



*Milled cereal straw accelerates earthworm (*Lumbricus terrestris*) growth more than selected organic amendments*

Article

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1 **Milled cereal straw accelerates earthworm (*Lumbricus terrestris*)**
2 **growth more than selected organic amendments**

3

4

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26 **Abstract**

27 Earthworms benefit agriculture by providing several ecosystem services. Therefore, strategies to
28 increase earthworm abundance and activity in agricultural soils should be identified, and encouraged.
29 *Lumbricus terrestris* earthworms primarily feed on organic inputs to soils but it is not known which
30 organic amendments are the most effective for increasing earthworm populations. We conducted
31 earthworm surveys in the field and carried out experiments in single-earthworm microcosms to
32 determine the optimum food source for increasing earthworm biomass using a selection of crop
33 residues and organic wastes available to agriculture. We found that although farmyard manure
34 increased earthworm populations more than cereal straw in the field, straw increased earthworm
35 biomass more than manures when milled and applied to microcosms. Earthworm growth rates were
36 positively correlated with the calorific value of the amendment and straw had a much higher calorific
37 value than farmyard manure, greenwaste compost, or anaerobic digestate. Reducing the particle size
38 of straw by milling to < 3 mm made the energy in the straw more accessible to earthworms. The
39 benefits and barriers to applying milled straw to arable soils in the field are discussed.

40

41 **Keywords** Earthworm, Straw, Manure, Food, Energy, Ecosystem service

42

43 **1. Introduction**

44 Earthworms are the most abundant animal, by biomass, in most soils (Lavelle and Spain, 2001) and
45 are responsible for providing numerous ecosystem services and functions (Blouin et al., 2013) that
46 benefit crop growth (Bertrand et al., 2015). Earthworms increase the rate of water infiltration
47 (Bouché and Al-Addan, 1997), the availability of nutrients (Devliegher and Verstraete, 1996), and can
48 increase crop yield by 25% (van Groenigen et al., 2014). Many agricultural practices such as tillage
49 (Chan, 2001), pesticide application (Pelosi et al., 2014), and the removal of crop residues (Karlen et
50 al., 1994) decrease the biomass and abundance of earthworm populations. Conversely, the addition of
51 organic amendments to soils increases earthworm populations in arable soils (Edwards and Lofty,
52 1982), even when tillage operations and pesticide applications are maintained (Blanchet et al., 2016;
53 Whalen et al., 1998).

54 Earthworm population dynamics can be explained by modelling the energy budgets of individuals
55 within a population and the interactions between individuals (Jager et al., 2006; Johnston et al., 2014a;
56 Johnston et al., 2014b). The models describe how individuals acquire and utilize energy, based on a
57 set of simple rules for metabolic organisation, treating individual earthworms as a system with a
58 closed mass and energy balance. Earthworms must reach a minimum mass to mature sexually and be
59 able to reproduce (Lofs-Holmin, 1983). The quantity of food supplied (assuming all else is equal)
60 also influences its reproduction rate because it converts food into offspring (Johnston et al., 2014b). It
61 is possible to reduce the time taken for earthworms to reach maturity and intensively rear earthworm
62 communities in laboratory cultures by optimising population density, temperature and moisture (Butt
63 et al., 1992; Lowe and Butt, 2007; Lowe and Butt, 2005). However, these parameters cannot be easily
64 manipulated in field populations.

65 The quality of food fed to laboratory reared earthworms affects earthworm biomass, time taken to
66 reach sexual maturity and cocoon production (Butt, 2011). There is also considerable evidence that
67 the abundance and biomass of earthworms in arable fields can be increased by the application of
68 organic amendments such as straw (Kennedy et al., 2013), poplar bark (Pérès et al., 1998) and cattle

69 slurry (Pommeresche and Løes, 2009). Reducing the particle size of organic amendments to < 2 mm
70 increases the growth rate of laboratory-reared earthworms (Boström and Lofs-Holmin, 1986; Lowe
71 and Butt, 2003). However, growth rate can differ to a large extent depending on the type of organic
72 amendment applied. For example, livestock manures increase earthworm populations more than
73 composts, reportedly because the organic carbon in the composts is more humified and stable due to
74 microbial degradation (Leroy et al., 2008). However, despite crop residues (e.g. cereal straw) being
75 less humified and less degraded by microorganisms at the time they are incorporated into the soil,
76 they do not seem to increase earthworm biomass to the same extent as livestock manures (Blanchet et
77 al., 2016).

78 In the UK, and many other nations, the availability of animal manures to cereal growers for land
79 application is limited because of the geographical distance between livestock and arable farms, as
80 evidenced by lower use of farmyard manure in the Eastern region (13% of crop and grass area),
81 compared to the South West region (41% of crop and grass area) (DEFRA, 2016). Therefore, we
82 investigated ways of increasing earthworm populations using cereal straw produced on most arable
83 farms and contemporary soil amendments that are becoming increasingly available in arable regions
84 (compost and anaerobic digestate). We hypothesised that earthworm biomass could be increased in
85 soils by manipulating the type(s) of organic amendment(s) applied and their particle size.

86

87 **2. Materials and Methods**

88 2.1. Field surveys

89 Earthworm surveys were carried out on two long term field experiments at Rothamsted Experimental
90 Farm near Harpenden, UK (51.813N, 0.381 E) during spring 2014. All 16 plots of the Long Term
91 Straw Incorporation Experiment, described by Powlson et al (2011) were surveyed. The experiment
92 has grown winter wheat continuously and had wheat straw incorporated annually for 28 years at a rate
93 of none, once, twice, and four times the yield of straw the previous year (approximately 0, 5, 10 and
94 20 t ha⁻¹) in a complete randomised block design (Table 1). A 2 m x 3 m area was designated

95 specifically for sampling on the southern end of each plot. Two earthworm surveys were conducted in
96 each plot (as described below), resulting in 32 surveys in total.

97 Selected plots on the Broadbalk experiment, described by Blair et al (2006), that have grown winter
98 wheat continuously for 171 years (apart from occasional fallow years) were also surveyed but, due to
99 the age of the experiment, treatments are not replicated. Surveys were conducted on four plots that
100 have either (i) received 35 t ha⁻¹ of farmyard manure annually for 171 years, (ii) received wheat straw
101 for the last 28 years by incorporating the straw of the previous crop harvested from the same plot
102 (approximately 5 t ha⁻¹), (iii) received both farmyard manure and wheat straw annually, as described
103 above, or (iv) received no manure or straw applications for at least 171 years. All plots received 144
104 kg N ha⁻¹ since 1852. A 1 m x 14 m area was designated specifically for sampling along the northern
105 edge of each plot and this area was divided into four equal sub-plots that are considered here
106 statistically as true replicates (Table 1). In each sub-plot two earthworm surveys were conducted,
107 resulting in 32 surveys in total.

