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

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

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13   **Abstract**

14   Many ecosystem services are sustained by the combined action of microscopic and macroscopic

15   organisms, and shaped by interactions between the two. However, studies tend to focus on only one

16   of these two components. We combined the two by investigating the impact of mesofauna on

17   microbial community composition and functioning in the context of a major ecosystem service: the

18   decomposition of dung. We compared bacterial communities of pasture soil and experimental dung

19   pats inhabited by one (*Aphodius*), two (*Aphodius* and *Geotrupes*), or no dung beetle genera. Overall,

20   we found distinct microbial communities in soil and dung samples, and that the communities

21   converged over the course of the experiment. Characterising the soil microbial communities

22   underlying the dung pats revealed a significant interactive effect between the micro- and

23   mesofauna, where the diversity and composition of microbial communities was significantly

24   affected by the presence or absence of dung beetles. The specific identity of the beetles had no

25   detectable impact, but the microbial evenness was lower in the presence of both *Aphodius* and

26   *Geotrupes* than in the presence of *Aphodius* alone. These differences in microbial community

27   composition were associated with altered functional profiles. Our study suggests that the presence

28   of mesofauna (dung beetles) will modify the microfauna (bacteria) of both dung pats and pasture

29   soil, including community diversity and functioning. In particular, the presence of dung beetles

30   promotes the transfer of bacteria across the soil-dung interface, resulting in increased similarity in

31   community structure and functioning. The results demonstrate that to understand how microbes

32   contribute to the ecosystem service of dung decomposition, there is a need to understand their

33   interactions with larger co-occurring fauna.

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

Introduction

36 Dung is a major input of nutrients and carbon into soil food webs, particularly in agricultural

37 systems (Aarons et al. 2009, Yoshitake et al. 2014). Dung also plays an important role in regulating

38 key soil ecosystem processes, such as nutrient cycling and organic matter decomposition (Van Der

39 Heijden et al. 2008; Wagg et al. 2014; Wall et al. 2010). There is thus a need to understand the

40 ecological factors that help or hinder the impact of dung on belowground functioning. However,

41 even though the 'brown' world of faecal-detritus interaction webs and decomposition processes

42 form a fundamental link between above and below ground biodiversity – and play a major role in

43 ecosystem functioning – brown interaction webs remain notoriously understudied as compared to

44 their green, plant-based equivalents (Nichols 2013).

45   Among the mesofauna involved in the faecal-detritus pathway, dung beetles (Coleoptera:

46 Scarabaeidae) have been a focal group for studies linking biodiversity to ecosystem functioning

47 (Nichols and Gardner 2011; Spector 2006). Dung beetles have been shown to contribute crucially to

48 key processes such as nutrient recycling and dung removal across ecosystem-types across the world

49 (Nichols et al. 2008), and the loss of dung beetle species or changes in beetle community structure

50 following habitat disturbance or environmental perturbations can have detrimental effects on

51 ecosystem functioning (Beynon et al. 2012; Larsen et al. 2005; Slade et al. 2011). Nonetheless, of

52 the benefits attributed to the beetles, only part of these derive from their own removal, burying or

53 digestion of the dung; an unknown fraction comes from the indirect effects of microbes. However,

54 interactions among dung, dung beetles, and soil and dung microbial communities are poorly

55 studied.

56   Among dung beetles, different functional groups have been hypothesized to have different

57 functional impacts (Rosenlew and Roslin 2008; Slade et al. 2007). Among the dominant dung

58 beetle groups of Northern Europe, large tunnelling *Geotrupes* remove and bury dung outside of the

59 pat, whereas the smaller dung-dwelling *Aphodius* are mainly active within and very close to the

60   dung pat (Hanski and Cambefort 1991; Roslin et al. 2014). We may therefore predict *a priori* that

61   these different taxa will have different impacts on both dung decomposition and on microbial

62   communities. By burying dung, tunnelers may break the dung-soil interface more efficiently than

63   the dung dwellers, whereas the dwellers may contribute to aerating the pats with their tunnels (cf.

64   Penttilä et al. 2013).

65   In this paper, we explore linkages between micro- and mesofaunal community composition

66   and their effects on ecosystem functioning. We compare the bacterial communities of pasture soil

67   and experimental dung pats inhabited by one (*Aphodius*), two (*Aphodius* and *Geotrupes*) or no dung

68   beetle genera. Specifically, we examine (1) how the microbial community in soil and dung is

69   affected by dung beetle activity, (2) how potential dung beetle-mediated changes in microbial

70   community structure are reflected in microbial functioning, and (3) whether overall, dung beetles

71   may serve as mobile links between above- and below-ground decomposition processes, thus

72   modifying the microbial contribution to dung decomposition.

73

74   **Methods**

75   *Dung beetle communities*

76   To explore the direct and indirect impacts of dung beetles on dung decomposition, we used

77   mesocosms to construct dung beetle communities of varying richness and relative abundance. These

78   communities were built from four common early-summer north temperate species: *Geotrupes*

79   *stercorarius* (Linnaeus, 1758), *Aphodius erraticus* (Linnaeus, 1758)*, Aphodius pedellus* (De Geer,

80   1774), and *Aphodius fossor* (Linnaeus, 1758). The number of species encountered per natural dung

81   pat in temperate regions is typically low (median 2 species per pat, range 1-8 in a sample of 797

82   dung pats from across Finland; recalculated from Roslin (2001)), so we constrained our experiments

83   to relatively small and thereby realistic species pools. Within experimental assemblages, the

84   abundance of each species reflected their abundance observed in the field (Rosenlew and Roslin

85   2008; Roslin and Koivunen 2001).

