# Taxonomic partitioning of root placement by depth in a prairie plant community

#### Christopher K. Black1,2, Scott A. Woolbright2,3, Taylor L. Pederson2, Christopher R. Sligar2, Evan H. DeLucia1,2

1. Department of Plant Biology, University of Illinois at Urbana-Champaign
2. Carl. R. Woese Institute for Genomic Biology, University of Illinois
3. University of Arkansas at Little Rock

## 

## Running Head

Prairie root partitioning by depth

## Corresponding Author

Evan H. DeLucia  
Department of Plant Biology  
University of Illinois at Urbana-Champaign  
265 Morrill Hall  
505 South Goodwin Ave.  
Urbana, IL 61801  
Phone: (217) 333-6177  
Fax: (217) 244-7246  
Email: delucia@illinois.edu

## Author contributions

CKB, SAW, EHD designed the experiment; CKB, SAW, TLP, CRS collected data; SAW, TLP, CRS developed laboratory protocols; CKB, SAW developed data analysis methods; SAW, EHD provided funding; CKB, SAW, TLP, CRS, EHD wrote the paper.

## Keywords

Niche partitioning, DNA metabarcoding, grassland, root community, biodiversity

## Abstract

TK.

## Introduction

In a rapidly changing world, one of the most pressing questions in ecology is which ecosystem properties promote stability and which lead to rapid state changes. It is now well recognized that diversity generally promotes resilience in ecosystem productivity (Tilman et al. 2001), partly through facilitation but primarily through functional complementarity that allows more effective partitioning and therefore more complete exploitation of niche space, producing a more stable overall allocation of resources across the community (Fornara and Tilman 2008, 2009, De Deyn et al. 2008). This implies that the resilience benefits of niche partitioning will only be realized if different species in the community posess functions that are actually complementary to each other. For example, N and water availability are often strongly anticorrelated in grassland soils, leading *Festuca ovina* and *Achillea millefolium* to extract N from different depths when competing in mixed-species stands than when they are grown alone, while *Phleum pratense* and *Trifolium pratense* showed no such vertical partitioning (Jumpponen et al. 2002).

In addition to functional complementarity, the resilience of grassland systems may rely on diversity in *spatial* partitioning as well. Prairie plant communities are noted for maintaining a large proportion of their biomass below ground (Jackson et al. 1996), and the importance of belowground interactions between species with differing root traits for determining community prductivity has long been recognized (Weaver 1919, Bardgett et al. 2014). Existing evidence suggests that grassland plant communities may be more more strongly structured belowground than aboveground (Kesanakurti et al. 2011, Hiiesalu et al. 2012, Wilson 2014) and that the physiological capacity to exploit soil resources is a strong predictor of competitive success (Tucker et al. 2011, Kroon et al. 2012, Hendriks et al. 2015). Therefore, to understand the potential responses of multispecies communities to destabilized conditions such as a changing climate, it is necessary to understand the physical placement of the different species in the community as well as the between-species differences in resource allocation and niche partitioning strategies.

Progress in understanding these belowground dynamics is slowed by the challenges of studying belowground environments, especially for mixed-species samples. Because harvesting root systems requires digging and root systems show extreme spatial variability that increases the sample size needed to characterize a system, all methods are laborious and most require destructive harvests, often in the form of massive excavations to identify roots by physically tracing them to the plant crown (Weaver and Voigt 1950). Visual identification of roots based on anatomical or morphological characteristics is sometimes possible (Wardle and Peltzer 2003) but is an inherently low-throughput method that requires a trained specialist and becomes much more challenging for very fine roots or highly diverse communities. Minirhizotron methods can provide some information on root placement, but have limited success at distinguishing between species (Rewald et al. 2012).

With the increasing availability of high-throughput DNA sequencing, molecular methods now provide a partial solution to this dilemma. DNA-based methods can use smaller samples and therefore less-destructive sampling techniques, allow distinctions between visually identical roots, and permit quantitative comparisons. Additionally, because DNA persists to some extent in dead tissue, sequencing potentially can integrate longer timescales by detecting species that are present in the community but were dormant or senesced at the time of sampling. The internal transcribed spacer between the 5.8S and 26S regions of the nuclear ribosomal DNA (ITS2) is a popular choice for barcoding of mixed root species recovered from soil: It is easily extracted from all plant groups, widely used in root sequencing (Jackson et al. 1999) and therefore well represented in databases, and short enough (~200 bases) to sequence on an Illumina platform. Although higher taxonomic resolution can be obtained from multiple barcodes (e.g. *rcbL* and *matK*; Staats et al. 2016), assigning identities to multiple barcodes in mixed samples is challenging (Rewald et al. 2012) and ITS2 alone provides comparable resolution to other single barcodes (Chen et al. 2010, Fahner et al. 2016).

