

1 **Belowground plant carbon and nitrogen exchange: plant-derived carbon inputs and pore**  
2 **structure formation**

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18 foliar labeling, Pore-size distributions, X-ray computed micro-tomography

19

20 **Abstract**

21 Belowground plant transfer of carbon (C) and nitrogen (N) can benefit soil ecosystems,  
22 increasing soil C gains and plant N availability, while improving soil pore structure. We explored  
23 such transfers among three plant species of North American prairie, where C and N were

24 transferred from a grass (*Panicum virgatum* L., switchgrass (Sgrass)) to either a legume  
25 (*Lespedeza capitata* Michx., bush clover (Bclover)), a forb (*Rudbeckia hirta* L., black-eyed Susan  
26 (BSforb)), or a mixture of the two. The plants were grown either with/out direct root contact, thus  
27 allowing assessment of the relative contributions of fungal- and root-based transfer pathways. The  
28 Sgrass was labelled with <sup>13</sup>C and <sup>15</sup>N, and C and N transfers were assessed by measuring isotope  
29 enrichment of roots and aboveground biomass of neighboring plants. Soil inputs of plant-derived  
30 C and N were assessed by isotope analyses of the rhizosphere soil. X-ray computed tomography  
31 was used for pore structure analyses. Carbon transfer was much higher in the presence of  
32 direct/close root contact between source and recipient plants, yet N transfers appeared to be mainly  
33 fungal driven. While C and N were readily transferred from Sgrass to other Sgrass and Bclover  
34 neighbors, transfers to BSforb were negligible. However, in a three species system, the presence  
35 of the legume enhanced C and N transfers to BSforb, suggesting non-additive influences of diverse  
36 plant community composition. The more plant-derived C and N was found in the rhizosphere of  
37 recipient plants, the greater C and N transfers through roots. Greater C and N transfers were  
38 associated with increases in 8-30 μm diameter pores and decreases in >150 μm pores. Summarily,  
39 diverse plant communities, especially those with legumes, increase C and N transfers, which then  
40 benefit soil C inputs and its protection via changes in pore structure.

41

#### 42 **Abbreviation**

43 CO<sub>2</sub>, carbon dioxide; μCT, X-ray computed micro-tomography; HC, Biofuel Cropping System  
44 Experiment site in Hickory Corners; ESC, Marginal Land Experiment site in Escanaba; Sgrass,  
45 switchgrass; Bclover, bush clover; BSforb, black-eyed Susan; Ø, diameter; HPLC, high-  
46 performance liquid chromatography; POM, particulate organic matter

## 47 **Introduction**

48           Diverse perennial vegetation enhances soil carbon (C) gains by increasing plant inputs and  
49 promoting soil organic matter stabilization (Lange et al., 2015; Sprunger and Robertson, 2018; Lee  
50 et al., 2023). It also improves plant nitrogen (N) availability by supporting greater biological N  
51 cycling (Mulder et al., 2002; Lange et al., 2019), subsequently enhancing soil health and fostering  
52 plant growth (Lal, 2016). Enhanced belowground interplant transfer of C and N in diverse  
53 vegetation systems are suggested as potential drivers of this phenomenon (Kravchenko et al.,  
54 2021b; Zheng et al., 2022). Carbon transfer to roots of adjacent plants was first documented in  
55 1960s (Björkman, 1960; Reid and Woods, 1969) and was later reported in plants of the same as  
56 well as different species (Ren et al., 2013; Gorzelak et al., 2020; Cahanovite et al., 2022). In  
57 addition to C transfer, intra- and inter-specific N transfer also occurs frequently in plant  
58 communities, likely supporting nutrient sharing (Høgh-Jensen and Schjoerring, 2000; Shao et al.,  
59 2020; Reay et al., 2022).

60           Transfer of both C and N primarily takes place via two key routes: directly through root-  
61 to-root contacts and/or through fungal networks (Thilakarathna et al., 2016; Hupe et al., 2021).  
62 The root-based transfer occurs when C and N are released from roots of a source plant as  
63 rhizodeposits, e.g., exudates (Jones et al., 2004; Badri and Vivanco, 2009). These compounds can  
64 be decomposed or transformed by soil microorganisms into simpler, inorganic or low-molecular-  
65 weight forms, e.g.,  $\text{HCO}_3^-$ , which can subsequently be taken up by roots of recipient plants  
66 (Biernath et al., 2008; Rasmussen et al., 2010). Such uptake can take place both when there is a  
67 direct contact between roots of source and recipient plants or when roots of the recipient plant  
68 encounter the released C and N somewhere within the soil. The fungal-based transfer occurs when  
69 roots of neighboring plants are connected through common arbuscular mycorrhizal networks

70 (Wipf et al., 2019). The symbiotic fungi obtain plant-derived C and N and utilize them to develop  
71 their hyphae (Rillig, 2004a; Parniske, 2008), which can serve as pathways for the movement or  
72 transfer of C and N between neighboring plants (Martins and Cruz, 1998; He et al., 2003). In  
73 addition, a direct uptake of C and N from decomposed fungi by the roots cannot be ruled out.

74 Belowground transfers depend on the identity of the plants acting as sources and/or  
75 recipients of the C and N inputs (Meding and Zasoski, 2008; Walder and van der Heijden, 2015;  
76 Montesinos-Navarro et al., 2017). For example, the identity of the neighboring recipient can  
77 substantially impact root growth and fungal development in the source plant (Belter and Cahill,  
78 2015; Mony et al., 2021), where the roots of the source plants respond to neighbors' roots in a  
79 manner contingent on the neighbor's specific identity (Falik et al., 2003; de Kroon, 2007).  
80 Likewise, the impact of neighboring plant species on the fungal community is influenced by  
81 functional traits of the source plant (Burrows and Pflieger, 2002; Mony et al., 2021), and the identity  
82 of the neighboring recipient plant affects the direction and magnitude of nutrient transfer via  
83 mycorrhizal networks (Meding and Zasoski, 2008).

84 Interplant C and N transfers in diverse perennial vegetation can affect soil C accumulation  
85 via a multitude of mechanisms, one of which is soil pore structure alterations (Zheng et al., 2022,  
86 2023). Plant roots influence soil structure through direct penetration, water extraction, and root  
87 exudation (Bengough et al., 2016; Oburger and Schmidt, 2016; Lee et al., 2024b). Roots occupy  
88 relatively large (e.g. > 100  $\mu\text{m}$  diameter) pores (Bauhus and Messier, 1999; An et al., 2022), where  
89 they deposit a variety of root-released C compounds as well as leave root residues upon senescence  
90 (Jones et al., 2004; Badri and Vivanco, 2009). Organic inputs into such pores tend to be susceptible  
91 to decomposition and loss as carbon dioxide ( $\text{CO}_2$ ) (Ruamps et al., 2011; Kravchenko et al., 2019a).  
92 Fungi, particularly mycorrhizal species, influence soil structure by producing gluing agents such

93 as glycoproteins (Agnihotri et al., 2022; Yudina and Kuzyakov, 2023). They also contribute to the  
94 C accumulation by releasing C and N-containing compounds into the soil as proteins, extracellular  
95 enzymes, and other secondary metabolites (Rillig, 2004b; Agnihotri et al., 2022). Carbon and N  
96 from senescent and decomposed hyphae further add to soil organic matter (Treseder and Holden,  
97 2013). Unlike compounds of root origin, the fungi-originated organics can be located not only in  
98 the large but also in very fine (e.g., ~ 10 µm diameter) pores accessible to fungal hyphae (Carlile,  
99 1995). Organic C in such fine pores tends to be better protected from further microbial  
100 decomposition (Keiluweit et al., 2016; Kravchenko et al., 2019a; Zheng et al., 2022). Recently,  
101 Kravchenko et al. (2021) demonstrated that interplant C transfer contributed to the formation of  
102 50-100 µm diameter pores, and in plant systems with greater C transfer, more newly added root C  
103 remained in the soil after short-term incubations (Zheng et al., 2022). These studies hypothesized  
104 that, by delivering C into smaller soil pores, fungi could facilitate C stabilization and reduce CO<sub>2</sub>  
105 loss compared to roots, which introduced C into larger pores where it could be more rapidly  
106 processed and emitted. However, since in these works the fungal-based transfer pathways were  
107 not distinguished from the root-based ones, these hypotheses have not been empirically tested yet.

