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Soil carbon accrual and biopore formation across a plant diversity gradient

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Abstract. Plant diversity promotes soil organic carbon (SOC) gains through intricate changes in root-soil interactions and their subsequent influence on soil physical and biological processes. We assessed SOC and pore characteristics of soils under a range of switchgrass-based plant systems, representing a gradient of plant diversity with species richness ranging from 1 to 30 species 12 years after their establishment. We focused on soil biopores as indicators of root activity legacy, measured using X-ray computed micro-tomography scanning, and explored biopore relationships with SOC accumulation.

Plant functional richness explained 29% of bioporosity and 36% of SOC variation, while bioporosity itself explained 36% of the variation in SOC. The most diverse plant system (30 species) had the highest SOC, while long-term bare soil fallow and monoculture switchgrass had the lowest. Of particular note was a two-species mixture of switchgrass (Panicum virgatum L.) and ryegrass (Elymus canadensis), which exhibited the highest bioporosity and achieved SOC levels comparable to those of the systems with 6 and 10 plant species, and were inferior only to the system with 30 species. We conclude that plant diversity may enhance SOC through biopore-mediated mechanisms and suggest a potential for identifying specific plant combinations that may be particularly efficient for fostering biopore formation and subsequently SOC sequestration.

1 Introduction

Plant diversity has been found to positively influence soil organic carbon (SOC) accumulation in various ecosystems, including grasslands (Lange et al., 2015; Sprunger and Robertson, 2018) and row crop agriculture (Liebman et al., 2013; McDaniel et al., 2014). Among the mechanisms through which higher plant diversity promotes SOC storage are i) high biomass and C inputs from roots (Yang and Tilman, 2020), ii) slower root decomposition in high diversity system due to increased root C:N ratios (Chen et al., 2017), and iii) higher microbial activity enhanced by belowground inputs, where greater quantities of plant-added C are being microbially processed and transformed into microbial biomass (Prommer et al., 2020; Lee et al., 2023) then necromass (Qian et al., 2023; Mou et al., 2024). This microbially-processed C is then protected through physico-chemical associations with soil minerals (Cotrufo et al., 2022). Moreover, in diverse plant communities belowground competition among plants with contrasting root architectures can lead to greater root proliferation

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through the soil matrix (Gersani et al., 2001; Bargaz et al., 2017; Wang et al., 2017). More extensive and dense root growth results in greater volumes of the soil matrix being exposed to inputs of new root-derived organic material and subsequent microbial activities (Kravchenko et al., 2019). However, spatial distributions and root morphological characteristics of neighboring plant species can vary (Bolte and Villanueva, 2006; Wang et al., 2014), thereby producing root-soil interactions unique to specific plant communities. Moreover, recent evidence has shown that root-soil interactions and plant-derived C gains can also be affected by soil pore structure (Quigley and Kravchenko, 2022; Lucas et al., 2023).

Analysis of root-soil interactions in diverse perennial plant communities in situ is extremely challenging due to the opaque nature of soil and the difficulty of carrying out long-term (e.g., multiyear) continuous rhizobox or greenhouse studies. Thus, much knowledge regarding impacts of diverse communities on root-soil interactions is based on speculations from the data on root volumes and architecture generated upon disturbing the system to procure the roots and upon conducting the root measurements after cleaning away the soil. In contrast, sampling intact soil from long-term field studies and visualizing root residues, particulate organic matter (POM), and pores via X-ray computed micro-tomography (µCT) can generate helpful information on root-soil interactions, augmenting data from destructive root system analyses (Helliwell et al., 2013).

