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Effects of different pastures management on dung beetle communities in a sub-mountainous landscape of central Italy: a multicomponent biodiversity and ecological process analysis

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A te vengo, balena che tutto distruggi ma non vinci:

fino all’ultimo lotto con te;

dal cuore dell’inferno ti trafiggo;

in nome dell’odio, vomito a te l’ultimo mio respiro

(Herman Melville)

…nulla si può tentare se non

stabilire l’inizio e la direzione di

una strada infinitamente lunga. La pretesa di

qualsiasi completezza sistematica e definitiva

sarebbe, se non altro, un’illusione. Qui il singolo

ricercatore può ottenere la perfezione

solo nel senso soggettivo che egli

comunichi tutto ciò che è riuscito a vedere.

(Georg Simmel)

Al coraggio

di un pettirosso

da combattimento

INDEX

RESUMEN

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Los escarabajos coprófagos (Coleoptera: Scarabaeoidea) son un grupo de insectos muy importante en términos de diversidad y biomasa en los pastizales de la cuenca del Mediterráneo. A través del uso de estiércol como recurso trófico y para nidificar, estos organismos están involucrados en numerosos procesos ecológicos relacionados con la degradación del estiércol, tales como en el ciclo de nutrientes, en la dispersión secundaria de semillas, contribuyen mejorando la estructura del suelo, en la reducción de emisión de gases de efecto invernadero, así como en el control de endo-ecto parásitos del ganado. A pesar de su importancia ecológica, los escarabajos coprófagos están amenazados por varios factores, entre los cuales se destacan los cambios en el manejo de la ganadería tradicional.

En las últimas décadas, la ganadería tradicional ha sufrido cambios radicales debidos principalmente a dos procesos: el abandono progresivo de la ganadería y su intensificación. Por un lado, el abandono progresivo de la ganadería tradicional es un factor clave en la conservación de los escarabajos coprófagos. Debido a la dependencia que este grupo de insectos tiene con el estiércol, este factor llevaría a una disminución del recurso trófico con repercusiones negativas para la comunidad coprófaga. Históricamente, los escarabajos coprófagos han dependido del uso de los excrementos de la megafauna salvaje, y desde su disminución, el estiércol de la ganadería doméstica ha sido su principal recurso trófico. A pesar de ello, en los últimos 50 años, la ganadería tradicional ha comenzado un proceso de abandono progresivo, sobre todo en las áreas marginales como las de montaña. Por otra parte, el uso/abuso de productos médico veterinarios para el control de los endoparásitos y ectoparásitos del ganado es otro factor importante en tema de conservación de la fauna coprófaga y está relacionado con la

intensificación de la ganadería. Estos compuestos médico veterinarios, entre los cuales la ivermectina destaca por su frecuancia en el uso y el efecto negativo en la fauna coprófaga, son eliminados progresivamente en las heces de los organismos. Estudios recientes han demostrado que los residuos de algunos de estos compuestos tienen efectos sub-letales y letales sobre la fauna coprófaga y sobre todo en los escarabajos.

A pesar de la importancia que estos dos factores pueden tener en la conservación de los escarabajos coprófagos, pocos estudios comparativos han investigado el impacto sobre las comunidades de este grupo y como consecuencia sobre el papel ecológico en los cuales están involucrados. Debido a este vacío de conocimiento, el objetivo general de esta tesis fue evaluar los efectos del abandono de la ganadería, su extensificación y uso histórico de productos médico veterinarios sobre las comunidades de escarabajos coprófagos usando medidas de biodiversidad y cuantificando la capacidad de remoción de estiércol en un paisaje sub-montano de la provincia de Pesaro-Urbino (Italia).

Después de una introducción general (Capítulo 1) en los capítulos 2 y 3 de esta tesis se abordan los efectos de los diferentes métodos de manejo de la ganadería y se analiza el impacto de estos factores sobre la diversidad alfa y beta, abundancia y biomasa, especies indicadoras, grupos funcionales y clases de tallas de las especies. En los capítulos 4 y 5 se evalúan los efectos de estos factores desde una perspectiva funcional, analizando la diversidad funcional de las comunidades y el desempeño que cada comunidad tiene en la remoción del estiércol. Así mismo, se analizaron las relaciones entre varias medidas de diversidad funcional y de diversidad clásicas (número de especies y exponencial de Shannon) y la relación entre la diversidad funcional y el proceso ecológico.

En este trabajo se colectaron un total de 156.936 individuos, pertenecientes a 58 especies de escarabajos coprófagos (3 Geotrupinae; 16 Scarabaeinae; 39 Aphodiinae) y por primera vez se cita *Calamosternus mayeri* (Pilleri, 1953) en la región Marche. Los resultados de este trabajo sugieren que el uso histórico de productos médico veterinarios tiene un impacto negativo y ubiquitario sobre todas las especies de la comunidad de estudio, llevando a un empobrecimiento de los ensambles y a una pérdida de abundancia y biomasa. No obstante se ha registrado el mantenimiento de la estructura de la comunidad. Estos resultados son corroborados igualmente cuando nos enfocamos en la diversidad funcional. En efecto, el uso histórico de productos médico veterinarios lleva a una pérdida en el número de roles funcionales dentro de la comunidad, sin embargo las abundancias se encuentran repartidas equitativamente dentro de cada nicho funcional. Todos estos efectos sobre la comunidad de escarabajos coprófagos debido al uso de productos médico veterinarios, al final quedan reflejados en una pérdida del 70% en la capacidad de remoción de estiércol, comparados con áreas donde estos productos no son aplicados.

La extensificación de la ganadería está representada por áreas que tienen una intensidad de pastoreo muy baja, las cuales pueden ser consideradas como la primera etapa hacia el abandono completo. En este sentido, los resultados de este trabajo demuestran como una simple reducción de la carga ganadera lleva a efectos negativos sobre las comunidades de escarabajos coprófagos, registrando una disminución en la diversidad alfa y en el número de especies indicadoras. Esta disminución en la cantidad de recurso trófico tiene implicaciones sobre la composición de la comunidad, favoreciendo especies oportunistas cuya biología le permite evitar la competencia, como por ejemplo; especies cleptoparásitas o especies cuyas larvas tienen un comportamiento saprófago. Este "efecto filtro" (*filter effect*) debido a la disminución de la cantidad trófica, está confirmado por los resultados de diversidad funcional, en los cuales se registró una disminución en el número de nichos funcionales y una fuerte redundancia por la presencia de nichos funcionales muy similares entre ellos. Los efectos sobre la comunidad de escarabajos coprófagos en los sitios con baja carga ganadera reflejan finalmente una pérdida en la capacidad de enterramiento del estiércol del 30-40% respecto a las comunidades en áreas con una mayor carga ganadera.

Los resultados de este trabajo muestran que en las áreas con abandono total de la ganadería se observó una disminución en el número de especies, en la abundancia y biomasa total, en el número de especies indicadoras, en la abundancia de las especies de gran tamaño y en las especies que no tienen un comportamiento de nidificación durante el periodo reproductivo. Sin embargo, el mantenimiento de una comunidad bien estructurada, subraya la importancia de dos factores locales: el excremento de la fauna silvestre y la mayor complejidad del hábitat debido a un proceso de invasión de árboles en el pasto. Este último factor es probablemente responsable de la presencia de especies indicadoras típicas de hábitats más cerrados como los bosques o matorrales. En cuanto a los resultados de diversidad funcional, estos confirman la interpretación del "efecto filtro" debido a la disminución en la cantidad de recurso trófico. En efecto, estos resultados muestran una unicidad funcional significativa en esta comunidad como consecuencia del fuerte cambio composicional que el abandono de la ganadería ha implicado, a pesar de una disminución en el número de nichos funcionales y en el mantenimiento de una buena estructura funcional. Así mismo, se registró una disminución en la capacidad de enterramiento del estiércol entre 27% y 47% respecto a las áreas con baja carga ganadera y con carga moderada por todos estos cambios cuali y cuantitativos en la comunidad de escarabajos coprófagos de los sitios abandonados.

Con respecto a los patrones de diversidad funcional y la capacidad de remoción del estiércol, los resultados no permiten generalizar el hecho de que la diversidad funcional sea el motor principal de este proceso ecológico en los escarabajos coprófagos. Esto se debe a que también comunidades con una buena estructura funcional (áreas con abandono de la ganadería y áreas con uso histórico de productos veterinarios) mostraron una tasa de enterramiento disminuida.

En conclusión, este trabajo corrobora la importancia que tiene el mantenimiento de la ganadería tradicional. Por ello, con el propósito de conservar comunidades con una alta diversidad y para mantener un elevado nivel de capacidad en la remoción del estiércol es importante la implementación de medidas en el manejo de la ganadería. Una forma correcta de gestión puede ser la ganadería ecológica, la cual permitiría mantener un nivel de carga moderado, así como el control en uso de compuestos médico veterinarios.

CHAPTER 1

General introduction and objectives

1.1. General introduction and objectives

The Mediterranean basin is one of the biodiversity hotspot of the world (Myers et al., 2000). This peculiarity derived from various factors such as geological history, biogeography, landscape complexity and human history (Blondel et al., 2010). Through landscape management by mean of fire-setting, clear-cutting, grazing by domestic livestock and ploughing, humans have designed the Mediterranean landscape for about 10,000 years (Braudel, 1985; Pons and Quézel, 1985; Butzer, 2005). Although some taxa have suffered negative consequences from these activities, the long term effects of land use practices produce a complex landscape that is characterized by a high biodiversity (Grove and Rackham, 2001).

Open grasslands and pastures (*saltus*) are among the richest habitat of Mediterranean landscape, and are important for several taxa such as plants (Wilson et al., 2012), invertebrates (Bourn and Thomas, 2002; Van Swaay, 2002) and vertebrates (Knopf and Samson, 1997). This habitat was mainly managed for domestic livestock grazing (Poux et al., 2009), which actions concur to maintain the habitat and its biodiversity (Dolek and Geyer, 2002). Although its key role in maintain biodiversity, this habitat become to suffer from about 1950' the effects of two antinomics processes: abandonment and intensification (Beaufoy et al., 1994; Donald et al., 2001). Abandonment of the traditional management practices such as extensive livestock grazing, is a complex process that start with a progressive extensification toward a total abandonment (Correia, 1993). This mainly occurs in the marginal areas such as mountainous and sub-mountainous, which are less productive (MacDonald et al., 2000). The grazing abandonment have some negative effects on grasslands biodiversity (Fadda et al., 2008; Pöyry et al. 2004), which depend on the tree and shrub encroachment that tend to homogenize the landscape (Eldridge et al., 2011), and to the loss of available energy from the system that was supplied by the livestock dung (Augustine and Frank, 2001). On the other part, the intensification of grazing management techniques is characterized by an overexploitation of pastures due to overgrazing (Papanastasis et al., 2002; Negro et al., 2011 and references therein), or by means of the abuse of Veterinary Medical products for the control of the livestock ecto- and endoparasites (Lumaret et al., 2012). In the last decades, this latter, showed a raising concern from scientific community due to its negative impact on the no target coprophilous fauna (Jacobs and Scholtz, 2015).

Dung beetle are among the most important insect group of pastureslands, and their label denotes a group of insect (Coleoptera, Scarabaeoidea) that share the same trophic niche, the coprophagy, and belongs to the subfamilies Aphodiinae, Scarabaeinae and Geotrupinae (Halffter and Matthews, 1966; Halffter and Edmonds, 1982).

Dung feeding behavior probably arose from saprophagy (Halffter, 1959; Cambefort, 1991). However, the time of the origin of this new trait is not definite (Halffter and Matthews, 1966; Krell, 2006; Gunter et al., 2016; Ahrens et al., 2014; Arillo and Ortuño, 2008; Chin and Gill, 1996; Sánchez et al., 2010; Sánchez and Genise, 2009; Philips, 2011). Indeed, even if several studies highlight that coprophagy developed mainly as a consequences of Tertiary mammal radiations (Scholtz and Chown, 1995; Ahrens et al., 2014; Arillo and Ortuño, 2008 and references therein), dinosaur's coprolites with dung beetle activity (Chin and Gill, 1996) and molecular data (Gunter et al., 2016), bring into question this hypothesis, advancing the idea that coprophagy already existed during the Cretaceous.

Despite the doubt about the origins of coprophagy, several certainty exists on the fact that the use on this trophic resource forced the evolution of this insect group (Halffter and Matthews, 1966; Halffter and Edmonds, 1982; Ahrens et al., 2014; Gunter et al., 2016). Indeed, dung is a very nutritional rich resource (Hanski, 1987; Holter, 2016) but which availability is very ephemeral in the time and in the space (Halffter and Matthews, 1966; Halffter and Edmonds, 1982; Hanski and Cambefort, 1991). This lead to mouthpart modification, develop of complex nesting behavior and phenological patterns (Halffter and Matthews, 1966; Halffter and Edmonds, 1982; Hanski and Cambefort, 1991).

Nesting behavior is one of the prominent features of dung beetles, that lead to a functional classification of the same. Dung beetles can be categorized into different functional groups based on their differential use of trophic resources during nesting (Bornemissza, 1976; Halffter and Matthews, 1966). Firstly, we can distinguish two main strategies: a) direct and immediate use of trophic resources without nest construction, and b) relocation – or at least manipulation – behavior with nest construction. In the first strategy, eggs are laid directly in the excrement, where, in general, the entire development process takes place (functional group: no nesting). The second strategy involves some nesting behavior, and larvae develop within brood mass or brood balls. We can discern three main classes of tactics (Halffter and Matthews, 1966; Bornemissza 1969, 1971; Halffter and Edmonds, 1982; Zunino and Palestrini, 1986; Zunino, 1991): a) endocoprid, involving the manipulation of the trophic resource, without its relocation. Eggs are laid in brood balls that remain within food source; b) paracoprid: eggs are laid in brood masses that adults previously buried in the soil under the trophic resource; c) telecoprid: eggs and larvae develop within brood balls or masses previously transported and buried some distance from the food source.

This strong relationship among excrement and dung beetle, involve them on many ecological processes related to the dung removing from the soil surface (Halffter and Matthews, 1966; Nichols et al., 2008). Indeed, they are directly or indirectly implicated in:

a) Nutrient cycle: a great part of the nutrient eaten by vertebrates was voided through the excrement (Steinfeld et al., 2006), with defecation rates that, in mammals, can reach the 40% of the ingestion rate (Blueweiss et al., 1978). Dung relocation underground, form part of the cycle that move organic matter, nutrients and energy. For example, nitrogen is mainly lost from livestock dung through volatilization (Steinfeld et al., 2006). By dung burial, dung beetle prevents nitrogen loss and increase soil fertility (Gillard, 1967; Yokoyama et al., 1991). In addition to the nitrogen, several authors highlight the importance of dung beetle activity in the increasing of several nutrients into the soils (P, K, Ca, Mg), as well as in the cation exchange capacity (Bertone et al., 2006; Galbiati et al., 1995; Lastro, 2006; Yamada et al., 2007).
b) Vegetation growth: several authors highlight the correlation between dung beetle activity and the increasing in vegetal biomass (Bornemissza and Williams, 1970; Borghesio, 1999; Bang et al., 2005; Lastro, 2006), plant height (Galbiati et al., 1995; Kabir et al., 1985), nitrogen and proteins contents (Bang et al., 2005; Macqueen and Beirne 1975).
c) Secondary seed dispersal: although dung beetle not use seed as trophic resource, during dung relocation they can disperse until 95% of the seed presents into the dung (Feer, 1999; Andresen and Levey, 2004; Andresen, 2002; Shepherd and Chapman, 1998). Moreover, this seed dispersal has a positive effect on the

germination (Feer, 1999; Andresen and Levey, 2004; Andresen, 1999, 2001; Shepherd and Chapman, 1998; Chambers and MacMahon, 1994; Estrada and Coates-Estrada, 1991; Andresen and Feer, 2005).

d) Diptera and parasites control: dung is a key resource for Diptera, Nematoda and Protozoa. By mean of their trophic and reproductive activity, dung beetles damages directly or indirectly this organisms. Several studies highlight the inverse relationship among the density of dung beetle, and that of parasites (Halffter and Matthews, 1966; Bryan, 1976; Fincher, 1973, 1975; Bergstrom, 1983; Miller et al., 1961; Mathison and Ditrich, 1999; Bishop et al., 2005; Bornemissza, 1970; Kühne, 1996).

e) Greenhouse gasses emission control: livestock production is among the major contributor of greenhouse gasses, due to enteric fermentation and fluxes from manure of dairy and beef (Tubiello et al., 2013). Dung beetle play an important role in reducing greenhouse gasses emission through the aeration and burial of dung pats (Penttilä et al., 2013). This effect was mainly related to the large reduction of CH_4, that dung beetle reduces digging holes in the dung pat, increase the availability of oxygen, increase aerobic decomposition, decrease anaerobic decomposition and reduce the methanogenesis (Penttilä et al., 2013; Slade et al., 2016; Hammer et al., 2016).

f) Soil characteristics improvement: during nesting, tunneler dung beetle remove a large quantities of soil, and produces tunnels until one meter of depth. This activity improves soil characteristics by increasing soil aeration, porosity, water infiltration and reducing bulk density (Halffter and Matthews, 1966; Bang et al., 2005; Brown et al., 2010).

The performance of this ecological processes are related to the dung beetle community attributes (abundance, biomass, species richness, Shannon diversity, species composition etc) and several studies have linked this metrics to the processes (Kudavidanage et al., 2012; Giraldo et al., 2011; Tyndale-Biscoe, 1994; Tixier et al., 2015; Yamada et al., 2007; Larsen et al., 2005; Braga et al., 2013, Slade et al., 2007, Nervo et al., 2014; Ortega-Martínez et al., 2016; Gregory et al., 2015; Gollan et al., 2013; Beynon et al., 2012; Kaartinen et al., 2013; Griffiths et al., 2015). However, in the last years, a new line of research, assert that ecological processes mostly depend on the functional diversity of the community, i.e. the type, quantity and relative abundance of 'functional traits' presents in the community (Díaz and Cabido, 2001; Díaz et al., 2007; Violle et al., 2007). Nevertheless, to our knowledge, the links between functional diversity and ecological processes was tested for dung beetles only by Griffiths et al., (2015).

Hence, the objectives of the present work were:

1) Investigate the effects of grazing intensity, historical use of veterinary medical products and their interactions, on dung beetle community attributes as: abundance, biomass, alpha diversity and species composition;
2) Investigate the effects of progressive grazing abandonment on dung beetle abundance, biomass, biomass classes, functional group, alpha diversity and species composition;
3) Investigate the effects of grazing intensity, historical use of veterinary medical products and their interactions, on dung beetle community from a functional standpoint, analyzing the functional diversity patterns and their relationship with

classical biodiversity analysis, and with the ecological process (dung burial capacity);

4) Investigate the effects of progressive grazing abandonment on dung beetle community from a functional standpoint, analyzing the functional diversity patterns and their relationship with classical biodiversity analysis, and with the ecological process (dung burial capacity).

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CHAPTER 2

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 components, 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

Traditional grazing, Organic farming, Livestock management, Ivermectin, Scarabaeidae

2.1. 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 and Rodriguez-Menendez, 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). 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 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.

2.2. 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 mm, with two dry periods, one in summer and another in winter (www.lavalledelmetauro.it/contenuti/geologia-clima/indice.html). 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 different areas 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' areas – 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-Brometum erecti* and *Festuco circummediterraneae-Arrhenatheretum elatioris* associations. These grasslands are mainly used by horses that were abandoned and have reverted to a wild state. The grazing intensity of these pastures is around 0.7 units of livestock/ha. The most common wood species are: *Fraxinus ornus* L., *Ostrya carpinifolia* Scop., *Quercus ilex* L., *Quercus pubescens* Willd., *Acer opalus* (Miller), *Pinus nigra* J.F. Arnold, *Crataegus monogyna* Jacq., *Juniperus oxycedrus* L., *Lonicera etrusca* G. Santi, *Spartium junceum* L., and *Rosa canina* L.

B) 'Moderate grazing, VMP-free' areas – 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* L., *Quercus petraea* (Matt.) Liebl., *Carpinus betulus*

L., *Ostrya carpinifolia*, *Fraxinus ornus* L., *Acer opalus*, *Quercus ilex*, *Sorbus domestica* L., *Corylus avellana* L. and *Fagus sylvatica* L.

C) 'Moderate grazing with VMPs' areas – 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* Huds., *Briza media* L., *Filipendula vulgaris* Moench, *Cyanus triumfettii* (All.) Dostál ex Á.Löve, *Plantago lanceolata* subsp. *lanceolata* (Mert. & Koch), *Luzula campestris* (L.) DC., *Scorzoneroides cichoriacea* (Ten.) Greuter, *Cynosurus cristatus* L., *Anthoxanthum odoratum* L. and *Carex caryophyllea* Latourr. The tree 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' areas – 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*, *Cyanus triumfettii*, *Plantago lanceolata* subsp. *lanceolata*, *Luzula campestris*, *Scorzoneroides cichoriacea*, *Cynosurus cristatus*, *Anthoxanthum 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* (L., 1758) and *Sus scrofa* L., 1758) is very similar among all the studied areas (M. Tonelli, 2013 personal observation).

In the areas with VMPs use, the farmers' interviews (M. Tonelli, 2013, unpublished data) highlighted that the VMPs have been use since 1990s until today. The main veterinary formulations that are used are based on Ivermectin and Pyrantel pamoate. The main preventive treatments are administrated in spring and in autumn but the data of application vary between each farmer. Moreover, additional treatments are applied as many times as there are parasitic stress. In the LGVMP areas, VMPs are only given to foals and adult animals with evident parasitic stress, but have a very intense historical use of VMPs.

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 quadrate 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; Dormont et al., 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 to specific level (see Supplemental Information 2, for more details).

Sampling completeness

The inventory completeness was evaluated using a sample coverage analysis (Chao and 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 and Jost, 2012). Measurements were taken using iNext v.1.0 (Hsieh et al., 2013).

Alpha diversity

Alfa diversity was calculated using the Hill numbers' family diversity (MacArthur, 1965; Hill, 1973; Jost, 2006, 2007) (see Supplemental Information 2, for more details). 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 (each order q separately) using a full factorial 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 posthoc 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 (see Supplemental Information 2, for more details). To calculate the total biomass of the dung beetle at each treatment, we multiplied the average biomass 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 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) (see Supplemental Information 2, for more detail on C_{qN} measures). 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 (McGeoch et al., 2002; Verdú et al., 2011). 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).

2.3. Results

A total of 148,668 individuals belonging to 57 species of dung beetle were collected (38 Aphodiidae, 16 Scarabaeidae, 3 Geotrupidae). This breaks down into: 122,611 specimens belonging to 42 species for the low grazing treatment (25 Aphodiidae, 15 Scarabaeidae, 2 Geotrupidae); 26,057 individuals belonging to 54 species for the moderate grazing treatment (35 Aphodiidae, 16 Scarabaeidae, 3 Geotrupidae); 128,616 specimens from 53 species for the VMP-free treatment (35 Aphodiidae, 16 Scarabaeidae, 2 Geotrupidae); and 20,052 individuals belonging to 41 species for the VMP-use condition (24 Aphodiidae, 14 Scarabaeidae, 3 Geotrupidae) (Supplemental Information 1).

The sample coverage estimator revealed that our inventories were 99% complete for each treatment (Supplemental Information 1). 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

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 for all *q* order (${}^{0}D$: $F_{[1,8]} = 62.227$, $P < 0.0001$; ${}^{1}D$: $F_{[1,8]} = 48.602$, $P < 0.0005$; ${}^{2}D$: $F_{[1,8]} = 34.131$, $P < 0.0005$), with Moderate grazing that have higher equivalent number of species (post-hoc Tukey test ${}^{0}D$: $P < 0.0005$; ${}^{1}D$: $P < 0.0005$; ${}^{2}D$: $P < 0.001$) (Fig. 1). For VMP use factor significant difference exist only for ${}^{0}D$ ($F_{[1,8]} = 242.23$, $P < 0.00001$) whereas no significant difference exist for ${}^{1}D$ ($\mathrm{F}_{[1,8]} = 0.062$, $P = 0.81$) and ${}^{2}D$ ($F_{[1,8]} = 0.041$, $P = 0.85$). Post-hoc Tukey test show that VMP free areas have more equivalent species that VMP use areas for ${}^{0}D$ ($P < 0.0005$) but not for ${}^{1}D$ ($P = 0.81$) and ${}^{2}D$ ($P = 0.85$). A small significant interaction between the two factors was identified only for ${}^{0}D$ ($F_{[1,8]} = 5.5$, $P = 0.047$), with post-hoc Tukey test that show significant difference between all experimental groups, with the MGECO areas having 1.11 equivalent species more than the LGECO sites, 1.34 more than MGVMP areas and 1.86 more than the LGVMP sites. Sites with LGECO had 1.21 equivalent species more than the MGVMP areas and 1.66 more than the LGVMP sites. The areas with MGVMP had 1.38 equivalent species more than LGVMP sites. No significant interaction between the two factors exists for ${}^{1}D$ ($F_{[1,8]} = 1.82$, $P = 0.214$) and ${}^{2}D$ ($F_{[1,8]} = 0.86$, $P = 0.381$), with post-hoc Tukey test that showed statistical differences only between MGECO areas and LGVMP and LGECO areas, whereas MGVMP had significantly more equivalent species than those of LGVMP and LGECO areas.

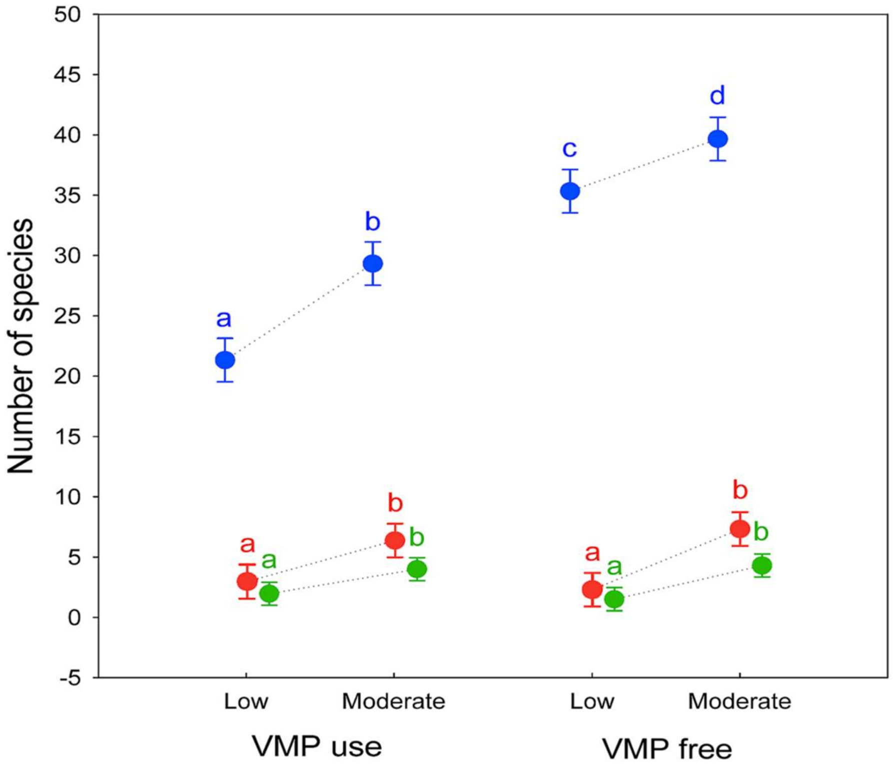

Figure 1: Alpha diversity of dung beetles using Hill numbers for different grazing intensity levels (low and moderate) and Veterinary Medical Products use (VMP use and VMP free) in sub-mountainous landscapes of Central Italy. ^{0}D (blue) correspond to species richness; ^{1}D (red) and ^{2}D (green) 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 $P < 0.05$).

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* (L., 1758) is an indicator of the VMP-free and low grazing sites, and *Onthophagus taurus* (Schreber, 1759) of the VMP-free and moderate grazing treatments.

Table 1: Dung beetle indicators of different livestock grazing management approaches. The numbers represent 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**
Aph	*Aphodius fimetarius* (Linnaeus, 1758)			90.5	
Aph	*Chilothorax conspurcatus* (Linnaeus, 1758)	93.7		95.9	
Aph	*Melinopterus consputus* (Creutzer, 1799)	97.3			
Aph	*Bodilopsis rufa* (Moll, 1782)		97.4		
Aph	*Calamosternus granarius* (Linnaeus, 1767)		83.3		
Aph	*Labarrus lividus* (Olivier, 1789)		76.4		
Aph	*Melinopterus prodromus* (Brahm, 1790)			99.7	
Aph	*Acanthobodilus immundus* (Creutzer, 1799)			76.1	
Aph	*Nimbus johnsoni* (Baraud, 1976)			79.4	
Aph	*Acrossus luridus* (Fabricius, 1775)			96	
Aph	*Aphodius foetidus* (Herbst, 1783)			83.3	
Aph	*Loraphodius suarius* (Faldermann, 1835)			90.4	
Aph	*Otophorus haemorrhoidalis* (Linnaeus, 1758)		100		
Aph	*Sigorus porcus* (Fabricius, 1792)	75.6			
Sca	*Onthophagus fracticornis* (Preyssler, 1790)		84.3		
Sca	*Onthophagus ruficapillus* Brullé, 1832			80.6	
Sca	*Onthophagus taurus* (Schreber, 1759)		91.3	89.8	
Sca	*Onthophagus coenobita* (Herbst, 1783)			91.3	
Sca	*Onthophagus opacicollis* Reitter, 1892			100	
Sca	*Bubas bison* (Linnaeus, 1767)			97.2	
Sca	*Copris lunaris* (Linnaeus, 1758)		87		
Geo	*Sericotrupes niger* (Marsham, 1802)			90.5	

Biomass and abundance of dung beetles

Significant differences in dung beetle biomass and abundance were obtained for the grazing intensity (*Wilks's lambda* = 0.138; $F_{[2,7]} = 21.87$; $P < 0.01$) and use of VMPs factors (*Wilks's lambda* = 0.17; $F_{[2,7]}=17.34$; $P < 0.05$) (Fig. 2). However, no differences were found in their interactions (*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.

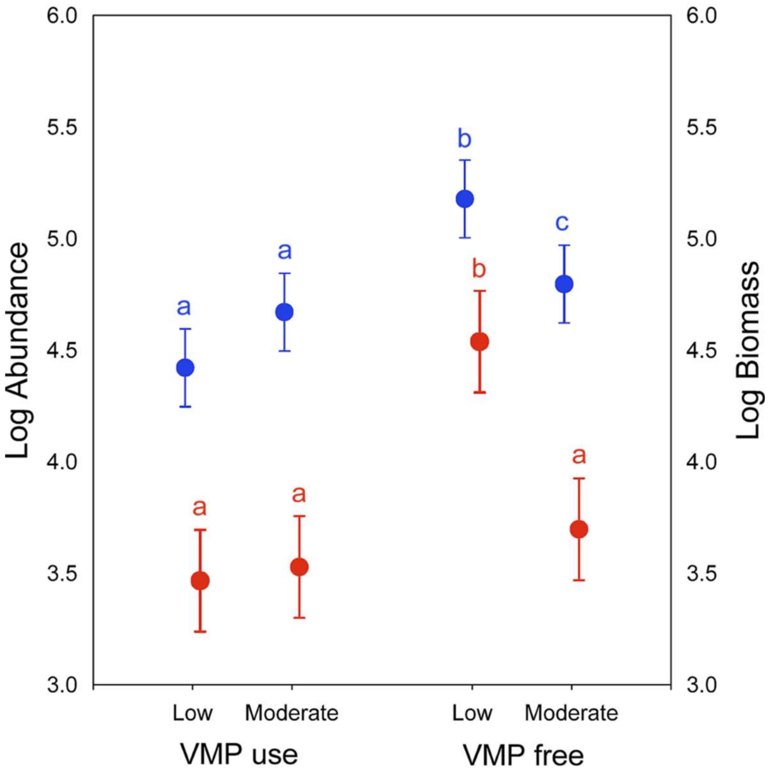

Figure 2: Dung beetle biomass (blue) and abundance (red) for different grazing intensity levels (low and moderate) and Veterinary Medical Products use (VMP use and VMP free) in sub-mountainous landscapes of Central Italy. Dots represents mean and bars represent standard errors. Different letters mean significant differences (post-hoc Tukey test $P < 0.05$).

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 (Fig. 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).

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			

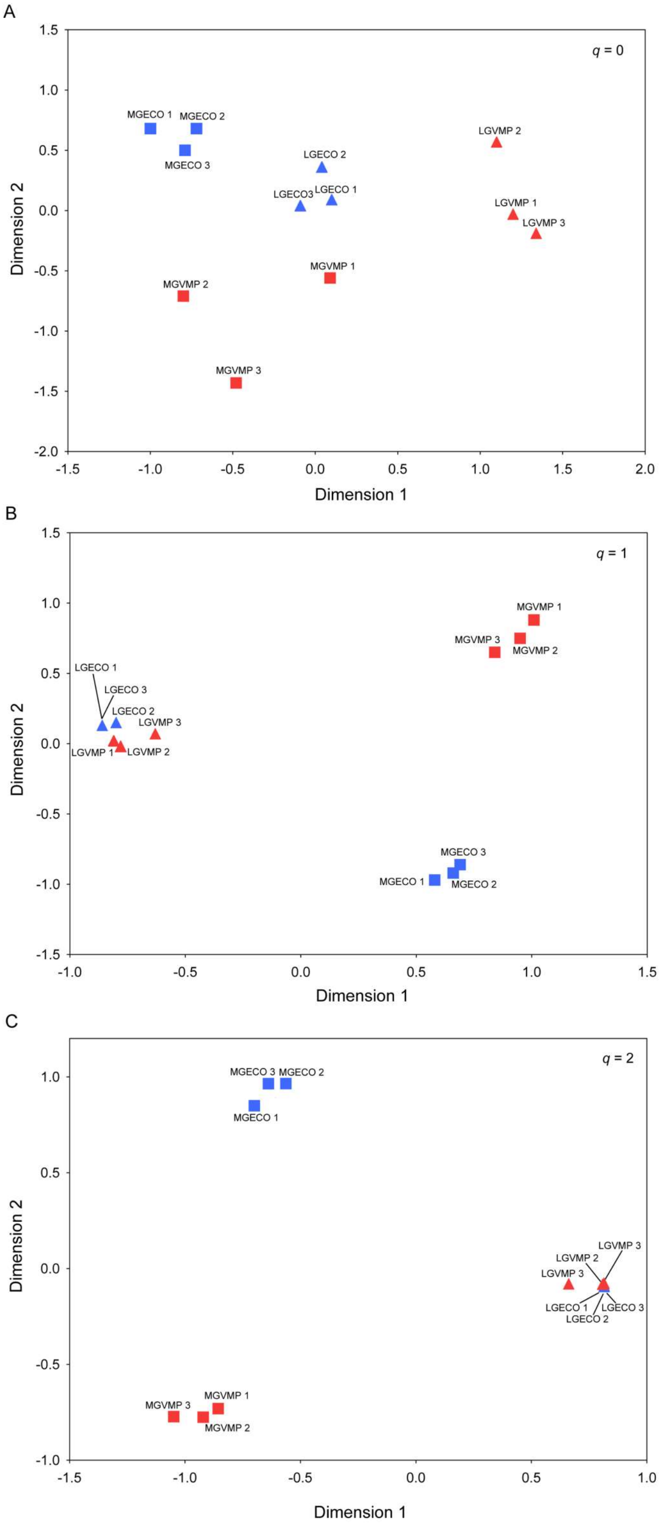

Figure 3: Multiple community similarity using Non-Metric Multidimensional Scaling (NMDS) ordination: A) generalised Sørensen index (C_{0N}): average proportion of shared species in each assemblage based on the incidence data; B) Horn entropy index (C_{1N}): proportion of shared species in an assemblage 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.

2.4. Discussion

Grazing intensity effects on dung beetle diversity

Our results support the hypothesis that a low grazing intensity have a negative effect on dung beetle diversity. Total domestic grazing abandonment is a know negative factor for dung beetle conservation (Jay-Robert et al., 2008; Verdú et al., 2000). However, our results highlighted that even a simple reduction in grazing intensity implies negative effects on dung beetle community in areas with a long grazing history such as the Mediterranean Region. Indeed, the areas with a moderate grazing intensity showed more alpha diversity than the low grazing intensity sites. Our results are consistent with those of other studies in other Mediterranean locations. For example, 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. In Southern France, Lumaret et al. (1992) explained that an increase of 260% in fresh dung availability, five years after a change of pasture management (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.

Furthermore, 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 the moderate grazing sites studied, three of the seven indicator species are paracoprids and, among them, one, *Copris lunaris* (L., 1758), is a large species. During breeding, *Copris lunaris* may bury about 100-165 g of dung (Klemperer, 1982; Martín-Piera and López-Colón, 2000). Thus, there is 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 beetle, as *C. lunaris*, can only to maintain well established populations 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). For example, 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 the low grazing areas studied have greater biomass and abundance. This may be explained by the dominance of two species, *Melinopterus consputus* (Creutzer, 1799) and *Onthophagus medius* (Kugelann, 1792). 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 (such as *M. consputus*) and highly competitive species such as small tunnellers (e.g. *O. medius*) (Horgan and Fuentes, 2005). The low grazed sites studied, 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* (Baraud, 1976)).

In terms of species composition of assemblages, beta diversity was strongly influenced by the quantity of the trophic resource at all *q* levels (Table 3); rare and abundant species were compositionally different between the assemblages obtained in the different grazing intensity treatments. These results implicate that grazing extensification lead to a change in dung beetle composition favouring more opportunistic species. This explanation is corroborated by the presence of three indicators species (*Melinopterus consputus*, *Chilothorax conspurcatus* and *Sigorus porcus*) characteristics of the low grazing areas that share an opportunistic behaviour. *Melinopterus consputus* and *C. conspurcatus* are dependent on the dung only during adult stage, whereas during larvae phase are saprophagous mainly (Verdú J.R. pers. observations); *Sigorus porcus* have a strong attitude to kleptoparasitism during both adult and larval stages (Dellacasa and Dellacasa, 2006).

Thus, dung beetles are strongly dependent to dung during their life cycle and our data support the hypothesis that even a simple reduction of its availability may have negative effects on the community. Less trophic resource availability lead to a compositional and structural impoverishment of the community with a loss of large body sizes dung beetles in favour of more opportunistic ones. Then, the fact that Mediterranean pastures suffer a continuous process of extensification, can be a factor of concern for the dung beetle conservation.

VMPs use effects on dung beetle community

Our results supported the hypothesis that the historical use of VMP substances have a negative effect on dung beetle diversity. The negative effect of VMP substances was relevant to all community parameters measured, such as alpha diversity, biomass, abundance, presence of indicator species and beta diversity. It has been documented that VMP-use shows 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; Lumaret et al., 2012; Wall and Beynon, 2012; Jacobs and Scholtz, 2015). Many essays show that VMPs negatively affect larval and adult survival of dung beetles, as well as some physiological processes such as reproductive, sensorial and locomotor capacities showing even negative repercussions in the dung decomposition (Wall and Strong, 1987; Lumaret et al., 1993; Wardhaugh et al., 2001; Verdú et al., 2015).

Here, we document that VMP-use sites studied showed 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. In South Africa, Krüger and Scholtz (1998) also showed that, under drought conditions, treatment with ivermectin led to a loss of dung beetle species. Beynon et al. (2012b) showed a reduction in dung beetle abundance and biomass in dung treated with ivermectin in the UK.

Unlike some studies (Krüger and Scholtz, 1998; Basto-Estrella et al., 2014; Hutton and Giller, 2003), we did not find a significant difference in ^{1}D ('common species number') and ^{2}D ('dominant species number') for the VMP-use factor. Give that macrocyclic lactones as ivermectin acting on a family of ligand-gated chloride channels gated by glutamate, which is shared by all Ecdysozoan (Geary and Moreno, 2012; Puniamoorthy et al., 2014), all dung beetles species should be sensible to ivermectin toxicity. Thus, the consequences on the assemblage structure may be differential based on the abundances of each species in each assemblage. Our data showed that less common species are first in disappearing in sites characterized by VMPs use, which explains the significant reduction in the number of species observed in these sites. At $q = 1$ and $q = 2$, however, differences are not observed between both treatments, so the reduction of the populations of the most common and dominant species took place of equitable way, which maintains similar measures of community structure (^{1}D and ^{2}D).

Accordingly to alpha diversity results, beta diversity was 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 more common and dominant species are not significantly different.

Our IndVal results showed how the VMP-use treatments have no indicator species. This means that no species were favoured by the use of these veterinary substances. In other words, the use of VMPs could affect all species and, apparently, no species could be resistant to VMP toxicity. These results agree with the explained above about diversity measures. In contrast, the VMP-free treatment had 14 indicator species.

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

Grazing intensity and VMPs interactions

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. Another explanation can be found in the halving of the sample size during the interaction analyses. This means that interactions between the two factors may have antagonistic effects on dung beetle assemblages, but more studies with greater sample size are needed on this issue.

Conclusions

The present analysis highlighted that the moderate grazing VMP-free treatment seems to be the more appropriate management system for maintaining a higher number of dung beetle species, as well as greater diversity and biomass. These results corroborated 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 diversity (Verdú et al., 2000). Furthermore, our results corroborated the hypothesis that both factors - low grazing intensity and VMP-use - have negative effects on dung beetle communities. Even a simple grazing intensity extensification may have negative impact on dung beetle, that is reflected in the compositional and structural impoverishment of the community. Our study strengthens the results about the environmental risk assessment made by Liebig et al. (2010) that concluded that the ivermectin use have an “unacceptable risk” for dung beetle fauna.

