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1 **Title: Substrate quality and not dominant plant community determines the vertical**  
2 **distribution and C assimilation of enchytraeids in peatlands**

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22

23 **Abstract**

- 24 1. Peatlands are important carbon (C) stores as a result of acidity, waterlogging  
25 conditions and low temperatures slowing decomposition rates. However, climate  
26 change is predicted to bring not only changes in abiotic conditions, but also the  
27 replacement of peat-forming vegetation, such as *Sphagnum* mosses, with  
28 vascular plants. In these systems, enchytraeid worms represent the dominant  
29 mesofaunal group, and previous climate change manipulations have shown that  
30 their abundances and vertical distribution are strongly influenced by temperature  
31 and moisture gradients, with important implications for C cycling. Therefore,  
32 determining their behavioural responses to changes in both abiotic and biotic  
33 factors is crucial to quantify their contribution to decomposition processes.
- 34 2. We investigated the importance of vertical edaphic gradients (soil moisture and  
35 labile C) on enchytraeids total numbers, vertical distribution and feeding activities  
36 by inverting intact soil cores and hence, reversing both substrate quality but  
37 leaving the microclimatic gradients intact. This manipulative experiment was set-  
38 up in two different peatland areas, one dominated by heather (*Erica mackaiana*)  
39 and another by mosses (*Sphagnum* sp.) to determine the influence of different  
40 plant functional growth forms on these responses.
- 41 3. Our results showed that most enchytraeid species were “stayers” (i.e. in the  
42 “Upturned cores”, they remained in the same layer where they are “Normally”  
43 located), and hence, their vertical distribution was more dependent on substrate  
44 quality than a change in microclimate. This response was more evident under  
45 drier conditions (i.e. the highest locations of the height gradient at the heather  
46 site) than at the wettest locations (i.e. the bottom of the slope and under mosses)  
47 due to more pronounced vertical gradients in substrate quality. Furthermore,  
48 radiocarbon measurements on enchytraeid tissues showed that to avoid

49 competition among the growing populations for the limited labile resources  
50 present in the top layers, enchytraeids selected older C sources.

51 4. These findings indicate that future climate change scenarios will not only bring  
52 important changes in the abiotic conditions (temperature and moisture) and biotic  
53 properties of peatlands (both vegetation composition and below-ground soil biota  
54 community structure and vertical distribution), but also alterations in the feeding  
55 preferences of key decomposers that could result in the mobilisation of previously  
56 unavailable C pools.

57 **KEYWORDS:** carbon storage, climate change, mesofauna, *Sphagnum* moss, vascular  
58 plants, vertical stratification

59

## 60 **1. Introduction**

61 In the northern hemisphere, peatlands occur in cold, acidic and water saturated  
62 environments that pose strong limitations to heterotrophic communities. Not only do low  
63 temperatures decrease reproduction rates of mesofauna (Briones, Ineson, & Pearce,  
64 1997), but acidity and waterlogging conditions are also the main factors responsible for  
65 the accumulation of phenolic compounds that inhibit microbial decomposing activities  
66 (Freeman, Ostle, & Kang, 2001; Mellegaård, Stalheim, Hormazabal, Granum, & Hardy,  
67 2009). However, more frequent warmer and drier spells, as predicted in several climate  
68 change scenarios, will result in these vulnerable systems retreating (e.g. Gallego-Sala  
69 et al., 2010; Crowther et al., 2016), in particular in lower latitudes where higher  
70 decomposition rates will not be fully compensated by increased photosynthetic activities  
71 (Gallego-Sala et al., 2018). Increased peat aeration that promotes aerobic  
72 decomposition, and the elimination of the enzymatic latch restricting the degradation of  
73 phenolic compounds (Freeman et al., 2001) are expected to cause their capacity to store  
74 carbon to become gradually reduced (Wu & Roulet, 2014).

75 In addition to alterations in the abiotic conditions, warmer and drier climates are also  
76 expected to alter plant community composition by displacing peat-forming vegetation,  
77 such as *Sphagnum* mosses, with ericaceous shrubs (Breeuwer et al., 2009). Shrub  
78 encroachment will not only affect plant cover composition but also soil biota activities by  
79 modifying the quality of the organic matter inputs (Ward et al. 2015). However, the effects  
80 of shrub encroachment on peat decomposition are still under debate, with several studies  
81 reporting both positive and negative effects on C storage. Thus, while some studies have  
82 shown that shrub expansion will lead to C losses (Hoorens, Stroetenga, & Aerts, 2010;  
83 DeMarco, Mack, & Bret-Harte, 2014; Walker et al., 2016), others indicated increased  
84 decomposition rates when shrubs are removed (Ward et al., 2013, 2015).

85 Because the position of the water table determines the activities of aerobic organisms,  
86 and the deeper layers of the peat contain lower amounts of labile carbon, soil  
87 communities tend to be concentrated in the surface layers. Consequently, changes in  
88 soil moisture conditions and nutrient quality alter their abundances and vertical  
89 distribution (Briones, Ostle, McNamara, & Poskitt, 2009; Krab et al., 2010; Andersen,  
90 Chapman & Artz, 2013; Ward et al., 2015; Ellers et al., 2018), with important effects on  
91 decomposition processes. For example, Ward et al. (2015) showed that shrub removal  
92 had cascading effects on soil fungal community composition, increased mesofauna  
93 abundance, and increased rates of N mineralization. Therefore, the effects of shrub  
94 encroachment on ecosystem functioning will strongly depend on the responses of soil  
95 organisms to the new environmental conditions.

96 In addition to altered soil organic matter decomposition rates, the expansion of  
97 vascular plants (including shrubs) in peatlands has been shown to promote the release  
98 of ancient carbon due to the positive effect of microbial priming on ecosystem respiration  
99 (Walker et al., 2016). This could represent an indication that previously stable carbon  
100 pools could become destabilised in response to changes in above-ground plant  
101 communities. In these systems, enchytraeids are the dominant mesofaunal group in

102 terms of biomass and they play a crucial role in C transformations by stimulating the  
103 breakdown of organic compounds and the release of CO<sub>2</sub> and DOC in peatlands  
104 (Briones, Ineson, & Poskitt, 1998; Cole, Bardgett, Ineson, & Adamson, 2002; Briones,  
105 Poskitt, & Ostle, 2004; Carrera, Barreal, Gallego, & Briones, 2009; Carrera, Barreal,  
106 Rodeiro, & Briones, 2011). In addition, it has been shown that at least some species are  
107 able to migrate downwards in response to drier conditions in the upper layers (Briones  
108 et al., 1997). This colonisation of the deeper layers has important implications for C  
109 turnover since greater amounts of older C are assimilated and released, as a  
110 consequence of their increased presence in the more humified layers (Briones, Ostle, &  
111 Garnett, 2007; Briones, Garnett, & Ineson, 2010). These findings provide clear evidence  
112 that abiotic and biotic changes could result in profound alterations in both the activities  
113 of the heterotrophic communities and the nature of the C pools to be mobilised.

