

# A deeper look at the relationship between root carbon pools and the vertical distribution of the soil carbon pool: response to reviewers

Anonymous Referee #1

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In this study, Dietzel et al. used an agronomic trial to study the linkage between root C input and the vertical distribution of Soil Organic Matter (SOM). Using a soil under corn cultivation for more than a century, they measured the SOM profile of this soil as well as the root material input and quality (C:N ratio) along the soil depth for both prairie and maize vegetation. They found that maize allocates a higher proportion of root input in deep soil layers and that it has a lower C:N ratio compared to prairie plants, which is quite classical. Further, they found that root C:N ratio increases with depth for all the treatments. This result is interesting and quite new from what I know. This suggests that deeper roots are dominated by transport root with highly sclerified tissues and poor absorptive proteins content compared to surface roots. Finally, they conclude that in moving from prairie to maize, a large, structural-tissue dominated root C pool with slow turnover, concentrated at shallow depths was replaced by a small, non-structural tissue dominated root C pool with fast turnover evenly distributed in the soil profile, suggesting that maize may allocate more root C input to the soil than prairies at deeper depths. This constitutes the strong portion of this manuscript.

Thank you, we too were excited to quantify differences in prairie and maize root allocation and find root C:N ratios increase with depth.

Based on the conceptual framework and the empirical results of the study of Cotrufo et al. (2015) about the formation of SOM, they also argue that their pattern of increasing root C:N ratio with depth could explain why an disproportionately large stock of SOM relative to root C inputs is found in deep soil. First, I found it quite tricky to conclude about the driver of such a global scale pattern from data of a case study like this.

Yes, since this is only one study, we avoid making any strong conclusions, but do suggest that root C:N ratio plays a role in development of the soil profile. Finding increases in root C:N ratios with depth is significant on its own, but we feel it is very important to put this finding in the context of larger scientific questions.

Beyond that, I am not convinced by this interpretation and I found their argumentation about this statement quite weak for several reasons related to logical contradiction and some misunderstanding about the work of Cotrufo as discussed into more detail below.

We feel we can do a better job communicating our proposed mechanism and we address these details below. We found your comments to be extremely helpful towards improving this manuscript and enjoyed getting a new perspective on many of the aspects we have wrestled with during writing. It seems we all have the same understanding of Cotrufo et al.'s conceptual models, but are used to thinking about these models under different environmental circumstances. One major assumption on which we do not completely agree is the likeliness of microbial by-products to be transported deeper in the soil profile. We hope the discussion below and additional references help to clarify the manuscript.

If I consider these two paths of SOM formation and your results together, I would consider that shallow root of high litter quality would supply high input of DOC that can be efficiently processed by soil microorganisms (high Carbon Use Efficiency [CUE]) and supply larger quantity of microbial by-products that can be then stabilized in soil microaggregates by mineral-binding, thus leading to higher C sequestration. In contrast, the deep root of poor quality (higher proportion of POM) will be least efficiently processed, thus leading to higher C lost by mineralization relative to SOM formation and ultimately lower C sequestration. This is thus not consistent with the pattern of the disproportionately large stock of SOM relative to root C inputs in deep soil.

Right! We find this inconsistency in patterns very interesting and a major motivation for the manuscript. Although the proposed relationship we describe between root C:N ratio and soil C profile development is not

46 immediately intuitive, the combination of MEMS and dissolved organic carbon (C) transport leads to a very  
47 possible mechanism behind a disproportionally large stock of SOM relative to root C inputs.

48 Further, Cotrufo et al. (2015) studied SOM formation over short-term scale whereas deep soil C is often  
49 hundreds to thousands year-old and highly microbially processed.

50 Yes, Cotrufo et al. have focused on short-term scales and their conceptual model is still hypothetical,  
51 however, what happens in the short-term is directly connected to what happens in the long term. The fact  
52 that deep soil C is highly microbially processed does not indicate where where the C originated.

53 Fontaine et al. (2007) found that deep soil C mineralization is strongly limited by energetic constraints.  
54 This slow turnover together with the DOC input from surface to deep soil layer documented by Rumpel and  
55 Kögel-Knabner (2010) could more likely explain the disproportionally large stock of SOM relative root C  
56 input is found in deep soil.

57 Both of these factors, and many more mentioned in the manuscript, contribute to the disproportionally large  
58 stock of SOM relative to root C input found in deep soil, but do not fully explain it and do not incorporate  
59 the role of root C:N ratio. The transport of DOC is especially important in our proposed mechanism and we  
60 spend some time on how roots at shallow depths vs. deeper depths contribute to this DOC.

61 I also pointed several methodological issues detailed below. Finally, I felt that you did not so much discussed  
62 how the root system of your different plant communities (maize vs. prairie) could explain the vertical C  
63 profile of your studied soil though this constitutes the strong part of your study to the linkage between root  
64 C input and the vertical distribution of SOM.

65 Thank you for the methodological questions below. We would have liked to spend more discussion on the  
66 root systems of maize vs. prairie, but felt that without measurements of the original soil C profile, discussion  
67 specific to change created by annual cropping systems would be challenged. However, we can strengthen this  
68 component in response to your comment.

69 Taken together, I think this manuscript will need important revisions to be acceptable for publication,  
70 especially by avoiding tricky extrapolation and misinterpretation and by refocused on the conclusion you can  
71 reasonably draw from your results. Clarify your scientific questions/hypotheses could also help to achieve  
72 this end.

73 Detailed discussion of the manuscript

74 P.1-L. 15. 'in moving from prairie to maize' If ?? I well understood your design, you studied soil root  
75 allocation on restored prairies that have maize cropping historical of >100 years. Therefore, would it not be  
76 more correct to talk about moving from maize to prairie.

77 We are referencing the historical shift from prairie to maize. I will change the wording for clarification.

78 P.1-L. 15. 'contribute' to what? To soil C stock? Please clarify. Alternatively, we could also talk about 'C  
79 allocation'.

80 Yes, soil C stock. We will clarify

81 P.1-L. 21. Please clarify what you mean by 'aboveground process'. Is really soil disturbance (tillage?) an  
82 aboveground process?

83 Will change 'aboveground processes' to 'soil management'.

84 P.1-L. 26-27. Is this definition really necessary here? I think it will be better placed in the Material and  
85 Methods section.

86 We included it here as we go on to use the definition in the introduction.

87 P.2-L.5. Please insert the Weaver citation

88 Thanks for catching this, we will insert the citation.

89 P.2-L.17. Why did you used 'Carbon:N' though you used 'C:N' just before.

90 We did not want to start a sentence with an abbreviation.

91 P.2-L.19-21. I do not clearly see how your experimental design give you a ‘unique perspective on characteristics  
92 of root inputs’ Please clarify.

93 We expect many of the characteristics reported here to be less detectable in well-established prairies systems,  
94 but you are right that prairie reconstruction in not entirely unique. We will replace "unique perspective" to  
95 "new perspective".

96 It would also be useful to indicate here the number of year since prairie restoration at the end of the study (5  
97 years not?).

98 Yes, this will be added here.

99 P.2-L.26-27. I did not understand the point of your second scientific question before to read the last extion of  
100 your discussion. Please be not explicit and precise on your purpose.

101 Thanks for this comment. You illustrate that we need to change some aspects of the introduction to make it  
102 understandable for an international audience. For example, in this instance we will simply say "perennial  
103 prairie" instead of "historical" and "annual cropping systems" instead of "current systems".

104 P.3-L.1-2. What about soil N concentration? This could be importabnt

105 We do have total soi N data and will include it here.

106 P.3-L.1-2. How many replicates (blocks)? 4? This information is crucial!

107 I’m sorry we did not include this, it will be added.

108 P.5-L.4-8. You used linear mixed models. Please state what factors are formulated as fixed or random effects  
109 in your models.

110 This information will be added.

111 P.5-L.15-23. Logistic model is used fit binary response variable. Therefore, I do not see the rationale to use  
112 Logistic model to fit root mass, which is a continuous variable. . .

113 We will add more details here. Logistic regression is used to fit binary response variables. We used a logistic  
114 function to fit the data and then statistically compared the parameters of each fit of the function as described  
115 in the book "Mixed Effect Models in S and S-plus", Pinheiro and Bates, 2000. We will add this citation.

116 P.5-L.19. It is not so clear to me what you mean by “root mass accumulation”. It is the difference in root  
117 mass between two sampling dates? Or is it cumulative root growth? But you did not measure it between all  
118 sampling dates, right? Please clarify. By the way, it not so clear what was the initial root mass stock and  
119 distribution prior to experimental set-up.

120 "Root mass accumulation" refers to root mass gained. We did not measure it between all sampling dates,  
121 rather we used the model we fit to predict values during times we did not make measurements.

122 P.5-L.29-30. We calculated root turnover constant as  $k = \text{root loss} / \text{root stock}$ . This computation is quite  
123 uncommon. Hence, Gill and Jackson (2000) calculated root turnover constant as  $k = \text{root gain} / \text{root stock}$ .  
124 In addition to be more standard method, I also found it clearer as root gains are directly obtained with the  
125 ingrowth core method while your root loss computation use root mass accumulation, which was not very well  
126 defined.

127 We can and will easily replace "root loss" with "root gain" in our equation.

128 P.7-L.9. Throughout the manuscript, we heavily use the ‘pool’. Though I found this term appropriate for  
129 distingue different component of the global soil C stock, I found the term ‘stock’ more suitable when talking  
130 about quantitative estimate.

131 We will change the text so that ‘pool’ is used only when discussing specific components of the global soil C  
132 stock.

133 P.6-L.6. There is no reference to Table 1 in the text.

134 Our apologies, we will correct this.

135 P.11-12. There is no reference to most of your tables and figures in this portion of your result section. . .  
136 You really need to clearly use reference to it for justify what you state in the text. In its current state, I do  
137 feel really difficult to follow your text.

138 We will add more references to make the text easier to follow.

139 P.10-Table 3. Is this really useful ? Figure 4 already provide this information. This table should be place in  
140 appendix. By the way, I found that there is quite too much table and figure in the article.

141 We will be happy to move Table 3 to the appendix and consider removing or combining some of the figures.

142 P.11-L.15. What you mean by input? Is it your root mass accumulation? Please clarify.

143 Root mass accumulation is root mass input - root mass loss. Input is how much root mass went into the soil.  
144 We will clarify this in the text.

145 P.12-L.6. What about soil N concentration and soil C:N ratio across soil depth and treatment? Isn't this  
146 information important is understand the root C:N profiles?

147 Yes, this information is important and we can include total (organic + inorganic) N values for this soil. While  
148 root C:N ratio increases with depth, soil C:N ratio decreases with depth, and it may be useful to discuss this  
149 relationship.

150 P.13-L7-8. 'a physical-transfer pathway whereby plant tissue is processed by soil microbes to its fullest extent,  
151 and then remains in the soil functionally inert'. Really? Cotrufo et al. (2015) actually talk about physical  
152 transfer of Particulate Organic Matter (POM) from litter to soil. POM is not functionally inert!

153 We will change this to better reflect Cotrufo's original language and refer instead to the "inherent chemical  
154 recalcitrance" of organic matter resulting from the physical-transfer pathway.

155 P.13-L.11-19. 'root decomposition in our study would have resulted in a gradient of microbially-derived  
156 to physically-derived organic matter from the top of the soil profile downward' Then this is not consistent  
157 with evidence that the contribution of microbial- and not root-derived C increases with depth (Rumpel and  
158 Kogel-Knabner, 2011) in contrast with what you stated L.15-16.

159 The sentence following this one in the manuscript is very important. The microbially-derived organic  
160 matter would be mobile and transported to deeper depths, contributing to the relatively immobile pool of  
161 physically-derived organic matter. This is very consistent with Rumpel and Kogel-Knaber (2011), as we  
162 eventually conclude.

163 I assume that DOC derived from soil surface can be mobile and move down the profile but a large portion can  
164 be stabilized in the surface and at least the SOM derived from deep root with high C:N ratio should be less  
165 microbial-derived given what you state. This point should be clarify. Moreover, the notion physically-derived  
166 SOM does not make sense, see my previous comment.

