as do percent C and N (TABLE I). The experimental site is managed by annual spring burning (typically late March), a common practice that controls woody vegetation and generally enhances productivity of the dominant perennial grasses.

*Sampling, DNA extraction, PCR amplification and sequencing.*—All soil cores were collected 25–30 Nov 2007 with a Geoprobe hydraulic soil corer (Geoprobe, Salina, Kansas)

with a 1 to one-half inch (3.8 cm) inner diam coring tool to a depth of 1 m, or until rock was encountered. The cores were taken encased in plastic sleeves, which were removed, capped and placed in a  $-20$  C freezer within 5 h of collection. Cores were sectioned into 10 cm depth increments, and individual sections were thawed in a refrigerator as they were processed and subsampled for various biological, physical and chemical analyses. In the present study we used six replicate cores taken from control (unsheltered) plots that represent the native tallgrass vegetation in absence of any experimental manipulations. Two approximately 0.5 g subsamples from each 0–10, 10–20, 30–40 and 50–60 cm deep strata from each of the six cores were sampled for genomic DNA extraction and transferred into MoBio Bead Tubes (UltraClean Soil DNA, MoBio Laboratories, Carlsbad, California). Because we expected that the fungal richness and diversity would decline with soil depth we reduced the sampling intensity in deeper strata. This also reduced the sample numbers and increased the expected sequencing depth per sample. The total DNA was extracted as instructed by the manufacturer and eluted in 100  $\mu$ L of buffer S5. All templates were quantified with an ND1000 spectrometer (NanoDrop Technologies, Wilmington, Delaware) and adjusted to a final 2.5 ng  $\mu$ L<sup>-1</sup> concentration for PCR amplification.

In direct 454 sequencing of internal transcribed spacer 2 (ITS2) amplicons we synthesized primer constructs that incorporated the 454 primers (Margulies et al. 2005) and ITS primers (ITS5 and ITS4, White et al. 1990) as described in Jumpponen and Jones (2009). Initial tests with the primer combination ITS1 and ITS4 (White et al. 1990) were unsuccessful and produced no detectable amplicons. The selected primer constructs combined 454 sequencing primer (A-primer) and the reverse primer (ITS4) with a five base-pair (bp) DNA-tag for postsequencing sample identification in between, or the DNA capture bead anneal primer (B-primer) for the emulsion PCR (emPCR) and the forward primer (ITS5). This primer choice resulted in reverse sequence across the ITS2 region.

After optimization for template concentration, MgCl<sub>2</sub> concentration and annealing temperature the ITS region was PCR-amplified in 25  $\mu$ L reactions. Each subsample was amplified in three reactions to account for heterogeneous amplification from the environmental template; each stratum from each of the six cores was represented by a total of six amplicons. Each reaction contained final concentrations or absolute amounts of reagents as follows: 200 nM each of forward and reverse primers, 5 ng template DNA, 200  $\mu$ M of each deoxynucleotide triphosphate, 2.5 mM MgCl<sub>2</sub>, 1 unit GoTaq Hot Start DNA polymerase (Promega, Madison, Wisconsin) and 5  $\mu$ L Green GoTaq Flexi PCR buffer (Promega, Madison, Wisconsin). PCR cycle parameters consisted of an initial denaturation at 94 C for 3 min, then 25 cycles of denaturation at 94 C for 1 min, annealing at 54 C for 1 min and extension at 72 C for 2 min, followed by a final extension step at 72 C for 10 min. All PCR reactions were performed on MasterCyclers (Eppendorf, Hamburg, Germany). Possible amplification of contaminants was determined with blank samples run through the extraction protocol simultaneously with the actual samples

and a negative PCR control in which the template DNA was replaced with sterile H<sub>2</sub>O. These remained free of visible amplicons.

Ten microliters each of the six amplicons for each stratum from each of the cores was combined and purified with an AmPure SPRI (AgenCourt Bioscience, Beverly, Massachusetts) magnetic PCR clean-up system. The cleaned PCR products again were quantified with the ND1000 spectrometer, 75 ng clean DNA per sample combined for sequencing and this pool adjusted to 10 ng  $\mu$ L<sup>-1</sup>. The pooled products were sequenced in a GS FLX sequencer (454 Life Sciences, Branford, Connecticut) at the Interdisciplinary Center for Biotechnology Research at University of Florida. Raw data are available at the Genome Short Read Archive (SRA) at the National Center for Biotechnology Information (NCBI) under accession number SRA009960.

*Bioinformatics and OTU designation.*—The sequence analysis follows that described in Jumpponen and Jones (2009) and Jumpponen et al. (2010). In brief, sequences were removed if they contained no valid primer sequence or DNA tag, contained ambiguous bases or were shorter than our threshold (200 bp). The remaining reads were aligned with CAP3 (Huang and Madan 1999) and assigned to operational taxonomic units (OTUs) at 89–99% similarity at 2% intervals using a minimum overlap of 100 bp. An example sequence for each OTU at 99% similarity is available at GenBank (accession numbers GU306179–GU317357). The data were parsed by sample to calculate the OTU frequencies for each sample. From this output SAS (SAS Institute Inc., Cary, NC) calculated richness and diversity indices.

*Diversity indices.*—Overall OTU richness (S) was calculated by summing the number of OTUs, including singletons, within each sample. Simpson's dominance ( $D = \sum p_i^2$ ) and Shannon's diversity ( $H' = -\sum p_i [\ln\{p_i\}]$ ) were calculated for each sample, where  $p_i$  is the frequency of occurrence of each OTU. Evenness was calculated as the ratio of Shannon's diversity and richness ( $H'/\ln[S]$ ). A final index of diversity, Fisher's alpha log-series (Fisher et al. 1943) was calculated by iterating the equation  $S/N = ([1 - x]/x)(-\ln[1 - x])$ , where S is richness and N is the total number of sequences within the sample. To explore organism coverage, species accumulation (rarefaction) curves were generated with EstimateS (Colwell 2006).

*OTU frequency.*—OTU frequencies were analyzed based on OTUs assigned at 95% similarity threshold. We chose 95% sequence identity because our analyses have indicated that the total number of OTUs tends to assume a steep, near exponential increase above 95% similarity (Jumpponen and Jones 2009), either attributable to fine scale intraspecific polymorphisms or sequencing errors inherent to the pyrosequencing platform (Quince et al. 2009). We used two strategies to identify taxa that might have differed across the strata. First, nonsingleton OTUs were analyzed as a proxy for species-level resolution for effects of sampling depth (stratum) using JMP 7.0.1 (SAS Institute, Cary, North Carolina). Second, all nonsingleton OTUs were manually assigned to genera, families or orders based on the top-

