Soil organic carbon, extracellular polymeric substances (EPS), and soil structural stability as affected by previous and current land-use

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Abstract

While soil microbial ecology, soil organic carbon (SOC) and soil physical quality are widely understood to be interrelated — the underlying drivers of emergent properties, from land management to biochemistry, are hotly debated. Biological binding agents, microbial exudates, or 'extracellular polymeric substances' (EPS) in soil are now receiving increased attention due to several of the existing methodological challenges having been overcome. We applied a recently developed approach to quantify soil EPS, as extracellular protein and extracellular polysaccharide, on the well-characterised soils of the Highfield Experiment, Rothamsted Research, UK. Our aim was to investigate the links between agricultural land use, SOC, transient binding agents known as EPS, and their impacts on soil physical quality (given by mean weight diameter of water stable aggregates; MWD). We compared the legacy effects from long-term previous land-uses (unfertilised grassland, fertilised arable, and fallow) which were established >50 years prior to investigation, crossed with the same current land-uses

established for a duration of only 2.5 years prior to sampling. Continuously fallow and grassland soils represented the poorest and greatest states of structural integrity, respectively. Total SOC and %N were found to be affected by both previous and current land-uses, while extractable EPS and MWD were driven primarily by the current land-use. Land-use change between these two extremes (fallow→grass; grass→fallow) resulted in smaller SOC differences (64% increase or 37% loss) compared to MWD (125% increase or 78% loss). SOC concentration correlated well to MWD (adjusted R^2 = 0.72) but the high SOC content from previous grassland was not found to contribute directly to the current stability ($p < 0.05$). Our work thus supports the view that certain distinct components of SOC, rather than the total pool, have disproportionately important effects on a soil's structural stability. EPS-protein was more closely related to aggregate stability than EPS-polysaccharide (p values of 0.002 and 0.027, respectively), and ranking soils with the 5 highest concentrations of EPS-protein to their corresponding orders of stability (MWD) resulted in a perfect match. We confirmed that both EPS-protein and EPS-polysaccharide were transient fractions: supporting the founding models for aggregate formation. We suggest that management of transient binding agents such as EPS —as opposed to simply increasing the total SOC content— may be a more feasible strategy to improve soil structural integrity and help achieve environmental objectives.

Keywords

Transient binding agents; microbial exudates; protein matrix bonding; exopolysaccharide; water stable aggregate (MWD); cation exchange resin (CER)

48 **1. Introduction**

49 *1.1. Land use, total SOC and soil structural stability*

50 It is well established that surface vegetation, as controlled by how land is managed,
51 has profound interactions with the underlying soil. In this way, land-use affects many of a soil's
52 fundamental properties including soil organic matter, and its primary constituent: soil organic
53 C (SOC; Jobbágy & Jackson, 2000). Managements that increase SOC in loamy soils enable
54 the development of microporous networks, which are more conducive to the transmission of
55 nutrients, water and air (Bacq-Labreuil et al., 2018; Guo et al., 2020). Without stability in soil
56 structure, these networks and aggregates collapse: restricting porosity, connectivity, nutrient
57 transport, infiltration and root penetration. Soils under arable crops are subjected to annual
58 cultivation and most of the above-ground biomass is harvested, compared to soils under
59 permanent vegetative cover, which have no such disturbance and benefit from increased
60 perennial inputs of C (Chapman et al., 2013; Wiesmeier et al., 2012). It follows that a change
61 in land use alters the balance between inputs and outputs and SOC adjusts to a new
62 equilibrium accordingly (Gregory et al., 2016; Johnston et al., 2009). Soil typically loses SOC
63 faster than it is gained (Johnston et al., 2009; Poeplau et al., 2011; Jones et al., 2016) and so
64 international efforts to increase SOC, such as the '4-per-mille' initiative (Chabbi et al., 2017)
65 are challenged by high frequencies in land-use change.

66 Alternative policies have been called for which focus more on improving soil physical
67 quality (Poulton et al., 2018) and understanding microbial processes contributing to C storage
68 (Liang, et al., 2017). The water-stability and size distribution of soil aggregates, as described
69 by their mean weight diameter (MWD) reflects the stability against physical stresses typically
70 imposed *in-situ* (Le Bissonais, 1996). Accordingly, MWD is commonly used to build an index
71 of soil health and/or quality. Regression models have gone as far as to explain aggregate
72 stability by simple SOC content (e.g. Kemper and Koch, 1966; Six et al., 2002; Soinne et al.,
73 2016) but the relationships between SOC and aggregate stability are not straightforward
74 (Denef and Six, 2005).

1.2 Organic binding agents

Both the quality and location of SOC, or more specifically 'binding agents' at the microscale are understood to be important factors determining the overall stability of a soil (Denef et al., 2007; Jensen et al., 2019). The seminal work of Tisdall and Oades (1982) was perhaps the early thesis on this. They proposed an aggregate hierarchy where smaller microaggregates, held together by *persistent* binding agents (organo-mineral associations), or *transient* binding agents (microbial exopolysaccharides) combine within macroaggregates and are stabilised by *temporary* binding agents such as roots and fungal hyphae. Whilst persistent agents were thought to be largely regulated by soil mineralogy and particle size, both the temporary and transient agents were proposed to be controlled by land-use (Tisdall and Oades, 1982). Golchin et al. (1994) further developed the theory to include microbially-dependent cycles of aggregate formation and dispersal, commencing with organic matter inputs from plant cover which stimulate microbial production of transient binding agents. This in turn facilitated the adsorption of aligned clay particles, by electrostatic attraction, forming central units of new stable aggregates. When readily-available C became depleted, their model predicted binding agents would no longer be produced and the aggregates would crumble when subjected to physical stress. Soil biology has since been shown to be a major contributor to soil aggregate stability (Watts et al., 2001; 2005; Jensen et al., 2019), which is highly responsive to management (Kabir, 2005) and especially C inputs (Hirsch et al., 2017).

1.3 Extracellular polymeric substances (EPS)

The production of exudates as biological binding agents is a salient feature of individual microorganisms attached to their physical habitat or co-habiting in groups called biofilms (Flemming and Wingender, 2010). This exuded matrix is composed of biopolymers more widely referred to as *extracellular polymeric substances* or 'EPS', a term we use hereafter. EPS is thought to be composed primarily of polysaccharides but proteins are a crucial functional component (Flemming et al., 2007). Evidence from water sciences shows that protein-rich EPS plays a central role in adhesion and stabilisation of microbial aggregates

102 (Guo et al., 2016). Indeed, *ex-situ* additions of bacteria selected for their propensity to exude
103 proteinaceous EPS (e.g. *Pseudomonas spp.*) have been shown to aggregate soil particles in
104 solution (Caesar-Tonthat et al., 2014; Krause et al., 2019). While extracellular polysaccharides
105 have long been assumed to be important for soil structure, studies of EPS in soil have been
106 held back by methodological challenges. For example, in the unique study of Tang et al.,
107 (2011) the addition of selective fungicides and bactericides were both shown to decrease soil
108 aggregate stability but the methods used to measure extracellular polysaccharides failed to
109 uncover any statistically significant link. Redmile-Gordon et al. (2014) investigated the 'hot
110 sulphuric acid' method used in the study of Tang et al. (2011) and found that the method also
111 co-extracted large amounts of intracellular biomass, and nonspecific extracellular SOC – likely
112 confounding all three classes of biological binding agent. Other investigators have attempted
113 to use hot water as an extractant but conceded that hot water was also not selective for
114 extracellular polysaccharides (Marchus et al., 2018).

115 Related work on the proteinaceous exudates of arbuscular mychorrhizal fungi, named
116 glomalin, or glomalin-related soil protein (GRSP) are believed to impart aggregate stability and
117 C storage (Wright and Upadhyaya, 1996; Bach et al., 2010). However, the autoclaving step
118 precludes any claim that these extracts represent extracellular material. Furthermore, extracts
119 of GRSP were shown to contain more proteins of bacterial than fungal origin (Gillespie et al.,
120 2011). Redmile-Gordon et al. (2013) proposed that it might be more practical to measure
121 changes in pools of categorically *extracellular* biopolymers as a biophysically distinct product
122 of the microbial biomass, rather than attempting to ascribe taxonomic origin to materials that
123 could be either intracellular or extracellular. The present lack of studies on EPS, linking soil
124 structural stability, or the effects of land-use change represents an opportunity for researchers
125 to advance our understanding of sustainable soil management in this rapidly expanding area
126 (Costa et al., 2018; Liang, et al., 2017).

