

## Article (refereed) - postprint

---

Burden, A.; Garbutt, R.A.; Evans, C.D.; Jones, D.L.; Cooper, D.M.  
Carbon sequestration and biogeochemical cycling in a saltmarsh subject to  
coastal managed realignment.

Copyright © 2013 Elsevier Ltd.

This version available <http://nora.nerc.ac.uk/500314/>

NERC has developed NORA to enable users to access research outputs wholly or partially funded by NERC. Copyright and other rights for material on this site are retained by the rights owners. Users should read the terms and conditions of use of this material at <http://nora.nerc.ac.uk/policies.html#access>

NOTICE: this is the author's version of a work that was accepted for publication in *Estuarine, Coastal and Shelf Science*. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in *Estuarine, Coastal and Shelf Science* (2013), 120. 12-20. [10.1016/j.ecss.2013.01.014](https://doi.org/10.1016/j.ecss.2013.01.014)

[www.elsevier.com/](http://www.elsevier.com/)

Contact CEH NORA team at  
[noraceh@ceh.ac.uk](mailto:noraceh@ceh.ac.uk)

1 **Carbon sequestration and biogeochemical cycling in a saltmarsh subject to coastal managed**  
2 **realignment**

3 A. Burden <sup>a\*</sup>, A. Garbutt <sup>a</sup>, C. Evans <sup>a</sup>, D. L. Jones <sup>b</sup>, D. Cooper <sup>a</sup>,

4 <sup>a</sup> *Centre for Ecology and Hydrology, Environment Centre Wales, Deiniol Road, Bangor, Gwynedd, LL57*  
5 *2UW, UK*

6 <sup>b</sup> *School of the Environment, Natural Resources & Geography, Bangor University, Bangor, Gwynedd,*  
7 *LL57 2UW, UK*

8 \* Corresponding author: Email: [anrd@ceh.ac.uk](mailto:anrd@ceh.ac.uk); Phone: +44 (0) 1248 374537 ; Fax: +44 (0) 1248  
9 362133

10 Keywords: saltmarsh; carbon sequestration; organic matter cycling; nutrient cycles; managed  
11 realignment

12 Regional index terms: UK, east coast, Tollesbury

13 **Abstract**

14 Globally, wetlands provide the largest terrestrial carbon (C) store, and restoration of degraded  
15 wetlands provides a potentially important mechanism for climate change mitigation. We examined  
16 the potential for restored saltmarshes to sequester carbon, and found that they can provide a  
17 modest, but sustained, sink for atmospheric CO<sub>2</sub>. Rates of C and nutrient cycling were measured and  
18 compared between a natural saltmarsh (high- and low-shore locations), claimed arable land on  
19 former high-shore saltmarsh and a managed realignment restoration site (high- and low-shore) in  
20 transition from agricultural land to saltmarsh 15 years after realignment, at Tollesbury, Essex, UK.  
21 We measured pools and turnover of C and nitrogen (N) in soil and vegetation at each site using a  
22 range of methods, including gas flux measurement and isotopic labelling. The natural high-shore site  
23 had the highest soil organic matter concentrations, topsoil C stock and below-ground biomass,  
24 whereas the agricultural site had the highest total extractable N concentration and lowest soil C/N  
25 ratio. Ecosystem respiration rates were similar across all three high-shore sites, but much higher in  
26 both low-shore sites, which receive regular inputs of organic matter and nutrients from the estuary.

27 Total evolution of  $^{14}\text{C}$ -isotopically labelled substrate as  $\text{CO}_2$  was highest at the agricultural site,  
28 suggesting that low observed respiration rates here were due to low substrate supply (following a  
29 recent harvest) rather than to inherently low microbial activity. The results suggest that, after 15  
30 years, the managed realignment site is not fully equivalent to the natural saltmarsh in terms of  
31 biological and chemical function. While above ground biomass, extractable N and substrate  
32 mineralisation rates in the high-shore site were all quite similar to the natural site, less dynamic  
33 ecosystem properties including soil C stock, C/N ratio and below-ground biomass all remained more  
34 similar to the agricultural site. These results suggest that reversion to natural biogeochemical  
35 functioning will occur following restoration, but is likely to be slow; we estimate that it will take  
36 approximately 100 years for the restored site to accumulate the amount of C currently stored in the  
37 natural site, at a rate of  $0.92 \text{ t C ha}^{-1} \text{ yr}^{-1}$ .

## 38 **1 Introduction**

39 Globally, wetlands provide the largest terrestrial carbon stores, and restoration of degraded  
40 wetlands provides a potentially important mechanism for climate change mitigation. To date, much  
41 research has focused on restoring degraded peatlands, for example through re-wetting. However,  
42 this research has highlighted uncertainties regarding its overall impact on C and greenhouse gas  
43 balances, due to the potential for enhanced release of  $\text{CH}_4$  following re-wetting (Strack et al., 2004;  
44 Baird et al., 2009). There is an increasing in the potential for restored coastal wetland systems to  
45 sequester large amounts of carbon (Craft et al., 2003; Shepherd et al., 2007; Santin et al., 2009;  
46 Livesley and Andrusiak, 2012). Additionally, restoring coastal wetlands may avoid the offsetting  
47 effects of enhanced methane production associated with peat re-wetting, due to the presence of  
48 sulphates which allows sulphate-reducing bacteria to outcompete methanogens for energy sources  
49 (Poffenbarger et al., 2011; Bartlett et al., 1987; Andrews et al., 2006). Therefore, per unit area,  
50 restoration of coastal wetlands such as saltmarshes may contribute more to C sequestration, and  
51 therefore to climate regulation, than peatlands. However, at present, evidence is sparse.

52           As well as carbon sequestration, saltmarshes provide a range of other ecosystem services.  
53   These include immobilisation of pollutants (e.g. retention of diffuse nutrient and faecal pollutants  
54   into accumulating sediments), flood defence and shore line erosion control and they are a significant  
55   reservoir of wild species diversity (Jones et al., 2011). However historically, human activity has  
56   focused on the land-claim ('reclamation') of saltmarsh for agriculture, and more recently for port  
57   development leading to an estimate by French (1997) that 25% of the world's intertidal estuarine  
58   habitat had been lost due to land claim. Accelerated sea-level rise also poses a threat to existing  
59   saltmarsh through coastal squeeze, as sea defences restrict their natural landward migration to  
60   higher elevation (Blackwell et al., 2004). Globally, efforts are now being made to restore and create  
61   saltmarshes to mitigate historic losses and on-going development. Since the early 1990s, the driving  
62   force for restoration was the unsustainable increasing cost of maintaining and upgrading existing sea  
63   defences (Andrews et al., 2006). However, managed realignment is also undertaken for purposes of  
64   habitat or biodiversity enhancement or restoration, for example in Europe, salt-marsh restoration  
65   allows government compliance with the European Union Habitats Directive (C.E.G., 1992) which  
66   states there should be 'no further net loss of coastal marsh' (UK Biodiversity Group, 1999). UK  
67   targets aim to create 2240 ha of saltmarsh between 1999 and 2015, primarily via a process known as  
68   'managed realignment'; the landward retreat of coastal defences and subsequent tidal inundation of  
69   previously-claimed agricultural land (Garbutt et al., 2006).

70           In general, managed realignment schemes in the UK and elsewhere have shown that, with  
71   relatively minimal pre-treatment and/or management of the area, allowing tidal ingress through a  
72   breach of the existing seawall onto low-lying agricultural land will quickly produce intertidal mudflats  
73   that are colonised by saltmarsh plants (French et al., 2000; Wolters et al., 2005). Managed  
74   realignment sites are sinks of sediment and, given time, representative saltmarsh plant, invertebrate  
75   and bird communities can become established (Garbutt et al., 2006). Newly created saltmarsh also  
76   acts as a natural sea defence by attenuating tidal amplitude (Pethick, 2002). Self-sustaining plant  
77   communities are often the primary goal of restoration efforts as they perform some of the desirable

78 functions of wetland ecosystems (Craft et al., 2002; Möller et al., 1999; van Andel, 1998). However,  
79 many physical and functional processes such as nutrient cycling in these sites are poorly understood,  
80 and it has yet to be shown that restored saltmarshes are functionally equivalent to referenced  
81 systems and therefore whether they do effectively compensate for the loss of habitat as intended. In  
82 particular, the capacity of managed realignment schemes to accumulate carbon following  
83 conversion from agricultural land to saltmarsh has not been fully quantified.

