

# *Legacy effects of grassland management on soil carbon to depth*

Article

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1 **Legacy effects of grassland management on soil carbon to depth**

2 Running header: Management and deep soil carbon

3

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18

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20 legacy effect, carbon inventory

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**22 Abstract**

23 The importance of managing land to optimise carbon sequestration for climate change  
24 mitigation is widely recognised, with grasslands being identified as having the potential to  
25 sequester additional carbon. However, most soil carbon inventories only consider surface  
26 soils, and most large scale surveys group ecosystems into broad habitats without considering  
27 management intensity. Consequently, little is known about the quantity of deep soil carbon  
28 and its sensitivity to management. From a nationwide survey of grassland soils to 1 m depth,  
29 we show that carbon in grasslands soils is vulnerable to management and that these  
30 management effects can be detected to considerable depth down the soil profile, albeit at  
31 decreasing significance with depth. Carbon concentrations in soil decreased as management  
32 intensity increased, but greatest soil carbon stocks (accounting for bulk density differences),  
33 were at intermediate levels of management. Our study also highlights the considerable  
34 amounts of carbon in sub-surface soil below 30cm, which is missed by standard carbon  
35 inventories. We estimate grassland soil carbon in Great Britain to be 2097 Tg C to a depth of  
36 1 m, with ~60% of this carbon being below 30cm. Total stocks of soil carbon ( $\text{t ha}^{-1}$ ) to 1 m  
37 depth were 10.7% greater at intermediate relative to intensive management, which equates to  
38  $10.1 \text{ t ha}^{-1}$  in surface soils (0-30 cm), and  $13.7 \text{ t ha}^{-1}$  in soils from 30-100 cm depth. Our  
39 findings highlight the existence of substantial carbon stocks at depth in grassland soils that  
40 are sensitive to management. This is of high relevance globally, given the extent of land  
41 cover and large stocks of carbon held in temperate managed grasslands. Our findings have  
42 implications for the future management of grasslands for carbon storage and climate  
43 mitigation, and for global carbon models which do not currently account for changes in soil  
44 carbon to depth with management.

**45 Introduction**

46 Permanent grasslands are found extensively across the temperate zone where they form the  
47 backbone of agricultural systems. Global land cover estimates for grasslands range between  
48 20-40% of the Earth (FAO 2015), and for the UK, constitute the largest category of land use  
49 at 36% of land cover (Carey et al. 2008). The multiple values of grassland ecosystems to  
50 mankind has long been recognised, ranging from direct benefits of agricultural production, to  
51 indirect ecosystem services such as the regulation of climate and water quality, and  
52 pollination services (Heidenreich 2009). As such, grasslands are arguably one of the most  
53 valuable biomes for ecosystem service provision, but also are among the most threatened by  
54 anthropogenic activities (Gibson 2008). Threats include increasing pressures to meet the  
55 food demands of more affluent and larger global populations, and to deliver concomitant  
56 multiple ecosystem services demanded by the sustainable intensification agenda (Garnett et  
57 al. 2013). Of the multiple ecosystem services provided by grasslands, climate regulation via  
58 soil carbon storage and sequestration, is highly valued (Heidenreich 2009).

59

60 In terms of terrestrial carbon storage, soils contain the largest global pool of terrestrial  
61 carbon, storing more carbon than is present in plant biomass and the atmosphere combined  
62 (Batjes 1996, Jobbagy and Jackson 2000). Temperate grasslands are the third largest global  
63 store of carbon in soils and vegetation (after wetlands and boreal forests), storing an  
64 estimated 304 Pg C, or 12.3% of global carbon (Royal Society 2001), most of which is in  
65 soil. Such carbon stocks are known to be vulnerable to changes in land use and  
66 intensification of agricultural management, with the conversion of croplands to permanent  
67 grassland generally increasing soil carbon, and the reverse change from grassland to  
68 croplands reducing soil carbon stocks (Guo and Gifford 2002, Smith et al. 2008). Moreover,  
69 such changes in land use can have a long lasting legacy effect on soil carbon, on a scale of

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70 decades to several hundreds of years, which is often slow to reverse (Dupouey et al. 2002,  
71 McLauchlan 2006, Smith 2014).

72

73 Grassland soil carbon has been shown to respond to changes in management intensity, with  
74 agricultural practices such as fertiliser application, irrigation, and livestock grazing affecting  
75 soil carbon stocks (Soussana et al. 2004, Smith et al. 2008, van Wesemael et al. 2010). In  
76 many parts of Europe, the intensity of grassland management has substantially increased  
77 since the 1950's. This has been driven largely by agri-environmental policy and farm  
78 subsidies, combined with technological innovations, leading to widespread legacy effects of  
79 long-term management on soils and vegetation. In addition to the documented effects of  
80 management on soil carbon (Guo and Gifford 2002, Smith et al. 2008), intensification of  
81 farming practices has led to widespread reductions in botanical diversity and loss of species-  
82 rich, traditionally managed grasslands, which now cover less than 3% of the area they did in  
83 the 1950's (Gamble et al. 2012). Furthermore, management intensification has caused major  
84 changes in plant functional composition, with intensively managed grasslands typically being  
85 dominated by fast-growing exploitative species, characterized by high specific leaf area  
86 (SLA) and leaf nitrogen concentration (LNC). This compares with less fertile, extensively  
87 managed grasslands that are dominated by slower-growing, conservative species of high leaf  
88 dry matter content (LDMC) and low leaf N content (Lavorel and Garnier 2002, de Vries et al.  
89 2012). Such shifts in plant functional composition are known to have important effects on  
90 soil nutrient cycling and carbon dynamics (de Vries et al. 2012, Grigulis et al. 2013, Manning  
91 et al. 2015), and LDMC has been correlated with soil fertility (Hodgson et al. 2011, Duru et  
92 al. 2012). However, there is a lack of knowledge on the relationship between soil carbon and  
93 management intensity with depth linked to the legacy of vegetation change.

