Succession of dung-inhabiting beetles and flies reflects the succession of dung-emitted volatile compounds

by Sladecek, F.X.J., Dötterl, S., Schäffler, I., Segar, S.T. and Konvicka, M.

DOI link to the version of record on the publisher's site

1   SUCCESSION OF DUNG-INHABITING BEETLES AND FLIES REFLECTS

2   THE SUCCESSION OF DUNG-EMITTED VOLATILE COMPOUNDS.

3

4   FRANTISEK XAVER JIRI SLADECEK[1,2*], STEFAN DÖTTERL[3], IRMGARD

5   SCHÄFFLER[3], SIMON TRISTRAM SEGAR [4], MARTIN KONVICKA [1,2]

6

7   *[1] Biology Centre of the Czech Academy of Sciences, Institute of Entomology, Branisovska 31,*

8   *370 05, Ceske Budejovice, Czech Republic, phone number: +420 606 420 548,*

9   *franzsladecek@gmail.com.*

10   *[2]Department of Zoology, Faculty of Science, University of South Bohemia, Branisovska 31,*

11   *370 05, Ceske Budejovice, Czech Republic, phone number: +420 606 420 548,*

12   *franzsladecek@gmail.com.*

13   *[3]Department of Biosciences, Plant Ecology, University of Salzburg, Salzburg, Austria*

14   *[4]Agriculture & Environment Department, Harper Adams University, United Kingdom*

15

16   **Abstract** – Chemical cues, such as volatile organic compounds (VOCs), are often essential for

17   insects when locating food. Contrary to communication between insects and plants, the

18   relationships between insect decomposers and the VOCs emitted by dung and carrion are

19   understudied. Such relationships may provide mechanistic understanding of the temporal axis

20   of community assembly processes in decomposing insect communities. We focused on the

21   temporal succession of volatiles released by cow dung pats and their potential influence on

22   dung-inhabiting insects. Using gas chromatography/mass spectrometry we identified the

23   spectrum and total amount of volatile compounds released from dung of one hour, one, two,

24   three, five and seven days old. We then related changes in the spectrum of VOCs to successional

25   patterns of dung-inhabiting beetles and flies. We detected 54 VOCs which formed two

successional groups with chemical turnover peaking in two-day-old dung. The early successional group consisted primarily of aliphatic alcohols and phenols, the late one of aliphatic esters, nitrogen- and sulfur-bearing compounds. Flies were predominately associated with the early successional group, mainly with 1-butanol. Beetles were associated predominately with the late successional group, mainly with dimethyl trisulfide. This association between insect and chemical successional patterns supports the idea that habitat filtering drives community assembly of dung-inhabiting insects on ageing resources. Moreover, the affinity of both insect groups to specific VOC groups provides a mechanistic explanation for the predictability of successional patterns found in dung inhabiting insect communities.

Key Words – Diptera, dung beetles, environmental filtering, ephemeral habitats, temporal segregations.

INTRODUCTION

51 The ability to locate food is of utmost importance for animals and it is achieved by utilizing

52 tactile cues, visual cues, chemical cues (smell, taste) or a combination of all three (Balkenius et

53 al. 2006; Karmakar et al. 2016; Milet-Pinheiro et al. 2015; Schneider et al. 2017). The

54 importance of chemical cues has been highlighted recently and there has been an increasing

55 number of studies, e.g., in the field of pollination, reporting that species rely on chemical cues

56 when looking for food, even in taxa previously considered to rely heavily on visual cues (Milet-

57 Pinheiro et al. 2015; Omura and Honda 2009; Primante and Dötterl 2010). Although there is an

58 abundance of studies focusing on importance of chemical cue utilization in herbivores,

59 including pollinators, and their predators (de Mendonca et al. 1999; Hulcr et al. 2006; Segura

60 et al. 2012), there are proportionally fewer studies focusing on decomposer communities

61 (Frederickx et al. 2012).

62   Insect decomposers locate their resources using volatile organic compounds (VOC)

63 (Frank et al. 2018; Wurmitzer et al. 2017), even to the extent that they can be deceived by plants

64 that mimic the scent of their natural food resources (Jürgens et al. 2006; Jürgens et al. 2013;

65 Marino et al. 2009; Midgley et al. 2015). Communities of insect decomposers inhabit decaying

66 ephemeral resource patches, such as dung and carrion, that are temporally unstable, yet energy

67 rich (Finn 2001). The physical properties of ephemeral resource patches change significantly

68 during their decomposition, e.g. decreasing dung moisture (Gittings and Giller 1998), which is

69 accompanied by changes in chemical composition and profile of volatile compounds emitted,

70 e.g. an increase in alcohols and phenols with time (Varma et al. 2018).

71   Such changes in VOC profiles should be the primary cue used by insect decomposer

72 communities to identify the specific resource ages and thus be a main mechanism of insect

73 succession along gradient of resource ageing. As such, they would play a major role in the

74 assembly of such communities. This would explain why succession of insect decomposers

75 already seems to reflect the habitat filtering assembly rule (Sladecek et al. 2017a) which is

76 based upon aggregation of species with similar traits induced by environmental conditions (e.g.

77 variation in chemical cues) (Keddy 1992; Kraft et al. 2015). Moreover, preferences of

78 decomposer species for certain resource ages seem to be conservative (Sladecek et al. 2013).

79 Specifically, dung beetle species retain preferences for dung of certain ages even when

80 introduced to communities that are not native for them (e.g. beetles from Europe to America)

81 (Wassmer 2014). We assume that these profile preferences indicate habitat suitable for foraging

82 or breeding (e.g. low dung moisture).

83   We are not aware of any study that focuses on the role of volatile organic compounds

84 in determining the assembly of the whole insect decomposer communities, which inhabit

85 ephemeral resource patches. Studies targeting the relations between decomposers and VOCs

86 emitted from their resource generally focus on responses to specific VOCs by few species

87 within a single genus or family (Frederickx et al. 2012; Podskalska et al. 2009; von Hoermann

88 et al. 2011; von Hoermann et al. 2013) or small subset of much larger community (Frank et al.

89 2018; Recinos-Aguilar et al. 2020) and the vast majority of them targeted the carrion-inhabiting

90 insect community. Nevertheless, there is an evidence for potential effects of VOCs on assembly

91 of insect communities along resource ageing. Abundances of carrion-visiting flies increased

92 with increased amounts of VOCs emitted (Recinos-Aguilar et al. 2020). The early successional

93 burying beetles (Silphidae) (Podskalska et al. 2009) and blowflies (Calliphoridae) (Frederickx

94 et al. 2012) prefer the oligosulfides characteristic of early-mid successional carrion, while late

95 successional hide beetles (Dermestidae) respond to carboxylic derivates that are mainly

96 released from more mature carrion (von Hoermann et al. 2011).

