Very fine roots differ among switchgrass (*Panicum virgatum* L.) cultivars and differentially affect soil pores and carbon processes

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Abstract

Switchgrass (*Panicum virgatum* L.) is a promising feedstock for biofuel production, with diverse cultivars representing several ecotypes adapted to different environmental conditions within the contiguous USA. Multiple field studies have demonstrated that monoculture switchgrass cultivation leads to slow to negligible soil carbon (C) gains, an outcome unexpected for such a deep-rooted perennial. We hypothesize that different switchgrass cultivars have disparate impacts on soil C gains, and one of the reasons is variations in physical characteristics of their roots, where roots directly and indirectly influence formation of soil pores. We tested this hypothesis at Great Lakes Bioenergy Research Center's research site in Michigan using two lowland cultivars (Alamo and Kanlow) and four upland cultivars (Southlow, Cave-in-Rock, Blackwell, and Trailblazer). Three types of soil samples were collected: 20cm diameter (Ø) intact cores used for root analyses; 5cm Ø intact cores subjected to X-ray computed tomography scanning used for pore characterization; and disturbed soil samples used for microbial biomass C (MBC) and soil C measurements. Path analysis was used to explore interactive relationships among roots, soil pores, and their impact on MBC, and ultimately, on soil C contents across six cultivars. The abundance of very fine roots (<200µm Ø) was positively associated with fractions of pores in the same size range, but negatively with distances to pores and particulate organic matter. Higher abundance of such roots also led to greater MBC, while greater volumes of medium pores (50-200µm Ø) and shorter distances to pores increased MBC. Results suggest that the greater proportion of very fine roots is a trait that can potentially stimulate soil C gains, with pore characteristics serving as links for the relationship between such roots and C gains. However, at present, ten years of cultivation generated no differences in soil C among the studied cultivars.

Introduction

Perennial grasses are more environmentally sustainable sources of bioenergy feedstock production than annual crops (Robertson et al., 2017; Gelfand et al., 2020) because they require fewer chemical inputs and generate fewer greenhouse gas emissions (Samson et al., 2005; Adler et al., 2007; Oates et al., 2016). Switchgrass (*Panicum virgatum* L.) is recognized as a viable biofuel feedstock due to its high biomass yield produced across a wide range of environmental and climatic conditions and thus a greater energy production potential compared to many other native perennial grasses (Parrish and Fike, 2005; Sanderson and Adler, 2008; Gelfand et al., 2020). In addition, modeling studies project this deep-rooted perennial to increase soil carbon (C) by as much as 0.78 Mg C ha^{-1} yr^{-1} in the eastern U.S. (McLaughlin et al., 2002; McLaughlin and Adams Kszos, 2005). Liebig et al. (2008) reported that 5-year switchgrass cultivation increased soil C by ~1.1 Mg C ha^{-1}yr^{-1} in Great Plains states of U.S. (Nebraska, South Dakota, and North Dakota). However, a number of recent field experiments showed that monoculture switchgrass lags behind other candidate perennial bioenergy cropping systems in its C gains, including mixtures of native grasses or restored prairie systems that involve switchgrass as one of the plant species (Yang et al., 2019; Lee et al., 2023; Perry et al., 2023).

Switchgrass is a phenotypically diverse species with genetic variation among divergent ecotypes and across environmental gradients of eastern North America (Casler et al., 2004, 2007; Lovell et al., 2021). Lowland ecotype switchgrass originates from the southern U.S., which has a warm and mesic climate, while the upland ecotype originates from more northern areas with a drier and colder climate (Vogel et al., 2005; Zhang et al., 2011). Switchgrass cultivars of both lowland and upland ecotypes have been selected for various bioenergy-related traits including biomass yield, winter mortality, and drought tolerance (Haque et al., 2009; Mitchell et al., 2012;

Lowry et al., 2019). We surmise that within-species diversity may also give rise to differences in soil C accrual, though Mosier et al. (2024) failed to find cultivar differences.

The plant's root system plays a critical role in plant contributions to soil C gains, and indeed, root characteristics of switchgrass cultivars differ substantially (de Graaff et al., 2013; Ulbrich et al., 2021; Mosier et al., 2024). Roots directly contribute to soil C gains by transferring organic C to the soil through their turnover and released rhizodeposits and exudates (Liang et al., 2018; Sokol et al., 2019; Panchal et al., 2022). The chemical composition of rhizodeposits and exudates is known to vary among different plant genotypes (Huang et al., 2014; Semchenko et al., 2021). In switchgrass, An et al. (2013) found that the concentration of exudates differed among 11 cultivars, and Li et al., (2022) found distinct differences in concentrations of exudates between lowland and upland ecotypes. Disparate root-derived C sources can have different influences on soil microorganisms (Emmett et al., 2017; Jiang et al., 2017), and several studies have documented varied impacts of different switchgrass cultivars on soil microbial biomass, microbial diversity, and microbial community composition (Sawyer et al., 2019; Roley et al., 2021; Ulbrich et al., 2021; da Costa et al., 2022).

Fine roots are particularly important for soil C cycling, contributing substantially to soil organic matter through their rapid turnover and subsequent decomposition; and their persistent C inputs can constitute 30-80% of soil C across various ecosystems (Ruess et al., 2003; Kalyn and Van Rees, 2006). Switchgrass roots <1 mm diameter (Ø) contributed to approximately 77% of the total root biomass, and such fine roots accounted for 50% of the total biomass in the 0-150 cm soil profile within the 0-20 cm profile (An et al., 2022), where C processing actively occurred (Henneron et al., 2022). Moreover, fine roots led to lower priming effect and greater soil C accumulation compared to coarser roots (de Graaff et al., 2013; Adkins et al., 2016). Since the biomass and length of such fine roots were found to differ among various switchgrass cultivars (de Graaff et al., 2013; Liu et al., 2016), we hypothesize that such differences can be an important cause leading to their distinct contributions to soil C processing and gains.

Roots also play a role in the formation of soil pore structure, defining size distributions and spatial locations of pores within the soil matrix (Bodner et al., 2014; Bacq-Labreuil et al., 2019; Helliwell et al., 2019; Lucas et al., 2022). That occurs both through direct impacts of roots on arrangement of soil particles and penetration of soil aggregates, and through water extraction (Angers and Caron, 1998; Bengough et al., 2016; Oburger and Schmidt, 2016). The size of the impact varies depending on root characteristics (Mahannopkul and Jotisankasa, 2019), because roots determine the water extraction strength (Assadollahi and Nowamooz, 2020).

Pores in tens to hundreds of µm Ø size range are especially relevant for microbial abundance and activity, and consequently, for the processing and protection of the newly added C (Strong et al., 2004; Kravchenko et al., 2019a; Franklin et al., 2021). Plants with a higher density of fine roots tend to form pore structures dominated by fine pores compared to plants with coarser roots (Bengough et al., 2016; Bodner et al., 2021). Spatial distributions of pores and organic C sources in soils define oxygen and nutrient supply for soil microbial functioning and concomitant fate of the C sources (Borer et al., 2018; Li et al., 2024; Scheidweiler et al., 2024). A ubiquitous spread of organic residues, likely roots in switchgrass, can promote the development of micro-habitats that foster microbial hotspots (Kuzyakov and Blagodatskaya, 2015; Li et al., 2024). The complex web of fine roots also can reduce the distance between soil pores, forming such pores throughout the areas occupied by the roots (Gyssels et al., 2005; Reubens et al., 2007). The proximity to pores governs microbial accessibility to C sources located on soil particles and regulates aeration for soil microorganisms (Dungait et al., 2012;

Schlüter and Vogel, 2016; Rohe et al., 2021), subsequently influencing their abundance and activity (Ekschmitt et al., 2008; Schlüter et al., 2019).

The abundance of soil microorganisms promoted by optimal habitats with accessible supplies of C can consequently lead to soil C accumulation upon their life and death. Microbially processed organic matter can be more easily stabilized by soil mineral surfaces than that of plant-originated C compounds (Grandy and Neff, 2008; Miltner et al., 2012). Microbial necromass also contributes significantly to C accumulation, being transformed and stabilized within the soil (Six et al., 2006; Miltner et al., 2012; Kallenbach et al., 2015; Liang et al., 2019).

Our objectives here are to quantify cultivar-level variations (i) in size distributions of switchgrass roots, (ii) in size distributions of soil pores and spatial patterns of pores and particulate organic matter (POM), and (iii) to estimate the impact of roots and pores on soil microbial biomass C (MBC), and ultimately, on soil C gains. We hypothesize that differences in root sizes among switchgrass cultivars lead to differences in sizes and spatial distributions of soil pores and POM fragments and consequently different MBC and soil C contents.

Materials and Methods

Experimental site and plant and soil sampling

The Great Lake Bioenergy Research Center's Switchgrass Variety Experiment used in this study is located at W.K. Kellogg Biological Station (42° 23'N, 85° 22'W), Michigan, United States. The soil of the experimental site belongs to the Kalamazoo series (fine-loamy, mixed, active, mesic Typic Hapludalf). For several years prior to the experiment's establishment, the field was in an alfalfa-soybean-maize rotation. In 2009, 12 switchgrass cultivars were established in a randomized complete block design with plots 4.6 m × 12.2 m arranged in four replicated blocks. After the establishment year, all plots were annually fertilized with 56 kg ha^{-1} of nitrogen as dry urea (46-0-0 NPK), and annually harvested post-frost following typical practices (Sanford et al., 2016).