86   Our previous work suggested that the presence of large tunnelling *Geotrupes* species have

87   larger effects on ecosystem functions than the species composition of small dung-dwelling

88   *Aphodius* (Kaartinen et al. 2013; Rosenlew and Roslin 2008). Here, we therefore focus on

89   comparisons between mesocosms containing both *Geotrupes stercorarius* and *Aphodius* (n = 20

90   mesocosms) and mesocosms containing only *Aphodius* species (n = 20 mesocosms). Three

91   mesocosms containing dung pats but no beetles were constructed as controls.

92

93   *Experimental setup*

94   The experiment was carried out on a grass sward reflecting a multiannual Finnish pasture, located in

95   Viikki, Helsinki, Southern Finland (60° 13' 31" N 25° 1' 0" E). Individual mesocosms were

96   constructed from plastic buckets with their base removed (cylinder 58 cm in diameter at ground

97   level, height 32cm, dug 20 cm into the ground). To prevent the beetles from escaping, the tops of

98   the mesocosms were covered with environmental mesh (1-mm aperture). The mesocosms were laid

99   out in a grid pattern, and the spatial distribution of replicates within each treatment was randomized

100   across the grid.

101   Dung beetles were collected from the pastures of the Koskis Manor in Salo, Southwestern

102   Finland (60° 22' 49" N 23° 17' 39" E) and Karjalohja (60° 11' 28" N 23° 40' 19" E) between 5-7th

103   June 2012. Beetles were stored in mixed-sex groups in moist paper at 5°C, until being assigned

104   randomly to treatments. Fresh, unmedicated cattle dung was collected from a closed cattle barn at

105   the Viikki Study and Research Farm, owned by the University of Helsinki. No animal in the herd

106   had been given antibiotics or antiparasitic treatments. All dung was homogenized before dividing

107   into 1.2 l experimental pats that were then applied to the mesocosms within 5 hours of collection.

108   Dung and beetles were added to the mesocosms on 8th June 2012. The experiment was run

109   for 60 days, roughly corresponding to the adult and larval lifecycle of the beetles included in the

110   experiment. To allow the beetles to emigrate rather than forcing them to artificially stay in the same

111   pat (cf. Roslin 2000), mesh tops were removed after 20 days. Vegetation inside the mesocosms was

112   kept low by manual trimming.

113

114   *Microbial measurements*

115   SAMPLING – To characterise the microbial community of dung and soil, samples were taken at the

116   early, mid- and late phase of the experiment. Sampling of soil and dung was differently timed due

117   to the successional processes involved. For the soil, the sampling was scheduled to cover the time

118   frame of other measurements (see below). For pats, we compressed the sampling, since dung pats

119   are already mostly decomposed and desiccated after four weeks, and by day 60, there is often only

120   the crust remaining (Kaartinen et al. 2013). Thus, from dung, samples were taken at day 0, 12 and

121   31 from the underside of the dung pat using a spatula. From soil, samples were taken on days 0, 12

122   and 60 from directly underneath the pat to 8–9 cm depth using a soil corer ($\varnothing$ 6 mm).

123   To account for heterogeneity within the pat and soil, each sample consisted of three

124   approximately 1-g dung or soil samples taken from different parts of the pat or the soil underneath.

125   The three replicate samples were collected into a sterile bag, placed immediately in a cool box,

126   homogenised and then transferred to a -80°C freezer within 1-8 hours after collection. To record the

127   microbial communities at the start of the experiment, on day 0, samples were taken only from six

128   control pats and from the soil in 12 mesocosms before the dung was added. As the dung was

129   homogenised before being placed in the mesocosms, we assumed that the starting microbial

130   communities were the same in all mesocosms.

131   DNA EXTRACTION AND COMMUNITY FINGERPRINTING WITH LH- PCR – For each sample, DNA was

132   extracted from 0.25 g of dung or soil with an MO BIO PowerSoil DNA Extraction Kit (MO BIO

133   Laboratories, Carlsbad, CA, USA), following the manufactures instructions with limited

134   modifications: the bead beating step was done with a FastPrep®-24 Instrument (MP-Biomedicals,

135   Illkirch, France) for 30 seconds at a speed of 4 m s^{-1}. At the last step, dung and soil samples were

136   eluted in 100 µl and 70 µl of elution solution, respectively. DNA concentrations were measured

137   with NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Willmington, DE, USA).

138   Bacterial communities were profiled using the LH-PCR fingerprint method as described in

139   Mikkonen et al. (2014) .The bacterial 16S rRNA gene was amplified with PCR primes fD1 (AGA

140   GTT TGA TCC TGG CTC AG) (Weisburg et al. 1991) and FAM-labelled primer PRUN518r (ATT

141   ACC GCG GCT GCT GG) (Muyzer et al. 1993). PCR reactions were carried out in a 25 µl volume

142   with 0.5 µl of DNA extract as a template. DNA extract from dung was diluted 1:10 in sterile water

143   to avoid inhibition. The PCR reaction mix included 1 U of Biotools Ultratools DNA polymerase (1

144   U µl^{-1}, Biotools, Spain), 0.3 µM of both primers (Oligomer, Finland), 0.2 mM of each dNTP (dNTP

145   Mix, 10 mM Each, Thermo Scientific Finland), 25 µg BSA (BSA acetylated, 10 mg ml^{-1}, Promega,

146   USA), and 1x Biotools reaction Buffer with 2 mM MgCl$_2$ (Biotools, Spain). PCR reactions were

147   carried out with the following program: initial denaturation at 94 °C for 5 min, followed by 30

148   cycles of 94 °C for 45 seconds, 55 °C for 1 minute, 72 °C for 1 minute and finalised with an

149   elongation step at 72 °C for 5 minutes. All PCR products were run on 1 % agarose gel and

150   visualised under UV light with ethidium bromide (Sigma-Aldrich, USA) to verify the quality and

151   quantity of the DNA.

