

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29

Article type : Articles

Running Head: Crop rotation meta-analysis

Title

Crop rotations for increased soil carbon: perenniality as a guiding principle

Authors

Alison E. King¹ and Jennifer Blesh^{1,2*}

¹ School for Environment and Sustainability,
University of Michigan
440 Church St.
Ann Arbor, MI 48109

² Corresponding author. Email: jblesh@umich.edu

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1002/eap.1648](https://doi.org/10.1002/eap.1648)

This article is protected by copyright. All rights reserved

Author Manuscript

31 **Abstract**

32

33 More diverse crop rotations have been promoted for their potential to remediate the range of
34 ecosystem services compromised by biologically simplified grain-based agroecosystems,
35 including increasing soil organic carbon (SOC). We hypothesized that functional diversity offers
36 a more predictive means of characterizing the impact of crop rotations on SOC concentrations
37 than species diversity *per se*. Furthermore, we hypothesized that functional diversity can either
38 increase or decrease SOC depending on its associated carbon (C) input to soil. We compiled a
39 database of 27 cropping system sites and 169 cropping systems, recorded the species and
40 functional diversity of crop rotations, SOC concentrations (g C kg soil^{-1}), nitrogen (N) fertilizer
41 applications ($\text{kg N ha}^{-1} \text{ yr}^{-1}$), and estimated C input to soil ($\text{Mg C ha}^{-1} \text{ yr}^{-1}$). We categorized crop
42 rotations into three broad categories: grain-only rotations, grain rotations with cover crops, and
43 grain rotations with perennial crops. We divided the grain-only rotations into two sub-categories:
44 cereal-only rotations, and those that included both cereals and a legume grain. We compared
45 changes in SOC and C input using mean effect sizes and 95% bootstrapped confidence intervals.
46 Cover cropped and perennial cropped rotations, relative to grain-only rotations, increased C input
47 by 42 and 23% and SOC concentrations by 6.3 and 12.5%, respectively. Within grain-only
48 rotations, cereal + legume grain rotations decreased total C input (-16%), root C input (-12%),
49 and SOC (-5.3%) relative to cereal-only rotations. We found no effect of species diversity on
50 SOC within grain-only rotations. N fertilizer rates mediated the effect of functional diversity on
51 SOC within grain-only crop rotations: at low N fertilizer rates ($\leq 75 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), the
52 decrease in SOC with cereal + legume grain rotations was less than at high N fertilizer rates.
53 Our results show that increasing the functional diversity of crop rotations is more likely to
54 increase SOC concentrations if it is accompanied by an increase in C input. Functionally diverse
55 perennial and cover cropped rotations increased both C input and SOC concentrations,
56 potentially by exploiting niches in time that would otherwise be unproductive – that is,
57 increasing the “perenniality” of crop rotations.

58

59

60 **Keywords**

61

62 Biodiversity, cover crops, cropping systems, functional diversity, meta-analysis, nitrogen
63 fertilizer, perennials, soil carbon, soil organic matter, sustainable agriculture

64

65

66 **Introduction**

67

68 Biologically simplified grain-based agroecosystems provide 50% of human calories worldwide
69 (FAOSTAT 2013) but create a host of environmental costs (Montgomery 2007, David et al.
70 2010, Heathcote et al. 2013), including diminished soil organic carbon (SOC) stocks (Guo and
71 Gifford 2002). Soil carbon (C) in agroecosystems has been the focus of renewed attention lately
72 (Lehmann and Kleber 2015, Paustian et al. 2016), because increasing SOC can mitigate a portion
73 of anthropogenic C emissions (Lal 2004a, 2004b) and improve both nutrient (Campbell and
74 Souster 1982, Booth et al. 2005) and water holding capacity of soils (Hudson 1994). The
75 concurrence of low diversity and low SOC proposes the question: does increasing the
76 biodiversity of agroecosystems increase SOC?

77 Soil C stocks are determined by both the fixed, edaphic properties of soil and by agroecosystem
78 management. Management practices regulate both C inputs to soil and C losses via respiration,
79 and SOC stocks represent the balance between these fluxes. Carbon input to soil, defined as the
80 difference between net primary productivity (NPP) and C exported in crop harvest, sometimes
81 predicts the effects of cropping systems on SOC (Rasmussen et al. 1980, Kong et al. 2005,
82 Novara et al. 2016, Sanderman et al. 2016). In some cases, however, lower total C input in one
83 cropping system corresponds to higher SOC (Gregorich et al. 2001, Kallenbach et al. 2015). In
84 these cases, other mechanisms are invoked to interpret cropping system effects on SOC, for
85 instance, that root tissues are preferentially stabilized in soil compared to shoot tissues (Puget
86 and Drinkwater 2001, Rasse et al. 2005, Kong and Six 2010), or that high quality plant tissues
87 (as characterized by their litter C chemistry; e.g., low C:N, low lignin:N) are preferentially
88 stabilized in SOC (Cotrufo et al. 2013). As data from intra-site cropping system comparisons
89 have not been aggregated, it is difficult to discern the relative prevalence of positive and negative
90 relationships between C input and SOC.

91 Crop rotation diversity has been previously linked to increased SOC (McDaniel et al. 2014),

92 however, to our knowledge no synthesis elaborates whether crop rotation diversity is also linked
93 to crop rotation C input. In order to predict the effect of crop rotation diversity on both C input
94 and SOC, it is necessary to re-evaluate the species diversity metrics originally employed in
95 biodiversity-ecosystem function research (Harper and Hawksworth 1994, Tilman et al. 2001).
96 Functional diversity is increasingly recognized as more predictive of ecosystem function than
97 species diversity (Petchey and Gaston 2006, Cadotte et al. 2011, Conti and Díaz 2013, Martin
98 and Isaac 2015, Wood et al. 2015). Although multiple metrics of functional diversity exist, plant
99 functional groups are often delineated based on representative individuals' functional traits –
100 those features of an organism's phenotype that determine its effect on ecosystem function –
101 because it can be impractical to measure traits for all individuals (Petchey and Gaston 2006).
102 With respect to SOC accrual, plant functional traits of interest include, at a minimum, above and
103 belowground NPP (Virto et al. 2012, Anderson-Teixeira et al. 2013) and plant tissue quality
104 (Wuest and Gollany 2013). We argue that a framework to link agroecosystem diversity to SOC
105 should articulate how C input, and its above and belowground components, change with the
106 inclusion of crop functional groups relevant to SOC accrual.

107 In natural grasslands, ecological theory elaborates the linkage between plant functional group
108 richness and SOC. This body of theory, however, does not fully capture C cycling dynamics in
109 agroecosystems for at least two reasons. First, it relies on interactions of functional groups in
110 space to explain increases in NPP with biodiversity, for example, through complementary
111 interactions such as facilitation or niche differentiation (Hector et al. 2001, Hooper et al. 2005,
112 Fornara and Tilman 2008, Steinbeiss et al. 2008). In most grain agroecosystems, however, the
113 potential for interspecific facilitation is limited because biodiversity occurs mostly in crop
114 rotations, with plant species isolated in time. Even facilitative effects over time, such as cereal
115 crops benefiting from the biologically fixed N from a previous legume crop (Peterson and
116 Russelle 1991), can be masked by N fertilizer inputs. Second, in agroecosystems, management
117 decisions – not at play in natural grasslands – also regulate C input. Farmers intentionally
118 manage crop species differently based on particular management goals, which can determine the
119 proportion of crop NPP that is returned to soil. For example, small grains such as oats could be
120 grown either as a cover crop or a harvested grain, but return approximately 50% more C input to
121 soil when managed as a cover crop (Bolinder et al. 2007).

122 Synthetic N fertilizer management is also a prominent feature of agroecosystems that can interact
123 with crop rotation diversity to affect SOC. N fertilizer inputs can decrease SOC by increasing C
124 mineralization from soil, or can increase SOC by increasing NPP. Holding crop species diversity
125 constant, the net effect of N fertilizer on SOC is still debated (Robertson et al. 2013), and is
126 likely context dependent, however most studies show an increase in SOC under N fertilization
127 (Alvarez 2005, Russell et al. 2009, Jian et al. 2016). Between crop rotations that differ in their
128 functional diversity, crop response to N fertilizer may follow the “stress gradient hypothesis,”
129 which proposes that facilitation is more important under conditions of greater environmental
130 stress (Brooker et al. 2005, Li et al. 2007, Tang et al. 2016), such as low N availability. For
131 instance, facilitative interactions between legume and non-legume crops are more important for
132 maintaining productivity in zero N fertilizer than in high N fertilizer systems (Russell et al.
133 2009). Such interactive effects between N fertilizer inputs and legumes on crop productivity
134 suggest that N fertilizer could mediate the relationship between crop rotation diversity and SOC.
135 For instance, at high N fertilizer rates, cereal grain rotations usually produce more residue input
136 (i.e., the non-harvested portion of aboveground NPP) than do rotations of mixed cereal grains
137 and legume grains. This could lead to higher SOC concentrations under continuous cereal grains
138 compared to rotations of mixed cereal and legume grains. At low N fertilizer rates, however,
139 cereal grain rotations might produce less biomass, performing more like rotations of mixed
140 cereals and legume grains in terms of residue production and SOC concentrations.