Four upland (Southlow, Cave-in-Rock, Blackwell, and Trailblazer) and two lowland (Alamo and Kanlow) cultivars were selected for this study. We conducted two sampling campaigns. First, three intact soil cores (20 cm height and 2 cm Ø), which we will refer to as tall cores, were collected within 10 cm from crowns of three randomly selected plants in each block, at 0-20 cm sampling depth. These tall cores were used for root scanning, and prior to analyses, they were kept at -20 °C. While it is known that roots of switchgrass plants can grow to substantial depths, most of the root biomass is often found within the topsoil across a comparable set of switchgrass varieties (Anderson-Teixeira et al., 2013; de Graaff et al., 2014; Mosier et al., 2024). Especially, switchgrass roots <500 μm Ø contributed to 77% of the total root biomass, and approximately 50% of the fine roots were located within 0-20 cm depth (An et al., 2022). Since the focus of our work was on the comparisons among the cultivars, root measurements in the topsoil were expected to provide most representative and accurate data for such comparisons. All cultivars were sampled in July of 2016. Belowground biomass of switchgrass is known to stabilize in the first two years since establishment, and seasonal changes in root biomass dynamics, particularly below the 5 cm soil depth, are reported as minimal (Garten et al., 2010; Yang et al., 2016). While some differences in cultivar phenology were present at the sampling time, no detectable impact on root systems was expected.

Second, in October 2019, two intact soil cores (5 cm height and 5 cm Ø), which will be referred to as short cores, and surrounding bulk soil were collected within 10 cm from crowns of randomly selected plants of each block. We decided to focus on the soil at 5-10 cm depth, because it represents the portion of the soil profile most significantly affected by the roots with the highest microbial biomass and soil organic C (Ma et al., 2000; Roosendaal et al., 2016). The short cores were subjected to X-ray computed micro-tomography (μCT) scanning, and the bulk soil was used for measurements of MBC and soil C. All short cores were stored at 4 °C until μCT scanning.

Root analyses

Detailed description of the processing of the tall cores are provided in Ulbrich et al. (2021). Briefly, soils of the tall cores were wet sieved (2 mm) with Nanopure (0.2 μM) water to separate roots from soil, and all visible roots were procured with tweezers. The cleaned roots were scanned with an Epson perfection V600 scanner (Epson America Inc., Long Beach, CA, USA) in a glass scanning bed with 200 ml of Nanopure water. Scanning resolution of root images was 75 μm.

Using RhizoVision software (version 2.0.3), binary images for roots and background were obtained. Then, non-root object filtering and hole filling were conducted to remove background noise and fill unsegmented holes in root portions of images, respectively. Tools in RhizoVision allowed us to identify different size Ø of roots using distance transformation and skeletonization of the root portions (Felzenszwalb and Huttenlocher, 2012). Upon the given scanning resolution, the minimum Ø size of detectable roots was 75 μm, thereby roots classified as the 75 μm size group is assumed to represent ~75-113 μm roots, and the 150 μm size group represents ~113-187 μm roots. Root volumes for each skeletal 2D root of the different Ø groups were calculated by multiplying the length of the root by the cross-sectional area (Seethepalli et al., 2021). Subsequently, volumes were used to determine size distributions of root volume fractions (mm^3) in different Ø groups per total core (root + soil) volume (mm^3). Root size groups finer than 500 µm Ø were only used in further analyses as such roots, which are generally defined as fine roots, are particularly important for soil C cycling (Ruess et al., 2003; Kalyn and Van Rees, 2006; de Graaff et al., 2013).

X-ray µCT scanning and image analyses

Soil pore characteristics were measured using short cores via X-ray µCT, a tool that allows for visualization of soil structure in its intact state (Udawatta et al., 2008; Vogel et al., 2010). Prior to X-ray µCT scanning, all short cores were brought to the matric potential of -28 kPa to ensure that >5 µm Ø pores were filled with air so easily detectable in the images. Thus, cores were first saturated for 24 hours on a water filled sand bath and then kept in a pressure chamber for two days at -28 kPa. The cores were scanned using an X-ray µCT instrument (North Star Imaging, X3000, Rogers, MN, USA) at the Horticulture Department of Michigan State University. The scanning resolution was 18 µm, achieved using the Subpix-mode of the scanner, and the projected energy level was 75 kV with 450 µA. Images from 3014 projections were reconstructed by the efX software (North Star, Rogers, MN, USA).

A schematic summary of the steps for image pre-processing and analyses is outlined in Figure 1. First, image pre-processing steps were conducted to remove artifacts and noise from 3D stacked soil images using ImageJ-Fiji software (Schindelin et al., 2012). In order to exclude sampling artifacts near the soil core walls, the images were cropped into 2.7 x 2.7 x 4.1 cm (1500 × 1500 × 2240 pixels) centrally located parallelograms. Then, we removed ring artifacts on the image polar domain using a stripe filter of the Xlib/Beat plugin. Finally, a 3D non-local mean filter (σ = 0.1) was applied to reduce the noise using scikit-image in Python (Darbon et al., 2008; Buades et al., 2011).

The pre-processed grayscale images were segmented into pore and solid binary images for size distributions and spatial locations of pores. Mean threshold values for the segmentation were obtained by averaging the thresholds between pore and solid phases derived from six segmentation methods (Otsu, Triangle, Huang, ISO, Li, and Moments) using SimpleITK in Python (Beare et al., 2018; Lucas et al., 2022). The rationale for averaging thresholds is to mitigate biases of the individual methods, thus enhancing accuracy in pore threshold calculation (Schlüter et al., 2014). The resolution level and steps of image-processing applied in this study allow us to reliably identify pores larger than 36 μm Ø.

POM segmentation was carried out with a U-Net (convolutional neural network) model under the deep learning engine pre-built in Dragonfly software (Ronneberger et al., 2015; Abadi et al., 2016; Makovetsky et al., 2018). The model was trained using two cores randomly selected from each experimental block (eight of total 48 cores). Seven frames with representative POM fragments in each of the selected cores were used as input, and two slices directly below and above the selected frames were also considered for generating segmentation outcomes. Then, the trained model was applied on the entire cores. Segmented POM images were visually inspected to ensure the integrity and accuracy of the process. The outcome of POM segmentation was de-noised by removing clusters < 4 voxels from the images. Then, distances from the locations of the segmented soil solid materials to the nearest pores and from such locations to the nearest POM fragments were determined using the ‘Distance Transform 3D’ approach in ImageJ-Fiji (Borgefors, 1996). Size distributions of pores in 3D binary images were determined by the

‘Local Thickness’ approach, based on the maximum inscribed sphere method (Hildebrand and Rüegsegger, 1997; Vogel et al., 2010) in ImageJ-Fiji.

To assess the relationships between size distributions of pores and roots based on volumes at comparable scales, pore Ø sizes were grouped into interval classes as similar as possible to the calculated Ø sizes of root fractions. For example, the 75 µm size group represents the ~36-108 µm pores and the 150 µm size group – the ~108-180 µm pores, etc. Then, pore fractions of each size group were determined by dividing the segmented pore volumes (mm^3) by the total cropped soil (pore + solid) volumes (mm^3). The > 500 µm Ø pores were not quantitatively assessed in this study because of high uncertainty of their estimation in relatively small and short cores.

Soil microbial biomass and total carbon measurements

We measured MBC by the chloroform fumigation-incubation method (Paul et al., 1999). Two sets of 10 g soil samples were prepared by adding sufficient water to reach 50% water holding capacity. The samples were pre-incubated for five days, after which one set was fumigated with ethanol-free chloroform vapor for 24 hours, while the other set remained unfumigated. Both sets were then incubated for 10 days in the dark at 20 °C. The emitted CO_2 was measured using Infrared Photoacoustic Spectroscopy (INNOVA Air Tech Instruments, Ballerup, Denmark) in the gas circulation mode. The difference in CO_2 emissions between the fumigated and non-fumigated samples was used to calculate the MBC. For soil C analysis, sieved and ground soil samples were analyzed using a CHNSO Elemental Analyzer (Costech Analytical Technologies, Valencia, CA, USA).

Statistical analysis

The differences in soil pores, root traits, MBC, and soil C contents were evaluated among six switchgrass cultivars and between two ecotypes using PROC MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA). All statistical models for comparisons among the six cultivars included fixed effects of cultivars and random effects of experimental blocks. The models for the analyses of the distance to pore and distance to POM data additionally included the random effects of soil cores nested within the blocks. For comparisons between two ecotypes, cultivars were considered as a random factor nested within the corresponding ecotypes. Models for root and pore size distributions included fixed effects of cultivars, root/pore size groups, and their interactions and another random effect of the cores nested within the blocks and cultivars. Root/pore size groups were treated as a repeated measure factor, and cores nested within cultivars were used as an error term for testing the cultivar effect and as a subject of the repeated measurement. The statistical models for comparisons between two ecotypes were similar to those used for cultivar comparisons, except that cultivars were treated as a random effect nested within the ecotype and contributing to the error term for testing for the ecotype effect.

For all datasets, normality of the residuals and homogeneity of the residual variances were assessed by examining histograms, normal probability plots, and side-by-side box plots of the residuals, and by conducting Levene's test for variances. Residuals were found to be normally distributed in all studied variables. Since residual variances among six cultivars or between two ecotypes were not significantly different at $\alpha = 0.1$ level in Levene's test, equal variance models were used in subsequent data analyses. Multiple comparisons among the cultivars or between the ecotypes were conducted using t-test.

