≛.	

1	Title
2	Pedoclimatic factors and management determine soil organic carbon and aggregation in
3	farmer fields at a regional scale
4	
5	Authors
6	Lucie Büchi ^{1,2,*} , Florian Walder ³ , Samiran Banerjee ³ , Tino Colombi ^{3,4} , Marcel G.A. van der
7	Heijden ^{3,4} , Thomas Keller ^{3,5} , Raphaël Charles ^{1,6} , Johan Six ⁷
8	
9	Affiliation
10	¹ Plant Production Systems, Agroscope, Nyon, Switzerland
11	² Natural Resources Institute, University of Greenwich, United Kingdom
12	³ Plant-Soil Interactions, Department of Agroecology and Environment, Agroscope,
13	Reckenholz, Switzerland
14	⁴ Department of Plant and Microbial Biology, University of Zurich, Switzerland
15	⁵ Soil and Environment, Swedish University of Agricultural Sciences, Uppsala, Sweden
16	⁶ Research Institute of Organic Agriculture FiBL, Lausanne, Switzerland
17	⁷ Department of Environmental Systems Science, Swiss Federal Institute of Technology ETH
18	Zurich, Zurich, Switzerland
19	
20	
21	*Corresponding author:
22	Lucie Büchi
23	Natural Resources Institute
24	University of Greenwich
25	Chatham Maritime
26	ME4 4TB

27	United Kingdom
28	L.A.Buchi@greenwich.ac.uk
29	
30	
31	
32	
33	

34 Abstract

35 The degradation of soil from agricultural land is a major threat to food security and a driver of global changes. Soil conservation systems are thus being promoted and/or adopted worldwide. 36 37 In this on-farm study conducted in Switzerland, we compared the effect of three cropping 38 systems – conventional with tillage, conventional without tillage (i.e. no-till) and organic 39 farming with tillage – on soil quality. Samples from 60 winter wheat fields belonging to these 40 three systems were analysed for soil carbon concentration, soil aggregate distribution and soil 41 biological properties (microbial carbon and mycorrhizal biomarkers), at three different depths 42 (0-5 cm, 5-20 cm and 20-50 cm). Information about cropping practices was collected through 43 surveys. The main differences in soil properties between systems occurred for the surface 44 layer (0-5 cm depth), with increased soil organic carbon concentration and stock under no-till 45 compared to the conventionally tilled fields. No-till and organic fields showed a higher mean 46 aggregate size and proportion of macroaggregates in the surface layer compared to tilled 47 conventional fields, with a greater amount of carbon in the large macroaggregates. However, large within-system variability was also observed, which tended to override differences 48 49 between systems. Across systems, clay content, microbial carbon, and the mycorrhizal PFLA 50 biomarkers were the major drivers of soil organic carbon concentration, clay to carbon ratio 51 and carbon accumulation in the large macroaggregate fraction. Aggregation at 0-5 cm was 52 mostly related to tillage depth, while climate variables and especially clay content played a 53 major role for deeper layers. Our results demonstrate that within the constraints set by soil 54 texture and climate, organic agriculture and no-till can contribute to improved soil carbon and 55 aggregation properties. Thus, we advocate for the identification of the main drivers of soil 56 quality in order to inform management and improve soil functioning in agricultural fields in 57 the long term.

- 58
- 59

60 Keywords

61 soil organic carbon, soil biological properties, tillage, cropping practices

63 **1. Introduction**

64 The massive increase in crop yield during the last century has come at a cost of degradation of agricultural soils (Tilman et al., 2002; Virto et al., 2015). Soil organic carbon content is 65 66 strongly related to many other crucial soil properties and is thus often used as a proxy for soil quality and functioning (Johannes et al., 2017; Schjønning et al., 2018; Wiesmeier et al., 67 68 2019; Baveye et al., 2020; Or et al, 2021). The loss of soil organic carbon is therefore a threat 69 to current and future soil quality, as well as a major driver of climate change (Lal et al., 2018). 70 So-called conventional farming systems, relying on intensive tillage and external inputs such 71 as mineral fertilisers and pesticides, have particularly impacted the soil quality, including 72 chemical, physical and biological degradation, and loss of soil organic carbon (Virto et al., 73 2015). Alternatives to conventional farming have been promoted to alleviate soil degradation 74 and the loss of soil organic carbon in arable systems. For example, reduced soil tillage, crop 75 diversification, the use of organic amendments and the optimisation of input use have been 76 shown to increase soil organic carbon (Merante et al., 2017; Williams et al., 2020). Among 77 those, reduction of soil tillage and the increase of organic inputs to the soil are the main 78 factors allowing to maintain or increase soil organic carbon content (Virto et al., 2012; Palm 79 et al., 2014; Mary et al., 2020).

80 Besides modifying specific practices, some farming systems as a whole have been promoted 81 with the aim of improving soil quality, but until now none has successfully addressed all of 82 the environmental challenges related to agriculture. Conventional no-till systems, while 83 achieving less soil disturbance thanks to the absence of tillage, usually rely more heavily on 84 herbicides. The effect of herbicides on soil life is still debated (Bünemann et al., 2006), and 85 some studies have shown negative effects of herbicides on microbial communities (e.g. 86 Druille et al., 2016; Helander et al., 2018) while some others have not shown any effects (e.g. 87 Kepler et al., 2020). However, herbicides are also known for other adverse effects on the 88 environment, for example to pollute groundwater and impact aquatic life (Schwarzenbach,

89 2006; Gregorio et al., 2012). On the other side, organic farming, while banning pesticide use 90 and synthetic inputs, usually involves high soil disturbance due to mechanical weeding and 91 tillage. In addition, both systems tend to have lower productivity (Knapp & van der Heijden, 92 2018). Lower productivity could result in lower biomass inputs to the soil, however, this may 93 also depend on other factors such as crop variety and a direct link between below and above 94 ground biomass could not always be inferred (Hirte et al, 2021). It is thus crucial to study how 95 these alternative systems compare to conventional farming in terms of soil quality. 96 Soil carbon concentration also depends on site or regional factors that cannot be managed, or 97 not easily, by farmers, such as soil texture or weather conditions. For example, clay 98 concentration is known to influence and constrain soil organic carbon content in a temperate 99 climate, through its ability to form stable complexes with carbon (Johannes et al., 2017). The 100 ratio of clay to carbon has thus been suggested as an indication of the potential of soil to store 101 carbon (Dexter et al., 2008; Merante et al., 2017) and as an indicator of soil structure 102 (Johannes et al., 2017). Furthermore, Dimassi et al (2014) have shown that carbon stocks 103 increase in wet years and decrease in drier years. Other site related soil properties, such as pH 104 and calcium concentration, have an impact on soil quality. For example, it has been shown 105 that calcium and aluminium concentration are drivers of soil organic carbon in tropical soils 106 (von Fromm et al., 2020). Therefore, the intrinsic characteristics of soils need to be taken into 107 account when investigating organic carbon sequestration potential of soils. 108 To ensure long term carbon storage, soil organic carbon needs to be stabilised. Several factors 109 govern the stabilisation and retention of soil carbon, of which soil aggregation and clay 110 complexation are central (Hassink 1997; Totsche et al., 2018). In soil, macroaggregates are 111 first formed when new organic matter is added to the soil and binding agents are produced by 112 microbes decomposing the newly added organic matter. With time, microaggregates are 113 formed within macroaggregates, leading to a hierarchy of aggregate fractions (Six et al., 114 2000a). It has been shown that increased soil aggregate size is directly related to organic

115 carbon protection (Six et al., 2000a). The cropping practices reducing soil organic carbon 116 content act mainly by reducing soil aggregation and aggregate size. In particular, soil tillage, 117 even when practised only once a year, has been shown to breakdown macroaggregates and 118 accelerate their turnover, leading to a decrease in mean aggregate size and to the production 119 of unstable fragments instead (Six et al., 2000a; Grandy & Robertson, 2006). Other important 120 factors also play a role in the formation or destruction of soil aggregates (Blanco-Canqui and 121 Lal, 2004; Six et al., 2004), some being manageable and some not. For example, soil 122 biological activity increases aggregation as earthworms, fungi and bacteria excrete substances fostering aggregation, as well as roots through rhizodeposition. Inorganic binding agents such 123 124 as calcium also promote the formation of aggregates. While weather-related variables such as 125 freezing-thawing and wetting-drying cycles could form or break down aggregates (Denef et 126 al., 2001; Blanco-Canqui and Lal, 2004; Six et al., 2004).

127 Previous studies have investigated the influence of either cropping systems (mostly organic vs 128 conventional, or no-till vs conventional) or tillage on soil organic carbon and aggregation (see 129 for example in the reviews by Leifeld and Fuhrer, 2010, and by Sun et al., 2020). However, a 130 comprehensive investigation of the relative importance of cropping system vs cropping 131 practices vs pedoclimatic conditions is still lacking. In addition, identifying the main drivers 132 of soil carbon and aggregates in soils from farmer fields, compared to on station experiments, 133 is also important to evaluate the opportunities for improved soil management to enhance soil 134 quality in the long term. The aim of the present study was to investigate the influence of three 135 cropping systems and cropping practices on soil organic carbon, aggregates and their 136 interaction. The study was conducted in a network of 60 farms belonging to conventional with 137 tillage, conventional with no-till and organic cropping with tillage systems in Switzerland. 138 The objectives of this study were: 1) to assess the difference in organic carbon content and 139 stock between cropping systems at different depths; 2) to compare aggregate size distribution 140 and the carbon accumulation in each aggregate fraction between cropping systems; and 3) to

investigate the main drivers of soil carbon and aggregate fraction distribution, using
quantitative descriptors of cropping practices, weather conditions and soil properties.

