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

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

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Abstract

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.

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

Introduction
Land use changes play a pivotal role in the loss of biodiversity (Sala et al. 2000). In the Mediterranean basin, starting about 10,000 years ago, the human population modified the landscape for agriculture and livestock grazing purposes (Blondel 2006). Passing through the different stages that have characterized each era (Vos and Meekes 1999), the basin has developed a complex “cultural landscape” (cfr. Farina 2000) that enables a large number of species to be maintained there (Myers et al. 2000). Semi-natural grasslands are one of the
keystone habitats of this landscape. They were developed and managed by man (Blondel et al. 2010) using extensive livestock grazing that prevented the homogenization of the landscape (Perevolotsky and Seligman 1998; Diacon-Bolli et al. 2012). This grazing also provides an energy input to the system through the cattle dung that was previously produced by wild herbivores.

In these semi-natural grasslands, dung beetles are among the most important groups within the dung fauna (Hanski and Cambefort 1991). Their bionomics involves them, directly and indirectly, in various ecological processes such as: nutrient cycles, vegetation development, secondary seed dispersion, and parasite control (Halffter and Matthews 1999; Nichols et al. 2008). Dung beetles fulfil all the characteristics of an ideal bioindicator taxon (Spector 2006, Halffter and Favila 1993), and have been used in a great number of studies on: habitat disturbance or conversion (Braga et al. 2013; Halffter and Arellano 2002; McGeoch et al. 2002); the natural environmental gradient (Jay-Robert et al. 1997; Romero-Alcaraz and Ávila 2000); and the vegetation and landscape structure (Numa et al. 2009; Verdú et al. 2011).

In the last few decades, extensive livestock management has undergone a rapid process of modification (Stoate et al. 2009). Italy has seen the progressive abandonment of traditional extensive grazing systems in favour of more intensive versions. Furthermore, from 1982 to 2010, Italian fields lost 20% of their heads of cattle (cows, sheep and horses), while the livestock of farms fell by about 71%. Nevertheless, the number of horses and sheep rose in the same period in valley areas (more than 13%) and hills (more than 12%), but fell by about 24% in mountain regions (ISTAT 2010). Moreover, the number cow herds across the country has decreased by about 35% in the last 28 years, with 70% of cows concentrated in the north of Italy in 2010. Indeed, in this part of the country, the number of cow heads/farm increased from 48 to 64
between 2000 and 2010 (ISTAT 2010; Sturaro et al. 2012). This has led to a situation where marginal areas are abandoned, but more productive locations can suffer from overgrazing. Another relevant factor related to intensification is the use/abuse of veterinary medical products (VMPs). These substances are widely utilized, with 194 tons of antiparasitic substances produced in the European Union in 2004 (Kools et al. 2008). VMP molecules such as ivermectin are poorly metabolized by cattle (McKellar and Gokbulut 2012) and are voided as unchanged residues in faeces (Floate et al. 2005; Lumaret et al. 1993). These residues have been demonstrated to have negative sub-lethal effects and ultimate lethal consequences on non-target dung fauna and, particularly, dung beetles (Verdú et al. 2015; Wardhaugh et al. 2001).

These three factors, i.e. grazing abandonment and intensification and VMP use, have been demonstrated to have negative effects on dung beetle biodiversity. Some studies have focused on the effects on dung beetles of grazing abandonment (Jay-Robert et al. 2008; Verdú et al. 2000; Carpaneto et al. 2005), overgrazing (Negro et al. 2011) and VMP use (for a review see: Beynon 2012; Lumaret and Errouissi 2002; Wall and Baynon 2012; Jacobs and Scholtz 2015).

When it comes to the impact of VMPs on dung beetles, however, the majority of research has been carried out in the laboratory, with the focus on the effects on a single or just a few species (Verdú et al. 2015; Cruz-Rosales et al. 2012; Hempel et al. 2006; Wardhaugh et al. 1988). Nevertheless, it is important to evaluate the impact of different grazing intensities in order to determine the optimum level for dung beetle conservation. This step is necessary because, increasingly, grazing activities are not being completely abandoned, but are instead suffering an ongoing process of extensification (*sensu* EUROSTAT: [http://ec.europa.eu/eurostat/statistics-explained/index.php/Glossary:Extensification](http://ec.europa.eu/eurostat/statistics-explained/index.php/Glossary:Extensification)). Furthermore, pollutants (i.e. VMPs) may interact with “natural stressors” (i.e. the quantity of the trophic resource), producing synergistic or
antagonistic effects (Folt et al. 1999; Laskowski et al. 2010). To our knowledge, no studies have evaluated the potential impact of the possible interaction of these two factors on dung beetle diversity.