Post-hoc power analysis was conducted to identify how many replications should have been taken to be able to detect as statistically significant differences in soil C contents among the six switchgrass cultivars (Stroup, 2002; Kravchenko and Robertson, 2011). The variance component, which was the estimate of block variance in this randomized complete block design, was estimated from the observed soil C contents. The size of the hypothesized difference used in the power analysis was 0.26% of soil C content. Then, the number of samples needed for statistical significance was calculated based on 0.05 probability of Type I error using PROC MIXED procedure in SAS.

Relationships among root and pore fractions within each individual size group, MBC, and soil C contents across all six cultivars were assessed using Pearson's correlation coefficients (*r*) via the PROC CORR procedure in SAS. The correlation analysis enabled us to assess the hypotheses that (1) roots of certain size groups contribute to the formation of soil pores of the same size range, (2) roots of certain size groups contribute to increases in MBC and soil C contents, and (3) pores of certain size groups contribute to increases in MBC and soil C contents. Correlations were subsequently used for path analysis.

Path analysis

Path analysis is a statistical approach that can infer causal relationships allowing for examination of direct and indirect effects among observed variables based on the theoretical model hypothesized by the researcher (Schumacker and Lomax, 1996; Grace, 2006). Indirect effects in path analysis are identified by estimating the relationship between two variables that is mediated by one or more intervening variables (Preacher and Hayes, 2004). Thus, we used it in this study to address hypotheses regarding relationships among root and pore traits and their direct and indirect contributions to soil MBC and C contents. Since the model for path analysis is constructed based on causal hypotheses between variables, the theoretical and empirical basis for these hypotheses should be provided by peer-reviewed literatures. Overall, we hypothesized that greater abundance of fine roots can lead to soil C gains by releasing more accessible C substrates, and by stimulating the formation of pores, which in turn supports more abundant microbial communities and facilitates greater soil C stabilization. Detailed descriptions of the individual hypotheses, along with the literature supporting their path formulation, are provided in Table 1.

The PROC CALIS procedure of SAS software was used for path analysis. A two-index presentation strategy was utilized for the path analysis model evaluation (Hu and Bentler, 1999). Model fitness and adequacy were determined through a chi-square test (χ^2) and goodness of fit index (GFI) (Bentler, 1990), and acceptable models are characterized by χ^2 test p values >0.05 and GFI >0.90 (Hu and Bentler, 1999; Eisenhauer et al., 2015). The strength of the paths was indicated using standardized coefficients (β). The rationale for using standardized coefficients is to facilitate comparisons of relative impacts upon the initially incommensurable variables (Kwan and Chan, 2011).

Results

Root and pore size distributions

Fraction of roots finer than 500 μm Ø did not show notable differences among the studied six switchgrass cultivars ($p = 0.074$) (Figure S1). However, there were differences in the root fraction of very fine size groups (75 and 150 μm Ø) among the six cultivars, while at sizes >200 μm Ø, the root fraction did not provide strong evidence for differences (Figure 2A). Specifically, Kanlow and Cave-in-Rock had the highest fraction of very fine roots, i.e., 0.0009 ±0.0001 mm^3/mm^3 in soil, followed by Southlow, Alamo, and Blackwell, while Trailblazer had the smallest fraction of very fine roots, i.e., 0.0005 ±0.0001 mm^3/mm^3 (Table S1).

Consistent with the root size results, soil pore fractions in medium size groups (75 and 150 µm Ø) also differed among the cultivars (Figure 2B) and, likewise, no differences were observed among cultivars in coarse pore size groups (>200 µm Ø) (Figure 3B). Soil under Cave-in-Rock had the greatest fraction of medium pores, i.e., 0.04 ±0.006 mm^3/mm^3 in soil, followed by Kanlow, Alamo, and Blackwell, with Southlow and Trailblazer having the smallest fractions with 0.02 ±0.005 mm^3/mm^3 (Table S1).

Root fractions in very fine size groups were positively associated with pore fractions of medium size groups ($r^2 = 0.21$ and $p = 0.023$ in 75 µm; $r^2 = 0.22$ and $p = 0.020$ in 150 µm Ø size group), while no correlations were observed between roots and pores of any other size groups (Figure 3A).

Distance to pores and POM

The average distance to pores was the largest in the soil under the Trailblazer cultivar, equal to 0.34 ±0.08 mm, while soils under the other five cultivars had similar distances to pores of 0.19 mm on average (Figure 4A). Overall, distances to pores in the soils under upland ecotype cultivars were 34% greater than those of lowland ecotype cultivars ($p = 0.019$).

The average distance to POM fragments was also the largest in the soil under Trailblazer, i.e., 0.82 ±0.12 mm, while Cave-in-Rock and two lowland cultivars, Alamo and Kanlow, had the lowest distances to POM of 0.54 ±0.17, 0.62 ±0.14, and 0.60 ±0.12 mm, respectively (Figure

4B). Overall, there were no differences in distances to POM between upland and lowland cultivars ($p = 0.314$) (Figure 4B).

Soil microbial biomass and carbon

MBC in the soils under Alamo and Kanlow, the two lowland switchgrass cultivars, was ~50-100% higher than under the other four cultivars; in particular MBC under Kanlow was more than double that under Blackwell (Figure 4C). Overall, MBC in soils under the cultivars of the lowland ecotype was 44% higher than that of the upland cultivars ($p = 0.002$). On the other hand, soil C contents were not different among the six cultivars, and the difference between the two ecotypes was also not significant ($p = 0.598$) (Figure 4D).

MBC across all six cultivars was positively associated with 75 µm Ø roots ($r^2 = 0.19$; $p = 0.022$) and with 75 µm Ø pores ($r^2 = 0.17$; $p = 0.036$), while MBC was not correlated with any other size groups of roots and pores >75 µm Ø (Figure 3B). MBC was negatively associated with the distance to pores ($r^2 = 0.13$; $p = 0.046$), whereas not associated with the distance to POM fragments (Table S2). Soil C was also positively associated with 75 and 150 µm Ø roots ($r^2 = 0.17$ and 0.31; $p = 0.035$ and 0.004, respectively), while no significant correlations were observed with any size groups of pores (Figure 3B). Soil C was negatively associated with the distance to POM fragments ($r^2 = 0.18$; $p = 0.024$), whereas not associated with the distance to pores (Table S2). Notably, soil C was positively associated with MBC across all six cultivars ($r^2 = 0.18$; $p = 0.025$).

Path analysis

The very fine roots (sum of 75 and 150 µm Ø size groups) had a direct positive impact on the fraction of medium pores (sum of 75 and 150 µm Ø size groups) ($\beta = 0.41$; $p = 0.008$), MBC ($\beta = 0.41$; $p = 0.003$), and soil C contents ($\beta = 0.50$; $p = 0.0009$), and negative impact on the distance to pores ($\beta = -0.47$; $p = 0.002$) and POM ($\beta = -0.52$; $p = 0.0003$) (Figure 5). Additionally, such roots indirectly influenced MBC by increasing the fraction of medium pores ($\beta = 0.19$; $p = 0.048$) and by decreasing the distances to pores ($\beta = -0.28$; $p = 0.021$) and POM ($\beta = -0.19$; $p = 0.042$). Soil C contents increased by a shorter distance to POM ($\beta = -0.21$; $p = 0.028$), while neither the fraction of medium pores nor the distance to pores appeared to directly influence soil C. Yet, increases in medium pore fractions and decreases in distances to pores and POM indirectly fostered increases in soil C due to the rise in MBC ($\beta = 0.30$; $p = 0.017$) (Figure 5).

Discussion

After seven years of continuous growth, variations were observed among the six switchgrass cultivars in volumes of very fine roots and of medium soil pores. Cultivars with a greater volume of very fine roots stimulated formation of medium soil pores compared to the cultivars with coarser root systems and led to a more ubiquitous spread of pores and POM within the soil matrix. Volumes of very fine roots were strongly positively associated with soil C and MBC. Abundance of the medium pores and spatial distribution patterns of the pore space were directly related to MBC, but not to soil C. While the cultivars of the lowland switchgrass ecotype increased soil MBC compared to that of the upland ecotype, in none of the studied lowland cultivars did soil C exceed that of upland cultivars.

Influence of very fine roots

Past comparisons of root systems among switchgrass cultivars generated contradictory results. For example, at experimental site in Michigan, Ulbrich et al. (2021) reported that cultivar Kanlow had relatively high specific root length (root length/weight) compared to other cultivars, while in Illinois Kanlow had lower specific root length than others (de Graaff et al., 2013). While a meta-analysis demonstrated that phylogenetic characteristics can be the largest driver of root traits (Valverde-Barrantes et al., 2017), the discrepancy suggests that root system development in switchgrass cultivars might be influenced by environmental factors (Lovell et al., 2021; Griffiths et al., 2022), that is, vary site-specifically.

The initial hypothesis for our path analysis focused on fine roots, generally defined as roots <500 µm Ø and well-established as key contributors to soil C cycling (de Graaff et al., 2013; McCormack et al., 2015; Adkins et al., 2016; Panchal et al., 2022). However, Kelly-Slatten et al. (2023) recently found that the differences in the abundance of the fine roots among Kanlow, Southlow, and Cave-in-Rock were minor, and this aligns with the lack of meaningful differences in fine roots observed here (Figure S1). As this study progressed, distinct variations were observed in the abundance of the specific range of fine roots (<200 µm Ø), referred to as very fine roots, across different switchgrass cultivars (Figure 2A & Table S1).