94

95 Another factor contributing to uncertainty over legacy effects of management and land use  
96 change is that most studies on soil carbon stocks are on more easily sampled surface soils.  
97 Even the IPCC recommend soil carbon accounting for the surface 30 cm of soil only (IPCC  
98 2006), and although they advocate sampling beyond 30cm, this is rarely done. As a result,  
99 soil carbon stocks at depth are largely ignored (Fontaine et al. 2007, Chapman et al. 2013),  
100 and little is known about the quantity of soil carbon at depth and how it responds to land  
101 management and associated vegetation change in grasslands (Jobbagy and Jackson 2000,  
102 Soussana et al. 2004, Fontaine et al. 2007). Given the substantial quantities of carbon in  
103 grassland soils, and the fact that grasslands worldwide are subject to increasing  
104 intensification of management, this represents a major gap in knowledge.

105

106 The overarching aim of our study was to quantify the distribution of soil carbon across  
107 English grasslands to depth (1 metre), and the relationship of surface and deep soil carbon to  
108 grassland management at a national scale. To do this, we carried out a nationwide survey of  
109 English grasslands. This included all the main grassland habitats found in Great Britain,  
110 namely acid, calcareous, mesotrophic and wet grassland, sampled across the broad range of  
111 soil and climatic conditions (de Vries et al. 2012, Manning et al. 2015), thereby  
112 encompassing a wide range of variation across a representative spatial domain (Smart et al.  
113 2012). Cumulative soil carbon stocks and mean depths from the survey were then used in  
114 conjunction with land cover data for matching grassland broad habitats from the Countryside  
115 Survey (Carey et al. 2008) to estimate total grassland soil carbon stocks in Great Britain to a  
116 depth of 1m, and to make comparisons with existing estimates of grassland soil carbon  
117 storage. Using two abundance-weighted leaf traits known to be indicators of soil fertility and  
118 management intensity (i.e., SLA and LDMC) (Hodgson et al. 2011; Grigulis et al. 2013), we

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119 also sought to test whether surface carbon stocks are more related to current plant functional  
120 composition than deeper carbon.

121

122

### 123 **Methods**

#### 124 *Field sampling.*

125 A survey of 180 permanent grasslands was conducted in summer 2010 from a range of acid,  
126 calcareous, mesotrophic and wet grasslands, using a network of sites located throughout  
127 England (Fig. 1) (de Vries et al. 2012, Manning et al. 2015). Sampling sites were in 60  
128 different geographical locations, from 12 broad regions of England. At each of the 60  
129 locations, three different fields were selected to give a gradient of management intensity of  
130 extensive, intermediate and intensive management. These triplicate sets of fields were sited  
131 on the same soil type, with similar topography and edaphic characteristics, and all three fields  
132 at the same location were sampled on the same day. The three management intensity  
133 classifications were based on expert judgement using information from consultations with  
134 farmers and land owners, and from vegetation surveys (de Vries et al. 2012, Manning et al.  
135 2015), and follow broad classifications that reflect dominant grassland systems in the UK and  
136 Europe (Tallowin and Jefferson 1999, Tallowin et al. 2005, Manning et al. 2015) ([Table 1](#)).

137 There are clearly many different factors involved in the intensification of grassland  
138 management, including increased use of fertilisers, increased disturbance through greater  
139 cutting and grazing intensities, and changes in the amount and diversity of plant-derived  
140 organic matter inputs to soil due to vegetation change. Given that it is not possible to  
141 disentangle the individual effects of these factors on soil carbon stocks at the national scale of  
142 sampling done here, we therefore compared grasslands subject to broadly defined levels of  
143 agricultural intensification; this approach has been used widely to identify broad-scale trends

144 in, and relationships between, vegetation and soil properties along gradients of management  
145 intensity (Bardgett and McAlister 1999, Grayston et al. 2004, Allan et al. 2015).

146

147 The extensively managed grasslands had relatively high plant diversity and high conservation  
148 status, typically received less than 25 kg N ha<sup>-1</sup> yr<sup>-1</sup> and, have been managed in a traditional,  
149 low intensity manner for many decades, with light grazing and annual cutting for hay. The  
150 intensively managed agriculturally improved grasslands had low plant diversity of mainly  
151 MG6 (*Lolium perenne* – *Cynosurus cristatus*) and MG7 (*Lolium perenne* leys and related  
152 grasslands) communities (Rodwell 1992). These intensively managed grasslands typically  
153 receive >100 kg N ha<sup>-1</sup> yr<sup>-1</sup>, and have been subjected to standard intensive management  
154 practices since the 1950's, with higher grazing pressures and more frequent cutting for silage  
155 than the extensively managed grasslands (Tallowin and Jefferson 1999, Tallowin et al. 2005,  
156 Critchley et al. 2007). The third category of grasslands is that of intermediate management  
157 intensity, which falls between the two other categories, having typical inputs of ~ 25-50 kg N  
158 ha<sup>-1</sup> yr<sup>-1</sup>, and intermediate levels of plant diversity, grazing and cutting (Table 1).

159

160 Soil cores (3.5 cm diameter) were taken from three random areas in each field, to 1 m depth  
161 (where possible) using an Eldeman auger, and divided into five depth increments: 0–7.5 cm,  
162 7.5–20 cm, 20–40 cm, 40–60 cm and 60–100 cm. A soil pit was dug at one location in each  
163 field and three bulk density cores (6 cm length x 6.3 cm diameter) were taken horizontally for  
164 each depth increment, from three different faces of the pit. This methodology builds on  
165 information from the previous sampling campaign at these locations, where soil was collected  
166 from the surface 7.5 cm only (de Vries et al. 2012, Manning et al. 2015).

