1   **Complementarity of dung beetle species with different**

2   **functional behaviours influence dung-soil carbon cycling**

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6   Rosa Menéndez*, [a], Paul Webb[a, b] and Kate H. Orwin[a, c]

7   [a] Lancaster Environment Centre, Lancaster University, Lancaster, UK

8   [b] Present address: 16 Wenning Avenue, High Bentham, N. Yorkshire, LA2 7LR

9   [c] Present address: 27 Delaware Cres, Russley, Christchurch, New Zealand

10   [*] Corresponding author: r.menendez@lancaster.ac.uk

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Abstract

Decomposition of large ungulate herbivore dung and its subsequent incorporation into the soil play key roles in carbon and nutrient cycling and are important for grassland productivity. Dung beetles contribute to the initial breakdown and transport of organic matter from the dung into the soil but how they interact with the microbial community to modify decomposition processes remains poorly understood.

Using a mesocosm experiment, we investigated the individual and interactive effect of two dung beetle species with contrasting functional behaviour (dweller species: *Agrilinus ater* (De Geer 1774) vs. tunneler species: *Typhaeus typhoeus* (Linneaus 1758)) on dung C cycling (CO_2 fluxes and C transfer through the soil profile) and resultant effects on microbial activity and biomass in the soil.

Both dung beetle species contributed significantly to dung removal, reducing the C lost through microbial respiration from the whole mesocosm. However, C concentrations measured in leachates from the mesocosm were only significantly higher in the presence of the tunneler species, indicating that tunnelling activity was required to increase C transfer down the soil profile. The combined effect of the two dung beetle species resulted in the highest soil microbial respiration from the soil and in particular in the 2-10 cm depth increment, suggesting positive complementarity effects between species with different functional behaviour.

 We conclude that the return of C in the form of dung in grasslands, coupled with the activity of a functionally diverse dung beetle assemblage, could result in short term fluctuations in soil microbial activity with important consequences for soil C cycling.

Key−words: CO_2 fluxes, complementarity, dung removal, functional diversity, microbial biomass, soil carbon.

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1. Introduction

In grasslands the decomposition of large ungulate herbivore dung and its incorporation

into soil play key roles in ecosystem carbon (C) and nutrient cycling. The way in which

dung is processed is thus important for the long-term sustainability of the grassland and

its productivity (Williams and Haynes, 1995; Zaman et al., 2002; Aarons et al., 2009;

Yoshitake et al., 2014). On pasture stocked at rates of 700 cow days ha^{-1} y^{-1}, dung

deposition adds around 22 t ha^{-1} of C (Bol et al., 2000), providing a significant input of C

to soil. This C input is thought to contribute to soil C stocks in temperate grasslands with

10-16% of cow-dung C incorporated into the soil in only two months (Bol et al., 2000;

Dungait et al., 2005). However, a significant proportion of dung-C is lost through

microbial respiration (Lovell and Jarvis, 1996; Chen et al., 2011; Grilo et al., 2011). In

addition, dung can stimulate microbial activity in the soil underneath the dung, resulting

in the loss of pre-existing soil C (Bol et al., 2003). Any factor that modifies microbial

decomposition of dung is therefore likely to have a strong influence on the retention of

dung- and soil-C in pasture soils. One such factor could be macro-invertebrates, which

are responsible for the initial breakdown and transport of organic matter from the dung

into the soil (Stevenson and Dindal, 1987; Lee and Wall, 2006).

In many regions a large proportion of dung removal is mediated by dung beetles,

which use the dung both for feeding and breeding (Yamada et al., 2007; Lee and Wall,

2006, Nichols et al., 2008). Adult dung beetles feed on the liquid part of fresh dung

(Holter, 2000) but some also create tunnels in the soil in which they store dung for further

feeding or for the creation of brood balls that host eggs and developing larvae (Cambefort

and Hanski, 1991). The activity of dung beetles (Owen et al., 2006) and that of other soil

macro-invertebrates such as earthworms (Hendriksen, 1997), has been reported to

62 increase the concentration of C in the upper soil horizon. Soil macro-invertebrates,

63 including dung beetles, have also been reported to strongly influence greenhouse gas

64 emissions (e.g. CO_2, N_2O and CH_4) from dung (Lubbers et al., 2013, Pentillä et al., 2013),

65 suggesting that these organisms influence microbial activity and dung decomposition

66 rates. The main mechanisms by which this might occur are: (1) by feeding on dung and

67 increasing the rate at which it dries out on the soil surface, which reduces the amount of

68 resource available for microbes, and (2) by burying dung, which increases its exposure to

69 soil microbes and changes the environment in which it is decomposed. Dung beetle

70 behaviour can also change the surface area of the dung, which alters its accessibility to

71 microbes and may influence the amount of C moved further down the soil profile in

72 leachate.

73   Although it is clear that dung beetles influence grassland C cycling, we lack any

74 real understanding of how dung beetle species with different functional behaviour and

75 interactions between them affect soil microbial biomass and activity. In temperate

76 grasslands dung beetles are typically subdivided into two main functional types, based on

77 their nesting behaviour: dweller species (adults and larvae feed inside the fresh dung

78 deposits) and tunneler species (adults dig tunnels in the soil under the dung deposit and

79 bury dung for feeding and nesting). Tunneler species have been shown to be much more

80 effective in dung removal than dweller species (Rosenlew and Roslin, 2008; Nervo et al.,

81 2014), but the consequences of this for soil microbial activity and biomass as well as

82 dung C retention in soils remains unknown. Furthermore, whether the two types of

83 species interact to have a non-additive effect on dung decomposition is unknown.

84   The aim of this study was to examine the individual and interacting effects of two

85 functionally contrasting dung beetle species (a tunneler and a dweller species) on

86 herbivore-dung decomposition, microbial biomass and respiration, and the incorporation

4

87 of C into the soil. To achieve this we used a mesocosm experimental approach that

88 allowed investigation of interactive effects of two types of dung beetle species under

89 controlled conditions. We test the following hypotheses: (1) dung processing and the

90 transfer of C from the dung through the soil profile will differ in the presence of the two

91 contrasting dung beetle species, being higher when tunneler beetles are present due to

92 their ability to bury large amounts of dung; (2) microbial respiration from dung deposits

93 will be modified by the action of dung beetles, being higher soon after the dung is

94 deposited as dung beetle activity will promote aerobic conditions within the dung, and

95 lower later on as beetle activity accelerates the drying process and depletes the amount of

96 dung resource available for microbes; (3) the interactive effects of the two functionally

97 contrasting dung beetle species on microbial respiration will be synergistic as a

98 consequence of greater physical processing of dung and a potentially larger more active

99 microbial community.