108         Here we specifically focused on interplant C and N transfers from switchgrass (*Panicum*  
109 *virgatum* L.), a C<sub>4</sub> grass species dominant in the North American prairie and considered a viable  
110 perennial cellulosic bioenergy crop (Gelfand et al., 2020). This deep-rooted perennial increases  
111 soil C when it is grown in diverse plant communities (Yang et al., 2019), thus making it a suitable  
112 model plant to explore the impact of interplant C and N transfer on soil C processes. Our first  
113 objective was to quantify C and N transfers from switchgrass source plants to neighbor plants of  
114 its own species or of two other species common in prairie communities: a legume - bush clover  
115 (*Lespedeza capitata* Michx) and a forb - black-eyed-Susan (*Rudbeckia hirta* L.), with a specific

116 focus on assessing the transfers via fungi-only vs. root-based plus fungal-based pathways. The  
117 second objective was to explore whether greater magnitude of transfers is associated with greater  
118 inputs of plant-derived C and N into the soil. The third objective was to examine whether and how  
119 the two transfer pathways alter soil pore structure in the experimental settings.

120

## 121 **Materials and Methods**

### 122 *Overview of the experiment*

123         The study was conducted as a greenhouse experiment, where plants of three species were  
124 grown in rhizoboxes filled with soil from two sources, described below (Table S1). In half of all  
125 rhizoboxes the plants could contact the neighbors via their roots through shared soil, while in the  
126 other half the plants were separated by root-impenetrable, but fungal hyphae-accessible nylon  
127 mesh barrier (35  $\mu\text{m}$ ) (Fig. 1A). It was assumed that in the absence of the barrier the C and N  
128 transfers could take place both via direct root contacts and via fungal interactions among the roots,  
129 while in the presence of the barrier only fungal-based transfers were possible. Hereafter we refer  
130 to them as barrier treatments. Each rhizobox consisted of three compartments, with one plant  
131 grown per compartment. The central compartment plants of each rhizobox, further referred to as  
132 source plants, were subjected to  $^{13}\text{C}$  and  $^{15}\text{N}$  labeling. The side compartment plants, referred to as  
133 recipient plants, were not labeled themselves and could have become  $^{13}\text{C}$  and  $^{15}\text{N}$  enriched only  
134 through belowground C and N transfer from the source plant.

135         At the end of the experiment, we opened the front panel of each rhizobox, and aboveground  
136 biomass, roots, and rhizosphere soil were destructively sampled for  $^{13}\text{C}$  and  $^{15}\text{N}$  analyses. In  
137 addition, intact soil cores were collected from each rhizobox and subjected to X-ray computed  
138 micro-tomography ( $\mu\text{CT}$ ) for soil pore structure characterization and particulate organic matter

139 (POM) segmentation (Fig. 1B). A representative picture of a rhizobox is in the Fig. 1C, and  
140 detailed descriptions of all experimental, sampling, and analyses components of the study are  
141 provided in subsequent sections.

142

### 143 *Soil and rhizobox preparation*

144 Two Alfisol soils with disparate characteristics were used (Table S1): a loam Typic  
145 Hapludalf and a sandy loam Inceptic Hapludalf. Both soils were collected from sites within the  
146 Great Lake Bioenergy Research Center (USA). The Typic Hapludalf soil originated from the  
147 Biofuel Cropping System Experiment site in Hickory Corners, Michigan, USA (42° 40'N,  
148 85°37'W) and will be referred to as HC soil, and the Inceptic Hapludalf soil originated from the  
149 Marginal Land Experiment site in Escanaba, ESC soil, Michigan, USA (45°76'N, -87°19'W). At  
150 each site the soil was collected from Ap horizon (5-20 cm) from conventionally managed  
151 agricultural fields. The collected soil was air-dried, sieved (2 mm), and thoroughly mixed, with  
152 small stones and visible plant residues removed.

153 A total of 88 rhizoboxes were constructed. Each rhizobox (30 cm tall, 54 cm wide, and 4  
154 cm thick) had three equally sized compartments with each compartment hosting a single plant (Fig.  
155 1). Three sides of the rhizobox were made from non-transparent plastic, while one side was  
156 transparent, which enabled visual monitoring of root growth. The transparent side was also  
157 removable, which facilitated the localization for root and soil sampling at the end of the study. The  
158 three compartments were separated by plastic dividers (30 cm tall, 2 cm wide, and 4 cm thick)  
159 (Fig. 1C). The dividers were either open between the compartments or covered by nylon mesh  
160 barriers (35  $\mu$ m, ELKO Filtering Co., Tamarac, FL, USA), restricting root-to-root contact, while  
161 still permitting passage of fungal hyphae (Fig. 1A). The rhizobox compartments as well as dividers

162 were filled with the prepared soil ensuring consistent bulk density of 1.3 g/cm<sup>3</sup> throughout the  
163 rhizobox, at the volumetric water content of 20%.

164

#### 165 *Plant treatments and combinations*

166 The three studied plants were switchgrass (*Panicum virgatum* L.), bush clover (*Lespedeza*  
167 *capitata* Michx), and black-eyed Susan (*Rudbeckia hirta* L.), hereafter referred to as Sgrass,  
168 Bclover, and BSforb, respectively. Sgrass growing in the central compartment was always the  
169 source plant, i.e., the plant that received <sup>13</sup>C and <sup>15</sup>N labeling. Neighboring plants were placed in  
170 the two side compartments, comprising the following four plant treatments: (1) two Sgrass  
171 neighbors, (2) two Bclover neighbors, (3) two BSforb neighbors, and (4) mixed neighbors, where  
172 one side compartment contained Bclover and the other BSforb (Table 1). The first three treatments  
173 will be referred to as “same-neighbor combination” and the last one as “mixed-neighbor  
174 combination”. The mixed-neighbor combination aimed at exploring the potential impact of a more  
175 diverse plant community. The recipient plants were randomly assigned to each side of each  
176 rhizobox, particularly for the boxes with two different recipient species (Fig. 1). Six replicated  
177 boxes with HC soil and five replicated boxes with ESC soil were constructed for each of the four  
178 plant treatments. Additional rhizoboxes hosting the three studied plants were prepared as non-  
179 isotope-labeled controls, and boxes without any plants were used as non-planted controls.