167

168 *Soil Analyses.*

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169 Soils were sieved (4 mm), oven dried at 60°C, ground using a ball mill, and analysed for total  
170 carbon by combustion and gas chromatography (Elementar Vario EL CN analyser). Total  
171 soil carbon (organic and inorganic) stocks per unit area ( $\text{g C cm}^{-3}$ ) were calculated from  
172 carbon concentrations (%C) and bulk density measures ( $\text{g cm}^{-3}$ ) for all depth increments. A  
173 further sample of the three surface soils (0 – 7.5 cm) collected from each field were pooled in  
174 equal proportions for separation into soil organic matter (SOM) fractions (n=180). SOM  
175 fractionation was by the flotation and sedimentation method using a sodium iodide (NaI)  
176 solution (Sohi et al. 2001, Sohi et al. 2005). This fractionation method distinguishes  
177 between: (a) a free SOM fraction (FR-SOM) at density  $< 1.80 \text{ g cm}^{-3}$  which represents  
178 discrete free organic particles located between stable soil aggregates; (b) an intra-aggregate  
179 SOM fraction (IA-SOM) at density  $< 1.80 \text{ g cm}^{-3}$  which represents discrete organic particles  
180 located within stable soil aggregates; and (c) a residual heavy organo-mineral fraction at  
181 densities  $> 1.80 \text{ g cm}^{-3}$ . Briefly, 7.5 g (dry wt) soil was added to 90 ml NaI at a density of  
182  $1.80 \text{ g cm}^{-3}$ , centrifuged for 30 minutes and the first floating FR-SOM fraction removed by  
183 suction. The remaining centrifuge pellet was then re-mixed with the NaI, sonicated for 195  
184 seconds, re-centrifuged for 30 minutes, and the second floating IA-SOM fraction removed by  
185 suction. The remaining pellet, thoroughly rinsed in water, forms the organo-mineral fraction.  
186 All soil fractions were dried, then ground in a ball mill and analysed for total C and N as  
187 above.

188

189 *Data analysis.*

190 Effects of depth and management on soil carbon concentration, bulk density and soil carbon  
191 stocks were tested by ANOVA using generalised linear models (SAS Enterprise Guide 4.3),  
192 with grassland type and region as factors. Cumulative soil carbon stocks to depth were  
193 estimated by a Bayesian mixed modelling approach (supporting information S1), with

194 random effects of region and location included plus a fixed effect of soil depth. Estimates of  
195 cumulative soil carbon stocks were summarised by the posterior distribution of values  
196 estimated for each soil depth and each level of management intensity, with the clustering of  
197 samples within locations accounted for in the mixed effects model. Abundance-weighted  
198 means for plant traits SLA and LDMC were calculated for sample plots using plant species  
199 data, derived from field surveys carried out in 2005 to help classify management intensity  
200 classifications, and paired with soil carbon measurements at each soil depth. Plant trait  
201 information was derived from the LEDA database based on UK values reported for each  
202 species (Kleyer et al. 2008). We modelled the between-location variation in total soil carbon  
203 at each depth increment in terms of the abundance weighted plant traits, testing linear and  
204 quadratic models at each depth; region and location were introduced as random effects.

205

### 206 *Upscaling*

207 Cumulative soil carbon stocks and soil depths from our survey were combined with the UK  
208 Countryside Survey land cover data (Carey et al. 2008) to estimate total grassland soil carbon  
209 in Great Britain to a depth of 1m. Grassland types and management categories from our  
210 survey were matched with the UK Countryside Survey (CS) broad habitat classifications of  
211 improved, neutral, calcareous and acid grasslands (Emmett et al. 2010). Our ‘intensively  
212 managed’ grassland category mapped to the CS category ‘improved grasslands’; our  
213 ‘intermediate’ grasslands mapped to CS ‘neutral’ grasslands; and, our ‘extensive’ grassland  
214 category mapped with the CS ‘acid’ and ‘calcareous’ grassland categories. These  
215 assumptions were successfully validated by comparing the total grassland soil carbon storage  
216 estimate from CS to a depth of 15cm with our modelled cumulative carbon stocks (see Fig.  
217 3). Cumulative soil carbon stocks for each management category were multiplied by the land  
218 cover area, and adjusted to account for the fact that not all grasslands surveyed had soil to the

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219 full 1m depth, to give an estimate of total grassland soil carbon stocks (supporting  
220 information S2).

221

222

## 223 **Results**

### 224 *Field survey*

225 Across all sites, the greatest concentrations of total carbon (% total C) were in surface soils to  
226 7.5 cm depth ( $F_{4,2096} = 288$ ,  $P < 0.0001$ ) (Table 2). Soil carbon concentration decreased with  
227 increasing management intensity ( $F_{2,2096} = 10.9$ ,  $P < 0.0001$ ), with significantly lower carbon  
228 in the most intensive relative to intermediate and extensively managed grasslands, based on  
229 all soils at all depths sampled (Table 2). Management effects were strongest in the surface  
230 7.5 cm soil (supporting information S3), where total carbon concentration decreased with  
231 intensification, being 19% and 25% lower in intensively managed than intermediate and  
232 extensively managed grasslands respectively (Table 2). The effects of management intensity  
233 on total % carbon decreased with depth, but were still significant to 40cm depth ( $P < 0.05$ )  
234 and weakly significant ( $P < 0.10$ ) at 60 cm depth. 70% of fields contained soils to 60cm  
235 depth, and 55% of all fields had soils to the full 1 m depth; therefore, the number of samples  
236 analysed decreased down the profile (Table 2). Those with deeper soils tended to be from  
237 more carbon rich mesotrophic and wet grasslands, hence the observed trend for increased  
238 mean carbon concentration at the 60-100 cm depth (Table 2).