152   PCR amplicons were separated by their length through capillary electrophoresis. Samples

153   for electrophoresis consisted of 14 µl of Hi-Di formamide (Hi-Di Formamide, Genetic Analysis

154   Grade, Applied Biosystems), 1 µl of 1/200 diluted self-made standard that had three known length

155   HEX-labelled PCR products (Tiirola et al. 2003) and 1–2 µl of PCR product. Samples were

156   denaturated in 98 °C for 3 minutes, then run in a ABI PRISM® 310 Genetic analyzer (Applied

157   Biosystems) as described in Mikkonen et al. (2011) with a 47 cm long sequencing capillary and

158   POP-6™ polyacrylamide as a polymer (Applied Biosystems). Raw data were scanned with program

159   GeneScan 3.7 (Applied Biosystems) and the data were further analysed with BioNumerics 6.0

160   (Applied Maths, Sint-Martens-Latem, Belgium) as described in Mikkonen et al. (2011). The active

161   area of the fingerprint was restricted to the expected PCR amplicon size 460-550 bp. FAM labelled

162   sample curves were normalized with the internal HEX-labelled standards. Average fingerprints

163   were created with the 'Create average fingerprint' script.

164

165   *Ecosystem functioning measurements*

166   To understand how dung beetles, microbes and their interactions affect ecosystem functioning, we

167   measured multiple functional properties associated with the decomposition process.

168   Dung mass loss was measured as cumulative weight loss over the 60 days of the experiment,

169   calculated from wet weights taken every 10 days. Changes in dung mass established by this method

170   will reflect both desiccation and actual dung removal and/or respiratory loss of mass by pat-

171   dwelling species (Kaartinen et al. 2013; Rosenlew and Roslin 2008; Wall and Strong 1987).

172   Nonetheless, by the end of the experiment the humidity of all dung pats will have equilibrated with

173   the environment, rendering remaining mass a valid measure of the overall fraction of mass

174   decomposed (see Kaartinen et al. 2013 for an in-depth treatment). Overall respiration (CO_2 fluxes)

175   was measured throughout the experiment using a closed chamber method and a portable EGM-4

176   infrared CO_2 analyser.

177   To investigate how different dung beetle communities affect the functional profile of

178   microbial communities, we used Biolog Ecoplates (Biolog Inc., Hayward, CA, USA). Each well of

179   the EcoPlates contains an individual substrate, with 31 carbon substrates overall. While the

180   substrates represent only a small fraction of those that might be available in natural environments,

181   the rate of breakdown of individual substrates gives an indication of the metabolic capacity of a

182   community (Garland 1997; Garland and Mills 1991). Dung and soil samples for inoculation were

183   collected at the end point of the experiment (dung: day 31, soil: day 60) as described above.

184   Samples were stored at 20°C overnight, then added to the EcoPlates and incubated for 5 days at

185   20°C. For each sample, 1 g of dung or soil was suspended in 4 ml (dung) or 8 ml (soil) of PBS

186   buffer (137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, at pH 7.4), and the homogenised

187   suspension was serially diluted in PBS. One set of the 31 carbon substrates was inoculated per

188   mesocosm by pipetting 150 µl of 10^{-4} diluted dung suspension or 10^{-3} diluted soil suspension into

189   the wells. Colour development was measured using an Infinite M200 microplate-reader (Tecan,

190   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.

191   We scored positive microbial growth if growth exceeded that observed in 95% of the water controls

192   (Gravel et al. 2011). Substrate usage (single Carbon Substrate Utilisation rates (sCSUR)) was

193   calculated as the area under the growth curve. The usage of substrates not exceeding water controls

194   was set to zero. As a measure of overall metabolic capacity, we defined the total substrate usage

195   across the Ecoplate, summed across all substrates (total carbon substrate utilisation rate (tCSUR)).

196   To pinpoint differences in the metabolic profile of different communities, we then divided the

197   substrates into five categories: carbohydrates, amino acids, carboxylic/acetic acids, polymers, and

198   amines/amides (Berga et al. 2012; Zak et al. 1994), and calculated mean substrate usage within each

199   category. The richness and diversity of substrate usage within each category was calculated as the

200   mean number, and the inverse of the Simpson Index (as above), respectively, of substrates showing

201   positive growth.

