Nesting strategies affect altitudinal distribution and habitat use in Alpine dung beetle communities

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The influence of altitude, habitat and nesting strategy on key parameters of ecosystem service provision in Alpine dung beetles

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Running head: Dung beetles along altitudinal gradients

34 **Abstract**

35 1. Dung beetles are key contributors to a suite of ecosystem services. Understanding the factors that

36   dictate their diversity is a necessary step towards preventing negative impacts of biodiversity loss.

37 2. We analysed Alpine dung beetle communities along altitudinal gradients to assess how different

38   components of the community, defined in terms of nesting strategy (dung-ovipositing Aphodidae

39   [DOAs], soil-ovipositing Aphodidae [SOAs] and two paracoprid [PAR] groups, Geotrupidae and

40   Onthophagini) and parameters relevant to ecosystem functioning (species richness, total biomass

41   and functional diversity relative to dung removal), are distributed, and to identify to which

42   environmental factors they respond.

43 3. Species richness declined with altitude. There was no significant variation in functional diversity, or

44   total biomass in relation to altitude. There were significant variations when considered by nesting

45   group: DOA species richness and biomass decreased, SOA biomass increased, and Geotrupidae

46   biomass showed a non-linear trend, as altitude increased.

47 4. Functional diversity and total species richness were positively related to vegetation cover. DOA

48   species richness was highest in forest and scrub, SOA species richness was highest in grassland and

49   PAR species richness was lowest in rocky areas.

50 5. Dung beetle species show different trends in species richness and biomass depending on nesting

51   strategy. Management to promote the dung beetle community should include maintenance of a

52   mosaic of habitat types. Given the importance of species richness and biomass to ecosystem

53   functioning, and the complimentary effect of different dung beetle groups, such a strategy will

54   protect and enhance the ecosystem services that Alpine dung beetles provide.

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57 **Key words.** Aphodidae, dung removal, ecosystem functioning, endocoprid, functional diversity,

58 Geotrupidae, Onthophagini, paracoprid.

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Introduction

Biodiversity is facing many pressures globally, leading to population declines, range contractions and species extinctions (Butchart *et al*., 2010), and consequent negative impacts on ecosystem services, causing a loss of ecosystem functioning (Naeem *et al*., 2012). Understanding the factors that dictate the diversity of key providers of ecosystem services is therefore a necessary step towards halting or even reversing damaging impacts of biodiversity loss.

Dung beetles contribute to a suite of important ecosystem services, including dung removal and decomposition, nutrient cycling, plant growth enhancement and seed dispersal (e.g. Nichols *et al*. 2008, Beynon *et al*., 2012; Braga *et al*., 2013). Dung beetle species show different nesting strategies, e.g. paracoprids dig tunnels below the dung mass in which they bury brood balls, and endocoprids brood their young inside the dung-mass itself, or at the soil-dung interface. The action of these different groups has been shown to have a complementary effect on dung removal rates (Beynon *et al*., 2012), although the contribution of individual species is not equal, larger species having a disproportionate effect (Rosenlew & Roslin, 2008). More generally, greater biomass has been shown to enhance decomposition rates for decomposer communities (O'Hea *et al*., 2010). A diverse and healthy dung beetle community is therefore likely to enhance ecosystem services, and this effect may be particularly important in perturbed ecosystems (Beynon *et al*., 2012).

Mountainous areas are likely to be particularly sensitive to environmental change. In the European Alps, negative impacts on biodiversity are expected due to increased disturbance (e.g. from leisure activities, Negro *et al*., 2009), changes in pastoral management (e.g. Marini *et al*., 2009) and elevational shifts in vegetation zones due to climate change (e.g. Cannone *et al*., 2007). Dung beetles are likely to be sensitive to such factors, in particular changes in livestock management (e.g. Tocco *et al*., 2013) and climate change (e.g. Menéndez *et al*., 2014). Most studies of dung beetles in European mountains have taken place in pastures at altitudes at or below the natural treeline (e.g. Jay-Robert *et al*., 2008; Negro *et al*., 2011, Tocco *et al*., 2013). However, there have been studies considering variations in overall abundance and diversity along altitudinal gradients up to 3000m (e.g. Jay-Robert *et al*., 1997).

In this paper, we advance the study of dung beetle ecosystem service provision by considering how key parameters, shown experimentally to be associated with dung removal, respond to environmental variations in the field, specifically by analyzing functional diversity, biomass and species richness along altitudinal gradients (c. 1900-2800m) in the European Alps. Functional diversity of dung beetles has been shown to be sensitive to land use changes (Barragàn *et al*., 2011) and specifically to grazing management in the Alps (Tocco *et al*., 2013), whilst biomass and species richness have been shown to be linked to dung removal rates, one of the key ecosystem functions provided by dung beetles (Beynon *et al*., 2012; Nervo *et*

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94   *al.*, 2014). Given the differing contributions to ecosystem functioning between dung beetle nesting groups

95   (e.g. Beynon et al., 2012), we also consider the effect of dung beetle nesting strategy on these parameters.

96   The altitudinal gradient approach can prove very useful in understanding the distributions of

97   species, as inferences can be drawn about larger-scale and longer-term processes from data collected at a

98   relatively small spatial scale (Hodkinson 2005). We are unaware of any studies that have considered dung

99   beetle functional diversity or biomass variations along altitudinal gradients in alpine (i.e. above the treeline)

100   habitat, although species richness gradients have been considered (e.g. Jay-Robert et al. 1997). For all

101   parameters, we consider both altitudinal gradients and habitat gradients, the latter being particularly

102   important in terms of informing management strategies in a landscape subject to several environmental

103   pressures. Furthermore, we control statistically for variations in trapping effort, variations in exposure time,

104   and variations in dung resources in the surrounding area of each sampling point, a potentially important

105   variable which has yet to be taken into account in studies of Alpine dung beetles. Our over-arching goals

106   are therefore to clarify how the different components of the assemblage, defined in terms of nesting

107   strategy and parameters relevant to ecosystem functioning, are distributed, and to identify to which

108   environmental factors they respond.