239

240 Soil bulk density increased with depth down the soil profile ( $F_{4,2011} = 57.5$ ,  $P < 0.0001$ ), and  
241 was also influenced by grassland management ( $F_{4,2011} = 25.9$ ,  $P < 0.0001$ ) (Table 3, Table  
242 S3.1). The strongest effects of management were in the top 20 cm of the profile, where bulk  
243 density was lowest in extensively managed and greatest in intensively managed grasslands,

244 and a trend for greater bulk density in intensively managed grasslands continued to 1 m  
245 depth. Total soil carbon stocks per unit area ( $\text{g C cm}^{-3}$ ), calculated from both the soil carbon  
246 concentration and bulk density, were also strongly influenced by depth, with greatest mean  
247 carbon stocks per unit area in the top 7.5 cm of the profile ( $F_{4,1965} = 228$ ,  $P < 0.0001$ ), and by  
248 management intensity ( $F_{2,2096} = 3.1$ ,  $P = 0.05$ ) (Fig. 2). Specifically, for all soils analysed,  
249 total soil C stocks ( $\text{g C cm}^{-3}$ ), were significantly greater in grasslands of intermediate  
250 management intensity relative to both the extensive and intensive managed grasslands (Fig.  
251 2.). When analysed by depth increments, soils from grasslands at intermediate levels of  
252 management had greater soil carbon stocks relative to extensive grasslands at 7.5 cm, and  
253 relative to intensive grasslands at 7.5 to 20cm depth ( $P < 0.05$ ), with a trend for greatest C  
254 stocks at intermediate levels of management detected to 60 cm depth ( $P = 0.1$ ) (supporting  
255 information S3). We estimated cumulative soil carbon storage per  $\text{m}^2$ , at 5 depth increments  
256 to 1 m depth (Table 4) using a Bayesian mixed effects model (supporting information S1).  
257 Uncertainties in carbon stock estimates increased with depth (Table 4), reflecting greater  
258 incremental size categories and smaller sample numbers because fewer profiles reached the  
259 full 1 m depth. This is a pattern commonly encountered with soil carbon measurements at  
260 depth (Syswerda et al. 2011), and in our sites, soils with samples present at 1m depth also  
261 tended to be those with greater carbon concentrations. Cumulative stocks of carbon to 1 m  
262 depth in grasslands with intermediate levels of management were 10.7 and 7.8% greater than  
263 for grasslands with intensive and extensive management respectively (Table 4). This  
264 unimodal relationship between soil carbon stocks and management is less apparent in other  
265 studies where bulk density has not been taken into account.  
266  
267 Although we measured total carbon in bulk soils, fractionation of surface soils (7.5 cm depth)  
268 showed that it was only the relatively labile soil organic matter (SOM) fractions that

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269 responded to management (Table 2). More specifically, the amount of carbon ( $\text{g C kg}^{-1}$  soil)  
270 in the free SOM, which represents  $\sim 15\%$  of soil mass (mean of all samples), reduced with  
271 increasing management intensity. The amount of carbon in the intra-aggregate SOM  
272 fractions ( $\sim 2\%$  of soil mass) was also lower in the intensive compared with the other two  
273 levels of management intensity. In contrast, the amount of carbon present in the recalcitrant  
274 organo-mineral fraction, which accounted for the largest remaining proportion of soil mass,  
275 did not respond to management intensity.

276

*277 Plant traits and soil carbon at varying depth*

278 Of 20 model tests (linear and quadratic fits attempted for two traits at five depths) only two  
279 significant relationships were found. These were for a unimodal and linear model between  
280 abundance-weighted SLA and soil carbon at 40 and 60cm depth, respectively. Consequently  
281 there was little evidence for consistent relationships with the plant traits SLA and LDMC and  
282 their systematic change down the soil profile.

283

**284 Discussion**

285 The aim of this study was to investigate the amount of soil carbon in grasslands to 1 m depth,  
286 and the sensitivity of soil carbon to management intensity at depth increments to 1 m. Our  
287 study was carried out at a national scale across a broad range of grasslands and soil  
288 conditions, thus extending knowledge from past studies into land management and deep  
289 carbon at the field scale. We reveal two key findings. First, we show that long-term changes  
290 in grassland management intensity have strongly influenced soil carbon, and that this effect is  
291 seen to considerable depths down the soil profile, albeit at decreasing significance with depth.  
292 Second, we show that considerable stocks of carbon are contained in sub-surface grassland  
293 soils, below the standard carbon inventory default depth of 30cm under tier 1 of the IPCC

294 (2006), suggesting that large stocks of unaccounted for carbon are sensitive to management  
295 change.

296

297 *Management intensity*

298 Our data show that intensive management has reduced the concentration of carbon in soil (%  
299 total C), and that soil carbon stocks per m<sup>2</sup> are greatest at intermediate levels of grassland  
300 management intensity. This sensitivity of soil carbon to management has been found in other  
301 grassland studies for surface soils in Europe (Soussana et al. 2004, Allard et al. 2007).

302 However, by sampling to 1 m depth from a large number of sites across a broad range of  
303 grassland and soil types, our study extends the knowledge of grassland management effects  
304 on soil carbon by demonstrating that, although management effects on soil carbon were  
305 strongest in surface soils, they are still observed to considerable depth down the soil profile,  
306 indicating that extensive stocks of soil carbon at depth are sensitive to management change.