The objective of this study was to identify the root species present at varying soil depths (0-100 cm) in a restored prairie in central Illinois, and to use these identities to infer differences in species roles for water usage, N uptake, and carbon storage. We used a DNA metabarcoding approach: we collected fine root samples by coring and extracted whole-community DNA for ITS2 amplicon library construction (Fliudigm Access Array) followed by sequencing (Illumina MiSeq 2x300), then identified the detected sequences to genus level by database search and compared the resulting abundance estimates against those from aboveground surveys.

## Methods

### Experimental site

Our experimental site is the University of Illinois Energy Farm (Urbana, Illinois, USA: 40.06N, 88.19W, elevation 220 m), a bioenergy research facility founded to compare the ecological and economic impacts of perennial grasses against those of conventional row crops when both are grown as feedstocks for fuel production. Four cropping systems are grown side-by-side; For this experiment we were interested in multispecies root communities and therefore sampled only from the five replicates (four 0.7 Ha plots plus one 3.8 Ha plot) of the prairie treatment. The site has a continental climate with a mean annual temperature of 11°C and approximately 1 m of precipitation annually. It is established on deep, highly fertile Mollisol soils (Argiudolls, mapped as Dana, Flanagan and Blackberry silt loam) and was used for agriculture for at least 100 years before establishment of the current experiment. For futher details on the establishment and management of the site, see previous work by (Zeri et al. 2011, Smith et al. 2013, Masters et al. 2016).

The prairie plots were established in 2008 by seeding with a mix of 28 species native to Illinois Tbl. 1. Species were chosen to balance four stated goals: Maximum biomass production, ecosystem resilience, trophic diversity, and locally adapted species. Seeds were obtained from Pizzo and Associates (Leland IL), treated at planting time with a mycorrhizal inoculum (“AM 120”; Reforestation Technologies International; Gilroy CA) that is primarily *Glomus intraradices* (Neal Anderson, RTI Inc.; personal communication), and overseeded with a spring oat (*Avena sativa*) cover crop. The plots were mowed after senescence each year and the above-ground biomass was baled and removed. 25 of these 28 species remained detectable in 2011 (Feng and Dietze 2013) and in 2013 a total of 32 species were censused in the plots Tbl. 1.

### Sample collection

To characterize the spatial distribution of species with depth, we collected mixed root samples on July 15-18 of 2013, after most late-season grasses were well emerged but before most early-season plants began to senesce. At 24 locations (one in each quadrant of each 0.7 Ha plot, two in each quadrant of the 3.8 Ha plot), we used an 8 cm bucket auger to collect roots and soil from five depth increments (0-10, 10-30, 30-50, 50-75, and 75-100 cm) of 3 cores within a 2 m radius. We pooled all three cores from each location, collected a ~0.5-1 kg subsample of mixed roots and soil from each depth, and returned the remaining material to the holes. The resulting 120 samples were stored on ice in 1-gallon Ziplok bags for transport to the laboratory, then frozen at -80 °C the same day and stored until further analysis.

To characterize the genetic diversity of our target species and generate a mock community for use as a sequencing control, we collected voucher specimens on August 31 and September 1, 2013. For each of the 33 plant species present in aboveground surveys(X. Feng, unpublished data), we located 3-5 individuals, identified them to species by leaf and flower morphology, and used a trowel to extract roots still attached to their well-identified crown. We pooled all roots from each species, placed them in Ziplok bags, placed them on ice for transport to the laboratory, and froze them at -80 °C the same day for storage until further analysis.

### Root recovery

To separate roots from soil, we thawed mixed samples overnight at 4 °C, then screened them through a 2 mm sieve followed by manually picking all visible roots using forceps. The picking process took about 30-90 minutes per sample and all sieves, forceps, and gloves were wiped with ethanol immediately before use to minimize contamination by non-sample DNA. After root picking, a subsample of the root-free bulk soil was collected and lyophilized, then ground and and combusted to determine total carbon and nitrogen content (4010 CHNSO Elemental Analyzer; Costech, Valencia CA).