141 We conducted a meta-analysis of crop rotation experiments to test the hypothesis that increasing
142 diversity in grain-based cropping systems is more likely to increase SOC if it includes crop
143 functional groups that increase C input. We use *crop rotation* to refer specifically to the sequence
144 of crop species grown in a field over time, reserving *cropping system* to describe an entire set of
145 farm management practices (e.g., crop rotation, tillage regime, nutrient source). In each crop
146 rotation, we classified crops into *functional groups* according to both physiological traits of crops
147 and management factors that are linked to total, root, and shoot C input (Table 1). To identify
148 functional groups, we delineated two common crop functional types independent of
149 management, i) *grass or legume*, and ii) *annual or perennial*. These functional types align with
150 plant traits relevant for SOC accrual: grasses, because they do not need to invest energy in
151 biological N fixation, generally have higher yields (Bolinder et al. 2007, Monfreda et al. 2008,
152 Iizumi et al. 2014), but lower quality residues (NRCS 2011), than do legumes. Perennial crops

153 produce more readily-stabilized root tissue than do annual crops (Bolinder et al. 2007, Anderson-
154 Teixeira et al. 2013). Since crop harvest is an important pathway of C export from
155 agroecosystems, we included a third functional type controlled by management: iii) *harvested or*
156 *non-harvested crops*. The resulting functional groups of crops represented most possible
157 combinations of these six functional types, and with them we constructed crop rotation
158 typologies (Table 2) and estimated both change in SOC and change in C input between crop
159 rotation typologies. Specifically, we addressed the following questions: Does increasing the
160 species or functional diversity of crop rotations 1) increase SOC concentrations and 2) increase C
161 input? And, 3) Does the effect of crop rotation diversity on SOC depend on N fertilizer
162 application rates?

163 **Methods**

164

165 *Literature search and screening criteria*

166 We performed a search of Web of Science [v.5.16] on October 28, 2014 using the search terms
167 (crop* rotation OR crop* system OR agroecosystem) AND (soil carbon OR soil organic matter)
168 AND (long-term OR field) NOT (greenhouse OR pot), which returned 563 hits. Criteria for
169 inclusion were at least two levels of crop species diversity (e.g., 1 and 2 species, or 2 and 4
170 species), or at least two levels of functional group diversity (e.g., cereals and cereals + perennial
171 crop). All crop rotations included at least one grain crop; agroforestry and pasture systems were
172 beyond the scope of this study.

173

174 *Data collection and compilation*

175 Forty-nine publications, representing 27 cropping system sites, met criteria for inclusion in the
176 study (Appendix S1, Table S1). For each experimental site we recorded elevation, latitude,
177 percent clay, depth of soil sampling, and time between site establishment and soil sampling. For
178 sites that did not report elevation, we used the Geoplaner database (<http://www.geoplaner.com/>)
179 to find elevations. For sites where the soil texture class was reported, but not percent clay, we
180 used the average clay concentration in the texture class.

181

182 For each cropping system, species diversity was recorded as the total number of crop species
183 present in the rotation. We recorded crop rotation length in years (the number of years before

184 return to an arbitrary entry point in the rotation), and N application rate (kg N ha^{-1}) for each crop
185 in the rotation. We calculated N fertilizer rate averaged over the crop rotation ($\text{kg N ha}^{-1} \text{ yr}^{-1}$) as
186 the sum of N fertilizer applied to each crop divided by length of the rotation in years. We were
187 unable to estimate N input from biological N fixation because legume biomass was rarely
188 reported. We excluded cropping systems with external C additions from the database to avoid
189 confounding the effect of manure or compost with crop rotation diversity. Tillage regimes were
190 inadequately reported in the studies to be used as an independent variable in the analysis.

191
192 To create crop rotation typologies based on functional diversity of crops, we first assigned each
193 crop to a functional type: 1) grass or legume, 2) annual or perennial, and 3) harvested or non-
194 harvested (hereafter, “cover crop”), (Table 1). Cover crops were classified as any crop not
195 burned, grazed, or harvested for grain, hay, or stover. As all harvested, annual crops were either
196 legumes or grasses, we aggregated them into a broader functional group “grains” for some
197 analyses. At one site, oat was harvested for both grain and straw (Porter et al. 2003); we
198 categorized the oat as an annual grain crop and did not count residue C inputs. We excluded
199 rotations with horticultural crops (potatoes, tomatoes) and oilseeds (flax, canola) because their
200 low frequency in the database precluded constructing viable typologies with them. Using the
201 above groups for individual crops, we created several typologies of crop rotations between which
202 to construct pair-wise comparisons within sites (Table 2). For a given pair-wise comparison, the
203 control rotation had lower functional group richness than the treatment rotation.

204
205 As response variables, we recorded SOC on a concentration (g C kg^{-1} soil) and an areal (Mg C
206 ha^{-1}) basis. While we planned to report SOC on both an areal and concentration basis, only 44%
207 of sites reported C on an areal basis or provided bulk density estimates for conversion. As 87%
208 of sites reported SOC on a concentration basis, we used SOC on a concentration basis in our
209 analyses. Where possible, SOC reported on areal basis was converted to concentration by
210 estimating bulk density from tillage regime and soil texture (Chen et al. 1998). Values reported
211 as soil organic matter (SOM) were converted to SOC by dividing by 1.72 (Soil Survey Division
212 Staff 1993). We took values reported as total C to be organic C as soil pH was less than 6 in
213 almost all cases (Izaurrealde et al. 2001). We used SOC values from 30 cm depth and shallower.
214 Where SOC was reported for multiple depth increments above 30 cm, SOC concentrations were

215 calculated as depth-weighted averages (Johnson and Curtis 2001). Only soils sampled at least
216 three years after site establishment were included. In some cases, we used Data Thief[®] software
217 to extract data from figures.

218 Of the 27 sites that met our selection criteria, six also replicated all cropping systems at different
219 N fertilizer application rates and 13 replicated all cropping systems under different tillage
220 regimes. In order to construct pair-wise comparisons for bootstrapped confidence intervals, for
221 each site with an N fertilizer gradient, we selected a moderate N fertilizer level. For experiments
222 with two rates of N fertilizer application (where zero N rate is not considered a level), we used
223 the higher level; with three levels of N fertilizer application, we used the middle level; and with
224 four levels, we used the third highest level. For sites testing tillage regimes, crop rotations were
225 paired only if they shared the same tillage regime. For sites without consistent tillage regimes,
226 tillage regimes could differ between treatment and control in pair-wise comparisons. This
227 approach allowed us to avoid an over-representation of pair-wise comparisons from sites that
228 tested crop rotation diversity replicated across multiple N fertilizer levels or tillage regimes.
229 When exploring whether N fertilizer could mediate the effect of crop rotation on SOC, however,
230 we included all possible pair-wise comparisons at N gradient sites.

231

232

233 *Carbon input*

234

235 Only 22% of cropping system sites reported C input parsed by root and shoot, or crop yields, so
236 to estimate C input ($\text{Mg C ha}^{-1} \text{ yr}^{-1}$), we used crop yield data from FAO coupled with C
237 allocation coefficients (Appendix S1, Table S2). We used crop yields specific to each site's
238 country, averaged over 1994-2014, unless crop yields were not reported, in which case we used
239 an average of crop yields from all other countries. To convert yield to dry matter we multiplied
240 crop yield by 0.85 for cereals (Ellis and Roberts 1980), forages (AARD, 2016), and legume
241 grains other than soybean (Roberts and Abdalla 1968), and by 0.87 for soybean (Iowa State
242 University Extension 2008). Crop biomass ($\text{Mg dry matter ha}^{-1}$) was assumed to be 45% C.
243 Carbon input of shoots, roots, and root exudates were estimated using allocation coefficients
244 (Voisin et al. 2002, Ozpinar and Baytekin 2006, Bolinder et al. 2007, Munoz-Romero et al. 2011,
245 Ju et al. 2015); see Appendix S1, Table S2. C input from grain crops was estimated as the sum of

246 C from shoot residues (total aboveground C – harvested C), roots, and root exudates; C input of
247 hays and forages was estimated as the sum of root and root exudate C; and C input from cover
248 crops was estimated as whole plant C input (aboveground biomass, roots, and root exudate C).
249 For crops that were harvested for both grain and residue (e.g., corn harvested for both grain and
250 stover), we estimated C input as root and exudate C. Some perennial crops in the database were
251 present for more than one year, however we counted standing root biomass as C input only once.
252 To include root exudates over multiple growing seasons, we estimated that root exudates in years
253 after the first were equal to those in the first year (65% of standing root biomass (Bolinder et al.,
254 2007)). To calculate average C input over a rotation cycle ($\text{Mg C ha}^{-1} \text{ yr}^{-1}$), we summed C input
255 for each crop and divided by the number of years in the rotation cycle.

256

257 *Statistical analyses*

258 Three broad (“pooled”) crop rotation typologies (Table 2) representing different combinations of
259 crop functional groups emerged during data analysis: 1) “grain-only” rotations that had only
260 harvested, annual grains; 2) “cover cropped” rotations that included a cover crop in rotation with
261 harvested, annual grains, and 3) “perennial cropped” rotations that included a harvested perennial
262 crop in rotation with harvested, annual grains. Within the “grain-only” typology, we identified
263 narrower rotation typologies that were composed only of cereal grains (“cereals”), or those that
264 included both legume grains and cereals (“cereals + legume grains”). Rotations of only legume
265 grains were not adequately reported to be included as an independent category. The “cover
266 crop” and “perennial crop” functional groups were not sufficiently represented in the database to
267 compare levels of species diversity within them for their effects on SOC or C input.

268

269 We tested three sets of control-treatment comparisons. In all instances, the control rotation was
270 the less diverse rotation. To test the effect of crop rotation functional diversity on SOC
271 concentrations and C input, we used: i) “cereal” – “cereal + legume grain”, ii) “grain-only” –
272 “cover cropped”, and iii) “grain-only” – “perennial cropped”. A relatively low proportion of
273 cropping system experiments in our dataset included rotations with cover crops or perennial
274 crops. We therefore compared “cover crop” and “perennial crop” to a more inclusive control
275 typology of “grain-only” rotations (Table 2), which included both “cereal” and “cereal + legume
276 grain” typologies. To test for the effects of species diversity on SOC concentrations and C input,

277 we split the “grain-only” group into constituent levels of species diversity and used i) “one
278 species” – “two species” and ii) “two species” – “three species”. There was insufficient data to
279 test one versus three species. To test whether N fertilizer inputs mediated the effect of functional
280 diversity on SOC, we used two main control-treatment groups: i) “cereal” – “cereal + legume
281 grain”, and ii) “grain-only” – “cover cropped or perennial rotations”. Cover cropped and
282 perennial cropped rotations exercised a similar effect on SOC concentrations, and combining
283 these categories, both with small sample sizes, allowed a more robust assessment of the
284 mediating effects of N fertilizer. Within each of these two broad groups, we parsed observations
285 into low N rates (both treatment and control groups received less than or equal to $75 \text{ kg N ha}^{-1} \text{ yr}^{-1}$)
286 and high N rates (both treatment and control groups received more than $75 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). We
287 included all possible pair-wise comparisons at N gradient sites.