180

#### 181 *Plant growth*

182 Seeds of each plant species were first germinated in seed starter trays for three weeks, and  
183 the seedlings that developed their first leaves were transplanted to the center of each compartment  
184 in rhizoboxes, one plant per compartment. The plants were grown for four months in the

185 rhizoboxes with HC soil and for four months and two weeks in the rhizoboxes with ESC soil. All  
186 plants were fertilized using Hoagland's solution after transplanting and then, again, one month  
187 later. Two months after transplanting, the plants in ESC soil boxes showed signs of phosphorous  
188 deficiency, and hence, were additionally fertilized with 134 mg of  $\text{KH}_2\text{PO}_4$  per box (equivalent to  
189 56 kg P/ha). Each plant in its own compartment was separately watered with approximately 0.5 L  
190 of reverse osmosis water every day. The temperature in the greenhouse was regulated to maintain  
191 a maximum of 29 °C during daylight hours and a minimum of 20 °C during the night, accompanied  
192 by 16 hours of artificial fluorescent light.

193         Initially, the boxes were kept tilted at a 60° angle with the transparent side down to  
194 stimulate root growth towards the transparent side and to enable visual observations of the root  
195 growth. After two months the boxes were moved to a vertical (90°) position to ensure that roots  
196 explored the rest of the soil and to increase root interactions through the dividers.

197

#### 198 *<sup>13</sup>C pulse and <sup>15</sup>N foliage labeling*

199         In order to trace the belowground C and N transfer from the source plants, all source Sgrass  
200 plants in the central compartment were pulse-labeled with  $^{13}\text{CO}_2$  and foliage-labelled with  
201  $^{15}\text{NH}_4\text{NO}_3$ . The labeling for both elements was performed three times for the rhizoboxes with HC  
202 soil and four times for those with ESC soil. In all cases the last labeling event took place one month  
203 before plant termination.

204         Pulse labeling for  $^{13}\text{C}$  enrichment of the source plants took place in labeling chambers.  
205 Before placing the rhizoboxes into the labeling chambers, the recipient plants of each rhizobox  
206 were covered with light-impenetrable plastic bags, while the central Sgrass remained uncovered.  
207 This setup ensured that only the uncovered source Sgrass plant was able to photo-assimilate  $^{13}\text{C}$

208 from the produced  $^{13}\text{CO}_2$ , and the recipient plants were not conducting photosynthesis. This setup  
209 has been successfully implemented by our team in the past (Kravchenko et al., 2021b; Zheng et  
210 al., 2022). For the  $^{13}\text{CO}_2$  pulse labeling, we utilized 98%  $^{13}\text{C}$ -enriched  $\text{NaHCO}_3$  solution. The  
211 solution was mixed with  $\text{H}_2\text{SO}_4$  to produce  $^{13}\text{CO}_2$ , releasing 88 mg of  $^{13}\text{C}$  per pulse event per  
212 labeling chamber. Upon the  $^{13}\text{CO}_2$  production, the chamber was sealed for six hours in each pulse  
213 labeling event. The chamber was equipped with ventilators to facilitate even distribution of  $^{13}\text{CO}_2$ ,  
214 and thermometers were installed to continuously monitor the internal temperature. To eliminate  
215 any potential heat stress, the lower portions of the chamber exteriors were insulated with ice. Upon  
216 opening the chamber, rhizoboxes were immediately moved to the environment with controlled  
217 lighting and ample ventilation to support normal plant photosynthesis and to protect the recipient  
218 plants from potential  $^{13}\text{C}$  assimilation through  $^{13}\text{CO}_2$  respiration of the source plants.

219 For  $^{15}\text{N}$  enrichment of the source plants, we conducted foliage labeling with 45%  $^{15}\text{N}$ -  
220 enriched  $\text{NH}_4\text{NO}_3$  (Chu et al., 2004; Shao et al., 2020). One undamaged leaf of each source Sgrass  
221 plant was gently inserted into a 2-ml tube containing 1.5 ml of 5% (v/w)  $^{15}\text{NH}_4\text{NO}_3$  solution (6.38  
222 g  $^{15}\text{N}$  per L). We aimed at synchronizing the  $^{13}\text{C}$  and  $^{15}\text{N}$  labeling events, thus, the  $^{15}\text{N}$  labeling  
223 events took place 48 hours each  $^{13}\text{CO}_2$  pulse-labeling event and ended 72 hours after inserting the  
224 leaf. This provided sufficient time for the leaf of the source Sgrass plant to absorb the  $^{15}\text{NH}_4\text{NO}_3$   
225 solution. The inserted Sgrass leaf, along with the labeling tube, was carefully removed to avoid  
226 any dripping of  $^{15}\text{NH}_4\text{NO}_3$  from the leaf and contaminating the soil.

227

#### 228 *Location-specific sampling and analyses for roots and rhizosphere soil*

229 Root and surrounding rhizosphere soil samples were collected from four locations on the  
230 transparent panel side in each box, two per each recipient compartment (Fig. 1A). One of the

231 sampling locations was close (~2 cm) to the source compartment, while the other was far (~15 cm)  
232 from the border of the source compartment; we refer to them, respectively, as locations close and  
233 far from the source Sgrass plant (location “R” in Fig. 1A). Between each location’s sampling, the  
234 sampling tools were thoroughly cleaned and sanitized with 70 % ethanol to eliminate cross  
235 contamination. All collected root and soil samples were stored in 5 ml tubes at 4 °C before the  
236 preparation for  $^{13}\text{C}$  and  $^{15}\text{N}$  analyses.

237         Root sample preparation consisted of cleaning the roots from the attached soil and drying  
238 them. For that, 1.8 ml of 0.05M  $\text{CaCl}_2$  was added to each tube containing the root and the tube was  
239 sonicated for five minutes. Then, the roots were brushed to remove any remaining soil particles,  
240 and dark-brown, older roots were excluded from the collection. After that, the roots were oven-  
241 dried at 30 °C for two days before further analyses.

242         Rhizosphere soil preparation consisted of cleaning, drying, and grinding the soil. For  
243 cleaning we manually removed visible roots and organic debris from each tube containing  
244 rhizosphere soil. The rhizosphere soil was oven-dried at 30 °C for two days, and then ground using  
245 an iron-ball mill.

246         An elemental analyzer (Vario ISOTOPE CUBE, Elementar Americas Inc., Ronkonkoma,  
247 NY, USA) coupled to an isotope ratio mass spectrometer (Isoprime Vision, Elementar Americas  
248 Inc., Ronkonkoma, NY, USA) was utilized for  $^{13}\text{C}$  and  $^{15}\text{N}$  analysis. Isotope ratios ( $^{13}\text{C}/^{12}\text{C}$  and  
249  $^{15}\text{N}/^{14}\text{N}$ ) in the root and soil samples were calculated using  $^{13}\text{C}$  and  $^{15}\text{N}$  enrichment expressed as  
250  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (‰) and the PeeDee Belemnite and AIR standard, respectively (Fry, 2006). Then,  
251  $^{13}\text{C}$  and  $^{15}\text{N}$  atom % values of each sample were calculated from the isotope ratios, and the atom %  
252 excess values were obtained by subtracting the  $^{13}\text{C}$  and  $^{15}\text{N}$  atom % in the non-labeled root and  
253 rhizosphere soil samples.