We then rinsed all roots in three changes of sterile water, with the final rinse including 10 minutes of sonication to dislodge any residual soil from the the root surface, then lyophilized all samples and stored them at room temperature. Single-species root voucher samples were treated identically to the mixed root samples, with the exception that bulk soil had been removed at collection time and therefore no sieving or hand-picking steps were necessary.

### DNA extraction and amplification

To maximize extraction of DNA from tough root tissue, we ground all samples once in a dry mortar and pestle at room temperature, then again in liquid nitrogen to a very fine powder. We then weighed ~100 mg of tissue from each sample and extracted whole DNA using a Powersoil-htp isolation kit (Mo Bio Laboratories, Carlsbad CA) according to the manufacturer’s directions, including an optional initial bead-beating step. We then performed a post-extraction cleanup using materials from the same kit (E. Adams, Mo Bio; personal communication): We diluted the DNA to a volume of 100 µL with DNAse-free water, added 50 µL of bead beating solution and inverted to mix, then added 25 µL each of solutions C2 and C3, inverted to mix, and centrifuged at 10000 xg for 2 minutes. We then collected the supernatant, added 2 volumes of solution C4, vortexed, and loaded the sample onto a spin filter. Finally, we washed with 500 µL of solution C5 and eluted with 50 µL of C6.

After extraction, we submitted whole DNA to the W.M. Keck Center (Urbana, IL, USA) for amplification and sequencing. In addition to DNA extracted from mixed samples, we included nominally pure extracts of root DNA from four species with high aboveground abundance (*Andropogon gerardii*, *Sorghastrum nutans*, *Silphium perfoliatum*, and *Elymus nutans*; Feng 2014), water extractions as a negative control on the DNA extraction + PCR + sequencing process taken as a whole, and a mock community of DNA from 29 species combined in equimolar quantity plus one species (*Heliopsis helianthoides*) at twice the concentration of the others. The second internal transcribed spacer of the nuclear ribosome gene (ITS2) was amplified from each sample by microfluidic PCR using the Fluidigm Access Array chip and sequenced by synthesis for 2x301 paired-end cycles (MiSeq, V3 chemistry, Illumina Inc, San Diego CA). The 5’ linker construct for each sequence consisted of Illumina flowcell-binding primer i5, Fluidigm linker pad CS1, and plant-specific ITS2 primer S2F (Chen et al. 2010) for a final foward linker sequence of 5'-AATGATACGGCGACCACCGAGATCT-ACACTGACGACATGGTTCTACA-ATGCGATACTTGGTGTGAAT. The 3’ linker construct for each sequence consisted of the Illumina flowcell-binding primer i7, a 10-base oligonucleotide barcode that was unique for each sample, Fluidigm linker pad CS2, and plant-specific ITS2 primer S3R (Chen et al. 2010) for a final reverse linker sequence of 5'-CAAGCAGAAGACGGCATACGAGAT-XXXXXXXXXX-TACGGTAGCAGAGACTTGGTCT-GACGCTTCTCCAGACTACAAT, where XXXXXXXXXX indicates the variable barcode sequence.

### Data processing

The raw Illumina read files were preprocessed by sequencing center staff before delivery by using CASAVA 1.8 to remove sequencing primers, PhiX reference reads, and reads from unrelated samples sequenced in the same flowcell lane. We then used cutadapt 1.8.1 (Martin 2011) to trim primers, discard all reads that did not begin with the expected primer, and trim 3’ bases with a Phred quality score below 20. We then joined the overlapping ends of each read using using the RDP maximum likelihood algorithm (Cole et al. 2013) as implemented in Pandaseq 2.10 (Masella et al. 2012) using a minimum alignment quality of 0.8, a minimum assembled length of 25 bases, and a minimum overlap of at least 20 “bits saved” (corresponds to ~10 bases; see Cole et al. 2013). We then used the split\_libraries\_fastq.py script in QIIME 1.9.1 (Caporaso et al. 2010) to assign barcodes to sequence identities.

