1 **Disentangling the 'brown world' faecal-detritus interaction web:**

2 **dung beetle effects on soil microbial properties**

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15 **Abstract**

16 Many ecosystem services are sustained by the combined action of microscopic and macroscopic
17 organisms, and shaped by interactions between the two. However, studies tend to focus on only one
18 of these two components. We combined the two by investigating the impact of macrofauna on
19 microbial community composition and functioning in the context of a major ecosystem process: the
20 decomposition of dung. We compared bacterial communities of pasture soil and experimental dung
21 pats inhabited by one (*Aphodius*), two (*Aphodius* and *Geotrupes*), or no dung beetle genera. Overall,
22 we found distinct microbial communities in soil and dung samples, and that the communities
23 converged over the course of the experiment. Characterising the soil microbial communities
24 underlying the dung pats revealed a significant interactive effect between the microflora and
25 macrofauna, where the diversity and composition of microbial communities was significantly
26 affected by the presence or absence of dung beetles. The specific identity of the beetles had no
27 detectable impact, but the microbial evenness was lower in the presence of both *Aphodius* and
28 *Geotrupes* than in the presence of *Aphodius* alone. Differences in microbial community
29 composition were associated with differences in substrate usage as measured by Ecoplates.
30 Moreover, microbial communities with similar compositions showed more similar substrate usage.
31 Our study suggests that the presence of macrofauna (dung beetles) will modify the microflora
32 (bacteria) of both dung pats and pasture soil, including community diversity and functioning. In
33 particular, the presence of dung beetles promotes the transfer of bacteria across the soil-dung
34 interface, resulting in increased similarity in community structure and functioning. The results
35 demonstrate that to understand how microbes contribute to the ecosystem process of dung
36 decomposition, there is a need to understand their interactions with larger co-occurring fauna.

37 **Keywords:** ecosystem functioning, below-ground biodiversity, dung decomposition

38 **Introduction**

39 Dung is a major input of nutrients and carbon into soil food webs, particularly in agricultural
40 systems (Aarons et al. 2009, Yoshitake et al. 2014). Dung also plays an important role in regulating
41 key soil ecosystem processes, such as nutrient cycling and organic matter decomposition (Van Der
42 Heijden et al. 2008; Wagg et al. 2014; Wall et al. 2010). There is thus a need to understand the
43 ecological factors that help or hinder the impact of dung on belowground functioning. However,
44 even though the 'brown' world of faecal-detritus interaction webs and decomposition processes
45 form a fundamental link between above and below ground biodiversity – and play a major role in
46 ecosystem functioning – brown interaction webs remain notoriously understudied as compared to
47 their green, plant-based equivalents (Nichols 2013).

48 Among the macrofauna involved in the faecal-detritus pathway, dung beetles (Coleoptera:
49 Scarabaeidae) have been a focal group for studies linking biodiversity to ecosystem functioning
50 (Nichols and Gardner 2011; Spector 2006). Dung beetles have been shown to contribute crucially to
51 key processes such as nutrient recycling and dung removal across ecosystem-types across the world
52 (Nichols et al. 2008), and the loss of dung beetle species or changes in beetle community structure
53 following habitat disturbance or environmental perturbations can have detrimental effects on
54 ecosystem functioning (Beynon et al. 2012; Larsen et al. 2005; Slade et al. 2011). Nonetheless, of
55 the benefits attributed to the beetles, only part of these derive from their own removal, burying or
56 digestion of the dung; an unknown fraction comes from the indirect effects of microbes. However,
57 interactions among dung, dung beetles, and soil and dung microbial communities are poorly
58 studied.

59 Among dung beetles, different functional groups have been hypothesized to have different
60 functional impacts (Rosenlew and Roslin 2008; Slade et al. 2007). Among the dominant dung
61 beetle groups of Northern Europe, large tunnelling *Geotrupes* remove and bury dung outside of the
62 pat, whereas the smaller dung-dwelling *Aphodius* are mainly active within and very close to the

63 dung pat (Hanski and Cambefort 1991; Roslin et al. 2014). We may therefore predict *a priori* that
64 these different taxa will have different impacts on both dung decomposition and on microbial
65 communities. By burying dung, tunnelers may break the dung-soil interface more efficiently than
66 the dung dwellers, whereas the dwellers may contribute to aerating the pats with their tunnels (cf.
67 Penttilä et al. 2013).

68 In this paper, we explore linkages between microfloral and macrofaunal community
69 composition and their effects on ecosystem functioning. We compare the bacterial communities of
70 pasture soil and experimental dung pats inhabited by one (*Aphodius*), two (*Aphodius* and
71 *Geotrupes*) or no dung beetle genera. Specifically, we examine (1) how the microbial community in
72 soil and dung is affected by dung beetle activity, (2) how potential dung beetle-mediated changes in
73 microbial community structure are reflected in microbial functioning, and (3) whether overall, dung
74 beetles may serve as mobile links between above- and below-ground decomposition processes, thus
75 modifying the microbial contribution to dung decomposition.

76

77 **Methods**

78 *Dung beetle communities*

79 To explore the direct and indirect impacts of dung beetles on dung decomposition, we used
80 mesocosms to construct dung beetle communities of varying richness and relative abundance. These
81 communities were built from four common early-summer north temperate species: *Geotrupes*
82 *stercorarius* (Linnaeus, 1758), *Aphodius erraticus* (Linnaeus, 1758), *Aphodius pedellus* (De Geer,
83 1774), and *Aphodius fossor* (Linnaeus, 1758). The number of species encountered per natural dung
84 pat in temperate regions is typically low (median 2 species per pat, range 1-8 in a sample of 797
85 dung pats from across Finland; recalculated from Roslin (2001)), so we constrained our experiments
86 to relatively small and thereby realistic species pools. Within experimental assemblages, the

87 abundance of each species reflected their abundance observed in the field (Rosenlew and Roslin
88 2008; Roslin and Koivunen 2001).