254

255 *Sampling from rhizobox compartments: plant biomass and soil properties*

256         After collecting roots and rhizosphere soil samples from the four locations as described in  
257 the previous section, the entire aboveground biomass was clipped and collected. Due to the  
258 extensive branching of roots across the three rhizobox compartments, it was not feasible to collect  
259 the total belowground biomass. Instead, the biomass of major roots was collected by plucking out  
260 the crown of roots from the opened panel of each rhizobox and then by gently washing away the  
261 soil with DI water. As this method could capture the majority of root biomass, we refer this  
262 biomass to as just root biomass. The collected aboveground and root biomass were oven-dried at  
263 60 °C for three days and weighed.

264         After the biomass removal, bulk soil from all three compartments of each rhizobox was  
265 collected and used for measurements of total C, total N, and inorganic N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) contents  
266 in soil. For total C and N, soil samples were homogenized, 2 mm-sieved, ground, and analyzed by  
267 a CHNSO Elemental Analyzer (Costech Analytical Technologies, Valencia, CA, USA). For the  
268  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , salicylate-cyanurate method (Sinsabaugh et al., 2000) and vanadium method  
269 (Doane and Horwath, 2003) were used for 10 g of the homogenized soil with spectrophotometry  
270 (Victor3 1420 Multilabel Counter, Perkin Elmer, Waltham, MA, USA) at 630 and 540 nm,  
271 respectively.

272

273 *Sampling from rhizobox dividers: fungal biomass and soil pore structure*

274         Intact soil micro-cores (2 cm height and 0.8 cm diameter) were taken from the dividers in  
275 between the rhizobox compartments at 15 cm depth (Location “S” in Fig. 1A). In the same-  
276 neighbor combinations with the same two recipient species (Table 1), the core was collected only

277 from one (randomly selected) rhizobox side. In the mixed-neighbor combination with Belover and  
278 BSforb recipients, two cores, one per each rhizobox side, were collected. A total of 104 intact cores  
279 were obtained and air-dried prior to  $\mu$ CT scanning.

280 After collecting the cores, the remaining bulk soil from two dividers of each rhizobox was  
281 collected to measure ergosterol concentration. Ergosterol is a sterol compound found only in  
282 fungal cell membranes, thus, its content in the soil is used as an indicator of fungal biomass  
283 (Gessner, 2020). Ergosterol was measured using a high-performance liquid chromatography  
284 (HPLC) (Agilent, Santa Clara, CA, USA) equipped with a diode array detector at 282 nm (Gong  
285 et al., 2001). Two grams of soil from the dividers and 10 ml of methanol were mixed in a 15 ml  
286 centrifuge tube. Then, the tube was vortexed for 10 seconds, sonicated for 15 minutes in a  
287 sonication bath (CODYSON, Shnzhen, China), and centrifuged at 5000 rpm for 15 minutes. Two  
288 milliliters of the supernatant were collected from the tube and filtered through a 0.22  $\mu$ m polyvinyl  
289 difluoride syringe filter (Merck Millipore, Burlington, MA, USA). Forty microliters of the filtrate  
290 were eluted through a 150 x 4.6 mm C<sub>18</sub> column (Avantor, Radnor Township, PA, USA) at 25 °C  
291 and at a flow rate of 1 ml per minute with a mobile phase of HPLC grade methanol. The column  
292 pressure was maintained at 660 – 700 psi. The retention time of ergosterol was 15.5 – 16.5 minutes  
293 under the described conditions.

294

#### 295 *X-ray $\mu$ CT scanning and image analysis*

296 The air-dried intact cores were scanned at the 13-BM-D station of the GeoSoilEnviroCARS  
297 sector, Advanced Photon Source, Argonne National Laboratory (Lemont, IL, USA), with an  
298 energy of 30 keV and the scanning resolution of 5.7  $\mu$ m. Original images were cropped to focus  
299 on the central portions of the cores, avoiding areas potentially subjected to scanning and sampling

300 artifacts. The stack of the cropped images was  $4.6 \times 4.6 \times 8.0$  mm ( $800 \times 800 \times 1400$  pixels) in  
301 size. Then, the cropped images were denoised using 3D non-local mean filter ( $\sigma = 0.08$ ) (Darbon  
302 et al., 2008; Buades et al., 2011) implemented in scikit-image of Python (Walt et al., 2014).

303 The filtered images were used to identify POM and pores. The POM was segmented using  
304 a U-Net (convolutional neural network) model under the deep learning engine pre-built in  
305 Dragonfly software (Ronneberger et al., 2015; Makovetsky et al., 2018). The segmentation model  
306 was trained using 16 cores randomly selected from the entire set of 104 cores. We used five slices  
307 with representative POM fragments in each of the selected cores as training inputs, and two slices  
308 directly below and above the selected frames were also considered for generating segmentation  
309 outcomes. Then, the trained model was applied to the entire set of cores. The segmented POM  
310 images were visually inspected to ensure the integrity and accuracy of the process. The outcome  
311 of POM segmentation was denoised by removing clusters with less than four voxels from the  
312 images.

313 While care has been taken to remove visible plant residues from soil prior to the experiment,  
314 still, a complete removal was not possible. Thus, we used POM fragments in the non-planted  
315 control soil as a baseline, representative of the inherent residues that remained in the soil. Newly  
316 grown roots in the studied rhizoboxes were the only new source of POM, thus comparisons with  
317 the non-planted controls enabled us to quantify the amount of residues originated from newly  
318 grown roots.

319 The pores were segmented using the filtered images via Otsu method in SimpleITK of  
320 Python (Yaniv et al., 2018; Lucas et al., 2022), and then the previously identified POM was  
321 subtracted from pore images to ensure that only air-filled pores were part of the finalized images

322 of soil pore space (Fig. 1C). The scanning resolution and the applied image filtering allow us to  
323 reliably identify pores with  $>8 \mu\text{m}$  diameters ( $\emptyset$ ).

324 The finalized pore images were used to compute pore size distributions using 'Local  
325 Thickness' tool in Image-Fiji software (Schindelin et al., 2012), an approach based on the  
326 maximum inscribed sphere method (Hildebrand and Rügsegger, 1997; Vogel et al., 2010). We  
327 decided to classify the segmented pores into three  $\emptyset$  size ranges, namely 8-30  $\mu\text{m}$ , 30-150  $\mu\text{m}$ ,  
328  $>150 \mu\text{m}$   $\emptyset$ , because pores 30-150  $\mu\text{m}$  are often found to be associated with high microbial  
329 abundance and activity (Nunan et al., 2003; Strong et al., 2004; Li et al., 2024). For each size range,  
330 pore fractions were determined by dividing the pore volumes ( $\text{mm}^3$ ) by the total cropped soil (pore  
331 + POM + solid) volumes ( $\text{mm}^3$ ).

332

### 333 *Statistical analysis*

334 Data analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA), and  
335 statistical models were fitted using PROC MIXED. The models for measures of atom %  $^{13}\text{C}$  and  
336  $^{15}\text{N}$  excess in roots and rhizosphere soil of recipient plants included fixed effects of barrier  
337 treatments (barrier and non-barrier), four plant treatments of recipient plant species (Table 1), two  
338 sampling locations (close vs. far from the source plant), and their interactions. Statistical models  
339 for aboveground and root biomass, image-based POM fraction and soil porosity, ergosterol, atom %  
340  $^{13}\text{C}$  and  $^{15}\text{N}$  excess in shoots, and soil inorganic N contents ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) included the fixed  
341 effects of the barrier treatments, plant treatments, and their interactions. Statistical models for pore  
342 fractions of three size ranges (8-30  $\mu\text{m}$ , 30-150  $\mu\text{m}$ , and  $>150 \mu\text{m}$   $\emptyset$ ) in the total soil volume  
343 additionally included the fixed effect of size ranges, which were treated as a repeated measure  
344 factor. In all models, replicated rhizoboxes nested within soil types, barrier treatments, and plant

345 treatments were used as an error term for testing their effects and as a subject of the repeated  
346 measurement.