167 Yes, DOC can be stabilized in the soil surface, but the proportion of C stabilized depends on soil type and level  
168 of C saturation. We will add a reference to Castellano (2015) to support this idea. These prairie-formed soils  
169 do not have C concentrations as high as historical levels, but total C concentrations are still at 2.8 percent,  
170 indicating a reduced capacity for additional C stabilization. In this environment, microbial by-products are  
171 likely to be part of the soil solution and easily transported to deeper depths with greater capacity for C  
172 stabilization. We will make this clearer in the manuscript.

173 P.13-L.12-14. 'Soil organic matter at the soil surface would be vulnerable to transport to greater depth  
174 as dissolved organic C whereas physically-transferred soil organic matter at depth would be relatively  
175 immobile'. If you read carefully Cotrufo (2015), she stated that DOC derived from litter is preceded by soil  
176 microorganisms and the microbial by-products are then stabilized in soil microagregates by mineral-binding.  
177 This mineral-stabilized SOM is thus actually less mobile than POM, in contrast with what you stated.

178 Microbial by-products are very mobile until they are stabilized. When and where they are stabilized depends  
179 on the soil conditions. In the mechanism we propose, microbial by-products reach the deeper profile and are  
180 stabilized there. This is consistent with findings that proportion of microbial-derived SOM increases with  
181 depth.

182 P.13-L.16-19. Exsudates are highly labile compounds that are very quickly preceded by soil microorganisms.  
183 Once metabolized, they are much less mobile. Therefore, they probably represent a minor fraction DOC  
184 moving down the profile and that could form deep SOC.

185 Thank you, we should provide clarification that exudates quickly move into microbial pools. However, the fate  
186 of the C after that again depends on the environmental capacity for microbial by-products to be stabilized.

187 P.13-L.27-29 'By the sixth year of reconstructed prairie establishment, root C pool equilibrium was reached  
188 and prairies began making substantial annual contributions to the soil organic matter pool above 30 cm,  
189 although the fraction of organic matter that remained in the soil is unknown' You have information on root  
190 litter decomposition and soil organic matter turnover, so cannot state anything about SOM formation or  
191 stock. All you can see this you likely have higher root litter input that could eventually increase SOM stock.

192 You are absolutely right. This will be fixed by changing 'contributions' to 'inputs'.

193 P.13-L.35-37. Probably, but this is quite speculative. . .

194 It is indeed speculative, but the most reasonable answer given the evidence available.

195 P.14-L.10. 'contributed more C' This is unclear.

196 Will change to 'had greater C inputs'.

197 References Cotrufo, M. F., Soong, J. L., Horton, A. J., Campbell, E. E., Haddix, M. L., Wall, D. H. &  
198 Parton, W. J. (2015) Formation of soil organic matter via biochemical and physical pathways of litter mass  
199 loss. *Nature Geosci*, 8, 776-779.

200 Fontaine, S., Barot, S., Barre, P., Bdioui, N., Mary, B. & Rumpel, C. (2007) Stability of organic carbon in  
201 deep soil layers controlled by fresh carbon supply. *Nature*, 450, 277-U10.

202 Gill, R. A. & Jackson, R. B. (2000) Global patterns of root turnover for terrestrial ecosystems. *New*  
203 *Phytologist*, 147, 13-31.

204 Rumpel, C. & Kögel-Knabner, I. (2010) Deep soil organic matter a key but poorly understood component of  
205 terrestrial C cycle. *Plant and Soil*, 338, 143-158.

206 Castellano, M. J., Mueller, K. E., Olk, D. C., Sawyer, J. E. and Six, J.: Integrating plant litter quality, soil  
207 organic matter stabilization, and the carbon saturation concept, *Global Change Biology*, 21(9), 3200-3209,  
208 doi:10.1111/gcb.12982, 2015.

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210 Anonymous Referee #2

211 Received and published: 17 April 2017

212 Dietzel et al. report on a root study conducted at a field experiment where continuous corn is compared to  
213 reconstructed fertilized and unfertilized prairie stands. They have measured: 1) root profiles to depth of 1  
214 m at the end of the growth season for six consecutive years, 2) root production (by regrowth cores) for 2  
215 growing seasons to 30 cm depth, 3) root and soil C and N concentrations to 1 m depth. Extracting root for  
216 multiple growing seasons, multiple soils layers and multiple replicated treatments is by no means easy, and the  
217 soil science community can certainly benefit from such precious data. The authors report interesting findings:  
218 1) the C/N ratio of root material increases with depth, which has potential implications for soil C storage, 2)  
219 the maize root profile is more uniform with depth than that of prairie species (confirmative), 3) fertilization  
220 of the reconstructed prairie greatly decreases root biomass.

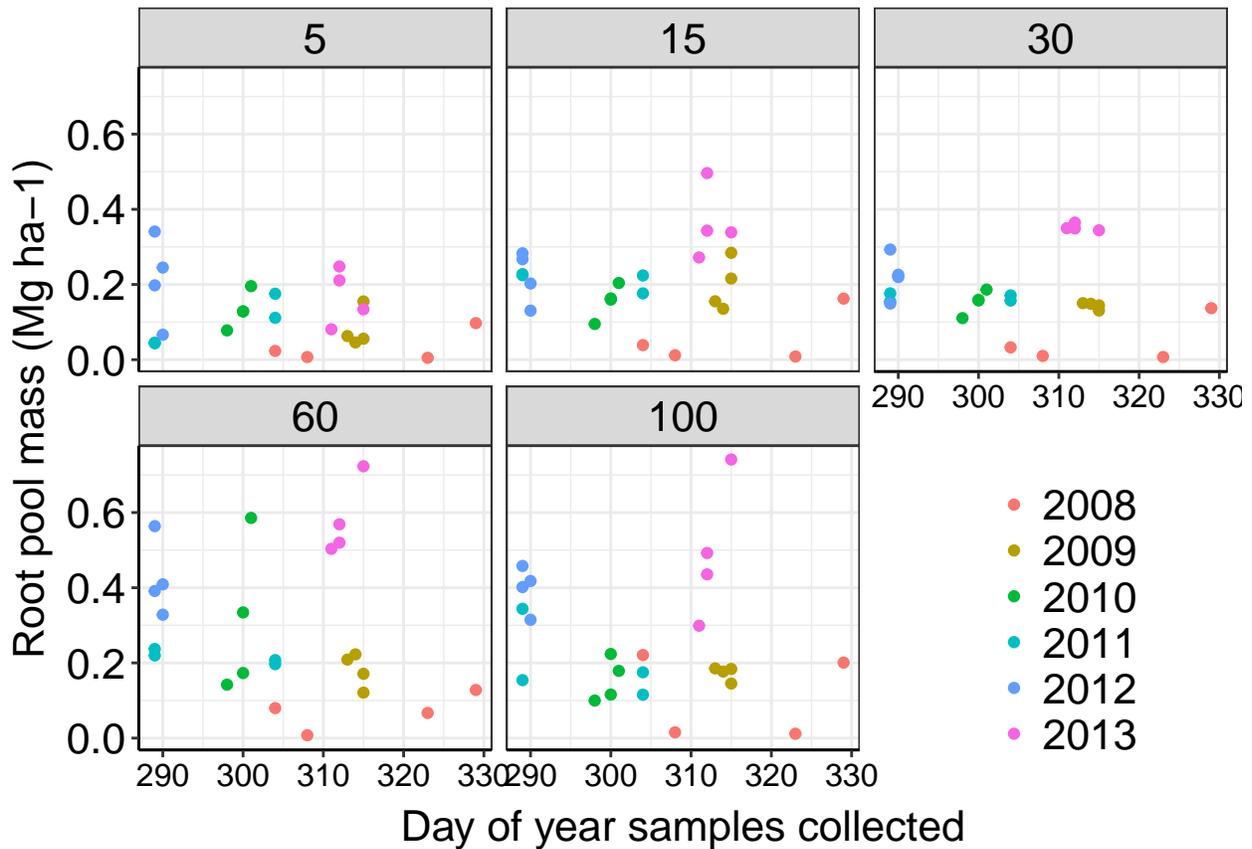
221 However, I have some significant concerns with the study:

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1) The continuous accumulation of maize roots throughout the 6-year period is quite troubling (Fig A3). The authors provide one reference (Dupont 2014) stating that intact prairie root (not maize) can be found in soil several years after cultivation. However, they ignore the substantial literature on maize roots that clearly indicates that maize roots decompose rapidly in soils, starting with the classical study of Mengel and Barber in 1974 (Agron. J. 66: 341-344) and several studies that have followed. Actually, Mengel and Barber (1974) state that root length and fresh weight decrease rapidly after maize has reached the reproductive stage. Here, Dietzel et al. themselves state in the abstract about maize roots that they are “non-structural-tissue dominated root C pool with fast turnover”. They also indicate that the site was apparently under maize soybean rotation prior to starting the experiment, so why was there no accumulated maize root biomass at the start of the experiment (if the root accumulation theory is correct)? Unfortunately, in the present study the roots were not sampled at the same time each year (from early October to early November), and the accumulation of maize roots the last two years also corresponds to the 2 earliest sampling. A possible explanation is that the roots actually decomposed quickly in the field and that by sampling a month earlier by the end of the six-year period a greater number of non-decomposed roots were retrieved. Effects of inter-annual climatic variability on root growth is another potentially contributing effect. There are three implications from this: 1) apparent maize-root accumulation in the field over 6 years is probably an artefact, 2) the pool and rate modelling of Fig 3 and 4 is not justified (it did not bring much to the paper anyway), 3) the paper should have included a much more throughout review of the literature about maize-root dynamics in field soils.

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Thank you very much for bringing this to our attention. The possibility of such as artifact led us to additional analysis of our data. During this analysis, we found that the dates included in the methods section were not correct. We are very sorry for this mistake. Please find below a figure that illustrates that time of sampling was most likely not a contributing factor in the accumulation of root mass.



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You bring up some other useful points here, thank you. We can certainly include more papers on the decomposition of maize roots. Our values for maize biomass taken after maize harvest are typically 10

248 percent of maize root values taken at maturity. This is in line with studies that have found that maize  
249 roots decompose rapidly. However, decomposition rate is an exponential function, with rapid decomposition  
250 occurring early and a slower rate of decomposition occurring later. Several months after maize maturity we  
251 are most likely sampling during a period of slower root decomposition.

252 We also questioned the lack of accumulated root mass from years previous to our experiment. We were  
253 careful to collect maize root samples 20 cm from our maize row and to plant our rows within 2-3 cm of the  
254 previous year's row. We assumed that the cropping legacy from years past would have left a more homogenous  
255 distribution of roots than the one we implemented.

256 2) The maize-root C profile is presented 3 times in the paper: 1) Fig 1 b, 2) Fig 2, and  
257 3) Fig. A3. The 3 figures are in the same units (Mg ha-1), but I could not reconcile the data between them.  
258 The 2013 data of fig. 1 b (with largest root accumulation in the top soil) do not seem to correspond to  
259 the 2013 data of figure A3, which seems to show highest maize-root biomass in the deeper soil.

260 You are correct in not being able to reconcile the figures in the paper (1b and 2) with the figures in the  
261 appendix (A3) because the depth increments are not the same. Figure A3 shows the actual data, mass  
262 collected at 0-5, 5-15, 15-30, 30-60, and 60-100 cm depths. For example, the deepest depth increment is 40 cm  
263 long, resulting in high values relative to 5-15 cm, only 10 cm long. We used the values taken in these depth  
264 increments to break distribution into 5 cm increments, as described in P6, L 3-4. This gave a more accurate  
265 depiction of the distribution of both roots and organic C through the soil profile, shown in Figs. 1 and 2.

266 In addition, the maize root profile appears more even in Fig.2 than in Fig. 1 b, while it should be exactly the  
267 opposite (e.g. the 5-10 cm should have about half of the 0-5 cm in fig 2, if extrapolated from fig 1). Or are  
268 the two figures exactly the same? But why figure 2 then? The 3 figures should have been reconciled and  
269 presented as one main figure in logical units, and then the results compared to the literature.