To assign sequences to taxonomic units, we used a de novo clustering approach. We dereplicated sequences and removed singletons and suspected PCR chimeras using VSEARCH 2.0.4 (Rognes et al. 2016), extracted full-length ITS2 variable regions using ITSx 1.0.11 (Bengtsson Palme et al. 2013), clustered the results using VSEARCH with a similarity threshold of 99%, and assigned taxonomy to each cluster using BLAST+ (Camacho et al. 2009) against the GenBank nt database. We then mapped taxonomy for the full dataset by using VSEARCH to search the full sequence file (including singletons and chimeras) against these cluster centroids, also with a similarity threshold of 99%. After taxonomy assignment, we collapsed all clusters assigned as the same phylotype (species, genus, or family depending on the analysis of interest) into single taxon groups using the phyloseq 1.16.2 package (McMurdie and Holmes 2013) in R 3.3.1 (R Core Team 2016), then corrected for between-sample differences in sequencing depth by transforming raw read counts for each taxon group into sample proportions. Taxa with a mean abundance less than 1% per sample were removed from plots, but included in mutivariate analyses.

To visualize the effects of depth and C/N content on root community composition, we performed nonmetric multidimensional scaling followed by permutational MANOVA using the adonis function of vegan 2.4-1 (Oksanen et al. 2016) in R 3.31 (R Core Team 2016), using Jaccard distance as the response variable; depth, C, and N as environmental variables; ‘plot:location’ as a blocking effect.

Full analysis scripts and raw sequence data are available online at (Dryad URL TK).

## Results

The MiSeq run and sequence cleanup produced ample, high-quality sequences for the planned anlysis: The raw file contained 1286163 plant ITS2 reads, of which 730235 were successfully end-paired, quality filtered, and assigned back from barcodes to samples. These clean reads contained 494505 unique sequences, of which 459986 were observed only once in the dataset (singletons). Of the 34519 sequences observed at least twice, 2219 were identified as probable or borderline PCR chimeras by vsearch. These chimeric sequences accounted for a total of 8526 reads, or ~1.2% of the raw dataset. A further 75 sequences were identified as incomplete or undetectable ITS2 regions by ITSx, leaving a total of 32225 ITS2 sequences to be clustered at 99% similarity into 1347 OTUs, which we then used as reference sequences at 99% similarity to map the 730235 reads (singletons included) of the cleaned sequence file, for a final sequence-by-sample table of 576650 reads. Sample coverage was excellent, with 110 of the 120 mixed root samples having enough reads (1355-8998) for confident analysis. There was no apparent change in total read count between samples from differing depths.

After obtaining best-match barcode identities for each of the 1347 OTUs clustered at 99%, we collapsed OTUs to phylotypes by combining groups of OTUs that all yielded the same taxon as their top BLAST hit. When we collapsed at the species level, we obtained 158 phylotypes that were identified as coming from 70 genera across 16 families, which is notably higher than the 32 species, 22 genera, and 6 families known from site vegetation surveys Tbl. 1. Many samples contained high read counts from several different species of genera that have only one known species at the site Figs. S2, S3. From this we infer that, at least with current databases, ITS2 barcode identities are may be more reliable at the genus level than the species level.

We observed more reads from grasses at increasing depths, both relative to other functional types (Fig. 3) and in absolute abundance (Fig. **??**). Forbs from the Asteraceae declined somewhat with depth, while reads from legumes were consistent across depths.

When grouped by genus, additional differences between forbs were visible (Fig. S4, Fig. **??**): Within the Asteraceae, Ratidida (fibrous rooted) and Siplhium (tap-rooted) appear to trade off, with Ratidida less prevalent at middle depths than either shallow or deep layers while Silphium was rare at the surface but most common at middle depths. The fibrous-rooted genera Coreopsis, Solidago, Symphotrichum, and the shallow-taprooted Taraxacum, all declined with depth. All genera of grasses were generally higher in abundance at deeplayers than near the surface, but Panicum, Elymus, and Sorghastrum had their greatest abundance at 75 cm while Andropogon and Schizachrium both continued to increase all the way to 100 cm.

…talk about weighting by total root mass here; likely that all species have greatest mass at the surface but that forbs win for overall total while grasses have small but ecologically important deep pools.