84 This study measures and compares biogeochemical functioning between a 15 year old managed  
85 realignment site in a state of transition from agricultural land to saltmarsh, relative to adjacent areas  
86 of natural saltmarsh and arable land on former saltmarsh. Our three main objectives were: 1) to  
87 compare general soil characteristics between the restored saltmarsh, natural saltmarsh and  
88 agricultural sites; 2) to quantify and compare the organic matter, carbon and nitrogen pools at all  
89 sites, and estimate how far soil carbon stocks at the restored site have progressed along a trajectory  
90 between its former agricultural condition and the natural saltmarsh; 3) to investigate potential  
91 differences in the dynamics of organic matter cycling by measuring in situ ecosystem respiration and  
92 carbon mineralisation rates.

## 93 **2 Materials and methods**

### 94 **2.1 Site description**

95 This study was undertaken at the Tollesbury managed realignment site, adjacent natural marshes  
96 and arable land of the Blackwater Estuary, south-east England (51°46'N, 0°51'E, Fig. 1) in July 2010.  
97 The 21-ha restoration site had originally been a saltmarsh, but was claimed for agriculture in the late  
98 18th century (Boorman et al., 1997). The sea defences were breached in August 1995, leaving a 50-  
99 m wide opening and allowing tidal ingress to the site for the first time in over 150 years. The  
100 construction of a new sea defence landward of the old embankment prevented tidal flooding of the  
101 neighbouring arable fields which were claimed from saltmarsh at the same time as the managed  
102 realignment site. The altitude of the site ranges from 0.9 m to 3.0 m above Ordnance Datum (OD),  
103 with the major part of the site lying below 2.0 m OD (Garbutt et al., 2006). Mean high water neap

104 (MHWN) and mean high water spring (MHWS) tide levels for the Blackwater estuary are 1.50 m and  
105 2.60 m OD, respectively (Pye and French, 1993).

106 There are two dominant plant communities within the managed realignment site. Above  
107 1.75m the upper part of the site (referred to as 'restored high') is dominated by a species poor  
108 *Puccinellia maritima* dominated community with occasional *Atriplex portulacoides*, *Spergularia*  
109 *media* and *Suaeda maritima*. At the same elevation on the adjacent natural saltmarshes (referred to  
110 as 'natural high') the plant communities are characterised by a diverse mix of saltmarsh plant species  
111 with abundant *Limonium vulgare* and *P. maritima*, frequent *Salicornia europaea* agg. and  
112 *Sarcocornia perennis* and occasional *Armeria vulgare*, *A. portulacoides*, *S. maritima* and *Triglochin*  
113 *maritima*. Below 1.75m the lower part of the site (referred to as 'restored low') is dominated by  
114 *Spartina anglica* and abundant *S. europaea* agg. At the same elevation on the adjacent natural  
115 saltmarshes (referred to as 'natural low') the plant communities are dominated by *S. europaea* agg.  
116 with occasional *S. maritima*. Landward of the managed realignment site land claimed from saltmarsh  
117 in the 1800s is farmed for wheat (*Triticum aestivum*) and other crops.

## 118 **2.2 Experimental design**

119 Two different elevations (2.5 and 1.75 m above OD) within the managed realignment site were  
120 chosen to best represent the dominant plant communities as described above. The equivalent  
121 elevations on the adjacent marshes were determined through a topographic survey. Elevation was  
122 used as a surrogate for tidal inundation to ensure that the plant communities within the de-  
123 embankment sites and reference marshes received equivalent submergence frequencies, and was  
124 checked by observing the depth and extent of the incoming tide for each site. No visual differences  
125 were observed. At the time of survey, the wheat crop had been harvested from the agricultural land  
126 adjacent to the site, however, the soil had not been tilled for subsequent crops and stubble  
127 remained at the surface.

128 A split-plot experimental design was used with six locations sampled at each site location.  
129 Sampling locations were situated at the same elevation for the two high shore sites, and for the two

130 low shore sites. The six sampling locations at each site were in two clusters of three, with the two  
131 clusters separated by 150m and within-cluster spacing of 10m. This arrangement provides an  
132 estimate of spatial variability (Fig. 1). With six sampling locations in two high shore, two low shore  
133 and one agricultural site, this gave 30 individual sample sites in all. Soil cores (4 cm diameter by 30  
134 cm depth) were taken from within the footprint of each of the greenhouse gas monitoring chambers  
135 (see below) after the third day of gas sampling. Each core was split into 3 sections (0-10 cm, 10-20  
136 cm and 20-30 cm) which were analysed separately. A second soil core was taken for below ground  
137 plant biomass measurements only. All field work took place in July 2010.

### 138 **2.3 Soil characteristics**

139 Electrical conductivity (EC) and pH were measured in 1:1 (w/v) soil:distilled water extracts (Smith  
140 and Doran, 1996). Moisture content was determined by measuring the weight loss after drying the  
141 soil at 105°C overnight. Organic matter content was determined as the percent weight loss after  
142 ignition overnight at 375°C. Available ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) were determined in 1:5  
143 (w/v) soil: 0.5 M  $\text{K}_2\text{SO}_4$  extracts following the method described in Jones et al. (2005) and the  
144 colorimetric analysis procedures of Mulvaney (1996) and Miranda et al. (2001). Water soluble  
145 phosphorous (P), sodium (Na), potassium (K) and calcium (Ca) were determined using 1:5 (w/v)  
146 soil:distilled water extracts following shaking (1 h) and centrifugation (6000 g, 15 min) to remove  
147 particulate material. P was determined colorimetrically using the molybdate blue/ascorbic acid  
148 procedure of Murphy and Riley (1962) while Na, K and Ca were determined with a PFP7 Flame  
149 Photometer (Jenway flame photometer (Bibby Scientific Ltd, Staffs, UK). Soluble humic substances in  
150 the water extracts were estimated by measuring the UV absorbance of the extracts at 254 nm (US-  
151 EPA, 2005). Bulk density was measured using bulk density rings with a volume of 45.2 cm<sup>3</sup>. Samples  
152 were collected from the soil surface only. Samples were dried at 105°C for 72 hours and the dry  
153 mass divided by the volume of the bulk density ring. Total soil C and N were measured by  
154 combustion on a TruSpec CN Analyser (Leco Corp, St Joseph, MI).

155

156 The soil carbon pool was estimated by multiplying the bulk density by the percentage carbon figures.  
157 It was therefore only estimated for the surface of the soil where bulk density values were available.  
158 The restored high marsh per year increase in carbon was derived by taking the difference in the soil  
159 carbon pool between the agricultural and restored high shore sites and dividing by the number of  
160 years since managed realignment (i.e. 15 y). The estimate of how long it would take for the soil  
161 carbon pool of the restored high shore site to become equivalent to the natural high shore site was  
162 calculated by dividing the difference between the agricultural and natural high shore site soil carbon  
163 pool by the per year increase of the restored high shore site, assuming that this was equivalent to  
164 the agricultural site pre-restoration.

