

1 Article

2 Grasslands and croplands have different microbial 3 biomass carbon levels per unit of soil organic carbon

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10 **Abstract:** Primarily using cropped systems, previous studies have reported a positive linear
11 relationship between microbial biomass carbon (MBC) and soil organic carbon (SOC). We
12 conducted a meta-analysis to explore this relationship separately for grasslands and croplands
13 using available literature. Studies were limited to those using fumigation-extraction for MBC for
14 field samples. Trials were noted separately where records were distinct in space or time. Grasslands
15 were naturally occurring, restored, or seeded. Cropping systems were typical of the temperate zone.
16 MBC had a positive linear response to increasing SOC that was significant in both grasslands
17 ($P < 0.001$; $r^2 = 0.76$) and croplands ($P < 0.001$; $r^2 = 0.48$). However, MBC increased 2.5-fold more steeply
18 per unit of increasing SOC for grassland soils, as compared to the corresponding response in
19 cropland soils. Expressing MBC as a proportion of SOC across the regression overall, slopes
20 corresponded to 2.7% for grasslands and 1.1% for croplands. The slope of the linear relationship for
21 grasslands was steeper significantly ($P = 0.0013$) than for croplands. The difference between the two
22 systems is possibly caused by a greater proportion of SOC in grasslands being active rather than
23 passive, relative to that in croplands, with that active fraction promoting the formation of MBC.

24 **Keywords:** soil health; soil quality; meta-analysis; organic matter; active fraction; linear regression

25

26 1. Introduction

27 Microbial biomass carbon (MBC) comprises all microorganisms in soil and is typically measured
28 as the carbon released in response to microbe death following chloroform fumigation [1]. MBC
29 changes with greater agility than soil organic carbon (SOC) in response to residue management [2].
30 SOC includes both a passive fraction that is chemically or physically protected from breakdown over
31 several decades or centuries, as well as an active fraction that is mineralized seasonally or within a
32 few years [3]. MBC was correlated closely with the active fraction, which was determined as up to
33 5% of SOC recovered as CO₂ over 45 days of incubation under laboratory conditions [4]. MBC acts as
34 a pool of organic carbon that contributes cyclically to immobilization and release of minerals during
35 formation and breakdown [5].

36 Soil health and soil quality are often considered as synonyms [6]. However, soil health places
37 more emphasis on biological activity in soil, such as the contribution of soil organisms to nutrient
38 cycling [7]. Either term is typically defined in terms of vegetation productivity, possibly along with
39 productivity of grazers, the capacity of soil to decompose residues, or the provision by soil of other
40 ecological services [8]. A soil property employed effectively as an indicator of soil health should allow
41 for prediction in advance of that production or service. SOC is considered a valuable indicator of soil

42 health in agricultural soils [9]. MBC has been suggested as another possible indicator [10]. SOC and
43 MBC have sometimes been combined with other measurements to form an index of soil quality [11-
44 13]. If MBC is to be used to evaluate soil health or soil quality, then the relationship between MBC
45 and SOC needs to be understood clearly.

46 A positive linear relationship was reported for MBC plotted against SOC for a combination of
47 mostly crop trials along with a small number of grassland trials [14]. A similar correlation was also
48 found for each of two sets of exclusively crop trials [15]. MBC as a percentage of SOC calculated on a
49 case-by-case basis for many studies was reported as lower for cropland at 1.6-1.7%, compared to 1.9-
50 2.2% in the combined category of grassland and savanna [11]. However, pasture was lower still at
51 1.3-1.6% [16]. In contrast, MBC as a percentage of total soil C was relatively high and in the range 1.6-
52 2.9% for poorly drained pasture in the south-west of the UK [17,18]. MBC as a proportion of SOC also
53 varies for agricultural systems. MBC was found to be 1.2% of SOC in paddy rice in China [19]. Crops
54 under moldboard, disk, rotary, and chisel tillage regimens in Iran had 1.0-2.4% of SOC in MBC [20],
55 whereas the percentage of SOC in MBC was similar under barley after six years of either
56 conventional-tillage and zero-till treatments in Alberta [21]. Greater MBC is expected in grasslands,
57 because croplands are reported to have reduced soil quality and less microbial activity associated
58 with soil disturbance and incomplete vegetation cover in time and space [22]. Thus, given the positive
59 relationship between MBC and SOC from the literature, analysis of published studies was
60 undertaken to test the hypothesis that grasslands have more MBC than croplands relative to the
61 amount of SOC present.