270 Fig. 1b and Fig.2 are drawn from the same dataframe. Fig. 1 shows distribution patterns that, as you point  
271 out, are not visible in Fig. 2, especially for maize. We use Fig. 1 to discuss root and soil C distribution in  
272 the soil profile. Figure 2 shows absolute differences in root pool mass among treatments, not visible in Fig. 1.  
273 Fig. 2 is used to point out and discuss these basic differences.

274 3) Data from the root regrowth cores are not clearly presented, but used for a direct extrapolation of a root  
275 turnover rate in the top 30 cm. Summary data (without statistics) are presented in g m-2 in Table 4,  
276 making it difficult to compare to the root pool data presented in Mg ha-1. The maize root productivity  
277 appears low and I am missing a coherent evaluation of the C input in the context of published studies.

278 Sorry these data are not clear, we will improve this in the paper as well as change the units to correspond  
279 with other reported units. The maize root productivity is low because samples were taken 2-3 months after  
280 maize maturity, which we will also highlight.

281 4) The implications for C storage presented in this paper are largely hypothetical and somewhat contra-  
282 dictory. The present paper contains no significant result to link root biomass profiles to soil C profiles.  
283 While it is OK to briefly elaborate about possible implication of a higher root C/N ratio with depth,  
284 this should not be the main part of the discussion, which should instead focus on actual significant  
285 results.

286 The implications for C storage presented in this paper are partly hypothetical, but are complex and novel  
287 enough to warrant extensive discussion. The mystery of the disproportionately large stock of SOM relative to  
288 root C input found in deep soil has been unsolved since first noticed over 100 years ago and root C:N ratio  
289 increase with depth is a new and useful piece of information. We do not have direct evidence to link root  
290 biomass profiles to soil C profiles, but what we do have, combined with what we are able to model, is better  
291 than anything that has been previously published.

292 In addition, I could not reconcile the two ideas presented here about the effect of root C/N ratio on C storage  
293 in soils. On the hand, the authors argue that a lower C/N ratio for maize root favours C storage in soil as  
294 compared to prairie roots (p14, line 11-12). On the other hand, they also argue that an increasing C/N ratio  
295 of roots with depth in the soil profile also favours C storage (e.g. p1, line 10-12). The potential attempt to  
296 reconcile these two contradictory effects of root C/N ratio on soil C was unconvincing (p 14).

297 We will reword P1, line 10-12 - "In all treatments we found that root C:N ratios increased with depth, which  
298 may help explain why an unexpectedly large proportion of soil organic C is found below 20 cm." It is meant to  
299 suggest that increasing C:N ratios with depth play a role in an unexpectedly large proportion of soil organic  
300 C found below 20 cm, not that the increase in C:N ratio directly contributes C storage. The relationship  
301 between C:N ratios and C storage is not necessarily intuitive, as we later describe in the text.

302 In conclusion, Dietzel et al. have collected an impressive data set on maize and prairie roots following  
303 maize-soybean rotation. The dataset appears to suffer from some artefacts, but root studies are difficult and  
304 shortcomings could have been acknowledged.

305 Thank you.

306 The data themselves are neither clearly presented nor sufficiently discussed in light of the literature. A main  
307 finding is largely ignored, i.e. the dramatic reduction of root biomass by fertilization in prairie systems.

308 Indeed, apart from the figures, we did include only three sentences on this finding. The effect of N fertilization  
309 on perennial root biomass is well-known (Troughton 1960, Thornley 1972, Gregory 2007) and we did not  
310 consider this plant response to be particularly important to a soils audience.

311 By contrast, the authors focus on an uncertain modelling and non-verifiable considerations about the effect of  
312 root C/N ratio on soil C. A focus on significant results and discussion of these results in light of the literature  
313 would have better served this study

314 While modelling is always uncertain, the fits of the models we used were very good and we stand behind the  
315 conclusions drawn from this effort. The effect of root C:N ratio on soil C may never be verifiable because it is  
316 a centuries-long process that is difficult to measure, but root C:N ratio plays some role in the development  
317 of the soil C profile. This manuscript is the very first to question this role and work with existing data  
318 to propose a mechanism by which root C:N ratio contributes to soil C profile development. It is our hope  
319 that by focusing our paper on root C:N ratios and current soil C profiles, we are inspiring and supporting  
320 future studies that will examine this important relationship more closely. Merely commenting on this possible  
321 relationship in a manuscript focused on a comparison of maize and prairie roots would not reach the intended  
322 audience or provide direction for related experiments.

323 Gregory, P.: Plants Roots: Growth, Activity and Interaction with Soils, in Plant, Roots and the Soil, pp. 5-7,  
324 Blackwell Publishing Ltd., 2007.

325 Thornley, J. H. M.: A balanced quantitative model for root: shoot ratios in vegetative plants,  
326 *Annals of Botany*, 36(145), 431-441, 1972. Troughton, A.: Further studies on the relationship  
327 between shoot and root systems of grasses, *Journal of the British Grassland Society* 15, 41-47, 1960.

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329 M. W. I. Schmidt michael.schmidt@geo.uzh.ch Received and published: 11 April 2017

330 *A note upfront from the submitting person: This review was prepared by Nadja Huber and Mirjam Mächler,*  
331 *both master students in geography at the University of Zurich. The review was part of an exercise during a*  
332 *second semester master level seminar on "the biogeochemistry of plant-soil systems in a changing world",*  
333 *which I organize. We would like to highlight that the depth of scientific knowledge and technical understanding*  
334 *of these reviewers represents that of master students. We enjoyed discussing the manuscript in the seminar,*  
335 *and hope that our comments will be helpful for the authors.*

336 That is so great that you discussed our paper as a group! What a great exercise and these comments are just  
337 the sort of thing we hoped would happen when submitting a paper to Soil. Thanks a lot for your comments.  
338 Hearing from many people really makes obvious which portions of the paper are difficult to understand and  
339 where the most improvement is needed.

340 Dietzel et al. start with the fundamental statement, that soil organic carbon and root mass are disproportion-  
341 ately distributed in soils, supposing that root mass has a direct influence on soil carbon pool. As a matter  
342 of fact, in a depth below 20cm half of all soil organic C in soils can be found where just a third of all root  
343 mass is. There is no clear answer to the question, why there is such a large difference between the two C  
344 pools. Dietzel et al. mention that temperature, moisture, O<sub>2</sub>, soil texture and soil C values are part of the  
345 explanation of this discrepancy. Still, the C:N ratio as part of it has always been neglected in previous studies.

346 Indeed, decreases in root C:N ratio with depth has been an unknown factor.

347 The paper therefore specifically concentrates on a more detailed look at the properties of C pools. For this  
348 purpose the authors examined soil C and root C pools in three different cropping systems. Continuous maize,  
349 multispecies prairie and N-fertilized multispecies prairie. Research questions asked were the following: “1)  
350 How does the quantity, distribution, and C:N ratio of the root C pool differ with depth and between these  
351 native perennial and non-native annual ecosystems and 2) what do these differences in input tell us about  
352 the historical belowground ecosystem under which these soils developed and the systems and will these soils  
353 continue to change?” To answer these questions the authors conducted a field experiment over six years in  
354 Boone County, IA, USA. With this field experiment the authors were able to show that an increase in root  
355 C:N ratio with depth is a potentially important factor determining the distribution of C in the soil profile.  
356 The authors consider the root pool C:N ratios to be sufficiently important that they result in a greater maize  
357 C contributions to soil organic matter than prairie C below 20 cm.

358 Objective 1 (root quality and quantity with depth) was discussed in detail in the manuscript.

359 During the discussion in the classroom, however, it became clear that objective 2 and the related discussion  
360 confused all of us. We did not understand i) why the “historical belowground ecosystem” is important

361 Reviewer 1 also brought this to our attention. This sentence needs to be reworded to be more explicit for a  
362 broader audience. The 'historical belowground ecosystem' is the prairie systems under which the soils formed.

363 and ii) how objective 2 relates to the presented results. The question if these soils will continue to change is  
364 rhetoric (soil always continue to develop) and very unspecific.

365 Yes, this part of the sentence will also be reworded. We did not mean 'if', but 'how' soils will continue to  
366 change under annual cropping systems compared to reconstructed perennial systems.

367 The corresponding discussion section (4.3) is very short and speculative. Are root “turnover” (with a lifetime  
368 of a few years) and soil organic carbon turnover (decades to centuries) somehow related?

369 They are definitely related. Where does soil organic carbon come from? As roots cease to be roots, they turn  
370 into CO<sub>2</sub> or organic matter, influencing soil organic carbon turnover on the scale of days to centuries, even  
371 thousands of years for soils that have been occupied by roots for thousands of years.

372 Probably they are not. Coincidence is not causation. We wondered if objective 2 and section 4.3 are needed  
373 at all. If you think they are, please elaborate this part of the manuscript.

374 Yes, we will certainly elaborate on this.

375 Detailed comments: We did not fully understand the link between C:N and root depth. Do you mean that  
376 C:N ratios increase with depth depending on species or on individual plants?

377 As we move deeper into the soil, root C:N ratios increase for all plants in this study.

378 P. 3, line 26: what does “sampling by replicate block from 31 October-25 November 2008” mean? Did you  
379 sample repeatedly? Explanation of “replicate block”-approach needed.

380 Yes, this is not clear and we will add more details.

381 P. 4, line 11/12: is there a difference between root measurements and root data?

382 Yes, there is. We should not have used them interchangeably here. Thanks for catching that.

383 P. 4, line 24/25: why different storage? Further explanation desired.

384 We explain that storage was different because soil from the first year was part of an incubation experiment.

385 P. 5, line 28: why the period between April 1st and November 30th to calculate the average root mass  
386 accumulation? Are these official dates? Further explanation needed.

387 These dates are the approximate window for plant growth in our region. We will include that information in  
388 the manuscript.

389 Table p. 10: unclear -> explanation of upper/lower case letters and meaning of those letters is missing; it  
390 could be part of the description of the table

391 This information is in the caption. Perhaps it did not come through with the format you were looking at.

392 Explanation pro glimmix on page 5/14 but not in table description.

393 Thank you, but not sure what you are looking for here.

394 P. 13, line 3: increase in root pool C:N ratio has not been reported previously in the literature: We would  
395 appreciate some information about previous research which focused on a related topic

396 We literally could not find any literature related to root pool C:N ratio.

397 P. 14, line 3/4: the pattern of distribution of what? Do you mean the vertical distribution of roots? What is  
398 place in this context?

399 Pattern of distribution of roots. We will add this to the text.

400 P. 14, line 30-32: For us, this sentence is very long and difficult to understand. No significant changes in soil  
401 C (changes in quantity or stocks?) at any depth but differences in quantity?

402 We will split this sentence up into segments that are easier to understand. No changes in soil C despite  
403 changes in root quantity.

404 “implementation of annual cropping systems”: Do you refer to line 19? Experimental location was a site of  
405 cultivation under annual crops for over 100 years.

406 These soils were dominated by prairies for 10,000 years, so the shift to annual cropping systems 100 years ago  
407 is still relatively recent in terms of soil development. This information is important for us to add to the text.

408 Remarks concerning formal structures (typos, figures etc.):

409 P. 3, line 2: typo: 11 mg kg<sup>-1</sup>

410 Thanks.

411 Figure p. 6: why not making a title with total C, root C maize, etc. instead of letters a,b,c -> would be more  
412 clear and consistent with the following figures

413 Yes, we can change this.

414 Figures p.8 + 9: legend can be improved -> no units -> unclear & colors are not suitable for black/white  
415 printing

416 We will add units to the legend and we used to different linetypes for different treatments in black and white  
417 printing, but see now that is not obvious.