89 Our previous work suggested that the presence of large tunnelling *Geotrupes* species have
90 larger effects on ecosystem functions than the species composition of small dung-dwelling
91 *Aphodius* (Kaartinen et al. 2013; Rosenlew and Roslin 2008). Here, we therefore focus on
92 comparisons between mesocosms containing both *Geotrupes stercorarius* and *Aphodius* (n = 20
93 mesocosms) and mesocosms containing only *Aphodius* species (n = 20 mesocosms). Three
94 mesocosms containing dung pats but no beetles were constructed as controls.

95

96 *Experimental setup*

97 The experiment was carried out on a grass sward reflecting a multiannual Finnish pasture, located in
98 Viikki, Helsinki, Southern Finland (60° 13' 31" N 25° 1' 0" E). Individual mesocosms were
99 constructed from plastic buckets with their base removed (cylinder 58 cm in diameter at ground
100 level, height 32cm, dug 20 cm into the ground). To prevent the beetles from escaping, the tops of
101 the mesocosms were covered with environmental mesh (1-mm aperture). The mesocosms were laid
102 out in a grid pattern, and the spatial distribution of replicates within each treatment was randomized
103 across the grid.

104 Dung beetles were collected from the pastures of the Koskis Manor in Salo, Southwestern
105 Finland (60° 22' 49" N 23° 17' 39" E) and Karjalohja (60° 11' 28" N 23° 40' 19" E) between 5-7th
106 June 2012. Beetles were stored in mixed-sex groups in moist paper at 5°C, until being assigned
107 randomly to treatments. Fresh, unmedicated cattle dung was collected from a closed cattle barn at
108 the Viikki Study and Research Farm, owned by the University of Helsinki. No animal in the herd
109 had been given antibiotics or antiparasitic treatments for at least a year. All dung was homogenized

110 before dividing into 1.2 l experimental pats that were then applied to the mesocosms within 5 hours
111 of collection.

112 Dung and beetles were added to the mesocosms on 8th June 2012. The experiment was run
113 for 60 days, roughly corresponding to the adult and larval lifecycle of the beetles included in the
114 experiment. To allow the beetles to emigrate rather than forcing them to artificially stay in the same
115 pat (cf. Roslin 2000), mesh tops were removed after 20 days. Vegetation inside the mesocosms was
116 kept low by manual trimming.

117

118 *Microbial measurements*

119 SAMPLING – To characterise the microbial community of dung and soil, samples were taken at the
120 early, mid- and late phase of the experiment. Sampling of soil and dung was differently timed due
121 to the successional processes involved. For the soil, the sampling was scheduled to cover the time
122 frame of other measurements (see below). For pats, we compressed the sampling, since dung pats
123 are already mostly decomposed and desiccated after four weeks, and by day 60, there is often only
124 the crust remaining (Kaartinen et al. 2013). Thus, from dung, samples were taken at day 0, 12 and
125 31 from the underside of the dung pat using a spatula. From soil, samples were taken on days 0, 12
126 and 60 from directly underneath the pat to 8–9 cm depth using a soil corer (⌀ 6 mm).

127 To account for heterogeneity within the pat and soil, each sample consisted of three
128 approximately 1-g dung or soil samples taken from different parts of the pat or the soil underneath.
129 The three replicate samples were collected into a sterile bag, placed immediately in a cool box,
130 homogenised and then transferred to a -80°C freezer within 1-8 hours after collection. To record the
131 microbial communities at the start of the experiment, on day 0, samples were taken only from six
132 control pats and from the soil in 12 mesocosms before the dung was added. As the dung was

133 homogenised before being placed in the mesocosms, we assumed that the starting microbial
134 communities were the same in all mesocosms.

135 DNA EXTRACTION AND COMMUNITY FINGERPRINTING WITH LH- PCR – For each sample, DNA was
136 extracted from 0.25 g of dung or soil with an MO BIO PowerSoil DNA Extraction Kit (MO BIO
137 Laboratories, Carlsbad, CA, USA), following the manufactures instructions with limited
138 modifications: the bead beating step was done with a FastPrep®-24 Instrument (MP-Biomedicals,
139 Illkirch, France) for 30 seconds at a speed of 4 m s^{-1}. At the last step, dung and soil samples were
140 eluted in 100 µl and 70 µl of elution solution, respectively.
141 Bacterial communities were profiled using the LH-PCR fingerprint method as described in
142 Mikkonen et al. (2014) .The bacterial 16S rRNA gene was amplified with PCR primes fD1 (AGA
143 GTT TGA TCC TGG CTC AG) (Weisburg et al. 1991) and FAM-labelled primer PRUN518r (ATT
144 ACC GCG GCT GCT GG) (Muyzer et al. 1993). PCR reactions were carried out in a 25 µl volume
145 with 0.5 µl of DNA extract as a template. DNA extract from dung was diluted 1:10 in sterile water
146 to avoid inhibition. The PCR reaction mix included 1 U of Biotools Ultratools DNA polymerase (1
147 U μl^{-1}, Biotools, Spain), 0.3 µM of both primers (Oligomer, Finland), 0.2 mM of each dNTP (dNTP
148 Mix, 10 mM Each, Thermo Scientific Finland), 25 µg BSA (BSA acetylated, 10 mg ml^{-1}, Promega,
149 USA), and 1x Biotools reaction Buffer with 2 mM $MgCl_2$ (Biotools, Spain). PCR reactions were
150 carried out with the following program: initial denaturation at 94 °C for 5 min, followed by 30
151 cycles of 94 °C for 45 seconds, 55 °C for 1 minute, 72 °C for 1 minute and finalised with an
152 elongation step at 72 °C for 5 minutes. All PCR products were run on 1 % agarose gel and
153 visualised under UV light with ethidium bromide (Sigma-Aldrich, USA) to verify the quality and
154 quantity of the DNA.