202

203   *Analyses*

204   DIFFERENCES BETWEEN SUBSTRATES AND SAMPLING PERIODS – The final sampling date differed

205   between soil and dung (see above), so temporal patterns were analysed separately for the two

206   substrates. To describe the microbial community, microbial operational taxonomic units (OTUs)

207   were defined as peaks in the LH-PCR traces, and OTU richness was defined as the total number of

208   OTUs in each profile. To identify peaks, LH-PCR traces were first smoothed by fitting a cubic

209   spline using the default settings in the smooth.spline function in the base stats package of R (R

210   Development Core Team 2013). OTUs were then delimited by identifying the peaks and valleys in

211   the trace. We used relative peak area as a proxy of relative abundance, and calculated Simpson

212   indices (D=1/sum of the squared relative abundances) to describe the diversity and evenness

213   (1/D/species richness) in each sample. For microbial OTU diversity and richness, we built

214   generalised linear models with normally and Poisson-distributed errors, respectively. Each response

215   was modelled as a function of the dung beetle community (*Aphodius* only, *Aphodius* and

216   *Geotrupes,* No Dung Beetles) and day (12, 31 or 60) as categorical fixed effects. In all cases, we

217   started from the full model including all main effects and their interactions, then removed non-

218   significant interactions until arriving at the minimum adequate model, for which results are

219   presented.

220

221   INTERACTIVE EFFECTS OF MICROBES AND DUNG BEETLES ON ECOLOGICAL FUNCTIONING – As both

222   dung beetle and microbial community composition varied in our experiment, we took a multistep

223   approach to examine their respective contributions to decomposition processes:

224   To establish whether the presence of *Aphodius*, or of *Aphodius* and *Geotrupes* affected

225   microbial community composition *per se*, we used permutational multivariate analysis of variance

226   (permutational MANOVA) calculated using the Bray-Curtis dissimilarity index. Statistical tests

227   were calculated using the R function *adonis* in the package *vegan* (Blackwood et al. 2007; Oksanen

228   et al. 2009), and communities visualised using nonmetric multidimensional scaling (NMDS)

229   implemented in *metaMDS* in *vegan*.

230   To examine whether similarity in microbial community composition was reflected in

231   similarity in function, we used Mantel tests to compare matrices of Bray-Curtis dissimilarity in LH-

232   PCR profiles (at day 30 and 60 for Ecoplates and at day 12 for dung decomposition and CO_2 fluxes)

233   to matrices describing similarity in 1) substrate usage on Ecoplates (similarity described by the

234   Bray-Curtis metric at the end of the experiment; 2) dung decomposition, measured as the slope of

235   the regression of dung mass loss on time (with similarity described by Euclidean distance) and 3)

236   CO_2 flux (using the average of fluxes from day 10 and 14, as no flux data was collected on day 12,

237   and again describing similarity by Euclidean distance). In each case, we compared the observed

238   Pearson correlation coefficient to values generated by 999 permutations. A significant association

239   would signal that communities more similar in structure were also more similar in function than

240   expected by chance alone. All analyses were carried out in R version 3.0.1 (R Development Core

241   Team 2013).

242

243   **Results**

244   *Microbial community composition*

245   Overall, distinct microbial communities were found in soil and dung samples, and there were

246   significant temporal changes in community composition (Table 1, Fig.1). Over the course of the

247   experiment and with the drying-out of the dung, the microbiome of the soil and of the dung

248   converged (Fig.1). Further analysis of soil collected from beneath the dung pats indicated that

249   microbial community composition of soil under dung pats was significantly affected by the

250   presence of dung beetles (Table 1a), whereas the specific identity of the beetles (*Aphodius* or

251 *Geotrupes*) had no further detectable impact on this comparison (Table 1b). Within dung, dung

252 beetles had no detectable effect on microbial community composition (Table 1c).

253   The presence of dung beetles also affected the microbial diversity observed in the soil

254 underneath dung pats. Soil microbial diversity significantly changed with the identity of the beetles

255 ($F_{2,82}$= 3.80, P=0.03). Microbial diversity was lower in the presence of both *Aphodius* and

256 *Geotrupes* than in the presence of *Aphodius* alone. There was no significant effect of day on soil

257 microbial diversity ($F_{1,82}$= 2.44, P>0.1). Although there were no significant effects of day or dung

258 beetle treatment on species richness (P>0.9 in both cases), the evenness of the microbial

259 communities was impacted by the dung beetle treatments ($F_{2,82}$= 3.79, P=0.03), and was lowest

260 when both dung beetle genera were present. Dung microbial OUT richness and diversity did not

261 significantly differ over time or among the dung beetle treatments (P>0.4 in all cases).

262

263 *Microbial functioning*

264 The microbial communities in soil and dung were associated with different functional profiles as

265 measured by the Ecoplates (Fig. 2). However, the presence or absence of dung beetles, or the

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

267 (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

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

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

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

271 in all cases).

272   Carbon substrate utilisation rates (sCSUR's) in dung and soil differed among substrates

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

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

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

276   *Geotrupes* had higher utilisation sCSUR's (Fig. 3a). In soil, the presence of dung beetles did not

277   increase utilisation rates ($F_{2,208}$=0.0308, P=0.97; Fig. 3b).

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

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

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

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

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

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

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

285   significantly positively correlated with similarities in dung microbial community composition

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

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

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

289

290   **Discussion**

291   Our results demonstrate an important interaction between dung beetles and microbial communities

292   in dung and soil, providing a link in biogeochemical cycling in agricultural systems. While the

293   microbial communities of dung and soil are initially different, they converge over time on the

294   pasture. During this process, dung beetle communities modify some aspects of both microbial

295   community structure and functioning in both the dung pats and in the soil underneath them. By

296   doing so, we suggest that the beetles may serve as mobile links between decomposition processes

297   occurring above and below ground. Thus, the bioturbation process offered by beetles may serve to

298   homogenise both microbial community structure and functioning across the soil-surface boundary.