418 P. 13, line 36: typo: this

419 Thanks.

# Minor Revisions List

- [X] Check for hypothetical conclusions that are too strong
- [X] Strengthen empirical links between roots & soil C where possible
- [X] Mention Fontaine (2007) energy restraints
- [X] Discuss different root systems and vertical distribution more
- [X] Clarify scientific questions/hypotheses
- [X] Especially the second question
- [X] Include total soil N data
- [X] Fixed vs. random effects in my stat models
- [X] Change root loss and root gain in the equation
- [X] Reference Table 1
- [X] Move table 3 to appendix
- [X] Correct dates in methods section
- [X] Clearly present root regrowth data
- [X] Check figure/table numbers
- [X] Improve figure linetypes and legends
- [X] Add Weaver, Castellano, Fontain in references
- [X] Check input vs. gain language
- [X] Change table to Mg/ha
- [X] All other details included in response

# A deeper look at the relationship between root carbon pools and the vertical distribution of the soil carbon pool

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**Abstract.** Plant root material makes a substantial contribution to the soil organic carbon (C) pool, but this contribution is disproportionate below 20 cm, where 30% of root mass and 50% of soil organic C is found. Root carbon inputs changed drastically when native perennial plant systems were shifted to cultivated annual plant systems. We used the reconstruction of a native prairie and a continuous maize field to examine both the relationship between root carbon and soil carbon and the fundamental rooting system differences between the vegetation under which the soils developed versus the vegetation under which the soils continue to change. In all treatments we found that root C:N ratios increased with depth, ~~which may help explain and this plays a role in~~ why an unexpectedly large proportion of soil organic C is found below 20 cm. Measured root C:N ratios and turnover times along with modelled root turnover dynamics showed that in ~~moving the historical shift~~ from prairie to maize, a large, structural-tissue dominated root C pool with slow turnover, concentrated at shallow depths was replaced by a small, non-structural-tissue dominated root C pool with fast turnover evenly distributed in the soil profile. These differences in rooting systems suggest that while prairie roots contribute more C to the soil than maize at shallow depths, maize may contribute more C to ~~the soil C stocks~~ than prairies at deeper depths.

## 1 Introduction

Prairie-formed Mollisols support some of the world's most productive agriculture, but declines in levels of soil organic matter threaten the reliability of this production. Soil organic matter losses coincide with a shift from perennial plant systems to annual cropping systems that introduced frequent tillage, subsurface drainage, and differences in organic matter inputs, including considerably different rooting systems (Davidson and Ackerman, 1993; Huggins et al. 1998; Guo and Gifford, 2002). The effects of changes in ~~aboveground processes~~ ~~soil management~~ such as increased soil disturbance and aeration, addition of fertilizers, and changes in residue amount and quality have often been cited as primary factors in the changes of soil organic matter from native levels (Buyanovsky et al. 1987, Huggins et al. 1998, David et al. 2009, Gregory et al. 2016). The role played by changes in rooting systems, on the other hand, is difficult to study and has received less attention.

In this paper, we distinguish between a root C pool defined as C found in any material that can still be visually identified as a root and a soil organic C pool defined as the rest of the soil organic C. Root growth allows the placement of plant tissue directly into the soil, creating a root C pool as deep as the rooting system occupies. Some studies suggest that root C pool size and soil organic C pool size have a direct relationship and that most soil organic matter is derived from roots (Balesdent and Balabane 1996, Rasse et al. 2005, Kong and Six 2010). This would mean that a change in root inputs, such as that engendered by switching from annual to perennial systems,

would have a direct impact on soil organic matter even deep into the soil profile. However, few direct comparisons of annual and perennial rooting systems have been made and our understanding of soil C dynamics decreases as soil depth increases.

On average, half of the world's soil C is found below 20 cm (Rumpel and Kogel-Knabner, 2011). However, only 30% of the world's roots are found below 20 cm (Jobbagy and Jackson, 2000). In the central US, this phenomenon was observed as early as 1935 when Weaver found 41-74% of the total soil organic matter, but only 23-29% of the total root mass in a tallgrass prairie were below 20 cm. Similarly, Gill and colleagues (1999) found 77% of total soil organic matter, but only 43% of total root mass below 15 cm in a shortgrass steppe. Although this disproportionate relationship between root and soil C distribution has been known for some time, no widely accepted explanation exists to explain the magnitude of difference between the amount of C in the root pool and the amount of C in the soil pool (Gill et al. 1999, Rumpel and Kogel-Knabner 2011).

Many factors interact to determine how much C is transferred between pools and how much C remains in a particular pool. Soil temperature, moisture, and O<sub>2</sub> availability (Gill and Burke, 2002), and energy availability (Fontaine 2007) are the most important environmental variables controlling the rate of decomposition (Gill and Burke, 2002) and soil texture and existing soil C levels determine the length of time C remains in the soil (Six et al. 2002; Rasse et al. 2005). The C:N ratio of the organic matter being decomposed also plays a key role in both the rate of decomposition and the fate of the decomposed organic matter, with higher C:N ratios leading to slower decomposition (Silver and Miya 2001) and fewer microbial by-products (Cotrufo 2015). Temperature, moisture, O<sub>2</sub>, soil texture, and soil C levels all vary with soil depth and contribute to partial explanations of the size discrepancy between root and soil C pools. However, previous studies that measured roots and/or organic matter with depth have neglected to report the change of root C:N ratio with depth (Tufekcioglu et al. 2003, Beniston et al. 2014). Carbon:N ratio differences between maize and prairie root C pools are also unknown. A more-detailed look at properties of root C pools is needed.

We examined the belowground reconstruction over six years of native vegetation on a Mollisol after >100 years of annual cropping to gain a unique perspective on characteristics of root inputs that would not necessarily be detected in established prairie systems, but that contribute to dynamics of the belowground ecosystem. We examined differences between maize and reconstructed prairie root pools to a depth of one meter to serve two separate, but related, purposes: 1) inform our understanding of the impacts of shifting millions of hectares from perennial to annual vegetation, and 2) contribute to an explanation of why levels of soil organic C found below 20 cm are greater than expected based on root distribution. In comparing the root C pool of a reconstructed prairie system to the root C pool of a maize cropping system we asked the following questions: 1) how does the quantity, distribution, and C:N ratio of the root C pool differ with depth and between these native perennial and non-native annual ecosystems? 2) what do these differences in inputs tell us about the differing effects of the historical belowground perennial prairie ecosystem under which these soils developed and the annual cropping systems under which these soils continue to change?

## 2 Materials and Methods

### 2.1 Site Conditions and Experimental Design

We conducted the experiment in Boone County, IA, USA on the Iowa State University Agronomy and Agricultural Engineering Research Farm (41°55'N, 93°45'W). Soils at the site were primarily Webster silty clay loam (fine-loamy, mixed, superactive, mesic Typic Endoaquolls) and Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls). The 60-year mean growing season precipitation 11 km from the site was 720 mm. Prior to initiation of the field experiment in 2008, the site was used for maize and

soybean production and was planted with soybean in 2007. Soil sampling to 15 cm in November 2007 indicated mean soil pH was 6.7, mean soil C concentration (via dry combustion analysis) was 30 g kg<sup>-1</sup>, mean extractable phosphorus concentration (via Bray-1 procedure) was 11 mg kg<sup>-1</sup>, and mean extractable potassium (via Mehlich-3 procedure) was 141 mg kg<sup>-1</sup>.

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Experimental plots were 27 m x 61 m, replicated 4 times, and were arranged as a spatially balanced complete block design (van Es et al., 2007). The three cropping systems used for the present study were continuous maize with annual removal of grain and about 50% of the stover (hereafter maize), reconstructed multispecies prairie with annual aboveground biomass removal (hereafter unfertilized prairie), and N-fertilized reconstructed multispecies prairie with annual aboveground biomass removal (hereafter fertilized prairie). All of the treatments were managed without tillage. Conventional farm machinery was used for planting, fertilization, crop protection, and harvest operations. Herbicides were not used in the prairie systems except for a small number of spot treatments for Canada thistle (*Cirsium canadense*) control, and the timing and frequency of herbicide use in the annual cropping systems varied among treatments.

Both prairie treatments were sown on 19 May 2008 with the same custom seed mix obtained from Prairie Moon Nursery (Winona, MN, USA) that contained 31 species, including C3 and C4 grasses and leguminous and non-leguminous forbs. All species were perennial and sourced from within 240 km of the experiment site. The composition of the seed mix by weight was 12% C3 grasses, 56% C4 grasses, 8% legumes, and 24% non-leguminous forbs. A detailed description of the prairie plant community compositions can be found in Jarchow and Liebman (2013). The fertilized prairie treatment received no fertilizer in 2008 (the establishment year), but was fertilized at a rate of 84 kg N ha<sup>-1</sup> year<sup>-1</sup> in all subsequent years. This fertilizer rate was chosen because it was similar to the maximum rate of pre-planting N fertilization recommended for maize (Blackmer et al., 1997) and the expected N removal in the harvested biomass of perennial grasses grown in the area (Heggenstaller et al., 2009).

The maize used was a 104-day relative maturity hybrid (Agrigold 6325 VT3) with transgenes for glyphosate resistance, corn borer (*Ostrinia nubilalis*) resistance, and corn rootworm (*Diabrotica* spp.) protection. Maize was planted following standard practices (Abendroth et al., 2011) in rows spaced 76 cm apart at a seeding rate between 79,500 seeds ha<sup>-1</sup> and 82,500 seeds ha<sup>-1</sup>, depending on the year. Fertilizer rates for corn were based on soil testing results (Blackmer et al. 1997) and varied from 123 to 200 kg N ha<sup>-1</sup>, depending on the year.

## 2.2 Data Collection

### 2.2.1 Soil Collection

Soil cores were taken to 1 m depth in all plots each year using a hydraulic soil probe (Giddings Machine Co., Windsor, CO, USA) after all crop and prairie plots were harvested. Sampling occurred by replicate block from 31 October-25 November 2008, 9-11 November 2009, 25-28 October 2010, 28-31 October 2011, 16-17 October 2012, and 7-11 ~~October~~November 2013.

In 2008, two cores were taken per plot. A 0-30 cm fraction was taken with a 10.2 cm internal diameter soil probe; the 30-100 cm fractions of the cores were taken within the same hole as the 0-30 cm fraction, but with a smaller soil probe. In Blocks 1 and 4, the internal diameter of the core was 6.0 cm. In Blocks 2 and 3, the internal diameter of the core was 5.2 cm. In 2009 and 2010, four cores were taken per plot. The 0-30 cm fraction of the cores was taken with a 10.2 cm internal diameter soil probe; the 30-100 cm fractions

of the cores were taken directly below the 0-30 cm fraction with a 5.1 cm internal diameter probe. In 2011-2013, four cores were taken per plot, and the entire core was taken with a 5.1 cm internal diameter probe.

Soil cores were ultimately divided into three or five depth increments. In 2008, depth increments were 0-30 cm, 30-60 cm, and 60-100 cm. In 2009-2013 depth increments were 0-5 cm, 5-15 cm, 15-30, cm, 30-60 cm, and 60-100 cm. Following division and extraction from the field, soil cores were stored at 5°C until processing was initiated.

Each year, 60-100 g of root-free soil was removed from each depth increment, air-dried, and archived in airtight containers at room temperature. In 2008 and 2013, this soil was ground on a roller-mill and organic C content was determined by catalytic oxidation and CO<sub>2</sub> measurement with NDIR in an Elementar TOC Cube at Brookside Laboratories, Inc. (New Bremen, OH, USA).

### 10 2.2.2 Root Pool Collection

Two sets of root pool measurements were collected: a) end-of-season root [measurements](#) for each year (depth 0 to 100 cm) and b) in-season root measurements during 2010 and 2011 (depth 0-30 cm). The first, described in this section, was used to track changes in the root C pools over all six years and the second, described in section 2.2.3, was used to quantify annual root C contributions in 2010 and 2011.