114 Therefore, in this study, we intended to determine the relative contributions of  
115 moisture and substrate quality factors on the vertical movements of enchytraeids, and  
116 whether these responses could be linked to changes in plant communities. We used the  
117 same experimental approach developed by Krab et al. (2010) and turned peat cores with  
118 intact vegetation (either dwarf shrubs or mosses) upside down. This reverses the  
119 substrate quality gradient, but leaves temperature and moisture gradients intact, i.e. it  
120 exposes the layers with lower quality substrates to the same abiotic conditions that are  
121 normally experienced by the top young layers. By giving the opportunity to the soil  
122 organisms to re-establish their “original” vertical pattern or to stay where they are  
123 (“movers” and “stayers”, respectively, according to Krab et al.’s terminology), it is  
124 possible to determine if direction of their migration is mainly driven by microclimate or  
125 substrate quality changes.

126 We hypothesised that vertical distribution of enchytraeids is primarily controlled by  
127 moisture conditions (Briones et al., 1997), whereas the quality of the organic matter will  
128 have a more important effect on their population sizes (i.e. less nutritious food will sustain

129 smaller population sizes; Briones et al., 2010). Consequently, we anticipated higher  
130 mesofaunal abundances under mosses than under shrubs (Ward et al., 2013; Juan-  
131 Ovejero et al., 2019), and also under more oxic conditions at the upper layers of both  
132 normally orientated and upturned cores; Krab et al., 2010). Under these assumptions,  
133 we also expected a greater assimilation of recently deposited C sources (i.e. labile C)  
134 from the top layers of both orientation treatments (i.e. in their original “normally  
135 orientated” position, and when turned upside down (“upturned”); Krab et al., 2010) under  
136 the wetter environments provided by *Sphagnum*, but a greater consumption of older C  
137 substrates in the case of the manipulation treatments under shrubs. This would be a  
138 consequence of the drier conditions where these woody plants proliferate, and the lower  
139 quality of the litter (Bragazza, Parisod, Buttler, & Bardgett, 2013; Ward et al., 2013),  
140 which will force these organisms to access different C sources in order to sustain their  
141 populations.

142

## 143 **2. Materials and methods**

### 144 *2.1 Field site and experimental set-up*

145 The field site was in a peatland at Serra do Xistral in the Galicia region (NW Spain). This  
146 area has been included in the European Natura-2000 network as a Site of Community  
147 Importance (SIC) since December 2004, and as a Special Area of Conservation (SAC)  
148 since March 2014. Climatic records from the nearest meteorological station of Fragavella  
149 (43°27'16.56"N, 7°26'46.5"W; 710 m a.s.l.; Meteogalicia, Xunta de Galicia:  
150 <http://www.meteogalicia.es/>) indicate that, for the period 2003-2013, mean monthly  
151 temperature was 10.3 °C, ranging from 3.2 °C (winter) to 16.8 °C (summer) and that the  
152 average annual rainfall for the same period was 1467 mm.

153 The investigation was carried out at two different peatland habitats with functionally  
154 different plant communities (vascular *versus* non-vascular): an Atlantic wet heathland

155 (EU Habitats Directive - Temperate Atlantic wet heaths code 4020\*) where the Mackay's  
156 heath *Erica mackayana* dominates (43°27'17"N, 7°29'22"W; 663 m a.s.l.), and a nearby  
157 active raised bog (EU Habitats Directive – Active raised bogs code 7110\*) covered by  
158 *Sphagnum* species (43°27'18"N, 7°29'23"W; 660 m a.s.l.). Using the methodological  
159 approach proposed by Krab et al. (2010), we selected two sample locations situated  
160 along the slope at each habitat (higher (H) and lower (L) sample plots) to create a slight  
161 soil moisture gradient.

162 In July 2013, six intact soil cores (10 cm diameter x 9 cm deep) were excavated at  
163 each sampling plot and half were placed back in their normal (original) position (N),  
164 whereas the remaining ones were re-inserted in an upturned position (U) (2 vegetation  
165 sites x 2 sampling plots x 2 core orientations x 3 replicates = 24 soil cores). Following  
166 the same methodology designed by Krab et al. (2010), we also chose to work with an  
167 open system (instead of incubating the cores in an enclosed system (e.g. PVC pipes)),  
168 and we only placed a 2 mm mesh under the cores. This approach not only ensured the  
169 free movements of the mesofauna (< 2 mm body diameter) and avoided any disruptions  
170 in the water and temperature exchanges with the surroundings, but also minimised  
171 disturbance at the time of harvesting. These cores were left in the field for 4 months to  
172 allow for enchytraeids to relocate and reproduce.

173 In addition, three extra cores from each site were obtained for initial determinations of  
174 enchytraeid population sizes and soil water conditions using the methodologies  
175 described below.

176

## 177 *2.2 Final sampling and sample processing*

178 In November 2013, the cores were carefully excavated and each one horizontally sliced  
179 in the field into three horizontal layers (0-3, 3-6 and 6-9 cm). Sub-samples from each soil  
180 layer were used for: (i) enchytraeid extractions (O'Connor, 1955) and species



181 determinations using appropriate keys (Schmelz and Collado, 2010), and with results  
182 expressed as ind./dm<sup>3</sup> (Krab et al., 2010); (ii) soil water content by oven drying the fresh  
183 soil sample (2-4 g) at 105°C to constant weight, and with the results being expressed as  
184 a percentage, iii) microbial biomass carbon (MBC) by means of the fumigation-extraction  
185 method (Vance, Brookes, & Jenkinson, 1987), with the results expressed as mg g<sup>-1</sup> dw  
186 soil, and iv) extractable dissolved organic carbon (DOCex) using 0.5 M K<sub>2</sub>SO<sub>4</sub>, with the  
187 results expressed as mg C g<sup>-1</sup> soil dry weight. The soil extracts (fumigated, non-  
188 fumigated and K<sub>2</sub>SO<sub>4</sub>) were filtered (7-9 µm, FilterLabR, Ref. 1252) and analysed using  
189 a continuous flow autoanalyzer (Sievers Innovox TOC Analyzer).