288
289 We explored the response of SOC to species and functional group diversity by calculating an
290 effect size using the response ratio of treatment to control ($r = SOC_t / SOC_c$). We log
291 transformed response ratios ($R = \ln(r) = \ln(SOC_t) - \ln(SOC_c)$) in order to perform the meta-
292 analysis on normally distributed data. Although weighted meta-analysis provides a more
293 rigorous assessment of treatment differences by assigning greater weight to observations with
294 larger sample sizes and correspondingly lower variances (Gurevitch and Hedges 1999), very few
295 studies reported any measure of variance for SOC. We therefore performed an unweighted
296 meta-analysis, in which the variance assigned to all response ratios was 1. Around the mean
297 effect size for each control-treatment comparison, we created bias-corrected bootstrapped 95%
298 confidence intervals with 5,000 iterations using MetaWin software Version 2.0 (Rosenburg et al.
299 1997). We recognize non-independence of observations from the same site; however, beyond
300 selecting a subset of N rates and tillage practices for some sites, we did not exclude observations
301 from the database. Instead, when sites were well-represented for a given pair-wise comparison,
302 we compared the within-group effect size for that site to the cumulative effect size for that
303 comparison, excluding observations from the well-represented site. Since no differences in
304 effect sizes were found, we proceeded with inclusion of all observations to construct
305 bootstrapped confidence intervals. We considered a treatment effect to be significant if its 95%
306 CI did not overlap zero, and treatments to be significantly different from each other if their 95%
307 CIs did not overlap (Gurevitch and Hedges 1999).

308

309 We used multiple linear regression to explore whether the effect of crop rotation diversity on
310 SOC was mediated by site-specific environmental factors. We used forward selection of
311 independent variables: latitude (absolute values, used as a proxy for climate (Kallenbach and
312 Grandy 2011)), percent clay content, elevation, mean SOC concentration at the site, pH, years
313 elapsed between sampling and the beginning of the trial, and greatest depth sampled above
314 30cm, and we used site as a random effect. We ran the mixed effects model using the *lmer*
315 function in the R package lme4 (Bates et al. 2014). We used the response ratios of SOC as the
316 independent variable, and we used two categories of control-treatment pairs: i) “cereals” –
317 “cereal + legume grain”, and ii) “grain-only” – “cover cropped or perennial” rotations. Models
318 were compared using Akaike Information Criterion (AIC) values, with lower AIC values
319 indicating the preferred model.

320 **Results**

321 Our search returned 563 hits, and of these, 49 publications reporting on 27 cropping system
322 studies met the criteria for inclusion in our analysis. Most cropping system experiments were
323 located in North America (63%) with others spread across South America, Europe, Australia,
324 and Asia (Table 3). Forty-one percent of cropping system experiments only tested rotations of
325 annual harvested grains, while a subset of experiments tested the inclusion of cover crops (37%)
326 or perennials (30%). The experimental sites focused heavily on three major grain species: corn,
327 soybean (a grain legume), and wheat. Of the 167 crop rotations in the database, only 13 did not
328 include one of these species. The median time from site establishment to soil sampling for the
329 database was 14 years.

330

331 The most effective crop rotations for increasing SOC concentrations were those that included the
332 functional groups *perennial crop* or *cover crop*: relative to crop rotations of only grains,
333 perennial cropped and cover cropped rotations increased SOC concentrations by 6.2 and 12.5%
334 respectively (Figure 1). Within grain-only rotations, however, including a legume grain in
335 rotation with cereal grains decreased SOC concentrations by 5.3% compared to rotations of only
336 cereal grains (Figure 1). Increasing the species diversity of grain-only rotations without adding
337 perennial crops or cover crops had no detectable effect on SOC concentrations (Figure 2).

338

339 The negative effect of legume grains on SOC concentrations was not strongly mediated by the
340 species of cereal grain in the control rotation. Cereal + legume grain rotations, when compared to
341 either a corn monoculture or to a wheat monoculture, did not differ significantly in their SOC
342 concentrations (Figure 3). Compared to grain-only rotations that included multiple species of
343 cereal grains (“miscellaneous”), incorporating legume grains into rotations decreased SOC
344 concentrations by 7.5% (Figure 3; “misc grain” versus cereal + legume).

345

346 Crop rotations with functional groups of crops that increased total C input (cover cropped and
347 perennial cropped rotations) increased SOC concentrations relative to a control (Figure 1).
348 Similarly, crop rotations that decreased C input relative to control corresponded to a decrease in
349 SOC concentrations (Figure 1). Changes in shoot C input between control-treatment
350 comparisons did not correspond to changes in SOC (Figure 1), but changes in root C input
351 between pairwise comparisons did correspond to changes in SOC. Increasing the species
352 diversity of grain-only rotations had no effect on total or shoot C input, and was associated with
353 a only a small increase in root C input compared to the increase observed under cover cropped
354 and perennial cropped rotations (Figure 2).

355

356 Cover cropped and perennial cropped rotations increased SOC relative to grain-only rotations
357 regardless of their N fertilizer rates, however their mean effect was slightly greater when N
358 fertilizer rates were low ($\leq 75 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, Figure 4). Cereal + legume grain rotations
359 decreased SOC more under high N fertilizer rates compared to low N fertilizer rates (Figure 4).

360

361 For the pair-wise comparison of cereal versus cereal + legume grain rotations, all environmental
362 moderators were retained during model selection, however, only elevation, latitude, and site-
363 level SOC concentrations significantly moderated the negative effect of legume grains on SOC
364 (Appendix S1, Table S3, marginal $R^2 = 0.16$). At higher elevations, further from the equator, and
365 at higher site-level SOC concentrations, incorporating legume grains into a cereal grain rotation
366 had less of a negative effect on SOC concentrations (Appendix S1, Table S3). In contrast, no
367 environmental variables were found to moderate the positive effect of perennial crops or cover
368 crops on SOC (Appendix S1, Table S4, marginal $R^2 = 0.06$).

369

370

371 **Discussion**

372

373 Prescriptions for multifunctional agriculture trend toward an overarching principle: increase
374 biodiversity. While compelling in its simplicity, this prescription will be most effectively
375 operationalized if biodiversity is categorized into functional groups or traits relevant to the
376 ecosystem functions, or services, of interest (Petchey and Gaston 2006, Jackson et al. 2007,
377 Finney and Kaye 2016). In our analysis of agroecosystems, we extended two common plant
378 functional types (legume or grass, and annual or perennial) to include a functional type
379 controlled by management, designating each crop as either a harvested crop or as a cover crop.
380 Although plant functional traits are considered better predictors of ecosystem function than are
381 groups (Martin and Isaac 2015, Wood et al. 2015), most studies in our database did not report
382 individual plant traits. Nevertheless, the functional groups defined here capture key plant traits
383 of interest to SOC accrual, i.e., total and root C input to soil. Designating crop functional groups
384 allowed us to delineate typologies of grain-based crop rotations according to their functional
385 diversity. We found that increasing the richness of functional groups in crop rotations was only
386 sufficient for increasing SOC levels when certain functional groups were included: perennial
387 crops and cover crops. Compared to grain-only rotations, cover cropped rotations increased
388 SOC by 6.3%, corresponding to approximately 2.9 Mg C ha⁻¹, while perennial cropped rotations
389 increased SOC by 12.5%, corresponding to approximately 5.7 Mg C ha⁻¹ in the top 20 cm of soil.
390 Within grain-only rotations, diversifying a rotation of cereal grains by adding legume grains
391 decreased SOC levels. The functional groups of crops effective for increasing SOC, cover crops
392 and perennial crops, were associated with increases in total and root C input to soil.

393

394 Do relative levels of C input between cropping systems predict their effects on SOC? Although
395 the bootstrapped confidence intervals from our meta-analysis reveal a general trend affirming
396 this relationship, there were noteworthy exceptions: in 16% of observations, decreases in C input
397 were accompanied by increases in SOC, as has been found previously (Gregorich et al. 2001,
398 Kallenbach et al. 2015). These observations were located at older sites, with an average site age
399 of 23 years, compared to 13 years for observations in which changes in C input corresponded to

400 changes in SOC, or 11 years for the 16% of observations in which SOC decreased despite
401 increases in C input. Indeed, it is increasingly recognized that a complex suite of interacting
402 phenomena govern the residence time of C inputs to soil (Schmidt et al. 2011), including plant
403 tissue quality (Cotrufo et al. 2013), microbial community growth efficiencies (Bradford et al.
404 2013, Kallenbach et al. 2015), oxygen availability (Keiluweit et al. 2016), soil temperature (Frey
405 et al. 2013), and bulk density (Wang et al. 2015). When diversifying crop rotations, many if not
406 all of these features necessarily vary in tandem. Increasing the functional group diversity of crop
407 rotations can decrease the quantity but increase the quality of C inputs (e.g., from a wheat –
408 wheat to a wheat – pea rotation), or may entail changing the frequency or intensity of disturbance
409 via tillage (Crews et al. 2016, King and Hofmockel 2017). Studies included in our database did
410 not report the necessary information to test to what extent these other mechanisms are
411 responsible for changes in SOC between diverse and less diverse crop rotations. Studies only
412 rarely reported yield data, so we made conservative use of country-level FAO data by restricting
413 our analysis to estimates of relative levels of C input within sites. Therefore, our analysis
414 provides grounds for broad generalizations about the relationship between crop rotation
415 diversity, C input, and SOC.

