The soil pore structure encountered by roots affects plant-derived carbon inputs and fate

Maik Lucas1,2, James P. Santiago3, Jinyi Chen4, Andrey Guber1 and Alexandra Kravchenko1

1Department of Plant, Soil and Microbial Sciences, DOE Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, MI 48824, USA; 2Department of Soil System Sciences, Helmholtz Centre for Environmental Research – UFZ, Halle (Saale), 06110, Germany; 3Plant Resilience Institute and MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA; 4Department of Plant Protection, Nanjing Agricultural University, Nanjing, 210095, China

Summary

- Plant roots are the main supplier of carbon (C) to the soil, the largest terrestrial C reservoir. Soil pore structure drives root growth, yet how it affects belowground C inputs remains a critical knowledge gap.
- By combining X-ray computed tomography with 14C plant labelling, we identified root–soil contact as a previously unrecognised influence on belowground plant C allocations and on the fate of plant-derived C in the soil.
- Greater contact with the surrounding soil, when the growing root encounters a pore structure dominated by small (<40 μm Ø) pores, results in strong rhizodeposition but in areas of high microbial activity. The root system of Rudbeckia hirta revealed high plasticity and thus maintained high root–soil contact. This led to greater C inputs across a wide range of soil pore structures. The root–soil contact Panicum virgatum, a promising bioenergy feedstock crop, was sensitive to the encountered structure. Pore structure built by a polyculture, for example, restored prairie, can be particularly effective in promoting lateral root growth and thus root–soil contact and associated C benefits.
- The findings suggest that the interaction of pore structure with roots is an important, previously unrecognised, stimulus of soil C gains.

Introduction

Approximately 1 : 3 of the carbon (C) photo-assimilated by plants from the atmosphere is transferred belowground (Jones et al., 2009; Pausch & Kuzyakov, 2018), making the Earth’s soils a massive C reservoir (Friedlingstein et al., 2022). The lion’s share of these inputs, however is being lost to the atmosphere as CO2, with only c. 5% of the total photo-assimilated C remaining in the soil (Jones et al., 2009; Pausch & Kuzyakov, 2018), and most of what remains are further degraded by microbes over time. Live root inputs via rhizodeposition, that is, mucilage, root cap cells, exudates, and lysates, are an important component of stable soil organic matter (Rasse et al., 2005; Gherardi & Sala, 2020; Gregory, 2022). Such inputs can be 2–13 times more efficient than inputs from dead roots, aka root litter, in generating both the rapidly utilisable C and the slowly metabolised mineral-associated C (Sokol et al., 2019). Maximising the live root inputs is a viable strategy for increasing the mean residence time of soil C (Poepplau et al., 2021).

Plant species differ, sometimes substantially, in their soil C inputs and their effects on soil C cycling, due to a variety of factors, with perenniality, C3 : C4 status, and root architecture just a few to mention. Many species-related root traits, for example, root lengths, diameters, and branching, are particularly important for soil C inputs as well as C stabilisation (Poirier et al., 2018). However, they are typically investigated under controlled conditions, such as well-sieved soil, potting soil mixes, or litter bags, and not in soils with intact structures (Poirier et al., 2018). Yet, it is the local variations in structural properties encountered by the roots as they navigate through the intact soil that greatly affects root growth patterns and architecture (Colombi et al., 2018; Lucas, 2022). How such local variations influence the quantities of C that a root puts underground remains largely enigmatic.

Soil pore structure, that is, size distributions, shapes, and connectivity of soil pores (Rabot et al., 2018), can affect root-derived C inputs and their fate in multiple ways. First, pore structure interacts with root growth. This interaction is mutual where roots change the structure of soil pores, but also, the pore structure changes root growth (Lucas et al., 2019a). A root can grow along pores larger than its size, encountering little resistance and thus navigating soil layers with high bulk density without marked changes to an existing arrangement of soil pores (White & Kirkegaard, 2010; Gao et al., 2016; Lucas et al., 2019a). Alternatively, a root can grow into a dense soil matrix, overcoming the penetration resistance, and reorganising the pore space as it goes, resulting in the densification of its immediate surroundings and creating biopores upon its death and decomposition (Bruand et al., 1996; Lucas et al., 2019b).

These different modes of interaction between roots and pore structure have profound effects on the rhizosphere (Vetterlein...
et al., 2020; Lucas, 2022) and define where within the soil matrix the root C inputs are deposited and how physically accessible they are for subsequent microbial decomposition (Erktan et al., 2020). The impacts of such interactions are very complex and sometimes contradictory. For example, plant-accessible pores in the few tens of μm size range can receive large quantities of plant-derived C (Quigley et al., 2018; Quigley & Kravchenko, 2022). The abundance and connectivity of such pores enable enhanced root growth, as observed in many well-structured soils, and subsequent high cumulative C inputs benefit soil C gains (Dexter, 1991; Stirzaker et al., 1996; Colombi et al., 2019). But, on the other hand, an absence of easily accessible pores can also lead to elevated C inputs, since roots grown into dense poorly-structured soil matrix increase rhizodeposition via mucilage production (Iijima & Kono, 1992; Jones et al., 2009). To further complicate the matters, root exudates, a substantial part of all rhizodeposits, are released passively, responding to root-to-soil gradients (Jones et al., 2009). Hence, their quantities might be stimulated by better root contact with the surrounding soil matrix, also largely a function of soil pore structure.

The goal of this study is to explore how root interactions with the soil pore structure affect the additions of photo-assimilated C to the soil and the fate of the added C. We hypothesise that plant species with disparate root architectures may naturally differ in how they react to a specific pore structure with disparate consequences for C inputs and protection. Thus, we selected two plant species with distinctly different root characteristics, namely Rudbeckia hirta (black-eyed Susan) and Panicum virgatum (switchgrass, Fig. 1a). The short-lived perennial forb R. hirta is an Asteraeae, with a fibrous root system and a large amount of small laterals (Levang-Brilz & Biondini, 2003). P. virgatum is a perennial grass with a massive, coarse root system and low root branching (Weaver, 1968; McLaughlin & Adams Kzos, 2005). While P. virgatum can have a tremendous effect on soil pore structure (Juyal et al., 2021), the small roots of R. hirta might not affect it in a measurable way (Judd et al., 2015).

The detailed characterisation of the soil pore structure, quantification of the new root growth within it, and assessments of the pore characteristics of the rhizosphere created by the ingrowing roots were made possible by the use of X-ray computed microtomography (μCT). Labelling the growing plants with $^{14}$C enabled us to explore multiple aspects of new C additions. Specifically, we assessed the quantities of plant-derived C inputs; traced localities of the new C placement within rhizosphere, rhizoplane (i.e. root surfaces), roots, and in the soil solution from pores of different size ranges; monitored losses of newly added C as CO$_2$ and quantified the amounts of newly added C remaining in the soil (Fig. 1b).

Materials and Methods

Study area and sampling

The experimental site used in this study was established in 2013 in Oregon, WI, USA in a randomised complete block design with four replications. The soil samples were taken from two vegetation systems: monoculture switchgrass (variety Cave-in-rock) and restored prairie (an 18-species mix including Panicum virgatum L. and Rudbeckia hirta L. along with other forbs, grasses, and legumes). It has been demonstrated that after multiple years of uninterrupted growth the vegetation communities of the two systems can develop distinctly different soil pore characteristics (Kravchenko et al., 2019), providing pore structure contrast needed for this study. Please refer to Supporting Information Methods S1 for additional details on the soil sampling and the design and management of the experiment.