155 PCR amplicons were separated by their length through capillary electrophoresis. Samples
156 for electrophoresis consisted of 14 µl of Hi-Di formamide (Hi-Di Formamide, Genetic Analysis
157 Grade, Applied Biosystems), 1 µl of 1/200 diluted self-made standard that had three known length

158 HEX-labelled PCR products (Tiirola et al. 2003) and 1–2 µl of PCR product. Samples were
159 denaturated in 98 °C for 3 minutes, then run in a ABI PRISM® 310 Genetic analyzer (Applied
160 Biosystems) as described in Mikkonen et al. (2011) with a 47 cm long sequencing capillary and
161 POP-6™ polyacrylamide as a polymer (Applied Biosystems). Raw data were scanned with program
162 GeneScan 3.7 (Applied Biosystems) and the data were further analysed with BioNumerics 6.0
163 (Applied Maths, Sint-Martens-Latem, Belgium) as described in Mikkonen et al. (2011). The active
164 area of the fingerprint was restricted to the expected PCR amplicon size 460-550 bp. FAM labelled
165 sample curves were normalized with the internal HEX-labelled standards.

166

167 *Ecosystem functioning measurements*

168 To understand how dung beetles, microbes and their interactions affect ecosystem functioning, we
169 measured multiple functional properties associated with the decomposition process.

170 Dung mass loss was measured as cumulative mass loss over the 60 days of the experiment,
171 calculated from wet weights taken every 10 days. Changes in dung mass established by this method
172 will reflect both desiccation and actual dung removal and/or respiratory loss of mass by pat-
173 dwelling species (Kaartinen et al. 2013; Rosenlew and Roslin 2008; Wall and Strong 1987).
174 Nonetheless, by the end of the experiment the humidity of all dung pats will have equilibrated with
175 the environment, rendering remaining mass a valid measure of the overall fraction of mass
176 decomposed (see Kaartinen et al. 2013 for an in-depth treatment). Overall respiration (CO_2 fluxes)
177 was measured throughout the experiment using a closed chamber method and a portable EGM-4
178 infrared CO_2 analyser.

179 To investigate how different dung beetle communities affect the functional profile of
180 microbial communities, we used Biolog Ecoplates (Biolog Inc., Hayward, CA, USA). Each well of
181 the EcoPlates contains an individual substrate, with 31 carbon substrates overall. While the

182 substrates represent only a small fraction of those that might be available in natural environments,
183 the rate of breakdown of individual substrates gives an indication of the metabolic capacity of a
184 community (Garland 1997; Garland and Mills 1991). Dung and soil samples for inoculation were
185 collected at the end point of the experiment (dung: day 31, soil: day 60) as described above.
186 Samples were stored at 20°C overnight, then added to the EcoPlates and incubated for 5 days at
187 20°C. For each sample, 1 g of dung or soil was suspended in 4 ml (dung) or 8 ml (soil) of PBS
188 buffer (137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, at pH 7.4), and the homogenised
189 suspension was serially diluted in PBS. One set of the 31 carbon substrates was inoculated per
190 mesocosm by pipetting 150 µl of 10^{-4} diluted dung suspension or 10^{-3} diluted soil suspension into
191 the wells. Colour development was measured using an Infinite M200 microplate-reader (Tecan,
192 Groedig, Austria) at OD_{590} nm at 0 h, 24 h, 30 h, 48 h, 54 h, 72 h, 102 h and 126 h after inoculation.
193 We scored positive microbial growth if growth exceeded that observed in 95% of the water controls
194 (Gravel et al. 2011). Substrate usage (single Carbon Substrate Utilisation rates (sCSUR)) was
195 calculated as the area under the growth curve. The usage of substrates not exceeding water controls
196 was set to zero. As a measure of overall metabolic capacity, we defined the total substrate usage
197 across the Ecoplate, summed across all substrates (total carbon substrate utilisation rate (tCSUR)).
198 To pinpoint differences in the metabolic profile of different communities, we then divided the
199 substrates into five categories: carbohydrates, amino acids, carboxylic/acetic acids, polymers, and
200 amines/amides (Berga et al. 2012; Zak et al. 1994), and calculated mean substrate usage within each
201 category. The richness and diversity of substrate usage within each category was calculated as the
202 mean number, and the inverse of the Simpson Index (as above), respectively, of substrates showing
203 positive growth.
204
205 *Analyses*

206 DIFFERENCES BETWEEN SUBSTRATES AND SAMPLING PERIODS – The final sampling date differed
207 between soil and dung (see above), so temporal patterns were analysed separately for the two
208 substrates. To describe the microbial community, microbial operational taxonomic units (OTUs)
209 were defined as peaks in the LH-PCR traces, and OTU richness was defined as the total number of
210 OTUs in each profile. To identify peaks, LH-PCR traces were first smoothed by fitting a cubic
211 spline using the default settings in the smooth.spline function in the base stats package of R (R
212 Development Core Team 2013). OTUs were then delimited by identifying the peaks and valleys in
213 the trace. We used relative peak area as a proxy of relative abundance, and calculated Simpson
214 indices (D=1/sum of the squared relative abundances) to describe the diversity and evenness
215 (1/D/species richness) in each sample. For microbial OTU diversity and richness, we built
216 generalised linear models with normally and Poisson-distributed errors, respectively. Each response
217 was modelled as a function of the dung beetle community (*Aphodius* only, *Aphodius* and
218 *Geotrupes,* No Dung Beetles) and day (12, 31 or 60) as categorical fixed effects. In all cases, we
219 started from the full model including all main effects and their interactions, then removed non-
220 significant interactions until arriving at the minimum adequate model, for which results are
221 presented.
222