109

110   **Methods**

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112   The study was carried out in 2010 and 2011 at five sites in the mountains of the western Italian Alps in Val

113   Gressoney, Val Grande, Val Chisone, Val Troncea and Val Argentera (Fig. 1). At each site, four sampling

114   points were identified along gradients ranging from c. 1700m to c. 2800m in altitude, each one in a

115   different habitat type: larch forest, scrub, alpine meadow and grass/rock mosaics. Between four and eight

116   hang-bait traps (Palestrini *et al*., 2008), baited with c. 200g of fresh cow dung, were placed at each point at

117   a minimum distance of 20m. Traps were first set between mid- June and mid-July and were left for variable

118   periods between visits (range 9 – 26 days). Note was made of any traps destroyed or removed. Dung

119   beetles trapped were stored in 70% alcohol before later identification in the laboratory.

120   At each point, the percentage cover of different broadly-defined habitat types was estimated

121   within 100m radius of the point: canopy, shrubs, field-layer vegetation, bare rock. The presence of dung on

122   the first visit (i.e. dung of the previous year) and the presence of livestock within the current year at a point

123   were noted. The topographic variables altitude, slope, easting and northing were extracted for each point

124   from a Digital Terrain Model in GIS. Easting and northing were expressed as an index between 1 (facing

125   directly south or east) and -1 (facing directly north or west) following Bradbury *et al*., (2011). Site

126   descriptions and precise locations are given in the Supplementary Material (Table S1).

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128   *Dung beetle nesting group*

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130 Dung beetles were defined into groups according to nesting strategy and taxonomy, based on Beynon *et al*.

131 (2012): dung-ovipositing Aphodidae (DOAs; species that live in the dung pad itself, and which either lay

132 eggs freely in the dung, or fashion brood balls into the dung pad), soil-ovipositing Aphodidae (SOAs; species

133 that construct the nest immediately below the dung pad in the soil-dung surface) and paracoprids (PARs;

134 species that deposit dung containing their eggs at the end of tunnels beneath the dung pad). Within

135 paracoprids, there is also considerable variation in ecology and morphology (Jay-Robert et al. 2008)

136 according to phylogenetic group, so for most analyses we also further divide paracoprids into Geotrupidae

137 (GEO) and Scarabaeidae, which in our case is represented only by the Onthophagini (ONT).

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139 *Dung beetle parameters*

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141 We considered three measures to describe the dung beetle community following Negro *et al*. (2011) and

142 Tocco *et al*. (2013): functional diversity (FD), species richness, and biomass. FD is a component of

143 biodiversity that refers to the range and value of organismal traits that influence ecosystem properties

144 (Hooper et al. 2002), and can be calculated by combining a matrix of species occurrence with data on

145 species traits. We focused on dung removal capability by taking into account four functional traits

146 following Tocco *et al.* (2013), using an ecomorphological approach in order to calculate the functional

147 diversity index (Petchey & Gaston, 2006). Functional traits used were nesting behaviour (as above), weight,

148 and two prothoracical leg allometries, all expected to be linked to dung removal efficiency. Dry weight,

149 body size (maximum width of the pronotum) and linear measurements of the prothoracical tibia for ten

150 randomly selected specimens within each species were recorded. These traits were then used in

151 conjunction with abundance data to calculate FD per trap, using the vegan package in R 3.03 (Oksanen *et*

152 *al*., 2013). This translates species traits into pairwise distances among species from which clustering trees

153 of the traits are derived (Oksanen *et al*., 2013). FD is the total branch length in a tree connecting all

154 species, and high values of FD indicate communities with a high diversity of functional traits.

155   The total biomass of dung beetles per trap was estimated by multiplying the abundance of each

156 species by its mean mass, and then calculating the sum over all species. As nesting group is intrinsic to the

157 expression of FD, this parameter was calculated only at the whole community level, and not specifically by

158 nesting group. Biomass and species richness were calculated for all groups combined, and for each nesting

159 group separately.

160

161 *Statistical Methods*

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163   The data were analysed primarily through GLMMs which accounted for multiple observations from the

164   same locations by specifying site, point and (for FD and species richness) trap as random factors (see

165   below). Many dung beetle species show seasonal variations in abundance (e.g. Tocco *et al*., 2013). Sites

166   had variable sampling periods (total trapping days varying from 54 to 95 days per site), so 'month' was

167   fitted as a three-level factor (i.e. June/July, August and September) in each model in addition to altitude,

168   habitat and/or group category variables (see below) in order to account for seasonal effects. All analyses

169   were initially carried out on all species combined. For biomass and species richness, the analytical

170   approaches were then repeated including the different nesting groups, and the interaction between

171   nesting group and the environmental variable of interest. Where there was a significant interaction, the

172   analysis was carried out on the groups separately.

173   Species richness is typically related non-linearly to sampling effort (Magurran, 2004), which varied

174   between visits due to trap loss. Therefore, species richness was analysed at the level of the individual trap

175   for each visit. Species accumulation curves were derived for each site using the vegan package in R in order

176   to assess sampling completeness. Species richness contributes to FD, and it is expected that FD will be

177   similarly sensitive to variation in trapping effort, therefore it was also analysed at the level of the individual

178   trap. Species richness and FD were analysed in relation to environmental variables using respectively the

179   glmer command (Poisson-distributed data) and the lmer command (normally distributed data) in the

180   package lme4 (Bates *et al*., 2013) in R 3.0.1 (R Development Core Team, 2013). Non-independence of traps

181   within points and points within sites was accounted for by specifying each as a nested random factor,

182   specifically trap nested within point nested within site. Note FD can only be calculated where a species is

183   present (a single species equates to FD = 0 for a given trap), so this analysis had a lower sample size (there

184   were 38 trap/visits where no dung beetles were captured, leaving a total of 338 traps from 69 point/visits)

185   compared to other analyses. There was a significant positive linear effect of trapping effort on the total

186   biomass per point (z = 2.59, P = 0.015), so mean biomass was expressed per trap for each point and visit for

187   the analysis, allowing a simplified model structure, with point nested within site defined as random factors.

188   The lmer command was used for analysis, after applying a square-root transformation to normalise the

189   data.

190   For all analyses, a statistical hypothesis testing framework was adopted, whereby model reduction

191   was carried out on the full model by sequentially deleting non-significant terms until a minimum adequate

192   model of significant fixed effects remained. For normally distributed data (FD and biomass), P-values were

193   obtained from *F*-tests using the lmerTest package in R. For Poisson models (species richness), P-values

194   were based on *z*-tests.

195   The period between visits varied from 9 to 26 days. The first part of the analysis was therefore to

196   determine if the number of days for which a trap was left between visits (termed exposure time) was

197   related to a given parameter, as longer exposure times might result in more individuals and more species,

198 which would then have to be taken into account in subsequent models. The next goal was then to describe

199 the altitudinal variation in abundance, richness and diversity, hence the model contained altitude, altitude^2

200 (to assess non-linear distributions), month, and exposure time if appropriate.