190 In addition, enchytraeid tissue samples were prepared for radiocarbon (<sup>14</sup>C) analysis  
191 following Briones and Ineson (2002). Briefly, enchytraeids were freeze-dried and  
192 combusted inside sealed quartz tubes, and the CO<sub>2</sub> cryogenically recovered and split  
193 into aliquots. One aliquot of CO<sub>2</sub> was used for <sup>13</sup>C measurement using isotope ratio mass  
194 spectrometry (Thermo Fisher Delta V, Germany), with results expressed as δ<sup>13</sup>C (<sup>13</sup>C/<sup>12</sup>C  
195 ratio in ‰ units relative to the Vienna Pee Dee Belemnite standard). A second aliquot of  
196 recovered CO<sub>2</sub> was converted to graphite by Fe/Zn reduction (Slota, Jull, Linick, & Toolin,  
197 1987) and analysed at the Scottish Universities Environmental Research Centre  
198 Accelerator Mass Spectrometry Facility. Radiocarbon concentrations are expressed as  
199 %modern after normalisation of the measured <sup>14</sup>C enrichment of each sample relative to  
200 δ<sup>13</sup>C of -25‰ (Stuiver & Polach, 1977). The average age of the enchytraeid carbon since  
201 fixation was determined by comparing the bomb-<sup>14</sup>C contents to an annual record of  
202 recent atmospheric <sup>14</sup>C concentration (Levin, Hammer, Kromer, & Meinhardt, 2008;  
203 Calibomb website: <http://calib.org/CALIBomb/>).

204

205 *2.3 Statistical analyses*

206 Data were log-transformed prior to performing the parametric analyses to meet the  
207 normality and homogeneity criteria. However, even after transformation, DOCex still  
208 failed to meet the homogeneity criterion (Shapiro-Wilk and Kolmogorov-Smirnov tests:  $p$   
209  $< 0.05$ ). Visual inspection of the data (normal probability plots) indicated that this variable  
210 exhibited a positively skewed distribution with heavy tails (Skewness  $> 1$  and Kurtosis  $>$   
211  $3$ ), which was reduced after log-transformation. Therefore, we used the log-transformed  
212 values in all statistical analyses to keep consistency in the choice of data transformations  
213 throughout the study (Onofri, Carbonell, Piepho, Mortimer, & Cousens, 2010).

214 One-way Analysis of Variance (ANOVA) was used to test for significant differences  
215 between initial and final values of soil water contents and initial and final densities of  
216 enchytraeids in relation to sampled habitats and sample locations. In the case of the  
217 abundances of individual enchytraeid species, these analyses were only performed for  
218 the most abundant species and if they were present in at least two replicates of any given  
219 treatment so a mean and standard error could be estimated.

220 Because conditions within the same core are not independent, repeated measures  
221 ANOVA was used to test for significant differences in the final values of the three  
222 dependent variables investigated between treatments, with habitat, slope location and  
223 core orientation as fixed factors and soil layers as a repeated factor. Thereafter, post hoc  
224 tests were carried out on one-way ANOVAs with core orientation combined with soil layer  
225 as a factor for site and slope treatments separately (Krab et al., 2010). This allowed us  
226 to test for significant differences between the same soil layers on the two core  
227 orientations ('Normal' versus 'Upturned') at each sample plot of the height gradient  
228 ('High' versus 'Low') at each habitat. Separation of means was determined using Tukey's  
229 HSD (honestly significant difference) test ( $\alpha = 0.05$ ).

230 Linear regressions were used to investigate the dependence of enchytraeid  
231 population numbers with soil water content, microbial and extractable carbon.

232 All statistical analyses were performed using SAS 9.3 (SAS Institute Inc., 2011).

233

### 234 3. Results

#### 235 3.1 Peat soil water contents and enchytraeid abundances

236 Initial soil water contents in the top 9 cm of peat differed between the two habitats, with  
237 lower values being measured under the Atlantic heath than under mosses (46% vs. 90%  
238 on average; ANOVA<sub>habitat</sub>:  $F = 826.05$ ,  $p < 0.0001$ ; Fig. 1). These differences in peat  
239 wetness were reflected in the total abundances of enchytraeids (albeit not significant),  
240 with their population sizes being five times greater under *Sphagnum* moss than under  
241 heather ( $> 2,000$  vs.  $< 400$  ind./dm<sup>3</sup> on average; Fig. 1).

242 Furthermore, the effect of the height gradient (i.e. the two sample plots along the  
243 slope) was significant at the *Sphagnum* bog only (ANOVA<sub>slope</sub>:  $F = 27.08$ ,  $p = 0.0065$ ),  
244 with averaged soil water contents of 87% and 93% being measured at the higher (H) and  
245 lower (L) sample locations, respectively (Fig. 1). This resulted in a significant difference  
246 in enchytraeid densities by showing the highest values at SH (with population numbers  
247 being 18 times higher at SH than at SL;  $p = 0.0022$ ; Fig. 1). Despite the lack of a moisture  
248 gradient at the heath site, enchytraeid abundances at the highest point (EH) were twice  
249 those recorded at the lowest location (EL) ( $p > 0.05$ ; Fig. 1).

250 These differences in soil water content between habitats and locations along the slope  
251 were also observed at the time of harvest. Thus, the peat under heather was again drier  
252 than that under mosses (70% vs. 84% on average; ANOVA<sub>habitat</sub>:  $F = 131.97$ ,  $p < 0.0001$ ;  
253 Fig. 1), but on this sampling occasion enchytraeids population sizes at the former site  
254 were nearly twice those recorded under mosses (2061 vs. 1083 on average;  $p = 0.0273$ ).  
255 Again, at the *Sphagnum* site, the peat was significantly ( $p = 0.0010$ ) wetter at SL  
256 ( $88.7 \pm 1.05\%$ ) than at SH ( $80.1 \pm 0.81\%$ ), but resulted in very similar densities of  
257 enchytraeids at both sample locations along the height gradient. In contrast, at the

258 habitat with shrubs, despite the water contents of the peat being very similar at EH and  
259 EL ( $70.0 \pm 0.91\%$  and  $70.5 \pm 0.75\%$ , respectively; Fig. 1), enchytraeid densities at EL were  
260 double those found at EH ( $2285.6 \pm 302.2$  and  $802.77 \pm 200$  ind./dm<sup>3</sup>, respectively;  $p =$   
261  $0.0241$ , Fig. 1).

262

### 263 *3.2 Biological responses to changes in the abiotic vertical gradients*

264 The four-month field incubation resulted in habitat (*Erica* and *Sphagnum*), slope location  
265 (H and L) and core orientation (N and U) having a significant effect on enchytraeids  
266 distribution among the different soil layers (Table 1). In the case of the *Erica* habitat, the  
267 majority of the individuals were significantly concentrated at the top layer (0-3 cm) of the  
268 normally orientated cores at both slope locations (i.e. EHN and ELN), which also had the  
269 highest soil water content (Fig. 2a). In contrast, the enchytraeid populations at the  
270 *Sphagnum* habitat showed no obvious preference for a particular layer, especially at the  
271 bottom of the slope (SL) where they were evenly distributed along the soil profile (Fig.  
272 2b). This was possibly a reflection of similar water content in the three peat layers at SLN  
273 (Fig. 2b).

274 Peat layer and its interaction with vegetation type were the only factors to have a  
275 significant effect on microbial biomass (Table 1). Accordingly, the uppermost peat layer  
276 of the normally orientated cores also showed the highest microbial biomass at both  
277 habitats and decreased with depth (Figs. 2c,d), although at the *Erica* site microbial  
278 communities were also densely packed in the 3-6 cm layer (Fig. 2c).

