Effects of grazing intensity and the use of veterinary medical products on dung beetle biodiversity in the sub-mountainous landscape of Central Italy

Mattia Tonelli [1, 2] , **José R. Verdú** [Corresp., 2] , **Mario E. Zunino** [1, 3]

[1] Department of Pure and Applied Science, University of Urbino, Urbino, Italy

[2] I.U.I. CIBIO, Universidad de Alicante, Alicante, Spain

[3] School of Biodiversity, Asti University Centre for Advanced Studies, Asti, Italy

Corresponding Author: José R. Verdú
Email address: jr.verdu@ua.es

Grazing extensification and intensification are among the main problems affecting European grasslands. We analyze the impact of grazing intensity (low and moderate) and the use of veterinary medical products (VMPs) on the dung beetle community in the province of Pesaro-Urbino (Italy). Grazing intensity is a key factor in explaining the diversity of dung beetles. In the case of the alpha diversity component, sites with a low level of grazing activity – related in a previous step to the subsequent abandonment of traditional farming – is characterized by a loss of species richness ($q = 0$) and a reduction in alpha diversity at the levels $q = 1$ and $q = 2$. In the case of beta diversity, sites with a different grazing intensity show remarkable differences in terms of the composition of their species assemblages. The use of VMPs is another important factor in explaining changes in dung beetle diversity. In sites with a traditional use of VMPs, a significant loss of species richness and biomass is observed, as is a notable effect on beta diversity. In addition, the absence of indicator species in sites with a historical use of VMPs corroborates the hypothesis that these substances have a ubiquitous effect on dung beetles. However, the interaction between grazing activity and VMPs when it comes to explaining changes in dung beetle diversity is less significant (or is not significant) than the main effects (each factor separately) for alpha diversity, biomass and species composition. This may be explained if we consider that both factors affect the various species differently. In other words, the reduction in dung availability affects several larger species more than it does very small species, although this does not imply that the former are more susceptible to injury caused by the ingestion of dung contaminated with VMPs. Finally, in order to prevent negative consequences for dung beetle diversity, we propose the maintenance of a moderate grazing intensity and the rational use of VMPs. It is our view that organic management can prevent excessive extensification while providing an economic stimulus

to the sector. Simultaneously, it can also prevent the abuse of VMPs.

1 **Effects of grazing intensity and the use of veterinary medical products on dung beetle**

2 **biodiversity in the sub-mountainous landscape of Central Italy**

4 Mattia Tonelli[1,2], José R. Verdú[2], Mario Zunino[1,3]

6 [1] Department of Pure and Applied Science (DiSPeA; previously DiSTeVA), University of

7 Urbino "Carlo Bo", 61029 Urbino (PU), Italy

8 [2] I.U.I. CIBIO, Universidad de Alicante, San Vicente del Raspeig, 03069 Alicante, Spain

9 [3] School of Biodiversity, Asti University Centre for Advanced Studies, Asti, Italy

11 **Abstract**

12 Grazing extensification and intensification are among the main problems affecting European

13 grasslands. We analyze the impact of grazing intensity (low and moderate) and the use of

14 veterinary medical products (VMPs) on the dung beetle community in the province of Pesaro-

15 Urbino (Italy). Grazing intensity is a key factor in explaining the diversity of dung beetles. In the

16 case of the alpha diversity components, sites with a low level of grazing activity – related in a

17 previous step to the subsequent abandonment of traditional farming – is characterized by a loss

18 of species richness ($q = 0$) and a reduction in alpha diversity at the levels $q = 1$ and $q = 2$. In the

19 case of beta diversity, sites with a different grazing intensity show remarkable differences in

20 terms of the composition of their species assemblages. The use of VMPs is another important

21 factor in explaining changes in dung beetle diversity. In sites with a traditional use of VMPs, a

22 significant loss of species richness and biomass is observed, as is a notable effect on beta

23   diversity. In addition, the absence of indicator species in sites with a historical use of VMPs

24   corroborates the hypothesis that these substances have an ubiquitous effect on dung beetles.

25   However, the interaction between grazing activity and VMPs when it comes to explaining

26   changes in dung beetle diversity is less significant (or is not significant) than the main effects

27   (each factor separately) for alpha diversity, biomass and species composition. This may be

28   explained if we consider that both factors affect the various species differently. In other words,

29   the reduction in dung availability affects several larger species more than it does very small

30   species, although this does not imply that the former are more susceptible to injury caused by the

31   ingestion of dung contaminated with VMPs.

32   Finally, in order to prevent negative consequences for dung beetle diversity, we propose

33   the maintenance of a moderate grazing intensity and the rational use of VMPs. It is our view that

34   organic management can prevent excessive extensification while providing an economic

35   stimulus to the sector. Simultaneously, it can also prevent the abuse of VMPs.

37   **Keywords**

38   Livestock management, traditional grazing, organic farming, ivermectin, Scarabaeidae

40   **Introduction**

41   Land use changes play a pivotal role in the loss of biodiversity (Sala et al., 2000). In the

42   Mediterranean basin, starting about 10,000 years ago, the human population modified the

43   landscape for agriculture and livestock grazing purposes (Blondel, 2006). Passing through the

44   different stages that have characterized each era (Vos and Meekes, 1999), the basin has

45   developed a complex "cultural landscape" (cfr. Farina, 2000) that enables a large number of

46   species to be maintained there (Myers et al., 2000). Semi-natural grasslands are one of the

47   keystone habitats of this landscape. They were developed and managed by man (Blondel et al.,

48   2010) using extensive livestock grazing that prevented the homogenization of the landscape

49   (Perevolotsky and Seligman, 1998; Diacon-Bolli et al., 2012). This grazing also provides an

50   energy input to the system through the cattle dung that was previously produced by wild

51   herbivores.

52   In these semi-natural grasslands, dung beetles are among the most important groups within

53   the dung fauna (Hanski and Cambefort, 1991). Their bionomics involves them, directly and

54   indirectly, in various ecological processes such as: nutrient cycles, vegetation development,

55   secondary seed dispersal, and parasite control (Halffter and Matthews, 1999; Nichols et al.,

56   2008). Dung beetles fulfil all the characteristics of an ideal bioindicator taxon (Spector, 2006,

57   Halffter and Favila, 1993), and have been used in a great number of studies on: habitat

58   disturbance or conversion (Braga et al., 2013; Halffter and Arellano, 2002; McGeoch et al.,

59   2002); the natural environmental gradient (Jay-Robert et al., 1997; Romero-Alcaraz and Ávila,

60   2000); and the vegetation and landscape structure (Numa et al., 2009; Verdú et al., 2011).

61   In the last few decades, extensive livestock management has undergone a rapid process of

62   modification (Stoate et al., 2009). Italy has seen the progressive abandonment of traditional

63   extensive grazing systems in favour of more intensive versions. Furthermore, from 1982 to 2010,

64   Italian fields lost 20% of their heads of cattle (cows, sheep and horses), while the livestock of

65   farms fell by about 71%. Nevertheless, the number of horses and sheep rose in the same period

66   in valley areas (more than 13%) and hills (more than 12%), but fell by about 24% in mountain

67   regions (ISTAT, 2010). Moreover, the number cow herds across the country has decreased by

68   about 35% in the last 28 years, with 70% of cows concentrated in the north of Italy in 2010.

69   Indeed, in this part of the country, the number of cow heads/farm increased from 48 to 64

70   between 2000 and 2010 (ISTAT, 2010; Sturaro et al., 2012). This has led to a situation where

71   marginal areas are abandoned, but more productive locations can suffer from overgrazing.

72   Another relevant factor related to intensification is the use/abuse of veterinary medical products

73   (VMPs). These substances are widely utilized, with 194 tons of antiparasitic substances produced

74   in the European Union in 2004 (Kools et al., 2008). VMP molecules such as ivermectin are

75   poorly metabolized by cattle (McKellar and Gokbulut, 2012) and are voided as unchanged

76   residues in faeces (Floate et al., 2005; Lumaret et al., 1993). These residues have been

77   demonstrated to have negative sub-lethal effects and ultimate lethal consequences on non-target

78   dung fauna and, particularly, dung beetles (Verdú et al., 2015; Wardhaugh et al., 2001).

79   These three factors, i.e. grazing abandonment and intensification and VMP use, have been

80   demonstrated to have negative effects on dung beetle biodiversity. Some studies have focused on

81   the effects on dung beetles of grazing abandonment (Jay-Robert et al., 2008; Verdú et al., 2000;

82   Carpaneto et al., 2005), overgrazing (Negro et al., 2011) and VMP use (for a review see:

83   Beynon, 2012; Lumaret and Errouissi, 2002; Wall and Baynon, 2012; Jacobs and Scholtz, 2015).

84   When it comes to the impact of VMPs on dung beetles, however, the majority of research has

85   been carried out in the laboratory, with the focus on the effects on a single or just a few species

86   (Verdú et al., 2015; Cruz-Rosales et al., 2012; Hempel et al., 2006; Wardhaugh and Rodriguez-

87   Menendez, 1988). Nevertheless, it is important to evaluate the impact of different grazing

88   intensities in order to determine the optimum level for dung beetle conservation. This step is

89   necessary because, increasingly, grazing activities are not being completely abandoned, but are

90   instead suffering an ongoing process of extensification (*sensu* EUROSTAT:

91   http://ec.europa.eu/eurostat/statistics-explained/index.php/Glossary:Extensification).