The abundance of very fine roots was the influential factor for microbial biomass and soil C (Figure 5). The very fine roots also indirectly affected them by contributing to volumes of medium (50-200 µm Ø) pores and spatial patterns in both pores and POM, the latter expressed via distances to pores and POM (Figure 5). Fine roots typically provide greater amounts of root exudates and rhizodeposits to soil (Xu and Juma, 1994; Paterson and Sim, 1999; Zhang et al., 2022). Such labile C sources as well as fine roots themselves can be preferentially used by soil microorganisms and contribute significantly to soil organic matter formation through their rapid turnover and subsequent decomposition (Ruess et al., 2003; Kalyn and Van Rees, 2006). While in the past many studies focused on a broadly defined size group of fine roots as those <1.0-2.5 mm Ø (Steinaker and Wilson, 2008; de Graaff et al., 2013; Sprunger et al., 2017; Sehgal et al., 2021), findings from our study indicate that in monoculture switchgrass systems, it is only the roots <200 µm Ø, i.e., very fine roots, that are particularly influential in promoting microbial biomass and concomitant soil C production (Figure 3 & 5).

We found a greater volume of very fine roots in lowland than in the upland cultivars (Figure 2A & Table S1). A single exception was Cave-in-Rock, the upland cultivar with a volume of very fine roots comparable to that of lowland cultivars (Figure 2A & Table S1). Cave-in-Rock, as a relatively drought-sensitive cultivar, possibly extends its fine roots more aggressively to promote soil water access, thereby navigating this environmental challenge with its fine roots (McCully, 1999; Liu et al., 2015; Fort and Freschet, 2020). Differences in very fine roots among our cultivars led to differences in formation of soil pores and different spatial distribution of the pores and POM through the soil matrix (Figure 2B, 4A, 4B, & 5). Root thickness can significantly influence a root's capacity to penetrate soil (Chimungu et al., 2015; Paez-Garcia et al., 2015), and differences in this trait are known to result in distinct pore structures, with prevalence of certain pore size classes (Bodner et al., 2014, 2021). Positive correlations between very fine roots and medium pores among six switchgrass cultivars support the notion that cultivars with greater volumes of very fine roots form greater volumes of medium pores (Figure 3A). Lowland cultivars possibly lead to the formation of more intricate soil pore networks through their fine roots (Gyssels et al., 2005; Reubens et al., 2007), and thus distances between individual pores are shorter than for those of upland cultivars (Figure 4A).

Roots are the major source of soil POM, thus shorter distances between the nearest POM fragments in soils under Kanlow and Cave-in-Rock (Figure 4B), which were the two cultivars with the greatest volumes of very fine roots (Figure 2A & Table S1), presumably resulted from a more uniform spread of root residues throughout the soil (Bengough et al., 2016; Bodner et al., 2021). Indeed, a significantly shorter distance was found in soils under prairie vegetation, which was known to have extensive root systems (Sprunger et al., 2017), compared to that under switchgrass (Cave-in-Rock) in a field adjacent to this study (Figure S2). Negative correlations between volumes of very fine roots and distances to pores and POM further support this conclusion (Table S2).

Influence of medium pores and their spatial distribution

Our results demonstrate that medium pores and distances to pores did not directly impact measured soil C gains, even though they promoted soil microbial biomass, which was positively associated with soil C (Figure 5 & Table S2). This is consistent with a direct effect of medium pores on MBC and an indirect effect of such pores on soil C gains that were recently observed in monoculture switchgrass (Cave-in-Rock) cultivated across a wide range of low fertility soils of the U.S. Midwest (Lee et al., 2023). However, even though lowland switchgrass cultivars greatly increased the proportion of medium pores as compared to the upland cultivars (Figure 2B & Table S1), such increases did not translate into measured soil C gains (Figure 4D).

Pores in 30-180 µm Ø, which is very close to the range of medium pores in this study, are suggested as optimal microbial habitats (Kravchenko et al., 2019b), since such pores facilitate high microbial activity by ensuring oxygen and water flows, while allowing for easy access to organic matter (Rawlins et al., 2016; Keiluweit et al., 2018). Since pores of this size range were also identified as the primary sites for rhizodeposition (Quigley and Kravchenko, 2022), the abundance of such pores likely facilitated the microbial growth in the soils under the lowland cultivars (Figures 2B & 4C).

Another two key links between very fine roots and microbial biomass were the distances from soil solid materials to the nearest pores and to the nearest POM fragments (Figure 5). The shorter distances to pores and POM likely benefited microbial habitats, because such distances imply closer proximity of microbes to water, oxygen, and C sources (Raynaud and Nunan, 2014; Rohe et al., 2021; Bickel and Or, 2023). Therefore, we can surmise that the indirect contribution of the extensive root systems in the two lowland cultivars as well as in Cave-in-Rock (Figure 2A) to increases in soil microbial biomass stemmed from decreasing distances to pores and POM (Figures 4A & 4B). Shorter distances to POM can allow easier translocation of the processed C during POM decomposition into surrounding soil (Védère et al., 2020; Schlüter et al., 2022), likely contributing to soil C gains (Figure 5).

Negligible soil C gains in switchgrass cropping systems

Neither the two lowland cultivars nor Cave-in-Rock, the cultivars that promoted several of the hypothesized drivers of soil C gains, including greater volumes of very fine roots and medium pores, higher MBC, and shorter distances to POM and pores (Figure 5 & Table S2), led to measurably greater soil C gains than other cultivars in this study (Figure 4D). Our results add to a substantial body of research reporting very slow to negligible soil C gains in monoculture switchgrass systems (Garten and Wullschleger, 2000; Liebig et al., 2005; Bates et al., 2022), suggesting that 10 years were not a sufficiently long term for soil C gains to build under the studied cultivars. In fact a 13-year study of switchgrass, recently conducted in a field adjacent to this experiment, also observed that switchgrass only barely increased soil C contents since its installation (~0.06 Mg C ha^{-1} yr^{-1}) (Perry et al., 2023). Another recent study, conducted in the same experimental site, showed that nine different switchgrass cultivars continuously grown for 11 years had no significant impact on soil C contents (Mosier et al., 2024).

There is an apparent discrepancy between the lack of the detected increases in soil C contents and the implications of path analysis suggestions that such increases should have taken place, at least in the cultivars with finer root systems (Figure. 5). We believe the discrepancy is due to lower statistical power of the experimental work, i.e., the attempts to detect relatively small changes in these soils C gains, given high variability, have been hampered by relatively small sample size. Note that the 4 replicated blocks, i.e., *n* of 4, of the current experiment is a common practice ubiquitously followed in field experimentation. Yet, *post-hoc* power analysis of the soil C data in this study suggests that at least three more replicated blocks, i.e., *n* of 7, would be required for detecting a statistically significant difference with the power of 75%, and six more blocks, i.e., *n* of 10, with the power > 90% (Table S3). In other words, the nuanced relationship between switchgrass cultivars and C gains, as revealed by the path analysis with multifaceted factors, emphasizes the need to examine soil C gains with a greater number of replications to better test the effects of switchgrass cultivars on soil C. Alternatively, as these switchgrass stands mature and cultivars continue to differentially accumulate soil C, differences should become evident with fewer replicates in next few years. Expanding the replication size, as suggested by the power analysis, will also be important for better parameterizing process-level models that, like our path analysis, also predict significant soil C gains under long-term switchgrass cultivation (McLaughlin et al., 2002; McLaughlin and Adams Kszos, 2005; Martinez-Feria and Basso, 2020).

Conclusions

Our study elucidates complex yet pivotal relationships among root traits, soil pore structure, and microbial biomass for the potential accumulation of soil C using six switchgrass cultivars with different root traits, representing two distinct ecotypes. Results suggest that switchgrass cultivars with greater volumes of very fine roots have a greater capacity for soil C accumulation, mediated by increases in medium pores and decreases in distances to pore and POM that affect concomitant increases in MBC. However, 10 years were insufficient to document measurable differences in soil C gains among cultivars. Overall, this study provides critical insights for the relative impacts of root traits and pore structure for soil C gains in bioenergy crop cultivation.

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References

Abadi, M., Barham, P., Chen, J., Chen, Z., Davis, A., Dean, J., Devin, M., Ghemawat, S., Irving, G., Isard, M., Kudlur, M., Levenberg, J., Monga, R., Moore, S., Murray, D.G., Steiner, B., Tucker, P., Vasudevan, V., Warden, P., Wicke, M., Yu, Y., Zheng, X., 2016. TensorFlow: A System for Large-Scale Machine Learning. Presented at the 12th

USENIX Symposium on Operating Systems Design and Implementation (OSDI 16), pp. 265–283.