279 Upturning the cores resulted in more significant alterations of the vertical distribution  
280 of the enchytraeids than in the case of microbes (Table 1 and Fig. 2). Accordingly, at the  
281 lowest sample location of the *Erica* site (ELU), enchytraeids moved to the most humified  
282 layer (6-9 cm, now closer to the surface) that also contained less water (Fig. 2a). A similar  
283 behaviour, albeit non-significant, was also observed at the SH location (Fig. 2b). In

284 contrast, microbial populations remained mainly associated with the layer they were  
285 originally found in both normally and upturned cores at both sites (Figs. 2c,d).

286 The taxonomical identification of the enchytraeid populations revealed that species  
287 richness was high, with a total number of 14 different taxa being identified and with some  
288 requiring further taxonomical confirmation (Table S1). However, only four of them  
289 (*Cognettia sphagnetorum*, *Cognettia cognettii*, *Cernosvitoviella aggtelekiensis* and  
290 *Cernosvitoviella* sp. 1) were well represented at the investigated sites (>350 ind./dm<sup>3</sup>).  
291 Furthermore, *C. sphagnetorum* was the most abundant species at the two habitats,  
292 whereas the remaining species were only found, or showed a higher abundance, at one  
293 of the two habitats (Table S1).

294 Vegetation type did not have a significant effect on the species vertical distribution  
295 and the height gradient only had a significant effect on the distribution patterns of  
296 *Fridericia* sp., which was only present under heather (Tables 2 and S1). However, the  
297 vertical stratifications of several enchytraeid species were significantly affected by the  
298 orientation of the core (Table 2). By using the same terminology adopted by previous  
299 researchers to describe the re-distribution patterns (Krab et al. 2010), we observed that  
300 the three most abundant enchytraeid species found at the *Erica* site were “stayers” (i.e.  
301 in the “Upturned cores”, they remained in the same layer where they are “Normally”  
302 located), at highest location of the slope (Figs. 3a-c). However, at the lowest sample  
303 location (EL), two species (*C. sphagnetorum* and *C. aggtelekiensis*) migrated to the layer  
304 closer to the surface when the cores were upturned, despite the peat being “older” (6-9  
305 cm of the “normally” positioned cores; Figs. 3a,c).

306 A similar upward migration of the most abundant species at the investigated area, *C.*  
307 *sphagnetorum*, was also observed under the *Sphagnum* moss, at the highest location of  
308 the height gradient ( $p < 0.05$ ; Fig. 3d). Furthermore, another species present in the peat  
309 moss, *Cernosvitoviella* sp. 1, was also a “mover” species at SLU by migrating down to

310 colonise the deeper soil layer in the upturned cores (0-3 cm in the “Normally” orientated  
311 cores; Fig. 3f).

312 Finally, some species behaving as “stayers” or “movers” in the heather habitat,  
313 showed no clear response to the treatment manipulations at the *Sphagnum* site  
314 (“inconsistent”; Figs. 3d,e).

315

### 316 *3.3 Effects of changes in the abiotic and biotic vertical gradients on labile C assimilation* 317 *and leaching*

318 The <sup>14</sup>C content of the C assimilated by enchytraeids was enriched compared to the  
319 contemporary atmospheric carbon dioxide (Fig. 4a), with their bulk <sup>14</sup>C signatures  
320 indicating that they were assimilating organic matter which was, on average, about 5.5  
321 years old. Neither habitat, core orientation nor soil depth had a significant effect on the  
322 age of their assimilated diets, but sample location along the height gradient did (Table  
323 3). Thus, at both habitats, when the cores were incubated at the highest location,  
324 enchytraeids showed a clear preference for younger C sources (< 7 years old), than  
325 when they were feeding at the lowest locations (Fig. 4a), where they appeared to  
326 consume a greater range of substrates (ranging between 4 and 12 years old).

327 In addition, the orientation of the cores had a marginal significant effect ( $p = 0.0532$ )  
328 on the age of their assimilated organic sources (Table 3). Accordingly, enchytraeids in  
329 the uppermost layer (0-3 cm) of the normally orientated cores, appeared to consume  
330 younger substrates than those feeding in the deeper layers; however, when the cores  
331 were upturned the enchytraeids feeding in this top layer (but now far from the peat  
332 surface) selected more <sup>14</sup>C enriched sources (Fig. 4b).

333 The amount of extractable dissolved organic carbon (DOC<sub>ex</sub>) significantly decreased  
334 with soil depth, with the response being significantly different according to habitat type  
335 and sample location, but not with core orientation (Table 2). Thus, higher DOC<sub>ex</sub> exports

336 were recorded from all three layers at the lowest sample locations and from the deeper  
337 layer (6-9 cm) under mosses than under shrubs (Fig. 5).

#### 338 **4. Discussion**

339 Enchytraeids require a water film for their movement (Briones et al., 1997) and therefore,  
340 the wetter conditions provided by *Sphagnum* mosses provide a more suitable  
341 environment for completing their life cycles. Consequently, lower abundances of these  
342 invertebrates were observed when the water content of the peat was too low, such as  
343 the one measured at the habitat with shrubs at the start of the experiment (<47%).  
344 Furthermore, our observations at this drier habitat also showed that significant increases  
345 in soil moisture contents of up to 70% (when compared to its initial summer values)  
346 promoted higher enchytraeid reproduction rates than in the nearly water saturated  
347 environments under *Sphagnum*. This partly contradicts our first hypothesis and too much  
348 water in the peat could have similar detrimental effects as too dry conditions. The  
349 observed significant negative relationship between enchytraeid abundances and water  
350 content ( $p = 0.0069$ ; Fig. S1) indicated that soil moisture values >85% also increase  
351 enchytraeid mortality rates, and earlier work has shown that these invertebrates cannot  
352 tolerate extended periods of anoxia (Healy, 1987).

353 Although enchytraeids can migrate downwards in response to changes in soil  
354 moisture, their populations in the field tend to be concentrated in the top layers where  
355 they can access a wider range of organic substrates (Briones et al., 1997; Briones &  
356 Ineson, 2002). In agreement with this, previous results have also concluded that the  
357 quality of the organic matter could be the main limitation for survival in the deeper layers  
358 during their vertical migrations (Briones et al., 2010). Therefore, upturning the cores and  
359 placing the top layer closer to the water table (or in contact with the water level) ensures  
360 high values of both soil moisture and labile substrates, and would provide more  
361 favourable conditions for the activities of these invertebrates. Accordingly, at the Atlantic  
362 heath, our results confirmed that enchytraeids remained at the 0-3 cm layer of the

363 upturned cores, resulting in the majority of the species being classified as “stayers”  
364 (*sensu* Krab et al., 2010). This means that most individuals preferred the uppermost top  
365 layer of the upturned cores, where wet conditions, but more importantly more labile C  
366 (as DOCex) and microbial biomass were present. In particular, two species (*Cognettia*  
367 *sphagnetorum* and *C. cognettii*) benefited from turning the cores upside down and their  
368 populations increased by two-fold or more when the cores were inverted than in their  
369 natural orientation. Therefore, as hypothesised, the higher quality of the organic matter  
370 deposited in the top layer can sustain larger population sizes and that changing the  
371 orientation of this substrate quality gradient did not alter this response. However, at the  
372 wettest locations of this habitat, some species behaved as “movers (up)” (*sensu* Krab et  
373 al. 2010) and many individuals migrated to the most humified layer now close to the  
374 surface. Despite this deeper layer having the lowest water content measured at this  
375 habitat at the time of harvest ( $\approx 65\%$ ), it contained similar contents of labile C (as DOCex)  
376 to the top layers (Fig. 5a), and reinforces the idea that substrate quality is the main driver  
377 for the observed migration responses.