#### 165 **2.4 Gas flux measurements**

166 Ecosystem CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O emissions were measured using dark static chambers within two hours  
167 of high water. Gas sampling with the static chambers was carried out on 3 successive days to take  
168 account of temporal variability. Placement of the static chambers was marked with canes allowing  
169 return to the exact same position on consecutive days. On the first day both clusters of 3 at each  
170 land use/elevation combination were sampled giving 30 sample locations in total. On days two and  
171 three only one cluster of each land use/elevation combination was sampled, giving 15 sample  
172 locations on the second and third days. After placement of the chambers on the soil surface, gas  
173 samples were taken with a syringe from each chamber after 0, 15, 30, 45 and 60 minutes, injected  
174 into evacuated gas chromatograph vials and analysed within one week. In addition to these samples,  
175 duplicate samples were taken from one chamber for each treatment to test reproducibility. All gas  
176 samples were analysed using a Perkin Elmer Clarus 500 Gas Chromatograph (GC) equipped with a  
177 Poropak QS (80-100 mesh) analytical column and turbomatrix 40 headspace auto analyser. N<sub>2</sub>O was  
178 detected using an electron capture detector (ECD) at 400°C, sample oven at 40°C; CH<sub>4</sub> was detected  
179 using a flame ionisation detector (FID) at 375°C, sample oven at 40°C equipped with a methaniser.  
180 Carrier gas pressure was 138 kPa, and injection pressure 160 kPa, all other controls were as Perkin  
181 Elmer standard setup. The GC was calibrated using bottled gas with a known concentration of CO<sub>2</sub>,

182 CH<sub>4</sub> and N<sub>2</sub>O (CryoService Ltd., Worcester, UK) and this gas was used for quality control (QC) at set  
183 points throughout each sample run. Gas fluxes were then calculated on an hourly basis using the  
184 following calculations:

$$185 \quad C_m = (C_v \times M \times P) / (R \times T)$$

186 where  $C_m$  is the mass per volume (expressed as mg m<sup>-3</sup>),  $C_v$  is the gas concentration (expressed as  
187 mg dm<sup>-3</sup>),  $M$  is the molecular weight of the trace species (e.g. 12 for Carbon),  $P$  is the barometric  
188 pressure,  $T$  is the chamber temperature in Kelvin (°C + 273.15) at time of sample, and  $R$  is the gas  
189 constant.

190 The per hour flux ( $F$ , mg m<sup>-2</sup> h<sup>-1</sup>) was then calculated by:

$$191 \quad F = V \times C_{rate} / A$$

192 where  $V$  is the internal volume of the enclosure including collar volume (expressed as m<sup>3</sup>),  $A$  is the  
193 area of the collar enclosed surface (m<sup>2</sup>),  $C_{rate}$  is the change in gas concentration (i.e.  $C_m t_1 - C_m t_0$ )  
194 over the enclosure period.

## 195 **2.5 Carbon substrate mineralisation**

196 A <sup>14</sup>C-isotopically labelled C substrate was used to estimate carbon mineralisation rates in soil as  
197 described in Simfukwe et al. (2011). The C substrate consisted of <sup>14</sup>C-labelled shoots of *Lolium*  
198 *perenne* (L.) with a specific activity of 12.3 kBq g<sup>-1</sup>. The <sup>14</sup>C-enrichment of *Lolium perenne* plant  
199 material was performed by pulse labelling with <sup>14</sup>CO<sub>2</sub> at a constant specific activity according to Hill  
200 et al. (2007). To characterise the <sup>14</sup>C label in the plant material, a sequential chemical fractionation  
201 was performed according to Jones and Darrah (1994). Briefly, 50 mg of finely ground plant material  
202 was sequentially extracted in 8 ml deionised water for 30 min at 85°C, 8 ml 20% ethanol for 30 min  
203 at 80°C, 5 ml 0.3% HCl for 3 h at 95 °C and 5 ml 1 M NaOH for 1 h at 95 °C. After each extraction  
204 step, the sample was centrifuged (5000 *g*, 15 min), the supernatant removed and its <sup>14</sup>C content  
205 determined using Optiphase 3<sup>®</sup> Scintillation fluid (PerkinElmer, Waltham, MA) and a Wallac 1404  
206 Liquid Scintillation Counter (PerkinElmer Corp., Waltham, MA). For each soil, 10 g was placed into a  
207 sterile 50 cm<sup>3</sup> polypropylene container and 100 mg of the <sup>14</sup>C-labelled complex C substrate was then

208 added to the soil. A vial containing 1 M NaOH was then placed above the soil and the polypropylene  
209 containers hermetically sealed. The  $^{14}\text{CO}_2$  capture efficiency of the NaOH traps was >95%. The soils  
210 were then placed in the dark in a climate-controlled room (10°C) and the NaOH traps exchanged  
211 every 3 days for 24 days. The  $^{14}\text{CO}_2$  in the NaOH traps was determined by liquid scintillation counting  
212 as described above.

213 Of the total  $^{14}\text{C}$  contained in the plant material and subsequently added to soil,  $32.9 \pm 1.5\%$   
214 was extractable by water,  $4.2 \pm 0.2\%$  by ethanol,  $16.8 \pm 0.6\%$  by HCl,  $27.5 \pm 0.4\%$  by NaOH and  $18.5 \pm$   
215  $2.2\%$  was insoluble residue. These components approximately correspond to the readily  
216 decomposable or neutral-detergent soluble C (water and ethanol soluble), cellulose and  
217 hemicellulose (HCl soluble) and lignin (NaOH soluble and insoluble) fractions of organic matter  
218 respectively (Domisch et al., 1998; Ekschmitt et al., 2008; Moorhead and Sinsabaugh, 2006).

## 219 **2.6 Statistical analysis**

220 A linear mixed effects model (lme) was used to describe the data using R version 2.13.2  
221 ( $y \sim \text{Site} * \text{Depth}, \text{random} = \sim 1 | \text{Location/sample}$ ). We also fitted models excluding respectively the  
222 depth and site effects to test the need for their inclusion in the model. On all but one occasion both  
223 regime and depth were significant ( $p < 0.05$ ), confirming the need for both fixed effects to be  
224 included. For each variable we also tested for the significance of differences between each regime  
225 and depth pair. Separate analyses by depth and by regime were also carried out. A similar approach  
226 was taken for the gas measurements replacing 'depth' with 'day' in the analysis. As there was no  
227 significant difference in gas measurements between days within sites, the average flux over the 3  
228 days of measurement was analysed. For the carbon substrate mineralisation, the final data points  
229 were used in the lme model (i.e. total evolution of  $^{14}\text{CO}_2$  within incubation period of 25 days –  
230 expressed as % of  $^{14}\text{C}$ -substrate added to the soil). This approach enabled the raw data to be  
231 analysed accounting for replication at the level of the experimental unit or site ( $n=5$ ). For analysis we  
232 used the  $\log_{10}$  of all variables other than pH.

## 233 3 Results and discussion

### 234 3.1 General soil characteristics

235 There were significant differences ( $p < 0.05$ ) between sites in all of the soil properties measured. Soil  
236 conductivity was highest at the natural high shore marsh, lowest at the agricultural site, and  
237 intermediate at the restored high shore site (averages of 12.08, 0.14 and 4.40  $\text{mS cm}^{-1}$  respectively,  
238 Table 1). These large differences highlight the influence of seawater on both the natural and  
239 restored sites, and of freshwater on the agricultural site. There was no significant difference  
240 between the restored and natural low shore sites ( $p = 0.582$ ) and all sites showed a decrease in  
241 conductivity with depth. The sodium, potassium and calcium data, as would be expected, reflected  
242 the differences observed in the conductivity data – they were all significantly ( $p \leq 0.009$ ) higher at  
243 the natural high shore site and lowest at the agricultural site ( $p \leq 0.010$ , Table 1).