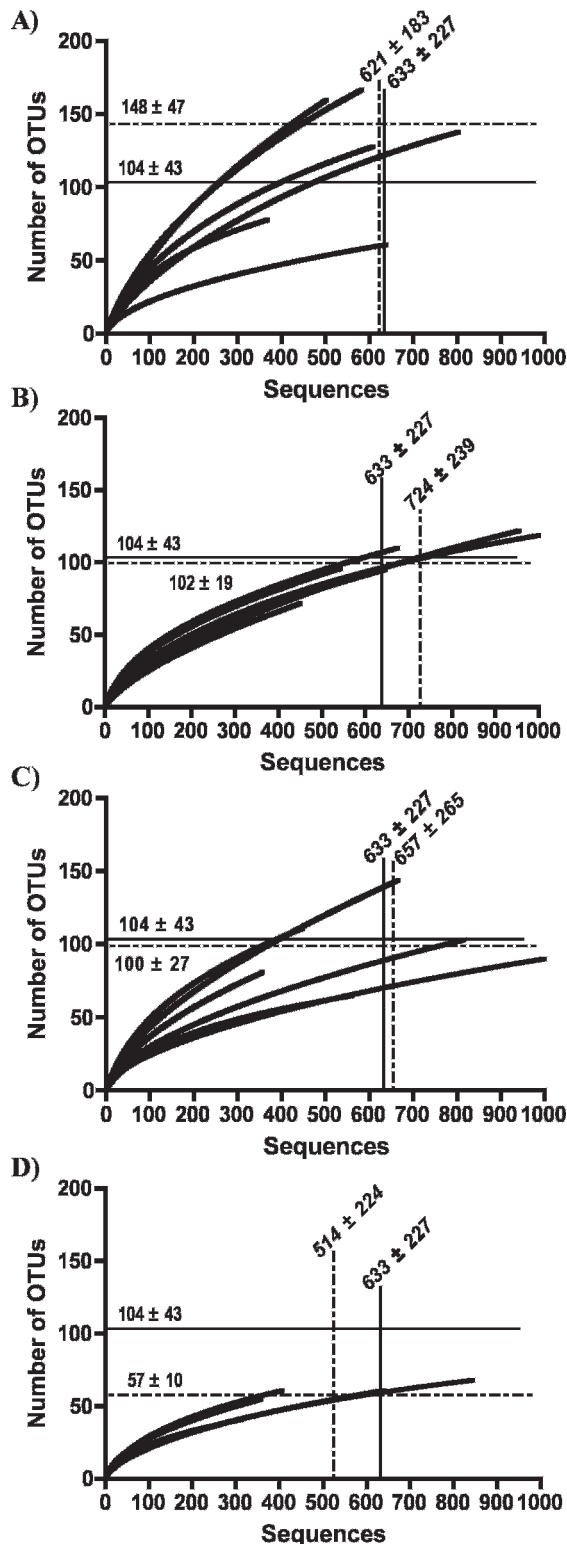


FIG. 1. Species accumulation curves for soil cores divided into A. 0–10 cm; B. 10–20 cm; C. 30–40 cm; D. 50–60 cm strata. Horizontal lines indicate the number of OTUs (mean  $\pm$  1 SD;  $n = 6$ ), vertical lines indicate the number of sequences obtained (mean  $\pm$  1SD;  $n = 6$ ), solid lines are the grand means across the entire dataset, dashed lines are the means for a particular stratum.

ranked BLAST matches (Zhang et al. 2000, SUPPLEMENTAL TABLE I), OTU frequencies summed for each core and stratum and taxon and analyzed for effects of depth (stratum). We acknowledge that the inaccuracies in database annotations (Nilsson et al. 2009, Vilgalys 2003) as well as the separate accessioning of teleomorph and anamorph genera present potential problems. Nonetheless because unambiguous sequence alignment across the ITS2 region is difficult this let us better summarize results and reduce the data volume. To minimize the number of environmental “unculturable fungus” matches to our queries we additionally applied an Entrez limit (Eukarya[ORGANISM] NOT environmental samples[FILTER] NOT unculturable[ALL FIELDS]). To improve the reliability of our taxon assignment through BLAST queries we removed reads whose assignment was based on short overlap ( $< 80\%$  coverage) or low sequence identity ( $< 90\%$  similarity) among the query and match sequences.

*Phylogenetic assignment of short MPS sequence reads.*—To improve the BLAST-based taxon assignment of the short 454 sequences we selected example sequences representing the most abundant OTUs that responded positively to sampling depth. The sequences within an OTU were selected so that where possible they represented different cores and different strata within a core. The orientation of these sequences was corrected and they were aligned with reference reads obtained from GenBank (<http://www.ncbi.nlm.nih.gov/>) in Sequencher (4.6, GeneCodes Corp., Ann Arbor, Michigan). The alignments were analyzed in MEGA4 (Tamura et al. 2007) with neighbor joining (NJ) and maximum parsimony (MP). All positions containing alignment gaps and missing data were eliminated in pairwise sequence comparisons (pairwise deletion option) in the NJ analyses. All alignment gaps were treated as missing data in the MP analyses. The robustness of the acquired topologies was assessed by 1000 bootstrap replicates in both NJ and MP analyses.

*Statistical analyses.*—We considered each soil core to represent an independent vertical sequence. The dependent variables were analyzed with linear regression against the depth in JMP 7.0.1 (SAS Institute, Cary, North Carolina). To examine community differences among the strata genus, family and order frequencies were analyzed in PC-ORD (4.1, McCune and Mefford 1999). We chose these analyses because we were concerned that the disproportionate effect of low frequency OTUs might have driven the community differences. Pairwise community distances were estimated with Sørensen (Bray-Curtis) index and analyzed by nonparametric multidimensional scaling (NMS, Mather 1976). The optimal number of dimensions ( $k$ ) was selected based on Monte Carlo test of significance at each level of dimensionality comparing 40 runs with empirical data against 50 randomized runs with a step-down in dimensionality from 6 to 1 and a random seed starting value. For all levels of taxonomic resolution, the  $k \geq 2$  dimensional solutions yielded similar results and produced solutions with stress values smaller than those in randomized runs ( $P = 0.0196$ ). Accordingly the two-dimensional solutions were selected and the data re-ordinated with  $k = 2$  configuration.