378 Similarly, at the site dominated by mosses, enchytraeids responded negatively to the  
379 high levels of moisture and to the low levels of both labile C and microbial biomass  
380 measured at this site by reducing their population sizes. However, individual species  
381 showed a greater variability in their responses to changes in the microclimate and  
382 substrate quality, when compared to the *Erica* habitat. For example, the dominant  
383 species, *C. sphagnetorum*, which behaved as a “stayer” or “mover (up)” under shrubs,  
384 was classified as “mover (up)” or “inconsistent” under non-vascular plants, and  
385 *Cernosvitoviella* sp. 1 collected only under *Sphagnum* behaved as a “stayer” at SH but  
386 as a “mover (down)” at SL. This contrasting pattern could reflect specific differences in  
387 their preferential diets, or a more distinct burrowing behaviour. Thus, while *C.*  
388 *sphagnetorum* responses were more clearly linked to a greater availability of labile  
389 substrates and more suitable moisture conditions, the second species was strongly



390 associated with the deeper layers independently of their substrate quality or  
391 microclimate. In support of this, previous laboratory incubations have reported slow  
392 growth and weight losses of *C. sphagnetorum* on *Sphagnum* (Standen, 1978; Latter &  
393 Howson 1978), and that *Cernosvitoviella* spp. are indicators of badly aerated wet soils  
394 (Graefe & Schmelz, 1999), confirming that although *Sphagnum* can provide a suitably  
395 moist environment, particularly for the less drought-resilient species, its poor food value  
396 can condition their survival (Standen & Latter, 1977).

397 Consistent with previous studies, microbial biomass decreased with depth due to the  
398 presence of more labile organic matter and higher redox states typically found near the  
399 surface of peat profiles (Blodau & Moore, 2002; Blodau, Basiliko, & Moore, 2004;  
400 Basiliko, Blodau, Roehm, Bengtson, & Moore, 2007; Kim et al., 2016; Zhong et al., 2017).  
401 However, this vertical gradient in microbial communities was more pronounced at the  
402 Atlantic heath than at the *Sphagnum* habitat, and was not significantly related to the  
403 gravimetric peat moisture content nor the amount of DOCex present in the peat. This  
404 suggests that different factors, such as vegetation type, might have a greater influence  
405 on microbial activity in peatlands, in agreement with previous studies (Trinder, Johnson,  
406 & Artz, 2008; Straková et al., 2011; Preston, Smemo, McLaughlin, & Basiliko, 2012;  
407 Ward et al., 2015; Robroek et al., 2015, 2016).

408 Despite several studies reporting that enchytraeids are mostly microbial grazers (e.g.  
409 Didden, 1993), we did not find a positive relationship between the abundance of these  
410 two groups and hence, a higher microbial biomass does not necessarily imply a higher  
411 abundance of these invertebrates, at least in the investigated systems. A recent review  
412 by Gajda, Gorgoń, & Urbisz (2017) concluded that several feeding strategies exist within  
413 the family Enchytraeidae, and depending on the species, they can be classified as  
414 primary decomposers or secondary decomposers/sapro-microphytophages. In  
415 agreement with this, the observed increases in the enchytraeid population numbers in  
416 the top layers of the upturned cores also resulted in a shift in their feeding behaviour,

417 and a greater consumption of “older” C sources was observed. Previous studies have  
418 shown that competitive pressures could drive this response (Briones et al., 2007).  
419 Consequently, although these organisms tend to feed preferentially on the most recently  
420 deposited C substrates at the top layer, when competition for labile substrates is high  
421 (due to a greater number of individuals but also microorganisms) they can access less  
422 labile sources in order to sustain their growing populations. This so-called “feeding  
423 flexibility” (Briones, 2014) allows these invertebrates to survive adverse abiotic and biotic  
424 conditions, at least for short-term periods. Therefore, in disagreement with our second  
425 hypothesis, plant identity did not have a significant effect on the age of the C being  
426 mobilised and the presence of vascular plants did not change enchytraeids feeding  
427 behaviour, despite more individuals competing for the available resources. Instead, they  
428 preferred to stay in the “crowded” top layer where more microbial C and DOCex was  
429 present. However, in agreement with previous findings (Briones et al., 2007, 2010), in  
430 order to sustain their population increases, they selected different aged C sources.

431 Taken together, these findings seem to indicate that, with independence of the plant  
432 communities above-ground, peat physico-chemical conditions are the most important  
433 factors regulating enchytraeids’ population sizes and vertical distribution. Therefore, the  
434 effects of the projected expansion of vascular plants on C dynamics in these systems  
435 will strongly depend on the interactions between climate, resource quality and the  
436 responses of the decomposer communities (Briones, McNamara, Poskitt, Crow, & Ostle,  
437 2014). Our field incubation experiment and other previous experimental studies provide  
438 a clear evidence that changes in decomposition rates due to climatic and/or plant  
439 community changes are linked to soil fauna vertical distribution (Briones et al., 2010;  
440 Krab et al., 2010). If environmental conditions become too dry, decomposition is likely to  
441 be transferred to the deeper layers and result in the mobilisation of previously unavailable  
442 C pools (Briones et al., 2010; Walker et al., 2016). This will not only lead to positive  
443 feedbacks to climate change, but the future of our C stocks will become critically

444 threatened. Our findings highlight the need to include soil biodiversity responses  
445 (including vertical distribution) as well as climate-driven vegetation changes when  
446 predicting the future carbon storage potential of these ecosystems.

447

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**Table 1** Results from repeated measures ANOVA for final enchytraeid densities (ind./dm<sup>3</sup>), microbial biomass (mg C g<sup>-1</sup> soil) and extractable DOC (mg C g<sup>-1</sup> soil) measured in each treatment (three peat layers (0-3, 3-6 and 6-9 cm), two soil core orientations (i.e. Normally positioned or Upturned), two locations along a slope (i.e. High or Low) and two habitats). Significance multivariate test is Wilks' lambda test.