143

144

145 **2. Materials and Methods**

146 2.1 Field selection

147 Samples were collected in 2016 from 60 fields (>1 hectare) distributed across the Swiss 148 Plateau (Supplementary Material Figure S1A). All soils were classified as Cambisol, and 149 were derived from Quaternary moraine. All fields were cultivated with winter wheat, sown in 150 autumn 2015. Twenty fields corresponded to conventional farming, with soil tillage (mainly 151 ploughing) and use of pesticides (mainly herbicides and fungicides) (called thereafter 152 'conventional' fields), 20 fields were conventional no-till fields, with continuous no-tillage for 153 more than 5 years (called thereafter 'no-till' fields). Finally, 20 fields were organically 154 certified for more than 5 years, with soil tillage (called thereafter 'organic' fields). The field 155 selection, characteristics and practices were described in Büchi et al. (2019). Based on this article, one field was moved from the no-till category to the conventional one for all the 156 157 analyses presented here.

158

159 2.2 Soil sampling

The main soil sampling took place between the 20th of April and the 27th of May 2016. In each field, in a sampling zone of 300-400 m², 15-20 soil cores were taken with a hand auger for four different depths, 0-5, 5-20, 20-25, 25-50 cm. For each depth, all individual samples were pooled together to form a unique composite sample for each field and stored in a plastic bag. The soil was then cleaned from plant and animal debris and sieved at 8 mm. Part of the sample was then air dried for aggregate fractionation, while another part was sieved at 2 mm and dried at 40°C for 72h for nutrient analyses. The remaining part was sieved at 2 mm and
stored in a cold room for microbial analyses.

Bulk density was determined in the same sampling zone in parallel to the core sampling for all fields. At five different places, undisturbed soil cores (100 cm³) were taken in the centre of each layer, at 0-5, 10-15, 20-25, 35-40 cm, with a soil sample ring kit. Samples were then dried at 105°C for 24h and weighed to determine bulk density. The median value of the five cylinders was used to represent each depth.

An additional sampling for mycorrhiza analysis took place between the 2nd and 23th of June 2016. In each field, ten soil cores were taken for the depth 0-20 cm with a hand auger and pooled to constitute a composite sample. These samples were kept in a cooling box during transportation and then stored in the lab at 4°C before further processing. Soil samples were then sieved at 5 mm, homogenised and 50 mL subsamples, cleaned from plant and animal debris by hand, were stored at -20°C.

179

180 2.3 Soil analyses

181 For each soil sample of the first sampling, texture, soil organic carbon (SOC), pH and total

182 calcium (Ca) were measured according to the Swiss standard methods (Agroscope, 1996).

183 The clay to carbon ratio was obtained by dividing clay content by SOC.

184 Soil aggregate fractionation was done following Six et al. (1998). A sample of about 80 g of

air-dried soil was rehydrated in deionised water, and then successively sieved at 2000 µm,

186 250 µm and 53 µm. Four different fractions were thus obtained, large macroaggregates (2000

187 μm - 8000 μm), small macroaggregates (250 μm - 2000 μm), microaggregates (53 μm - 250

188 μ m) and silt and clay (< 53 μ m). Each fraction was then dried at 60°C for 72h, then weighted

and prepared for nutrient analysis. Total carbon and nitrogen concentration of each fraction

190 were determined by dry combustion (CN-628 Elemental Determinator; LECO Corp., St

191 Joseph, MN).

192 Microbial biomass carbon estimates by chloroform-fumigation-extraction were carried out 193 according to Vance et al. (1987) on the soil samples of the main soil sampling. Extracted 194 organic C was determined by infrared spectrometry after combustion (DIMA-TOC 100, 195 Dimatec, Essen, Germany), soil microbial biomass was then calculated according Joergensen 196 (1996). 197 The soil samples from the second sampling were analysed for phospholipid fatty acids 198 (PFLA), according to a modified version of Bligh and Dyer method (Bligh and Dyer, 1959). 199 The PLFA 16:1 ∞ 5 was used as a marker for arbuscular mycorrhizal fungi (Olsson et al., 200 1999), and employed in this study as a potential explanatory variable for soil carbon content 201 and aggregation. 202 In tilled soil, the plough depth would in general be around 20 cm. The soil properties from the 203 depths 20-25 cm and 25-50 cm were thus averaged, using their respective bulk density as 204 weights, to obtain values for a composite 20-50 cm layer. Results are therefore presented for 205 three depths: 0-5 cm, 5-20 cm and 20-50 cm. 206

207

208 2.4 Data analyses

All analyses were performed using R 3.6.3 (R Core Team, 2020).

210 Carbon stocks were computed for each layer as the product of carbon content, bulk density

and layer thickness. In addition to individual depths, carbon stock for the composite layer 0-

212 20 cm and 0-50 cm were calculated, using the maximal equivalent soil mass (ESM) method

213 for the plough layer (0-20 cm) and using the minimal ESM method for the whole depth (0-50

214 cm) (Lee et al., 2009).

215 To estimate the global level of aggregation of each layer, mean weight diameter (MWD) was

216 computed as the weighted mean of each aggregate size class average size and their respective

relative weight proportion. C accumulation in each aggregate size class was obtained by
multiplying their relative weight by their respective C concentration.

219 Differences in soil properties (bulk density, SOC, C stocks and MWD) between cropping 220 systems were tested using analyses of covariance, using clay content as a quantitative 221 covariate and cropping system (conventional, no-till, organic) as a fixed factor. Clay 222 concentration and clay-carbon ratio were tested with one way ANOVA. Tests showing a 223 significant (p < 0.05) effect of cropping systems were followed by least-squares mean test 224 ('lsmeans' R package; Lenth, 2016) to differentiate the individual cropping systems. The 225 analyses were performed independently for each depth (0-5 cm, 5-20 cm, 20-50 cm). 226 Differences between layers within each cropping systems were tested using the same methods 227 (ANOVA followed by least-squares mean test). 228 Differences in aggregate related variables (relative weight and C accumulation) were tested 229 using two-factors analyses of variance with cropping systems and fractions (four levels: large 230 and small macroaggregates, microaggregates, silt and clay) as fixed factors. In case of 231 significant interactions (i.e., different value for each fraction, depending on cropping system) 232 (p<0.05), pairwise post-hoc Tukey tests were performed separately for each fraction. The

analyses were performed independently for each depth.

234

235 2.5 Linear regressions and R^2 decomposition

To investigate the main drivers of soil properties beyond a priori system definitions, additional analyses were performed across the three cropping systems. For each depth, the influence of several explanatory variables (see description below) on soil organic carbon concentration 'SOC', clay to carbon ratio 'CCR', mean weight diameter 'MWD' and carbon accumulation in the large macroaggregate fraction 'CAM' was tested using multiple linear regressions. These linear regressions were followed by a R² decomposition, according to 'Img' method from 'rlaimpo' R package (Grömping, 2006), to assess the importance of each

explanatory variable. The explanatory variables were chosen for their known links to soilorganic carbon and aggregate formation and persistence.

245 The explanatory variables that were initially considered were clay concentration ('clay'), sand 246 concentration, total calcium ('calc'), pH, number of freezing-thawing days (period: from 247 01.10.2015 to the date of soil sampling in April-May 2016, definition: number of days where 248 the minimum temperature is below 0°C and the maximum temperature above 0°C), mean air 249 temperature ('temp', from 01.07.2015 to the date of soil sampling in April-May 2016), total 250 precipitation ('rain', period: from 01.07.2015 to the date of soil sampling in April-May 2016), 251 soil tillage intensity (sum of the STIR ratings (USDA, 2012) of each tillage or weeding 252 implement used, period: harvest of the previous crop to soil sampling), mean number of 253 tillage and weeding interventions ('nbTW', period: five-year crop rotation), usual maximum 254 tillage depth ('depthT'), crop rotation diversity ('cropDiv', calculated as the number of 255 different crops (main and cover) during the five-year rotation), presence of rotational leys 256 ('nbLeys'), organic matter input from crop residues ('cropOrg'), amendments, and both 257 ('totOrg', period: five-year crop rotation), nitrogen inputs (mineral and total 'totN'), microbial 258 carbon ('microb', at soil sampling) and mycorrhizal AMF biomarker ('amf', measured one 259 month after soil sampling). The weather data were retrieved from the nearest local weather 260 station. Detailed explanations about how the variables linked to cropping practices were 261 calculated are given in Büchi et al. (2019).