We surmise that information on biopores from µCT scanning can be of particular relevance for assessing root impacts and root-soil interactions in perennial plant systems. Biopores are the soil pores originated from biological activity such as plant root growth and the movement of earthworms and other soil fauna (Dexter, 1986; Blackwell et al., 1990). Root-originated biopores, formed either by growth of living roots or by decomposition of old roots, are particularly significant as they are 40 times more abundant, especially in subsoils, compared to earthworm biopores (Banfield et al., 2018). Root biopores are tubular, round-shaped channels with sizes ranging from a few micron to several centimeters (Kautz, 2015). Since root inputs are primarily introduced into the soil through the biopores, they have >2.5 times higher soil C contents compared to bulk soil (Banfield et al., 2017). Rapid microbial decomposition of plant residues and accumulation of microbial residues observed in root biopores (Banfield et al., 2018) suggest that biopore characteristics may reflect the physical preferences and biochemical processes involved in the transformation and accumulation of plant-derived C.

Reuse of existing biopores by newly grown roots is a commonly observed process in annual crops such as wheat, fodder radish, and spring barley (White and Kirkegaard, 2010; Wahlström et al., 2021). Switchgrass, a North American prairie grass, currently actively explored as a potential bioenergy feedstock (Larnaudie et al., 2022; Zegada-Lizarazu et al., 2022), is known for its particularly active use of old root channels (i.e., biopore reuse), especially when grown in monoculture (Lucas et al., 2023). Because of their continuous reuse, accompanied by repeated influxes of new C and stimulated microbiota, biopores in perennial plant systems can be viewed as hotspots of C processing and are both a product of historic root-soil interactions and a current arena of such interactions. X-ray µCT is particularly suitable for biopore investigation (Wendel et al., 2022), enabling the examination of accumulated evidence of past root-soil interactions.

We hypothesize that in systems with varying levels of plant diversity enhanced biopore formation and their greater abundance can stimulate C gains. Here we examine first, how a plant diversity gradient comprised of 1 to 30 North America prairie species can shape soil pore characteristics, especially biopores, and second, whether these characteristics are associated with SOC levels accumulated over the prior 12-years.

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2 Materials and Methods

2.1 Experimental site and soil sampling

Soil samples were collected from the Cellulosic Biofuel Diversity Experiment site established in 2008 at Kellogg Biological Station (KBS, 42°23′47″ N, 85°22′26″ W), a part of the KBS Long-Term Ecological Research (LTER) program (Robertson and Hamilton, 2015). The soil is fine-loamy, mixed, mesic, Typic Hapludalf (Kalamazoo loam). The experiment consists of twelve plant systems representing a 12-point gradient of plant diversity, six of which were used in this study. Specifically, we sampled a bare soil system, which was kept free of vegetation since 2016 after 8 years of continuous corn (CE1), a monoculture switchgrass (Panicum virgatum, L.; var. Southlow) system (CE7), a mixture of two grass species, namely, switchgrass and Canadian rye (Elymus canadensis, L.) (CE8), a mixture of six native grasses (CE9), a mixture of six native grasses and four forbs (CE10), and a mixture of six native grasses and 24 forbs (CE12). Plant species of each system are listed in Supplementary Table S1. The experiment is in a randomized complete block design with four replicated 9.1 m x 27.4 m plots for each plant system.

Aboveground biomass of the plots, except for CE1 (bare soil), was sampled every fall from 2010-2019 (Fig. S1a), and the data of 2018 and 2019 was used for this study (Fig. S1b). The entire aboveground biomass from each plot was harvested with a mini combine, leaving 10-15 cm of stubble, and weighed and subsampled for moisture content determination.

For soil pore analysis, intact soil cores (5 cm diameter (Ø) and 5 cm height) were taken from the 7-12 cm depth interval in July 2019. Loose soil adjacent to each core was also procured for measurements of other soil characteristics. Two soil cores were collected from each plot, for a total of 48 soil cores (6 systems x 4 replicate plots x 2 cores per plot).

2.2 Soil characteristics measured using destructively sampled soil

Soil moisture at the time of core sampling was determined gravimetrically using a 20 g subsample of loose soil immediately upon collection. The remaining loose soil samples were air-dried for 2 days and sieved to < 2 mm for further analysis.