	F	P
<i>Enchytraeid densities</i>		
Layer	24.62	< 0.0001
Layer x orientation	8.96	0.0028
Layer x slope	4.06	0.0389
Layer x habitat	31.4	< 0.0001
Layer x orientation x slope	1.32	0.2971
Layer x orientation x slope x habitat	1.99	0.0990
<i>Microbial biomass</i>		
Layer	23.87	< 0.0001
Layer x orientation	0.98	0.3991
Layer x slope	0.90	0.4294
Layer x habitat	25.71	< 0.0001
Layer x orientation x slope	0.58	0.5736
Layer x orientation x slope x habitat	1.64	0.1721
<i>Extractable DOC</i>		
Layer	8.58	0.0033
Layer x orientation	1.49	0.2566
Layer x slope	0.02	0.9787
Layer x habitat	11.40	0.0010
Layer x orientation x slope	0.25	0.7797
Layer x orientation x slope x habitat	0.93	0.4885

**Table 2.** Results from repeated measures ANOVA for the layer effects, the interactions between layer and either core orientation, location along the slope or vegetation type, the interaction between layer, core orientation and height treatment and the interaction between the four investigated factors.

	Layer		Layer x orientation		Layer x slope		Layer x vegetation		layer x orientation x slope		layer x orientation x slope x vegetation	
	F	P	F	P	F	P	F	P	F	P	F	P
<i>Cognettia sphagnetorum</i>	24.17	< 0.0001	9.14	0.0025	3.21	0.0689	10.46	0.0014	0.49	0.6201	2.37	0.0542
<i>Cognettia cognettii</i>	4.15	0.0368	0.78	0.4779	0.92	0.4181	2.71	0.0992	0.33	0.7248	0.35	0.9044
<i>Cognettia hibernica</i>	6.38	0.0099	0.53	0.6012	1.07	0.3683	7.11	0.0067	0.14	0.8666	0.68	0.6640
<i>Cognettia clarae</i>	5.70	0.0144	5.62	0.0146	0.25	0.7791	5.70	0.0144	0.26	0.7733	1.82	0.1297
<i>Cognettia glandulosa</i>	5.18	0.0195	0.71	0.5085	0.05	0.9507	5.18	0.0195	0.6	0.5622	0.44	0.8457
<i>Cognettia</i> sp.	4.77	0.0249	1.34	0.2922	0.26	0.7728	3.17	0.0712	1.43	0.2708	1.08	0.3947
<i>Fridericia</i> sp.	0.82	0.4576	8.00	0.0043	8.33	0.0037	0.82	0.4576	3.87	0.0442	4.94	0.0013
<i>Achaeta</i> sp. 1	1.57	0.2413	0.00	0.9962	2.34	0.1305	1.57	0.2413	1.87	0.1887	1.25	0.3080
<i>Achaeta</i> sp. 2	0.91	0.4229	0.33	0.7246	0.33	0.7267	0.91	0.4229	0.92	0.4206	0.51	0.7937
<i>Cernosviella aggtelekiensis</i>	2.89	0.0865	15.12	0.0003	0.30	0.7477	4.07	0.0387	4.94	0.0225	5.01	0.0012
<i>Cernosvitoviella</i> sp. 1	9.41	0.0022	1.40	0.2772	3.21	0.0693	9.41	0.0022	1.65	0.2245	2.04	0.0917
<i>Cernosvitoviella</i> sp. 2	2.11	0.1558	0.13	0.8802	0.67	0.5282	2.11	0.1558	1.83	0.1950	0.86	0.5330
<i>Mesenchytraeus</i> sp.	0.98	0.3975	25.61	< 0.0001	0.40	0.6778	2.24	0.1405	3.51	0.0562	6.10	0.0003
<i>Marionina</i> sp.	2.22	0.1434	0.55	0.5906	0.56	0.5849	0.89	0.4316	1.69	0.2185	0.53	0.7835

**Table 3** Results from ANOVA for the  $^{14}\text{C}$  content of enchytraeids' tissues (%modern C) at the end of the field incubation period.

	F	P
Habitat	12.25	0.0728
Slope	69.08	0.0142
Orientation	1.50	0.3456
Layer	0.08	0.9260
Layer x habitat	7.01	0.1249
Layer x slope	1.46	0.4061
Layer x orientation	17.79	0.0532
Layer x orientation x slope	2.39	0.3086
Layer x orientation x habitat	0.18	0.8999
Layer x slope x habitat	1.80	0.3772

## Figure legends

**Figure 1.** Initial (black) and final (white) values of enchytraeid densities (ind./dm<sup>3</sup>; horizontal bars) and water contents (%; circles) of the sampled peat profile (0-9 cm) measured along the two locations along the slope (High (H) or Low (L)) at each habitat (*Erica* (E) or *Sphagnum* (S)). Different letters indicate significant differences between treatments (lowercase) on each sampling occasion and between sampling occasions per each treatment (uppercase) ( $p < 0.05$ ; Tukey's HSD comparison of means). Values are means and standard errors.

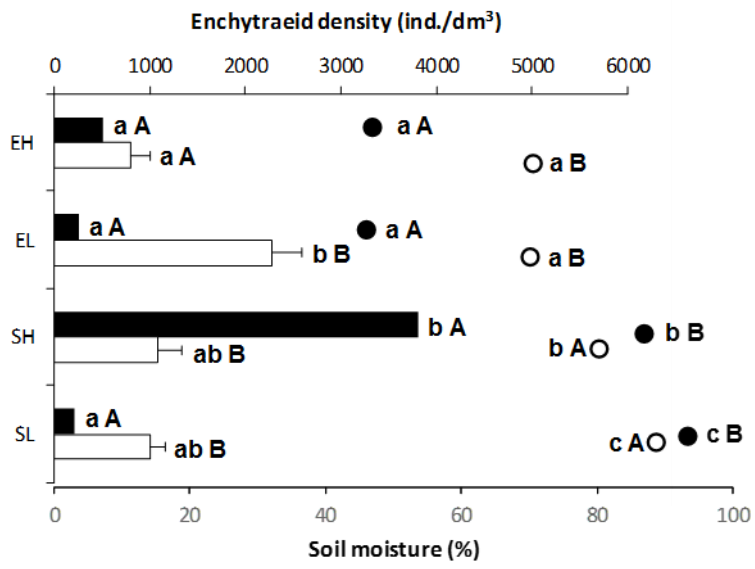
**Figure 2.** Final values of enchytraeid densities (ind./dm<sup>3</sup>) combined with those of soil water contents (a,b), and microbial biomass carbon (c,d), measured in three different layers (0–3, 3–6 and 6–9 cm deep; bars) of the soil core treatments (two core orientations: “Normally” positioned (N) or “Upturned” (U) and two sample locations: High (H) or Low (L)), at (a,c) *Erica* (E) and (b,d) *Sphagnum* (S). The order of the three bars per treatment represents the position of the different layers at time of harvest. Different letters indicate significant differences between treatments (Tukey's HSD comparison of means,  $p < 0.05$ ). Values are means and standard errors.