97   Our study connects the changes in volatile compounds emitted with the successional

98 composition of the insect decomposer community of dung-inhabiting insects. We sampled both

99 VOCs and insect community in field, using cow dung as a model resource. Cow dung is

100 inhabited by a wide array of organisms, among which beetles and flies play the main ecological

4

role in dung degradation (Wu and Sun 2010), either by destroying dung directly (Slade et al. 2007) or by facilitating the activity of other dung-inhabiting biota (Holter 1979; Stevenson and Dindal 1987). Succession in both beetles (Hanski and Koskela 1977; Sladecek et al. 2013) and flies (Hammer 1941; Sladecek et al. 2017b) has been well studied. For succession of dung-emitted VOCs, we are aware of just one study carried out *in vitro* (Varma et al. 2018). The *in vitro* approach has some limitations, the omission of soil microbiota likely has serious implications relating to volatile emissions, especially since they remove anaerobic microbita (Perez-Valera et al. 2019), and thus probably their VOCs (Saito et al. 2018). Our study therefore represents the first exploration of volatiles as emitted from dung during succession in natural conditions.

We asked the following questions:

1) Do the total amount of VOCs (total volumes of VOCs in samples in $ng.min^{-1}$, similarly to abundances in animal/plant communities), compound richness (number of VOCs emitted, similarly to species richness in animal/plant communities) and VOCs composition (multivariate analysis of VOC changes in samples, similarly to changes in species composition in animal/plant communities) change along dung pat ageing?

2) Do abundance and species richness of beetles and flies correlate to compound richness and total amount of VOCs during ageing of dung pats?

3) Are changes in insect community affected by the turnover of VOC with aging?

We expect that the dynamics of dung emitted VOCs will differ to those found by (Varma et al. 2018). In particular, we expect a significant input of alcohols and phenols as produced by gut microbiota (Saito et al. 2018). This microbiota shall later be suppressed by soil microbiota in natural conditions (Perez-Valera et al. 2019), and thus VOCs emitted shall change from anaerobic products (alcohols and phenols) to products of aerobic organisms. Similarly to evidence from carrion communities (Frederickx et al. 2012; Podskalska et al. 2009; von

126 Hoermann et al. 2011), we expect that insect abundances and species richness will change as to

127 reflect both the total amount of VOCs and compound richness. Overall, we expect that dung

128 VOCs will provide a reliable cue for dung-inhabiting insects and explain, at least in part, their

129 successional patterns.

130

131

132 <div align="center">METHODS AND MATERIALS</div>

133 Study site

134 We carried out this study on a 23 ha pasture, 10 km west of Ceske Budejovice, Czech Republic

135 (48°59'2.4"N, 14°24'34.957"E), Central Europe. This pasture hosts a permanent herd of 30

136 adult cows and has been continuously grazed for decades. It is situated at 380 m a.s.l., in a

137 region with a mean annual temperature of 8.1 °C, mean annual precipitation of 620 mm, and a

138 vegetation season spanning from March to October (Sladecek et al. 2013).

139

140 Volatile sampling

141 We sampled the volatiles released by dung in June 2015 (22 – 29th June) from cow dung pats

142 of 1.5 litres volume. The pats were artificially created to ensure that they were of the same age

143 at the time of their creation and of the same volume (Krell 2007). We collected fresh dung

144 immediately after defecation from 20 stalled cows, to avoid any potential insect colonization in

145 the barn where those cows are stalled, and homogenized it. We then created five dung pats at

146 the study site, forming a line with pats placed 10 m apart. We used clean metallic tools to

147 manipulate the dung in order to avoid / minimize contamination potentially induced by using

148 plastic equipment. Each of the pats represented a replicate, of which we repeatedly collected

149 volatiles at one hour, one, two, three, five and seven days after the pats establishment, summing

150 up to 30 samples of volatiles.

151   We sampled the volatiles by placing a glass funnel of 9 cm diameter on the surface of a

152   dung pat. We then trapped volatiles into the adsorbent tubes (quartz microvials, 15 mm length,

153   2 mm inner diameter) filled with 1.5 mg of Tenax-TA 60–80 and 1.5 mg of Carbotrap 20–40

154   (both Supelco), fixed in the tube with glass wool. We sampled volatiles by sucking air with

155   volatiles into those tubes with a 9V operated membrane pump (G12/01 EB, Rietschle Thomas,

156   Puchheim, Germany) (Dötterl et al. 2006; Mitchell et al. 2015). We adjusted the flow by a flow

157   meter to 200 ml.min^{-1}. To validate that our volatile samples are truly emitted by dung, we

158   sampled two blank control samples of volatiles (two per each five dung age samples, e.g. two

159   for samples of 1hour dung age, two for samples of 1 day dung age, etc.) from the meadow using

160   the same setup, with the glass funnel above the grass layer (~ 10 cm above ground). After

161   preliminary trials using dung of various ages, we decided to sample one hour old dung for five

162   minutes, while we sampled other dung ages, which released smaller amounts of volatiles, for

163   ten minutes.

164

165   Chemical analyses

166   We analyzed the volatile samples using gas chromatography/mass spectrometry (GC/MS) to 1)

167   determine the concentration of volatiles released ("Total amount of VOCs", i.e. volume of

168   VOCs in samples, similarly to abundance of species in animal/plant communities) and 2)

169   indentify the volatile compounds present ("compound richness", similarly to species richness

170   in animal/plant communities); and in dung of various ages. We carried out GC/MS analyses

171   using an automatic thermal desorption (TD) system (TD-20, Shimadzu, Kyoto, Japan) coupled

172   to a Shimadzu GCMS-QP2010 Ultra equipped with a ZB-5 fused silica column (5% phenyl

173   polysiloxane; 60 m, i.d. 0.25 mm, film thickness 0.25 μm, Phenomenex). For more details on

174   GC/MS and column settings see Heiduk et al (2016). We ran the samples with a 1:1 split and a

175   constant helium carrier gas flow of 1.5 ml/min. The GC oven temperature started at 40°C, then

176 increased by 6°C/min to 250°C and held for 1 min. The MS interface worked at 250°C. Mass

177 spectra were taken at 70 eV (EI mode) from m/z 30 to 350. GC/MS data were processed using

178 the GCMSolution package, Version 2.72 (Shimadzu Corporation 2012). We tentatively

179 identified the compounds by using both, the mass spectral libraries ADAMS (2007), FFNSC 2,

180 W9N11, ESSENTIAL OILS (available in MassFinder 3), and the Kovats retention indices of

181 the compounds (based on n-alkane series). We only considered compounds which had a

182 calculated Kovats index ± 10 compared to various data bases (Adams 2007; El-Sayed 2018;

183 Nist11). Some of the components were confirmed by comparison of mass spectra and retention

184 times with standard components available in the reference collection of the Plant Ecology Lab

185 of the University of Salzburg. For quantitative analysis, we injected VOCs, 100 ng each of ca.

186 150 components, among them monoterpenes, aliphatic, and aromatic compounds, into the GC-

187 MS system. We used the mean of the peak areas (total ion current) of these compounds to

188 estimate the total amount of scent available in the scent samples collected from the pats (see Etl

189 et al. 2016). The total amount of VOCs was standardized to the amount of VOCs trapped per

190 minute, to control for different sampling times (see above).

191

192 Insect Sampling

193 We sampled both adult beetles and adult flies from artificially created dung pats of 1.5 litres

194 volume, which we created in the same manner as we did with the pats for volatile sampling.