108 Earthworm surveys were conducted by excavating a 20x20x20 cm cube of soil, bringing it back to the
109 on-site laboratory and sorting it to find all the earthworms and identify them following (Sherlock,
110 2012). Deep burrowing (anecic) earthworms were extracted by pouring a 5 L aqueous solution
111 containing 6 g l⁻¹ of Colman's mustard flour, following (Bartlett et al., 2008; Murchie and Gordon,
112 2013) into the excavated hole and waiting up to 1 hour to collect any emerging earthworms. All
113 earthworms were washed by submerging them in water, blotted dry, identified to the species level and
114 then its mass determined. All adults and some juveniles were identified but if the species of an
115 earthworm was unclear then it was classified as 'unidentified'.

116

117 **Table 1** An outline of the individual experiments conducted in this investigation.

Experiment	Field/Laboratory	No. of treatments	Factors	No. of replicates	No. of units
Long Term Straw Incorporation Experiment	Field	4	Straw rate 0, 5, 10 and 20 t ha ⁻¹	4	16
Broadbalk	Field	4	Organic matter type Farmyard manure, straw, mixture, nil	4 †	16
Microcosm experiment 1	Laboratory	65	Organic matter type Straw, farmyard manure, anaerobic digestate, compost Organic matter rate 0, 2, 4, 6 and 8 g C kg ⁻¹ soil Straw-manure mixtures	4	260
Microcosm experiment 2	Laboratory	11	Straw type Wheat straw, barley straw Straw rate 0, 2, 4, 6, 8 and 10 g kg ⁻¹ month ⁻¹	4	44
Microcosm experiment 3	Laboratory	17	Straw particle size <1 mm, <3 mm, 1 cm and chopped Straw rate 0, 2, 4, 6 and 8 g kg ⁻¹ month ⁻¹	4	68

118 † Subplots are considered here as true replicates

119 2.2. Microcosm experiments

120 2.2.1. Materials

121 A silty clay loam soil of the Batcombe Series (Avery and Catt, 1995), a Chromic Luvisol according to
122 FAO classification, was collected from Fosters field of Rothamsted Experimental Farm. Fosters field
123 has been in continuous arable production for more than 200 years. and has a soil organic carbon
124 content of 14.3 g kg⁻¹ (Johnston et al., 2009). The soil was air dried and sieved to < 2 mm.

125 Barley and wheat straw was also sourced from Rothamsted Experimental Farm. Farmyard manure
126 was obtained from a farm with a mixed single suckling beef herd that is housed inside during the
127 winter in bullock yards. Greenwaste compost was obtained from Organic Recycling Ltd. Anaerobic
128 digestate was obtained from Staples Vegetables Ltd. and comprises the fibre portion of a brassica
129 waste and maize-fed digester. All organic amendments were sampled shortly after delivery and air
130 dried prior to being milled, to the sizes described below, using a Christy Turner Lab Mill and a <
131 1mm sample analysed for N and C concentration using a LECO TruMac Combustion Analyser, and
132 for gross energy by Sciantec Analytical Services Ltd. using a PAR 6100Bomb Calorimeter..
133 Properties of the amendments used are given in Table 2 and can be seen in Figure 1.

134 **Table 2** Properties of soil amendments used in microcosm experiments.

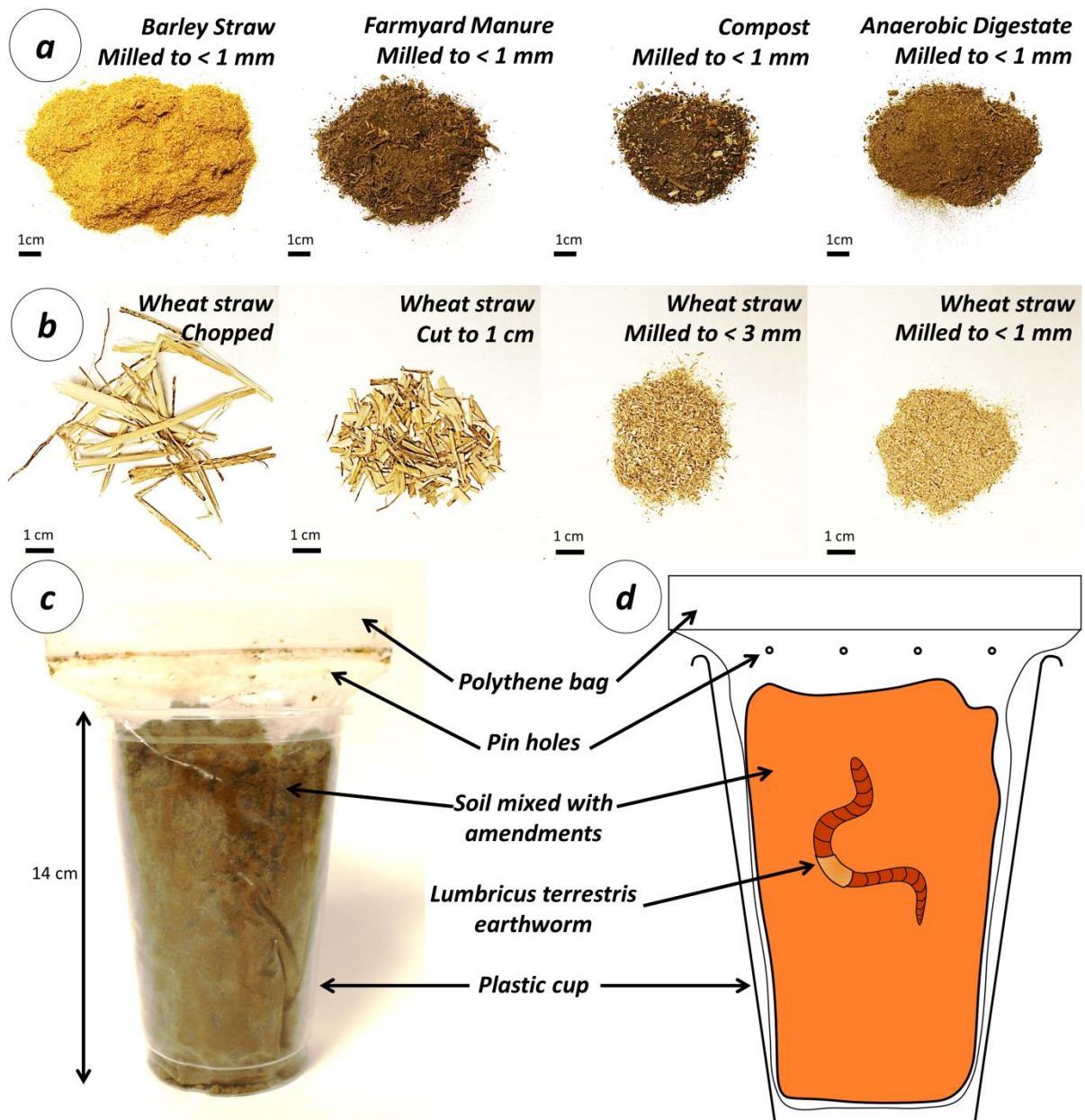
Soil amendment	%N	%C	C:N	Gross energy (kJ g ⁻¹)
Barley Straw	0.50 (0.003)	46 (0.09)	92	17.0
Farmyard Manure	2.7 (0.008)	31 (0.04)	11	12.5
Anaerobic Digestate	2.4 (0.013)	42 (0.23)	17	11.5
Compost	1.4 (0.022)	29 (0.88)	21	8.0
Wheat Straw	0.53 (0.003)	45 (0.10)	84	16.4