The aim of this study was to analyze the effects of grazing intensity and the use of VMPs on dung beetle diversity in the sub-mountainous landscape of Central Italy. Comparing areas with different grazing intensities (low and moderate) and those with a historical use or non-use of VMPs (used as a proxy of intensification), we attempt to answer the following four questions:

A) what is the effect of grazing intensity and VMP use on: dung beetle alpha diversity at different Hill numbers or levels ($q = 0$, $q = 1$, and $q = 2$), abundance and biomass? B) What is the possible interaction between these factors with respect to dung beetle diversity? C) Are there any indicator species for a particular form of treatment? D) What are the effects on the composition of dung beetle assemblages (beta diversity)? Our hypothesis is that a low level of grazing intensity and the use of VMPs have negative effects on dung beetle biodiversity, resulting in changes in alpha and beta diversity and biomass, and favouring the presence of some species that may act as indicators of a particular form of pasture management. Moreover, we hypothesize that the effects of low grazing intensity and VMP use are worse in combination than alone.

Materials and Methods

Study area and experimental design

The study was carried in the sub-mountainous area of the Pesaro-Urbino province in the Marche region, Italy. The provincial climate falls into the temperate Köppen categories (Cfa and Cfb). The average annual temperature is around 12°C, with a minimum average of around 3.5°C in winter and a maximum average of 21°C in summer. Average annual precipitation is around 930
The soil is calcareous.

To evaluate the effects of grazing intensity and VMP use, we designed a 2x2 full factorial design with three replications for each treatment. We identified sites with: a VMP-free, low grazing intensity; a VMP-free, moderate grazing intensity; a VMP-use, low grazing intensity; and a VMP-use, moderate grazing intensity.

A) ‘Low grazing, VMP-free’ sites – LGECO - (Pietralata pastures; 43°39’33.64’’N; 12°42’27.65’’E). These secondary grasslands, located between 750 and 900 m a.s.l., are represented by the *Brizo mediae-Bromerum* and *Festuco circummediterraneae-Arrhenatheretum elatioris* associations. These grasslands are used by horses that were abandoned and have reverted to a wild state. The grazing intensity of this pasture is about 0.7 units of livestock/ha.

The most common wood species are: *Fraxinus ornus*, *Ostrya carpinifolia*, *Quercus ilex*, *Quercus pubescens*, *Acer opalus obtusatum*, *Pinus nigra*, *Crataegus monogyna*, *Juniperus oxycedrus*, *Lonicera etrusca*, *Spartium junceum*, and *Rosa canina*.

B) ‘Moderate grazing, VMP-free’ sites – MGECO - (Montebello pastures; 43°43’13, 83’’N; 12°45’19,98’’E). These grasslands are located between 500 and 600 m a.s.l. within the Gino® Girolomoni Cooperativa Agricola. The pastures are used by cows according to organic farming rules with grazing rotation. The grazing intensity is about 1.5 units of livestock/ha. The herbaceous association falls within the *Brizo mediae-Brometum erecti* group. The spontaneous arboreal vegetation is prevalently comprised of *Quercus pubescens*, *Quercus cerris*, *Quercus petraea*, *Carpinus betulus*, *Ostrya carpinifolia*, *Fraxinus ornus*, *Acer opalus*, *Quercus ilex*, *Sorbus domestica*, *Corylus avellana* and *Fagus sylvatica*. 
C) ‘Moderate grazing with VMPs’ sites – MGVMP - (Catria pastures; 43°30’23.39’’N; 12°39’22.39’’E). These grasslands are used by cows and horses and have a historical grazing tradition. The farmers there highlighted that VMPs have long been used and this convention continues to today. The unit of livestock/ha is about 1.5 and there is no sign of overgrazing. These pastures are referred to the association Brizo mediae-Brometum erecti, where the most abundant species are Bromus erectus, Briza media, Filipendula vulgaris, Centaurea triumfetti subsp. aligera, Plantago lanceolata subsp. sphaerostachya, Luzula campestre, Leontodon cichoraceus, Cynosurus cristatus, Anthoxanhtum odoratum and Carex caryophyllea. The arboreal species are represented by the Scutellario columnare-Ostryetum carpinifolia association. The sampling sites are located between 800 and 1000 m a.s.l.