195 While collecting the dung used for insect sampling, cows were also fed with hay/silage, the

196 same food offered to the cows while collecting dung for volatile sampling.

197 We sampled beetles during five sampling sessions in 2009 (11 – 29 April, 17 May – 4

198 June, 4 – 22 July, 15 August – 2 September and 23 September – 11 October), and flies during

199 three sampling sessions in 2011 (23 April – 1 May, 16 – 24 July, 26 August – 3 September) and

200 three sampling sessions in 2012 (9 – 17 May, 27 July – 4 August, 14 – 22 September). We

201 floated the beetles from the dung pats and the underlying portion of soil in a bucket of water.

202 We sampled adult flies from the dung pats' surface using a sweep net. Both beetles and flies

203 were collected from 1, 2, 3, 5 and 7 day old dung pats, while flies were additionally sampled

204 from 1 hour old pats (each successive time was replicated five times in each season). In

205 temperate communities, there are virtually no beetles presented in 1 hour old dung (personal

206 observation), while adult flies reach their maximum abundance on dung 1 hour or less old

207 (Hammer 1941; Mohr 1943; Sladecek et al. 2017b). For additional details on insect sampling,

208 see Sladecek et al. (2013; 2017b).

209   To create a unified dataset from the three separate samplings, we pooled our insect and

210 volatile data. To merge insect data (6 dung ages, 5 replications, 5 (beetles) and 6 (flies) sampling

211 seasons) with the volatile data (6 dung ages, 5 replications), we created "average" insect

212 communities for individual dung ages and replications across seasons to match the insect data

213 with volatile data. Therefore the abundance of flies in 1 hour old dung in replication 1 was a

214 mean abundance of fly abundances in 1 hour old dung in replication 1 across 6 sampling

215 seasons. We conserved the replication structure in our insect data, assigning the respective

216 replication in volatile data to it (e.g. replication 1 in our insect data was associated with

217 replication 1 in our volatile data). In total, we thus obtained 30 samples of insect + volatile data

218 (6 dung ages, 5 replications).

219

220

221

222 Statistical analyses

223 All data used in this paper are available at the figshare.com repository

224 (https://doi.org/10.6084/m9.figshare.7039553.v1) (Sladecek et al. 2018).

225

9

226 1) Do the total amount of VOCs, compound richness and VOCs composition change along

227 dung pat ageing?

228 To analyze changes in total amount of VOCs and compound richness across dung age class, we

229 fitted a Generalized Linear Model (GLM) with total amount of scent captured or compound

230 richness as response variables and dung pat age as explanatory variable; in R 4.0.3 (Team

231 2020). We used Gamma (total amount of VOCs; with log as link function) and Poisson

232 (compound richness) distributions of errors for the two models. We validated our models using

233 the DHARMa package (Hartig 2019), checking especially for overdispersion in model with

234 Poisson distribution. We did not include dung pat identity as random effect factor in the models

235 as it explained negligible variance ($<10^{-6}$) in the Generalized Linear Models with Mixed effects

236 that were originally calculated (lme4 package) (Bates et al. 2015).

237   We analyzed the temporal changes in VOCs composition along dung pat ageing using

238 Detrended Canonical Correspondence Analysis (DCCA), with detrending by second order

239 polynomial, using CANOCO 5 for Windows (Ter Braak and Smilauer 2012). DCCA is a

240 multivariate technique suitable for datasets with unimodal response variables along predictor

241 variable gradient (Leps and Smilauer 2003). In addition, it also eliminates the 'arch effect'

242 artefact (which was present in the volatile data in non-detrended Canonical Correspondence

243 Analysis that we computed first). We log (x+1) transformed total amounts of individual VOCs

244 (total volume of each VOC in each sample) prior to the analysis. We used dung pat age as a

245 predictor variable and affinity to replication was included as a block covariate (equivalent to

246 random effect in regressions). We then assessed statistical significance using Monte Carlo

247 permutation tests (999 permutations). Finally, we used Ward's hierarchical clustering in R 4.0.3

248 (Team 2020) to identify successional groups of compounds. This clustering was performed

249 using the compound position (optimum) on the first canonical axis computed by DCCA. Such

250 optima numerically represent compound position on dung age gradient.

251

252   2) Do abundance and species richness of beetles and flies correlate to compound richness and

253   total amount of VOCs during ageing of dung pats?

254   To test the relationship between beetle and fly abundance/species richness and the total amount

255   of VOCs/compound richness, we ran eight different generalized linear models (GLMs) in R

256   4.0.3 (Team 2020). Each model consisted of one of the above insect related variables (e.g. fly

257   species richness) as the response variable and with the explanatory variable total amount of

258   VOCs or compound richness (a total of eight combinations). All models had a Poisson

259   distribution of errors. We then validated each model using DHARMa (Hartig 2019), to check

260   for overdispersion. Since overdispersion was high in all our models, we fitted final models with

261   quasipoisson distribution of errors.

262

263   3) Are changes in insect community composition affected by changes in the composition of

264   volatiles emitted by dung?

265   First, we analysed the successional patterns of beetles and flies with Detrended Canonical

266   Correspondence Analysis (DCCA) in CANOCO 5 for Windows (Ter Braak and Smilauer

267   2012). Separate DCCAs were fitted for beetle and fly community composition, with

268   successional time as an predictor variable. We assessed the statistical significance using Monte

269   Carlo permutation test (999 permutations).

270   We then analyzed the potential effect of dung emitted volatiles on the dung-inhabiting

271   insect community at two scales. First we ran a Canonical Correspondence Analysis (CCA) in

272   CANOCO 5 for Windows (Ter Braak and Smilauer 2012) with the multivariate insect data as

273   the response variable. For predictors, we used our VOC data. We chose the volatiles with the

274   highest potential influence on insect community using forward selection of predictor variables

275   in CANOCO 5 for Windows (Ter Braak and Smilauer 2012). We then ran another CCA with

276 insect data as response variables and successional groups of volatiles, retrieved in question one

277 as the predictor variables. Again, we assessed statistical significance using Monte Carlo

278 permutation test (999 permutations).

279

280   RESULTS

281 We recorded a total of 54 dung volatile compounds representing; one aromatic heterocyclic

282 compound, one ionon, two aldehydes, two esters, two ketons, two sulfur-bearing compounds,

283 four terpenoids, four nitrogen-bearing compounds, five aliphatic alcohols, and seven phenols.

284 We were not able to fully identify 24 compounds (Table S1), which were mostly suggested to

285 be derivates of carboxylic acids, although with low support for specific compound identity. We

286 collected a total of 28,284 beetle individuals from 86 species (five families) and 5,139 fly

287 individuals from 37 species and morpho species (17 families).

288

289 1) Do the total amount of VOCs, compound richness and VOCs composition change along dung

290 pat ageing?