92   Furthermore, pollutants (i.e. VMPs) may interact with "natural stressors" (i.e. the quantity of the

93   trophic resource), producing synergistic or antagonistic effects (Folt et al., 1999; Laskowski et

94   al., 2010). To our knowledge, no studies have evaluated the potential impact of the possible

95   interaction of these two factors on dung beetle diversity.

96   The aim of this study was to analyze the effects of grazing intensity and the use of VMPs on

97   dung beetle diversity in the sub-mountainous landscape of Central Italy. Comparing areas with

98   different grazing intensities (low and moderate) and those with a historical use or non-use of

99   VMPs (used as a proxy of intensification), we attempt to answer the following four questions: A)

100 what is the effect of grazing intensity and VMP use on: dung beetle alpha diversity at different

101 Hill numbers or levels ($q = 0$, $q = 1$, and $q = 2$), abundance and biomass? B) What is the possible

102 interaction between these factors with respect to dung beetle diversity? C) Are there any

103 indicator species for a particular form of treatment? D) What are the effects on the composition

104 of dung beetle assemblages (beta diversity)? Our hypothesis is that a low level of grazing

105 intensity and the use of VMPs have negative effects on dung beetle biodiversity, resulting in

106 changes in alpha and beta diversity and biomass, and favouring the presence of some species that

107 may act as indicators of a particular form of pasture management. Moreover, we hypothesize that

108 the effects of low grazing intensity and VMP use are worse in combination than alone.

109

110 **Materials and Methods**

111 *Study area and experimental design*

112 The study was carried in the sub-mountainous area of the Pesaro-Urbino province in the Marche

113 region, Italy. The provincial climate falls into the temperate Köppen categories (Cfa and Cfb).

114 The average annual temperature is around 12 °C, with a minimum average of around 3.5°C in

115   winter and a maximum average of 21 °C in summer. Average annual precipitation is around 930

116   mm, with two dry periods, one in summer and another in winter (www.pesarourbinometeo.it).

117   The soil is calcareous.

118   To evaluate the effects of grazing intensity and VMP use, we designed a 2x2 full factorial

119   design with three replications for each treatment. We identified different areas with: a VMP-free,

120   low grazing intensity; a VMP-free, moderate grazing intensity; a VMP-use, low grazing

121   intensity; and a VMP-use, moderate grazing intensity.

122   A) 'Low grazing, VMP-free' areas – LGECO - (Pietralata pastures; 43°39'33.64''N;

123   12°42'27.65''E). These secondary grasslands, located between 750 and 900 m a.s.l., are

124   represented by the *Brizo mediae-Brometum erecti* and *Festuco circummediterraneae-*

125   *Arrhenatheretum elatioris* associations. These grasslands are mainly used by horses that were

126   abandoned and have reverted to a wild state. The grazing intensity of these pastures is around 0.7

127   units of livestock/ha. The most common wood species are: *Fraxinus ornus* L., *Ostrya*

128   *carpinifolia* Scop., *Quercus ilex* L., *Quercus pubescens* Willd., *Acer opalus* (Miller), *Pinus nigra*

129   J.F. Arnold*, Crataegus monogyna* Jacq., *Juniperus oxycedrus* L., *Lonicera etrusca* G. Santi,

130   *Spartium junceum* L.*,* and *Rosa canina* L.

131   B) 'Moderate grazing, VMP-free' areas – MGECO - (Montebello pastures; 43°43'13. 83''N;

132   12°45'19.98''E). These grasslands are located between 500 and 600 m a.s.l. within the Gino®

133   Girolomoni Cooperativa Agricola. The pastures are used by cows according to organic farming

134   rules with grazing rotation. The grazing intensity is about 1.5 units of livestock/ha. The

135   herbaceous association falls within the *Brizo mediae-Brometum erecti* group. The spontaneous

136   arboreal vegetation is prevalently comprised of *Quercus pubescens, Quercus cerris* L.*, Quercus*

137 *petraea* (Matt.) Liebl*., Carpinus betulus* L*., Ostrya carpinifolia, Fraxinus ornus* L., *Acer opalus*,

138 *Quercus ilex*, *Sorbus domestica* L*., Corylus avellana* L. and *Fagus sylvatica* L.

139   C) 'Moderate grazing with VMPs' areas – MGVMP - (Catria pastures; 43°30'23.39''N;

140 12°39'22.39''E). These grasslands are used by cows and horses and have a historical grazing

141 tradition. The farmers there highlighted that VMPs have long been used and this convention

142 continues to today. The unit of livestock/ha is about 1.5 and there is no sign of overgrazing.

143 These pastures are referred to the association *Brizo mediae-Brometum erecti*, where the most

144 abundant species are *Bromus erectus* Huds., *Briza media* L., *Filipendula vulgaris* Moench,

145 *Cyanus triumfettii* (All.) Dostál ex Á.Löve, *Plantago lanceolata* subsp. *lanceolata* (Mert. &

146 Koch), *Luzula campestris* (L.) DC., *Scorzoneroides cichoriacea* (Ten.) Greuter, *Cynosurus*

147 *cristatus* L., *Anthoxanthum odoratum* L. and *Carex caryophyllea* Latourr. The tree species are

148 represented by the *Scutellario columnare-Ostryetum carpinifolia* association. The sampling sites

149 are located between 800 and 1000 m a.s.l.

150   D) 'Low grazing with VMPs' areas – LGVMP - (Nerone pastures; 43°32'07.27''N;

151 12°33'26.13''E). These grasslands are grazed by horses that represent a grazing intensity of

152 about 0.5 units of livestock/ha. These sites have been submitted to the historical and intensive

153 use of VMPs from about the 1990s. Today, VMPs are only given to foals and adult animals with

154 evident parasitic stress. The grass associations of these pastures are *Asperulo purpureae-*

155 *Brometum erecti* and *Brizo mediae-Brometum erecti*, with the principal species being: *Bromus*

156 *erectus, Briza media, Filipendula vulgaris, Cyanus triumfettii, Plantago lanceolata* subsp.

157 *lanceolata, Luzula campestris, Scorzoneroides cichoriacea, Cynosurus cristatus, Anthoxanthum*

158 *odoratum* and *Carex caryophyllea*. The arboreous species are dominated by the *Scutellario*

159 *columnare-Ostryetum carpinifolia* association. The sampling sites are located between 800 and

160 1000 m a.s.l.

161 The density of wild fauna (i.e. *Capreolus capreolus* (L., 1758) and *Sus scrofa* L., 1758) is

162 very similar among all the studied areas (Tonelli, personal observation, 2013).

163 In the areas with VMPs use, the farmers' interviews (Tonelli, unpublished data) highlighted

164 that the VMPs have been use since 1990s until today. The main veterinary formulations that are

165 used are based on Ivermectin and Pyrantel pamoate. The main preventive treatments are

166 administrated in spring and in autumn but the data of application vary between each farmer.

167 Moreover, additional treatments are applied as many times as there are parasitic stress. In the

168 LGVMP areas, VMPs are only given to foals and adult animals with evident parasitic stress, but

169 have a very intense historical use of VMPs.

170

171 *Dung beetle trapping*

172 For each treatment, we selected three sampling sites separated by at least 500 m to ensure

173 independence among the replicates. In each site, we placed a 50 x 50 m quadrate with four pitfall

174 traps at the corners; two traps were baited with cow dung (about 500 cm^3) and two with horse

175 dung (about 500 cm^3) to maximize differential species attraction (Barbero et al., 1999; Dormont

176 et al., 2004, 2007). The dung used for the trapping was collected from organic farming that was

177 VMP free. We filled the pitfall traps with propylene glycol (50%) to preserve the dung beetles

178 we collected. The traps were left active for 48 h in each sampling period. The sampling was

179 repeated about every 15 days from June 2013 to November 2013 and in May and June 2014. We

180 excluded rainy days in order to prevent any interference with the trapping. The total number of

181 traps used was 48, and we collected a total of 528 samples (4 traps x 3 sampling points x 4

182   treatments x 11 sampling periods). The dung beetles were identified to specific level (see

183   Supplemental Information 2, for more details).

184

185   *Sampling completeness*

186   The inventory completeness was evaluated using a sample coverage analysis (Chao and Jost,

187   2012). This is a measure of sample completeness, and reveals the proportion of the total number

188   of individuals in a community that belong to the species represented in the sample. The sample

189   coverage formula uses information about sample size, singletons and doubletons (Chao and Jost,

190   2012). Measurements were taken using iNext v.1.0 (Hsieh et al., 2013).

191

192   *Alpha diversity*

193   Alfa diversity was calculated using the Hill numbers' family diversity (MacArthur, 1965; Hill,

194   1973; Jost, 2006, 2007) (see Supplemental Information 2, for more details). In order to

195   characterize the complete species abundance distribution and provide full information about its

196   diversity, we computed the diversity of the orders 0, 1 and 2 for each replication of each

197   treatment for the two factors (grazing intensity and VMP use). We then analyzed these results

198   (each order q separately) using a full factorial generalized linear model in order to evaluate the

199   main effect of the two factors and highlight any interactions. Pairwise comparisons were made

200   using the Tukey post-hoc test. The diversity profile was produced with SpadeR (Chao et al.,

201   2015) and the generalized linear model with the Statistica 7.0 package (StatSoft, 2004).