15 Root extraction from the soil began by washing the soil samples described in 2.2.1 in wire mesh tubes (0.28 mm mesh) for 3 h in an elutriator (Wiles et al., 1996). Roots were removed from the remaining soil by suspending the air-dried sample in water and collecting the roots, which floated, with sieves followed by manually removing any remaining non-root material that was present in the samples. Any plant crowns that were present in the samples were removed and were not considered to be root biomass. Roots were then dried at 70°C for at least 4 h before being weighed. All root samples were ground to 2 mm with a centrifugal mill and concentrations of C and  
20 N were determined by combustion analysis at the Soil and Plant Analysis Laboratory at Iowa State University (Ames, IA, USA).

### 2.2.3 In-season Root Growth

In 2010 and 2011, root biomass was measured with an in-situ growth core approach (Neill 1992) to capture only those roots growing within the measurement year. After fall harvest in 2009 and 2010, eight 10.2-cm-diam soil cores were taken to 30 cm depth in each plot and brought to the laboratory. Holes created in the field were held open during the winter by capped 10.2 cm PVC piping. In the  
25 laboratory, cores were divided into 10 cm sections and virtually all roots were removed by hand. Soil was stored in intact cores at 30°C for the first year of the experiment (intended to be used for an incubation experiment) and 4°C in sealed plastic bags for second year of the experiment. The differences in storage conditions did not have an apparent effect on the outcome of the experiment. At the end of winter while plants were still dormant, the root-free soil was returned to its original location in the field in 10 cm depth increments. Soil was packed to imitate the surrounding bulk density, approximately 1.4 g cm<sup>-3</sup>. Root-free zones were located randomly within prairie  
30 plots and at 20 cm from maize rows. Eight root-free areas were situated within each plot, allowing duplicate sampling at four time points throughout the growing season. Two 4-cm-diameter soil cores were taken within each 10.2-cm-diam root-free area to a 30 cm

depth at each root sampling date. Bulk soil was washed from the roots with water using a soil elutriator (Wiles et al., 1996), roots were dried at 60° C for 24 hours, non-root biomass was removed from the roots by hand, and roots were weighed.

### 2.3 Data Analysis

Root pool mass for the entire meter depth was calculated by summing together the root mass for each depth increment of an entire core and whole core root masses were compared between treatments within each year using contrasts within a linear mixed effect model in the R package nlme (Pinheiro et al. 2013). Treatment differences within depths within years and differences between treatments within depths within years for root biomass were also made with contrasts with linear mixed effects models using proc glimmix in SAS (SAS Institute, 2011) Block x treatment and block x trt x core interactions were treated as random effects.

Because root mass in 2008 was measured at three increments (0-30 cm, 30-60 cm, and 60-100 cm) instead of the five increments used later in the experiment (0-5 cm, 5-15 cm, 15-30 cm, 30-60 cm, and 60-100 cm), 2008 root mass for 0-5 cm, 5-15 cm, and 15-30 cm depths was estimated by multiplying the average 2009-2013 depth distribution proportions by the 2008 0-30 cm increment. No important comparisons were made using this estimated data, but the data were used as a starting point for graphing C:N ratios in different depth increments and fitting curves to root accumulation. Carbon:N ratios were compared between treatments within years within depths and between years within treatments within depths using proc glimmix in SAS.

Root mass measured at the end of each growing season was subset by depth increment and each subset was fit by both a logistic model and a linear model for each plot. Logistic models and linear models were compared against each other using Akaike's Information Criterion (AIC) and the model with the lowest AIC was chosen. The AIC was not greatly different for any of the comparisons, but the logistic model had the best fit for every depth. Model fits and comparisons were done using the R package nlme (Pinheiro et al. 2013) and methods are explained in more detail by Pinheiro and Bates (2000).

The first derivative of the logistic model was used to calculate the daily rate of root mass accumulation, or the net gain of root mass each day. Parameters from the logistic model were used to predict both amount and rate of accumulation for each day for each depth in each plot of the experiment. These predictions were averaged for each treatment and plotted. The annual mean rate was calculated by averaging accumulation rates across each growing season for each depth in each plot. Comparisons of rates between treatments within depths and within years and comparisons of rates between depths within treatments within years were made with proc glimmix in SAS.

Models used to fit root mass over time did not accurately reflect within year biomass fluctuations caused by the start and stop of plant growth and freezing and thawing of soil, rather these curves were used to compare long-term trends in root mass accumulation. Accordingly, the daily rate of root mass accumulation was also inaccurate, but very useful to compare relative accumulation rates among treatments and soil depths. An average daily root mass accumulation rate was calculated by considering the period of possible root growth and decomposition to be between April 1st and November 30<sup>th</sup> (the approximate growing season) of each year.

In-situ root measurements in 2010 and 2011 combined with differences in root pool masses at 30 cm over these years were used to calculate a root turnover constant (k) and root mean residence time (mrt) using the equations  $k = \frac{\text{input} - \text{loss}}{\text{pool}}$  and  $\text{mrt} = 1/k$ . Root pool loss during each year was calculated as the difference between the mass accumulated during that year and the gain measured by

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in-situ growth cores, input values were calculated from the in-situ growth cores and functional growth analysis described by Dietzel and Liebman (2015). The root mass measured at the end of each year was the pool value.

The height and volume of root samples varied among depth increments, making visual comparisons among depths, such as 0-5 cm and 60-100 cm, difficult. Thus, splines were fit to the data and integrated by 5 cm depths to facilitate visualization of root and soil organic

C distribution in the soil profile.

### 3 Results

Table 1. Soil characteristics measured at the establishment of the experiment.

Depth	Bulk Density (g cm <sup>-3</sup> )	pH	Total C (%)	Total N (%)	Sand (%)	Silt (%)	Clay (%)
0-5	1.28	6.36	2.81	0.24	37.5	36.8	25.8
5-15	1.41	5.85	2.55	0.22	37.5	36.0	26.6
15-30	1.50	5.94	2.14	0.18	35.4	35.8	28.9
30-60	1.45	NA	1.23	0.11	NA	NA	NA
60-100	1.60	NA	0.95	0.05	NA	NA	NA

Our study site had soil characteristics typical for a Webster silty clay loam. Both total C and total N decreased with depth, but maintained a relatively constant relationship (mean C:N ratio = 11.6) until 60-100 cm, where the C:N ratio was 19 (Table 1).

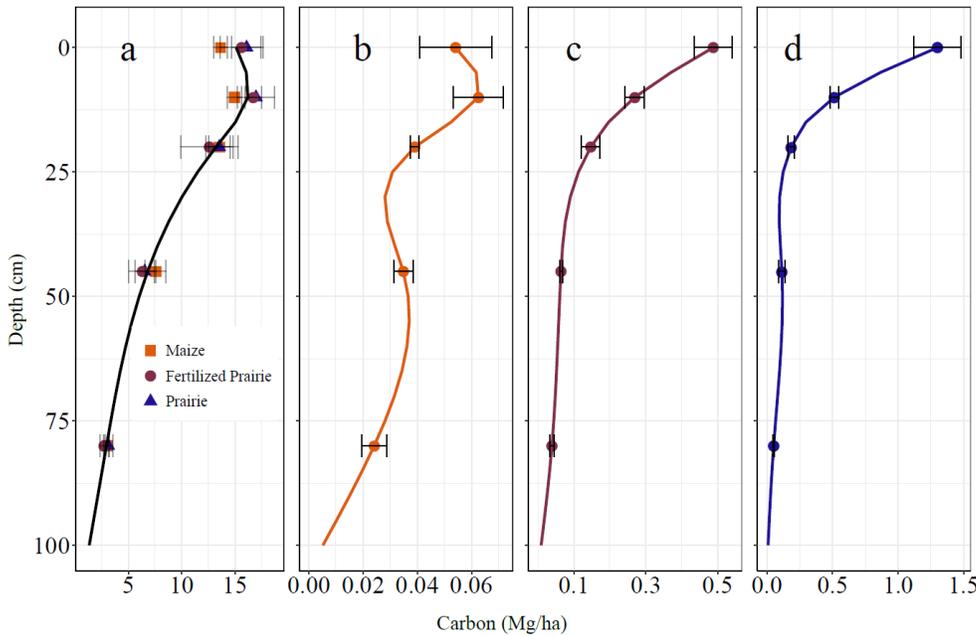


Figure 1. a) Total soil carbon with each treatment represented by a point and the site average represented by a solid line, b) maize root carbon, c) fertilized prairie root carbon, d) unfertilized prairie root carbon measured in 2013, six years after establishment of the experiment. Different x-axes scales are used to emphasize similarities and differences in profile distribution patterns, not absolute mass

amounts (see Fig 2).

The total amount of organic C found in the soil 6 years after establishment of the experiment did not differ among treatments at any depth (Fig 1), nor was it different from initial total organic C levels measured at the beginning of the experiment (data not shown). Half of the total soil organic C was found below 20 cm (Table 2). The pattern of vertical soil C distribution was similar to the pattern of maize root distribution, not prairie root distribution (Fig 1).

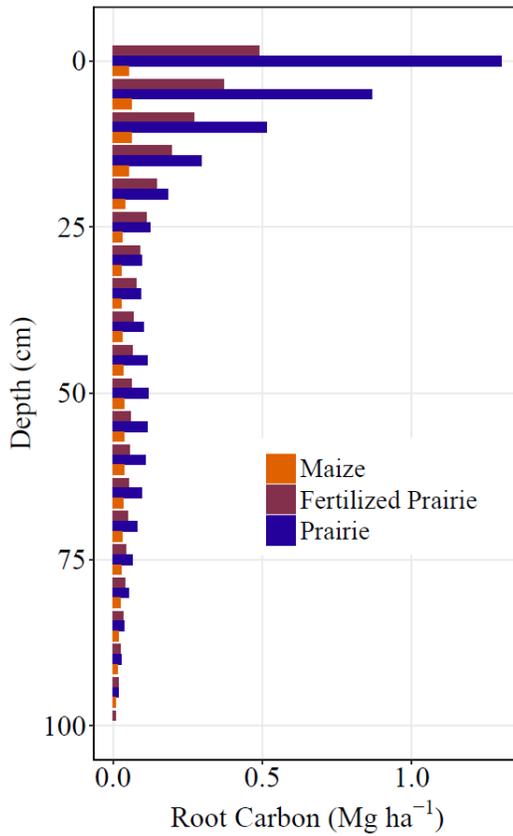
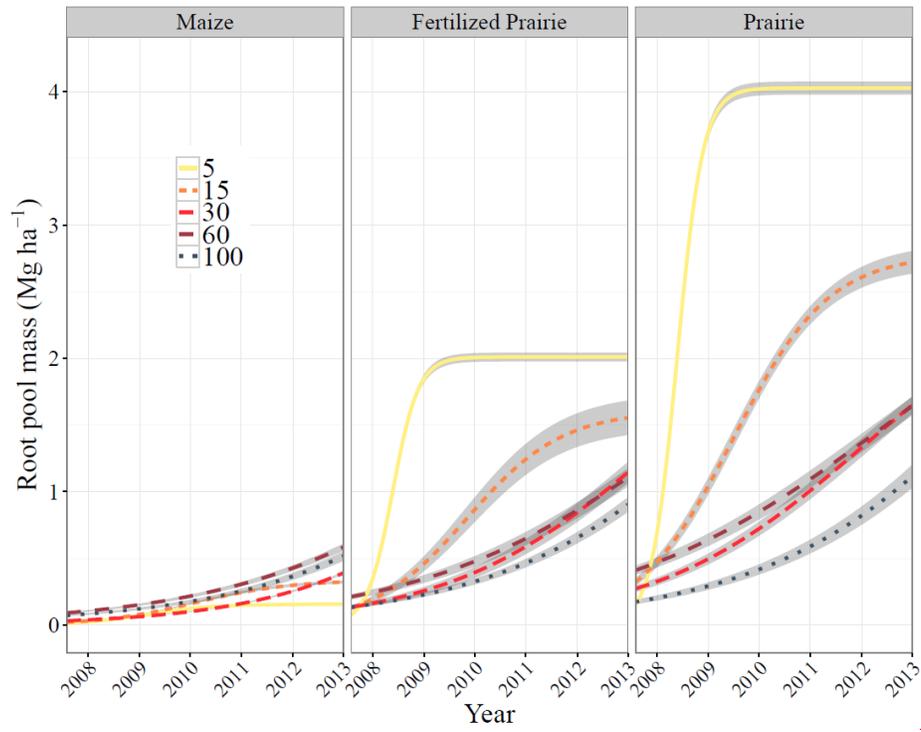


Figure 2. Absolute difference in root C pools six years after prairie establishment.