135 Mean of three replicate samples. Standard errors in brackets.

136

137 *L. terrestris* (anecic) earthworms were obtained commercially from wormsdirectuk.co.uk to ensure an
138 abundant supply of specimens of similar size and age. They were in good condition (i.e. well
139 hydrated), responsive (determined by assessing their response to a physical stimuli to the anterior),

140 were all clitellate, and had mean masses of 1.7 g (SD: 0.39, n = 372). Earthworms were equilibrated to
 141 our laboratory conditions, following Fründ et al., (2010), in a culture made from the same silty clay
 142 loam soil (Fosters field, Rothamsted) used in the experiments and fed with Irish Moss Peat, following
 143 Spurgeon et al., (2000) at approximately 1g earthworm⁻¹ week⁻¹ for more than one week prior to
 144 addition to experimental microcosms.



145
 146 **Figure 1** Amendments and experimental microcosms. Photographs (a) of barley straw, farmyard
 147 manure, compost and anaerobic digestate after grinding to < 1 mm and (b) wheat straw after
 148 chopping, cutting to 1 cm, milling to < 3 mm and milling to < 1 mm. Scale bars indicate 1 cm.
 149 Photograph (c) and schematic (d) of the experimental setup of microcosms for determining the effect
 150 of amendments on changes in earthworm biomass.

151 *2.2.2. Microcosm experimental design*

152 Experimental microcosms were constructed using polyethene bags and 1 pint (0.57 litre) plastic
153 drinking cups (Figure 1). Soil was wetted up to 70% of the water holding capacity and a treatment
154 applied, as described below, before 500g (dry wt.) of soil was added to each polythene bag. A pin was
155 used to perforate the top of each plastic bag to allow the circulation of air. The bag was placed in the
156 plastic drinking cup to ensure at least 10 cm depth of soil for the earthworms to burrow (Lowe and
157 Butt, 2005). The mass of a single earthworm was determined before it was added to each microcosm
158 at the start of the experiment. This stocking density is below the 3-5 adult worms l⁻¹ rate
159 recommended by Lowe and Butt (2005) so it is unlikely that the earthworms were stressed due to a
160 lack of space. Experimental microcosms were arranged in a complete randomised block design in a
161 controlled environment chamber, in constant darkness at 15°C. Earthworms were removed from the
162 microcosms by destructive sampling and thorough mixing of the soil every 2 weeks for the duration
163 of the experiment to ensure that the removal of each earthworm had an equal impact on the soil
164 structure and the position of the food in each microcosm. Earthworms were washed by submerging
165 them in deionised water, blotted dry, their mass determined, and then returned to the same microcosm.

166

167 *2.2.3. Microcosm experiment 1: Comparing amendments and straw-amendment mixtures*

168 Before earthworms were added to the experimental microcosms, soil was thoroughly mixed with five
169 rates of < 1mm milled farmyard manure, compost, or anaerobic digestate (Table 3), each relating to 0,
170 2, 4, 6 and 8 g C kg⁻¹ soil (13 treatments). Each of these 13 treatments was further amended and
171 thoroughly mixed with < 1mm milled straw at five rates, also relating to 0, 2, 4, 6 and 8 g C kg⁻¹ soil.
172 Each of the resulting 65 treatments was replicated four times comprising a total of 260 experimental
173 microcosms (Table 1). No further applications of organic amendments were made to the pots after this
174 initial addition. Every two weeks of the 12 week duration of the experiment the earthworms were
175 removed from the microcosms, their mass determined, and returned. The soil was homogenised each
176 time the earthworm was removed.

177 **Table 3** Rates of organic amendment applied in microcosm experiment 1.

Rate gC kg ⁻¹	Barley Straw g kg ⁻¹	Farmyard Manure g kg ⁻¹	Anaerobic Digestate g kg ⁻¹	Compost g kg ⁻¹
0	0	0	0	0
2	4.4	6.5	4.8	6.8
4	8.7	13.0	9.6	13.6
6	13.1	19.5	14.4	20.5
8	17.4	26.0	27.3	19.2

178

179 *2.2.4. Microcosm experiment 2: Comparing wheat and barley straw*

180 After earthworms were added to the experimental microcosms and had burrowed into the soil, the
181 microcosms were amended with six rates of either wheat or barley straw milled to < 1mm by adding
182 the straw to the surface of the pot. Every two weeks, when the earthworm was removed and its mass
183 determined, any straw remaining on the surface was mixed in with the soil and then, after the
184 earthworm was returned to the microcosm and burrowed into the soil, a new application was made to
185 the soil surface. Each straw was applied at a rate of 0, 2, 4, 6, 8 and 10 g kg⁻¹ month⁻¹, resulting in 11
186 treatments, and replicated four times, resulting in a total of 44 experimental microcosms (Table 1).
187 The experiment was continued for 10 weeks.

188

189

190 *2.2.5. Microcosm experiment 3: Comparing wheat straw particle size*

191 After the earthworm was added to the experimental microcosms and had burrowed into the soil, the
192 soil was amended with four rates of wheat straw that had either been (i) milled to < 1mm, (ii) milled
193 to < 3 mm, (iii) chopped to 1cm pieces using scissors, or (iv) been chopped with a bale chopper to
194 approximately 10 cm pieces, analogous to the chopping of straw behind a combine harvester. Straw
195 was applied every two weeks for 16 weeks, in the same manner as in Experiment 2 at rates of 0, 2, 4,
196 6 and 8 g kg⁻¹ month⁻¹, each replicated four times, resulting in 17 treatments and 68 experimental
197 microcosms (Table 1).

198 2.3. Statistical analysis

199 All statistical analysis was carried out in Genstat, version 16.2.0.11713. Analysis of Variance
200 (ANOVA) and Fisher's least significant difference test were employed to test significant differences
201 between treatments at a single time point. Repeated Measures ANOVA was used to discriminate
202 between treatments of microcosm experiments when data from all time points was included in the
203 analysis. In all cases normality was checked by inspecting the residual plots and homoscedasticity
204 confirmed using Bartlett's test ($P > 0.05$).