62 2. Materials and Methods

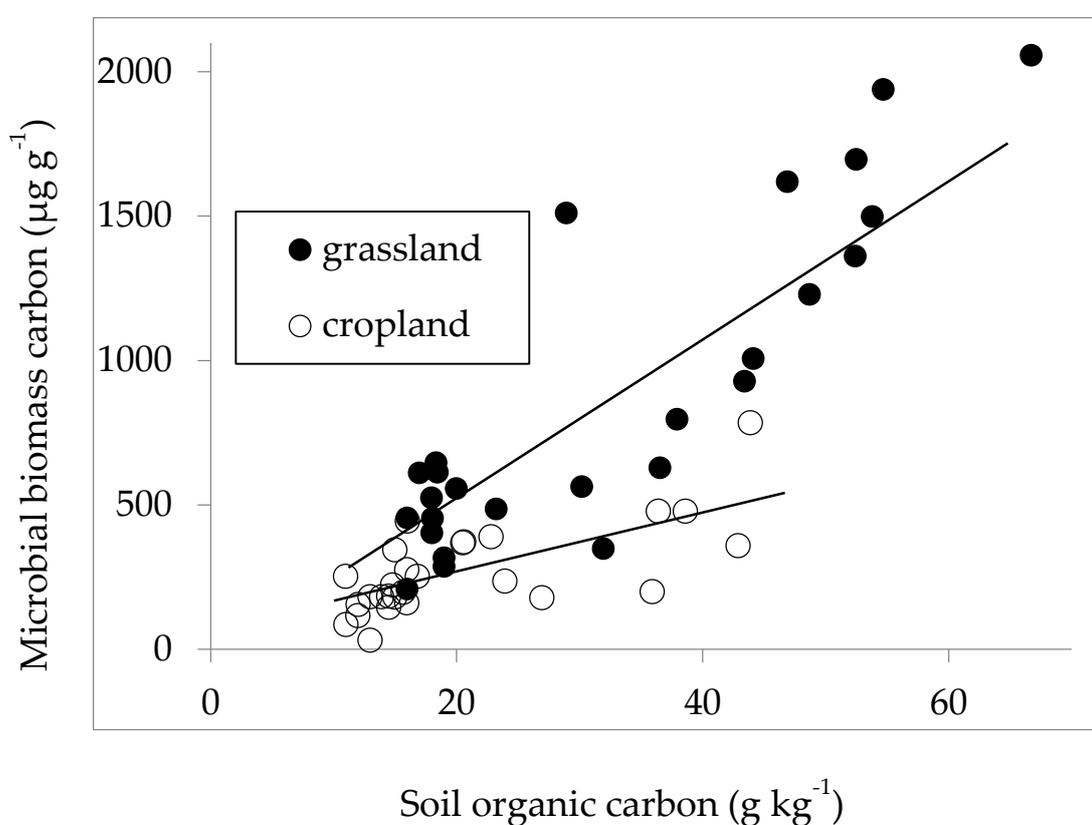
63 A literature search was conducted in late 2016 via internet search engine and keywords for
64 studies reporting both SOC and MBC for field-collected soils from grasslands, croplands, or both.
65 Only studies using the fumigation extraction (FE) method [23] for MBC were included in the meta-
66 analysis. Excluded methods to determine MBC were: the fumigation incubation (FI) method [24,25],
67 the substrate-induced respiration (SIR) method [26], microwave extraction [27], ultraviolet
68 absorption [28], and direct chloroform extraction [29]. FI was omitted, because the carbon flush
69 released by FE was only 73% of that released by FI [23]. SIR gives different MBC values compared to
70 FE, although correlation is claimed for MBC values from these two methods [30]. A period of
71 incubation is needed to undertake SIR and FI, and so the most commonly used method to determine
72 MBC has been FE [31]. Even though promise was offered by the microwave method and the direct
73 extraction method for MBC, few studies have taken these approaches [32,33]. Data for organic matter
74 were converted to SOC using the following relationship: organic matter = 1.724*SOC [34]. Many
75 papers with data for MBC were necessarily omitted, because neither SOC nor organic matter was
76 reported. MBC decreases with soil depth [35]. Thus, where studies used multiple depths, only the
77 surface layer was included. Sample depths among studies were 0-5 cm, 0-10 cm, or 0-15 cm.