307

308 We suggest that both biological and physical mechanisms contributed to the greater  
309 cumulative carbon stocks observed at intermediate levels of grassland management. Soil  
310 carbon sequestration is dictated by processes controlling the balance of carbon inputs and  
311 carbon outputs. Carbon inputs are derived from primary productivity through photosynthetic  
312 uptake of carbon, input to soil in the form of root exudates and litter; plus additional inputs  
313 from animal manure. Carbon losses are through a range of biological and physical processes  
314 including respiration, decomposition, erosion, leaching and removal of biomass by harvesting  
315 or grazing animals. Because we measured total rather than organic soil carbon, it is possible  
316 that differences in total soil carbon are due, in part, to variations in inorganic carbon, from  
317 liming or the influence of parent bedrock, particularly in calcareous soils. It is also possible  
318 that black carbon (charcoal) made up a small proportion of the total carbon observed

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319 (Manning et al. 2015). However, by sampling triplicate groups of fields from the same  
320 bedrock across a management intensity gradient, we suggest that these effects would be  
321 minimal.

322

323 There are clearly many factors involved in the intensification of grassland management, such  
324 as fertiliser addition, the intensity of grazing and biomass removal, and compaction  
325 associated with livestock and machinery, and it is difficult to separate out the effects of each  
326 of these in terms of their influence on soil carbon. Such management factors also impact on  
327 plant community composition, which is known to influence soil carbon dynamics (De Deyn  
328 et al. 2008, Manning et al. 2015). We did not directly measure plant productivity,  
329 decomposition, or other processes involved in carbon cycling. However, it has been shown  
330 in other studies that extensive grassland management can reduce soil carbon accumulation  
331 over time as plant productivity declines in response to nutrient limitation (Allard et al. 2007),  
332 and that intensive management involving the application of large amounts of fertiliser can be  
333 detrimental to soil carbon (Mack et al. 2004, Soussana et al. 2004, Schipper et al. 2007).  
334 Conversely, the application of fertiliser in modest amounts in intermediate grasslands has  
335 been shown to enhance soil carbon (Conant et al. 2011, Leifeld et al. 2011, Smith 2014),  
336 which is likely related to an increase in primary productivity without over-stimulating  
337 decomposition, although, as pointed out, the effect of other concomitant management factors  
338 cannot be ruled out.

339

340 In addition to changes in bulk soil carbon, we also found that different fractions of carbon in  
341 surface soils to 7.5 cm depth varied in their sensitivity to management. In particular, both the  
342 labile free SOM and intra-aggregate SOM fractions were reduced by management  
343 intensification. While these two SOM fractions make up less than a quarter of total soil mass,

344 they are of vital importance to soil carbon storage as they contain a greater concentration of  
345 carbon than the recalcitrant organo-mineral fraction and account for 31% of soil carbon in  
346 intensively managed soils, rising to 37% and 44% of soil carbon in intermediate and  
347 extensively managed grassland soils respectively. We found no evidence of a systematic  
348 relationship between total soil carbon at varying depth and community weighted values of  
349 two key leaf traits (i.e. SLA and LDMC) that are known to be responsive to grassland  
350 intensification and to mediate effects on carbon storage via differences in the  
351 decomposability of plant material (De Deyn et al. 2008). We expected that the relationship  
352 between leaf traits and soil carbon would be strongest at the soil surface and decline in  
353 strength with soil depth, reflecting historic plant traits prior to intensification in the last 60  
354 years. Using the same network of grassland sites, Manning et al (2015) investigated  
355 relationships between plant traits and soil carbon fractions in surface soil (7.5 cm soil depth)  
356 and found that only the labile carbon fraction was partially explained by community weighted  
357 SLA, with soil carbon stocks being greatest under vegetation with thick and/or dense leaves.  
358 In contrast, the less active fractions, which make up the bulk of the carbon pool, were better  
359 explained by abiotic factors, including pH and climate (Manning et al. 2015). Our results  
360 indicate that the lack of correlation between leaf traits and soil carbon propagates down the  
361 soil profile, suggesting that the mediating effect of current plant trait variation on soil carbon  
362 is weaker than the long-term effect of other environmental controls on the production and  
363 storage of carbon at depth.

364

365 Differences in carbon stocks between management levels were also strongly influenced by  
366 variations in soil bulk density, which increased as management intensified and with depth.  
367 This increase in bulk density with more intensive management is likely largely explained by  
368 soil compaction from a greater use of machinery and trampling from grazing animals

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369 (Gifford and Roderick 2003, Batey 2009), but also the greater proportion of higher density  
370 soil fractions in fertilised soils (Fornara et al. 2011). The combination of low carbon  
371 concentration with high bulk density in intensive grasslands, and high carbon concentration  
372 with low bulk density in extensive grasslands, contributed to the equivalence of carbon stocks  
373 observed at high and low levels of management intensity, and their lower levels relative to  
374 intermediate intensity. This highlights a dilemma in comparing soil carbon stocks both  
375 spatially and temporally, in that soil carbon stocks calculated per unit area could be increased  
376 by simple physical compaction without a change in soil carbon concentration, particularly if  
377 measured at shallow sampling depths. Gifford and Roderick (2003) propose an alternative  
378 way of calculating soil carbon based on soil mass rather than volume to avoid this problem of  
379 bulk density change associated with land use. For comparison, we estimated cumulative  
380 carbon stocks on a soil mass rather than volume basis, using approximated soil mass values  
381 (supporting information). On this approximated soil mass basis, differences in cumulative  
382 carbon stocks between intermediate and extensive grasslands were reduced, whereas the  
383 difference in cumulative carbon stocks for intensive relative to both intermediate and  
384 extensive grasslands increased, to an estimated 15% less carbon in intensively managed  
385 grasslands (supporting information). This differs from our findings on a bulk density basis,  
386 where soil carbon stocks were greatest under intermediate compared with both intensive and  
387 extensive intensity of management, but highlights the strong detrimental effect of intensive  
388 management on grassland soil carbon stocks. However, these results need to be treated with  
389 caution as calculations were made retrospectively on approximated soil mass values without  
390 following the recommended protocol (Gifford and Roderick 2003), and did not meet the  
391 requirement for samples to be taken from adjacent positions (Chapman et al. 2013).