205

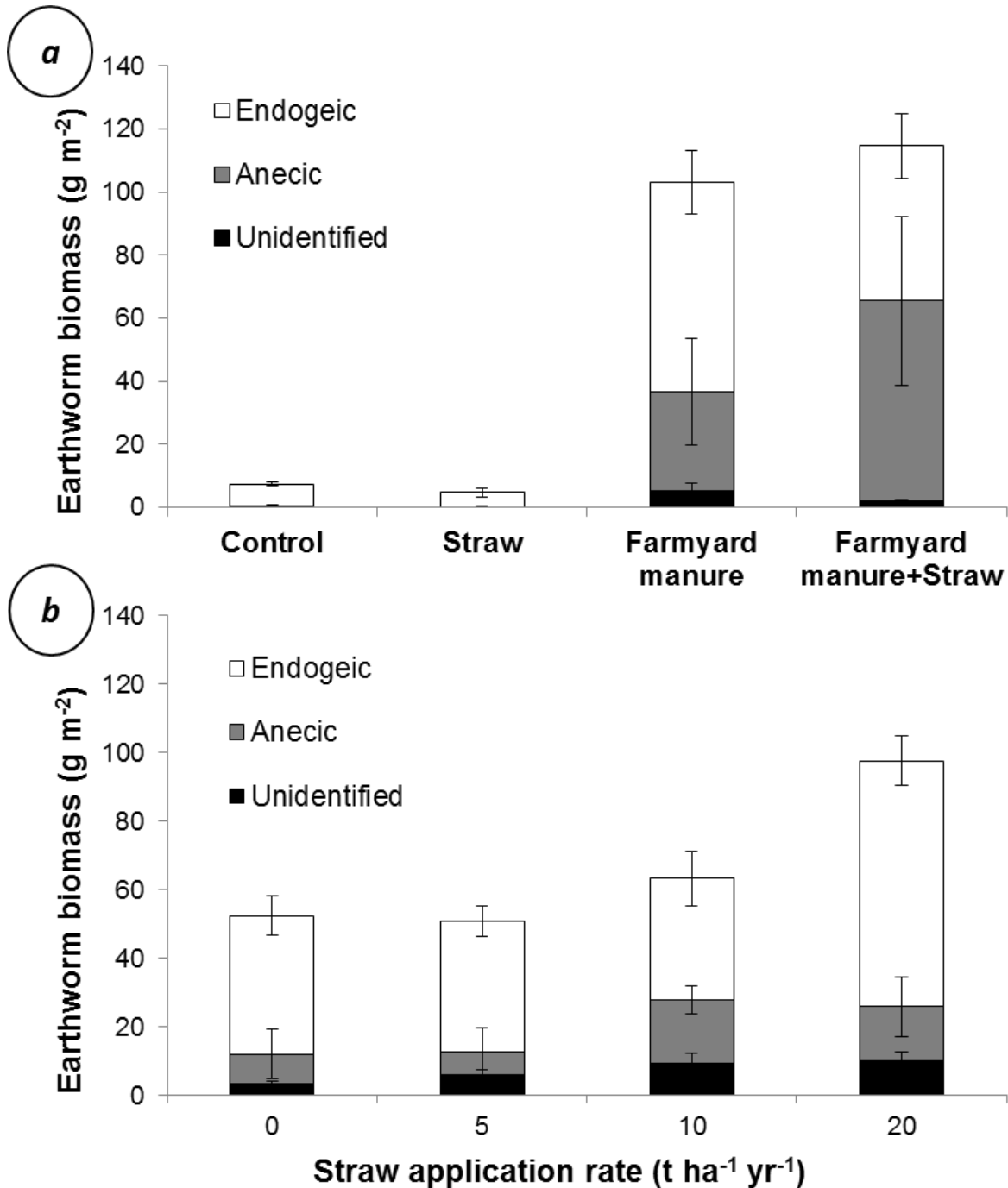
206 **3. Results**

207 3.1. Field surveys

208 Farmyard manure significantly ($p < 0.001$) increased the biomass of earthworms in the Broadbalk plots
209 (Figure 2a). This increase was due to a significantly greater biomass and number of endogeic
210 ($p < 0.001$), anecic ($p < 0.05$), mature ($p < 0.01$) and juvenile ($p < 0.01$) earthworms in the farmyard
211 manure treatments (see Table A1 and A2). Straw had no significant effect on the earthworm
212 population in the Broadbalk experiment and there were no significant interactions between straw and
213 farmyard manure on earthworm abundance or biomass.

214 Only the highest rate of straw application resulted in significantly ($p < 0.05$) greater earthworm
215 abundance and biomass (Figure 2b) of the Long Term Straw Incorporation Experiment and this was
216 reflected by a significantly ($p < 0.05$) greater abundance of both juvenile and mature earthworms (see
217 Table A3 and A4). This difference is largely due to a significantly greater number and biomass
218 ($p < 0.01$) of endogeic earthworms in the 20 t ha⁻¹ treatment. Although we found a significantly greater
219 number of anecic earthworms in the 10 t ha⁻¹ and 20 t ha⁻¹ treatments, compared to the 5 t ha⁻¹ and 0 t
220 ha⁻¹ plots, there was no significant difference in the biomass of anecic earthworms between any of the
221 treatments.

222 Because both earthworm surveys were conducted at different times, they cannot be compared with
 223 one another statistically since the results of earthworm surveys are highly dependent on the
 224 temperature and moisture of the soil (Eggleton et al., 2009)



225 **Figure 2** Biomass of endogeic, anecic, and unidentified earthworms determined by surveys of plots
 226 on (a) the Broadbalk field experiment and (b) the Long Term Straw Incorporation Experiment at
 227 Rothamsted Experimental Farm. Each bar is the average of four replicate plots or subplots with two
 228 pseudoreplicate surveys conducted per plot/subplot. Error bars are standard errors of the mean.
 229
 230