Total soil C and N were measured by combustion analysis using an elemental CN analyzer (Costech Analytical Technologies Inc., CA, USA). Soil C mineralization was measured via 10 d incubation: 10 g of air-dried soil were brought to 20% gravimetric moisture, placed in a beaker that was then placed in a 450 mL Mason jar with ~5 mL of purified water on the bottom for maintaining high humidity within the jar. Mason jars were kept in the dark at 20 °C for 10 days, and CO_2 concentration in the headspace was measured for each jar using Infrared Photoacoustic Spectroscopy (INNOVA Air Tech Instruments, Denmark).

2.3 Soil core scanning and pore structure analysis

Soil cores were subjected to X-ray computed micro-tomography (North Star Imaging, X3000, Rogers, USA) to visualize and quantify soil pore structure. The scanning was conducted with a projection energy level of 75 KV

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and 450 µA, with 2880 projections per scan. 3D reconstruction of the images was computed using efX-CT software (North Star Imaging, Rogers, USA) obtaining a final scanning resolution of 18.2 µm.

Reconstructed images were processed using Fiji software (Schindelin et al., 2012) and simpleITK package in Python (Beare et al., 2018). A series of image pre-processing steps was conducted using Fiji and its Xlib plugin (Münch and Holzer, 2008). Specifically, images were cropped into 1500 x 1500 pixels with a height of 2240 pixels to remove artifacts near core edges. Then, a 2D non-local filter (sigma=0.1) was applied to reduce noise.

For pore segmentation, threshold values were obtained from eight segmentation methods, i.e., Otsu, Kitler, Huang, Triangle, ISO, Li, Renyi, and Moments. Outliers that exceeded >1 standard deviation of the mean were removed. This approach enabled us to minimize the side effects of using one specific thresholding method and ensured robustness of the segmentation (Schlüter et al., 2014). Hereafter, we refer to the >18.2 µm Ø pores as visible pores, and their total volume as visible porosity. Pore size distributions of visible pores were determined by the Local Thickness method embedded in Fiji, which is based on maximal inscribed spheres approach (Silin and Patzek, 2006). For biopore identification, the images were subjected to Tubeness filtering in Fiji to detect tubular type pores of different radius. Detailed procedures for biopore segmentation are publicly available (https://github.com/Maik-Lu/Roots_and_Biopores). Total volumes of visible pores and biopores were presented as visible porosity and bioporosity. Surface area of biopores was calculated by applying assumption that the biopore shape is cylindrical in a given radius, and the average distance to biopores was calculated using the Euclidean Distance Transform (3D) function in Fiji.

2.4 Plant diversity indicators

Two plant diversity indicators were used in this study: i) plant species richness, and ii) plant functional richness (Díaz and Cabido, 2001). Plant species richness was represented by the number of plant species in each treatment, i.e., 0, 1, 2, 6, 10, and 30 for CE1, CE7, CE8, CE9, CE10, and CE12, respectively. For plant functional richness we adopted the ecological concept of plant functional types, used to simplify plant diversity and behavior in ecological models (McMahon et al., 2011). Specifically, we followed the approach used in grassland studies (Mangan et al., 2011; Spiesman et al., 2018) by separating species based on different photosynthetic pathways (C3 vs. C4), and leaf shape (broad-leaf vs. grasses) to form three functional groups, namely, C3 grasses, C4 grasses, and forbs. Based on species characteristics (Table S1), plant functional richness of each treatment was equal to 0, 1, 2, 2, 3, and 3 for CE1, CE7, CE8, CE9, CE10, and CE12, respectively.

2.5 Statistical analysis

The statistical model for soil C, N, C:N ratio, 10-day mineralization, visible porosity, and bioporosity data consisted of plant system as a fixed effect, and experimental blocks and blocks by systems interaction as random effects (Milliken and Johnson, 2009). The latter term, in essence, represents experimental plots and was used as an error term to test the plant system effect. The statistical model for the aboveground biomass measured during two consecutive years further included year and its interaction with the plant system as fixed factors. The statistical model for pore and biopore size distribution data also included the size and size by plant system interaction as

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fixed effects, and soil core nested within the plant system and experimental plot as the random effect. Normality was checked by visual inspection of normal probability plots, and when found violated, the data were subjected to either square root or lognormal transformation prior to the analyses. The equal variance assumption was tested by Levene's test, and when found to be violated, the unequal variance model was fitted using the approach suggested by Milliken and Johnson (2009).