291 Both total amount of VOCs (Deviance = -3.208, P < 0.001) and compound richness (F = -3.89,

292 P = 0.007) decreased along dung pat ageing (Fig. 1A) (model coefficients: Table 1). Total

293 amount of VOCs decreased from one hour old pats to two days old pats. There was a subsequent

294 peak of total amount of VOCs in three days old dung pats followed by a relatively steady

295 decline, with a lower total amount of VOCs in five and seven day old pats (Fig. 1B). The

296 compound richness decreased more steadily with its lowest values in two days old dung, while

297 the compound richness was almost identical in one and three days old pats (Fig. 1B).

298   The composition of volatile compounds was significantly structured along dung pat

299 ageing (F = 14.6, P = 0.001, the first horizontal axis explains 35.5 % of variability in VOC

300 data). Ward's clustering along the successional axis resulted into two major clusters: 1) early-

12

successional compounds that occurred until two days of dung pat age; and 2) late-successional compounds that occurred after two days of dung pat age (Fig. 2, Fig. S1). Samples from early successional stages were dominated by aliphatic and aromatic alcohols, such as 1-butanol, phenol, and p-cresol. Some of the compounds, such as 3-methylbutanal, were even found only in samples collected from pats one hour or one day old. Dung from late successional stages released mainly nitrogen- (e.g. 2,6-dimethylpiridine) and sulfur-bearing compounds (dimethyl trisulfide), and aromatic esters (isobutyl benzoate). Such stages also released unknown compounds in relatively high amounts (UNK 1365 and 1378). See Fig. S2 for changes of amounts per sample in individual VOCs over the course of dung pat ageing.

2) Do abundance and species richness of beetles and flies correlate to compound richness and total amount of VOCs during ageing of dung pats?

Fly abundance and species richness were both positively correlated with total amount of VOCs (fly abundance: Deviance = 92.160, P = 0.025; fly species richness: Deviance = 6.658, P = 0.016) and compound richness (fly abundance: Deviance = 281.600, P < 0.001; Fly species richness: Deviance = 16.957, P < 0.001) (Table 2). For beetles, only beetle species richness was marginally negatively correlated with compound richness (Deviance = 33.291, P = 0.051). Otherwise, we found a very weak negative association between total amount of VOCs and beetle abundance (Deviance = 35.558, P = 0.487), species richness (Deviance = 26.941, P = 0.080), and between beetle abundance and compound richness (Deviance = 10.202, P = 0.710).

3) Are changes in insect community affected by the turnover of compounds with aging?

Both beetle (F = 16.7, P = 0.001, all canonical axes explain 42.11% of variability) and fly (F = 10.0, P = 0.001, all canonical axes explain 26.38% of variability) communities were significantly structured by dung pat age. The majority of fly species reached their highest

326 abundance in dung that was 1 hour to 2 days old (Fig. S3). In contrast, the majority of beetle

327 species reached their highest abundances in dung older than 2 days (Fig. S3).

328   Among individual volatile compounds, 1-butanol and dimethyl trisulfide had the

329 strongest correlation with insect community structure ($F = 21.9$, P = 0.001, all canonical axes

330 explain 61.89% of variability) (Fig 3A). Fly species were predominantly associated with 1-

331 butanol, with the exception of few late successional groups (e.g. Chironomidae), while beetles

332 were predominantly associated with dimethyl trisulfide.

333   As we previously identified two prominent successional groups of volatiles (early and

334 late), we analysed their effect on insect community composition. Insect community was

335 significantly structured by volatile successional groups ($F = 5.5$, P = 0.001, all canonical axes

336 explain 29% of variability). Fly species were predominantly associated with early successional

337 volatiles, again with exception of few very late successional species/families (Fig. 3B), while

338 beetles were predominantly associated with late successional volatiles, with exception of few

339 very early successional species (e.g. *Aphodius erraticus* and *Onthophagus joanne*).

340

341

342

343   DISCUSSION

344 As we expected, the pattern of volatile organic compounds sampled under natural conditions

345 differ markedly from that retrieved from *in vitro* sampling (Varma et al. 2018). Our field

346 samples contained two prominent successional groups of dung emitted volatiles. The early

347 successional group comprised primarily of aliphatic alcohols and phenols (present in dung less

348 than 48 hours old). Late successional group comprised mainly of aliphatic esters, nitrogen and

349 sulphur bearing compounds (present in dung older than 48 hours). In contrast to our

350 expectations, only fly abundance and species richness were negatively associated with the total

14

351 amount of scents captured, primarily due to a decrease in early sucessional VOCs amounts in

352 48 hours old dung. Finally, mostly early successional fly species were associated with early

353 successional VOC compounds (represented by 1-butanol) while later occurring beetles were

354 associated with late successional VOC compounds (represented by dimethyl trisulfide).

355

356 **Succession of volatiles emitted from dung**

357 The turn-over between early and late successional group of volatiles seems to reflect the

358 interchange in dung microbial community. During dung ageing the early successional anaerobic

359 microbial community, originating in defecators' gut, gets replaced by aerobic microbiota

360 originating in soil (Perez-Valera et al. 2019). Such replacement is facilitated by burrowing

361 activity of macrofauna, primarily by dung-inhabiting insects (Stevenson and Dindal 1987).

362 This explains the exclusive presence of aliphatic alcohols and phenols in early succession,

363 which are either by-products of anaerobic sugar assimilation (alcohols) (Boumba et al. 2008;

364 Dekeirsschieter et al. 2009; Forbes and Perrault 2014) or products of protein asimilation

365 (phenols) (Saito et al. 2018). Late successional group of compounds could be a result of

366 combined activity of insects and aerobic microbiota. A direct contribution by insects to the pool

367 of late successional compounds is highly probable, yet only indirect evidence is available at

368 present. The majority of unidentified volatiles are likely derivates of carboxylic acids, which in

369 turn constitute insect pheromones (Burger et al. 2002). Among other late successional

370 compounds, terpenoids, citrals and their derivates were found to be products of insect defensive

371 glands, especially in Staphylinidae (Dettner and Schwinger 1982; Huth and Dettner 1990), who

372 are the most species rich group of dung-inhabiting beetles at our study site (Sladecek et al.

373 2013). Finally, the nitrogen- and sulphur- bearing compounds could be a result of nucleotide

374 and protein degradation of insects killed by predators or previous anaerobic microbiota

375 (Dekeirsschieter et al. 2009). These connections should be tested directly in future studies.

376   The interchange in anaerobic and aerobic microbial community might explain the

377   pattern in the total amount of VOCs and compound richness, which both decreased between 1

378   hour and 48 hours old dung and peaked in dung 72 hours old before steadily decreasing again.

379   Our results are therefore in strong contrast to previous studies of compound turnover in dung

380   (Varma et al. 2018), which reported general increase in total amounts of VOCs until 7-14 days.

381   It is important to note, however, that the study of Varma (2018) presents results of *in vitro*

382   experiments, which lack aerobic soil bacteria and insects. Thus, differences between the two

383   studies might be linked to the prescence/absence of insects and as a consequence the prescence

384   or absence of soil bacteria. Further field experiments with exclusion of insects are needed to

385   investigate this assumption.