202

203   *Dung beetle biomass and abundance*

204   We tested the statistical difference in dung beetle total biomass and abundance using a full

205   factorial multivariate generalized linear model with the Statistica 7.0 package (StatSoft, 2004)

206   after log transformation of the dependent variable. Pairwise comparisons were made using the

207   Tukey post-hoc test. The average biomass of each species was calculated using the formula

208   'Biomass = 0.010864 x Length$^{3.316}$' suggested by Lobo (1993). Ten individuals of each species

209   (when available) were measured to obtain the average species length (see Supplemental

210   Information 2, for more details). To calculate the total biomass of the dung beetle at each

211   treatment, we multiplied the average biomass of each species by the number of individuals

212   collected and added these numbers together.

213

214   *Beta diversity*

215   We analyzed whether grazing intensity and VMP use had any effect on the composition of the

216   dung beetle assemblages. We first calculated an index of multiple community similarity of the

217   two factors (using $q = 0, 1, 2$) among all the replicates. This produced six similarity matrices (3 q

218   order x 2 factors). Based on these matrices, Non-Metric Multidimensional Scaling (NMDS) were

219   constructed and analyzed using a Permanova test (Anderson, 2001) to evaluate the statistical

220   significance of each factor for the composition of the dung beetle assemblages at each q level.

221   We computed the multiple community similarity of each treatment with a multiple-assemblage

222   abundance-based overlap measure C_{qN} (Chao et al., 2008) (see Supplemental Information 2, for

223   more detail on C_{qN} measures). Similarity matrices were computed using SpadeR (Chao et al.,

224   2015). A Permanova test was performed using the Permanova+ add-on for PRIMER v.7

225   (Anderson et al., 2008; Clarke and Gorley, 2015). Interaction between the factors was also

226 evaluated. A total of 999 unrestricted permutations of raw data were computed. The P values

227 were calculated using the Bonferroni correction in all cases.

228

229 *Indicator species*

230 The indicator value method (Dufrêne and Legendre, 1997) was computed for each factor to

231 identify the indicator species of a particular treatment. This method is used to quantify the value,

232 as a bioindicator, of a set of taxa. In relation to a given species, it combines the measurement of

233 the degree of specificity (how much the species tends to be abundant in a particular ecological

234 state) with the measurement of the degree of fidelity (how much the species tends to be present

235 inside a determined ecological state) with respect to a given ecological status (McGeoch et al.,

236 2002; McGeoch and Chown, 1998; Dufrêne and Legendre, 1997). The indicator values range

237 from 0 (no indication) to 100 (perfect indication). Species with significant ($P < 0.05$) IndVal

238 results above 70% were considered to be indicator species for the given treatment. Species with

239 an intermediate IndVal between 45% and 70% were considered to be detector species (McGeoch

240 et al., 2002; Verdú et al., 2011). Indicator species are highly characteristic of a particular

241 ecological state (treatment) and may decline rapidly under other ecological conditions up to the

242 point of disappearance. Detector species have a different degree of preference for different

243 ecological states, and relative changes in their abundance across states may be indicative of the

244 direction in which change is occurring (McGeoch et al., 2002). The analysis was performed

245 using PC-Ord 5 (McCune and Mefford, 1999).

246

247 **Results**

248   A total of 148,668 individuals belonging to 57 species of dung beetle were collected (38

249   Aphodiidae, 16 Scarabaeidae, 3 Geotrupidae). This breaks down into: 122,611 specimens

250   belonging to 42 species for the low grazing treatment (25 Aphodiidae, 15 Scarabaeidae, 2

251   Geotrupidae); 26,057 individuals belonging to 54 species for the moderate grazing treatment (35

252   Aphodiidae, 16 Scarabaeidae, 3 Geotrupidae); 128,616 specimens from 53 species for the VMP-

253   free treatment (35 Aphodiidae, 16 Scarabaeidae, 2 Geotrupidae); and 20,052 individuals

254   belonging to 41 species for the VMP-use condition (24 Aphodiidae, 14 Scarabaeidae, 3

255   Geotrupidae) (Supplemental Information 1).

256   The sample coverage estimator revealed that our inventories were 99% complete for each

257   treatment (Supplemental Information 1). This indicates that only 1% of the individuals in a

258   community belong to species not represented in our samples. We can thus consider our samples

259   to be complete, and we have utilized empirical data for the diversity analysis and comparisons.

260

261   *Alpha diversity*

262   Alpha diversity showed a large decrease in the effective number of species as the *q* order

263   increased, indicating a high degree of dominance in the studied assemblages. There are

264   significant differences in alpha diversity due to the grazing intensity for all *q* order (^0D: $F_{[1,\,8]} =$

265   62.227, $P < 0.0001$; ^1D: $F_{[1,\,8]} = 48.602$, $P < 0.0005$; ^2D: $F_{[1,\,8]} = 34.131$, $P < 0.0005$), with

266   Moderate grazing that have higher equivalent number of species (post-hoc Tukey test ^0D: $P <$

267   0.0005; ^1D: $P < 0.0005$; ^2D: $P < 0.001$) (Figure 1).

268   For VMP use factor significant difference exist only for ^0D ($F_{[1,\,8]} = 242.23$, $P < 0.00001$)

269   whereas no significant difference exist for ^1D ($F_{[1,\,8]} = 0.062$, $P = 0.81$) and ^2D ($F_{[1,\,8]} = 0.041$, P

270    $= 0.85$). Post-hoc Tukey test show that VMP free areas have more equivalent species that VMP

271   use areas for ^0D ($P < 0.0005$) but not for ^1D ($P = 0.81$) and ^2D ($P = 0.85$). A small significant

272   interaction between the two factors was identified only for ^0D ($F_{[1,\,8]} = 5.5$, $P = 0.047$), with post-

273   hoc Tukey test that show significant difference between all experimental groups, with the

274   MGECO areas having 1.11 equivalent species more than the LGECO sites, 1.34 more than

275   MGVMP areas and 1.86 more than the LGVMP sites. Sites with LGECO had 1.21 equivalent

276   species more than the MGVMP areas and 1.66 more than the LGVMP sites. The areas with

277   MGVMP had 1.38 equivalent species more than LGVMP sites.

278   No significant interaction between the two factors exists for ^1D ($F_{[1,\,8]} = 1.82$, $P = 0.214$) and

279   ^2D ($F_{[1,\,8]} = 0.86$, $P = 0.381$), with post-hoc Tukey test that showed statistical differences only

280   between MGECO areas and LGVMP and LGECO areas, whereas MGVMP had significantly

281   more equivalent species than those of LGVMP and LGECO areas.

282

283   *Indicator values of species*

284   The IndVal analysis (Table 1) for the grazing intensity factor revealed 10 indicator species:

285   three for the low grazing treatment and seven for the moderate grazing treatment. For the VMP-

286   use factor, 14 indicator species were identified, all with respect to the VMP-free treatment. Two

287   VMP-free indicator species were also indicator species of some treatments for the grazing

288   intensity factor: *Chilothorax conspurcatus* (L., 1758) is an indicator of the VMP-free and low

289   grazing sites, and *Onthophagus taurus* (Schreber, 1759) of the VMP-free and moderate grazing

290   treatments.

291

292   *Biomass and abundance of dung beetles*

...

293   Significant differences in dung beetle biomass and abundance were obtained for the grazing

294   intensity (*Wilks's lambda* = 0.138; $F_{[2,7]}$ = 21.87; $P < 0.01$) and use of VMPs factors (*Wilks's*

295   *lambda* = 0.17; $F_{[2,7]}$=17.34; $P < 0.05$) (Figure 2). However, no differences were found in their

296   interactions (*Wilks's lambda* = 0.28; $F_{[2,7]}$ = 9.13; $P = 0.09$). The post-hoc Tukey test showed that

297   the LGECO treatment had a higher dung beetle biomass and abundance than the LGVMP,

298   MGECO and MGVMP treatments, whereas the MGECO treatment had more biomass than the

299   LGVMP treatment.

300

301   *Beta diversity*

302   Multiple-assemblage abundance-based similarity measures (C_qN) showed a clear aggrupation

303   between sites characterised by both factors studied. For each q level, Non-Metric

304   Multidimensional Scaling (NMDS) plots represent a clear ordination of sites based on grazing

305   activity and VMP use (Figure 3). The Permanova test showed significant differences in beta

306   diversity for the grazing intensity factor at each q order of similarity matrix (Table 2). For the

307   VMP-use factor, the Permanova test showed a significant compositional impact only for $q = 0$,

308   whereas it was not significant when species abundance was taken into account, i.e. for q =1 and q

309   =2. Furthermore, the interaction between the two factors was significant only for the similarity

310   matrix of order $q = 0$, but was not significant for $q = 1$ and $q = 2$ (Table 2).

311

312   **Discussion**

313   *Grazing intensity effects on dung beetle diversity*

314   Our results support the hypothesis that a low grazing intensity have a negative effect on dung

315   beetle diversity. Total domestic grazing abandonment is a recognised negative factor for dung

316 beetle conservation (Jay-Robert et al., 2008; Verdú et al., 2000). However, our results

317 highlighted that even a simple reduction in grazing intensity implies negative effects on dung

318 beetle community in areas characterised by a long grazing history, such as the Mediterranean

319 Region. Indeed, the areas with a moderate grazing intensity showed more alpha diversity than the

320 low grazing intensity sites. Thus, our results were consistent with those of other studies in

321 different Mediterranean locations. For example, Lobo et al. (2006), in Spain, showed that the

322 quantity of dung in a radius of 2 km and the presence of a flock are key factors in determining

323 the local variation in dung beetle species richness and abundance. In Southern France, Lumaret

324 et al. (1992) explained that an increase of 260% in fresh dung availability, five years after a

325 change of pasture management (from sheep to cows), caused an increase in species richness from

326 38 to 42 species. In Italy, Carpaneto et al. (2005) showed that after 13 years, the abandonment of

327 the sheep grazing system in the Rome urban area led to a loss of 53% of the dung beetle species,

328 especially those with a large body size.