100

101 **2. Materials and methods**

102 *2.1. Experimental design*

103 To examine the effects of dung beetles with contrasting functional behaviour on soil

104 microbial respiration, biomass and dung decomposition rates, we set up a mesocosm

105 experiment at Hazelrigg Field Station in Lancaster (54°00′49.35″N/ 2°46′30.68″W).

106 Treatments consisted of no dung (Soil only treatment), dung only (Dung treatment), dung

107 plus the dweller species (Dweller species treatment), dung plus tunneler species (Tunneler

108 species treatment), and dung plus both species (Dweller & Tunneler species treatment).

109 The dweller species was *Agrilinus ater* (De Geer 1774) and the tunneler species was

110 *Typhaeus typhoeus* (Linneaus 1758). These two species are common in grazed grasslands

111   in the area and use dung for both adult and larval feeding. We used a complete random

112   block design with five replicates of each treatment.

113   Experimental mesocosms were constructed from 11 cm diameter sections of

114   plastic pipe and were 25 cm in length to allow the tunnelling species to construct nests

115   underneath the dung. Soil (silt loam of the Brickfield 2 association (Avis and Harrop,

116   1983), %C = 3.05, %N = 0.26, pH = 6.1) was collected from the field station and sieved

117   to 4 mm. After sieving, the soil was homogenized and the same amount (1350 g) added to

118   each mesocosm. The bottom end of the pipe was covered with fine weave plastic netting

119   material to hold in the soil while allowing water to flow through the mesocosm. Plastic 45

120   mm garden mesh was formed into a cylinder and inserted into the soil at the top of the

121   mesocosms, extending to approximately 9 cm above the top of the pipe. This acted as a

122   support for a covering of fine weave plastic netting material, with a removable lid. The

123   space created at the top of the pipe allowed the dung balls to sit on the top of the soil and

124   be exposed to natural weather conditions. The fine weave plastic netting allowed the sun

125   and rain to take effect as well as preventing colonization of the dung by flies and other

126   beetles. The lid covers were removed from all mesocosms after 20 days, once it was

127   certain that the dung had ceased to attract other organisms, and to allow the beetles to

128   leave the dung before their food resource had been completely consumed, representing

129   natural behaviour (Koskela, 1972).

130   *Agrilinus ater* specimens, along with the dung used for the experiment, were

131   collected from a sheep-grazed field on Bailrigg Farm in Lancaster (54°01′08.40″N/

132   2°47′25.98″W). Tunnelling beetles (*T. typhoeus*) were collected from rough cattle grazing

133   fields at Warton Crag Nature Reserve (54°09′07.53″N/ 2°46′45.49″W). Sheep dung was

134   used for the experiment; it was collected fresh and free of beetles from the field and

135   mixed together in a bucket and then formed into balls of equal size (mean wet weight of

136   199.79 ± 0.02 g), and frozen for 48 hours. Dung was fully defrosted before being added to

137   the appropriate mesocosms. Twenty representative dung balls were also oven dried at

138   60°C to obtain a dry weight. Beetles were added to appropriate treatments with numbers

139   of *A. ater* determined through allometric scaling of biomass, such that $B = M^{3/4}$ (where B

140   is the metabolic rate and M is body mass) (West et al., 1997). This allowed species

141   assemblage numbers to be more representative of those found in the field than through

142   biomass alone and also to standardise biomass across treatments. For treatments

143   containing *A. ater* equal numbers of males and females were added (28 individuals per

144   mesocosm in the single species treatment and 14 individuals in the mixed-species

145   treatment). For treatments containing *T. typhoeus,* only females were included (2 in the

146   single species treatment and 1 in the mixed-species treatment). Mesocosms were sealed

147   with the mesh lids immediately after the beetles were added. The experiment was set up

148   on the 28th May 2010 and allowed to run for 6 weeks in the field to enable both adult and

149   larval dung beetle effects on the dung to be included (Rosenlew and Roslin, 2008).

150

151   *2.2. Dung removal*

152   At the end of the experiment, dung remaining on the soil surface was removed from the

153   mesocosms and weighed before being dried in an oven at 60ºC and then reweighed. The

154   amount of dung lost during the experiment was assessed in two ways: (a) comparison

155   between the original and final wet mass of each dung ball allowed us to measure the

156   amount of dung lost due to both dung removal by beetle activity and the drying effects of

157   time (Rosenlew and Roslin, 2008; Slade et al., 2007) and (b) the dried weight of fresh

158   dung balls allowed the original dry mass of each dung ball to be estimated and

159   comparison of the original and final dry masses allowed estimation of the actual amount

160   of dung removed by beetle and microbial activity.

161

162 *2.3. In situ CO_2 fluxes*

163 Microbial activity was measured as CO_2 fluxes at weekly intervals; from seven days after

164 mesocosms were set up and up to 35 days. Gas fluxes were measured using a portable

165 IRGA EGM-4 with an SRC-1 soil respiration chamber placed directly onto the top of the

166 mesocosms. Measurements were taken over a maximum of 120 s, and sampling was

167 started at 10 a.m. on each measurement day. In addition, immediately after the final

168 measurement, a further measurement was taken following the removal of any dung

169 remaining on the soil surface to allow microbial activity in the soil alone to be measured.

170 *2.4. Soil carbon measured in leachates*

171 The effect of dung beetles on C movement through the soil profile was measured by

172 collecting leachates from the bottom of each mesocosm. Leachates were collected

173 throughout the experimental period, the morning after any rain events. Due to the

174 unusually low rainfall for this period, mesocosms were watered (simulating 20 mm

175 rainfall events) on two occasions corresponding with week 2 and 4 of the experiment (on

176 the 11th and 25th of June, respectively). Leachates were collected in bottles through a

177 funnel attached to perforated saucers placed underneath mesocosms. Once collected,

178 leachates were filtered through Whatman filter paper no. 1, refrigerated, and analyzed

179 within one week. Total dissolved C was determined using a Shimadzu 5000A TOC

180 analyser. The majority of C in the leachates was organic (94 %).