## References

Anderson-Teixeira, K. J., M. D. Masters, C. K. Black, M. Zeri, M. Z. Hussain, C. J. Bernacchi, and E. H. DeLucia. 2013. Altered Belowground Carbon Cycling Following Land-Use Change to Perennial Bioenergy Crops. Ecosystems 16:508–520.

Bardgett, R. D., L. Mommer, and F. T. De Vries. 2014. Going underground: root traits as drivers of ecosystem processes. TRENDS in Ecology and Evolution 29:692–699.

Bengtsson Palme, J., M. Ryberg, M. Hartmann, S. Branco, Z. Wang, A. Godhe, P. Wit, M. Sánchez García, I. Ebersberger, F. Sousa, A. Amend, A. Jumpponen, M. Unterseher, E. Kristiansson, K. Abarenkov, Y. J. K. Bertrand, K. Sanli, K. M. Eriksson, U. Vik, V. Veldre, and R. H. Nilsson. 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods in Ecology and Evolution 4:914–919.

Camacho, C., G. Coulouris, V. Avagyan, N. Ma, J. Papadopoulos, K. Bealer, and T. L. Madden. 2009. BLAST+: architecture and applications. BMC Bioinformatics 10:421.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight. 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7:335–336.

Chen, S., H. Yao, J. Han, C. Liu, J. Song, L. Shi, Y. Zhu, X. Ma, T. Gao, X. Pang, K. Luo, Y. Li, X. Li, X. Jia, Y. Lin, and C. Leon. 2010. Validation of the ITS2 Region as a Novel DNA Barcode for Identifying Medicinal Plant Species. Plos One 5:e8613.

Cole, J. R., Q. Wang, J. A. Fish, B. Chai, D. M. McGarrell, Y. Sun, C. T. Brown, A. Porras-Alfaro, C. R. Kuske, and J. M. Tiedje. 2013. Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucleic Acids Research 42:D633–D642.

De Deyn, G. B., J. H. C. Cornelissen, and R. D. Bardgett. 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. Ecology Letters 11:516–531.

Fahner, N. A., S. Shokralla, D. J. Baird, and M. Hajibabaei. 2016. Large-Scale Monitoring of Plants through Environmental DNA Metabarcoding of Soil: Recovery, Resolution, and Annotation of Four DNA Markers. Plos One 11:e0157505.

Feng, X. 2014. Productivity, physiology, community dynamics, and ecological impacts of a grassland agro-ecosystem: integrating field studies and ecosystem modeling. PhD thesis, University of Illinois At Urbana-Champaign; University of Illinois At Urbana-Champaign.

Feng, X., and M. Dietze. 2013. Scale dependence in the effects of leaf ecophysiological traits on photosynthesis: Bayesian parameterization of photosynthesis models. New Phytologist 200:1132–1144.

Fornara, D. A., and D. Tilman. 2008. Plant functional composition influences rates of soil carbon and nitrogen accumulation. Journal of Ecology 96:314–322.

Fornara, D. A., and D. Tilman. 2009. Ecological mechanisms associated with the positive diversity-productivity relationship in an N-limited grassland. Ecology 90:408–418.

Hendriks, M., J. M. Ravenek, A. E. Smit Tiekstra, J. W. Paauw, H. Caluwe, W. H. Putten, H. Kroon, and L. Mommer. 2015. Spatial heterogeneity of plant–soil feedback affects root interactions and interspecific competition. New Phytologist 207:830–840.

Hiiesalu, I., M. Öpik, M. Metsis, L. LILJE, J. Davison, M. Vasar, M. Moora, M. Zobel, S. D. Wilson, and M. Pärtel. 2012. Plant species richness belowground: higher richness and new patterns revealed by next‐generation sequencing. Molecular Ecology 21:2004–2016.

Jackson, R. B., J. G. Canadell, J. R. Ehleringer, H. A. Mooney, O. E. Sala, and E. D. Schulze. 1996. A global analysis of root distributions for terrestrial biomes. Oecologia 108:389–411.

Jackson, R. B., L. A. Moore, W. A. Hoffmann, W. T. Pockman, and C. R. Linder. 1999. Ecosystem rooting depth determined with caves and DNA. Proceedings Of The National Academy Of Sciences Of The United States Of America 96:11387–11392.

Jumpponen, A., P. Högberg, K. Huss-Danell, and C. P. H. Mulder. 2002. Interspecific and spatial differences in nitrogen uptake in monocultures and two‐species mixtures in north European grasslands. Functional Ecology 16:454–461.