386   In contrast to carrion, which emits the highest total amounts of VOCs during the mid

387   to mid-late phases of its ageing (Dekeirsschieter et al. 2009; Paczkowski et al. 2015; Stadler et

388   al. 2015), dung emits the highest compound richness and highest total amount of VOCs at the

389   earliest stages. In dung, insect activity leads to lower emission of VOCs via moisture reduction

390   and hardening the dung crust (Skidmore 1991), although the crust is still permeable to dung

391   emitted VOCs (Laubach et al. 2013). In carrion, insect activity leads to increase of compound

392   richness and total amount of VOCs due to increase of carrion surface (Matuszewski et al. 2011).

393   In contrast to dung, there are more distinct successional stages along carrion ageing when it

394   comes to the composition of compounds emitted (Dekeirsschieter et al. 2009; Paczkowski et al.

395   2015; Stadler et al. 2015). Indeed there are rather conflicting results for both the identification

396   of individual stages and the succession of carrion VOCs (namely what are very early and very

397   late successional compounds) making a comparison between dung and carrion VOCs

398   succession, in terms of volatile compounds, impossible for now.

399

400   **Interplay of insect and dung-released VOC succession**

401   The most peculiar result of our study is that the two most abundant and species-rich dung-

402   inhabiting insect groups reflect the separation between early and late successional compounds.

403   As dung-inhabiting insects depend on chemical cues when locating their food (Frank et al.

404   2018; Wurmitzer et al. 2017), the preference for either early or late successional compounds

405   may allow recognition of environmental conditions suitable for their activity (habitat filtering)

406   (Keddy 1992; Kraft et al. 2015). Alternatively, it may help to avoid inter-specific competition

407   (niche differentiation) (Silvertown 2004), or to assist in locating dung pats with a high number

408   of prey (resource availability) (Hulcr et al. 2006; Segura et al. 2012).

409   The habitat filtering scenario appears as the most probable, at least in temperate

410   communities, where beetles co-occur with fly larvae, the main potential competitors for

411   coprophagous beetles during succession (Sladecek et al. 2017a). In this case, the early

412   successional compounds, their compound richness and total amount of scent captured, may

413   signal to adult flies that the dung is fresh, without a firm crust (personal observation). Such

414   dung pats are ideal feeding and oviposition resources for adult flies (Erzinclioglu 1996;

415   Hammer 1941). The late successional compounds could signal to the adult flies that there is a

416   hard crust on the dung pat with empty galleries underneath (unsuitable for oviposition)

417   (Skidmore 1991). Additionally, such galleries host an ever increasing community of beetle

418   predators which feed on adult flies (Hanski and Koskela 1977; Sladecek et al. 2013). For

419   beetles, in general, the early successional compounds could signal a lethal level of dung

420   moisture (Whipple et al. 2013). Moreover, individual early successional compounds were found

421   to attract fewer beetles than complete bouquets of volatiles released from dung (Frank et al.

422   2018; Wurmitzer et al. 2017). The late successional compounds might provide a cue, signalling

423   prey density to dung-inhabiting predators. Indeed predators inhabiting ephemeral resource

424   patches are well known to use chemical signals for prey localization (Hulcr et al. 2006; Segura

425   et al. 2012).

426   Despite the apparent association between both insect groups and their respective VOC

427   successional groups (e.g. flies and early successional volatiles), there were some exceptions

428   from this pattern. Such exceptions are species that are usually better/worse in dealing with dung

429   characteristics (e.g. dung moisture) than other species from their group. These exceptions

430   included fly species that develop in very old dung that resembles other decaying materials (e.g.

431   humus), like dipteran Chironomidae species (Hammer 1941). Moreover, some beetles oviposit

432   directly into the soil and are as such able to avoid lethal dung moisture in fresh dung pats (e.g.

433   *Aphodius erraticus*) (Gittings and Giller 1997). Both such exceptions further support the idea

434   that habitat filtering dominates this system.

435   Distinct separation of dung-inhabiting insects among early (represented by 1-butanol)

436   and late (represented by dimethyl trisulfide) successional groups of volatiles suggests that dung-

437   inhabiting insects might be associated with more complex clusters of VOCs. This is in

438   agreement with contemporary studies pinpointing dung beetle preference to complex bouquets

439   rather than to single compounds (Frank et al. 2018; Wurmitzer et al. 2017). In contrast, there

440   are finer scale beetle preferences for volatiles emitted from carrion of different age, namely the

441   burying beetles and mid-successional oligosulfides (Kalinova et al. 2009; Podskalska et al.

442   2009; von Hoermann et al. 2013), and late successional hide beetles and carboxylic derivates

443   (von Hoermann et al. 2011). The association between dung beetles and dimethyl trisulfide is,

444   however, a pertinent result because dimethyl trisulfide attracts beetles to carrion (Podskalska et

445   al. 2009), which could explain why dung-inhabiting beetles (both coprophages and predators)

446   are also attracted to carrion (Kadlec et al. 2019; Weithmann et al. 2020). It is also one of the

447   main compounds used by plants for deceptive pollination (Jürgens et al. 2006), which could

448   explain why they are deceived by such plants (Sakai and Inoue 1999).

449

450   **Conclusions and directions for future studies**

18

451   We present another piece of evidence supporting the hypothesis that the succession of dung-

452   inhabiting insects is driven by habitat filtering (Sladecek et al. 2017a), with dung emitted

453   volatiles used as a signal for other dung conditions (dung moisture, etc.). Our data also suggest

454   a point at which microbes originating in the herbivores gut are naturally surpassed by the

455   activity of aerobic soil microbes. It appears that this switch is facilitated by insects burrowing

456   and aerating the dung (48 – 72 hours old dung) (Perez-Valera et al. 2019).

457   For the first time we demonstrate a correlation between the structure of the insect decomposer

458   community and its dynamics, and the volatile organic compounds emitted from their resource.

459   This connection is likely to play a crucial role in insect community assembly.

460   Future studies should A) manipulate the availability of insects in natural conditions to

461   test which dung volatiles are produced or induced by the availability insects; and B) investigate

462   the relationship between insects and volatiles in different dung types (e.g. horse, sheep) and

463   other types of habitat (e.g. carrion, rotting fruit) to further test our hypothesis of habitat filtering-

464   driven succession in ephemeral resource patches (Sladecek et al. 2017a).

465

466   **Data accessibility**

467   Data are available from the figshare.com repository (doi:10.6084/m9.figshare.7039553.v1)

468   (Sladecek et al. 2018).

469   **Supplementary Materials**

470   Table S1: Dung emitted volatile compounds sampled in our study.

471   Figure S1: Hierarchical clustering of dung volatile compounds along the gradient of dung pat

472   ageing.

473   Figure S2: Total amount of each VOC per dung ages.

474   Figure S3: DCCA ordination diagrams presenting the successional patterns of dung-inhabiting

475   beetles and flies.