### 244 3.2 Plant biomass and soil organic matter, C and N pools

245 The restored high shore site was found to have approximately twice as much above ground plant  
246 biomass compared to the natural high shore site ( $p = 0.037$ , Fig. 2) due to it being dominated by a  
247 monoculture of *P. maritima*. On the other hand, there was 16 times more below ground plant  
248 biomass in the natural high shore site than at any of the other sites sampled (average of 11.5  $\text{kg m}^{-2}$   
249 compared to 0.7  $\text{kg m}^{-2}$  for the other four sites; Fig. 2) due to the species-rich vegetation consisting  
250 of long lived perennials with woody tap roots. This translated into the natural high shore site soil  
251 having significantly greater ( $p < 0.05$ ) organic matter content (and therefore less mineral material)  
252 than all other sites at all three depths (average of 21.8% compared to an average of 5.2% for the  
253 other four sites, Table 1); this appears consistent with data collected from created *Spartina*  
254 *alterniflora* marshes along the North Carolina coast (Craft, 2000), which showed that macro-organic  
255 matter (MOM) content increased with age of the created marsh. The agricultural site had  
256 significantly lower ( $p < 0.05$ ) soil organic matter content than all other sites at depth 0-10 cm ( $p \leq$   
257 0.004) but was not significantly different ( $p < 0.05$ ) to the two restored sites at depths 10-20 cm and  
258 20-30 cm. This suggests that even after 15 years of inundation, soils below 10 cm depth retain

259 properties characteristic of the agricultural soil – an idea also supported by Craft (2000) in  
260 constructed *Spartina alterniflora* saltmarshes in North Carolina. Spencer et al. (2008) found similar  
261 evidence within a restored site 8 years after managed realignment, and hypothesized that the relic  
262 land surface may have formed an aquaclude which prevents vertical soil water movement. This idea  
263 is further supported in the current study by the pH and humic substances data, which both increased  
264 with depth in the restored high shore site to values that were more comparable to the agricultural  
265 site than the natural high shore site (supplementary Table 1).

266 The natural and restored low shore sites had the lowest soil carbon pool (average of 13.7  
267 and 10.9 kg m<sup>-3</sup> respectively, Fig. 2) and were not significantly different from each other (p = 0.260).  
268 This is not surprising as the low shore sites are both inundated daily and are dominated by pioneer  
269 annual species. In contrast, the natural high marsh site had a significantly greater soil carbon pool  
270 than all other sites (p ≤ 0.016, average of 31.1 kg m<sup>-3</sup>), whilst the restored high shore and agricultural  
271 sites had a similar soil carbon pool (p = 0.621) of 22.1 and 20.7 kg m<sup>-3</sup> respectively (Fig. 2). This  
272 suggests that there has been, at best, only a small overall increase in the soil carbon pool of the  
273 restored high-shore site in the 15 years since managed realignment, and that the site is thus likely to  
274 take many more years to accumulate an equivalent carbon store to the natural system.

275 Assuming that the restored high site previously resembled the agricultural site (see  
276 methods), the estimated rate of carbon accumulated at the Tollesbury managed realignment site  
277 was calculated to be 92.4 g C m<sup>-3</sup> yr<sup>-1</sup>, or 0.92 t C ha<sup>-1</sup> yr<sup>-1</sup>. This is within the estimated UK saltmarsh  
278 carbon sequestration range of 0.64 – 2.19 t C ha<sup>-1</sup> yr<sup>-1</sup> proposed by Cannell et al. (1999). We  
279 therefore estimate that it would take approximately 100 years for the restored site to accumulate  
280 the amount of carbon currently stored in the natural site. This is similar to the figure estimated by  
281 Craft et al. (2003) of up to 70 years for the total organic carbon pool to become equivalent to natural  
282 within a created *S. alterniflora* marsh. The 100 year estimate also corresponds that of Crooks et al.  
283 (2002) for the length of time it could take vegetation in realignment sites to resemble that of natural  
284 marshes, which (as noted above) has a substantial influence on the size of the soil organic matter

285 pool. It has been suggested, however, that the organic matter formed in constructed marshes  
286 contains a greater proportion of labile organic compounds (Craft et al., 2003) which are turned over  
287 more quickly by the microbial community and could result in constructed or restored wetlands being  
288 less effective at sequestering carbon over time; this is considered further below.

289 The C/N ratio of the soil was significantly greater at the natural high shore site than all other  
290 sampling sites ( $p \leq 0.004$ , Table 1). The restored high marsh site had slightly (albeit not significantly)  
291 higher C/N than the agricultural site, which is again consistent with the interpretation that this site is  
292 slowly transitioning towards the soil conditions currently observed at the natural marsh. This accords  
293 with other terrestrial data that suggest it takes a very long time, even centuries, to reverse historic  
294 enrichment of nutrient poor pools.

295 Measured total inorganic nitrogen at the agricultural site ( $2.46 \text{ mg kg}^{-1}$  dry weight, 97.7% as  
296 oxidised N, Table 1) was 2.5 times higher than at any of the other sites, and significantly higher ( $p <$   
297  $0.05$ ) at all sites except the natural high shore site. This is indicative of both nutrient enrichment  
298 (wheat is cultivated at this site and is presumably fertilised) and aerobic conditions, both of which  
299 favour nitrification and therefore increased nitrate versus ammonium concentrations. In contrast,  
300 ammonium concentrations were at least four times higher at all other sites when compared to the  
301 agricultural site, and ammonium was the dominant form of mineral N at the natural high, managed  
302 high and managed low sites. These data suggest that managed realignment, which has led to the  
303 reinstatement of anaerobic soil conditions that promote denitrification and leaching, has resulted in  
304 rapid decreases in extractable nitrate levels. On the other hand, the presence of extractable  
305 ammonium in all samples from all these sites ( $0.27 - 0.92 \text{ mg kg}^{-1}$  dry weight, Table 1) suggests a  
306 continued supply of mineral N via organic matter mineralisation in both natural and restored  
307 conditions, consistent with the relatively low measured C/N ratios of soil organic matter (Table 1).  
308 The fact that ammonium concentrations were not significantly higher in the restored high shore site  
309 compared to the natural high site suggests that, despite soil C/N remaining lower in the restored

310 site, N mineralisation rates (and therefore nutrient N supply) may have returned to pre-agricultural  
311 levels following managed realignment.

### 312 **3.3 Dynamics of organic matter cycling**

313 CH<sub>4</sub> and N<sub>2</sub>O fluxes were near-zero for all sites and on all sampling occasions, with concentrations at  
314 or close to ambient air concentrations (Table 2) – a result reflecting those of Livesley and Andrusiak  
315 (2012) who found that CH<sub>4</sub> and N<sub>2</sub>O fluxes were close to zero in temperate saltmarsh in south  
316 eastern Australia. No significant between-site differences were observed. CH<sub>4</sub> production is known  
317 to be inhibited by the presence of sulphate (e.g. from seawater), due to competition between  
318 sulphate reducing bacteria and methanogens (Bartlett et al., 1987; Andrews et al., 2006).  
319 Poffenbarger et al. (2011) proposed that the salinity regime required for methane flux to be  
320 negligible was 18 µg l<sup>-1</sup> – well within the expected range of salinity at Tollesbury. However, a similar  
321 study of a restored saltmarsh in the estuary of the River Torridge, Devon, UK, 6 months after  
322 managed realignment, concluded that managed realignment could result in increased production of  
323 N<sub>2</sub>O, due the combination of high residual soil nitrogen levels from the agricultural site, and the  
324 reinstatement of dry-wet cycles following tidal reconnection (Blackwell et al., 2010). The River  
325 Torridge experiment was laboratory-based, and measured fluxes over simulated tidal cycles, which  
326 may explain the difference in results (an average of 0.65 mg N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup> compared to zero in our  
327 study). However, if tidal pumping – tidal forcing of seawater into the coastal aquifer – does in fact  
328 flush CO<sub>2</sub> and other carbon forms out of re-flooded soils (an idea proposed by Kathilankal et al.,  
329 2008) then our results should demonstrate maximum gaseous flux as they were taken within two  
330 hours prior to high tide. Given the limited number of sampling occasions, we cannot draw clear  
331 conclusions about the overall magnitude of N<sub>2</sub>O flux at the Tollesbury sites, other than to note that  
332 no evidence of a measurable flux was recorded at any of the sites on any of the sampling occasions.