78 Designations used by authors in the source papers to distinguish trials were as follows: time since
79 grassland restoration, grazing system, stocking density, slope position, forage species sown, and
80 sample date. For cropland locations, corresponding categories were current crop, tillage regimen,
81 crop rotation, and farm. Within North America, locations were dispersed across the regions of the
82 Great Lakes, the Mid-West, and the Great Plains. Worldwide, additional locations were dispersed
83 across southern Brazil, Germany, the Netherlands, and the south-western part of the UK.

84 Grassland and cropland systems were investigated separately using linear regression of MBC as
 85 the dependent variable and SOC as the independent variable. The significance of regression lines was
 86 evaluated using analysis of variance [36]. Slopes were calculated initially as $\mu\text{g g}^{-1}/\text{g kg}^{-1}$, in order to
 87 retain the units most frequently used historically for MBC and SOC. However, slopes of SOC versus
 88 MBC were also expressed with MBC as a proportion of SOC in percentage form, because of the
 89 frequent use in the literature of this proportion. Slopes for grassland and cropland were compared
 90 by t-test [36].

92 3. Results

93 The relationship between MBC and SOC was sufficiently robust to give linear regressions for
 94 both grassland and cropland (Fig. 1). Linear regressions were found even though source trials were



116 **Figure 1.** Microbial biomass carbon in relation to soil organic carbon for grassland and cropland.
 117 Respective linear regressions are: for grassland, $y = 27.2x - 31.7$ with $r^2 = 0.76$ and $n = 28$; and for
 118 cropland, $y = 10.7x + 48.2$ with $r^2 = 0.48$ and $n = 27$.

119
 120 drawn from a wide-ranging variety of field situations and from across a considerable range of the
 121 temperate world (Table 1). Grasslands varied considerably in character, including both virgin and
 122 restored prairie, grazed pasture, and various other grass systems that were either native or sown
 123 (Table 1). Cropping systems were also varied, including soybean, cereals, corn, and cotton (Table 1).
 124 Many studies identified in our literature search were excluded from our meta-analysis, because they
 125 did not meet our criteria of using the FE method to determine MBC, or because they did not report
 126 SOC or organic matter. We retained our criteria rather than expanding our analysis to encompass
 127 other methods to determine MBC, because the choice of method modifies the value obtained for MBC

128 and could be expected to obscure relationships to SOC. MBC increased significantly in response to
 129 increasing SOC in both grassland (Table 2) and cropland (Table 3). MBC in cropland increased from
 130 210 $\mu\text{g g}^{-1}$ at 15 g kg^{-1} SOC to only 530 $\mu\text{g g}^{-1}$ at 45 g kg^{-1} SOC (Fig. 1). In contrast, MBC in grassland
 131 increased from 440 $\mu\text{g g}^{-1}$ at 15 g kg^{-1} SOC to 1190 $\mu\text{g g}^{-1}$ at 45 g kg^{-1} , thereafter increasing further to
 132 1800 $\mu\text{g g}^{-1}$ at 65 g kg^{-1} SOC (Fig. 1). The slope of increase of MBC in response to increasing SOC was
 133 2.5-fold higher in grassland at 27.2 ($\mu\text{g g}^{-1}$)/(g kg^{-1}) compared to 10.7 ($\mu\text{g g}^{-1}$)/(g kg^{-1}) for cropland (Fig.
 134 1). The slope for grassland was significantly ($t=3.399$; $df=51$; $P=0.0013$) greater than for cropland. The
 135 slopes as presented for plots of SOC versus MBC correspond to MBC as a proportion of SOC as 2.7%
 136 for grassland and 1.1% for cropland.

137 Slopes (Fig. 1) were recalculated with a restricted x-axis ranging from SOC of 15-49 g kg^{-1} in order
 138 to evaluate relationships without inclusion of values at distance from common x-axis range, yet at
 139 the same time retaining samples both equitable and sizeable of $n=21$ for grassland and $n=20$ for
 140 cropland. Although not presented graphically, such recalculation gave significant regressions of $y =$
 141 $24.2x + 20.1$ for grassland ($P<0.001$) and $y = 9.3x + 92.8$ for cropland ($P=0.004$). The recalculated slopes
 142 remained different statistically ($t=2.347$; $df=37$; $P=0.024$).

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Table 1. Source data for the meta-analysis.