476

477

478

479

480

481

482

483

484

485

486

487

References

Adams RP (2007) Identification of Essential Oil Components by Gas Chromatography/Mass
    Spectrometry. Allured Publishing Corporation, Carol Streams

Balkenius A, Rosen W, Kelber A (2006) The relative importance of olfaction and vision in a
    diurnal and a nocturnal hawkmoth J Comp Physiol 192:431-437 doi:10.1007/s00359-
    005-0081-6

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using
    lme4 Journal of Statistical Software 67:1-49

Boumba VA, Ziavrou KS, Vougiouklakis T (2008) Biochemical pathways generating post-
    mortem volatile compounds co-detected during forensic ethanol analyses Forensic Sci
    Int 174:133-151 doi:10.1016/j.forsciint.2007.03.018

Burger BV, Petersen WGB, Weber WG, Munro ZM (2002) Semiochemicals of the
    Scarabaeinae. VII: Identification and synthesis of EAD-active constituents of

20

501   abdominal sex attracting secretion of the male dung beetle, *Kheper subaeneus* J Chem

502   Ecol 28:2527-2539 doi:Doi 10.1023/A:1021440220329

503   de Mendonca F, Vilela E, Eiras A, Sant'Ana A (1999) Resposta de Cosmopolites sordidus

504   (Germar) (Coleoptera, Curculionidae) aos voláteis da planta hospedeira e de adultos

505   coespecíficos em olfatômetro Rev Bras Zool 16:123-128

506   Dekeirsschieter J, Verheggen FJ, Gohy M, Hubrecht F, Bourguignon L, Lognay G, Haubruge

507   E (2009) Cadaveric volatile organic compounds released by decaying pig carcasses

508   (Sus domesticus L.) in different biotopes Forensic Sci Int 189:46-53

509   doi:10.1016/j.forsciint.2009.03.034

510   Dettner K, Schwinger G (1982) Defensive secretions of three oxytelinae rove beetles

511   (Coleoptera: Staphylinidae) J Chem Ecol 8:1411-1420 doi:10.1007/BF01403104

512   Dötterl S, Jürgens A, Seifert K, Laube T, Weissbecker B, Schutz S (2006) Nursery pollination

513   by a moth in *Silene latifolia*: the role of odours in eliciting antennal and behavioural

514   responses New Phytol 169:707-718 doi:10.1111/j.1469-8137.2005.01509.x

515   Erzinclioglu Z (1996) Blowflies. The Richmond Publishing Co, Slough

516   Etl F, Berger A, Weber A, Schönenberger J, Dötterl S (2016) Nocturnal Plant Bugs Use cis-

517   Jasmone to Locate Inflorescences of an Araceae as Feeding and Mating Site J Chem

518   Ecol 42:300-304 doi:10.1007/s10886-016-0688-9

519   Finn J (2001) Ephemeral resource patches as model systems for diversity-function

520   experiments OIKOS 92:363-366

521   Forbes SL, Perrault KA (2014) Decomposition Odour Profiling in the Air and Soil

522   Surrounding Vertebrate Carrion Plos One 9 doi:ARTN e95107

523   10.1371/journal.pone.0095107

524   Frank K, Bruckner A, Bluthgen N, Schmitt T (2018) In search of cues: dung beetle attraction

525   and the significance of volatile composition of dung Chemoecology 28:145-152

526   doi:10.1007/s00049-018-0266-4

527   Frederickx C, Dekeirsschieter J, Verheggen FJ, Haubruge E (2012) Responses of *Lucilia*

528   *sericata* Meigen (Diptera: Calliphoridae) to Cadaveric Volatile Organic Compounds J

529   Forensic Sci 57:386-390 doi:10.1111/j.1556-4029.2011.02010.x

530   Gittings T, Giller P (1997) Life history traits and resource utilisation in an assemblage of

531   north temperate *Aphodius* dung beetles (Coleoptera: Scarabaeidae) Ecography 20:55-

532   66

533   Gittings T, Giller P (1998) Resource quality and the colonisation and succession of

534   coprophagous dung beetles Ecography 21:581-592

535   Hammer O (1941) Biological and ecological investigations on flies associated with pasturing

536   cattle and their excrement Vidensk Medd Fra Dansk Naturh Foren 105:1-257

537   Hanski I, Koskela H (1977) Niche relations among dung-inhabiting beetles Oecologia 28:203-

538   231

539   Hartig F (2019) DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed)

540   Regression Models.

541   Holter P (1979) Effect of dung-beetles (*Aphodius* spp) and earthworms on the disappearance

542   of cattle dung Oikos 32:393-402

543   Hulcr J, Ubik K, Vrkoc J (2006) The role of semiochemicals in tritrophic interactions between

544   the spruce bark beetle Ips typographus, its predators and infested spruce J Appl

545   Entomol 130:275-283 doi:10.1111/j.1439-0418.2006.01069.x

546   Huth A, Dettner K (1990) Defense Chemicals from Abdominal Glands of 13 Rove Beetle

547   Species of Subtribe Staphylinina (Coleoptera, Staphylinidae, Staphylininae) J Chem

548   Ecol 16:2691-2711 doi:Doi 10.1007/Bf00988079

549 Jürgens A, Dötterl S, Meve U (2006) The chemical nature of fetid floral odours in stapeliads

550 (Apocynaceae-Asclepiadoideae-Ceropegieae) New Phytol 172:452-468

551 doi:10.1111/j.1469-8137.2006.01845.x

552 Jürgens A, Wee SL, Shuttleworth A, Johnson SD (2013) Chemical mimicry of insect

553 oviposition sites: a global analysis of convergence in angiosperms Ecol Lett 16:1157-

554 1167 doi:10.1111/ele.12152

555 Kadlec J, Mikatova S, Maslo P, Sipkova H, Sipek P, Sladecek FXJ (2019) Delaying insect

556 access alters community composition on small carrion: a quantitative approach

557 Entomol Exp Appl 167:729-740 doi:10.1111/eea.12826

558 Kalinova B, Podskalska H, Ruzicka J, Hoskovec M (2009) Irresistible bouquet of death-how

559 are burying beetles (Coleoptera: Silphidae: Nicrophorus) attracted by carcasses

560 Naturwissenschaften 96:889-899 doi:10.1007/s00114-009-0545-6

561 Karmakar A, Mukherjee A, Barik A (2016) Floral volatiles with colour cues from two

562 cucurbitaceous plants causing attraction of Aulacophora foveicollis Entomol Exp Appl

563 158:133-141 doi:10.1111/eea.12395

564 Keddy PA (1992) Assembly and Response Rules - 2 Goals for Predictive Community

565 Ecology J Veg Sci 3:157-164 doi:Doi 10.2307/3235676

566 Kraft NJB, Adler PB, Godoy O, James EC, Fuller S, Levine JM (2015) Community assembly,

567 coexistence and the environmental filtering metaphor Funct Ecol 29:592-599

568 doi:10.1111/1365-2435.12345

569 Krell FT (2007) Dung Beetle Sampling Protocols. Denver Museum of Nature and Science,

570 USA

571 Laubach J, Taghizadeh-Toosi A, Gibbs SJ, Sherlock RR, Kelliher FM, Grover SPP (2013)

572 Ammonia emissions from cattle urine and dung excreted on pasture Biogeosciences

573 10:327-338 doi:10.5194/bg-10-327-2013

574   Leps J, Smilauer P (2003) Multivariate analysis of ecological data using CANOCO.

575   Cambridge university press, Cambridge, UK

576   Marino P, Raguso R, Goffinet B (2009) The ecology and evolution of fly dispersed dung

577   mosses (Family Splachnaceae): Manipulating insect behaviour through odour and