D) ‘Low grazing with VMPs’ sites – LGVMP - (Nerone pastures; 43°32’07.27’’N; 12°33’26.13’’E). These grasslands are grazed by horses that represent a grazing intensity of about 0.5 units of livestock/ha. These sites have been submitted to the historical and intensive use of VMPs from about the 1990s. Today, VMPs are only given to foals and adult animals with evident parasitic stress. The grass associations of these pastures are Asperulo purpureae-Brometum erecti and Brizo mediae-Brometum erecti, with the principal species being: Bromus erectus, Briza media, Filipendula vulgaris, Centaurea triumfetti subsp. aligera, Plantago lanceolata subsp. sphaerostachya, Luzula campestre, Leontodon cichoraceus, Cynosurus cristatus, Anthoxanhtum odoratum and Carex caryophyllea. The arboreous species are dominated by the Scutellario columnare-Ostryetum carpinifolia association. The sampling sites are located between 800 and 1000 m a.s.l.

The density of wild fauna (i.e. Capreolus capreolus and Sus scrofa) is very similar among all the studied areas (Tonelli, personal observation, 2013).
Dung beetle trapping

For each treatment, we selected three sampling sites separated by at least 500 m to ensure independence among the replicates. In each site, we placed a 50 x 50 m quadrat with four pitfall traps at the corners; two traps were baited with cow dung (about 500 cm$^3$) and two with horse dung (about 500 cm$^3$) to maximize differential species attraction (Barbero et al. 1999; Dormont et al. 2004, 2007). The dung used for the trapping was collected from organic farming that was VMP free. We filled the pitfall traps with propylene glycol (50%) to preserve the dung beetles we collected. The traps were left active for 48 h in each sampling period. The sampling was repeated about every 15 days from June 2013 to November 2013 and in May and June 2014. We excluded rainy days in order to prevent any interference with the trapping. The total number of traps used was 48, and we collected a total of 528 samples (4 traps x 3 sampling points x 4 treatments x 11 sampling periods). The dung beetles were identified according to Baraud (1992) for the Scarabaeinae and Geotrupinae subfamilies. Aphodiinae were identified following Dellacasa and Dellacasa (2006). Biralus mahunkaorum (Ádám, 1983), Onthophagus mediuss (Kugelann, 1792) and Aphodius fimetarius (Linnaeus, 1758) were identified following the work, respectively, of Rössner and Fery (2014), Rössner et al. (2010) and Miraldo et al. (2014), respectively. The species of the ovatus group (i.e. Onthophagus ruficapillus Brullé, 1832, Onthophagus joannae Goljan, 1953, Onthophagus grossepunctatus Reitter, 1905) were identified using the genitalia characters proposed by Martin-Piera and Zunino (1986) based on the work of Binaghi et al. (1969).

Sampling completeness
The inventory completeness was evaluated using a sample coverage analysis (Chao & Jost 2012). This is a measure of sample completeness, and reveals the proportion of the total number of individuals in a community that belong to the species represented in the sample. The sample coverage formula uses information about sample size, singletons and doubletons (Chao & Jost 2012). Measurements were taken using iNext v.1.0 (Hsieh et al. 2013).

**Alpha diversity**

Alpha diversity was calculated using the Hill numbers’ family diversity (McArthur 1965; Hill 1973; Jost 2006, 2007). These diversity measures are parameterized by the order $q$. The $q$ parameter determines the sensitivity of the index with respect to rare or abundant species. For example, the diversity of $q = 0$ is completely insensitive to species abundance and so corresponds to species richness; for $q = 1$, species are weighted proportionally to their relative abundance and correspond to the Shannon entropy exponential; for $q = 2$ the index is disproportionately sensitive to common species and corresponds to the reciprocal of the Simpson index (Jost 2006, 2007). The results of these indices are expressed in terms of the “effective number of species”, i.e. the number of equally abundant species needed to produce the same value of the diversity measure (Jost 2006, 2007). These measures conform to the replication principle introduced by Hill (1973).