First, univariate regressions between the four response variables (soil organic carbon 'SOC', clay to carbon ratio 'CCR', mean weight diameter 'MWD' and carbon accumulation in the large macroaggregate fraction 'CAM') and each explanatory variable were performed and only variables showing at least one significant correlation (at p<0.1) with at least one of the response variable and depth were included in the model. Correlations between explanatory variables was also checked (Supplementary Material Table S1) and highly redundant variables (>0.7 or <0-.7) were removed when related to the same category. Thus, the variables

279	3. Results
278	
277	
276	for one of the fields.
275	These analyses were performed on 59 fields only, as some explanatory variables were missing
274	regressions.
273	fertilisation (correlated with total nitrogen inputs) were not included in the multivariate
272	amendments (not significant and correlated with total organic inputs) and mineral nitrogen
271	number of tillage and weeding interventions and tillage depth), organic inputs from
270	and correlated with temperature), soil tillage intensity (not significant and correlated with
209	sand concentration (correlated with clay), number of freezing-thawing days (not significant

c c ·

41.

(, · · · · · ·

280 3.1 Bulk soil properties

200

In general, substantial variability of soil properties was observed between fields within
cropping systems. Clay concentration varied widely, between 10% and 48% across all fields
and depths, with an overall mean of 22%. No differences in clay concentration were observed
between systems or between layers within systems (Table 1, Supplementary Material Figure
S1B).

Bulk density varied between 0.89 g/cm³ and 1.66 g/cm³ across all fields and layers and

showed significant differences between systems only for the 5-20 cm layer (p=0.003), with a

higher mean value in no-till (1.36 g/cm³) compared to conventional (1.26 g/cm³) and organic

289 (1.22 g/cm³) systems (Table 1). An increase in bulk density with depth was found for all three

- systems (on average, from 1.21 g/cm^3 at the surface to 1.46 g/cm^3 at 20-50 cm) (Table 1).
- 291 Soil organic carbon concentration SOC varied between 2.9 g/kg and 56.3 g/kg across all fields
- and depths. The three cropping systems showed significant differences in terms of SOC for
- 293 the 0-5 cm layer (p=0.012) but not for the 5-20 cm (p=0.231) and 25-50 cm layers (p=0.129)
- 294 (Table 1). In the uppermost layer (0-5 cm depth), SOC was significantly higher for the no-till

295 system compared to the conventional system, with the organic system intermediate and not 296 different from the other two systems (Table 1). Different depth-distribution patterns of SOC 297 were observed for the different systems. No-till system showed decreasing concentration with 298 depth, in contrast to conventional and organic systems that had more homogeneous SOC 299 concentrations in the two first layers (0-5 and 5-20 cm) (Table 1). 300 The clay to organic carbon ratio differed between systems for the 0-5 cm layer, with lower 301 values for the no-till (clay/SOC = 10) compared to conventional system (clay/SOC = 14), 302 with organic having intermediate value with a clay/SOC ratio of 12 (Table 1, Figure 1). In 303 addition, the clay to carbon ratio increased with depth. 304 The mean carbon stock across all systems was 47.8 t/ha for 0-20 cm (equivalent soil mass 305 used for the calculation of carbon stock: 2961 t/ha) and 72.2 t/ha for 0-50 cm (minimal 306 equivalent soil mass: 5387 t/ha), with high variability within systems (Figure 2). In the 307 uppermost layer (0-5 cm depth), differences in SOC stock between systems were observed 308 with higher values in no-till fields and lower in conventional fields (Figure 2, Table 1). No 309 significant differences were observed for the other depths and for topsoil (0-20 cm depth) and 310 total depth (0-50 cm) (Table 1). 311

312

313 3.2 Aggregate size distribution and C and N accumulation in aggregates

Overall, macroaggregates (large: 2000 μ m - 8000 μ m and small: 250 μ m - 2000 μ m) were the dominating fraction (compared to microaggregates: 530 μ m - 250 μ m, and silt and clay: < 53 μ m), representing 77% of the total, across all systems and layers (Figure 3). Significant difference in aggregate size distribution between systems was observed only in the 0-5 cm layer. In this layer, conventional system had fewer large macroaggregates than the no-till and organic systems (Figure 3). As a consequence, the conventional system had a higher proportion of small macroaggregates than the other two systems, and higher proportion of 321 microaggregates than no-till. No significant differences were observed for the 'silt and clay' 322 fractions. In all the other layers, the three systems showed a similar aggregate size distribution 323 (Figure 3). Following a similar pattern, mean weight diameter (MWD) was significantly 324 different between systems for the 0-5 cm layer (p<0.001), with a higher value for no-till and 325 organic systems compared to the conventional system (Table 1). MWD was markedly lower 326 in the subsoil layer (20-50 cm) for all three systems (Table 1). However, as with carbon, 327 MWD showed high within system variability at all depths (Figure 4). 328 The concentration of carbon in each aggregate fraction showed a tendency to lower concentration in the micro aggregate fractions in all management by depth combinations 329 330 (Supplementary Material Figure S2). The amount of soil organic carbon accumulated in each 331 aggregate fraction (i.e., equivalent of carbon stock in each fraction) was different between 332 systems for the top layer (0-5 cm depth), in which the amount of carbon accumulated in the 333 large macroaggregate fraction was higher in no-till and organic systems compared to 334 conventional (Figure 5). No significant differences were observed for the other fractions or 335 layers.

336

337 3.3 Drivers of soil organic carbon and aggregate properties

338 Multiple linear regressions were performed to investigate the main drivers explaining the soil 339 carbon and aggregate results, via four response variables: soil organic carbon 'SOC', clay to 340 carbon ratio 'CCR', mean weight diameter 'MWD' and carbon accumulation in the large macroaggregate fraction 'CAM' (Figure 6 and Supplementary Material Table S2). Fourteen 341 342 explanatory variables were retained to build the models and assess their contribution in terms of \mathbb{R}^2 . Figure 6 shows how the partial \mathbb{R}^2 decomposed across the 14 variables, grouped into 343 344 four main categories: 1. site-related, unmanageable pedoclimatic variables: clay content, 345 temperature and rainfall; 2. site-related, partially manageable variables: pH and calcium 346 concentration; 3. site-related, partially indirectly manageable variables: soil biological

347 properties, microbial carbon and mycorrhizal marker, and 4. directly manageable variables:348 cropping practices.

For SOC, total R^2 was high for all depths (>80%). The variance decomposition of the R^2 349 350 showed, for all depths, that, along with clay (28-37%), the biological variables microbial 351 carbon ('microb') and mycorrhizal marker ('amf') accounted together for the highest part of 352 R^{2} (32-38%) (Figure 6 and Supplementary Material Table S2), both with a positive impact on 353 SOC. However, the contribution of 'amf' decreased with depth and was significant only at the 354 0-5 cm depth, while that of 'microb' increased with depth (Figure 6). Other variables had negligible contributions in terms of \mathbb{R}^2 , but pH showed a significant negative slope in the 355 356 multiple regression for 0-5 cm and 5-20 cm.

For CCR, total R^2 was between 61% and 71%. Clay played a minor role in terms of R^2 , but

358 was significant in the model at the three depths. As for SOC, biological variables 'microb'

and 'amf' accounted for the highest part of R^2 (28%-38% in total), with 'amf' significant only

360 for 0-5 cm. Effect of cropping practices accounted for 19% of R^2 at 0-5 cm, but only for 13%

at 5-20 cm and 8% at 20-50 cm, while effect of weather variables increased from 8% at 0-5

362 cm to 19% at 20-50 cm.

For MWD, total R² was lower than for SOC but increased with depth (59% at 0-5 cm, 66% at 363 5-20 cm, 78% at 20-50 cm). The decomposition of R^2 showed a clear contrast between the 364 365 uppermost layer (0-5 cm) and the deeper ones (5-20 cm and 20-50 cm). Clay explained only 9% at 0-5 cm, but 45% at 5-20 cm and 64% at 20-50 cm. In contrast, the R² associated to the 366 367 other explanatory variables was 50% at 0-5 cm, but only 22% at 5-20 cm and 14% at 20-50 368 cm. For the 0-5 cm layer, cropping practices explained the largest part of the variance, with 369 tillage depth 'depthT' being the most important variable (16%, negative slope), followed by 370 nitrogen inputs 'totN' (7%, negative slope) (Figure 6). At 5-20 cm, after clay, weather 371 variables were the most important, with temperature (8%, negative slope) and rainfall (6%, positive slope) accounting for the highest partial R^2 . At 20-50 cm, except from clay, the other 372

variables in the model explained only 14% of the variability, with significant slopes for
temperature (3%, negative) and 'microb' (3%, positive).

For CAM, total R^2 was high for all depths (>80%). The part explained by clay increased from 375 376 30% at 0-5 cm to 56% at 5-20 cm and 48% at 20-50 cm. After clay, 'microb' accounted for the highest part of R², for all depths (17%, 12%, 18%, positive slopes) (Figure 6). At 0-5 cm, 377 378 'amf' also showed high contribution (14%, positive slope), followed by tillage depth 'depthT' 379 (7%, negative slope), calcium concentration (4%, positive slope) and pH (4%, negative slope). 380 At 5-20 cm, temperature (5%, negative slope) and calcium (4%, positive slope) were also 381 significant. At 20-50 cm, beside clay and 'microb', the only other almost significant variable 382 was calcium (2%, positive slope, p=0.09).