To create a variety of contrasting pore structures, we generated four types of ingrowth cores (15 cores of each type): namely, intact and sieved cores from each of the switchgrass and prairie system. All ingrowth cores were held by perforated covers with large perforations (4 mm Ø) to not inhibit root growth (Fig. S1d–f). The intact ingrowth cores were prepared by taking them out of their original field holders and enclosing them within the perforated covers. The sieved cores were prepared by sieving individual field cores (2 mm) and then repacking their soil into perforated covers. Sieving drastically affected pore characteristics, thus greatly expanding the range of pore structures for the study, yet, it did not change other soil properties. To ensure that the intact and sieved cores differ only in terms of their pore structure, all roots and stones from the original field soil core collected on the sieve were mixed back before repacking.

Experimental design

For the plant growth experiment, 12 pots (15 cm Ø and 20 cm height) were filled with sieved soil (see Methods S1) to 1.34 g cm$^{-3}$ bulk density, the average bulk density measured in the field. During the filling process, four ingrowth cores representing every combination of soil structure (sieved vs intact) and soil origin (switchgrass vs prairie-soil) were placed into the pots at 10–15 cm depth (Figs 1a, S1). The use of the ingrowth cores enabled us to achieve high resolution of the μCT scans, providing pore structure data of high quality. At the same time, keeping the ingrowth cores within large pots ensured unrestricted root growth. Our experimental settings can be regarded as a split-root design, where exposing the cores with different pore structures to the roots of the same plant minimised variations due to plant effects, for example, plant development stages or photo-assimilation rates. The cores were placed to ensure they were within the same depth of the pot and at the same distances to the pot walls.

Pre-germinated seedlings were planted into the centres of the pots at an equal distance to all ingrowth cores. The plants were grown for 48 d in a growth chamber. Detailed information on the growing conditions is provided in Methods S2.

$^{14}$C labelling

In order to assess the quantities, locations, and decomposition losses of the photo-assimilated C added by plants to the ingrowth cores with contrasting pore structures, we labelled the plants with $^{14}$C–CO$_2$ according to Santiago et al. (2021). Briefly, LI-COR 6800 portable photosynthesis system (Li-Cor, Lincoln, NE,
USA) with an attached 3 × 3 cm chamber was used to feed plant leaf’s with CO₂ (Fig. S1g). For P. virgatum two to three leaf blades were laid side by side and clamped into the chamber. Leaves of R. hirta were large enough to fill the whole chamber, and only one fully mature leaf was clamped. Before labelling, stable photosynthetic rates were achieved by feeding the leaves with unlabelled CO₂. For feeding with labelled CO₂, a constant flow from a pressurised 14CO₂ tank ensured a concentration of >40 μmol mol⁻¹ of labelled CO₂ in the chamber. The pressurised tank contained 5% unlabelled CO₂ in O₂ mixed with 14C-CO₂, created by acidifying NaH¹⁴CO₃. The final radioactivity of ¹⁴CO₂ used was 1 Bq mmol⁻¹. To ensure a distribution of ¹⁴C in the whole root system (Pausch & Kuzyakov, 2011) as well as sufficient amounts of detectable ¹⁴C in the rhizosphere, the plants were labelled 11 d and 3 d before harvest. The photosynthetic activity was logged every 5 min and used to calculate the total radioactivity taken up by the plant over the labelling time of 2.5 h. Both plants took up ¹⁴C in the amounts sufficient for the subsequent analysis (Table S1).

**Harvest and ¹⁴C analyses**

During the harvest, the aboveground plant tissue was collected, cut into pieces and dried (60°C) overnight. The pots were cut open, and the ingrowth cores were carefully removed, with ingrowing roots carefully cut-out at the core surfaces.

The upper cap of the cores was removed, and two intact soil samples (2 cm Ø and 2.5 cm height) were taken into plastic tubes and pushed into the soil (Fig. 1b). One of the two samples was used for quantifying ¹⁴C with the soil solution. The centrifugation method (Russell & Richards, 1939) enabled us to derive soil solution from two different pore size classes with equivalent diameters of >35 μm (referred here to DOC from macropores >40 μm Ø) and 2–35 μm (referred here to DOC from small pores <40 μm Ø). While the first size class is associated with increased microbial activity, the latter was shown to be important for C storage (Bouckaert et al., 2013; Kravchenko & Guber, 2017; Kravchenko et al., 2019). The other intact soil sample was used for determining ¹⁴C–CO₂ losses during 30-d soil incubation. Note that to ensure precise ¹⁴C–CO₂ analyses, we did not measure total CO₂ release on a subsample of the trapping solution and could, therefore, not capture priming effects, that is, the release of native C through increased microbial activity.

To explore where else within the soil (aside from the rhizosphere) the newly added ¹⁴C was deposited, we collected nine microsamples (0.1 cm³) from referenced locations within each intact soil core. The microsamples were taken on a regular square grid (Fig. S1h) and are referred to further on as ¹⁴C grid samples (Figs 1b, S1h).
The remaining soil of the ingrowths cores was used to procure the ingrown roots in order to characterise $^{14}$C of the root tissues, rhizoplane, and rhizosphere. The roots were carefully taken out of the remaining soil with tweezers, and rhizosphere and rhizoplane fractions were obtained following the procedures described by Lucas et al. (2018).

For details on the sampling procedure as well as the radioactivity evaluation, see Methods S3. The $^{14}$C activities of each compartment (e.g. rhizosphere and root) in different cores are normalised based on the total assimilated $^{14}$C of the plant within a given container to describe the relative pathways of the added $^{14}$C-label. To ensure that the $^{14}$C-activities are not misinterpreted due to differences in root growth, we also present data based on the length of the roots found within each core.

X-ray μCT scanning and image analyses

All intact and sieved ingrowth cores were μCT scanned twice – before and after the ingrowth experiment at a resolution of 18.2 μm. The images were reconstructed following the procedures from Lucas et al. (2022). To follow the root growth path, the μCT images taken before and after the growth experiment were registered using elastix (Klein et al., 2010; Shamonin, 2013) as described in Lucas et al. (2020a). After the registration, 1850 × 1850 × 2300 voxel cubes were cut from the centres of the cores’ images in Fiji (Ollion et al., 2013) to remove artefacts along the core walls. In addition, a contrast enhancement (saturation value of 0.35) was performed, and the bit depth was reduced to 8-bit. After this, a non-local means filter was used (Darbon et al., 2008; Buades et al., 2011) with scikit-image (van der Walt et al., 2014) in Python (van Rossum & Drake, 2009).

Lucas et al. (2020b) showed that to representatively describe pore volumes, the pores need to have a diameter two to three times the scanning resolution. Thus, we focused on pores > 40 μm Ø, hereafter referred to as macropores, while soil volumes dominated by pores < 40 μm Ø, hereafter, will be referred to as soil matrix. For pore segmentation, we used the Otsu algorithm (Otsu, 1979). Biopores and roots were segmented according to the workflow of Lucas et al. (2022). The respective IMAGEJ scripts can be found on ‘https://github.com/Mai-Lu/Roots_and_Biopores’. See Methods S4 and Fig. S2 for details on the root segmentation approach. To assess pore characteristics of the soil matrix, that is, < 40 μm pores, we conducted μCT scanning and subsequent analyses of several intact subsamples at c. 5 μm resolution. For that, three intact subsamples (0.8 cm Ø and 0.8 cm height) were taken from an additional set of intact cores of both vegetation systems and subjected to X-ray μCT scanning at the Advanced Photon Source (APS), Argonne National Laboratory (scanning and analyses details are provided in Methods S5).

By identifying the root residues in the μCT scans of intact ingrowth cores before the experiment and then examining the same residues on the scans obtained after the experiment, we were able to quantify the residue decompositon and relate it to the properties of the surrounding soil. Newly grown roots were separated from the old root residues by image subtraction (Fig. 1c). The volume of degraded old roots was obtained by subtracting the root image after the experiment, that is, with only new roots and degraded root residues, from the root image before the experiment, that is, with non-degraded root residues.