347 Note that we regarded the two studied soils as a source of random variation, representing  
348 soil variability among Alfisols under agricultural land use in the studied region. Thus, a random  
349 effect of the soil was included in all statistical models.

350 The assumptions of normality and variance homogeneity were assessed by examining  
351 histograms, normal probability plots, and side-by-side box plots of the residuals, and by  
352 conducting Levene's test for variances. Except for atom %  $^{13}\text{C}$  and  $^{15}\text{N}$  excess in roots, the  
353 residuals were found to be normally distributed and the equal variance assumption not violated for  
354 all studied variables. As the normality assumption for  $^{13}\text{C}$  and  $^{15}\text{N}$  excess in roots and rhizosphere  
355 soil was not met, the variables were log transformed, and due to the negative values in the excesses,  
356 a constant value was added to the data prior to the transformation.

357 When the interactions between the studied factors were found statistically significant, we  
358 used simple F-tests with slicing followed by multiple comparisons with *t*-tests. The effects and  
359 differences were reported as statistically significant at  $p < 0.05$ . Additionally, to determine the  $^{13}\text{C}$   
360 and  $^{15}\text{N}$  enrichment in recipient roots, soil, and shoots and changes in pore and POM fractions  
361 between the source and recipients, we performed another series of *t*-tests to compare them with  
362 those of non-isotope-labelled controls and in non-planted controls, respectively. Linear  
363 correlations among the studied response variables were assessed using Pearson's correlation  
364 coefficients via PROC CORR procedure in SAS.

365

## 366 **Results**

367 *Plant and fungal growth and soil inorganic N*

368 Three recipient neighbor species differed in their aboveground and root biomass, with  
369 Bclover plants being much smaller than Sgrass and BSforb ( $p < 0.001$ ) (Fig. S1). The presence of  
370 barriers did not affect aboveground and root biomass for any of the recipient species or the source  
371 Sgrass (Table S2 & Fig. S2). Fractions of POM identified by X-ray  $\mu$ CT within the meshed barrier  
372 dividers were similar to those identified in the non-planted control soil, while in the absence of  
373 barriers, POM fractions were  $>1.5$ -3 times greater than in the non-planted control (Fig. S3 & Table  
374 S3). Since root growth is the main source of new POM in the study, we conclude that the mesh  
375 barriers were effective in keeping away the roots and in eliminating direct root interactions as  
376 needed for testing the study's hypotheses on fungal-based C and N transfers.

377 Ergosterol concentrations in the soil within mesh barrier dividers were comparable to those  
378 in the absence of barriers across all recipient species (Table S4), and both were significantly higher  
379 than those in the non-planted control soil ( $p < 0.001$ ) (Fig. S4). Since ergosterol concentration in  
380 the soil is indicative of fungal biomass, we conclude that fungal networks were successfully  
381 established in our experimental setup during plant growth and that the mesh barriers did not  
382 substantially restrict fungal growth.

383 Soil under Bclover had greater inorganic N ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) contents compared to the soils  
384 under Sgrass and BSforb (Fig. S5). Soil inorganic N contents under Bclover were similar in the  
385 same-neighbor and mixed-neighbor combination (Fig. S5). Moreover, the inorganic N was not  
386 affected by the barrier treatment (Table S5).

387

388  *$^{13}\text{C}$  and  $^{15}\text{N}$  excesses in roots of recipient plants*

389  $^{13}\text{C}$  enrichment of recipient roots at locations close to the source Sgrass was generally  
390 greater than that at locations far from the source ( $p < 0.001$ ), and the presence of the barrier led to

391 an overall lower  $^{13}\text{C}$  enrichment ( $p < 0.01$ ) (Table S6). Specifically, in the same-neighbor  
392 combinations, in the absence of barriers, the roots of the recipient Sgrass plants were  $^{13}\text{C}$  enriched  
393 at both close and far locations ( $p < 0.01$ ) (Fig. 2A). In the presence of the barrier, the Sgrass  
394 recipient's roots were enriched only at close locations ( $p < 0.01$ ) (Fig. 2A). In the same-neighbor  
395 combinations, the  $^{13}\text{C}$  root enrichment of recipient Bclover plants was detected in both close and  
396 far locations, both in the presence and in the absence of the barrier ( $p < 0.05-0.01$ ) (Fig. 2A). On  
397 the contrary, the roots of recipient BSforb plant were not  $^{13}\text{C}$  enriched at either close or far  
398 locations, and either in the presence or absence of the barrier ( $p > 0.05$ ) (Fig. 2A).

399 In the mixed-neighbor combination, the  $^{13}\text{C}$  excess in the roots of Bclover and BSforb  
400 recipients (Fig. 2B) was overall similar to those of their respective same-neighbor combinations  
401 (Fig. 2A). For example, just as in the same-neighbor combinations, the  $^{13}\text{C}$  excess in Bclover  
402 recipients was greater than that of the BSforb recipients. The sizes of the barrier effects were also  
403 comparable. Yet, notably, roots of BSforb recipients in the mixed-neighbor combination were  $^{13}\text{C}$   
404 enriched at the close location (Fig. 2B), while BSforb roots were not  $^{13}\text{C}$  enriched in the same-  
405 neighbor combination (Fig. 2A). In addition, at the far location in the mixed-neighbor combination,  
406 the  $^{13}\text{C}$  excess in the roots of Bclover recipients was greater than that in far locations of any other  
407 recipient species.

408 Similar to  $^{13}\text{C}$  results, the  $^{15}\text{N}$  enrichment of recipient roots was higher at locations close  
409 to the source Sgrass than at the far locations ( $p < 0.01$ ) (Table S6). However, unlike  $^{13}\text{C}$ , the barrier  
410 effect on  $^{15}\text{N}$  enrichment was not statistically significant ( $p > 0.05$ ). Overall, the  $^{15}\text{N}$  enrichment of  
411 recipient roots was more pronounced than the  $^{13}\text{C}$  enrichment (Fig. 2). Specifically, for both Sgrass  
412 and Bclover recipients in the same-neighbor combinations, large  $^{15}\text{N}$  enrichment of the roots was  
413 common at both close and far locations not only in the absence but also in the presence of the

414 barrier ( $p < 0.05-0.01$ ) (Fig. 2C). Roots of BSforb recipients were also  $^{15}\text{N}$  enriched at the close  
415 locations without barriers in the same-neighbor combination ( $p < 0.05$ ) (Fig. 2C), even though their  
416  $^{13}\text{C}$  enrichment was negligible ( $p > 0.05$ ) (Fig. 2A). In the mixed-neighbor combination, BSforb  
417 roots were  $^{15}\text{N}$  enriched even at the far locations ( $p < 0.05$ ) (Fig. 2D), while it was  $^{15}\text{N}$  enriched  
418 only at the close locations in the same-neighbor combination (Fig. 2C).

419 At close locations,  $^{13}\text{C}$  and  $^{15}\text{N}$  excesses in the roots of the recipient plants were positively  
420 correlated in Sgrass (in the absence of the barriers) and in Bclover (both in barrier and non-barrier  
421 treatments) neighbors. No correlations between  $^{13}\text{C}$  and  $^{15}\text{N}$  in roots of the recipients were observed  
422 at far locations.