To explore associations of soil characteristics with pore and biopore data, the porosity and bioporosity from two intact cores of each plot were averaged, followed by linear regression analysis. All statistical analyses were conducted using SAS 9.4 software, using PROC MIXED and PROC REG procedure. Results are reported as statistically significant at $p<0.05$ and as trends at $p<0.1$. P-values < 0.1, < 0.05, and < 0.01 are marked with *, **, and ***, respectively.

3 Results

3.1 Plant biomass and soil C characteristics of the studied plant systems

Total aboveground biomass (2018-2019) tended to be higher in the most diverse systems (CE10 and CE12) than in grass-only systems (CE8 and CE9), while the monoculture switchgrass (CE7) was intermediate ($p < 0.10$, Fig. S1b). Total aboveground biomass was weakly correlated with plant species richness (Fig. S2a) and not correlated with plant functional richness (Fig. S2b). Total aboveground biomass was not correlated with SOC contents (Fig. S2c).

The plant systems with the highest diversity (CE12) had markedly higher SOC as compared to the rest of the systems (Fig. 1a). However, an increase in plant diversity from a two-species (CE8) to six-species (CE9) and then to a ten-species (CE10) system did not affect SOC (Fig. 1a). Soil C:N ratio was smallest in monoculture switchgrass (CE7), and greatest in high diversity systems (CE10 and CE12) ($p< 0.01$, Fig. S3). Carbon mineralization was highest in CE12, followed by bare soil (CE1), and CE8 (Fig. 1c). C mineralization was lowest in CE7 and CE10.

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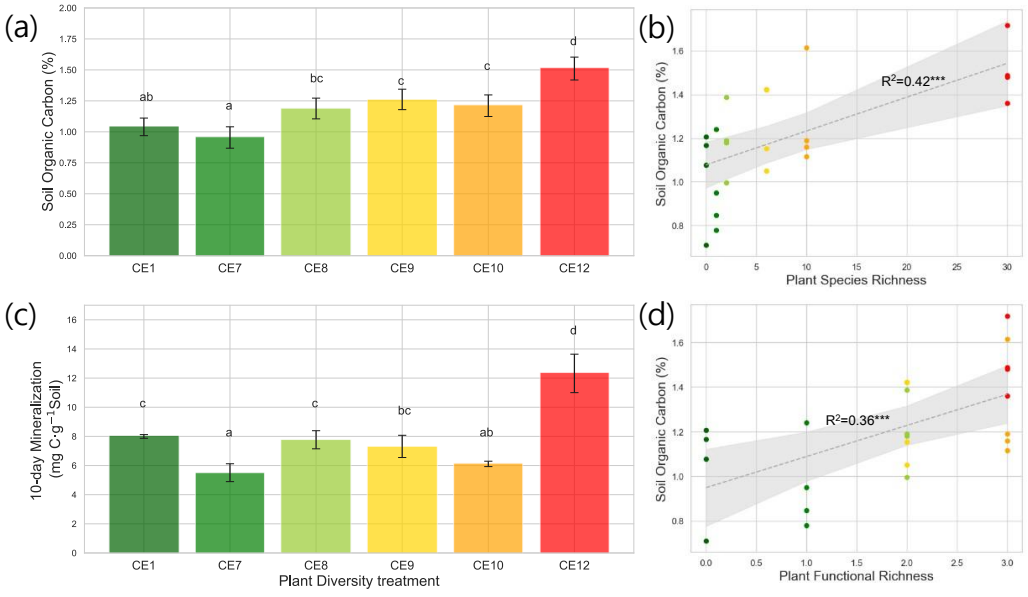

Figure 1: Soil Organic Carbon (SOC) content (a) and 10-day mineralization (c) across different plant diversity systems. Correlations between SOC and Plant Species Richness (b), as well as Plant Functional Richness (d), are shown. Letters indicate significant differences between plant diversity treatments ($p < 0.05$). Dotted gray lines in (b) and (d) represent fitted regression models, with light gray shaded area denoting 95% confidence interval. R2 values are provided for each model. Asterisks (***) indicate statistically significant regression models at $p < 0.01$.