**Figure 3.** Final densities of the most abundant enchytraeid species (ind./dm<sup>3</sup>) collected from the three peat layers (0–3, 3–6 and 6–9 cm deep; bars) of the soil cores either “Normally” positioned (N) or “Upturned” (U) along the two sample locations, either High (H) or Low (L) at (a,b,c) *Erica* (E) and (d,e,f) *Sphagnum* (S). Species type of response are characterized as either ‘Stayer’, ‘Mover (down)’, ‘Mover (up)’ or ‘Inconsistent’ (*sensu* Krab et al. 2010). The order of the three bars per treatment represents the position of the different layers at time of harvest. Different letters indicate significant differences in enchytraeid densities between treatments (Tukey's HSD comparison of means,  $p < 0.05$ ). Values are means and standard errors.

**Figure 4.**  $^{14}\text{C}$  enrichment in enchytraeid tissues at the end of the field incubation period. Means and standard error values are shown for: (a) the two sample locations (High (H) or Low (L)) and two core orientations (“Normally” positioned (N) or “Upturned” (U)) at *Erica* (E, black columns) and *Sphagnum* (S, white columns); (b) the three peat layers (0–3, 3–6 and 6–9 cm deep; bars) of the “Normally” positioned (N) and “Upturned” (U) cores. The order of the three bars per treatment represents the position of the different layers at time of harvest. Dashed line represents the contemporary atmosphere  $^{14}\text{C}$  content.

**Figure 5.** Final concentrations of extractable dissolved organic carbon ( $\text{mg C g}^{-1}$  soil) measured in three different layers (0–3, 3–6 and 6–9 cm deep; bars) of the soil core treatments (two core orientations: “Normally” positioned (N) or “Upturned” (U) and two sample locations: High (H) or Low (L)), at (a) *Erica* (E) and (b) *Sphagnum* (S). The order of the three bars per treatment represents the position of the different layers at time of harvest. Different letters indicate significant differences in enchytraeid densities between treatments (Tukey’s HSD comparison of means,  $p < 0.05$ ). Values are means and standard errors.