223 INTERACTIVE EFFECTS OF MICROBES AND DUNG BEETLES ON ECOLOGICAL FUNCTIONING – As both
224 dung beetle and microbial community composition varied in our experiment, we took a multistep
225 approach to examine their respective contributions to decomposition processes:
226 To examine the changes in microbial community composition within the dung and soil
227 beneath it we tested whether the degree of community dissimilarity (Bray-Curtis pairwise
228 dissimilarities) depended on the substrate (dung or soil) or on time, or on their interaction.
229 Microbial community dissimilarity was calculated as the ratio of ((mean dissimilarity within dung
230 or soil) / (mean dissimilarity between dung and soil)) dissimilarity over time (calculated for day 0,

231 12, 31/60). We then established whether the presence of *Aphodius*, or of *Aphodius* and *Geotrupes*
232 affected microbial community composition *per se*. We used permutational multivariate analysis of
233 variance (permutational MANOVA) calculated using the Bray-Curtis dissimilarity index for both of
234 these analyses. Statistical tests were calculated using the R function *adonis* in the package *vegan*
235 (Blackwood et al. 2007; Oksanen et al. 2009), and communities were visualised using nonmetric
236 multidimensional scaling (NMDS) implemented in *metaMDS* in *vegan*.

237 To examine whether similarity in microbial community composition was reflected in
238 similarity in function, we used Mantel tests to compare matrices of Bray-Curtis dissimilarity in LH-
239 PCR profiles (at day 30 and 60 for Ecoplates and at day 12 for dung decomposition and CO_2 fluxes)
240 to matrices describing similarity in 1) substrate usage on Ecoplates (similarity described by the
241 Bray-Curtis metric at the end of the experiment; 2) dung decomposition, measured as the slope of
242 the regression of dung mass loss on time (with similarity described by Euclidean distance) and 3)
243 CO_2 flux (using the average of fluxes from day 10 and 14, as no flux data was collected on day 12,
244 and again describing similarity by Euclidean distance). In each case, we compared the observed
245 Pearson correlation coefficient to values generated by 999 permutations. A significant association
246 would signal that communities more similar in structure were also more similar in function than
247 expected by chance alone. Linear models were used to test if cumulative CO_2 fluxes or cumulative
248 dung mass loss (both log-transformed to meet the assumptions of normality) differed among the
249 dung beetle communities (*Aphodius* only, *Aphodius* and *Geotrupes,* No Dung Beetles). All analyses
250 were carried out in R version 3.0.1 (R Development Core Team 2013).

251

252 **Results**

253 *Microbial community composition*

254 Overall, distinct microbial communities were found in soil and dung samples, and there were
255 significant temporal changes in community composition (Table 1, Fig.1, Supplementary material,
256 Appendix 1, Fig. A1). Over the course of the experiment and with the drying-out of the dung, the
257 microbiome of the soil and of the dung converged (Fig.1, Supplementary material, Appendix 1, Fig.
258 A1). The degree of community dissimilarity depended on the substrate (dung or soil) ($F_{1,180}$= 30.65,
259 P=0.001), and on time ($F_{1,180}$= 43.64, P=0.001). The interaction between the substrate and time was
260 also highly significant ($F_{1,180}$= 39.18, P=0.001), indicating that the degree of dissimilarity among
261 the substrates is dependent on the sampling date. Further analysis of soil collected from beneath the
262 dung pats indicated that microbial community composition of soil under dung pats was significantly
263 affected by the presence of dung beetles (Table 1a), whereas the specific identity of the beetles
264 (*Aphodius* or *Geotrupes*) had no further detectable impact on this comparison (Table 1b). Within
265 dung, dung beetles had no detectable effect on microbial community composition (Table 1c).
266 The presence of dung beetles also affected the microbial diversity observed in the soil
267 underneath dung pats. Soil microbial diversity significantly changed with the identity of the beetles
268 ($F_{2,82}$= 3.80, P=0.03). Microbial diversity was lower in the presence of both *Aphodius* and
269 *Geotrupes* than in the presence of *Aphodius* alone. There was no significant effect of day on soil
270 microbial diversity ($F_{1,82}$= 2.44, P>0.1). Although there were no significant effects of day or dung
271 beetle treatment on species richness (P>0.9 in both cases), the evenness of the microbial
272 communities was impacted by the dung beetle treatments ($F_{2,82}$= 3.79, P=0.03), and was lowest
273 when both dung beetle genera were present. Dung microbial OTU richness and diversity did not
274 significantly differ over time or among the dung beetle treatments (P>0.4 in all cases).
275
276 *Microbial functioning*
277 The microbial communities in soil and dung were associated with different functional profiles as
278 measured by the Ecoplates (Fig. 2). However, the presence or absence of dung beetles, or the

279 particular dung beetle taxa involved had no further detectable impact on this difference

280 (MANOVA: dung: $F_{2,42}$= 1.14, P=0.29; soil: $F_{2,40}$= 0.7, P=0.8). When the effect of dung beetles on

281 microbial activity in dung and soil was analysed in further detail (number of substrates utilised,

282 diversity of substrates utilised, total substrate utilisation rate (tCSUR), proportion of substrate

283 categories), the presence of dung beetles had no significant effect on soil microbial activity (P> 0.08

284 in all cases).

285 Carbon substrate utilisation rates (sCSURs) in dung and soil differed among substrates

286 (dung: $F_{4,208}$=21.94 , P>0.001; soil: $F_{4,208}$=10.87, P>0.001), with polymers having the highest rates

287 and amines the lowest (Fig. 3a, b). There were also significant differences among the dung beetle

288 treatments in sCSUR in the dung ($F_{2,208}$=3.38 , P=0.04). In dung, mesocosms with *Aphodius* and

289 *Geotrupes* had higher utilisation sCSURs (Fig. 3a). In soil, the presence of dung beetles did not

290 increase utilisation rates ($F_{2,208}$=0.03, P=0.97; Fig. 3b).