392

393 *Carbon stocks at depth*

394 Our study revealed that considerable stocks of soil carbon are contained in sub-surface  
395 grassland soils below the standard carbon inventory default depth of 30cm (IPCC 2006).  
396 Using Great Britain as a case study, we combined our model of cumulative soil carbon stocks  
397 across a range of grasslands and management intensities with land cover data (Carey et al.  
398 2008) for comparable grassland categories, to estimate total grassland soil carbon storage to 1  
399 m depth. Cumulative soil carbon stocks were adjusted for depth, based on our survey results  
400 of a decreasing proportion of grasslands having soil to the full sampling depth, with 55% of  
401 all fields having soil to the full 1 m depth (Table 4). From this, we estimate a total carbon  
402 stock in British grassland soils of 2097 Tg C to a depth of 1 m (Fig. 3). This is over three  
403 times the amount for soil organic carbon estimated by the latest Countryside Survey to 15 cm  
404 depth (660 Tg), and more than double the amount when extrapolated to the standard IPCC  
405 recommended carbon accounting depth of 30 cm (estimated at 880 Pg, Fig. 3). Of this figure  
406 of 2097 Tg C for grassland carbon storage to 1 m depth, the greatest proportion of grassland  
407 soil carbon stocks (1130 Tg C) were in improved grasslands, which account for the largest  
408 land cover of all grasslands in Great Britain (Carey et al. 2008) and are most likely to contain  
409 soil to 1 m depth. Total stocks of soil carbon to 1 m depth (Table S2.2) were 10.7% greater  
410 at intermediate relative to intensive management, which equates to  $10.1 \text{ t ha}^{-1}$  in surface soils  
411 to 30 cm depth, and an additional  $13.7 \text{ t ha}^{-1}$  in soils from 30-100 cm depth.

412

413 At shallower soil depths, our soil carbon values of  $76$  and  $97 \text{ t ha}^{-1}$  at 15 and 30 cm depths  
414 respectively, are in line with those reported elsewhere for the UK, of  $48\text{-}90 \text{ t ha}^{-1}$  at 15 cm  
415 and  $100 \text{ t ha}^{-1}$  at 30 cm depth (Bradley et al. 2005, Emmett et al. 2010, Chapman et al. 2013).  
416 However, our estimation of grassland soil carbon stocks of 2097 Tg C to 1 m depth is  
417 considerably greater than that of Bradley et al. (2005), estimated at 1345 Tg C for UK  
418 pasture, and of Chapman (2013), who reported  $138 \text{ t ha}^{-1}$  for improved and  $185 \text{ t ha}^{-1}$  for

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419 semi-natural grasslands, compared with our value of at  $229 \text{ t ha}^{-1}$  at 1 m depth. This suggests  
420 that grassland carbon stocks at depth may be greater than previously thought. However,  
421 extrapolation of data needs to be treated with caution, due to the high variability of carbon  
422 stocks at depth and regional differences between management and grassland types (Maskell  
423 et al. 2013), and our modelling results explicitly quantify the uncertainty associated with our  
424 estimates and how the uncertainty increases with depth (Table 4). Differences in soil carbon  
425 accounting at depth between studies are likely due to increased uncertainties caused by lower  
426 sample numbers and greater incremental size categories (Syswerda et al. 2011), and  
427 differences between land use classifications; for example, Bradley et al (2005) estimate a  
428 further  $2015 \text{ Tg C}$  to 1m depth in their semi natural category. Our data also show that around  
429 60% of the total grassland soil carbon stocks to 1 m depth are found in sub-surface soils  
430 below 30 cm depth. Previous estimates, albeit for organic not total carbon, commonly quote  
431 a 50/50 split between surface and sub-surface (30-100 cm) soil carbon (Batjes 1996, Jobbagy  
432 and Jackson 2000, Schils et al. 2008), although 60% sub-surface organic carbon has been  
433 previously estimated for some grassland soils (Hiederer 2009).

434

435 Our data clearly reveal an important stock of carbon in grassland soil at depth, which is  
436 unrecognised in current surface soil carbon accounting (IPCC 2006, Emmett et al. 2010).  
437 Despite the tier 1 default soil sampling depth being 30 cm, the IPCC advocate sampling  
438 beyond 30 cm depth, although in likelihood this is rarely done. Our findings provide clear  
439 support for the IPCC recommendation of the need for deeper C sampling, not only to improve  
440 soil C inventories, but also to take into account changes in soil carbon at depth due to  
441 management intensification.

442

443 *Conclusion*

444 In conclusion, the findings of our national-scale study, which encompassed a broad range of  
445 grasslands and environmental conditions, show that carbon in grasslands soils is vulnerable to  
446 management, and that the legacy of long-term grassland management impacts on soil carbon  
447 to considerable depth down the profile. Moreover, we highlight the presence of substantial  
448 stocks of carbon in sub-surface soil in grasslands, which are not accounted for by the majority  
449 of standard carbon inventories (IPCC 2006, Emmett et al. 2010, Sanderman et al. 2011),  
450 indicating that large stocks of unaccounted for soil carbon are sensitive to changes in land  
451 management intensity. Our finding suggest potential future benefits for soil carbon  
452 sequestration alongside biodiversity through extensification of the most highly managed and  
453 fertilised grasslands, given that soil carbon concentrations decrease with management  
454 intensity, and that cumulative stocks of soil carbon to depth when bulk density was accounted  
455 for were greatest in grasslands managed at intermediate levels of intensity and plant diversity.  
456 Given the global extent of managed grasslands, these findings not only have implications for  
457 their future management for soil carbon storage and climate mitigation, that would benefit  
458 future global carbon targets (United Nations FCCC 2015), but also for global carbon models  
459 which need to take account of deep soil carbon stocks, and changes in this soil carbon at  
460 depth due to management.