1.4 Methodological developments for extracting EPS

The qualitative challenges described above suggest a priority for methods that do not co-extract large amounts of 'non-target' organic matter (intracellular or extracellular). The only method reported to meet these criteria to date is the 'cation exchange resin' (CER) method initially developed by Frolund et al. (1996) and adapted for application in soil by Redmile-Gordon et al. (2014). In the method for soils, a dilute divalent metal salt ($CaCl_2$) is used to remove the easily soluble organic fraction. This fraction is highly variable in soil depending on rainfall and moisture content immediately prior to sampling. It is important that the preliminary extractant is not monovalent to maintain EPS stability prior to subsequent removal using CER. The resin extraction step works by reducing binding between negatively charged moieties of the polymeric matrix that are otherwise bridged by divalent cations and releases shorter chains into the extraction buffer (Sheng et al., 2010). This same method was also found to be suitable for EPS in soils dominated by trivalent metal cations (Wang et al., 2019).

The extracellular specificity of the soil EPS extraction method was confirmed using measurements of soil microbial adenosine 5′-triphosphate (ATP; Redmile-Gordon et al., 2014). Their findings concurred with investigators in aquatic sciences (e.g. Takahashi et al., 2009; Ge et al., 2010) that cell-lysis during CER extraction was minimal. To address concerns regarding extracellular contamination, Redmile-Gordon et al. (2015) traced stable isotopes of N through the microbial biomass, showing that CER preferentially extracted synthesised EPS over extracellular SOC of more dubious origin. Colorimetric measurements of EPS protein and polysaccharide in the soil extracts were also corroborated by GC-MS measurements of volatile derivatives, adding support to the more affordable colorimetric determination methods that are normally applied.

1.5 Aims and objectives

Our objectives were to investigate the effect of land use or management changes on soil EPS at the field scale and relate these findings to concomitant changes in soil aggregate stability as a fundamental soil physical property. Importantly, the combinations of 'previous'

154 and 'current' land-uses in this long-term experiment would provide a contrast in the proportion
155 of transient binding agents relative to the total organic matter pool: with 'previous' and 'current'
156 each representing a deficit, and abundance, of land-use-driven binding agents, respectively.
157 Accordingly, we tested three hypotheses: (1) The mixed ley/grass land-use would support the
158 greatest production of microbial EPS, followed by arable, and fallow managements,
159 respectively. (2) EPS concentrations would be more affected by short-term application of the
160 most conducive management than by 'legacy effects' from previous managements – even
161 when that previous management had been applied continuously for a duration of over 50
162 years. (3) These same EPS concentrations would be significantly correlated to an indicator of
163 soil physical quality *i.e.* aggregate stability.

164 **2. Material and methods**

165 *2.1 Experimental site*

166 This study was conducted on the Highfield experiment at Rothamsted Research, UK
167 (Figure 1; global coordinates 51.804, –0.363) where contrasting land-uses were established
168 previously (duration >50 years) and recently (2.5 years prior to sampling). There is an annual
169 mean temperature (1992–2014) and rainfall (1981–2010) of 10.2 °C and 718 mm, respectively
170 (Scott et al., 2014). The soil is classified as a stagnogleyic paleo-argillic brown earth
171 (Batcombe series), equivalent to a Chromic Luvisol (IUSS Working Group WRB, 2015) with
172 silty clay loam texture (Avery and Catt, 1995). Mineralogy is dominated by expanding 2:1 clays
173 (randomly interstratified montmorillonite and vermiculite). Mineralogy and particle size did not
174 differ significantly between treatments allowing the effect of contrasting land-use to be
175 examined without confounding effects of mineralogy or particle size (Jensen et al., 2019).
176 *[Suggested position for Table 1 (soil properties) and Figure 1 (experimental site plan)]*
177 Long-term ley-arable and bare fallow experiments at the site provided 'previous' grassland
178 and arable (both since 1949, in 50 × 7 m plots in a randomized complete block design with
179 four blocks) and 'previous' bare fallow (since 1959, in adjacent land totalling *c.* 1400 m^2) land-

180 uses, as described elsewhere (Gregory et al., 2016). The grassland management supports
181 predominantly rye grass (*Lolium perenne* L.) and clover (*Trifolium* spp.), mown twice in
182 summer with arable represented by winter wheat (*Triticum aestivum* L.) formerly in rotation
183 with winter oats (*Avena sativa* L.). Hay and straw residues are removed after cutting. The
184 fallow treatment is maintained by mouldboard ploughing and cultivating two to four times a
185 year. Arable plots receive 220 kg N ha^{-1} annually (primarily as ammonium nitrate, with
186 occasional ammonium sulphate to supply crop S). Selected properties of the long-term
187 treatments are given in Table 1.

188 In October 2008, three split-plots (10 × 6 m each) were established on each previous
189 grassland and arable treatment in all four blocks, though in the present study we only focussed
190 on the first three. Three sets of three split-plots (of the same size) were established on the
191 adjacent fallow treatment to achieve the same replication. In the split-plots, the same three
192 treatments (grassland, arable and fallow) were established as 'current' management
193 treatments. Thus, the experimental design comprises 9 treatments triplicated in 27 split-plots
194 (3 previous × 3 current management treatments × 3 field replicates). Each of the 9 treatments
195 are therefore characterised by both a *previous* and a *current* management component. This
196 temporal separation implicitly establishes a contrast in the abundance of management-driven
197 transient binding agents (with transient agents being more abundant under the current
198 managements which favour them). Hereafter we use the terms 'grass', 'arable' and 'fallow' to
199 describe the management treatments and the symbol → to indicate where a previous
200 management has been changed to a new current management. For example, grass→arable
201 indicates the treatment previously under grass, but currently under arable.

202 *2.2 Sampling and analysis*

203 Soil samples were collected from three random locations in each of the 27 split-plots
204 in February 2011 with a 75-mm diameter auger to a depth of 52 mm. To reduce within-plot
205 variation as much as practicable, sub-samples were bulked to make a single sample per plot.
206 A natural 3-5 mm aggregate fraction was teased from the bulk after partial-drying in a dark,

207 20 °C ventilated chamber for 18 h to prevent wet-smearing of aggregates upon handling.
208 Roots and macrofauna were removed by hand. The majority of the 3-5 mm fraction was further
209 air-dried in preparation for physical and chemical analyses while portions of the moist 3-5 mm
210 aggregates were crumbled to pass through a 2 mm sieve and chilled to 4 °C overnight. EPS
211 was then extracted as described by Redmile-Gordon et al. (2014). Accordingly, readily soluble
212 organic material was removed from about 3.0 g moist soil (2.5 g dry weight equivalent of soil
213 from each plot) using 0.01 M $CaCl_2$ at a 1:10 soil:solution ratio, in 50 mL capacity
214 polypropylene centrifuge tubes. These tubes were packed in ice and placed on an end-end
215 reciprocating shaker set to 120 rev. min^{-1}. Following this they were centrifuged at 3200 *g* for
216 30 min. Supernatant was discarded. EPS was then extracted from the remaining pellet. This
217 was done by re-suspending the pellet in extraction buffer (2 mM $Na_3PO_4.12H_2O$ (0.760 g L^{-1}),
218 4 mM $NaH_2PO_4.H_2O$ (0.552 g L^{-1}), 9 mM NaCl (0.526 g L^{-1}), 1 mM KCl (0.0746 g L^{-1}), adjusted
219 to pH 7 with 1 M HCl and pre-cooled to 4 °C. CER (Dowex 'Marathon C' sodium form, strongly
220 acidic, 20-50 mesh) was prewashed twice in the above buffer, and 15.98 g CER was added
221 together with the 25 mL buffer. This amount of CER was determined on the basis of 178 mg
222 CER mg^{-1} SOC, as required by the soil with highest SOC content. Tubes were shaken for 2 h,
223 and then centrifuged at 4000 *g* for 30 min. The supernatant containing EPS was transferred
224 into new tubes, frozen in liquid N_2, and stored at -80 °C prior to analysis. Total polysaccharide
225 and uronic acids were quantified as described by DuBois et al. (1956) and Mojica et al. (2007),
226 respectively. Extracted protein was measured using a Lowry based technique with reagent
227 concentrations modified for microplate format as described by Redmile-Gordon et al. (2013),
228 except that no additions of model polyphenolics or dilutions of soil extracts were required. As
229 in previous work, EPS-protein was analysed by comparison to standards of bovine serum
230 albumin (BSA; Sigma A7906) prepared in EPS buffer. Redmile-Gordon et al., (2013) tested
231 the approach proposed by Frolund et al. (1995) to correct for interferences in the extracts. The
232 interference of non-proteinaceous chromogens in natural soil extracts was well described.
233 Accordingly, the interference from non-proteinaceous chromogens in the soil extracts was
234 corrected for by i) measuring the absorbance of a second set of samples using Lowry reagents