The results could have an application for sustainable farmland management, highlighting that an incorrect grazing management of the pastures could be a strong effect on dung beetle community (e.g. number of species, biomass, composition), and so in the correct function of ecosystem processes performed by dung beetle as nutrient cycles, vegetation development, secondary seed dispersion, and parasite control (Nichols et al., 2008; Nervo et al., 2014; Beynon et al., 2012a; Larsen et al., 2005). Then, 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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Supplementary Material S1. Number of individuals of dung beetle species collected from each treatment of the two factors. For each treatment, inventory completeness was also reported according to the INext estimators. LG: low grazing; MG: moderate grazing; ECO: VMP-free; VMP: with the use of VMPs.

Family	Species	ECO	VMP	LG	MG	Total
Aph	*Acanthobodilus immundus* (Creutzer, 1799)	21	2	4	19	23
Aph	*Acrossus luridus* (Fabricius, 1775)	217	9	170	56	226
Aph	*Acrossus rufipes* (Linnaeus, 1758)	0	4	0	4	4
Aph	*Agrilinus constans* (Duftschmid, 1805)	1	0	0	1	1
Aph	*Agrilinus convexus* (Erichson, 1848)	23	0	0	23	23
Aph	*Aphodius coniugatus* (Panzer, 1795)	4	2	5	1	6
Aph	*Aphodius fimetarius* (Linnaeus, 1758)	124	13	119	18	137
Aph	*Aphodius foetidus* (Herbst, 1783)	9	0	3	6	9
Aph	*Biralus mahunkaorum* (Ádám, 1983)	0	1	1	0	1
Aph	*Bodilopsis rufa* (Moll, 1782)	42	779	21	800	821
Aph	*Bodiloides ictericus* (Laicharting, 1781)	11	0	0	11	11
Aph	*Calamosternus granarius* (Linnaeus, 1767)	20	2	0	22	22
Aph	*Calamosternus mayeri* (Pilleri, 1953)	1	0	1	0	1
Aph	*Chilothorax conspurcatus* (Linnaeus, 1758)	749	32	732	49	781
Aph	*Chilothorax lineolatus* (Illiger, 1803)	3	0	2	1	3
Aph	*Chilothorax paykulli* (Bedel, 1907)	53	0	0	53	53
Aph	*Colobopterus erraticus* (Linnaeus, 1758)	1005	1964	1706	1263	2969
Aph	*Coprimorphus scrutator* (Herbst, 1789)	6	167	3	170	173
Aph	*Esymus merdarius* (Fabricius, 1775)	103	3	102	4	106
Aph	*Esymus pusillus* (Herbst, 1789)	19	8	14	13	27
Aph	*Eurodalus paracoenosus* (Balthasar & Hrubant, 1960)	2	0	0	2	2
Aph	*Labarrus lividus* (Olivier, 1789)	219	310	125	404	529
Aph	*Limarus zenkeri* (Germar, 1813)	2	0	0	2	2
Aph	*Loraphodius suarius* (Faldermann, 1835)	47	5	34	18	52
Aph	*Melinopterus consputus* (Creutzer, 1799)	94951	6449	98709	2691	101400
Aph	*Melinopterus prodromus* (Brahm, 1790)	7289	20	1135	6174	7309
Aph	*Melinopterus reyi* (Reitter, 1892)	16	0	0	16	16
Aph	*Melinopterus stolzi* (Reitter, 1906)	2	0	0	2	2
Aph	*Nialus varians* (Duftschmid, 1805)	9	0	0	9	9
Aph	*Nimbus contaminatus* (Herbst, 1783)	464	341	470	335	805
Aph	*Nimbus johnsoni* (Baraud, 1976)	20	1	7	14	21
Aph	*Nimbus obliteratus* (Panzer, 1823)	2309	260	2170	399	2569
Aph	*Otophorus haemorrhoidalis* (Linnaeus, 1758)	21	39	0	60	60
Aph	*Phalacronothus biguttatus* (Germar, 1824)	4	0	1	3	4
Aph	*Planolinus fasciatus* (Olivier, 1789)	1	0	1	0	1
Aph	*Sigorus porcus* (Fabricius, 1792)	234	102	254	82	336
Aph	*Teuchestes fossor* (Linnaeus, 1758)	0	11	0	11	11
Aph	*Trichonotulus scrofa* (Fabricius, 1787)	192	54	192	54	246
Sca	*Bubas bison* (Linnaeus, 1767)	103	3	14	92	106
Sca	*Caccobius schreberi* (Linnaeus, 1767)	5	8	2	11	13
Sca	*Copris lunaris* (Linnaeus, 1758)	15	8	3	20	23
Sca	*Euoniticellus fulvus* (Goeze, 1777)	4955	2401	4359	2997	7356
Sca	*Onthophagus coenobita* (Herbst, 1783)	168	16	36	148	184
Sca	*Onthophagus fracticornis* (Preyssler, 1790)	3157	5881	1418	7620	9038
Sca	*Onthophagus grossepunctatus* Reitter, 1905	110	0	0	110	110
Sca	*Onthophagus illyricus* (Scopoli, 1763)	1	1	1	1	2
Sca	*Onthophagus joannae* Goljan, 1953	26	7	31	2	33
Sca	*Onthophagus lemur* (Fabricius, 1781)	324	66	74	316	390
Sca	*Onthophagus medius* (Kugelann, 1792)	10151	666	10216	601	10817
Sca	*Onthophagus opacicollis* Reitter, 1892	36	0	12	24	36

Sca	*Onthophagus ruficapillus* Brullé, 1832	178	6	7	177	184
Sca	*Onthophagus taurus* (Schreber, 1759)	710	81	69	722	791
Sca	*Onthophagus verticicornis* (Laicharting, 1781)	122	165	149	138	287
Sca	*Sisyphus schaefferi* (Linnaeus, 1758)	10	9	11	8	19
Geo	*Sericotrupes niger* (Marsham, 1802)	229	24	75	178	253
Geo	*Geotrupes spiniger* Marsham, 1802	123	125	153	95	248
Geo	*Trypocopris vernalis apenninicus* Mariani, 1958	0	7	0	7	7
	Total species (S)	53	41	42	54	57
	Total individuals (N)	128616	20052	122611	26057	148668
	Sampling coberture (%)	99	99	100	99	

Supplementary Material S2. Supporting text to Materials and Methods topics.

Dung beetle identification

Scarabaeinae and Geotrupinae subfamilies have been identified according to Baraud (1992). Aphodiinae subfamily were identified following Dellacasa and Dellacasa (2006). *Biralus mahunkaorum* (Ádám, 1983), *Onthophagus medius* (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 Martín-Piera and Zunino (1986) based on the work of Binaghi et al. (1969).

Alpha diversity

The Hill numbers' family diversity are measures 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).

Dung beetle biomass and abundance

Fewer than 10 specimens of the following species 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); *Nialus varian*s (Duftschmid, 1805) (4); and *Amidorus thermicola* (Sturm, 1800) (7).

Beta diversity

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 ${}^{q}Gp/{}^{q}Gs$. 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.

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CHAPTER 3

Effects of progressive grazing abandonment on dung beetle biodiversity in a central Italian province: nesting behaviour and body size matters.

Tonelli, M., Verdú, J.R., Zunino, M., *submitted*. Effects of progressive grazing abandonment on dung beetle biodiversity in a central Italian province: nesting behaviour and body size matters. Biodiversity and Conservation

Abstract

Dung beetles are an essential group of insect species inhabiting semi-natural grasslands in Europe. Despite their ecological importance, some species face local threats mainly due to changes in land use practices such as the abandonment of pasturelands. The aim of this study was to analyze the impact of progressive abandonment of grazing lands comparing areas that represent a range of trophic resource availability: i) abandoned, ii) low and ii) moderate grazing intensity.

In order to analyze the effects of the abandonment of pasturelands we used: i) alpha diversity measures based on Hill numbers (q = 0, 1 and 2), ii) total dung beetle biomass and abundance, iii) dung beetle biomass between classes (small, medium, large), iv) dung beetle composition at intrahabitat and interhabitat levels using multiple assemblage abundance based overlap measures (C_{qN}) at different q levels (q = 0, 1 and 2), v) a IndVal analysis to determine the existence of species indicators of each grazing level and finally, vi) the impact on different functional groups was evaluated.

Pastureland abandonment induced a notable decrease in both alpha diversity and dung beetle biomass, up to -22% and -78% respectively. From a functional standpoint, the effects of grazing land abandonment varied according to the functional groups and biomass classes of the dung beetles, with non-nesting species and larger species proving more susceptible to local extinction. The presence of more and larger indicator species in the moderate grazing intensity pastures corroborates our results. Moreover, beta diversity was affected by grazing intensity. Dung beetle species found in abandoned sites were typical of shrub and forest habitats, which could be accounted for by an incipient transformation of the vegetation structure due to shrub and tree encroachment.

In order to preserve dung beetle communities, traditional pasturelands management with moderate intensity grazing should be maintained.

Keywords

Scarabaeoidea, grazing management, body size, functional group, alpha diversity, biomass.

3.1. Introduction

Dung beetles are an important group of insects in terms of diversity and biomass in grassland habitats in the Mediterranean Basin (Lumaret and Kirk, 1991). Using dung during feeding and nesting they are linked to numerous ecosystem services dependent on dung degradation and burying (Nichols et al., 2008). Dung removal from the pasture surface and its reincorporation into the soil supporting nutrient cycles (Bertone et al., 2006) improve the physical structure of the soil (Brown et al., 2010), reduce greenhouse gas emissions from dung (Slade et al., 2016) and the livestock's ecto and endoparasites (Bishop et al., 2005; Fincher, 1975) and improve secondary seed dispersal and germination (Andresen and Levey, 2004). Despite their ecological importance, dung beetles are globally threatened (Nichols et al., 2007; Kryger, 2009). In the Mediterranean Basin, 150 endemic dung beetle species have been reported, 14% of which (n = 21) are at risk of extinction (IUCN, 2016). The principal threats to dung beetles in the Mediterranean Basin include changes in land use, habitat degradation, urbanization, abuse of medical veterinary products used for livestock, and grazing land abandonment (IUCN, 2016). It is noteworthy that some dung beetle functional groups, namely rollers, appear to be more threatened than others (Lobo, 2001; Carpaneto et al., 2007) and that dung beetles with large body size seem to be more prone to extinction than their smaller counterparts (Larsen et al., 2005).

Grazing land abandonment is a key factor affecting dung beetle conservation. Due to their dependence on dung, pastureland abandonment leads to a loss of trophic resources with negative effects on dung beetle communities (Carpaneto et al., 2005). Dung beetles depended on wild megafauna excrement for millions of years (Ahrens et al., 2014), but as this resource diminished, domesticated livestock became the beetles' principal trophic resource (Barnosky, 2008; Sandom et al., 2014). Livestock grazing is one of the main forces that has shaped the Mediterranean environment (Blondel, 2006), transforming it into a heterogeneous and hyperdiverse area (Myers et al., 2000). However, after a period of approximately 10,000 years of traditional grazing, livestock management has changed dramatically over the last fifty years mainly-driven by the abandonment of pasturelands.

The abandonment of grazing lands is a process that mainly occurs in marginal areas (mountain and submountain areas), which are less productive than valleys. In Italy from 1982 to 2010, there was a 20% reduction in livestock (cows, sheep and horses), the number of farms fell by about 71% and the number of horses and sheep decreased by approximately12% and 24% in hill and mountain regions, respectively (ISTAT, 2010).

Grazing abandonment has been shown to have many negative effects on numerous taxa such as plants (Peco et al., 2006), birds (Suarez-Seoane et al., 2002), butterflies (Pöyry et al., 2004), gastropods (Baur et al., 2006) and Orthoptera (Marini et al., 2009). However, few studies have explicitly investigated its effects on dung beetle communities (but see: Jay-Robert et al., 2008; Carpaneto et al., 2005). In literature, there are some reports on the effects that different habitats, selected as 'successional stages after abandonment' have on dung beetle communities (Tocco et al., 2013; Macagno and Palestrini, 2009; Negro et al., 2011) and on temporal variation of trophic resource availability (Lumaret et al., 1992; Carpaneto et al., 2005). Even less is known about the synchronous effect of variations in livestock density as an indicator of the quantity of trophic resources (dung) (but see Jay-Robert et al., 2008; Kadiri et al., 1997).

The aim of this study was to investigate the effects of the progressive abandonment of grazing lands on dung beetle diversity in sub-mountainous grasslands in Central Italy. Comparing areas that represent a range of trophic resource availability (abandoned, low intensity grazing and moderate grazing), we attempted to answer the following questions: a) What is the effect of progressive grazing land abandonment on dung beetle alpha diversity? b) What is the impact of pastureland abandonment on dung beetle community biomass and abundance? Are changes in biomass and abundance responses to trophic resource availability? Are different biomass classes (from small to large) of dung beetles affected differently by grazing intensity? Are there indicator species of each particular grazing intensity? Does grazing intensity have differential effects on dung beetle functional group species richness and abundance? What are the effects of grazing intensity on dung beetle beta diversity within and between different grassland management systems? Our hypothesis is that progressive pastureland abandonment has negative effects on dung beetle diversity, resulting in changes in alpha diversity, beta diversity and biomass, while favouring the presence of some species that could act as indicators of a particular kind of pasture management. Moreover, we hypothesize that functional groups are affected differently according to their nesting behaviour.

3.2. Materials and Methods

Study area

The study was carried in sub-mountainous areas of the Pesaro-Urbino province in the Marche-region of Central Italy. The climate of the province falls into the temperate Köppen's categories (Cfa and Cfb). The average annual temperature is around 12 °C with an average minimum of around 3.5 °C in winter and average maximum of 21 °C in summer. Average annual precipitation is around 930 mm with two dry periods, one in summer and another in winter (www.lavalledelmetauro.it/contenuti/geologia-clima/indice.html). The soil is calcareous. The arboreous vegetation in the study sites is dominated by *Quercus ilex* L., *Quercus pubescens* Willd., *Quercus cerris* L., *Ostrya carpinifolia* Scop. and *Fraxinus ornus* L. whereas the herbaceous vegetation belongs to the *Brizo mediae-Brometum erecti* and to *Asperula purpureae-Brometum erecti* phyto-associations.

To evaluate the effect of progressive pastureland abandonment we compare sites with a range of grazing activity from abandoned to moderate grazing intensity:

a) 'Abandoned' (Calamello-Paravento pastures; 43°30'43,00''N; 12°40'58,68''E): These pastures, once used by cows and sheep, are located between 550 and 750 m a.s.l. and were abandoned about fifteen years ago. Today, these pastures are only used by wildlife fauna such as roe deer (*Capreolus capreolus* L., 1758), wild boar (*Sus scrofa* Linnaeus, 1758) and fallow deer (*Dama dama* L., 1758). Due to grazing abandonment, these pastures are undergoing a process of shrub and tree encroachment principally by *Quercus ilex* L., *Spartium junceum* L. and *Rosa canina* L. (Tonelli, M., personal observation, 2013).

b) ‘Low Intensity Grazing’ (Pietralata pastures; 43°39’33,64’’N; 12°42’27,65’’E): These secondary grasslands are located between 750 and 900 m a.s.l. and are used by approximately a forty head herd that were abandoned and have reverted to a wild state. The livestock density in this pasture is approximately 0.7 Livestock unit/ha.

c) ‘Moderate Intensity Grazing’ (Montebello pastures; 43°43’13.83’’N; 12°45’19.98’’E). These pastures, used by livestock bred according to organic farming guidelines, are located between 500 and 600 m a.s.l. on the ®Gino Girolomoni Cooperativa Agricola farmlands. The livestock density in this pasture is about 1.5 Livestock Unit/ha.

Sampling design and dung beetles trapping

We selected three sampling sites in each of the three areas and used standardized methodology to sample the dung beetles (Lobo et al., 1988). Four pitfall traps were used for each sampling site with at least 50 m between the traps (Larsen and Forsyth, 2005) and 500 m between the sampling sites in order to maintain their spatial independence and avoid pseudoreplication (Silva and Hernández, 2015b). At each sampling site, half of the traps were baited with cow dung while the other half was baited with horse dung to control for differential species attraction (Barbero et al., 1999; Dormont et al., 2004, 2007). In the abandoned area, we randomly placed about 10 dung pats in the area around the trapping site. This procedure is recommended to prevent the bias of excessive attractiveness of traps, due to the scarcity of trophic resources for dung beetles in the area (Lobo et al., 1998).

The dung used for the sampling was ivermectin free because this substance may alter dung attraction (Wardhaugh and Mahon, 1991; Holter et al., 1993; Floate, 2007; Errouissi and Lumaret, 2010; Webb et al., 2010). In order to preserve the insects, we filled the pitfall traps with propylene glycol (50%). During each sampling period the traps were left active for 48 h and samples were collected approximately every 15 days from June 2013 to November 2013 and in May and June 2014. Dung beetles were identified at species level according to Baraud (1992) for Scarabaeinae and Geotrupinae subfamilies. Aphodiinae were identified according to Dellacasa and Dellacasa (2006). Some cryptic species or difficult species complexes were identified referring to specific works (Rössner and Fery, 2014; Rössner et al., 2010; Miraldo et al., 2014; Martín-Piera and Zunino, 1986).

3.3. Data analysis

Sampling completeness

The inventory completeness was evaluated using a sample coverage analysis (Chao and Jost, 2012). It is a measure of sample completeness, giving the proportion of the total number of individuals in a community that belong to the species represented in the sample. Sample coverage can be estimated very accurately and efficiently using information contained in the sample itself (sample size, singletons and doubletons) and it allows us to compare different communities of equally complete sample coverage without any need for rarefaction (Chao and Jost, 2012) The iNext software v.1.0 was used for these analyses (Hsieh et al., 2013).

Alpha diversity

In order to characterize the alpha diversity of the areas we computed mean alpha diversity applying the formulas proposed by Jost (2006, 2007). This diversity measures are parameterized by the order q, which determines the sensitivity of the index to rare or abundant species (${}^{0}D$ = species richness; ${}^{1}D$ = exponential of Shannon entropy; ${}^{2}D$ =reciprocal of Simpson index). These measures make the results comparable because their units consist of "effective number of species" and conform with the replication principle introduced by Hill (1973). Alpha diversity was analyzed based on abundance and biomass data to evaluate their possible differential response to the trophic resource availability gradient (Nichols et al., 2007). The alpha diversity measures were performed with SpadeR online (Chao et al., 2015). Average alpha diversity was assessed with empirical data using Generalized Linear Models and performing a Tukey post hoc test for multiple pairwise comparisons using the Statistica 7.0 package (StatSoft, 2004). The P values were calculated using the Bonferroni correction in all cases.

Dung beetle abundance and biomass

We tested the statistical difference in dung beetle total abundance and biomass using a Generalized Linear Model with the Statistica 7.0 package (StatSoft, 2004) after log transformation of the dependent variable. Pairwise comparisons were made between the areas with different livestock density using the Tukey post hoc test. The P values were calculated using the Bonferroni correction in all cases.

The average biomass of each species was calculated using the formula Biomass = 0.010864 x Lenght$^{3.316}$ of Lobo (1993). To calculate the total biomass of dung beetles at each area we multiplied the average biomass of each species by the total number of specimens of the particular species that were collected and added together.

Furthermore, we analysed the distribution of biomass within three biomass classes according to parameters in literature (Campos and Hernández, 2013; Silva and Hernández, 2015a): Species with < 10 mg of biomass were classified as 'small species'; species between 10 – 100 mg were categorized as 'medium-sized species'; species more than 100 mg of biomass were considered 'large species'.

The Generalized Linear Model with Statistica 7.0 package (StatSoft, 2004) was used after log transformation of dependent variable. Multiple pairwise comparisons were made for each biomass class between different areas using the Tukey post hoc test. The *P* values were calculated using the Bonferroni correction in all cases.

Beta diversity

We analysed beta diversity within (intra habitat) and between (inter habitat) different grazing areas using the multiple assemblage abundance based overlap measure C_{qN} (Chao et al., 2008). This is a measure of the average percentage of overlapped species in a community, where q is a parameter that determines the measure's sensitivity to the species' relative abundance, and N is the number of assemblages (Chao et al., 2012; Jost et al., 2011). This measure ranges from 0 when all assemblages are completely different, and 1 when they are identical. Hence, its inverse is an estimate of beta diversity.

To estimate intra habitat beta diversity we calculated C_{qN} (for $q = 0, 1, 2$) among the replication of each area. C_{qN} indices and their 95% confidence intervals were performed with SpadeR (Chao et al., 2015), which use a bootstrap method based on 200 replications in order to estimate the confidence intervals.

To evaluate the effect of grazing intensity on dung beetle beta diversity (between treatment beta diversity) a Permanova test (Anderson, 2001) was applied to C_{qN} (with $q = 0, 1, 2$) similarity matrices. Similarity matrices were computed using SpadeR Online (Chao et al., 2015). The Permanova test was performed using Permanova+ add-on for Primer 7 (Anderson et al., 2008; Clarke and Gorley, 2015). 999 unrestricted permutations of raw data were computed.

Indval

The Indicator Value Method (Dufrêne and Legendre, 1997) was used to identify some indicator species of a particular grazing management. It combines the measurement of the degree of specificity (pattern of relative abundance) with the measurement of the degree of fidelity (pattern of incidence) of a given species to a given ecological status (McGeoch et al., 2002; McGeoch and Chown, 1998; Dufrêne and Legendre, 1997). IndVal results range from 0 (no indication) to 100 (perfect indication). Species may be categorized as indicator or detector species based on their IndVal value (Verdú et al., 2011). Species with significant ($P < 0.05$) results above 70% were considered as indicator species for the given ecological conditions. Species with intermediate IndVal, between 45% and 70%, were considered detector species. Analysis was performed using PC-Ord 4 (McCune and Mefford, 1999).

Functional group analysis

Dung beetles can be categorized into different functional groups based on their differential use of trophic resources during nidification (Bornemissza, 1976; Halffter and Matthews, 1966).

Firstly, we can distinguish two main strategies: a) direct and immediate use of trophic resources without nest construction, and b) relocation – or at least manipulation – behavior with nest construction. In the first strategy, eggs are laid directly in the excrement, where, in general, the entire development process takes place (functional group: no nesting). The second strategy involves some nesting behavior, and larvae develop within brood mass or brood balls. We can discern three main classes of tactics (Halffter and Matthews, 1966; Bornemissza, 1969, 1971; Halffter and Edmonds, 1982; Zunino and Palestrini, 1986; Zunino, 1991): a) endocoprid, involving the manipulation of the trophic resource, without its relocation. Eggs are laid in brood balls that remain within food source; b) paracoprid: eggs are laid in brood masses that adults previously buried in the soil under the trophic resource; c) telecoprid: eggs and larvae develop within brood balls transported and buried some distance from the food source.

We compared functional group species richness and biomass in the different grazing conditions using the GLM test and the Tukey post hoc test was performed for pairwise comparison using the Statistica 7.0 package (StatSoft, 2004). The biomass was Log transformed before the analysis was performed. Due to the presence of 0 in the biomass matrix of the telecoprid functional group, for this category we used the following formula to transform the data to Log data: x’=Log(x+1) (Podani, 2007).

3.4. Results

A total of 136,884 specimens belonging to 56 species of dung beetle were collected. 8,268 specimens belonging to 38 species were collected in the abandoned area, 113,650 specimens representing 41 species were collected in the low grazing area and 14,966 specimens belonging to 47 species were collected in the moderate grazing area.

The sample coverage estimator indicated that our inventories were 99% complete for each site. Hence, we can consider our empirical data to be complete and adequate for further analysis and comparisons (see Supplementary Material S1 for details).

Alpha diversity

Grazing intensity was found to have a significant effect on dung beetle alpha diversity (*Wilks's lambda* = 0.0004; $F_{[10,4]} = 20.08$; $P < 0.01$), with a significant progressive loss of mean species richness comparing the moderate grazing area (${}^{0}D$ = 39.67) to the abandoned area (${}^{0}D$ = 30.67), and an intermediate value in the low grazing area (${}^{0}D$ = 35.33) (Fig. 1).

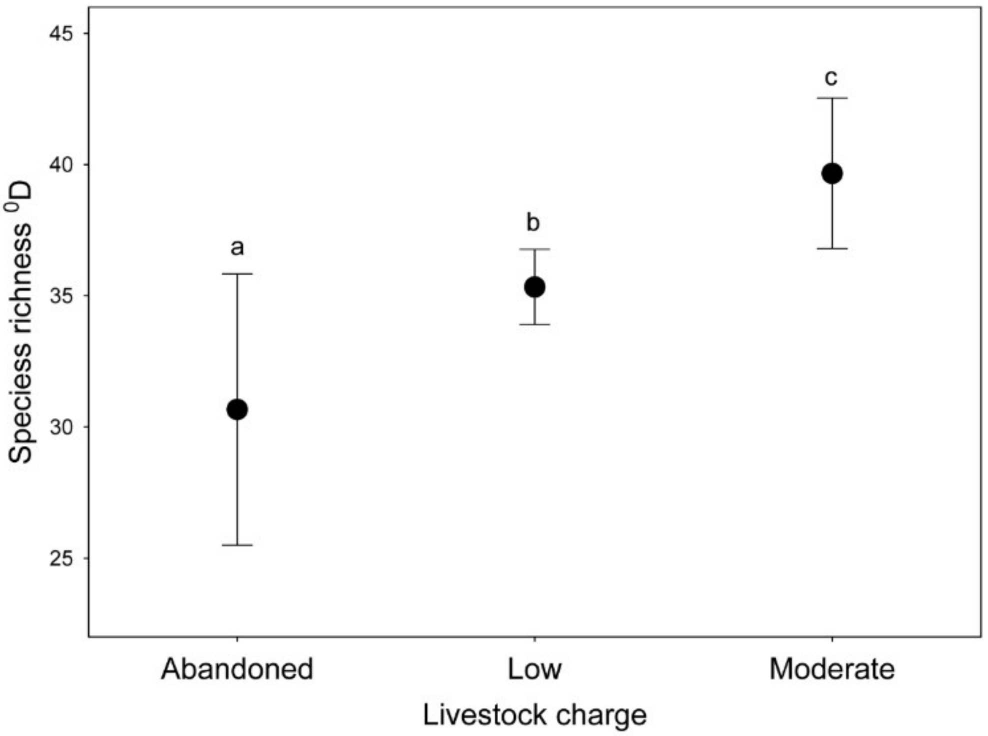

Figure 1. Species richness (^0D) of dung beetles for different grazing intensity levels (abandoned, low and moderate) in sub-mountainous landscapes of Central Italy. Dots represent mean and bars represent standard errors. Different letters mean significant differences (post-hoc Tukey test $P < 0.05$).

When alpha diversity (^1D and ^2D) was evaluated considering species abundance, no significant differences were found between moderate ($^1D = 7.33$; $^2D = 4.3$) and abandoned ($^1D = 7.93$; $^2D = 5.43$) sites (^1D: $P = 0.6$; ^2D: $P = 0.19$), whereas significant differences were revealed between abandoned and low ($^1D = 2.3$; $^2D = 1.5$) grazing areas (^1D: $P < 0.001$; ^2D: $P < 0.01$) and between moderate and low grazing sites (^1D: $P < 0.001$; ^2D: $P < 0.01$) (Fig. 2).

When alpha diversity (^1D and ^2D) was assessed considering biomass, no significant differences between low ($^1D = 5.3$; $^2D = 3.63$) and abandoned ($^1D = 7.20$; $^2D = 5.27$) areas (^1D: $P = 0.116$; ^2D: $P = 0.069$) were observed, whereas significant differences were found between moderate ($^1D = 9.55$; $^2D = 7.13$) and low grazing areas

(^{1}D: $P < 0.001$; ^{2}D: $P < 0.005$). Lastly, significant differences were observed for ^{2}D (P = 0.044) but not for ^{1}D (P = 0.058) between moderate and abandoned sites (Fig. 2).

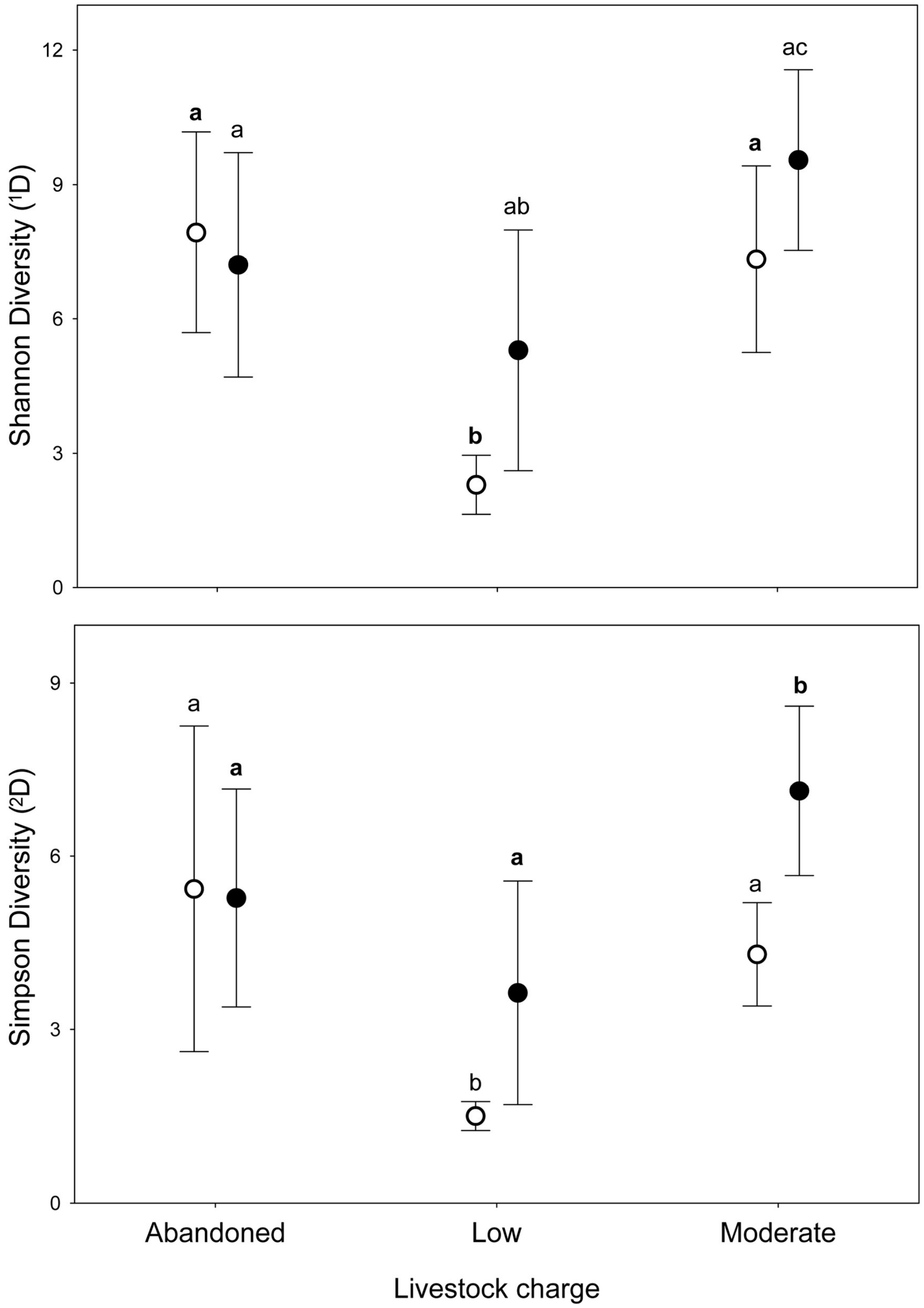

Figure 2. Alpha diversity using Hill numbers of dung beetles for different grazing intensity levels (abandoned, low and moderate) in sub-mountainous landscapes of Central Italy. Shannon Diversity ^{1}D (a) and Simpson Diversity ^{2}D (b) are the alpha diversity measures of q = 1 and q = 2, respectively. Empty dots represent alpha diversity calculated with abundance data; black dots represent alpha diversity calculated with biomass data. Dots represent mean and bars represent standard errors. Different letters mean significant differences (post-hoc Tukey test $P < 0.05$).

Biomass and abundance

Progressive grazing abandonment led to significant differences in total dung beetle biomass and abundance (*Wilks's lambda* = 0.0076; $F_{[4,10]} = 26.16$; $P < 0.0001$). The low grazing area showed higher biomass and abundance than the abandoned (biomass $P < 0.001$; abundance $P < 0.0005$) and moderate areas (biomass $P < 0.05$; abundance $P < 0.001$), whereas the moderate grazing area showed significantly greater biomass than the abandoned area ($P < 0.05$), but not more abundance ($P = 0.127$) (Fig. 3).

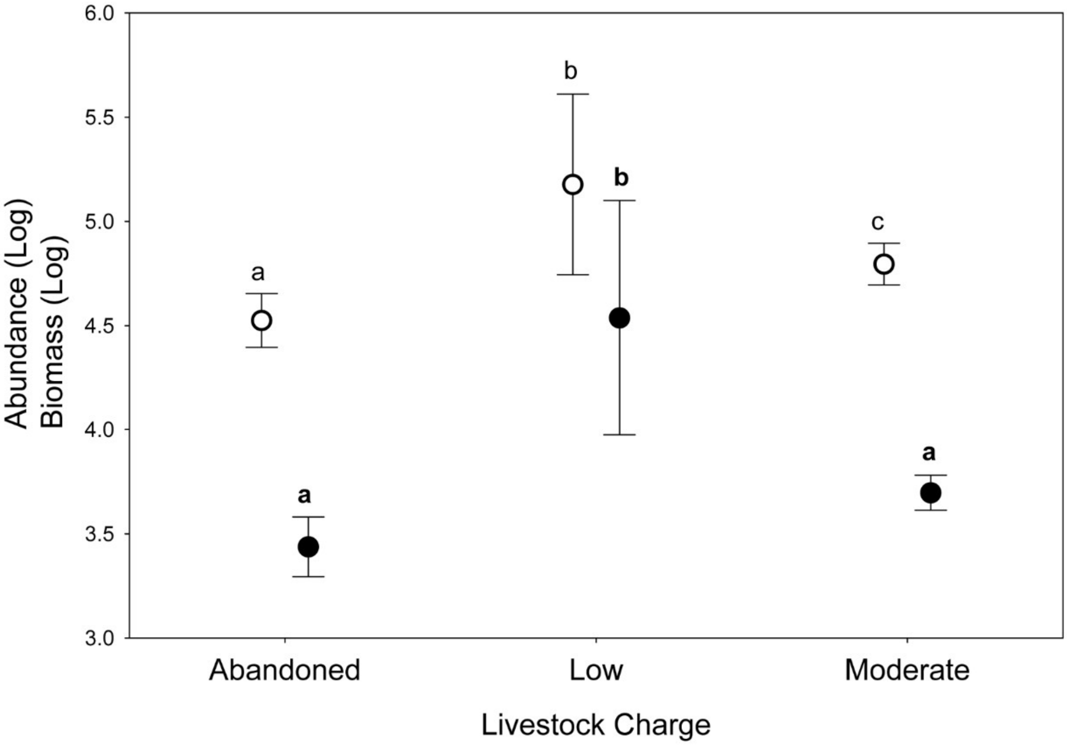

Figure 3. Dung beetle total biomass (empty dots) and abundance (black dots) for different grazing intensity levels (abandoned, low and moderate) in sub-mountainous landscapes of Central Italy. Dots represent mean and bars represent standard errors. Different letters mean significant differences (post hoc Tukey test $P < 0.05$).

Biomass classes were influenced by the progressive grazing abandonment (*Wilks's lambda* = 0.003, $F_{[6,8]} = 22.559$, $P < 0.0005$).

The abandoned area had fewer small beetles than the low ($P < 0.0005$) and moderate grazing areas ($P < 0.001$), fewer medium-sized beetles than low grazing area

($P < 0.05$) but no difference in medium-sized beetles compared to the moderate grazing area ($P = 0.213$). Moreover, abandoned area showed fewer large dung beetles than the low ($P < 0.005$) and moderate ($P < 0.001$) grazing areas. The low grazing area showed more small ($P < 0.01$) and medium-sized ($P < 0.005$) beetles compared to the moderate grazing site, but there were no significant differences among these sites with regard to large beetles ($P = 0.119$) (Fig. 4). Therefore, small and large beetles accounted for the main differences in dung beetle biomass that were observed in the grazed and abandoned areas. Moreover, the equitability of biomass classes diminished continuously from moderate to abandoned areas.

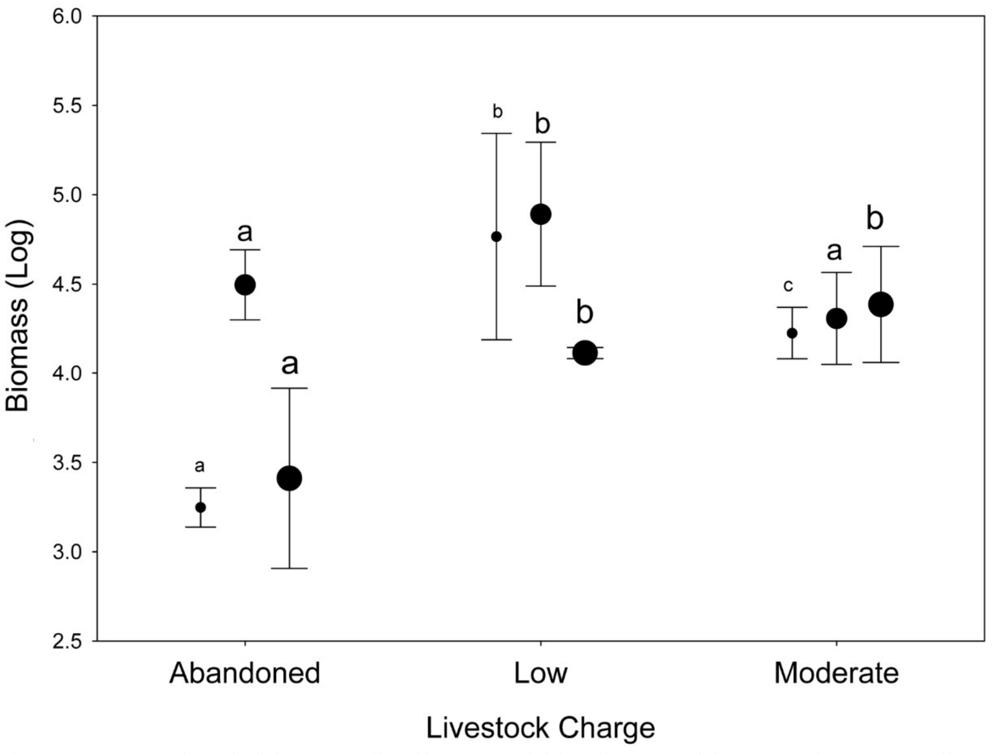

Figure 4. Dung beetle biomass distribution within different biomass classes for different grazing intensity levels (abandoned, low and moderate) in sub-mountainous landscapes of Central Italy. Biomass classes (mg): large > 100 (large dots); medium = 10–100 (medium dots); small < 10 (small dots). Dots represent means and bars represent standard errors. Different letters mean significant differences (post hoc Tukey test $P < 0.05$).

Indicator values (IndVal)

The IndVal analysis (Table 1) showed a total of 22 indicator species and 2 detector species. 3 indicator species (*Onthophagus joannae* Goljan, 1953, *Onthophagus verticicornis* (Laicharting, 1781), *Sisyphus schaefferi* (L., 1758)) were found in the abandoned pastures, 7 indicator species and 1 detector species (*Sigorus porcus* (F., 1792)) were found in the low grazing site and 12 indicator species and 1 detector species (*Onthophagus opacicollis* Reitter, 1892) were found in the moderate grazing area.

Table 1: Dung beetle species with significant value of IndVal (p<0.05). Species with IndVal values higher than 70% (in bold) were considered indicator species. Species with IndVal values between 45% and 70% were considered detector species. AB: abandoned; LG: Low grazing; MG: Moderate grazing.

Indicator species	**AB**	**LG**	**MG**
Onthophagus joannae Goljan, 1953	**96.9**		
Onthophagus verticicornis (Laicharting, 1781)	**89.5**		
Sisyphus schaefferi (Linnaeus, 1758)	**98.9**		
Aphodius fimetarius (Linnaeus, 1758)		**74.5**	
Chilothorax conspurcatus (Linnaeus, 1758)		**93.6**	
Esymus merdarius (Fabricius, 1775)		**98.1**	
Melinopterus consputus (Creutzer, 1799)		**97.3**	
Nimbus contaminatus (Herbst, 1783)		**99.1**	
Onthophagus medius (Kugelann, 1792)		**98.9**	
Trichonotulus scrofa (Fabricius, 1787)		**99.5**	
Agrilinus convexus (Erichson, 1848)			**100**
Bodilopsis rufa (Moll, 1782)			**70**
Bubas bison (Linnaeus, 1767)			**88.3**
Calamosternus granarius (Linnaeus, 1767)			**95.2**
Chilothorax paykulli (Bedel, 1907)			**100**
Copris lunaris (Linnaeus, 1758)			**80**
Melinopterus prodromus (Brahm, 1790)			**83.6**
Melinopterus reyi (Reitter, 1892)			**100**
Onthophagus coenobita (Herbst, 1783)			**73.2**
Onthophagus ruficapillus Brullé, 1832			**85.1**
Onthophagus taurus (Schreber, 1759)			**82**
Sericotupes niger (Marsham, 1802)			**73.7**
Sigorus porcus (Fabricius, 1792)		67.6	
Onthophagus opacicollis Reitter, 1892			63.2

Functional groups

The functional groups analysis highlights significant difference in species richness (*Wilks's lambda* = 0.014, $F_{[6, 8]} = 5.72$, $P < 0.05$) and biomass (*Wilks's lambda* = 0.0076, $F_{[6, 8]} = 13.94$, $P < 0.001$). With respect to functional group species richness (Fig. 5a), abandoned grazing areas had significantly fewer non-nesting species than moderate ($P < 0.01$) and low grazing ($P < 0.05$) areas, whereas no significant difference in non-nesting species richness was found between moderate and low grazing areas ($P = 0.067$). As regards the number of species of paracoprid and telecoprid, no significant differences were detected among the areas.