299   Below, we will address each of these observations in turn.

300   Dung is a major source of nutrients and carbon into soil food webs, particularly in

301   agricultural systems (Aarons et al. 2009; Yoshitake et al. 2014). Microbial activity is a key driver

302   behind soil carbon and nutrient cycling (Falkowski et al. 2008), and has been extensively studied,

303   for example in the context of carbon storage (Trivedi et al. 2013). Contrasting with such studies is a

304   major body of literature focusing on the role of macroscopic invertebrates in the decomposition of

305   dung. Among such taxa, dung beetles have been identified as the most important invertebrate

306   contributors to dung decomposition in temperate agricultural grasslands (Lee and Wall 2006).

307   Despite the evident potential to incorporate microbial processes into studies of dung beetles, the

308   link between dung beetles, dung and soil microbes and biogeochemical cycling has never been

309   explicitly explored. With global increases in cattle farming, and hence greenhouse gas emissions

310   from agriculture (Bellarby et al. 2013; FAO 2006), it is important to examine the processes

311   contributing to the decomposition of cattle dung.

312   Our study revealed substantial differences in the microbial communities of dung and soil –

313   and also differences in microbial functioning among these strata. Initial differences in the

314   microbiome of the dung and the soil reflect both the specific composition of the substrate (cattle

315   fodder versus soil) and the specific conditions prevailing in the digestive tract of the ruminants (de

316   Menezes et al. 2011; Kim et al. 2011). After the dung is deposited in the pasture, the microbiome of

317   the pat is exposed to ambient conditions and eventually converges towards that of the soil – as

318   paralleled by increasing convergence of functioning. On this process, the dung beetles left an

319   imprint. In particular, in terms of community structure, microbial evenness was lower in the

320   presence of both *Aphodius* and *Geotrupes* than in the presence of *Aphodius* alone. However, the

321   presence of dung beetles and their community composition had little effect on affect overall

322   microbial functioning in either dung or soil, although utilisation rates of certain substrate categories

323   increased when dung beetles were present. In particular, amines were utilised more when dung

324   beetles were present and carbohydrates had higher utilisation rates when both *Aphodius* and

325 *Geotrupes* were present than with *Aphodius* alone, thus yielding a different functional profile of

326 microbial communities in the presence versus absence of beetles. One possible explanation for this

327 contrast with *a priori* expectations is that the soil samples were taken close to the surface

328 (maximum depth 9cm), and that the effects of the tunnelling by *Geotrupes* may thus be more

329 pronounced deeper in the soil profile. Future studies will be targeted at resolving such effects.

330 Regardless of the factors giving rise to it, large overall variation in microbial community

331 composition both within and between substrates (soil versus dung) and time periods directly

332 translated to differences in functional rates. Significant association between similarities in microbial

333 community composition and substrate usage add to associations observed for the main function of

334 dung decomposition, where more similar microbial communities were also more similar in terms of

335 how quickly they disposed of dung. Both patterns attest to a general relationship between microbial

336 community composition and functioning (Bell et al. 2009; Bell et al. 2005).

337 Our study suggests that the presence of mesofauna (dung beetles) will modify the

338 microfauna (microbes), including its diversity and functioning. In particular, the presence of dung

339 beetles appears promote the transfer of microbes across the soil-surface interface, and result in

340 increased similarity in both community structure and functioning. However, the specific impact of

341 dung beetle groups and interactions between them is less clear. While the patterns reported here

342 apply to aerobic bacteria, we propose that an added focus on the anaerobic part of the community –

343 and on associated functions like methane emissions (see Penttilä et al. 2013) – may prove a

344 particularly interesting avenue for further research.

345

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354   the field and lab, EMS and TB analysed the data, and all authors contributed to the writing of the

355   manuscript.

356

357   **References**

358   Aarons, S., O'Connor, C., Hosseini, H., Gourley, C. P. 2009. Dung pads increase pasture

359   production, soil nutrients and microbial biomass carbon in grazed dairy systems. - Nutr Cycl

360   Agroecosyst 84:81-92. doi: 10.1007/s10705-008-9228-5.

361   Bell, T., Gessner, M. O., Griffiths, R. I., McLaren, J. R., Morin, P. J., van der Heijden, M., van der

362   Putten, W. 2009. Microbial biodiversity and ecosystem functioning under controlled

363   conditions and in the wild. - Biodiversity, Ecosystem Functioning, and Human Wellbeing:

364   An Ecological and Economic Perspective:121-133

365   Bell, T., Newman, J. A., Silverman, B. W., Turner, S. L., Lilley, A. K. 2005. The contribution of

366   species richness and composition to bacterial services. - Nature 436:1157-1160

367   Bellarby, J., Tirado, R., Leip, A., Weiss, F., Lesschen, J. P., Smith, P. 2013. Livestock greenhouse

368   gas emissions and mitigation potential in Europe. - Global Change Biology 19:3-18. doi:

369   10.1111/j.1365-2486.2012.02786.x.

370   Berga, M., Székely, A. J., Langenheder, S. 2012. Effects of Disturbance Intensity and Frequency on

371   Bacterial Community Composition and Function. - PLoS ONE 7:e36959. doi:

372   10.1371/journal.pone.0036959.