System	Trials	Location	Field Use	Reference
Grassland	9	IL, USA	Restored prairie	[37]
Grassland	8	MB, Canada	Grazed pasture	[38]
Grassland	4	Devon, UK	Grazed pasture	[39]
Grassland	3	PA, USA	Native grassland	[40]
Grassland	2	MO, USA	Long-term Timothy	[41]
Grassland	1	MO, USA	Virgin prairie	[41]
Grassland	1	Lake Constance, Germany	Grassland	[42]
Cropland	10	IA, USA	Agricultural fields	[43]
Cropland	6	Parana, Brazil	Maize, wheat, cotton, soybean	[44]
Cropland	5	MO, USA	Maize, soybean	[41]
Cropland	3	Stuttgart, Germany	Winter wheat	[42]
Cropland	2	IL, USA	Maize, soybean	[37]
Cropland	1	Lovinghoeve, Netherlands	Arable	[42]

145

146 **Table 2.** Linear regression analysis of variance for the plot of microbial biomass carbon as
 147 dependent variable against soil organic carbon for grassland.

Source	DF	SS	MS	F	P
Regression	1	6998352	6998352	84.4	<0.001
Residual	26	2155244	82894		
Total	27	9153595			

148 **Table 3.** Linear regression analysis of variance for the plot of microbial biomass carbon as
 149 dependent variable against soil organic carbon for cropland.

Source	DF	SS	MS	F	P
Regression	1	305486	305486	22.98	<0.001
Residual	25	332338	13294		
Total	26	637824			

150

151 4. Discussion

152 The slope of the relationship between MBC as a function of SOC found here as 10.7 ($\mu\text{g g}^{-1}$)
153 $/(\text{g kg}^{-1})$ for cropland compares well to corresponding values previously reported. Moore et al.
154 [15] found slopes of 7.9 ($\mu\text{g g}^{-1}$) $/(\text{g kg}^{-1})$ for 1996 data and 10.1 ($\mu\text{g g}^{-1}$) $/(\text{g kg}^{-1})$ for 1997 data in
155 cropland, whereas Carter [14] reported a slope of 13.6 ($\mu\text{g g}^{-1}$) $/(\text{g kg}^{-1})$ for a graph of 35 crop trials
156 combined with five grassland trials in the same graph. We are not aware of any previously
157 published report of this linear relationship exclusively for grassland systems, shown here to
158 have a slope of 27.2 ($\mu\text{g g}^{-1}$) $/(\text{g kg}^{-1})$. Expression of these slopes with MBC as a proportion of SOC,
159 found here to be 2.7% for grassland and 1.1% for cropland, are consistent with the report of a
160 greater value of this proportion in grassland and savanna, relative to cropland, as calculated on
161 a trial-by-trial basis [16]. However, the values for MBC as a proportion of SOC from our slopes
162 were more widely separated between systems than in the case of Xu et al. [16], who reported
163 averages of 1.5% in pasture, 1.7% in cropland, and 2.1% in grassland and savanna. Selective use
164 within our meta-analysis of a single method for MBC determination likely reduced variability
165 and gave clearer separation between grassland and cropland systems.

166 The mechanism for a steeper increase in MBC per unit of SOC in grasslands as compared
167 to croplands likely relates to a difference in the quality of the SOC between the two systems.
168 Chemical estimation of the active fraction of SOC has used permanganate oxidation [45], and
169 this method has been applied to soil quality evaluation [46]. However, recent work illustrates
170 that the fraction of SOC determined by permanganate appears to contribute primarily to SOC
171 sequestration, whereas a closer correspondence to nutrient availability in the short term is
172 shown by the SOC fraction disposed to mineralization during incubation studies [47]. Similarly,
173 the fraction of SOC oxidized with dichromate was associated with larger aggregates of grassland
174 and forest, rather than the small aggregates of croplands [48]. Use of density fractions and size
175 fractions to characterize SOC from recent residue inputs shows that the nitrogen concentration
176 and nitrogen availability of the SOC are affected by not only the fractionation method, but also
177 by the quality and placement of the residue and by the environmental conditions that speed or
178 slow the breakdown [49]. Compared to continuous grass cover with 64% of SOC in the active
179 fraction, corresponding values ranged from 11–45% among various crops [50]. Only 3–8% of SOC
180 was found in the active fraction of the soils of the corn belt of the USA, this fraction being derived
181 from corn residues and having a residence time of 100 days [51]. Loss of SOC under cultivation
182 is attributed to soil aeration, breakdown of larger aggregates, and reduced residue inputs [52–
183 54]. Loss of MBC in cropland relates to reduced rhizodeposition, which is higher in grasslands
184 due to greater root biomass [55]. The quality of the microbial community also changes in
185 response to land use. Fungi increased relative to bacteria following prairie restoration, relative
186 to a maize control [56]. Similarly, greater fungal representation was seen for microbes under
187 reduced tillage [57]. Rotation provides aboveground diversity that stimulates microbial
188 functional activity in soil [58].