329   Furthermore, the decrease in the number of indicator species observed in function of the

330 decrease in grazing intensity supported our hypothesis. We encountered seven and three species

331 with significant IndVal values for the moderate and low grazing areas, respectively. This fact

332 suggest that a reduced quantity of a trophic resource can favour a limited number of species.

333 Moreover, it is interesting to note that in the moderate grazing sites studied, three of the seven

334 indicator species are paracoprids and, among them, one, *Copris lunaris* (L., 1758), is a large

335 species. During breeding, *Copris lunaris* may bury about 100-165 g of dung (Klemperer, 1982;

336 Martín-Piera and López-Colón, 2000). Thus, there is a positive relationship between body size

337 and dung mass burial (Doube et al., 1988; Larsen et al., 2005; Slade et al., 2007), which supports

338   the idea that large paracoprid dung beetle, as *C. lunaris*, can only to maintain well established

339   populations if the trophic resource is relatively abundant.

340   Our results on the grazing intensity factor can be explained by the species-energy

341   relationship (Gaston, 2000; Wright, 1983; Hawkins et al., 2003), i.e. the lower the level of

342   (trophic) energy available, the smaller the number of species that an area can support (Evans et

343   al., 2005). For example, Tshikae et al. (2013) explicitly tested the species–energy relationship for

344   dung beetles across an arid and trophic resource gradient in Botswana. Their results showed that

345   the species richness, diversity and biomass of the dung beetle diminish with a decrease in

346   available (trophic) energy.

347   However, it is interesting to note that the low grazing areas studied showed greater biomass

348   and abundance. This may be explained by the dominance of two species, *Melinopterus consputus*

349   (Creutzer, 1799) and *Onthophagus medius* (Kugelann, 1792). Both species may alter the

350   diversity pattern of this treatment by means of a competitive exclusion (Hardin, 1960). The low

351   quantity of the trophic resource available in this site perturbed the dung beetle community,

352   favouring generalist r-strategic species (such as *M. consputus*) and highly competitive species

353   such as small tunnellers (e.g. *O. medius*) (Horgan and Fuentes, 2005). The low grazed sites

354   studied, in fact, showed more biomass but fewer species than the moderately grazed areas. The

355   same results were reported in the Rome urban area (Italy) by Carpaneto et al. (2005), who found

356   a decrease in the number of species and a rise in total biomass, with the dominance of one

357   species of Aphodinae with the same explosive reproductive strategy (i.e. *Nimbus johnsoni*

358   (Baraud, 1976)).

359   In terms of species composition of assemblages, beta diversity was strongly influenced by the

360   quantity of the trophic resource at all q levels (Table 3); rare and abundant species were

361   compositionally different between the assemblages obtained in the different grazing intensity

362   treatments. These results implicate that grazing extensification lead to a change in dung beetle

363   composition favouring more opportunistic species. This explanation is corroborated by the

364   presence of three indicators species (*Melinopterus consputus*, *Chilothorax conspurcatus* and

365   *Sigorus porcus*) characteristics of the low grazing areas that share an opportunistic behaviour.

366   *Melinopterus consputus* and *C. conspurcatus* are dependent on the dung only during adult stage,

367   whereas during larvae phase are saprophagous, mainly (Verdú J.R. personal observations);

368   *Sigorus porcus* have a strong attitude to kleptoparasitism during both adult and larval stages

369   (Dellacasa and Dellacasa, 2006).

370   Thus, dung beetles are strongly dependent to dung during their life cycle and our data support

371   the hypothesis that even a simple reduction of its availability may have negative effects on the

372   community. Less trophic resource availability lead to a compositional and structural

373   impoverishment of the community with a loss of large body sizes dung beetles in favour of more

374   opportunistic ones. Then, the fact that Mediterranean pastures suffer a continuous process of

375   extensification, can be a factor of concern for the dung beetle conservation.

376

377   *VMPs use effects on dung beetle community*

378   Our results supported the hypothesis that the historical use of VMP substances have a negative

379   effect on dung beetle diversity. The negative effect of VMP substances was relevant to all

380   community parameters measured, such as alpha diversity, biomass, abundance, presence of

381   indicator species and beta diversity. It has been documented that VMP-use shows a variety of

382   lethal and sub-lethal effects on non-target fauna depending on the molecule, doses, mode of

383   administration, environmental factors and insect species in question (Lumaret and Errouissi,

384 2002; Lumaret et al., 2012; Wall and Beynon, 2012; Jacobs and Scholtz, 2015). Many essays

385 show that VMPs negatively affect larval and adult survival of dung beetles, as well as some

386 physiological processes such as reproductive, sensorial and locomotor capacities showing even

387 negative repercussions in the dung decomposition (Wall and Strong, 1987; Lumaret et al., 1993;

388 Wardhaugh et al., 2001; Verdú et al., 2015).

389   Here, we document that VMP-use sites studied showed significantly fewer species and a

390 reduced biomass compared to the VMP-free sites. Our results agree with other studies that have

391 explored the impact of VMPs in the field. For example, in southern Ireland, Hutton and Giller

392 (2003) observed a lower number of species and a reduced abundance of dung beetles in intensive

393 and rough grazing farms compared to organic farms. In South Africa, Krüger and Scholtz (1998)

394 also showed that, under drought conditions, treatment with ivermectin led to a loss of dung

395 beetle species. Beynon et al. (2012b) showed a reduction in dung beetle abundance and biomass

396 in dung treated with ivermectin in the UK.

397   Unlike some studies (Krüger and Scholtz, 1998; Basto-Estrella et al., 2014; Hutton and

398 Giller, 2003), we did not find a significant difference in ^1D ('common species number') and ^2D

399 ('dominant species number') for the VMP-use factor. Give that macrocyclic lactones as

400 ivermectin acting on a family of ligand-gated chloride channels gated by glutamate, which is

401 shared by all Ecdysozoan (Geary and Moreno, 2012; Puniamoorthy et al., 2014), all dung beetles

402 species should be sensible to ivermectin toxicity. Thus, the consequences on the assemblage

403 structure may be differential based on the abundances of each species in each assemblage. Our

404 data showed that less common species are first in disappearing in sites characterized by VMPs

405 use, which explains the significant reduction in the number of species observed in these sites. At

406 $q = 1$ and $q = 2$, however, differences are not observed between both treatments, so the reduction

407   of the populations of the most common and dominant species took place of equitable way, which

408   maintains similar measures of community structure (^1D and ^2D).

409   Accordingly to alpha diversity results, beta diversity was influenced by the use of VMPs only

410   for $q = 0$. This means that the two assemblages are different in terms of 'rare' species, whereas

411   the more common and dominant species are not significantly different.

412   Our IndVal results showed how the VMP-use treatments have no indicator species. This

413   means that no species were favoured by the use of these veterinary substances. In other words,

414   the use of VMPs could affect all species and, apparently, no species could be resistant to VMP

415   toxicity. These results agree with the explained above about diversity measures. In contrast, the

416   VMP-free treatment had 14 indicator species.

417   Our results are congruent with those of Puniamoorthy et al. (2014), which show that

418   ivermectin sensitivity is an ancient trait affecting potentially all Ecdysozoan (moulting animals)

419   species. This corroborates the hypothesis that the use of VMPs may have a ubiquitous, negative

420   effect on dung beetle fauna. The fact that no species were found to be indicator species in the

421   areas with VMP-use could be due to the irrational use of these substances throughout the year.

422

423   *Grazing intensity and VMPs interactions*

424   Interesting results were highlighted by the interactions between the two factors. Contrary to our

425   hypothesis, the interactions terms were less significant, or no more significant, than the main

426   effects (each factor separately) for alpha diversity, biomass and species composition. This could

427   be explained if we consider that both factors affect different forms of each species. In other

428   words, the decrease in dung availability affects several bigger species more than the very small

429   species, but this does not imply that the former are more susceptible to injury caused by the

430   ingestion of dung contaminated with VMPs. Another explanation can be found in the halving of

431   the sample size during the interaction analyses. This means that interactions between the two

432   factors may have antagonistic effects on dung beetle assemblages, but more studies with greater

433   sample size are needed on this issue.

434

435   *Conclusions*

436   The present analysis highlighted that the moderate grazing VMP-free treatment seems to be the

437   more appropriate management system for maintaining a higher number of dung beetle species, as

438   well as greater diversity and biomass. These results corroborated the notion that, in a

439   Mediterranean context with a long history of grazing, traditional management techniques with a

440   moderate grazing intensity have a positive effect on dung beetle diversity (Verdú et al., 2000).

441   Furthermore, our results corroborated the hypothesis that both factors - low grazing intensity and

442   VMP-use - have negative effects on dung beetle communities. Even a simple grazing intensity

443   extensification may have negative impact on dung beetle, that is reflected in the compositional

444   and structural impoverishment of the community. Our study strengthens the results about the

445   environmental risk assessment made by Liebig et al. (2010) that concluded that the ivermectin

446   use have an "unacceptable risk" for dung beetle fauna.