In order to characterize the complete species abundance distribution and provide full information about its diversity, we computed the diversity of the orders 0, 1 and 2 for each replication of each treatment for the two factors (grazing intensity and VMP use). We then analyzed these results using a full factorial multivariate generalized linear model in order to evaluate the main effect of the two factors and highlight any interactions. Pairwise comparisons
were made using the Tukey post-hoc test. The diversity profile was produced with SpadeR (Chao et al. 2015) and the generalized linear model with the Statistica 7.0 package (StatSoft, 2004).

Dung beetle biomass and abundance

We tested the statistical difference in dung beetle total biomass and abundance using a full factorial multivariate generalized linear model with the Statistica 7.0 package (StatSoft, 2004) after log transformation of the dependent variable. Pairwise comparisons were made using the Tukey post-hoc test. The average biomass of each species was calculated using the formula ‘Biomass = 0.010864 x Length^{3.316}’ suggested by Lobo (1993). Ten individuals of each species (when available) were measured to obtain the average species length. Fewer than 10 specimens of the following were measured (the figure in brackets is the number of specimens measured):

- Calamosternus mayeri (Pilleri, 1953) (1);
- Limarus zenkeri (Germar, 1813) (1);
- Melinopterus stolzi (Reitter, 1906) (1);
- Nimbus johnsoni (Baraud, 1976) (1);
- Phalacronotus biguttatus (Germar, 1824) (1);
- Planolinus fasciatus (Olivier, 1789) (1);
- Trypocopris vernalis apenninicus Mariani, 1958 (4);
- Nialis varians (Duftschmid, 1805) (4); and
- Amidorus thermicola (Sturm, 1800) (7). To calculate the total biomass of the dung beetle at each treatment, we multiplied the average weight of each species by the number of individuals collected and added these numbers together.

Beta diversity

We analyzed whether grazing intensity and VMP use had any effect on the composition of the dung beetle assemblages. We first calculated an index of multiple community similarity of the two factors (using $q = 0, 1, 2$) among all the replicates. This produced six similarity matrices ($3 q$ factors).
order x 2 factors). Based on these matrices, Non-Metric Multidimensional Scaling (NMDS) were constructed and analyzed using a Permanova test (Anderson 2001) to evaluate the statistical significance of each factor for the composition of the dung beetle assemblages at each $q$ level.

We computed the multiple community similarity of each treatment with a multiple-assemblage abundance-based overlap measure $C_qN$ (Chao et al. 2008). Like the Hill numbers, $q$ is the same parameter that we used for alpha diversity and $N$ is the number of assemblages (sites). When $q = 0$, $C_0N$ is equivalent to the multiple community version of the classical Sørensen index; when $q = 1$, $C_1N$ corresponds to the multiple community version of the Horn homogeneity measure, and when $q = 2$, $C_2N$ is equivalent to the multiple community version of the Morisita-Horn similarity index (Chao et al. 2012). For the integer values of $q$ between 2 and $N$, the overlap measures $C_qN$ have a simple statistical interpretation as the ratio of two probabilities $\frac{\#Gp}{\#Gs}$. The numerator is the probability that $q$ randomly sampled individuals belong to the same species given that they did not all come from the same assemblage. The denominator is the probability that $q$ randomly sampled individuals belong to the same species given that they are all drawn from the same assemblage. This interpretation shows the depth of the measure: when $q = 2$ only the pairwise similarity is considered, but when $q = 3$ the measure also takes into account species that are shared by three assemblages (Jost et al. 2011). This measure ranges from 0, when all the assemblages are completely different in terms of species composition, to 1 when they are identical. In summary, the measure $C_qN$ quantifies the effective average overlap per community, i.e. the average percentage of overlapped species in an assemblage. Its inverse is an estimate of the beta diversity. Similarity matrices were computed using SpadeR (Chao et al. 2015). A Permanova test was performed using the Permanova+ add-on for PRIMER v.7 (Anderson et al. 2008; Clarke and Gorley, 2015). Interaction between the factors was also evaluated. A total of
999 unrestricted permutations of raw data were computed. The $P$ values were calculated using the Bonferroni correction in all cases.