Pore size distribution (PSD) and pore connectivity were obtained from the pore binary images. To compute the PSD in Fiji, the local thickness method (Hildebrand & Rueggeger, 1997) was used. A size thresholding on the PSD (pores larger than 0.1 mm) was labelled using the connected component labelling from the plugin BoneJ2 plugin (v.7.10, Domander et al., 2021). This image was used to calculate the $\Gamma$-indicator, a metric of pore connectivity (Lucas et al., 2020b).

To describe in which pore structure roots grew, the image of the newly grown roots was used as a mask on the segmented image from before the experiment. The physical properties of the rhizosphere were calculated using the Euclidean Distance Transform in Fiji, as shown in Lucas et al. (2019a). To determine root–soil contact, we calculated mean values (total macropore, matrix and narrow macropore volume, i.e. pores < 150 μm Ø) as percentages of the total volume of the rhizosphere up to 0.1 mm distance to the root surfaces. This distance was determined from iterative comparing the correlation of soil matrix density with $^{14}$C measurements, accounting for root–soil contact changes due to root shrinkage (Koebenick et al., 2018).

Statistics

The data were analysed using a linear mixed model approach implemented in the lme4-package (Bates et al., 2015) of R (v.4.1.1). The statistical model consisted of fixed effects of the plant (P. virgatum vs R. hirta), soil structure (sieved vs intact), and soil origin (switchgrass-soil vs prairie-soil) and their interactions. Random effects consisted of the planted pots, used as an error term for testing the plant effect, and of the ingrowth cores nested within the pots, used as an error term for testing the effects of the soil structure and origin. The assumptions of normality and homogeneity of variances were assessed using normal probability plots of the residuals and Levene’s tests for equal variances, respectively. When the normality assumption was found to be violated, the data were log-transformed (as in the case of all $^{14}$C results). When the interactions between the studied factors were found to be statistically significant (P < 0.05), slicing of the interaction, aka simple effect $\overline{t}$-tests, were conducted, followed, when significant, by $t$-tests for multiple comparisons among the mean values.

Results

The effect of pore structure on root growth

After 8 yr of implementation, soils taken from the two perennial plant systems diverged in their biological and structural characteristics but remained similar in terms of chemical composition (Table S2). The sieved cores exhibited significantly higher macroporosity than the intact cores (c. 20% vs 5%) (Fig. S3a), with a large proportion of narrow (40–150 μm Ø) macropores (Fig. S3c) but similar pore connectivity (Fig. S3b). The capability
of long-term monoculture switchgrass vegetation to increase biopores volumes (Rachman et al., 2004) was clearly pronounced, and soil from the long-term monoculture switchgrass had >1.6 times higher biopore volumes than prairie (2.8 Vol% vs 1.7 Vol %, Fig. S3d). The differences in pores structure mainly occurred in the macropore space, while the bulk density and volumes of pores in 5–40 μm Ø size range of the two vegetation systems were similar (Table S3).

While both plant species performed well during the 2 months of the experiment (Table S4; Fig. S1b,c), R. hirta developed significantly higher belowground biomass than P. virgatum (Table S3). Most, that is, 59–65%, of R. hirta roots grew into the soil matrix, regardless of the structure (sieved vs intact soil) or origin (switchgrass vs prairie) of the encountered soil (Fig. 2c). Only 35–45% of P. virgatum roots grew into the soil matrix when encountering the sieved soil of both origins and the intact soil of switchgrass origin (Fig. 2b). However, P. virgatum dedicated a substantially higher share of its roots (65%) to the soil matrix when growing into the intact cores of prairie origin (Fig. 2f).

Both plants markedly preferred utilising existing biopores. While biopores made up only 2–3% of the total soil volume, 18% of R. hirta roots were found in them, in switchgrass- and prairie-soils alike. P. virgatum even more distinctly preferred the biopores of its ‘familiar’ soil – 35% of its roots grew into the biopores in the intact switchgrass-soil (Fig. 2f). Yet, a surprisingly low (only 17%) proportion of P. virgatum roots was found in the biopores of the intact prairie-soil.

In total, R. hirta developed >5 times greater root length density, that is, root length per volume of soil, than P. virgatum (Fig. 3a); and significantly higher total root branch density, that is, number of lateral roots per soil volume, than P. virgatum (Fig. 3b). However, when encountering the intact pore structure, both plants reacted similarly – that is, they developed significantly higher branch densities there than in the sieved soil (Fig. 3b). Further, P. virgatum root diameters were the smallest when growing into the intact prairie-soil, while there were no significant differences in the diameters of R. hirta roots (Fig. 3c).

The mean root diameter within cores was not associated with the share of roots growing into the soil matrix (Fig. S4).

Pore connectivity appeared to play an important role in defining whether the roots grew into existing macropores and/or biopores or into the soil matrix (Fig. 3d). For both plant species and
in all studied pore structure systems, lower pore connectivity was associated with greater growth into the soil matrix and a subsequently increased root–soil contact (Fig. 3e). But when the roots preferentially grew into the macropores and biopores, the contact decreased. Notably, a markedly higher growth of *P. virgatum* roots into the soil matrix in the prairie-soil (Fig. 2f) corresponded to its significantly higher root–soil contact there as compared to all other soil treatments, while *R. hirta* maintained high root–soil contact throughout all pore structures (Fig. 3e).

Pore characteristics in root vicinity

Rhizosphere porosity was the highest in the immediate vicinity (<100 μm) of the roots, decreasing with the distance (Fig. 2d,g). It should be noted that narrow (40–150 μm Ø) macropores either completely dominated the direct vicinity of the roots or constituted a substantial portion of the rhizosphere volumes there.

The plant effects on the rhizosphere porosity differed in sieved vs intact soils. In the sieved soil, the rhizosphere around *P. virgatum* roots was dominated by wide macropores (>150 μm Ø) (Fig. 2g), while narrow macropores (40–150 μm Ø) dominated the rhizosphere of *R. hirta* (Fig. 2d). In the intact soil the rhizosphere characteristics of the two plants were more similar: for both, there was a substantial presence of wide macropores in the intact switchgrass-soil and dominance of narrow macropores in the intact prairie-soil. Interestingly, only in the intact prairie-soil, where *P. virgatum* grew into the soil matrix to the same extent as *R. hirta* (Fig. 2c,f), the pore characteristics of *P. virgatum*’s rhizosphere became similar to those of *R. hirta* (Fig. 2d,g).

Carbon translocation into the soil

*Rudbeckia hirta* had a higher photosynthesis rate, assimilated more 14C, retained a lower proportion of assimilated 14C in the shoots,
and transferred much more of its assimilated $^{14}$C belowground than $P$. virgatum (Table S1). There was, however, a notable exception to this overall pattern: the $^{14}$C allocated by $P$. virgatum into the roots and its surroundings (i.e. rhizosphere and rhizoplane) through rhizodeposition was comparable to that of $R$. hirta when $P$. virgatum grew into the intact prairie-soil (Figs 4b, S5).

It is expected that the roots actively growing at a time of a $^{14}$C pulse are the ones that will be most enriched (Pausch & Kuzmany, 2011), hence contributing the most to $^{14}$C enriched rhizodeposition. However, the positive association between $^{14}$C in the rhizodeposition and root–soil contact (Fig. 4a) still held even when the $^{14}$C of rhizodeposits was standardised by the $^{14}$C of the roots (Fig. S6). This suggests that areas of high root–soil contact were eliciting greater quantities of new C rhizodeposits from roots with a wide range of $^{14}$C levels and growth stages.

The species differences in terms of the quantities of newly assimilated C transferred into the soil appeared to be driven by a greater root length density of $R$. hirta (Fig. 3a). When expressed on a per unit of root length, the newly assimilated C within the roots of the two species was similar (Fig. S7a). However, even per unit of root length, $R$. hirta translocated more of the new C into its rhizoplane than $P$. virgatum (Fig. S7b). Even though the soil origin did not influence C translocation and C soil inputs of $R$. hirta, it did matter for $P$. virgatum when grown into the prairie-soil. $P$. virgatum transported in total three times more of its assimilated $^{14}$C to its roots and released five times more $^{14}$C in its rhizoplane than when it grew into the switchgrass-soil (Fig. S7a,b).