Regarding functional group biomass (Fig. 5b), the low grazing site showed significantly more non-nesting biomass than the moderate ($P < 0.05$) and abandoned sites ($P < 0.001$), whereas the moderate area had more non-nesting biomass than the abandoned site ($P < 0.001$). With respect to paracoprid biomass, the low grazing area had significantly more biomass than the moderate ($P < 0.05$) and abandoned areas ($P < 0.001$), whereas the moderate grazing area showed more paracoprid biomass than the abandoned site ($P < 0.05$). Telecoprid biomass was found to be significantly higher in the abandoned area than it was in the moderate ($P < 0.05$) and low ($P < 0.05$) areas, whereas no statistical difference was found between low and moderate areas with respect to telecoprid biomass ($P = 0.97$).

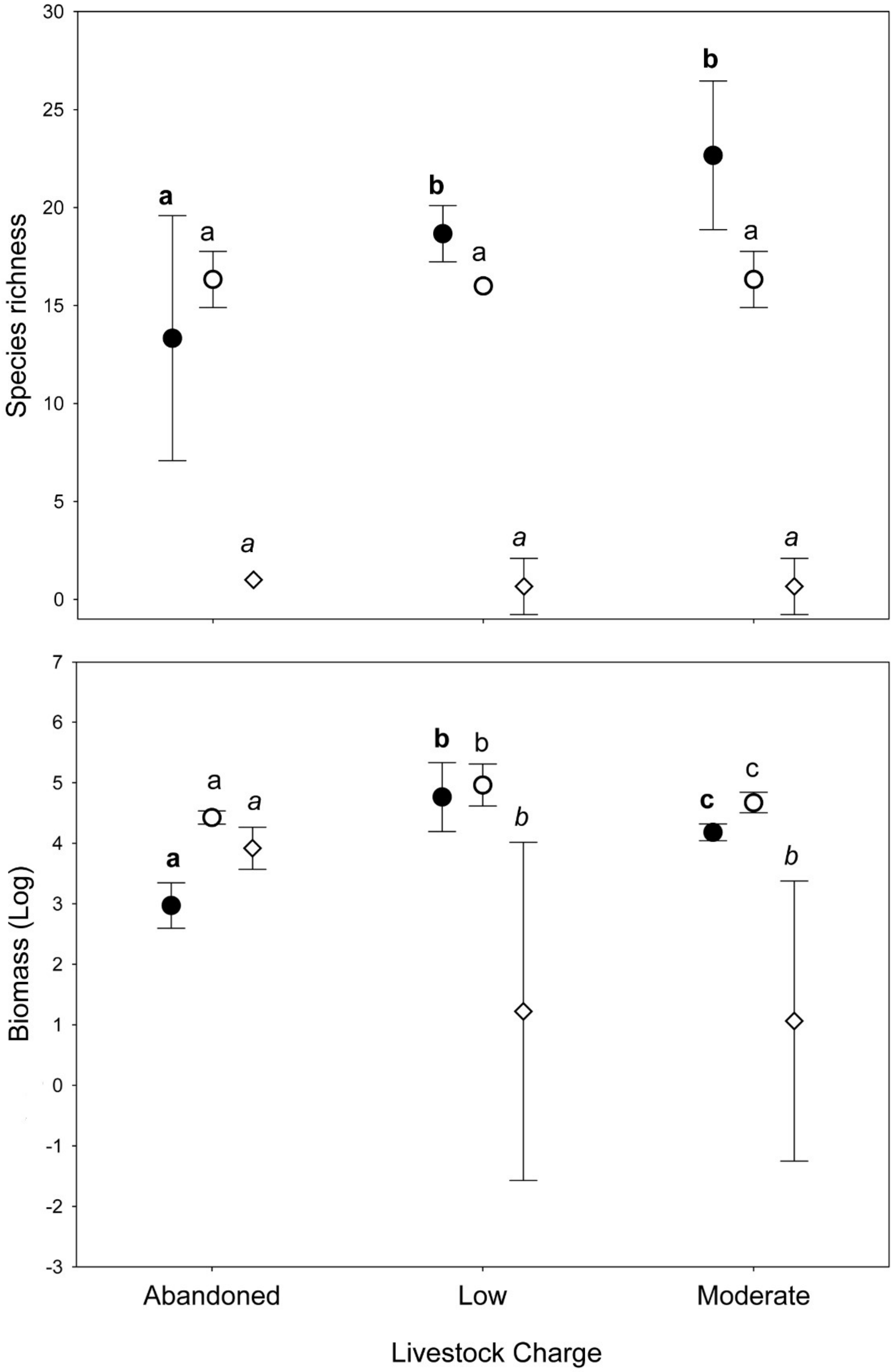

Figure 5. Species richness (a) and biomass (b) of functional groups of dung beetles for different grazing intensity levels in sub-mountainous landscapes of Central Italy. Black dots represent no nesting functional group; empty dots represent paracoprid functional group; empty rhombuses represent telecoprid functional group. Dots represent mean and bars represent standard errors. Different letters mean significant differences (post hoc Tukey test $P < 0.05$).

Beta diversity

Intra habitat beta diversity analysis (Fig. 6) showed that all areas increase their within-similarity from C_{03} to C_{13}. For C_{23} each area showed a particular behaviour: C_{23} rose steadily in the low grazing area, it remained relatively stable in the moderate grazing area showing only a slight-decrease, and it decreased more markedly in the abandoned area. Hence, the abundant species composition is homogeneously distributed within the low and moderate grazing areas, whereas it is less homogeneously distributed in the abandoned area. As regards intra habitat beta diversity for C_{03}, there was no difference among the three areas. With respect to C_{13} and C_{23}, the three areas showed significantly different intra habitat beta diversity. The abandoned area showed the highest internal beta diversity, followed by the moderate and low grazing areas.

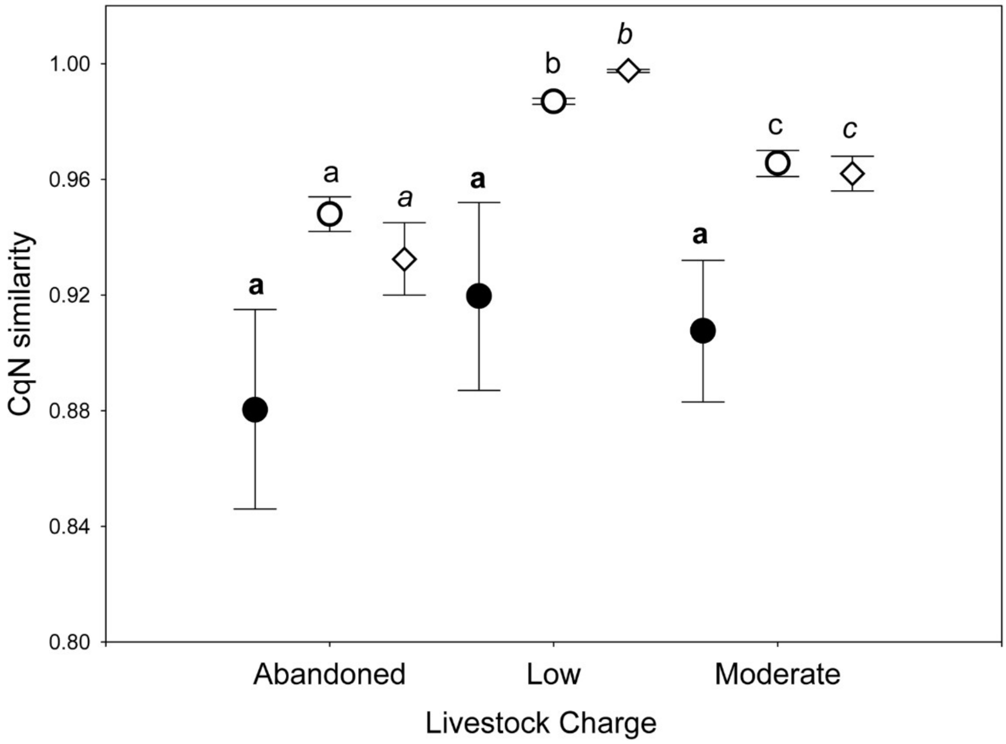

Figure 6: Intra habitat beta diversity of dung beetles for different grazing intensity levels (abandoned, low and moderate) in sub-mountainous landscapes of Central Italy, using multiple assemblage abundance based overlap measure C_{qN} (C_{03} = black dots; C_{13} = empty dots; C_{23} = empty rhombus). Dots represent mean and bars represent 95% confidence intervals. Different letters mean significant differences (post hoc Tukey test $P < 0.05$).

The Permanova test for beta diversity among the areas showed significant differences at each order of q of the C_{qN} similarity matrix (Table 2). Hence, composition of rare and abundant species was shown to be affected by grazing intensity.

Table 2: PERMANOVA results for the grazing intensity factor. q indicates the value by which we calculated the similarity matrix used in the PERMANOVA test. GI= Grazing intensity

Sensitive parameter	Source	df	SS	MS	Pseudo-F	P (perm)
$q = 0$	GI	2	9900.8	4950.4	1.0075	0.004
	Res	6	29480	4913.4		
	Total	8	39381			
$q = 1$	GI	2	10127	5063.3	1.0324	0.003
	Res	6	29426	4904.3		
	Total	8	39553			
$q = 2$	GI	2	10256	5127.8	1.0456	0.007
	Res	6	29425	4904.1		
	Total	8	39680			

3.5. Discussion

Effects on dung beetle richness and population size

Progressive grazing abandonment leads to a loss of species richness for ${}^{0}D$ of up to—22%, whereas the total abundance loss varies between –45% to –93%. The decrease in the number of indicator species observed with the decrease in grazing intensity support our hypothesis. We encountered 13, 8 and 3 species with a significant IndVal value for moderate, low and abandoned sites, respectively. This means that fewer trophic resources favour only a limited number of species.

This 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 and individuals that an area can support (Evans et al., 2005). In fact, areas with more available energy (in this context, trophic energy) can support more individuals, allowing species to obtain higher population sizes, reducing extinction rates and enhancing species richness (Evans et al., 2005). A recent work by Tshikae et al. (2013) reinforces our findings and our interpretation. They explicitly tested the species – energy relationship using dung beetles across an aridity and trophic resource gradient in Botswana. Their results are in line with our findings, namely that the richness, diversity and abundance of dung beetle populations diminish with the decreasing availability of (trophic) energy.

This also confirms concerns over grazing abandonment in Europe. Due to the long history of grazing in this area (Blondel, 2006), its abandonment results in the lack of a key element in these ecosystems, and dung beetles are not able to maintain viable communities only relying on wild ungulate droppings (Jay-Robert et al., 2008) in agro-ecosystems, or manure from domestic animal such as dogs (Carpaneto et al., 2005) in suburban areas. The quantity of dung seems to impact the dung beetle community locally (Lobo et al., 2006), and they show a strong resilience in their capacity to recuperate community richness and abundance after an increase in fresh dung availability (Lumaret et al., 1992). However, the continuous decline of some European species (Lobo, 2001, Carpaneto et al., 2007) may lead to their extinction from the regional pool, preventing such community recovery.

Effects on dung beetle biomass and biomass classes

The grazed areas favoured the presence of more total biomass and large dung beetles, while the abandoned site showed a loss of dung beetle biomass of up to –78%.

This is due to the fact that the largest dung beetles need more dung for feeding and nesting. Indeed, there is a positive relationship between body size and dung burial (Doube et al., 1988; Larsen et al., 2005; Slade et al., 2007; Nervo et al., 2014). For example, during breading, *Bubas bison* may bury 197 g of dung (Lumaret and Kirk, 1987), whereas *Copris lunaris* may bury about 100-165 g of dung (Klemperer, 1982; Martín-Piera and López-Colón, 2000). Due to a minimum threshold regarding the amount of dung needed to achieve pupation and to the fact that larvae that are allowed to feed longer exhibit higher pupation success (Shafiei et al., 2001), larger beetles may not find the minimum amount of trophic resources in the abandoned area to allow the survival of a stable population. Specifically, this area may prove to be inadequate to sustain larger beetles because of: a) insufficient quantities of available dung in the pasture and b) differences between domestic cattle and wildlife fauna (boar, deer) in terms of single excrement sizes and spatial distribution. Our findings therefore support the idea that the quantity and the configuration of trophic resources is a key factor for maintaining viable dung beetle communities and that large dung beetles may survive only if trophic resources are abundant (Lumaret et al., 1992) and with an important mass that permit to relocate a certain quantity of dung in a single horizontal relocation (telecoprids) or under a single dung mass (paracoprids). This is confirmed by the presence of 3 indicator species (*Bubas bison* (Linnaeus, 1767), *Copris lunaris* (Linnaeus, 1758) and *Sericotrupes niger* (Marsham, 1802)) in the moderate grazing area, which are among the largest species in the regional species pool.

Another result that bears further investigation is the variation in the equitability of biomass within biomass classes for each grazing intensity area. We showed that when a trophic resource becomes a limiting factor, there is a dominance of some biomass classes.

This means that the more limited the available trophic resources become, the fewer the opportunities will be for species to share those resources and consequently only some body size classes will be favoured. The simultaneous presence of different body size classes seems to suggest the sharing of resources by dung beetles (Nervo et al., 2014); however, our data suggest that this phenomenon does not occur when trophic resources fall below threshold levels.

Effects on community structure

The most interesting result is the differential response of alpha diversity measures when calculated by means of abundance or biomass data. It is noteworthy to observe the positive variation of ${}^{1}D$ and ${}^{2}D$ when considering biomass instead of abundance in low and moderate grazed areas, whereas these values diminished in the abandoned area. Moreover, considering abundance, the abandoned area showed the highest values of ${}^{1}D$ and ${}^{2}D$. These results were reversed when considering biomass with moderate areas showing the highest values for ${}^{1}D$ and ${}^{2}D$. These results may be explained by the fact that abundance data do not consider the difference in species sizes, and all species are equally considered. However, we clearly demonstrated that the scarcity of trophic resources may have a differential effect on dung beetle biomass classes. This differential effect may also have affected diversity metrics even when it was not being considered. We therefore propose using both biomass and abundance data because they are differentially sensitive to trophic resource availability. The same proposal was made by Nichols et al. (2007), who stated 'biomass is indicative of the total available resource and may decline with disturbance even as abundance increases'.

However, the high level of alpha diversity found in the abandoned area for ${}^1D$ and ${}^2D$ (comparable to moderate grazing areas) is noteworthy and is consistent with the results of Kadiri et al. (1997) obtained in southern France. We agree with their explanation that communities in areas with a low level of resources should have a high level of codominant species, whereas areas with a higher level of trophic resources are dominated by a small number of species (Kadiri et al., 1997; Tilman, 1982). This results highlight the importance of wild fauna presence in the abandoned area, which provide dung that can maintain a well-diversified community although impoverished (low species richness). In other words, grazing abandonment lead to a species richness decrease and contemporary to a community composition change (see below) where a well-diversified community of habitat or trophic specialized species was maintained.

The lowest diversity value for ${}^1D$ and ${}^2D$ in the low grazing site may be explained by the overwhelming dominance of two species, *Melinopterus consputus* (Creutzer, 1799) and *Onthophagus medius* (Kugelann, 1792). These two species may alter the diversity profile of this site by means of competitive exclusion (Hardin, 1960). The low quantity of trophic resources available in this site has affected the dung beetle community by favoring generalist r-strategic species (*Melinopterus consputus*) and high competitive species such as medium size tunnelers (*Onthophagus medius*) (Horgan and Fuentes, 2005). The maximum dung beetle density that we found in the low grazing area within the experimental dung pat of about 0.5 l was 329 *Onthophagus medius* and 10,082 *Melinopterus consputus* specimens (Tonelli M., personal observation, 2015). These values are higher than the estimated threshold for such competition occurring in the field (Finn and Gittings, 2003). These results are consistent with Kadiri et al. (1997), who in an area in France with a similar grazing intensity, found a diminished value of equitability due to variations in the relative frequencies of few species. Analogous results were reported in an investigation of an urban area in Rome (Italy) by Carpaneto et al. (2005), who found a decrease in the number of species with the dominance of one species of Aphodinae (i.e. *Nimbus johnsoni* (Baraud, 1976)), showing the same explosive reproductive success of *M. consputus*.

Finally, the comparison of our results and other similar European areas (i.e. Kadiri et al., 1997) indicate that more studies with more intervals of grazing intensity levels are needed because dung beetle diversity appears to be sensitive to small differences in trophic resource availability.

Effects on functional groups

Grazing abandonment differentially affects dung beetles as a function of nesting behavior. Generally, as regards grazed areas, abandoned areas showed a loss of non-nesting species richness and abundance; however, they showed higher telecoprid abundance and similar paracoprid species richness. In general terms, food relocation behavior is a fundamental trait that allows species to avoid competition for an ephemeral resource such as dung (Halffter and Matthews, 1966; Halffter and Edmonds, 1982; Zunino, 1991). Such a behavioral strategy guarantees a certain amount of dung for feeding and nesting (Zunino, 1991); hence, telecoprid and paracoprid species may survive in our abandoned site with few food resources because they relocate dung. On the contrary, non-nesting species are largely prevented from maintaining viable populations because they need a certain quantity of exposed dung for adult feeding and, even more importantly, for the development of their larvae, which are free-living in, or immediately below, the dung pat (Lobo, J.M., personal communication, 2016). These results were confirmed by the IndVal results, which showed that 12 of the total 21 indicator species of grazed areas (Low + Moderate) were non-nesting species, whereas no indicator species of the abandoned area was non-nesting.

Effects on community composition

Beta diversity among areas is strongly influenced by the quantity of trophic resources at all q levels; hence, rare and abundant species are compositionally different among different grazing intensity areas. In accordance with Lobo et al. (2006), our study showed how the quantity of available dung for dung beetle communities is an important factor in determining dung beetle composition. Grazing abandonment therefore led to a change in dung beetle composition due to the differential impact on biomass classes and functional groups, favouring the presence of more opportunistic species (Tonelli et al., 2017).

However, the difference in dung beetle composition may be explained by factors other than the quantity of resources *per se*. In fact, due to the lack of high grazing intensity, the abandoned pasture begins to be colonized by shrubs and trees; hence, the habitat may be considered more complex than a pure grassland. Habitat heterogeneity is an important factor in determining dung beetle biodiversity patterns (Negro et al., 2011). Our results show how dung beetle community metrics are sensitive to this incipient and progressive change in the habitat structure. The higher internal beta diversity of the abandoned area and its high alpha diversity for ${}^{1}D$ and ${}^{2}D$ (similar to the moderate grazing area) could be an index of this intermediate, more complex, condition. The same result was found by Numa et al. (2009), who showed how a heterogeneous landscape (grassland habitat surrounded by a forest or shrubland landscape) has more within-beta diversity than a homogeneous one (grassland habitat surrounded by a grassland landscape). This is in agreement with the idea that heterogeneous habitats may support more potential niches for a functionally diverse suite of species than less complex habitats (Klopfer and McArthur, 1960; Lassau et al., 2005). This interpretation is corroborated by the IndVal results for the abandoned site, where the indicator species were previously recognized to have a clear preference (although not exclusive) for more closed habitats. For example, *Onthophagus verticicorni*s prefer grassland and garigue habitats in southern France (Lumaret and Kirk, 1987); *Onthophagus joannae* was recognised as a pasture indicator in alpine areas (Macagno and Palestrini, 2009; Tocco et al., 2013) but in Mediterranean France prefer more closed habitats (Lumaret and Kirk, 1987); *Sisyphus schaefferi* have a large range of habitat preferences that vary from pastures and garigues (Lumaret and Kirk, 1987; Lumaret, 1990) to forests (Verdú et al., 2011). These results support our interpretation of an incipient impoverishment of the dung beetle community driven by grazing abandonment, which leads to a loss of trophic resources and a changing habitat and, consequently, to a community composition shift toward specialized species.

3.6. Conclusion and conservation implications

Grazing abandonment resulted in a loss of alpha diversity of up to–22% and to a loss of dung beetle biomass of up to –78%, with large beetles and species which do not display nesting behaviour appearing to be the most compromised. The non-random impact on dung beetle body-sizes is reflected in the differential response of abundance and biomass to trophic resource availability, suggesting that both abundance and biomass data should be used in future studies.

Many studies report that the abandonment of a pasturelands contributes to accelerate the regeneration of woodlands (Harmer et al., 2001; Smit and Olff, 1998). From our standpoint, this is another problem for the conservation of dung beetle fauna. Indeed, it is widely recognized that forested habitats in Europe have fewer dung beetle species, abundance and diversity than pasture lands (Macagno and Palestrini, 2009; Tocco et al., 2013; Negro et al., 2011; Verdú et al., 2000; Jay-Robert et al., 2008; Lumaret and Kirk, 1987). We showed that dung beetles of the abandoned site were sensitive to this incipient habitat change, showing the presence of indicator species typical of shrub and woodlands. These results reinforce the idea that dung beetles are good bio-indicators whose preservation depends heavily on the presence of a high level of trophic resources used during their feeding and nesting.

Hence, maintaining grazing lands with a moderate level of grazing is a key factor in the conservation of grasslands and dung beetle communities.

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Supplementary Material S1 – Number of individuals of dung beetle species collected in each area. Also inventory completeness were reported according to the INext estimator. AB: Abandoned; LG: Low grazing; MG: Moderate grazing. Functional group: NN = No nesting; P = Paracoprid; T = Telecoprid. Biomass classes (mg): B1 > 100; B2 = 10-100; B3 < 10.

Species	**Functional group**	**Biomass class**	**AB**	**LG**	**MG**	**Total**
Acanthobodilus immundus (Creutzer, 1799)	NN	B3	0	4	17	21
Acrossus luridus (Fabricius, 1775)	NN	B2	3	162	55	220
Agrilinus constans (Duftschmid, 1805)	NN	B3	0	0	1	1
Agrilinus convexus (Erichson, 1848)	NN	B3	0	0	23	23
Amidorus thermicola (Sturm, 1800)	NN	B2	2			2
Aphodius coniugatus (Panzer, 1795)	NN	B2	5	4		9
Aphodius fimetarius (Linnaeus, 1758)	NN	B2	21	108	16	145
Aphodius foetidus (Herbst, 1783)	NN	B2	2	3	6	11
Bodilopsis rufa (Moll, 1782)	NN	B3	8	7	35	50
Bodiloides ictericus (Laicharting, 1781)	NN	B3	0		11	11
Bubas bison (Linnaeus, 1767)	P	B1	0	12	91	103
Caccobius schreberi (Linnaeus, 1767)	P	B3	17	2	3	22
Calamosternus granarius (Linnaeus, 1767)	NN	B3	1	0	20	21
Calamosternus mayeri (Pilleri, 1953)	NN	B3	0	1	0	1
Chilothorax conspurcatus (Linnaeus, 1758)	NN	B3	0	701	48	749
Chilothorax lineolatus (Illiger, 1803)	NN	B3	0	2	1	3
Chilothorax paykulli (Bedel, 1907)	NN	B3	0	0	53	53
Colobopterus erraticus (Linnaeus, 1758)	P	B3	44	463	542	1049
Coprimorphus scrutator (Herbst, 1789)	P	B2	6	3	3	12
Copris lunaris (Linnaeus, 1758)	P	B1	0	3	12	15
Esymus merdarius (Fabricius, 1775)	NN	B3	1	102	1	104
Esymus pusillus (Herbst, 1789)	NN	B3	10	14	5	29
Euoniticellus fulvus (Goeze, 1777)	P	B2	2903	3738	1217	7858
Euorodalus paracoenosus (Balthasar & Hrubant, 1960)	NN	B3	0	0	2	2
Geotrupes spiniger Marsham, 1802	P	B1	17	65	58	140
Labarrus lividus (Olivier, 1789)	NN	B3	142	120	99	361
Limarus zenkeri (Germar, 1813)	NN	B3	0	0	2	2
Loraphodius suarius (Faldermann, 1835)	NN	B3	0	33	14	47
Melinopterus consputus (Creutzer, 1799)	NN	B3	134	92563	2388	95085
Melinopterus prodromus (Brahm, 1790)	NN	B3	81	1126	6163	7370
Melinopterus reyi (Reitter, 1892)	NN	B3	0	0	16	16
Melinopterus stolzi (Reitter, 1906)	NN	B3	0	0	2	2
Nialus varians (Duftschmid, 1805)	NN	B3	0	0	9	9
Nimbus contaminatus (Herbst, 1783)	NN	B3	1	461	3	465
Nimbus johnsoni (Baraud, 1976)	NN	B3	0	6	14	20
Nimbus obliteratus (Panzer, 1823)	NN	B3	435	2155	154	2744
Onthophagus coenobita (Herbst, 1783)	P	B2	22	29	139	190

Onthophagus fracticornis (Preyssler, 1790)	P	B2	1021	999	2158	4178
Onthophagus grossepunctatus Reitter, 1905	P	B3	44	0	110	154
Onthophagus illyricus (Scopoli, 1763)	P	B2	0	1	0	1
Onthophagus joannae Goljan, 1953	P	B3	824	26	0	850
Onthophagus lemur (Fabricius, 1781)	P	B2	297	41	283	621
Onthophagus medius (Kugelann, 1792)	P	B2	57	10100	51	10208
Onthophagus opacicollis Reitter, 1892	P	B3	2	12	24	38
Onthophagus ruficapillus Brullé, 1832	P	B3	23	7	171	201
Onthophagus taurus (Schreber, 1759)	P	B2	80	62	648	790
Onthophagus verticicornis (Laicharting, 1781)	P	B2	1038	61	61	1160
Otophorus haemorrhoidalis (Linnaeus, 1758)	NN	B3	12	0	21	33
Phalacronothus biguttatus (Germar, 1824)	NN	B3	2	1	3	6
Planolinus fasciatus (Olivier, 1789)	NN	B3	1	1	0	2
Sericotrupes niger (Marsham, 1802)	P	B1	7	55	174	236
Sigorus porcus (Fabricius, 1792)	NN	B3	59	198	36	293
Sisyphus schaefferi (Linnaeus, 1758)	T	B2	942	7	3	952
Teuchestes fossor (Linnaeus, 1758)	P	B2	1	0	0	1
Trichonotulus scrofa (Fabricius, 1787)	NN	B3	1	192	0	193
Trypocopris vernalis apenninicus Mariani, 1958	P	B1	2	0	0	2
Total species (S)			**38**	**41**	**47**	**56**
Total individuals (N)			**8268**	**113650**	**14966**	**136884**
INext (%)			**99**	**99**	**99**	

CHAPTER 4

The effects of grazing intensity and historical veterinary medical products use on dung beetle functional diversity and ecological process

4.1. Introduction

Biodiversity loss and its alteration at regional and local level is a world-wide problem of this century (Barnosky et al., 2011). Besides being an ethical problem, this biodiversity crisis offers us some practical challenges (Tilman, 2000). Indeed, it is widely accepted that biodiversity is one of the principal engine of ecosystem functioning (Hooper et al., 2005; Loreau, 2000; Loreau et al., 2001) and its loss may have negative effects on their performance lead to an alteration of productivity, decomposition rate and nutrient cycling (Cardinale et al., 2012).

Herbivores accelerate the nutrient turnover directly by excreting nutrients in a form readily available for uptake by microbes and plants (Doughty et al., 2016; Hobbs, 1996). Animal excrement is a very important element of the nutrient cycle processes because dung is rich in nutrients such as carbohydrates, nitrogen, carbon, vitamins and minerals (Hanski, 1987; Holter, 2016). However, the majority of this nutrients are lost by volatilization and are not available for soil uptake (Steinfeld et al., 2006). Because of their dependence on vertebrate dung, dung beetles are considered a keystone taxon due to their functional role. By feeding and nesting with dung they are involved in several ecosystem processes (Nichols et al., 2008). They are very important for nutrient cycling (Yamada et al., 2007; Bertone et al., 2006), seed dispersal (Slade et al., 2007; Andersen and Feer, 2005) and control of vertebrate parasites (Gregory et al., 2015; Nichols and Gómez, 2014). Although with some variation depending on the type of process considered, the majority of these ecological functions are dependent on the amount of buried dung (Braga et al., 2013; Slade et al., 2007).

Several studies have investigated the link between dung beetle community attribute and ecosystem processes, highlighting the relative importance of species richness (Beynon et al., 2012; Gollan et al., 2013), richness and type of functional groups (Slade et al., 2007; Beynon et al., 2012), species body size (Larsen et al., 2005; Nervo et al., 2014), community abundance and biomass (Braga et al., 2013; Gollan et al., 2013). However, some of this study are made with artificial laboratory communities that may prevent to infer their results toward real world (i.e. Beynon et al., 2012; Nervo et al., 2014; Manning et al., 2016). So, although there are many studies on the dung beetle community structure, the majority does not add an empirical study on the processes but try to deduct them from the biodiversity metrics with a great risk of mistake (Braga et al., 2013; Gollan et al., 2013).

In the last years, an approach based on functional diversity was implemented (Díaz and Cabido, 2001; Tilman et al., 2014). Functional diversity was defined as “the kind, range and relative abundance of functional traits present in a given community” (Díaz et al., 2007) where the functional traits are “any morphological, physiological or phenological feature measurable at the individual level, from the cell to the whole-organism level, without reference to the environment or any other level of organization” (Violle et al., 2007). This approach is increasingly applied in the recent years because numerous evidences highlight the possibility that the ecological processes are more influenced by the functional diversity rather than species richness (Díaz and Cabido, 2001; Tilman, 2001; Hooper et al., 2005; Hillebrand and Matthiessen, 2009; Scherer-Lorenzen, 2009).

Our previous work (Tonelli et al., 2017), demonstrate the impact of the low grazing intensity and the veterinary medical products (VMPs) use on dung beetle biodiversity: alpha and beta diversity, large beetle and abundance are all affected by these two factors. The aims of the present study were to investigate the impact of grazing intensity and VMPs use from a functional standpoint. We attempt to answer the following questions: i) what are the effects of VMPs use, grazing intensity and their interaction on dung beetle functional diversity? ii) Is species richness a good proxy for functional diversity metrics? iii) What is the effects of grazing intensity and VMPs use on the dung beetle ecological process (i.e. dung burial)? and iv) can functional biodiversity measures acts as a proxy of ecological process?

We have been analyzed functional diversity using annual data (whole community) in order to evaluate the general impact of grazing intensity and VMPs. Moreover, we analyzed the community data from a dung burial experiment (spring and autumn) in order to investigate the potential link among functional diversity and the ecological process using data on real communities.

4.2. Materials and Methods

Study area

The study was carried in sub-mountains areas of Pesaro-Urbino province in the Marche region, Italy. Provincial climate falls into the temperate Köppen's categories (Cfa and Cfb). The average annual temperature is around 12 °C with average minimum of around 3.5 °C in winter and average maximum of 21 °C in summer. Average annual precipitation is around 930 mm with two driest periods, one in summer and another in winter (www.lavalledelmetauro.it/contenuti/geologia-clima/indice.html). Soil is calcareous. The arboreous vegetation of the study sites is dominated by *Quercus ilex* L., *Quercus pubescens* Willd., *Quercus cerris* L., *Ostrya carpinifolia* Scop. and *Fraxinus ornus* L. whereas the herbaceous vegetation belongs to the *Brizo mediae-Brometum erecti* and to *Asperula purpureae-Brometum erecti* phyto-associations.

To evaluate the effects of grazing intensity and VMP use on functional diversity, we designed a 2x2 full factorial design with three replications for each treatment. We identified different areas 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' areas – 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 mainly used by horses that were abandoned and have reverted to a wild state. The grazing intensity of these pastures is around 0.7 units of livestock/ha.

B) 'Moderate grazing, VMP-free' areas – 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.

C) 'Moderate grazing with VMPs' areas – 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. The sampling sites are located between 800 and 1000 m a.s.l.

D) 'Low grazing with VMPs' areas – 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 sampling sites are located between 800 and 1000 m a.s.l.

The density of wild fauna (i.e. *Capreolus capreolus* (L., 1758) and *Sus scrofa* L., 1758) is very similar among all the studied areas (Tonelli, personal observation, 2013).

In the areas with VMPs use, the farmers' interviews (Tonelli, unpublished data) highlighted that the VMPs have been use since 1990s until today. The main veterinary formulations that are used are based on Ivermectin and Pyrantel pamoate. The main preventive treatments are administrated in spring and in autumn but the data of application vary between each farmer. Moreover, additional treatments are applied as many times as there are parasitic stress. In the LGVMP areas, VMPs are only given to foals and adult animals with evident parasitic stress, but have a very intense historical use of VMPs.

Dung beetle trapping

For each treatment, we selected three sampling sites separated by at least 500 m to ensure independence among the replicates (Silva and Hernández, 2015). In each site, we placed a 50 x 50 m quadrate 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 to specific level.

Functional diversity analysis

Twenty-four traits were selected and measured in order to analyze functional diversity (See Annex 2 for more detail on traits selection and measurement): fresh beetle biomass, 6 body morphological traits, 8 mouthparts morphological traits, and 9 bionomical traits.

In order to highlight the effect of grazing intensity and IVM use on dung beetle functional diversity, we measure four indexes that measure different aspects of dung beetle community: *FD* is an estimate of the functional richness (the number of functional niches present s in a community) based on dendrogram length (Petchey and Gaston, 2006). The same measure was also weighted by species abundance performing the *wFD* index (Pla et al., 2012). Two indices that are sensible to the distribution of the functional niches within the functional space of the community was used: *FEve* (Villéger et al., 2008), and *FDis* (Laliberté and Legendre, 2010). *FEve* measures the regularity of spacing between species in the trait space and also the evenness of species abundance, whereas *FDis* is the average distance of individual species to the centroid of all species in the community trait space taken into account the relative abundances of species for computing the weighted centroid. All measures were calculated using the software FDiveristy (Casanoves et al., 2011). These measures were calculated after normalization of traits and the application of Principal Components Analysis in order to reduce matrix dimensionality. We used the first 13 axes of PCA that contains the 96% of total variance. The PCA was calculated with the software PAST 3.14 (Hammer et al., 2001).

The effects of grazing intensity, VMPs use and their interaction on functional diversity measures was evaluate using a full factorial generalized linear model. Pairwise comparisons were made using the Tukey post-hoc test. This statistical analysis was performed with Statistica 7.0 package (StatSoft, 2004). The relationship among species richness, exponential of Shannon diversity (^{1}D) and functional diversity metrics was investigate using Pearson correlation test performed with Statistica 7.0 package (StatSoft, 2004). Data about species richness and exponential of Shannon derived from Tonelli et al. (2017).

Dung removal experiment

Dung removal experiment was performed twice, once in in autumn (15-16/10/2015) and another in spring (17-18/5/2016). We select this sampling periods because a previous research highlighted that they contain 83% of number of species and 65% of total abundance of the community (Tonelli, unpublished data). We selected eight sampling sites (replicates) for each treatment for a total 32 sampling points (8 replicate x 4 treatments). In order to avoid interference and pseudoreplication problems, the minimum distance between replicate was been 100 m (Larsen and Forsyth, 2005; Silva and Hernández, 2015).

At each sampling site we buried one plastic container (Ø = 40 cm; h= 20 cm) up to the soil level, filled for 2/3 with site soil. A mean of 590 g (Standard deviation ± 39 g) pile of homogenised and mixed cow and horse fresh dung (1:1 proportion) was placed at each plastic container. Dung was homogenised after a 48 h of freezing in order to kill any dung beetle, predators or Dipteran larvae that may alter the results (O'Hea et al., 2010). For each dung pile, a standard quantity (20 g) of fresh dung was sampled in order to calculate its humidity contents and then its initial dry weight. Fresh dung was VMPs free because this substance may alter dung attraction (Webb et al., 2010). Dung was placed in the field between 06:00 and 12:00 for both sampling dates. Plastic containers were recovered after 24h of exposition and was transported to the laboratory in order to collect the intact dung. All soil particles attached to the dung have been eliminated with the help of pliers.

When the number of dung beetle into the intact dung was high, the intact dung was placed in a dark rectangular plastic container with a light bulb connected to one extremity. Under the light bulb it was made a hole where a plastic jar with a funnel at its opening was placed in order to prevent the return of the beetles into the dung. Dung was left 24h inside the boxes and finally rechecked in order to eliminate the remaining beetles. Intact dung was finally dried at 90 °C to a constant weight. Intact dung dry weight was then subtracted to initial dry weight in order to calculate the percentage of dry weight dung removed [(grams of removed dry dung/grams of initial dry dung)*100].

The community attributes (abundance, biomass, species richness, exponential of Shannon diversity ^{1}D, *FD*, *wFD*, *FEve* and *FDis*) were calculated also for the dung removal experiment in order to evaluate the real impact of dung beetle community on the ecological process. Total biomass was calculated using average fresh biomass of each species, and multiplying it by their abundance (See Annex 2 for more detail).

The effects of grazing intensity, VMPs use and their interaction on percentage of dry dung removed and community attributes were evaluated using a full factorial generalized linear model. Pairwise comparisons were made using the Tukey post-hoc test. All statistical analysis was performed with Statistica 7.0 package (StatSoft, 2004).

4.3. Results

Functional diversity and community attribute relationship

The historical use of VMPs showed a general significant effect on functional diversity (*Wilks's lambda* = 0.0077; $F_{[4,5]}$ = 161.15; $P < 0.00001$), that was drive by *FD* which showed an higher value in the areas without any use of VMPs ($P < 0.0005$), and *FEve* ($P < 0.005$) which reversely showed an higher value in the areas with VMPs use. Whereas no significant difference exists for *wFD* ($P = 0.207$), and *FDis* ($P = 0.22$) (Fig. 1).

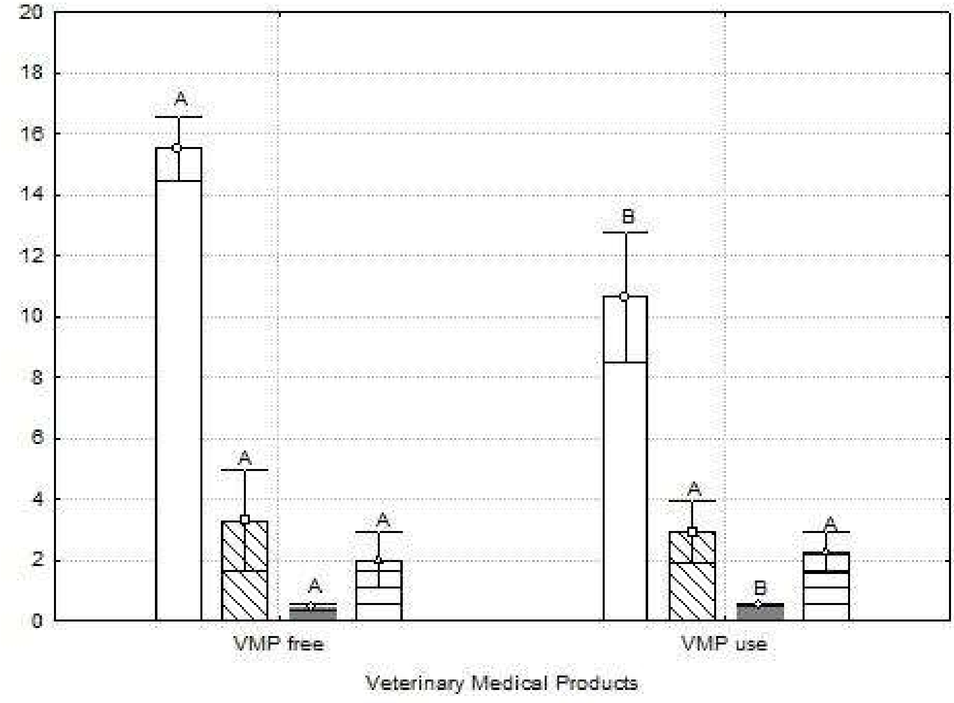

Figure 1: Functional diversity indices of dung beetle communities inhabit sites with different Veterinary Medical Products use (VMP use and VMP free) in sub-mountainous landscapes of Central Italy. *FD* is show in white, *wFD* oblique lines, *FEve* in grey and *FDis* horizontal bars. Dots represents mean and bars represent standard errors. Different letters mean significant differences (post-hoc Tukey test P < 0.05).