3.2 Biopore characteristics

Plant systems affected pore size distributions (Fig. 2a), where CE8, CE10, and CE12 had the lowest volumes of < 200 µm diameter pores and the highest volumes of >400 µm diameter pores, while an opposite trend was observed for CE1, CE7, and CE9 systems. Biopores, which was segmented based on its tubular morphology, tended to be larger than regular pores of arbitrary shapes (Fig. 2). For example, while the mode (i.e., the most frequent value) pore diameter of the entire pore size distribution was ~100 µm (Fig. 2a), for biopores the modal pore diamater was ~300 µm (Fig. 2b).

Visible porosity (pores of > 18.2 µm diam.) was highest in CE9 followed by CE8 (Fig. 3a). In contrast to visible porosity, bioporosity was the highest in CE8, which is comprised of switchgrass and Canadian ryegrass (Fig. 3b). In addition to CE8, throughout the entire range of biopore sizes, CE10 and CE12 also had consistently higher biopore volumes (Fig. 2b) as well as higher total bioporosity than the rest of the systems (Fig. 3b). Total surface areas of biopores (all pores of < 1500 µm diam.) were highest in CE7 and CE8, and lowest in CE1 ($p < 0.05$, Fig. 4a). Average distance to biopores showed an opposite trend from surface area, i.e., farthest in CE1 and CE9 and shortest in CE8 ($p < 0.05$, Fig. 4d).

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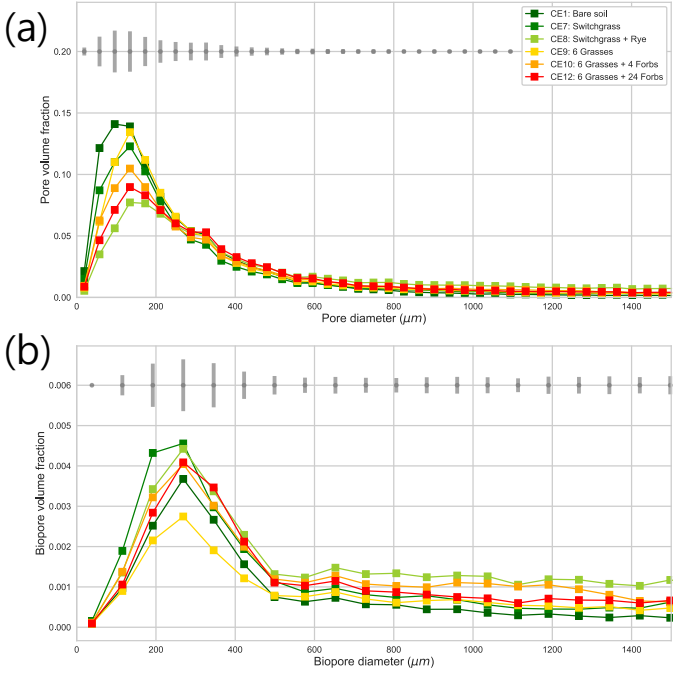

Figure 2: Pore size distribution (a) and biopore size distribution (b) in different plant diversity systems. Gray bars indicate the least significant difference (LSD) of each pore diameter.