235 without $CuSO_4$, and ii) applying the mathematical correction as presented by Frolund et al.,
236 (1995) *i.e.*

237 $$Abs_{protein} = 1.25(Abs_A - Abs_B) \quad (1)$$

238 Where Abs_A is the absorbance given for soil extracts using Lowry reagents, Abs_B is the
239 absorbance given by the set analysed using Lowry reagents without added $CuSO_4$, and
240 $Abs_{protein}$ is the theoretical absorbance due to protein, which is compared against a standard
241 curve of BSA standards made in EPS extraction buffer.

242 SOC and total N concentrations were determined on the 3-5 mm fraction which was
243 firstly air-dried, and fine-milled (< 355 µm) before dry combustion in a Leco TruMac
244 Combustion Analyser (LECO Corp., St Joseph, MI, USA) after carbonate removal with HCl.
245 The MWD (µm) of stable 3-5 mm aggregates was obtained following the procedure for slaking
246 by 'fast wetting' described by Le Bissonnais (1996; ISO, 2012). We selected the 'fast wetting'
247 component of the test as this assesses the response of soil to a highly-disaggregating force,
248 as controlled by the strength of aggregates and their wetting rate.

249 *2.3 Statistical analyses*

250 All statistical analyses were completed using the GenStat (18th Edition) programme
251 (VSN International Ltd., Hemel Hempstead, UK). We first assessed whether the residuals
252 were normally distributed, and transformed (log_{10}) the SOC, N and MWD variates accordingly.
253 The complexity of the previous treatments in the Highfield experiments prior to the imposition
254 of the current treatments was recently described by Gregory et al. (2016). Following their
255 approach, we tested for significant effects of previous and current management treatments on
256 the variates measured using analysis of variance (ANOVA) with the following model
257 structures:

258

259 treatment: (experiment / previous) * current (1)

260 block: (experiment · block) / plot / split-plot (2)

261

262 The 'previous' treatment factor was first nested (/) within an 'experiment' factor which allocated
263 data according to whether it derived from either the original ley-arable or the bare fallow
264 experiment, prior to forming the cross-products (*) with the 'current' treatment factor. Likewise,
265 the block model confirmed that there was no single block factor, but rather there were separate
266 blocks for the different 'experiments' interacting (·). By convention in a factorial design, we
267 compared the effects of the 'previous' and 'current' land use main factors (treatments
268 averaged over their current and previous land use, respectively; n = 9), and their interaction
269 (n = 3). We deemed treatment differences to be 'significant' if the probability (p) of the ANOVA
270 variance ratio statistic (F) was < 0.05. We also conducted regression analysis to test for
271 significant relationships between SOC, EPS and MWD variates.

272 **3. Results**

273 As an individual factor, previous land-use had no significant legacy effects on soil
274 aggregate stability (MWD) or EPS (Table 2). However, there was strong legacy effect on total
275 SOC and N concentrations which decreased significantly in the order grass > arable > fallow
276 ($p < 0.05$; Table 2). A significant effect was also observed for current management with SOC
277 and N ($p < 0.05$) all increasing under grass with the exception that there was no significant
278 difference ascribed to current management between the SOC concentrations currently under
279 arable and fallow (Table 2). Moreover, the extractable EPS-protein, EPS-polysaccharide and
280 EPS-uronic acid pools were all strongly affected by current management, with the variates
281 being significantly greater under grass compared to either arable or fallow ($p < 0.05$), which
282 did not differ significantly. Stable aggregate MWD was also only influenced by current
283 management (Table 2), being significantly greater for soil under grass compared to arable,
284 and significantly greater under arable compared to fallow ($p < 0.05$; Table 3). Under the
285 scheme of Le Bissonnais (1996), soils currently under grass, arable and fallow are classed as
286 'stable', 'unstable' and 'very unstable', respectively.

287 [Suggested position for Table 2]

288 The only variate that revealed a significant interaction between previous and current land-use
289 was MWD ($p = 0.035$; Table 2). Within each previous management treatment, MWD showed
290 statistically significant differences ($p < 0.05$) as a result of current land-use: MWD being
291 highest for grass, intermediate for arable, and lowest for fallow within just 2.5 years since the
292 current land-uses were established. Plots previously under grass showed enhanced structural
293 stability when the current land-use was also grass (grass→grass).

294 [Suggested position for Table 3]

295 Significant regression models ($p < 0.05$) were found for MWD ($\log_{10}$) as a function of SOC,
296 EPS-protein and EPS-polysaccharide (adjusted $R^2 = 0.72$, 0.30, and 0.15, respectively; Table
297 3). Accordingly, these are presented as Figure 2.

298 [Suggested position for Figure 2]

299 Notably, all soils under grass appeared to exhibit MWDs in excess of those predicted by the
300 regression between MWD and SOC (Figure 2). If it is assumed that the measured organic
301 substances contribute to an increase in MWD (as is supposed; Tisdall and Oades, 1982) then
302 the upper limit of the magnitude of the 'binding effect' per unit binder on a weight-for-weight
303 basis (w/w) can be compared by standardising units SOC to the same scale as EPS
304 parameters (Table 3). Standardising all to mg binder g^{-1} soil gives mass-adjusted coefficients
305 for SOC, EPS-polysaccharide, and EPS-protein of 0.027, 1.6, and 3.6, respectively.

306 [Suggested position for Figure 3]

307 Since there was a significant previous–current land use interaction on MWD (Table 2)
308 we explored the links to SOC and EPS-protein by plotting the two most significant predictors
309 of MWD on the natural scale (Figure 3). This provides visual clarity that something subtler than
310 total SOC is affecting the MWD, and that appreciable changes in aggregate stability have
311 been achieved within 2.5 year's application of the new managements. For example, soils
312 previously under grass retained high SOC contents, but the MWDs of these treatments
313 (grass→arable and grass→fallow) were only about half those of the arable→grass soils.
314 Furthermore, arable→grass soils contained less SOC than grass→arable (Figure 3a).
315 Accordingly, prediction of the expected stability order, based on ranking of SOC content was

316 successful in only 3 out of 9 cases with a mismatch occurring between SOC rank and MWD
317 order for most land-use combinations (Figure 3a). In contrast, EPS-protein (Figure 3b) enabled
318 better prediction of rankings for management-driven stabilities across the range of stability
319 classes from 'very stable' to 'unstable' (Figure 3b): being successful in 6 out of 9 cases (Figure
320 3b). This relationship was more apparent with the more stable soils, where rank of EPS-protein
321 matched the order of the top five structural stabilities: greatest EPS-protein for the greatest
322 MWD, second greatest EPS-protein for the second greatest MWD, and so on.