There are significant difference in functional diversity due to grazing intensity (*Wilks's lambda* = 0.021; $F_{[4,5]} = 58.19$; $P < 0.0005$) with moderate intensity that showed higher level for all the functional diversity indices (*FD*, $P < 0.0005$; *wFD*, $P < 0.0005$; *FEve*, $P < 0.05$; *FDis*, $P < 0.0005$) (Fig. 2). A significant interaction occur between the two factors (*Wilks's lambda* = 0.041; $F_{[4,5]} = 29.18$; $P < 0.005$), but it seem an antagonistic effect due to its lower effect compared with the main effect of each factor alone.

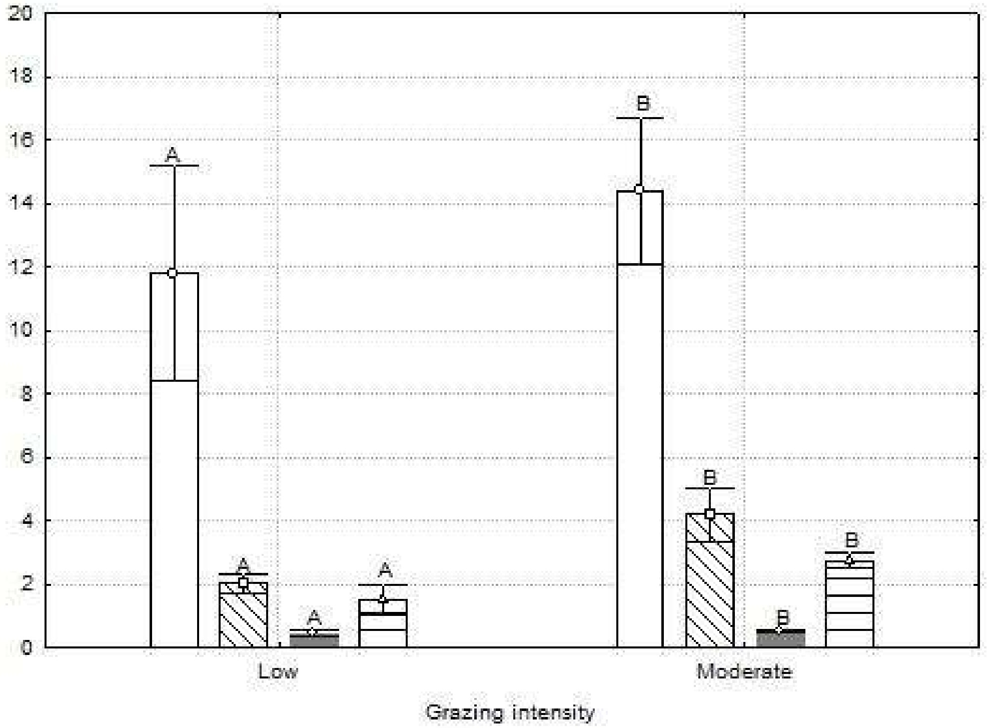

Figure 2: Functional diversity indices of dung beetle communities inhabit sites with different grazing intensity levels (low and moderate) in a sub-mountainous landscape of Central Italy. *FD* is show in white, *wFD* oblique lines, *FEve* in grey and *FDis* horizontal bars. Dots represents mean and bars represent standard errors. Different letters mean significant differences (post-hoc Tukey test P < 0.05).

Positive significant correlation exists between species richness and *FD*, the same occur among ^{1}D, *wFD* and *FDis*, and also between *FEve* and *FDis* (Table 1).

Table 1. Pearson correlation matrix for the community biodiversity metrics. Number in bold indicate significant correlation between variables ($P < 0.05$).

	Species richness	^{1}D	*FD*	*wFD*	*FEve*	*FDis*
Species richness	1.00					
^{1}D	0.43	1.00				
FD	**1.00**	0.42	1.00			
wFD	0.53	**0.97**	0.52	1.00		
FEve	-0.33	0.45	-0.34	0.44	1.00	
FDis	0.18	**0.93**	0.18	**0.86**	**0.58**	1.00

Dung removal and Functional Diversity

We totally analyzed 60 samples of dung pile because 4 samples (2 for spring VMPs use and 2 for spring VMPs free) are lost due to cow trampling. On average, 16.81% (Standard deviation ± 12,64%) of dry dung was buried in the 24h of the experiment. The grazing intensity significantly affect dung removal process ($F_{[1,55]} = 18.66$; $P < 0.0001$) with the moderate grazing community that can degrade almost twice (mean 21.3% ± 2.6 Std. Err.) dung respect to low grazing area (mean 12.4% ± 1.8 Std. Err.) (Post-hoc Tukey test $P < 0.0005$). There was significant difference in dung burial capacity due to VMPs use ($F_{[1,55]} = 82.26$; $P < 0.00001$) with the area without any use of VMPs that show more than three times (25.9% ± 1.7 Std. Err.) the dung burial capacity in respect of VMP use areas (7.7% ± 1.5 Std. Err.) (Post-hoc Tukey test $P < 0.0005$). A non-significant interaction exist between grazing intensity and VMPs use factors ($F_{[1,55]} = 0.002$; $P = 0.967$) (Fig. 3).

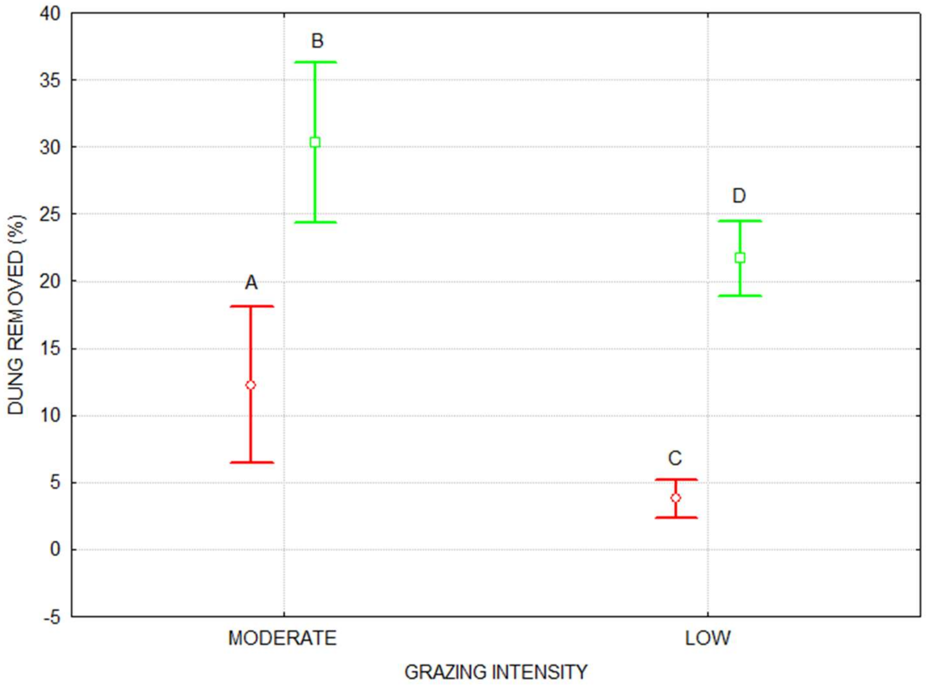

Figure 3: Dry dung removed (%) after 24h by dung beetle communities inhabit sites with different grazing intensity levels (low and moderate) and Veterinary Medical Products use (VMP use and VMP free) in sub-mountainous landscapes of Central Italy. Dung removed in sites with VMPs use are shown in red, whereas the dung removed in sites without any use of VMPs are in green. Dots represents mean and bars represent standard errors. Different letters mean significant differences (post-hoc Tukey test $P < 0.05$).

During dung removal experiment, we totally sampled 50,593 specimens belonging to 32 species. In the area VMPs Free 49,322 specimens belonging to 31 species was found whereas in the area with historical use of VMPs only 1,271 specimens belonging to 19 species were found. In the low grazing intensity areas 45,381 specimens belonging to 23 species was found, whereas in the moderate grazing intensity areas 5,212 specimens belonging to 30 species was trapped (Table 2).

Table 2. Number of individuals of dung beetle species collected during dung burial experiment from each treatment of the two factors. For each treatment, total abundance and total species richness was also reported. LG: Low grazing intensity; MG: Moderate grazing intensity; VMPs Free: areas without use of veterinary medical products; VMPs Use: areas with an historical use of veterinary medical products.

Species	**LG**	**MG**	**VMPs Free**	**VMPs use**	**TOTAL**
Acrossus luridus (Fabricius, 1775)	2	15	17	0	17
Agrilinus convexus (Erichson, 1848)	1	1	2	0	2
Aphodius fimetarius (Linnaeus, 1758)	1	5	4	2	6
Aphodius foetidus (Herbst, 1783)	0	33	33	0	33
Bubas bison (Linnaeus, 1767)	0	49	49	0	49
Caccobius schreberi (Linnaeus, 1767)	1	5	2	4	6
Calamosternus granarius (Linnaeus, 1767)	0	24	24	0	24
Chilothorax conspurcatus (Linnaeus, 1758)	2	0	2	0	2
Colobopterus erraticus (Linnaeus, 1758)	22	147	84	85	169
Esymus merdarius (Fabricius, 1775)	0	2	2	0	2
Esymus pusillus (Herbst, 1789)	6	6	6	6	12
Euoniticellus fulvus (Goeze, 1777)	5	1141	1119	27	1146
Geotrupes spiniger Marsham, 1802	1	3	4	0	4
Melinopterus consputus (Creutzer, 1799)	43032	277	43295	14	43309
Melinopterus prodromus (Brahm, 1790)	7	125	112	20	132
Nimbus contaminatus (Herbst, 1783)	829	2	831	0	831
Nimbus obliteratus (Panzer, 1823)	93	16	107	2	109
Onthophagus coenobita (Herbst, 1783)	0	6	6	0	6
Onthophagus fracticornis (Preyssler, 1790)	235	2200	1731	704	2435
Onthophagus grossepunctatus Reitter, 1905	1	144	144	1	145
Onthophagus joannae Goljan, 1953	6	0	1	5	6
Onthophagus lemur (Fabricius, 1781)	44	35	66	13	79
Onthophagus medius (Kugelann, 1792)	905	318	957	266	1223
Onthophagus ruficapillus Brullé, 1832	6	177	179	4	183
Onthophagus taurus (Schreber, 1759)	1	338	336	3	339
Onthophagus verticicornis (Laicharting, 1781)	178	107	175	110	285
Othophorus haemorrhoidalis (Linnaeus, 1758)	0	3	3	0	3
Sericotrupes niger (Marsham, 1802)	0	8	8	0	8
Sigorus porcus (Fabricius, 1792)	1	9	10	0	10
Sisyphus schaefferi (Linnaeus, 1758)	2	6	7	1	8
Trichonotulus scrofa (Fabricius, 1787)	0	9	6	3	9
Trypocopris vernalis apenninicus Mariani, 1958	0	1	0	1	1
Total abundance	**45381**	**5212**	**49322**	**1271**	**50593**
Species richness	**23**	**30**	**31**	**19**	**32**

The historical use of VMPs affect community metrics (*Wilks's lambda* = 0.404; $F_{[8,46]} = 8.49$; $P < 0.00001$) with species richness ($P < 0.0005$), abundance ($P < 0.005$) and biomass ($P < 0.0005$) were significantly diminished in areas with VMPs use, but ${}^{1}D$ not showed significant variation ($P = 0.215$). Three functional diversity measure resulted not significant different between VMPs Use and VMPs Free areas for Tukey post hoc test (*wFD*, $P = 0.113$; *FEve*, $P = 0.148$; *FDis*, $P = 0.358$). *FD* was strongly diminished due to

VMPs use, with a mean value less than half compared with areas without any use of VMPs ($P < 0.0005$).

Grazing intensity affect community metrics (*Wilks's lambda* = 0.548; $F_{[8,46]}$=4.74; $P < 0.0005$) with species richness ($P < 0.0005$), ^{1}D ($P < 0.005$), *FD* ($P < 0.0005$), *wFD* ($P < 0.0005$); *FEve* ($P < 0.05$) and *FDis* ($P < 0.001$) that showed higher values in moderate grazing areas compared with low grazing areas. Reversely, abundance ($P < 0.05$) showed higher value in low grazing areas, whereas biomass showed no significant differences ($P = 0.831$).

A significant interaction occur between the two factors (*Wilks's lambda* = 0.587; $F_{[8,46]}$ = 4.05; $P < 0.005$), but it seem an antagonistic effect due to its lower effect compared with the main effect of each factor alone.

Because the biodiversity metrics of the whole community (annual data) maintained the same pattern in the dung removal experiment, we are entitled to use this lasts in order to evaluate the possible effect of the functional diversity on ecological process.

4.4. Discussion

Several studies have investigated the effects of grazing intensity and VMPs use on dung beetle communities, using "classical" biodiversity measures (i.e. species richness, Shannon index) and total abundance/biomass data to reach their conclusions (Krüger and Scholtz, 1998; Carpaneto et al., 2005; Jay-Robert et al., 2008; Basto-Estrella et al., 2014; Tonelli et al., 2017). However, the impacts of these factors have not been explored from a functional point of view. In this study we investigate for the first time the effects of grazing intensity and historical use of Veterinary Medical Products from a functional standpoint, comparing different functional diversity metrics and the ecological process of dung beetle communities from various pastures management.

We showed that species richness and ${}^{1}D$ are good proxies of *FD* and *wFD* respectively, due to their strong linear correlations. These results are in accordance with several studies that investigate this relationship from several taxa (Micheli and Halpern, 2005; Heino, 2008; Bihn et al., 2010; Mayfield et al., 2010; Mouchet et al., 2010; Biswas and Mallik, 2011; Lohbeck et al., 2012), even if the shape and the intensity of this correlation seems to be context dependent (Díaz and Cabido, 2001; Fonseca and Ganade, 2001; Mayfield et al., 2010; Biswas and Mallik, 2011). This linear relationship denotes a high level of functional complementarity and hence a low level of redundancy among our community of dung beetles: each species belongs to different functional niches, and its extinction leads to a loss of its functional role into the community. This suggest a concern about the conservation of dung beetles, because is know that a lack of functional redundancy may be an indication of lack of resilience of the system (Laliberté et al., 2010). Moreover, these results raise some concerns about the quantity and the types of functional traits that must be used in functional diversity analysis. It is know that strong correlation between traits (Naeem and Wright, 2003), selection of only categorical traits (Tsianou and Kallimanis, 2016) and the high number of traits (Cadotte et al., 2011) produce low redundant functional diversity output. Hence the traits must to be selected with care, and on biological basis. However, the context dependency of functional diversity-species richness correlation (Mayfield et al., 2010; Biswas and Mallik, 2011) and the non-random loss of species (Larsen et al., 2005), suggests to use the functional diversity measures in order to investigate their behavior in several situations (Cadotte et al., 2011), and obtain complementary information.

In the areas with an historical use of VMPs a loss of *FD* occurred. This is due by the fact that VMPs use reduce the quantity of functional niche presents in the community. This impact is linked to the loss of species richness, which is strongly correlated with *FD*, and reflect a high level of traits complementarity among the species. The impact of these veterinary substances on functional diversity, however, seems a random action that affect indifferently the functional traits types and their abundance, reflecting in the maintenance of functional structure as showed by the *wFD*, *FEve* and *FDis* measures. Even if several authors (Cornwell et al., 2006; Pakeman, 2011; de Bello et al., 2013) proposed that a loss of the richness of functional niches is due to an environmental filter effect (only adapted traits can remain in a perturbed environment), we do not have any evidence that this is the case. Indeed, the Ivermectin acts indistinctly on all dung beetle species (Puniamoorthy et al., 2014; Tonelli et al., 2017) and there is not any reason that some selected traits were more affected. In other words, in the study region, ivermectin impacts ubiquitously on dung beetle populations by decreasing their abundance, maintaining the community structure (even functionally) and lead to a loss of rare species (Tonelli et al., 2017).

On the contrary, grazing intensity affects all functional diversity metrics, with moderate grazing areas that showed higher values for *FD*, *wFD*, *FEve* and *FDis*. This can be due to the fact that a reduced quantity of trophic resource lead to a loss of the *FD* due to species loss. It is possible that this factor has been acted as a filter for the community, allowing only some functional niche to remaining in this area. Indeed, our previous research highlight the fact that the loss of trophic resource differentially impacts on dung beetle community, preventing the presence of large body size species and promoting the presence of opportunistic dung beetles (Tonelli et al., 2017). The contemporary decreasing in the functional structure, as highlighted by *wFD*, *FEve* and *FDis*, point out a strong functional redundancy of this community, due to the strong similarity in the functional niche of the species that belong to this community.

The absence of studies that investigate the effects of grazing intensity and VMPs use on dung beetle functional diversity, prevent us to make precise comparisons and drawn strong conclusions. However, comparing our results with other studies that investigate the effects of environmental stressor (i.e. land use change) on functional diversity of dung beetle, we found accordance and discrepancies. For example, Edwards et al. (2014) found that oil palm plantation in Southeast Asian forest lead to a decrease in the number of functional niches and their eveness due to the loss of forest specialists and a shift of dominance toward disturbance tolerant species. Audino et al. (2014) found a loss of number of functional niches in secondary and primary forest of Brazil compared with a degraded area, but *FEve* and *FDis* not showed significant differences. The same results were found by Barragán et al. (2011) in Mexico, although the loss of functional richness was found in degraded areas. Hence, this variability in the results highlight the need for more studies that investigate the effect of several environmental stressors on dung beetle functional diversity.

The fact that interaction term between grazing intensity and VMPs use was less significant than the main effect may be a corroboration that they act differently on dung beetle community (Tonelli et al., 2017). Indeed, functional diversity analysis highlight that the loss of trophic resource availability act as a filter allowing to only some functional niche to persist in this areas. On the other part, VMPs use seem to impact dung beetle population by diminished their abundance without differential effect on some particular functional niche.

Are functional diversity metrics a good surrogate for ecological process? Several studies use the functional diversity measures in order to forecast the impacts on ecological functioning (Barragán et al., 2011; Edwards et al., 2014). This because a large extent of literature highlight that functional diversity is strongly related with ecological processes (Díaz and Cabido, 2001; Lefcheck and Duffy, 2015). However, in some cases this naïve equation has been questioned due to the context dependence of Biodiversity-Ecological Functioning relationship (Hiddink et al., 2009; Needham et al., 2011). Our results support, almost partially, the fact that functional diversity measures not always reflect completely the impacts on ecological process and then highlight the necessity to evaluate empirically the process rather than deduce it only from community attribute.

Indeed, in the areas with an historical use of VMPs, although only a decrease in functional richness (*FD*) exist with a maintenance of functional structure (*wFD*, *FEve* and *FDis*), 70% of dung burial capacity was lost. Then in these areas a neutral mechanism may occur, where functional diversity is not very important and total dung beetle biomass and abundance act as driver of ecological process (Kudavidanage et al., 2012; Giraldo et al., 2011; Tyndale-Biscoe, 1994; Tixier et al., 2015; Yamada et al., 2007). However, another direct mechanism may occur in the VMPuse areas that prevent to bury the dung efficiently. We know that dung beetle that feed on contaminated dung show an acute toxicity that prevent them to perform basics activities (Verdú et al., 2015). Even at low concentration, Ivermectin cause a reducing muscle force in adults of *Scarabaeus cicatricosus* Lucas, 1846 that limit its interaction with the environment. Others researches show that ivemerctin affect dung beetle reproductive behavior diminishing the number of brood mass buried (Ridsdill-Smith, 1988; Cruz Rosales et al., 2012; Dadour et al., 2000) and the weight of brood masses (Cruz Rosales et al., 2012). All this effects may lead to the limited influence that dung beetles have on the dung removal for dung pat contaminated with IVM (Beynon et al., 2012). Hence, although in our study the experimental dung pats are not contaminated with IVM, it could be that the beetles in that areas, by normally feeding with contaminated dung, are intoxicated by the substance that make them unable to carry out their activities.

The contrary occur for the grazing intensity factor, which results showed a higher level of abundance and a comparable level of biomass in the low grazing area, but this is not reflected in the ecological process that is half less efficient. These results seem to disagree with much other (Kudavidanage et al., 2012; Giraldo et al., 2011; Tyndale-Biscoe, 1994; Tixier et al., 2015; Yamada et al., 2007) that highlight the importance of total community abundance and biomass in the ecological process. Hence, in this case, the difference may be due to the functional niche of the species here present. For example, the loss of large dung beetle (i.e. *Bubas bison*) from low grazing areas may explicate this loss of dung burial capacity. In fact, exist a positive relation between body size and dung burial capacity, namely larger dung beetle can bury a greater amount of dung (Larsen et al., 2005; Braga et al., 2013; Slade et al., 2007; Nervo et al., 2014; Ortega-Martínez et al., 2016). Moreover, in the low grazing areas, opportunistic small dwellers dung beetles are favored (i.e. *Melinopterus consputus* (Creutzer, 1799)). *Melinopterus consputus* is a very small dweller species (about 4 mg of fresh weight) that not present a relocation behavior. It feed within dung pat or at the soil-dung interface where only a little amount of dung may be buried shallowly by the pedoturbation due of the great quantity of individuals (Tonelli, M., personal observation, 2015). Then, not always high density of small beetle compensates for dung removal efficiency as report in various studies (Nervo et al., 2014; Ortega-Martínez et al., 2016) because this depend on their functional niche (Braga et al., 2013; Slade et al., 2007). Then, further studies are necessary in order to investigate the relationship between dung beetle biomass and abundance with ecological process, even because this community attribute respond differently to environmental changes (Tonelli et al., *submitted*). In accord with Braga et al. (2013), our results highlight the necessity to evaluate empirically the process rather than deduce it only from community attribute, because do not exists a general and universal relationship.

The absence of interaction between factors for dung burial process is in line with community attributes that not present some interaction with grazing intensity and VMPs use factors.

Thus, we would like to stress that the loss of dung burial capacity (as a consequences of biodiversity pattern alteration) due to VMPs use is worse respect to the loss due to grazing intensity. In fact, although the amount of dung buried in the low grazing areas is little, this seems proportionate to the quantity of dung produced by cattle; approximately half of the number of the cattle, correspond to about half of the buried dung respect to moderate grazing areas. This is not true for VMPs use areas where, regardless of the number of cattle, there is always a minor amount of buried dung. These results implicate that in the VMPs pastures a real risk of dung accumulation exist.

Our results have some conservationist and management implication. In fact, the quantity of dung buried is a representation of many others ecological processes such as seed dispersion, nutrient recycling, pedoturbation and parassite suppression (Slade et al., 2007; Braga et al., 2013; Gregory et al., 2015) and the loss of this ecosystem functions can be very detrimental to the preservation of the health of the entire pasture.

4.5. Conclusions

We demonstrate that grazing intensity affect all the parameters of dung beetle functional diversity, probably due the filter effect of this factor on some functional niches. This resulted in a loss (-42%) in dung burial capacity in the low grazing areas even if this last show a greater dung beetle abundance.

On the contrary, the sites with VMPs use show 70% less dung burial capacity although its functional structure was maintained compared with sites without VMPs application. These results highlight the risk of dung accumulation in pastures where VMPs are used, whereas are of less concern in areas low grazed because of the proportionality between grazing intensity and buried dung.

Moreover, we would to stress the necessity of evaluate empirically the ecological process rather than deduce it only from functional diversity measures, because do not exists a general and universal relationship.

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CHAPTER 5

The effects of progressive grazing abandonment on dung beetle functional diversity and ecological process

5.1. Introduction

Species today show an extinction rate a thousand times higher than the natural background value (Lawton and May, 1995; Pimm et al., 1995; Pimm and Raven, 2000). One of the principal factors of this biodiversity loss is the land-use change (Sala et al., 2000). However, most of the studies evaluated the impact of land use intensification (Flynn et al., 2009; Lindenmayer et al., 2012), while the abandonment of traditional human activities, such as extensive grazing, received less attention (MacDonald et al., 2000). Moreover, the influence of land use change on biodiversity is often studied using "classical" biodiversity metrics (e.g. species richness and Shannon diversity) (Newbold et al., 2015), which consider all species as equivalent and are not sensible to species life-history differences. However, changes in the environmental conditions may impact differentially on each species and, acting as a filter, permitting only a narrow range of traits to persist (Laliberté et al., 2014). Several measures of functional diversity were developed, which allow to evaluate the communities considering the differences among the species (Mouchet et al., 2010; Pla et al., 2012). Functional diversity was defined as "the kind, range and relative abundance of functional traits present in a given community" (Díaz et al., 2007) where the functional traits are "any morphological, physiological or phenological feature measurable at the individual level, from the cell to the whole-organism level, without reference to the environment or any other level of organization" (Violle et al., 2007). These measures were mechanistically linked to ecosystem process performance and community assembly rules (Díaz and Cabido, 2001; Spasojevic and Suding, 2012) highlighting the importance of considering species difference in ecological studies.

Dung beetle community provides an excellent model to evaluate the impact of grazing abandonment on functional diversity. Due to the functional difference among species we can evaluate the impact of environmental stress on their functional diversity and, consequently, attempt to investigate the relationship between functional diversity and dung beetle ecosystem process. Indeed, dung beetles are involved in many ecological processes as nutrient cycling, vegetation growth, soil structure, and dung removal (Nichols et al., 2008). The capacity of dung beetles to maintain a high level of this ecological process, however, is influenced by various community attributes whose decline would lead to a reduction of the community functionality. Among the most important attributes of dung beetle community linked to the ecological processes there are: species richness (Larsen et al., 2005; Beynon et al., 2012; Gollan et al., 2013; Slade et al., 2011), richness and type of functional groups (Slade et al., 2007; Beynon et al., 2012), species body size (Larsen et al., 2005; Nervo et al., 2014), community abundance and biomass (Braga et al., 2013; Gollan et al., 2013; Tixier et al., 2015; Giraldo et al., 2011). However, few studies evaluated the link between dung beetle functional diversity and ecological process (Griffiths et al., 2015). Furthermore, several studies extrapolate the consequences for the ecological process performance only studying the functional diversity metrics (Barragán et al., 2011; Edwards et al., 2014). This absence of empirical evidence about the real impact on the ecological process may conduce to worrying mistakes from a management and conservation point of view (Braga et al., 2013), because this relationship cannot be generalized (Griffiths et al., 2015).

In a previous work in the same study area (Tonelli et al., *submitted*), we showed that progressive grazing abandonment lead to a differential impact on dung beetle species depending on their body size and nesting behavior. This provides us a perfect case in order to evaluate if grazing abandonment impact also on dung beetle functional diversity.

The aims of the present study were to investigate the impact of progressive grazing abandonment on dung beetle communities from a functional standpoint. We attempt to answer the following questions: i) what are the effects of progressive grazing abandonment on dung beetle functional diversity? ii) what are the relationship between classical biodiversity metrics and functional diversity metrics? iii) What are the repercussions of grazing abandonment on the dung beetle ecological process (dung burial)? and iv) can differences in the functional biodiversity measures explain the differences in the ecological process?

5.2. Matherials and Methods

Study area

The study was carried in sub-mountains areas of Pesaro-Urbino province in the Marche region, Italy. Provincial climate falls into the temperate Köppen's categories (Cfa and Cfb), with average annual temperature around 12 °C (average min 3.5 °C – average max 21 °C). Average annual precipitation is around 930 mm with one driest period in summer and another in winter (www.lavalledelmetauro.it/contenuti/geologia-clima/indice.html). The arboreous vegetation of the study sites is dominated by *Quercus ilex* L., *Quercus pubescens* Willd., *Quercus cerris* L., *Ostrya carpinifolia* Scop. and *Fraxinus ornus* L. whereas the herbaceous vegetation belongs to the *Brizo mediae-Brometum erecti* and to *Asperula purpureae-Brometum erecti* phyto-associations. Soil is calcareous.

To evaluate the effect of the progressive pasture abandonment we compare sites with different livestock charge from abandoned to moderate grazing intensity:

a) 'Abandoned' (Calamello-Paravento pastures; 43°30'43,00''N; 12°40'58,68''E): These pastures were abandoned about fifteen years ago, and are located between 550 and 750 m a.s.l. Today, these pastures are only populated by wildlife fauna such as roes (*Capreolus capreolus* Linnaeus, 1758), wild boars (*Sus scrofa* Linnaeus, 1758) and fallow deers (*Dama dama* Linnaeus, 1758). These pastures are undergoing a process of shrubs and trees encroachment principally by *Quercus ilex*, *Spartium junceum* L. and *Rosa canina* L. (Tonelli, M., personal observations, 2013).

b) 'Low Grazing Charge' (Pietralata pastures; 43°39'33,64''N; 12°42'27,65''E): These secondary grasslands are located between 750 and 900 m a.s.l. The livestock charge of this pasture is about 0.7 Livestock unit/ha.

c) Moderate Grazing Charge (Montebello pastures; 43°43'13,83''N; 12°45'19,98''E): these pastures are located between 500 and 600 m a.s.l. whitin the ®Gino Girolomoni Cooperativa Agricola. These pastures are used by cows bred according to the organic farming rules. The livestock charge of this pasture is about 1.5 Livestock Unit/ha.

The density of wild fauna (i.e. *Capreolus capreolus* (L., 1758) and *Sus scrofa* (L., 1758) is very similar among all the studied areas (Tonelli, personal observations, 2013).

Dung beetle trapping

The sampling was repeated about every 15 days from June 2013 to November 2013 and in May and June 2014. For each treatment, we selected three sampling sites separated by at least 500 m to ensure independence among the replicates (Silva and Hernández, 2015). Four pitfall traps spaced at least 50 m are placed at each site and were baited with cow and horse dung (about 500 cm^3) to maximize differential species attraction (Barbero et al., 1999; Dormont et al., 2004, 2007). We filled the pitfall traps with propylene glycol (50%) to preserve the dung beetles we collected. Due to the scarcity of dung in the neighbourhood of abandoned area, we randomly placed about 10 dung pats in the area around the trapping site. This was in order to avoid the bias of excessive attractiveness of traps (Lobo et al., 1998). The traps were left active for 48 h in each sampling period. The dung beetles were identified to specific level.

Functional diversity analysis

Twenty-four traits were selected and measured in order to analyze functional diversity (See Annex 2 for more detail on traits selection and measurement). To test the hypothesis that progressive grazing abandonment negatively impact dung beetle functional diversity, we used five indexes that measure different aspects of dung beetle community: the richness component (*FD*), the structure component, which reflect the abundance distribution (*wFD*, *FEve* and *FDis*) and the functional unicity of the community (*FSpe*). *FD* (Petchey and Gaston, 2006) and its weighted version *wFD* (Pla et al., 2012), respectively measures the total length of the branches of a functional dendrogram and how the abundances are distributed within it. *FD* reflect the range of the functional niches present into the community and therefore indicate the number of ways in which species interact with the environment (Mason et al., 2005). Two indices that are sensible to the functional niches abundance distribution within the functional space of the community were used: *FEve* (Villéger et al., 2008), and *FDis* (Laliberté and Legendre, 2010). *FEve* measures the regularity of spacing between species in the trait space and also the evenness of species abundance, whereas *FDis* is the average distance of individual species to the centroid of all species in the community trait space taken into account the relative abundances of species for computing the weighted centroid. *wFD*, *FEve* and *FDis*, identify if the resource is evenly exploited by the functional niches of the community (Mason et al., 2005). Moreover, we used an index of functional specialization, *FSpe*, which measure how functionally unique a community is relative to the regional pool of species (Bellwood et al., 2006).

All measures were calculated using the software FDiveristy (Casanoves et al., 2011). These measures were calculated after normalization of traits and the application of Principal Components Analysis in order to reduce matrix dimensionality. We used the first 13 axes of PCA that contains the 96% of total variance. The PCA was calculated with the software PAST 3.14 (Hammer et al., 2001).

The effects of grazing intensity were evaluated using a generalized linear model. Pairwise comparisons were made using the Tukey post-hoc test. This statistical analysis was performed with Statistica 7.0 package (StatSoft, 2004). The relationship among species richness, exponential of Shannon diversity (^1D) and functional diversity metrics was investigate using Pearson correlation test performed with Statistica 7.0 package (StatSoft, 2004). Data about species richness and exponential of Shannon (^1D) derived from Tonelli et al. (*submitted*).

Experimental design and analysis of dung removal

The basic procedure was identical to that described in the chapter 4 where further details are given. Here, we briefly explicate the mains information. Dung removal experiment was performed twice, once in autumn (15-16/10/2015) and another in spring (17-18/5/2016). We selected this sampling period because prior studies highlighted that they contain 79% of number of species and 68% of abundance of the community (Tonelli, unpublished data). We selected eight sampling sites (replicates) for each treatment spaced by at least 100 m (Larsen and Forsyth, 2005; Silva and Hernández, 2015). At each sampling site we placed a plastic container where a mean of 595 g (Standard deviation ± 40 g) pile of dung, of known humidity, was positioned. Plastic containers were left active on the field 24 h, after which intact dung and dung beetles were recovered to be, respectively, identified and weighed (dry weight). The percentage of dry weight dung removed was calculated as: [(grams of removed dry dung/grams of initial dry dung)*100]. We randomly placed 10 dung pats around sampling sites of abandoned area as described in the dung beetle trapping heading.

The community attributes (abundance, biomass, species richness, exponential of Shannon index ^{1}D, *FD*, *wFD*, *FEve*, *FDis* and *FSpe*) were calculated also for the dung removal experiment in order to evaluate the real impact of dung beetle community on the ecological process. Total biomass was calculated using average fresh biomass of each species (see annex 2 for more detail), and multiplying it by their abundance.

The effects of progressive grazing abandonment on percentage of dry dung removed and community attributes were evaluated using a generalized linear model. Pairwise comparisons were made using the Tukey post-hoc test. All statistical analysis was performed with Statistica 7.0 package (StatSoft, 2004).

5.3. Results

Functional diversity and community metrics relationships

Progressive grazing abandonment have significant effect on dung beetle functional diversity (*Wilks's lambda* = 0.00006; $F_{[10, 4]} = 49.68$; $P < 0.001$) with a significant progressive loss of FD from moderate grazing areas, to low and abandoned ones (moderate vs. low $P < 0.05$; moderate vs. abandoned $P < 0.0005$; low vs. abandoned $P < 0.01$) (Fig. 1).

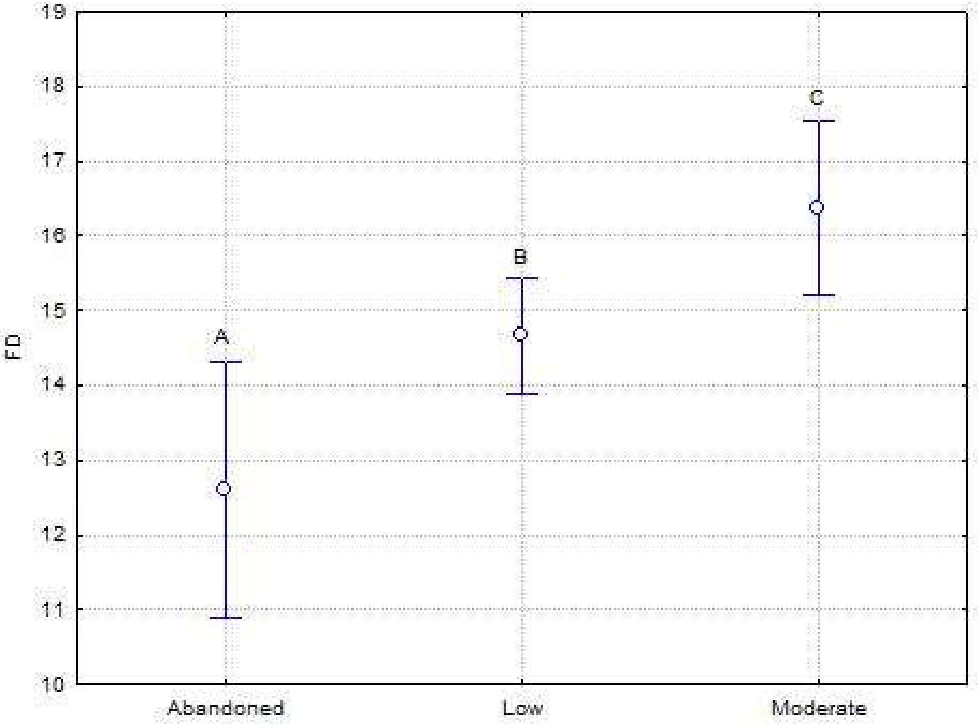

Figure 1: Functional richness component, expressed as *FD*, of dung beetle communities inhabit areas with different grazing intensity (abandoned, low and moderate) in sub-mountainous landscapes of Central Italy. Dots represents mean and bars represent standard errors. Different letters mean significant differences (post-hoc Tukey test $P < 0.05$).

On the contrary, *wFD* and *FEve* showed a comparable value between moderate and abandoned areas (*wFD*, $P = 0.219$; *FEve*, $P = 0.498$), that are both higher compared to low grazing area (*wFD*, $P < 0.001$; *FEve*, $P < 0.01$) (Figure 2). Respect to *FDis*, abandoned areas showed the higher value compared to moderate (*P* < 0.005) and low grazing sites (*P* < 0.0005), whereas moderate grazing area showed significant high level respect to low grazing site (*P* < 0.0005) (Fig. 2).

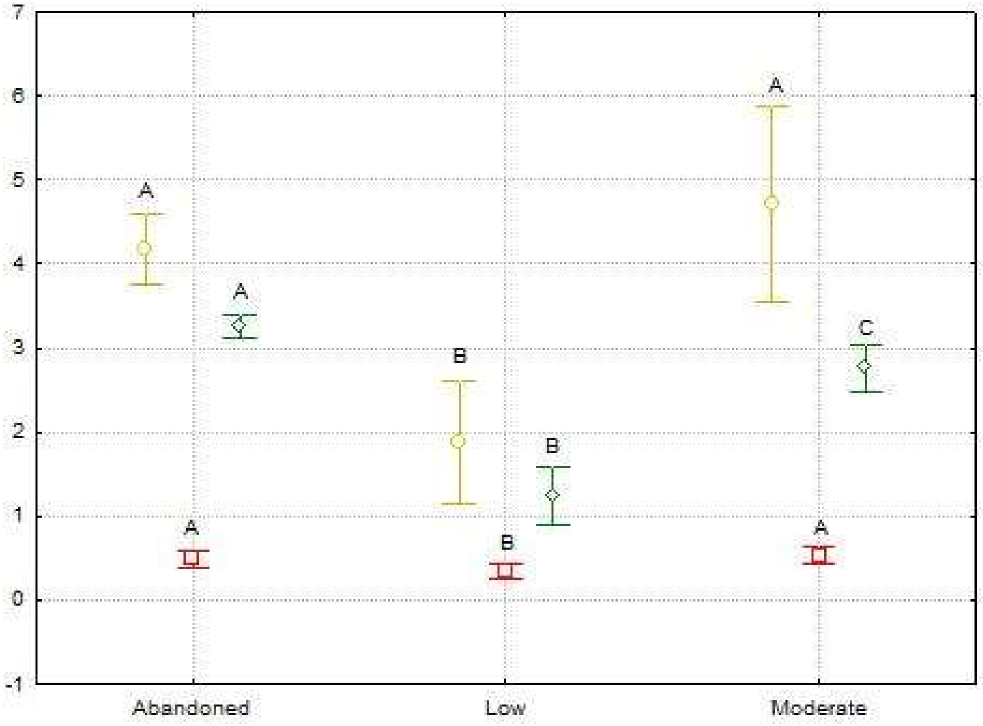

Figure 2: Functional structure component, expressed as *wFD* (yellow dots), *FEve* (red squares) and *FDis* (green diamonds), of dung beetle communities inhabit areas with different grazing intensity (abandoned, low and moderate) in sub-mountainous landscapes of Central Italy. Dots, squares and diamonds represents mean and bars represent standard errors. Different letters mean significant differences (post-hoc Tukey test *P* < 0.05).

FSpe measure show a general trend of increasing from moderate to low and abandoned areas, with abandoned areas that have significant higher value respect to low (*P* < 0.005) and moderate grazing areas (*P* < 0.001), whereas no significant difference exist between moderate and low grazing areas (*P* = 0.569) (Fig. 3).

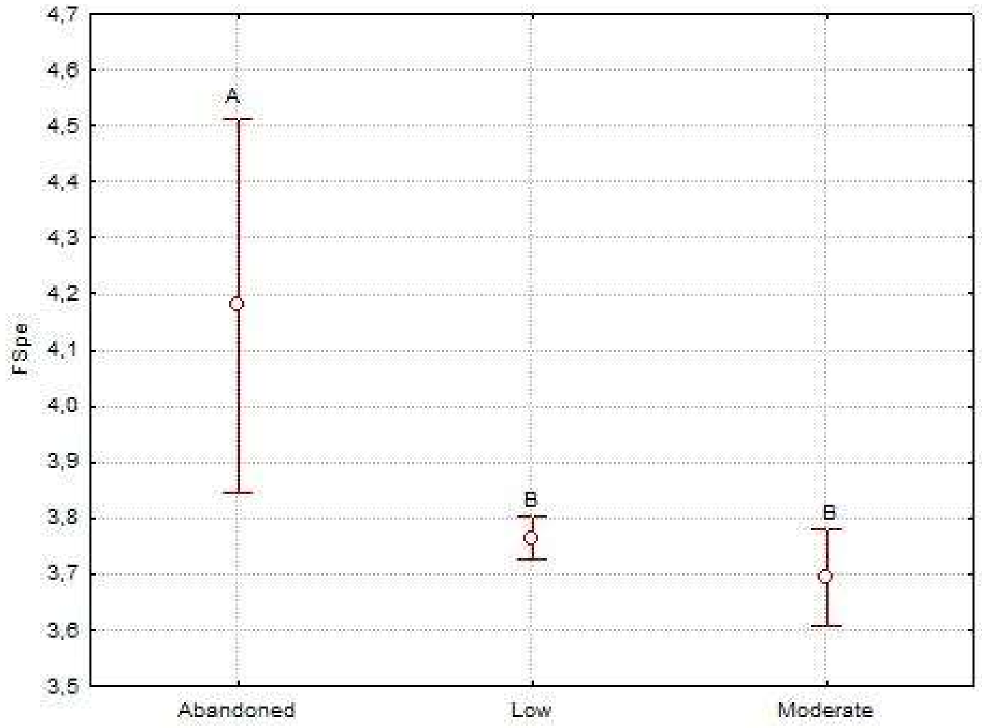

Figure 3: Functional unicity component, expressed as *FSpe*, of dung beetle communities inhabit areas with different grazing intensity (abandoned, low and moderate) in sub-mountainous landscapes of Central Italy. Dots represents mean and bars represent standard errors. Different letters mean significant differences (post-hoc Tukey test $P < 0.05$).