333 Ecosystem respiration ( $R_{eco}$ ) measurements using dark chambers indicated substantial CO<sub>2</sub>  
334 production rates, with the highest fluxes recorded for the restored low shore site, followed by the  
335 natural low shore site (Fig. 3). Overall, between-site differences were significant ( $p < 0.001$ )

336 suggesting that both of the low shore sites were more microbially active, turning over carbon inputs  
337 at a higher rate than either of the high shore sites. The natural high shore site had the lowest CO<sub>2</sub>  
338 flux but was not significantly lower than the restored high shore or agricultural site ( $p \geq 0.3$ )  
339 suggesting that rates of carbon cycling among these three sites were similar. There was some  
340 indication however of an inverse relationship between  $R_{\text{eco}}$  and C/N for these three sites, consistent  
341 with higher rates of carbon turnover at the more nutrient-rich agricultural site. It is likely that lower  
342  $R_{\text{eco}}$  values at the agricultural site relative to the low shore sites are a consequence of reduced  
343 substrate supply following the harvesting of the crop (see below). Based on our measurements, we  
344 did not detect a clear influence of substrate quality on respiration rates, as suggested by Craft et al.  
345 (2003), although we acknowledge that the dataset only covers a short time-period.

346         The total evolution of <sup>14</sup>C-isotopically labelled C substrate as CO<sub>2</sub> (expressed as % of <sup>14</sup>C-  
347 substrate added to the soil) was highest at the agricultural site (Fig. 4). This result contrasts with the  
348 low measured ecosystem respiration flux but, as noted above, the respiration measurements  
349 occurred after crop harvesting at the site, when substrate inputs would have been very low. As well  
350 as the direct production of CO<sub>2</sub> from plant respiration, the presence or absence of vegetation has  
351 been shown to significantly affect microbial activity in soils via the supply of litter inputs and root  
352 exudates for microbial respiration (Oburger and Jones, 2009). The observation that added substrate  
353 was rapidly respired at the agricultural site indicates that potential rates of microbial carbon  
354 turnover are higher than those observed at the time of sampling.

355         Among the natural and restored sites, mineralisation of <sup>14</sup>C-labelled substrate was  
356 consistently lower, and the restored high shore site was not significantly different to the natural high  
357 shore site ( $p = 0.107$ , Fig. 4). As the added substrate was of high molecular weight carbon, results  
358 should provide an insight into differences in carbon sequestration potential between sites, on the  
359 basis that this material has the potential to be retained in the soil rather than respired, whereas low  
360 molecular weight fractions would likely be utilised by the microbial community in all sites. The  
361 results suggest an overall slower turnover rate, and therefore greater carbon storage potential, in

362 the natural site, when compared to the agricultural soil. The absence of a significant difference in  
363 turnover rates between natural and restored high shore sites further suggests that, 15 years post-  
364 restoration, rates of carbon sequestration at the Tollesbury managed realignment site have now  
365 returned to those characteristic of the natural marsh.

366 At both low shore sites, measured substrate utilisation rates were higher than at the natural  
367 and restored high shore (significantly different at  $p < 0.05$  for all combinations other than the  
368 restored high versus natural low sites,  $p = 0.218$ , Fig. 4), but lower than at the agricultural site.  
369 Higher substrate utilisation rates at the low versus high shore natural and restored sites are  
370 consistent with the ecosystem respiration measurements, again suggesting faster carbon turnover  
371 rates at these sites. Higher extractable nitrate and phosphate, and lower soil C/N ratios, further  
372 suggest that this rapid turnover is linked to higher nutrient availability, possibly due to tidal recharge  
373 of nutrients.

#### 374 **4 Conclusions**

375 In the UK, managed realignment is primarily undertaken for purposes of habitat and biodiversity  
376 enhancement or restoration, or for coastal defence. Carbon and nutrient cycling are rarely  
377 considered when these schemes are developed and monitored, let alone used as success criteria  
378 (which currently only consider vegetation development). However, with a growing policy emphasis  
379 on the wider ecosystem service implications of land-management, it is clear that enhanced carbon  
380 sequestration could provide an additional benefit resulting from restoration, whilst reversion of the  
381 nitrogen cycle to the low-nutrient levels characteristic of natural ecosystems may be a prerequisite  
382 for full vegetation recovery. Our data suggest that managed realignment reduces nitrogen  
383 mineralisation rates towards those of natural saltmarsh levels, but that soil C/N ratios remain well  
384 below those of the natural site, suggesting that complete recovery to natural conditions may be far  
385 slower. Similarly, the soil carbon pool of the restored site was more similar to the agricultural site  
386 than the natural marsh, suggesting that there has been at best only a small overall increase in the  
387 carbon pool of the restored high-shore site in the 15 years since managed realignment. On the other

388 hand, carbon mineralisation rates at the restored site were similar to the natural site, and lower  
389 than the agricultural site, suggesting that the soil carbon pool of the restored site will ultimately  
390 converge with that of the natural marsh. Our calculations predict that this will take approximately  
391 100 years.

## 392 **5 Acknowledgements**

393 The authors would like to thank Sarah Hodgson for soil quality assessment analysis, Stephanie Ellis  
394 for the mineralisation analysis and Marc Brouard for LOI, biomass and bulk density analysis. Also our  
395 thanks go to Philip Stickler and David Watson (Cartographic Unit, Department of Geography,  
396 University of Cambridge) who assisted with the production of figure 1.

## 397 **References**

- 398 Andrews, J. E., Burgess, D., Cave, R. R., Coombes, E. G., Jickells T. D., Parkes, D. J., Turner, R. K., 2006.  
399 Biogeochemical value of managed realignment, Humber estuary, UK. *Science of the Total*  
400 *Environment* 371, 19–30.
- 401 Baird, A., Holden, J., and Chapman, P., 2009. A Literature Review of Evidence on Emissions of  
402 Methane in Peatlands. Defra Project SP0574.
- 403 Bartlett, K.B., Bartlett, D.S., Harriss, R.C., Sebacher, D.I., 1987. Methane emissions along a salt-marsh  
404 salinity gradient. *Biogeochemistry* 4, 183–202.
- 405 Blackwell, M.S.A., Hogana, D.V., Maltbya, E., 2004. The short-term impact of managed realignment  
406 on soil environmental variables and hydrology. *Estuarine, Coastal and Shelf Science* 59, 687-  
407 701.
- 408 Blackwell, M.S.A., Yamulki, S., Bol, R. 2010. Nitrous oxide production and denitrification rates in  
409 estuarine intertidal saltmarsh and managed realignment zones. *Estuarine, Coastal and Shelf*  
410 *Science* 87, 591-600.
- 411 Boorman, L.A., Garbutt, A., Barratt, D., Myhill, D., Eversham, B., Reading, C., Cox, R., Rothery, P.  
412 1997. Large scale experimental managed realignment, vol. 1, Tollesbury, Essex. Institute of  
413 Terrestrial Ecology, Huntingdon.

414 Cannell, M.G., Milne, R., Hargreaves, K.J., Brown, T.A., Cruickshank, M.M., Bradley, R.I., Spencer, T.,  
415 Hope, D., Billett, M.F., Adger, W.N., Subak, S. 1999. National Inventories of Terrestrial Carbon  
416 Sources and Sinks: The UK Experience. *Climate Change*, 42, 505–530.

417 C.E.G., 1992. Council Directive 92/43/EEC of May 1992 on the Conservation of Natural Habitats and  
418 of Wild Fauna and Flora. *Official Journal of the European Communities*: L 206.

419 Crooks, S., Schutten, J., Sheern, G.D., Pye, K., Davy, A.J. 2002. Drainage and elevation as factors in  
420 the restoration of saltmarsh in Britain. *Restoration Ecology* 10, 591–602.

421 Craft, C. 2000. Co-development of wetland soils and benthic invertebrate communities following  
422 saltmarsh creation. *Wetlands Ecology and Management* 8, 197-207.

423 Craft, C., Broome, S., Campbell, C. 2002. Fifteen years of vegetation and soil development after  
424 Brackish-water marsh creation. *Restoration Ecology* 10, 248-258.

425 Craft, C., Megonigal, P., Broome, S., Stevenson, J., Freese, R., Cornell, J., Zheng, L., Sacco, J. 2003. The  
426 pace of ecosystem development of constructed *Spartina alterniflora* marshes. *Ecological*  
427 *Applications* 13, 1417 – 1432.

428 Domisch, T., Finer, L., Karsisto, M., Laiho, R., Laine, J., 1998. Relocation of carbon from decaying litter  
429 in drained peat soils. *Soil Biology & Biochemistry* 30, 1529-1536.