383

384

385 **4. Discussion**

386 4.1 Influence of cropping systems on soil carbon and aggregation

387 Overall, this study showed little differences between cropping systems in terms of soil carbon 388 and aggregation, except for the surface soil layer (0-5 cm depth). This may be due to a large 389 within-system variability, which is common in on-farm studies compared to on-station field 390 experiments. However, it might also be due to the soil protection guidelines followed in Swiss 391 agriculture, which incentivise the use of diversified crop rotations and cover crops, and thus 392 help maintaining a reasonably good soil quality in conventional systems (Dupla et al., 2021). 393 Clay content was a strong driver for carbon and aggregate properties, and variability in clay 394 content within systems could partly explain the lack of observed differences between systems. 395 This shows that soil organic carbon related variables should always be interpreted together 396 with clay content to avoid any spurious conclusions. Clay mineralogy also plays an important 397 role for the stabilisation of soil organic carbon (Singh et al., 2018), but this was not assessed 398 in this study, as no differences in clay mineralogy between cropping systems was expected.

399 This should however be the focus of future studies aimed at disentangling the effect of 400 management from that of site-related factors. This also reinforces clay content as a major 401 driver of soil organic carbon content, as shown by many studies (Hassink, 1997; Merante et 402 al., 2017; Li et al., 2020a,b), due to its ability to stabilize organic carbon (Dexter et al., 2008). 403 However, other variables such as exchangeable calcium and iron or aluminium oxyhydroxides 404 could better reflect the potential of soil carbon stabilisation in certain soils (Rasmussen et al., 405 2018; Pihlap et al., 2021). A recent study has also shown that calcium and aluminium were 406 stronger drivers of soil organic carbon than clay in tropical soils in sub-Saharan Africa (von 407 Fromm et al., 2020). These studies together, thus, suggest that for each pedoclimatic context, 408 several soil properties need to be considered to assess the potential of carbon sequestration in 409 soils.

410 The differences between cropping systems observed in the uppermost soil layer (0-5 cm 411 depth) is in accordance with other studies, showing that topsoil is more sensitive than subsoil 412 to management (e.g., Novelli et al., 2017). The superficial layer of the soil is expected to be more affected by cropping practices, especially in no-till systems, where the absence of tillage 413 414 induces a stratification of most soil properties with depth (Franzluebbers 2002), whereas tilled 415 systems tend to have more homogenous properties within the tilled layer. This strong 416 stratification of soil properties was also observed here for the no-till fields. Despite being a 417 thin layer, the surface layer is at the interface with the atmosphere and plays a major role in 418 soil quality through soil stabilisation, water infiltration ability and potential role in the 419 reduction of erosion (Franzluebbers 2002). Since the topsoil is more prone to erosion, 420 accumulation of carbon in the surface of no-till fields, while improving soil quality, can also 421 put soil carbon at higher risk of loss during major disturbance events. 422 In this study, no-till systems had higher soil organic carbon concentrations and stocks in the 423 topsoil (0-5 cm depth) compared to conventional systems, while organic systems had

424 intermediate values. However, no difference in carbon stocks was observed for the topsoil (0-

425 20 cm) and total soil profile (0-50 cm). This shows, in accordance with other studies (e.g. 426 Virto et al., 2012; Mary et al., 2020), that the reduction of tillage alone does not necessarily 427 lead to an increase in carbon stocks across the profile. These studies have shown that the 428 amount of organic inputs to the soil is the main driver explaining differences in carbon stocks 429 between systems (Virto et al., 2012; Mary et al., 2020). In our study, the organic fields did not 430 show any significant increase in carbon stocks compared to conventional fields. This could be 431 explained by the absence of difference in external organic matter inputs between the cropping 432 systems, along with reduced biomass production and yield in the organic fields studied here 433 (Büchi et al., 2019). In contrast, the aggregate mean weight diameter of organic fields was 434 similar to no-till fields, and higher than conventional fields in the uppermost soil layer (0-5 435 cm). This indicates that some practices may offset the negative effect of tillage on soil 436 aggregate (see section 4.2 below).

437 The measured organic carbon stocks and clay to carbon ratios highlight a potential for 438 increasing soil organic carbon in the studied fields. At 0-5 cm, 23 fields out of 60 achieved a 439 clay/carbon ratio <10, indicating good soil quality and the potential complexion of all 440 available clay with carbon (Johannes et al., 2017; Merante et al., 2017; Schjønning et al., 441 2018). These fields with 'good' soil quality according to Johannes et al. (2017), while mainly 442 observed in no-till (14 fields), also appeared in the organic (5 fields) and conventional 443 systems (4 fields). This shows that good soil quality can be achieved in all cropping systems. 444 However, most fields presented clay/carbon values >10, meaning the likely presence of non-445 complexed clay and thus the potential to increase organic carbon storage. The average value 446 for clay/SOC ratios for the conventional fields (0-5 cm) was 14, which is above the threshold 447 limit of 13 defined by Johannes et al. (2017) corresponding to degraded soil structural quality. 448 The clay/carbon ratio increased with depth, showing an even higher potential for carbon 449 increase in subsoils.

450 An average of 47.7 t C/ha for 0-20 cm and 72.1 t C/ha for 0-50 cm is currently stored in the 451 60 fields analysed here. An increase in carbon concentration allowing to reach a clay/carbon 452 ratio of 10 for all fields would roughly increase this quantity to 66.7 t C/ha for 0-20 cm and 453 122.7 t C/ha for 0-50 cm. This would represent a significant potential to store large amounts 454 of carbon in arable fields in the lowlands of Switzerland. Achieving such an increase in 455 carbon storage would contribute to improving soil quality and to the global effort towards 456 mitigation of climate change through carbon sequestration in agricultural soils (Smith et al., 457 2008; Lal et al., 2018). However, the strategies to practically increase soil organic carbon at 458 depth to such a degree remain unclear.

In addition, climate also plays an important role in determining the maximum potential of carbon sequestration, as mineralisation rate is directly influenced by soil moisture and temperature (Jobbagy and Jackson, 2000; Curtin et al., 2012). A clay to carbon ratio of 10 may thus not be achievable under all climates, but previous studies indicates that this should be the case in Switzerland (Johannes et al., 2017). Furthermore, changing the focus from sole carbon storage to the overall improvement of soil quality and functions might be a more promising approach as advocated in recent studies (Poulton et al., 2017; Baveye et al., 2020).

466

467 *4.2 Main drivers of soil organic carbon and aggregation*

468 Our results indicated that some fields had potentially a better long-term protection of soil 469 organic carbon compared to others, as a large mean weight diameter of aggregates, proportion 470 of large macroaggregates and accumulation of carbon in these large macroaggregates are 471 known to improve carbon protection and thus reduce its potential loss (Six et al., 2000b). As 472 our results showed that the type of cropping system was not the sole driver of differences in 473 soil carbon and aggregates, we assessed the main drivers among a set of continuous variables 474 across all fields without considering their cropping system 'label'. Six main factors have been 475 shown to influence soil aggregation (Blanco-Canqui and Lal, 2004; Six et al., 2004): 1.

476 environmental variables, 2. inorganic binding agents, 3. soil microorganisms, 4. cropping 477 practices such as tillage, 5. soil fauna, and 6. roots. In this on-farm study, we tested the 478 relative importance of variables belonging to the first four of these six categories. Rainfall and 479 mean temperature were used as representative environmental variables (freezing-thawing days 480 was highly correlated with temperature and thus discarded). Total calcium concentration is a 481 known binding agent (Six et al., 2004), which was assessed here together with pH. Microbial 482 carbon and mycorrhizal biomarker were used to test the effect of soil microorganisms. We 483 also included several cropping practices variables, related to crop diversity, ley cultivation, 484 tillage intensity, amount of organic inputs and nitrogen inputs. However, earthworm 485 abundance and diversity were not assessed here, although it has been shown to be an 486 important driver of soil aggregation (Fonte et al., 2007; Sheehy et al., 2019; Guhra et al., 487 2020).

488

489 <u>Environmental variables and inorganic binding agents</u>

490 As previously discussed, clay concentration was a major driver of soil organic carbon 491 concentration in this study. Rainfall tended to be positively associated with aggregate size, perhaps due to washing off or erosion of small aggregates, or indirectly through positive 492 493 influence on soil biological activity. Nevertheless, dry-wet cycles, which were not 494 investigated here, have been shown to be more relevant to explain aggregation (Denef et al., 495 2001; Cosentino et al., 2006; Harrison-Kirk et al., 2014). Mean temperature during the 496 previous autumn and winter was negatively associated with aggregation. Previous studies 497 have shown that frost could either decrease or increase aggregation, depending on soil water 498 content, freezing intensity, soil type (Edwards, 1991; Lehrsch et al., 1993; Lehrsch, 1998; Six 499 et al., 2004).

500 While not accounting for a large part of the variance, pH and sometimes calcium

501 concentration appeared as significant for almost all carbon related variables. Soil pH of arable

fields is among the most frequently managed soil properties, and liming is therefore regularly used to correct this and improve soil structure. The impact of these variables on soil carbon and aggregation and how these could be managed to improve soil quality deserves thus further investigations.