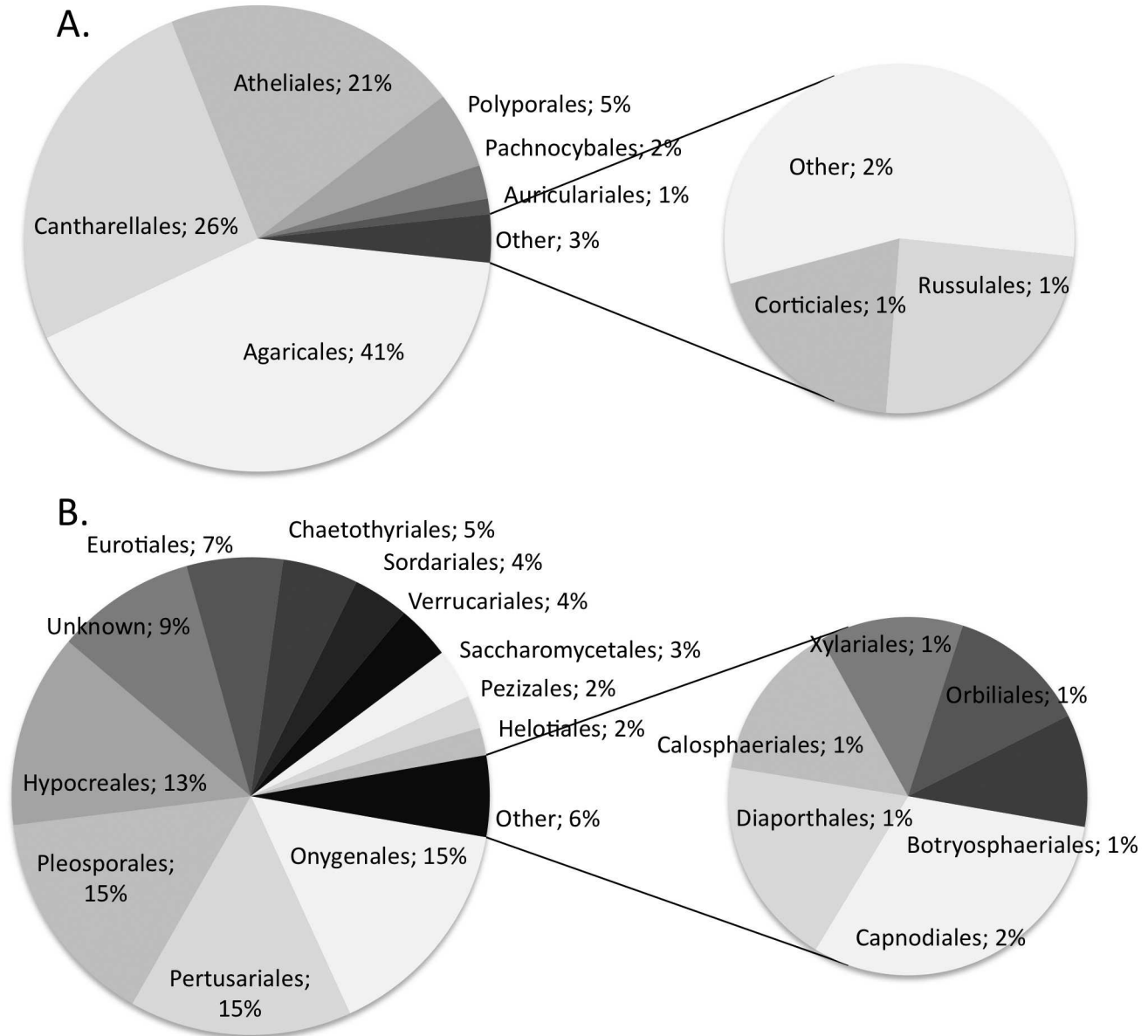


FIG. 2. Proportional distribution of fungal taxa identified from tallgrass prairie soil cores. A. Sequence assignment of basidiomycetes by order. B. assignment of ascomycetes by order.

## RESULTS

*General data characterization.*—After quality control we retained 14578 high quality sequences across the 24 samples for an average of  $633 \pm 227$  (mean  $\pm$  1SD) sequences per sample. The number of sequences was invariable among the strata (one-way ANOVA:  $F_{3,24} = 0.79$ ,  $P = 0.5148$ ) and cores (one-way ANOVA:  $F_{5,24} = 1.27$ ,  $P = 0.3198$ ), indicating that our presequencing pooling was accurate across the samples. Similarly the total number of sequences did not correlate with the sampling depth (linear regression: slope =  $-2.55 \pm 2.53$ ;  $t = -1.01$ ;  $P =$

0.3247), indicating that any observed patterns that correlated with sampling depth were not driven by unequal sequencing effort.

At 95% sequence similarity our data represented 597 nonsingleton OTUs and 554 singletons. On a sample level the observed richness estimators ranged on average from nearly 150 distinct OTUs in the topmost soil to 57 in soil 50–60 cm deep (see FIG. 1 for means and standard errors). Despite our sequencing depth of up to  $> 1000$  reads per sample, we did not saturate the richness in any of our samples, not even in those with lowest observed richness (FIG. 1A–D). Similarly extrapolative richness estimators (ACE,

TABLE II. Linear regression analyses of total richness (S), OTU singletons and various diversity indices (Fisher's  $\alpha$ , Simpsons 1/D, and Shannon's  $H'$  and dominance Simpson's D or Evenness  $H'/\ln S$ )

	Estimate St. $\pm$ Err. <sup>a</sup>	Student's $t^c$	Lower 95% CI	Upper 95% CI
Richness (S) $\downarrow^b$				
Intercept	152.86 $\pm$ 12.73	12.01***	126.39	179.33
DEPTH	-1.56 $\pm$ 0.35	-4.47***	-2.29	-0.83
Singletons				
Intercept	15.16 $\pm$ 3.26	4.65***	8.37	21.94
DEPTH	-0.17 $\pm$ 0.09	-1.86 <sup>ns</sup>	-0.35	0.02
Fisher's $\alpha$ $\downarrow^b$				
Intercept	62.04 $\pm$ 6.90	8.99***	47.68	76.39
DEPTH	-0.76 $\pm$ 0.19	-4.03***	-1.16	-0.37
Simpson's dominance (D)				
Intercept	0.08 $\pm$ 0.04	2.24*	0.01	0.16
DEPTH	0.001 $\pm$ 0.001	1.47 <sup>ns</sup>	-0.001	0.003
Simpson's diversity (1/D) $\downarrow^b$				
Intercept	18.91 $\pm$ 3.74	5.06***	11.14	26.69
DEPTH	-0.22 $\pm$ 0.10	-2.11*	-0.43	-0.002
Shannon's diversity ( $H'$ ) $\downarrow^b$				
Intercept	3.78 $\pm$ 0.23	16.45***	3.30	4.26
DEPTH	-0.02 $\pm$ 0.01	-3.17**	-0.03	-0.01
Evenness ( $H'/\ln S$ )				
Intercept	0.75 $\pm$ 0.04	18.56***	0.66	0.83
DEPTH	-0.001 $\pm$ 0.001	-1.74 <sup>ns</sup>	-0.004	0.0004

<sup>a</sup> Each analysis fits a model  $Y = \text{Intercept} + \text{Depth } X$ , where  $Y$  is the response variable,  $X$  is the depth and the Intercept and Depth are the intercept and slope terms with their respective standard errors, 95% confidence intervals and Student's  $t$ -tests displayed in the table.

<sup>b</sup> Arrows indicate the direction of the estimated response with increasing depth.

<sup>c</sup> ns  $P > 0.05$ , \*  $0.01 < P \leq 0.05$ , \*\*  $0.001 < P \leq 0.01$ , \*\*\*  $P \leq 0.001$ .

Chao1 and first-order Jackknife; not shown) nearly always more than doubled the observed richness (S), indicating that the coverage in our assessment was still inadequate.

We assigned OTUs to taxa with the aid of BLAST and aimed to reduce environmental sequences with Entrez filters. Of the 14 384 sequences (98.67% of all retained sequences) that could be placed with this strategy, 14 336 (99.67%) were assigned to kingdom Fungi. The few remaining, nonfungal sequences represented Cercozoa, Proteobacteria, Viridiplantae, Alveolata, Euglenozoa and Metazoa. These nonfungal sequences are likely to be either artifacts, erroneously annotated reads or might represent novel organisms. For example the queries that returned Cercozoan reads were of low coverage ( $< 35\%$ ) and therefore unlikely to represent true Cercozoan affinities. In contrast the queries that returned reads within Viridiplantae represented true nonfungal targets (OTUs 1746 and 2342 were assigned to *Arabidopsis thaliana* [accession number AC006837]

and *Spartina densiflora* [AJ489786]) or erroneous annotations (OTU 2412 query returned *Begonia ravenii* [accession number AJ491250] but has a likely affinity within *Tilletiopsis* as indicated by the number of Entylomatales accessions that followed *Begonia*).

Among the fungi basidiomycetes (54%) dominated, followed by ascomycetes (41%), zygomycetes (3%, including those assigned to the basal lineages), glomeromycetes (1%) and chytridiomycetes (1%). Basidiomycetes were distributed across 20 orders and were dominated by Agaricales, Cantharellales and Atheliales (FIG. 2A). The ascomycetes were distributed relatively evenly across 35 orders and dominated by Onygenales, Pertusariales, Pleosporales, and Hypocreales (FIG. 2B). The majority of taxa belonging to the basal fungal lineages were not assigned to orders. Those that were belonged to Mortierellales, Mucorales and Endogonales. Glomeromycota were dominated by Diversisporales, followed by Glomerales, Archeosporales and Paraglomerales. Chytridiomycota comprised Chytridiales, Spizellomycetales and Rhizophydiales.