416
417 Cover crops and perennials were both associated with increases in SOC (Figure 1), concomitant
418 with increases in total C input, which in many cases were facilitated by increased time of living
419 soil cover, or perenniality. Adding a cover crop to a crop rotation – all other features of the
420 rotation being equal – uses a niche for primary production that would have otherwise been
421 fallow, increasing both shoot and root C input above the grain-only rotation. Similarly, a
422 perennial crop, while sometimes replacing a grain crop, can use the beginning and end of
423 temperate growing seasons that most grain crops, excluding winter wheat, would not harness for
424 plant growth. Although the perennial cropped rotations returned less shoot C to soil than the
425 grain-only rotations did, they overcame the difference with increased root C inputs (Figure 1),
426 which include both root biomass and root exudate inputs. Root C inputs were likely responsible
427 for the increase in SOC reported here, as they have elsewhere been shown to have stronger
428 effects on SOC accumulation than shoot C inputs (Puget and Drinkwater 2001, Rasse et al.
429 2005). Our estimation of shoot C input from perennial hay crops, based on Bolinder (2007), is
430 likely an underestimate, as perennial crops in different experimental sites were hayed

431 inconsistently during the first season of growth (e.g., Porter et al., 2006; Robinson, 1996). Both
432 perennial and cover crops capitalize on windows of time that would have otherwise been
433 unproductive, thereby increasing total C input to soil. Globally, there is enormous biological
434 potential to leverage these windows of time for plant growth, as most of the world's croplands
435 produce one or fewer harvests per year (Siebert et al. 2010), which, in grain-based cropping
436 systems, leaves half or more of the year unused for primary productivity and potential C input to
437 soil.

438
439 It is possible that the increase in SOC with perennial cropping systems could be due in part to
440 decreases in tillage frequency (West and Post 2002) that may occur under perennial cropping.
441 We were not able to test for this effect in our analysis, however: due to the inconsistent reporting
442 of tillage regimes in the literature, we relied on two broad categories (till and no-till), which
443 could mask variability in tillage frequency. Thus, although studies in the database compared
444 annual grain and perennial cropped systems across ostensibly similar tillage regimes (by chance
445 rather than design; see methods in *Data Collection and Compilation*), perennial cropped systems
446 were in most cases subject to lower tillage frequency than annual grain cropping systems. At two
447 sites, however, both perennial cropped and annual grain systems were no-till (Basamba et al.
448 2006, dos Santos et al. 2011). It is also possible that tillage frequency has little direct effect on
449 SOC. Deeper sampling than used in initial tillage studies (down to 40cm) generally reveals that
450 reducing tillage frequency redistributes but does not significantly increase SOC throughout the
451 soil profile (Baker et al. 2007, Blanco-canqui and Lal 2008, Luo et al. 2010).

452
453 Within grain-only rotations, the transition from a rotation of only cereal grains to a rotation
454 including both cereal grains and legume grains, often, but not always, represents a tradeoff
455 between quantity and quality of C input. Legume grains produce less biomass than do most
456 cereals (Monfreda et al. 2008, Iizumi et al. 2014) and return correspondingly less crop residue to
457 soil. Given that many legume crops also have higher quality tissues than do cereals (Johnson et
458 al. 2007, NRCS 2011), their tissues could be expected to be more efficiently stabilized in soil
459 due to increases in microbial substrate use efficiency (Cotrufo et al. 2013, Castellano et al.
460 2015), potentially counteracting the effects of their lower residue quantity and enabling them to
461 maintain SOC levels similar to or greater than cereal-only cropping systems. In our meta-

462 analysis, however, grain-only cropping systems with legume grains maintained mean SOC levels
463 slightly below those of cereal-only cropping systems. The stabilization efficiencies of litter may
464 still differ in the direction predicted by this framework, however it may not be adequate to
465 counteract the differences in total C input.

466
467 The crop functional groups used in this analysis encompassed considerable variability in average
468 crop NPP. For instance, both wheat and corn were classified as cereal grains, even though wheat
469 contributed on average 17% less C input than did corn. When comparing cereal grain rotations
470 to cereal + legume grain rotations, however, the effect of legume grains in decreasing SOC was
471 comparable regardless of the cereal grain in the control rotation (Figure 3). Similarly, cover
472 crops and perennials were compared to grain-only rotations that could include either cereals or
473 legume grains. Cover crops and perennial crops increased SOC concentrations relative to grain-
474 only control even when the control included a legume grain in rotation (data not shown),
475 suggesting that cover crops and perennial crops can counteract the neutral or negative effect of
476 annual legume grains on SOC levels.

477
478 In general, crop rotation functional group diversity had a stronger effect on SOC than did N
479 fertilizer levels (Figure 4). At low N fertilizer rates, however, grain-only rotations that included a
480 legume grain were more similar in SOC concentrations to cereal grain rotations. This may be due
481 to poor productivity and low C input of the cereal grain rotation at low N fertilizer levels.
482 Simultaneously, in the cereal + legume grain rotation, consistent with the stress gradient
483 hypothesis (Brooker et al. 2005, Li et al. 2007), the legume grain may increase cereal yield
484 relative to cereal only rotations. Indeed, at zero N fertilizer, corn yields from a corn-soy rotation
485 can double those of a corn-corn rotation (Shah et al. 2003, Mallarino and Ortiz-Torres 2006,
486 Stanger and Lauer 2008). Among the perennial and cover cropped rotations, almost all (95%)
487 included leguminous species exclusively or in mixtures, suggesting that at low N fertilizer rates,
488 they may increase production of residue from grain crops relative to those of the grain-only
489 control by providing a N source. This facilitative interaction could explain the slightly greater
490 effect of (mostly leguminous) perennial crops and cover crops on SOC at low N fertilizer levels
491 (Figure 4).

492

493 Within grain-only rotations, we found no effect of increasing species diversity on SOC (Figure
494 2), consistent with McDaniel et al. (2014). All other features of a rotation being equal,
495 exchanging one species of annual grain for another has limited potential to increase SOC levels
496 despite differences among species in total NPP. Previous studies have explored whether, within
497 grain-only rotations, increasing the frequency of cropping (e.g., reducing fallow time) can
498 increase SOC levels. At some sites, decreasing fallow increased SOC (Gan et al. 2012, Yang et
499 al. 2012, Shrestha et al. 2013), however a synthesis by West and Post (2002) of 11 paired
500 comparisons found no effect of decreasing fallow time on SOC. Our results were not likely
501 confounded by grain cropping frequency since the grain-only cropping systems in our database
502 were relatively homogeneous: more than 80% of grain-only rotations had only one crop grown
503 per year. Although our database did not allow us to compare the effect of increasing cropping
504 frequency in annual grain systems relative to other practices, we speculate that increasing
505 cropping frequency of annual grain crops likely has less potential to remediate SOC levels than
506 do cover crops and perennials, due to grain crops' lower total C return compared to cover crops
507 and lower investment in root tissue compared to perennial crops.

508
509 Agricultural soils, by virtue of the quantity of C they contain, represent a potential leverage point
510 by which to manage the C cycle and mitigate global climate change (Paustian et al. 2016).
511 Nevertheless, predicting the potential of different cropping systems to mitigate climate change
512 will require an integrated approach. The impact of cropping systems on SOC needs to be
513 integrated with the greenhouse gas footprint of their associated agricultural practices, including
514 those used to power tillage operations and manufacture agrochemical inputs. A cropping system
515 with a slight negative effect on SOC, such as cereal + legume grain compared to cereal only,
516 may yet be associated with greater climate change mitigation potential if the inclusion of a
517 legume grain sufficiently reduces the use of energy-intensive synthetic N fertilizer. Conversely, a
518 cover crop, if it increases emissions of N₂O, a powerful greenhouse gas, from soil (Basche et al.
519 2014), may offset some of the climate change mitigation potential it achieves through soil C
520 storage. A change in existing cropping systems may also lead to indirect effects on soil C storage
521 via land use change. For instance, substituting perennial crops for annual grains could cause an
522 increase in market prices for grain crops and subsequent expansion of annual grain cropping
523 systems into previously uncultivated land (*sensu* Lark et al., (2015); Plevin et al., (2010)). While

524 any cropping system can give rise to trade-offs vis-à-vis its climate change mitigation potential,
525 leguminous perennial crops and cover crops are particularly promising because they have the
526 potential to both increase SOC levels and to reduce the need for synthetic N fertilizer.

527
528 Functionally diverse crop rotations need to also be considered for the broad suite of benefits they
529 offer compared to grain-only crop rotations. Functionally diverse crop rotations create
530 variability in planting and harvesting times, and therefore in the availability of resources for
531 weeds and pests, which can interfere with weed and pest cycles and reduce the need for herbicide
532 and pesticide applications (Onstad et al. 2003, Davis et al. 2012). Crop rotations with functional
533 groups that extend the time of living soil cover can also enhance agroecosystem N retention and
534 reduce nitrate losses from soil via leaching (Randall et al. 1997, Tonitto et al. 2006, Syswerda et
535 al. 2012, Blesh and Drinkwater 2013). As a result, crop rotations with high phenological
536 diversity and perennality – that is, rotations that better mimic the natural grasslands that grain
537 agriculture usually supplants – are likely best equipped to supply a broad suite of ecosystem
538 services.

539
540 Our synthesis revealed opportunities to advance both testing and reporting of crop rotations for
541 SOC accrual. First, we found relatively few cropping system sites testing the typologies of crop
542 rotations – cover cropped and perennial cropped – able to provide some of the greatest
543 environmental benefits in terms of SOC storage. Due to this low testing frequency, we
544 aggregated crop functional types (legume, grass) within perennial and cover cropped rotations,
545 however, it may be that plant functional diversity in mixed species cover crops and perennial
546 crops can influence their provisioning of ecosystem services (Smith et al. 2014, Storkey et al.
547 2015, White et al. 2017). Cover crops and perennial crops are better-suited to growing in species
548 mixtures than are grain crops, leaving open the possibility for leveraging functional diversity in
549 mixed-species cover crops or perennial forages to provide SOC storage and other environmental
550 services (Wortman et al. 2012, Finney and Kaye 2016, Blesh 2017). Second, we were able to
551 estimate N inputs from synthetic N fertilizer, but not N inputs from biological N fixation, leaving
552 unexplored the role that legume N fixation may play in mediating the effect of crop functional
553 diversity on SOC. Such effects may be difficult to isolate, however, as N input from leguminous
554 fixation would be confounded with plant tissue quantity and quality. Finally, we relied on crop

555 functional groups that only roughly captured plant traits of interest for SOC accrual. In the
556 future, species-specific reporting of plant traits, including tissue biochemistry, could allow for
557 more specific prescriptions about the features of crop rotation functional diversity most effective
558 for increasing SOC.