181

182 *2.5. Basal microbial respiration and microbial biomass*

183 At the end of the experiment, soil within the mesocosms was harvested from the 0-2 cm

184 and the 2-10 cm depth increments, as dweller activity is restricted to the dung and the first

185 cm of soil while tunnelers activity will also affect deeper soil horizons. These soil

186   samples were used to measure the effects of the beetles on soil microbial activity and

187   biomass at different depths. Soil subsamples from each mesocosm and depth increment

188   were adjusted to 40% moisture content and 2 g dry weight equivalent weighed into

189   MacCartney bottles. Bottles were incubated overnight at 17 ºC, and then sealed. Gas

190   samples (0.5 mL) were injected into an IRGA (model ADC-225-MK3; Analytical

191   Development Co. Ltd, Hoddesdon, UK) 1 and 4 hours after sealing to give a measure of

192   basal respiration. Microbial biomass was measured using substrate-induced respiration

193   (SIR). The methods used were the same as for basal respiration except 0.02 g of glucose

194   was added to each soil subsample before sealing, and gas samples were taken 1 and 3

195   hours after sealing (West and Sparling, 1986).

196

197   *2.6. Data analysis*

198   To test the effects of dung beetle assemblages and time on CO_2 fluxes and changes in

199   dissolved carbon in leachates over the six week period we used Linear Mixed Models

200   (LMMs), with treatment and time as fixed factors, and mesocosm identity nested within

201   block as a random factor to account for our experimental design and the repeated

202   measures nature of the measurements. Fluxes of CO_2 were logarithmic transformed to

203   improve normality. The significance of fixed effects and interactions was assessed by

204   sequential deletion from the maximal model using maximum likelihood parameter

205   estimation. Deviance change between models with and without individual terms was

206   tested using chi-squared (χ^2) tests. The final model, including significant fixed effects and

207   the random effects, was re-fitted under REML parameter estimation (Zuur et al., 2009).

208   At the end of the experiment, differences between treatments of dung mass loss

209   (measured as wet and dry weight), the overall amount of dissolved C lost in leachates, the

210   CO_2 flux from each mesocosm with and without remaining dung, and soil microbial

211 respiration and biomass at different soil depth increments were analysed using LMMs

212 with block as a random and treatment as fixed factors. If normality or equal variance was

213 not met data were log transformed for analysis. For soil microbial basal respiration and

214 SIR in the 2-10 cm depth increment the presence of 2 outliers meant that even after

215 transformations, data did not have a normal distribution. We therefore carried out a non-

216 parametric test (Kruskal Wallis) for this response variable, and also conducted a LMM

217 after removal of the outliers. To test for evidence of complementarity between dung

218 beetle species we compared the observed effect of both species together (mixed species

219 treatment) with that expected based on the single species treatments. Expected effects

220 were calculated by dividing the effect of each species in the single species treatments by

221 two, as the density of beetles in the mixed group treatment was half of that in the single

222 species treatment, and then adding them together. This calculation was performed for

223 each block; we then used LMM to test for significant differences between observed and

224 expected effects. All analyses were conducted using the R statistical software (R

225 Development Core Team, 2011) and all LMMs were fitted using the 'lme4' package

226 (Bates et al., 2012).The 'R' package 'effects' (Fox, 2015) was used to calculate upper and

227 lower 95% confidence intervals to determine significant differences between levels of the

228 fixed factors.

229

230 **3. Results**

231 *3.1. Dung removal*

232 The contribution of dung beetles to dung removal was measured as wet and dry mass lost

233 at the end of the experiment. Decrease in dung weight, measured as % mass loss of the

234 initial dung wet weight, significantly differed between treatments ($\chi^2 = 20.350$, df = 3, p <

235 0.001) with higher loss in treatments containing the tunneler species (tunneler species

236 only and dweller & tunneler species treatment) compared to the dung only control

237 treatment (Fig. 1a). The tunneler species alone treatment also reduced dung wet mass

238 much more than the dweller species alone (Fig. 1a) but there was no difference in wet

239 dung mass loss between the dweller species alone and the dung alone treatment (Fig. 1a).

240 When dung weight loss was expressed on a dry weight basis, the presence of beetles of

241 any species significantly increased mass loss over that in the dung alone treatment ($\chi^2 =$

242 25.934, df = 3, p < 0.001, Fig. 1b), but no differences were found between different beetle

243 treatments.

244

245 *3.2. In situ CO_2 fluxes*

246 We measured the individual and combined effect of dung beetle species on microbial

247 respiration from the mesocosms over time. Microbial respiration from the mesocosms

248 with dung added was higher than the soil only treatment throughout the 6-week

249 experimental period, with the magnitude of this difference declining over time (Fig. 2).

250 Microbial respiration differed significantly between treatments but the nature of that

251 difference depended on the week of measurement (Table 1). In comparison with the dung

252 only treatment, dung beetles significantly decreased microbial respiration three and four

253 weeks after the experiment began but had no effect during the first and the last two weeks

254 of the experiment (Fig. 2 & Fig.3a). At the end of the experiment, after the dung balls

255 were removed, soil microbial respiration significantly differed between treatments ($\chi^2 =$

256 19.291, df = 4, p < 0.001). Microbial respiration in the soil was increased by the presence

257 of dweller and tunneler beetle species together compared with the soil only, dung only

258 and dung colonised by the dweller species only treatments (Fig. 3b). Moreover, there was

259 a significant synergistic positive effect between the two dung beetle species, with higher

260 observed CO_2 fluxes when both species were together in comparison to that expected

261   based on an additive effect (mean ± SE: observed= 0.67 ± 0.08, predicted = 0.46 ± 0.04;

262     $\chi^2 = 6.450$, df = 1, p < 0.011). The presence of the tunneler beetle species (without the

263   dweller beetle species) increased soil microbial respiration significantly over the soil only

264   treatments but not over the dung only or dweller species only treatments (Fig. 3b).

265   Finally, no significant differences in soil microbial respiration were found between the

266   dweller species only, dung only and soil only treatments (Fig. 3b).

267

268   *3.3. Soil carbon measured in leachates*

269   We measured the amount of soluble C lost in leachates to assess the individual and

270   combined effects of the two dung beetle species on the transfer of dung C through the soil

271   profile. Total rainfall recorded during the period between leachate measurements in

272   weeks 2, 4 and 6 was 37 mm, 24 mm, and 69 mm, respectively. The amount of dissolved

273   C measured in leachates increased with time, and differed between treatments, but no

274   significant interaction was found between week and treatment (Fig. 4a, Table 1).