Positive significant correlation exists between species richness and *FD*, the same occur among ${}^{1}D$, *wFD*, *FEve* and *FDis*, and also between *wFD*, *FEve* and *FDis* and finally, between *FEve* and *FDis*. On the contrary, negative significant relationship exists between *FSpe* and species richness and *FD* (Table 1).

Table 1. Pearson correlation matrix for the community biodiversity metrics. Number in bold indicate significant correlation between variables ($P < 0.05$).

	Species richness	${}^{1}D$	*FD*	*wFD*	*FEve*	*FDis*	*FSpe*
Species richness	1.00						
${}^{1}D$	-0.06	1.00					
FD	**1.00**	-0.1	1.00				
wFD	0.16	**0.96**	0.14	1.00			
FEve	0.12	**0.79**	0.1	**0.84**	1.00		
FDis	-0.24	**0.96**	-0.28	**0.89**	**0.84**	1.00	
FSpe	**-0.88**	0.4	**-0.9**	0.2	0.21	0.56	1.00

Dung buried and Functional diversity

We totally analyzed 44 samples of dung pile because 4 samples (1 in abandoned area, 1 in low grazing area and 2 in moderate grazing areas) are lost due to domestic and wild mammals trampling. On average, 22,47% (Standard deviation ± 9,72%) of dry dung was buried in the 24h of the experiment.

The progressive grazing abandonment have a strong impact on dung burial process ($F_{[2,41]}$=12.18; $P < 0.0001$). There is a general trend of loss of dung burial capacity with the decrease of grazing intensity, with the moderate grazing area that show the higher value (30.33% ± 2.77 Std. Err.), follow by the low grazing area (21.68% ± 1.3 Std. Err.) and by the abandoned area (15.93% ± 1.94 Std. Err.). However, these differences are significant only between moderate grazing area and low grazing area (Post-hoc Tukey test $P < 0.05$), and between moderate grazing area and abandoned area (Post-hoc Tukey test $P < 0.0005$). No significant difference exists between low grazing area and abandoned area (Post-hoc Tukey test $P = 0.13$) (Fig. 4).

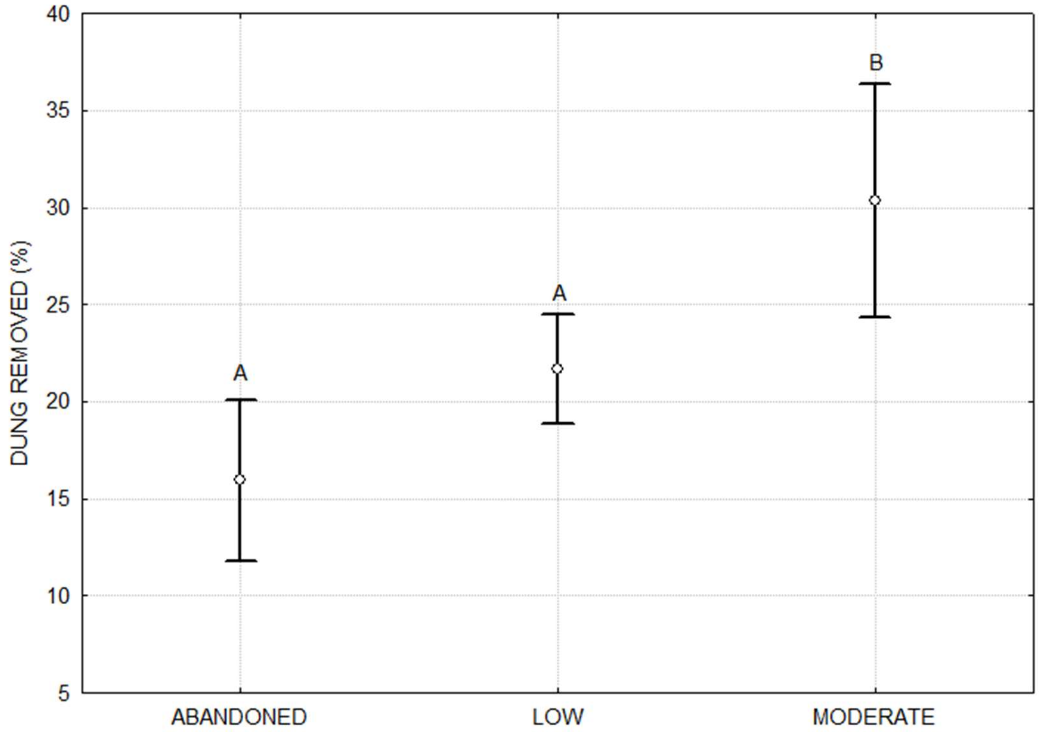

Figure 4: Dry dung removed (%) after 24h by dung beetle communities inhabit areas with different grazing intensity levels (abandoned, low and moderate) in sub-mountainous landscapes of Central Italy. Dots represents mean and bars represent standard errors. Different letters mean significant differences (post-hoc Tukey test $P < 0.05$).

During dung removal experiment, we totally sampled 49,679 specimens belonging to 32 species. In the moderate grazing intensity areas 4,038 specimens belonging to 28 species was found, in the low grazing areas 45,284 specimens belonging to 20 species were found, whereas in the abandoned areas 357 specimens belonging to 13 species were found (Table 2).

Table 2. Number of individuals of dung beetle species collected during dung burial experiment from each area of different grazing intensity. Total abundance and total species richness was also reported. MG: Moderate grazing intensity; LG: Low grazing intensity; AB: grazing abandoned.

Species	**MG**	**LG**	**AB**	**Total**
Acrossus luridus (Fabricius, 1775)	15	2	0	17
Agrilinus convexus (Erichson, 1848)	1	1	0	2
Aphodius fimetarius (Linnaeus, 1758)	4	0	0	4
Aphodius foetidus (Herbst, 1783)	33	0	0	33
Bodilopsis rufa (Moll, 1782)	0	0	1	1
Bubas bison (Linnaeus, 1767)	49	0	0	49
Caccobius schreberi (Linnaeus, 1767)	1	1	0	2
Calamosternus granarius (Linnaeus, 1767)	24	0	0	24
Chilothorax conspurcatus (Linnaeus, 1758)	0	2	0	2
Colobopterus erraticus (Linnaeus, 1758)	79	5	0	84
Esymus merdarius (Fabricius, 1775)	2	0	0	2
Esymus pusillus (Herbst, 1789)	6	0	0	6
Euoniticellus fulvus (Goeze, 1777)	1117	2	0	1119
Geotrupes spiniger Marsham, 1802	3	1	2	6
Melinopterus consputus (Creutzer, 1799)	274	43021	1	43296
Melinopterus prodromus (Brahm, 1790)	107	5	0	112
Nimbus contaminatus (Herbst, 1783)	2	829	2	833
Nimbus obliteratus (Panzer, 1823)	15	92	0	107
Onthophagus coenobita (Herbst, 1783)	6	0	14	20
Onthophagus fracticornis (Preyssler, 1790)	1502	229	83	1814
Onthophagus grossepunctatus Reitter, 1905	144	0	15	159
Onthophagus joannae Goljan, 1953	0	1	111	112
Onthophagus lemur (Fabricius, 1781)	22	44	24	90
Onthophagus medius (Kugelann, 1792)	93	864	1	958
Onthophagus ruficapillus Brullé, 1832	173	6	0	179
Onthophagus taurus (Schreber, 1759)	335	1	0	336
Onthophagus verticicornis (Laicharting, 1781)	0	175	54	229
Othophorus haemorrhoidalis (Linnaeus, 1758)	3	0	0	3
Sericotrupes niger (Marsham, 1802)	8	0	1	9
Sigorus porcus (Fabricius, 1792)	9	1	0	10
Sisyphus schaefferi (Linnaeus, 1758)	5	2	48	55
Trichonotulus scrofa (Fabricius, 1787)	6	0	0	6
Total abundance	**4038**	**45284**	**357**	**49679**
Species richness	**28**	**20**	**13**	**32**

The progressive grazing abandonment affected community metrics also during dung burial experiment (*Wilks's lambda* = 0.070; $F_{[18,66]} = 10.173$; $P < 0.00001$). Species richness was significantly higher in the moderate grazing areas respect to low ($P < 0.005$) and abandoned areas ($P < 0.0005$), between which there was no significant difference (*P*

= 0.5). Exponential of Shannon index ^{1}D was higher in the moderate grazing areas respect to low grazing areas ($P < 0.05$), whereas no differences exist between moderate and abandoned areas ($P = 0.93$), and between low grazing sites and abandoned ones ($P = 0.077$). Low grazing areas have higher abundance than moderate ($P < 0.005$) and abandoned areas ($P < 0.005$), between which no significance difference exists ($P = 0.942$). Considering biomass, moderate and low grazing areas have comparable values ($P = 0.127$), but both showed higher values respect to abandoned ones ($P > 0.001$). Regarding *FD*, moderate grazing intensity areas showed significant higher values respect to low ($P < 0.005$) and abandoned areas ($P < 0.0005$), between which no significant difference exists ($P = 0.558$). Considering *wFD*, low grazing areas showed the lowest values respect to Moderate ($P < 0.005$) and abandoned areas ($P < 0.05$), between which no significant difference was highlighted ($P = 0.492$). *FEve* showed comparable values among all areas, without significant differences. Regarding *FDis*, low grazing areas showed the lowest value respect to moderate ($P < 0.005$) and abandoned areas ($P < 0.01$), that on the contrary have comparable values between them ($P = 0.792$). *FSpe* was higher in the moderate grazing areas compared to low ($P < 0.0005$) and abandoned areas ($P < 0.005$), moreover, this last, showed significant higher values respect to low grazing areas ($P < 0.0005$).

5.4. Discussion

Whole community functional diversity and diversity metrics relationship

We showed that *FD* had a strongly linear relationship with species richness, hence each new species added to the community possess a unique functional niche. This means that the dung beetle community of study area have high level of functional complementarity (low functional redundancy). This is coherent with several other studies that investigate this relationship among several taxa (Micheli and Halpern, 2005; Petchey et al., 2007; Heino, 2008; Flynn et al., 2009; Bihn et al., 2010; Mayfield et al., 2010; Mouchet et al., 2010; Biswas and Mallik, 2011; Pakeman, 2011; Gerisch et al., 2012; Lohbeck et al., 2012; Luck et al., 2013; Griffiths et al., 2015). It was stressed that this strongly linear relationship it is not universal (Díaz and Cabido, 2001) and depend on several factors, such as number of traits (Cadotte et al., 2011), types of traits (Tsianou and Kallimanis, 2016), intensity and type of environmental disturb (Biswas and Mallik, 2011; Luck et al., 2013), and the number of species into the community (Luck et al., 2013). We selected a similar number of quantitative and qualitative traits basing on their ecological implication (cfr. Annex 2). Hence, this suggest that our results are not dependent on the methodology used, but provides an indication of really no redundant dung beetle communities. The same linear relationship among species richness and *FD* in communities submitted to different type and intensity of environmental stress (cfr. Chapter 4) reinforce our interpretation. However, more studies with more variation in species richness are needed in order to outline stronger conclusions.

The same may be said for the relationship among exponential of Shannon index (^{1}D) and *wFD*, *FEve* and *FDis*. This depend to the fact that the great species complementarity, when was weighted for species abundance, follow the same pattern. However, it is interesting to note the absence of relationship between ^{1}D and *FEve* in the community analyzed in the Chapter 4, whereas a significant correlation exists for the community investigated in the present areas. This mean that the relationship among functional and classical biodiversity metrics are context dependent (Díaz and Cabido, 2001; Fonseca and Ganade, 2001; Mayfield et al., 2010; Biswas and Mallik, 2011) and would be appropriate to evaluate empirically this association instead of relying on theoretic generalizations.

Among all functional indices tested, the most interesting seem the *FSpe*, which showed only a negative correlation with species richness and *FD*, and became the best candidate as useful index which can produce complementary information.

Hence, we suggest that functional diversity measure may be a useful method that can give us additional information about communities because they are, almost in part (i.e. *FSpe*), not completely related with classical biodiversity measures, and because this relationship vary among communities (cfr. Chapter 4).

FD diminished from moderate to abandoned, due to species loss. However, the functional structure of moderate and abandoned area, showed a well-structured community from a functional point of view whereas low grazing intensity area showed a lower value for *FEve*, *wFD* and *FDis*. Moreover, functional specialization (*FSpe*) showed a trend of increase from moderate, to low and abandonment. This results corroborate our previous interpretation (cfr. Chapter 4; Tonelli et al., *submitted*) that both, the quantity and the quality of trophic resource, act as an environmental filter, allowing only some functional niche to remaining in each area. Probably, from moderate to low grazing intensity a differential impact on dung beetle functional niches lead to a loss of functional richness, and by promoting the presence of more opportunistic dung beetles a decreasing in the functional structure occur. This highlight a strong functional redundancy in the low grazing area community. Considering the abandoned area, a community impoverishment was detected, but this is linked to the change of community with more specialized species.

This corroborate our previous observations that grazing abandonment act as environmental filter, and only some species can remain in these areas. For example, even if in abandoned area we lost no-nesting and large body size species (Tonelli et al., *submitted*), closed habitat specialist's species begin dominant probably due to shrub and tree encroachment. Also the type of available trophic resource in the abandoned area (roe deer and wild boar) may has determine the functional shift toward a more peculiar community from a functional point of view.

It is interesting the high values of *wFD*, *FEve* and *FDis* in the abandoned area. This mean that, although the community is impoverished from a functional point of view, the abundance within the functional space are well distributed. This is probably linked to the scarcity of trophic resource that lead to a limiting similarity in the community (MacArthur and Levins, 1967). Indeed, limiting similarity favors functional dissimilarity among species within a community, producing highest functional diversity values (Mouillot et al., 2007). Namely, high levels of functional diversity (*wFD*, *FEve* and *FDis*) will be associated to a high degree of niche differentiation among species: the most abundant species are very dissimilar and weakly compete (Mouchet et al., 2010).

Dung removal and functional diversity

In this study we demonstrate that progressive grazing abandonment indirectly affect the dung beetle process by affecting community structure and composition. Moderate grazing area show 40% more dung buried respect to low grazing area, and 90% more dung removed then abandoned one, whereas low grazing area have about 36% more buried dung respect to abandoned one, even if this difference was not significant.

The difference among buried dung in the areas, however, seem due to particular difference in the community attributes, and no generalization on the importance of functional diversity are allowed.

Very interesting is the low value of buried dung in low grazing area respect to moderate grazing one. Indeed, this area has more total dung beetle abundance and comparable biomass respect to moderate grazing area. Then our results appear in conflict with many other studies that identify these two parameters as the most important in controlling the dung removal (Nervo et al., 2014; Ortega-Martínez et al., 2016; Slade et al., 2007; Braga et al., 2013; Giraldo et al., 2011; Kudavidanage et al., 2012; Gollan et al., 2013; Beynon et al., 2012; Tixier et al., 2015; Tyndale-Biscoe, 1994; Kaartinen et al., 2013). We think that the major quantity of buried dung in the moderate grazing area was due principally to its higher number of species, higher alpha diversity and to its higher functional diversity (in terms of both functional richness and functional structure). The ecological mechanisms which may explain this result is the "niche complementarity", namely species complement each other in the resource use and should lead to a more efficient resource acquisition (Hooper et al., 2005; Tilman et al., 2014; Scherer-Lorenzen, 2009). This mechanism was empirically tested for dung beetle with corroborant results toward our interpretation. Beynon et al. (2012) for example, found a decrease of 7-8% in the removed dung by a single species compared with experimental unit with two or three species maintaining total biomass constant. Their results highlight that even a small difference in the number of species may result in a significant effect on the ecological process. Similar results are highlight by Manning et al. (2016) which tested the relative contributions of single and multiple dung beetle species toward various ecological process. The multiple presence of dung beetle species performed as well as the most functionally single species experimental unit for each of the process considered. Slade et al. (2007), even if tested for dung beetle functional group richness instead of the number of species, showed that the treatment with more functional groups performs better than the best single functional group treatment. Nervo et al. (2014) showed how biomass heterogeneity (i.e. the equal representation of different body size classes within the same functional group) lead to a more efficient dung removal in the short term, probably due to resource partitioning by species with different body size. Then our results support the hypothesis that high alpha diversity and functional diversity are between the principal engine of ecological processes between these two areas.

On the contrary, the differences in the dung buried between moderate and abandoned area seem due to their difference in dung beetle total biomass. In fact, abandoned area has comparable functional diversity structure respect to moderate one (*wFD*, *FEve*, *FDis*), and similar abundance and exponential of Shannon index. The difference exists only among species richness, biomass, *FD* and *FSpe*, where moderate grazing area show the highest values. In this case the interpretation was complex because the absence of clear pattern in the community attributes differences, we do not disentangle the effect of the number of functional niche (species richness and *FD*), from that of the biomass, which mean a neutral contribution of functional diversity (Gagic et al., 2015).

It is noteworthy the non-significant difference in dung burial capacity between low and abandoned site. Indeed, low grazing area have more abundance and biomass, that is known to have a strong effect on dung removal capacity (Larsen et al., 2005; Nervo et al., 2014; Ortega-Martínez et al., 2016; Slade et al., 2007; Braga et al., 2013; Giraldo et al., 2011; Kudavidanage et al., 2012; Gollan et al., 2013; Beynon et al., 2012; Tixier et al., 2015; Tyndale-Biscoe, 1994; Kaartinen et al., 2013), that are reflected in the 36%

more capacity in the dung removal. In this case, hence, the non-significant difference may be due to the functional diversity of abandoned areas, which showed a well-developed functional structure (*wFD*, *FDis*) respect to low grazing intensity. However, we think that, in this case, also the *FSpe* have played an important role. Indeed, the abandoned area is characterized by a higher abundance of the only roller species of the whole species regional pool (i.e. *Sisyphus schaefferi*). Even if various studies found that rollers are less efficient than tunnelers at dung removal (Slade et al., 2007; Kudavidanage et al., 2012), they are very performant when are coupled with tunnelers (Slade et al., 2007) as in our case. Moreover, the short permanence of the dung at the field (24h) may have played an important role. In fact, in general terms, *S. schaefferi* is one of the first species that colonize the dung pat and is able to shape and remove a brood ball in a short time (Tonelli, M., peronal observation, 2015). On the other hand, tunnelers, need more time because they require to digging a tunnel under the dung pat prior to relocate the dung.

Finally, we would stress that, even if ecological processes seem to be proportionate to the quantity of dung deposited on pasture, the general trend of loss of biodiversity may have long term negative effect on the dung removal capacity. Indeed, in the short term the numerically dominant species may buffer against the loss of alpha and functional diversity (the case of low grazing intensity area) but the loss of complementarity among less common species may diminish the ecological performance of the community in the long term (Smith and Knapp, 2003), as proposed by Slade et al. (2011). Moreover, higher community diversity may support higher levels of multifunctionality of the ecosystems (Soliveres et al., 2016). Furthermore, we would to stress the necessity of evaluate empirically the ecological process, at the dung pat level, rather than deduce it only from functional diversity measures, because do not exists a general and universal relationship, as showed in our previous research (cfr. Chapter 4).

5.5. Conclusions

Progressive abandonment showed a pattern that may be interpreted as an environmental filter. Passing from a functional rich and well-structured community (moderate grazing area), the first step was a loss of all functional diversity components (low grazing area) due to the niche filling mainly by opportunistic species. When the pasture was totally abandoned, a functional unique and well-structured community develop, probably linked to the habitat changes and to the available trophic resource (in terms of quantity and quality). We demonstrate that progressive grazing abandonment affect the dung burial process performed by dung beetle community. There is a loss of dung burial capacity of 28.5% and 47.5% passing from moderate grazing intensity to low grazing intensity and total abandonment respectively. Different mechanisms in the way in which dung beetle community can sustain ecological function are highlighted, with functional structure, total biomass and presence of performant dominant species that seem the major contributors, even if they seem context dependent. Although the quantity of dung removed seem to be proportional to the quantity of dung deposed on pasture, care must be taken to the possible negative effects on long term processes due to the loss of biodiversity caused by the grazing abandonment.

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CHAPTER 6

General discussion and conclusions

6.1. Provincial dung beetle community

During our studies that attempt to evaluate the effects of progressive grazing abandonment, VMPs use and their interaction on dung beetle assemblages from Pesaro-Urbino Province, we totally trapping 156,936 specimens belonging to 58 species (3 Geotrupinae; 16 Scarabaeinae; 39 Aphodiinae). Considering the Marche Region this number of species represents the 61% of the total regional pool (Ballerio et al., 2014; Bellucci et al., 2008; Carpaneto et al., 1994; Dellacasa and Dellacasa, 2006), including the 68% of Aphodiinae, 38% of Geotrupinae and 53% of Scarabaeinae occurring in the Region. Among the Aphodiinae *Calamosternus mayeri* (Pilleri, 1953), was quoted for the first time from the Marche Region (Tonelli et al., 2016). These data showed the importance of pastures around the Pesaro-Urbino Province to maintain a high level of dung beetle diversity. *Melinopterus consputus* (Creutzer, 1799) was the most abundant species, representing the 64.7% of the total abundance. We also highlight that, for the entire studied area, only 8 species have presented trophic preference toward horse or cow dung (cfr. Annex 1). *Aphodius coniugatus* (Panzer, 1795), *A. fimetarius* (Linnaeus, 1758), *Bodilopsis rufa* (Moll, 1782), *Colobopterus erraticus* (Linnaeus, 1758), *Esymus pusillus* (Herbst, 1789) and *Geotrupes spiniger* (Marsham, 1802) were significantly more attracted by cow dung, whereas *Chilothorax conspurcatus* (Linnaeus, 1758) and *Labarrus lividus* (Olivier, 1789) were more attracted by horse dung. These data corroborate the results of previous works, which reported the same trophic preference for the examined species (Dormont et al., 2004, 2007; Dellacasa and Dellacasa, 2006; Martín-Piera and López-Colón, 2000). However, more studies in additional areas are needed, due to the geographical variation of trophic preference (Barbero et al., 1999).

6.2. The effects of VMPs use

Our results report that an historical use of VMPs may affect negatively all the species of the community as shown by the absence of indicator species. It is likely that VMPs affect all the species by decreasing their abundance, with consequent loss of rare species, but maintaining the structure of the community. This is confirmed by the fact that the dung beetle composition between areas with use or not use of VMPs is different only for the rare species, whereas the common and dominant ones maintain a comparable composition. The loss of species, abundance and biomass are consistent with other studies (Hutton and Giller, 2003; Krüger and Scholtz, 1998; Beynon et al., 2012), but unlike our results, some authors found an impact on Shannon diversity index (Hutton and Giller, 2003; Krüger and Scholtz, 1998; Basto-Estrella et al., 2014). As probably different impacts depend on factors that have not been considered in our study, such as the frequency of treatment, the dosage, the type of molecules, the way of administration, the period of application and climatic conditions (Lumaret and Errouissi, 2002; Lumaret et al., 2012; Wall and Beynon, 2012; Jacobs and Scholtz, 2015; Adler et al., 2016), there is a high necessity to essay more in depth the usage of VMPs.

From a functional standpoint, the use of VMPs leads to a loss of the number of functional roles (*FD*), even though the functional structure of the community was maintained, probably because the usage of VMPs do not affect specific functional traits, fostering the homogeneous distribution of the abundance of the functional roles into the functional space. Hence, in our region, VMPs use appears to indistinctly impacts all the dung beetle functional niches, with no reason that specific traits were more affected than others.

Finally, the impact of VMPs use on dung beetle communities is reflected in the loss of about 70% of the dung burial capacity, which is of concern considering the possible over-accumulation of dung piles in the pastures. This result may be due to two mechanisms: a) alterations of dung beetle community attributes (abundance and biomass, number of species, number of functional niches) and b) intoxication of the dung beetles, making them unable to carry out their activities.

6.3. The effects of grazing extensification

Low grazing intensity can be considered one of the step of grazing extensification that leads progressively to a total abandonment (Baudry, 1991). In our studies we found that even a simple reduction of grazing intensity implies negative effects on dung beetle community. This grazing extensification leads to a loss of alpha diversity (species richness, ^{1}D and ^{2}D) and in the number of favored species (indicator species). Moreover, we observed a shift in the community composition that fostered more opportunistic species (r-strategy reproduction) and those adopting particular behavioral strategies to avoid competition with other dung-feeder insects (saprophagous larvae or cleptoparasitim). Although our results are consistent with other studies (Kadiri et al., 1997; Carpaneto et al., 2005; Jay-Robert et al., 2008; Lobo et al., 2006; Lumaret et al., 1992; Tshikae et al., 2013), new surveys that consider more intervals of grazing intensity are needed, as dung beetle diversity appears to be very sensitive even to small differences in the availability of trophic resources.

From a functional point of view, grazing extensification leads to a loss of functional diversity in terms of both functional richness and functional structure. Indeed, it is likely that even the loss of a little amount of trophic resource may act as a filter, allowing only species with particular functional traits to maintain viable populations in this area. The loss of large body size species, and the advantages for the opportunistic ones, which increase the functional redundancy of the community, seem to support these findings.

Generally, the changes in the dung beetle community caused by grazing extensification, produced a loss of dung burial capacity of about 30-40% in the low grazing area compared to those with moderate grazing activity. From a management point of view, this result appears to be of little concern compared to the impact of VMPs use. Indeed, the dung burial capacity of the community occurring in the low grazing areas, seems to be proportional to the amount of dung produced by the cattle.

6.4. Effects of the grazing abandonment

Domestic grazing abandonment leads to a loss of species richness, total abundance and biomass, number of favored species (indicator species), loss of large body size beetles and loss of no nesting species. All these results are consistent with those obtained by other authors (Tshikae et al., 2013; Jay-Robert et al., 2008; Lobo et al., 2006; Lumaret et al., 1992; Kadiri et al., 1997). However, the domestic grazing abandonment maintains a well-structured community, which is demonstrated by the high level of ${}^{1}D$ and ${}^{2}D$ that emphasize the importance of the wild fauna excrements to maintain a diverse assemblage, contrary to what Jay-Robert et al. (2008) suggested for Southern France. Generally, abandonment leads to the impoverishment of the communities where only medium sized beetles, which perform food relocation for nesting, can maintain viable populations.

Moreover, a shift toward a fauna which prefer closed habitat was found, which might be indication of an incipient tree and shrub encroachment due to grazing abandonment.

From a functional standpoint, grazing abandonment caused a loss in the number of functional roles (*FD*), but the functional structure was maintained because the abundances of each functional role are evenly distributed. This is probably due to a mechanism of limiting similarity triggered by the scarcity of resources. Furthermore, the functional uniqueness of abandoned sites, as showed by the *FSpe* measure, corroborates the idea that the loss of trophic resource act as a filter that impoverishes and changes the composition of the community.

Compared with low and moderate grazing intensity sites, the community occurring in the abandoned areas showed a low dung burial performance that vary from about -27% to -47%. These results seem to be a consequence of the qualitative and quantitative changes in the community attributes.

6.5. Relationship among classical and functional biodiversity measures

In both studies reported in chapter 4 and chapter 5, we found a robust correlation between species richness and *FD*, and between ^{1}D and *wFD*, and this is in accord with other studies (Micheli and Halpern, 2005; Petchey et al., 2007; Heino, 2008; Flynn et al., 2009; Bihn et al., 2010; Mayfield et al., 2010; Mouchet et al., 2010; Biswas and Mallik, 2011; Pakeman, 2011; Gerisch et al., 2012; Lohbeck et al., 2012; Luck et al., 2013; Griffiths et al., 2015). Although several methodological or context dependent factor can interfere with this relationship (e.g. number of traits, types of traits, intensity and type of environmental disturb, number of species) (Díaz and Cabido, 2001; Fonseca and Ganade, 2001; Mayfield et al., 2010; Biswas and Mallik, 2011; Naeem and Wright, 2003; Tsianou and Kallimanis, 2016; Cadotte et al., 2011; Luck et al., 2013), we suggest that our results can be interpreted as a real absence of functional redundancy in the dung beetle community. This is because we selected the functional traits on biological basis, using a similar number of quantitative and qualitative traits, and tested this relationship for two dung beetle assemblages that suffered different environmental stressors. However, we would like to stress the usefulness of the functional diversity metrics for two reasons: firstly, some of them are little related with other measures and can provide complementary information (i.e. *FEve* and *FSpe*); and secondly, for some metrics, the correlation is context dependent (e.g. *FEve*). Hence, for every study that intends to evaluate the functional diversity, it is recommended to always test its relationships with classical biodiversity measures.

6.6. Are the functional diversity patterns related to the ecological process?

While several studies (Díaz and Cabido, 2001; Hooper et al., 2005; Lefcheck and Duffy, 2015) suggest that functional diversity is more related to ecological processes than classical biodiversity metrics (e.g. species richness, total abundance, Shannon index etc.), we cannot generalize this statement. Indeed, the patterns of variation of functional diversity do not always follow the patterns of variation of the ecological processes.

For example, ‘VMPs use areas’ and ‘abandoned areas’, have a well-developed functional structure, but they have a low capacity of dung burial compared with ‘VMPs free areas’ and ‘Moderate grazing intensity’ areas. Although more analyses are needed in order to evaluate the relative importance of each community variable on ecological process, we suggest to empirically evaluate this relationship than extrapolating it from community biodiversity patterns (Braga et al., 2013).

6.7. General conclusions

According to the main results of the present Thesis, we provide general conclusion as follows:

1) VMPs use appears to have a ubiquitous impact on the dung beetle species here involved. Considering that all dung beetle species should be sensitive to ivermectin, its irrational use leads to a loss of dung beetle abundance, biomass and in some cases in the number of species;
2) Due to the equitable effects of VMPs on dung beetle species, the consequent impacts are different according to the abundance of each species. This was recorded in the loss of least common species, and in maintained community structure thanks to common and dominant species;
3) From a functional standpoint, the use of VMPs seems to affect indistinctly all dung beetle functional traits, which led to the loss of the number of functional roles (*FD*) but, at the meantime, in a well-developed functional structure of the dung beetle community;
4) The impacts of VMPs use on dung beetle communities are reflected by the loss of about 70% of the dung burial capacity, which may be due either to an alteration of dung beetle community attributes or to the intoxication of dung beetle individuals;

5) Grazing extensification leads to loss of the alpha diversity and to the change of the composition of the dung beetle community, fostering more opportunistic species. Occupying all the available trophic resource, these species alter the structure of the community;
6) From a functional point of view, grazing extensification leads to the loss of the functional diversity in terms of both functional richness and functional structure. This can be interpreted as environmental filter, where only the species with particular functional traits are able to maintain viable populations;
7) Grazing abandonment was related with the loss of species richness, abundance, biomass, large body size species and no-nesting dung beetles. This because the amount and configuration of trophic resources are key factors for maintaining viable dung beetle communities. Large-sized dung beetles may survive only if trophic resources are abundant (whole pasture) and the single dung pat is sufficiently large to allow for food relocation during nesting. The no-nesting species could be affected because adults need exposed dung to feed on and to look for suitable environment for the development of their larvae;
8) Grazing abandonment caused changes in the dung beetle composition. This may be due to the trees and shrubs encroachment and/or to the presence of species with particular traits to exploit the available dung (roe deer and wild boar). These interpretations are corroborated by the presence of species that prefer closed habitat, and to the increase of the functional unicity (*FSpe*) within the community of the abandoned area;

9) Generally, progressive grazing abandonment (extensification and total abandonment) produced a loss of dung burial capacity from 27% to 47%;
10) A robust correlation between dung beetle species richness and *FD*, and between ^{1}D and *wFD* was found, indicating an absence of functional redundancy in the dung beetle community;
11) As dung burial follows local idiosyncrasies, the patterns of variation of functional diversity do not always follow the patterns of variation of the ecological processes. This highlights the need to empirically evaluate this ecological process.

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ANNEX 1

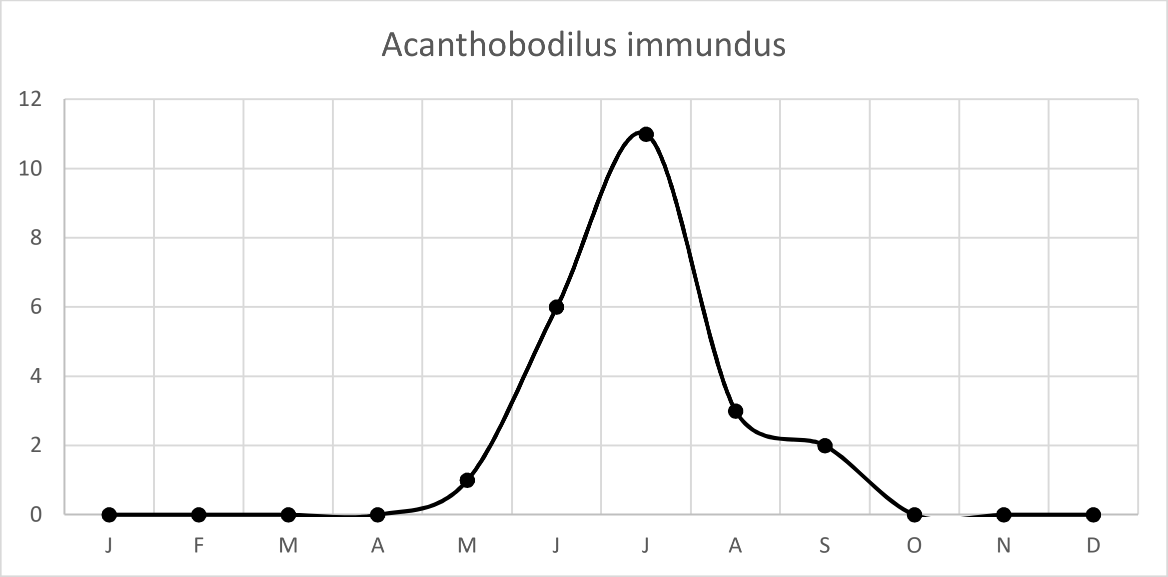
Catalog, biology and phenology of the species

Acanthobodilus immundus (Creutzer, 1799)

BIOLOGY: This species is strongly linked to open habitats such as xeric pastures and grasslands with Mediterranean influence from 0 to 2000 m a.s.l. (Dellacasa and Dellacasa, 2006; Galante, 1983). Is more frequent on clayey and calcareous soils (Lumaret, 1990; Sullivan et al., 2016). It is a tipical dweller species that not show a nesting behaviour and laying eggs directly within the dung pat, where the entire ontogenetic development take place.

A. immundus show a preference toward dung with high hydric content such as cow dung, but is encountered also in horse, sheep, goat and human dung (Dellacasa and Dellacasa, 2006; Veiga, 1998; Lumaret, 1990). Lumaret (1990) highlight the strong preference toward cow dung (64%) respect to horse dung (18%) in France, whereas in southern Spain, Martín-Piera and Lobo (1996) describe it as a generalist species without any preference with regard to various types of dung such as horse, cow, wild boar, badger, deer, fallow deer, lynx, fox and human. In our study area this species (N=23) show a preference toward cow dung (74%), respect to horse dung (26%), but it is not significant (IndVal=29.6; $P = 0.225$).

PHENOLOGY: our phenological data show the begin of activity in spring, with a population maximum in summer (July), and a slow decline toward autumn, when no specimens was encountered. This pattern is strongly coincident with other European areas where this species shows a tipical summer maximum (Veiga, 1982; Lumaret, 1990; Dellacasa and Dellacasa, 2006; Carpaneto et al., 2011; Agoglitta et al., 2012). Overwinter as adult or third instar larvae, and can have more than one generation at year with favourable conditions (Veiga, 1982; Dellacasa and Dellacasa, 2006).

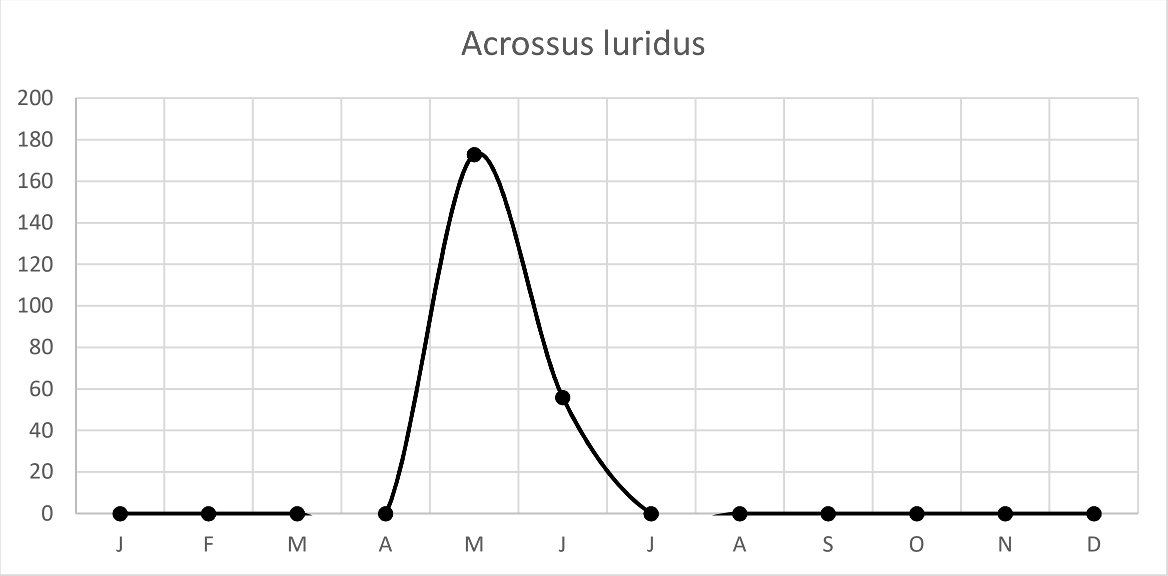


Acrossus luridus (Fabricius, 1775)

BIOLOGY: this is an oligotopic species with a preference for exposed xeric pastures (Dellacasa and Dellacasa, 2006; Veiga, 1998; Lumaret and Kirk, 1987; 1991; Verdú et al., 2011) until 2000 m a.s.l. even if above the 1500 m a.s.l. the altitude begins to be a limiting factor (Veiga, 1998; Dellacasa and Dellacasa, 2006). Its nesting behaviour is similar to that of some species of the genus *Trox* Fabricius (Family: Trogidae); the females laying the eggs at the soil-dung interface and the larvae buring their owns tunnels in the soil that fill with the dung deposed at the soil surface (Lumaret, 1983). Because of its euriphagy, it can be found in various dung type, although show a preference toward dung with medium hydric content such as sheep and goat, and is less frequent in the cow and horse dung (Veiga, 1998; Lumaret, 1990; Dellacasa and Dellacasa, 2006; Carpaneto et al., 2005). It was found occasionally also in human, dog and fox dung, and below a dead sheep (Dellacasa and Dellacasa, 2006; Lumaret, 1990; Veiga, 1998; Veiga, 1985). Pittino (2001) cited the presence of *A. luridus* specimens into the *Spermophilus citellus macedonicus* Fraguedakis-Tsolis, 1977 burrows in Macedonia, whereas Ziani and Moradi Gharakhloo (2011) found it inside burrows of rodents in Iran. *A. luridus* remains were found into the pellets of *Milvus milvus* (Linnaeus, 1758) in Wales, and in the stomach contents of *Pluvialis apricaria* (Linnaeus, 1758) in Britain (Young, 2015). In southern France, *A. luridus* seem more attracted by dung contamined with ivermectin (Errouissi and Lumaret, 2010).

Contrary to the above-cited literature, we found this species (N=229) more frequently in cow dung (71%) respect to horse dung (29%), although without a significant preference (IndVal=47.5; *P* = 0.269).

PHENOLOGY: our data show a narrow population peak in spring (May) that abrupt diminsh toward the summer. This pattern is strongly coincident with other European areas, confirming that this species is strictly springly (Veiga, 1998; Lumaret, 1990; Sowig and Wassmer, 1994; Dellacasa and Dellacasa, 2006). Overwinter at the adult phase and has only one generation per year (Veiga, 1998; Lumaret, 1990; Dellacasa and Dellacasa, 2006).