291 Differences in the composition of microbial communities as resulting from either dung

292 beetle treatment or substrate (dung or soil) were correlated with differences in functional rates.

293 Overall, we found a significant positive correlation between similarities in microbial community

294 composition and similarities in substrate usage across dung and soil samples collected on days 31

295 and 60, respectively (Mantel test: r=0.17, P=0.008). This significant association was also evident

296 when the data were broken down into samples from dung (r=0.14, P=0.05) *versus* soil (r=0.21,

297 P=0.025), as collected on single dates. The similarity in the rate of dung decomposition was also

298 significantly positively correlated with similarities in dung microbial community composition

299 (r=0.21, P=0.005), but not with similarity in soil microbial community composition (r =-0.001,

300 P=0.52). Similarities in CO_2 fluxes were not detectably associated with similarities in either the soil

301 or dung microbial communities (r=0.01, P=0.41 versus r=0.08, P=0.21, respectively). There were

302 no detectable differences among dung beetle treatments for cumulative CO_2 fluxes ($F_{2,30}$=0.27,

303 P=0.76), or cumulative dung mass loss ($F_{2,40}$=2.97, P=0.063) (Supplementary Material, Appendix 1,
304 Fig. A2).

305

306 **Discussion**

307 Our results demonstrate an important interaction between dung beetles and microbial communities
308 in dung and soil, providing a link in biogeochemical cycling in agricultural systems. While the
309 microbial communities of dung and soil are initially different, they converge over time on the
310 pasture. During this process, dung beetle communities modify some aspects of both microbial
311 community structure and functioning in both the dung pats and in the soil underneath them. By
312 doing so, we suggest that the beetles may serve as mobile links between decomposition processes
313 occurring above and below ground. Thus, the bioturbation process offered by beetles may serve to
314 homogenise both microbial community structure and functioning across the soil-surface boundary.
315 Below, we will address each of these observations in turn.

316 Dung is a major source of nutrients and carbon into soil food webs, particularly in
317 agricultural systems (Aarons et al. 2009; Yoshitake et al. 2014). Microbial activity is a key driver
318 behind soil carbon and nutrient cycling (Falkowski et al. 2008), and has been extensively studied,
319 for example in the context of carbon storage (Trivedi et al. 2013). Contrasting with such studies is a
320 major body of literature focusing on the role of macroscopic invertebrates in the decomposition of
321 dung. Among such taxa, dung beetles have been identified as the most important invertebrate
322 contributors to dung decomposition in temperate agricultural grasslands (Lee and Wall 2006).
323 Despite the evident potential to incorporate microbial processes into studies of dung beetles, the
324 link between dung beetles, dung and soil microbes and biogeochemical cycling has never been
325 explicitly explored. With global increases in cattle farming, and hence greenhouse gas emissions
326 from agriculture (Bellarby et al. 2013; FAO 2006), it is important to examine the processes
327 contributing to the decomposition of cattle dung.

328 Our study revealed substantial differences in the microbial communities of dung and soil – 329 and also differences in microbial functioning among these strata. Initial differences in the 330 microbiome of the dung and the soil reflect both the specific composition of the substrate (cattle 331 fodder versus soil) and the specific conditions prevailing in the digestive tract of the ruminants (de 332 Menezes et al. 2011; Kim et al. 2011). After the dung is deposited in the pasture, the microbiome of 333 the pat is exposed to ambient conditions and eventually converges towards that of the soil – as 334 paralleled by increasing convergence of functioning. On this process, the dung beetles left an 335 imprint. In particular, in terms of community structure, microbial evenness was lower in the 336 presence of both *Aphodius* and *Geotrupes* than in the presence of *Aphodius* alone. However, the 337 presence of dung beetles and their community composition had little effect on affect overall 338 microbial functioning in either dung or soil. This may in part be due to the caveats associated with 339 using Ecoplates to assess functional profiles. Importantly, Ecoplates measure potential substrate 340 usage rather than actual substrate usage. Thus, if there are a lot of inactive bacteria in the soil, some 341 may proliferate when added to suitable substrates on the Ecoplates. In this case, there will be no 342 difference in substrate use on the plates (as it will be high everywhere), despite pronounced 343 differences in the field (where it will be variably high and low depending on whether the bacteria 344 are active or inactive). We suggest that as a next step RNA sequencing is used to reflect gene 345 activity in the field.

346 Utilisation rates of certain substrate categories did, however, increase when dung beetles 347 were present. In particular, amines were utilised more when dung beetles were present and 348 carbohydrates had higher utilisation rates when both *Aphodius* and *Geotrupes* were present than 349 with *Aphodius* alone, thus yielding a different functional profile of microbial communities in the 350 presence versus absence of beetles. One possible explanation for this contrast with *a priori* 351 expectations is that the soil samples were taken close to the surface (maximum depth 9cm), and that

352 the effects of the tunnelling by *Geotrupes* may thus be more pronounced deeper in the soil profile.
353 Future studies will be targeted at resolving such effects.

354 Regardless of the factors giving rise to it, large overall variation in microbial community
355 composition both within and between substrates (soil versus dung) and time periods directly
356 translated to differences in functional rates. Significant association between similarities in microbial
357 community composition and substrate usage add to associations observed for the main function of
358 dung decomposition, where more similar microbial communities were also more similar in terms of
359 how quickly they disposed of dung. Both patterns attest to a general relationship between microbial
360 community composition and functioning (Bell et al. 2009; Bell et al. 2005).

361 Our study suggests that the presence of macrofauna (dung beetles) will modify the
362 microflora (microbes), including its diversity and functioning. In particular, the presence of dung
363 beetles appears promote the transfer of microbes across the soil-surface interface, and result in
364 increased similarity in both community structure and functioning. However, the specific impact of
365 dung beetle groups and interactions between them is less clear. While the patterns reported here
366 apply to aerobic bacteria, we propose that an added focus on the anaerobic part of the community –
367 and on associated functions like methane emissions (see Penttilä et al. 2013) – may prove a
368 particularly interesting avenue for further research.