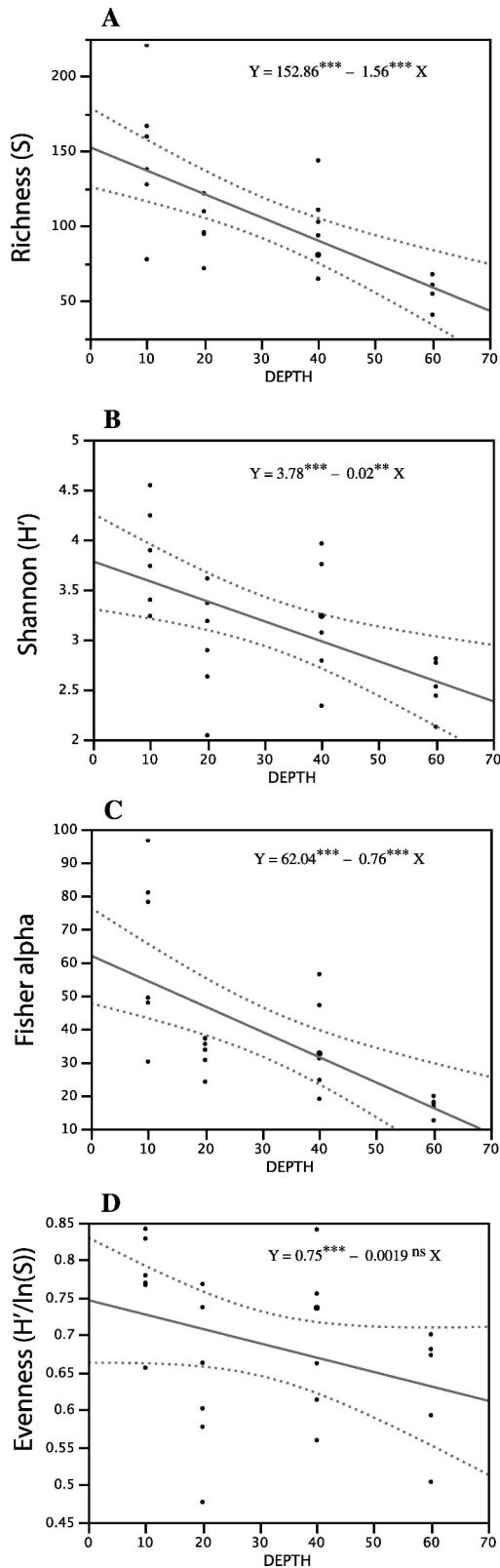


FIG. 3. Richness and diversity of fungal communities as a function of soil depth. Solid lines indicate the linear regression; the dashed lines indicate the 95% confidence intervals. A. OTU richness or number of detected species

*Fungal communities across soil profiles.*—As we expected, most richness and diversity estimators showed a declining trend with increasing depth (TABLE II). Richness, Fisher's  $\alpha$ , Simpson's  $1/D$  and Shannon's  $H'$  declined (FIG. 3A–D), whereas Simpson's dominance ( $D$ ) and evenness were not affected (TABLE II). There were no examples of genera, families or orders that tended to increase in frequency with greater depth. In contrast the relative abundance of most taxa, regardless of the taxon assignment (genus, family or order), tended to decline with increasing depth unless they were too infrequent to analyze. On the level of an OTU, 25 showed a significant declining trend in abundance whereas eight tended to increase with the increasing depth (TABLE III). (Examples of these responses are shown in SUPPLEMENTAL FIG. 1.)

Those OTUs that showed increasing trends with soil depth represented mainly uncultured ascomycetes (OTUs 426, 1003, 1603, 1954, 2072, 2081, 2142, 2292) or were placed within Pucciniomycetes (OTU1710, assigned to genus *Pachnocybe*) by BLAST queries. Our limited phylogenetic analyses on the most abundant and most strongly responding OTUs supported these assignments or provided further details on some OTU assignments. In the MP analyses (FIG. 4A) the environmental 454 reads exemplifying OTU1710 clustered on a well supported clade with members of Septobasidiaceae and Pachnocybaceae, whereas the NJ analyses assigned (80% bootstrap support) this OTU as a sister group of *Pachnocybe ferruginea* (Pachnocybaceae). OTU1954 was confirmed to have ascomycetous affinities, likely within Venturiaceae (FIG. 4B). The example reads clustered on a clade that included mitosporic *Fusicladium africanum* in addition to uncultured, unidentified fungi. However this clade remained unsupported in the analyses. OTU2142 was assigned within Nectriaceae after exclusion of those GenBank sequences that contained “environmental sample” as a filter (FIG. 4C). Our MP and NJ analyses supported this assignment and placed the environmental example reads on a clade that included mitosporic *Cylindrocarpum destructans*. Note however that the three *C. destructans* accessions did not form a monophyletic group. The assignment of most OTUs could not be improved by further analyses. For example OTU2072

(S). B. Shannon-Wiener diversity index ( $H'$ ). C. Fisher's alpha ( $\alpha$ ). D. Evenness ( $H'/\ln(S)$ ). Note that although evenness shows a declining trend the slope for this term is not significant ( $P = 0.0968$ ). ns  $P > 0.05$ , \*  $0.01 < P \leq 0.05$ , \*\*  $0.001 < P \leq 0.01$ , \*\*\*  $P \leq 0.001$ .

TABLE III. Linear regression analyses of those OTUs that showed significant responses to sampling depth