231 3.2. Microcosm experiments

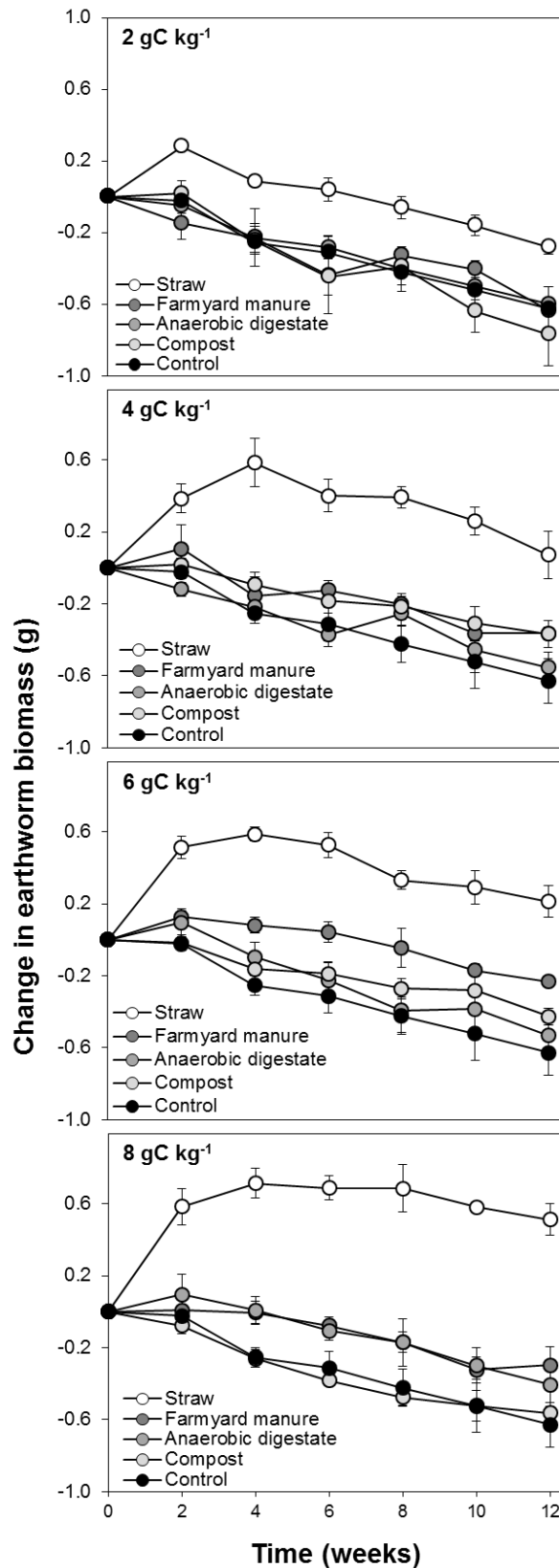
232 Across all three microcosm experiments there was a 92% survival rate over the duration of the
233 experiments (which ranged from 10 weeks to 16 weeks depending on the individual experiment). The
234 high survival rate indicates that the experimental conditions were suitable for culturing the
235 earthworms, even when starvation conditions were imposed in the control treatments. Units in which
236 mortality occurred were excluded from the dataset and treated as missing data during statistical
237 analysis.

238

239 *3.2.1. Microcosm experiment 1: Comparing amendments and straw-amendment mixtures*

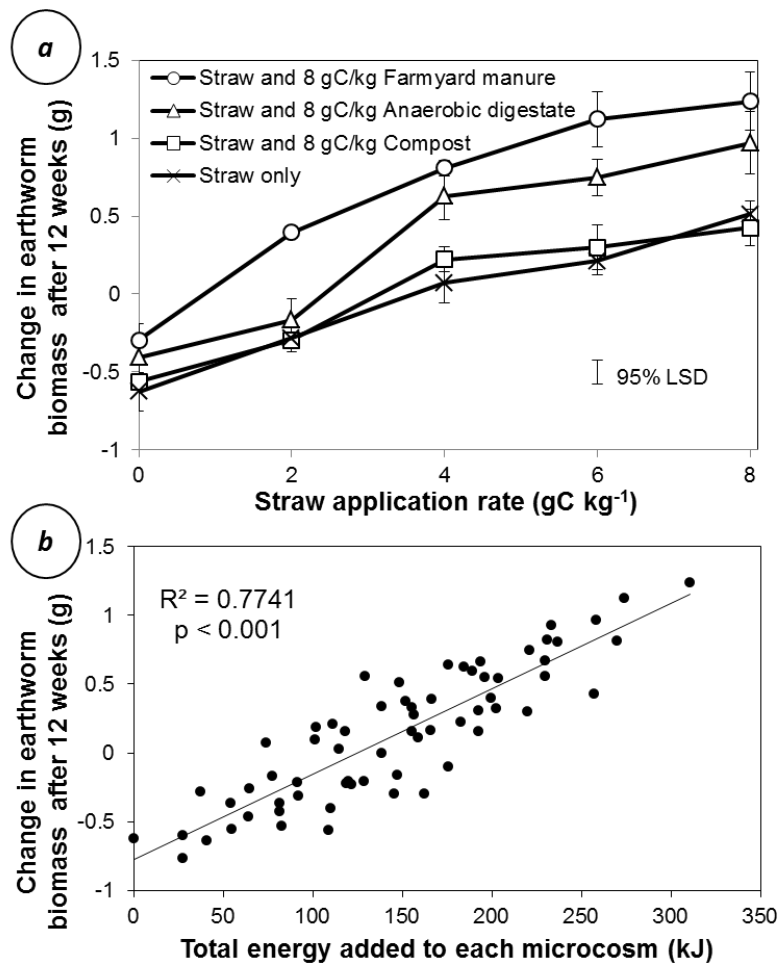
240 The change in earthworm biomass over the 12 week course of the experiment for all 65 treatments
241 treatment is presented in Figure A1 and displayed for selected treatments in Figure 3. The addition of
242 manures (farmyard manure, compost and anaerobic digestate: $P < 0.001$), the rate of manure
243 amendment ($P < 0.05$), and rate of straw amendment ($P < 0.001$), all significantly affected earthworm
244 biomass during the experiment, with high rates resulting in greater earthworm biomass. The
245 amendments increased earthworm biomass, relative to the unamended control, in the order straw >
246 farmyard manure > anaerobic digestate > compost (Figure 3).

247 Straw out-performed all of the other amendments, increasing earthworm biomass by 37% after 12
248 weeks at the rate of 8 g C kg^{-1} , compared to decreases of 17%, 23% and 28% for farmyard manure,
249 anaerobic digestate and compost, respectively (Figure 3). There was, however, a significant ($P <$
250 0.001) interaction between manure rate and straw rate. The positive impact of organic amendments
251 (particularly farmyard manure and anaerobic digestate) on earthworm biomass was greater when
252 applied in combination with straw (Figure 4a). We found a significant ($P < 0.001$) positive correlation
253 between the quantity of energy added to the soil within the organic amendments and the resulting
254 change in earthworm biomass over the 12 week duration of the experiment (Figure 4b) which was
255 stronger ($R^2 = 0.77$) than the relationship between %C and change in earthworm biomass ($R^2 = 0.66$).



256

257 **Figure 3** Change in the biomass of *Lumbricus terrestris* earthworms over the course of a 12 week.
 258 Either no food (i.e. control treatments), straw, farmyard manure, anaerobic digestate, or compost was
 259 added to each microcosm at the start of the experiment at a rate equivalent to 2, 4, 6 and 8 g C kg⁻¹.
 260 Each data point is the mean of four replicates. Error bars are standard errors of the mean.