369

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376

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379 manuscript.
380

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491
492 Supplementary material (Appendix oik.1 at <www.oikosjournal.org/readers/appendix>). Appendix
493 1.

494 Table 1. Results of permutational MANOVAs of community composition (measured as arcsine

495 square-root transformed relative abundance) in two substrates (soil versus dung) as functions of

496 sampling dates and treatments.

497

Term	Df	F	P value
1a: Soil			
Day[a]	1,82	27.61	**0.001**
Treatment[b]	2,82	2.78	**0.013**
1b: Soil – Dung only controls removed[c]			
Day	1,77	29.62	**0.001**
Dung beetle treatment	1,77	1.45	0.18
1c: Dung			
Day	1,82	48.81	**0.001**
Dung beetle treatment	2,82	1.8	0.087

498 [a]Day 12 & 31 for dung and Day 12 and 60 for soil.
499 [b]Three treatments: mesocosms with 1) *Aphodius* only, 2) *Geotrupes* present, 3) Controls with dung
500 but no dung beetles.
501 [c]Only mesocosms with 1) *Aphodius* only and 2) *Aphodius* & *Geotrupes* present.
502 All Day by Dung beetle treatment interactions were non-significant (P>0.1 in all cases).

503

504 **Figure Legends**

505 Figure 1. NMDS plots highlighting the changes in the dung (blue points) and soil (red points)
506 microbial community composition over time. The three panels show different points in time, with
507 the complete dataset (grey points) included for reference. Symbols identify mesocosms with *G.*
508 *stercorarius* present (■) versus mesocosms with only *Aphodius* species present (▲).Control
509 mesocosms with dung but no dung beetles are indicated with the symbol +. On day 0, samples
510 were taken only from the six control pats and from the soil in 12 mesocosms before the dung was
511 added (see methods).

512

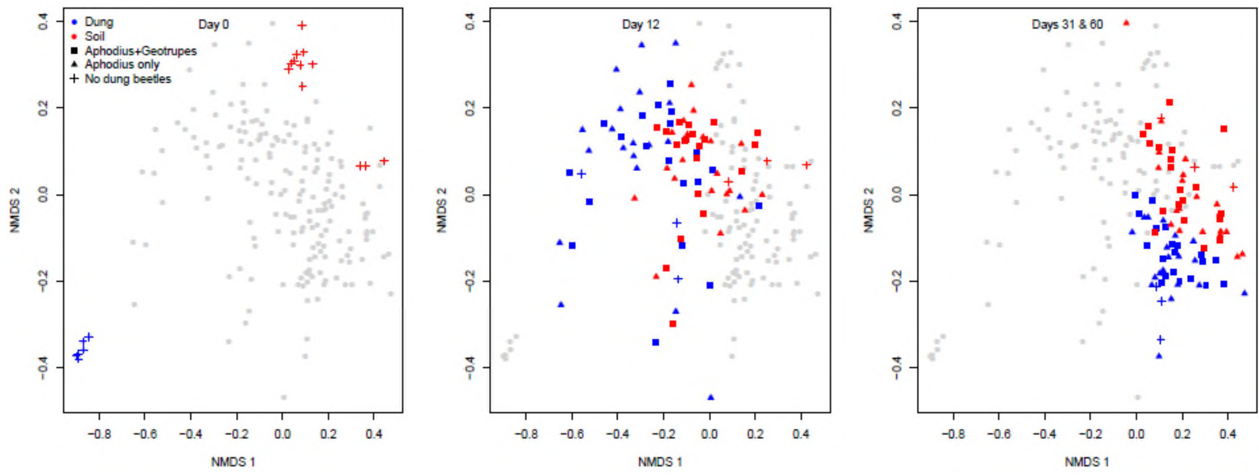
513 Figure 2. NMDS plot showing the utilisation of carbon substrates (based on sCSURs of Ecoplate
514 substrates) in dung (day 31) and soil (day 60) in mesocosms with *Aphodius* and *Geotrupes*
515 *stercorarius* present (■);mesocosms with only *Aphodius* present (▲) and control mesocosms with
516 dung but no dung beetles present (+).

517

518 Figure 3. Microbial activity and functioning measured as mean single carbon substrate utilisation
519 rates (sCSUR) in a) dung (a) and b) soil in the presence of different dung beetle communities.
520 Shown are means ±SE.