201 The next step was to identify other environmental correlates of species parameters. There was a

202 high level of inter-correlation between most of the habitat variables considered. PCA was therefore used to

203 describe community variation in relation to continuous environmental variables across the gradient

204 (canopy, shrubs, grass, rock, easting, northing, slope and altitude). The princomp command from the vegan

205 package (Oksanen *et al*., 2013) was used, specifying the scale option. The relationships between habitat

206 gradients, as measured by the derived PCA axes, and dung beetle parameters were analysed using the

207 modelling approaches described above. These models also included the categorical fixed factors presence

208 of livestock and presence of dung. Nesting group and the interaction between nesting group and each PCA

209 axis was also included to assess whether different groups showed different responses to the habitat

210 gradient.

211 Altitude may be a proxy for a multitude of effects operating at various scales (Hodkinson, 2005). If

212 such factors are important, it may be expected that altitude models will perform better than habitat

213 models. If habitat models are better, this suggests that dung beetle communities may be significantly

214 influenced by habitat management. This was assessed by comparing model performance of both full

215 models and reduced models between those for altitude and those for habitat using Akaike's Information

216 Criterion corrected for small sample size (AICc; Burnham & Anderson, 2002).

217

218 **Results**

219

220 One point at the highest altitude in Val Grande was abandoned due to repeated losses of all traps, leaving a

221 total of 19 points sampled. For the five sites, altitudinal range was between 297m and 813m. There were a

222 total of 510 trap/visits placed (i.e. the sum of the number of times an individual trap was set across all sites

223 and visits), but 104 traps (20.4%) were destroyed or removed (due mostly to suspected large wild and

224 domestic mammals), leaving a total sample size of 406 trap/visits and 76 point/visits. Rates of trap loss

225 varied between sites from 11.3% (n = 88 traps set) in Val Argentera to 45.6% (n = 68) in Val Grande.

226 A total of 24 species and 12218 individuals of three families (Aphodiidae, Scarabaeidae and

227 Geotrupidae) were captured (a summary of the numbers and occurrence of each species caught, and their

228 nesting group, is given in Supplementary Material Table S2). The total number of species recorded in the

229 different sites was: Val Gressoney = 17, Val Grande = 9, Val Chisone = 19, Val Troncea = 20, Val Argentera =

230 11. Accumulation curves showed adequate sampling in all except Val Grande, which experienced the

231 highest rate of trap loss (Supplementary Material Fig. S1). This site was omitted from subsequent analyses,

232 leaving a total of 369 trap/visits and 69 point/visits, although results were generally very similar when

233 including these sites (full model details for models derived from four sites, and from all five sites, are given

234 in Supplementary Material Table S3).

235   There was no significant effect of exposure time on species richness (z = 1.54, P = 0.12) or biomass

236 ($F_{1,55.2}$ = 0.02, P = 0.93), but there was a significant negative effect on FD ($F_{1,193.7}$ = 4.03, P = 0.032). The

237 general weak effect of exposure was probably because the dung bait only has a finite period during which it

238 is attractive (Lumaret & Stiernet, 1991). Exposure time was included in all subsequent models for FD, but

239 was not considered further for biomass or species richness.

240

241 *Distribution along the altitudinal gradient*

242

243 There was a decrease in species richness with increasing altitude (z = -2.21, P = 0.027; Fig. 2A). There was

244 no significant relationship between altitude and either FD or biomass. For species richness, there were only

245 two species in ONT, therefore this was combined with GEO for the analyses. There was a significant

246 interaction between altitude and nesting group indicated by significant differences between levels (test

247 relative to PARs: AltA*DOAs, z = -8.00, P < 0.001; AltA*SOAs, z = 6.404, P < 0.001), so the three groups

248 were subsequently analysed separately. There was a significant decrease in species richness of DOAs with

249 increasing altitude (z = -5.58, P < 0.001; Fig. 2B), but no significant variation in the richness of SOAs. PARs

250 showed a non-linear trend (Fig. 2C), with significant effects of both altitude (z = 2.75, P = 0.006) and

251 altitude^2 (z = -2.81, P = 0.005). When considering only GEO, results were very similar in that there was also

252 an overall interaction, and there were significant effects of both altitude (z = 3.96, P < 0.001) and altitude^2

253 (z = -4.00, P < 0.001). FD and most measures of species richness showed a decrease from July to September,

254 with the exception of DOA richness (highest in August) and richness of paracoprid groups (PAR, GEO and

255 ONT), none of which showed significant seasonal variation (Table S3).

256   There was a significant interaction between altitude and nesting group on biomass ($F_{3,253.7}$ = 10.85,

257 P < 0.001). SOAs showed a significant increase in biomass with altitude ($F_{1,13.7}$ = 8.72, P = 0.011; Fig. 3A),

258 whereas there was a significant decrease in DOAs ($F_{1,14.3}$ = 29.67, P < 0.001; Fig. 3B), but no significant effect

259 in GEO or ONT. There was no significant effect of altitude when considering biomass of all paracoprids

260 combined ($F_{1,15.3}$ = 1.24, P = 0.28). Seasonal trends in biomass broadly followed those of species richness,

261 although both PAR and ONT biomass showed significantly higher values in July (Table S3).

262

263 *Associations with habitat*

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265 The first two axes of the PCA explained 85.5% of variation in the data. The first axis described a gradient

266 from open to closed habitat, being positively correlated with canopy (biplot score = 0.66) and negatively

267 with grass (-0.63). The second axis was related to vegetation cover in general, being positively correlated

with grass cover (0.53) and to a lesser extent canopy (0.23), but negatively with rock cover (-0.80). The bi-plot of habitat scores for the first two axes is shown in Fig. 4. The topographic variables had little influence on these axes.