	BLAST affinity <sup>a</sup>	Term <sup>b</sup>	Estimate $\pm$ St. Err. <sup>a</sup>	Student's $t^d$	Lower 95%	Upper 95%
OTU 9 ↓	<i>Sordaria tomento-alba</i> [AY681195]	Intercept	$6.2 \times 10^{-3} \pm 1.7 \times 10^{-3}$	3.74**	$2.8 \times 10^{-3}$	$9.7 \times 10^{-3}$
—		DEPTH	$-1.3 \times 10^{-4} \pm 0.4 \times 10^{-4}$	-2.85**	$-2.2 \times 10^{-4}$	$-0.3 \times 10^{-4}$
OTU 36 ↓	Uncultured basidiomycete [DQ672273]	Intercept	$2.4 \times 10^{-3} \pm 0.7 \times 10^{-3}$	3.26**	$0.9 \times 10^{-3}$	$4.0 \times 10^{-3}$
—	<i>Hygrocybe hypohaemacta</i> [EU435150]	DEPTH	$-5.0 \times 10^{-5} \pm 2.0 \times 10^{-5}$	-2.52*	$-9.1 \times 10^{-5}$	$-0.9 \times 10^{-5}$
OTU 44 ↓	<i>Entrophospora infrequens</i> [U94714]	Intercept	$5.8 \times 10^{-3} \pm 2.1 \times 10^{-3}$	2.76*	$1.4 \times 10^{-3}$	$10.2 \times 10^{-3}$
—		DEPTH	$-1.2 \times 10^{-4} \pm 0.6 \times 10^{-4}$	-2.12*	$-2.4 \times 10^{-4}$	$-0.2 \times 10^{-5}$
OTU 50 ↓	<i>Cercophora coprophila</i> [AY999136]	Intercept	$3.0 \times 10^{-3} \pm 1.1 \times 10^{-3}$	2.73*	$0.7 \times 10^{-3}$	$5.3 \times 10^{-3}$
—		DEPTH	$-6.2 \times 10^{-5} \pm 2.9 \times 10^{-5}$	-2.10*	$-1.2 \times 10^{-5}$	$-0.1 \times 10^{-5}$
OTU 61 ↓	<i>Epicoccum nigrum</i> [DQ981396]	Intercept	$2.0 \times 10^{-3} \pm 0.7 \times 10^{-3}$	2.97**	$0.7 \times 10^{-3}$	$3.4 \times 10^{-3}$
—		DEPTH	$-4.0 \times 10^{-5} \pm 1.8 \times 10^{-5}$	-2.27*	$-7.7 \times 10^{-5}$	$-0.3 \times 10^{-5}$
OTU 71 ↓	Uncultured fungus [DQ420990]	Intercept	$1.7 \times 10^{-3} \pm 0.5 \times 10^{-3}$	3.53**	$0.7 \times 10^{-3}$	$2.7 \times 10^{-3}$
—	Fungal endophyte [EU685980]	DEPTH	$-3.1 \times 10^{-5} \pm 1.3 \times 10^{-5}$	-2.44*	$-8.8 \times 10^{-5}$	$-0.5 \times 10^{-5}$
OTU 89 ↓	Fungal sp. [EU816397]	Intercept	$2.6 \times 10^{-3} \pm 0.9 \times 10^{-3}$	3.03**	$0.8 \times 10^{-3}$	$4.5 \times 10^{-3}$
—		DEPTH	$-5.3 \times 10^{-5} \pm 2.3 \times 10^{-5}$	-2.30*	$-1.0 \times 10^{-5}$	$-0.5 \times 10^{-5}$
OTU 106 ↓	<i>Apodus deciduus</i> [AY681199]	Intercept	$3.2 \times 10^{-3} \pm 1.2 \times 10^{-3}$	3.13**	$1.1 \times 10^{-3}$	$5.3 \times 10^{-3}$
—		DEPTH	$-6.2 \times 10^{-5} \pm 2.7 \times 10^{-5}$	-2.31*	$-1.1 \times 10^{-5}$	$-0.6 \times 10^{-5}$
OTU 137 ↓	Uncultured Dothideomycete [EU680546]	Intercept	$9.8 \times 10^{-3} \pm 3.3 \times 10^{-3}$	2.97**	$3.0 \times 10^{-3}$	$16.7 \times 10^{-3}$
—	Fungal endophyte [FJ450027]	DEPTH	$-2.0 \times 10^{-4} \pm 0.9 \times 10^{-4}$	-2.18*	$-3.7 \times 10^{-4}$	$-0.1 \times 10^{-4}$
OTU 167 ↓	Uncultured fungus [DQ420990]	Intercept	$2.6 \times 10^{-3} \pm 0.6 \times 10^{-3}$	4.19***	$1.3 \times 10^{-3}$	$3.8 \times 10^{-3}$
—	Fungal endophyte [EU685980]	DEPTH	$-5.1 \times 10^{-5} \pm 1.6 \times 10^{-5}$	-3.15**	$-8.5 \times 10^{-5}$	$-1.7 \times 10^{-5}$
OTU 211 ↓	<i>Terfezia</i> sp. [DQ061109]	Intercept	$3.6 \times 10^{-3} \pm 1.2 \times 10^{-3}$	3.00**	$1.1 \times 10^{-3}$	$6.0 \times 10^{-3}$
—		DEPTH	$-6.2 \times 10^{-5} \pm 3.1 \times 10^{-5}$	-2.23*	$-1.3 \times 10^{-5}$	$-0.5 \times 10^{-5}$
OTU 228 ↓	Uncultured fungus [DQ421205]	Intercept	$3.3 \times 10^{-3} \pm 0.9 \times 10^{-3}$	3.66**	$1.5 \times 10^{-3}$	$5.2 \times 10^{-3}$
—	Ascomycete sp. [DQ092523]	DEPTH	$-6.1 \times 10^{-5} \pm 2.4 \times 10^{-5}$	-2.51*	$-1.1 \times 10^{-5}$	$-1.1 \times 10^{-5}$
OTU 237 ↓	<i>Pyrenochaeta lycopersici</i> [AY649585]	Intercept	$2.5 \times 10^{-3} \pm 0.9 \times 10^{-3}$	2.95**	$0.8 \times 10^{-3}$	$4.3 \times 10^{-3}$
—		DEPTH	$-5.0 \times 10^{-5} \pm 2.3 \times 10^{-5}$	-2.19*	$-9.7 \times 10^{-5}$	$-0.3 \times 10^{-5}$
OTU 263 ↓	Uncultured fungus [FJ386864]	Intercept	$4.5 \times 10^{-3} \pm 1.5 \times 10^{-3}$	3.06**	$1.4 \times 10^{-3}$	$7.5 \times 10^{-3}$
—	<i>Mortierella alpina</i> [AJ271630]	DEPTH	$-9.0 \times 10^{-5} \pm 3.9 \times 10^{-5}$	-2.33*	$-1.7 \times 10^{-5}$	$-1.0 \times 10^{-5}$
OTU 266 ↓	Uncultured fungus [DQ421295]	Intercept	$3.7 \times 10^{-3} \pm 1.1 \times 10^{-3}$	3.23**	$1.3 \times 10^{-3}$	$6.0 \times 10^{-3}$
—	<i>Entophycis</i> sp. [DQ273782]	DEPTH	$-7.4 \times 10^{-5} \pm 3.0 \times 10^{-5}$	-2.45*	$-1.3 \times 10^{-5}$	$-1.1 \times 10^{-5}$
OTU 271 ↓	<i>Alternaria</i> sp. [FJ465171]	Intercept	$2.8 \times 10^{-3} \pm 0.9 \times 10^{-3}$	2.98**	$0.8 \times 10^{-3}$	$4.6 \times 10^{-3}$
—	<i>Alternaria</i> sp. [FJ466719]	DEPTH	$-5.2 \times 10^{-5} \pm 2.5 \times 10^{-5}$	-2.12*	$-1.0 \times 10^{-5}$	$-0.1 \times 10^{-5}$
OTU 415 ↓	Uncultured Pezizomycotina [EF027382]	Intercept	$2.7 \times 10^{-3} \pm 0.8 \times 10^{-3}$	3.15**	$0.9 \times 10^{-3}$	$4.5 \times 10^{-3}$
—	Fungal endophyte [AY700129]	DEPTH	$-5.4 \times 10^{-5} \pm 2.3 \times 10^{-5}$	-2.36*	$-1.0 \times 10^{-5}$	$-0.7 \times 10^{-5}$
OTU 426 ↓	Ascomycota sp. [FJ008694]	Intercept	$1.5 \times 10^{-3} \pm 2.1 \times 10^{-3}$	-0.73 <sup>ns</sup>	$-5.9 \times 10^{-3}$	$2.8 \times 10^{-3}$
—		DEPTH	$1.2 \times 10^{-4} \pm 0.6 \times 10^{-4}$	2.08*	0.0	$2.3 \times 10^{-4}$
OTU 611 ↓	Uncultured fungus [DQ420837]	Intercept	$4.6 \times 10^{-3} \pm 1.0 \times 10^{-3}$	4.51***	$2.5 \times 10^{-3}$	$6.7 \times 10^{-3}$
—	Epacris endophyte [AY268201]	DEPTH	$-8.5 \times 10^{-5} \pm 2.7 \times 10^{-5}$	-3.17**	$-1.4 \times 10^{-5}$	$-2.9 \times 10^{-5}$
OTU 729 ↓	<i>Phoma pinodella</i> [AB369504]	Intercept	$8.5 \times 10^{-3} \pm 2.3 \times 10^{-3}$	3.63**	$3.6 \times 10^{-3}$	$13.3 \times 10^{-3}$
—		DEPTH	$-1.6 \times 10^{-4} \pm 0.6 \times 10^{-4}$	-2.51*	$-2.8 \times 10^{-4}$	$-0.3 \times 10^{-4}$
OTU 740 ↓	<i>Fusarium solani</i> [EF152426]	Intercept	$7.2 \times 10^{-3} \pm 1.7 \times 10^{-3}$	4.22***	$3.7 \times 10^{-3}$	$10.8 \times 10^{-3}$