506

507 Soil microorganisms

508 Our analyses showed that for the variables linked to organic carbon (i.e., bulk soil organic 509 carbon concentration, clay/carbon ratio and accumulation of carbon in the large 510 macroaggregates), the explanatory variables accounting for the major part of variability, 511 besides clay content, were the biological variables microbial biomass carbon and the 512 abundance of mycorrhizal fungi. This major role of biological variables contrasts with recent 513 findings of Li et al (2020a) in Australia, who found only little impact of biological variables 514 (microbial diversity and enzyme activity) on soil carbon and nitrogen. The interrelation 515 between soil organic carbon and soil biology is well known (McGill et al., 1975; Kögel-516 Knabner, 2002; Kallenbach et al., 2016; Paul, 2016), but our results did not allow us to 517 identify if it was high microbial biomass and activity that promoted SOC formation or vice 518 versa.

519 In contrast, these biological variables did not appear as the principal factors explaining the 520 degree of aggregation itself (i.e. mean weight diameter). although bacteria and fungi have 521 been shown to promote aggregate formation and stabilisation (Bossuyt et al., 2001; Six et al., 2004; Costa et al., 2018). Fungi, and particularly mycorrhizal fungi, play an important role in 522 523 macroaggregate formation as the hyphae allow to stick soil particles together (Bossuyt et al., 524 2001; Six et al., 2004; Wilson et al., 2009). Bacteria are also involved in microaggregate 525 formation and stabilisation through the secretion of extracellular polymeric substances that 526 aggregate particles (Six et al., 2004; Costa et al., 2018).

528 Cropping practices

For the mean aggregate size in the surface layer (0-5 cm), it is notable that the partial R^2 of 529 clay was only 9% while it represented most of the R^2 for the deeper layers (see Figure 6). 530 531 Apart from clay concentration, variables linked to tillage and fertilisation (for the 0-5 cm 532 layer), and to weather (for the 5-20 cm layer) played a significant role in explaining soil 533 aggregation. Aggregation decreased with increasing tillage depth and nitrogen fertilisation, in 534 accordance with previous observations (Six et al., 2000a for tillage, Fonte et al., 2009 for 535 nitrogen fertilisation). However, the influence of tillage was observed only at 0-5 cm, which is 536 in contrast to several studies showing that tillage is one of the major drivers of reduced 537 aggregation down to the plough depth (Mikha & Rice, 2004; Six et al., 2004; Grandy & 538 Robertson, 2006). 539 Crop diversity and biomass inputs to the soil (either through crop residue or amendment 540 inputs) have previously been demonstrated to play a role in soil aggregation (Mikha & Rice, 541 2004; Cates et al., 2016; Abiven et al., 2009). 'Perennialisation' is also sometimes mentioned 542 as a driver for soil aggregation (Cates et al., 2016; Panettieri et al., 2017; Jensen et al., 2019), 543 and was tested here using the number of years with leys in the rotation in the model.

However, none of these variables were major variables explaining the mean weight diameteror carbon accumulation in our study.

546

547 Potential additional drivers

Interestingly, the total R² for mean aggregate size (weight diameter) for the 0-5 cm layer was low, and lower than for carbon. This indicates that some drivers of aggregation were probably not captured in this study. In addition to earthworms, another important known driver of aggregation that was not studied here is plant roots and their exudates (Baumert et al., 2018). This could also potentially explain the surprising results that organic systems had similar soil organic carbon and aggregation as the no-till systems despite higher tillage intensity and

similar organic inputs in organic fields (Büchi et al., 2019). Cates et al. (2016) showed that 554 555 higher tillage intensity and lower biomass inputs in organic systems could explain lower 556 aggregation and carbon accumulation. Other studies have shown higher aggregation in 557 organic systems but only under reduced tillage (Loaiza Puerta et al., 2018). Some additional 558 analyses done on a subset of fields of this study have shown a tendency to higher root biomass 559 in the organic fields (on a 0-25 cm depth), probably due to several reasons including 560 management, varietal choice and higher weed biomass (Hirte et al., 2020). Another study on 561 the same fields has shown higher root microbial network complexity in organic fields than 562 conventional and no-till (Banerjee et al., 2019), and the role of this diversity and complexity 563 in aggregate formation is a potential lead that would require further investigations. This, 564 together with potentially higher earthworm biomass in organic fields and increased presence 565 of levs in the rotation, could explain the results observed here.

566

567 4.3 Potential for management of soil quality

568 Our analysis showed that unmanageable pedoclimatic factors played a major role in 569 explaining variability in soil organic carbon concentration and related properties across all 570 depths. This shows the key role of on-farm studies, that allow assessing soil quality within 571 coherent farming systems and sets of practices and on a range of pedoclimatic conditions, 572 while on-station experiments usually test individual practices separately in unique or few 573 pedoclimatic conditions for all treatments, sometimes neglecting their vital role in setting 574 boundaries for soil quality. A recent study from Dupla et al. (2021) also demonstrated 575 important discrepancies between soil quality assessment between on-farm and on-station 576 studies. Our results agree with recent findings from Li et al. (2020ab), who also showed an 577 important role of climate and soil type for shaping physico-chemical soil properties in 578 Australia. Indirectly manageable properties such as microbial and mycorrhiza presence also 579 played an important role in our study, while more directly manageable properties such as soil

580 pH and calcium concentration, and cropping practices only played a minor role. Only the 581 mean weight diameter at the 0-5 cm layer was related to cropping practices and is thus 582 directly manageable by farmers. Subsoil properties were primarily explained by clay content 583 and weather and were little influenced by soil management and cropping systems. Altogether, 584 these results show that when comparing fields with different pedoclimatic conditions, the 585 potential of cropping system classification to explain differences in soil quality is only low. In 586 contrast, according to the local pedoclimatic conditions, the use of practices promoting soil 587 biological properties may benefit soil quality as a whole, while no strong direct link between 588 specific cropping practices and soil aggregate could be demonstrated here.

589

590

591 **5. Conclusions**

592 Based on a network of 60 farmer fields in Switzerland, this study demonstrated that traditional 593 cropping system classification (conventional, no-till, organic) only explained differences in 594 soil organic carbon concentration and aggregation size distribution in the surface soil layer, 595 but not in the deeper layers. Clay content was a one of the main driver of almost all assessed 596 soil properties, and thus the potential to increase soil organic carbon storage was primarily 597 determined by soil texture, and climate sometimes. However, many fields had proportionally 598 more clay than carbon, indicating a potential for increasing carbon sequestration regardless of 599 the cropping system. Our results suggest that the specificities of each field in terms of 600 location, climate, soil type and management are more important in determining soil properties 601 than cropping systems labels. This advocates for the identification and consideration of the 602 main drivers of soil quality beyond a priori classification to inform management decision and 603 improve soil functionality in agricultural fields.

- 604
- 605

606 Acknowledgements

- 607 The authors thank Cindy Bally, Britta Jahn-Humphrey, Diane Bürge, Florent Georges,
- 608 Arianne Greppin, Julia Hess, Kexing Liu, Marcel Meyer, Loïck Müllauer and Hélène Suss for
- their help for field and lab work, and Juliane Hirte for statistical advice. We thank all the
- 610 participating farmers for their support and confidence.
- 611 This study was funded by the Swiss National Science Foundation in the framework of the
- 612 National Research Program 'Sustainable Use of Soil as a Resource' (NRP 68) [grant 406840-
- 613 161902].
- 614

616 **References**

617	Abiven, S., Menasseri, S., Chenu, C., 2009. The effects of organic inputs over time on soil
618	aggregate stability — A literature analysis. Soil Biol. Biochem. 41, 1–12.
619	http://dx.doi.org/10.1016/j.soilbio.2008.09.015.
620	Agroscope, 1996. Méthodes de référence des stations de recherche Agroscope, volume 1:
621	Analyse de terre et du substrat pour conseil de fumure.
622	Banerjee, S., Walder, F., Büchi, L., Meyer, M., Held, A.Y., Gattinger, A., Keller, T., Charles,
623	R., van der Heijden, M., 2019. Agricultural intensification reduces microbial network
624	complexity and the abundance of keystone taxa in roots. The ISME Journal,
625	doi.org/10.1038/s41396-019-0383-2
626	Baumert, V.L., Vasilyeva, N.A., Vladimirov, A.A., Meier, I.C., Kögel-Knabner, I., Mueller,
627	C.W., 2018. Root exudates induce soil macroaggregation facilitated by fungi in
628	subsoil. Front. Environ. Sci. 6:140, doi: 10.3389/fenvs.2018.00140
629	Baveye, P.C., Schnee, L.S., Boivin, P., Laba, M., Radulovich, R., 2020. Soil Organic Matter
630	Research and Climate Change: Merely Re-storing Carbon Versus Restoring Soil
631	Functions. Front. Environ. Sci. 8, 579904. doi: 10.3389/fenvs.2020.579904
632	Blanco-Canqui, H., Lal, R., 2004. Mechanisms of carbon sequestration in soil aggregates,
633	Critical Reviews in Plant Sciences, 23:6, 481-504, doi: 10.1080/07352680490886842
634	Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification.
635	Canadian Journal of Biochemistry and Physiology 37, 911-917.
636	Bossuyt, H., K. Denef. J. Six, S.D. Frey, R. Merckx, and K. Paustian. 2001. Influence of
637	microbial populations and residue quality on aggregate stability. Appl. Soil Ecol.,
638	16:195-208.
639	Büchi, L., Georges, F., Walder, F., Banerjee, S., Keller, T., Six, J., van der Heijden, M.,
640	Charles, R., 2019. The hidden side of cropping system classification: differences and