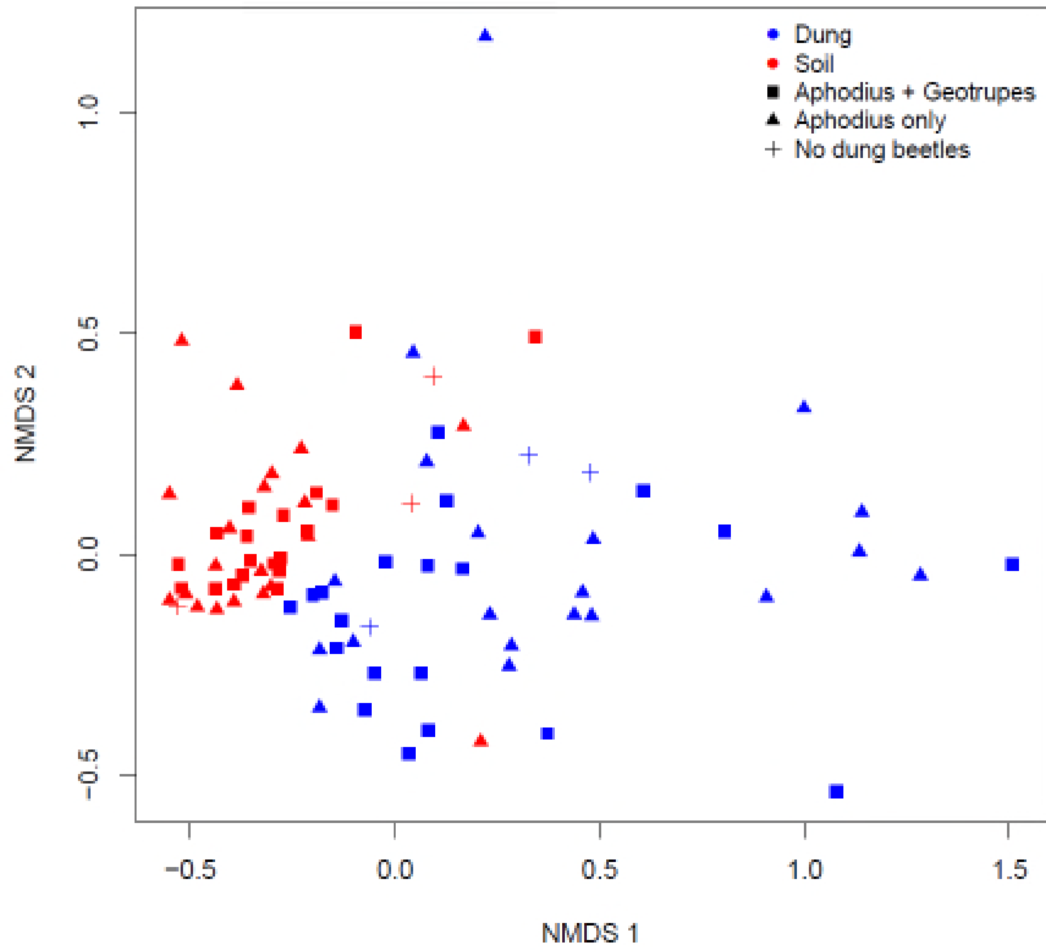
521

522



523 Figure 1.

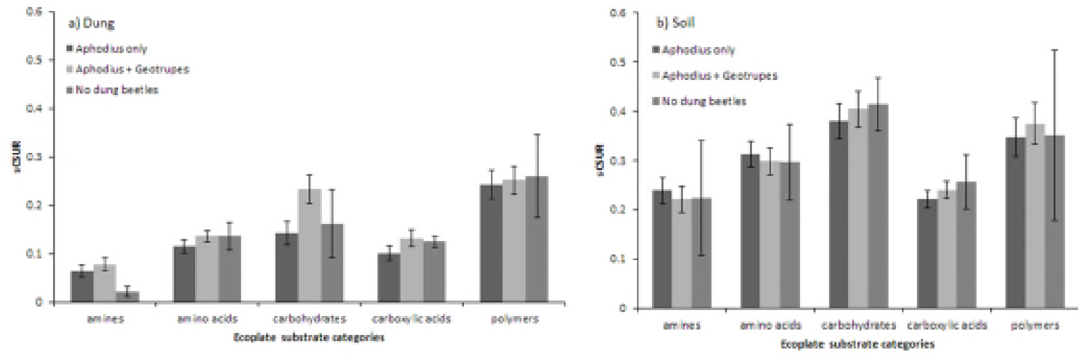
524



525

526 Figure 2.

527



528

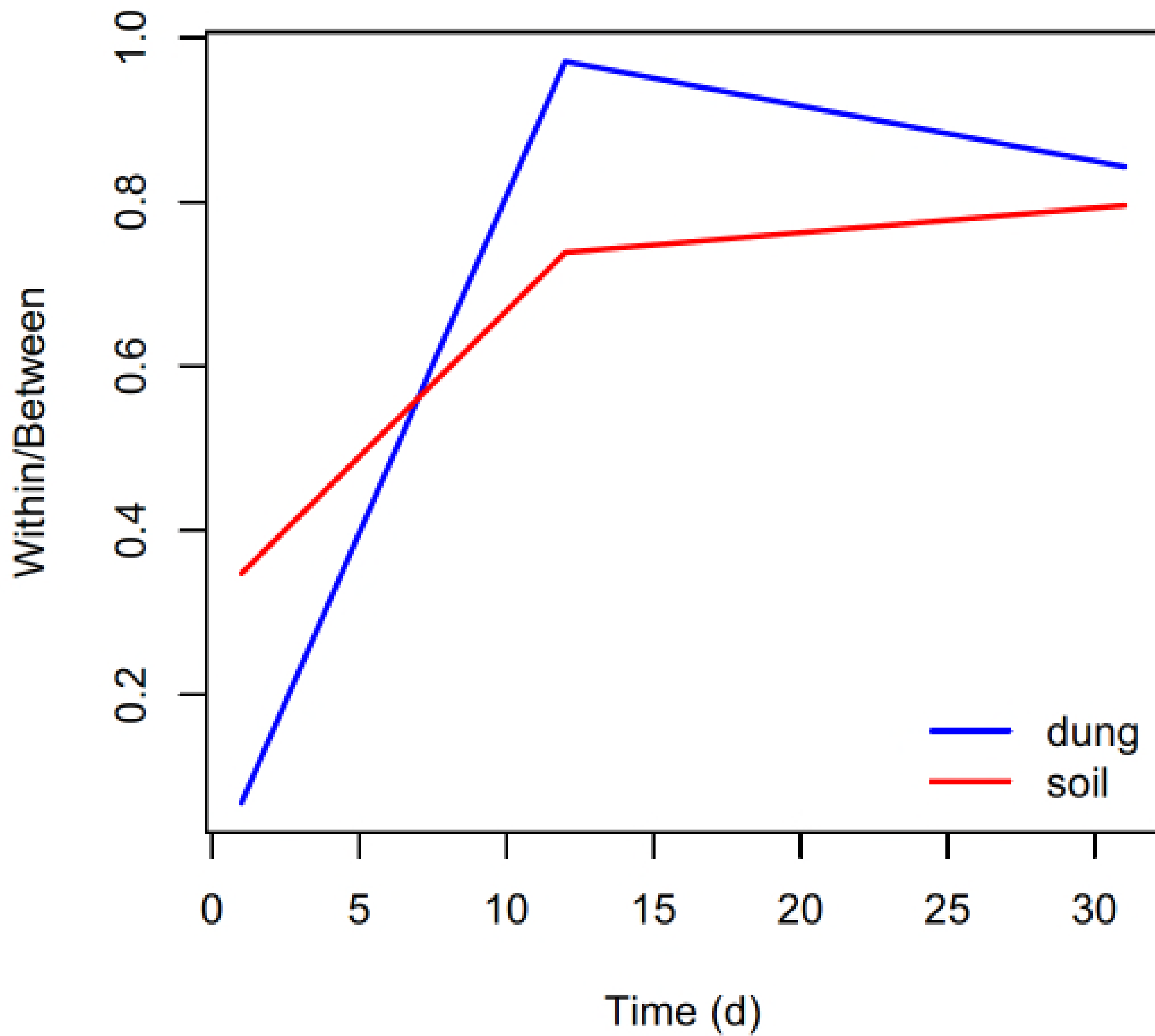
529 Figure 3.