Adkins, J., Jastrow, J.D., Morris, G.P., Six, J., de Graaff, M.-A., 2016. Effects of switchgrass cultivars and intraspecific differences in root structure on soil carbon inputs and accumulation. Geoderma 262, 147–154. doi:10.1016/j.geoderma.2015.08.019

Adler, P.R., Grosso, S.J.D., Parton, W.J., 2007. Life-Cycle Assessment of Net Greenhouse-Gas Flux for Bioenergy Cropping Systems. Ecological Applications 17, 675–691. doi:10.1890/05-2018

An, Q.-Q., Wang, S.-Q., Kang, J.-Y., Wang, Z., Chen, Y.-L., Xu, B.-C., 2022. Fine root distribution and morphological characteristics of switchgrass under different row spacings on semi-arid Loess Plateau, China. Archives of Agronomy and Soil Science 68, 1–17. doi:10.1080/03650340.2020.1820490

An, Y., Ma, Y., Shui, J., 2013. Switchgrass root exudates have allelopathic potential on lettuce germination and seedling growth. Acta Agriculturae Scandinavica, Section B — Soil & Plant Science 63, 497–505. doi:10.1080/09064710.2013.810770

Anderson-Teixeira, K.J., Masters, M.D., Black, C.K., Zeri, M., Hussain, M.Z., Bernacchi, C.J., DeLucia, E.H., 2013. Altered Belowground Carbon Cycling Following Land-Use Change to Perennial Bioenergy Crops. Ecosystems 16, 508–520. doi:10.1007/s10021-012-9628-x

Angers, D.A., Caron, J., 1998. Plant-induced Changes in Soil Structure: Processes and Feedbacks. Biogeochemistry 42, 55–72. doi:10.1023/A:1005944025343

Assadollahi, H., Nowamooz, H., 2020. Long-term analysis of the shrinkage and swelling of clayey soils in a climate change context by numerical modelling and field monitoring. Computers and Geotechnics 127, 103763. doi:10.1016/j.compgeo.2020.103763

Bacq-Labreuil, A., Crawford, J., Mooney, S.J., Neal, A.L., Ritz, K., 2019. Cover crop species have contrasting influence upon soil structural genesis and microbial community phenotype. Scientific Reports 9, 7473. doi:10.1038/s41598-019-43937-6

Bates, C.T., Escalas, A., Kuang, J., Hale, L., Wang, Y., Herman, D., Nuccio, E.E., Wan, X., Bhattacharyya, A., Fu, Y., Tian, R., Wang, G., Ning, D., Yang, Y., Wu, L., Pett-Ridge, J., Saha, M., Craven, K., Brodie, E.L., Firestone, M., Zhou, J., 2022. Conversion of marginal land into switchgrass conditionally accrues soil carbon but reduces methane consumption. The ISME Journal 16, 10–25. doi:10.1038/s41396-021-00916-y

Beare, R., Lowekamp, B., Yaniv, Z., 2018. Image Segmentation, Registration and Characterization in R with SimpleITK. Journal of Statistical Software 86, 8. doi:10.18637/jss.v086.i08

Bengough, A.G., Loades, K., McKenzie, B.M., 2016. Root hairs aid soil penetration by anchoring the root surface to pore walls. Journal of Experimental Botany 67, 1071–1078. doi:10.1093/jxb/erv560

Bentler, P.M., 1990. Comparative fit indexes in structural models. Psychological Bulletin 107, 238–246. doi:10.1037/0033-2909.107.2.238

Beschoren da Costa, P., Benucci, G.M.N., Chou, M.-Y., Van Wyk, J., Chretien, M., Bonito, G., 2022. Soil Origin and Plant Genotype Modulate Switchgrass Aboveground Productivity and Root Microbiome Assembly. mBio 13, e00079-22. doi:10.1128/mbio.00079-22

Bickel, S., Or, D., 2023. Aqueous habitats and carbon inputs shape the microscale geography and interaction ranges of soil bacteria. Communications Biology 6, 1–10. doi:10.1038/s42003-023-04703-7

Bodner, G., Leitner, D., Kaul, H.-P., 2014. Coarse and fine root plants affect pore size distributions differently. Plant and Soil 380, 133–151. doi:10.1007/s11104-014-2079-8

Bodner, G., Mentler, A., Keiblinger, K., 2021. Plant Roots for Sustainable Soil Structure Management in Cropping Systems, in: The Root Systems in Sustainable Agricultural Intensification. John Wiley & Sons, Ltd, pp. 45–90. doi:10.1002/9781119525417.ch3

Borer, B., Tecon, R., Or, D., 2018. Spatial organization of bacterial populations in response to oxygen and carbon counter-gradients in pore networks. Nature Communications 9, 769. doi:10.1038/s41467-018-03187-y

Borgefors, G., 1996. On Digital Distance Transforms in Three Dimensions. Computer Vision and Image Understanding 64, 368–376. doi:10.1006/cviu.1996.0065

Buades, A., Coll, B., Morel, J.-M., 2011. Non-Local Means Denoising. Image Processing On Line 1, 208–212. doi:10.5201/ipol.2011.bcm_nlm

Casler, M.D., Vogel, K.P., Taliaferro, C.M., Ehlke, N.J., Berdahl, J.D., Brummer, E.C., Kallenbach, R.L., West, C.P., Mitchell, R.B., 2007. Latitudinal and Longitudinal Adaptation of Switchgrass Populations. Crop Science 47, 2249–2260. doi:10.2135/cropsci2006.12.0780

Casler, M.D., Vogel, K.P., Taliaferro, C.M., Wynia, R.L., 2004. Latitudinal Adaptation of Switchgrass Populations. Crop Science 44, 293–303. doi:10.2135/cropsci2004.2930

Chimungu, J.G., Loades, K.W., Lynch, J.P., 2015. Root anatomical phenes predict root penetration ability and biomechanical properties in maize (Zea Mays). Journal of Experimental Botany 66, 3151–3162. doi:10.1093/jxb/erv121

Darbon, J., Cunha, A., Chan, T.F., Osher, S., Jensen, G.J., 2008. Fast nonlocal filtering applied to electron cryomicroscopy, in: 2008 5th IEEE International Symposium on Biomedical Imaging: From Nano to Macro. Presented at the 2008 5th IEEE International Symposium on Biomedical Imaging (ISBI 2008), IEEE, Paris, France, pp. 1331–1334. doi:10.1109/ISBI.2008.4541250

de Graaff, M.-A., Jastrow, J.D., Gillette, S., Johns, A., Wullschleger, S.D., 2014. Differential priming of soil carbon driven by soil depth and root impacts on carbon availability. Soil Biology and Biochemistry 69, 147–156. doi:10.1016/j.soilbio.2013.10.047

de Graaff, M.-A., Six, J., Jastrow, J.D., Schadt, C.W., Wullschleger, S.D., 2013. Variation in root architecture among switchgrass cultivars impacts root decomposition rates. Soil Biology and Biochemistry 58, 198–206. doi:10.1016/j.soilbio.2012.11.015

Dungait, J.A.J., Hopkins, D.W., Gregory, A.S., Whitmore, A.P., 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. Global Change Biology 18, 1781–1796. doi:10.1111/j.1365-2486.2012.02665.x

Eisenhauer, N., Bowker, M.A., Grace, J.B., Powell, J.R., 2015. From patterns to causal understanding: Structural equation modeling (SEM) in soil ecology. Pedobiologia 58, 65–72. doi:10.1016/j.pedobi.2015.03.002

Ekschmitt, K., Kandeler, E., Poll, C., Brune, A., Buscot, F., Friedrich, M., Gleixner, G., Hartmann, A., Kästner, M., Marhan, S., Miltner, A., Scheu, S., Wolters, V., 2008. Soil-carbon preservation through habitat constraints and biological limitations on decomposer activity. Journal of Plant Nutrition and Soil Science 171, 27–35. doi:10.1002/jpln.200700051

Emmett, B.D., Youngblut, N.D., Buckley, D.H., Drinkwater, L.E., 2017. Plant Phylogeny and Life History Shape Rhizosphere Bacterial Microbiome of Summer Annuals in an Agricultural Field. Frontiers in Microbiology 8.

Felzenszwalb, P.F., Huttenlocher, D.P., 2012. Distance Transforms of Sampled Functions. Theory of Computing 8, 415–428. doi:10.4086/toc.2012.v008a019

Fort, F., Freschet, G.T., 2020. Plant ecological indicator values as predictors of fine-root trait variations. Journal of Ecology 108, 1565–1577. doi:10.1111/1365-2745.13368

Franklin, S.M., Kravchenko, A.N., Vargas, R., Vasilas, B., Fuhrmann, J.J., Jin, Y., 2021. The unexplored role of preferential flow in soil carbon dynamics. Soil Biology and Biochemistry 161, 108398. doi:10.1016/j.soilbio.2021.108398

Garten, C.T., Smith, J.L., Tyler, D.D., Amonette, J.E., Bailey, V.L., Brice, D.J., Castro, H.F., Graham, R.L., Gunderson, C.A., Izaurralde, R.C., Jardine, P.M., Jastrow, J.D., Kerley, M.K., Matamala, R., Mayes, M.A., Metting, F.B., Miller, R.M., Moran, K.K., Post, W.M., Sands, R.D., Schadt, C.W., Phillips, J.R., Thomson, A.M., Vugteveen, T., West, T.O., Wullschleger, S.D., 2010. Intra-annual changes in biomass, carbon, and nitrogen dynamics at 4-year old switchgrass field trials in west Tennessee, USA. Agriculture, Ecosystems & Environment 136, 177–184. doi:10.1016/j.agee.2009.12.019

Garten, C.T., Wullschleger, S.D., 2000. Soil Carbon Dynamics beneath Switchgrass as Indicated by Stable Isotope Analysis. Journal of Environmental Quality 29, 645–653. doi:10.2134/jeq2000.00472425002900020036x