**Placement and fate of photo-assimilated C in the soil**

Surprisingly, despite much higher root length (Fig. 3a) and $^{14}$C-activity in the roots, rhizoplane, and rhizosphere of $R$. hirta as compared to those of $P$. virgatum (Fig. 4b; Table S1), the $^{14}$C activity in the grid samples of the two plants was similar (Fig. S5e). Even more surprising was that the more $P$. virgatum roots grew into the soil matrix, the lower was the $^{14}$C-activity observed in the grid samples (Fig. 4g), and some of the highest grid $^{14}$C corresponded to the cores where most of the $P$. virgatum roots grew into the existing biopores (Fig. 4g).

When expressed on a per unit of root length basis, $^{14}$C in the pore solution tended to be higher in $P$. virgatum than in $R$. hirta (Fig. S7d,e). While $^{14}$C-DOC from $R$. hirta was not affected by either soil structure or soil origin, we found especially high $^{14}$C-DOC in 2–40 $\mu$m Ø pores when $P$. virgatum grew into the intact prairie-soil, both in total and on a per unit of root length basis (Figs S5g, S7e).

Consistent with its high root biomass (Fig. 3a) and high $^{14}$C rhizodeposition (Fig. 4a), $R$. hirta's soil had significantly more $^{14}$CO$_2$ emitted during the incubation than the soil of $P$. virgatum (Fig. 4d). Also, in total, more $^{14}$C-SOC remained in $R$. hirta's soil at the end of the incubation (Fig. S5d). Greater root–soil contact appeared to stimulate the processing of new C and its losses as CO$_2$, as suggested by positive association between root–soil contact and $^{14}$CO$_2$ (Fig. 4c). For both plant species roots, the higher the rhizodeposition, the more of the newly added C remained in the soil after incubation (Fig. 4e).

The degradation of roots found in the prairie-soil was roughly double that of the switchgrass-soil, equal to 67% and 34%, respectively (Fig. 5). Consistent with the positive correlations between the root–soil contact and $^{14}$CO$_2$ (Fig. 4c), a positive correlation was also observed between the volumes of degraded old roots and the root–soil contact (Fig. 5a).

**Discussion**

Our findings suggest that for the two studied plant species, the local variations in soil pore structure influence not only the root growth patterns but also the quantities of C deposited by the roots into the soil, as well as its microbial processing. Yet, these influences depend on the root architecture of the plants. Specifically, in this study, they were negligible for the fine-root dominated $R$. hirta while major for the coarse-rooted $P$. virgatum.

Greater root growth into the soil matrix enhanced root–soil contact.

Root plasticity allows the plant to adapt to the changes in environmental conditions of the heterogenous soil matrix (Morrison et al., 2017; Lippold et al., 2022; Glass et al., 2023). Both plants adapted their root morphology to the local structures they encountered (Fig. 3a–e). Especially $P$. virgatum created much thicker roots in the sieved cores compared to the intact structures (Fig. 3c). Roots respond to increased penetration resistance with decreasing root elongation and root thickening, which may result in a better ability to grow into denser soil matrix, while increased macroporosity was shown to have an opposite effect (Clark et al., 2003; Bengough et al., 2011; Tracy et al., 2012). However, the high water content, the bulk density of 1.34 g cm$^{-3}$ (Table S3) and the high macroporosity in the sieved cores make it unlikely that the roots were restricted (Jones, 1983; Valentine et al., 2012).

As expected (Stirzaker et al., 1996; Colombi et al., 2017), both plant species preferred to follow the path of least resistance and tended to choose pores with high connectivity (Fig. 3d). Lower pore connectivity stimulated root growth into the soil matrix (Fig. 3d), where greater exploration of the matrix by the roots led to stronger root–soil contact (Fig. 3e). Yet, the two species substantially differed in how they explored the soil space. Roots of $R$. hirta easily and readily grew into both macropores and the soil matrix, regardless of the soil structure they encountered (Fig. 2c), leading to an overall high root–soil contact. The root system of the dicot $R$. hirta is known for substantial quantities of lateral roots (Levag-Brilz & Biondini, 2003), a trait demonstrated when growing into the intact soil cores of this study (Fig. 3b). Laterals extending from a root located within a large pore presumably hit the surrounding soil at a nearly perpendicular angle (Jin et al., 2013) to reach water resources (Bao et al., 2014), resulting in substantial growth of $R$. hirta roots into the soil matrix, as observed in our study.

The pore structure encountered by the roots of $P$. virgatum had a notable influence on how it explored the soil. When $P$. virgatum encountered the pore structure created by the prairie vegetation, 65% of the small and predominately lateral roots (Fig. 3b,c) developed and grew into the soil matrix, as opposed
to just 36–48% in all other structures (Fig. 2f) and resulting in high root–soil contact levels (Fig. 3e). On the contrary, *P. virgatum* tended to avoid the soil matrix when growing into the other structures, that is, into sieved soils or into the intact soil of its own, that is, switchgrass origin, where it had a greater share of roots in macropores than *R. hirta* (Fig. 2c,f). Similarly,
Z. maize was shown to develop a large share of lateral roots within and around loamy, dense macroaggregates, comprising only a small volume of macropores (> 40 μm), while in the otherwise sandy matrix, roots were mainly found in macropores with a low number of laterals (Lippold et al., 2022). In the intact soil of the switchgrass origin, P. virgatum also had a particularly strong preference for biopores (Fig. 2b). The biopores were mostly likely built by previous P. virgatum plants; thus, the newly growing roots were just following the old ‘familiar’ pathways (Video S1, White & Kirkegaard, 2010).

Differences in root growth patterns of P. virgatum affected the pore structure of its rhizosphere (Fig. 2g). In the sieved soils of both switchgrass and prairie origin and in the intact switchgrass-soil, wide macropores prevailed in root vicinity with a concomitant significant reduction in root–soil contact (Fig. 3c).

Influence of roots and pores on the fate of soil C inputs

Our findings demonstrated that while soil C inputs from R. hirta, do not depend on the type of pore structure that is encountered by their roots (Figs 4a, S5); yet for P. virgatum the pore structure matters. Growth into the soil matrix and subsequently increased root–soil contact apparently influence: the amount of C released into the root’s surroundings (Fig. 4a); the microbial processing of the plant-derived C (Fig. 4c); and the spatial distribution patterns of this C (Fig. 4g).

High 14C-rhizodeposition of R. hirta coincided with a substantial portion of its extensive root system growing into the soil matrix and thus with a high root–soil contact (Fig. 4a). On the other hand, 14C-rhizodeposition of P. virgatum was similar to that of R. hirta only in the intact prairie-soil, where P. virgatum’s roots explored the matrix as much as those of R. hirta. The most likely explanations of this phenomenon are: that the growth into the soil matrix requires additional mucilage production (Iijima & Kono, 1992); and that the enhanced root–soil contact increases exudation (Jones et al., 2009). Our results from these two species with contrasting root systems suggest that root–soil contact might be an important driver stimulating plants to increase their C inputs (Fig. 6); however, future experimentation with a wider range of plant species will be paramount for assessing the universality of its role.

The differences in the chemical composition of the old roots of P. virgatum encountered in the switchgrass-soil and of the old roots of a variety of unidentified plant species in the prairie-soil were the likely important contributors to the observed difference in root decomposition of the two plants (Kim et al., 2022). Yet, growth into the soil matrix and resultant greater root–soil contact also promoted microbial respiration (Fig. 4c) and root decomposition (Fig. 5). Microbial respiration depends on the spatial organisation and connectivity of pathways between decomposers and organic compounds (Nunan et al., 2017; Mbé et al., 2022). Root growth into the soil matrix apparently minimises distances between microbial decomposers and root-derived organic inputs, optimising the movement of labile organic compounds and enzymes (Fig. 6).