10 Six years after the establishment of the experiment, the unfertilized prairie root C pool was almost 6 times greater than the maize root C pool and the fertilized prairie root C pool was 3.5 times greater than the maize root C pool over a 1 m depth. Twenty-eight percent of the unfertilized prairie root C pool, 37% of the fertilized prairie root C pool and 62% of the maize root C pool was found below 20 cm (Fig 2, Table 2).

Table 2. Root pool and soil organic C found above and below 20 cm.

Treatment	Depth (cm)	Root C (Mg)	Soil C (Mg)	Root C (proportion)	Soil C (proportion)
Maize	0-20	0.27	71.17	0.38	0.44
	20-100	0.43	89.97	0.62	0.56
Unfertilized Prairie	0-20	3.16	79.14	0.72	0.48
	20-100	1.26	85.00	0.28	0.52
Fertilized Prairie	0-20	1.47	76.66	0.63	0.50
	20-100	0.85	76.54	0.37	0.50



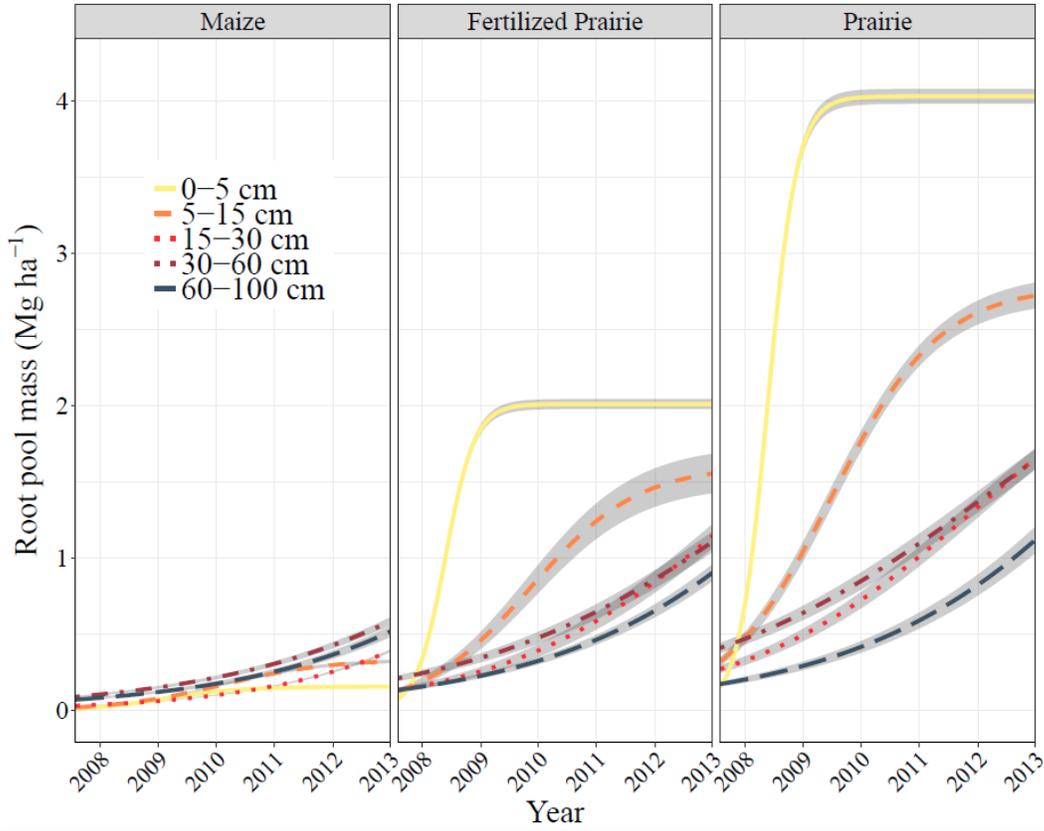
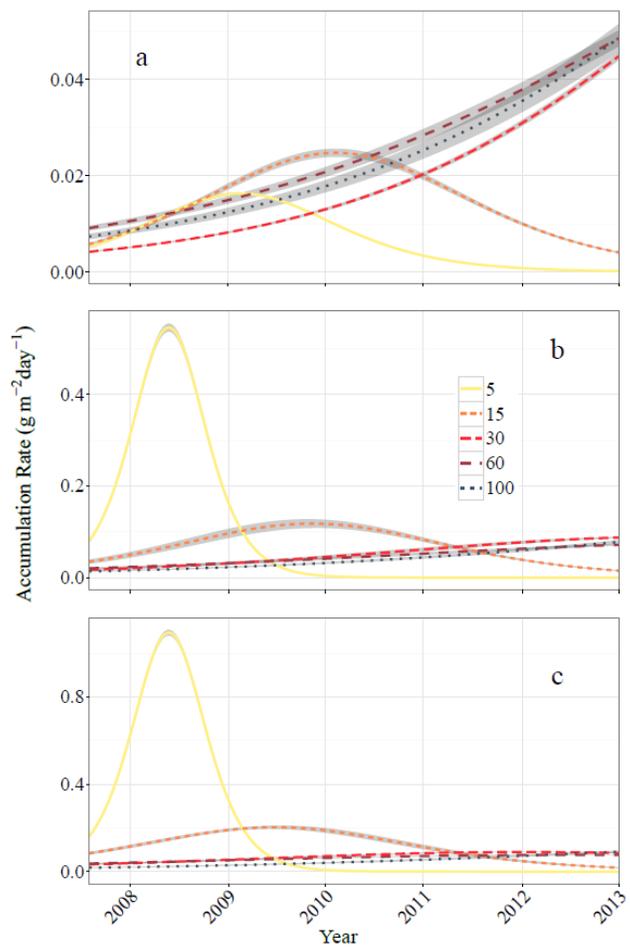


Figure 3. Modeled accumulation of root pool mass over six years at 0-5 cm, 5-15 cm, 15-30 cm, 30-60 cm, and 60-100 cm. Grey shading represents one standard error of the mean. Seasonal effects are smoothed.



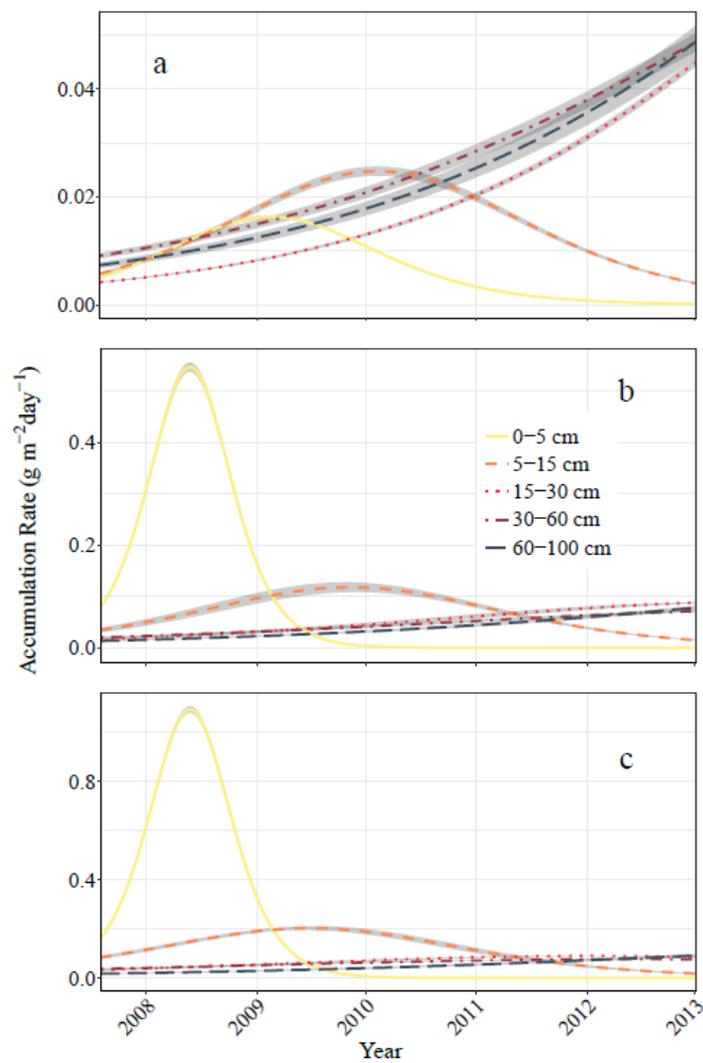


Figure 4. Modeled rates of root pool mass accumulation over 6 years in a) continuous maize, b) fertilized prairie and c) unfertilized prairie at 0-5 cm, 5-15 cm, 15-30 cm, 30-60 cm, and 60-100 cm. Grey shading represents one standard error of the mean. Different y-axes are used to emphasize similarities and differences in timing as well as to make within treatment relationships easier to see. Seasonal effects are smoothed.

5

Table 3. Root pool accumulation rates averaged across each growing season ( $\text{g m}^{-2}\text{day}^{-1}$ ). Differences in lowercase letters indicate significant differences between depths within treatments within years (read up and down). Differences in uppercase letters indicate differences between treatments within depths within years (read left to right).

Year	Depth (cm)	Maize ( $\text{g m}^{-2}\text{day}^{-1}$ )	Fertilized Prairie ( $\text{g m}^{-2}\text{day}^{-1}$ )	Unfertilized Prairie ( $\text{g m}^{-2}\text{day}^{-1}$ )
2008	0-5	0.007 a C	0.205 a B	0.411 a A
	5-15	0.007 a C	0.044 b B	0.102 b A
	15-30	0.005 a C	0.019 e B	0.036 e A
	30-60	0.010 a C	0.025 e B	0.058 a A
	60-100	0.008 a B	0.015 e AB	0.019 a A
2009	0-5	0.015 a C	0.315 a B	0.632 a A
	5-15	0.016 a C	0.087 b B	0.177 b A
	15-30	0.007 a C	0.029 e B	0.051 e A
	30-60	0.015 a C	0.036 e B	0.084 d A
	60-100	0.012 a B	0.021 e AB	0.027 e A
2010	0-5	0.013 a A	0.011 d AB	0.021 d A
	5-15	0.024 a D	0.117 a B	0.197 a A
	15-30	0.012 a C	0.042 be B	0.067 e A
	30-60	0.020 a C	0.047 b B	0.090 b A
	60-100	0.016 a BC	0.030 e AB	0.037 d A
2011	0-5	0.005 a A	0.000 e AB	0.000 e A
	5-15	0.022 a D	0.093 a B	0.131 a A
	15-30	0.018 a C	0.058 b B	0.082 b A
	30-60	0.027 a C	0.056 b B	0.068 e A
	60-100	0.023 a C	0.041 b AB	0.051 d A
2012	0-5	0.001 e A	0.000 e A	0.000 d A
	5-15	0.012 b D	0.048 b B	0.061 b A
	15-30	0.028 a D	0.074 a B	0.089 a A
	30-60	0.034 a B	0.058 b A	0.041 e B
	60-100	0.033 a D	0.056 b B	0.068 b A
2013	0-5	0.000 b A	0.000 e A	0.000 e A
	5-15	0.005 b B	0.019 d A	0.023 b A
	15-30	0.041 a D	0.086 a A	0.087 a A
	30-60	0.041 a B	0.052 e A	0.022 b C
	60-100	0.045 a C	0.074 b B	0.087 a A

Prairie rooting systems were established sequentially in the soil profile from the top down (Fig 4). The top five cm of the root pool peaked in the first full year of growth and then reached an equilibrium during the second full year of growth with large year-to-year variability given the sensitivity of this thin surface layer to environmental conditions (Fig A1-A3). The next soil layer, from 5-15 cm, had the greatest increase in root pool mass during the second full year of prairie growth, whereas, in contrast, the 15-30 cm and 30-60 cm depths didn't reach peak rates of root pool accumulation until five and six years after establishment, with no indication of when accumulation would cease. In the unfertilized prairie, rates of root pool accumulation in the 60-100 cm of the soil in the sixth year were greater than all other depths with no sign of slowing down. Fertilized prairie also had a high rate of root pool accumulation at 60-100 cm in the sixth year with no sign of decreasing (Fig 4).