Acrossus rufipes (Linnaeus, 1758)

BIOLOGY: this is a species with a high humidity requirement. Landin (1961) had experimentally demonstrated that little humidity decrease lead to the death of specimens. This humidity requirement explicates its orofilous character, expecially during dry summer months (Galante, 1983; Veiga, 1998; Dellacasa and Dellacasa, 2006). Probably its humidity need is a key factor also in habitat selection, considering that it can vary from forest (Wassmer, 1995; Borghesio et al., 2001; Tocco et al., 2013; Kamiński et al., 2015) to pasture (Lumaret, 1990; Negro et al., 2011), while some authors consider it a generalist species (Galante, 1983; Macagno and Palestrini, 2009). Humidity need may also explicate its night activity (Kamiński et al., 2015).

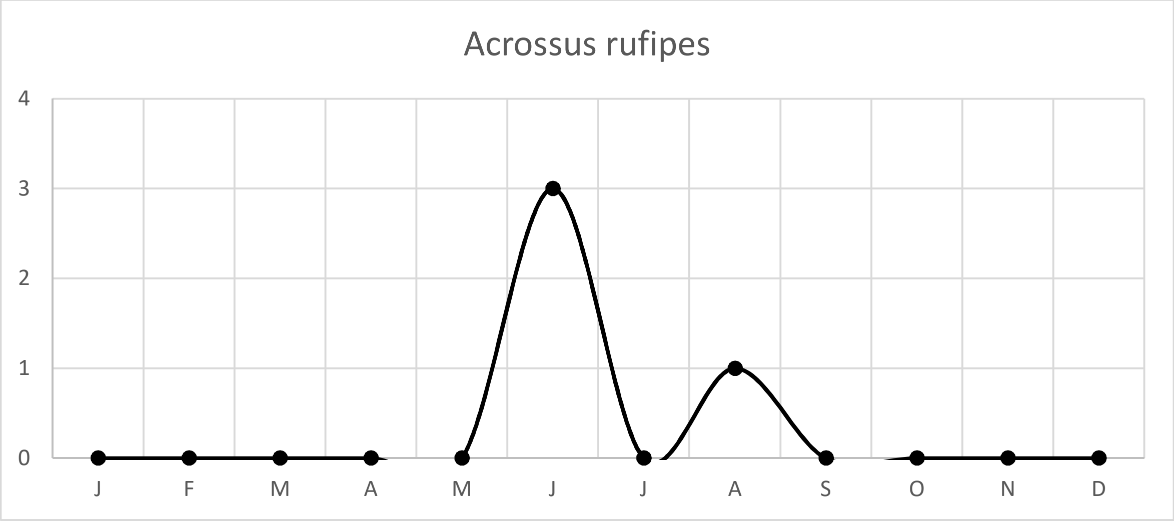
This species shows a rudimental nesting behavior. At the end of the summer, females dig short tunnels below the excrement (10-50 mm deep), on the bottom of which laying a group of 5-11 eggs into chambers that are subsequently sealed. After hatching, the first instar larvae moved up into the dung. The second instar larvae burrowed 10-20 mm into the soil by digging its own tunnel, whereas the third instar larvae moved back and forth between soil and dung, gradually extending the vertical shafts to a depth of 40-50 mm. At the end of October, the third instar larvae prepare their own cell at 60-120 mm deep, within which they overwintering (Klemperer, 1980; Dellacasa and Dellacasa, 2006). During the dispersion into the soil, the larvae, can colonize and exploit the brood masses of Geotrupes spiniger acting as facultative kleptoparasite (Klemperer, 1980).

Also the trophic requirements of *A. rufipes* appear to be related to its high need of humidity. Indeed, it is one of the earlier sucessional species that prefeer the dung in the first 2 days after deposition (Psarev, 2001a; Gittings and Giller, 1998) and show a strong preference toward highly hydrated excrement such as cow dung (Veiga, 1998; Lumaret, 1990; Borghesio et al., 2001; Errouissi et al., 2004; Dormont et al., 2004; Wassmer, 1995; Galante, 1983) of which is able to ingest only the smaller particles (10-14 μm) (Holter, 2000). Occasionally it can also be found in horse dung, human dung, bear dung, sheep dung and alpine ibex (Carpaneto and Fabbri, 1984; Muona and Viramo, 1995; Lumaret, 1990; Borghesio et al., 2001; Dormont et al., 2007), whereas Matuszewski et al. (2008) detect its presence below a pig cadaver in Western Poland forest. Slay et al. (2012) mentioning the discovery of some specimens inside the caves of Virginia and east Tennessee (Holsinger and Culver, 1988). Dung contaminated with ivermectin seems to have no effects on adult survival, but significantly reduced the number of eggs per female of *A. rufipes* (O'Hea et al., 2010).

Even if we found few specimens (N=4) in our area of study, they showed an exclusive preference toward cow dung (100%), but it is not significant (Indval 13.3; *P* = 0.486)

This species is part of the diet of many vertebrates including *Corvus corone* L. (Horgan and Berrow, 2004), *Pyrrhocorax pyrrhocorax* (Meyer et al., 1994), *Milvus milvus, Sylvia communis, Corvus frugilegus, Corvus monedula, Eptesicus serotinus, Vulpes vulpes* (Young, 2015) and *Rhinolophus ferrumequinum* (Kervyn et al., 2009).

PHENOLOGY: our data show a summer activity from June to August that coincide with other European areas corroborating its summery feature (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006) with only one generation at year (Veiga, 1998). Overwinter as third instar larvae (Klemperer, 1980), but under favorables conditions, even as an adult (Dellacasa and Dellacasa, 2006).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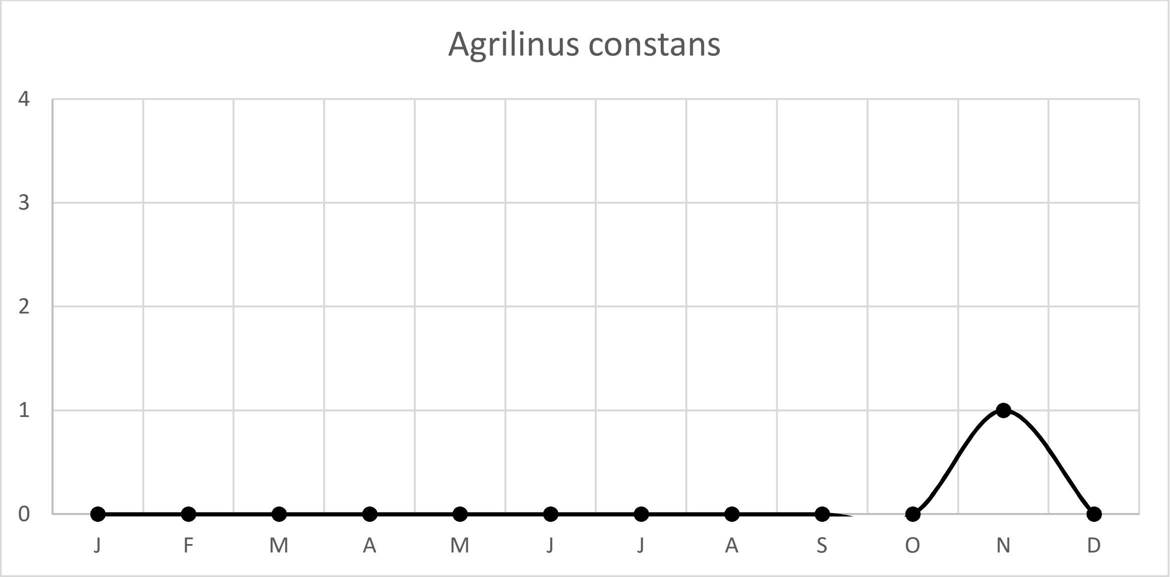
Agrilinus constans (Dufschmid, 1805)

BIOLOGY: *Agrilinus constans* is a oligotopic species that not present a habitat preference, considering that it can be found in exposed pastures, closed garigues and woodland (Lumaret and Kirk, 1987; Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006), but always with a high level of humidity. It is a typical dweller species that not present any nesting behavior. Generally, the oviposition take place into the intermediate zone of the dung pat, where eggs and larvae encounter the optimal environment for its development, whereas the pupation happen at the dung-soil intephase (Lumaret, 1975; Lumaret, 1990; Dellacasa and Dellacasa, 2006).

Due to its moisture requirement, this species show a strong preference toward cow dung, although it can be found in sheep, horse, human, cervids and lagomorphs (Dellacasa and Dellacasa, 2006; Veiga, 1998; Lumaret, 1990; Dormont et al., 2004, 2010). Dormont et al. (2010) experimentally demonstrated that its dung preference is an innate character and that the presensce of conspecific specimens increase dung actrattivenes, while the presence of other species decreases it. Although we found only 1 specimen during our study, this was encountered in cow dung in line the the previous literature. Due to this singular finding, this species not show significant dung preference (Indval 6.7; $P = 1.000$).

A. constans seems more attracted by dung contaminated with ivermectin from cow treated with slow-release bolouses (Errouissi and Lumaret, 2010). The effects of Ivermectin on *A. constans* was investigated repeatedly, showing a LC50 of 0.59-0.88 mg/kg of dry dung (Hempel et al., 2006; Lumaret et al., 2007) and a negative effect on adult emergence until 143 days after a cattle treatment with ivermectin sustained-release bolus (Errouissi et al., 2001). However, the use of *Duddingtonia flagrans* for control of gastro-intenstinal nematode larvae of ruminants, did not alter the development and the survival of *A. constans* (Paraud et al., 2007).

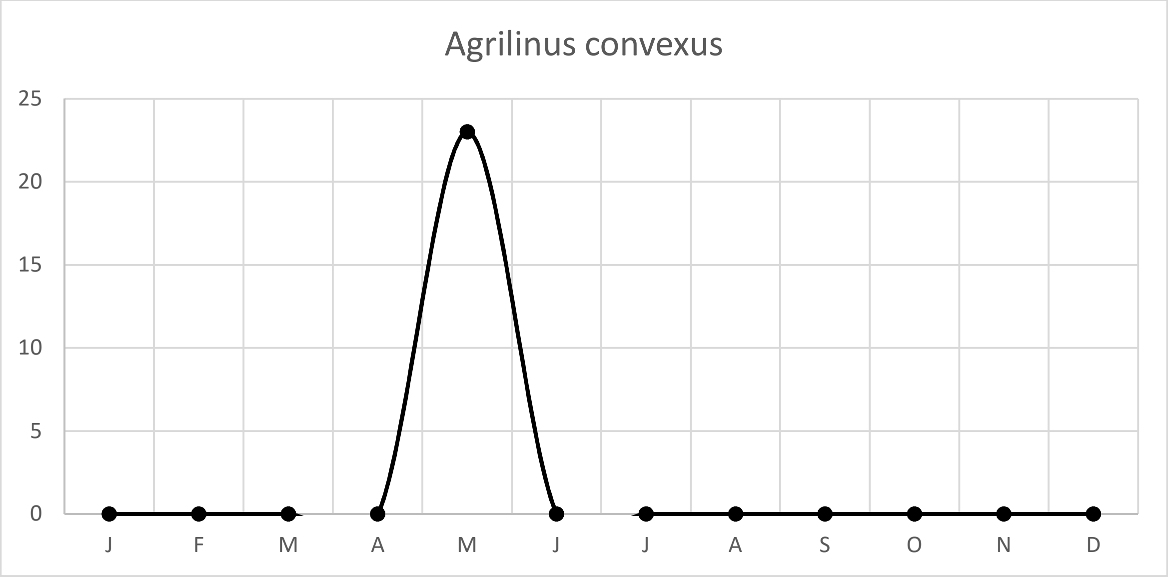
PHENOLOGY: the only specimens we collected prevent us to establish its phenological pattern, but is in line with its phenological activity in other European areas that is tipical winterly with some speciemens until early spring (Lumaret and Kirk, 1987; Dellacasa and Dellacasa, 2006; Lumaret, 1990; Agoglitta et al., 2012; Lumaret and Kirk, 1991; Veiga, 1998). Indeed, this species apperas after the first autumn rains and the number of individuals decreases slowly toward the end of the winter (Veiga, 1998; Lumaret, 1990). Lumaret and Kirk, (1991) explicate that its winterly phenology may be due to its humidity requirement.



Agrilinus convexus (Erichson, 1848)

BIOLOGY: this is a oligotopic species, that prefeer exposed pasture habitat (Dellacasa and Dellacasa, 2006; Barbero et al., 1999). It shows preference toward sheep dung, although may be found in other dung type as horse, cow, bear and in rotting vegetables (Carpaneto and Fabbri, 1984; Carpaneto et al., 2005; Dellacasa and Dellacasa, 2006). Although not significant (Indval 15.7; *P* = 0.569), we encountered the 78% of the specimens in cow dung, and the remaining 22% in horse dung (N=23). Our data on trophic preference are in line with Galante (1983) that encountered this species principally in cow dung.

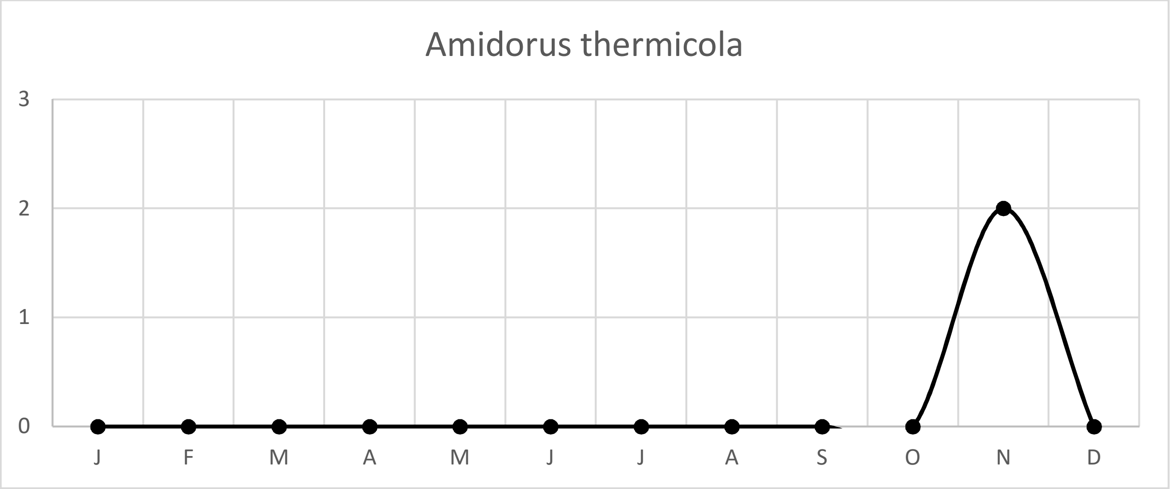
PHENOLOGY: our phenological data show that this species is narrowly restricted to spring months, in accord with Dellacasa and Dellacasa (2006).



Amidorus thermicola (Sturm, 1800)

BIOLOGY: this is a oligotopic species that preferring open habitat such as pastures with calcareous draining soils (Dellacasa and Dellacasa, 2006; Lumaret, 1990; Veiga, 1998; Dellacasa and Kirgiz, 2002). *A. thermicola* show a strong preference toward sheep dung (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006), but it can be found with lower frequency even in human, horse, cow and goat dung (Lumaret, 1990; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008). We encountered only 2 specimens, both in horse dung, but the low number prevent us to highlight any trophic preference (Indval 6.7; *P* = 1.000).

PHENOLOGY: the low number of specimens captured in our study prevent us to highlight any phenological pattern. However, the two specimens were captured in autumn, that seem the season of major activity for this species (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006; Dellacasa and Kirgiz, 2002; Agoiz-Bustamante, 2008), followed by a second peak of activity in the spring (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006; Dellacasa and Kirgiz, 2002;) that has led Veiga (1998) to hypothesis the presence of two generations per year.



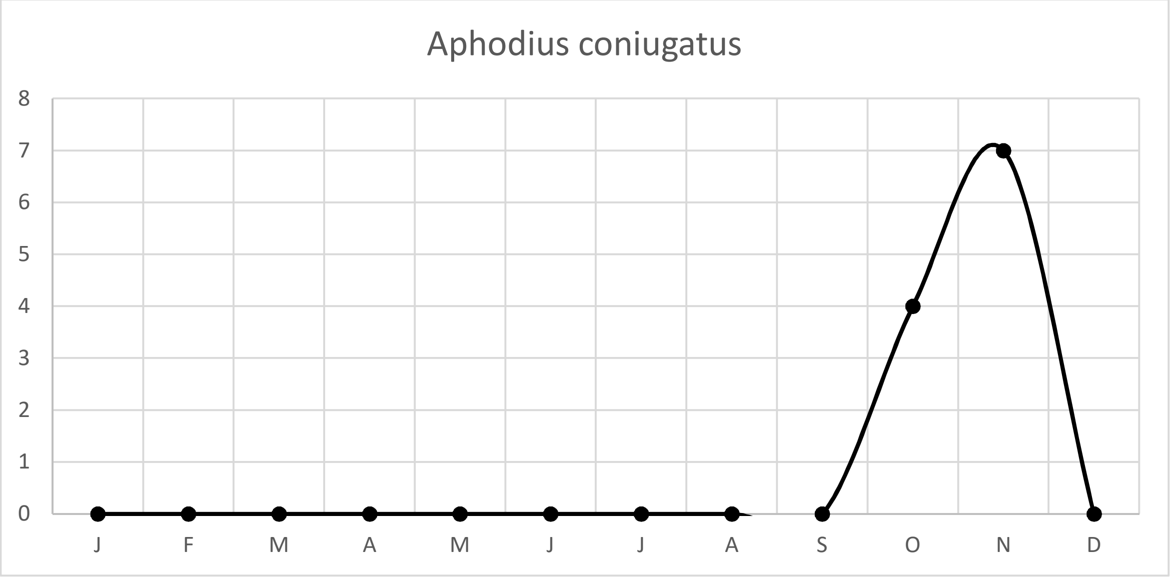
Aphodius coniugatus (Panzer, 1795)

BIOLOGY: this species shows a preference toward open pastures in mountainous environments due to its high humidity requirement. Indeed, it seems not tolerate hot-arid climates, where it became strongly orofilous (Veiga, 1998; Dellacasa and Dellacasa, 2006; Lumaret, 1990). Romero-Samper and Martín-Piera (2007) had studied its nesting behavior under laboratory conditions: eggs were layd during April and May into the dung pat or at soil-dung interphase. After hatching, the larvae remain into the dung that use for feeding. After 39-45 days from hatching, the third instar larvae become the pupal cell construction, that can be modelled into the dung, at soil-dung interphase or into the soil below the dung pat at 4-6 cm of deep. After 12-16 days the adults stage emerged and remain for 8 days into the pupal cell. Under laboratory conditions, the adults remained inactive until autumn, aestivating at some cm deep into the soil.

Due to its hydric requirement, this species strongly prefeer cow dung (Veiga, 1998; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008), although it can be found in horse dung, and occasionally in sheep, goat and human dung (Veiga, 1998). We found 91% of the total specimens (N=11) in cow dung and the remaining 9% into the horse dung, confirming its strong preference toward cow dung. Moreover, this preference was significant (Indval 42.4; $P = 0.022$).

This species was found into rodent burrows in Iran (Ziani and Moradi Gharakhloo, 2011), but this finding must be regarded as accidental and does not allow to consider the species as pholeophiles or pholeobionts.

PHENOLOGY: various authors conincide in identify two generations per year, one in spring and other in autumn coinciding with rainy periods and mild temperatures (Veiga, 1998; Lumaret, 1990; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008). Due to our sampling period, we identify only the autumn generation, which overwinter as adult, whereas the spring generation overwinter as larvae (Romero-Samper and Martín-Piera, 2007; Agoiz-Bustamante, 2008).



Aphodius fimetarius (Linnaeus, 1758)

BIOLOGY: this is a diurnal (Kamiński et al., 2015) generalist species that colonize evry type of environment with every type of soil (Galante, 1983; Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006; Negro et al., 2011), although seem to prefeer open pastures habitat (Lumaret, 1990; Wassmer, 1995; Dormont et al., 2007; Verdú et al., 2011; Zamora et al., 2007; Macagno and Palestrini, 2009; Tocco et al., 2013). *A. fimetarius* is a euryphagous species, that feeding on any dung type such as cow, horse, sheep, goat, cervids, human, lagomorphs, dog, fox, marmot, donkey and bear (Dellacasa and Dellacasa, 2006; Carpaneto and Fabbri, 1984; Borghesio et al., 2001; Agoiz-Bustamante, 2008), moreover it is found into dunghills, potatoes, mushrooms, rotting vegetables (Landin, 1961; Lumaret, 1990), pig carrion in Poland forest (Matuszewski et al., 2008) and rodent burrows in Iran (Ziani and Moradi Gharakhloo, 2011). Despite its wide trophic spectrum, several studies found a preference toward cow dung (Galante, 1983; Lobo, 1985; Wassmer, 1995; Errouissi et al., 2004; Dormont et al., 2007). *A. fimetarius* is capable of colonizing the dung of all ages (Borghesio et al., 2001; Psarev, 2001a) albeit with a good amount of residual moisture (Gittings and Giller, 1998). Its euryphagy and its wide successional colonization capacity can be explicated by its capacity to eat relatively big dung particles (until 18 μm) compared to other Aphodiinae species (Holter, 2000).

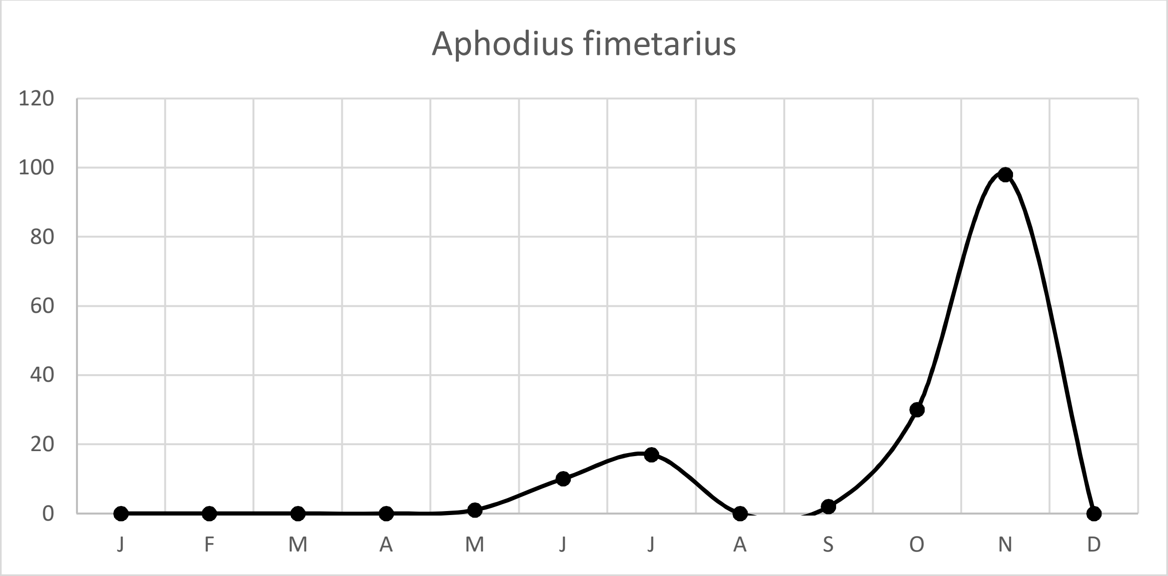
Our data (N=158) on trophic preference are in line with the above cited literature, and show a significant preference toward cow dung (72%) compared to horse dung (28%) (IndVal 67.3; $P = 0.036$). This species seems most attracted by dung contaminated with ivermectin (Floate, 2007; Errouissi and Lumaret, 2010; Sutton et al., 2014) and Doramectin (Floate, 2007).

This is a typical dweller species that laying the eggs into the dung pat, where the entire larval development take place, whereas the pupation can take place into the soil or at dung-soil interphase (Romero-Samper and Martín-Piera, 2007).

Aphodius fimetarius forms part of the diet of a large variety of animals such as: *Corvus frugilegus, Larus ridibundus, Corvus corone, Tyrannus tyrannus, Tyrannus verticalis, Myiarchus crinitus, Contopus virens, Nuttallornis borealis, Sayornis phoebe, Sayornis nigricans, Epidonax virescens, Epidonax flaviventris, Epidonax traillii, Epidonax minimus, Eremophila alpestris, Progne subis, Iridoprocne bicolor, Petrochelidon pyrrhonota, Hirundo rustica, Riparia riparia, Corvus brachyrhynchos, Turdus migratorius, Hylocichla ustulata, Hylocichla guttata, Sialia sialis, Sialia currucoides, Anthus spinoletta, Sturnus vulgaris, Vireo olivaceus, Vireo philadelphicus, Vireo gilvus, Vireo flavifrons, Vireo solitarius, Sturnella neglecta, Quiscalus quiscula, Pheucticus ludovicianus, Guiraca caerulea, Passerculus princeps, Melospiza melodia, Falco subbuteo, Lyrurus tetrix, Tetrao urogallus, Grus grus, Pluvialis apricaria, Eudromias morinellus, Tringa totanus, Scolopax rosticola, Cursorius cursor, Larus ridibundus, Martula urbica, Lanius senator, Sylvia communis, Corvus frugilegus, Pica pica* (Horgan and Berrow, 2004; Tryjanowski et al., 2003; Young, 2015).

PHENOLOGY: *Aphodius fimetarius* is active throughout the year, although principally during spring and autumn (Lumaret, 1990; Wassmer, 1994; Veiga, 1998; Dellacasa and Dellacasa, 2006; Sladecek et al., 2013). Generally, the generational maximum happens in autumn (Wassmer, 1994; Palestrini et al., 1995; Borghesio et al., 2001; Anlaş et al., 2011). The phenology reflects the generation cycle of this species, that show from two to three generation per year depending on the climatic conditions (Lobo, 1985; Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006): the first generation emergence in spring-summer, whereas the second emerge in autumn (Lumaret, 1990; Borghesio et al., 2001). Usually, overwinter as adult in the septentrional areas, while in the southern areas may overwinter as any ontogenetic stage (Schmidt, 1935; Landin, 1961; Wassmer, 1994; Borghesio et al., 2001; Dellacasa and Dellacasa, 2006).

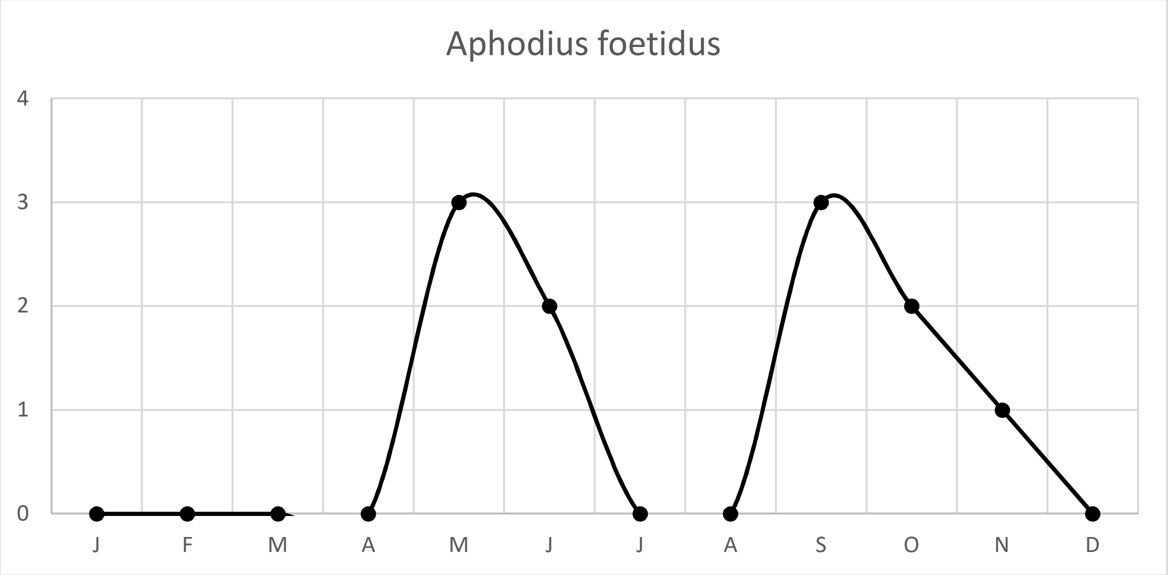
Our data are consistent with the literature, showing a first small peak during spring-summer, and the maximum generational peak during autumn, coinciding with the adult emergence.



Aphodius foetidus (Herbst, 1783)

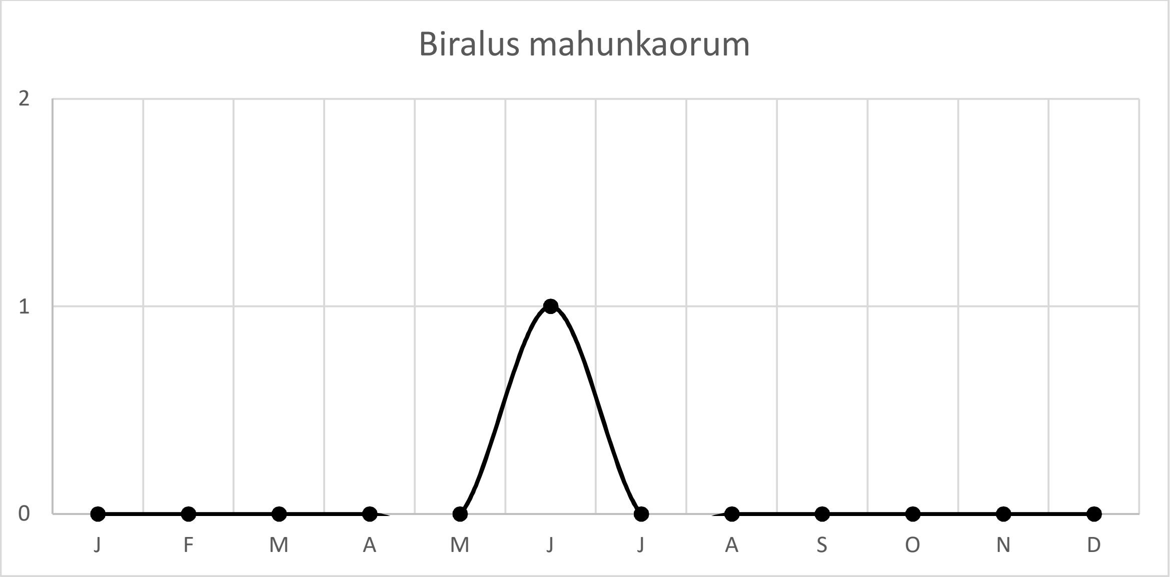
BIOLOGY: *Aphodius foetidus* is an oligotopic species that prefeer open mediterranean habitats as pasture surrounded by shrubland or forest landscape (Veiga, 1998; Romero-Alcaraz and Ávila, 2000; Numa et al., 2012) with well draining soils (Lumaret, 1990; Dellacasa and Dellacasa, 2006). It is a coprophagous species, that not show any trophic preference and may be found in dung of sheep, horse, human, lagomorphs, dog and dunghills (Lumaret, 1990; Veiga, 1998; Carpaneto et al., 2005; Dellacasa and Dellcasa, 2006; Agoiz-Bustamante, 2008). It was found in rotting vegetables and roe deer carrion (Horion, 1958; Van Wielink, 2004). The low number of individual captured during our study (N=11) does not allow to highlight any significant trophic preference (Indval 21.2; $P = 0.598$) although we found that cow dung is a little more attractive (64%) compared to horse dung (36%). We consider this species as a tunneler species because in the nesting phase the adult dig short gallery under dung pat where laying the eggs (Verdú J.R. pers. comm. 2016).

PHENOLOGY: in its areal this species may be found all around the year but with an activity mainly during spring and autumn with a period of inactivity during summer months (Lumaret, 1990; Veiga, 1998; Verdú, 1998; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008). Although some authors highlight the presence of two generations per year (Veiga, 1998; Dellacasa and Dellacasa, 2006) Verdú (1998) consider this species as univoltine with the oviposition during autumn months and the adult emergence during spring months. Due to the low number of specimens captured during our study, we can not define the phenological pattern of this species with details. However, our data suggest the presence of a period of inactivity during summer months, and two activity peaks during autumn (reproductive period) and spring (adult emergence period); in concordance with above cited literature. *Aphodius foetidus* overwinter as third instar larvae or as adult (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006).



Biralus mahunkaorum **Adam, 1983**

BIOLOGY: recently, Rössner and Fery (2014), highlight that several European quotes for *Biralus satellitius*, actually belong to *B. mahunkaorum*. Therefore, we refrain from defining the biological characteristics of this species in the absence of reliable data relating to it. Moreover, we found only one specimen in June (in cow dung) that prevent us to define any phenological or trophic preference pattern (IndVal 6.7; *P* = 1.000).



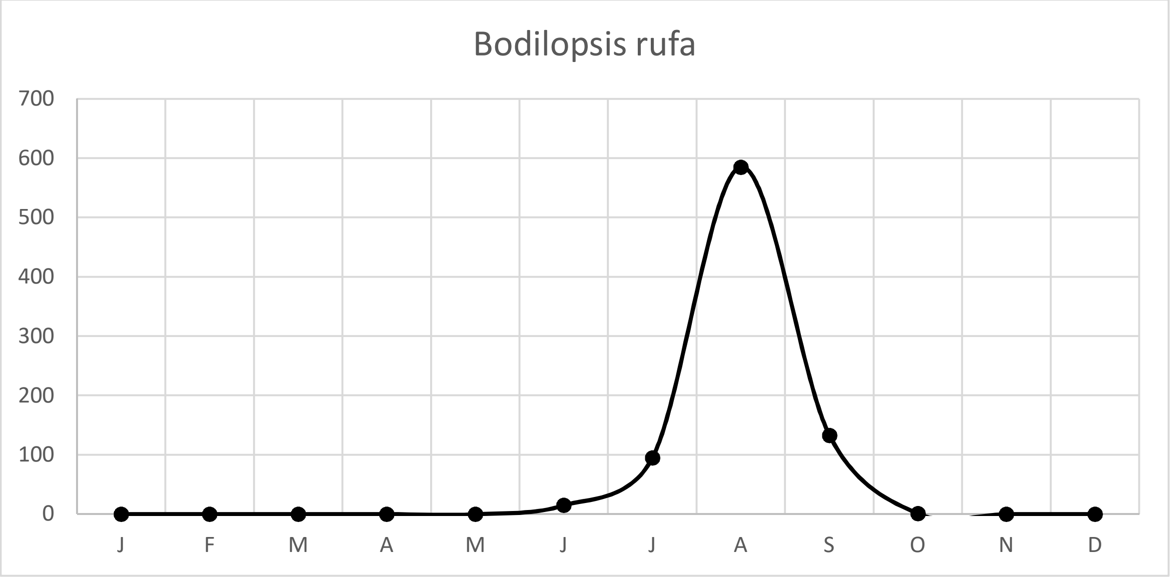
Bodilopsis rufa (Moll, 1782)

BIOLOGY: *Bodilopsis rufa* is a eurytopic diurnal (Kamiński et al., 2015) species that seem generalist in term of habitat preference and can colonize open pastures or forested environments (Galante, 1983; Lumaret, 1990; Wassmer, 1995; Dellacasa and Dellacasa, 2006; Macagno and Palestrini, 2009; Negro et al., 2011). It is strongly linked to humid environments and soils (Galante, 1983; Lumaret, 1990; Dellacasa and Dellacasa, 2006), that is reflected to its high preference toward dung with high moisture content as cow dung (Galante, 1983; Lumaret, 1990; Gittings and Giller, 1998; Dellacasa and Dellacasa, 2006; Bebermans et al., 2016) that colonize at "mid-age" after its deposition (Gittings and Giller, 1998; Sladecek et al., 2013). It can be found also in dung provenient from human, horse, sheep, marmot and bear (Carpaneto and Fabbri, 1984; Ziani, 2003; Dellacasa and Dellacasa, 2006). There was one quote about its founding under pig carrion (Matuszewski et al., 2008). Our data (N=829) corroborate the preference of *Bodilopsis rufa* toward cow dung (97%) compared with horse dung (3%) (IndVal 84.5; $P = 0.001$).

Borghesio and Palestrini, (2002) show how *Bodilopsis rufa* presents a variable nesting behavior from dweller to tunneler, probably according to different environmental conditions. Under laboratory conditions this species showed reproductive activity from mid August to late September, during which adult females laid from 10 to 35 eggs in a single dung pat. 25% of their observation highlight that eggs had been laid in the dung, just under the external crust of the pat, while on the remaining 75% observations, they had been laid in underground burrows dug under the pat. Burrows consisted in a vertical shaft, 1-3 cm long with 1-3 spherical cells departing radially from the end of the shaft, each one containing an egg, but no dung. Eggs hatched 3 days after deposition and young larvae reached the dung where they feed. After 21-35 days from eggs deposition the larvae abandoned the dung and moved to underground cells at a depth of 3-15 cm where pupation occurred. Both larvae hatched from eggs laid in the soil and those from eggs laid in the dung eventually pupated in underground cells (Borghesio and Palestrini, 2002).

This species was found as part of the diet of *Lanius collurio* and *Corvus corone* (Tryjanowski et al., 2003; Horgan and Berrow, 2004).

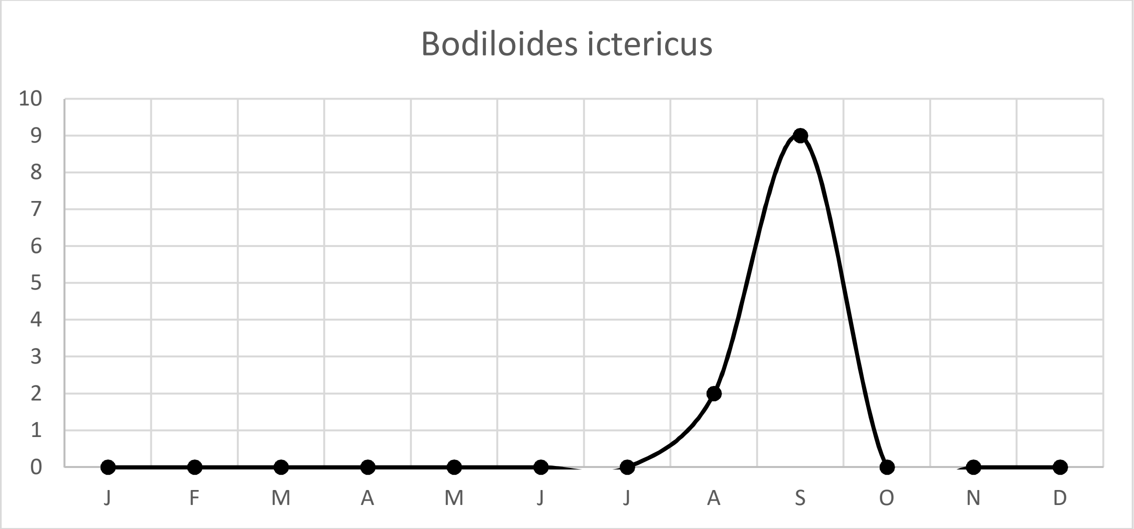
PHENOLOGY: this is a tipical summerly species that is active from May to October with a generational maximum during July and September (Lumaret, 1990; Wassmer, 1994; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008). Although several author suggest that overwinter as third instar larvae (Lumaret, 1990; Dellacasa and Dellacasa, 2006), other ones highlight the possibility of overwintering as adult phase (Wassmer, 1994; Borghesio and Palestrini, 2002). Our phenological data are in accordance with previous literature, showing its activity peak during summer months, probably coincident with its reproductive phase.



Bodiloides ictericus **(Laicharting, 1781)**

BIOLOGY: eurytopic species but that prefeer open dry habitat as exposed pastures (Zunino, 1982; Lumaret, 1990; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006) on silty and clayey soils (Lumaret, 1990). It is a typical dweller species that laying eggs into the dung (González-Megías and Sánchez-Piñero, 2003). This specie is considered as widely euryphagous (Zunino, 1982) that can colonize any type of dung such as: cow, human, horse and sheep (Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008). However, some authors highlight its preference toward cow dung (Lumaret, 1990; Martín-Piera and Lobo, 1996; Veiga, 1998), with which our data (N=11; 100% in cow dung) coincide (Indval 13.3; $P = 0.501$).

PHENOLOGY: this species has only one generation per year, with an activity peak during summer (Lumaret, 1990; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008; Agoglitta et al., 2012). The nesting seems occour during autumn (González-Megías and Sánchez-Piñero, 2003). The ontogenetic cycle need seven months (Veiga, 1982) and overwinter as third instar larvae (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006). Although the number of specimens encountered during our study is low, the phenological data are in agreement with the literature and reflects its ontogenetic cycle.


Bodiloides ictericus
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Bubas bison (Linnaeus, 1767)

BIOLOGY: *Bubas bison* is a crepuscular and nocturnal species (Lumaret and Kirk, 1987) that colonize prefereably open habitat such as pastures (Lumaret, 1990; Martín Piera and López-Colón, 2000; Verdú et al., 2011). This species shows a strong preference toward cow dung, although it is capable to nesting even with horse dung (Klemperer, 1981), and occasionally it can be found in dung of sheep, human, carnivorous and omnivorous (Lumaret 1990; Martín Piera and Lobo, 1996; Martín Piera and López-Colón, 2000; Carpaneto et al., 2005). Although not significant (IndVal 33.5; $P = 0.449$), our data (N=106) are in accordance with other studies, showing the preference toward cow dung (72%) compared with horse dung (28%).