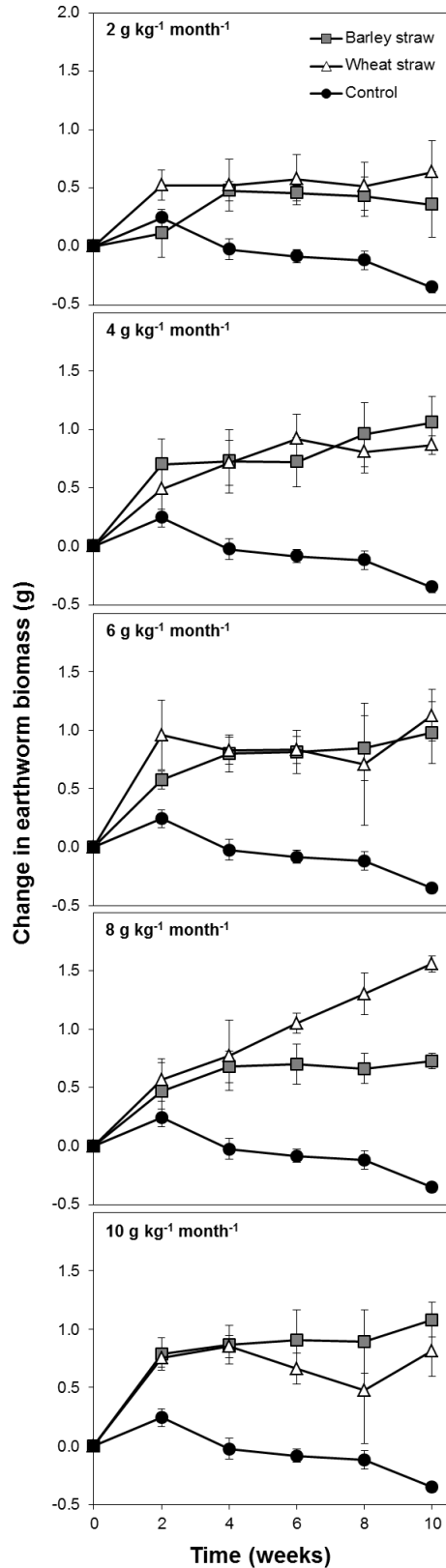
261

262 **Figure 4** Change in the biomass of *Lumbricus terrestris* earthworms over the course of a 12 week
 263 experiment where barley straw and organic amendments (farmyard manure, anaerobic digestate and
 264 compost) were added individually and in combination at rates equivalent to 0, 2, 4, 6 and 8 g C kg⁻¹.
 265 The figure demonstrates (a) the significantly greater change in biomass resulting from farmyard
 266 manure and anaerobic digestate applications to earthworms already receiving straw, and (b) the
 267 significant positive relationship between the energy of amendments fed to each earthworm and the
 268 change in earthworm biomass. Each data point is the mean of four replicates. Error bars are standard
 269 errors of the mean.

270

271 3.2.2. Microcosm experiment 2: Comparing wheat and barley straw

272 The addition of either barley or wheat straw significantly ($p < 0.001$) increased the biomass of
 273 earthworms in the experimental microcosms and earthworm biomass was significantly ($p < 0.05$)
 274 greater when higher rates of straw were applied. However, there was no significant difference in the
 275 change in earthworm biomass due to the type of straw applied to the soil. Since the energy contents of
 276 these two types of straw are similar (barley straw has 17.0 and wheat straw has 16.4 kJ g⁻¹: Table 2) it
 277 seems that the energy in each straw is equally accessible to the earthworms.



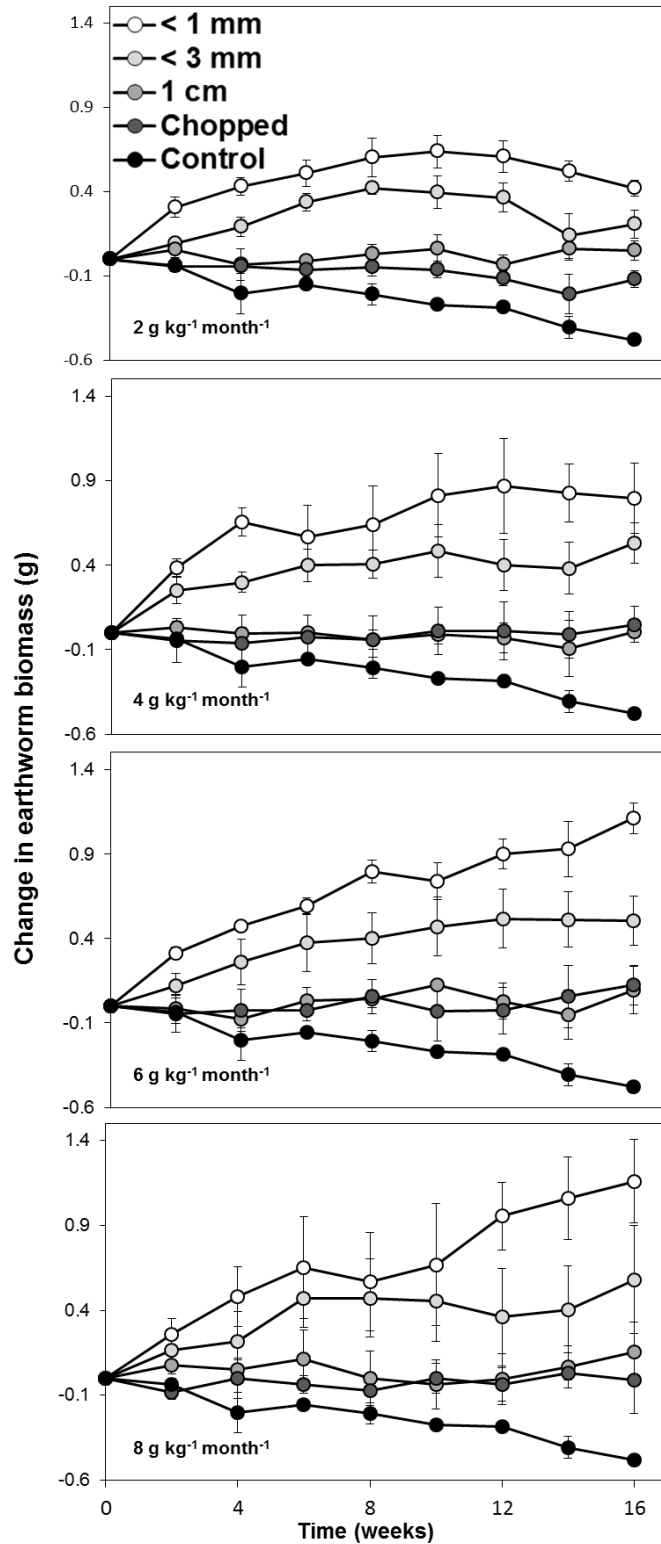
278

279 **Figure 5** Change in the biomass of *Lumbricus terrestris* earthworms over the course of a 10 week
 280 microcosm experiment are receiving no food (i.e. control treatments), wheat straw or barley straw at a
 281 rate of 2, 4, 6, 8 or 10 g kg⁻¹ week⁻¹ applied to the surface of the microcosm. Each data point is the
 282 mean of four replicates. Error bars are standard errors of the mean.