423 In contrast to recipient roots,  $^{13}\text{C}$  enrichment in recipient plants' shoots was absent in all  
424 plant and barrier treatments ( $p > 0.05$ ) (Figs. S6A & S6B), while shoots of most recipient plants  
425 were strongly enriched with  $^{15}\text{N}$  in most plant and barrier treatments ( $p < 0.05-0.01$ ) (Figs. S6C &  
426 S6D).

427

#### 428 *$^{13}\text{C}$ and $^{15}\text{N}$ excesses in rhizosphere soil of recipient plants*

429 The mesh barriers led to lower  $^{13}\text{C}$  enrichment in rhizosphere soil of recipient plants ( $p$   
430  $< 0.001$ ) (Table S7), and the  $^{13}\text{C}$  enrichment at locations close to the source Sgrass was generally  
431 greater than that at locations far from the source ( $p < 0.001$ ) (Table S7). Specifically, when Sgrass  
432 and Bclover were the recipient species in the same-neighbor combinations, in the absence of  
433 barriers, rhizosphere soil was  $^{13}\text{C}$  enriched only at close locations ( $p < 0.01$ ) (Fig. 4A). In the  
434 mixed-neighbor combination, the results of  $^{13}\text{C}$  excess in the rhizosphere soil of Bclover and  
435 BSforb recipients (Fig. 4B) were very similar to those of their respective treatments in same-

436 neighbor combinations (Fig. 4A), having greater  $^{13}\text{C}$  excess in Bclover recipients than that of the  
437 BSforb recipients at the close locations.

438 The  $^{15}\text{N}$  enrichment of the rhizosphere soil at the close locations was also higher than at  
439 the far ones ( $p < 0.01$ ) (Table S7). Similar to the  $^{13}\text{C}$  enrichment in the rhizosphere soil,  $^{15}\text{N}$   
440 enrichment was detected at the close locations without barriers in same-neighbor combinations ( $p$   
441  $< 0.05$ ) (Fig. 4C). The presence of the barrier generally led to lower soil  $^{15}\text{N}$  enrichment ( $p < 0.01$ )  
442 (Table S7), with the exception of BSforb recipients ( $p > 0.05$ ) (Fig. 4C). Notably, in the mixed-  
443 neighbor combination, the  $^{15}\text{N}$  enrichment of Bclover rhizosphere soil was detected in the presence  
444 of barriers ( $p < 0.05$ ) (Fig. 4D), while it was absent in the same-neighbor combinations (Fig. 4C).

445 In the absence of barriers, at locations close to the source Sgrass,  $^{13}\text{C}$  excesses in  
446 rhizosphere soil were positively associated with  $^{13}\text{C}$  excesses in roots in all three recipient species,  
447 when there were no barriers between the source and recipients ( $p < 0.05-0.001$ ) (Fig. 5A). When  
448 barriers were present, significant positive association between the rhizosphere soil and root  $^{13}\text{C}$   
449 excesses was observed only in Sgrass recipient ( $p < 0.001$ ). The  $^{15}\text{N}$  excess in rhizosphere soil at  
450 the close location were also positively associated with the  $^{15}\text{N}$  excess in roots in both barrier  
451 conditions in all three recipient species ( $p < 0.05-0.01$ ), except for that in Sgrass recipients in the  
452 presence of barriers ( $p > 0.05$ ) (Fig. 5B). Within the rhizosphere soil of recipient plants, significant  
453 correlations between  $^{13}\text{C}$  and  $^{15}\text{N}$  excesses were observed only in Bclover recipient both in the  
454 presence and the absence of barriers ( $p < 0.01$ ) (Fig. 3B). No correlations were observed at far  
455 locations.

456

457

458 *Pores in the soil between source Sgrass and recipient plants*

459 In the absence of mesh barriers, soil within the dividers between the source and recipient  
460 compartments tended to have greater fractions of fine pores (8-30  $\mu\text{m}$   $\emptyset$ ) than that in the presence  
461 of barriers (Fig. 6). Specifically, Sgrass and Bclover recipients of same-neighbor combinations  
462 had, respectively, 70% and 50% greater fractions of fine pores in the absence than in the presence  
463 of barriers ( $p < 0.01$ ), and approximately 75% greater fractions of fine pores than the soil of non-  
464 planted controls ( $p < 0.001$ ) (Fig. 6A & 6B). In contrast, fractions of medium (30-150  $\mu\text{m}$   $\emptyset$ ) and  
465 coarse pores ( $> 150 \mu\text{m}$   $\emptyset$ ) in soils with Sgrass and Bclover recipients were approximately 20% ( $p$   
466  $< 0.01$ ) and 60% ( $p < 0.001$ ) smaller than those in the non-planted control soil, respectively. Notably,  
467 unlike Sgrass recipient, for Bclover recipient pore differences from non-planted control soil  
468 occurred even in the presence of barriers (Fig. 6B). For BSforb recipient fine and coarse pore  
469 fractions were, respectively, greater ( $p < 0.001$ ) and smaller ( $p < 0.01$ ) than those of the non-planted  
470 control only in the absence of barrier in the mixed-neighbor combination (Fig. 6D).

471 In the same-neighbor combinations fractions of fine pores were greater in the soil of  
472 Bclover and Sgrass recipients compared to those of BSforb recipients. However, in the mixed  
473 combination, soil of BSforb recipients was comparable to the other ones (Fig. 6). Note that soil  
474 porosities (pores  $> 8 \mu\text{m}$   $\emptyset$ ) measured using the images were similar among all plant treatments  
475 and between the two barrier treatments (Fig. S7).

476 In the absence of barriers, POM fractions were positively associated with fractions of fine  
477 pores for all three recipient plant species ( $r^2 = 0.47$  and  $p < 0.01$ ;  $r^2 = 0.38$  and  $p < 0.05$ ;  $r^2 = 0.41$   
478 and  $p < 0.05$  in Sgrass, Bclover, and BSforb, respectively). Alternatively, they were negatively  
479 associated with fractions of coarse pores ( $r^2 = 0.45$  and  $p < 0.001$ ;  $r^2 = 0.34$  and  $p < 0.05$ ;  $r^2 = 0.33$   
480 and  $p < 0.05$  in Sgrass, Bclover, and BSforb, respectively) (Fig. S8).

481

482 **Discussion**

483 Carbon and N from the labeled switchgrass were transferred to neighboring plants via both  
484 fungal-only and root-based belowground pathways. However, the transfer of Sgrass-assimilated C  
485 via fungal-only pathway was markedly lower than the transfer by the roots; and the extent of the  
486 C transfer depended on the identity of the neighboring recipient. In contrast, substantial transfer of  
487 Sgrass-derived N occurred through both fungal-only and the root-based pathways, and the transfer  
488 through the root-based pathway was present regardless of the identity of the recipient plants.  
489 Surprisingly, not only the identity of the neighboring recipient, but also the identity of the other  
490 plant community member affected the transfers. Sgrass-assimilated C and N in the rhizosphere of  
491 the recipients were positively correlated with those in the recipients' roots. Changes in pore size  
492 distributions in the soil between the source and recipient plants co-occurred with detectable C  
493 transfers.