275   Treatments containing the tunneler beetle species alone lost more dissolved C in leachate

276   than any other treatment (Fig. 4a).

277   The total amount of dissolved C in leachates over the entire experimental period

278   was significantly greater in dung colonised by the tunneler beetle species alone ($\chi^2 =$

279   30.545, df = 4, p < 0.001) than in any other treatment (Fig. 4b). Treatments containing the

280   dweller and tunneler beetle species together also resulted in higher overall losses of

281   dissolved C than in soil only, as did dung alone (Fig. 4b). Dung with the dweller beetle

282   species only, however, had no detectable effect over soil alone (Fig 4b).

283

284   *3.4. Soil microbial respiration and biomass*

285   We measured microbial basal respiration and SIR in the soil at two depth increments to

286   assess the individual and combined effect of the two dung beetle species on soil microbial

287   activity and biomass respectively. In the 0-2 cm soil depth increment the level of

288   microbial respiration significantly differed between treatments (χ^2= 22.648, df = 4, p <

289   0.001). The presence of dung beetles significantly increased microbial respiration

290   compared to the soil alone treatment (Fig. 5a). Despite numerical increases, the presence

291   of beetles did not increase microbial respiration enough to cause a significant difference

292   between beetle treatments and dung alone (Fig. 5a). A similar trend was found for

293   microbial biomass, with SIR values being higher in the upper 2 cm of soil in mesocosms

294   with dung added compared to the soil only treatment, but no difference found between

295   any other treatment ($\chi^2 = 27.255$, df = 4, p < 0.001).

296   In the 2-10 cm soil depth increment the combined activity of the dweller

297   and tunneler beetle species together resulted in a significantly higher microbial

298   respiration compared with the soil only, dung only and dung colonised by the dweller

299   beetle species only treatments (Fig. 5b; LMM without 2 outliers: $\chi^2 = 29.699$, df = 4, p <

300   0.001; Kruskal Wallis non-parametric test including 2 outliers: $\chi^2 = 15.399$, df = 4, p <

301   0.005). In addition, there was a significant synergistic effect between dung beetle species,

302   with higher observed CO_2 fluxes when both species were together in comparison to that

303   expected based on an additive effect (mean ± SE: observed= 1.20 ± 0.19, predicted = 0.81

304   ± 0.13; $\chi^2 = 3.881$, df = 1, p < 0.049). In contrast, there was no significant difference in

305   SIR between treatments in the 2-10 cm depth increment (LMM without 2 outliers: $\chi^2 =$

306   5.663, df = 4, p = 0.226; Kruskal Wallis non parametric test including 2 outliers: $\chi^2 =$

307   7.492, df = 4, p = 0.112).

308

309   **4. Discussion**

310 Our results showed that both the presence of dung beetles, and in some cases, the

311 interaction between the two dung beetle species, affected the fate of dung C by

312 influencing microbial activity and C transfer into the soil. Both types of beetles reduced

313 the C lost through microbial respiration from the dung deposits and increased microbial

314 respiration from the soil. However, the differential use of dung by the dweller and

315 tunneler species meant that the presence of tunneler beetles was required to significantly

316 increase C transfers through the soil profile and enhance microbial respiration deeper in

317 the soil. Interestingly, the combined effect of the two dung beetle species resulted in a

318 synergistic, positive effect on soil microbial respiration. This suggests that

319 complementarity between species with contrasting functional behaviour could be

320 important for facilitating dung transfer into the soil and stimulating microbial activity.

321

322 *4.1. Dung removal*

323 In general, the tunneler beetle species was the most effective in removing dung; their

324 presence resulted in an 80 % loss of wet mass and in 21 % loss of dry mass compared to

325 that caused by the dweller beetle species (77.8% wet mass and 16% dry mass loss).

326 However, this superiority was only significant when measured as wet mass loss,

327 suggesting that this result was largely driven by differences in the effect of each type of

328 dung beetle on moisture loss from the dung rather than actual organic matter removal.

329 Owen et al. (2006) found that after 40 days dung pads colonised by *Aphodius fossor*

330 beetles (a dweller species) showed no differences in external surface area and moisture

331 content compared with uncolonised dung and beetles were observed to re-entered dung

332 pads from the underside of the pad, near the dung-soil interface, a behaviour that would

333 conserve moisture. Sustained dung moisture could result in high levels of food

334 availability and optimal moisture conditions for larval survival in the dung. In contrast,

335 tunnelling species physically remove dung and transport it under the soil for adult feeding

336 and larval provision, which accelerates water evaporation from the dung deposit (Brown

337 et al., 2010). Our results therefore suggest that in cold temperate grasslands dwellers can

338 be as functionally important as tunnelers for dung mass removal, rather than less

339 important as previously suggested (Rosenlew and Roslin, 2008, Nervo et al., 2014).

340

341 *4.2. Microbial activity and biomass*

342 Dung beetles significantly affected how dung-C was processed by microbes. Mesocosm

343 respiration rates during the third and fourth week of the experiment were significantly

344 lower in the presence of dung beetles compared to the dung alone treatment. This was

345 probably because the physical breakdown of dung by beetle activity reduced resource

346 availability for the microbes in the dung deposit and accelerated dung desiccation,

347 reducing microbial activity (Penttilä et al., 2013). In addition, it appeared that the tunneler

348 beetle species increased soil microbial activity underneath the dung (Fig 3b), and

349 increased the amount of dissolved C leached from the dung (Fig. 4). Together, this

350 suggests that tunnelling activity facilitates the transfer and use of dung-C in the soil to a

351 greater extent than when only the dweller beetle species was present.