373   Beynon, S. A., Mann, D. J., Slade, E. M., Lewis, O. T. 2012. Species-rich dung beetle communities

374   buffer ecosystem services in perturbed agro-ecosystems. - Journal of Applied Ecology

375   49:1365-1372. doi: 10.1111/j.1365-2664.2012.02210.x.

376   Blackwood, C. B., Hudleston, D., Zak, D. R., Buyer, J. S. 2007. Interpreting Ecological Diversity

377   Indices Applied to Terminal Restriction Fragment Length Polymorphism Data: Insights

378   from Simulated Microbial Communities. - Applied and Environmental Microbiology

379   73:5276-5283. doi: 10.1128/aem.00514-07.

380   de Menezes, A. B., Lewis, E., O'Donovan, M., O'Neill, B. F., Clipson, N., Doyle, E. M. 2011.

381   Microbiome analysis of dairy cows fed pasture or total mixed ration diets. - FEMS

382   Microbiology Ecology 78:256-265

383   FAO2006. Livestock's long shadow, environmental issues and options. Food and Agriculture

384   Organization of the United Nations, Rome, Italy.

385   Garland, J. L. 1997. Analysis and interpretation of community-level physiological profiles in

386   microbial ecology. - FEMS Microbiology Ecology 24:289-300. doi: 10.1016/s0168-

387   6496(97)00061-5.

388   Garland, J. L., Mills, A. L. 1991. Classification and Characterization of Heterotrophic Microbial

389   Communities on the Basis of Patterns of Community-Level Sole-Carbon-Source Utilization.

390   - Applied and Environmental Microbiology 57:2351-2359

391   Gravel, D., Bell, T., Barbera, C., Bouvier, T., Pommier, T., Venail, P., Mouquet, N. 2011.

392   Experimental niche evolution alters the strength of the diversity-productivity relationship. -

393   Nature 469:89-92

394   Hanski, I., Cambefort, Y. (eds) (1991) Dung Beetle Ecology. Princeton University Press, Princeton,

395   New Jersey.

396   Kaartinen, R., Hardwick, B., Roslin, T. 2013. Using citizen scientists to measure an ecosystem

397   service nationwide. - Ecology 94:2645-2652. doi: 10.1890/12-1165.1.

398   Kim, M., Morrison, M., Yu, Z. 2011. Status of the phylogenetic diversity census of ruminal

399   microbiomes. - FEMS Microbiology Ecology 76:49-63

400   Larsen, T. H., Williams, N. M., Kremen, C. 2005. Extinction order and altered community structure

401   rapidly disrupt ecosystem functioning. - Ecology Letters 8:538-547

402   Lee, C. M., Wall, R. 2006. Cow-dung colonization and decomposition following insect exclusion. -

403   Bulletin of Entomological Research 96:315-322. doi: 10.1079/ber2006428.

404   Mikkonen, A., Lappi, K., Wallenius, K., Lindström, K., Suominen, L. 2011. Ecological inference

405   on bacterial succession using curve-based community fingerprint data analysis,

406   demonstrated with rhizoremediation experiment. - FEMS Microbiology Ecology 78:604-

407   616. doi: 10.1111/j.1574-6941.2011.01187.x.

408   Mikkonen, A., Santalahti, M., Lappi, K., Pulkkinen, A.-M., Montonen, L., Suominen, L. 2014.

409   Bacterial and archaeal communities in long-term contaminated surface and subsurface soil

410   evaluated through coextracted RNA and DNA. - FEMS Microbiology Ecology 90:103-114

411   Muyzer, G., De Waal, E. C., Uitterlinden, A. G. 1993. Profiling of complex microbial populations

412   by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified

413   genes coding for 16S rRNA. - Applied and Environmental Microbiology 59:695-700

414   Nichols, E. 2013. Fear begets function in the 'brown' world of detrital food webs. - Journal of

415   Animal Ecology 82:717-720. doi: 10.1111/1365-2656.12099.

416   Nichols, E., Spector, S., Louzada, J., Larsen, T. H., Amezquita, S., Favila, M., The Scarabaeinae

417   Research Network 2008. Ecological functions and ecosystem services of Scarabaeine dung

418   beetles: a review. - Biological Conservation 141:1461-1474

419   Nichols, E. S., Gardner, T. A. 2011. Dung beetles as a candidate study taxon in applied biodiversity

420   conservation research. - Ecology and evolution of dung beetles:267-291

421   Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G. L., Solymos, P., Stevens, M. H. H.,

422   Wagner, H. 2009. vegan: Community Ecology Package.

423   Penttilä, A., Slade, E. M., Simojoki, A., Riutta, T., Minkkinen, K., Roslin, T. 2013. Quantifying

424   Beetle-Mediated Effects on Gas Fluxes from Dung Pats. - PLoS ONE 8:e71454. doi:

425   10.1371/journal.pone.0071454.

426   R Development Core Team 2013. R: A language and environment for statistical computing. R

427   Foundation for Statistical Computing, Vienna, Austria.

428   Rosenlew, H., Roslin, T. 2008. Habitat fragmentation and the functional efficiency of temperate

429   dung beetles. - Oikos 117:1659-1666. doi: 10.1111/j.1600-0706.2008.16904.x.