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642

**643 Supporting Information**

644 S1. Methods for modelling cumulative soil carbon.

645 S2. Upscaling cumulative soil carbon to the whole of Great Britain.

646 S3. Statistical effects for soil carbon and bulk density.

647 S4. Estimating soil C stocks on soil mass basis.

648 S5. BUGS code for modelling cumulative soil carbon stocks.

649

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650 **Tables**

651 **Table 1.** Typical values for grassland management practices, including fertiliser use, grazing  
 652 management (LU = livestock units), cutting regimes, and plant species diversity for the three  
 653 levels of management intensity.

	<b>Extensive</b>	<b>Intermediate</b>	<b>Intensive</b>
Fertiliser application rate	< 25 kg N ha <sup>-1</sup> yr <sup>-1</sup>	25 – 50 kg N ha <sup>-1</sup> yr <sup>-1</sup>	> 100 kg N ha <sup>-1</sup> yr <sup>-1</sup>
Grazing	Set stocking or continuous grazing at stocking rates generally below 1.0 LU ha <sup>-1</sup>	Set stocking or continuous grazing at stocking rates up to 1.5 LU ha <sup>-1</sup>	Rotational/paddock grazing commonly used with stocking rates of 2.0 – 3.5+ LU ha <sup>-1</sup>
Cutting	Generally one cut in mid – late summer for hay or haylage. Regrowth generally grazed in late summer/autumn	Generally one cut in mid-summer for silage or haylage. Regrowth generally grazed in late summer/autumn	Two – three silage cuts per year (May, July, September/October)
<sup>1</sup> Plant diversity	High diversity (Mean 21 sp. m <sup>-2</sup> )	Intermediate Diversity (Mean 15 sp. m <sup>-2</sup> )	Low Diversity (Mean 10 sp. m <sup>-2</sup> )

654

655 <sup>1</sup>Data on plant species diversity are derived from De Vries et al. (2012).

656 **Table 2.** Effects of management intensity on soil carbon, measured as (a) % C of bulk soil  
 657 and (b) C content of soil fractions in surface soils.

	Soil depth (cm)	(n)	Extensively managed	Intermediate management	Intensively managed	
<b>(a) Bulk soil carbon concentration (%C)</b>						
Depth (cm)	0 – 7.5	(515)	11.53 ( $\pm$ 0.54) <sup>a</sup>	10.60 ( $\pm$ 0.54) <sup>b</sup>	8.59 ( $\pm$ 0.44) <sup>c</sup>	**
	7.5 – 20	(499)	8.18 ( $\pm$ 0.59) <sup>a</sup>	7.77 ( $\pm$ 0.53) <sup>a</sup>	6.13 ( $\pm$ 0.47) <sup>b</sup>	**
	20 – 40	(446)	6.48 ( $\pm$ 0.84) <sup>ab</sup>	6.34 ( $\pm$ 0.74) <sup>a</sup>	5.59 ( $\pm$ 0.72) <sup>b</sup>	**
	40 – 60	(359)	6.85 ( $\pm$ 1.28)	6.51 ( $\pm$ 1.12)	5.35 ( $\pm$ 0.96)	*
	60 – 100	(277)	9.76 ( $\pm$ 1.66)	7.25 ( $\pm$ 1.43)	7.55 ( $\pm$ 1.37)	
<b>(b) Carbon content of soil fractions in surface soils (0-7.5 cm) (g C kg<sup>-1</sup> soil)</b>						
	Free SOM		43.9 ( $\pm$ 9.1) <sup>a</sup>	34.3 ( $\pm$ 8.9) <sup>b</sup>	23.6 ( $\pm$ 7.9) <sup>c</sup>	**
	Intra-aggregate SOM		3.0 ( $\pm$ 0.5) <sup>a</sup>	2.6 ( $\pm$ 0.3) <sup>a</sup>	1.8 ( $\pm$ 0.2) <sup>b</sup>	**
	Organo-mineral		59.9 ( $\pm$ 4.0)	62.3 ( $\pm$ 3.9)	56.7 ( $\pm$ 3.8)	

658 Values are means  $\pm$  s.e. Significant management effects shown by \*\* ( $P < 0.05$ ) and \*

659 ( $P < 0.10$ ), with letters denoting differences between the 3 levels of management.

## Management and deep soil carbon

660 **Table 3.** Soil bulk density ( $\text{g cm}^{-3}$ ), for grassland soils at each of five depth increments  
 661 sampled, by management intensity and depth.

Soil depth (cm)	(n)	Extensively managed	Intermediate management	Intensively managed	
0 – 7.5	(486)	0.64 ( $\pm$ 0.02)	0.73 ( $\pm$ 0.02)	0.83 ( $\pm$ 0.02)	**
7.5 – 20	(464)	0.85 ( $\pm$ 0.02)	0.91 ( $\pm$ 0.02)	0.98 ( $\pm$ 0.02)	**
20 - 40	(399)	1.06 ( $\pm$ 0.03)	1.00 ( $\pm$ 0.03)	1.06 ( $\pm$ 0.03)	**
40 - 60	(362)	1.05 ( $\pm$ 0.04)	1.06 ( $\pm$ 0.04)	1.13 ( $\pm$ 0.03)	**
60 - 100	(300)	1.02 ( $\pm$ 0.05)	1.05 ( $\pm$ 0.04)	1.09 ( $\pm$ 0.04)	**

662 Values are means  $\pm$  s.e. Significant management effects shown by \*\* ( $P < 0.05$ ).

663

664 **Table 4.** Mean cumulative soil carbon (kg C m<sup>-2</sup>).