323 All treatments previously under grass showed the greatest changes in SOC, with
324 losses outweighing the loss or gain of any other permutation. This was not the case with EPS-
325 protein (Figure 3b) where the magnitude of increase from fallow (fallow→grass) was similar to
326 the loss of EPS-protein when transitioning from the reverse permutation (grass→fallow).
327 There were greater losses of MWD, SOC and EPS-protein associated with transition to fallow
328 (grass→fallow) over arable (grass→arable). Both transitions resulted in aggregates being
329 classed as 'unstable' compared to those remaining under grass which were 'very stable' (Le
330 Bissonnais, 1996). In contrast, soils previously under arable showed very little loss in SOC or
331 EPS-protein content (1.64 to 1.56%; and 185 to 176 μg g^{-1}) when converted to fallow, but the
332 decrease in MWD was much greater: from 584 to 395 μm with a change of class from
333 'unstable' to 'very unstable' (Le Bissonnais, 1996).

334 The greatest total improvement in MWD was seen with transitions from arable to grass
335 (increase of 590 μm), and while the SOC content of this treatment was less than the SOC
336 content of the reverse grass→arable treatment, the EPS-protein was indeed greater, and the
337 MWD was much greater too (at 1174 'medium' *vs.* 684 μm 'unstable'; Le Bissonnais, 1996).
338 Likewise comparing the grass→fallow with fallow→grass treatment changes we see that the
339 former retained a considerably greater SOC concentration (2.33 *vs.* 1.43%) but a considerably
340 lesser EPS-protein concentration (157 *vs.* 191 μg g^{-1}), while the latter had the greater EPS-
341 protein concentration and structural stability as indicated by MWD (494 *vs.* 595 μm; Table 2).

342 We established that there was no significant interaction effect between previous ×
343 current land use on EPS fractions, however we did note some unexpected observations. EPS-

polysaccharide decreased with a transition from fallow to arable, as did EPS-protein (Table 2), but apart from these soils previously managed as fallow, there were no other similarities in land-use responses between these EPS pools. EPS-polysaccharide and EPS-uronic acid both unexpectedly increased when converted to the reduced C-input fallow (Table 2; arable→fallow; 318 to 375 μg EPS-polysaccharide; 167 to 194 μg EPS-uronic acid g^{-1} soil). Accordingly, the reverse fallow→arable conversion decreased all EPS fractions, but increased the MWD (Table 2). Additionally, the greatest MWD of all land-uses (grass→grass) was associated with merely average EPS-polysaccharide concentrations.

4. Discussion

4.1 Effects of land-use and land-use change on SOC, EPS and MWD

Continuous grassland clearly resulted in the greatest SOC, EPS and structural stability (MWD). We found total SOC concentration to be the best *overall* predictor of MWD according to the adjusted R^2 (Table 3). However, the maximum possible contribution of SOC to MWD was relatively low when considered on an equivalent mass basis: about 60 or 140 x smaller than that of EPS-polysaccharide or EPS-protein, respectively (Table 3). This stands to reason as not all SOC will have a binding effect. This also suggests that the relationship may not always be sensitive enough to describe how small changes in SOC quantity or quality affect a soil's stability. The lack of a decisive relationship between SOC and aggregate stability has been identified before (Tisdall and Oades, 1982; Liu et al., 2005; Denef et al., 2007; Sarker et al., 2018) and we explore this further in our data.

We found greater scattering in EPS-protein's relationship to MWD from the data shown in Figure 2b compared to Figure 2a, which could be due to variability introduced during the extraction of EPS prior to measurement, incomplete extraction, and/or reflect some natural underlying variability in the relationship. Indeed, one of the strengths of the total SOC measurement is the complete combustion of C in the analysis. Nonetheless, comparisons of Figures 3a and 3b show that EPS-protein may be more mechanistic in the formation —or maintenance— of the more stable aggregates in response to land-use and land-use change.

371 For example, total SOC (Figure 3a) did not explain the mean stability order of land-use
372 combinations, with a mismatch occurring between SOC rank and MWD rank in most cases.
373 In contrast, EPS-protein, which was controlled by current land-use (Table 1), described the
374 stability order more accurately (Figure 3b). From a biofilm perspective this might be expected,
375 because proteinaceous moieties in EPS are widely known to mediate biological attachment to
376 abiotic surfaces (e.g. Taglialegna et al., 2016). A notable example of these impacts on stability
377 at odds with total SOC is found in the extreme land-use changes between grass and fallow.
378 While grass→fallow retained considerably greater SOC than fallow→grass (2.33 *vs.* 1.43%),
379 the latter yielded more EPS-protein (191 vs. 157 μg g^{-1}) and with this, significantly greater
380 structural stability (MWD; 595 vs. 494 μm; Table 2).

381 There were examples where considerable decreases in MWD were not fully explained
382 by either SOC or EPS. Treatments previously under arable showed very little loss in SOC
383 when converted to fallow (1.64 to 1.56%, a relative decrease of 5%), but the decline in soil
384 physical stability (MWD) was much greater and significant ($p < 0.05$) from 584 μm to 395 μm:
385 a relative decrease of 32%, or transition from qualitative classes 'unstable' to 'very unstable'
386 (Le Bissonnais, 1996). In this instance, the loss of EPS-protein was similar to SOC (185 to
387 176 μg EPS g^{-1}; relative decrease of 5%). This decline is likely to be more related to differences
388 in *temporary* binding agents (plant roots and hyphae) because increased tillage is well-known
389 known for its disruptive influence on fungal hyphae in fallow soils (Kabir, 2005). This
390 magnitude of decline in aggregate stability is also similar to that found by Blankinship et al.
391 (2016) where plants were manually removed from a seasonally dry grassland. We also found
392 examples where a considerable *increase* in MWD was not fully explained by either SOC or
393 EPS. The transition from arable to grass corresponded to increases of 7-48% in the EPS
394 fractions and a 46% increase in SOC but a much greater (101%) increase in MWD. As above
395 this suggests temporary binding agents were also implicated. Grass leys in arable rotation are
396 widely recognised for their capacity to improve soil physical quality, which is understood to be
397 largely owed to improvements in both temporary and transient binding agents (Tisdall and
398 Oades, 1982). Accordingly, the size of the microbial biomass and abundance of fungi at our

399 experimental site were reported by other authors to increase in the order fallow>arable>grass
400 (Gregory et al., 2009; Hirsch et al., 2009; Wu et al., 2012). In the present study, the only
401 binding agents measured were *transient*. However, most roots were removed in the
402 preparation (Section 2.2) and so we assume that fungal hyphae likely accounted for the
403 majority of *temporary* binding effects.

404 Considering the positive interaction between previous and current land-uses observed
405 for MWD ($p < 0.05$; Table 2; Figure 3a) it is apparent that the beneficial effects of current grass
406 and arable land-uses on MWD must have been enhanced by specific conditions at the time of
407 land-use change (owed to the preceding 50 year's land-use). Root establishment of the grass
408 ley and winter wheat (2.5 years prior to sampling) would have clearly benefitted from the soil
409 physical and chemical properties originating from the previous grass land-use (Table 1).
410 Increased plant cover and/or reduced tillage therefore can be seen to have provided positive
411 feedback for the subsequently beneficial land-uses. Nevertheless, no measurable legacy
412 effects or interactions were detected for any of the extracted EPS (Table 2) confirming that
413 the EPS themselves were *transient*. Furthermore, it is not likely that *persistent* binding agents
414 declined substantially in the 2.5 years following land-use change, because organo-mineral
415 associations tend to be resistant to oxidation and microbial metabolism (Kögel-Knabner et al.,
416 2008). Therefore, we expect that this interaction effect is due to *temporary* binding agents co-
417 existing at the time of sampling. Both temporary and transient binding agents are
418 predominantly composed of relatively 'labile' carbon compounds, which are a temporally
419 unstable pool known to decline relative to total SOC in response to reduced plant cover and
420 increased tillage (Haynes, 2000).