TABLE III. Continued

	BLAST affinity <sup>a</sup>	Term <sup>b</sup>	Estimate ± St. Err. <sup>a</sup>	Student's <i>t</i> <sup>d</sup>	Lower 95%	Upper 95%
—		DEPTH			-2.4×10 <sup>-4</sup>	-0.5×10 <sup>-4</sup>
<b>OTU 1003</b> ↓ <sup>c</sup>	Uncultured fungus [DQ4211173]	Intercept	-4.8×10 <sup>-4</sup> ± 5.9×10 <sup>-4</sup>	-3.17**	-16.8×10 <sup>-4</sup>	7.8×10 <sup>-4</sup>
—	<i>Aspicilia verruculosa</i> [EU057941]	DEPTH	3.6×10 <sup>-5</sup> ± 1.6×10 <sup>-5</sup>	2.32*	0.4×10 <sup>-5</sup>	6.9×10 <sup>-5</sup>
<b>OTU 1201</b> ↓ <sup>c</sup>	Uncultured fungus [FJ213516]	Intercept	7.4×10 <sup>-3</sup> ± 2.4×10 <sup>-3</sup>	3.08**	2.4×10 <sup>-3</sup>	12.4×10 <sup>-3</sup>
—	<i>Cladosporium cladosporioides</i> [EU563957]	DEPTH	-1.5×10 <sup>-4</sup> ± 0.6×10 <sup>-4</sup>	-2.29*	-2.8×10 <sup>-4</sup>	-0.1×10 <sup>-4</sup>
<b>OTU 1257</b> ↓ <sup>c</sup>	Uncultured ascomycete [AY970195]	Intercept	7.0×10 <sup>-2</sup> ± 1.5×10 <sup>-2</sup>	4.81***	4.0×10 <sup>-2</sup>	10.1×10 <sup>-2</sup>
—	<i>Cylindrosporium lauri</i> [EU035414]	DEPTH	-1.1×10 <sup>-3</sup> ± 0.4×10 <sup>-3</sup>	-2.89**	-1.9×10 <sup>-3</sup>	-0.3×10 <sup>-3</sup>
<b>OTU 1597</b> ↓ <sup>c</sup>	Uncultured ascomycete [AY970195]	Intercept	7.7×10 <sup>-3</sup> ± 2.3×10 <sup>-3</sup>	3.43**	3.1×10 <sup>-3</sup>	12.4×10 <sup>-3</sup>
—	<i>Venturia cerasi</i> [EU035452]	DEPTH	-1.5×10 <sup>-4</sup> ± 0.6×10 <sup>-4</sup>	-2.59*	-2.8×10 <sup>-4</sup>	-0.3×10 <sup>-4</sup>
<b>OTU 1603</b> ↓ <sup>c</sup>	Uncultured basidiomycete [AY970288]	Intercept	-1.1×10 <sup>-2</sup> ± 2.1×10 <sup>-2</sup>	-0.53 ns	-5.6×10 <sup>-2</sup>	3.3×10 <sup>-2</sup>
—	<i>Clitocybe subditopoda</i> [EU669216]	DEPTH	1.3×10 <sup>-3</sup> ± 0.6×10 <sup>-3</sup>	2.30*	0.1×10 <sup>-3</sup>	2.5×10 <sup>-3</sup>
<b>OTU 1608</b> ↓ <sup>c</sup>	Uncultured fungus [EU826902]	Intercept	2.9×10 <sup>-2</sup> ± 0.6×10 <sup>-2</sup>	4.90***	1.7×10 <sup>-2</sup>	4.2×10 <sup>-2</sup>
—	Zygomycete sp. [EU428773]	DEPTH	-4.7×10 <sup>-4</sup> ± 1.6×10 <sup>-4</sup>	-2.97**	-7.9×10 <sup>-4</sup>	-1.4×10 <sup>-4</sup>
<b>OTU 1710</b> ↓ <sup>c</sup>	<i>Pachnocybe ferruginea</i> [DQ241473]	Intercept	-9.5×10 <sup>-3</sup> ± 10.6×10 <sup>-3</sup>	-0.89 ns	-31.4×10 <sup>-3</sup>	12.5×10 <sup>-3</sup>
—		DEPTH	6.6×10 <sup>-4</sup> ± 2.8×10 <sup>-4</sup>	2.35*	0.8×10 <sup>-4</sup>	12.4×10 <sup>-4</sup>
<b>OTU 1824</b> ↓ <sup>c</sup>	Uncultured fungus [EU826902]	Intercept	2.1×10 <sup>-3</sup> ± 0.6×10 <sup>-3</sup>	3.77**	1.0×10 <sup>-3</sup>	3.3×10 <sup>-3</sup>
—	Zygomycete sp. [EU428773]	DEPTH	-3.3×10 <sup>-5</sup> ± 1.5×10 <sup>-5</sup>	-2.20*	-6.4×10 <sup>-5</sup>	-0.2×10 <sup>-5</sup>
<b>OTU 1954</b> ↓ <sup>c</sup>	Uncultured fungus [AM260882]	Intercept	-1.0×10 <sup>-2</sup> ± 0.7×10 <sup>-2</sup>	-1.40 ns	-2.6×10 <sup>-2</sup>	0.5×10 <sup>-2</sup>
—	Ascomycete sp. [FJ008694]	DEPTH	7.1×10 <sup>-4</sup> ± 2.0×10 <sup>-4</sup>	3.60**	3.0×10 <sup>-4</sup>	11.2×10 <sup>-4</sup>
<b>OTU 2072</b> ↓ <sup>c</sup>	Uncultured fungus [DQ421173]	Intercept	-7.4×10 <sup>-2</sup> ± 3.9×10 <sup>-2</sup>	-1.92 ns	-15.5×10 <sup>-2</sup>	0.6×10 <sup>-2</sup>
—	<i>Aspicilia verruculosa</i> [EU057941]	DEPTH	4.4×10 <sup>-3</sup> ± 1.0×10 <sup>-3</sup>	4.28***	2.3×10 <sup>-3</sup>	6.5×10 <sup>-3</sup>
<b>OTU 2081</b> ↓ <sup>c</sup>	Uncultured fungus [DQ421173]	Intercept	-2.2×10 <sup>-3</sup> ± 1.5×10 <sup>-3</sup>	-1.43 ns	-5.3×10 <sup>-3</sup>	1.0×10 <sup>-3</sup>
—	<i>Aspicilia verruculosa</i> [EU057941]	DEPTH	1.3×10 <sup>-4</sup> ± 0.4×10 <sup>-4</sup>	3.12**	0.4×10 <sup>-4</sup>	2.1×10 <sup>-4</sup>
<b>OTU 2142</b> ↓ <sup>c</sup>	Uncultured Nectriaceae [EU754942]	Intercept	-5.8×10 <sup>-4</sup> ± 10.1×10 <sup>-4</sup>	-0.57 ns	-26.7×10 <sup>-4</sup>	15.1×10 <sup>-4</sup>
—	<i>Neonectria radicitola</i> [FJ841036]	DEPTH	6.6×10 <sup>-5</sup> ± 2.7×10 <sup>-5</sup>	2.47*	1.1×10 <sup>-5</sup>	12.1×10 <sup>-5</sup>
<b>OTU 2292</b> ↓ <sup>c</sup>	Uncultured ascomycete [AY970195]	Intercept	-2.2×10 <sup>-3</sup> ± 2.4×10 <sup>-3</sup>	-0.92 ns	-7.2×10 <sup>-3</sup>	2.8×10 <sup>-3</sup>
—	Ascomycota sp. [FJ008694]	DEPTH	1.5×10 <sup>-4</sup> ± 0.6×10 <sup>-4</sup>	2.29*	0.1×10 <sup>-4</sup>	2.8×10 <sup>-4</sup>

<sup>a</sup> BLAST affinity is the top most match to a megablast query. If the blast results with and without Entrez filter differ, the latter of the two for each OTU identifies the match when those sequences with the filter "Environmental sample = TRUE" were omitted.

<sup>b</sup> Each analysis fits a model Y = Intercept + Depth X, where Y is the OTU frequency, X is the depth and the Intercept and Depth are the intercept and slope terms.

<sup>c</sup> Arrows indicate the direction of the frequency response.