494

495 *Differences in C vs. N transfers*

496 Both C and N were transferred via root-based and fungal-based pathways (Fig. 2). However,  
497 the extent of the fungal-based C transfer through barriers was much less compared to that through  
498 the root-based pathways, whereas N transfers via the two pathways were comparable. It should be  
499 noted that the experimental settings of this study did not allow for isolation of the root-based  
500 transfer only, and the root-based pathway tested here included transfers through both root contact  
501 and fungal networks, making the observed difference in C vs. N transfers even more remarkable.

502 The discrepancy between C and N results can be partially attributed to the differences in  
503 forms and mobility of C and N compounds that mycorrhizal networks obtain from source roots.  
504 Mycorrhizal fungi efficiently transport smaller molecules such as amino acids,  $\text{NH}_4^+$ , and  $\text{NO}_3^-$

505 (Parniske, 2008; Xie et al., 2022). This contrasts with larger C compounds such as glycogen  
506 granules and lipid droplets, synthesized by fungi from sucrose and hexose obtained from the source  
507 plant (Pfeffer et al., 1999; Keymer et al., 2017). Such C-rich molecules can have greater challenges  
508 for transport through hyphal networks compared to smaller compounds due to their large size and  
509 structural complexity (Wang et al., 2018; Salvioli di Fossalunga and Novero, 2019). Thus, the  
510 observed discrepancy between C and N (Fig. 2) suggests that the transfer of the C-rich compounds  
511 may require more direct pathways, i.e., direct involvement of roots.

512         The relatively weaker barrier effects observed in N transfer compared to the pronounced  
513 barrier effects in C transfer (Fig. 2 & Table S6) may also be attributed to the high demand and  
514 rapid uptake of root-released N by soil organisms. Once N is released into the soil, it tends to be  
515 immediately assimilated by them (Jones et al., 2005; Kuzyakov and Xu, 2013), leaving little  
516 available N for direct uptake by the roots of neighboring plants (Luo et al., 2006), leading to higher  
517 relative contribution of the fungal-based N transfer observed here (Figs. 2C & 2D).

518         It is also possible that the  $^{13}\text{C}$ -containing compounds transported from the plant to the  
519 arbuscular mycorrhizal fungi were not fully assimilated into the connected recipient plants but  
520 were instead retained within the fungal mycelium located within the recipients' roots (Pfeffer et  
521 al., 2004). Indeed, the shoots of recipient plants were not  $^{13}\text{C}$  enriched, i.e., did not receive the C  
522 that was transferred from the source Sgrass (Figs. S6A & S6B), suggesting that the transferred C  
523 was not substantially utilized by recipient plants for further growth. This explains the negligible  
524  $^{13}\text{C}$  enrichment in roots at locations far from the source in the presence of barriers (Figs. 2A & 2B).

525         In contrast to C, plant roots utilized the N transferred via mycorrhizal networks for their  
526 biomass production (Govindarajulu et al., 2005; Parniske, 2008), and enriched  $^{15}\text{N}$  in the recipients'  
527 shoots observed here (Figs. S6C & S6D), indeed, indicates that the neighbors used N of Sgrass

528 origin for their growth. Therefore, high  $^{15}\text{N}$  enrichment in recipient roots at far locations (Figs. 2C  
529 & 2D) could stem from the use of N received at close locations to build other roots. Moreover, the  
530 potential reach of mycorrhizal hyphae can extend farther than 13 cm from their symbiotic root  
531 (Muneer et al., 2020; Shen et al., 2020), forming extensive hyphal networks, well distributed  
532 throughout the soil (Miller et al., 1995). Given that legumes are particularly effective at promoting  
533 the development of mycorrhizal hyphae (Burrows and Pflieger, 2002; Liu et al., 2021), the  
534 possibility of direct hyphal connections facilitating fungal-based C transfer from source Sgrass to  
535 recipient Bclover roots at far locations cannot be ruled out (Fig. 3A).

536

#### 537 *Inputs of C and N from source plants into rhizosphere*

538 In the absence of the barrier, a substantial  $^{13}\text{C}$  enrichment was common in rhizosphere soils  
539 of Sgrass and Bclover recipients of same-neighbor combinations and of Bclover recipients of the  
540 mixed-neighbor combination (Figs. 2A & 2B). The higher were  $^{13}\text{C}$  signatures in recipient roots,  
541 the more of the Sgrass-originated C was detected in the rhizosphere (Fig. 5A). This also aligns  
542 with the finding by Kravchenko et al. (2021), demonstrating that C transfer to neighboring plants  
543 could increase inputs of plant-derived organic compounds into the soil. Roots release organic  
544 compounds as rhizodeposits including exudates (Jones et al., 2009), and such root-derived  
545 compounds and decomposed root and fungal biomass substantially contribute to soil organic C  
546 pools (Jones et al., 2004; Badri and Vivanco, 2009). Our results suggest that the presence of  
547 direct/close contacts between neighboring roots is an important driver stimulating the inputs of  
548 plant-derived organic compounds into the rhizosphere.

549 Interestingly, unlike the sizeable  $^{15}\text{N}$  transfers to neighbors' roots both in the presence and  
550 absence of barriers and both at close and far locations (Figs. 2C & 2D), the  $^{15}\text{N}$  enrichment in the

551 rhizosphere soil was detected only in the absence of the barrier and only in close locations (Figs.  
552 4C & 4D). This suggests that some of the source-plant-derived N was released into rhizosphere  
553 and intercepted by the recipient roots (Govindarajulu et al., 2005; Biernath et al., 2008). Moreover,  
554 fungal hyphae take up N as  $\text{NO}_3^-$  and  $\text{NH}_4^+$  (López-Pedrosa et al., 2006) and then transport it as  
555 arginine, the compound known as relatively stable against release (Govindarajulu et al., 2005).  
556 This implies that minimal N was released into the rhizosphere at the far locations, even though  
557 transfers still occurred there.

558         It should be noted that the pulse labeling approach used in this study likely underestimated  
559 the full extent of C and N transfers and their subsequent inputs into the soil, particularly those  
560 arising from longer-term processes such as fungal turnover or persistent root exudation.  
561 Continuous labeling (Pang et al., 2021) over longer time periods would provide a more integrated  
562 assessment of both immediate and persistent dynamics of interplant C and N exchange in these  
563 perennial plants. Moreover, highly controlled greenhouse settings do not reflect the complexity of  
564 environmental conditions experienced by the plants in the field, including variability in soil  
565 moisture and microbial activity (Chung et al., 2019). Future field-based studies are needed to  
566 validate and expand upon these findings in more realistic environments.

567

#### 568 *Influence of recipient species on C and N transfer*

569         The Bclover neighbor experienced greater C transfers taking place at far distances than  
570 BSforb (Figs. 2A & 2B). Legumes are known to enrich soil N (Guan et al., 2014; Mounier et al.,  
571 2014) and promote mycorrhizal hyphal development (Burrows and Pflieger, 2002; Liu et al., 2021).  
572 Indeed, soil  $\text{NO}_3^-$  and  $\text{NH}_4^+$  contents under Bclover recipient plants were greater than those under  
573 BSforb recipients in the mixed-neighbor combination (Figs. S5B & S5D), which likely imply that

574 the higher N availability could stimulate preferential Sgrass root growth and/or hyphal  
575 development toward the locally N-enriched zones.