421 These combined findings above support the claim of Jensen et al. (2019) that distinct
422 components of SOC, rather than size of the total pool, have a disproportionate effect on
423 aggregate stability. We also observed a larger range, and greater maximum total SOC
424 between previous land-use classes than between current ones (Table 2). This suggests that
425 total SOC content was primarily owed to previous land-use, and moreover that the bulk of
426 SOC in soil previously under grass persisted > 2.5 years. This is supported by historically

fallow plots containing the least SOC whereas historically grass-covered plots contain the most (Table 2). While the concentration of 'persistent' or 'older' SOC cannot be used as a surrogate for *persistent binding agents*, it is important that this large quantity of organic material associated with previous land-uses does not appear to contribute significantly to the stability of soils in our study (see MWD; Table 2; $p = 0.137$). Furthermore, this form —or location— of SOC was not readily available for production of microbial EPS.

In accordance with the present findings, Redmile-Gordon et al. (2015) took soils from the same grass→arable plots in February 2012 and found that the relatively high SOC concentration did not support microbial assimilation of inorganic N ($^{15}NH_4{}^{15}NO_3$) provided in the laboratory. Neither was there any exudation of ^{15}N labelled EPS into the extracellular space *unless* ^{15}N was accompanied with simultaneous inputs of labile C. The above study highlighted the importance of fresh C inputs for EPS production, which is reflected in the present findings of elevated quantities of EPS-protein, EPS-polysaccharide and EPS-uronic acids in soils under dense vegetation cover at the time of sampling (i.e. grass; Table 2). Moreover, these findings reinforce the notion of a 'turning point' where older SOC, which is presumably more decomposed or physically occluded, ceases to be a viable source of C to measurably support exudation of EPS. This is further illustrated by the considerably greater EPS concentrations (protein, polysaccharide and uronic acid) under fallow→grass compared to grass→fallow (Table 2) and thus supports the theory presented by Golchin et al. (1994). Our findings show that measurement of 'transient' binding agents using the method of Redmile-Gordon et al., (2014) can add useful depth and contrast to measures of total SOC.

4.2 SOC vs. biological binding agents as drivers of soil stability

There remains a small paradox to be settled in that SOC shows a good correlation to aggregate stability, while the previous land-managements that contribute the majority of this SOC were not directly related to the MWD observed (Table 2; $p = 0.137$). However, as discussed in section 4.1, a positive interaction arising from the previous grass land-use was driving improved soil quality generally, for example with high SOC contributing a much

454 reduced bulk density (Table 1). More successful plant establishment in 2008 would therefore
455 be expected, and is confirmed by yield measurements taken by other investigators at the same
456 site (e.g. Hirsch et al., 2017). This increased plant biomass would in turn have increased the
457 abundance of associated binding agents such as roots and hyphae - contributing to the MWD
458 measured at the site. Importantly, this clarifies an important point that residual SOC needs no
459 *direct* contribution to soil stability to indirectly contribute to a good R^2 for MWD via positive
460 feedback effects. This example illustrates the case that large quantities of 'inactive' SOC can
461 contribute relatively small concentrations of temporary and transient binding agents, which are
462 overwhelmed by the contributions from current land-use (Table 2; Figure 3b).
463 While it could be argued that EPS production may also have been boosted by the same
464 factors presented in Table 1, this fraction is sufficiently 'transient' that any increases in EPS
465 that occurred previous to the moment of land-use change were no longer detectable at the
466 time of sampling (Table 2). However, there would have been an abundance of EPS in plots
467 formerly under grass, which interestingly corresponds to the increase in exchangeable
468 multivalent cations (Ca^{2+} and Mg^{2+}) under this land-use (Table 1). As discussed in section 4.1,
469 fresh C was previously seen to be vital for production of EPS in these soils. Multivalent cations
470 not only bridge carboxylic acid moieties within the EPS, but when sufficiently concentrated,
471 contribute electrostatic interactions between the EPS and negatively charged mineral surfaces
472 to form considerably stable bonds (Berne et al., 2015). We propose that electrostatic bonding
473 between clays and biota could therefore depend on soil biota being able to maintain production
474 of EPS to retain anchorage of these cations. Our data suggest that the majority of all stabilising
475 effects subsided within 2.5 years of transition away from the high-C grass land-use, with MWD
476 of grass→arable becoming similar to that of continuous arable by the time of measurement
477 (Table 2; LSD 0.16 $\log_{10}$ µm).

478 *4.3 EPS-protein vs. EPS-polysaccharide*

479 While Tisdall and Oades (1982) claimed that extracellular polysaccharides would be
480 the most important transient binding agents in soil, investigations of biofilms in other

environments show that protein and polysaccharide pools are not mutually exclusive, with glycoproteins typically being abundant, and essential, in bonding (Flemming et al., 2007). Among the transient EPS parameters measured here, EPS-protein showed the greatest coefficient of regression and R^2 against MWD (Table 3). Proteinaceous moieties in the EPS matrix are especially recognised for their contribution to stable biofilm structure (Vlamakis et al., 2013). The relatively low R^2 for polysaccharide and protein (0.15, and 0.30, respectively) are expected because the contributions of *temporary* (and *persistent*) binding agents are, of course, excluded.

With regard to the mechanisms underlying the apparently EPS-protein driven stabilisation in the more stable soils (Fig. 3b), BslA proteins exuded from the model soil organism (*Bacillus subtilis*) were found to self-assemble extracellularly: providing architectural stability and protecting the otherwise fragile polysaccharides against disruption from fast wetting (Arnaouteli et al., 2016). Earlier work found that hydrophobic amino acids —most notably phenylalanine and tyrosine— were characteristic of community EPS produced in response to a hydrophilic source of C (Redmile-Gordon et al., 2015). Tyrosine has since been shown to be of mechanistic importance in the formation of adhesive peptides, which impart strength and elasticity to 'living glues' produced by soil organisms (Zhang et al., 2019). The elevated concentrations of proteinaceous EPS in soils currently under grass (Table 2) is perhaps not surprising given that increased availability of plant polysaccharides is known to trigger production of extracellular proteins in the laboratory (Beauregard et al., 2013). However, given that in the present study a) these pools of EPS are transient, and b) solar radiation was at a seasonal low, it is somewhat surprising that these increases in community EPS were still measurable in the field by extraction in the temperate midwinter. Whether this is indicative of basal winter photosynthesis supporting sufficient rhizodeposit-C or a minimum turnover rate equal to several months we cannot say. Nonetheless, the majority do not remain extractable for more than 2.5 years, with previous land-use having no direct effect on the size of this pool ($p = 0.823$; Table 2).

508 Exceptions to the straightforward relationship of [increased plant-C] + [decreased
509 tillage] = [greater EPS] are seen with land-use changes between fallow and arable.
510 Fallow→arable conversion, with its implicit increases in C-inputs and decreased tillage,
511 showed marked declines in EPS-polysaccharide and uronic acid fractions amounting to -51
512 and -41 μg EPS g^{-1} soil, respectively (Table 2). Likewise, arable→fallow conversion had
513 notably increased EPS-polysaccharide and EPS-uronic acid fractions compared to its
514 arable→arable counterpart, despite clearly greater C inputs from winter wheat and
515 comparatively reduced tillage. Precedent exists for two feasible explanations. Firstly, the
516 increased solar radiation for cyanobacteria and other photoautotrophs on the fallow surface
517 may enable sufficient production of extracellular polysaccharides to be detected in the bulk
518 soil. Cyanobacterial polysaccharides can stabilise soil surface crusts (Cania et al., 2019) but
519 are apparently not highly influential for the stability of bulk soil (Table 2). Secondly, and it
520 seems more likely, the inorganic N fertiliser applied annually to these arable plots somehow
521 reduced the efficiency of microbial EPS production in the bulk soil (mass of EPS per unit
522 microbial ATP), as shown previously by Redmile-Gordon et al. (2015). In every other sense,
523 the arable land-use represents the 'intermediate' of individual factors contributing to the
524 contrasting land-use pressures, i.e. tillage frequency, C inputs, duration of vegetative cover,
525 and pH (Table 1). Wu et al. (2012) also reported intermediate soil microbial biomass
526 concentrations, as expected between grass and fallow. Nonetheless, the arable land-use is
527 exceptional in that inorganic N fertiliser is applied annually. Our data therefore support the
528 conceptual model proposed by Redmile-Gordon et al. (2015) that while C is a fundamental
529 requirement for significant EPS production, surplus inorganic N acts as a 'switch': shifting soil
530 microbial investment of C from EPS to intracellular investment for growth of cells.