447   The results could have an application for sustainable farmland management, highlighting that

448   an incorrect grazing management of the pastures could be a strong effect on dung beetle

449   community (e.g. number of species, biomass, composition), and so in the correct function of

450   ecosystem processes performed by dung beetle as nutrient cycles, vegetation development,

451   secondary seed dispersion, and parasite control (Nichols et al., 2008; Nervo et al., 2014; Beynon

452   et al., 2012a; Larsen et al., 2005). Then, the loss of dung beetle biodiversity can have a negative

453   impact on various ecosystem processes (Nichols et al., 2008), with harmful effects on pastures.

454   Finally, we suggest that organic farming with a moderate grazing intensity could have a

455   positive effect on dung beetle conservation. This farming management approach may contribute

456   to this by avoiding pasture abandonment, conferring an economic stimulus (Willer and Lernoud,

457   2016) and controlling for the excessive use of VMPs (Hutton and Giller, 2003). Further studies

458   in different biogeographical and bioclimatic regions are, however, needed to assess the impact of

459   the long-term use of VMPs on dung beetles.

460

461   **Acknowledgements**

462   Sally-Ann Ross checked the English version of the manuscript.

463

464   **References**

465   Anderson, M.J., 2001. A new method for a non-parametric multivariate analysis of variance.

466   Austral Ecology, 26(1): 32-46.

467   Anderson, M.J., Gorley, R.N., Clarke, K.R. 2008. PERMANOVA+ for PRIMER: Guide to

468   Software and Statistical Methods: PRIMER-E: Plymouth, UK.

469   Barbero, E., Palestrini, C., Rolando, A., 1999. Dung beetle conservation: effects of habitat and re

470   source selection (Coleoptera: Scarabaeoidea). Journal of Insect Conservation, 3: 75-84.

471   Basto-Estrella, G.S., Rodríguez-Vivas, R.I., Delfín-González, Reyes-Novelo, E., 2014. Dung

472   beetle (Coleoptera: Scarabaeinea) diversity and seasonality in response to use of

473   macrocyclic lactones at cattle ranches in the Mexican neotropics. Insect Conservation and

474   Diversity, 7: 73-81.

475   Beynon, S.A., 2012. Potential environmental consequences of administration of anthelmintics to

476   sheep. Veterinary Parasitology, 189: 113-124.

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

478   communities buffer ecosystem services in perturbed agro-ecosystems. Journal of Applied

479   Ecology, 49(6): 1365-1372.

480   Beynon, S.A., Peck, M., Mann D.J., Lewis, O.T., 2012b. Consequences of alternative and

481   conventional endoparasite control in cattle for dung-associated invertebrates and ecosystem

482   functioning. Agriculture, Ecosystem and Environment, 162: 36-44.

483   Blondel, J., 2006. The 'design' of Mediterranean landscapes: a millennial story of humans and

484   ecological systems during the historic period. Human Ecology, 34(5): 713-729.

485   Blondel, J., Aronson, J., Bodiou, J.-Y., Boeuf, G., 2010. The Mediterranean Region: Biological

486   Diversity in Space and Time. Second Edition. Oxford University Press Inc., New York.

487   Braga, R.F., Korasaki, V., Andresen, E., Louzada, J., 2013. Dung beetle community and

488   functions along a habitat-disturbance gradient in the Amazon: a rapid assessment of

489   ecological   functions   associated   to   biodiversity.   PLoS   ONE,   8(2):   e57786.

490   doi:10.1371/journal.pone.0057786

491   Carpaneto, G.M., Mazziotta, A., Piattella, E., 2005. Changes in food resources and conservation

492   of scarab beetles: from sheep to dog dung in a green urban area of Rome (Coleoptera,

493   Scarabaeoidea). Biological Conservation, 123: 547-556.

494   Chao, A., Jost, L., 2012. Coverage-based rarefaction and extrapolation: standardizing samples by

495   completeness rather than size. Ecology, 93(12): 2533-2547.

496   Chao, A., Jost, L., Chiang, S.-C., Jiang, Y.-H., 2008. A two-stage probabilistic approach to

497   multiple-assemblage similarity indices. Biometrics, 64: 1178-1186.

498 Chao, A., Ma, K.H., Hsieh, T.C., 2015. The Online Program SpadeR: Species-richness

499   Prediction and Diversity Estimation in R. Program and User's Guide published at

500   http://chao.stat.nthu.edu.tw/blog/software-download/.

501 Clarke, K.R., Gorley, R.N. 2015. PRIMER v7: User Manual/Tutorial, PRIMER-E: Plymouth.

502 Cruz-Rosales, M., Martínez, I.M., López-Collado, J., Vargas-Mendoza, M., González-

503   Hernández, H., Fajersson, P., 2012. Effect of ivermectin on the survival and fecundity of

504   *Euoniticellus intermedius* (Coleoptera: Scarabaeidae). Revista de Biologia Tropical, 60(1):

505   333-345.

506 Dellacasa, G., Dellacasa, M., 2006. Coleoptera Aphodiidae, Aphodiinae. Fauna d'Italia Vol.

507   XLI, Coleoptera Aphodiidae Aphodiinae. Calderini de Il Sole 24 Ore. Milán. Italia. 484 pp.

508 Diacon-Bolli, J., Dalang, T., Holderegger, R., Bürgi, M., 2012. Heterogeneity fosters

509   biodiversity: linking history and ecology of dry calcareous grasslands. Basic and Applied

510   Ecology, 13: 641-653.

511 Dormont, L., Epinat, G., Lumaret J.-P., 2004. Trophic preferences mediated by olfactory cues in

512   dung beetles colonizing cattle and horse dung. Environmental Entomology, 33(2): 370-377.

513 Dormont, L., Rapior, S., McKey, D.B., Lumaret J.-P., 2007. Influence of dung volatiles on the

514   process of resource selection by coprophagous beetles. Chemoecology, 17: 23-30.

515 Doube, B.M., Giller, P.S., Moola, F., 1988. Dung burial strategies in some South African coprine

516   and onitine dung beetles (Scarabaeidae: Scarabaeinae). Ecological Entomology, 13: 251-

517   261.

518 Dufrêne, M., Legendre, P., 1997. Species assemblages and indicator species: the need for a

519   flexible asymmetrical approach. Ecological Monographs, 67(3): 345-366.

520   Evans, K.L., Warren, P.H., Gaston, K.J., 2005. Species-energy relationships at the

521   macroecological scale: a review of the mechanisms. Biological Reviews, 80(1): 1-25.

522   Farina, A., 2000. The cultural landscape as a model for the integration of ecology and

523   economics. BioScience, 50(4): 313-320.

524   Floate, K.D., Wardhaugh, K.G., Boxall, A.B.A., Sherratt, T.N., 2005. Fecal residues of

525   veterinary parasiticides: nontarget effects in the pasture environment. Annual Review of

526   Entomology, 50: 153-179.

527   Folt, C.L., Chen, C.Y., Moore, M.V., Burnaford, J., 1999. Synergism and antagonism among

528   multiple stressors. Limnology and Oceanography, 44(3, part 2): 864-877.

529   Gaston, K.J., 2000. Global patterns in biodiversity. Nature, 405: 220-227.

530   Geary, T.G., Moreno, Y., 2012. Macrocyclic lactone anthelmintics: spectrum of activity and

531   mechanism of action. Current Pharmaceutical Biotechnology, 13: 866-872.

532   Halffter, G., Arellano, L., 2002. Response of dung beetle diversity to human-induced changes in

533   a tropical landscape. Biotropica, 34(1): 144-154.

534   Halffter, G., Favila, E., 1993. The Scarabaeinae (Insecta: Coleoptera) an animal group for

535   analyzing, inventorying and monitoring biodiversity in tropical rainforest and modified

536   landscapes. Biology International, 27: 15-21.

537   Halffter, G., Matthews, E.G., 1999. The natural history of dung beetles of the subfamily

538   Scarabaeinae. Medical Books, Palermo, 313 pp.

539   Hanski, I., Cambefort, Y., (Eds.) 1991. Dung Beetle Ecology. Princeton University Press,

540   Princeton, NJ, 481 pp.

541   Hardin, G., 1960. The competitive exclusion principle. Science, 131: 1292-1297.

542 Hawkins, B.A., Field, R., Cornell, H.V., Currie, D.J., Guegan, J., Kaufman, D.M., Kerr, J.T.,

543   Mittelbach, G.G., Oberdorff, T., O'Brien, E.M., Porter, E.E., Turner J.R.G., 2003. Energy,

544   water, and broad-scale geographic patterns of species richness. Ecology, 84(12): 3105-

545   3117.

546 Hempel, H., Scheffczyk, A., Schallnaß, H.-J., Lumaret, J.-P., Alvinerie, M., Römbke, J., 2006.

547   Toxicity of four veterinary parasiticides on larvae of the dung beetle *Aphodius constans* in

548   the laboratory. Environmental Toxicology and Chemistry, 25(12): 3155-3163.

549 Hill, M.O. 1973. Diversity and evenness: a unifying notation and its consequences. Ecology, 54:

550   427-432.

551 Horgan, F.G., Fuentes, R.C., 2005. Asymmetrical competition between Neotropical dung beetles

552   and its consequences for assemblage structure. Ecological Entomology, 30: 182-193.

553 Hsieh, T. C., K. H. Ma, and A. Chao. 2013. iNEXT online: interpolation and extrapolation

554   (Version 1.0) [Software]. Available from http://chao.stat.nthu.edu.tw/blog/software-

555   download/.