- 641 similarities in cropping practices between conventional, no-till and organic systems.
 642 European Journal of Agronomy 109, 125920.
- Bünemann, E. K., Schwenke, G. D., Van Zwieten, L., 2006. Impact of agricultural inputs on
 soil organisms—a review. Australian Journal of Soil Research 44, 379-406.
- Cates, A.M., Ruark, M.D., Hedtchke, J.L., Posner, J.L., 2016. Long-term tillage, rotation and
 perennialization effects on particulate and aggregate soil organic matter. Soil and
 Tillage Research, 155, 371-380.
- 648 Cosentino, D., Chenu, C., Le Bissonnais, Y., 2006. Aggregate stability and microbial
- 649 community dynamics under drying–wetting cycles in a silt loam soil. Soil Biology650 and Biochemistry 38, 2053-2062.
- Costa O.Y.A., Raaijmakers, J.M., Kuramae, E.E., 2018. Microbial extracellular polymeric
 substances: ecological function and impact on soil aggregation. Frontiers in
 Microbiology 9, 1636.
- Curtin, D., Beare, M.H., Hernandez-Ramirez, G., 2012. temperature and moisture effects on
 microbial biomass and soil organic matter mineralization, Soil Science Society of
 America Journal 76, 2055–2067.
- 657 Denef, K., Six, J., Bossuyt, H., Frey, S.D., Elliott, E.T., Merckx, R., Paustian, K., 2001.
- Influence of dry–wet cycles on the interrelationship between aggregate particulate
 organic matter and microbial community dynamics. Soil Biol. Biochem. 33, 1599–
 1611
- Dexter, A.R., Richard, G., Arrouays, D., Czyz, E.A., Jolivet, C., Duval, O., 2008. Complexed
 organic matter controls soil physical properties. Geoderma 144, 620–627.
- 663 Dimassi, B., Mary, B., Wylleman, R., Labreuche, J., Couture, D., Piraux, F., Cohan, J.-P.,
- 664 2014. Long-term effect of contrasted tillage and crop management on soil carbon
 665 dynamics during 41 years. Agric. Ecosyst. Environ. 188, 134–146.

666	Druille, M., García-Parisi, P.A., Golluscio, R.A., Cavagnaro, F.P., Omacini, M., 2016.
667	Repeated annual glyphosate applications may impair beneficial soil microorganisms
668	in temperate grassland. Agriculture, Ecosystems & Environment 230, 184-90.
669	Dupla, X., Gondret, K., Sauzet, O., Verrecchia, E., Boivin, P., 2021. Changes in topsoil
670	organic carbon content in the Swiss leman region cropland from 1993 to present.
671	Insights from large scale on-farm study. Geoderma 400, 115125.
672	Edwards, L.M., 1991. The effect of alternate freezing and thawing on aggregate stability and
673	aggregate size distribution of some Prince Edward Island soils. J. Soil Sci. 42, 193-
674	204.
675	Fonte, S.J., Kong, A.Y.Y., Kessel, C. van, Hendrix, P.F., Six, J., 2007. Influence of
676	earthworm activity on aggregate-associated carbon and nitrogen dynamics differs
677	with agroecosystem management. Soil Biology & Biochemistry 39, 1014-1022.
678	doi:10.1016/j.soilbio.2006.11.011
679	Fonte, S.J., Winsome, T., Six, J., 2009a. Earthworm populations in relation to soil organic
680	matter dynamics and management in California tomato cropping systems. Applied
681	Soil Ecology 41, 206–214. doi:10.1016/j.apsoil.2008.10.010
682	Franzluebbers, A.J., 2002. Soil organic matter as an indication of soil quality. Soil TillageRes.
683	66, 95–106.
684	Grandy, A.S., Robertson, G.P., 2006. Aggregation and organic matter protection following
685	tillage of a previously uncultivated soil. Soil Sci. Soc. Am. J. 70, 1398–1406.
686	Gregorio, V., Büchi, L., Anneville, O., Rimet, F., Bouchez, A., Chèvre, N., 2012. Risk of
687	herbicide mixtures as a key parameter to explain phytoplankton fluctuation in a great
688	lake: The case of Lake Geneva, Switzerland. Ecotoxicology 21, 2306-2318.
689	Grömping, U., 2006. Relative Importance for Linear Regression in R: The Package relaimpo.
690	Journal of Statistical Software, 17, 1-27.

- Guhra, T., Stolze, K., Schweizer, S., Totsche, K.U., 2020. Earthworm mucus contributes to
 the formation of organo-mineral associations in soil. Soil Biology and Biochemistry
 145, 107785.
- Harrison-Kirk, T., Beare, M.H., Meenken, E.D., Condron, L.M., 2014. Soil organic matter
- 695and texture affect responses to dry/wet cycles: Changes in soil organic matter696fractions and relationships with C and N mineralisation. Soil Biology and
- 697 Biochemistry 74, 50-60.
- Hassink, J., 1997. The capacity of soils to preserve organic C and N by their association withclay and silt particles. Plant and Soil 191: 77-87.
- Helander, M., Saloniemi, I., Omacini, M., Druille, M., Salminen, J.P., Saikkonen, K., 2018.
 Glyphosate decreases mycorrhizal colonization and affects plant-soil feedback. Sci
 Total Environ 642, 285-91.
- Hirte, J., Walder, F., Hess, J., Büchi, L., Colombi, T., van der Heijden, M., Mayer, J., 2020.
- Enhanced root carbon allocation through organic farming is restricted to topsoils.
 Science of the Total Environment, doi: 10.1016/j.scitotenv.2020.143551
- Jensen, J.L., Schjønning, P., Watts, C.W., Christensen, B.T., Peltre, C., Munkholm, L.J.,
- 707 2019. Relating soil C and organic matter fractions to soil structural stability.
 708 Geoderma 337, 834-843.
- Jobbagy, E.G., Jackson, R.B., 2000. The vertical distribution of soil organic carbon and its
 relation to climate and vegetation. Ecological Applications 10, 423-436.
- Joergensen, R. G., 1996. Quantification of the microbial biomass by determining ninhydrinreactive N. Soil Biol. Biochem. doi:10.1016/0038-0717(95)00141-7
- Johannes, A., Matter, A., Schulin, R., Weisskopf, P., Baveye, P.C., Boivin, P., 2017. Optimal
 organic carbon values for soil structure quality of arable soils. Does clay content
- 715 matter? Geoderma 302, 14–21.

716	Kallenbach, C.M., Frey, S.D., Grandy, A.S., 2016. Direct evidence for microbial-derived soil
717	organic matter formation and its ecophysiological controls. Nature Communications
718	7, 13630.
719	Kepler, R.M., Epp Schmidt, D.J., Yarwood, S.A., Cavigelli, M.A., Reddy, K.N., Duke, S.O.,
720	Bradley, C.A., Williams, M.M., Buyer, J.S., Maul, J. E., 2020. Soil microbial
721	communities in diverse agroecosystems exposed to the herbicide glyphosate. Applied
722	and Environmental Microbiology 86, e01744-19. doi.org/10.1128/AEM.01744-19
723	Knapp, S., van der Heijden, M.G.A., 2018, A global meta-analysis of yield stability in organic
724	and conservation agriculture, Nature Communications 9, 3632.
725	Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial
726	residues as inputs to soil organic matter. Soil Biol. Biochem. 34, 139–162.
727	Lal, R., Smith, P., Jungkunst, H.F., Mitsch, W.J., Lehmann, J., Nair, P.K.R, McBratney, A.B.,
728	de Moraes Sá, J.C., Schneider, J., Zinn, Y.L., Skorupa, A.L.A, Zhang, HL.,
729	Minasny, B., Srinivasrao, C., Ravindranath, N.H., 2018. The carbon sequestration
730	potential of terrestrial ecosystems. Journal of Soil and Water Conservation 73, 145A-
731	152A. doi:10.2489/jswc.73.6.145A
732	Lee, J., Hopmans, J.W., Rolston, D.E., Baer, S.G., Six, J., 2009. Determining soil carbon
733	stock changes: Simple bulk density corrections fail. Agric. Ecosyst. Environ. 134,
734	251-256.
735	Lehrsch, G.A., Sojka, R.E., Jolley, P.M., 1993. Freezing effects on aggregate stability of soils
736	amended with lime and gypsum. In: Poesen, J.W.A., Nearing, M.A. (Eds.), Soil
737	Surface Sealing and Crusting. Catena Verlag, Cremlingen-Destedt, Germany, pp.
738	115–127.
739	Lehrsch, G.A., 1998. Freeze-thaw cycles increase near-surface aggregate stability. Soil Sci.
740	163, 63–70.