559

560

561 **Conclusions**

562

563 More diverse crop rotations have the potential to provide a broad suite of ecosystem services
564 compared to biologically simplified grain-based agroecosystems, including increasing SOC. Our
565 results indicate that a framework to link crop rotation diversity to SOC should articulate how C
566 input changes with changes in the functional diversity of crop rotations. In addition to commonly
567 used functional types for crops (annual or perennial, grass or legume), we included a
568 management component to functional diversity: harvested or non-harvested (cover crops), to
569 capture the effect of harvest practices on crop C input. The most effective crop rotations for
570 increasing SOC concentrations relative to grain-only rotations were those that included a
571 perennial crop or a cover crop. Perennial cropped and cover cropped rotations performed
572 similarly in their ability to increase SOC, and their effects were more pronounced in low N input
573 systems. Perennial crops, by increasing root C input over the rotation cycle, provide C input in a
574 form known to be most readily stabilized in soil. Cover crops, by definition, return all of their
575 biomass to soil, increasing both shoot and root C input compared to rotations with only harvested
576 annual grains. In most systems, cover crops and perennial crops can also harness niches of time
577 at the beginning and end of the growing season, increasing the available time for primary
578 productivity. For crop rotations to increase soil C, multiple complementary heuristics can guide
579 discussion: increasing functional diversity with cover crops and perennials, increasing C input,
580 especially root C input, and increasing perenniality, the time of living soil cover.

581 **Acknowledgments**

582 The authors would like to thank two anonymous reviewers for feedback that improved the
583 manuscript. This research was supported by the School for Environment and Sustainability at the
584 University of Michigan.

585

586 **Literature Cited**

- 587 AARD (Alberta Agriculture and Rural Development). 2016. Bale Moisture - Frequently Asked
588 Questions. [http://www1.agric.gov.ab.ca/\\$department/deptdocs.nsf/all/faq9757](http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/faq9757).
- 589 Alvarez, R. 2005. A review of nitrogen fertilizer and conservation tillage effects on soil organic
590 carbon storage. *Soil Use and Management* 21:38–52.
- 591 Anderson-Teixeira, K. J., M. D. Masters, C. K. Black, M. Zeri, M. Z. Hussain, C. J. Bernacchi,
592 and E. H. DeLucia. 2013. Altered Belowground Carbon Cycling Following Land-Use
593 Change to Perennial Bioenergy Crops. *Ecosystems* 16:508–520.
- 594 Baker, J. M., T. E. Ochsner, R. T. Venterea, and T. J. Griffis. 2007. Tillage and soil carbon
595 sequestration—What do we really know? “Agriculture, Ecosystems and Environment”
596 118:1–5.
- 597 Basamba, T. A., E. Barrios, E. Amézquita, I. M. Rao, and B. R. Singh. 2006. Tillage effects on
598 maize yield in a Colombian savanna oxisol: Soil organic matter and P fractions. *Soil &
599 Tillage Research* 91:131–142.
- 600 Basche, A. D., F. E. Miguez, T. C. Kaspar, and M. J. Castellano. 2014. Do cover crops increase
601 or decrease nitrous oxide emissions? A meta-analysis. *Journal of Soil and Water
602 Conservation* 69:471–482.
- 603 Bates, D., M. Mächler, B. Bolker, and S. Walker. 2014. Fitting Linear Mixed-Effects Models
604 using lme4. eprint arXiv:1406.5823 67:51.
- 605 Blanco-canqui, H., and R. Lal. 2008. No-Tillage and Soil-Profile Carbon Sequestration : An On-
606 Farm Assessment. *Soil Science Society of America Journal* 72:693–701.
- 607 Blesh, J. 2017. Functional traits in cover crop mixtures: biological nitrogen fixation and
608 multifunctionality. *Journal of Applied Ecology*.
- 609 Blesh, J., and L. E. Drinkwater. 2013. The impact of nitrogen source and crop rotation on
610 nitrogen mass balances in the Mississippi River Basin. *Ecological Applications* 23:1017–
611 1035.
- 612 Bolinder, M. A., H. H. Janzen, E. G. Gregorich, D. A. Angers, and A. J. VandenBygaart. 2007.
613 An approach for estimating net primary productivity and annual carbon inputs to soil for
614 common agricultural crops in Canada. *Agriculture, Ecosystems and Environment* 118:29–

- 615 42.
- 616 Booth, M. S., J. M. Stark, and E. Rastetter. 2005. Controls on nitrogen cycling in terrestrial
617 ecosystems: a synthetic analysis of literature data. *Ecological Monographs* 75:139–157.
- 618 Bradford, M. A., A. D. Keiser, C. A. Davies, C. A. Mersmann, and M. S. Strickland. 2013.
619 Empirical evidence that soil carbon formation from plant inputs is positively related to
620 microbial growth. *Biogeochemistry* 113:271–281.
- 621 Brooker, R., Z. Kikvidze, F. I. Pugnaire, R. M. Callaway, P. Choler, C. J. Lortie, and R.
622 Michalet. 2005. The importance of importance. *Oikos* 109:63–70.
- 623 Cadotte, M. W., K. Carscadden, and N. Mirotchnick. 2011. Beyond species: Functional diversity
624 and the maintenance of ecological processes and services. *Journal of Applied Ecology*
625 48:1079–1087.
- 626 Campbell, C. A., and W. Souster. 1982. Loss of organic matter and potentially mineralizable
627 nitrogen from Saskatchewan soils due to cropping. *Canadian Journal of Soil Science*
628 65:651–656.
- 629 Castellano, M. J., K. E. Mueller, D. C. Olk, J. E. Sawyer, and J. Six. 2015. Integrating plant litter
630 quality, soil organic matter stabilization, and the carbon saturation concept. *Global Change*
631 *Biology* 21:3200–3209.
- 632 Chen, Y., S. Tessier, and J. Rouffignat. 1998. Soil bulk density estimation for tillage systems and
633 soil textures. *Transactions of the ASABE* 41:1601–1610.
- 634 Conti, G., and S. Díaz. 2013. Plant functional diversity and carbon storage - an empirical test in
635 semi-arid forest ecosystems. *Journal of Ecology* 101:18–28.
- 636 Cotrufo, M. F., M. D. Wallenstein, C. M. Boot, K. Denef, and E. Paul. 2013. The Microbial
637 Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition
638 with soil organic matter stabilization: do labile plant inputs form stable soil organic matter?
639 *Global Change Biology* 19:988–995.
- 640 Crews, T. E., J. Blesh, S. W. Culman, R. C. Hayes, E. S. Jensen, M. C. Mack, M. B. Peoples, and
641 M. E. Schipanski. 2016. Going where no grains have gone before: From early to mid-
642 succession. *"Agriculture, Ecosystems and Environment"* 223:223–238.
- 643 David, M. B., L. E. Drinkwater, and G. F. McIsaac. 2010. Sources of Nitrate Yields in the
644 Mississippi River Basin. *Journal of Environment Quality* 39:1657.
- 645 Davis, A. S., J. D. Hill, C. A. Chase, A. M. Johanns, and M. Liebman. 2012. Increasing

646 Cropping System Diversity Balances Productivity, Profitability and Environmental Health.
647 PLoS ONE 7:e47149.

648 Ellis, R., and E. Roberts. 1980. The influence of temperature and moisture on seed viability
649 period in Barley (*Hordeum distichum* L.). *Annals of Botany* 45:31–37.

650 FAOSTAT. 2013. Food and Agriculture Organization of the United Nations.

651 Finney, D. M., and J. P. Kaye. 2016. Functional diversity in cover crop polycultures increases
652 multifunctionality of an agricultural system. *Journal of Applied Ecology*.

653 Fornara, D. A., and D. Tilman. 2008. Plant Functional Composition Influences Rates of Soil
654 Carbon and Nitrogen Accumulation. *Journal of Ecology* 96:314–322.

655 Frey, S. D., J. Lee, J. M. Melillo, and J. Six. 2013. The temperature response of soil microbial
656 efficiency and its feedback to climate. *Nature Publishing Group* 3:1–4.

657 Gan, Y., C. Liang, C. A. Campbell, R. P. Zentner, R. L. Lemke, H. Wang, and C. Yang. 2012.
658 Carbon footprint of spring wheat in response to fallow frequency and soil carbon changes
659 over 25 years on the semiarid Canadian prairie. *European Journal of Agronomy* 43:175–
660 184.

661 Gregorich, E. G., C. F. Drury, and J. A. Baldock. 2001. Changes in soil carbon under long-term
662 maize in monoculture and legume-based rotation. *Canadian Journal of Soil Science* 81:21–
663 31.

664 Guo, L. B., and R. M. Gifford. 2002. Soil carbon stocks and land use change: a meta analysis .
665 *Global Change Biology* 8:345–360.

666 Gurevitch, J., and L. Hedges. 1999. Statistical Issues in Ecological Meta-Analyses. *Ecology*
667 80:1142–1149.

668 Harper, J. L., and D. L. Hawksworth. 1994. Biodiversity: measurement and estimation Preface.
669 *Philosophical Transactions: Biological Sciences Biodiversity: Measurement and Estimation*
670 345:5–12.

671 Heathcote, A. J., C. T. Filstrup, and J. A. Downing. 2013. Watershed Sediment Losses to Lakes
672 Accelerating Despite Agricultural Soil Conservation Efforts. *PLoS ONE* 8:e53554.

673 Hector, A., B. Schmid, C. Beierkuhnlein, M. C. Caldeira, M. Diemer, P. G. Dimitrakopoulos, J.
674 A. Finn, H. Freitas, P. S. Giller, J. Good, R. Harris, P. Hogberg, K. Huss-Danell, J. Joshi, A.
675 Jumpponen, C. Korner, P. W. Leadley, M. Loreau, A. Minns, C. P. H. Mulder, G.
676 O'Donovan, S. J. Otway, J. S. Pereira, A. Prinz, D. J. Read, M. Scherer-Lornzen, E. D.