283 3.2.3. *Microcosm experiment 3: Comparing wheat straw particle size*

284 The presence ($p < 0.001$), rate ($p < 0.05$), and particle size ($p < 0.001$) of straw all significantly affected
285 the change in earthworm biomass over the 16 week duration of the experiment (Figure 6). After 16
286 weeks, the change in earthworm biomass in the chopped straw or 1 cm straw treatments was
287 significantly ($p < 0.05$) greater than the control treatments, which saw a decrease in biomass of
288 approximately 0.5 g per earthworm. However, the increase in earthworm biomass due to applying
289 straw cut to 1 cm pieces was not significantly ($p > 0.05$) different to the increase due to the straw
290 chopped with a bale chopper. Milling the straw to < 3 mm particles increased earthworm biomass by
291 17%, 29%, 36% and 42% when applied at rates of 2, 4, 6 and 8 g kg⁻¹ month⁻¹, respectively. These
292 increases were significantly ($p < 0.05$) greater than those observed in treatments where straw was cut to
293 1 cm (4%, 1%, 7% and 11%) or chopped with the bale chopper (-7%, 6%, 8% and 3%), when applied
294 at rates of 2, 4, 6 and 8 g kg⁻¹ month⁻¹. Milling to < 1 mm particles significantly increased the
295 earthworm biomass by 31%, 50%, 89% and 81% when applied at rates of 2, 4, 6 and 8 g kg⁻¹ month⁻¹,
296 respectively. These increases in earthworm biomass were significantly ($p < 0.05$) greater than bale
297 chopping or 1 cm cutting at all rates and significantly ($p < 0.05$) greater than milling to < 3 mm at rates
298 of 6 and 8 g kg⁻¹ month⁻¹.

299



300

301 **Figure 6** Change in the biomass of *Lumbricus terrestris* earthworms over the course of a 16 week
 302 microcosm experiment are receiving no food (i.e. control treatments) or wheat straw with particle size
 303 < 1mm, < 3 mm, 1cm or chopped to pieces approximately 10 cm in length applied to the surface of
 304 microcosms every two weeks at a rate equivalent to 2, 4, 6 or 8 g kg⁻¹ month⁻¹. Each data point is the
 305 mean of four replicates. Error bars are standard errors of the mean.

306

307 **4. Discussion**

308 4.1. *L. terrestris* growth depends on energy content of amendments

309 We found that straw increased the growth rate of *L. terrestris* to a greater extent than organic manures
310 in the laboratory (Figure 3). Growth rates could be explained by a positive correlation between the
311 total energy content of a soil amendment and the change in earthworm biomass (Figure 4b). This
312 correlation is a strong indication that (when all food is ground to the same size and therefore
313 accessible to *L. terrestris*) the calorific value of food is an important factor concerning the growth rate
314 of earthworms. This assertion is supported by observations of laboratory-reared compost earthworms
315 that the nutritional benefits of food is only supplied by cellular mass, that earthworm growth and
316 survival cannot be supported by nutrients alone (Neuhauser et al., 1980), and that paper sludge is a
317 better food source for earthworms than horse manure (Fayolle et al., 1997).

318 For all the organic manures used in our experiments (farmyard manure, compost and anaerobic
319 digestate), organisms had already partially used the substrate as an energy source prior to addition to
320 the soil: The manure has passed through the gut of a cow and both the compost and the anaerobic
321 digestate have been metabolised by thermophilic microorganisms under aerobic and anaerobic
322 conditions, respectively. During each of these processes energy is used by the organisms in question
323 (and, in the case of anaerobic digestion by burning the biogas produced). In each case much of the
324 labile energy (i.e. the compounds that are easiest to metabolise) will have been used first. What was
325 left in the final product that was added to the microcosms in this experiment contained less energy and
326 proportionally more recalcitrant energy than the plant material used to generate the manure, compost
327 or digestate. Therefore, even if all the food supplied to the earthworms is accessible (i.e. small enough
328 to ingest), not all of the energy in the food can be metabolised quickly.

329 The lability of the energy in an amendment depends, not only on the particle size (physical
330 availability), but also on the chemical composition of the substrate (chemical availability). Materials
331 that have a high cellulose/lignin ratio contain more labile energy than materials that have a low
332 cellulose/lignin ratio (McKendry, 2002). Earthworms can produce endogenous cellulase in their gut

333 (Nozaki et al., 2009), which may be responsible for much of the straw degradation, and subsequent
334 increase in *L. terrestris* biomass, observed in our microcosm experiments.

335

336 4.2. Organic manures support larger earthworm populations in the field than straw, but straw
337 contains more energy

338 Cereal straw applied to the field plots at a rate commensurate with standard farm practice (~5 t ha⁻¹ yr⁻¹)
339 had no significant impact on the size of the earthworm population in the Broadbalk experiment and
340 the Long Term Straw Incorporation experiment, even when applications were made annually for
341 decades (Figure 2). The observations agree with those of Eriksen-Hamel et al. (2009) who observe no
342 effect of crop residue management on earthworm populations and Stroud et al. (2017) who observe no
343 effect of cover cropping on *L. terrestris* midden abundance. Tian et al. (1993) observed greater
344 earthworm populations when crop residues were surface applied to soils in the humid tropics but
345 populations were negatively correlated with the lignin:nitrogen ratio of the residues, which indicates
346 that the earthworms gain more nutrition from easily digestible residues.

347 In the Long Term Straw Incorporation experiment only annual applications of wheat straw that were
348 four times the rates harvested (~20 t ha⁻¹ yr⁻¹) resulted in an increase (86%) in earthworm biomass
349 whereas the annual application of 35 t ha⁻¹ of farmyard manure increased the earthworm biomass by
350 1290% on the Broadbalk experiment. Assuming 25% dry matter (Powlson et al., 2012) and an energy
351 content of 12.5 kJ g⁻¹ (Table 2), 35 t ha⁻¹ farmyard manure provides approximately 109 GJ ha⁻¹ of
352 energy to the soil, whereas 20 ha⁻¹ of wheat straw provides approximately 279 GJ ha⁻¹, assuming 85%
353 dry matter (Powlson et al., 2008) and an energy content of 16.4 kJ g⁻¹ (Table 2). Our field
354 observations indicate that although the long term incorporation of very high quantities of straw is
355 capable of increasing earthworm populations, application rates commensurate to standard farm
356 practice do not appear to have any impact on the size of the earthworm community and that, per kJ
357 added to the soil, farmyard manure applications are a more efficient way of stimulating earthworm
358 growth.