However, even when the roots grow into the soil matrix, there always remains a gap between the root and the soil surface, which can be attributed to root shrinkage (Carminati et al., 2013) and to geometrical reasons, that is, the packing of round-shaped soil particles at the flat root surface (Koebenick et al., 2019). Formed by narrow macropores (Fig. 2d,g), this gap can facilitate oxygen flow and greater microbial activity (van Veelen et al., 2019). High abundance of narrow macropores can be particularly important for processing the newly added plant C as these pores may provide optimal micro-environmental habitats for microbial decomposers and often are characterised by greater enzyme activity and C turnover (Bouckaert et al., 2013; Kravchenko et al., 2019).

Spatial distribution of the plant-derived C is driven by where the plants deposit it. Thus, new C has been previously reported to be positively associated with an abundance of root-accessible macropores (Quigley et al., 2018; Quigley & Kravchenko, 2022). Our results support this notion. Indeed, greater P. virgatum growth into the macropores corresponded to higher 14C in the soil solution extracted from macropores (> 40 μm Ø, Fig. S7d), while the highest 14C in small (2–40 μm Ø) pores was observed when P. virgatum grew predominately into the soil matrix (Fig. S7e).

However, surprisingly, more of P. virgatum’s 14C was found to be ubiquitously distributed through the soil (as can be surmised from soil 14C grid data) when its roots predominantly grew into the macropores (Fig. 4g). Occurrence of newly photo-assimilated C so far away from the roots could not be simply explained by diffusion away from the root-hosting macropores, as those pores were mostly air-filled during the plant growth (Schlüter et al., 2022). We propose a hypothetical explanation suggesting that the 14C transport in these cases was facilitated by fungi (Fig. 6). Fungal hyphae can be important vectors of C transport into the dense soil matrix (Vidal et al., 2018; Witzgall et al., 2021). Colonisation by mycorrhiza enables a plant to gain resources from a large soil volume with relatively low C translocation into the soil (Veresoglou et al., 2012). Fungi grow preferentially into larger (> 100 μm), air-filled pores (Otten et al., 2001; Soufan et al., 2018), and their growth is stimulated by greater pore volume and connectivity (Erkten et al., 2020). Preferential root growth into such well-connected macropores (Fig. 3c) presumably provided ideal conditions for fungal hyphae colonisation and respective food chain for the distribution of C products throughout the soil. Mycorrhiza fungi can have a large positive effect on P. virgatum growth (Hestrin et al., 2021), and their presence in the biopores of the switchgrass-soil might compensate for decreased root growth and low root–soil contact and facilitate aboveground plant growth (Schröder-Moreno et al., 2012). Interestingly, P. virgatum seemed not only to modify its C inputs into the rhizosphere depending on the encountered pore structure but also apparently changed the relationships with the microbial community of the surrounding soil – possibly, relying more on the bacterial activity in its immediate rhizosphere when the root–soil contact was good while utilising the extended fungal network when the root–soil contact was poor. Future experimental work will be needed to test this hypothesis.
Although root growth into the soil matrix and the subsequently increased root–soil contact (Fig. 3d) apparently benefited plant residue decomposition (Fig. 5) and C losses (Fig. 4c), the good root–soil contact was still advantageous for photoassimilated C to remain protected in the soil, as attested by our 30-d incubation results (Fig. 4e). Processing of the new C inputs by microorganisms and subsequent conversion of the decomposition products into mineral-associated organic C is one important pathway of soil C gains (Cotrufo et al., 2015), while direct stabilisation of plant inputs or microbial extracellular products and necromass is the other major route (Craig et al., 2022).

However, regardless of the stabilisation pathway, the long-term storage potential of the newly added C depends on protection from further decomposition (Schmidt et al., 2011; Dungait et al., 2012). Enhanced root–soil contact increases microbial respiration (Fig. 4c), but it also enables larger quantities of root-derived C products to diffuse into the dense soil matrix surrounding the plant roots (Fig. 2d,g, also demonstrated in Schlüter et al., 2022). Much longer incubation times than the typical 30-d employed by our study would be needed to quantify the long-term C storage. Yet, the rhizosphere is known to densify upon subsequent root growth, with its porosity and permeability being reduced, while binding opportunities for C compounds increase (van Veelen et al., 2019), therefore providing optimal settings for creating mineral-associated organic matter for long-term storage (Fig. 6).

Concluding remarks and general implications
Comparisons between sieved switchgrass and prairie-soils enabled us to assess the role of non-structural effects, that is, the inherent differences in chemical and microbial properties. In the studied soil (silt-loam Alfisol), the non-structural effects were of no consequence for *R. hirta*’s root growth and inputs and played only a minor role for *P. virginatum* (Figs 3, 4, S5).

Comparisons between sieved and intact soils enabled us to assess the role of the structural effects. The strength of the structural effect depended on the plant species and on the soil’s origin/vegetation history (Figs 4, S5): it was negligible for *R. hirta* in both soils, and it also was unsubstantial in the switchgrass-soil for both plants. Yet, in the prairie-soil the structural effect played a
major role for \textit{P. virgatum} in terms of its influence on where the roots grew and how much C they placed into the soil. When the pore structure of the intact prairie-soil was destroyed by sieving, \textit{P. virgatum}'s growth patterns, root-derived C inputs, and their processing changed dramatically. Our findings contribute to the explanation of an apparent paradox of \textit{P. virgatum} being a positive influence on C gains in polyculture systems (Yang et al., 2019), while demonstrating a very slow, at best, C accrual as a monoculture (Kantola et al., 2017; Chatterjee et al., 2018), despite its extensive root system (McLaughlin & Adams Kszos, 2005; Chimento et al., 2016). Plant community of restored prairie with its diverse root systems establish a broad PSD with fewer large biopores (Fig. S1c,d, Bodner et al., 2014), arguably an ideal pore structure for C sequestration. When encountering that structure, the roots of \textit{P. virgatum} were not trapped within wide macropores (as was the case when they grew into the switchgrass-soil), but explored the soil matrix, maintained good root–soil contact, with subsequent soil C input and protection benefits (Figs 3, 4).

The use of the ingrowth cores within large pots enabled us to follow the root growth of a single plant into different structures with high resolution and to capture a large part of the total root system. While this work should be followed by further experiments with a wide range of plant species and soils, consistently high inputs of root-derived C from \textit{R. hirta} across all studied soil structures indicate the possibility of stimulating soil C gains by cultivating plants with certain root characteristics. Root systems that maintain high root–soil contact can potentially improve C accumulation across a wide range of soil pore structures.

Acknowledgements

This research was funded in part by the Great Lakes Bioenergy Research Center, US Department of Energy, Office of Science, Office of Biological and Environmental Research under award no. DE-SC0018409, by the NSF DEB Program (award no. 1904267), by the NSF LTER Program (DEB 1027253) at the Kellogg Biological Station, and by Michigan State University AgBioResearch.

We thank Maxwell Oerther for his great support during the whole experiment, Jinho Lee for his help on the field site and soil chemical analysis and Michelle Quigley for her support during the X-ray CT scans. We are indebted to Chelsea Mamott of the AgBioResearch.

We thank GeoSoilEnviroCARS (The University of Chicago, Sector 13), Advanced Photon Source (APS), Argonne National Laboratory, and most notably, M. L. Rivers for the possibility and assistance with synchrotron CT measurements. We thank the anonymous reviewers for their suggestions, which greatly improved the manuscript.

Competing interests

None declared.


Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Photographs taken during the plant growth and after plant harvest.

Fig. S2 Relationship of root length densities estimated with X-ray μCT and disturbed.

Fig. S3 Pore characteristics of the cores with intact or sieved soils of switchgrass or prairie origin before plant growth.

Fig. S4 Pore characteristics of the cores with intact or sieved soils of switchgrass or prairie origin before plant growth.