677 Schulze, A. S. D. Siamantziouras, E. M. Spehn, A. C. Terry, and A. Y. Troumbis. 2001.
678 Plant diversity and productivity experiments in European Grasslands. *Science*:1–6.

679 Hooper, D. U., F. S. I. I. I. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton,
680 D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, J. Setälä, A. J. Symstad, J. Vandermeer,
681 and D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: a consensus of
682 current knowledge:3–35.

683 Hudson, B. D. 1994. Soil organic matter and available water capacity. *Journal of Soil and Water*
684 *Conservation* 49:189–194.

685 Iizumi, T., M. Yokozawa, G. Sakurai, M. I. Travasso, V. Romanerikov, P. Oettli, T. Newby, Y.
686 Ishigooka, and J. Furuya. 2014. Historical changes in global yields: Major cereal and
687 legume crops from 1982 to 2006. *Global Ecology and Biogeography* 23:346–357.

688 Iowa State University Extension. 2008. Soybean Drying and Storage.

689 Izaurralde, R. C., W. B. McGill, J. A. Robertson, N. G. Juma, and J. J. Thurston. 2001. Carbon
690 balance of the Breton Classical plots over a half a century. *Soil Sci. Soc. Am. J.*:431–441.

691 Jackson, L. E., U. Pascual, and T. Hodgkin. 2007. Utilizing and conserving agrobiodiversity in
692 agricultural landscapes. “Agriculture, Ecosystems and Environment” 121:196–210.

693 Jian, S., J. Li, J. Chen, G. Wang, M. A. Mayes, K. E. Dzantor, D. Hui, and Y. Luo. 2016. Soil
694 extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization:
695 A meta-analysis. *Soil Biology and Biochemistry* 101:32–43.

696 Johnson, D. W., and P. S. Curtis. 2001. Effects of forest management on soil C and N storage :
697 meta analysis 140.

698 Johnson, J. M. F., N. W. Barbour, and S. L. Weyers. 2007. Chemical Composition of Crop
699 Biomass Impacts Its Decomposition. *Soil Science Society of America Journal* 71:155.

700 Ju, C., R. J. Buresh, Z. Wang, H. Zhang, L. Liu, J. Yang, and J. Zhang. 2015. Root and shoot
701 traits for rice varieties with higher grain yield and higher nitrogen use efficiency at lower
702 nitrogen rates application. *Field Crops Research* 175:47–55.

703 Kallenbach, C., and A. S. Grandy. 2011. Controls over soil microbial biomass responses to
704 carbon amendments in agricultural systems: A meta-analysis. *Agriculture, Ecosystems and*
705 *Environment* 144:241–252.

706 Kallenbach, C. M., A. S. Grandy, S. D. Frey, and A. F. Diefendorf. 2015. Microbial physiology
707 and necromass regulate agricultural soil carbon accumulation. *Soil Biology and*

708 Biochemistry 91:279–290.

709 Keiluweit, M., P. S. Nico, M. Kleber, and S. Fendorf. 2016. Are oxygen limitations under
710 recognized regulators of organic carbon turnover in upland soils ? Biogeochemistry
711 127:157–171.

712 King, A. E., and K. S. Hofmockel. 2017. Diversified cropping systems support greater microbial
713 cycling and retention of carbon and nitrogen. Agriculture, Ecosystems & Environment
714 240:66–76.

715 Kong, A. Y. Y., and J. Six. 2010. Tracing Root vs. Residue Carbon into Soils from Conventional
716 and Alternative Cropping Systems. Soil Science Society of America Journal 74:1201–1210.

717 Kong, A. Y. Y., J. Six, D. C. Bryant, R. F. Denison, and C. van Kessel. 2005. The Relationship
718 between Carbon Input, Aggregation, and Soil Organic Carbon Stabilization in Sustainable
719 Cropping Systems. Soil Science Society of America Journal 69:1078–1085.

720 Lal, R. 2004a. Soil Carbon Sequestration Impacts on Global Climate Change and Food Security.
721 Science 304:1623–1627.

722 Lal, R. 2004b. Soil carbon sequestration to mitigate climate change. Geoderma 123:1–22.

723 Lark, T. J., J. Meghan Salmon, and H. K. Gibbs. 2015. Cropland expansion outpaces agricultural
724 and biofuel policies in the United States. Environmental Research Letters 10:44003.

725 Lehmann, J., and M. Kleber. 2015. The contentious nature of soil organic matter. Nature.

726 Li, L., S.-M. Li, J.-H. Sun, L.-L. Zhou, X.-G. Bao, H.-G. Zhang, and F.-S. Zhang. 2007.
727 Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on
728 phosphorus-deficient soils. Proceedings of the National Academy of Sciences of the United
729 States of America 104:11192–6.

730 Luo, Z., E. Wang, and O. J. Sun. 2010. Can no-tillage stimulate carbon sequestration in
731 agricultural soils? A meta-analysis of paired experiments. Agriculture, Ecosystems and
732 Environment 139:224–231.

733 Mallarino, A. P., and E. Ortiz-Torres. 2006. A long-term look at crop rotation effects on corn
734 yield and response to nitrogen fertilization. Pages 1–92006 Integrated Crop Management
735 Conference - Iowa State University.

736 Martin, A. R., and M. E. Isaac. 2015. Plant functional traits in agroecosystems: A blueprint for
737 research. Journal of Applied Ecology 52:1425–1435.

738 McDaniel, M. D., L. K. Tiemann, and A. S. Grandy. 2014. Does agricultural crop diversity

739 enhance soil microbial biomass and organic matter dynamics? A meta-analysis. *Ecological*
740 *Applications* 24:560–570.

741 Monfreda, C., N. Ramankutty, and J. A. Foley. 2008. Farming the planet: 2. Geographic
742 distribution of crop areas, yields, physiological types, and net primary production in the
743 year 2000. *Global Biogeochemical Cycles* 22:n/a-n/a.

744 Montgomery, D. R. 2007. Soil erosion and agricultural sustainability. *PNAS* 104:13268–13272.

745 Munoz-Romero, V., L. Lopez-Bellido, and R. J. Lopez-Bellido. 2011. Faba bean root growth in
746 a Vertisol: Tillage effects. *Field Crops Research* 120:338–344.

747 Novara, A., I. Poma, M. Sarno, G. Venezia, and L. Gristina. 2016. Long-Term Durum Wheat-
748 Based Cropping Systems Result in the Rapid Saturation of Soil Carbon in the
749 Mediterranean Semi-arid Environment. *Land Degradation and Development* 619:612–619.

750 NRCS, U. 2011. Carbon to Nitrogen Ratios in Cropping Systems:1–2.

751 Onstad, D. W., D. W. Crwoder, S. A. Isard, E. Levine, J. L. Spencer, M. E. O’neal, S. T.
752 Ratcliffe, M. E. Gray, L. W. Bledsoe, C. D. Di Fonzo, J. B. Easley, and C. R. Edwards.
753 2003. Does Landscape Diversity Slow the Spread of Rotation-Resistant Western Corn
754 Rootworm (Coleoptera: Chrysomelidae)? *Environmental Entomology* 32:992–1001.

755 Ozpinar, S., and H. Baytekin. 2006. Effects of tillage on biomass, roots, N-accumulation of vetch
756 (*Vicia sativa* L.) on a clay loam soil in semi-arid conditions. *Field Crops Research* 96:235–
757 242.

758 Paustian, K., J. Lehmann, S. Ogle, D. Reay, G. P. Robertson, and P. Smith. 2016. Climate-smart
759 soils. *Nature* 532:49–57.

760 Petchey, O. L., and K. J. Gaston. 2006. Functional diversity: Back to basics and looking forward.
761 *Ecology Letters* 9:741–758.

762 Peterson, T. A., and M. P. Russelle. 1991. Alfalfa and the nitrogen cycle in the Corn Belt.
763 *Journal of Soil and Water Conservation* 46:229–235.

764 Plevin, R. J., M. O’Hare, A. D. Jones, M. S. Torn, and H. K. Gibbs. 2010. Greenhouse Gas
765 Emissions from Biofuels’ Indirect Land Use Change Are Uncertain but May Be Much
766 Greater than Previously Estimated. *Environmental Science and Technology* 44:8015–8021.

767 Porter, P., D. Allan, K. Crookston, M. Harbur, K. Olson, and D. Wyse. 2003. Organic and other
768 management strategies with two- and four-year crop rotations in Minnesota. *Agronomy*
769 *Journal* 95:233–244.