Gelfand, I., Hamilton, S.K., Kravchenko, A.N., Jackson, R.D., Thelen, K.D., Robertson, G.P., 2020. Empirical Evidence for the Potential Climate Benefits of Decarbonizing Light Vehicle Transport in the U.S. with Bioenergy from Purpose-Grown Biomass with and without BECCS. Environmental Science & Technology 54, 2961–2974. doi:10.1021/acs.est.9b07019

Grace, J.B., 2006. Structural Equation Modeling and Natural Systems. Cambridge University Press, Cambridge. doi:10.1017/CBO9780511617799

Grandy, A.S., Neff, J.C., 2008. Molecular C dynamics downstream: The biochemical decomposition sequence and its impact on soil organic matter structure and function. Science of The Total Environment, BIOGEOCHEMISTRY OF FORESTED ECOSYSTEM - Selected papers from BIOGEOMON, the 5th International Symposium on Ecosystem Behaviour, held at the University of California, Santa Cruz, on June 25–30, 2006 404, 297–307. doi:10.1016/j.scitotenv.2007.11.013

Griffiths, M., Wang, X., Dhakal, K., Guo, H., Seethepalli, A., Kang, Y., York, L.M., 2022. Interactions among rooting traits for deep water and nitrogen uptake in upland and lowland ecotypes of switchgrass (Panicum virgatum L.). Journal of Experimental Botany 73, 967–979. doi:10.1093/jxb/erab437

Gyssels, G., Poesen, J., Bochet, E., Li, Y., 2005. Impact of plant roots on the resistance of soils to erosion by water: a review. Progress in Physical Geography: Earth and Environment 29, 189–217. doi:10.1191/0309133305pp443ra

Haque, M., Epplin, F.M., Taliaferro, C.M., 2009. Nitrogen and Harvest Frequency Effect on Yield and Cost for Four Perennial Grasses. Agronomy Journal 101, 1463–1469. doi:10.2134/agronj2009.0193

Helliwell, J.R., Sturrock, C.J., Miller, A.J., Whalley, W.R., Mooney, S.J., 2019. The role of plant species and soil condition in the structural development of the rhizosphere. Plant, Cell & Environment 42, 1974–1986. doi:10.1111/pce.13529

Henneron, L., Balesdent, J., Alvarez, G., Barré, P., Baudin, F., Basile-Doelsch, I., Cécillon, L., Fernandez-Martinez, A., Hatté, C., Fontaine, S., 2022. Bioenergetic control of soil carbon dynamics across depth. Nature Communications 13, 7676. doi:10.1038/s41467-022-34951-w

Hildebrand, T., Rüegsegger, P., 1997. A new method for the model-independent assessment of thickness in three-dimensional images. Journal of Microscopy 185, 67–75. doi:10.1046/j.1365-2818.1997.1340694.x

Hu, L., Bentler, P.M., 1999. Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. Structural Equation Modeling: A Multidisciplinary Journal 6, 1–55. doi:10.1080/10705519909540118

Huang, X.-F., Chaparro, J.M., Reardon, K.F., Zhang, R., Shen, Q., Vivanco, J.M., 2014. Rhizosphere interactions: root exudates, microbes, and microbial communities. Botany 92, 267–275. doi:10.1139/cjb-2013-0225

Jiang, Y., Li, S., Li, R., Zhang, J., Liu, Y., Lv, L., Zhu, H., Wu, W., Li, W., 2017. Plant cultivars imprint the rhizosphere bacterial community composition and association networks. Soil Biology and Biochemistry 109, 145–155. doi:10.1016/j.soilbio.2017.02.010

Kallenbach, C.M., Grandy, A.S., Frey, S.D., Diefendorf, A.F., 2015. Microbial physiology and necromass regulate agricultural soil carbon accumulation. Soil Biology and Biochemistry 91, 279–290. doi:10.1016/j.soilbio.2015.09.005

Kalyn, A.L., Van Rees, K.C.J., 2006. Contribution of fine roots to ecosystem biomass and net primary production in black spruce, aspen, and jack pine forests in Saskatchewan. The Fluxnet-Canada Research Network: Influence of Climate and Disturbance on Carbon Cycling in Forests and Peatlands 140, 236–243. doi:10.1016/j.agrformet.2005.08.019

Keiluweit, M., Gee, K., Denney, A., Fendorf, S., 2018. Anoxic microsites in upland soils dominantly controlled by clay content. Soil Biology & Biochemistry.

Kelly-Slatten, M.J., Stewart, C.E., Tfaily, M.M., Jastrow, J.D., Sasso, A., de Graaff, M.-A., 2023. Root traits of perennial C4 grasses contribute to cultivar variations in soil chemistry and species patterns in particulate and mineral-associated carbon pool formation. GCB Bioenergy 15, 613–629. doi:10.1111/gcbb.13041

Kravchenko, A.N., Guber, A.K., Razavi, B.S., Koestel, J., Blagodatskaya, E.V., Kuzyakov, Y., 2019a. Spatial patterns of extracellular enzymes: Combining X-ray computed micro-tomography and 2D zymography. Soil Biology and Biochemistry 135, 411–419. doi:10.1016/j.soilbio.2019.06.002

Kravchenko, A.N., Guber, A.K., Razavi, B.S., Koestel, J., Quigley, M.Y., Robertson, G.P., Kuzyakov, Y., 2019b. Microbial spatial footprint as a driver of soil carbon stabilization. Nature Communications 10, 3121. doi:10.1038/s41467-019-11057-4

Kravchenko, A.N., Robertson, G.P., 2011. Whole-Profile Soil Carbon Stocks: The Danger of Assuming Too Much from Analyses of Too Little. Soil Science Society of America Journal 75, 235–240. doi:10.2136/sssaj2010.0076

Kuzyakov, Y., Blagodatskaya, E., 2015. Microbial hotspots and hot moments in soil: Concept & review. Soil Biology and Biochemistry 83, 184–199. doi:10.1016/j.soilbio.2015.01.025

Kwan, J.L.Y., Chan, W., 2011. Comparing standardized coefficients in structural equation modeling: a model reparameterization approach. Behavior Research Methods 43, 730–745. doi:10.3758/s13428-011-0088-6

Lee, J.H., Lucas, M., Guber, A.K., Li, X., Kravchenko, A.N., 2023. Interactions among soil texture, pore structure, and labile carbon influence soil carbon gains. Geoderma 439, 116675. doi:10.1016/j.geoderma.2023.116675

Li, X., Sarma, S.J., Sumner, L.W., Jones, A.D., Last, R.L., 2022. Switchgrass Metabolomics Reveals Striking Genotypic and Developmental Differences in Specialized Metabolic Phenotypes. Journal of Agricultural and Food Chemistry 70, 8010–8023. doi:10.1021/acs.jafc.2c01306

Li, Z., Kravchenko, A.N., Cupples, A., Guber, A.K., Kuzyakov, Y., Philip Robertson, G., Blagodatskaya, E., 2024. Composition and metabolism of microbial communities in soil pores. Nature Communications 15, 3578. doi:10.1038/s41467-024-47755-x

Liang, C., Amelung, W., Lehmann, J., Kästner, M., 2019. Quantitative assessment of microbial necromass contribution to soil organic matter. Global Change Biology 25, 3578–3590. doi:10.1111/gcb.14781

Liang, J., Zhou, Z., Huo, C., Shi, Z., Cole, J.R., Huang, L., Konstantinidis, K.T., Li, X., Liu, B., Luo, Z., Penton, C.R., Schuur, E.A.G., Tiedje, J.M., Wang, Y.-P., Wu, L., Xia, J., Zhou, J., Luo, Y., 2018. More replenishment than priming loss of soil organic carbon with additional carbon input. Nature Communications 9, 3175. doi:10.1038/s41467-018-05667-7

Liebig, M.A., Johnson, H.A., Hanson, J.D., Frank, A.B., 2005. Soil carbon under switchgrass stands and cultivated cropland. Biomass and Bioenergy 28, 347–354. doi:10.1016/j.biombioe.2004.11.004

Liebig, M.A., Schmer, M.R., Vogel, K.P., Mitchell, R.B., 2008. Soil Carbon Storage by Switchgrass Grown for Bioenergy. BioEnergy Research 1, 215–222. doi:10.1007/s12155-008-9019-5

Liu, C., Lou, L., Deng, J., Li, D., Yuan, S., Cai, Q., 2016. Morph-physiological responses of two switchgrass (Panicum virgatum L.) cultivars to cadmium stress. Grassland Science 62, 92–101. doi:10.1111/grs.12119

Liu, Y., Zhang, X., Tran, H., Shan, L., Kim, J., Childs, K., Ervin, E.H., Frazier, T., Zhao, B., 2015. Assessment of drought tolerance of 49 switchgrass (Panicum virgatum) genotypes using physiological and morphological parameters. Biotechnology for Biofuels 8, 152. doi:10.1186/s13068-015-0342-8