**Indicator species**

The indicator value method (Dufrêne and Legendre, 1997) was computed for each factor to identify the indicator species of a particular treatment. This method is used to quantify the value, as a bioindicator, of a set of taxa. In relation to a given species, it combines the measurement of the degree of specificity (how much the species tends to be abundant in a particular ecological state) with the measurement of the degree of fidelity (how much the species tends to be present inside a determined ecological state) with respect to a given ecological status (McGeoch et al. 2002; McGeoch and Chown 1998; Dufrêne and Legendre, 1997). The indicator values range from 0 (no indication) to 100 (perfect indication). Species with significant ($P < 0.05$) IndVal results above 70% were considered to be indicator species for the given treatment. Species with an intermediate IndVal between 45% and 70% were considered to be detector species. Indicator species are highly characteristic of a particular ecological state (treatment) and may decline rapidly under other ecological conditions up to the point of disappearance. Detector species have a different degree of preference for different ecological states, and relative changes in their abundance across states may be indicative of the direction in which change is occurring (McGeoch et al. 2002). The analysis was performed using PC-Ord 5 (McCune and Mefford 1999).

**Results**
A total of 148,668 individuals belonging to 57 species of dung beetle were collected. This breaks down into: 122,611 specimens belonging to 42 species for the low grazing treatment; 26,057 individuals belonging to 54 species for the moderate grazing treatment; 128,616 specimens from 53 species for the VMP-free treatment; and 20,052 individuals belonging to 41 species for the VMP-use condition (Supplementary Material S1).

The sample coverage estimator revealed that our inventories were 99% complete for each treatment. This indicates that only 1% of the individuals in a community belong to species not represented in our samples. We can thus consider our samples to be complete, and we have utilized empirical data for the diversity analysis and comparisons.

**Alpha diversity**

The alpha diversity showed a large decrease in the effective number of species as the $q$ order increased, indicating a high degree of dominance in the studied assemblages. There are significant differences in alpha diversity due to the grazing intensity (Wilks’s lambda = 0.08; $F_{[3,6]} = 21.88; P < 0.01$) and use of VMPs (Wilks’s lambda = 0.02; $F_{[3,6]} = 83.67; P < 0.0001$).

However, a non-significant interaction between the two factors was identified (Wilks’s lambda = 0.30; $F_{[3,6]} = 4.78; P = 0.39$). The post-hoc Tukey test for the grazing intensity factor showed a significant difference between the low and moderate grazing intensity for $^0D (P < 0.001)$, $^1D (P < 0.001)$ and $^2D (P < 0.001)$, indicating a negative effect of a low level of grazing on alpha diversity. In terms of VMP-use, significant factor differences were only found for $^0D (P < 0.001)$, and not for the other alpha diversity levels ($^1D$ and $^2D$). The post-hoc Tukey test for the interaction between the two factors (Figure 1) showed that for $^0D$, all the treatments had significant differences, with the MGECO sites having 1.11 equivalent species more than the
LGECO sites, 1.34 more than the MGVMP sites and 1.86 more than the LGVMP sites. Sites with LGECO had 1.21 equivalent species more than the MGVMP sites and 1.66 more than the LGVMP sites. The sites with MGVMP had 1.38 equivalent species more than LGVMP.

For $1D$ and $2D$, the post-hoc Tukey test showed that statistical differences only exist between the areas MGECO and LGVMP and LGECO, whereas the sites with MGVMP had significantly more equivalent species than those with LGVMP and LGECO (Figure 1).

**Indicator values of species**

The IndVal analysis (Table 1) for the grazing intensity factor revealed 10 indicator species: three for the low grazing treatment and seven for the moderate grazing treatment. For the VMP-use factor, 14 indicator species were identified, all with respect to the VMP-free treatment. Two VMP-free indicator species were also indicator species of some treatments for the grazing intensity factor: *Chilothorax conspurcatus* is an indicator of the VMP-free and low grazing sites, and *Onthophagus taurus* of the VMP-free and moderate grazing treatments.