Kesanakurti, P. R., A. J. Fazekas, K. S. Burgess, D. M. Percy, S. G. Newmaster, S. W. Graham, S. C. H. Barrett, M. Hajibabaei, and B. C. Husband. 2011. Spatial patterns of plant diversity below-ground as revealed by DNA barcoding. Molecular Ecology 20:1289–1302.

Kroon, H. de, M. Hendriks, J. van Ruijven, J. Ravenek, F. M. Padilla, E. Jongejans, E. J. W. Visser, and L. Mommer. 2012. Root responses to nutrients and soil biota: drivers of species coexistence and ecosystem productivity. Journal of Ecology 100:6–15.

Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17:10.

Masella, A. P., A. K. Bartram, J. M. Truszkowski, D. G. Brown, and J. D. Neufeld. 2012. PANDAseq: paired-end assembler for illumina sequences. BMC Bioinformatics 13:31.

Masters, M. D., C. K. Black, I. B. Kantola, K. P. Woli, T. Voigt, M. B. David, and E. H. DeLucia. 2016. Soil nutrient removal by four potential bioenergy crops: Zea mays, Panicum virgatum, Miscanthus× giganteus, and prairie. Agriculture, Ecosystems & Environment 216:51–60.

McMurdie, P. J., and S. Holmes. 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. Plos One 8:e61217.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O’Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, and H. Wagner. 2016. vegan: Community ecology package.

R Core Team. 2016. R: A language and environment for statistical computing. Version 3.3.1. R Foundation for Statistical Computing, Vienna, Austria.

Rewald, B., C. Meinen, M. Trockenbrodt, J. E. Ephrath, and S. Rachmilevitch. 2012. Root taxa identification in plant mixtures – current techniques and future challenges. Plant and Soil 359:165–182.

Rognes, T., T. Flouri, B. Nichols, C. Quince, and F. Mahé. 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584.

Smith, C. M., M. B. David, C. A. Mitchell, M. D. Masters, K. J. Anderson-Teixeira, C. J. Bernacchi, and E. H. DeLucia. 2013. Reduced Nitrogen Losses after Conversion of Row Crop Agriculture to Perennial Biofuel Crops. Journal of Environment Quality 42:219.

Staats, M., A. J. Arulandhu, B. Gravendeel, A. Holst-Jensen, I. Scholtens, T. Peelen, T. W. Prins, and E. Kok. 2016. Advances in DNA metabarcoding for food and wildlife forensic species identification. Analytical and Bioanalytical Chemistry 408:4615–4630.

Tilman, D., P. B. Reich, J. Knops, D. Wedin, T. Mielke, and C. Lehman. 2001. Diversity and productivity in a long-term grassland experiment. Science 294:843–845.

Tucker, S. S., J. M. Craine, and J. B. Nippert. 2011. Physiological drought tolerance and the structuring of tallgrass prairie assemblages. Ecosphere 2.

Wardle, D. A., and D. A. Peltzer. 2003. Interspecific interactions and biomass allocation among grassland plant species. Oikos 100:497–506.

Weaver, J. E. 1919. The Ecological Relations of Roots. Carnegie Institution of Washington, Washington.

Weaver, J. E., and J. W. Voigt. 1950. Monolith method of root-sampling in studies on succession and degeneration. Botanical Gazette 111:286–299.

Wilson, S. D. 2014. Below‐ground opportunities in vegetation science. JOURNAL OF VEGETATION SCIENCE 25:1117–1125.

Zeri, M., K. J. Anderson-Teixeira, G. C. Hickman, M. D. Masters, E. H. DeLucia, and C. J. Bernacchi. 2011. Carbon exchange by establishing biofuel crops in Central Illinois. Agriculture, Ecosystems & Environment 144:319–329.