Lovell, J.T., MacQueen, A.H., Mamidi, S., Bonnette, J., Jenkins, J., Napier, J.D., Sreedasyam, A., Healey, A., Session, A., Shu, S., Barry, K., Bonos, S., Boston, L., Daum, C., Deshpande, S., Ewing, A., Grabowski, P.P., Haque, T., Harrison, M., Jiang, J., Kudrna, D., Lipzen, A., Pendergast, T.H., Plott, C., Qi, P., Saski, C.A., Shakirov, E.V., Sims, D., Sharma, M., Sharma, R., Stewart, A., Singan, V.R., Tang, Y., Thibivillier, S., Webber, J., Weng, X., Williams, M., Wu, G.A., Yoshinaga, Y., Zane, M., Zhang, L., Zhang, J., Behrman, K.D., Boe, A.R., Fay, P.A., Fritschi, F.B., Jastrow, J.D., Lloyd-Reilley, J., Martínez-Reyna, J.M., Matamala, R., Mitchell, R.B., Rouquette, F.M., Ronald, P., Saha, M., Tobias, C.M., Udvardi, M., Wing, R.A., Wu, Y., Bartley, L.E., Casler, M., Devos, K.M., Lowry, D.B., Rokhsar, D.S., Grimwood, J., Juenger, T.E., Schmutz, J., 2021. Genomic mechanisms of climate adaptation in polyploid bioenergy switchgrass. Nature 590, 438–444. doi:10.1038/s41586-020-03127-1

Lowry, D.B., Lovell, J.T., Zhang, L., Bonnette, J., Fay, P.A., Mitchell, R.B., Lloyd-Reilley, J., Boe, A.R., Wu, Y., Rouquette, F.M., Wynia, R.L., Weng, X., Behrman, K.D., Healey, A., Barry, K., Lipzen, A., Bauer, D., Sharma, A., Jenkins, J., Schmutz, J., Fritschi, F.B., Juenger, T.E., 2019. QTL × environment interactions underlie adaptive divergence in switchgrass across a large latitudinal gradient. Proceedings of the National Academy of Sciences 116, 12933–12941. doi:10.1073/pnas.1821543116

Lucas, M., Nguyen, L.T.T., Guber, A., Kravchenko, A.N., 2022. Cover crop influence on pore size distribution and biopore dynamics: Enumerating root and soil faunal effects. Frontiers in Plant Science 13, 928569. doi:10.3389/fpls.2022.928569

Ma, Z., Wood, C.W., Bransby, D.I., 2000. Impacts of soil management on root characteristics of switchgrass. Biomass and Bioenergy 18, 105–112. doi:10.1016/S0961-9534(99)00076-8

Mahannopkul, K., Jotisankasa, A., 2019. Influences of root concentration and suction on Chrysopogon zizanioides reinforcement of soil. Soils and Foundations 59, 500–516. doi:10.1016/j.sandf.2018.12.014

Makovetsky, R., Piche, N., Marsh, M., 2018. Dragonfly as a Platform for Easy Image-based Deep Learning Applications. Microscopy and Microanalysis 24, 532–533. doi:10.1017/S143192761800315X

Martinez-Feria, R., Basso, B., 2020. Predicting soil carbon changes in switchgrass grown on marginal lands under climate change and adaptation strategies. GCB Bioenergy 12, 742–755. doi:10.1111/gcbb.12726

McCormack, M.L., Dickie, I.A., Eissenstat, D.M., Fahey, T.J., Fernandez, C.W., Guo, D., Helmisaari, H.-S., Hobbie, E.A., Iversen, C.M., Jackson, R.B., Leppälammi-Kujansuu, J., Norby, R.J., Phillips, R.P., Pregitzer, K.S., Pritchard, S.G., Rewald, B., Zadworny, M., 2015. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. New Phytologist 207, 505–518. doi:10.1111/nph.13363

McCully, M.E., 1999. ROOTS IN SOIL: Unearthing the Complexities of Roots and Their Rhizospheres. Annual Review of Plant Physiology and Plant Molecular Biology 50, 695–718. doi:10.1146/annurev.arplant.50.1.695

McLaughlin, S.B., Adams Kszos, L., 2005. Development of switchgrass (Panicum virgatum) as a bioenergy feedstock in the United States. Biomass and Bioenergy 28, 515–535. doi:10.1016/j.biombioe.2004.05.006

McLaughlin, S.B., de la Torre Ugarte, D.G., Garten, C.T., Lynd, L.R., Sanderson, M.A., Tolbert, V.R., Wolf, D.D., 2002. High-Value Renewable Energy from Prairie Grasses. Environmental Science & Technology 36, 2122–2129. doi:10.1021/es010963d

Miltner, A., Bombach, P., Schmidt-Brücken, B., Kästner, M., 2012. SOM genesis: microbial biomass as a significant source. Biogeochemistry 111, 41–55. doi:10.1007/s10533-011-9658-z

Mitchell, R., Vogel, K.P., Uden, D.R., 2012. The feasibility of switchgrass for biofuel production. Biofuels 3, 47–59. doi:10.4155/bfs.11.153

Mosier, S., Kelly, L., Ozlu, E., Robertson, G.P., 2024. Switchgrass (Panicum virgatum L.) cultivars have similar impacts on soil carbon and nitrogen stocks and microbial function. GCB Bioenergy 16, e13125. doi:10.1111/gcbb.13125

Oates, L.G., Duncan, D.S., Gelfand, I., Millar, N., Robertson, G.P., Jackson, R.D., 2016. Nitrous oxide emissions during establishment of eight alternative cellulosic bioenergy cropping systems in the North Central United States. GCB Bioenergy 8, 539–549. doi:10.1111/gcbb.12268

Oburger, E., Schmidt, H., 2016. New Methods To Unravel Rhizosphere Processes. Trends in Plant Science, Special Issue: Unravelling the Secrets of the Rhizosphere 21, 243–255. doi:10.1016/j.tplants.2015.12.005

Paez-Garcia, A., Motes, C.M., Scheible, W.-R., Chen, R., Blancaflor, E.B., Monteros, M.J., 2015. Root Traits and Phenotyping Strategies for Plant Improvement. Plants 4, 334–355. doi:10.3390/plants4020334

Panchal, P., Preece, C., Peñuelas, J., Giri, J., 2022. Soil carbon sequestration by root exudates. Trends in Plant Science 27, 749–757. doi:10.1016/j.tplants.2022.04.009

Parrish, D.J., Fike, J.H., 2005. The Biology and Agronomy of Switchgrass for Biofuels. Critical Reviews in Plant Sciences 24, 423–459. doi:10.1080/07352680500316433

Paterson, E., Sim, A., 1999. Rhizodeposition and C-partitioning of Lolium perenne in axenic culture affected by nitrogen supply and defoliation. Plant and Soil 216, 155–164. doi:10.1023/A:1004789407065

Paul, E.A., Harris, D., Klug, M., Ruess, R., 1999. The determination of microbial biomass, in: Standard Soil Methods for Long-Term Ecological Research. Oxford University Press, New York, NY, pp. 291–317.

Perry, S., Falvo, G., Mosier, S., Robertson, G.P., 2023. Long-term changes in soil carbon and nitrogen fractions in switchgrass, native grasses, and no-till corn bioenergy production systems. Soil Science Society of America Journal n/a. doi:10.1002/saj2.20575

Preacher, K.J., Hayes, A.F., 2004. SPSS and SAS procedures for estimating indirect effects in simple mediation models. Behavior Research Methods, Instruments, & Computers 36, 717–731. doi:10.3758/BF03206553

Quigley, M.Y., Kravchenko, A.N., 2022. Inputs of root-derived carbon into soil and its losses are associated with pore-size distributions. Geoderma 410, 115667. doi:10.1016/j.geoderma.2021.115667

Rawlins, B.G., Wragg, J., Reinhard, C., Atwood, R.C., Houston, A., Lark, R.M., Rudolph, S., 2016. Three-dimensional soil organic matter distribution, accessibility and microbial respiration in macroaggregates using osmium staining and synchrotron X-ray computed tomography. SOIL 2, 659–671. doi:10.5194/soil-2-659-2016

Raynaud, X., Nunan, N., 2014. Spatial Ecology of Bacteria at the Microscale in Soil. PLOS ONE 9, e87217. doi:10.1371/journal.pone.0087217

Reubens, B., Poesen, J., Danjon, F., Geudens, G., Muys, B., 2007. The role of fine and coarse roots in shallow slope stability and soil erosion control with a focus on root system architecture: a review. Trees 21, 385–402. doi:10.1007/s00468-007-0132-4

Robertson, G.P., Hamilton, S.K., Barham, B.L., Dale, B.E., Izaurralde, R.C., Jackson, R.D., Landis, D.A., Swinton, S.M., Thelen, K.D., Tiedje, J.M., 2017. Cellulosic biofuel contributions to a sustainable energy future: Choices and outcomes. Science 356, eaal2324. doi:10.1126/science.aal2324

Rohe, L., Apelt, B., Vogel, H.-J., Well, R., Wu, G.-M., Schlüter, S., 2021. Denitrification in soil as a function of oxygen availability at the microscale. Biogeosciences 18, 1185–1201. doi:10.5194/bg-18-1185-2021

Roley, S.S., Ulbrich, T.C., Robertson, G.P., 2021. Nitrogen Fixation and Resorption Efficiency Differences Among Twelve Upland and Lowland Switchgrass Cultivars. Phytobiomes Journal 5, 97–107. doi:10.1094/PBIOMES-11-19-0064-FI

Ronneberger, O., Fischer, P., Brox, T., 2015. U-Net: Convolutional Networks for Biomedical Image Segmentation, in: Navab, N., Hornegger, J., Wells, W.M., Frangi, A.F. (Eds.), Medical Image Computing and Computer-Assisted Intervention – MICCAI 2015, Lecture Notes in Computer Science. Springer International Publishing, Cham, pp. 234–241. doi:10.1007/978-3-319-24574-4_28

Roosendaal, D., Stewart, C.E., Denef, K., Follett, R.F., Pruessner, E., Comas, L.H., Varvel, G.E., Saathoff, A., Palmer, N., Sarath, G., Jin, V.L., Schmer, M., Soundararajan, M., 2016.