578   visual cues Symbiosis 47:61-76

579   Matuszewski S, Bajerlein D, Konwerski S, Szpila K (2011) Insect succession and carrion

580   decomposition in selected forests of Central Europe. Part 3: Succession of carrion

581   fauna Forensic Sci Int 207:150-163

582   Midgley JJ, White JDM, Johnson SD, Bronner GN (2015) Faecal mimicry by seeds ensures

583   dispersal by dung beetles Nat Plants 1 doi:Artn 15141

584   10.1038/Nplants.2015.141

585   Milet-Pinheiro P, Ayasse M, Dötterl S (2015) Visual and Olfactory Floral Cues of Campanula

586   (Campanulaceae) and Their Significance for Host Recognition by an Oligolectic Bee

587   Pollinator Plos One 10 doi:ARTN e0128577

588   10.1371/journal.pone.0128577

589   Mitchell TC, Dötterl S, Schaefer H (2015) Hawk-moth pollination and elaborate petals in

590   Cucurbitaceae: The case of the Caribbean endemic Linnaeosicyos amara Flora 216:50-

591   56 doi:10.1016/j.flora.2015.08.004

592   Mohr CO (1943) Cattle droppings as ecological units Ecol Monogr 13:275-298

593   Omura H, Honda K (2009) Behavioral and electroantennographic responsiveness of adult

594   butterflies of six nymphalid species to food-derived volatiles Chemoecology 19:227-

595   234 doi:10.1007/s00049-009-0024-8

596   Paczkowski S, Nicke S, Ziegenhagen H, Schutz S (2015) Volatile Emission of Decomposing

597   Pig Carcasses (Sus scrofa domesticus L.) as an Indicator for the Postmortem Interval J

598   Forensic Sci 60:S130-S137 doi:10.1111/1556-4029.12638

599   Perez-Valera E, Kyselkova M, Ahmed E, Sladecek FXJ, Goberna M, Elhottova D (2019)

600   Native soil microorganisms hinder the soil enrichment with antibiotic resistance genes

601   following manure applications Sci Rep-Uk 9 doi:Artn 6760

602   10.1038/S41598-019-42734-5

603   Podskalska H, Ruzicka J, Hoskovec M, Salek M (2009) Use of infochemicals to attract

604   carrion beetles into pitfall traps Entomol Exp Appl 132:59-64 doi:10.1111/j.1570-

605   7458.2009.00871.x

606   Primante C, Dötterl S (2010) A Syrphid Fly Uses Olfactory Cues to Find a Non-Yellow

607   Flower J Chem Ecol 36:1207-1210 doi:10.1007/s10886-010-9871-6

608   Recinos-Aguilar YM, Garcia-Garcia MD, Malo EA, Cruz-Lopez L, Cruz-Esteban S, Rojas JC

609   (2020) The Succession of Flies of Forensic Importance Is Influenced by Volatiles

610   Organic Compounds Emitted During the First Hours of Decomposition of Chicken

611   Remains J Med Entomol 57:1411-1420 doi:10.1093/jme/tjaa064

612   Saito Y, Sato T, Nomoto K, Tsuji H (2018) Identification of phenol- and p-cresol-producing

613   intestinal bacteria by using media supplemented with tyrosine and its metabolites

614   Fems Microbiol Ecol 94 doi:ARTN fiy125

615   10.1093/femsec/fiyD25

616   Sakai S, Inoue T (1999) A new pollination system: Dung-beetle pollination discovered in

617   *Orchidantha inouei* (Lowiaceae, Zingiberales) in Sarawak, Malaysia Am J Bot 86:56-

618   61 doi:Doi 10.2307/2656954

619   Segura DF, Viscarret MM, Ovruski SM, Cladera JL (2012) Response of the fruit fly

620   parasitoid *Diachasmimorpha longicaudata* to host and host-habitat volatile cues

621   Entomol Exp Appl 143:164-176 doi:10.1111/j.1570-7458.2012.01246.x

622   Schneider ER et al. (2017) Molecular basis of tactile specialization in the duck bill P Natl

623   Acad Sci USA 114:13036-13041 doi:10.1073/pnas.1708793114

624   Silvertown J (2004) Plant coexistence and the niche Trends Ecol Evol 19:605-611

625   doi:10.1016/j.tree.2004.09.003

626   Skidmore P (1991) Insects of the British cow-dung community. Field Studies Council,

627   Slade E, Mann D, Villanueva J, Lewis O (2007) Experimental evidence for the effects of

628   dung beetle functional group richness and composition on ecosystem function in a

629   tropical forest J Anim Ecol 76:1094-1104

630   Sladecek F, Dötterl S, Schäffler I, Segar S, Konvicka M (2018) Succession of dung-inhabiting

631   beetles and flies reflects the succession of dung-emitted volatile compounds.

632   doi:10.6084/m9.figshare.7039553.v1

633   Sladecek FXJ, Hrcek J, Klimes P, Konvicka M (2013) Interplay of succession and seasonality

634   reflects resource utilization in an ephemeral habitat Acta Oecol 46:17-24

635   Sladecek FXJ, Segar ST, Lee C, Wall R, Konvicka M (2017a) Temporal Segregation between

636   Dung-Inhabiting Beetle and Fly Species Plos One 12:e0170426

637   doi:10.1371/journal.pone.0170426

638   Sladecek FXJ, Sulakova H, Konvicka M (2017b) Temporal segregations in the surface

639   community of an ephemeral habitat: Time separates the potential competitors of

640   coprophilous Diptera Entomol Sci 20:111-121 doi:10.1111/ens.12240

641   Stadler S, Desaulniers JP, Forbes SL (2015) Inter-year repeatability study of volatile organic

642   compounds from surface decomposition of human analogues Int J Legal Med

643   129:641-650 doi:10.1007/s00414-014-1024-y

644   Stevenson BG, Dindal DL (1987) Functional Ecology of Coprophagous Insects - a Review

645   Pedobiologia 30:285-298

646   Team RC (2020) R: A Language and Environment for Statistical Computing. Vienna, Austria

647   Ter Braak CJF, Smilauer P (2012) Canoco reference manual and user's guide: software for

648   ordination, version 5.0. Microcomputer Power, Ithaca, NY

649   Varma VS et al. (2018) Diet Supplementation With Pomegranate Peel Extract Altered

650   Odorants Emission From Fresh and Incubated Calves' Feces Front Sustain Food S 2

651   doi:Unsp 33

652   10.3389/Fsufs.2018.00033

653   von Hoermann C, Ruther J, Reibe S, Madea B, Ayasse M (2011) The importance of carcass

654   volatiles as attractants for the hide beetle *Dermestes maculatus* (De Geer) Forensic Sci

655   Int 212:173-179 doi:10.1016/j.forsciint.2011.06.009

656   von Hoermann C, Steiger S, Muller JK, Ayasse M (2013) Too Fresh Is Unattractive! The