359 4.3. Organic manure/straw mixtures reveal a synergistic interaction in microcosm experiments, but
360 not under field conditions

361 We show (Figure 4a) that the combination of straw with manures (farmyard manure and anaerobic
362 digestate) resulted in the farmyard manure and anaerobic digestate increasing *L. terrestris* biomass
363 more than when manures were applied without straw. This synergistic interaction could occur due to
364 both the straw and manure containing compounds or elements that only provide a benefit to growth
365 when ingested together. Alternatively, the presence of a mixture of amendments may have accelerated
366 the rate of microbial decomposition and thus increased the lability of the energy in the amendments to
367 the earthworm, based on the idea that a greater diversity of organic inputs to soils accelerates residue
368 decomposition (Cong et al., 2015; McDaniel et al., 2014). Despite this significant interaction between
369 crop residues and manures in microcosms, these interactions could not be confirmed in the field.
370 Although we found a greater earthworm biomass in the plot of the Broadbalk field experiment that
371 received both straw and farmyard manure, compared to the manure-only plot (Figure 2a), this
372 interaction was not statistically significant.

373

374 4.4. Milling straw appears to result in a more accessible energy source for earthworms

375 Although there were no significant differences in *L. terrestris* growth in treatments where straw was
376 chopped to 1 cm pieces and treatments in which straw was chopped to ~10 cm stalks, milling the straw
377 to <3 mm did accelerate growth, and this growth rate was further increased by milling to <1 mm
378 (Figure 6). The beneficial effect of reducing the particle size of food for earthworm consumption on
379 growth rate has been observed in both organic manures (Lowe and Butt, 2003) and crop residues
380 (Boström and Lofs-Holmin, 1986). Lowe and Butt (2003) showed that the milling of separated cattle
381 solids to < 1 mm increased the mass of *Allolobophora chlorotica* and *L. terrestris* compared to
382 unmilled controls by 185 and 54%, respectively after 18 weeks incubation. Boström and Lofs-Holmin
383 (1986) showed that reducing the size of barley straw and roots from 10 mm to 0.2-1 mm resulted in
384 increases in the growth rate of *Aporrectodea caliginosa*, and that a further reduction to < 0.2 mm
385 resulted in even greater growth rates. Our field observations indicate that earthworms are seemingly

386 unable to ingest straw applied to the soil as long stalks and were thus unable to access the majority of
387 the calories in this food source directly. Therefore, we hypothesise that the incorporation of crop
388 residues with smaller particle size may directly result in a short-term increase in the biomass of *L.*
389 *terrestris* in the field.

390 Whalen and Parmelee (1999) recorded *L. terrestris* growth rates to be much lower in the field,
391 compared to the laboratory, despite similar moisture and temperature conditions. Since the food
392 supplied to their laboratory-reared earthworms was first crushed into 2 cm fragments (Whalen and
393 Parmelee, 1999), this may have resulted in particle sizes that *L. terrestris* was able to ingest. Eriksen-
394 Hamel et al. (2009) noted that the incorporation of corn or barley residues in a sandy or clayey soil,
395 respectively, did not significantly affect earthworm biomass in the field. However, when intact soil
396 cores from these field plots were brought into the laboratory, the plots that were subjected to
397 minimum tillage operations (harrowing or chisel ploughing) resulted in the greatest earthworm
398 biomass response to residue application, compared to cores from conventional tillage (moldboard
399 plough/disk harrow) or no tillage plots. The authors suggest that the minimum tillage operations may
400 have reduced the particle size of the residues and made them more palatable to earthworms. Minimum
401 tillage operations also mix straw with soils and provide better substrate distribution in the top few
402 centimetres of the soil compared to ploughing, which buries a mat of straw at depth and is associated
403 with reductions in anecic earthworm biomass (Chan, 2001).

404

405 4.5. Reducing the particle size of straw applied to soil in the field may increase *L. terrestris* 406 populations

407 Approximately 850 Tg of wheat residues alone are produced every year, globally (Talebnia et al.,
408 2010) which represents a considerable energy resource (3872 TWh: more than the entire UK annual
409 energy consumption) and our data indicates that applying these residues to the soil has little impact on
410 the populations of earthworms, an important soil ecosystem engineer. The long-term addition of straw
411 to the soil is however, linked to increased levels of labile C which in turn is correlated with increase
412 aggregate stability and water infiltration (Blair et al., 2006). While we have demonstrated that milling

413 crop residues and applying them to soils in the laboratory does seem to considerably increase the
414 growth rates of *L. terrestris* reared in microcosms, there are several barriers to applying this
415 knowledge in the field to increase earthworm populations in arable soils.

416 Milling straw requires a significant input of energy and thus has a financial cost associated with it.
417 Mani et al., (2004) compared the energy required to mill barley and wheat straw using a hammer mill
418 and found that while they were similar, wheat straw required slightly less energy, which is consistent
419 with our anecdotal observations that wheat straw appears to be more brittle. Considering that we
420 observed no significant difference between the barley straw and wheat straw on the growth rate of *L.*
421 *terrestris* (Figure 5), and that the total energy content of both straws was similar (Table 2), we
422 propose that either residue is a suitable candidate for field applications. Based on an application rate
423 of 5 t ha⁻¹ and an energy requirement of 37 kWh t⁻¹ to mill wheat straw at 8.3% moisture content
424 through a 1.6 mm screen (Mani et al., 2004), the energy investment to mill all the wheat straw
425 harvested from a field would be approximately 185 kWh ha⁻¹, or 666 MJ ha⁻¹. This value compares
426 with an estimated 100 – 1000 MJ ha⁻¹ used to plough arable soils (Bailey et al., 2003; Patterson et al.,
427 1980). If the surface application of straw reduced to < 1.6 mm by a hammer mill (perhaps attached to
428 a combine harvester) increased earthworm populations to the extent that their activities negated
429 mechanical cultivations due to their beneficial soil biological engineering (Bender et al., 2016) then
430 crops of similar yield could potentially be grown with a lower input of energy and labour.

431 Although our laboratory experiments have revealed that milling crop residues can result in rapid
432 accelerations in growth rate of individual *L. terrestris* earthworms in microcosms containing a single
433 macroinvertebrate, it will be difficult to sustain this level of growth in the field because the milled
434 residues have a higher surface area and will likely be metabolised by the entire soil biological
435 community much more quickly than chopped straw. It may therefore be appropriate to apply milled
436 straw to the field in staged applications throughout the year; applying greater quantities when
437 earthworms are most active. Returning milled residues with multiple applications would likely
438 increase the energy expended and may increase soil compaction by increasing the number of tractor
439 passes. Our future experiments will focus on determining whether staged applications of milled straw

440 can increase earthworm populations in the field and whether this practice can sustainably be
441 incorporated into arable agricultural practice.

442

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