**Biomass and abundance of dung beetles**

Significant differences in dung beetle biomass and abundance were obtained for the grazing intensity ($\text{Wilks’s lambda }= 0.138; F_{[2,7]} = 21.87; P < 0.01$) and use of VMPs factors ($\text{Wilks’s lambda }= 0.17; F_{[2,7]} = 17.34; P < 0.05$). However, no differences were found in their interactions ($\text{Wilks’s lambda }= 0.28; F_{[2,7]} = 9.13; P = 0.09$). The post-hoc Tukey test showed that the LGECO treatment had a higher dung beetle biomass and abundance than the LGVMP, MGECO and MGVMP treatments, whereas the MGECO treatment had more biomass than the LGVMP treatment.
Beta diversity

Multiple-assemblage abundance-based similarity measures ($C_qN$) showed a clear aggrupation between sites characterised by both factors studied. For each $q$ level, Non-Metric Multidimensional Scaling (NMDS) plots represent a clear ordination of sites based on grazing activity and VMP use (Figure 3). The Permanova test showed significant differences in beta diversity for the grazing intensity factor at each $q$ order of similarity matrix (Table 2). For the VMP-use factor, the Permanova test showed a significant compositional impact only for $q = 0$, whereas it was not significant when species abundance was taken into account, i.e. for $q = 1$ and $q = 2$. Furthermore, the interaction between the two factors was significant only for the similarity matrix of order $q = 0$, but was not significant for $q = 1$ and $q = 2$ (Table 2).

Discussion

Our results support the hypothesis that a low grazing intensity and the use of VMP substances have a negative effect on dung beetle biodiversity. The areas with a moderate grazing intensity have more alpha diversity than the low grazing intensity sites. Our results are consistent with those of other studies in Europe. Lobo et al. (2006) in Spain showed that the quantity of dung in a radius of 2 km and the presence of a flock are key factors in determining the local variation in dung beetle species richness and abundance. Lumaret et al. (1992) explained that an increase of 260% in fresh dung availability, five years after a change of pasture management in Southern France (from sheep to cows), caused an increase in species richness from 38 to 42. In Italy, Carpaneto et al. (2005) showed that after 13 years, the abandonment of the sheep grazing system in the Rome urban area led to a loss of 53% of the
dung beetle species, especially those with a large body size. The decrease in the number of indicator species that occurred with a decrease in grazing intensity supports our hypothesis. We encountered seven and three species with significant IndVal values for the moderate and low grazing areas, respectively. This means that a reduced quantity of a trophic resource can favour a limited number of species. Moreover, it is interesting to note that in our moderate grazing sites, three of the seven indicator species are paracoprids and, among them, one, *Copris lunaris*, is a large species. During breeding, *Copris lunaris* may bury about 100-165 g of dung (Klemperer 1982; Martin-Piera and López-Colón 2000). There is thus a positive relationship between body size and dung mass burial (Doube et al. 1988; Larsen et al. 2005; Slade et al. 2007), which supports the notion that large paracoprid dung beetles can only survive if the trophic resource is abundant. Our results on the grazing intensity factor can be explained by the species-energy relationship (Gaston 2000, Wright 1983, Hawkins et al. 2003), i.e. the lower the level of (trophic) energy available, the smaller the number of species that an area can support (Evans et al. 2005). Tshikae et al. (2013) explicitly tested the species–energy relationship for dung beetles across an arid and trophic resource gradient in Botswana. Their results showed that the species richness, diversity and biomass of the dung beetle diminish with a decrease in available (trophic) energy.

However, it is interesting to note that our low grazing areas have greater biomass and abundance. This may be explained by the dominance of two species, *Melinopterus consputus* and *Onthophagus medius*. Both species may alter the diversity pattern of this treatment by means of a competitive exclusion (Hardin 1960). The low quantity of the trophic resource available in this site has perturbed the dung beetle community, favouring generalist r-strategic species (*Melinopterus consputus*) and highly competitive species such as small tunnellers (*Onthophagus*...
medius) (Horgan and Fuentes 2005). Our low grazed sites, in fact, have more biomass but fewer species than the moderately grazed areas. The same results were reported in the Rome urban area (Italy) by Carpaneto et al. (2005), who found a decrease in the number of species and a rise in total biomass, with the dominance of one species of Aphodinae with the same explosive reproductive strategy (i.e. Nimbus johnsoni).