657   Attraction of Newly Emerged *Nicrophorus vespilloides* Females to Odour Bouquets of

658   Large Cadavers at Various Stages of Decomposition Plos One 8 doi:ARTN e58524

659   10.1371/journal.pone.0058524

660   Wassmer T (2014) Seasonal Occurrence (Phenology) of Coprophilous Beetles (Coleoptera:

661   Scarabaeidae and Hydrophilidae) from Cattle and Sheep Farms in Southeastern

662   Michigan, USA Coleopts Bull 68:603-618

663   Weithmann S, von Hoermann C, Schmitt T, Steiger S, Ayasse M (2020) The Attraction of the

664   Dung Beetle Anoplotrupes stercorosus (Coleoptera: Geotrupidae) to Volatiles from

665   Vertebrate Cadavers Insects 11 doi:10.3390/insects11080476

666   Whipple SD, Cavallaro MC, Hoback WW (2013) Immersion Tolerance in Dung Beetles

667   (Coleoptera: Scarabaeidae) Differs among Species but Not Behavioral Groups

668   Coleopts Bull 67:257-263

669   Wu X, Sun S (2010) The roles of beetles and flies in yak dung removal in an alpine meadow

670   of eastern Qinghai-Tibetan Plateau Ecoscience 17:146-155

671   Wurmitzer C, Bluthgen N, Krell FT, Maldonado B, Ocampo F, Muller JK, Schmitt T (2017)

672   Attraction of dung beetles to herbivore dung and synthetic compounds in a

673   comparative field study Chemoecology 27:75-84 doi:10.1007/s00049-017-0232-6

674

675

676

677

678

679

680

681

682

683

684 Table 1. Model coefficients for GLMs focusing on trends in the total amount of VOCs and

685 compound richness along the gradient of dung pat ageing.

686 All models were fitted with Poisson distribution of errors. amount = total amount of VOCs (in

687 ng.min^{-1}), richness = compound richness, Est. = slope estimate for relation between total

688 amount of scents captured /compound richness and dung age, Std.Err. = standard error of mean

689 for Est., z = z value for each model, p = statistical significance, Null. D. (df) = null deviance of

690 each model (degrees of freedom), Res. D. (df) = residual deviance of each model (degrees of

691 freedom), GoF = goodness of fit estimate for each model, calculated as; GoF = 1- (residual

692 deviance/null deviance).

693

	Est.	Std.Err.	z	p	Null. D. (df)	Res. D. (df)	GoF
amount	-0.003	0.001	-3.894	<0.001	35.631(29)	20.011(28)	0.438
richness	-0.006	0.002	-3.099	0.004	9.920(29)	7.258(28)	0.268

694

695

696

697

698

699

700

701

702

703

704

705

706 Table 2. Model coefficients for GLMs focusing on the relationship between the community of

707 dung-inhabiting insects and dung-released VOCs.

708 All models were fitted with quasipoisson distribution of errors. Two sets of four models were

709 fitted; four models with insect (beetles, flies) abundances as response variables and total amount

710 of VOCs as explanatory variable, and four models with insect species richness response

711 variables and compound richness as explanatory variable. Est. = slope estimate for relation

712 between dung-inhabiting insects' abundance/species richness and total amount of scents

713 captured /compound richness, Std.Err. = standard error of mean for Est., t = t value for each

714 model, p = statistical significance, Null. D. (df) = null deviance of each model (degrees of

715 freedom), Res. D. (df) = residual deviance of each model (degrees of freedom), GoF = goodness

716 of fit estimate for each model, calculated as; GoF = 1- (residual deviance/null deviance).

	Est.	Std.Err.	t	P	Null. D. (df)	Res. D. (df)	GoF
Insects ~ total amount of VOCs							
Beetle abundance	-0.009	0.014	-0.684	0.500	2789.300(29)	2753.800(28)	0.013
Beetle richness	-0.019	0.011	-1.691	0.102	432.950(29)	406.010(28)	0.062

Fly abundance	0.037	0.016	2.343	0.026	631.080(29)	538.920(28)	0.146
Fly richness	0.014	0.006	2.468	0.020	39.744(29)	33.086(28)	0.168
Insects ~ compound richness							
Beetle abundance	-0.008	0.022	-0.372	0.713	2789.300(29)	2779.100(28)	0.004
Beetle richness	-0.032	0.016	-1.944	0.062	432.950(29)	399.660(28)	0.077
Fly abundance	0.120	0.027	4.518	<0.001	631.080(29)	349.480(28)	0.446
Fly richness	0.038	0.008	4.525	<0.001	39.744(29)	22.787(28)	0.427

Figure 1: Total amount of VOCs and compound richness of volatiles released from dung throughout dung pat ageing.

A) GLM curves for total amount of VOCs (solid line) and compound richness (dashed line) of volatiles released from dung throughout dung pat ageing. Grey bands represent the standard error of the mean.

B) Mean total amount of VOCs (light grey) and compound richness (dark grey) from individual dung pat ages. Error bars represent the standard error of the mean.

Time (Hours) = time after dung pat creation on the pasture

Figure 2: DCCA ordination presenting changes in dung-emitted volatile compounds profile along dung pat ageing.

The arrow represents the dung pat age as a quantitative predictor, black triangles represent individual dung ages plotted as the supplementary variable, grey stars represent the early successional compounds (with highest abundance until 2 days old dung), white diamonds represent the late successional compounds (with highest abundance in dung older than 2 days). Classification into early and late successional groups is based upon clustering of individual

736 compounds on the horizontal canonical axis (their relation with dung age variable) as presented

737 in Fig. S1. For abbreviations of individual volatiles see Table S1

738 Figure 3: CCA ordinations of relations between dung-emitted volatiles and dung-inhabiting

739 insects.

740 Arrows represent compound/volatiles' successional groups used as predictors, symbols

741 represent insect species or morphospecies (triangle = beetles, star = flies). Named insect species

742 represent exceptions in general patterns (e.g. beetles that are, unlike the majority of beetle

743 species, not associated with late successional volatiles). Such species are, for beetles: Adisti =

744 *Aphodius distinctus*, Aerrat = *Aphodius erraticus*, Cuni = *Cercyon unipunctatus*, Ojoan =

745 *Onthophagus joanne*, Pmarg = *Philonthus marginatus*; for flies: Chir = Chironomidae spp.,

746 Dolicho = Dolichopodidae spp., Hnig = *Hebecnema nigricolor*, Scat = Scatopsidae spp.

747 A) Relation between insect community structure and two compounds with highest impact on

748 insect community. Those two compounds were chosen from the whole pool of compounds by

749 forward selection (see methodology for question 3).

750 B) Relation between insect community and successional groups of volatiles (early and late

751 successional groups). Early VOCs = total amount of scents captured for early successional

752 compounds (e.g. aliphatic alcohols, phenols), Late VOCs = total amount of scents captured

753 for late successional compounds (e.g. esters, sulphur-bearing compounds). For membership of

754 individual compounds within those successional groups see Table S1.

755