FD was significantly positively related to PCA2 ($F_{1,11.6}$ = 11.58, P = 0.003; Fig. 5A), indicating lower values in habitat with greater rock cover. For species richness, there was a positive relationship with PCA2 (z = 5.01, P < 0.001; Fig. 5B). There were significant interactions between nesting group and both PCA1 (test relative to PARs: PCA1*DOA, z = 9.63, P < 0.001; PCA2*SOA, z = -8.18, P < 0.001) and PCA2 (PCA2*DOA, z = 3.39, P < 0.001; PCA2*SOA, z = -4.80, P < 0.001), so groups were analysed separately (figures are given in the Supplementary Material). DOAs had higher species richness at higher values of PCA1, i.e. more closed habitats (z = 5.24, P < 0.001), and at higher values of PCA2 i.e. well vegetated habitat (z = 5.96, P < 0.001), although species richness was lower when dung was present from the previous year (z = -2.42, P = 0.016, parameter estimate = -0.197 ± 0.081). SOAs conversely showed a negative association with PCA1 (z = -2.68, P = 0.007). They also showed a positive association with PCA2 (z = 1.98, P = 0.047) and a higher species richness when livestock were present (z = 2.52, P = 0.012, parameter estimate = 0.342 ± 0.136). PAR species richness was significantly higher at higher values of PCA2 (z = 5.23, P = 0.001), significantly higher when livestock were present (z = 2.71, P = 0.007, parameter estimate = 0.352 ± 0.137), but significantly lower when dung from the previous year was present (z = -2.35, P = 0.019 parameter estimate = -0.311 ± 0.133). However, when only GEO was considered, there was no significant effect of any habitat variable.

There was no overall effect of habitat on biomass, but there was a significant interaction between PCA1 and nesting group ($F_{2,253.2}$ = 12.67, P < 0.001). Separate analyses showed that the two endocoprid groups had differing responses to the habitat gradient (see Supplementary Material Fig. S3), DOAs increasing in biomass with PCA1 ($F_{1,11.1}$ = 15.00, P = 0.003) and PCA2 ($F_{1,16.9}$ = 4.45, P = 0.050), but SOAs decreasing in biomass with PCA1 ($F_{1,10.3}$ = 14.87, P = 0.003). There was no significant effect of habitat in either GEO or ONT, nor when both paracoprid groups were combined.

Model comparison

In the majority of cases, the habitat model was better (i.e. lower AICc) than the altitude model for species richness, the single exception being for the Geotrupidae full model (Table 1), whereas the altitude models clearly performed better for biomass (when environmental variables were included in the minimum adequate models, which was not always the case). FD was the only parameter to show inconsistencies between ΔAICc for the full model and reduced model, the altitude model being better for the former and the habitat model for the latter.

Discussion

There was a significant decrease in species richness, but no evidence of variation in FD or biomass of dung beetles, in relation to altitude. There were, however, significant variations in biomass and species richness in relation to altitude when considered by nesting group, but these patterns were often opposing. We are unaware of previous studies that have considered variations in the FD or biomass of dung beetle communities along altitudinal gradients. There is no consistent pattern across studies that have considered species richness in relation to altitude, with evidence of decreases (e.g. Jay-Robert *et al*., 1997; Lobo *et al*., 2007), increases (Negro *et al*., 2011), non-linear effects (Escobar *et al*., 2005) or no effect (Romero-Alcaraz & Ávila, 2000). The lack of stronger effects of altitude in our study seems likely to have been due to differing responses of the nesting groups, where DOAs showed a decreasing trend in species richness and biomass with altitude, and SOAs showed no effect on species richness but an increase in biomass with altitude. Paracopids showed a non-linear trend in biomass with altitude, a result largely driven by variations in Geotrupidae biomass. Jay-Robert *et al*. (1997) found a decrease in paracoprids, but an increase in all endocoprids, with altitude. Some of the variability in the findings of our and other studies is likely to result both from the length of the gradient and the maximum altitude considered, but geographic (Errouissi *et al*., 2004), year-to-year (Jay-Robert *et al*., 1997) and sampling period (see below) variation in the response to the altitudinal gradient may also be important.

Both FD and species richness were lower when rock cover was higher, demonstrating that dung beetles are linked to well-vegetated areas. Species richness of SOA and PAR was also higher when livestock were present, suggesting that resources in the area local to the sampling location will influence positively the number of species caught and will not therefore have a competition effect on the pitfall traps. There was a negative effect of presence of dung from the previous year on the species richness of DOAs. Given the role of dung beetles in dung decomposition, this result may indicate a slower rate of dung decomposition in sites where dung beetles are scarce – for example, in their absence, dung has been shown to take up to four years to fully decompose (Walters, 2008). A scarcity could be caused by particular local meteorological conditions, or could also arise if livestock in the area had been treated with pesticides such as avermectins, which may be passed into the dung which then becomes less attractive to the dung beetles (e.g. Webb et al. 2010).

Different groups also responded differently to habitat gradients, both in terms of species richness and biomass, in particular DOAs and SOAs showed opposing trends: both the richness and biomass of SOAs was higher in open grassland, but for DOAs they were higher in closed habitats. The different responses to habitat and altitude may be influenced by the physical factors affecting the dung. In particular, the opposing trends of DOAs and SOAs are probably driven by the different rates of dung dessication in closed and open habitats. DOAs require largely intact dung pads for a high rate of breeding success, and they are

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338   likely to be more sensitive to dung dessication, hence their apparent preference for closed habitats and

339   lower elevations. On the other hand, the burying activity of SOAs may mean they are less sensitive to

340   higher rates of dung drying in open habitats. We suggest that the location of eggs and larvae on or under

341   the soil surface is an adaptation to the harsh, high altitude environmental conditions, which enables SOAs

342   to occur in great numbers in dung pads at high altitude (in keeping with the increase of SOA biomass with

343   increasing altitude). Moreover, since dung drying makes the microhabitat structurally more suitable for

344   predators to invade (Slansky & Rodriguez, 1987), the DOA strategy might result in greater exposure to

345   predators in open habitats.

346   All paracoprids combined (PAR) showed higher species richness in vegetated habitats (i.e. PCA2),

347   but otherwise, they did not show significant variation in relation to habitat gradients. Paracoprids excavate

348   relatively deep tunnels beneath the dung pad, and hence they avoid rocky areas with little or no soil. More

349   broadly, models for paracoprids, including those separately for Geotrupidae and Onthophagini, tended to

350   explain less variation in the data compared to DOAs and SOAs (see pseudo-R^2 values in Table S3). This may

351   be because paracoprid distribution is more closely linked to the ability to excavate deep tunnels, and

352   therefore to soil structure (e.g. Sowig 1995), rather than broader measures of habitat variation.

353   Habitat models were better for describing patterns in FD and species richness, whereas altitude

354   models were better for describing biomass (Table 1). This suggests that habitat management alone is likely

355   to have major effects on species richness of Alpine dung beetle communities. However, the biomass, which

356   will be in part dictated by abundance and in part by the presence of larger species, is likely also to be

357   strongly linked to other factors along the altitudinal gradient (Hodkinson, 2005) – in other words, the

358   broad-scale habitat measures considered here may have been too coarse to identify key small-scale habitat

359   elements for certain components of the community. Further research into effects of micro-habitat and

360   micro-climate on dung beetles at high altitude would help resolve these issues.