Beta diversity in our study is strongly influenced by the quantity of the trophic resource at all $q$ levels (Table 3); rare and abundant species are compositionally different between the grazing intensity treatments. According to the findings of Lobo et al. (2006), our study showed that the quantity of available dung for dung beetle assemblages is an important factor in determining dung beetle composition.

We document that our VMP-use sites have significantly fewer species and a reduced biomass compared to the VMP-free sites. Our results agree with other studies that have explored the impact of VMPs in the field. For example, in southern Ireland, Hutton and Giller (2003) observed a lower number of species and a reduced abundance of dung beetles in intensive and rough grazing farms compared to organic farms. Krüger and Scholtz (1998) also showed that, under drought conditions in South Africa, treatment with ivermectin led to a loss of dung beetle species. Basto-Estrella et al. (2014) showed a loss of dung beetle species in a Mexican farm with macrocyclic lactone use, but they also found greater dung beetle abundance in this farm compared to farms that did not use macrocyclic lactones. According to these authors, this variation was probably due to an attracting/repelling effect on the dung beetle. Beynon et al. (2012b) showed a reduction in dung beetle abundance and biomass in dung treated with ivermectin in the UK. VMP-use may have a variety of lethal and sub-lethal effects on non-target fauna depending on the molecule, doses, mode of administration, environmental factors and
insect species in question (Lumaret and Errouissi 2002; Wall and Beynon 2012; Jacobs and Scholtz 2015). Many essays on the dung beetle show that VMPs negatively affect larval and adult survival, as well as some physiological processes such as reproductive, sensorial and locomotor capacities, and this has negative repercussions for dung decomposition (Wall and Strong 1987; Lumaret et al. 1993; Verdú et al. 2015; Wardhaugh et al. 2001). Unlike some studies (Krüger and Scholtz 1998; Basto-Estrella et al. 2014; Hutton and Giller 2003), we did not find a significant difference in alpha diversity ($^{1}D$ and $^{2}D$) for the VMP-use factor.

A recent piece of research shows that very low doses of ivermectin cause higher adult mortality and physiological stress in *Scarabaeus cicatricosus*, and this prevents them from performing basic biological activities (Verdú et al. 2015). Some models estimate a reduction of about 25-35% of the population of dung beetle species in the next generation following a single treatment with some VMPs (Wardhaugh et al. 2001; Sherratt et al. 1998).

Our IndVal results showed how the VMP-use treatments have no indicator species. This means that no species were favored by the use of these veterinary substances. In other words, the use of VMPs affects all species and, apparently, no species are resistant to VMP toxicity. In contrast, the VMP-free treatment had 14 indicator species, 10 of which were larger than the median body mass value of the entire community (data not reported). It has been documented that large dung beetles are more prone to extinction due to environmental stress (Larsen et al. 2005). Our results are congruent with those of Puniamoorthy et al. (2014), which show that ivermectin sensitivity is an ancient trait affecting potentially all Ecdysozoan (moulting animals) species. This corroborates the hypothesis that the use of VMPs may have a ubiquitous, negative effect on dung beetle fauna. The fact that no species were found to be bioindicators in the areas with VMP-use could be due to the irrational use of these substances throughout the year.
Onthophagus taurus is an indicator of the VMP-free and moderate grazing treatments. We suggest that Onthophagus taurus may be taken into account as a useful general bioindicator of the state of an environment, since it seems to be sensitive to various environmental stressors. Beta diversity is influenced by the use of VMPs only for \( q = 0 \). This means that the two assemblages are different in terms of rare species, whereas the abundant ones are not significantly different. Accordingly, some (rare) species cannot remain in a disturbed habitat, whereas dominant species may be less perturbed.

Interesting results were highlighted by the interactions between the two factors. Contrary to our hypothesis, the interactions terms were less significant, or no more significant, than the main effects (each factor separately) for alpha diversity, biomass and species composition. This could be explained if we consider that both factors affect different forms of each species. In other words, the decrease in dung availability affects several bigger species more than the very small species, but this does not imply that the former are more susceptible to injury caused by the ingestion of dung contaminated with VMPs. This means that interactions between the two factors may have antagonistic effects on dung beetle assemblages, but more studies are needed on this issue.