430   Roslin, T. 2000. Dung beetle movements at two spatial scales. - Oikos 91:323-335

431   Roslin, T. 2001. Large-scale spatial ecology of dung beetles. - Ecography 24:511-524

432   Roslin, T., Forshage, M., Ødegaard, F., Ekblad, C., Liljeberg, G. 2014. Nordens dyngbaggar.

433   Hyonteistarvike TIBIALE, Oy, Helsingfors.

434   Roslin, T., Koivunen, A. 2001. Distribution and abundance of dung beetles in fragmented

435   landscapes. - Oecologia 127:69-77

436   Slade, E. M., Mann, D. J., Lewis, O. T. 2011. Biodiversity and ecosystem function of tropical forest

437   dung beetles under contrasting logging regimes. - Biological Conservation 144:166-174

438   Slade, E. M., Mann, D. J., Villanueva, J. F., Lewis, O. T. 2007. Experimental evidence for the

439   effects of dung beetle functional group richness and composition on ecosystem function in a

440   tropical forest. - Journal of Animal Ecology 76:1094-1104

441   Spector, S. 2006. Scarabaeine Dung Beetles (coleoptera: Scarabaeidae: Scarabaeinae): An

442   Invertebrate Focal Taxon for Biodiversity Research and Conservation. - The Coleopterists

443   Bulletin 60:71-83. doi: 10.1649/0010-065x(2006)60[71:sdbcss]2.0.co;2.

444   Tiirola, M. A., Suvilampi, J. E., Kulomaa, M. S., Rintala, J. A. 2003. Microbial diversity in a

445   thermophilic aerobic biofilm process: analysis by length heterogeneity PCR (LH-PCR). -

446   Water research 37:2259-2268

447   Trivedi, P., Anderson, I. C., Singh, B. K. 2013. Microbial modulators of soil carbon storage:

448   integrating genomic and metabolic knowledge for global prediction. - Trends in

449   microbiology 21:641-651

450   Van Der Heijden, M. G. A., Bardgett, R. D., Van Straalen, N. M. 2008. The unseen majority: soil

451   microbes as drivers of plant diversity and productivity in terrestrial ecosystems. - Ecology

452   Letters 11:296-310. doi: 10.1111/j.1461-0248.2007.01139.x.

453   Wagg, C., Bender, S. F., Widmer, F., van der Heijden, M. G. A. 2014. Soil biodiversity and soil

454   community composition determine ecosystem multifunctionality. - Proceedings of the

455   National Academy of Sciences. doi: 10.1073/pnas.1320054111.

456   Wall, D. H., Bardgett, R. D., Kelly, E. 2010. Biodiversity in the dark. - Nature Geosci 3:297-298

457   Wall, R., Strong, L. 1987. Environmental consequences of treating cattle with the antiparasitic drug

458   ivermectin. - Nature 327:418-421

459   Weisburg, W. G., Barns, S. M., Pelletier, D. A., Lane, D. J. 1991. 16S ribosomal DNA

460   amplification for phylogenetic study. - Journal of bacteriology 173:697-703

461   Yoshitake, S., Soutome, H., Koizumi, H. 2014. Deposition and decomposition of cattle dung and its

462   impact on soil properties and plant growth in a cool-temperate pasture. - Ecological

463   Research:1-12. doi: 10.1007/s11284-014-1153-2.

464   Zak, J. C., Willig, M. R., Moorhead, D. L., Wildman, H. G. 1994. Functional diversity of microbial

465   communities: A quantitative approach. - Soil Biology and Biochemistry 26:1101-1108. doi:

466   10.1016/0038-0717(94)90131-7.

467

468

469

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

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

472   sampling dates and treatments.

473

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

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

479

480   **Figure Legends**

481   Figure 1. NMDS plots showing the changes in the dung (blue points) and soil (red points) microbial

482   community composition over time. The three panels show different points in time, with the

483   complete dataset (grey points) included for reference. Symbols identify mesocosms with *G.*

484   *stercorarius* present (■) versus mesocosms with only *Aphodius* species present (▲).Control

485   mesocosms with dung but no dung beetles are indicated with the symbol ＋. On day 0, samples

486   were taken only from the six control pats and from the soil in 12 mesocosms before the dung was

487   added (see methods).

488

489   Figure 2. NMDS plot showing the utilisation of carbon substrates (based on sCSURs of Ecoplate

490   substrates) in dung (day 31) and soil (day 60) in mesocosms with *Aphodius* and *Geotrupes*

491   *stercorarius* present (■);mesocosms with only *Aphodius* present (▲) and control mesocosms with

492   dung but no dung beetles present (＋).

493

494   Figure 3. Microbial activity and functioning measured as mean single carbon substrate utilisation

495   rates (sCSUR) in a) dung (a) and b) soil in the presence of different dung beetle communities.