**Figure 1**





**Figure 2**

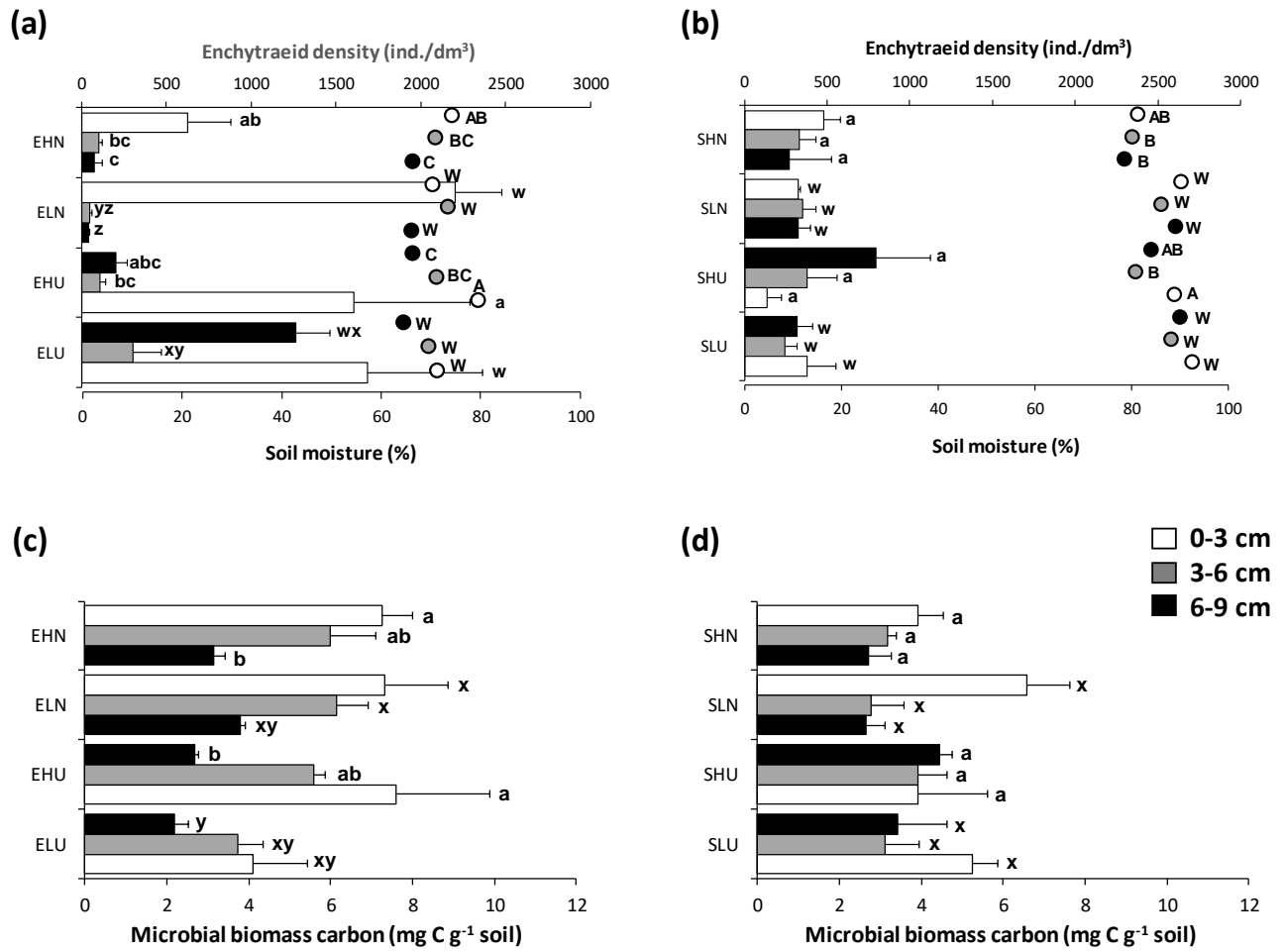


Figure 3

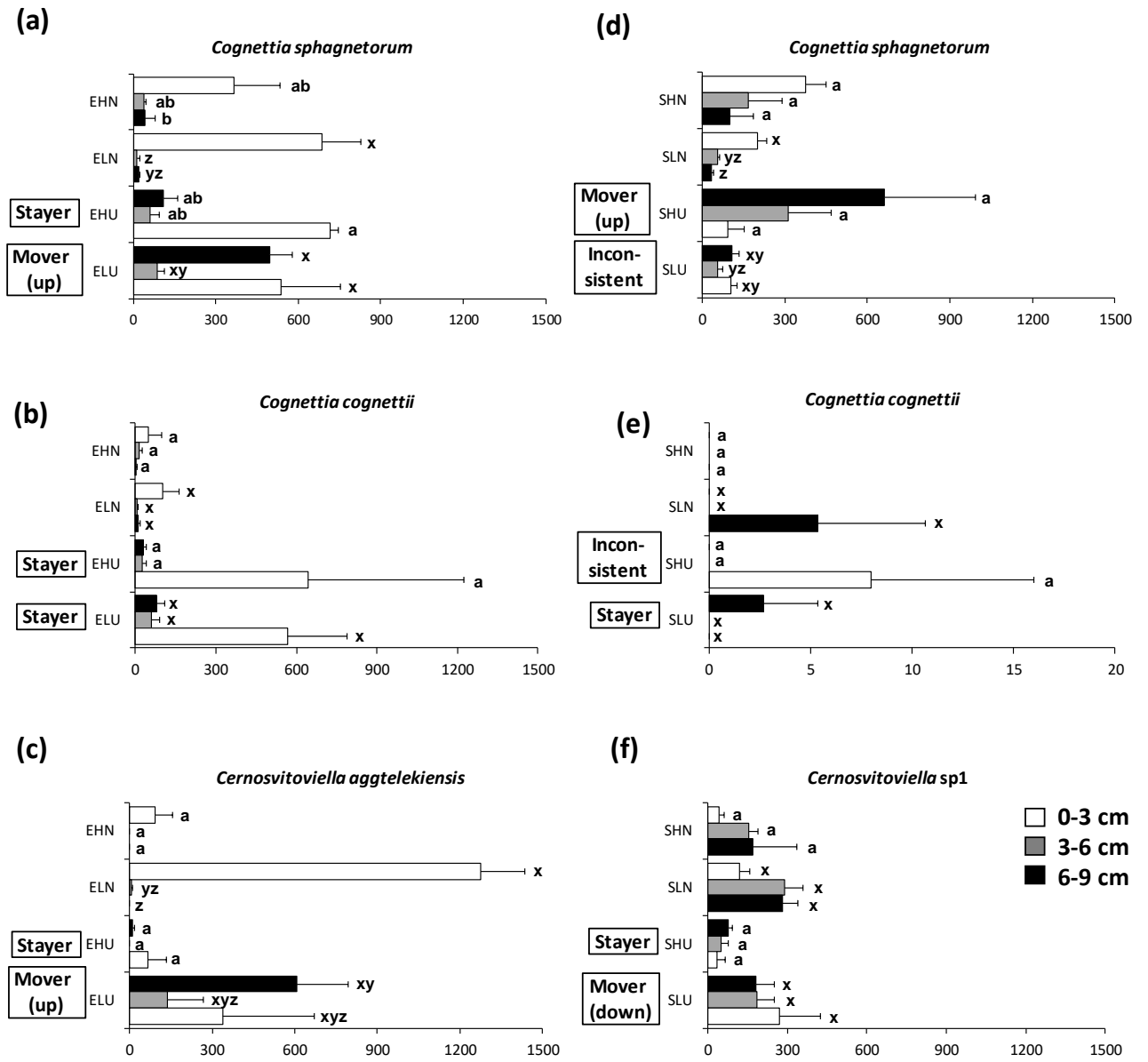


Figure 4

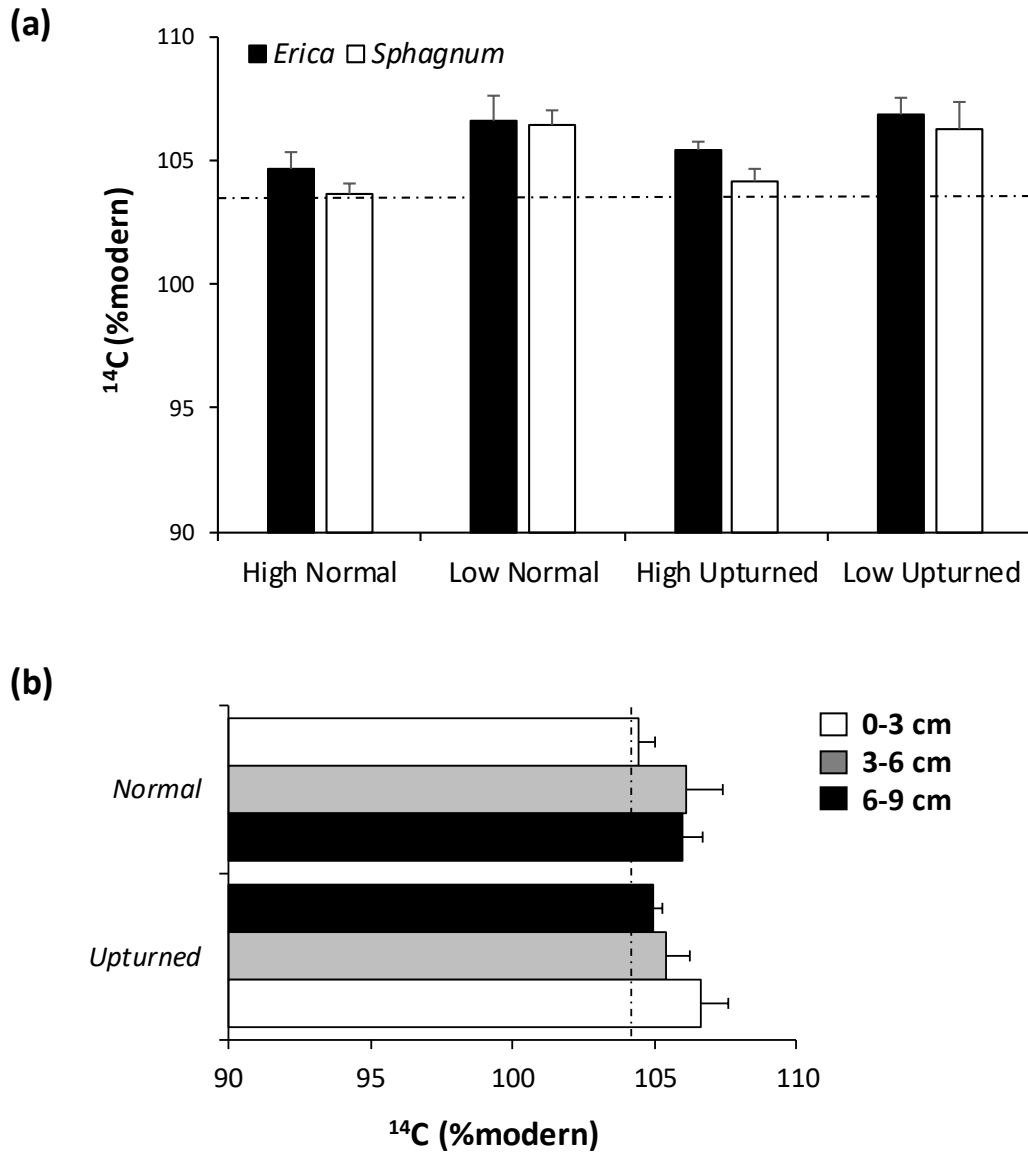


Figure 5