556 Hutton, S.A., Giller, P.S., 2003. The effects of the intensification of agriculture on northern

557   temperate dung beetle communities. Journal of Applied Ecology, 40: 994-1007.

558 ISTAT 2010. 6° Censimento generale dell'agricoltura. ISTAT: Roma.

559   http://censimentoagricoltura.istat.it/Jacobs C.T., Scholtz, C.H., 2015. A review on the

560   effect of macrocyclic lactones on dung-dwelling insects: Toxicity of macrocyclic lactones

561   to dung beetles. Onderstepoort Journal of Veterinary Research, 82(1): Art.#858, 8 pages.

562   http://dx.doi.org/ 10.4102/ojvr.v82i1.858

563 Jay-Robert, P., Lobo, J.M., Lumaret, J.-P. 1997. Elevational turnover and species richness
564   variation in European mountain dung beetle assemblages. Arctic, Antarctic, and Alpine
565   Research, 29: 196-205.

566 Jay-Robert, P., Niogret, J., Errouissi, F., Labarussias, M., Paoletti, E., Vázquez Luis, M.,
567   Lumaret, J.-P., 2008. Relative efficiency of extensive grazing vs. wild ungulates
568   management for dung beetle conservation in a heterogeneous landscape from Southern
569   Europe (Scarabaeinae, Aphodiinae, Geotrupinae). Biological Conservation, 141: 2879-
570   2887.

571 Jost, L., 2006. Entropy and diversity. Oikos, 113: 363–375.

572 Jost, L., 2007. Partitioning diversity into independent alpha and beta components. Ecology, 88:
573   2427-2439.

574 Klemperer, H.G., 1982. Parental behavior in *Copris lunaris* (Coleoptera, Scarabaeidae): care and
575   defense of brood balls and nest. Ecological Entomology, 7: 155-167.

576 Kools, S.A., Moltmann, J.F., Knacker, T., 2008. Estimating the use of veterinary medicines in
577   the European Union. Regulatory Toxicology and Pharmacology, 50: 59-65.

578 Krüger, K., Scholtz, C.H., 1998. Changes in the structure of dung insect communities after
579   ivermectin usage in a grassland ecosystem. I. Impact of ivermectin under drought
580   conditions. Acta Oecologica, 19(5): 425-438.

581 Larsen, T., Williams, N., Kremen, C., 2005. Extinction order and altered community structure
582   rapidly disrupt ecosystem functioning. Ecology Letters, 8: 538-547.

583 Laskowski, R., Bednarska, A.J., Kramarz, P.E., Loureiro, S., Scheil, V., Kudlek, J., Holmstrup,
584   M., 2010. Interactions between toxic chemicals and natural environmental factors – A
585   meta-analysis and case studies. Science of the Total Environment, 408: 3763-3774.

586 Liebig, M., Fernandez, Á.A., Blübaum-Gronau, E., Boxall, A., Brinke, M., Carbonell, G., Egeler,

587   P., Fenner, K., Fernandez, C., Fink, G., Garric, J., Halling-Sørensen, B., Knacker, T.,

588   Krogh, K.A., Küster, A., Löffler, D., Cots, M.Á.P., Pope, L., Prasse, C., Römbke, J.,

589   Rönnefahrt, I., Schneider, M.K., Schweitzer, N., Tarazona, J.V., Ternes, T.A.,

590   Traunspurger, W., Wehrhan, A., Duis, K., 2010. Environmental risk assessment of

591   Ivermectin: a case study. Integrated Environmental Assessment and Management, 6(1):

592   567-587.

593 Lobo, J.M., 1993. Estimation of dung beetle biomass (Coleoptera: Scarabaeoidea). European

594   Journal of Entomology, 90: 235-238.

595 Lobo, J.M., Hortal, J., Cabrero-Sañudo F.J., 2006. Regional and local influence of grazing

596   activity on the diversity of a semi-arid dung beetle community. Diversity and Distribution,

597   12: 111-123.

598 Lumaret, J.-P., Errouissi, F., 2002. Use of anthelmintics in herbivores and evaluation of risks for

599   the non target fauna of pastures. Veterinary Research, 33: 547-562.

600 Lumaret, J.-P., Errouissi, F., Floate, K., Römbke, J., Wardhaugh, K., 2012. A review on the

601   toxicity and non-target effects of macrocyclic lactones in terrestrial and aquatic

602   environments. Current Pharmaceutical Biotechnology, 13: 1004-1060.

603 Lumaret, J.-P., Galante, E., Lumbreras, C., Mena, J., Bertrand, M., Bernal, J.L., Cooper, J.F.,

604   Kadiri, N., Crowe, D., 1993. Field effects of ivermectin residues on dung beetles. Journal

605   of Applied Ecology, 30: 428-436.

606 Lumaret, J.-P., Kadiri, N., Bertrand, M., 1992. Changes in resources: consequences for the

607   dynamics of dung beetle communities. Journal of Applied Ecology, 29: 349-356.

608 MacArthur, R.H., 1965. Patterns of species diversity. Biological Reviews, 40(4): 510-533.

609 Martín-Piera, F., López-Colón, J.I. 2000. Coleoptera, Scarabaeoidea I. Fauna Ibérica, vol. 14.

610   Ramos, M.A., et al (Eds.). Museo Nacional de Ciencias Naturales. CSIC. Madrid. 528 pp.

611 McCune, B., Mefford M.J., 1999. Multivariate Analysis of Ecological Data, Version 4.0. MjM

612   Software, Gleneden Beach, Oregon, U.S.A.

613 McGeoch, M.A., Chown, S.L., 1998. Scaling up the value of bioindicators. Trends in Ecology

614   and Evolution, 13: 46-47.

615 McGeoch, M.A., van Rensburg, B.J., Botes, A., 2002. The verification and application of

616   bioindicators: a case study of dung beetles in a savanna ecosystem. Journal of Applied

617   Ecology, 39(4): 661-672.

618 McKellar, Q., Gokbulut, C., 2012. Pharmacokinetic features of the antiparasitic macrocyclic

619   lactones. Current Pharmaceutical Biotechnology, 13: 888-911.

620 Myers, N., Mittermeier, R.A., Mittermeier, C.G., de Fonseca G.A.B., Kent, J., 2000. Biodiversity

621   hotspots for conservation priorities. Nature, 403: 853-858

622 Negro, M., Rolando, A., Palestrini, C., 2011. The impact of overgrazing on dung beetle diversity

623   in the Italian Maritime Alps. Environmental Entomology, 40(5): 1081-1092.

624 Nervo, B., Tocco, C., Caprio, E., Palestrini, C., Rolando, A., 2014. The effects of body mass on

625   dung removal efficiency in dung beetles. PLoS ONE, 9(9): e107699. Doi:

626   10.1371/journal.pone.0107699

627 Nichols, E., Spector, S., Louzada, J., Larsen, T., Amezquita, S., Favila, M.E., 2008. Ecological

628   functions and ecosystem services provided by Scarabaeinae dung beetles. Biological

629   Conservation, 141(6): 1461-1474.

630   Numa, C., Verdú, J.R., Sánchez, A., Galante, E., 2009. Effect of landscape structure on the
631   spatial distribution of Mediterranean dung beetle diversity. Diversity and Distributions,
632   15(3): 489-501.

633   Perevolotsky, A., Seligman, N.G., 1998. Degradation of Mediterranean rangeland ecosystems by
634   grazing: Inversion of a paradigm. BioScience, 48: 1007-1017.

635   Pesaro Urbino Meteo. Available at: www.pesarourbinometeo.it (accessed 28 January 2016)

636   Puniamoorthy, N., Schäfer, M.A., Römbke, J., Meier, R. and W.U. Blanckenhorn. 2014.
637   Ivermectin sensitivity is an ancient trait affecting all Ecdysozoa but shows phylogenetic
638   clustering among sepsid flies. Evolutionary Applications, 7: 548–554.

639   Romero-Alcaraz, E., Ávila, J.M., 2000. Effect of elevation and type of habitat on the abundance
640   and diversity of scarabaeoid dung beetle (Scarabaeoidea) assemblages in a Mediterranean
641   area from southern Iberian peninsula. Zoological Studies, 39(4): 351-359.

642   Sala, O.E., Chapin III, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald,
643   E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A.,
644   Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M., Wall, D.H., 2000.
645   Global biodiversity scenarios for the year 2100. Science, 287: 1770-1774.

646   Slade, E.M., Mann, D.J., Villanueva, J.F., Lewis, O.T., 2007. Experimental evidence for the
647   effects of dung beetle functional group richness and composition on ecosystem function in
648   a tropical forest. Journal of Animal Ecology, 76: 1094-1104.

649   Spector, S., 2006. Scarabaeine dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae): an
650   invertebrate focal taxon for biodiversity research and conservation. The Coleopterists
651   Bulletin, Monograph Number, 5: 71-83.

652   StatSoft, Inc. (2004). STATISTICA (data analysis software system), version 7.

653   www.statsoft.com.

654   Stoate, C., Báldi, A., Beja, P., Boatman, N.D., Herzon, I., van Doorn, A., de Snoo, G.R., Rakosy,

655   L., Ramwell, C., 2009. Ecological impacts of early 21st century agricultural change in

656   Europe – A review. Journal of Environmental Management, 91: 22-46.

657   Sturaro, E., Cassandro, M., Cozzi, G., 2012. Sustainability of cattle farms in Italy. 20°

658   International Symposium, "Animal Science Days", Kranjska gora, Slovenia, September 19-

659   21, 2012.