Switchgrass ecotypes alter microbial contribution to deep-soil C. SOIL 2, 185–197. doi:10.5194/soil-2-185-2016

Ruess, R.W., Hendrick, R.L., Burton, A.J., Pregitzer, K.S., Sveinbjornssön, B., Allen, M.F., Maurer, G.E., 2003. COUPLING FINE ROOT DYNAMICS WITH ECOSYSTEM CARBON CYCLING IN BLACK SPRUCE FORESTS OF INTERIOR ALASKA. Ecological Monographs 73, 643–662. doi:10.1890/02-4032

Samson, R., Mani, S., Boddey, R., Sokhansanj, S., Quesada, D., Urquiaga, S., Reis, V., Ho Lem, C., 2005. The Potential of C4 Perennial Grasses for Developing a Global BIOHEAT Industry. Critical Reviews in Plant Sciences 24, 461–495. doi:10.1080/07352680500316508

Sanderson, M.A., Adler, P.R., 2008. Perennial Forages as Second Generation Bioenergy Crops. International Journal of Molecular Sciences 9, 768–788. doi:10.3390/ijms9050768

Sanford, G.R., Oates, L.G., Jasrotia, P., Thelen, K.D., Robertson, G.P., Jackson, R.D., 2016. Comparative productivity of alternative cellulosic bioenergy cropping systems in the North Central USA. Agriculture, Ecosystems & Environment 216, 344–355. doi:10.1016/j.agee.2015.10.018

Sawyer, A., Staley, C., Lamb, J., Sheaffer, C., Kaiser, T., Gutknecht, J., Sadowsky, M.J., Rosen, C., 2019. Cultivar and phosphorus effects on switchgrass yield and rhizosphere microbial diversity. Applied Microbiology and Biotechnology 103, 1973–1987. doi:10.1007/s00253-018-9535-y

Scheidweiler, D., Bordoloi, A.D., Jiao, W., Sentchilo, V., Bollani, M., Chhun, A., Engel, P., de Anna, P., 2024. Spatial structure, chemotaxis and quorum sensing shape bacterial biomass accumulation in complex porous media. Nature Communications 15, 191. doi:10.1038/s41467-023-44267-y

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nature Methods 9, 676–682. doi:10.1038/nmeth.2019

Schlüter, S., Leuther, F., Albrecht, L., Hoeschen, C., Kilian, R., Surey, R., Mikutta, R., Kaiser, K., Mueller, C.W., Vogel, H.-J., 2022. Microscale carbon distribution around pores and particulate organic matter varies with soil moisture regime. Nature Communications 13, 2098. doi:10.1038/s41467-022-29605-w

Schlüter, S., Sheppard, A., Brown, K., Wildenschild, D., 2014. Image processing of multiphase images obtained via X-ray microtomography: A review. Water Resources Research 50, 3615–3639. doi:10.1002/2014WR015256

Schlüter, S., Vogel, H.-J., 2016. Analysis of Soil Structure Turnover with Garnet Particles and X-Ray Microtomography. PLOS ONE 11, e0159948. doi:10.1371/journal.pone.0159948

Schlüter, S., Zawallich, J., Vogel, H.-J., Dörsch, P., 2019. Physical constraints for respiration in microbial hotspots in soil and their importance for denitrification. Biogeosciences 16, 3665–3678. doi:10.5194/bg-16-3665-2019

Schumacker, R.E., Lomax, R.G., 1996. A Beginner's Guide to Structural Equation Modeling: Fourth Edition, 2nd ed. Psychology Press, New York. doi:10.4324/9781410610904

Seethepalli, A., Dhakal, K., Griffiths, M., Guo, H., Freschet, G.T., York, L.M., 2021. RhizoVision Explorer: open-source software for root image analysis and measurement standardization. AoB PLANTS 13, plab056. doi:10.1093/aobpla/plab056

Sehgal, A., Sita, K., Rehman, A., Farooq, M., Kumar, S., Yadav, R., Nayyar, H., Singh, S., Siddique, K.H.M., 2021. Chapter 13 - Lentil, in: Sadras, V.O., Calderini, D.F. (Eds.), Crop Physiology Case Histories for Major Crops. Academic Press, pp. 408–428. doi:10.1016/B978-0-12-819194-1.00013-X

Semchenko, M., Xue, P., Leigh, T., 2021. Functional diversity and identity of plant genotypes regulate rhizodeposition and soil microbial activity. New Phytologist 232, 776–787. doi:10.1111/nph.17604

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and Fungal Contributions to Carbon Sequestration in Agroecosystems. Soil Science Society of America Journal 70, 555–569. doi:10.2136/sssaj2004.0347

Sokol, N.W., Kuebbing, Sara.E., Karlsen-Ayala, E., Bradford, M.A., 2019. Evidence for the primacy of living root inputs, not root or shoot litter, in forming soil organic carbon. New Phytologist 221, 233–246. doi:10.1111/nph.15361

Sprunger, C.D., Oates, L.G., Jackson, R.D., Robertson, G.P., 2017. Plant community composition influences fine root production and biomass allocation in perennial bioenergy cropping systems of the upper Midwest, USA. Biomass and Bioenergy 105, 248–258. doi:10.1016/j.biombioe.2017.07.007

Steinaker, D.F., Wilson, S.D., 2008. Phenology of fine roots and leaves in forest and grassland. Journal of Ecology 96, 1222–1229. doi:10.1111/j.1365-2745.2008.01439.x

Strong, D.T., Wever, H.D., Merckx, R., Recous, S., 2004. Spatial location of carbon decomposition in the soil pore system: Spatial location of carbon decomposition. European Journal of Soil Science 55, 739–750. doi:10.1111/j.1365-2389.2004.00639.x

Stroup, W.W., 2002. Power analysis based on spatial effects mixed models: A tool for comparing design and analysis strategies in the presence of spatial variability. Journal of Agricultural, Biological, and Environmental Statistics 7, 491–511. doi:10.1198/108571102780

Udawatta, R.P., Anderson, S.H., Gantzer, C.J., Garrett, H.E., 2008. Influence of Prairie Restoration on CT-Measured Soil Pore Characteristics. Journal of Environmental Quality 37, 219–228. doi:10.2134/jeq2007.0227

Ulbrich, T.C., Friesen, M.L., Roley, S.S., Tiemann, L.K., Evans, S.E., 2021. Intraspecific Variability in Root Traits and Edaphic Conditions Influence Soil Microbiomes Across 12 Switchgrass Cultivars. Phytobiomes Journal 5, 108–120. doi:10.1094/PBIOMES-12-19-0069-FI

Valverde-Barrantes, O.J., Freschet, G.T., Roumet, C., Blackwood, C.B., 2017. A worldview of root traits: the influence of ancestry, growth form, climate and mycorrhizal association on the functional trait variation of fine-root tissues in seed plants. New Phytologist 215, 1562–1573. doi:10.1111/nph.14571

Védère, C., Vieublé Gonod, L., Pouteau, V., Girardin, C., Chenu, C., 2020. Spatial and temporal evolution of detritusphere hotspots at different soil moistures. Soil Biology and Biochemistry 150, 107975. doi:10.1016/j.soilbio.2020.107975

Vogel, H.-J., Weller, U., Schlüter, S., 2010. Quantification of soil structure based on Minkowski functions. Computers & Geosciences 36, 1236–1245. doi:10.1016/j.cageo.2010.03.007

Vogel, K.P., Schmer, M.R., Mitchell, R.B., 2005. Plant Adaptation Regions: Ecological and Climatic Classification of Plant Materials. Rangeland Ecology and Management 58, 315–319. doi:10.2111/1551-5028(2005)58[315:PAREAC]2.0.CO;2

Xu, J.G., Juma, N.G., 1994. Relations of shoot C, root C and root length with root-released C of two barley cultivars and the decomposition of root-released C in soil. Canadian Journal of Soil Science 74, 17–22. doi:10.4141/cjss94-002

Yang, J., Worley, E., Ma, Q., Li, J., Torres-Jerez, I., Li, G., Zhao, P.X., Xu, Y., Tang, Y., Udvardi, M., 2016. Nitrogen remobilization and conservation, and underlying senescence-associated gene expression in the perennial switchgrass Panicum virgatum. New Phytologist 211, 75–89. doi:10.1111/nph.13898

Yang, Y., Tilman, D., Furey, G., Lehman, C., 2019. Soil carbon sequestration accelerated by restoration of grassland biodiversity. Nature Communications 10, 718. doi:10.1038/s41467-019-08636-w

Zhang, P., Wang, Y., Xu, L., Sun, H., Li, R., Zhou, J., 2022. Factors controlling the spatial variability of soil aggregates and associated organic carbon across a semi-humid watershed. Science of The Total Environment 809, 151155. doi:10.1016/j.scitotenv.2021.151155

Zhang, Y., Zalapa, J., Jakubowski, A.R., Price, D.L., Acharya, A., Wei, Y., Brummer, E.C., Kaeppler, S.M., Casler, M.D., 2011. Natural Hybrids and Gene Flow between Upland and Lowland Switchgrass. Crop Science 51, 2626–2641. doi:10.2135/cropsci2011.02.0104