Table 1: Plant species planted or found present during aboveground vegetation surveys of permanent quadrats in prairie restoration plots at the University of Illinois Energy Farm. Mean and standard deviation of stem abundance and percent cover across growing season 2012 (n = 30 quadrats \* 4 sampling days = 120) are realculated from X. Feng et al. (manuscript in prep).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Functional type | Family | Species | Planted? | % cover | sd |
| C3 grass | Cyperaceae | *Carex bicknellii* | y | 1.4 | 2.3 |
| C3 grass | Poaceae | *Elymus canadensis* | y | 2.2 | 3.4 |
| C4 grass | Poaceae | *Andropogon gerardii* | y | 34.3 | 13.7 |
| C4 grass | Poaceae | *Schizachyrium scoparium* | y | 3.0 | 3.4 |
| C4 grass | Poaceae | *Sorghastrum nutans* | y | 21.2 | 10.5 |
| Forb | Asteraceae | *Coreopsis palmata* | y | 0.1 | 0.5 |
| Forb | Asteraceae | *Coreopsis tripteris* | y | 4.9 | 6.1 |
| Forb | Asteraceae | *Echinacea pallida* | y | 0.1 | 0.4 |
| Forb | Asteraceae | *Felicia hirta* |  | 0.3 | 0.8 |
| Forb | Asteraceae | *Helianthus grosseserratus* | y | 7.2 | 8.2 |
| Forb | Asteraceae | *Heliopsis helianthoides* | y | 0.1 | 0.3 |
| Forb | Asteraceae | *Parthenium integrifolium* | y | 0.4 | 1.2 |
| Forb | Asteraceae | *Ratibida pinnata* | y | 8.3 | 5.4 |
| Forb | Asteraceae | *Rudbeckia hirta* |  | 0.0 | 0.2 |
| Forb | Asteraceae | *Rudbeckia subtomentosa* | y | 2.9 | 4.2 |
| Forb | Asteraceae | *Silphium integrifolium* | y | 1.6 | 2.8 |
| Forb | Asteraceae | *Silphium laciniatum* | y | 0.2 | 0.8 |
| Forb | Asteraceae | *Silphium perfoliatum* | y | 0.5 | 2.0 |
| Forb | Asteraceae | *Silphium terebinthinaceum* | y | 0.1 | 0.7 |
| Forb | Asteraceae | *Solidago canadensis* |  | 3.2 | 6.4 |
| Forb | Asteraceae | *Solidago rigida* | y | 1.3 | 2.7 |
| Forb | Asteraceae | *Symphyotrichum novae-angliae* | y | 4.0 | 3.8 |
| Forb | Asteraceae | *Taraxacum officinale* |  | 0.1 | 0.5 |
| Forb | Lamiaceae | *Monarda fistulosa* | y | 1.0 | 2.0 |
| Forb | Lamiaceae | *Pycnanthemum virginianum* | y | 0.6 | 0.8 |
| Forb | Plantaginaceae | *Penstemon digitalis* | y | 0.5 | 2.2 |
| Forb | Plantaginaceae | *Veronicastrum virginicum* | y | 0.2 | 0.8 |
| N fixer | Fabaceae | *Astragalus canadensis* | y | 0.0 | 0.2 |
| N fixer | Fabaceae | unidentified *Baptisia* sp. |  | 0.1 | 0.4 |
| N fixer | Fabaceae | *Baptisia alba* | y | 0.7 | 1.5 |
| N fixer | Fabaceae | *Dalea purpurea* | y | 0.2 | 0.6 |
| N fixer | Fabaceae | *Desmodium canadense* | y | 4.8 | 5.7 |
| N fixer | Fabaceae | *Lespedeza capitata* | y | 0.2 | 0.5 |

1Indicates a species present in the seed mix planted during plot establishment in 2008.

Figure 1: (a) total root mass of prairie plots measured in mid-August of 2011 Replotted from(Replotted from Anderson-Teixeira et al. 2013) and 2014 (Replotted from Black et al., in prep). Error bars show mean ± 1 standard deviation of 24 cores. Remaining panels show means of soil properties measured when the plots were established in 2008 replotted from(replotted from Smith et al. 2013): (b) soil texture; (c) soil organic C and N content; (d) soil bulk density.

Figure 1: (a) total root mass of prairie plots measured in mid-August of 2011 (Replotted from Anderson-Teixeira et al. 2013) and 2014 (Replotted from Black et al., in prep). Error bars show mean ± 1 standard deviation of 24 cores. Remaining panels show means of soil properties measured when the plots were established in 2008 (replotted from Smith et al. 2013): (b) soil texture; (c) soil organic C and N content; (d) soil bulk density.