531 *4.4 Implications for land-use and soil management strategies*

532 Most SOC (> 98%) in sieved soil taken from a grass→arable plot of the same long-
533 term experiment was shown to be non-living and inaccessible to the soil microbial biomass
534 (Redmile-Gordon et al., 2015). In the present study, the residual fraction of SOC from the

same previous land-use was also found to not be directly influential for soil structural stability (Table 2) with the primary binding agents operating at our study site being attributed to the influence of current land-use over the long-term previous land-use (Table 2). Taken together, these results suggest that soil biology, or biological binding agents are vital for favourable soil quality (MWD). The significant shifts in this reasonably 'holistic' measure of soil quality (Six et al., 2000) were achieved within a relatively short period (< 2.5 years). Management of biological binding agents, including EPS as a highly responsive *transient binding agent* may therefore be useful to rapidly improve soil structural stability to meet various environmental objectives. The approach of building a smaller more 'active' subset of total SOC may also help to avoid some of the seasonal risks associated with large pools of total SOC, including emissions of carbon dioxide, leaching of N overwinter, contamination of rivers, aquifers, and the subsequent indirect emissions of nitrous oxide arising from leached N (Reay et al., 2012).

5. Conclusion We confirmed that the primary binding agents affected by current land-use were transient and/or temporary. Direct effects of current mixed grass and clover, without fertiliser inputs, were more important for both soil physical stability, and EPS, than any legacy from a previous land-use (including greater SOC). Among the extracellular fractions studied, the stability of aggregates was most strongly related to increases in EPS-protein, less related to EPS-polysaccharide, and not related to uronic acids. We also demonstrated that measurement of transient binding agents, using a recently developed method, adds useful depth and contrast to measures of total SOC. We conclude that there may be significant environmental benefits to managing smaller specific 'functional' pools of C in agriculture and horticulture, rather than increasing total SOC *per se*.

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736

737 **Captions to figures (uploaded separately)**

738 **Fig. 1.** The Highfield experiment with previous grass (G), arable (A) (both since 1949, in 50
739 × 7 m plots) and bare fallow (F) (since 1959, in adjacent land totalling *c.* 1400 m^2) land uses,
740 with superimposed current grass (black), arable (grey) and fallow (white) land uses. The
741 experimental design comprises 9 treatments triplicated in 27 split-plots (3 previous × 3
742 current land uses × 3 field replicates). Plot numbers are given.
743

744 **Fig. 2.** Stable aggregate mean weight diameter (MWD) as a linear function of (a) soil organic
745 C concentration (y-axis), and (b) EPS-protein concentration. Current grass, arable and fallow
746 land uses are shown (black, grey and white symbols, respectively). See Table 3 for further
747 details of the linear regression analyses.
748

749 **Fig. 3.** Mean (*n*=3) (a) soil organic C concentration (y-axis), (b) EPS-protein concentration as
750 related to stable aggregate mean weight diameter (MWD; circles) for the nine previous ×
751 current land use combinations. Previous land use (x-axis) is shown in factorial combination
752 with current grass, arable and fallow land uses (black, grey and white symbols, respectively).
753 The diameter of each circle represents the stability (scale bar at 1000 µm MWD).

Table 1
Selected physical and chemical properties of the soil (upper 10 cm) at Highfield under long-term grass and arable (since 1949) and fallow (since 1959) land uses as measured in 2005 by Gregory et al. (2009) and Watts (personal communication) unless stated.

Property	Unit	Grass	Arable	Fallow
Sand	g kg^{-1}	179	189	178
Silt	g kg^{-1}	487	504	525
Clay	g kg^{-1}	333	306	297
Particle density	g cm^{-3}	2.46	2.57	2.61
Bulk density [a]	g cm^{-3}	0.99	1.44	1.32
Plastic limit	g kg^{-1}	484	225	208
Liquid limit	g kg^{-1}	663	364	319
Plasticity index	%	17.9	13.9	11.1
Linear shrinkage	%	11.0	10.0	8.2
Water content –1 kPa	g kg^{-1}	449	254	231
Water content –5 kPa	g kg^{-1}	407	244	219
Water content –30 kPa	g kg^{-1}	355	234	221
Water content –300 kPa	g kg^{-1}	178	126	115
Water content –1500 kPa	g kg^{-1}	176	115	99
Exchangeable Ca	$mmol_c$ kg^{-1}	61.8	23.8	10.3
Exchangeable Mg	$mmol_c$ kg^{-1}	6.0	3.5	4.2
Exchangeable Mn	$mmol_c$ kg^{-1}	11	9.1	4.2
Exchangeable K	$mmol_c$ kg^{-1}	4.2	1.4	0.8
Exchangeable Na	$mmol_c$ kg^{-1}	1.0	1.9	0.8
Cation exchange capacity	$mmol_c$ kg^{-1}	78.9	36.4	18.7
pH	–log (g $[H^+]$ L^{-1})	6.30	5.76	4.43
Electrical conductivity	mS cm^{-1}	0.21	0.08	0.05

[a] Gregory et al. (2016)

Table 2

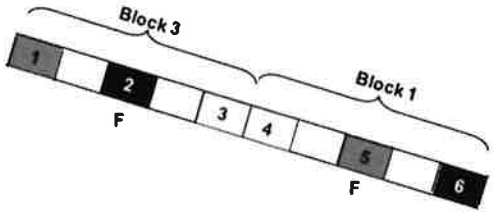
Mean ± standard error of the mean of soil organic C (SOC), total N (N), and soil extracellular polymeric substances (EPS) concentrations (protein, polysaccharide and uronic acid; μg EPS g^{-1} soil), and stable aggregate mean weight diameter (MWD), grouped by land uses (n=3), and their interaction (n=9). Where significant at p<0.05, the least significant differences (LSD) of means are given ('ns' indicates non-significance). Note that some variates were transformed by $\log_{10}$ firstly to normalise the distribution of residuals.

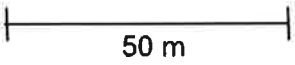
Land use		SOC			N			EPS						MWD		
								protein		polysaccharide		uronic acid				
		(%)		($\log_{10}$)%	(%)		($\log_{10}$)%			(μg g^{-1})				(μm)		($\log_{10}$)μm
Previous																
Grass		2.83	±0.27	0.439	0.26	±0.02	−0.598	188	±12	327	±16	144	±7	1157	±307	2.96
Arable		1.86	±0.13	0.262	0.18	±0.01	−0.753	186	±10	376	±24	203	±16	718	±124	2.81
Fallow		1.12	±0.09	0.038	0.11	±0.01	−0.974	143	±15	301	±27	180	±17	437	±52	2.61
p				0.017			0.035	0.823		0.285		0.086				0.137
LSD				0.100			0.129	ns		ns		ns				ns
Current																
Grass		2.49	±0.36	0.362	0.22	±0.03	−0.681	201	±11	387	±16	209	±16	1354	±276	3.06
Arable		1.73	±0.22	0.211	0.17	±0.02	−0.804	163	±16	298	±22	152	±11	573	±45	2.75
Fallow		1.59	±0.21	0.166	0.15	±0.02	−0.840	153	±11	319	±25	166	±15	384	±37	2.57
p				<0.001			<0.001	0.014		0.005		0.002				<0.001
LSD				0.053			0.052	31		50		28				0.07
Previous	*Current*															
Grass	Grass	3.67	±0.56	0.553	0.32	±0.05	−0.503	213	±21	346	±9	152	±4	2293	±388	3.35
	Arable	2.51	±0.19	0.397	0.23	±0.02	−0.632	195	±11	340	±24	152	±11	684	±43	2.83
	Fallow	2.33	±0.06	0.367	0.22	±0.01	−0.659	157	±20	294	±41	129	±19	494	±49	2.69
Arable	Grass	2.39	±0.09	0.377	0.22	±0.01	−0.653	198	±2	435	±13	248	±2	1174	±109	3.07
	Arable	1.64	±0.04	0.215	0.16	±0.00	−0.787	185	±31	318	±45	167	±34	584	±82	2.76
	Fallow	1.56	±0.03	0.194	0.15	±0.00	−0.818	176	±13	375	±37	194	±12	395	±27	2.60
Fallow	Grass	1.43	±0.05	0.155	0.13	±0.01	−0.888	191	±29	382	±31	227	±19	595	±61	2.77
	Arable	1.05	±0.06	0.021	0.10	±0.00	−0.992	111	±5	236	±3	136	±2	450	±42	2.65
	Fallow	0.87	±0.05	−0.063	0.09	±0.00	−1.041	126	±14	287	±46	177	±30	264	±1	2.42
p				0.988			0.988	0.597		0.133		0.073				0.035
LSD				ns			ns	ns		ns		ns				0.16