	<b>Extensively managed</b>	<b>Intermediate management</b>	<b>Intensively managed</b>
<b>Surface to 7.5cm depth</b>	6.61 (± 5.26-8.32)	7.14 (± 5.68-8.96)	6.45 (± 5.12-8.11)
<b>Surface to 20 cm depth</b> (97% of samples)	8.47 (± 6.75-10.65)	9.15 (± 7.29-11.47)	8.26 (± 6.56-10.37)
<b>Surface to 40 cm depth</b> (87% of samples)	12.59 (± 10.04-15.82)	13.60 (± 10.83-17.02)	12.28 (± 9.76-15.40)
<b>Surface to 60 cm depth</b> (70% of samples)	18.71 (± 14.90-23.54)	20.21 (± 16.07-25.36)	18.24 (± 14.53-22.92)
<b>Surface to 100 cm depth</b> (55% of samples)	41.38 (± 32.65-52.33)	44.62 (± 35.20-56.47)	40.30 (± 31.84-50.98)

665 Values are best estimates of the mean carbon stock where soil samples were present, with  
666 lower and upper credible intervals (2.5 and 97.5%tiles of the posterior distribution from a  
667 Bayesian mixed effects model).

668 **Figure captions**

669

670 **Figure 1.** Sampling locations in England (DeVries et al. 2012). Five farms were selected in  
671 each of the 12 regions, and three fields in each farm were sampled: one extensively managed,  
672 one of intermediate management and one intensively managed. Regions are: (a) Worcester,  
673 (b) Upper Thames, (c) Somerset, (d) Devon, (e) Cotswolds, (f) High Weald, (g) South  
674 Downs, (h) Breckland, (i) Dales meadows, (j) Yorkshire Ings, (k) Yorkshire Dales/South  
675 Lake District, (l) Lake District.

676

677 **Figure 2.** Total carbon per unit area in grassland soils ( $\text{g C cm}^{-3}$  soil). Shown for each of five  
678 depth increments sampled, with management intensity indicated by coloured bars: white for  
679 extensively managed, grey for intermediate management and black for intensively managed  
680 soils. Values are means  $\pm$  s.e.

681

682 **Figure 3.** Estimated cumulative total soil carbon stocks for grasslands in Great Britain (Tg  
683 C), adjusted for the availability of soil at depth. Shown in depth increments to 1 m, with  
684 management intensity indicated by colour: white for extensively managed, grey for  
685 intermediate management and black for intensively managed soils. The solid black circle  
686 shows the current GB estimate of soil carbon at 15 cm depth. Dashed lines indicate  
687 recommended soil sampling depths (GB Countryside Survey 15cm, IPCC at 30cm).

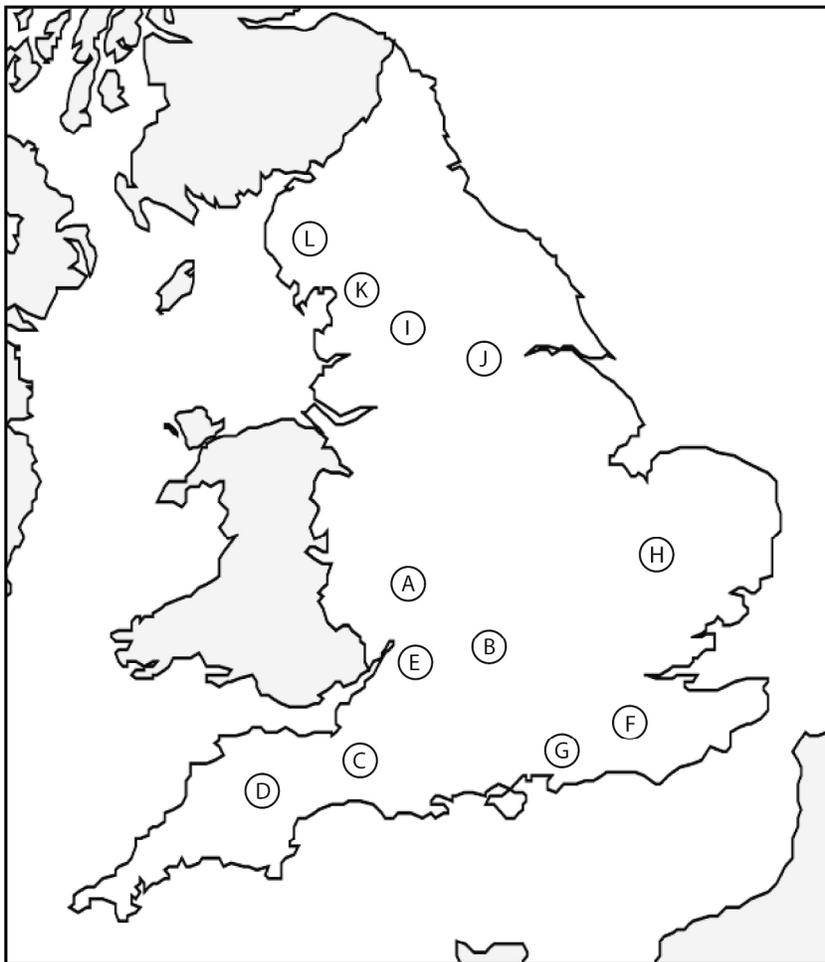


Figure 1  
209x234mm (300 x 300 DPI)