Maize root pool accumulation was almost always slower than prairie root pool accumulation with the exception of the top 5 cm after 2010, 60-100 cm before 2011 (not different from fertilized prairie), and a greater value in maize than unfertilized prairie at 30-60 cm in 2013. There was no difference in maize root pool accumulation among depths until 2011 when accumulation below 15 cm then began to exceed accumulation above 15 cm (Fig 4).

Table 34. Root turnover at 0-30 cm.

Year	Treatment	Input (Mg mha <sup>-2</sup> )	Pool (Mg mha <sup>-2</sup> )	k	mrt (years)
2010	Unfertilized Prairie	3.67	7.48	0.49 35	2.852.04
	Fertilized Prairie	1.46	2.31	0.37 63	2.741.58
	Maize	0.56	0.44	1.27 0.86	0.794.16
2011	Unfertilized Prairie	3.87	7.58	0.44 51	2.451.96
	Fertilized Prairie	1.68	3.42	0.33 49	3.022.03
	Maize	0.48	0.47	1.01 0.67	0.994.50

Prairie roots had a mean residence time (mrt) of 2.751.90 years in the top 30 cm of the profile when averaged across treatments and years (2010 and 2011). Maize roots turned over almost twice as fast as prairie roots when averaged across treatments and years (Table 3).

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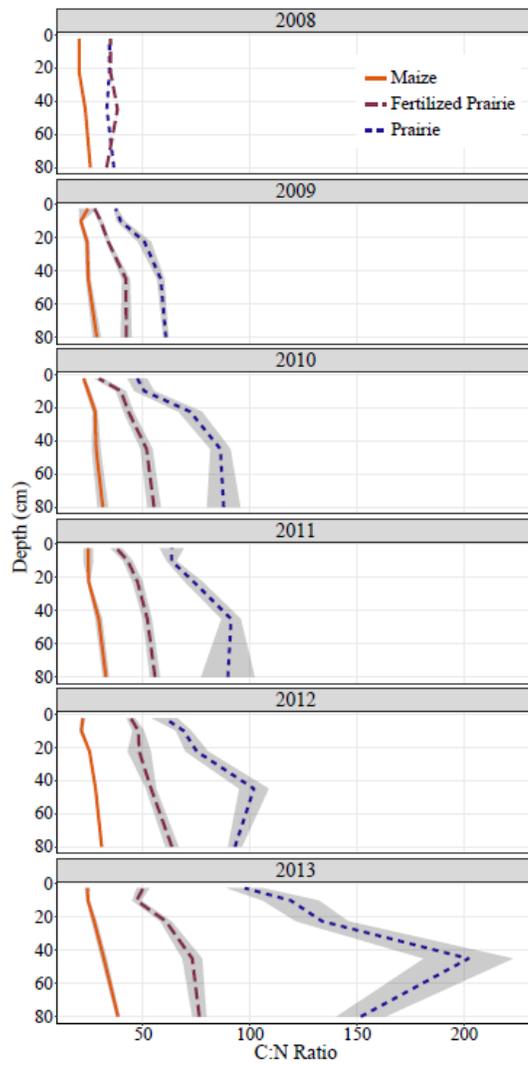


Figure 54. Root C:N ratios with depth over time. Grey shading represents one standard error of the mean.

Carbon:N ratios increased with depth in all treatments (Fig 5). Carbon to nitrogen ratios increased in both prairie treatments in every depth over time, although the increase in fertilized prairie was not always different between consecutive years. In all treatments, changes in C:N ratios were the result of both an increase in C content and a decrease in N content (data not shown). The maize root pool did not exhibit an increase in C:N ratio over time (Fig 5).

## 4 Discussion

### 4.1 Reconstruction of a prairie root C pool and implications for C contribution

An increase in root pool C:N ratio with depth has not been reported previously in the literature and not previously considered when trying to determine why a disproportionately large amount of soil C is found at depth when compared to root distribution. It has been recently theorized that plant tissue becomes organic matter through two different pathways: 1) a dissolved organic C-microbial pathway whereby plant litter is first processed by soil microbes and eventually transported and stabilized in the soil matrix as a microbial by-product, if the soil has the capacity to stabilize these compounds, and 2) a physical-transfer pathway whereby plant tissue is processed by soil microbes to its fullest extent, and then remains in the soil functionally inert until it remains in the soil due to inherent chemical recalcitrance (Cotrufo 2015). In the present study, the former pathway is more applicable to tissue dominated by non-structural compounds, such as that with lower C:N ratios found here at shallower depths, whereas the latter pathway applies to tissue dominated by structural compounds, indicated by high C:N ratios in root tissue at greater depth. Under this framework, root decomposition in our study would have resulted in a gradient of microbially-derived to physically-derived organic matter from the top of the soil profile downward. In turn, this would mean that soil organic matter at the soil surface would be vulnerable to transport to greater depth as dissolved organic C, whereas physically-transferred soil organic matter at depth would be relatively immobile. -This is a possible mechanism by which the amount of soil organic C found at depth is disproportionately large compared to the size of the root C pool. This mechanism would be particularly strong in soils that are high in C and have a reduced capacity for C stabilization nearer to the surface, such as soils formed under prairie vegetation (Castellano 2015). These findings are-is suggested mechanism is also consistent with evidence that the contribution of microbial- and not root-derived C increases with depth (Liang and Balser 2008, Rumpel and Kogel-Knabner, 2011). In addition to the less-structural root material found at shallow depths, these areas of concentrated roots produce labile exudates that are easily metabolized and transported deeper in the soil profile (Badri and Vivanco, 2009), again under conditions that do not favour immediate stabilization of the metabolites. While we did not measure root exudates, it is important to recognize that these mobile compounds also likely play an important role in the development of the soil organic C profile.

Because the root pool is made up of a combination of new, mature, ageing, and dead roots, an increase in its mass comes from root growth, live root retention, and inhibited root decomposition. The relatively quick rate of accumulation in the top 30 cm of soil was most likely a function of new root addition, which slowed as the system became more established (Fig 4). Slower increases at deeper depths than shallower depths may indicate that accumulation there is more dependent upon the carryover of roots from previous years than at shallower depths, although annual root inputs were not directly measured in this study.

By the sixth year of reconstructed prairie establishment, root C pool equilibrium was reached and prairies began making substantial annual contributions-inputs to the soil organic matter pool above 30 cm (Figs 3&4), although the fraction of organic matter that remained in the soil is unknown. This was indicated by the finding that the majority of prairie roots (75%) was found in this depth fraction, where mean residence time was measured to be 2.5-3-2 years (Tables 2 &3). The prairie root C pool at 0-5 cm reached an equilibrium and began steady root turnover in the third year after establishment, as indicated by very low rates of accumulation, and was likely able to contribute material to the soil organic matter pool at this time. Other prairie restorations have also found soil organic matter accumulation to be most rapid closer to the soil surface (O'Brien et al. 2010, Omonode and Vyn 2006).

Annual prairie root inputs were not measured below 30 cm, so turnover rates could not be calculated. However, continuous increases in the root pool at depth (Fig 3) due to root growth and retention indicate that root tissue loss to the soil was very low during this time and the mean residence time of roots at depth may have greatly exceeded those closer to the surface. This means that at depth, not only

was the root C pool substantially smaller than near the surface, but root material also became available to the soil much more slowly than near the surface. Indeed, DuPont et al. (2014) found intact prairie roots in the soil five years after conversion to annual wheat.

5 Nitrogen fertilization of prairies led to a smaller root pool at every depth, with lower rates of accumulation, and lower C:N ratios (Figs 2, 4, 5). However, fertilization did not affect the time until root systems were fully established or the turnover rate of roots in the top 30 cm (Fig 3). Differences between fertilized and unfertilized prairie showed that the pattern of root distribution was a function of nutrient availability and not a response to soil space conditions because fertilized prairie used half as much space as unfertilized prairie and still showed decreased accumulation above 30 cm over time (Figs 3 & 4).

#### 4.2 Quantity, distribution, and quality of root biomass differs in native perennial and non-native annual ecosystems

10 It is possible that maize roots had greater C input to the soil than did prairie roots below a certain depth. Maize root C pools were much smaller than prairie root C pools (Fig 2), but faster turnover times (Table 3) and lower C:N ratios (Fig 5) resulted in a greater proportion of the maize root C pool available for stabilization in the soil compared to the prairie root C pool. In the top 0-30 cm, the difference in mass between even the fertilized prairie and maize was too great to be overcome by faster turnover and greater carbon use efficiency, but the difference in root mass between the annual and perennial systems decreased with depth while the difference in C:N ratio increased and turnover times may have maintained the same relative relationship. Although we do not have measurements of distribution of soil C through the soil profile previous to cultivation, data from this experiment show that the pattern of soil C distribution is much more similar to the distribution of maize roots than the distribution of prairie roots (Fig. 1), demonstrating the importance of differing root systems in the development of the soil C profile.

#### 4.3 ~~What do these differences in inputs tell us about the perennial prairie ecosystem under which these soils developed and the annual cropping systems under which these soils continue to change~~What do these differences in inputs tell us about the historical belowground ecosystem under which these soils developed in comparison to the systems under which these soils continue to change?

25 The experimental location was a site of cultivation under annual crops for over 100 years, following ~10,000 years of perennial prairie systems. We do not have measurements of the pre-cultivation soil C profile, but other data from sites near our experiment (Guzman 2009, McGranahan et al. 2014) show that the soil C profile shifted from a pattern of having an exponential decrease in C with distance from the surface to a pattern of more uniform distribution of C with the highest point of C 10 cm below the surface (Fig 1). The loss of C in the soil surface after cultivation is well known and attributed to mass loss through soil erosion, increased mineralization of organic matter through tillage, and decreased belowground organic matter inputs (Davidson et al. 1993; Huggins et al. 1998). Less is documented about the change in soil carbon below 30 cm, but using a robust dataset, Veenstra et al. (2015) found soil organic C to increase below 35 cm after 50 years in maize and soybean cropping systems in Iowa, USA. Initial soil organic C measurements in that study were made ~50 years after these soils had already been converted to annual systems, preventing comparison to soil organic C levels at depth under native vegetation, but results from Veenstra et al. still show that Mollisols can and do gain soil organic C at deeper depths under maize and soybean systems. Similarly, David et al. (2008) and Follett et al. (2009) found cultivated sites that gained deep soil organic C relative to remnant prairies and grasslands.

Our relatively short-term study of 6 years did not detect significant changes in soil C at any depth (Fig 1), ~~but~~However, differences in quantity, distribution, and C:N ratios between the annual and perennial rooting systems we studied have important implications for how deep soil organic C may have changed and continues to change with the implementation of annual cropping systems. A large, structural-tissue dominated root C pool with slow turnover, concentrated at shallow depths was replaced by a small, non-structural-tissue dominated root C pool with fast turnover evenly distributed in the soil profile. The difference in size between these two pools has long been obvious, but often misleading for comparisons related to C accounting because differences in root turnover and tissue C:N ratio are often not taken into consideration. An exception, Omonode et al. (2006) discusses the possibility that slower turnover in perennial rooting systems may prevent expected increases in soil organic C compared to adjacent maize systems. The data presented here in the context of recent organic matter formation theory suggest that while differences in root C pool and soil organic C relationships in maize and prairie above 20 cm are predominately controlled by root biomass amount, root biomass amount is less of a factor below 20 cm.