361

362   *Caveats on interpretation*

363

364   There are a number of caveats that need to be made when interpreting the results.   First, the sampling for

365   the majority of points was carried out between July and mid-September. Other comparable studies have

366   often also included samples from October (e.g. Lumaret & Stiernet, 1991), or early June (e.g. Jay-Robert *et*

367   *al*., 1997), but there is much variation in sampling periods from published Alpine studies (e.g. May to

368   October, Jay-Robert *et al*., 2008; June and July only, Jay-Robert *et al*., 1997). Our study may have missed

369   some early and late species, and indeed for lower altitude points, species richness was towards the lower

370   end of the range recorded in other studies that considered different periods. Conversely, species richness

371   at the higher altitudes was higher than reported in some comparable studies (e.g. Lumaret & Stiernet,

372   1991; Palestrini *et al*., 2008). The conclusions here must therefore be considered as relevant to the summer

period. Second, there was a high rate of trap loss in some sites, which was probably mostly due to disturbance by livestock. For example, at Val Grande, the highest point suffered 100% trap loss due to the continued presence of sheep. This issue seems to have been particularly severe in our area compared to other similar studies, and may reflect a generally higher level of grazing. We were, however, able to account for the variation in trapping effort between sites statistically using nested random factors. Third, higher altitude species may be more adapted to, and hence more attracted to, dung of wild animals such as Alpine Ibex *Capra ibex* L. and Chamois *Rupicapra rupicapra* L., so sheep dung may have been a better alternative than the cow dung used as bait in the traps. Indeed, it has been shown to be preferred by a number of species recorded in our study (Dormont *et al.*, 2007). Nevertheless, the vast majority of studies cited here also used cow dung. Fourth, although we took a measure of dung resources from domestic animals into account, we did not measure dung of wild animals. Both the amount and diversity of dung could have affected local dung beetle communities. Further research on altitudinal patterns using different types of bait, and accounting for all dung resources at a given sampling point, would be interesting methodological topics to pursue.

Management implications

There are two general findings relevant to habitat management arising from this work. First, vegetated habitats are important. Whilst this may seem self-evident, such a simple relationship may become more important in the future if elevational treeline shifts continue at their current rate (Harsch *et al.*, 2009), because rocky habitats are generally predominant at higher altitudes, but development of vegetation in these areas is likely to be a slow process (e.g. Cannone *et al.*, 2007), resulting in asymmetric shifts in vegetation zones which are likely to have negative consequences for the biodiversity of Alpine grasslands (e.g. Chamberlain *et al.*, 2013). Second, different dung beetle species may show different trends in terms of richness and biomass depending on their nesting strategy. In terms of management to promote the dung beetle community, maintenance of a mosaic of habitat types would therefore seem necessary. Management such as targeted grazing (Tocco *et al.*, 2013) has the potential to maintain a landscape mosaic of open and closed habitat areas, particularly where treelines are advancing due to climate change and changes in grazing practices. Given the likely importance of species richness and biomass to ecosystem functioning, and given the likely complimentary effect of different dung beetle groups, such practices ultimately will protect and enhance the ecosystem services that Alpine dung beetles provide.

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Author contributions

The project was developed by DC and AR. Data were collected in the field by DC, CT, AL and SM. CT identified specimens and took trait measurements in the lab. CP advised on dung beetle sampling methods and identification. DC analysed the data and took the lead role in writing the paper. All co-authors contributed significantly to the submitted manuscript through discussions and additions to the text.

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Table 1. A comparison of performance of altitude and habitat models based on AICc. Full models were considered which included either altitude and altitude^2 (altitude models), or PCA1, PCA2, presence of cattle and presence of dung (habitat models). Month was in all models. ΔAICc is the difference in AICc between habitat and altitude models, where ΔAICc > 2 suggests altitude is the 'best' model and ΔAICc < -2 suggests habitat is the 'best' model. ΔAICc MAM is the same comparison but considering the final reduced models (see Table S3). In some cases, no environmental variables were significant, in which case ΔAICc MAM is zero (e.g. when only month was significant). Note that species richness was not analysed for Onthophagini.

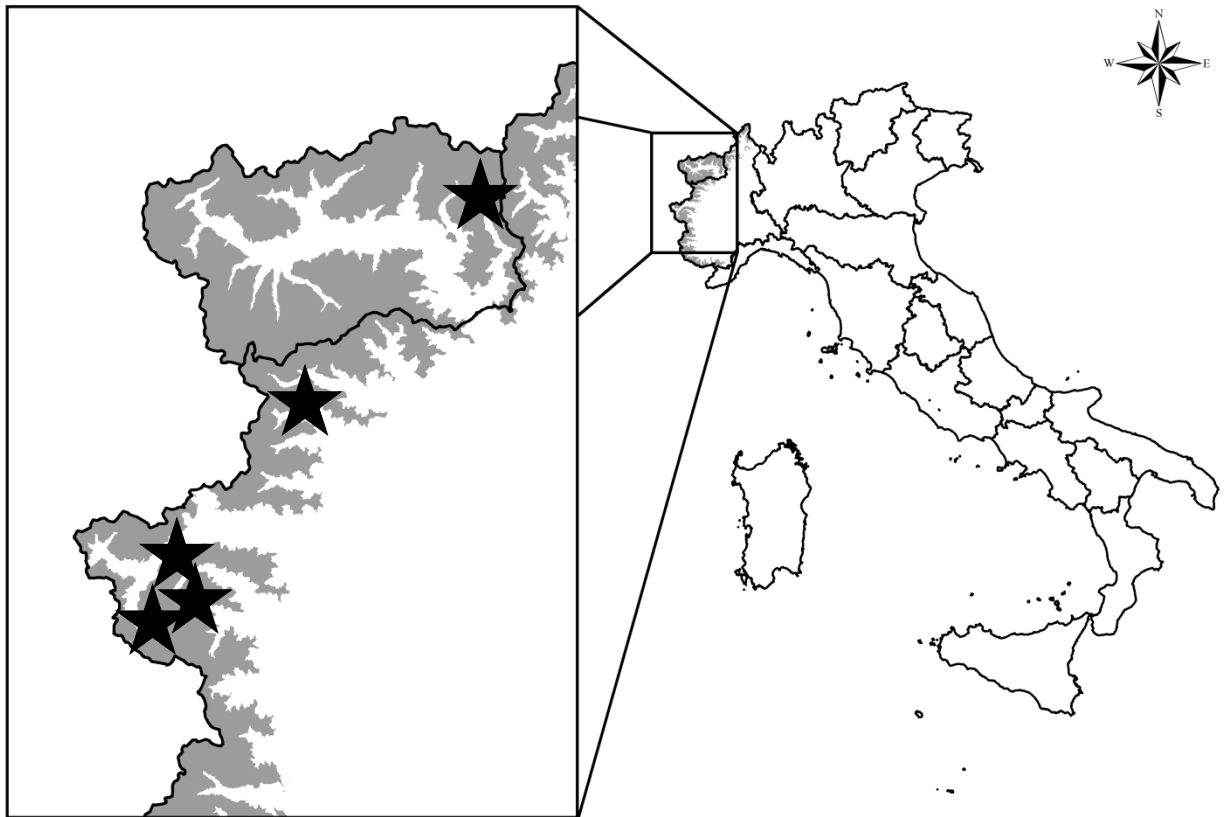| Group	Parameter	ΔAICc	ΔAICc MAM
All	FD	3.7	-2.3
	Species richness	-13.4	-12.4
	Biomass	31.7	0
Dung-ovipositing Aphodidae	Species richness	-12.7	-10.7
	Biomass	35.8	26.6
Soil-ovipositing Aphodidae	Species richness	-7.2	-6.3
	Biomass	32.9	7.9
Paracoprids (all)	Species richness	-2.7	-9.4
	Biomass	35.3	0
Geotrupidae	Species richness	5.88	0
	Biomass	35.4	0
Onthophagini	Biomass	36.4	0