Fig. S5 Distribution of 14C in intact and sieved cores of the switchgrass and prairie origin subjected to Panicum virgatum and Rudbeckia hirta growth.

Fig. S6 Relationship between root–soil contact and the ratio of 14C in rhizodeposition and 14C in roots.
Fig. S7 Distribution of $^{14}$C between the different compartments expressed on per unit of root length.

Methods S1 MLE Site and soil handling.

Methods S2 Plant growing conditions.

Methods S3 Radioactivity evaluation.

Methods S4 Validating root segmentation by destructive sampling.

Methods S5 Analyse of subsamples at Argonne national lab for pores $< 40 \text{ mm}$.

Table S1 $^{14}$C Labelling of the plants and translocation of the $^{14}$C label into shoots, roots, rhizoplane, and rhizosphere of *Rudbeckia hirta* and *Panicum virgatum*.

Table S2 Selected chemical properties of the soils under monoculture switchgrass and restored prairie vegetation systems.

Table S3 Selected physical properties of the soils under monoculture switchgrass and restored prairie vegetation systems.

Table S4 Root and shoot weights for the two investigated plant species and corresponding root : shoot ratios.

Video S1 Visualisation of root growth through different soil components.

Please note: Wiley is not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the New Phytologist Central Office.
New Phytologist Supporting Information

Article title: The soil pore structure encountered by roots affects plant-derived carbon inputs and fate
Authors: Maik Lucas, James P Santiago, Jinyi Chen, Andrey Guber, Alexandra Kravchenko
Article acceptance date: 05 July 2023
The following Supporting Information is available for this article:

Fig. S1 Photographs taken during the plant growth and after plant harvest.

Fig. S2 Relationship of root length densities estimated with X-ray μCT and disturbed.

Fig. S3 Pore characteristics of the cores with intact or sieved soils of switchgrass or prairie origin before plant growth

Fig. S4 Pore characteristics of the cores with intact or sieved soils of switchgrass or prairie origin before plant growth.

Fig. S5 Distribution of $^{14}$C in intact and sieved cores of the switchgrass and prairie origin subjected to P. virgatum and R. hirta growth.

Fig. S6 Relationship between root-soil contact and the ratio of $^{14}$C in rhizodeposition and $^{14}$C in roots.

Fig. S7 Distribution of $^{14}$C between the different compartments expressed on per unit of root length.

Table S1: $^{14}$C Labelling of the plants and translocation of the $^{14}$C label into shoots, roots, rhizoplane, and rhizosphere of R. hirta and P. virgatum

Table S2: Selected chemical properties of the soils under monoculture switchgrass and restored prairie vegetation systems.

Table S3 Selected physical properties of the soils under monoculture switchgrass and restored prairie vegetation systems.

Table S4: Root and shoot weights for the two investigated plant species and corresponding root/shoot ratios.
Methods S1 MLE Site and soil handling
Methods S2 Plant growing conditions
Methods S3 Radioactivity evaluation
Methods S4 Validating root segmentation by destructive sampling
Methods S5 Analyse of subsamples at Argonne national lab for pores < 40 µm
Video S1 Visualisation of root growth through different soil components
Fig. S1 Photographs taken during the plant growth and after plant harvest. (a) *R. hirta* and *P. virgatum* were planted into containers filled with 2-mm sieved soil, covered by gravel and grew in a growth chamber for 48 days. (b) *P. virgatum* and (c) *R. hirta* plants before harvest. The containers holded cylindric cores with soil of four contrasting pore structure characteristics. We transferred (d) intact and sieved soils of prairie and switchgrass origin into (e) perforated ingrowth cores to enable root growth and (f) buried them into the larger containers. To ensure that the intact and sieved cores differed from each other only in terms of pore structure, the sieved soil was packed to the same bulk density as the intact cores and all >2 mm inclusions, such as stones and large root residues were incorporated back into the sieved soil prior to packing. To enable tracing $^{14}$C into root/soil compartments (g) the plants were subjected to two pulses of $^{14}$C labelling and (h) the distribution of C of plant origin (i.e., $^{14}$C) within the soil was determined by grid sampling. To measured dissolved $^{14}$C in pores of two contrasting sizes we (i) transferred the soil samples into large Falcon Tubes for extraction of soil solution by centrifugation.
Fig. S2 Relationship of root length densities estimated with X-ray µCT and disturbed. Shown are the information for all roots analyzed (a) and roots larger than 0.2 mm (b). The regression the two parameters, a linear model with zero as intercept, between is shown by the green dashed line.
Fig. S3 Pore characteristics of the cores with intact or sieved soils of switchgrass or prairie origin before plant growth. (a) Macroporosity (i.e., the volume of > 40 μm Ø pores) and (b) pore connectivity of intact and sieved cores from prairie and switchgrass soils. (c) Pore size distributions in the intact and sieved cores, and (d) bioporosity in the intact cores from prairie and switchgrass soils. The Γ-indicator (pore connection probability) was calculated for large macropores (> 200 μm Ø), which are assumed to be easily followed by roots. Letters and stars mark the cases when differences among/between pore structures were statistically significant (p <0.05). Vertical line on (c) marks subdivision of the studied macropores into narrow (40-150 μm Ø) and wide (>150 μm Ø) ones.
Fig. S4 Pore characteristics of the cores with intact or sieved soils of switchgrass or prairie origin before plant growth. A linear mixed effect model found no statistically significant association between the mean root diameter and the share of roots growing into the matrix for the two plants (p-value >0.05).

Fig. S5 Distribution of $^{14}$C in intact and sieved cores of the switchgrass and prairie origin subjected to P. virgatum and R. hirta growth. Shown are the $^{14}$C activities in (a) roots, (b) rhizoplane (c) rhizosphere soil, (d) remaining in the soil after 30 days of incubation, (e) soil microsamples taken from each core on a regular grid (n=9), and in the soil solution from macropores (>40 µm Ø) (f) and small pores (< 40 µm Ø) (g), respectively. The letters indicate significant differences among the plant, structure, and soil combinations investigated by linear mixed effect models (p-value < 0.05). In addition, we report statistical significance of F-tests for the main effect of the plant species (P. virgatum vs. R. hirta) and simple F-tests (aka slicing) for the effects of structure (sieved vs. intact) and origin (prairie vs. switchgrass-soil) within each species (shown in their respective colors) and marked by ns, *, **, and *** for p-values > 0.1, < 0.05, < 0.01, and < 0.001, respectively. Error bars show the standard errors of the means.
Fig. S6 Relationship between root-soil contact and the ratio of $^{14}$C in rhizodeposition and $^{14}$C in roots. The solid line represents the statistically significant fitted linear mixed effect model for *P. virgatum* (p-value 0.01).
Fig. S7 Distribution of $^{14}$C between the different compartments expressed on per unit of root length. Shown are the $^{14}$C activity in (a) root, (b) rhizoplane (c) rhizosphere, and (d, e) in soil solution from macropores (> 40 µm) and small pores (< 40µm), respectively. The letters indicate significant differences among the plant, structure, and soil combinations investigated by linear mixed effect models (p-value < 0.05). In addition, we report statistical significance of F-tests for the main effect of the plant species (P. virgatum vs. R. hirta) and simple F-tests (aka slicing) for the effects of structure (sieved vs. intact) and origin (prairie vs. switchgrass-soil) within each species (shown in their respective colors) and marked by ns, *, **, and *** for p-values > 0.1, < 0.05, < 0.01, and < 0.001, respectively. Error bars show the standard errors of the means.
Table S1: 14C Labelling of the plants and translocation of the 14C label into shoots, roots, rhizoplane, and rhizosphere of R. hirta and P. virgatum. Mean photosynthesis rate and total 14C assimilated were measured by the Licor system during the two labelling events. Shown are mean values and standard errors of the mean (n=6). Significant differences between the species are indicated by the following: * for p-value < 0.05, *** for p-value < 0.001, as revealed by t-tests.