660   Tshikae, B.P., Davis, A.L.V., Scholtz, C.H., 2013. Species richness – Energy relationship and

661   dung beetle diversity across an aridity and trophic resource gradient. Acta Oecologica, 13:

662   71-82.

663   Verdú, J.R., Cortez, V., Ortiz, A.J., González-Rodríguez, E., Martinez-Pinna, J., Lumaret, J.-P.,

664   Lobo, J.M., Numa, C., Sánchez-Piñero, F., 2015. Low doses of ivermectin cause sensory

665   and locomotor disorders in dung beetles. Scientific Reports, 5: 13912. doi:

666   10.1038/srep13912

667   Verdú, J.R., Crespo, M.B., Galante, E., 2000. Conservation strategy of a nature reserve in

668   Mediterranean ecosystems: the effects of protection from grazing on biodiversity.

669   Biodiversity and Conservation, 9: 1707-1721.

670   Verdú, J.R., Numa, C., Hernández-Cuba, O., 2011. The influence of landscape structure on ants

671   and dung beetles diversity in a Mediterranean savanna-Forest ecosystem. Ecological

672   Indicators, 11(3): 831-839.

673   Vos, W., Meekes, H., 1999. Trends in European cultural landscape development: perspectives

674   for a sustainable future. Landscape and Urban Planning, 46: 3-14.

675   Wall, R., Beynon, S., 2012. Area-wide impact of macrocyclic lactone parasiticides in cattle

676   dung. Medical and Veterinary Entomology, 26: 1-8.

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

678   drug ivermectin. Nature, 327: 418–421.

679   Wardhaugh, K.G., Longstaff, B.C., Morton, R., 2001. A comparison of the development and

680   survival of the dung beetle, *Onthophagus taurus* (Schreb.) when fed on the faeces of cattle

681   treated with pour-on formulations of eprinomectin or moxidectin. Veterinary Parasitology,

682   99: 155–168.

683   Wardhaugh, K.G., Rodriguez-Menendez, H., 1988. The effects of the antiparasitic drug,

684   ivermectin, on the development and survival of the dung-breeding fly, *Orthelia cornicina*

685   (F.) and the scarabaeine dung beetles, *Copris hispanus* L., *Bubas bubalus* (Oliver) and

686   *Onitis belial* F. Journal of Applied Entomology, 106: 381–389.

687   Willer, H., Lernoud, J., (Eds.) 2016. The world of organic agriculture. Statistics and emerging

688   trends 2016. Research Institute of Organic Agriculture (FiBL), Frick, and IFOAM –

689   Organic International, Bonn.

690   Wright, D.H., 1983. Species-energy theory: an extension of species–area theory. Oikos 41: 496–

691   506.

Figure 1

Alpha diversity of dung beetles in sub-mountainous landscapes of Central Italy.

Alpha diversity of dung beetles using Hill numbers for both grazing intensity levels (low and moderate) and Veterinary Medical Products use (VMP use and VMP free). ^{0}D (blue) correspond to species richness; ^{1}D (red) and ^{2}D (green) are the alpha diversity indices of $q = 1$ and $q = 2$, respectively. Dots represents mean and bars represent standard errors. Different letters mean significant differences (post-hoc Tukey test $P < 0.05$, with Bonferroni correction).

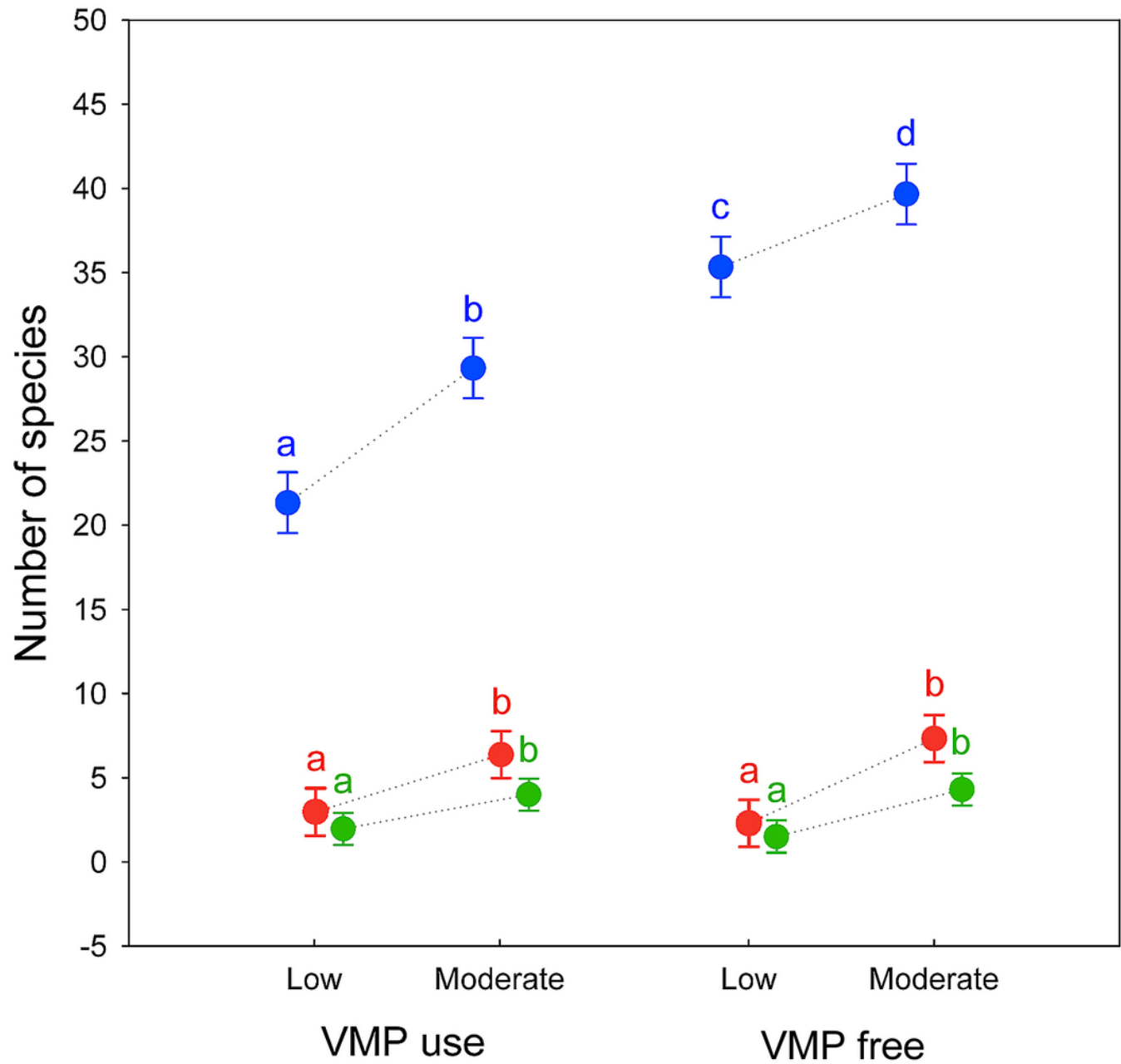



Figure 2

Dung beetle biomass and abundance in sub-mountainous landscapes of Central Italy.

Dung beetle biomass (blue) and abundance (red) for different grazing intensity levels (low and moderate) and Veterinary Medical Products use (VMP use and VMP free). Dots represents mean and bars represent standard errors. Different letters mean significant differences (post-hoc Tukey test $P < 0.05$, with Bonferroni correction).