530

531 **Supplementary Material**

532 Appendix 1.

533



534

535 Fig. A1. Changes in microbial community composition over the first 30 days of the experiment

536 (dung microbial samples were only taken up to day 30 (see Methods - Microbial measurements))

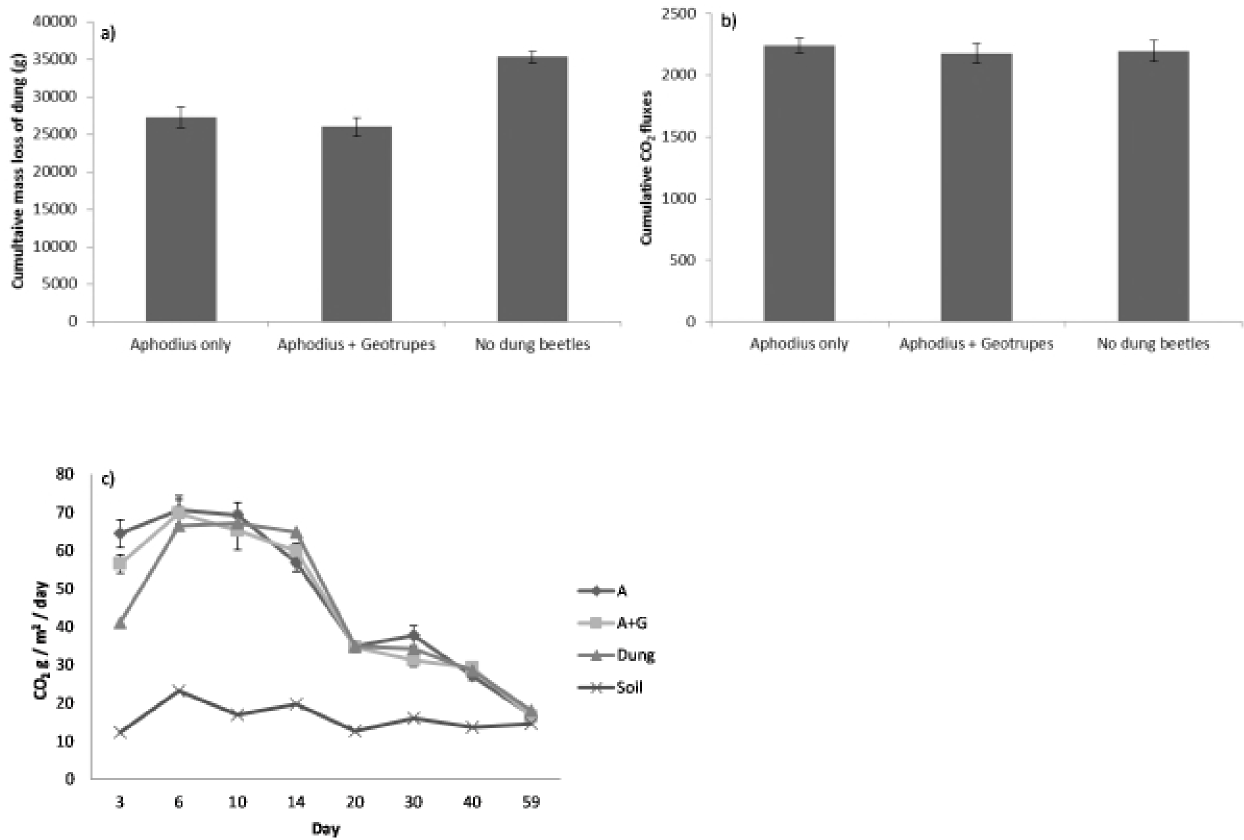
537 within the dung, and soil beneath it. Microbial community dissimilarity for dung and soil is shown

538 as the ratio of ((mean dissimilarity within dung or soil) / (mean dissimilarity between dung and

539 soil)) dissimilarity over time (in days). Values approaching one indicate that dung or soil

540 communities are indistinguishable.

541



542

543 Fig. A2. Changes in a) dung decomposition (measured as cumulative mass loss over the 60 days of

544 the experiment, calculated from wet weights taken every 10 days), b) cumulative CO_2 fluxes

545 (calculated from eight measurements over the 60 days of the experiment), and c) CO_2 fluxes over

546 time, with changes in dung beetle communities. There were no detectable differences among dung

547 beetle treatments for cumulative dung mass loss ($F_{2,40}$=2.97, P=0.063) or cumulative CO_2 fluxes

548 ($F_{2,30}$=0.27, P=0.76).