<sup>d</sup> ns *P* > 0.05, \* 0.01 < *P* ≤ 0.05, \*\* 0.001 < *P* ≤ 0.01, \*\*\* *P* ≤ 0.001.



could be assigned only among uncultured fungi and among those the example read placement received no support in either MP or NJ analyses (not shown). Similarly OTUs 1003 and 2081 could be aligned among well accessioned sequences representing Helotiaceae or lichenized families Pertusariaceae, Ramalinaeae and Physciaceae. However these affinities were either not supported or the NJ and MP analyses provided contradictory results (not shown).

In addition to differences in the frequencies among the commonly occurring OTUs, our NMS ordination separated the communities in different soil depths (FIG. 5) regardless of the chosen rank. The two ordination axes represented 75.0%, 63% and 64.9% of the variance in the dataset assigned on the level of an order, family and genus respectively. When the NMS scores for the two axes were analyzed in a one-way ANOVA, the first axis scores were never significantly different among soil depths ( $P > 0.7500$ ) whereas the second axis scores were always significantly or almost significantly different ( $P = 0.0445$ ,  $P = 0.0917$ ,  $P = 0.0277$  for order, family and genus analyses respectively). The post-hoc tests (Tukey's HSD) of the second axis scores indicated that only the two extreme strata had different communities whereas the communities detected in 10–20 cm and 30–40 cm deep soil did not differ from either the uppermost or lowest stratum (NMS for the genus level is an example in FIG. 5).

While causative associations cannot be inferred from ordination analyses such as the NMS used here, they do allow examination of correlations between community members and the environments within which those communities occur. Our NMS analyses suggest that, while the frequencies of most genera correlate with topmost strata (negative Axis 2 values in TABLE IV), relatively few correlate with the samples from deeper soils (positive Axis 2 values in TABLE IV). To illustrate the most strongly correlated genera we plotted five genera with most extreme negative and positive Axis 2 scores (FIG. 5). These analyses suggest that OTUs assigned to genera *Cudoniella* (Helotiales), *Cylindrosyndonium* (Venturiceae, Pleosporales), *Malassezia* (Malasseziales), *Phialocephala* (mitosporic Helotiales) and *Ramichloridium* (mitosporic Capnodiales) are correlated with deeper soils.

#### DISCUSSION

We combined 454 sequencing and DNA tagging to characterize soil-inhabiting fungal communities across a vertical gradient. Our analyses detected a large number of OTUs; at 95% sequence identity we captured an estimated 1151 distinct OTUs, half of

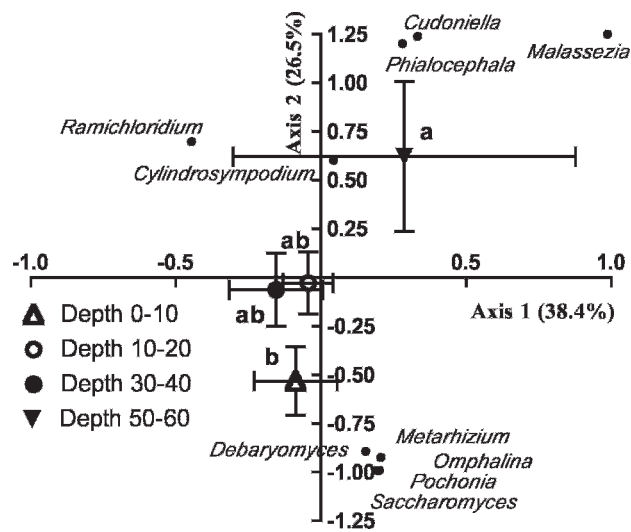


FIG. 5. Nonmetric multidimensional scaling (NMS) of genus frequencies observed across the four strata in the tallgrass prairie soil. The percentages next to the axes indicate the variance represented by that axis. Strata with different letters differ according to Tukey's HSD at  $\alpha < 0.05$  on the second ordination axis. Note that the one-way ANOVA indicated no stratum differences on the first ordination axis. Five genera with the most extreme scores on the Axis 2 are shown for the inference of taxa with potential correlation with stratum depth (TABLE IV).

which occurred only once in the dataset. Each of our experimental units representing a stratum within a core contained on average more than a hundred unique OTUs. While our sequencing depth was substantial (more than 600 sequences per sample on average and more than 1000 in the most deeply sequenced sample) it was inadequate to reach a point of saturation for the resident taxon richness. Recent data suggest that sequencing depths substantially greater—more than an order of magnitude greater—might be necessary to approach a plateau in the species accumulation curves for soil-inhabiting fungal communities (Buée et al. 2009). Similarly for bacterial communities in soil a far greater sequencing effort is required for thorough community characterization (Roesch et al. 2007). However it presently remains unclear what these species richness estimators mean because the 454 pyrosequencing platform may produce a substantial proportion of artifacts (Quince et al. 2009). We assume these biases to be stochastically distributed through the dataset and therefore likely to contribute to the noise instead of the signal that we were able to detect. It also is important to note that our analyses focus on the comparisons on the level of experimental unit and aim not to extrapolate ecosystem-wide richness estimators.

TABLE IV. NMS scores for the genera observed in the tallgrass prairie soil

Genus <sup>a</sup>	Axis 1 score	Axis 2 score
<b>Omphalina</b>	0.19474	-0.99129 <sup>c</sup>
<b>Pochonia</b>	0.19474	-0.99129 <sup>c</sup>
<b>Saccharomyces</b>	0.19474	-0.99129 <sup>c</sup>
<b>Metarhizium</b>	0.20613	-0.92408 <sup>c</sup>
<b>Debaryomyces</b>	0.15368	-0.89242 <sup>c</sup>
<i>Beauveriana</i>	0.3411	-0.81576
<i>Chaetomium</i>	0.3411	-0.81576
<i>Colletotrichum</i>	0.3411	-0.81576
<i>Discostroma</i>	0.3411	-0.81576
<i>Erythrobasidium</i>	0.3411	-0.81576
<i>Gracilacus</i>	0.3411	-0.81576
<i>Helicoma</i>	0.3411	-0.81576
<i>Neurospora</i>	0.3411	-0.81576
<i>Spizellomyces</i>	0.3411	-0.81576
<i>Cercophora</i>	0.0803	-0.81345
<i>Gibberella</i>	0.29687	-0.79074
<i>Cryptococcus</i>	0.09348	-0.77082
<i>Articulospora</i>	0.12953	-0.72933
<i>Tetracladium</i>	0.20886	-0.70999
<i>Apodus</i>	0.20422	-0.70994
<i>Entrophospora</i>	-0.07733	-0.70911
<i>Cordyceps</i>	-0.14651	-0.67894
<i>Aspergillus</i>	-0.16676	-0.67658
<i>Spirosphaera</i>	-0.09779	-0.64637
<i>Sordaria</i>	0.07988	-0.64579
<i>Hygrocybe</i>	-0.67796	-0.64454
<i>Epicoccum</i>	0.12328	-0.63745
<i>Phoma</i>	-0.03209	-0.62817
<i>Anthostomella</i>	-0.19652	-0.61063
<i>Nectria</i>	0.21914	-0.59525
<i>Aureobasidium</i>	0.06412	-0.59423
<i>Panaeolus</i>	-0.47954	-0.56924
<i>Mortierella</i>	-0.07319	-0.56342
<i>Monacrosporium</i>	0.01279	-0.55317
<i>Cistella</i>	-0.03285	-0.54689
<i>Arniium</i>	-0.0446	-0.54563
<i>Codinaeopsis</i>	-0.0446	-0.54563
<i>Preussia</i>	-0.0446	-0.54563
<i>Pycnidiophora</i>	-0.0446	-0.54563
<i>Stachybotrys</i>	-0.05873	-0.48639
<i>Fonsecaea</i>	0.24197	-0.48569
<i>Cephalotheca</i>	-0.06446	-0.48425
<i>Morganella</i>	-0.05669	-0.48064
<i>Bullera</i>	-0.06722	-0.47711
<i>Microdiplodia</i>	-0.13618	-0.46469
<i>Periconia</i>	-0.13965	-0.4428
<i>Marasmiellus</i>	0.03872	-0.44202
<i>Fusarium</i>	0.14157	-0.43986
<i>Alternaria</i>	0.12675	-0.42713
<i>Nigrospora</i>	-0.08703	-0.41707
<i>Corynascus</i>	-0.18059	-0.39851
<i>Dactylaria</i>	-0.18059	-0.39851
<i>Dioszegia</i>	-0.18059	-0.39851
<i>Sporisorium</i>	-0.18059	-0.39851
<i>Mycena</i>	-0.31713	-0.38529