Fig. 1. Map of study area showing location of each sampling site. Shading indicates altitudes above 1700m. From north to south the sites were Val di Gressoney, Valli di Lanzo, Val Chisone, Val Troncea, Val Argentera (see text for precise coordinates).
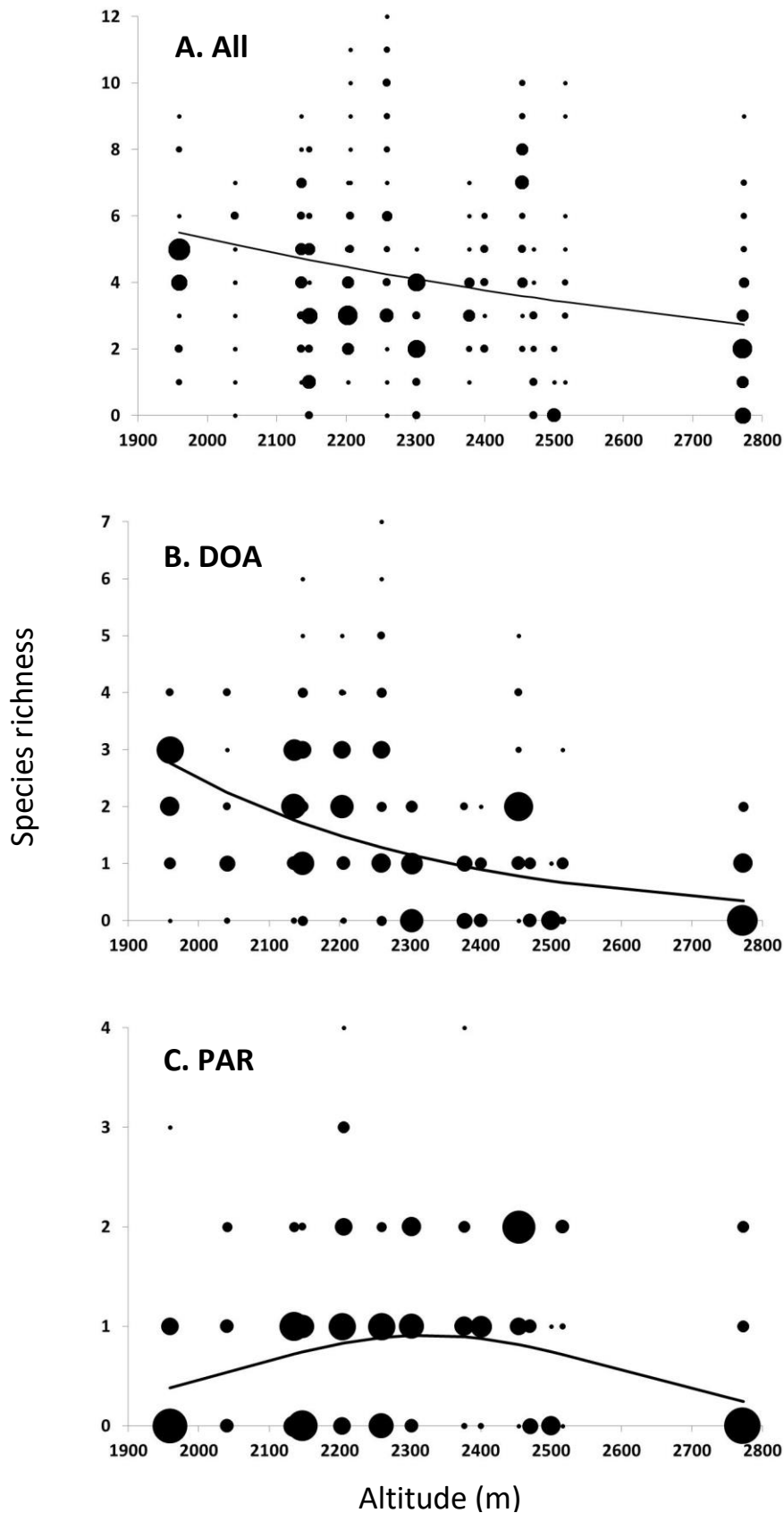
Fig. 2. Variation in species richness of dung beetles in relation to altitude. A. All species. B. Dung-ovipositing Aphodidae (DOA). C. Paracoprids (PAR). Lines fitted from Poisson GLMMs with site, point and trap fitted as random effects (n = 376). Symbol sizes represent the number of observations for a given point (range: A = 1 to 11; B = 1 to 26; C = 1 to 31).
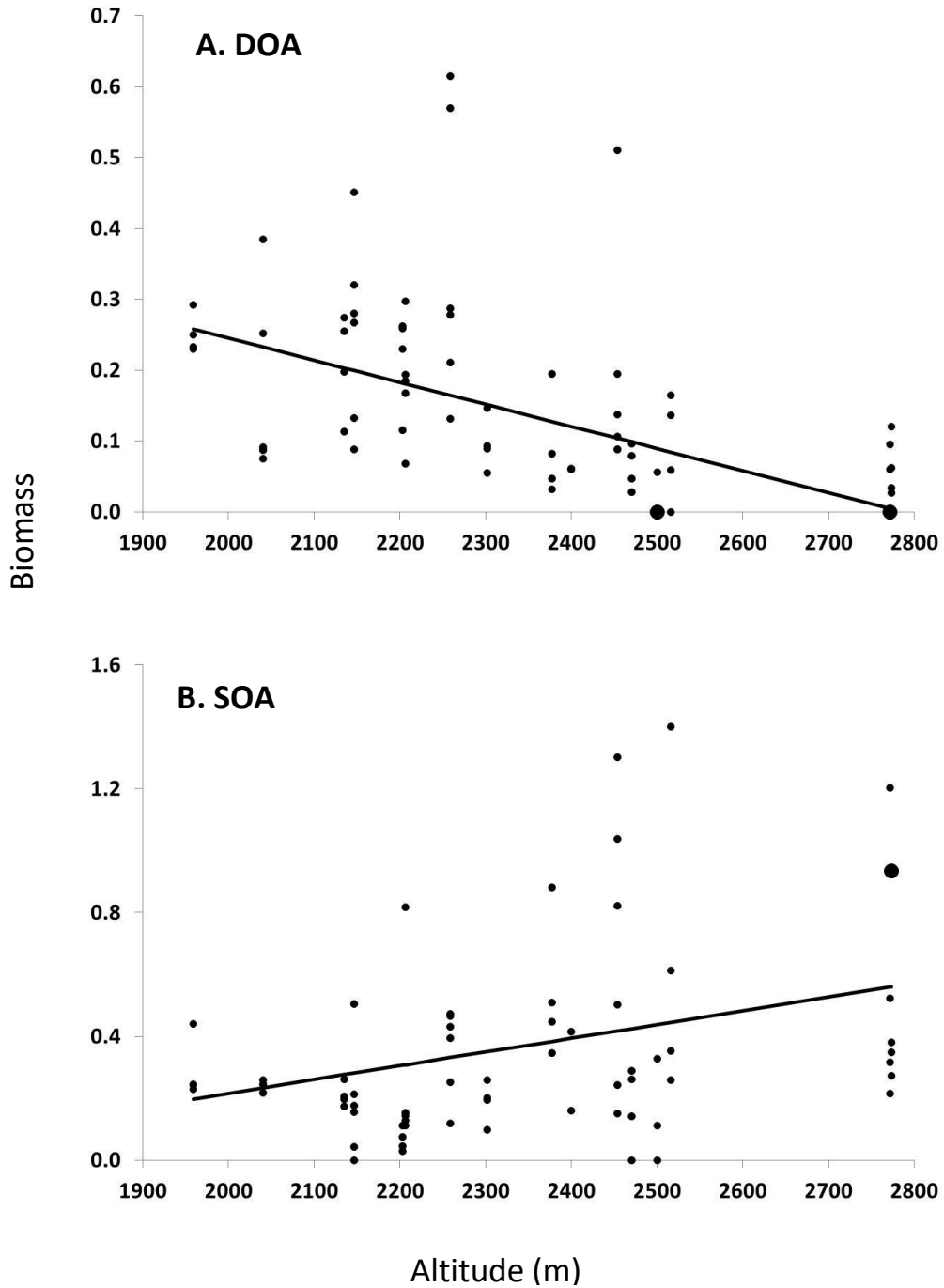