- Leifeld, J., Fuhrer, J., 2010. Organic Farming and Soil Carbon Sequestration: What Do We
 Really Know About the Benefits? AMBIO 39, 585–599.
- Li, J., Nie, M., Pendall, E., 2020a. Soil physico-chemical properties are more important than
 microbial diversity and enzyme activity in controlling carbon and nitrogen stocks
 near Sydney, Australia. Geoderma 366, 114201.
- Li, J., Nie, M., Powell, J.R., Bissett, A., Pendall, E., 2020b. Soil physico-chemical properties
 are critical for predicting carbon storage and nutrient availability across Australia.
 Environ. Res. Lett. 15, 94088.
- Lenth, R.V., 2016. Least-Squares Means: The R Package Ismeans. Journal of Statistical
 Software, 69, 1-33.
- 751 Loaiza Puerta, V., Pujol Pereira, E.I., Wittwer, R., van der Heijden, M., Six, J., 2018.
- 752 Improvement of soil structure through organic crop management, conservation tillage753 and grass-clover ley. Soil and Tillage Research, 180, 1-9.
- Mary, B., Clivot, H., Blaszczyk, N., Lebreuche, J., Ferchaud, F., 2020. Soil carbon storage
 and mineralization rates are affected by carbon inputs rather than physical
 disturbance: Evidence from a 47-year tillage experiment. Agriculture, Ecosystems
 and Environment 299, 106972.
- McGill, W. B., Shields, J. A. & Paul, E. A., 1975. Relation between carbon and nitrogen
 turnover in soil organic fractions of microbial origin. Soil Biol. Biochem. 7, 57e63
- 760 Merante, P., Dibari, C., Ferrise, R., Sánchez, B., Iglesias, A., Lesschen, J.P., Kuikman, P.,
- Yeluripati, J., Smith, P., Bindi, M., 2017. Adopting soil organic carbon management
 practices in soils of varying quality: implications and perspectives in Europe. Soil
 Tillage Res. 165, 95–106.
- Mikha, M.M., Rice, C.W., 2004. Tillage and manure effects on soil and aggregate-associated
 carbon and nitrogen. Soil Sci. Soc. Am. J. 68, 809. doi:
- 766 dx.doi.org/10.2136/sssaj2004.0809.

767	Novelli, L.E., Caviglia, O.P., Piñeiro, G., 2017. Increased cropping intensity improves crop
768	residue inputs to the soil and aggregate-associated soil organic carbon stocks. Soil and
769	Tillage Research 165, 128-136.
770	Olsson P, Thingstrup I, Jakobsen I, Bååth E., 1999. Estimation of the biomass of arbuscular
771	mycorrhizal fungi in a linseed field. Soil Biol Biochem. 31, 1879–87.
772	Or, D., Keller, T., Schlesinger, W.H., 2021. Natural and managed soil structure: On the fragile
773	scaffolding for soil functioning. Soil & Tillage Research 208, 104912.
774	Panettieri, M., Rumpel, C., Dignac, MF., Chabbi, A., 2017. Does grassland introduction into
775	cropping cycles affect carbon dynamics through changes of allocation of soil organic
776	matter within aggregate fractions? Sci. Total Environ. 576, 251–263. doi :
777	dx.doi.org/10.1016/j.scitotenv.2016.10.073.
778	Paul, E. A., 2016. The nature and dynamics of soil organic matter: plant inputs, microbial
779	transformations, and organic matter stabilization. Soil Biol. Biochem. 98, 109–126.
780	Pihlap, E., Steffens, M., Kögel-Knabner, I., 2021. Initial soil aggregate formation and
781	stabilisation in soils developed from calcareous loess. Geoderma 385, 114854.
782	Rasmussen, C., Heckman, K., Wieder, W.R., Keiluweit, M., Lawrence, C.R., Berhe, A.A.,
783	Blankinship, J.C., Crow, S.E., Druhan, J.L., Hicks Pries, C.E., Marin-Spiotta, E.,
784	Plante, A.F., Schädel, C., Schimel, J.P., Sierra, C.A., Thompson, A., Wagai, R.,
785	2018. Beyond clay: towards an improved set of variables for predicting soil organic
786	matter content. Biogeochemistry. doi:10.1007/s10533-018-0424-3
787	R Core Team, 2020. R: A language and environment for statistical computing. R Foundation
788	for Statistical Computing, Vienna, Austria.
789	Palm, C., Blanco-Canqui, H., DeClerck, F., Gatere, L., Grace, P., 2014. Conservation
790	agriculture and ecosystem services. Overview. Agric. Ecosyst. Environ. 187, 87–105.
791	Poulton, P., Johnston, J., Macdonald, A., White, R., Powlson, D., 2017. Major limitations to
792	achieving "4 per 1000" increases in soil organic carbon stock in temperate regions:

- Final Evidence from long-term experiments at Rothamsted Research, United Kingdom.Global Change Biology 24, 2563-2584.
- Schjønning, P., Jensen, J.L., Bruun, S., Jensen, L.S., Christensen, B.T., Munkholm, L.J.,
 Oelofse, M., Baby, S., Knudsen, L., 2018. The role of soil organic matter for
 maintaining crop yields: evidence for a renewed conceptual basis. Advances in
 Agronomy 150, 35-79.
- Schwarzenbach, R.P., Escher, B.I., Fenner, K., Hofstetter, T.B., Johnson, C.A., von Gunten,
 U., Wehrli, B., 2006. The challenge of micropollutants in aquatic systems. Science
 313, 1072–1077.
- Sheehy, J., Nuutinen, V., Six, J., Palojärvi, A., Knuutila, O., Kaseva, J., Regina, K., 2019.
 Earthworm *Lumbricus terrestris* mediated redistribution of C and N into large
 macroaggregate-occluded soil fractions in fine-textured no-till soils. Applied Soil
 Ecology 140, 26-34.

806 Singh, M., Sarkar, B., Sarkar, S., Churchman, J., Bolan, N., Mandal, S., Menon, M.,

- 807 Purakayastha, T.J., Beerling, D.J., 2018. Chapter Two Stabilization of Soil Organic
 808 Carbon as Influenced by Clay Mineralogy. Advances in Agronomy 148, 33-84.
- 809 Six, J., Bossuyt, H., Degryze, S., Denef, K. 2004. A history of research on the link between
- 810 (micro)aggregates, soil biota, and soil organic matter dynamics. Soil & Tillage
 811 Research, 79, 7–31
- Six, J., Elliott, E.T., Paustian, K., Doran, J.W., 1998. Aggregation and soil organic matter
 accumulation in cultivated and native grassland soils. Soil Sci. Soc. Am. J. 65:1367–
 1377.

815 Six, J., Elliot, E.T., Paustian, K., 2000a. Soil microaggregate turnover and microaggregate

816 formation: a mechanism for C organic under no-tillage agriculture. Soil Biol.

817 Biochem. 32, 2099–2103.

- Six, J., K. Paustian, E.T. Elliott, and C. Combrink. 2000b. Soil structure and soil organic
 matter: I. distribution of aggregate size classes and aggregate associated carbon. Soil
 Sci. Soc. Am. J., 64:681-689.
- 821 Smith, P., D. Martino, Z. Cai, D. Gwary, H. Jenzen, P. Kumar, B. McCarl, et al. 2008.
- 822 Greenhouse gas mitigation in agriculture. Philosophical Transactions of the Royal
 823 Society (B): Biological Sciences 363, 789-813.
- Sun, W., Canadell, J.G., Yu, L., Yu, L., Zhang, W., Smith, P., Fischer, T., Huang, Y., 2020.
 Climate drives global soil carbon sequestration and crop yield changes under
 conservation agriculture. Global Change Biology 26, 3325-3335.
- Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural
 sustainability and intensive production practices. Nature 418, 671–677.
- 829 Totsche, K.U., Amelung, W., Gerzabek, M.H., Guggenberger, G., Klumpp, E., Knief, C.,
- 830 Lehndorff, E., Mikutta, R., Peth, S., Prechtel, A., Ray, N., Kögel-Knabner, I., 2018.
- 831 Microaggregates in soils. J. Plant Nutr. Soil Sci. 181, 104–136.
- USDA, 2012. Revised Universal Soil Loss Equation, Version 2 (RUSLE2). Natural Resources
 Conservation Service.
- Vance, E. D., Brookes, P. C. & Jenkinson, D. S., 1987. An extraction method for measuring
 soil microbial biomass C. Soil Biol. Biochem. doi:10.1016/0038-0717(87)90052-6
- Virto, I., Barré, P., Burlot, A., Chenu, C., 2012. Carbon input differences as the main factor
 explaining the variability in soil organic C storage in no-tilled compared to inversion
 tilled agrosystems. Biogeochemistry 108, 17–26.
- 839 Virto, I., Imaz, M.J., Fernández-Ugalde, O., Gartzia-Bengoetxea, N., Enrique, A., Bescansa,
- P., 2015. Soil degradation and soil quality in western Europe: current situation and
 future perspectives. Sustainability 7, 313–365.