TABLE IV. Continued

Genus <sup>a</sup>	Axis 1 score	Axis 2 score
<i>Capronia</i>	-0.14031	-0.36949
<i>Candida</i>	1.86799	-0.36794
<i>Leptosphaeria</i>	0.19539	-0.36729
<i>Phialemonium</i>	0.19539	-0.36729
<i>Ramularia</i>	-0.10377	-0.35731
<i>Podospora</i>	-0.04845	-0.35666
<i>Cladosporium</i>	-0.107	-0.34644
<i>Rhexocercosporidium</i>	-0.32203	-0.30461
<i>Glomus</i>	-0.06532	-0.27859
<i>Hypocrea</i>	-0.1785	-0.27265
<i>Humicola</i>	-0.26528	-0.26713
<i>Phaeosphaeria</i>	-0.1515	-0.26149
<i>Schizothecium</i>	-0.04145	-0.26102
<i>Myrothecium</i>	-0.18111	-0.26097
<i>Lophiostoma</i>	-0.14305	-0.20788
<i>Davidiella</i>	-0.36171	-0.17937
<i>Curvularia</i>	-0.19403	-0.13942
<i>Flagelloscypha</i>	-0.548	-0.12251
<i>Penicillium</i>	0.07197	-0.11981
<i>Gliomastix<sup>b</sup></i>	-0.04466	-0.06571
<i>Lachnum</i>	-0.32875	0.16023
<i>Buergenerula</i>	-0.36639	0.20486
<i>Clitopilus</i>	-0.66829	0.2393
<i>Geomyces</i>	-0.66829	0.2393
<i>Schizophyllum</i>	-0.66829	0.2393
<i>Peziza</i>	-0.3641	0.29666
<i>Coniosporium</i>	-0.39291	0.34826
<i>Teratospora</i>	-0.06587	0.47766
<i>Trichoderma</i>	-0.06587	0.47766
<i>Ceratobasidium</i>	-0.11399	0.52488
<b>Cylindrosporium</b>	0.04292	0.60318 <sup>c</sup>
<b>Ramichloridium</b>	-0.44623	0.69825 <sup>c</sup>
<b>Phialocephala</b>	0.28039	1.20182 <sup>c</sup>
<b>Cudoniella</b>	0.33191	1.46486 <sup>c</sup>
<b>Malassezia</b>	0.98609	1.84349 <sup>c</sup>

<sup>a</sup>Taxa are arranged by the Axis 2 scores to emphasize those likely correlated with the topmost strata (low Axis 2 scores) or with deep strata (high Axis 2 scores).

<sup>b</sup>The cross line midtable emphasizes where the first axis intercepts the second axis.

<sup>c</sup>Boldfaced taxa are shown in FIG. 5; Axis 2 scores for *Malassezia* and *Cudoniella* were modified to illustrate them in FIG. 5.

Detected OTUs were broadly distributed across 35 ascomycetous and 20 basidiomycetous orders in addition to a small number of Glomeromycota and orders representing basal fungal lineages. Assignment of ecological roles to molecular OTUs is problematic. The taxa detected in our sampling cover a wide variety of orders, many of which include members that are lichenized (e.g. Lecanorales, Peltigerales, Pertusariales), pathogens (e.g. Capnodiales, Pucciniales, Ustilaginales), parasites (e.g. Entorrhizales,

Erysiphales), saprobes (e.g. Orbiliales, Sporidiobolales, Xylariales), or mutualistic root symbionts (e.g. Glomerales, Diversisporales, Archeosporales, Paraglomerales). Many of the observed taxa are likely present only as dormant propagules (e.g. foliar inhabitants such as Erysiphales and Mycosphaerellaceae) and probably do not participate in soil ecosystem functions. To account for only the actively functioning taxa approaches that more specifically target the active microbial communities are necessary. Such approaches include the reverse transcription of the ribosomal RNA or its precursors (see Anderson and Parkin 2007, Anderson et al. 2008).

Our results support our hypotheses and earlier observations that the majority of fungal species richness and diversity are concentrated in the topmost horizons in soil (Oehl et al. 2005, Rosling et al. 2003, Zajicek et al. 1986). However it is noteworthy that substantial community richness was maintained in the deeper soil. More importantly, our ordination analyses show that while adjacent soil strata may differ only slightly, distinct fungal communities occupy the topmost and deep soil strata. These broad community level differences are further supported by our analyses at the level of individual OTUs, particularly with respect to the fungi whose relative frequencies increased with sampling depth. While the functions of these fungi remain elusive and are difficult to pinpoint, our results underline the importance of including vertical strata in a community characterization (Fierer et al. 2003).

We attempted to assign sequences to taxa in the case of fungi that appeared more frequently in deeper soil strata. At the OTU level the linear regression analyses suggested that it is mainly uncultured fungi that showed preferences for deeper strata. The OTUs with affinities to well accessioned taxa represented mainly saprobes, parasites or pathogens. Venturiaceae (OTU1954) are mainly endophytes and weak parasites of plants, infecting usually aerial plant tissues. *Fusicladium africanum* that appears closely related to these environmental sequences has been isolated from *Eucalyptus* litter (Crous et al. 2007), suggesting a saprobic habit. Nectriaceae (represented by OTU2142) similarly infect plant tissues but are often pathogens or weak parasites. The OTU (OTU1710) with affinities within Pucciniomycetes was assigned within Septobasidiales and had likely affinities to either *Pachnocybe* or *Septobasidium*. The former is found on wood, whereas the latter include obligate parasites of insects (Henk and Vilgalys 2007).

When the OTUs were grouped by genus, the NMS analyses suggested a few taxa that positively correlated with the deeper soil (TABLE IV). As with the OTUs

that were inferred from the regression analyses, the reasons why these taxa are detected in the deeper soil strata remain unclear; for example two genera, *Malassezia* and *Cudoniella*, present a puzzle. In addition to our study *Malassezia* has been detected in the fungal communities from deep-sea sediments in the South China Sea (Lai et al. 2007). Its presence there, beyond anthropogenic contamination, remains a mystery. However this taxon is commonly associated with mammals as well as nematodes (Renker et al. 2003) and therefore can inhabit even deeper soil if appropriate hosts are present. *Cudoniella* is considered an aero-aquatic genus (Wang et al. 2005) among a number of other aquatic Helotiales (Goos 1987). Its presence in deeper soil may be attributable to long-maintained water saturation at these depths or simply spore transport to these strata with water infiltration through the soil profile. These correlations inferred from the regression and ordination analyses should be considered with caution. However our results emphasize the importance of further and more extensive sampling in the deeper soil horizons.

In conclusion we characterized fungal communities across a vertical gradient in tallgrass prairie soil with 454 pyrosequencing. While we fell short of saturation of species richness, we successfully tested hypotheses on species richness, diversity and community composition as a function of soil depth. Our results show that fungal community richness and diversity decline with soil depth. In addition our data show that the communities are distinct among the strata. Although many community members decline with soil depth, we were able to pinpoint some that increase. The ecology and nutritional modes of the fungi that are more abundant in deeper soil strata remain elusive. However we speculate that those fungi have access to their primary substrates in those strata and possess the environmental tolerance necessary to survive in those environments.

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