Table 3

Stable aggregate mean weight diameter ($(\log_{10})$ µm) as a linear function of soil organic C (SOC; %) and soil extracellular polymeric substances (EPS) concentrations (protein, polysaccharide and uronic acid; µg EPS g^{-1} soil). The table gives the constant (*a*) and coefficient (*b*) of the linear regression ($y = a + bx$), the probability level associated with the regression (*p*), and the adjusted proportion of the variance accounted for by the fit (Adjusted R_2). Note that for uronic acid, the adjusted R_2 is not calculable as the residual variance exceeded the variance of the response variate.

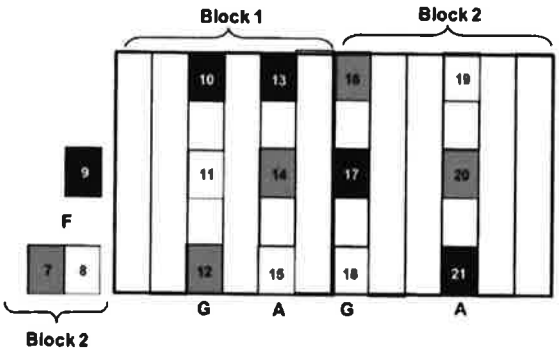
Statistic	SOC	EPS		
		protein	polysaccharide	uronic acid
Constant	2.29±0.07	2.17±0.18	2.26±0.23	2.64±0.21
Coefficient	0.26±0.03	0.0036±0.0010	0.0016±0.0007	0.0008±0.0011
p	<0.001	0.002	0.027	0.465
Adjusted R^2	0.719	0.301	0.149	not calculable

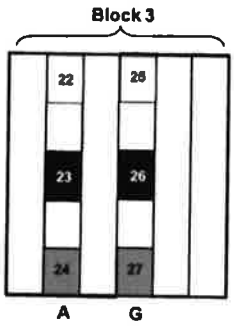


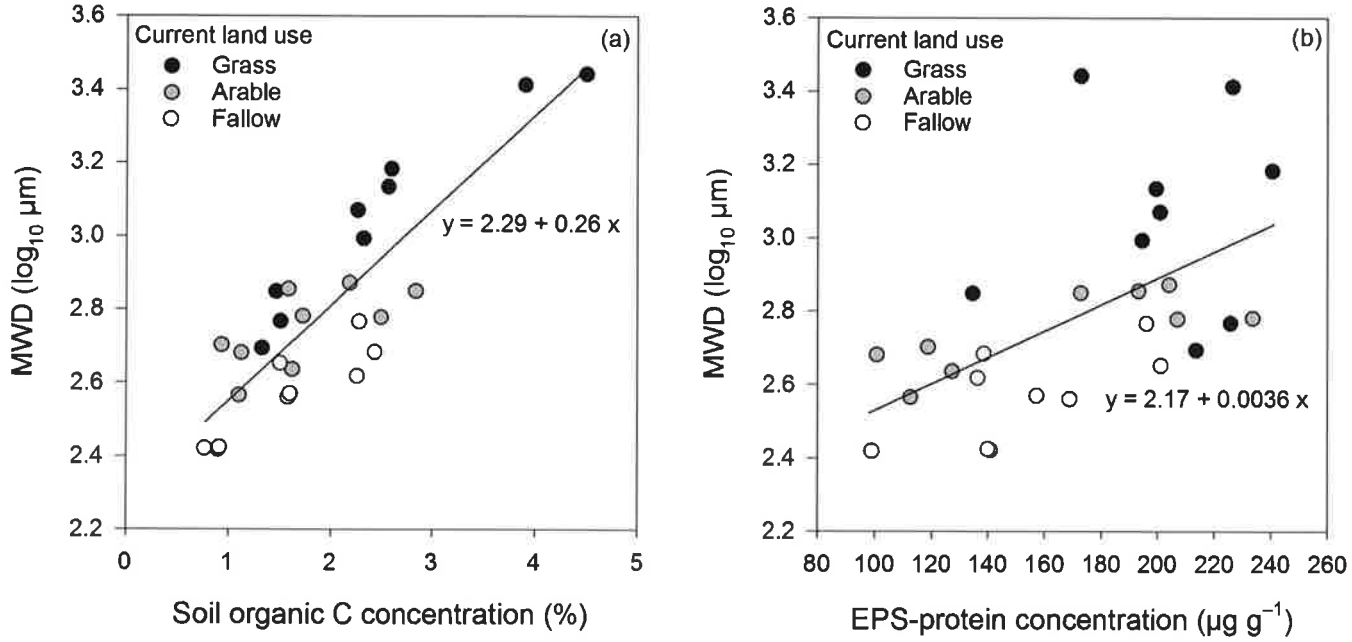


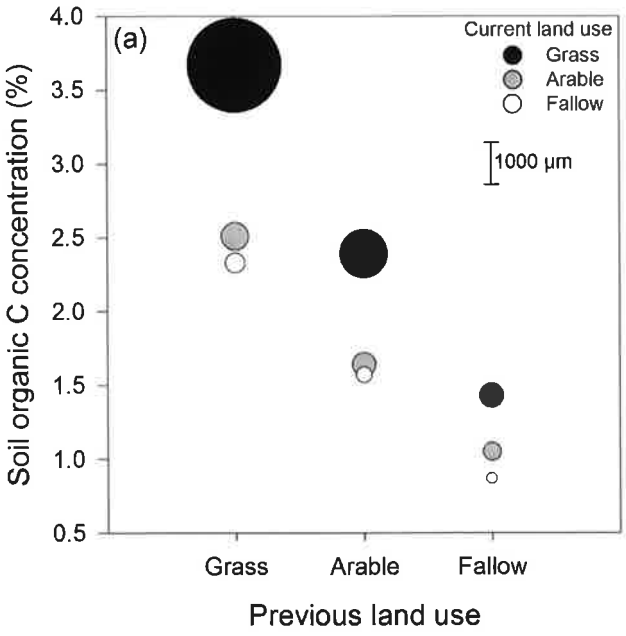
Current land use

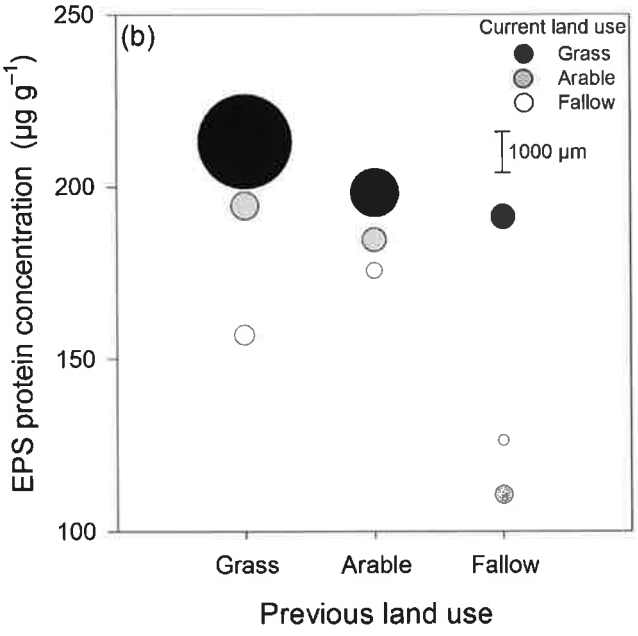
- Grass
- Arable
- Fallow






Current land use
Grass
Arable
Fallow
(a)
y = 2.29 + 0.26 x
MWD (log10 µm)
Soil organic C concentration (%)
(b)
y = 2.17 + 0.0036 x
EPS-protein concentration (µg g−1)


(a)
Current land use
Grass
Arable
Fallow
1000 µm
Soil organic C concentration (%)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Grass
Arable
Fallow
Previous land use


(b)
Current land use
Grass
Arable
Fallow
1000 µm
EPS protein concentration (µg g^{-1})
250
200
150
100
Grass
Arable
Fallow
Previous land use

Supplementary Material

Table S1

Full dataset of soil organic C (SOC), total N (N) and extracellular polymeric substances (EPS) concentrations (protein, polysaccharide and uronic acid), and stable aggregate mean weight diameter (MWD) variates under previous and current land uses and their interaction.