842	von Fromm, S.F., Hoyt, A.M., Acquah, G.E., Aynekulu, E., Berhe, A.A., Haefele, S.M.,
843	Lange, M., McGrath, S.P., Shepherd, K.D., Sila, A.M., Six, J., Towett, E.K.,
844	Trumbore, S.E., Vågen, TG., Weullow, E., Winowiecki, L.A., Doetterl, S., 2020.
845	Continental-scale controls on soil organic carbon across sub-Saharan Africa. SOIL
846	Discussions. doi: 10.5194/soil-2020-69
847	Wiesmeier, M., Urbanski, L., Hobley, E., Lang, B., von Lützow, M., Marin-Spiotta, E., van
848	Wesemael, B., Rabot, E., Liess, M., Garcia-Franco, N., Wollschläger, U., Vogel, H
849	J., Kögel-Knabner, I., 2019. Soil organic carbon storage as a key function of soils - A
850	review of drivers and indicators at various scales. Geoderma 333, 149-162.
851	Williams, H., Colombi, T., Keler, T., 2020. The influence of soil management on soil health:
852	An on-farm study in southern Sweden. Geoderma 360, 114010.
853	Wilson, G.W.T., Rice, C.W., Rillig, M.C., Springer, A., Hartnett, D.C., 2009. Soil
854	aggregation and carbon sequestration are tightly correlated with the abundance of
855	arbuscular mycorrhizal fungi: results from long- term field experiments. Ecology
856	letters 12, 452-461.
857	
858	

860 Table and figure legends

861

862 Table 1 Mean values and standard error of bulk density (g/cm3), clay concentration, soil 863 organic carbon (SOC, g/kg), clay/carbon ratio, carbon stock (t/ha) and mean weight diameter 864 (MWD, mm) for the three cropping systems. The 'p-value' column gives the p-values of the 865 effect of cropping systems for the analyses of variance, or covariance with clay content as 866 covariate. Lowercase letters indicate pairwise differences between cropping systems, for a 867 given layer. Uppercase letters indicate pairwise differences between layers, for a given 868 cropping system (p-values not shown for these analyses). Pairwise comparisons were assessed 869 with a Tukey HSD test, at p=0.05. n=20 for each cropping system. 870 871 Figure 1 Clay to (soil organic) carbon ratio for the three depths 0-5 cm (A.), 5-20 cm (B.) and 872 20-50 cm (C.), for the three cropping systems. Each 'bean' represents the density distribution 873 of the values, with the large black line showing the median of each group. In each panel, the 874 horizontal line represents the threshold value = 10 for the clay to carbon ratio. The lower the 875 ratio is, the better in terms of soil structural quality. Note that the y-axis scale is different for

876 each panel.

877

Figure 2 Carbon stocks for the three depths 0-5 cm (A.), 5-20 cm (B.) and 20-50 cm (C.), for
the three cropping systems. Each 'bean' represents the density distribution of the values, with
the large black line showing the median of each group.

881

Figure 3 Aggregate fraction distribution (mean ± 1 standard error, g aggregate/kg dry soil) for
each depth and cropping systems. 'conv': conventional systems, 'nt': no-till systems, 'org':
organic systems. Lowercase letters indicate pairwise differences between cropping systems,
for a given aggregate fraction. From bottom to top of each bar: large macroaggregates (2000)

µm - 8000 µm), small macroaggregates (250 µm - 2000 µm), microaggregates (53 µm - 250
µm), silt and clay (< 53 µm). The dashed lines represent a visual aid to compare the size of
the bar fractions.

889

Figure 4 Mean weight diameter for the three depths 0-5 cm (A.), 5-20 cm (B.) and 20-50 cm
(C.), for the three cropping systems. Each 'bean' represents the density distribution of the
values, with the large black line showing the median of each group.

893

Figure 5 Accumulation of carbon in the aggregate fractions (mean ± 1 standard error, g C/kg dry soil) for each depth and cropping systems. 'conv': conventional systems, 'nt': no-till systems, 'org': organic systems. Lowercase letters indicate pairwise differences between cropping systems, for a given aggregate fraction. From bottom to top of each bar: large macroaggregates (2000 µm - 8000 µm), small macroaggregates (250 µm - 2000 µm), microaggregates (53 µm - 250 µm), silt and clay (< 53 µm). The dashed lines represent a visual aid to compare the size of the bar fractions.

901

902 Figure 6 Total R² decomposition by variable type, for the multivariate regressions of soil 903 organic carbon content (SOC), clay to carbon ratio (clay/carbon, CCR), mean weight diameter 904 (MWD), and accumulation of carbon in large macroaggregate (CAM) across cropping 905 systems. Variable types: 1. site-related, unmanageable pedoclimatic variables: clay content, 906 temperature and rainfall, 2. site-related, partially manageable variables: soil chemical 907 properties pH and calcium concentration, site-related, partially indirectly manageable 908 variables: soil biology variables properties, microbial carbon and mycorrhiza marker, and 4. 909 directly manageable variables: cropping practices.

Table S1 Pearson correlation coefficients (lower triangle) and p-values (upper triangle)
between explanatory variables, for the three depths. Coefficients higher than 0.7 or lower than
-0.7 are highlighted in colour, with their respective p-values (colours according to variable
categories, as in Figure 6). Note that weather and cropping practices variables are not depthspecific and thus the values are the same for each depth

916

917**Table S2** Total \mathbb{R}^2 decomposition for the multivariate regressions of soil organic carbon918content (SOC), clay to carbon ratio (CCR), mean weight diameter (MWD), and accumulation919of carbon in large macroaggregate (CAM) across cropping systems and depths. R2 are given920first per categories and then for each individual variable. Significant slopes at p<0.05 in the</td>921multivariate regressions are indicated with a *, p-values between 0.05 and 0.1 are indicated922with a °.

923

Figure S1 A. Geographic distribution of the fields studied and B. Texture of the field topsoil
(0-20 cm) in the ISSS texture triangle. Blue points correspond to conventional systems, red
points to no till systems and green points to organic systems. This figure is adapted from
Büchi et al., 2019.

928

Figure S2 Carbon concentration in the aggregate fractions (mean ± 1 standard error, g C/kg aggregate) for each depth and cropping systems. 'conv': conventional systems, 'nt': no-till systems, 'org': organic systems. From bottom to top of each bar: large macroaggregates (2000 μ m - 8000 μ m), small macroaggregates (250 μ m - 2000 μ m), microaggregates (53 μ m - 250 μ m), silt and clay (< 53 μ m).

935 Table 1

		Conventional				No till			Organic		
		mean		se	mean		se	mean		se	p-value
*Bulk density [g/cm3]	0-5 cm	1.24	В	0.03	1.22	С	0.03	1.18	В	0.03	0.624
	5-20 cm	1.26 b	В	0.03	1.36 a	В	0.02	1.22 b	В	0.04	0.003
	20-50 cm	1.49	Α	0.02	1.47	Α	0.02	1.43	Α	0.03	0.391
clay [%]	0-5 cm	20.4		1.4	21.3		1.5	22.7		2.0	0.600
	5-20 cm	20.3		1.4	21.0		1.6	23.3		2.0	0.428
	20-50 cm	22.0		1.4	22.6		1.8	23.9		1.9	0.718
*Corg concentration [g/kg]	0-5 cm	15.2 b	Α	1.23	23.2 a	Α	2.47	20.5 ab	Α	2.42	0.012
	5-20 cm	14.4	Α	1.17	15.7	В	1.69	19.8	Α	2.42	0.231
	20-50 cm	9.1	В	0.92	8.4	С	0.94	12.5	В	2.12	0.129
clay/Corg	0-5 cm	14 a	В	1.0	10 b	В	0.8	12 ab	В	0.7	0.004
	5-20 cm	15	В	0.9	15	В	1.5	13	В	0.7	0.235
	20-50 cm	28	Α	2.5	34	Α	5.2	25	Α	2.9	0.232
*C stock [t/ha]	0-5 cm	6.5 b	-	0.5	9.9 a	-	1.1	8.8 ab	-	1.0	0.012
	5-20 cm	19.3	-	1.6	21.0	-	2.3	26.6	-	3.2	0.231
	20-50 cm	30.0	-	3.0	27.8	-	3.1	41.3	-	7.0	0.129
*cumulated C stock [t/ha]	0-20 cm	40.6	-	3.1	48.1	-	4.5	54.8	-	6.3	0.225
	0-50 cm	62.6	-	5.3	68.5	-	6.4	85.5	-	11.7	0.213
*MWD [mm]	0-5 cm	2.08 b	AB	0.13	3.20 a	Α	0.11	3.04 a	Α	0.16	0.000
	5-20 cm	2.28	Α	0.16	2.55	В	0.20	2.68	Α	0.17	0.441
	20-50 cm	1.83	В	0.16	1.85	С	0.23	2.06	В	0.23	0.899

* tested with clay as a covariate 936



Cropping systems

Cropping systems

Cropping systems



Cropping systems

Cropping systems

Cropping systems





Cropping systems

Cropping systems

Cropping systems