352   Interestingly, the presence of the dweller and tunneler beetle species together had

353 a synergistic, positive effect on soil microbial respiration. Complementarity and/or

354 facilitation in dung removal have been previously reported between functionally different

355 dung beetle species (Slade et al. 2007) and between dung beetles and other coprophagous

356 invertebrates (Holter, 1079; O'Hea et al., 2010). However, to our knowledge, this is the

357 first experimental evidence that such functional species complementarity promotes

358 microbial respiration and modifies C cycling. The amount of organic matter removed

359 from the dung deposit as well as the amount of C measured in leachates was similar in the

15

360 treatment with the dweller and tunneler beetle species together and with the tunneler

361 species only, so it appears that the mechanism behind this complementarity effect was not

362 due to resource availability. A possible explanation is that each species stimulated the

363 activity of different components of the microbial community. *Aphodius* beetles (dwellers)

364 have been reported to increase bacterial density through substrate mixing (Lussenhop et

365 al., 1980), while tunnelers have been shown to enhance fungal growth (Yokohama et al.,

366 1991). The complementarity effect was most evident in the 2-10 cm soil horizon, with the

367 dweller and tunneler species together being the only treatment to significantly increase

368 soil microbial activity above dung alone, as well as being 25% greater than the tunneler

369 species alone (Fig. 4b). That such functional complementarity occurs at this depth is

370 important for grassland productivity, as around 44% of grass root biomass is concentrated

371 in the top 10 cm of soil (Jackson et al., 1996). Further research is required to fully

372 understand this complementarity and its potential effects on pasture health.

373   Finally, despite the obvious effect of beetles on microbial activity, below the 0-2

374 cm depth increment there were no significant effects of dung or beetle treatments on soil

375 microbial biomass measured as SIR. This discrepancy between activity and biomass

376 results is consistent with the idea that, whereas microbial activity is influenced rapidly by

377 the input of labile C, soil microbial biomass is determined by the long term input of stable

378 organic C (Bardgett et al., 1998). Additionally, dry conditions during the experiment may

379 have affected the ability of microbes to process the extra C into biomass, resulting in no

380 significant differences among microbial biomass in treatments containing dung.

381

382 *4.3. Dissolved carbon transferred into the soil*

383 In general, the presence of dung increased the dissolved C content of leachates, consistent

384 with previous findings (Haynes and Naidu, 1998; Zaman et al., 2002; Arons et al., 2009;

385   Yoshitake et al., 2014). The additional effect of beetles on transfer of dung C deeper into

386   the soil was dependent on beetle functional behaviour, with the largest quantities of

387   dissolved C in leachates in the treatment with the tunneler beetle species only and the

388   lowest in the treatment with the dweller beetle species only. The higher C content of

389   leachates in the tunneler species alone treatment is likely due to facilitation of water flow

390   down the tunnels and water movement past the buried dung. Similarly, the burial of dung

391   beneath the soil surface by earthworms has been found to raise the levels of C in soil

392   (Hendriksen, 1997). The lower dissolved-C content of leachate from the dweller species

393   alone treatments may be due to these beetles promoting microbial respiration within

394   dung deposits, which could result in more C being lost through respiration and hence

395   lower C availability for leaching (Steven and Dindal, 1987). This is supported by the fact

396   that at the end of the experiment respiration was similar from all mesocosms with beetles,

397   but a greater proportion of this was from the dung in the dweller species alone treatment

398   (Fig. 3). However, the difference between dweller and tunneller beetle species in the

399   amount of C recorded in leachates suggests that although the tunneler species was more

400   efficient at transferring C into the soil, it may also have increased its vulnerability to loss

401   from the soil via leaching.

402   In conclusion, our results show that dung beetles and their functional behaviour

403   differently contribute to C transfer from the dung into the soil, affecting dung

404   decomposition, carbon cycling and soil microbial respiration. Importantly, the presence

405   of both types of species was needed to achieve the highest levels of soil microbial

406   respiration, suggesting complementarity between species with contrasting functional

407   behaviour in stimulating the soil microbial community. Together, this suggests that

408   changes in dung beetle assemblages could have a significant effect on the way in which

409   dung C is cycled in grasslands. It remains to be seen whether our results can be

410 transferred to other species, and whether differences in the relative abundance of

411 functional types can significantly affect dung C processing and retention in grassland

412 systems.

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562   Table 1. Results of linear mixed models (χ^2 provided, see methods) for the effect of week

563   of measurement and treatment on the microbial respiration (CO_2 flux) and the dissolved

564   carbon measured in leachates from the mesocosms. Mesocosms were nested within

565   blocks as random factors in the analysis to account for repeated measures. Total sample

566   size was 150.

567

	CO_2 flux			Dissolved carbon		
Term	χ^2	**df**	**p**	χ^2	**df**	**p**
Treatment	172.490	24	< 0.001	40.629	12	< 0.001
Week	208.830	25	< 0.001	97.033	10	< 0.001
Treatment*Week	112.080	20	< 0.001	6.210	4	0.624