189 Based on the meta-analysis reported herein, reduced MBC in cropland can be seen to
190 correspond not only to less SOC in croplands, but also to proportionately less MBC per unit of SOC
191 in croplands. The difference in the quantity of MBC per unit SOC reported here when comparing
192 croplands and grasslands stands in contrast to previous commentary, in which reduced MBC in

193 croplands was attributed solely to reduced SOC [59]. Based on the present meta-analysis, we note
194 that both the quantity and quality of the SOC in croplands appear to modify the MBC therein. The
195 SOC that remains in long-cultivated cropland soil is predominantly passive, with turnover on a
196 timescale of centuries to millennia [60]. The sensitivity of MBC to this loss of the active fraction of
197 SOC demonstrates that MBC is a measure more closely aligned to soil health than is SOC alone.

198 5. Conclusions

199 Our meta-analysis of data from the literature shows a positive relationship between MBC and
200 SOC. However, this relationship was different for contrasting ecosystem types: MBC increases 2.5-
201 times faster per unit of SOC in grassland, as compared to cropland. This outcome shows that MBC
202 offers promise an indicator of soil health, because elevated MBC is associated with the features that
203 accompany grassland versus cropland systems: more SOC, greater proportion SOC in the active
204 fraction, and greater microbial activity to promote nutrient availability and vegetation productivity.
205 This study supports our hypothesis that, relative to croplands, grasslands have more MBC per unit
206 of SOC. These results encourage the use of MBC for assessment of soil health broadly across locations
207 and land use systems.

208

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211 References

- 212 1. Powlson, D.S. The soil microbial biomass: before, beyond and back. In *Beyond the biomass*. Ritz,
213 K.; Dighton, J.; Giller, K.L. Eds. Wiley-Sayce, London, 1994; pp. 3-20.
- 214 2. Powlson, D.S.; Brookes, P.C.; Christensen, B.T. Measurement of soil microbial biomass provides
215 an early indication of changes due to straw incorporation. *Soil Biol. Biochem.* **1987**, *19*, 159-164.
- 216 3. Wander, M. Soil organic matter fractions and their relevance to soil function. In *Soil organic
217 matter in sustainable agriculture*. Magdoff, F.; Weil, R.R. Eds. CRC Press, Boca Raton, 2004; 67-
218 102.
- 219 4. Rovira, P.; Jorba M.; Romanya, J. Active and passive organic matter fractions in Mediterranean
220 forest soils. *Biol. Fertil. Soils* **2010**, *46*, 355-369.
- 221 5. Hassink J. Density fractions of soil macroorganic matter and microbial biomass as predictors of
222 C and N mineralization. *Soil Biol. Biochem.* **1995**, *27*, 1099-1108.
- 223 6. Doran, J.W.; Zeiss, M.R. Soil health and sustainability: managing the biotic component of soil
224 quality. *Appl. Soil Ecol.* **2000**, *15*, 3-11.
- 225 7. Anderson, T.-H. Microbial eco-physiological indicators to assess soil quality. *Agric. Ecosyst.
226 Environ.* **2003**, *98*, 285-293.
- 227 8. Karlen, D.L.; Mausbach, M.J.; Doran, J.W.; Cline, R.G.; Harris, R.F.; Schuman, G.E. Soil quality:
228 a concept, definition, and framework for evaluation (a guest editorial). *Soil Sci. Soc. Am. J.* **1997**,
229 *61*, 4-10.
- 230 9. Zornoza, R.; Acosta, J.A.; Bastida, F.; Domínguez, S.G.; Toledo, D. M.; Faz, A. Identification of
231 sensitive indicators to assess the interrelationship between soil quality, management practices
232 and human health. *Soil* **2015**, *1*, 173.
- 233 10. Gonzalez-Quinones, V.; Stockdale, E.A.; Banning, N.C.; Hoyle, F. C.; Sawada, Y.; Wherrett, A.D.;
234 Jones, D.L.; Murphy, D.V. Soil microbial biomass—interpretation and consideration for soil
235 monitoring. *Soil Res.* **2011**, *49*, 287-304.
- 236 11. Andrews, S.S.; Karlen, D.L.; Cambardella, C.A. The soil management assessment
237 framework. *Soil Sci. Soc. Am. J.* **2004**, *68*, 1945-1962.