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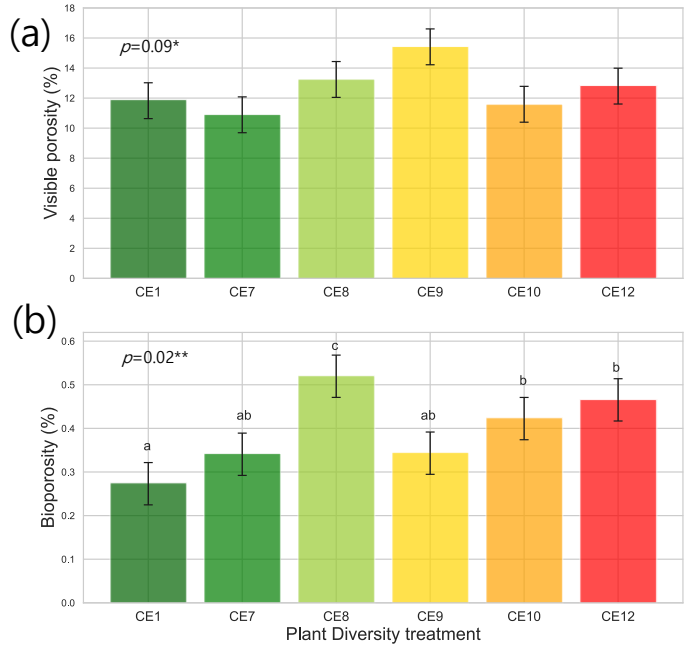

Figure 3: Visible porosity (a) and bioporosity (b) in different plant diversity systems. Asterisks * and ** indicate statistically significant differences among plant diversity treatments at p< 0.10 and 0.05, respectively. Letters indicate significant differences between plant diversity treatments (p< 0.05).

3.3 Correlation between plant diversity, pore characteristics, and soil organic carbon

Overall, plant species richness and plant functional richness were positively correlated with SOC, with plant species richness explaining 42% of SOC variation (p< 0.01, Fig. 1b) and plant functional richness 36% of SOC (p< 0.01, Fig. 1d). The surface area of biopores was positively correlated with bioporosity with an R2 of 0.50 (p< 0.01, Fig. 4b). On the other hand, average distance to biopores was negatively associated with bioporosity, with an R2 of 0.46 (p< 0.01, Fig. 4d). Visible porosity was not correlated with plant species richness (Fig. S4b). Bioporosity was significantly affected by plant species richness (p< 0.01), which explained 10% of bioporosity variation (Fig. S4a). In contrast to plant species richness, plant functional richness explained 29% of the variance in bioporosity (p< 0.05, Fig. 5a). Total visible porosity was not correlated with SOC (Fig. S4b), while bioporosity was positively correlated with SOC with an R2 of 0.36 (p< 0.01, Fig. 5b).

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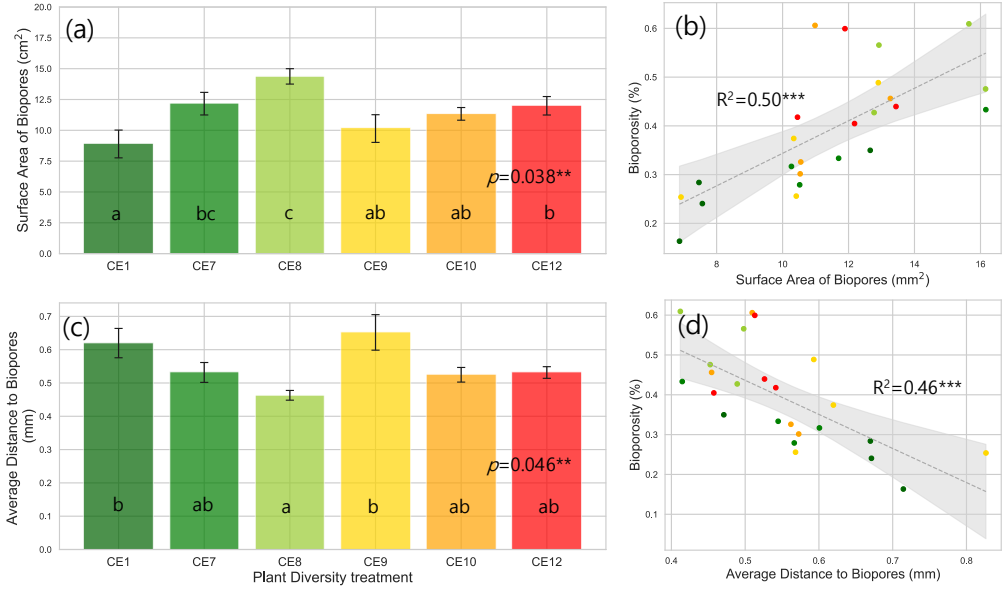