430 Ekschmitt, K., Kandeler, E., Poll, C., Brune, A., Buscot, F., Friedrich, M., Gleixner, G., Hartmann, A.,  
431 Kästner, M., Marhan, S., Miltner, A., Scheu, S., and Wolters, V. 2008. Soil carbon preservation  
432 through habitat constraints and biological limitations on decomposer activity. *Journal of Plant*  
433 *Nutrition and Soil Science* 171, 27-35.

434 French, 1997 P.W. *French, Coastal and Estuarine Management*, Routledge, London (1997).

435 French, C.E., French, J.R., Clifford, N.J., Watson, C.J. 2000. Sedimentation-erosion dynamics of  
436 abandoned reclamations: the role of waves and tides. *Continental Shelf Research* 20, 1711–  
437 1733.

438 Garbutt, R.A., Reading, C.J., Wolters, M., Gray, A.J., Rothery, P. 2006. Monitoring the development of  
439 intertidal habitats on former agricultural land after the managed realignment of coastal  
440 defences at Tollesbury, Essex, UK. *Marine Pollution Bulletin* 53, 155–164.

441 Hill, P. W., Marshall, C., Williams, G. G., Blum, H., Harmens, H., Jones, D. L. and Farrar, J. F. 2007. The  
442 fate of photosynthetically-fixed carbon in *Lolium perenne* grassland as modified by elevated  
443 CO<sub>2</sub> and sward management. *New Phytologist*, 173, 766–777.

444 Jones, D.L., Darrah, P.R., 1994. Simple method for <sup>14</sup>C labelling root material for use in root  
445 decomposition studies. *Communications in Soil Science and Plant Analysis* 25, 2737-2743.

446 Jones, D.L., Kemmitt, S.J., Wright, D., Cuttle, S.P., Bol, R. and Edwards, A.C. 2005. Rapid intrinsic rates  
447 of amino acid biodegradation in soils are unaffected by agricultural management strategy. *Soil*  
448 *Biology and Biochemistry* 37, 1267-1275.

449 Jones, L., Angus, S., Cooper, A., Doody, P., Everard, M., Garbutt, A., Gilchrist, P., Hansom, J., Nicholls,  
450 P., Pye, K., Ravenscroft, N., Rees, S., Rhind, P., Whitehouse, A. 2011. Coastal Margins. In: The  
451 UK National Ecosystem Assessment Technical Report. UK National Ecosystem Assessment,  
452 UNEP-WCMC, Cambridge.

453 Kathilankal, J.C., Mozdzer, T.J., Fuentes, J.D., D’Odorico, P., McGlathery, K.J., Zieman, J.C. 2008. Tidal  
454 influences on carbon assimilation by a saltmarsh. *Environmental research letters* 3, 044010.

455 Livesley, S.M., Andrusiak, S.M. 2012. Temperate mangrove and saltmarsh sediments are a small  
456 methane and nitrous oxide source but important carbon store. *Estuarine, Coastal and Shelf*  
457 *Science* 97, 19-27.

458 Miranda, K.M., Espey, M.G., Wink, D.A. 2001. A rapid, simple spectrophotometric method for  
459 simultaneous detection of nitrate and nitrite. *Nitric Oxide* 5, 62-71.

460 Möller, I., Spencer, T., French, J.R., Leggett, D.J., Dixon, A.M. 1999. Wave transformation over  
461 saltmarshes: a field and numerical modelling study from North Norfolk, England. *Estuarine,*  
462 *Coastal and Shelf Science* 49, 411-426.

463 Moorhead D.L, Sinsabaugh R.L. 2006. A theoretical model of litter decay and microbial interaction.  
464 Ecological Monographs 76(2), 151-174.

465 Mulvaney, R.L. 1996. Nitrogen-Inorganic Forms. In: Sparks, D.L. (Eds.), Methods of Soil Analysis:  
466 Chemical Methods. Part 3. Soil Science Society of America, Madison WI.

467 Murphy, J., Riley, J.P. 1962. A modified single solution method for the determination of phosphate in  
468 natural waters. Analytica Chimica Acta 27, 31-36.

469 Oburger, E., Jones, D.L. 2009. Substrate mineralization studies in the laboratory show different  
470 microbial C partitioning dynamics than in the field. Soil Biology and Biochemistry 41, 1951–  
471 1956.

472 Pethick, J. S. 2002. Estuarine and tidal wetland restoration in the United Kingdom: policy versus  
473 practice. Restoration Ecology 10, 431–437

474 Poffenbarger, H.J., Needelman, B.A., Megonigal, J.P. 2011. Salinity influence on methane emissions  
475 from tidal marshes. Wetlands 31, 831-842.

476 Pye, K. and French, P.W. 1993. Erosion and accretion processes on British saltmarshes. Volume 2.  
477 Database of British saltmarshes. Final report to MAFF, ES19B(2). London.

478 R Development Core Team. 2011. R: A language and environment for statistical computing. R  
479 Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL  
480 <http://www.R-project.org>

481 Santín, C., de la Rosa, J.M., Knicker, H., Otero, X.L., Álvarez, M.A., González-Vila, F.J. 2009. Effects of  
482 reclamation and regeneration processes on organic matter from estuarine soils and  
483 sediments. Organic Geochemistry 40, 931-941.

484 Simfukwe, P., Hill, P.W., Emmett, B.A., Jones, D.L., 2011. Soil classification provides a poor indicator  
485 of carbon turnover rates in soil. Soil Biology & Biochemistry 43, 1688-1696.

486 Shepherd, D., Burgess, D., Jickells, T., Andrews, J., Cave, R., Turner, R.K., Aldridge, J., Parker, E.R.,  
487 Young, E. 2007. Modelling the effects and economics of managed realignment on the cycling

488 and storage of nutrients, carbon and sediments in the Blackwater estuary UK. *Estuarine,*  
489 *Coastal and Shelf Science* 73, 355-367.

490 Smith, J.L., Doran, J.W. 1996. Measurement and use of pH and electrical conductivity for soil quality  
491 analysis. In: Doran, J.W., Jones, A.J. (Eds.), *Methods for Assessing Soil Quality*, Soil Sci. Soc. Am.  
492 Special Publication 49. Soil Science Society of America, Madison, WI.

493 Spencer, K.L., Cundy, A.B., Davies-Hearn, S., Hughes, R., Turner, S., MacLeod, C.L. 2008.  
494 Physicochemical changes in sediments at Orplands Farm, Essex, UK following 8 years of  
495 managed realignment. *Estuarine, Coastal and Shelf Science* 76, 608-619.

496 Strack, M., Waddington, J.M., Tuittila, E.S. 2004. Effect of water table drawdown on northern  
497 peatland methane dynamics: Implications for climate change. *Global Biogeochemical Cycles*  
498 18, GB4003.

499 UK Biodiversity Group, 1999. UK Biodiversity Group: Tranche 2, Action Plans. *Maritime Species and*  
500 *Habitats*, vol. V, English Nature, Peterborough.

501 US-EPA, 2005. Determination of total organic carbon and specific UV absorbance at 254 nm in  
502 source water and drinking water. Method 415/3. EPA Document #: EPA/600/R-05/055. US  
503 Environmental Protection Agency.

504 van Andel, J. 1998. Two approaches towards the relationship between plant species diversity and  
505 ecosystem functioning. *Applied Vegetation Science* 1, 9-14.

506 Wolters, M., Garbutt, A., Bakker, J.P. 2005. Salt-marsh restoration: evaluating the success of de-  
507 embankments in northwest Europe. *Biological Conservation* 123, 249–268.

508

509

510

511

512

513

514  
515  
516  
517  
518  
519  
520  
521  
522  
523  
524  
525  
526  
527  
528  
529  
530  
531  
532  
533  
534  
535  
536  
537  
538  
539

**Fig. 1.** Experimental design at the Tollesbury managed realignment site, adjacent natural marshes and arable land of the Blackwater Estuary, south-east England (51°46'N, 0°51'E). Each circle represents one sampling location where three replicates were taken. Within replicate distances were 10 m, and between sampling location distances were in the order of 150 m. Open circles = high marsh, closed circles = low marsh and grey circles = agricultural.