- 238 12. Bastida, F.; Zsolnay, A.; Hernandez, T.; Garcia, C. Past, present and future of soil quality indices:
239 a biological perspective. *Geoderma* **2008**, *147*, 159-171.
- 240 13. Erkossa, T.; Itanna, F.; Stahr, K. Indexing soil quality: a new paradigm in soil science
241 research. *Soil Res.* **2007**, *45*, 129-137.
- 242 14. Carter, M.R. The influence of tillage on the proportion of organic carbon and nitrogen in the
243 microbial biomass of medium-textured soils in a humid climate. *Biol. Fertil. Soils* **1991**, *11*, 135-
244 139.
- 245 15. Moore, J.M.; Klose, S.; Tabatabai, M.A. Soil microbial biomass carbon and nitrogen as affected
246 by cropping systems. *Biol. Fertil. Soils* **2000**, *31*, 200-210.
- 247 16. Xu, X.; Thornton, P.E.; Post, W.M. A global analysis of soil microbial biomass carbon, nitrogen
248 and phosphorus in terrestrial ecosystems. *Glob. Ecol. Biogeogr.* **2013**, *22*, 737-749.
- 249 17. Lovell, R.D.; Jarvis, S.C.; Bardgett, R.D. Soil microbial biomass and activity in long-term
250 grassland: effects of management changes. *Soil Biol. Biochem.* **1995**, *27*, 969-975.
- 251 18. Lovell, R.D.; Jarvis, S.C. Effect of cattle dung on soil microbial biomass C and N in a permanent
252 pasture soil. *Soil Biol. Biochem.* **1996**, *28*, 291-299.
- 253 19. Wang, F.E.; Chen, Y.X.; Tian, G.M.; Kumar, S.; He, Y.F.; Fu, Q.L.; Lin, Q. Microbial biomass
254 carbon, nitrogen and phosphorus in the soil profiles of different vegetation covers established
255 for soil rehabilitation in a red soil region of southeastern China. *Nutr. Cycling Agroecosyst.* **2004**,
256 *68*, 181-189.
- 257 20. Kabiri, V.; Raiesi, F.; Ghazavi, M.A. Tillage effects on soil microbial biomass carbon, SOM
258 mineralization and enzyme activity in a semi-arid calcixerepts. *Agric. Ecosyst. Environ.* **2016**, *232*,
259 73-84.
- 260 21. Franzluebbers A.J.; Arshad, M.A. Soil organic matter pools during early adoption of
261 conservation tillage in northwestern Canada. *Soil Sci. Soc. Am. J.* **1996**, *60*, 1422-1427.
- 262 22. Karlen, D.L.; Stott, D.E.; Cambardella, C.A.; Kremer, R.J.; King, K.W.; McCarty, G.W. Surface soil
263 quality in five midwestern cropland conservation effects assessment project watersheds. *J. Soil*
264 *Water Conserv.* **2014**, *69*, 393-401.
- 265 23. Vance, E.D., Brookes, P.C.; Jenkinson, D.S. An extraction method for measuring soil microbial
266 biomass C. *Soil Bio. Biochem.* **1987**, *19*, 703-707.
- 267 24. Jenkinson, D.S. Studies on the decomposition of plant material in soil. *J. Soil Sci.* **1966**, *17*, 280-
268 302.
- 269 25. Jenkinson, D.S. Determination of microbial biomass carbon and nitrogen in soil. In *Advances in*
270 *nutrient cycling in agricultural ecosystems*. Wilson, J.R. Ed. CAB International, Wallingford,
271 1988; pp. 368-386.
- 272 26. Anderson, J.P.E.; Domsch, K.H. A physiological method for the quantitative measurement of
273 microbial biomass in soils. *Soil Bio. Biochem.* **1978**, *10*, 215-221.
- 274 27. Islam, K.R.; Weil, R.R. Microwave irradiation of soil for routine measurement of microbial
275 biomass carbon. *Biol. Fertil. Soils* **1998**, *27*, 408-416.
- 276 28. Turner, B.L.; Bristow, A.W.; Haygarth, P.M. Rapid estimation of microbial biomass in grassland
277 soils by ultra-violet absorbance. *Soil Bio. Biochem.* **2001**, *33*, 913-919.
- 278 29. Gregorich, E.G.; Wen, G.; Voroney R.P.; Kachanoski, R.G. Calibration of a rapid direct
279 chloroform extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* **1990**,
280 *22*, 1009-1011.
- 281 30. Strecker, T.; Barnard, R.L.; Ni Claus, P.A.; Scherer-Lorenzen, M.; Weigelt, A.; Scheu, S.;
282 Eisenhauer, N. Effects of plant diversity, functional group composition, and fertilization on soil
283 microbial properties in experimental grassland. *Plos One* **2015**,
284 DOI:10.1371/journal.pone.0125678
- 285 31. Jenkinson, D.S.; Brookes, P.C.; Powlson, D.S. Measuring soil microbial biomass. *Soil Bio. Biochem.*
286 **2004**, *36*, 5-7.
- 287 32. Johnson, C.K.; Wienhold, B.J.; Doran, J.W.; Drijber, R.A.; Wright, S.F. Linking microbial-scale
288 findings to farm-scale outcomes in a dryland cropping system. *Precis. Agric.* **2004**, *5*, 311-328.