Figure 4: Surface area of biopores (a) and average distance to biopores (c) across different plant diversity systems. Correlations between bioporosity and surface area of biopores (b), as well as average distance to biopores (d), are shown. Letters indicate significant differences between plant diversity treatments (p< 0.05). Dotted gray lines in (b) and (d) represent fitted regression models, with light gray shaded area denoting 95% confidence interval. R2 values are provided for each model. Asterisks (***) indicate statistically significant regression models at p< 0.01.

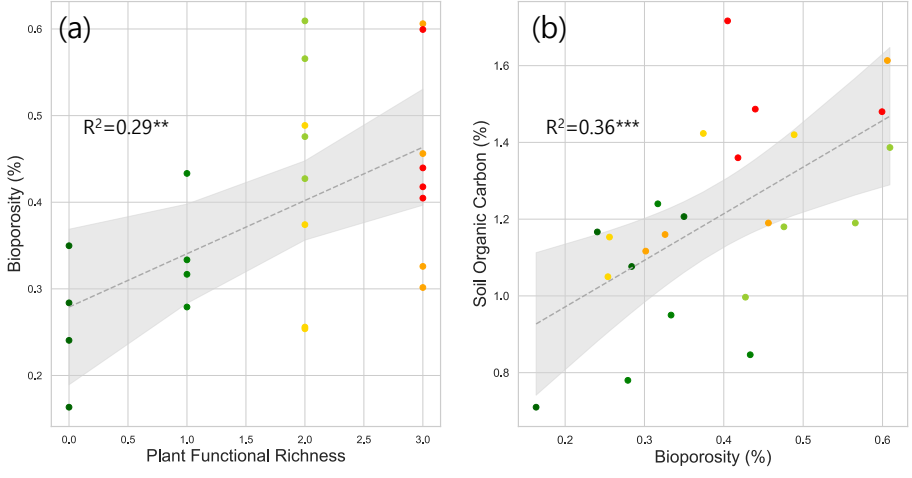

Figure 5: Correlation between plant functional richness and bioporosity (a), and between bioporosity and soil organic carbon (b). R^2 values are provided for each model. Dotted gray lines represent fitted regression models, with light gray shaded area denoting 95% confidence interval. Asterisks ** and * indicate statistically significant regression models at p< 0.05 and 0.01, respectively.**

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4 Discussion

4.1 Biopores link plant diversity and SOC accumulation

Consistent with our expectations and previous literature (Lange et al., 2015; Prommer et al., 2020; Yang and Tilman, 2020), 12 years of contrasting plant system was sufficient to develop differences in SOC among the studied plant systems, with the highest SOC observed in the most diverse plant system (Fig. 1a, b, and d). However, the lack of correlation between the plant aboveground biomass and SOC observed here (Fig. S2c) suggests that greater plant biomass did not itself significantly contribute to SOC increases. This lack of impact is even more obvious when comparing CE7 (monoculture) and CE8 (two-species system), where CE8 had the lowest aboveground biomass but its SOC was comparable to that from the systems with 3~5 times higher plant species richness and higher aboveground biomass (CE9 and 10, Fig. 1a and S1b).