Fig. 3. Variation in the biomass of different groups of dung beetles across an altitudinal gradient, defined according to reproductive strategy. A. Dung-ovipositing Aphodidae (DOA). B. Soil-ovispositing Aphodidae (SOA). Filled circles show mean biomass (g) per trap for each point and each visit, square-root transformed. The lines were fitted from linear mixed models which included site and sampling point as random effects. N = 69 point/visits. Larger symbols indicate 2 observations for a given point.
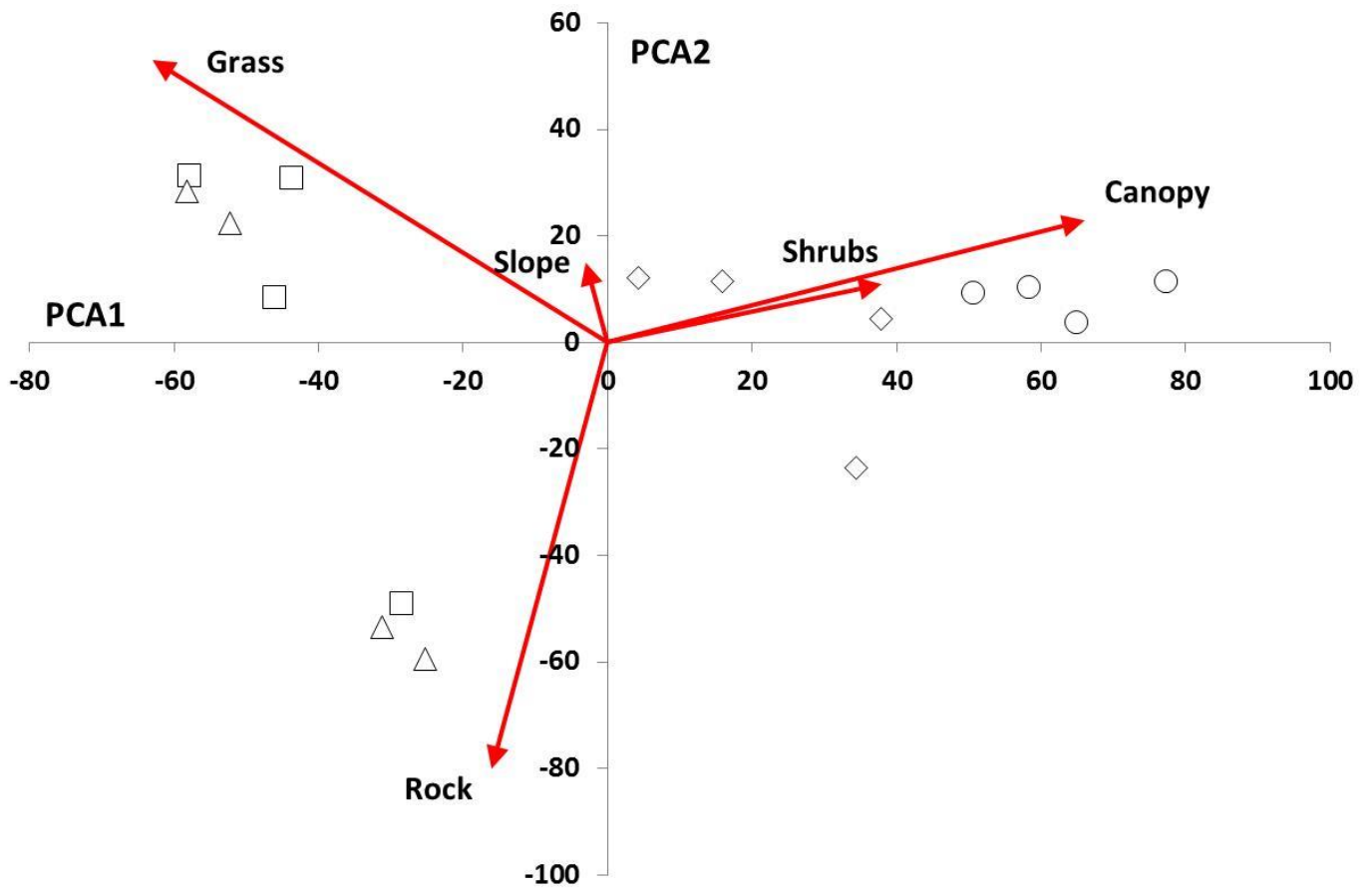
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Fig. 4. Bi-plot of PCA scores for the first two axes based on habitat data collected at 16 sampling points along altitudinal gradients at four sites. Each point is classified from 1 to 4 according to the rank in altitude at each site, and corresponds approximately to forest (circles), shrubs (diamonds), continuous