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569

570 **a)**
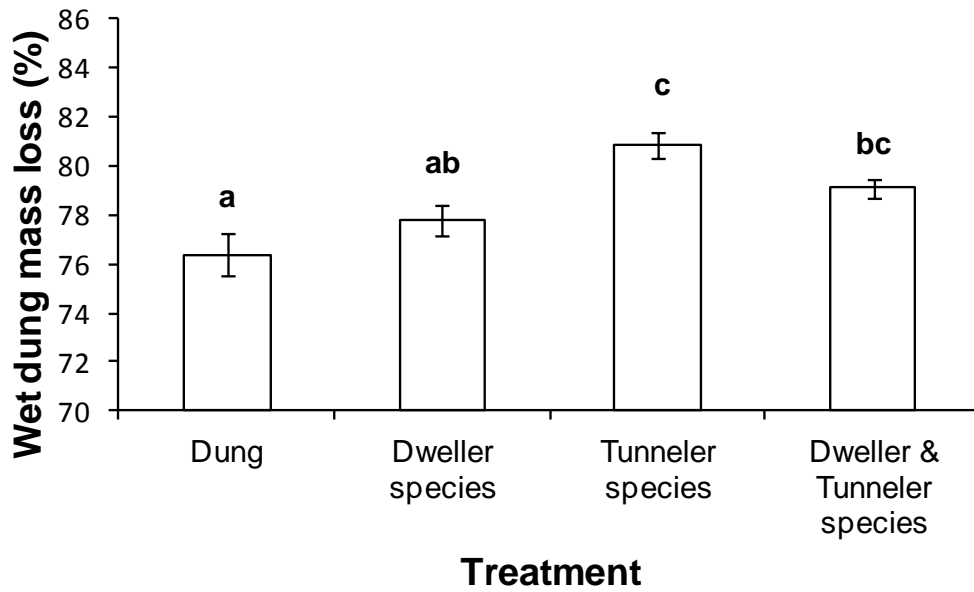
571

572 **b)**
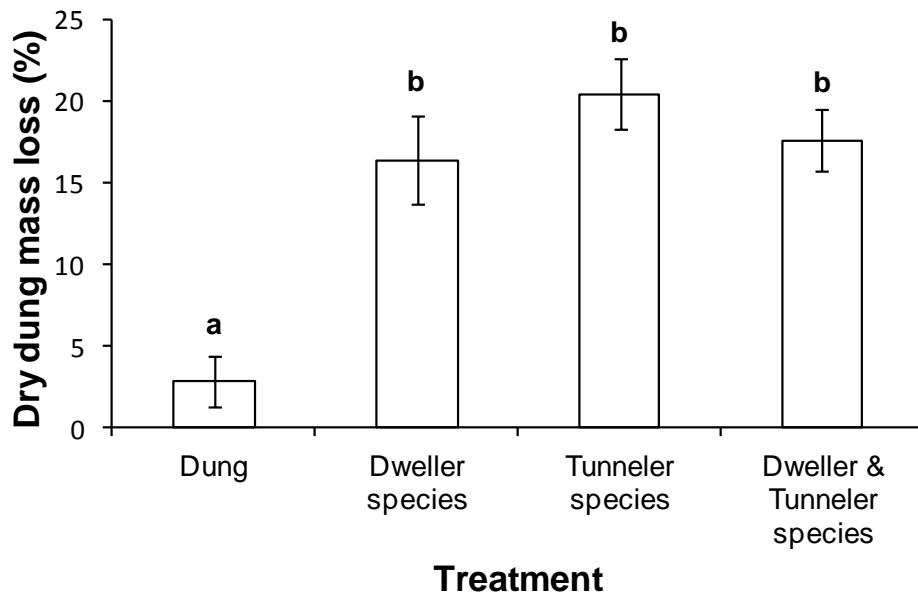
573

574 **Figure 1.** Effects of treatments on the amount of (a) wet mass and (b) dry mass of dung

575 lost at the end of the experiment. Bars represent mean ± SE. Different letters indicate

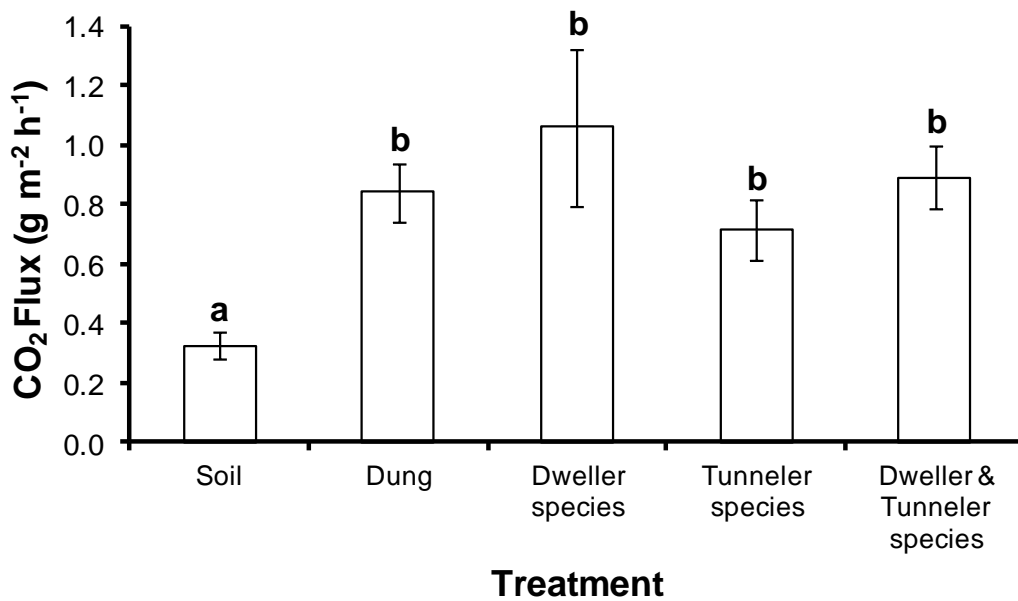576 significant differences between treatments based on 95% CI of parameters estimates.

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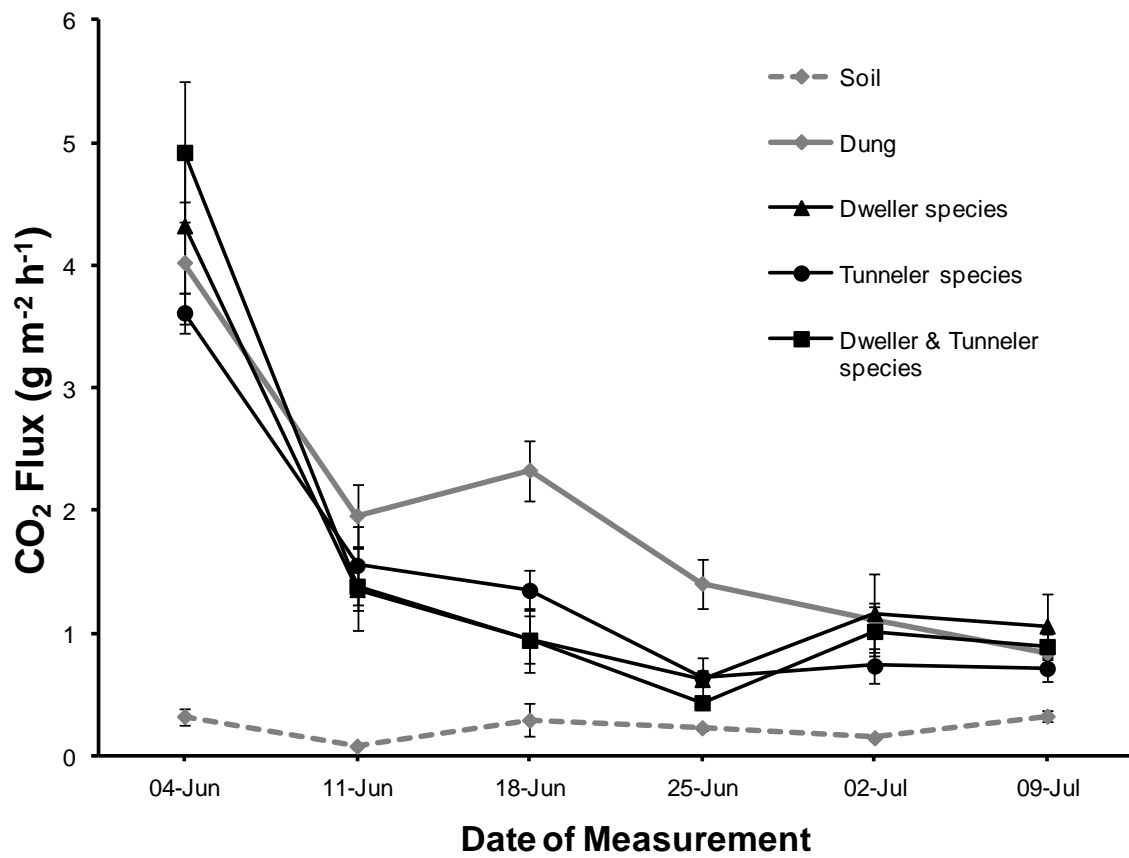
579