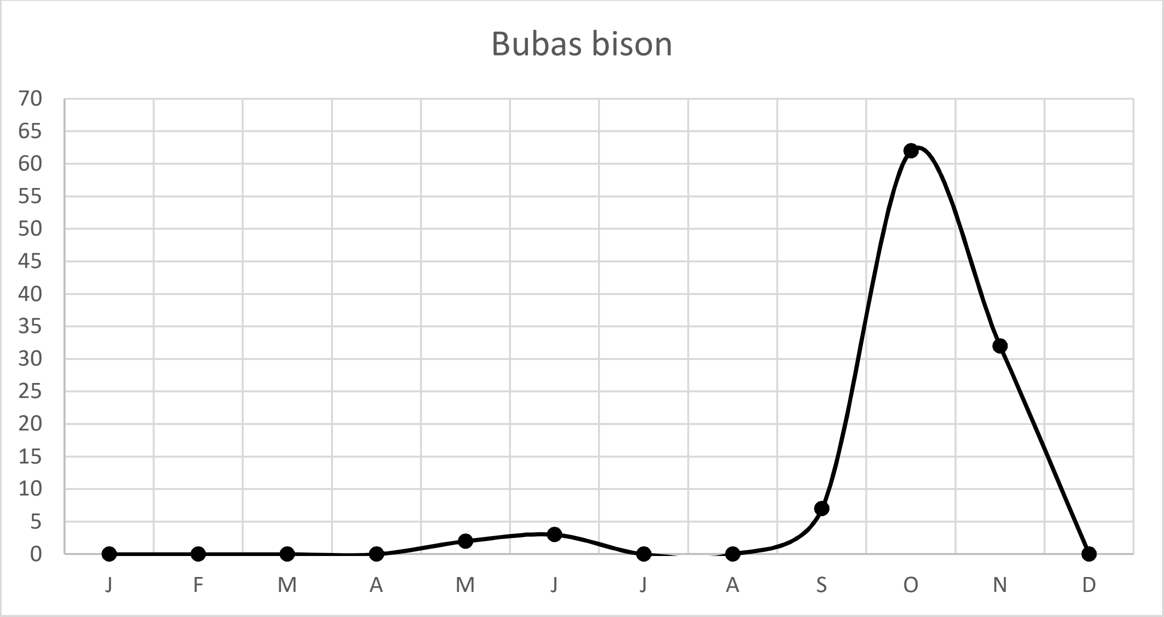
The nesting behavior was studied in detail by Klemperer (1981), Kirk (1983) and Palestrini et al. (2001), which stated that the female, alone or assisted by the male, dig multiple nests below dung pat. Each nest consists of one tunnel of about 50-100 mm deep, that lead to several (~3) brood masses (~50 g), where the female layd two eggs (one for each pole) whitin an incubation chamber (8-10 mm of diameter). The mean number of nests under each dung pat is about 5 and the mean dry weight of dung buried under a pad was therefore equal to 123,9 g. (Kirk, 1983). Such dung burial activity can determine a reduction in the viability of the *Cryptosporidium* oocystis up to 90% (Ryan et al., 2011). Under laboratory conditions, Zunino and Monteresino (1994) had demonstrated that this species can relocate the dung horizontally above soil surface when the burial is prevented.

Although a previsional model highlight the possibilities of an increase in its distributional range until 110% in a climate change scenario (Dortel et al., 2013), experimental works show that temperatures higher than 20° C lead to an increase in the mortality of *Bubas bison* eggs (Kirk and Kirk, 1990).

Bubas bison form part of the diet of several raptors such as *Tyto alba* (De Pablo, 2000), *Falco tinnunculus* (Fattorini et al., 2001; Costantini et al., 2005) and *Athene noctua* (Fattorini et al., 1999).

PHENOLOGY: *Bubas bison* is active from September to June with two demographic peaks during spring (March and April) and other in autumn (October and November) (Kirk, 1983; Lumaret, 1990; Martín Piera and López-Colón, 2000; Errouissi et al., 2009; Agolitta et al., 2012). Our data are in accordance with the literature, showing the presence of *Bubas bison* during spring months (although our tramping period may have prevented the registration of the spring peak) and the demographic peak during autumn.

All this data well reflects the reproductive cycle of this species. The adults of the new generation emerge from September to January and become to nesting and laid eggs. Regardless of when they were laid, all the eggs hatch at the same time in May (Kirk, 1983). Than, the spring peak correspond to the period of maximum reproductive activity, whereas the autumnal one to the adult emergence (Klemperer, 1981; Kirk, 1983; Kirk and Kirk, 1990; Lumaret, 1990; Lumaret and Kirk, 1991; Martín Piera and López-Colón, 2000; Palestrini et al., 2001).


Bubas bison
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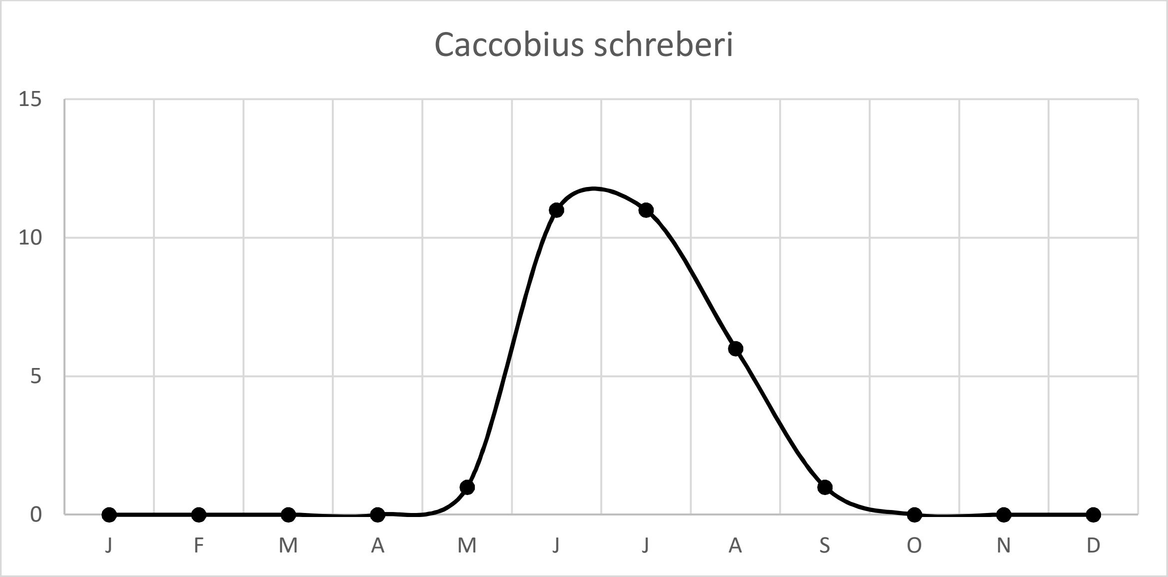
Caccobius schreberi (Linnaeus, 1767)

BIOLOGY: *Caccobius schreberi* is a diurnal species (Mena et al., 1989) typical of open habitat as pastures without arboreal vegetation (Lumaret and Kirk, 1987; Lumaret, 1990; Galante et al., 1991; Verdú et al., 2011) on clayey, silty or sandy soils (Lumaret, 1990; Sullivan et al., 2016). It is a polyphagous species that can be found in various excrements such as cow, horse, sheep, deer, fallow deer, wild boar, dog and human, although seem to prefer cow and horse dung indistinctly (Galante, 1979; Zunino, 1982; Lobo, 1985; Lumaret, 1990; Martín Piera and Lobo, 1996; Martín Piera and López-Colón, 2000; Dormont et al., 2004; Errouissi et al., 2004) and appear more attracted by dung contaminated with ivermectin (Errouissi and Lumaret, 2010). Ziani and Moradi Gharakhloo (2011) found it into rodent burrows in Iran. It was part of the diet of *Glareola pratincola* in Ukraine (Young, 2015).

Our data (N=30) about trophic preference show that there is not any preference toward cow (53%) or horse dung (47%) (IndVal 21.3; P = 0.887), in accordance with above cited literature.

Caccobius schreberi is a tunneler species that builds a compound nest where burying several brood masses of dung (15 x 10 mm), in each of which lays an egg (Lumaret, 1990).

PHENOLOGY: *Caccobius schreberi* have a spring-summer phenology with a maximum demographical between May and August (Galante, 1979; Lumaret and Kirk, 1987; Lumaret, 1990; Galante et al., 1991, 1995; Martín Piera and López-Colón, 2000; Sullivan et al., 2016). Eggs are laid during May and June, and after a rapid larval development (about 45 days), the adult of the new generation emerging (Lumaret, 1990). Our phenological data coincide with those of the above cited literature, and show the beginning of the activity in may, and is maintained until the end of the summer.



Calamosternus granarius (Linnaeus, 1767)

BIOLOGY: *Calamosternus granarius* show a great ecological plasticity (Galante, 1983; Lumaret, 1990; Wassmer, 1995; Veiga, 1998; Dellacasa and Dellacasa, 2006) and may be found from sea level until above 2000 m a.s.l. in any habitat type such as grasslands (Zunino, 1982; Lumaret and Kirk, 1987; Lumaret, 1990; Zamora et al., 2007; Romero-Samper and Lobo, 2009; Meijer et al., 2011; Verdú et al., 2011), shrublands (Lumaret and Kirk, 1987; Verdú et al., 2000) and forest (Macagno and Palestrini, 2009).

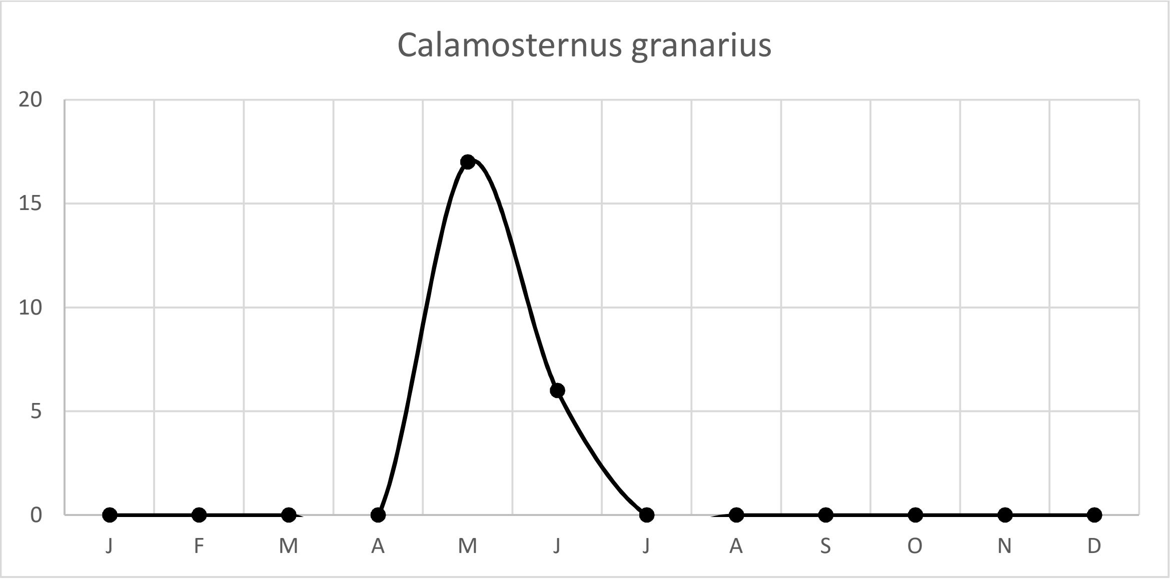
It is a tipical dweller species that laid eggs into the dung pat where the entire ontogenetical development occurs. *Calamosternus granarius* is a polyphagous species that can be found indistinctly in any dung type such as sheep, goat, horse, cow, human, lagomorphs, bear, wild boar (Galante, 1983; Lumaret, 1990; Martín Piera and Lobo, 1996; Dormont et al., 2004; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Romero-Samper and Lobo, 2009) and also in rotten vegetables and carrions (Landin, 1961; Carpaneto and Fabbri, 1984; Veiga, 1998; van Wielink, 2004). However, some authors have found its preference toward dung with lower hydric content such as sheep dung (Lumaret, 1990; Wassmer, 1995; Gittings and Giller, 1998; Veiga, 1998; Agoiz-Bustamante, 2008). Ziani and Moradi Gharakhloo (2011) found it into rodent burrows in Iran.

Our data (N=23) on trophic preference, although not significant (IndVal 34.8; *P* = 0.131), show a strong preference toward less humid dung such as horse dung (87%) compared with cow dung (13%) in accordance with above cited authors.

The presence of endectocides (Doramectin, Eprinomectin, Ivermectin and Moxidectin) increase the attractiveness of the dung toward *C. granarius* (Floate, 2007; Errouissi and Lumaret, 2010).

It is part of the diet of several species such as: *Corvus frugilegus, Capella gallinago, Tyrannus tyrannus, Contopus virens, Sayornis phoebe, Sayornis nigricans, Epidonax traillii, Tachycineta thalassina, Petrochelidon pyrrhonota, Riparia riparia, Corvus brachyrhynchos, Hylocichla guttata, Sialia sialis, Sialia mexicana, Sialia currucoides, Sturnus vulgaris, Vireo olivaceus, Vireo gilvus, Vireo flavifrons, Vireo griseus, Vireo solitarius, Sturnella neglecta, Pheucticus ludovicianus, Sericornis citreogularis, Sericornis frontalis, Bufo cognatus, Bufo compactilis, Bufo terrestris, Bufo w. woodhousii* and *Sceloporus graciosus,* (Young, 2015).

PHENOLOGY: it can be found throughout the year, but mainly during spring and summer months (Lumaret and Kirk, 1987; Lumaret, 1990; Wassmer, 1994; Palestrini et al., 1995; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008). According to Verdú (1998), the adult emergence occurs in March and the oviposition in May. Overwinter as adult (Lumaret, 1990; Dellacasa and Dellacasa, 2006). Our data are in accordance with previous literature, and show a demographic peak during spring months, probably during the maximum reproductive activity.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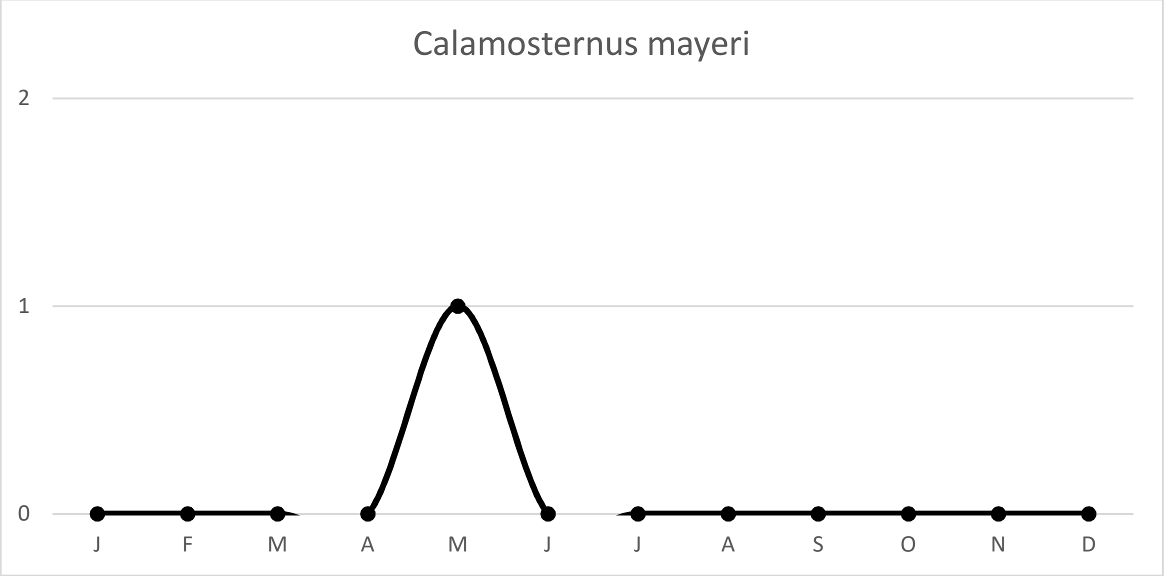
Calamosternus mayeri (Pilleri, 1953)

BIOLOGY: stenotopic species, exclusively on open and sunny habitats as exposed xeric pastures (Veiga, 1998; Dellacasa and Dellacasa, 2006). It seems to prefer dung with low moisture content such as sheep, goat and horse dung (Ávila and Sánchez-Piñero, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006), although may be found in cow, human and lagomorphs (Veiga, 1998).

During our study we found only one specimens (male) in cow dung, that represent the first finding of this species for the entire Marche region (Tonelli et al., 2016).

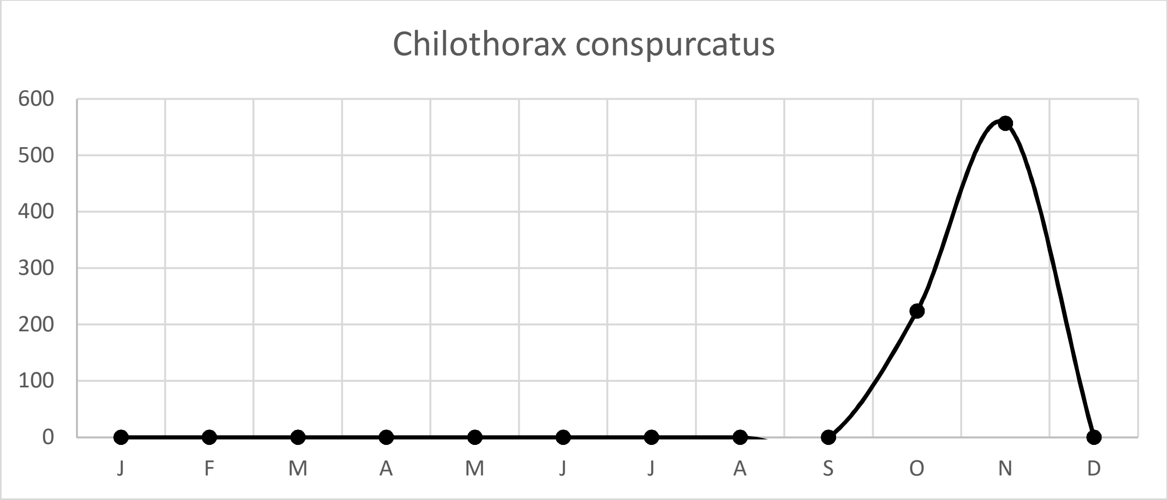
PHENOLOGY: it is a species with winter and spring activity (Veiga, 1998; Dellacasa and Dellacasa, 2006). We found the specimens on 15/5/2014.



Chilothorax conspurcatus (Linnaeus, 1758)

BIOLOGY: oligotopic species that prefeer sheltered pastures or forested habitats (Mariani, 1971; Veiga, 1998; Dellacasa and Dellacasa, 2006; Kamiński et al., 2015). It is a tipical dweller species, then all the ontogenetic development occurs into dung pat (Romero-Samper and Martín-Piera, 2007). *C. conspurcatus* may be found into several dung types such as horse, sheep, cow, cervids, wild boar (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008), although several authors highlight its preference toward horse dung (Lumaret, 1990; Dellacasa and Dellacasa, 2006). In accordance with this latter authors, we found (N=781) a strongly, if not exclusive, significant preference toward horse dung (99.6%) compared with cow dung (0.4%) (IndVal 59.8; $P = 0.001$).

PHENOLOGY: several authors highlight the possibility of two generations per year, one in spring and another in autumn (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006), with a demographic peak during the latter (Lumaret, 1990; Agoiz-Bustamante, 2008; Jay-Robert et al., 2008). Overwinter as adult (Lumaret, 1990; Dellacasa and Dellacasa, 2006). Because our sampling period not include the beginning of the spring, the first generation peak registration was prevented. However, we are in accord with the previous quoted authors for the demographic explosion during autumnal months.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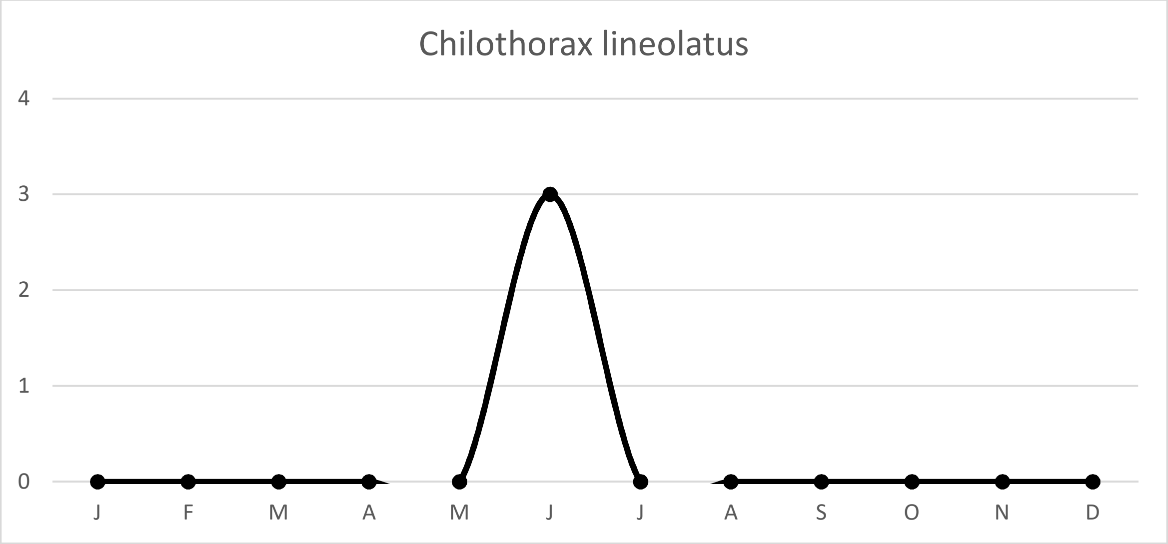
Chilothorax lineolatus (Illiger, 1803)

BIOLOGY: *Chilothorax lineolatus* is a oligotopic species with preference toward open habitats as exposed pasture with Mediterranean influence (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006; Verdù et al., 2011; Numa et al., 2012). It is a dweller species which ontogenetic development happen into the dung pat. It may be found into various type of dung as goat, sheep, horse, cow, dog, deer, fallow deer, and human (Lumaret, 1990; Martín-Piera and Lobo, 1996; Veiga, 1998; Carpaneto et al., 2005; Dellacasa and Dellacasa, 2006). There is not accordance in literature about its trophic preference that vary from sheep (Dellacasa and Dellacasa, 2006) to goat (Veiga, 1998) and cow (Ávila and Sánchez-Piñero, 1990; Lumaret, 1990; Martín Piera and Lobo, 1996).

We found only three specimens (66% horse dung; 33% cow dung), which prevents to outline any trophic preference for this species (Indval 8.9; $P = 1.000$).

PHENOLOGY: *Chilothorax lineolatu*s is a winter-springly species which population decrease slowly toward summer months (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006; Errouissi et al., 2009) that passing at the adult phase (Veiga, 1998). We only found three specimens at the end of the spring.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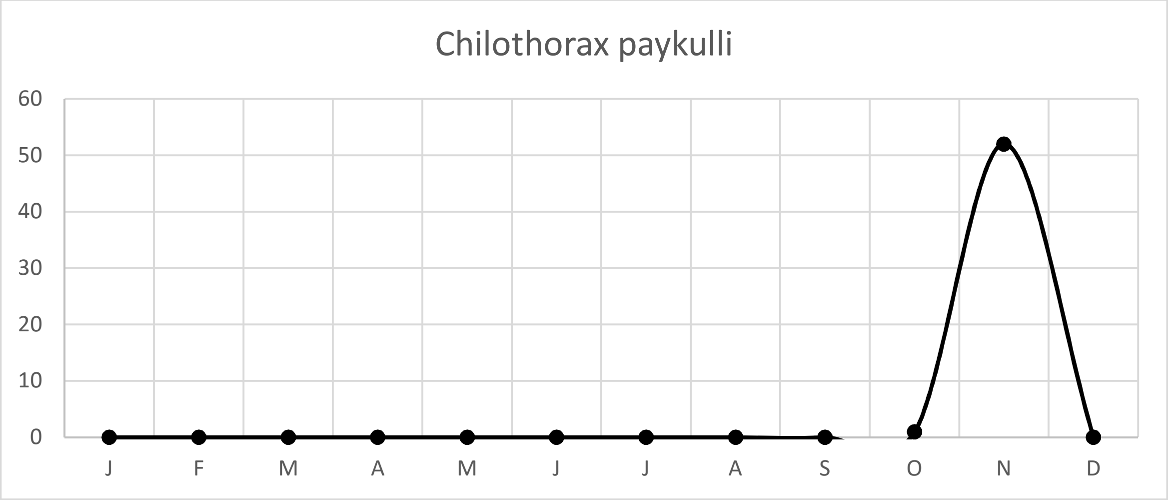
Chilothorax paykulli (Bedel, 1907)

BIOLOGY: *Chilothorax paykulli* is an oligotopic species that prefeer forested habitat or sheltered pasture with drained soils (Landin, 1961; Avila and Sánchez-Piñero, 1989, 1990; Wassmer, 1995; Hansen et al., 1998; Veiga, 1998; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008; Borowski et al., 2016). It is a polyphagous species that can be found in dung of sheep, cow, horse, lagomorphs, human, goat (Lumaret, 1990; Wassmer, 1995; Veiga, 1998; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008; Borowski et al., 2016) that colonize at when have higher moisture content (Sowig and Wassmer, 1994). It was found also in rotting vegetables (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006). We do not found a significant trophic preference (N=53; IndVal 18.1; $P = 0.346$) in accordance with previous authors, but a strongly trend toward horse dung (91%) was found comparing with cow dung (9%).

PHENOLOGY: this is a univoltine (Veiga, 1998; Wassmer, 1994) species typical of the cold months, that start its activity in autumn and slowly decrease toward the spring (Lumaret, 1990; Wassmer, 1994; Veiga, 1998; Dellacasa and Dellacasa, 2006). It may overwinter at the adult or egg phase (Landin, 1961; Lumaret, 1990; Wessmer, 1994). Our data confirm it maximum demographic during autumnal season.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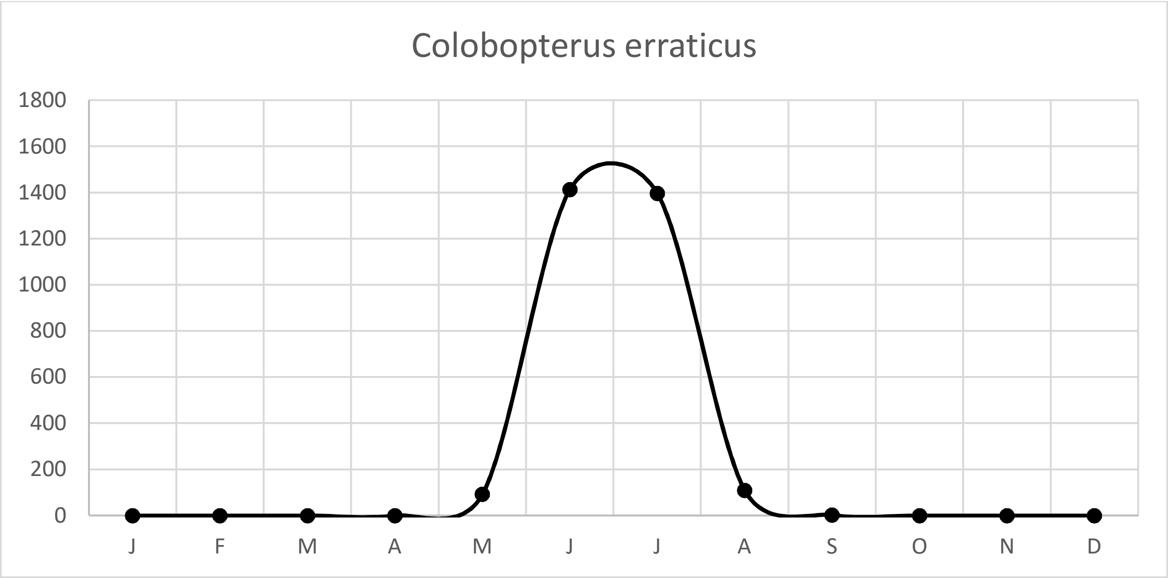
Colobopterus erraticus (Linnaeus, 1758)

BIOLOGY: this is a oligotopic diurnal (Psarev, 2001b) species with a strong preference toward open exposed pasture (Landin, 1961; Galante, 1983; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Macagno and Palestrini, 2009; Romero-Samper and Lobo, 2009; Negro et al., 2011; Verdú et al., 2011; Numa et al., 2012; Tocco et al., 2013). It may be found in various dung type such as cow, cervids, human, goat, horse, sheep, bear, wild boar, dog and marmot (Galante, 1983; Carpaneto and Fabbri, 1984; Lumaret, 1990; Martín-Piera and Lobo, 1996; Veiga, 1998; Dormont et al., 2004; Errouissi et al., 2004; Carpaneto et al., 2005; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008) although a strong preference toward cow dung was found all around its distributional range (Galante, 1983; Lumaret, 1990; Errouissi et al., 2004; Martín Piera and Lobo, 1996; Dormont et al., 2004; Borghesio et al., 2001) that colonize in the first period after its deposition (Gittings and Giller, 1998; Psarev, 2001a; Sladecek et al., 2013). The trophic preference and its early successional colonization stage may be due to its inability to feed on big dung particles (< 5 μm) (Holter, 2000). In accord with this authors we found a significant preference (N= 3013; IndVal 86.7; $P = 0.003$) toward cow dung (87%) compared with horse dung (13%). This species seems more attracted by dung contaminated with endectocide such as Doramectin, Eprinomectin, Ivermectin and Moxidectin (Floate, 2007; Errouissi and Lumaret, 2010; Sutton et al., 2014). *Colobopterus erraticus* is a tunneler species which female dig short galleries (n= 4-8; 3-10 cm deep) below dung pat, and laid one egg whitin a cell prepared near gallery. Subsequently each nest is supplied with dung needed for the entire larval development. After hatching, larvae move toward dung reserve in the gallery where the nymphosis occur (Rojewski, 1983). It was experimentally demonstrated that a temperature increase of 2.3° C would anticipate eggs laying of 4.1 days and the hatched of 7.2 days. Moreover, eggs dimensions' decrease of about 22% and the larvae of 33% (Wu and Sun, 2012)

It forms part of the diet of *Riparia riparia, Sylvia communis, Corvus frugilegus, Corvus monedula* and *Eptesicus serotinus* (Young, 2015)

PHENOLOGY: it is a species active during spring and summer months with demographic peak during June and July (Galante, 1979; Lumaret, 1990; Veiga, 1998; Borghesio et al., 2001; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008; Jay-Robert et al., 2008; Errouissi et al., 2009; Romero-Samper and Lobo, 2009; Sullivan et al., 2016). Although in some regions it shows two generations per year (Landin, 1961), at our latitudes only one generation exist (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006; Romero-Samper and Lobo, 2009). May overwinter as adult or third instar larvae buried 20-27 cm deep in the soil (Lumaret, 1990; Dellacasa and Dellacasa, 2006).

Our data confirm the presence of only one generation during spring and summer months with a peak in June and July, in accordance with above cited authors.



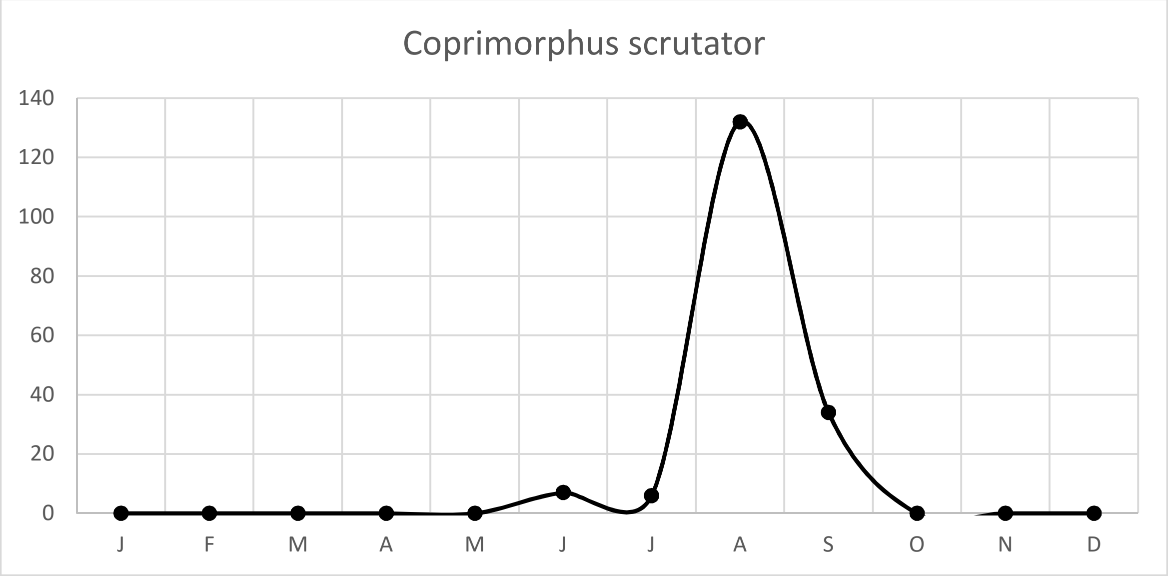
Coprimorphus scrutator (Herbst, 1789)

BIOLOGY: *Coprimorphus scrutator* is a stenotopic species which habitat requirement is restricted to open habitats as exposed pastures, on moist loamy and clayey soils (Galante, 1983; Lumaret, 1990; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008). Although this species can be found into various dung types as cow, horse, sheep and human, a general agreement exist about its strong preference toward cow dung (Galante, 1983; Lobo, 1985; Lumaret, 1990; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dormont et al., 2004; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008), that colonize at its intermediate state of hydration (Veiga, 1998; Dellacasa and Dellacasa, 2006). Our data (N=179) about trophic preference, although not significant (Indval 39.9; P = 0.191), are in accordance with above cited authors and highlight a trend of preference toward cow dung (75%) compared with horse dung (25%).

Coprimorphus scrutator is a tunneler species. It relocates its food and provisions nest. Nesting is of a primitive type, since the eggs are not laid directly in food source but in roughly cilindrical (13.6 x 9.2 mm) nest masses, built under the ground surface at about 1 cm depth. The egg is laid in a chamber (7 x 4 mm) located at the bottom of the nest mass. Each pair laid on average 7-8 eggs. Brood masses seemed to be abandoned after egg deposition. They might have been built by females only. (Palestrini and Barbero, 1994).

PHENOLOGY: it is a summerly species which activity start in late spring and decrease toward autumn with a demographic peak in August and September (Lumaret, 1990; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008; Jay-Robert et al., 2008). *Coprimorphus scrutator* have only one generation per year, that need seven months to complete its biological cycle (Veiga, 1998; Dellacasa and Dellacasa, 2006). Overwinter as third instar larvae (Dellacasa and Dellacasa, 2006).

Our phenological data are strongly in accordance with the literature, showing the beginning of the activity in late spring, with a population peak in midsummer, which fades slowly toward autumn.



Copris lunaris (Linnaeus, 1758)

BIOLOGY: *Copris lunaris* is a eurytopic nocturnal species (Mena et al., 1989; Lumaret, 1990) that colonize preferably open habitat such as pastures without arboreal vegetation (Zunino, 1982; Lumaret, 1990; Wassmer, 1995; Martín Piera and López-Colón, 2000). It shows a strong preference toward cow dung although it was found in horse, sheep, human and dog dung (Zunino, 1982; Lumaret, 1990; Wassmer, 1995; Martín Piera and López-Colón, 2000). Contrary to the literature, our data (N=23) about trophic preference are not significant (IndVal 20.9; $P = 0.69$), moreover we not found any trend of preference toward cow (52%) or horse dung (48%).

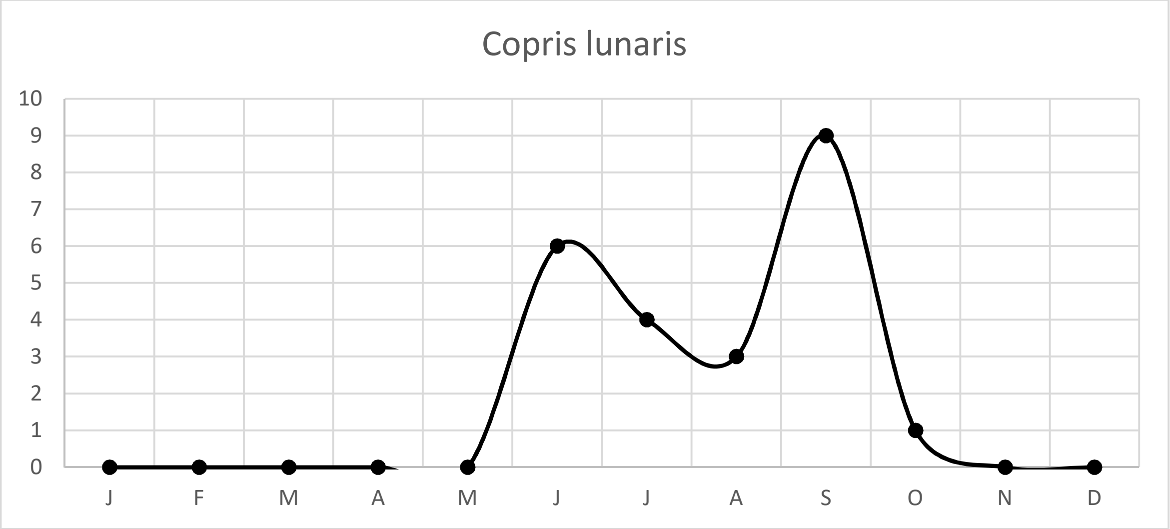
Copris lunaris is a tunneler species. Females, alone or helped by male, build a hypogeal nest below excrement (10-30 cm deep) where buried 100-300 g of dung mass from which subsequently will shape 3-9 ovoidal brood balls (15-28 g). Only one egg is laid in each brood ball and the oviposition include 4 phases: a) egg chamber formation in the apical pole of the brood ball, b) oviposition, c) closure of the chamber and d) formation of the porous area. The female remains inside the nest until emergence of the new adults, and providing parental care to their progeny by repairing damaged brood balls and defending them from disturbances. The normal nesting sequence consisted then of three phases: excavation and provisioning (3-5 days), then brood ball formation and oviposition (approximately 10 days), and finally brood care (approximately 90 days) (Klemperer 1982a, 1982b; Kirk and Feehan, 1984; Lumaret, 1990). Zunino and Monteresino (1994) had demonstrated that this species can relocate the dung horizontally above soil surface when the burial is prevented.

A previsional model highlight the possibilities of a decreasing in its distributional range until 84% in a climate change scenario (Dortel et al., 2013).

It has been documented that *Copris lunaris* form part of the diet of *Strix aluco, Coracias garrulous, Melanocorypha calandra, Corvus frugilegus, Athene noctua* and *Rhinolophus ferrumequinum* (Charrier and Vaslin, 2005; Kitowski and Pawlega, 2010; Young, 2015).

PHENOLOGY: it is a spring-summerly species which show an activity peak between May and September (Galante, 1979; Klemperer 1982a; Lumaret, 1990; Galante et al., 1991; Lumaret and Kirk, 1991; Wassmer, 1994; Galante et al., 1995; Martín Piera and López-Colón, 2000; Jay-Robert et al., 2008). Several authors highlight the presence of two activity peak, one in spring and other toward autumn (Galante, 1979; Wassmer, 1994; Lumaret and Kirk, 1991; Jay-Robert et al., 2008). This phenological pattern reflect the biological cycle of this species, which require 90 days (Klemperer, 1982a). Indeed, the demographic peak of spring correspond to the nesting period, whereas the second one represent the emergence of part of the adult of the new generation which will nesting the following spring (Galante, 1979; Klemperer 1982a; Lumaret and Kirk, 1991; Martín Piera and López-Colón, 2000). Overwinter at adult stage (Klemperer, 1982a).

Our phenological data correspond to the typical phenological pattern of this species. Indeed, we found the start of the activity during late spring early summer, which decrease during midsummer, and increase toward autumn probably due to the emergence of the new generation.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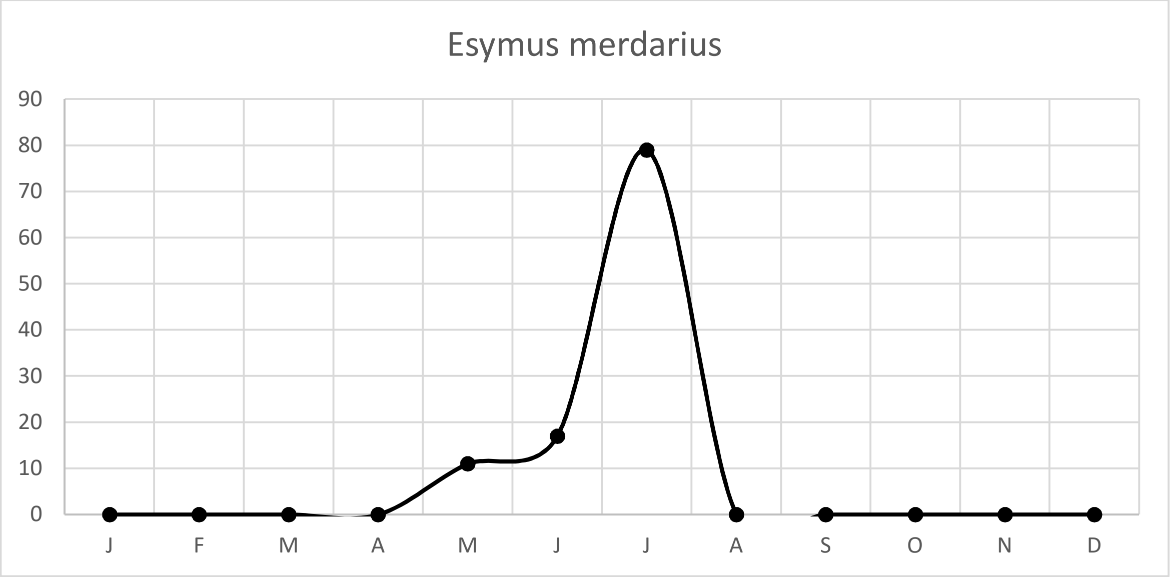
Esymus merdarius (Fabricius, 1775)

BIOLOGY: this is a oligotopic species that prefer open sunny habitat as exposed pastures with a good quantity of humidity (Lumaret, 1990; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006). *Esymus merdarius* is a tipical dweller species, which larval development occur totally into dung pat (Romero-Samper and Martín Piera, 2007).