grassland (squares) and high altitude grassland, rock and scree (triangles). Arrows show the loadings for each environmental variable. Note that the variables easting, northing and altitude had negligible scores (<5) on each axis and are not shown in the figure.
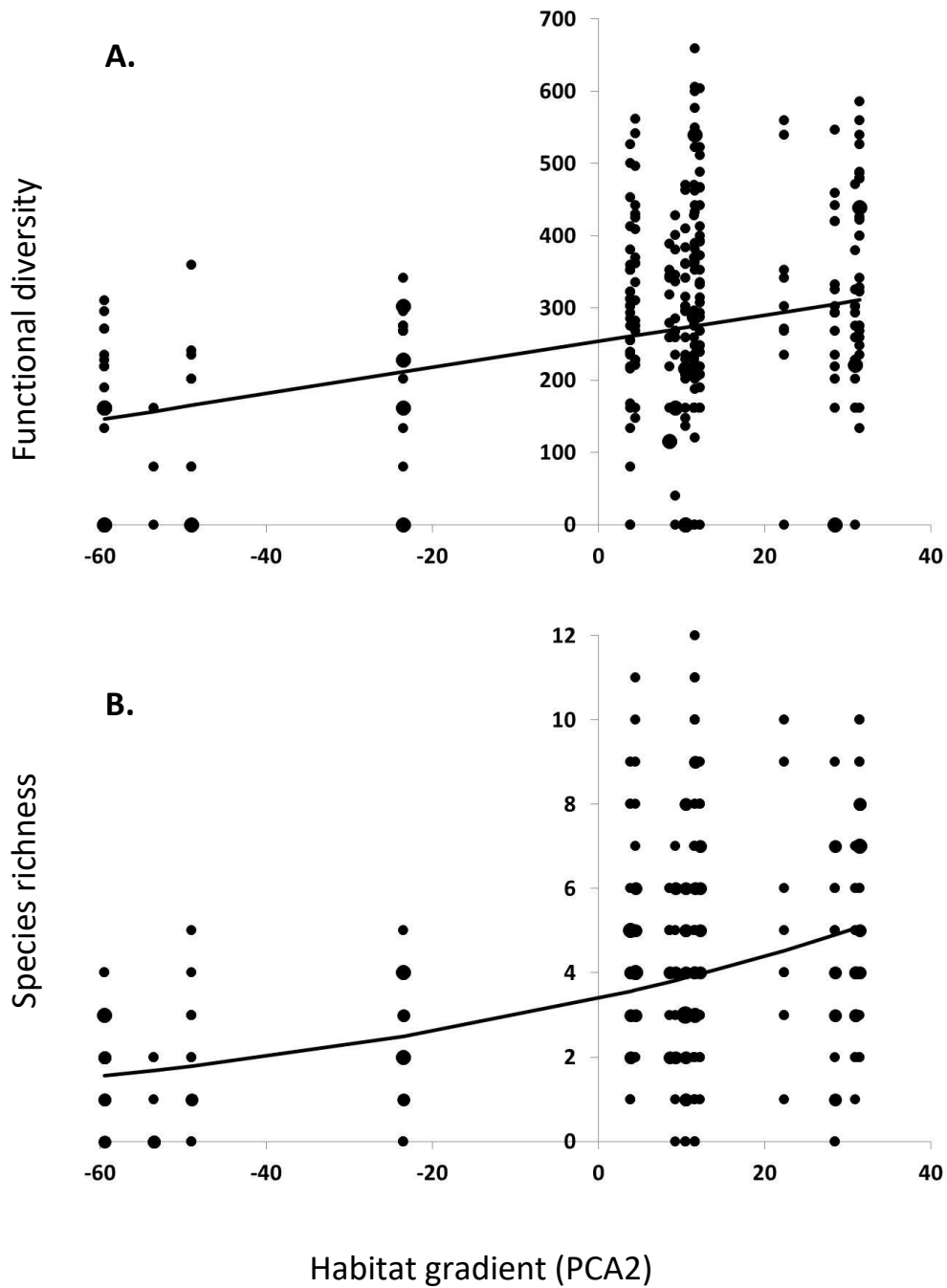
Fig. 5. Variation in dung beetle functional diversity and species richness in relation to a habitat gradient as measured by PCA score (PCA2 which represents a gradient from high rock cover to high vegetation cover. – see Fig. 4). A. Functional diversity per trap. B. Species richness (all species) per trap. Lines fitted from GLMMs with site, point and trap fitted as random effects (n = 376 trap). Symbol sizes represent the number of observations for a given point (range: A = 1 to 2; B = 1 to 4).