The present analysis highlights that the moderate grazing VMP-free treatment seems to be the best management system for maintaining a higher number of dung beetle species, as well as greater diversity and biomass. These results corroborate the notion that, in a Mediterranean context with a long history of grazing, traditional management techniques with a moderate grazing intensity have a positive effect on dung beetle biodiversity (Verdú et al. 2000). Furthermore, our results corroborate the hypothesis that both factors - low grazing intensity and VMP-use - have negative effects on dung beetle communities. The number of species, diversity,
biomass, and indicator species and composition are parameters affected by pasture management techniques. Dung beetle species richness and biomass are strongly related to the dung decomposition process (Nervo et al. 2014; Beynon et al. 2012a; Larsen et al. 2005). The loss of dung beetle biodiversity can have a negative impact on various ecosystem processes (Nichols et al. 2008), with harmful effects on pastures.

Finally, we suggest that organic farming with a moderate grazing intensity could have a positive effect on dung beetle conservation. This farming management approach may contribute to this by avoiding pasture abandonment, conferring an economic stimulus (Willer and Lernoud 2016) and controlling for the excessive use of VMPs (Hutton and Giller 2003). Further studies in different biogeographical and bioclimatic regions are, however, needed to assess the impact of the long-term use of VMPs on dung beetles.

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Figure 1

Alpha diversity of dung beetles

Alpha diversity of dung beetles using Hill numbers for different grazing intensity levels and VMP use in sub-mountainous landscapes of Central Italy. $^0D$ (blue) corresponds to species richness; $^1D$ (red) and $^2D$ (green) are the alpha diversity indices of $q = 1$ and $q = 2$, respectively. Different letters mean significant differences (post-hoc Tukey test $P < 0.05$).
Figure 2

Dung beetle biomass and abundance

Dung beetle biomass (blue) and abundance (red) for different grazing intensity levels and VMP use in sub-mountainous landscapes of Central Italy. Different letters mean significant differences (post-hoc Tukey test $P < 0.05$).
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. Areas where VMPs are used are shown in red, whereas the sites without any use of VMPs are in blue.
Table 1 (on next page)

Dung beetle indicators of different sites

Dung beetle indicators of different livestock grazing management approaches. The numbers represent IndVal values ($P < 0.05$). LG: low grazing; MG: moderate grazing; ECO: VMP free; VMP: VMP use.
| Indicator species                        | LG  | MG  | ECO | VMP |
|-----------------------------------------|-----|-----|-----|-----|
| *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|     |
| *Bubas bison* (Linnaeus, 1767)          |     |     |     | 97.2|
| *Calamosternus granarius* (Linnaeus, 1767) | 83.3|     |     |     |
| *Copris lunaris* (Linnaeus, 1758)      |     |     | 87  |     |
| *Labarrus lividus* (Olivier, 1789)      |     |     | 76.4|     |
| *Melinopterus prodromus* (Brahm, 1790) |     |     |     | 99.7|
| *Onthophagus coenobita* (Herbst, 1783) |     |     |     | 91.3|
| *Onthophagus fracticornis* (Preyssler, 1790) | 84.3|     |     |     |
| *Onthophagus ruficapillus* Brullé, 1832 |     |     | 80.6|     |
| *Onthophagus taurus* (Schreber, 1759)  |     |     | 91.3| 89.8|
| *Otophorus haemorroidalis* (Linnaeus, 1758) | 100 |     |     |     |
| *Sericotrupes niger* (Marsham, 1802)   |     |     | 90.5|     |
| *Acanthobodilus immundus* (Creutzer, 1799) |    |     | 76.1|     |
| *Acrossus luridus* (Fabricius, 1775)   |     |     | 96  |     |
| *Aphodius foetidus* (Herbst, 1783)     |     |     |     | 83.3|
| *Loraphodius suarius* (Faldermann, 1836) |     |     | 90.4|     |
| *Nimbus johnsoni* (Baraud, 1976)      |     |     | 79.4|     |
| *Sigorus porcus* (Fabricius, 1792)     |     |     | 75.6|     |
Onthophagus opacicollis Reitter, 1892

100
Table 2

Species compositional similitude among assemblages at different \( q \) values. The \( q \) values (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 |       |        |          |