<table>
<thead>
<tr>
<th>Plant</th>
<th>R. hirta</th>
<th>P. virgatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photosynthesis rate (14C-CO₂ µmol m⁻² s⁻¹)</td>
<td>16.89 ±0.49</td>
<td>14.01 ±0.82 *</td>
</tr>
<tr>
<td>Total 14C assimilated (kBq)</td>
<td>282.71 ±7.80</td>
<td>235.17 ±13.73 *</td>
</tr>
<tr>
<td>Shoot (% of total assimilated)</td>
<td>30.54 ±2.60</td>
<td>65.52 ±9.48 *</td>
</tr>
<tr>
<td>Root (% of total assimilated)</td>
<td>3.42 ±0.16</td>
<td>0.79 ±0.10 ***</td>
</tr>
<tr>
<td>Rhizoplane (% of total assimilated)</td>
<td>0.35 ±0.02</td>
<td>0.02 ±0.01 ***</td>
</tr>
<tr>
<td>Rhizosphere (% of total assimilated)</td>
<td>0.34 ±0.03</td>
<td>0.04 ±0.01 ***</td>
</tr>
</tbody>
</table>

± = standard error

Table S2 Selected chemical properties of the soils under monoculture switchgrass and restored prairie vegetation systems. Shown are mean values ± standard errors (n=4). The differences between the two systems were not statistically significant in t-tests for any of the reported soil characteristics with exception of Fe (p-value <0.05).

<table>
<thead>
<tr>
<th>Soil type</th>
<th>P, mg/kg</th>
<th>Ca, mg/kg</th>
<th>CEC</th>
<th>Cl, mg/kg</th>
<th>Cu, mg/kg</th>
<th>Mg, mg/kg</th>
<th>Mn, mg/kg</th>
<th>S, mg/kg</th>
<th>K, mg/kg</th>
<th>Zn, mg/kg</th>
<th>pH</th>
<th>TC%</th>
<th>TN%</th>
<th>Fe, mg/kg</th>
<th>Lime index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prairie</td>
<td>11.5 ±3.8</td>
<td>1385.0 ±120.9</td>
<td>10.1 ±1.1</td>
<td>70.5 ±22.8</td>
<td>3.8 ±0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Switchgrass</td>
<td>12.3 ±2.2</td>
<td>1320.8 ±74.6</td>
<td>9.2 ±0.7</td>
<td>53.3 ±4.6</td>
<td>3.7 ±0.2</td>
<td>Mg, mg/kg</td>
<td>Mn, mg/kg</td>
<td>S, mg/kg</td>
<td>K, mg/kg</td>
<td>Zn, mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prairie</td>
<td>342.3 ±63.4</td>
<td>95.9 ±12.8</td>
<td>4.8 ±0.9</td>
<td>101.0 ±17.8</td>
<td>5.4 ±0.8</td>
<td>Mg, mg/kg</td>
<td>Mn, mg/kg</td>
<td>S, mg/kg</td>
<td>K, mg/kg</td>
<td>Zn, mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Switchgrass</td>
<td>287.0 ±46.5</td>
<td>86.9 ±10.9</td>
<td>5.8 ±0.5</td>
<td>75.8 ±14.2</td>
<td>5.6 ±0.8</td>
<td>Mg, mg/kg</td>
<td>Mn, mg/kg</td>
<td>S, mg/kg</td>
<td>K, mg/kg</td>
<td>Zn, mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

± = standard error
Table S3 Selected physical properties of the soils under monoculture switchgrass and restored prairie vegetation systems. Shown are mean values ± standard error. The differences between the two systems were not statistically significant for any of the reported soil characteristics. Bulk density n=8, Pores 5-40 µm n=3

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Bulk density, g/cm³</th>
<th>Pores 5-40 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prairie</td>
<td>1.34 ±0.03</td>
<td>0.14 0.04</td>
</tr>
<tr>
<td>Switchgrass</td>
<td>1.34 ±0.05</td>
<td>0.13 0.03</td>
</tr>
</tbody>
</table>

± = standard error

Table S4 Root and shoot weights for the two investigated plant species and corresponding root/shoot ratios. Shown are mean values ± standard error for R. hirta and P. virgatum, n =6. Species comparison was conducted by t-tests.

<table>
<thead>
<tr>
<th>Plant</th>
<th>R. hirta</th>
<th>P. virgatum</th>
<th>p-value for species comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot weight (g)</td>
<td>2.66 ±0.48</td>
<td>1.55 ±0.12</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Root weight (g)</td>
<td>0.89 ±0.22</td>
<td>0.3 ±0.03</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Root/Shoot</td>
<td>0.33 ±0.05</td>
<td>0.21 ±0.04</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

± = standard error

Methods S1 MLE Site and soil handling
The DOE-Great Lakes Bioenergy Research Center’s (GLBRC) Marginal Land Experiment (MLE) is located in Oregon, Wisconsin [42.9661, -89.3561]. The soil is a well-drained Alfisol formed on loess (9.1% sand, 74.8% silt and 16.1 % clay) (Kasmerchak & Schaetzl, 2018). The
experimental site was ploughed in 2013 prior to establishment and no further ploughing was conducted in either of the systems. Detailed information on the design and management as well as its land use history of the MLE, can be found on https://lter.kbs.msu.edu/research/long-term-experiments/marginal-land-experiment.

A total of 60 intact cores were taken at 5-10 cm depth from the experimental site, with 7-8 cores from each replicated plot. Eight intact soil cores (5 cm Ø × 5 cm height) were collected from each plot of both vegetation systems at 10-15 cm depth in early 2021. The cores were taken using a soil core sampler (Soil Moisture Equipment Corp.) into acrylic cylinders located within the sampler. Two additional soil cores for bulk density and root length density measurements were also collected from each sampling location at the same soil depth. Bulk density was measured using the core method (Nimmo et al., 2023). To preserve the cores during transportation each core was closed on both ends with ridged foil caps and wrapped in several layers of plastic using duct tape. Additionally, ~3 kg of loose disturbed soil was taken from all plots from the same depth, air-dried and sieved through a 2 mm sieve. This soil was later used as a filling material of the plant pots.

The intact and sieved ingrowth soil cores for the experiment were prepared from the intact soil cores taken in the field as described above. For that, all intact cores (n=60) were first placed into a pressure chamber to bring them to -28 kPa matric potential. To prepare an intact ingrowth core the field core was taken out of the cylinder (Fig. S1d), moved into perforated covers (Fig. S1e), The cover consisted of a polypropylene tube with 4 mm Ø perforations (41% open area), which was cut open and stretched around the soil core, hot glued and closed by perforated caps on top and bottom (Fig. S1e).

The chemical properties of the soil were analyzed by the MSU Soil & Plant Nutrient Laboratory (East Lansing, Michigan, USA) after sieving soil by a 2 mm sieve. Soil pH was measured in a 1:1 soil and water slurry. Available phosphorus was determined by Bray-Kurtz P1 (weak acid) test. Concentrations of potassium, calcium, and magnesium for cation exchange capacity (CEC) and metals including zinc (Zn), manganese (Mn), iron (Fe), and copper (Cu) were measured by inductively coupled plasma (ICP) spectrophotometers after extracting them from soil samples.

Methods S2 Plant growing conditions
During packing of the plant pots, Hoagland-Solution (Hoagland & Arnon, 1950) was added at every 2 cm layer (1L in total) to assure a homogenize distribution of the basal fertilizer also in the depth of the Ingrowth cores. The soil surface of the pots was fully covered with a 1-cm thick layer of quartz gravel to reduce evaporation. The volumetric soil water content was set to 30% (corresponding to -28 kPa), and kept constant throughout the experiment by adding water periodically from the bottom of the pots through a 30-μm nylon mesh. Pregerminated plants of *P. virgatum* and *R. hirta* were transplanted to the pots (one per pot) and kept for 48 days in a growth chamber under the following conditions: 14 hr photoperiod with 400 μmol m$^{-2}$ s$^{-1}$ photosynthetically active radiation, and 25°C and 20°C day and night temperature. Additional 200 ml of the nutrient solution was added to the pots on the 10$^{th}$ and 20$^{th}$ days after transplanting. Three not planted control pots were assembled and treated in the same way as the planted pots throughout the experiment.