## 5 Conclusion

Soils are incredibly complex systems and biogeochemical processes that determine soil C storage happen over a long time and in places that are difficult to study without artifact-inducing disturbances. We have shown here that an increase in root C:N ratio with depth is a potentially important, and previously unconsidered, factor determining the distribution of C in the soil profile. This factor interacts with depth-determined differences in soil temperature, moisture, O<sub>2</sub>, soil texture, microbial communities, and existing soil C content and thus carries different significance in different environments. In our comparison of maize and reconstructed prairie systems, root pool C:N ratios may be sufficiently important that they result in greater maize C contributions to soil organic matter than prairie C contributions to soil organic matter below 20 cm. In these and many other herbaceous systems, an increase in root C:N ratio with an increase in depth may in part explain why 50% of soil organic C is found below 20 cm, while only 30% of root biomass is found in the same location. Elucidating the mechanisms determining soil C retention and addition is important as we strive to design systems that maintain and build soils that are productive and resilient. The role of roots and root composition, as well as the importance of soil organic C below 20 cm should be carefully considered in such designs.

## 25 Data and code availability

Data and code for this work is currently publicly stored in a GitHub repository. ~~During and after the review process, we will clean up the repository to include only relevant data and code, improve comments within the code, and write a thorough readme file to ensure the creation of a fully reproducible compendium. The GitHub repository will be linked with a Zenodo account, which will provide a DOI for the data and code, making the data easily discoverable and citable. Zenodo will also create a mirrored repository, backing up code and data in their own system. The DOI to find and access these files is 10.5281/zenodo.321910.~~

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Appendix A.

Curve fits used to generate predicted root accumulation for each depth. The mean and standard error of these curves are found in Fig. 3.

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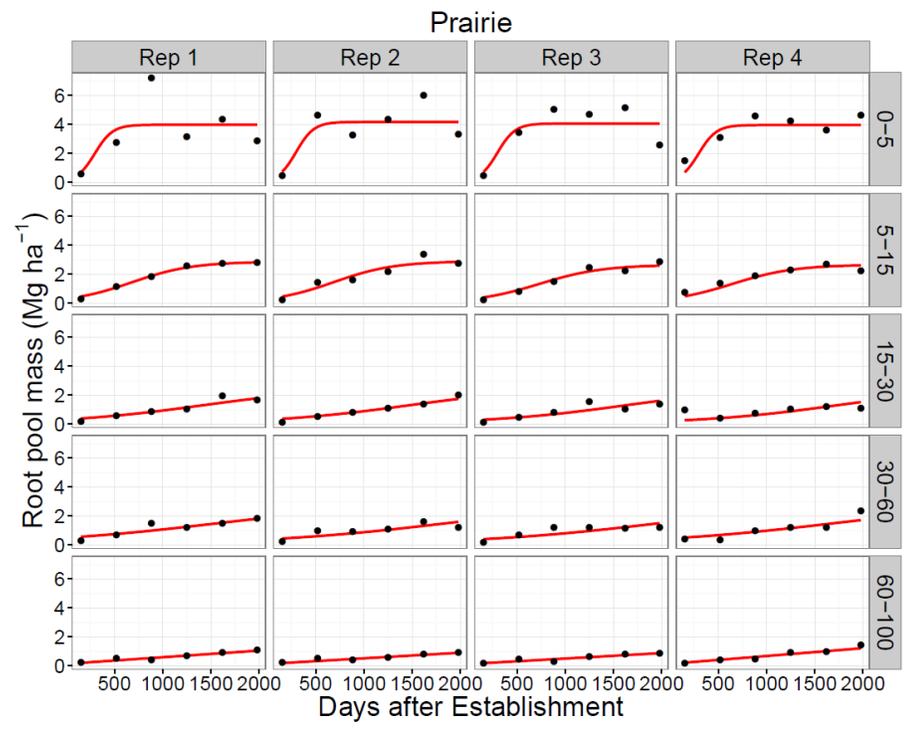


Fig A1. Logistic curves fit to root pool mass accumulation at each replication and depth increment in the prairie treatment.

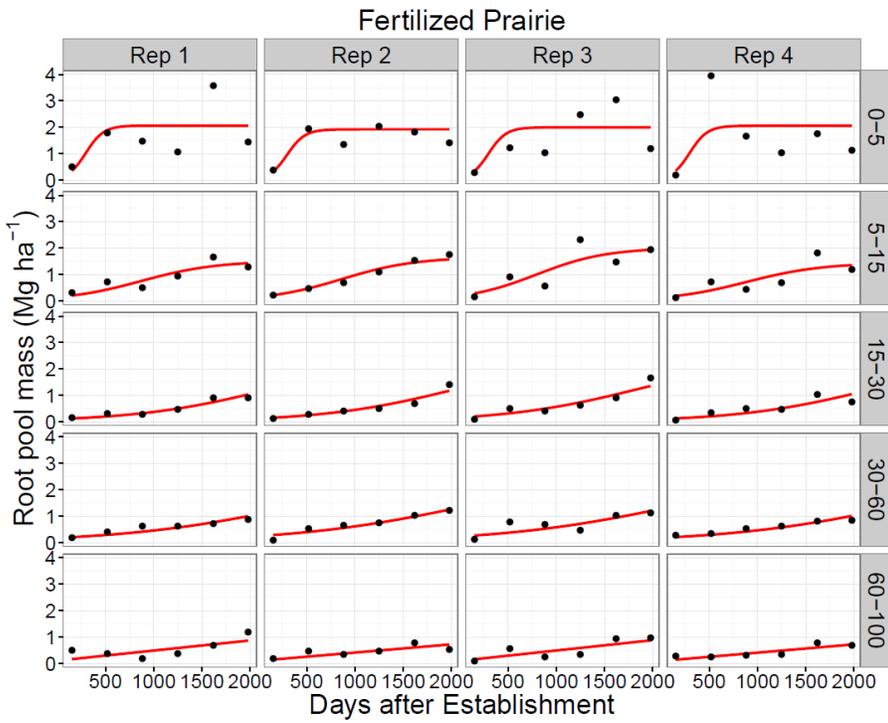


Fig A2. Logistic curves fit to root pool mass accumulation at each replication and depth increment in the fertilized prairie treatment.

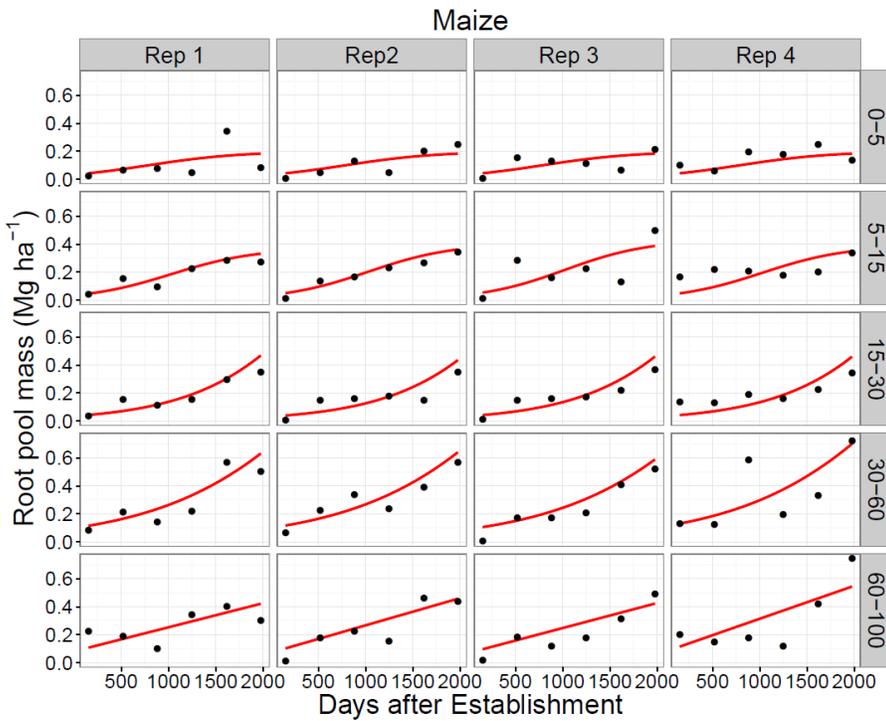


Fig A3. Logistic curves fit to root pool mass accumulation at each replication and depth increment in the maize treatment.

Table A1. Root pool accumulation rates averaged across each growing season ( $\text{g m}^{-2} \text{day}^{-1}$ ). Differences in lowercase letters indicate significant differences between depths within treatments within years (read up and down). Differences in uppercase letters indicate differences between treatments within depths within years (read left to right).

Year	Depth (cm)	Maize ( $\text{g m}^{-2} \text{day}^{-1}$ )	Fertilized Prairie ( $\text{g m}^{-2} \text{day}^{-1}$ )	Unfertilized Prairie ( $\text{g m}^{-2} \text{day}^{-1}$ )
2008	0-5	0.007 a C	0.205 a B	0.411 a A
	5-15	0.007 a C	0.044 b B	0.102 b A
	15-30	0.005 a C	0.019 c B	0.036 c A
	30-60	0.010 a C	0.025 c B	0.058 a A
	60-100	0.008 a B	0.015 c AB	0.019 a A
2009	0-5	0.015 a C	0.315 a B	0.632 a A
	5-15	0.016 a C	0.087 b B	0.177 b A
	15-30	0.007 a C	0.029 c B	0.051 c A
	30-60	0.015 a C	0.036 c B	0.084 d A
	60-100	0.012 a B	0.021 c AB	0.027 e A
2010	0-5	0.013 a A	0.011 d AB	0.021 d A

	<u>5-15</u>	<u>0.024</u>	a	D	<u>0.117</u>	a	B	<u>0.197</u>	a	A
	<u>15-30</u>	<u>0.012</u>	a	C	<u>0.042</u>	bc	B	<u>0.067</u>	c	A
	<u>30-60</u>	<u>0.020</u>	a	C	<u>0.047</u>	b	B	<u>0.090</u>	b	A
	<u>60-100</u>	<u>0.016</u>	a	BC	<u>0.030</u>	c	AB	<u>0.037</u>	d	A
	<u>0-5</u>	<u>0.005</u>	a	A	<u>0.000</u>	c	AB	<u>0.000</u>	e	A
	<u>5-15</u>	<u>0.022</u>	a	D	<u>0.093</u>	a	B	<u>0.131</u>	a	A
<u>2011</u>	<u>15-30</u>	<u>0.018</u>	a	C	<u>0.058</u>	b	B	<u>0.082</u>	b	A
	<u>30-60</u>	<u>0.027</u>	a	C	<u>0.056</u>	b	B	<u>0.068</u>	c	A
	<u>60-100</u>	<u>0.023</u>	a	C	<u>0.041</u>	b	AB	<u>0.051</u>	d	A
	<u>0-5</u>	<u>0.001</u>	c	A	<u>0.000</u>	c	A	<u>0.000</u>	d	A
	<u>5-15</u>	<u>0.012</u>	b	D	<u>0.048</u>	b	B	<u>0.061</u>	b	A
<u>2012</u>	<u>15-30</u>	<u>0.028</u>	a	D	<u>0.074</u>	a	B	<u>0.089</u>	a	A
	<u>30-60</u>	<u>0.034</u>	a	B	<u>0.058</u>	b	A	<u>0.041</u>	c	B
	<u>60-100</u>	<u>0.033</u>	a	D	<u>0.056</u>	b	B	<u>0.068</u>	b	A
	<u>0-5</u>	<u>0.000</u>	b	A	<u>0.000</u>	e	A	<u>0.000</u>	c	A
	<u>5-15</u>	<u>0.005</u>	b	B	<u>0.019</u>	d	A	<u>0.023</u>	b	A
<u>2013</u>	<u>15-30</u>	<u>0.041</u>	a	D	<u>0.086</u>	a	A	<u>0.087</u>	a	A
	<u>30-60</u>	<u>0.041</u>	a	B	<u>0.052</u>	c	A	<u>0.022</u>	b	C
	<u>60-100</u>	<u>0.045</u>	a	C	<u>0.074</u>	b	B	<u>0.087</u>	a	A

#### Competing interests

5 The authors have no competing interests.

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