Besides plant diversity, bioporosity was another characteristic consistent with SOC results of the study (Fig. 5b). Since the correlation between plant richness and SOC might have been amplified due to a wide range of plant richness (specifically driven by species richness = 30, Fig. 1b and d), bioporosity might be a better indicator of SOC. Moreover, the CE8 system with its unexpectedly high SOC turned out to have higher bioporosity than the other studied systems (Fig. 3b), a trend that was present for all studied biopore sizes (Fig. 2b). Carbon processing in root-originated biopores is more rapid than in bulk soil, since higher inputs of organic substrates (e.g., root exudates) promote growth and assimilation of C by soil microbes (Kuzyakov and Blagodatskaya, 2015) and consequently increases microbial necromass accumulation (Banfield et al., 2018).

The biopores in CE8 had the greatest surface area of biopores, followed by the most diverse system (Fig. 4a). The average distance to biopores was the shortest in CE8, followed by diverse systems (CE10 and CE12) (Fig. 4c). The surface area of biopores is critical as it represents the specific portion of the soil matrix that directly intercepts root-derived carbon inputs, i.e., rhizodeposits (Keller et al., 2021) and decomposed root residues processed by soil microbes (Kim et al., 2022). A shorter average distance to biopores indicates greater accessibility for root-derived carbon, facilitating its efficient transfer into the soil matrix.

Biopores are pore spaces that have been impacted by roots and thus reflect root growth, death, and resulting changes in the soil's physicochemical properties accumulated for 12 years. In ecosystems with high plant species diversity, the importance of biopore characteristics is further amplified. Diverse plant communities exhibit complex root architectures due to interspecific interactions, leading to varying quantities and spatial distributions of root-derived carbon. Increased root-originated bioporosity also indicates diversified root growth paths, which leads to strengthened root-soil contact (Lucas et al., 2023). A greater interface with the soil matrix (i.e., surface area) facilitates diffusion and storage of microbially processed C (Kravchenko et al., 2019), thereby contributing to increased SOC.

4.2 Future research directions: winning plant species combinations?

Interestingly, while bioporosity was not affected by the number of plant species within the system, it was positively correlated with the systems' functional diversity (Fig. 5a). This implies that greater diversity of functional groups – rather than a greater diversity of plant species per se –may lead to a more thorough exploration of the soil matrix in part through biopore formation, subsequently enhancing soil C accumulation.

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Moreover, certain combinations of specific plant species, e.g., CE8 (Switchgrass + Canadian rye), can be more "effective" in building biopores, and potentially furthering SOC accumulation through rapid processing of added substrates (Banfield et al., 2017; Banfield et al., 2018). We hypothesize that Canadian rye could be viewed as a species with a keystone effect on bioporosity and SOC accumulation. A "keystone effect" refers to beneficial effect of specific plant species on ecosystem function (Mills et al., 1993). For instance, legume species added to grasslands have been found to disproportionately affect biomass productivity and root C accrual (Minns et al., 2001; Fornara and Tilman, 2008; Lange et al., 2015; Yang et al., 2019) due to N assimilation by legumes and consequent utilization of that N by grasses (Minns et al., 2001; Mangan et al., 2011; Mou et al., 2024). Introduction of a C3 plant, i.e., Canadian rye, into a monoculture C4 switchgrass community may have altered switchgrass root growth and exudation patterns. Sensitivity of chemical composition of switchgrass root exudates to neighboring plant species (e.g., C3 Koeleria macrantha Ledeb.), and resultant increases in microbial biomass C and changes in bacterial diversity in the switchgrass rhizosphere have indeed been demonstrated before (Ulbrich et al., 2022). We see the need for further investigation into the role that individual members of grassland communities may play in stimulating soil pore structure development and soil C accumulation. Identifying keystone species enabling more efficient C accumulation can guide plant restoration and SOC accrual efforts.

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Code/Data availability

The datasets generated during and/or analyzed during the current study are available upon request.

Author contribution

K. K Formal analysis, investigation, Writing-original draft, visualization
M.G: Software, Validation, Writing-Review & Editing
G. P. R: Resources, Project administration, Writing-Review & Editing
A. K: Conceptualization, Resources, Writing-Review & Editing, Supervision, Funding acquisition

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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