**Methods S3 Radioactivity evaluation**

**Sampling of rhizosphere soil, rhizoplane and roots**

The roots were carefully taken out of the remaining soil with tweezers, and rhizosphere and rhizoplane fractions were obtained following the procedures described in (Lucas *et al.*, 2018). In short, roots were spread on filter papers, and cleaned from the adhering soil. The soil so removed was classified as rhizosphere soil and subsequently subjected to $^{14}$C determination. To obtain $^{14}$C from the roots’ rhizoplane the cleaned roots were placed into 30 ml tubes containing 15 ml of distilled water. The tubes were sonicated for 5 minutes, and the suspension solution procured after removing the roots was used for $^{14}$C in the rhizoplane determination. Roots and rhizosphere soil were oven-dried at 60°C overnight to derive dry root mass and the weight of the rhizosphere soil.

**$^{14}$C-DOC sampling**

The centrifugation method (Russell & Richards, 1939) enabled us to derive soil solution from two different pore size classes with equivalent diameters of > 35 μm (referred here to DOC from macropores > 40 μm Ø) and 2-35 μm (referred here to DOC from small pores < 40 μm Ø). To
calculate the needed angular velocity of the centrifuge, we used the equation from, which takes into account the nonlinear behaviour of water potential distribution within the soil sample:

\[
\psi = \frac{k \omega^2 L}{g} \left[ L - 3r_i \right]
\]

with \(\psi\), the water potential [kPa], the constant \(k\) (0.09807 kPa cm\(^{-1}\)), the sample length \(L\) [cm], the angular velocity \(\omega\) [s\(^{-1}\)], the radius of the outer boundary \(r_i\) [cm], and the gravity acceleration \(g\) of 981 cm s\(^{-1}\). To reduce the loss of soil from the sample, the bottom of the 2 cm subsamples used in centrifugation were closed by a perforated cap containing a filter paper of 200 \(\mu\)m mesh size. The samples were placed into a 50 ml falcon tube, which enabled to gain the soil solution after centrifugation (Fig. S2i). After each centrifugation of 1 hour, the soil solution was collected from the tube. Between the two centrifugation steps, the filter paper was replaced to reduce cross-contamination.

\(^{14}\)C-CO\(_2\) – Incubation experiment

The second 2-cm subsample from soil cores was placed into a mason jar with two LCS-vials in it. One was filled with 5 ml NaOH-solution, while the other contained 10 ml of water to assure that the sample does not dry out over the 30-days incubation. The jars were kept in the dark at approximately 20°C. The NaOH trapping solution was extracted and replaced after 3, 7 and 30 days via a septum. At the end of the incubation experiment, the soil was dried in an oven at 60°C to estimate the \(^{14}\)C activity remaining in the soil sample.

Radioactivity evaluation

The oven-dried aboveground plant tissue was mixed and five subsamples of mixed plant tissue were taken for the combustion, as well as approximately 1 g of soil from the incubation experiment. All other root and soil samples (including no plant controls and the 432 grid samples) as well as the rhizosphere soil were combusted in an oxidizer (PerkinElmer®, Boston, MA, Model A307). The \(^{14}\)CO\(_2\) released from the oxidizer was trapped in 20 ml of scintillation cocktail (Carbo-Sorb® E:Permafluor® E+, 1:1 [v/v], PerkinElmer, Groningen, Netherlands). For the solution samples of rhizoplane and NaOH-traps from the incubation, the solution was each mixed with Ultima GoldTM of a three-times-larger volume. The radioactivity of all samples was quantified with a liquid scintillation counter (PerkinElmer® Tri-Carb 4910TR Liquid Scintillation counter, PerkinElmer, Waltham, MA). The radioactivity of each sample was represented after subtracting
the background 14C-activity of the cocktail solutions (estimated with a standard error of 7.44%), plus 10 percent error margin. The total 14C-activity of the plant, root-biomass, rhizosphere and rhizoplane were divided by the total activity taken up by the plant to derive the relative amounts of the total assimilated. The activities for the belowground parts were extrapolated for the total soil volume, as for the belowground parts only the ingrowth cores were analysed. This extrapolation will underestimate the total belowground translocation of C by roots, as the largest root length densities can be assumed in the top of the soil, i.e. above the cores. Assuming this influence is the same for both plant species studied, the comparison between their total allocation of 14C will however be meaningful.

**Methods S4 Validating root segmentation by destructive sampling**

To validate the root segmentations, nine additional soil cores from each, switchgrass- and prairie-soil were taken to compare the root length derived from X-ray µCT images with the ones measured destructively. The cores were scanned and analysed as written above. Afterwards the soil of the cores was washed through a 1 mm sieve to collect roots. The roots were stored in ethanol, then were scanned on a flatbed scanner (Epson Perfection V850 pro) at 1200 dpi. The root length estimation was performed using the Rhizovision Explorer (V. 2.0.3 Seethepalli et al., 2021). The length densities showed a good agreement between the two methods (R²=0.76, p <0.001 for all roots and R²=0.91, p <0.001 for roots larger 0.2 mm Ø, Fig. S2). However, the estimation for all roots revealed general higher values analysed by X-ray µCT compared to destructively measured ones, while roots larger 0.2 mm Ø were slightly underestimated by X-ray µCT. This difference may be explained by brittle dead roots lost during the sieving process.

A big advantage off our method compared to destructive sampling is that we were able to differentiate between newly developed roots and old ones. While most of the root debris was easily to differentiate from new roots, others looked quite fresh but were not freshly grown roots (especially in Switchgrass-soil), which is in line with our findings. The root segmentation, which is here compared to the results of the destructive sampling, does include all kind of roots, including root debris, i.e. very short, half degraded root segments, which may partly flush through the fine sieve. Note that our main analyses are based on subtracting the image before the experiments, i.e.
which contains all these root segments, from the one after the experiment. The result is an image containing only new, fresh roots, i.e. roots which are easy to segment. Thus, the validation test can be seen as a worse case scenario. Indeed, visual inspection showed that we hardly missed any roots visible in the CT.

**Methods S5 Analyse of subsamples at Argonne national lab for pores < 40 µm**

After setting a matrix potential of -28 kPa six samples were randomly taken from the intact soil cores, which were not used as ingrowth cores for planted pots but used to extract subsamples. Three intact subsamples were from both vegetation systems were taken using small PE containers (0.8 cm Ø and 0.8 cm height) to analyze pores at high resolution at the bending magnet beam line, station 13-BM-D of the GeoSoilEnvironCARS (GSECARS) at the Advanced Photon Source (APS), Argonne National Laboratory (ANL). Images were collected with the Si (111) double crystal monochromator at 37.6 keV incident energy. Two-dimensional projections (1800) were taken with a 1 s exposure and reconstructed into a three-dimensional image consisting of 1,040 slices with a voxel size of 5.7 µm. Images were cut to volumes of 1500x1500x1000 after reconstruction. The images were processed similar to the larger ones, i.e. after, applying a non-local means filter (Darbon *et al.*, 2008; Buades *et al.*, 2011) with scikit-image (van der Walt *et al.*, 2014) in Python (van Rossum & Drake, 2009) to ensure a good automatic threshold detection for separating pores <40 µm Ø. For this the Otsu algorithm was used (Otsu, 1979), before computing the local thickness of the pores (Hildebrand & Rüegsegger, 1997) to extract only pores between 5.7 and 40 µm, which were not resolved in the larger images.

**References**