580   **Figure 2**. Effects of treatments on changes in microbial respiration (CO_2 flux) over the
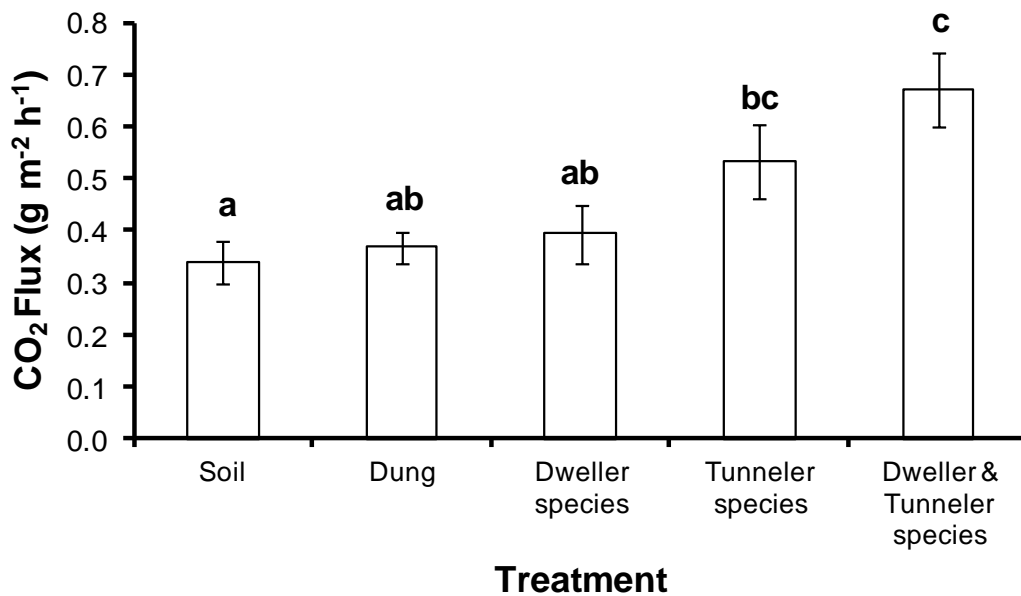
581   six weeks of the study. Bars represent mean ± SE.

582

27

583 **a)**

584

585 **b)**

586

587 **Figure 3.** Effects of treatments on microbial respiration (CO_2 flux) (a) from the whole

588 mesocosm and (b) from the soil after the remaining dung on the soil surface has been

589 removed at the end of the experiment. Bars represent mean ± SE. Different letters indicate

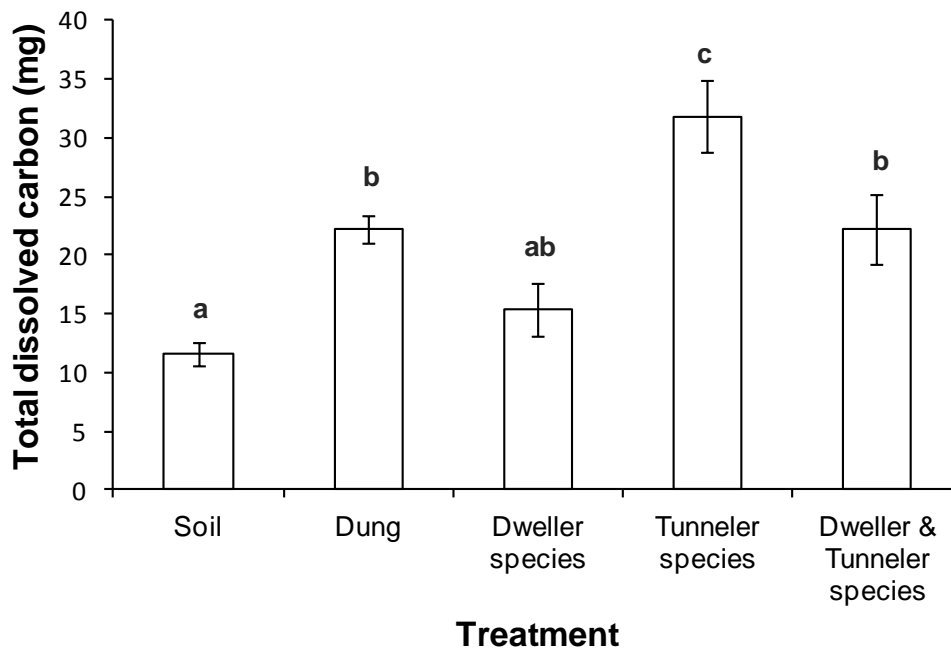590 significant differences between treatments based on 95% CI of parameters estimates.