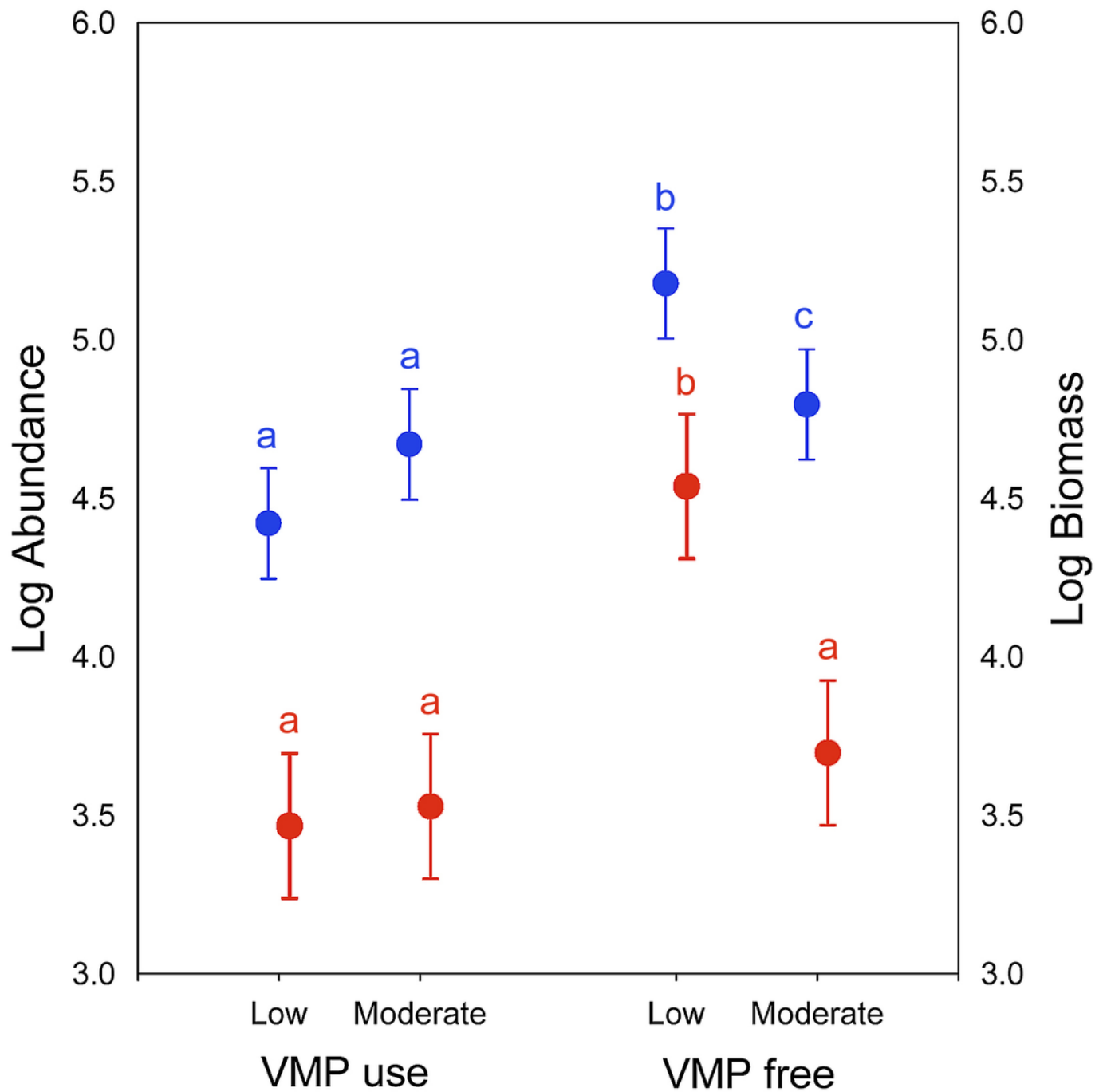



Figure 3

Beta diversity of dung beetles between sites.

Multiple community similarity using Non-Metric Multidimensional Scaling (NMDS) ordination: A) Generalised Sørensen index (C_{0N}): Average proportion of shared species in each assemblage based on the incidence data; B) Horn entropy index (C_{1N}): proportion of shared species in an assemblage based on abundance data; and C) Morisita-Horn index (C_{2N}): proportion of shared species in an assemblage based on abundance data of the most abundant (dominant) species. A two dimensional ordination was selected. Each point corresponds to a treatment replication. Squares correspond to moderate grazing areas and triangles to low grazing sites. Sites where VMPs are used are shown in red, whereas sites without any use of VMPs are in blue.

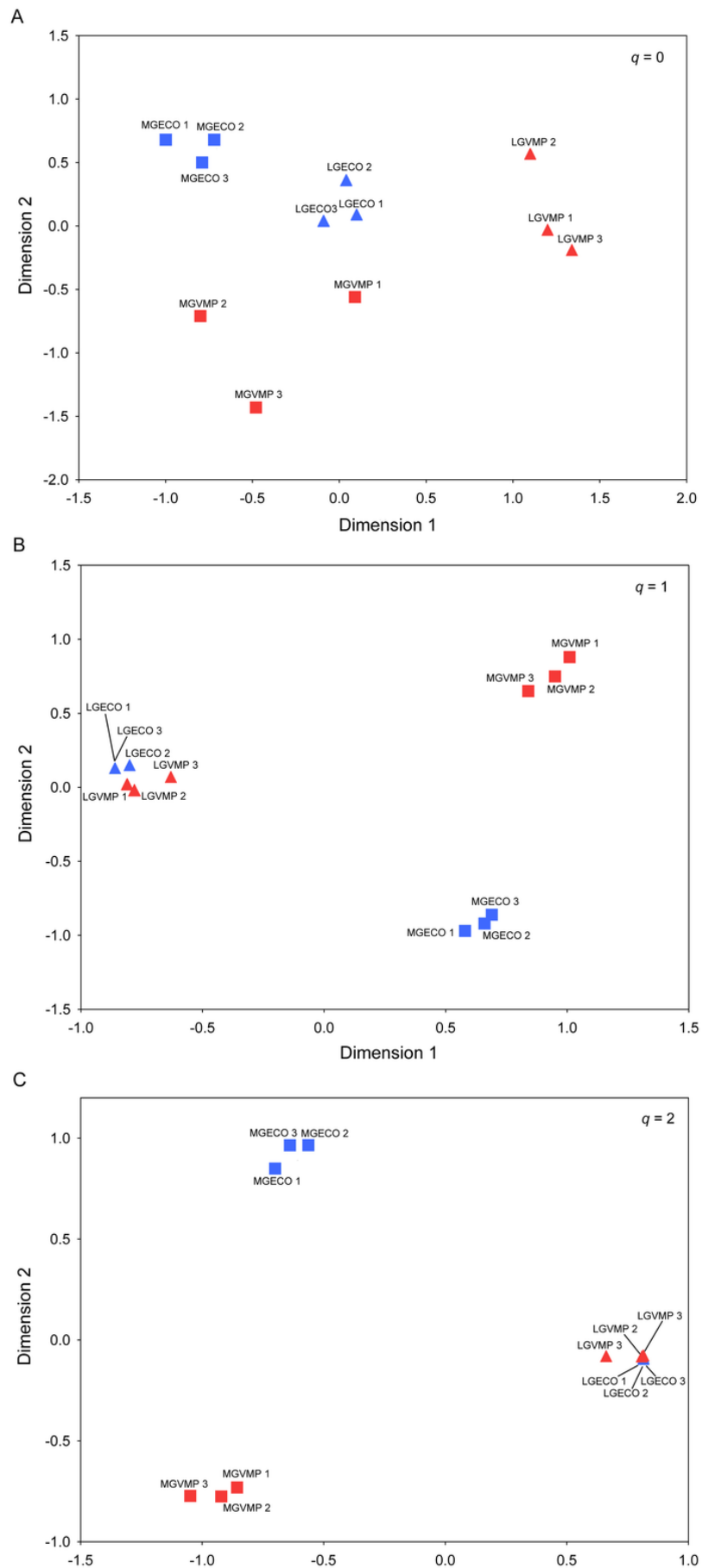
A

q = 0

B

q = 1

C

q = 2

Dung beetle indicators of different livestock grazing management techniques.

Numbers represent statistically significant IndVal values ($P < 0.05$). LG: low grazing; MG: moderate grazing; ECO: VMP free; VMP: VMP use.

Family	Indicator species	LG	MG	ECO	VMP
Aphodiidae					
	Aphodius fimetarius (Linnaeus, 1758)			90.5	
	Chilothorax conspurcatus (Linnaeus, 1758)	93.7		95.9	
	Melinopterus consputus (Creutzer, 1799)	97.3			
	Bodilopsis rufa (Moll, 1782)		97.4		
	Calamosternus granarius (Linnaeus, 1767)		83.3		
	Labarrus lividus (Olivier, 1789)		76.4		
	Melinopterus prodromus (Brahm, 1790)			99.7	
	Acanthobodilus immundus (Creutzer, 1799)			76.1	
	Nimbus johnsoni (Baraud, 1976)			79.4	
	Acrossus luridus (Fabricius, 1775)			96	
	Aphodius foetidus (Herbst, 1783)			83.3	
	Loraphodius suarius (Faldermann, 1836)			90.4	
	Otophorus haemorroidalis (Linnaeus, 1758)		100		
	Sigorus porcus (Fabricius, 1792)	75.6			
Scarabaeidae					
	Onthophagus fracticornis (Preyssler, 1790)		84.3		
	Onthophagus ruficapillus Brullé, 1832			80.6	
	Onthophagus taurus (Schreber, 1759)		91.3	89.8	
	Onthophagus coenobita (Herbst, 1783)			91.3	
	Onthophagus opacicollis Reitter, 1892			100	

Bubas bison (Linnaeus, 1767)	97.2
Copris lunaris (Linnaeus, 1758)	87
Geotrupidae	
Sericotrupes niger (Marsham, 1802)	90.5

Species compositional similitude among dung beetle assemblages.

The q levels (0, 1 and 2) indicate the value by which multiple community similarity matrices (C_{q3}) were calculated. GI = the grazing intensity factor; VMP = the VMP-use factor. P values are calculated using the Bonferroni correction.

Parameter	Source	df	SS	MS	Pseudo-F	P
	GI	1	4960.6	4960.6	1.0089	0.003
	VMP	1	4961.3	4961.3	1.009	0.003
$q = 0$	GI x VMP	1	4949.2	4949.2	1.0066	0.027
	Residuals	8	39336	4916.9		
	Total	11	54207			
	GI	1	5118.3	5118.3	1.043	0.003
	VMP	1	4966.5	4966.5	1.0121	0.225
$q = 1$	GI x VMP	1	4977.3	4977.3	1.0143	0.156
	Residuals	8	39259	4907.4		
	Total	11	54321			
	GI	1	5234.2	5234.2	1.0667	0.003
	VMP	1	5000.4	5000.4	1.0191	0.213
$q = 2$	GI x VMP	1	4984.2	4984.2	1.0158	0.258
	Residuals	8	39255	4906.9		
	Total	11	54474			