**Fig. 2.** Above and below ground biomass and calculated soil carbon pool measured at all 5 sites. Values represent means  $\pm$  standard deviation. The site effect was evaluated using a linear mixed effects model, the p value of which is displayed. Significant differences between site means are denoted by different letters.

**Fig. 3.** Ecosystem respiration ( $R_{eco}$ ) measured for all 5 sites. Values represent means  $\pm$  standard deviation. The site effect was evaluated using a linear mixed effects model, the p value of which is displayed. Significant differences between site means are denoted by different letters.

540 **Fig. 4.** Carbon mineralisation rates measured (as a % of total <sup>14</sup>C-substrate added) for all 5 sites.  
 541 Values represent means ± standard deviation. The site effect was evaluated using a linear mixed  
 542 effects model on the final data points (total evolution within incubation period of 25 days –  
 543 expressed as % of <sup>14</sup>C-substrate added to the soil), the *p* value of which is displayed. Significant  
 544 differences between site means are denoted by different letters.

545  
 546  
 547  
 548  
 549

550 **Table 1.** Soil properties measured at all 5 sites. Site means (n = 18) are presented ± standard  
 551 deviation. For bulk density n = 6. The site effect was evaluated using a linear mixed effects model.  
 552 Significant differences (*p* < 0.05) between site means are denoted by different letters.

553

	Agricultural		Restored		Natural		Restored		Natural	
			High		High		Low		Low	
pH	7.9 ± 0.5	<i>b</i>	7.2 ± 0.3	<i>c</i>	6.4 ± 0.2	<i>a</i>	7.6 ± 0.3	<i>bc</i>	7.8 ± 0.1	<i>b</i>
Soil conductivity (mS)	0.1 ± 0.1	<i>b</i>	4.4 ± 1.2	<i>c</i>	12.1 ± 2.7	<i>a</i>	5.9 ± 1.9	<i>c</i>	6.7 ± 0.6a	<i>b</i>
Total oxidised N (mg kg <sup>-1</sup> dry weight)	2.41 ± 1.28	<i>b</i>	0.08 ± 1.00	<i>a</i>	0.07 ± 0.07	<i>a</i>	0.11 ± 0.16	<i>a</i>	0.37 ± 0.27	<i>c</i>
Ammonium (mg kg <sup>-1</sup> dry weight)	0.06 ± 0.02	<i>b</i>	0.61 ± 0.58	<i>c</i>	0.92 ± 0.44	<i>a</i>	0.34 ± 0.25	<i>cd</i>	0.27 ± 0.19	<i>d</i>
Total inorganic N (mg kg <sup>-1</sup> dry weight)	2.46 ± 1.28	<i>a</i>	0.69 ± 0.57	<i>c</i>	0.99 ± 0.47	<i>ab</i>	0.46 ± 0.29	<i>c</i>	0.64 ± 0.32	<i>bc</i>
Bulk Density (g cm <sup>-3</sup> )	1.30 ± 0.13	<i>b</i>	0.84 ± 0.21	<i>c</i>	0.29 ± 0.03	<i>a</i>	0.42 ± 0.07	<i>a</i>	0.60 ± 0.03	<i>d</i>
Organic matter content (%)	3.8 ± 0.5	<i>b</i>	5.5 ± 1.2	<i>c</i>	21.8 ± 4.6	<i>a</i>	5.5 ± 1.3	<i>c</i>	6.0 ± 0.7	<i>c</i>
Humic substances (RAU cm <sup>-1</sup> )	0.90 ± 0.46	<i>b</i>	1.15 ± 0.85	<i>b</i>	0.33 ± 0.16	<i>a</i>	0.67 ± 1.07	<i>a</i>	0.15 ± 0.03	<i>c</i>
Sodium (g kg <sup>-1</sup> dry weight)	0.02 ± 0.01	<i>b</i>	4.18 ± 1.57	<i>c</i>	39.49 ± 10.63	<i>a</i>	7.59 ± 4.63	<i>e</i>	10.31 ± 1.87	<i>d</i>
Potassium (g kg <sup>-1</sup> dry weight)	0.02 ± 0.01	<i>b</i>	0.28 ± 0.09	<i>c</i>	1.75 ± 0.31	<i>a</i>	0.58 ± 0.29	<i>d</i>	0.64 ± 0.13	<i>d</i>
Calcium (g kg <sup>-1</sup> dry weight)	0.07 ± 0.05	<i>b</i>	0.24 ± 0.09	<i>c</i>	3.54 ± 0.87	<i>a</i>	0.50 ± 0.31	<i>c</i>	0.63 ± 0.08	<i>c</i>
Phosphate (mg kg <sup>-1</sup> dry weight)	3.42 ± 1.76	<i>b</i>	1.95 ± 0.35	<i>c</i>	1.02 ± 0.95	<i>a</i>	3.08 ± 1.14	<i>b</i>	3.38 ± 0.43	<i>b</i>

C (%)	1.7 ± 0.3 <sup>b</sup>	2.2 ± 0.4 <sup>b</sup>	9.7 ± 2.4 <sup>a</sup>	2.4 ± 0.7 <sup>b</sup>	2.2 ± 0.3 <sup>b</sup>
N (%)	0.17 ± 0.03 <sup>b</sup>	0.21 ± 0.03 <sup>c</sup>	0.72 ± 0.17 <sup>a</sup>	0.23 ± 0.05 <sup>c</sup>	0.25 ± 0.03 <sup>c</sup>
C/N ratio	10.0 ± 0.7 <sup>bc</sup>	10.5 ± 0.8 <sup>c</sup>	13.5 ± 0.7 <sup>a</sup>	10.0 ± 1.3 <sup>bc</sup>	8.7 ± 0.3 <sup>b</sup>

---

554

555

556

557

558

559 **Table 2.** Organic matter cycling measurements at all 5 sites. Gas flux site means (n = 12) are  
 560 presented  $\pm$  standard deviation. For carbon substrate mineralisation ( $^{14}\text{CO}_2$  evolution) n = 6. The site  
 561 effect was evaluated using a linear mixed effects model. Significant differences ( $p < 0.05$ ) between  
 562 site means are denoted by different letters. Non significant results are recorded as *ns* ( $p > 0.05$ ).  
 563

	Agricultural	Restored High	Natural High	Restored Low	Natural Low
$\text{CO}_2$ flux ( $\text{mg m}^{-2} \text{h}^{-1}$ )	$69.8 \pm 39.1$ <sup>a</sup>	$48.5 \pm 57.5$ <sup>a</sup>	$18.7 \pm 26.1$ <sup>a</sup>	$615.1 \pm 200.4$ <sup>c</sup>	$264.6 \pm 117.8$ <sup>b</sup>
$\text{CH}_4$ flux ( $\text{mg m}^{-2} \text{h}^{-1}$ )	$0.02 \pm 0.12$ <sup>ns</sup>	$-0.01 \pm 0.04$ <sup>ns</sup>	$-0.01 \pm 0.03$ <sup>ns</sup>	$0.08 \pm 0.08$ <sup>ns</sup>	$0.02 \pm 0.02$ <sup>ns</sup>
$\text{N}_2\text{O}$ flux ( $\text{mg m}^{-2} \text{h}^{-1}$ )	$-0.02 \pm 0.04$ <sup>ns</sup>	$0.00 \pm 0.06$ <sup>ns</sup>	$-0.02 \pm 0.06$ <sup>ns</sup>	$0.00 \pm 0.06$ <sup>ns</sup>	$-0.02 \pm 0.06$ <sup>ns</sup>
$^{14}\text{CO}_2$ evolution (% evolved of total $^{14}\text{C}$ -substrate added)	$24.2 \pm 1.2$ <sup>b</sup>	$14.6 \pm 2.2$ <sup>ac</sup>	$12.3 \pm 1.1$ <sup>a</sup>	$19.9 \pm 2.8$ <sup>d</sup>	$16.3 \pm 2.4$ <sup>c</sup>

564 **Supplementary table 1.** Soil properties measured at all 5 sites by depth. Individual depth means by site (n = 6) are presented  $\pm$  standard deviation. Dry wt  
 565 indicates dry weight.