496   Shown are means ±SE.

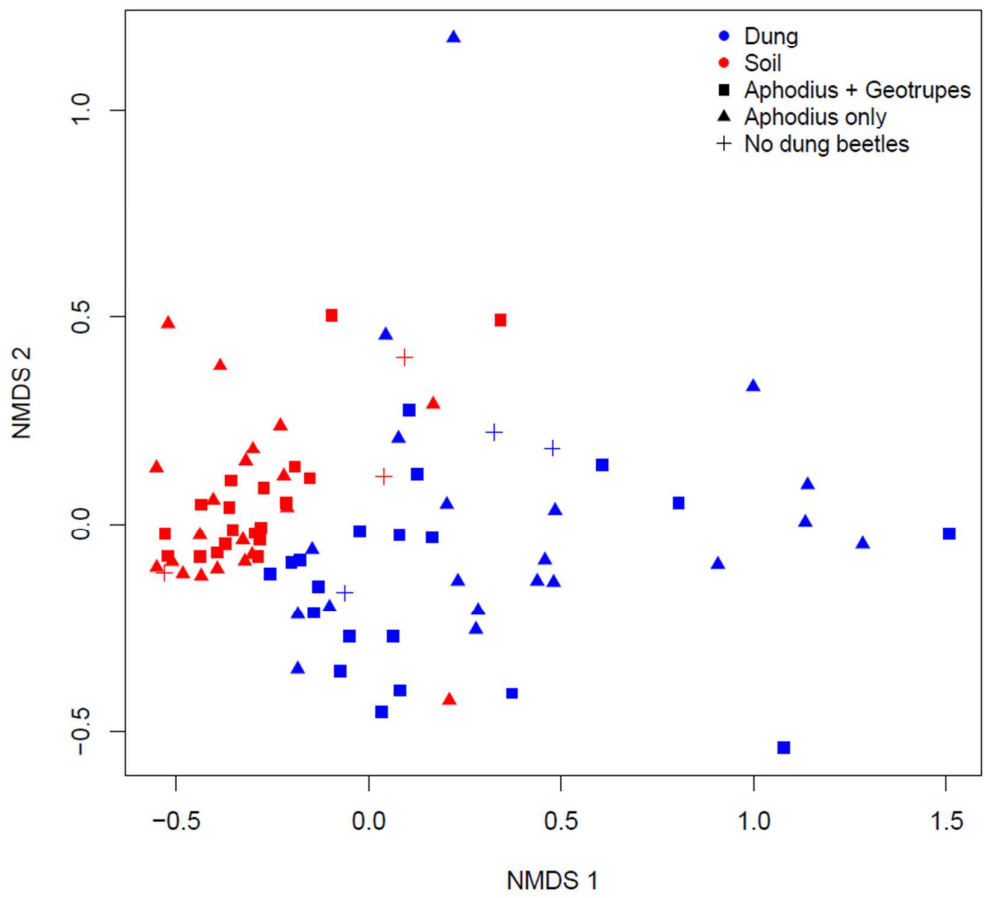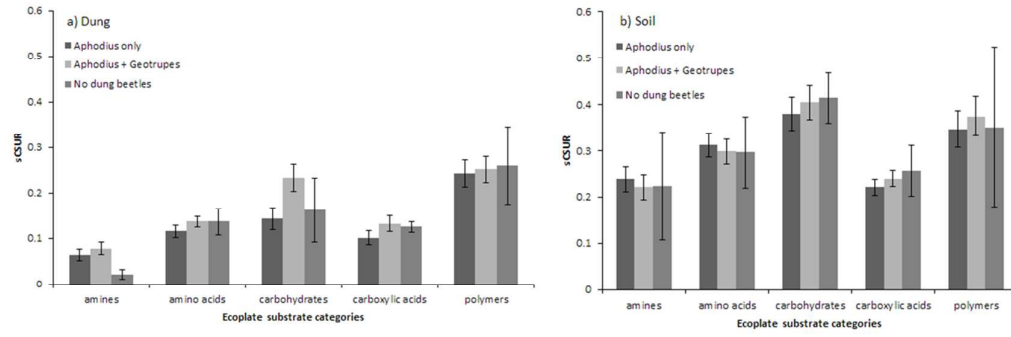497
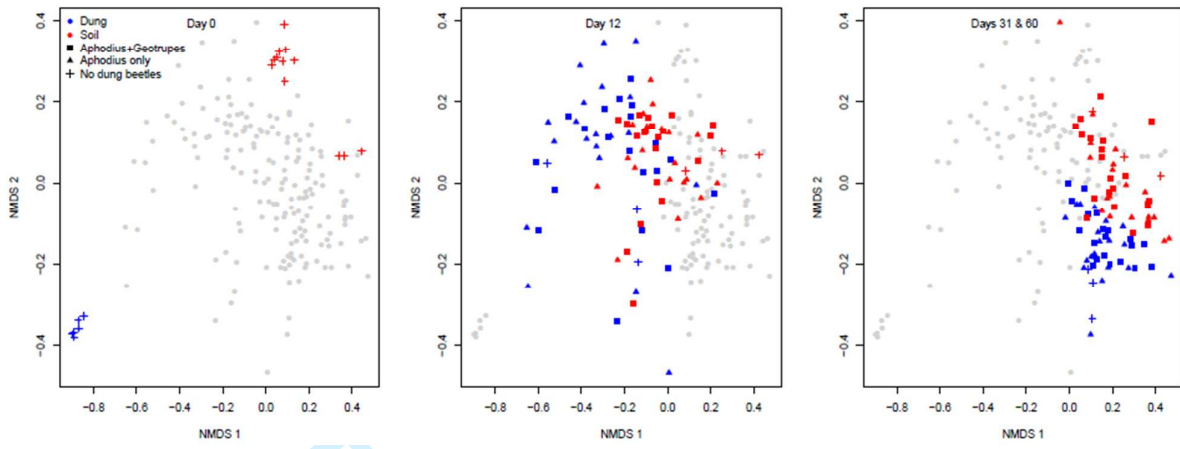
498

499   Figure 1.

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