591

592 **a)**

593
594
595 **b)**
596
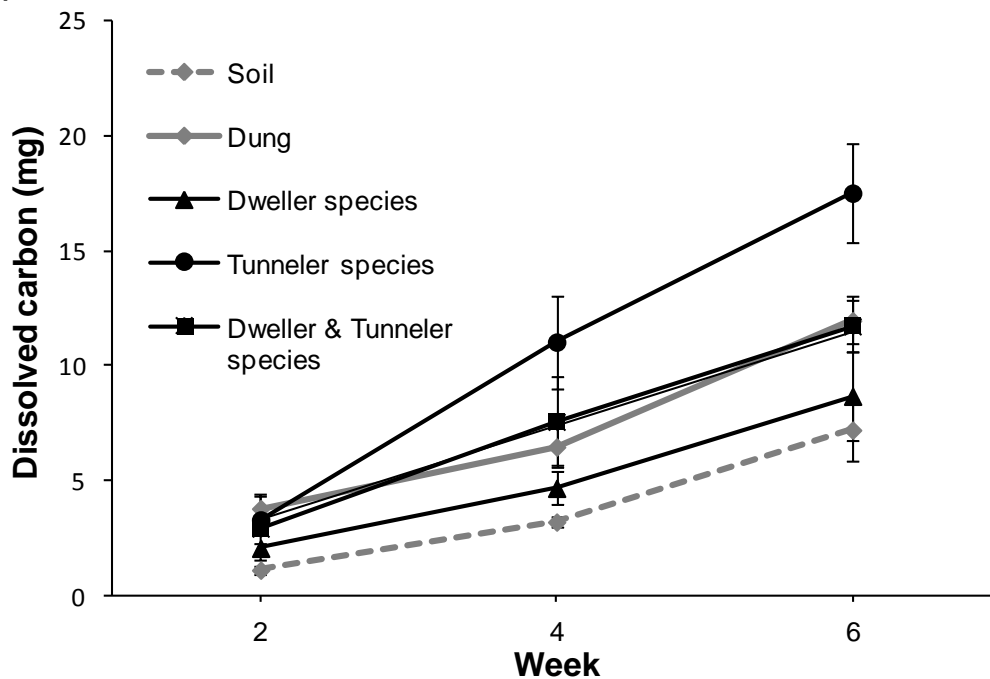
597
598 **Figure 4**. Effects of treatments on (a) changes in dissolved carbon collected from

599 leachates over the six week duration of the study (measures taken every 2 weeks) and (b)

600 total amount of dissolved carbon collected from leachates throughout the experiment.

601 Bars represent mean ± SE. Different letters indicate significant differences between

602 treatments based on 95% CI of parameters estimates.

603

604 **a)**
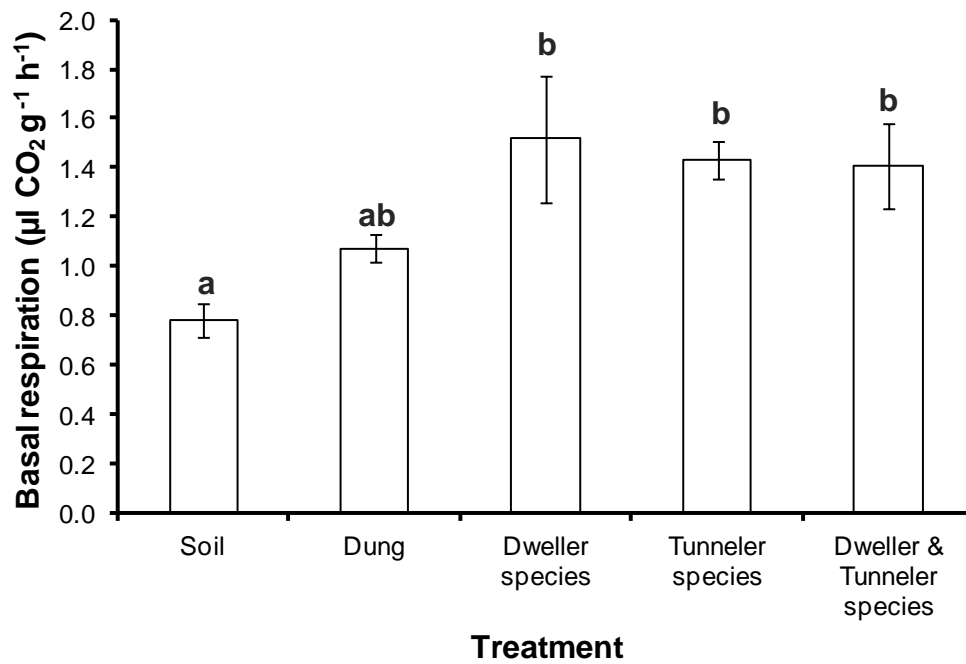
605

606 **b)**
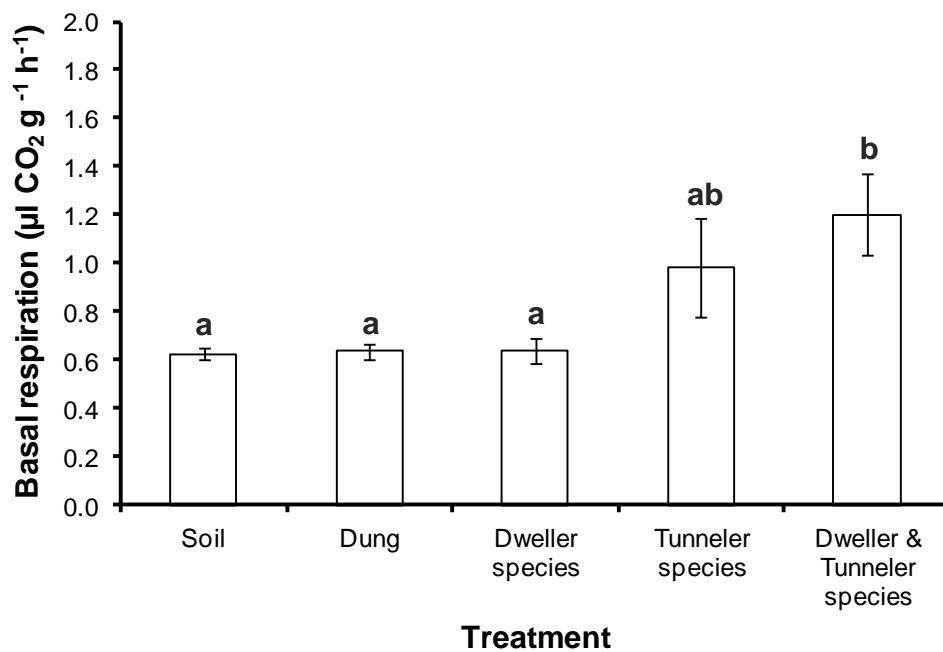
607

608 **Figure 5.** Effects of treatments on microbial basal respiration in (a) the 0-2 cm soil depth

609 increment and (b) the 2-10 cm soil depth increment. Bars represent mean ± SE. Different

610 letters indicate significant differences between treatments based on 95% CI of parameters

611 estimates.