Figure 2: Correlation between percent aboveground cover (horizonal axis) and read proportion per root sample (vertical axis). Points are genus means for one experimental plot (N=5 plots); bars are 1 standard errors in each direction. Root proportions are averaged across all depths.

Figure 2: Correlation between percent aboveground cover (horizonal axis) and read proportion per root sample (vertical axis). Points are genus means for one experimental plot (N=5 plots); bars are 1 standard errors in each direction. Root proportions are averaged across all depths.

Figure 3: Relative abundance (fraction of reads from each sample) as a function of sample depth for each observed plant family. Sequences were clustered at 99% similarity and identified to species according to the closest BLAST match against the Genbank nt database. Taxa were then collapsed by family and groups with a mean abundance less than 1% per sample were removed for plotting.

Figure 3: Relative abundance (fraction of reads from each sample) as a function of sample depth for each observed plant family. Sequences were clustered at 99% similarity and identified to species according to the closest BLAST match against the Genbank nt database. Taxa were then collapsed by family and groups with a mean abundance less than 1% per sample were removed for plotting.

Figure 4: Nonmetric multidimensional scaling plot showing centroids for all detected species. Red: Poaceae. Green: Asteraceae. Blue: Fabaceae. Black: Other families. Grey crosses: Low-abundance species, unlabeled for figure clarity. Pink arrows show best fits for environmental vectors of depth in soil, organic C and N content, and C:N ratio.

Figure 4: Nonmetric multidimensional scaling plot showing centroids for all detected species. Red: Poaceae. Green: Asteraceae. Blue: Fabaceae. Black: Other families. Grey crosses: Low-abundance species, unlabeled for figure clarity. Pink arrows show best fits for environmental vectors of depth in soil, organic C and N content, and C:N ratio.

Appendix: Supplemental figures

Figure S1: Read counts obtained from water controls, binned by assigned genus. Each panel shows reads from a separate aliquot of DNA-free water processed simultaneously through the same DNA extraction, PCR, and sequencing pipeline as the root samples. Notice that the y-axis shows raw read counts (not sample proportions as in other figures) and that the scale differs between panels.

Figure S1: Read counts obtained from water controls, binned by assigned genus. Each panel shows reads from a separate aliquot of DNA-free water processed simultaneously through the same DNA extraction, PCR, and sequencing pipeline as the root samples. Notice that the y-axis shows raw read counts (not sample proportions as in other figures) and that the scale differs between panels.

Figure S2: Genus identities of reads obtained from a mock community of DNA obtained from known-species root samples. Green dots show proportion of template DNA added to the mixture. Black bars show the proportion of reads obtained. Panels are technical replicates, each separately amplified and sequenced from the same aliquot of mixed DNA.

Figure S2: Genus identities of reads obtained from a mock community of DNA obtained from known-species root samples. Green dots show proportion of template DNA added to the mixture. Black bars show the proportion of reads obtained. Panels are technical replicates, each separately amplified and sequenced from the same aliquot of mixed DNA.

Figure S3: Species identities of reads obtained from (top row) DNA extracted from single-species root samples, and (second two rows) DNA from the Andropogon gerardii sample spiked with 1%, 5%, or 10% Elymus canadensis or Sorghastrum nutans DNA. Notice that the barcoding assignment identifies the dominant sequence from the Andropogon gerardii sample as Solidago, not a grass!

Figure S3: Species identities of reads obtained from (top row) DNA extracted from single-species root samples, and (second two rows) DNA from the “*Andropogon gerardii*” sample spiked with 1%, 5%, or 10% *Elymus canadensis* or *Sorghastrum nutans* DNA. Notice that the barcoding assignment identifies the dominant sequence from the “*Andropogon gerardii*” sample as *Solidago*, not a grass!

Figure S4: Relative abundance (fraction of reads from each sample) as a function of sample depth for each observed plant genus. Sequences were clustered at 99% similarity and identified to species according to the closest BLAST match against the Genbank nt database. Taxa were then collapsed by genus and groups with a mean abundance less than 1% per sample were removed for plotting.

Figure S4: Relative abundance (fraction of reads from each sample) as a function of sample depth for each observed plant genus. Sequences were clustered at 99% similarity and identified to species according to the closest BLAST match against the Genbank nt database. Taxa were then collapsed by genus and groups with a mean abundance less than 1% per sample were removed for plotting.