566

Depth (cm)	Agricultural			Restored High			Natural High			Restored Low			Natural Low		
	0-10	10-20	20-30	0-10	10-20	20-30	0-10	10-20	20-30	0-10	10-20	20-30	0-10	10-20	20-30
pH	7.6 $\pm$ 0.3	7.8 $\pm$ 0.5	8.3 $\pm$ 0.2	7.0 $\pm$ 0.1	7.1 $\pm$ 0.2	7.4 $\pm$ 0.3	6.4 $\pm$ 0.2	6.4 $\pm$ 0.2	6.5 $\pm$ 0.3	7.4 $\pm$ 0.3	7.6 $\pm$ 0.3	7.7 $\pm$ 0.2	7.8 $\pm$ 0.1	7.8 $\pm$ 0.1	7.8 $\pm$ 0.2
Soil conductivity (mS)	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.0	5.6 $\pm$ 0.7	4.4 $\pm$ 0.8	3.2 $\pm$ 0.8	15.0 $\pm$ 1.1	11.8 $\pm$ 1.5	9.4 $\pm$ 1.4	8.3 $\pm$ 0.7	5.4 $\pm$ 0.6	4.1 $\pm$ 0.5	7.1 $\pm$ 0.4	6.8 $\pm$ 0.6	6.2 $\pm$ 0.4
Total oxidised N (mg kg <sup>-1</sup> dry wt)	2.43 $\pm$ 1.22	2.24 $\pm$ 1.47	2.62 $\pm$ 1.38	0.05 $\pm$ 0.02	0.11 $\pm$ 0.15	0.07 $\pm$ 0.09	0.13 $\pm$ 0.08	0.06 $\pm$ 0.05	0.03 $\pm$ 0.02	0.25 $\pm$ 0.23	0.05 $\pm$ 0.05	0.04 $\pm$ 0.02	0.65 $\pm$ 0.23	0.30 $\pm$ 0.11	0.15 $\pm$ 0.12
Ammonium (mg kg <sup>-1</sup> dry wt)	0.07 $\pm$ 0.03	0.05 $\pm$ 0.02	0.05 $\pm$ 0.02	0.38 $\pm$ 0.33	0.33 $\pm$ 0.18	1.13 $\pm$ 0.71	0.86 $\pm$ 0.27	0.98 $\pm$ 0.62	0.92 $\pm$ 0.44	0.45 $\pm$ 0.20	0.18 $\pm$ 0.05	0.40 $\pm$ 0.35	0.34 $\pm$ 0.15	0.17 $\pm$ 0.10	0.30 $\pm$ 0.27
Total inorganic N (mg kg <sup>-1</sup> dry wt)	2.50 $\pm$ 1.24	2.29 $\pm$ 1.47	2.67 $\pm$ 1.39	0.44 $\pm$ 0.33	0.44 $\pm$ 0.32	1.19 $\pm$ 0.66	0.99 $\pm$ 0.34	1.04 $\pm$ 0.65	0.95 $\pm$ 0.43	0.70 $\pm$ 0.14	0.23 $\pm$ 0.06	0.44 $\pm$ 0.35	0.99 $\pm$ 0.17	0.47 $\pm$ 0.18	0.45 $\pm$ 0.25
Organic matter content (%)	4.1 $\pm$ 0.3	3.9 $\pm$ 0.5	3.3 $\pm$ 0.6	6.6 $\pm$ 1.1	5.0 $\pm$ 0.4	4.8 $\pm$ 0.9	24.4 $\pm$ 3.4	23.2 $\pm$ 3.8	17.8 $\pm$ 3.9	6.7 $\pm$ 0.8	4.9 $\pm$ 1.0	4.8 $\pm$ 1.3	5.8 $\pm$ 0.5	6.2 $\pm$ 1.0	5.9 $\pm$ 0.4
Humic substances (RAU cm <sup>-1</sup> )	0.89 $\pm$ 0.36	1.04 $\pm$ 0.67	0.69 $\pm$ 0.10	0.51 $\pm$ 0.49	1.00 $\pm$ 0.70	1.92 $\pm$ 0.72	0.45 $\pm$ 0.16	0.30 $\pm$ 0.12	0.23 $\pm$ 0.12	0.16 $\pm$ 0.04	0.38 $\pm$ 0.16	1.47 $\pm$ 1.63	0.17 $\pm$ 0.01	0.14 $\pm$ 0.02	0.14 $\pm$ 0.03
Sodium (g kg <sup>-1</sup> dry wt)	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	5.53 $\pm$ 1.28	3.99 $\pm$ 1.34	3.01 $\pm$ 1.02	47.36 $\pm$ 10.66	39.90 $\pm$ 7.38	31.19 $\pm$ 7.65	13.23 $\pm$ 3.09	5.79 $\pm$ 1.57	3.76 $\pm$ 1.05	11.17 $\pm$ 1.55	10.44 $\pm$ 2.05	9.31 $\pm$ 1.76
Potassium (g kg <sup>-1</sup> dry wt)	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01	0.01 $\pm$ 0.00	0.36 $\pm$ 0.06	0.26 $\pm$ 0.07	0.21 $\pm$ 0.06	1.92 $\pm$ 0.35	1.66 $\pm$ 0.26	1.65 $\pm$ 0.27	0.88 $\pm$ 0.28	0.49 $\pm$ 0.13	0.36 $\pm$ 0.10	0.66 $\pm$ 0.04	0.65 $\pm$ 0.16	0.62 $\pm$ 0.16
Calcium	0.08 $\pm$ 0.07	0.06 $\pm$ 0.04	0.06 $\pm$ 0.03	0.33 $\pm$ 0.07	0.22 $\pm$ 0.05	0.16 $\pm$ 0.04	4.14 $\pm$ 0.77	3.57 $\pm$ 0.74	2.90 $\pm$ 0.71	0.89 $\pm$ 0.11	0.38 $\pm$ 0.12	0.22 $\pm$ 0.05	0.70 $\pm$ 0.06	0.62 $\pm$ 0.06	0.57 $\pm$ 0.06

(g kg<sup>-1</sup> dry wt)

Phosphate	2.95 ± 0.69	4.30 ± 2.64	2.81 ± 0.77	2.05 ± 0.45	1.75 ± 0.09	2.05 ± 0.38	0.91 ± 0.57	1.10 ± 1.27	1.05 ± 1.05	4.40 ± 0.84	2.63 ± 0.56	2.21 ± 0.38	3.35 ± 0.40	3.53 ± 0.37	3.27 ± 0.55
(mg kg <sup>-1</sup> dry wt)															
C	1.8 ± 0.3	1.8 ± 0.3	1.5 ± 0.5	2.6 ± 0.2	2.1 ± 0.3	1.9 ± 0.2	10.9 ± 1.1	11.2 ± 1.3	7.1 ± 2.1	2.7 ± 0.4	2.2 ± 0.6	2.2 ± 1.0	2.3 ± 0.1	2.2 ± 0.4	2.1 ± 0.4
N	0.18 ± 0.02	0.18 ± 0.02	0.15 ± 0.03	0.24 ± 0.02	0.20 ± 0.02	0.19 ± 0.01	0.81 ± 0.10	0.81 ± 0.11	0.54 ± 0.16	0.27 ± 0.04	0.22 ± 0.04	0.21 ± 0.06	0.26 ± 0.02	0.25 ± 0.03	0.24 ± 0.04
C:N	9.9 ± 0.5	10.1 ± 0.6	9.8 ± 1.3	11.0 ± 0.7	10.5 ± 0.8	10.0 ± 0.6	13.5 ± 0.7	13.8 ± 0.7	13.1 ± 0.6	9.8 ± 0.6	10.0 ± 1.2	10.1 ± 2.0	8.6 ± 0.3	8.9 ± 0.4	8.7 ± 0.3

---

567

568

569

570

571

572

573