Land use		Block	Plot	Split-plot	SOC	N	EPS			MWD
Previous	Current						protein	polysaccharide	uronic acid	
					(%)			($\mu g\ g^{-1}$)		(μm)
Grass	Grass	1	4	10	4.500	0.386	172.54	338.84	145.46	2768.57
Grass	Grass	2	6	17	3.900	0.349	226.46	364.06	151.83	2587.72
Grass	Grass	3	9	26	2.600	0.230	240.50	335.17	157.66	1524.06
				Mean	*3.667*	*0.322*	*213.17*	*346.02*	*151.65*	*2293.45*
Grass	Arable	1	4	12	2.186	0.204	204.10	366.75	158.35	744.61
Grass	Arable	2	6	16	2.840	0.266	172.58	292.39	130.74	707.66
Grass	Arable	3	9	27	2.497	0.234	207.00	359.51	165.75	599.74
				Mean	*2.508*	*0.235*	*194.56*	*339.55*	*151.61*	*684.00*
Grass	Fallow	1	4	11	2.285	0.211	196.00	366.98	163.26	584.45
Grass	Fallow	2	6	18	2.439	0.230	138.51	290.60	124.38	482.65
Grass	Fallow	3	9	25	2.260	0.217	136.36	224.40	98.01	414.90
				Mean	*2.328*	*0.219*	*156.96*	*293.99*	*128.55*	*494.00*
Arable	Grass	1	5	13	2.570	0.240	199.42	455.70	244.38	1362.67
Arable	Grass	2	7	21	2.264	0.211	200.85	438.84	246.90	1175.54
Arable	Grass	3	8	23	2.323	0.217	194.46	410.06	251.60	983.72
				Mean	*2.386*	*0.223*	*198.24*	*434.87*	*247.63*	*1173.98*
Arable	Arable	1	5	14	1.723	0.166	233.58	291.52	140.03	603.67
Arable	Arable	2	7	20	1.621	0.155	127.19	256.28	126.29	432.25
Arable	Arable	3	8	24	1.584	0.169	193.15	405.56	235.28	715.86
				Mean	*1.643*	*0.163*	*184.64*	*317.79*	*167.20*	*583.93*
Arable	Fallow	1	5	15	1.585	0.154	168.71	325.95	174.81	364.27
Arable	Fallow	2	7	19	1.604	0.152	157.13	350.64	190.59	371.89
Arable	Fallow	3	8	22	1.505	0.150	201.16	447.40	216.15	449.17
				Mean	*1.565*	*0.152*	*175.67*	*374.66*	*193.85*	*395.11*
Fallow	Grass	1	1	2	1.463	0.128	134.58	331.83	209.57	705.15
Fallow	Grass	2	2	6	1.507	0.141	225.71	437.72	264.38	585.13
Fallow	Grass	3	3	9	1.326	0.120	213.51	374.96	206.77	494.25
				Mean	*1.432*	*0.130*	*191.27*	*381.50*	*226.91*	*594.84*
Fallow	Arable	1	1	1	1.125	0.108	100.68	229.42	139.56	479.84
Fallow	Arable	2	2	5	0.934	0.097	118.74	237.02	135.56	503.87
Fallow	Arable	3	3	7	1.101	0.101	112.59	240.20	133.63	367.60
				Mean	*1.053*	*0.102*	*110.67*	*235.55*	*136.25*	*450.44*
Fallow	Fallow	1	1	3	0.908	0.094	98.82	195.51	124.95	262.58
Fallow	Fallow	2	2	4	0.777	0.084	140.71	335.48	229.02	263.91
Fallow	Fallow	3	3	8	0.919	0.095	139.76	329.90	175.98	265.82
				Mean	*0.868*	*0.091*	*126.43*	*286.96*	*176.65*	*264.10*

Table S2

The analysis of variance (ANOVA) table for the soil organic C (SOC), total N (N) and extracellular polymeric substances (EPS) concentrations (protein, polysaccharide and uronic acid), and stable aggregate mean weight diameter (MWD) variates with the structures outlined in equations 1-2, showing the effect of previous (P) and current (C) land uses and their interaction. The table gives the degrees of freedom (df) associated with the factor (first number) and its residual (comma separated), together with the variance ratio statistic (F) and its probability level (p). Where significant at $p<0.05$, the standard error of differences (SED) and the least significant differences (LSD) of means are given. Note that some variates were firstly transformed by $\log_{10}$ to normalise the distribution of residuals.

Variate	Unit	Factor	df	F	p	SED	LSD
SOC	$(\log_{10})\%$	P	1,2	57.42	0.017	0.023	0.100
		C	2,12	35.47	<0.001	0.024	0.053
		P × C	2,12	0.01	0.988		
N	$(\log_{10})\%$	P	1,2	26.76	0.035	0.030	0.129
		C	2,12	24.66	<0.001	0.024	0.052
		P × C	2,12	0.01	0.988		
EPS- protein	$\mu g\ g^{-1}$	P	1,2	0.06	0.823		
		C	2,12	6.23	0.014	14.3	31.1
		P × C	2,12	0.54	0.597		
EPS- polysaccharide	$\mu g\ g^{-1}$	P	1,2	2.09	0.285		
		C	2,12	8.38	0.005	23.0	50.0
		P × C	2,12	2.40	0.133		
EPS- uronic acid	$\mu g\ g^{-1}$	P	1,2	10.12	0.086		
		C	2,12	10.51	0.002	12.9	28.2
		P × C	2,12	3.29	0.073		
MWD	$(\log_{10})\mu m$	P	1,2	5.81	0.137		
		C	2,12	131.49	<0.001	0.031	0.067
		P × C	2,12	4.50	0.035	0.069	0.155

Table S3

Regression statistics of stable aggregate mean weight diameter ($(\log_{10})$ μm) as a linear function of soil organic C (SOC; %) and soil extracellular polymeric substances (EPS) concentrations (protein, polysaccharide and uronic acid; μg EPS g^{-1} soil). The table gives the constant (*a*) and coefficient (*b*) of the linear regression ($y = a + bx$), the variance ratio statistic (*F*), the probability level associated with the regression (*p*), the degrees of freedom (df) and mean square error (MSE) of the residual, and the adjusted proportion of the variance accounted for by the fit (Adjusted R_2). Note that for uronic acid, the adjusted R_2 is not calculable as the residual variance exceeded the variance of the response variate.

Statistic	SOC	EPS		
		protein	polysaccharide	uronic acid
Constant	2.29±0.07	2.17±0.18	2.26±0.23	2.64±0.21
Coefficient	0.26±0.03	0.0036±0.0010	0.0016±0.0007	0.0008±0.0011
F	67.49	12.21	5.56	0.55
p	<0.001	0.002	0.027	0.465
df	25	25	25	25
MSE	0.020	0.051	0.062	0.074
Adjusted R^2	0.719	0.301	0.149	not calculable

Declarations of interest statement

Soil organic carbon, extracellular polymeric substances (EPS), and soil structural stability as affected by previous and current land-use

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