- 770 Porter, P., D. Allan, K. Crookston, M. Harbur, K. Olson, and D. L. Wyse. 2006. Overview of the
771 University of Minnesota Variable Input Crop Management Systems (VICMS) Trails:1–18.
- 772 Puget, P., and L. E. Drinkwater. 2001. Short-term dynamics of root- and shoot-derived carbon
773 from a leguminous green manure. *Soil Science Society of America Journal* 65:771–779.
- 774 Randall, G. W., D. R. Huggins, M. P. Russelle, D. J. Fuchs, W. W. Nelson, and J. L. Anderson.
775 1997. Nitrate Losses through Subsurface Tile Drainage in Conservation Reserve Program,
776 Alfalfa, and Row Crop Systems. *J. Environ. Qual.* 26:1240–1247.
- 777 Rasmussen, P. E., R. R. Allmaras, C. R. Rohde, and J. Roager N C. 1980. Crop residue
778 influence on soil carbon and nitrogen in a wheat-fallow system. *Soil Sci. Soc. Am. J.*
779 44:596–600.
- 780 Rasse, D. P., C. Rumpel, and M.-F. Dignac. 2005. Is soil carbon mostly root carbon?
781 Mechanisms for a specific stabilisation. *Plant and Soil* 269:341–356.
- 782 Roberts, E. H., and F. H. Abdalla. 1968. The Influence of Temperature, Moisture, and Oxygen
783 on Period of Seed Viability in Barley, Broad Beans, and Peas. *Annals of Botany* 32:97–117.
- 784 Robertson, G. P., T. W. Bruulsema, R. J. Gehl, D. Kanter, D. L. Mauzerall, C. A. Rotz, and C. O.
785 Williams. 2013. Nitrogen–climate interactions in US agriculture. *Biogeochemistry* 114:41–
786 70.
- 787 Robinson, C. A. 1996. Cropping system and nitrogen effects on Mollisol organic carbon. *Soil*
788 *Sci. Soc. Am. J.* 60:264–269.
- 789 Rosenburg, M. S., D. C. Adams, and J. Gurevitch. 1997. Metawin statistical software for meta-
790 analysis. Sinauer Associate, Sunderland, MA, US.
- 791 Russell, A. E., C. A. Cambardella, D. A. Laird, D. B. Jaynes, and D. W. D. W. Meek. 2009.
792 Nitrogen fertilizer effects on soil carbon balances in Midwestern U.S. agricultural systems.
793 *Ecological Applications* 19:1–13.
- 794 Sanderman, J., C. Creamer, W. T. Baisden, M. Farrell, and S. Fallon. 2016. Greater soil carbon
795 stocks and faster turnover rates with increasing agricultural productivity. *SOIL*
796 *Discussions*:1–31.
- 797 dos Santos, N. Á. colas Z., J. Dieckow, C. Bayer, R. Molin, N. Favaretto, V. Pauletti, and J. T.
798 Piva. 2011. Forages, cover crops and related shoot and root additions in no-till rotations to
799 C sequestration in a subtropical Ferralsol. *Soil & Tillage Research* 111:208–218.
- 800 Schmidt, M. W. I., M. S. Torn, S. Abiven, T. Dittmar, G. Guggenberger, I. A. Janssens, M.

801 Kleber, I. Kögel-Knabner, J. Lehmann, D. A. C. Manning, P. Nannipieri, D. P. Rasse, S.
802 Weiner, and S. E. Trumbore. 2011. Persistence of soil organic matter as an ecosystem
803 property. *Nature* 478:49–56.

804 Shah, Z., S. H. Shah, M. B. Peoples, G. D. Schwenke, and D. F. Herridge. 2003. Crop residue
805 and fertiliser N effects on nitrogen fixation and yields of legume – cereal rotations and soil
806 organic fertility 83:1–11.

807 Shrestha, B. M., B. G. McConkey, W. N. Smith, R. L. Desjardins, C. a Campbell, B. B. Grant,
808 and P. R. Miller. 2013. Effects of crop rotation, crop type and tillage on soil organic carbon
809 in a semiarid climate. *Canadian Journal of Soil Science* 93:137–146.

810 Siebert, S., F. T. Portmann, and P. Döll. 2010. Global patterns of cropland use intensity. *Remote*
811 *Sensing* 2:1625–1643.

812 Smith, R. G., L. W. Atwood, and N. D. Warren. 2014. Increased productivity of a cover crop
813 mixture is not associated with enhanced agroecosystem services. *PLoS ONE* 9.

814 Soil Survey Division Staff. 1993. *Soil Survey Manual*. Page (United States Department of
815 Agriculture, Ed.). Handbook n. U.S. Gov. Print. Off., Washington, DC.

816 Stanger, T. F., and J. G. Lauer. 2008. Corn Grain Yield Response to Crop Rotation and Nitrogen
817 over 35 Years:643–650.

818 Steinbeiss, S., H. Bebler, C. Engels, V. M. Temperton, N. Buchmann, C. Roscher, Y. Kreuziger,
819 J. Baade, M. Habekost, and G. Gleixner. 2008. Plant diversity positively affects short-term
820 soil carbon storage in experimental grasslands. *Global Change Biology* 14:2937–2949.

821 Storkey, J., T. Döring, J. Baddeley, R. Collins, S. Roderick, H. Jones, and C. Watson. 2015.
822 Engineering a plant community to deliver multiple ecosystem services. *Ecological*
823 *Applications* 25:1034–1043.

824 Syswerda, S. P., B. Basso, S. K. Hamilton, J. B. Tausig, and G. P. Robertson. 2012. Long-term
825 nitrate loss along an agricultural intensity gradient in the Upper Midwest USA. *Agriculture,*
826 *Ecosystems and Environment* 149:10–19.

827 Tang, X., S. A. Placella, F. Dayd????, L. Bernard, A. Robin, E. P. Journet, E. Justes, and P.
828 Hinsinger. 2016. Phosphorus availability and microbial community in the rhizosphere of
829 intercropped cereal and legume along a P-fertilizer gradient. *Plant and Soil* 407:119–134.

830 Tilman, D., P. B. Reich, J. Knops, D. Wedin, T. Mielke, and C. Lehman. 2001. Diversity and
831 Productivity in a Long-Term Grassland Experiment. *Science*:843–845.

- 832 Tonitto, C., M. B. David, and L. E. Drinkwater. 2006. Replacing bare fallows with cover crops in
833 fertilizer-intensive cropping systems: A meta-analysis of crop yield and N dynamics.
834 *Agriculture, Ecosystems and Environment* 112:58–72.
- 835 Virto, I., P. Barré, A. Burlot, and C. Chenu. 2012. Carbon input differences as the main factor
836 explaining the variability in soil organic C storage in no-tilled compared to inversion tilled
837 agrosystems. *Biogeochemistry* 108:17–26.
- 838 Voisin, A.-S., C. Salon, N. G. Munier-Jolain, and B. Ney. 2002. Effect of mineral nitrogen on
839 nitrogen nutrition and biomass partitioning between the shoot and roots of pea (*Pisum*
840 *sativum* L.). *Plant and Soil* 242:251–262.
- 841 Wang, Y., M. Boelter, Q. Chang, R. Duttman, K. Marx, J. F. Petersen, and Z. Wang. 2015.
842 Functional dependencies of soil CO₂ emissions on soil biological properties in northern
843 German agricultural soils derived from a glacial till. *Acta Agriculturae Scandinavica*
844 *Section B-Soil and Plant Science* 65:233–245.
- 845 West, T. O., and W. M. Post. 2002. Soil Organic Carbon Sequestration Rates by Tillage and
846 Crop Rotation: A Global Data Analysis. *Soil Sci. Soc. Am. J.* 66:1930–1946.
- 847 White, C. M., S. T. DuPont, M. Mautau, D. Hartman, D. M. Finney, B. Bradley, J. C. LaChance,
848 and J. P. Kaye. 2017. Managing the trade off between nitrogen supply and retention with
849 cover crop mixtures. *Agriculture, Ecosystems & Environment* 237:121–133.
- 850 Wood, S., D. Karp, F. DeClerck, C. Kremen, S. Naeem, and C. Palm. 2015. Functional traits in
851 agriculture: Agrobiodiversity and ecosystem services. *Trends in Ecology and Evolution*:1–
852 9.
- 853 Wortman, S. E., C. A. Francis, and J. L. Lindquist. 2012. Cover crop mixtures for the western
854 Corn Belt: Opportunities for increased productivity and stability. *Agronomy Journal*
855 104:699–705.
- 856 Wuest, S. B., and H. T. Gollany. 2013. Soil Organic Carbon and Nitrogen After Application of
857 Nine Organic Amendments. *Soil Science Society of America Journal* 77:237.
- 858 Yang, X., W. Ren, B. Sun, and S. Zhang. 2012. Effects of contrasting soil management regimes
859 on total and labile soil organic carbon fractions in a loess soil in China. *Geoderma* 177–
860 178:49–56.
- 861
- 862

863

Supporting Information

864

Additional supporting information may be found in the online version of this article at

865

<http://onlinelibrary.wiley.com/doi/10.1002/eap.xxxx/supinfo>

866

867

Data Availability

868

Data available from the University of Michigan Deep Blue Data repository:

869

<https://dx.doi.org/10.7302/Z2K072FC>

870

Author Manuscript

Table 1. Crop functional types (grass or legume, annual or perennial, and harvested or non-harvested). The crop functional groups used in the meta-analysis are shown in italics.

	Harvested		Non-harvested	
	Grass	Legume	Grass	Legume
Annual	<i>Cereal</i>	<i>Legume grain</i>	<i>Cover Crop*</i>	
Perennial	<i>Perennial crop[‡]</i>		€	

*Cover crops included grasses, legumes, and grass-legume mixtures. We combined these categories due to the low overall frequency of cover crops in the database. Most (68%) crop rotations tested only legume cover crops; 23% tested both legumes and grasses, and 9% tested only grass cover crops.

‡All perennial crops included legumes, and 78% of perennial crops were comprised of a single legume species, alfalfa. The remaining perennial crops were legume mixtures (11%) or legume + grass mixtures (11%).

€No sites reported non-harvested perennial crops.

Table 2. Criteria for assigning crop rotation functional typologies.

Crop rotation functional typologies			
<i>pooled</i>	<i>narrow</i>	Crops always included	Crops never included
"grain-only"	"cereals"	cereals	legume grains, cover crops, perennial crops
	"cereals + legume grains"	cereals and legume grains	cover crops, perennial crops

“cover cropped or perennial”	“cover cropped”	cover crop and grain crop	perennial crop
	“perennial cropped”	perennial crop and grain crop	cover crop

Table 3. Summary of cropping system experiments in the database, by location and purpose of the experiment.