- 289 33. Seita, R.; Verma, S.L.; Marschner P. Measuring microbial biomass carbon by direct extraction —
290 comparison with chloroform fumigation-extraction. *Eur. J. Soil Biol.* **2012**, *53*, 103-106.
- 291 34. Nelson, D.W.; Sommers, L.E. Total carbon, organic carbon, and organic matter. In *Methods of*
292 *soil analysis. Part 3. Chemical methods*; Sparks, D.L. Ed.; Soil Science Society of America,
293 Madison, WI, USA, 1996; pp. 961-1010.
- 294 35. Iyyemperumal, K.; Israel, D.W.; Shi, W. Soil microbial biomass, activity and potential nitrogen
295 mineralization in a pasture: Impact of stock camping activity. *Soil Biol. Biochem.* **2007**, *39*, 149-
296 157.
- 297 36. Zar, J.H. *Biostatistical analysis* 5th ed.; Prentice Hall: Upper Saddle River, NJ., USA, 2010.
- 298 37. Allison, V.J.; Miller, R.M.; Jastrow, J.D.; Matamala, R.; Zak, D.R. Changes in soil microbial
299 community structure in a tallgrass prairie chronosequence. *Soil Sci. Soc. Am. J.* **2005**, *69*, 1412-
300 1421.
- 301 38. Banerjee, M.R.; Burton, D.L.; McCaughey, W.P.; Grant, C.A. Influence of pasture management
302 on soil biological quality. *J. Range Man.* **2000**, *53*, 127-133.
- 303 39. Lovell, R.D.; Jarvis, S. C. Soil microbial biomass and activity in soil from different grassland
304 management treatments stored under controlled conditions. *Soil Biol. Biochem.* **1998**, *30*, 2077-
305 2085.
- 306 40. Corre, M.D.; Schnabel, R.R.; Stout, W.L. Spatial and seasonal variation of gross nitrogen
307 transformations and microbial biomass in a Northeastern US grassland. *Soil Biol. Biochem.*
308 **2002**, *34*, 445-457.
- 309 41. Jordan, D.; Kremer, R.J.; Bergfield, W.A.; Kim, K.Y.; Cacio, V. N. Evaluation of microbial
310 methods as potential indicators of soil quality in historical agricultural fields. *Biol. Fertil. Soils*
311 **1995**, *19*, 297-302.
- 312 42. Friedel, J.K.; Scheller, E. Composition of hydrolysable amino acids in soil organic matter and soil
313 microbial biomass. *Soil Biol. Biochem.* **2002**, *34*, 315-325.
- 314 43. Klose, S.; Tabatabai, M.A. Urease activity of microbial biomass in soils. *Soil Biol. Biochem.*
315 **1999**, *31*, 205-211.
- 316 44. Balota, E.L.; Colozzi-Filho, A.; Andrade, D.S.; Dick, R.P. Microbial biomass in soils under
317 different tillage and crop rotation systems. *Biol. Fertil. Soils* **2003**, *38*, 15-20.
- 318 45. Weil, R.R.; Islam, K.R.; Stine, M.A.; Gruver, M.A.; Samson-Liebig, S.E. Estimating active carbon
319 for soil quality assessment: a simplified method for laboratory and field use. *Am. J. Altern. Agric.*
320 **2003**, *18*, 3-17.
- 321 46. Veum, K.S.; Goyne, K.W.; Kremer, R.J.; Miles, R.J.; Sudduth, K. A. Biological indicators of soil
322 quality and soil organic matter characteristics in an agricultural management continuum.
323 *Biogeochem.* **2014**, *117*, 81-99.
- 324 47. Hurisso, T.T.; Culman, S.W.; Horwath, W.R.; Wade, J.; Cass, D.; Beniston, J.W.; Bowles, T.M.;
325 Grandy, A.S.; Franzluebbers, A.J.; Schipanski, M.E.; Lucas, S.T.; Ugarte, C.M. Comparison of
326 permanganate-oxidizable carbon and mineralizable carbon for assessment of organic matter
327 stabilization and mineralization. *Soil Sci. Soc. Am. J.* **2016**, *80*, 1352-1364.
- 328 48. Liu, M.-Y.; Chang, Q.-R.; Qi, Y.-B.; Liu, J.; Chen, T. Aggregation and soil organic carbon fractions
329 under different land uses on the tableland of the Loess Plateau of China. *Catena* **2014**, *115*, 19-28.
- 330 49. Gregorich, E.G.; Beare, M.H.; McKim, U.F.; Skjemstad, J.O. Chemical and biological
331 characteristics of physically uncomplexed organic matter. *Soil Sci. Soc. Am. J.* **2006**, *70*, 975-985.
- 332 50. Hsieh, Y.-P. Pool size and mean age of stable carbon in cropland. *Soil Sci. Soc. Am. J.* **1992**, *56*,
333 460-464.
- 334 51. Collins, H.P.; Elliott, E.T.; Paustian, K.; Bundy, L.G.; Dick, W.A.; Huggins, D.R.; Smucker, A.J.M.;
335 Paul, E.A. Soil carbon pools and fluxes in long-term corn belt agroecosystems. *Soil Biol. Biochem.*
336 **2000**, *32*, 157-168.
- 337 52. Burke, I.C.; Yonker, C.M.; Parton, W.J.; Cole, C.V.; Schimel, D.S.; Flach, K. Texture, climate, and
338 cultivation effects on soil organic matter content in US grassland soils. *Soil Sci. Soc. Am. J.*
339 **1989**, *53*, 800-805.