576 Non-legume plants in a diverse plant community can rapidly deplete the available N in the  
577 soil which can in turn result in higher N<sub>2</sub> fixation by companion legumes (Viera-Vargas et al.,  
578 1995). Such stimulatory interactions between legumes and non-legumes may increase the total N  
579 uptake by plants in the community (Nyfeler et al., 2011). Moreover, legumes synergistically  
580 interact with non-legume species having fibrous root systems (grasses), increasing soil nutrient  
581 transformation and transport as well as soil's capability to support plant biomass production  
582 (Mahieu et al., 2009; Chapagain and Riseman, 2014). Even though the increase in the root biomass  
583 of BSforb in the mixed-neighbor combination – compared with the same-neighbor combination –  
584 was not statistically significant (Figs. S1C & S1D), N transfer still occurred at the far location (Fig.  
585 2D). This result suggests that high plant diversity can facilitate more efficient N transfer, aided by  
586 arbuscular mycorrhizal fungi forming symbiotic relationships with non-leguminous plants.

587 In the absence of barrier, the BSforb roots were <sup>13</sup>C enriched in the mixed-neighbor  
588 combination (Fig. 2B), while it did not happen in the only BSforb neighbors' case (Fig. 2A). Past  
589 findings (Kravchenko et al., 2021; Zheng et al., 2022) demonstrated that identities of the source  
590 and neighbor plants in Sgrass-based plant mixtures impact the magnitude of the interplant C  
591 transfer. Our result points to an impact from not only neighbor identities, but also from neighbor  
592 interactions, where C transfer to one of the neighbors, i.e., BSforb, was influenced by the identity  
593 of the other neighbor and differed depending on whether that was another BSforb or a Bclover  
594 plant. While further experimentation with various plant combinations is needed, this finding  
595 demonstrates the complexity of the drivers influencing C transfer in diverse plant communities  
596 and a potentially non-additive stimulation of such transfers there.

597

598 *Fine pore formation and a potential for C storage*

599         The response of pore size distributions within the rhizobox dividers to plant neighbor and  
600 barrier effects (Fig. 6) mirrored the pattern observed in C transfers (Fig. 2). Specifically, whenever  
601 significant C transfers occurred, changes in pore size distributions, namely, increases in volumes  
602 of fine (8–30  $\mu\text{m}$   $\text{\O}$ ) and decreases in volumes of coarse pores (>150  $\mu\text{m}$   $\text{\O}$ ), were detected. Since  
603 the image-based porosity (i.e., pores >8  $\mu\text{m}$   $\text{\O}$ ) showed no significant differences among plant and  
604 barrier treatments or between planted and non-planted soils (Fig. S7), the observed alterations in  
605 pore size distributions within the planted soil are likely attributable to the reorganization of existing  
606 pore spaces rather than an overall increase in the total pore volume.

607         Root growth can lead to soil compaction in its vicinity with either full or partial collapses  
608 of coarse pores (Aravena et al., 2011; Pandey et al., 2021). Subsequent root's senescence and  
609 shrinking can result in formation of air-gaps (Phalempin et al., 2021; Lee et al., 2024a),  
610 contributing to fine pore formation around root residues (Aravena et al., 2011; Liu et al., 2022).  
611 Positive correlations between POM fractions and fine pores, along with the reduction in coarse  
612 pores, support the notion that roots were the main driver of the observed changes in pore structure  
613 (Fig. S8).

614         Yet, notably, for Bclover neighbors the changes in pore-size distributions occurred even in  
615 the presence of the barrier (Figs. 6B & 6D), again coinciding with high C and N transfers (Fig. 2).  
616 This result may suggest a potential role of fungal networks developed between source Sgrass plants  
617 and their legume neighbor. It is possible that extensive fungal hyphae, particularly those of  
618 mycorrhizal fungi, contributed to changes in soil structure by producing polysaccharides and  
619 glycoproteins (e.g., glomalin) that bind soil particles (Rillig, 2004b; Rillig and Mummey, 2006).

620 Mafla-Endara et al. (2021) were able to visually observe a passage opening by hyphae which  
621 squeezed through the borders of a pore space, creating a new fine pore. While direct evidence was  
622 not collected in this study, these mechanisms may help to explain the observed modifications in  
623 pore size distributions.

624 Fine (8-30  $\mu\text{m}$   $\text{\O}$ ) pores, development of which was related to interplant C transfer (Figs.  
625 2 & 6), play a critical role in long-term soil C storage. They are too small to host large microbial  
626 communities and have limited oxygen availability, which can hinder microbial activity and slow  
627 organic matter decomposition (Bailey et al., 2017; Franklin et al., 2021). Plant combinations that  
628 facilitated greater C transfers might increase soil C accumulation within such newly formed fine  
629 pores close to roots, releasing more C into the rhizosphere (Fig. 5A). Pores of this size range are  
630 readily accessible to fungal hyphae (2-20  $\mu\text{m}$   $\text{\O}$ ) (Smith and Smith, 2011), and hyphae can release  
631 or deposit their root-originated organic compounds into these microenvironments. Even though  
632 this study did not directly measure microbial activity or community composition contributing to  
633 soil C stabilization within fine pores, recent research strongly supports this notion. For example,  
634 enzyme activities assessed through zymography were significantly higher in areas with 30-150  $\mu\text{m}$   
635 pores but diminished in pores  $< 30 \mu\text{m}$  (Kravchenko et al., 2019b), indicating reduced microbial  
636 activity in fine pores. Similarly, new C turnover in large ( $> 60 \mu\text{m}$ ) pores was positively associated  
637 with  $\beta$ -glucosidase activity, reflecting faster decomposition rates in large pores compared to  
638 smaller ones (Kravchenko et al., 2021a). In addition, pore-specific 16S/ITS sequencing studies  
639 reported distinct microbial communities occupying different pore sizes, each with unique life  
640 strategies and functions (Benucci et al., 2023; Li et al., 2024). Ding et al. (2024) combined 16S  
641 rDNA sequencing with  $^{13}\text{C}$ -phospholipid fatty acid analysis and demonstrated that  $> 100 \mu\text{m}$  pores  
642 promoted active microbial communities decomposing  $^{13}\text{C}$ -labeled maize straw, supporting the idea

643 that smaller pores limit microbial access and activity, while larger pores serve as hotspots for  
644 microbial processing. Quigley and Kravchenko (2022) also found positive correlations between  
645 root-derived C and fine pores, which were not accessible to roots, further supporting the notion  
646 that fungi-derived C could enter such pores and be protected from further decomposition.

647

## 648 **Conclusions**

649 The greenhouse experiment with  $^{13}\text{C}$  and  $^{15}\text{N}$  isotope labeling and tracing allowed detecting  
650 belowground C and N transfers among prairie plants. The root-based pathway, which involved  
651 both direct/close root contacts and fungal networks, resulted in much greater C transfer than the  
652 fungal-based pathway, suggesting the importance of direct root contact. However, N was  
653 effectively transferred via fungal network-only pathway as well. Notably, in the most diverse  
654 mixture (three species) the legume species (Bclover) increased the transfer of both C and N not  
655 only to themselves but also to another neighbor (BSforb). Direct/close root contacts promoted the  
656 input of transferred C and N into the rhizosphere soil and stimulated the formation of fine pores in  
657 all plant combinations. Given the importance of fine pores for soil C protection, these findings  
658 suggest that coupled enhanced C and N transfers with stimulation of fine pores – driven by root  
659 interactions and aided by fungal networks – is one of the mechanisms through which legumes  
660 promote C gains in diverse plant communities.

661

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671

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