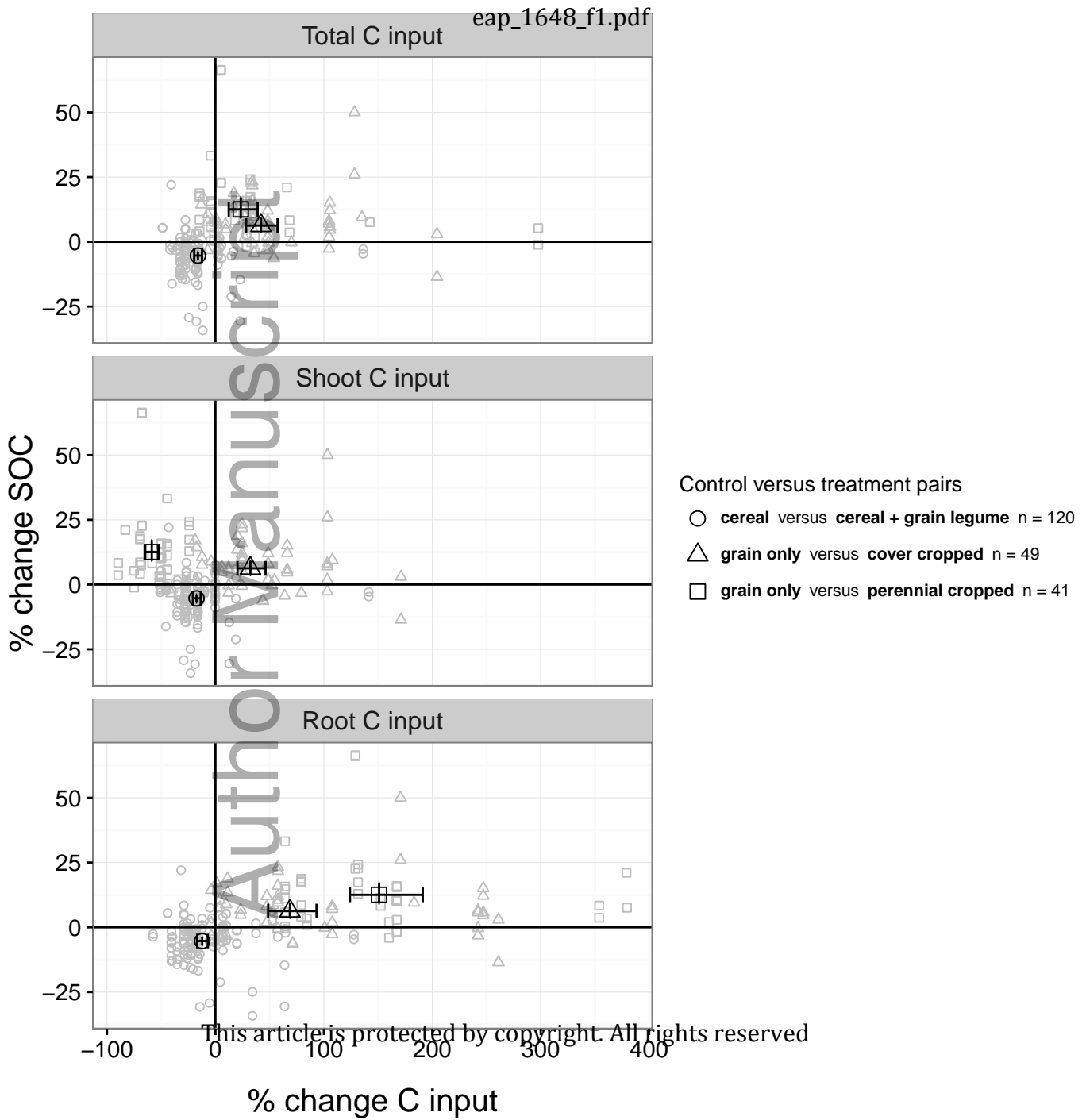
Data base	Sites	Percent of Total
Total sites used	27	
North America	17	63
South America	5	19
Europe	1	4
Asia	3	11
Africa	1	4
Only tested grain-only rotations	11	41
Assessed cover cropped rotations	10	37
Assessed perennial cropped rotations	8	30

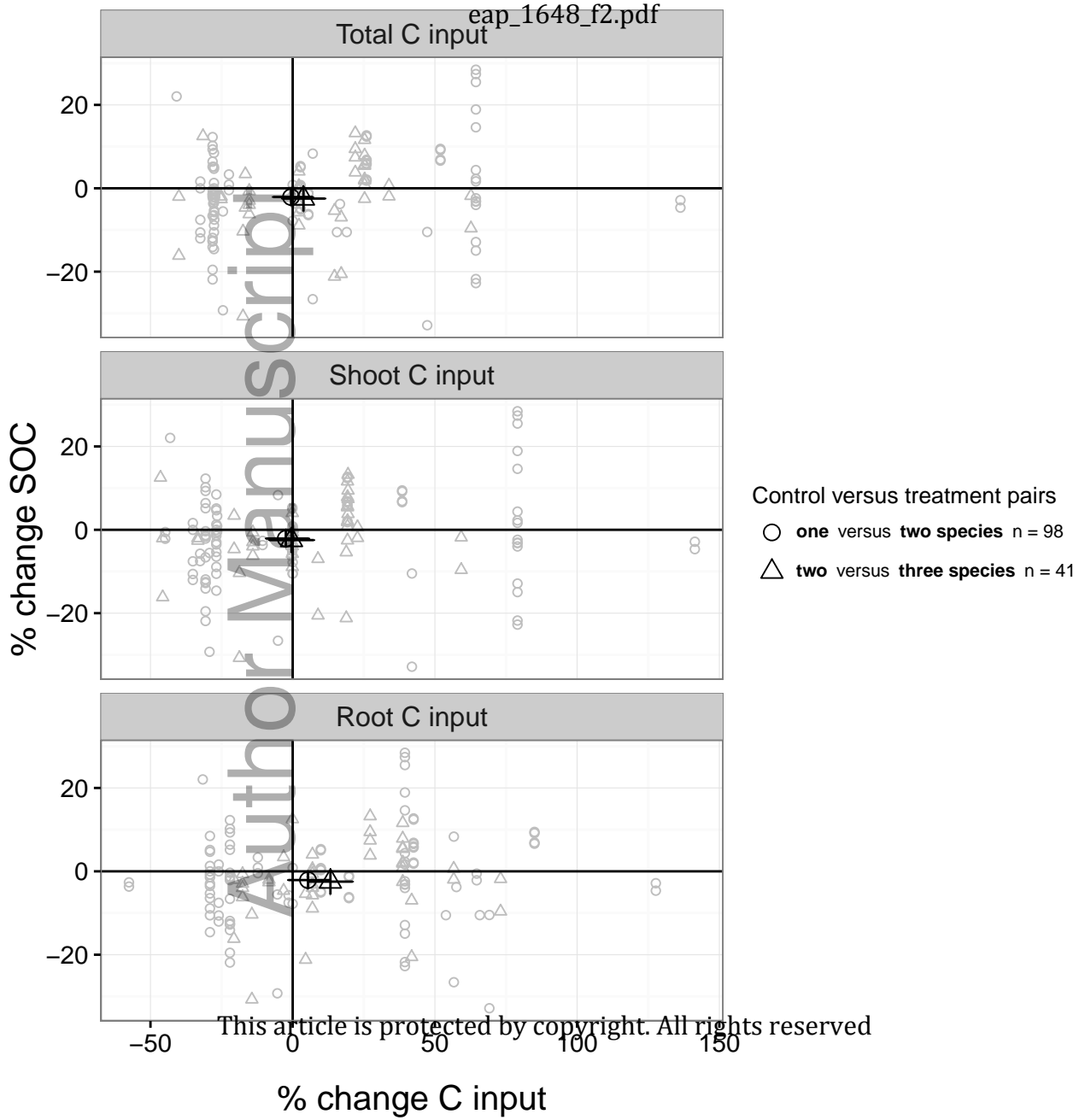
Figure 1. Percent change in C input and SOC between pair-wise comparisons of crop rotation functional group diversity. Gray points represent individual control-treatment comparisons, and black points with bars represent the mean effect size and 95% bootstrapped confidence intervals within each group of control-treatment pairs. Levels were considered significantly different from each other on an axis if their 95% CIs did not overlap. C input was estimated in $\text{Mg C ha}^{-1} \text{ yr}^{-1}$; root C input includes root exudates. SOC was measured in g SOC kg^{-1} soil. In the root C input panel, two points at % change C input = 933 were removed for legible representation on a non-log transformed axis.

Figure 2. Percent change in C input and SOC between pair-wise comparisons of species diversity within grain-only crop rotations. Gray points represent individual control-treatment comparisons, and black points with bars represent the mean effect size and 95% bootstrapped confidence intervals within each group of control-treatment pairs. Levels were considered significantly different from each other on an axis if their 95% CIs did not overlap. CIs overlapping the zero line indicate no difference in SOC or C input between levels of species diversity. C input was estimated in $\text{Mg C ha}^{-1} \text{ yr}^{-1}$; root C input included root exudates. SOC was measured in g SOC kg^{-1} soil.

Figure 3. Percent change in SOC within cereal grain versus cereal + legume grain comparisons. Levels differed in the species of cereal grain in the control rotation (e.g., “corn” indicates the rotation was continuous corn, “misc grain” indicates the rotation was composed of miscellaneous cereal grains). Levels were considered significantly different from each other if their 95% CIs did not overlap, and different from zero if their 95% CIs did not overlap zero.

Figure 4. Percent change in SOC with crop rotation functional diversity at varying levels of N fertilizer rates. Rates were divided into two categories: i) ‘high: N fertilizer > 75 ’ are pair-wise comparisons in which both treatment and control received $> 75 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, and ii) ‘low: N fertilizer ≤ 75 ’ are pair-wise comparisons in which both treatment and control received $\leq 75 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Percent changes were considered significantly different from each other if their 95% CIs did not overlap. CIs that overlap zero indicate no change in SOC at a given level of functional diversity and N fertilizer rate.





corn versus **cereal + legume grain** (18)

wheat versus **cereal + legume grain** (30)

misc grain versus **cereal + legume grain** (74)

This article is protected by copyright. All rights reserved

-10

0

10

% Change SOC

cereal + legume grain
cap_1648_us4.pdf

low: ≤ 75

(47)

high: > 75

(39)

grain-only versus cover or perennial cropped

low: ≤ 75

(70)

high: > 75

(22)

This article is protected by copyright. All rights reserved

% Change SOC

N fertilizer, $\text{kg N ha}^{-1} \text{ yr}^{-1}$

Author Manuscript