- 340 53. Brady, N.C.; Weil, R.R. The nature and properties of soils, 13th ed.; Prentice Hall, Upper Saddle
341 River, NJ, USA, 2002.
- 342 54. Shi, X.P.; Li, X.G.; Long, R.J.; Singh, B.P.; Li, Z.T.; Li, F.M. Dynamics of soil organic carbon and
343 nitrogen associated with physically separated fractions in a grassland-cultivation sequence in
344 the Qinghai-Tibetan plateau. *Biol. Fertil. Soils* **2010**, *46*, 103-111.
- 345 55. Agbenin, J.O.; Adeniyi, T. The microbial biomass properties of a savanna soil under improved
346 grass and legume pastures in northern Nigeria. *Agric. Ecosyst. Environ.* **2005**, *109*, 245-254.
- 347 56. Bailey, V.L.; Smith, J.L.; Bolton, H. Fungal-to-bacterial ratios in soils investigated for enhanced
348 C sequestration. *Soil Biol. Biochem.* **2002**, *34*, 997-1007.
- 349 57. Mbuthia, L.W.; Acosta-Martínez, V.; Debryun, J.; Schaeffer, S.; Tyler, D.; Odoi, E.; Eash, N. Long
350 term tillage, cover crop, and fertilization effects on microbial community structure, activity:
351 implications for soil quality. *Soil Biol. Biochem.* **2015**, *89*, 24-34.
- 352 58. Tiemann, L.K.; Grandy A.S.; Atkinson, E.E.; Marin-Spiotta, E.; McDaniel, M.D. Crop rotational
353 diversity enhances belowground communities and functions in an agroecosystem. *Ecol. Lett.*
354 **2015**, *18*, 761-771.
- 355 59. Wardle, D.A. A comparative assessment of factors which influence microbial biomass carbon
356 and nitrogen levels in soil. *Biol. Rev.* **1992**, *67*, 321-358.
- 357 60. Carter, M.R. Analysis of soil organic matter storage in agroecosystems. In Structure and organic
358 matter storage in agricultural soils; Carter, M.R.; Stewart, B.A., Eds.; CRC Press, Boca Raton, FL,
359 USA, 1996. pp. 3-11.
- 360
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