It may be found in several dung type such as cow, horse, sheep, goat and human (Lumaret, 1990; Martín Piera and Lobo, 1996; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dormont et al., 2004; Carpaneto et al., 2005; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008), but there is not accordance in the literature about its trophic preference that change from human (Martín Piera and Lobo, 1996), to sheep (Lumaret, 1990; Dellacasa and Kirgiz, 2002), horse (Veiga 1998; Dormont et al., 2004) and cow (Veiga 1998; Agoiz-Bustamante, 2008). Our data (N=107) are in accordance with the literature considering that we do not found any preference (Indval 17.8; $P = 0.775$) toward cow (47%) or horse dung (53%).

It was quoted as part of the diet of *Apus apus* and *Phyrocorrax phyroccorax* (Young, 2015).

PHENOLOGY: *Esymus merdarius* is a species which activity start after the first spring rains and decrease toward midsummer, with demographic peaks in April-May (Lumaret, 1990; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008; Jay-Robert et al., 2008). It has only one generation per year and overwinter as adult or third instar larvae (Landin, 1961; Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006). Our phenological data are in little discordance with previous literature, because we have recorder the generational peak during summer in July. However, this may be due to the climatic conditions of our area that is more temperate compared to the areas of study of the above cited literature. Indeed, Lumaret (1990) highlight that *Esymus merdarius* has a shifted phenology toward summer in the north of France, compared with south of France.

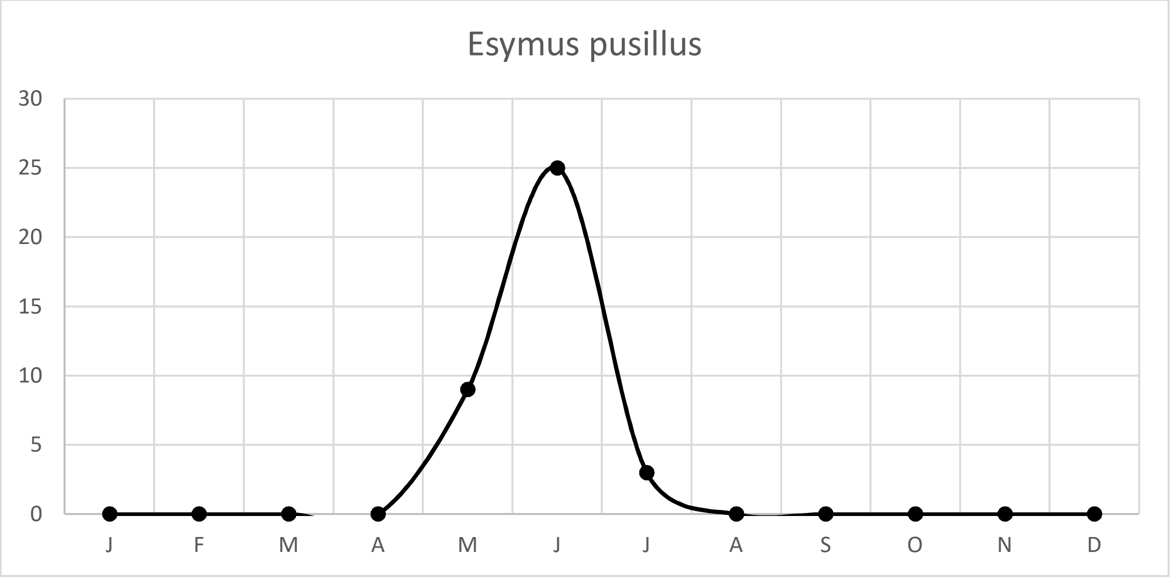


Esymus pusillus (Herbst, 1789)

BIOLOGY: *Esymus pusillus* is a eurytopic generalist (Negro et al., 2011) species that can be found in several habitats from open pastures (Roslin, 2000; Borghesio et al., 2001) to woodland (Barbero et al., 1999). It can be found in several dung type such as cow, horse, sheep, goat, human and marmot (Falcoz, 1915; Galante, 1983; Lumaret, 1990; Wassmer, 1995; Veiga, 1998; Borghesio et al., 2001; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008) that colonize in the first 3-4 days after its deposition (Psarev, 2001a). Our data (N=37) highlight a strong significant preference (IndVal 61.4; $P = 0.006$) toward cow dung (84%) compared with horse dung (16%), in accordance with Veiga (1998).

It is a dweller species during feeding, but during nesting eggs were laid singly in spaces, not coated with dung, in the soil beneath dung pat (10.3 ± 9.73 mm depth) (Yoshida and Katakura, 1992). However, because no dung relocation exists, we consider this species as no nesting dweller species.

PHENOLOGY: *Esymus pusillus* have a tipical spring-summer phenology, although in some part of its distrubutional range show a second peak, corresponding to a second generation, during autumn (Galante, 1983; Lumaret, 1990; Wassmer, 1994; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008; Jay-Robert et al., 2008). Overwinter as adult (Lumaret, 1990; Dellacasa and Dellacasa, 2006). Our data confirm the presence of only one generation in our study area, which start the activity during spring and rapidly decrease toward midsummer, probably due to the intolerance toward high temperatures (Veiga, 1998; Dellacasa and Dellacasa, 2006).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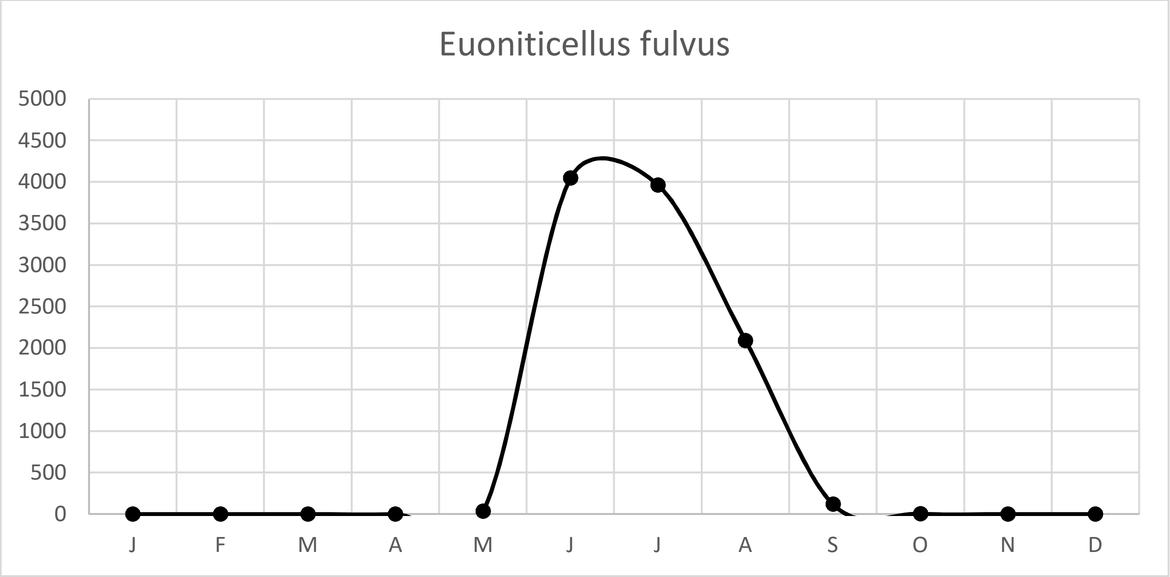
Euoniticellus fulvus (Goeze, 1777)

BIOLOGY: *Euoniticellus fulvus* is a diurnal (Mena et al., 1989; Lumaret and Kirk, 1987) eurytopic species that colonize preferently open habitat as exposed pastures (Zunino, 1982; Lumaret and Kirk, 1987; Lumaret, 1990; Galante et al., 1991; Barbero et al., 1999; Lobo et al., 2001; Macagno and Palestrini, 2009; Verdú et al., 2011) on moist sandy and clayey soils (Lumaret, 1990; Sullivan et al., 2016). It shows a strong preference toward cow (Galante, 1979; Lumaret, 1990; Barbero et al., 1999; Martín Piera and López-Colón, 2000; Errouissi et al., 2004) and horse dung (Rodríguez-Romo et al., 1988; Dormont et al., 2004) that colonize until 3-4 days after its deposition (Lobo, 1992). However, it may be found in several dung type such as sheep, fallow deer, goat, pig, wild boar, human, deer and dog (Barbero et al., 1990; Martín Piera and Lobo, 1996; Carpaneto et al., 2005). Ziani and Moradi Gharakhloo (2011) found it into rodent burrows in Iran. The maximum diameter of ingested particles of dung is about 12-16 µm (Holter et al., 2002). Several authors highlight that *E. fulvus* seem more attracted by dung contaminated with endectocide (Wardhaugh and Mahon, 1991; Errouissi and Lumaret, 2010), which lead to increased mortality and delayed maturation of newly emerged adults (Lumaret et al., 1993; Wardaugh et al., 1993; Wardaugh et al., 1998; Wardhaugh et al., 2001a).

We do not found significant trophic preference (N=10259; IndVal 59.7; P = 0.172), although a slight trend toward horse dung (60%) compared to cow dung (40%) was found.

Euoniticellus fulvus is a tunneler species which nest consist in a short gallery dug below dung pat, at the end of which the female buries a certain amount of dung and laid one egg. Several authors highlight that *E. fulvus* nests's are composed, and very similar to that of some *Onthophagus* species (Lumaret, 1990; Halffter and Edmonds, 1982; Martín Piera and López-Colón, 2000).

PHENOLOGY: this is a species which activity start in spring, show a demographic peak in summer, and rapidly decrease toward autumn (Galante, 1979; Lumaret, 1990; Galante et al., 1991; Galante et al., 1995; Jay-Robert et al., 2008; Errouissi et al., 2009; Agoglitta et al., 2012; Sullivan et al., 2016). The eggs laying occur during May-July (Galante, 1979; Lumaret, 1990). The larval development is fast, and the new generation emerge after 45 days that overwintering until the next spring (Galante, 1979; Lumaret and Kirk, 1991; Martín Piera and López-Colón, 2000).

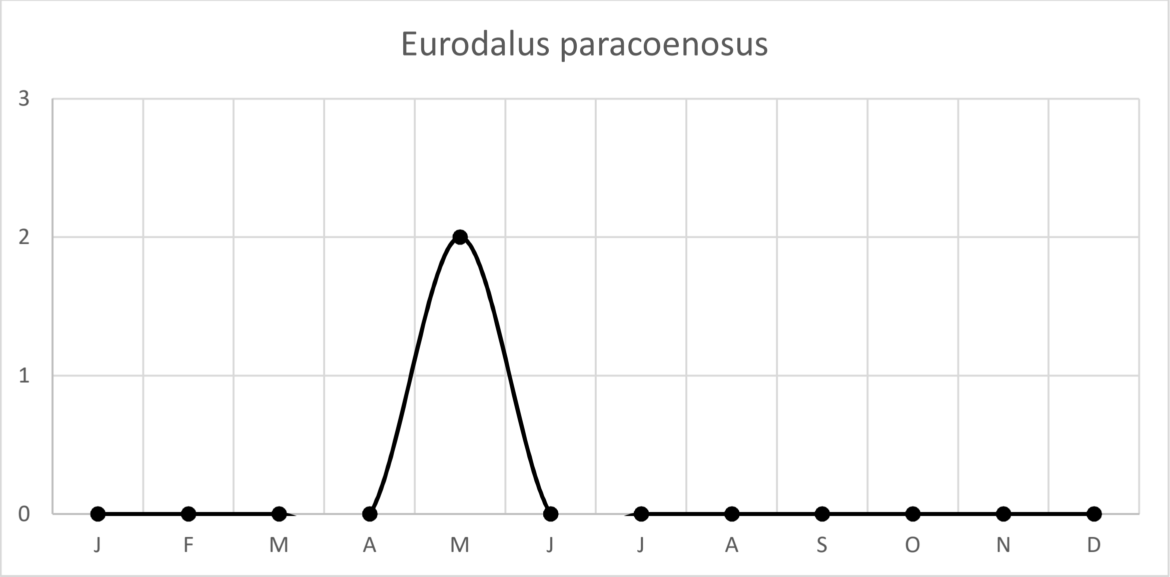


Euorodalus paracoenosus (Balthasar & Hrubant, 1960)

BIOLOGY: *Euorodalus paracoenosus* is a oligotopic species that prefer dry and open habitat (Zunino, 1982; Lumaret and Kirk, 1987; Lumaret, 1990; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006), although Barbero et al. (1999) highlight its preference toward wooded areas in north Italy. It is a dweller species which ontogenetic development occur into dung pat. It may be found in several dung types such as cow, horse, sheep, goat, deer, human, dog and fox (Zunino, 1982; Lumaret, 1990; Veiga, 1998; Barbero et al., 1999; Dellacasa and Kirgiz, 2002; Dormont et al., 2004; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008). Brussaard (1987) highlight that it can act as kleptocoprid of *Thyphaeus typhoeus* brood masses. It shows a great variability in dung preference across its distributional range, which vary from sheep (Lumaret, 1990; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006), goat (Veiga, 1998), horse (Dormont et al., 2004), deer (Barbero et al., 1999) and cow (Zunino, 1982; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008). It seems more attracted by dung contaminated with ivermectin (Errouissi and Lumaret, 2010).

We only found two specimens in horse dung, which prevent to highlight any trophic preference (IndVal 6.7; $P = 1.000$).

PHENOLOGY: This species shows a phenology springly phenology, which activity decrease toward midsummer (Zunino, 1982; Lumaret and Kirk, 1987; Lumaret, 1990; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008; Jay-Robert et al., 2008). We only found two specimens of this species, which however coincides with its demographic peak in spring.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Geotrupes spiniger (Marsham, 1802)

BIOLOGY: *Geotrupes spiniger* is a nocturnal species (Lumaret, 1990) that colonize open habitat as pastures (Lumaret, 1990; Byk, 2011) on silty and clayey moist soils (Lumaret, 1990). Although, Wassmer (1995) found it as typical of wodded areas, whereas Macagno and Palestrini (2009) as habitat generalists.

It shows a strong preference toward cow dung (Lumaret, 1990; Wassmer, 1995; Borghesio et al., 2001) that can colonize at various days (1-25) after deposition (Gittings and Giller, 1998; Sladecek et al., 2013). It may be found also in a variety of dung types as horse, human, sheep, fox, dunghills (Lumaret, 1990) and also in carrions (van Wielink, 2004; Matuszewski et al., 2008). The maximum dung particles that it can eat have a diameter of 60-90 μm (Holter, 2004).

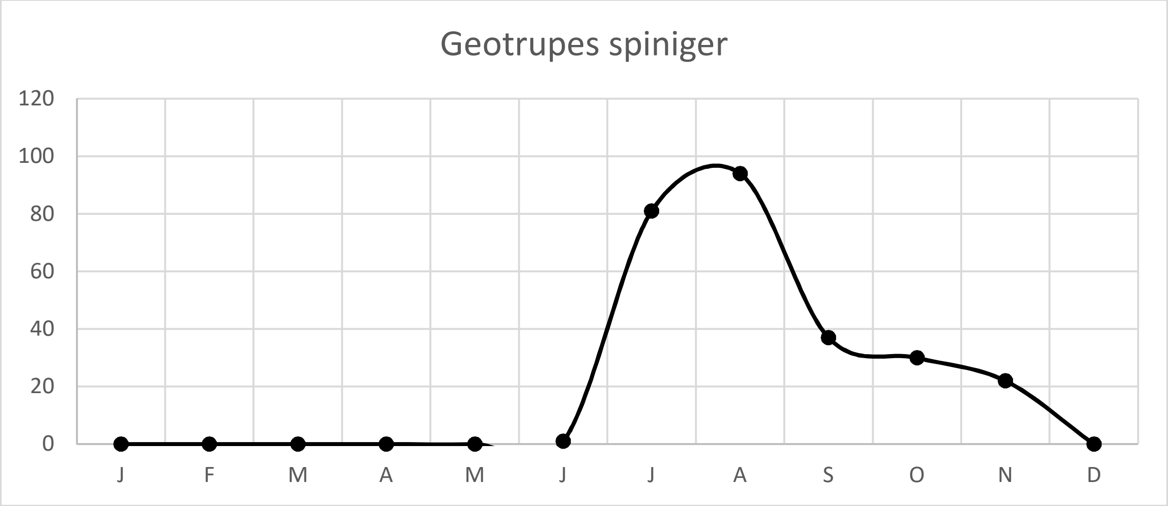
We found a significant preference (N=265; IndVal 65.7; $P = 0.046$) toward cow dung (66%) compared to horse dung (34%), in accordance with previous authors.

G. spiniger is a tunneler species. Costruction of the brood burrow begins with the excavation of a vertical gallery (50-250 mm depth) leading to a horizontal tunnel-like brood chamber. The latter is filled with dung to make a horizontal brood mass (100 mm long) which provides sufficient food for the future larva. The egg is laid at the distal end of the brood mass just after the start of this provisioning phase. The vertical shaft above each brood mass is sealed with soil. In this way a series of brood masses is formed by the female working either alone or in cooperation with the male. Under favourable conditions the female lays about 20 eggs. (Klemperer, 1979, 1980; Kühne, 1996). The larvae are capable to repaired damages in their cells (Klemperer, 1978).

Geotrupes spiniger is part of the diet of *Meles meles*, *Athene noctua*, *Falco tinnunculus*, *Milvus milvus*, *Asio otus* and *Rhinolophus ferrumequinum* (Pigozzi, 1991; Young, 2015).

PHENOLOGY: the activity of *G. spiniger* start in July when adults emrges, and begin to laying eggs in the second half of September until November-December (Lumaret, 1990; Lumaret and Kirk, 1991; Wassmer, 1994; Kühne, 1996; Borghesio et al., 2001; Agoglitta et al., 2012). The larval development lasted about 3 months on average, the prepupal phase 2 weeks, and the pupal stage 4 weeks. The young beetles remained in the pupal cell about 10 days (Klemperer, 1978). The total period of developmenr until leaving the pupal cell averaged about 5-6 months. In the same year, or sometimes only in the following year, the young beetles start breeding after a period of "maturation feeding" (Klemperer, 1979). ln the meantime they normally dig short, vertical food shafts weekly, which they fill with dung for their own nutrition. (Kühne, 1996). Overwinter as larvae or pupae, although is possibly also as adult (Lumaret and Kirk, 1991).

Our data are in accordance with the literature, showing the start of activity of *G. spiniger* during July, probably in coincidence with new generation emergence. This peak slowly decreases toward autumn when probably most of the indivduals are occupied in the nesting.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Labarrus lividus (Olivier, 1789)

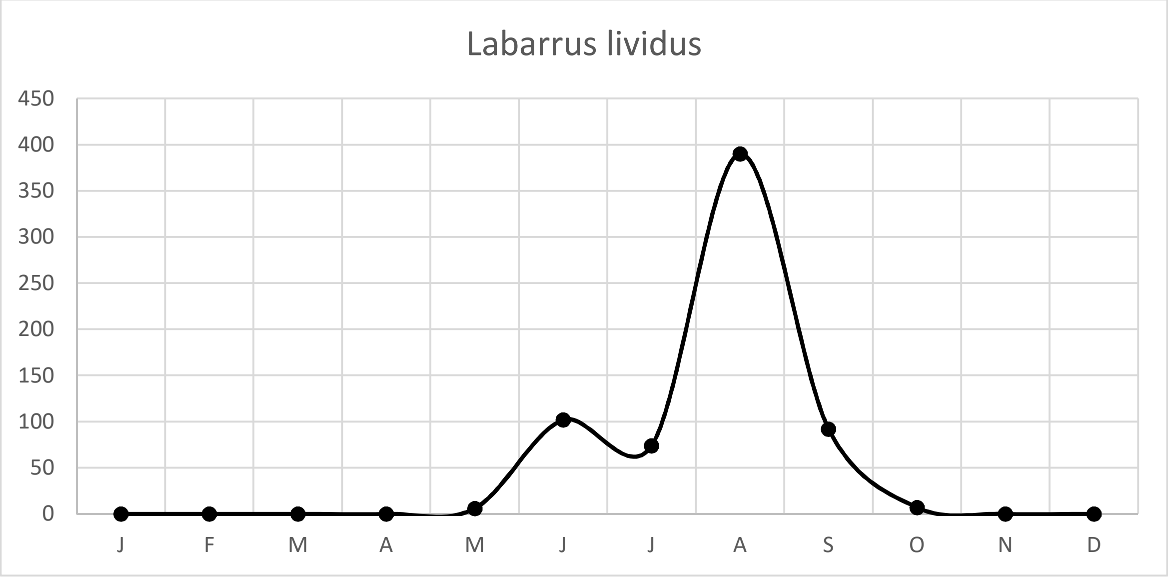
BIOLOGY: *Labarrus lividus* is a nocturnal (Verdú, 1998) oligotopic species which prefeer open habitats such as exposed pastures (Verdú, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Romero-Samper and Lobo, 2009). It is a polyphagous species that can be found in any type of dung such as horse, cow, sheep, dunghills, lagomorphs, human, wild boar (Lumaret, 1990; Martín Piera and Lobo, 1996; Veiga, 1998; Verdú, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008), and also in rotting vegetables (Lumaret, 1990). However, several authors highlight its strong preference toward horse dung (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006). We are in accordance with these latter authors, because we found a strong preference (N=671; IndVal 92.2; $P = 0.001$) toward horse dung (98%) compared with cow dung (2%).

It is a dweller species which larval development occur into dung pat, although nynphosis happen in the soil under dung pat (Romero-Samper and Martín Piera, 2007). In Africa, it can act as kleptoparasite (Rougon and Rougon, 1983).

Labarrus lividus form part of the diet of *Petrochelidon pyrrhonota*, *Corvus brachyrhynchos*, *Stelia mexicana*, *Vireo philadelphicus*, *Richmondena cardinalis*, *Lichenostomus penicillatus*, *Artamus superciliosus* (Young, 2015).

PHENOLOGY: this species is tipical of spring and summerly months (Lumaret, 1990; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008; Romero-Samper and Lobo, 2009; Agoglitta et al., 2012; Sullivan et al., 2016). Its activity starts in spring, and the oviposition occurs during March and April, whereas the larval development take place during the rest of the spring. Adults emerge during midsummer

(Verdú, 1998). It can overwinter as adult (Agoiz-Bustamante, 2008). We found a strong correlation between its biological cycle and our phenological data. Indeed, its start the activity during May-July and show a demographical peak in August, probably in coincidence with new adult emergence.



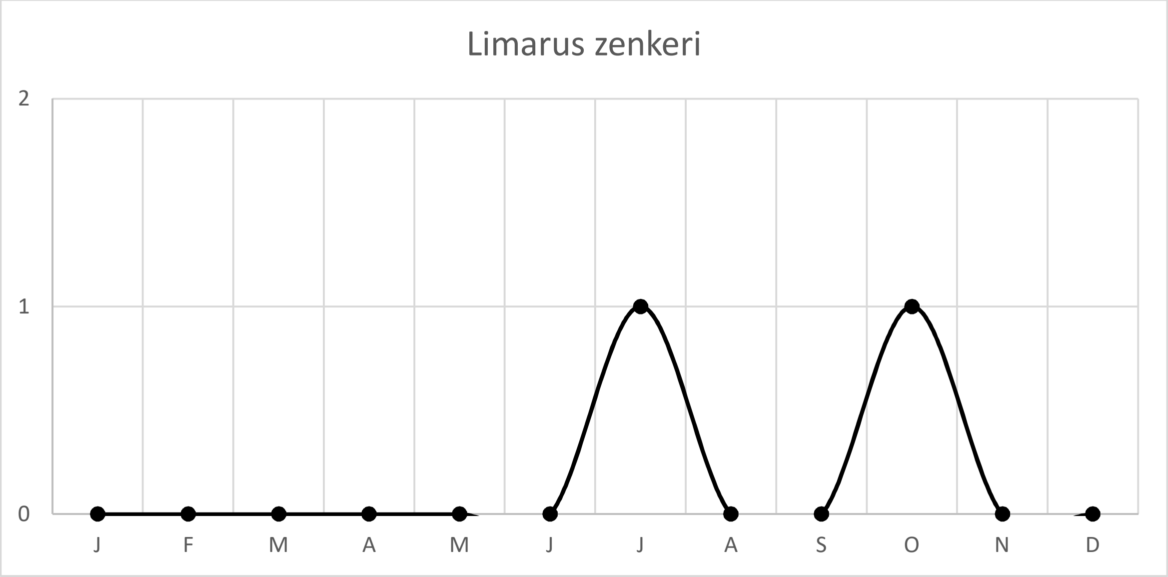
Limarus zenkeri (Germar, 1813)

BIOLOGY: its a heterotopic crepuscular species (Lumaret, 1990), which colonize open pastures in its septentrional distributional area (Landin, 1961), whereas prefer more closed habitat in the southern ones, as in our latitudes (Lumaret, 1990; Wassmer, 1995; Veiga, 1998; Dellacasa and Dellacasa, 2006; Macagno and Palestrini, 2009; Tocco et al., 2013; Borowski et al., 2016), mainly on sandy soils (Lumaret, 1990). It can be found in various dung type as cow, horse, sheep, human, cervids, roe deer, deer, wild boar and bear (Carpaneto and Fabbri, 1984; Lumaret, 1990; Veiga, 1998). It seems to prefer less moist dung as sheep and deer (Lumaret, 1990; Dellacasa and Dellacasa, 2006)

We found only two specimens in horse dung. Although the low number of individuals prevents us to highlight any trophic preference (Indval 13.3; $P = 0.466$), the fact that they have been sampled only on horse manure seems to be in agreement with its slightly moist dung requirements.

PHENOLOGY: *L. zenkeri* begin its activity in spring, but it can be found mainly during summer from July to September (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006; Borowski et al., 2016). It overwinters as third instar larvae (Lumaret, 1990; Dellacasa and Dellacasa, 2006).

We are prevented to highlight any phenological pattern due to the scarsity of sampled specimens.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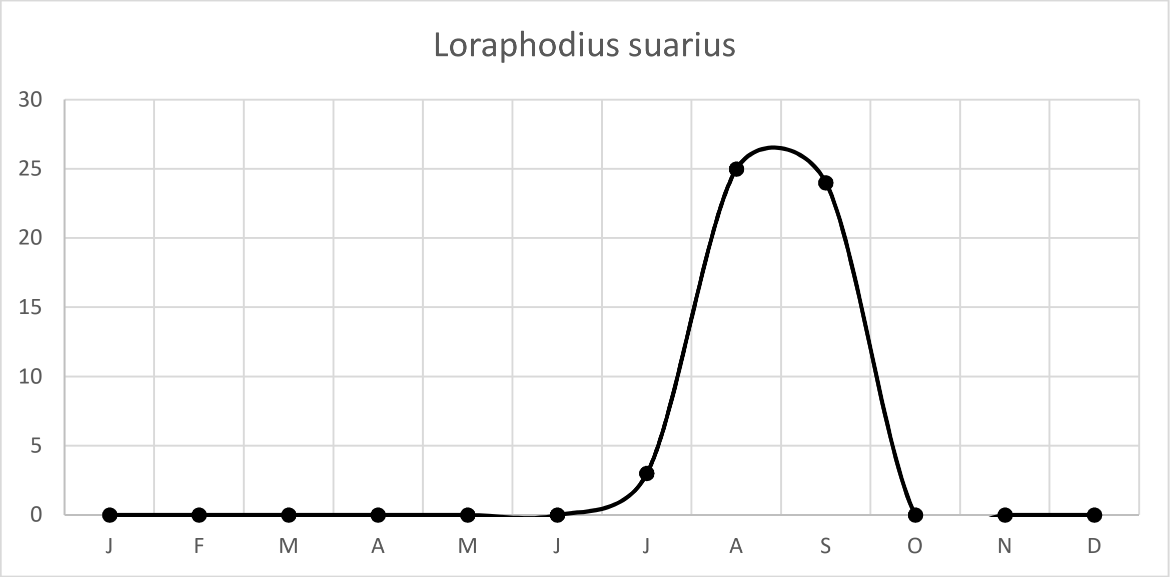
Loraphodius suarius (Feldermann, 1835)

BIOLOGY: *L. suarius* is an oligotopic species that colonize preferably open habitat as exposed pastures (Lumaret, 1990; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006) on moist soils (Lumaret, 1990). It is a polyphagous species that seem to prefer horse dung (Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006), but can be found in different dung types such as cow, human, dog and also on rotting vegetation (Lumaret, 1990; Král and Malý, 1993; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006). Although we do not found significant trophic preference (N= IndVal; 33.8; *P* = 0.392), a little predilection toward horse dung (63%) compared with cow dung (37%) seem to exist.

PHENOLOGY: *L. suarius* occurring in summer, mainly in September, and rapidly disappear toward autumn (Lumaret, 1990; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006). The activity of this species in our province, reflect that recorded by above cited authors in other study areas. The activity starts in July, show a peak during August and September, and totally disappear in October.



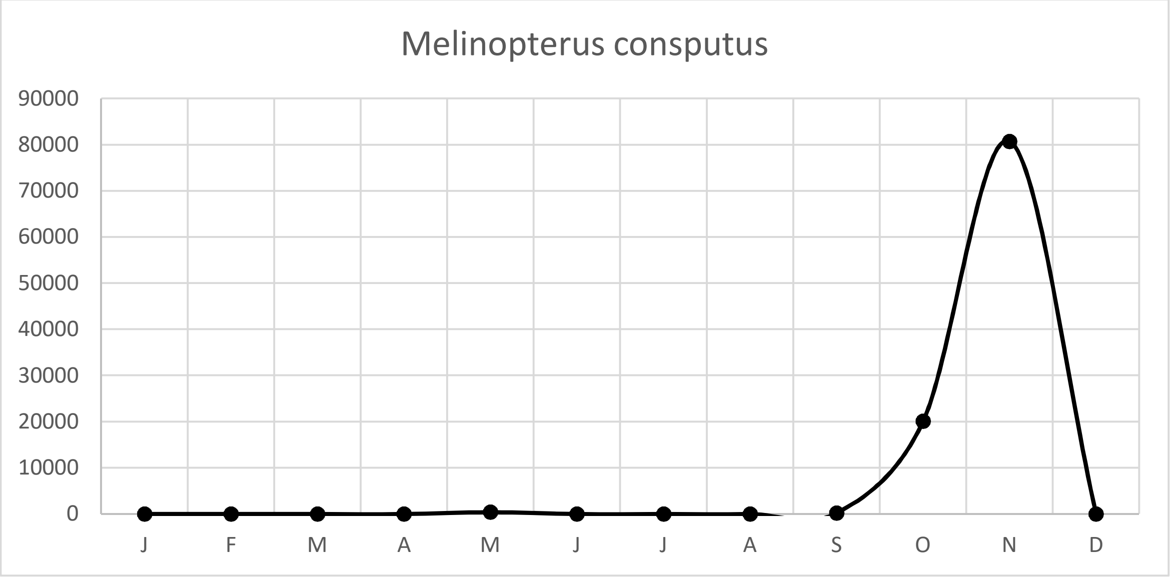
Melinopterus consputus (Creutzer, 1799)

BIOLOGY: it is a eurytopic species that prefer open habitat such as xeric pastures on calcareous soils (Avila and Sánchez-Piñero, 1989; Lumaret, 1990; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Verdú et al., 2011). It is a polyphagous species that can be found in every dung type such as cow, horse, goat, sheep, human, fox, bear, deer, dog and pig (Carpaneto and Fabbri, 1984; Avila and Sánchez-Piñero, 1989; Lumaret, 1990; Veiga, 1998; Carpaneto et al., 2005; Dellacasa and Dellacasa, 2006; Dormont et al., 2007; Agoiz-Bustamante, 2008). It seems to not have a stable trophic preference, considering that it has show different preference across its ditributional range such as for deer (Dormont et al., 2007), sheep (Avila and Sánchez-Piñero, 1989) and cow (Veiga, 1998). In accordance with above cited literature, we do not found any significant trophic preference (N=101'534; IndVal 60.2; $P = 0.618$), even if more individual are trappend in horse dung (60%) compared with cow dung (40%). It is noteworthy that adult phase is coprophagousm whereas the larval phase is saprophagous (Verdú, J.R., pers. comm. 2016).

M. consputus form part of the diet of *Tringa tetanus*, *Scolopax rosticola*, *Cursorius cursor* (Young, 2015). Some larvae were found into *Bubas bison* brood masses (Kirk, 1983). In the southern part of Spain, it was found into a cave (Pérez et al., 2011).

PHENOLOGY: it is a typical autumnal and winterly species, which have a demographical peak in October-November (Avila and Sánchez-Piñero, 1989; Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006; Jay-Robert et al., 2008; Agoglitta et al., 2012; Sullivan et al., 2016), sometimes with explosive adult emergence (Agoglitta et al., 2012). The activity reaches the end of the spring, and disappear in summer (Lumaret, 1990;

Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008). We found the begin of its activity in September-October, a demographic peak during November and we recorded the tail of activity in May, in accordance with above cited authors.



Melinopterus prodromus (Brahm, 1790)

BIOLOGY: *M. prodromus* is a eurytopic species that prefer open habitat as exposed pastures (Lumaret, 1990; Wassmer, 1995; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006) on clayey or silty-clay soils (Lumaret, 1990; Dellacasa and Dellacasa, 2006). Polyphagous species that can be found in every type of dung such as human, lagomorphs, sheep, horse, cow, dog and badger (Lumaret 1990; Hancox, 1991; Wassmer, 1995; Veiga, 1998; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008; Bebermans et al., 2016), that colonize during the first days after its deposition (Gittings and Giller, 1998) when it is very moist (Sladecek et al., 2013). Moreover, it was found in rotting vegetables (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006) and carrions (van Wielink, 2004; Matuszewski et al., 2008). It was quoted into rodent burrows (Falcoz, 1915). There is not accordance in literature about its trophic preference, that vary from sheep (Wassmer, 1995), horse (Bebermans et al., 2016) and human dung (Veiga, 1998). We do not found a significant trophic preference (N=7390; IndVal 55.7; P = 0.213), although a strong evidence of predilection toward horse dung (93%) compared with cow dung (7%) was found.

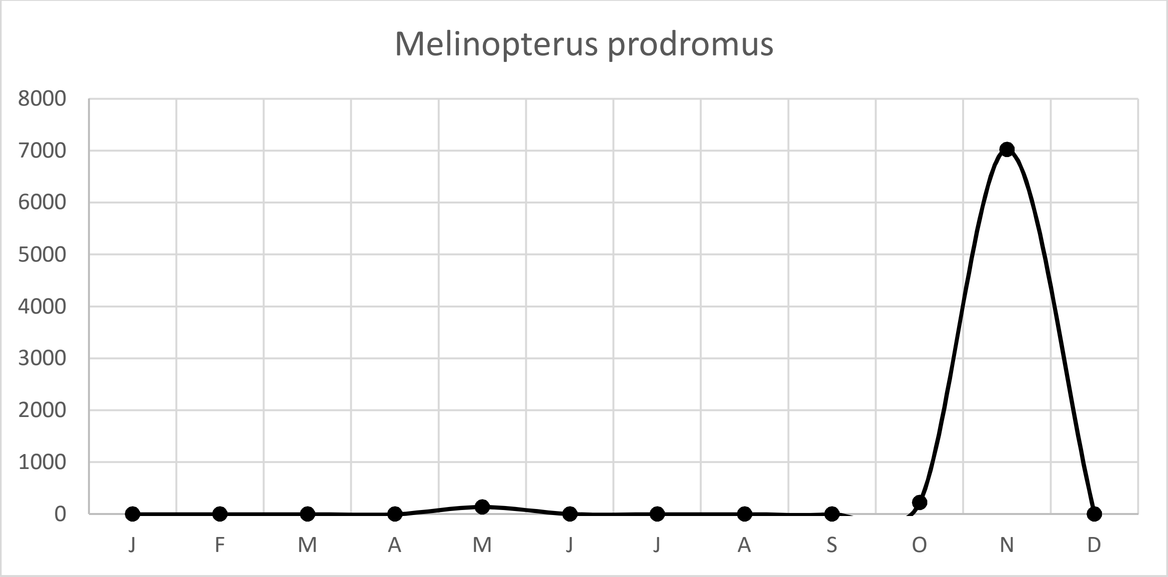
M. prodromus seem to avoid dung contaminated with eprinomectin and doramectin (Floate, 2007; Webb et al., 2010) and it is attracted toward dung contaminated with moxidectin (Floate, 2007). The acctractivenes results toward dung contaminated with ivermectin seem inconsistent: Floate, (1998) show that *M. prodromus* avoid dung contaminated with ivermectin, Sutton et al. (2014) that it is more attracted, whereas Floate (2007) highlight that it is more attracted by contaminated dung in autumn, but that avoid it in spring.

It is a dweller species during feeding, but the eggs are laid outside the dung pat and its larvae are saprophagous (Gittings and Giller, 1997).

M. prodromus form part of the diet of *Hirundo rustica*, *Hylocichla guttata*, *Milvus milvus*, *Pluvialis apricaria*, *Motacilla cinerea*, *Prunella atrogularis*, *Myophonus coeruleus*, *Turdus ericetorum*, *Corvus frugilegus*, *Corvus corone cornix* (Horgan and Berrow 2004; Young, 2015)

PHENOLOGY: this species has two generations per year, one in autumn and other in spring (Stebnicka, 1973; Lumaret, 1990; Wassmer, 1994; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008; Jay-Robert et al., 2008; Agoglitta et al., 2012; Borowski et al., 2016). Overwinter as adult (Dellacasa and Dellacasa, 2006).

Contrary to some authors that found its major activity period in spring (Lumaret, 1990; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006) we found the demographic peak during autumn. However, this may be due to the fact that our sampling period started in May, prevented us to record any previous activity of early spring.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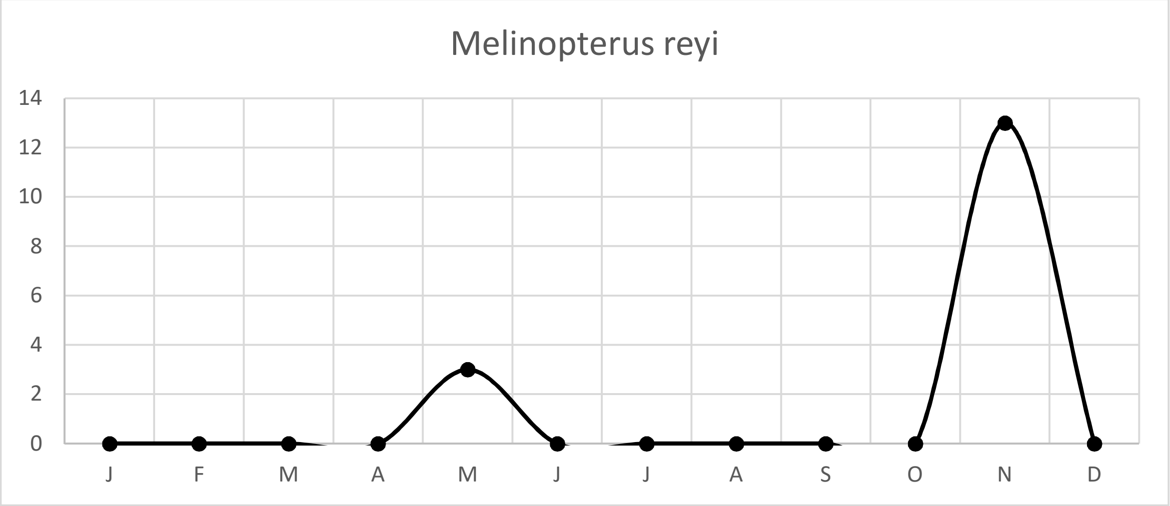
Melinopterus reyi (Reitter, 1892)

BIOLOGY: *M. reyi* is an oligotopic species, which prefer open habitat as pastures (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006) on sandy and silty soils (Lumaret, 1990; Dellacasa and Dellacasa, 2006). It is a dweller species, which can be found in cow, horse, sheep and human dung (Lumaret, 1990; Král and Malý, 1993; Veiga, 1998; Dellacasa and Dellacasa, 2006). Although we do not found significant preference (N=16; IndVal 5.0; *P* = 1.000), a trend toward horse dung (75%) preference exist compared with cow dung (25%). This is in accordance with Král and Malý (1993), but discordant with Veiga (1998) and Dellacasa and Dellacasa (2006).

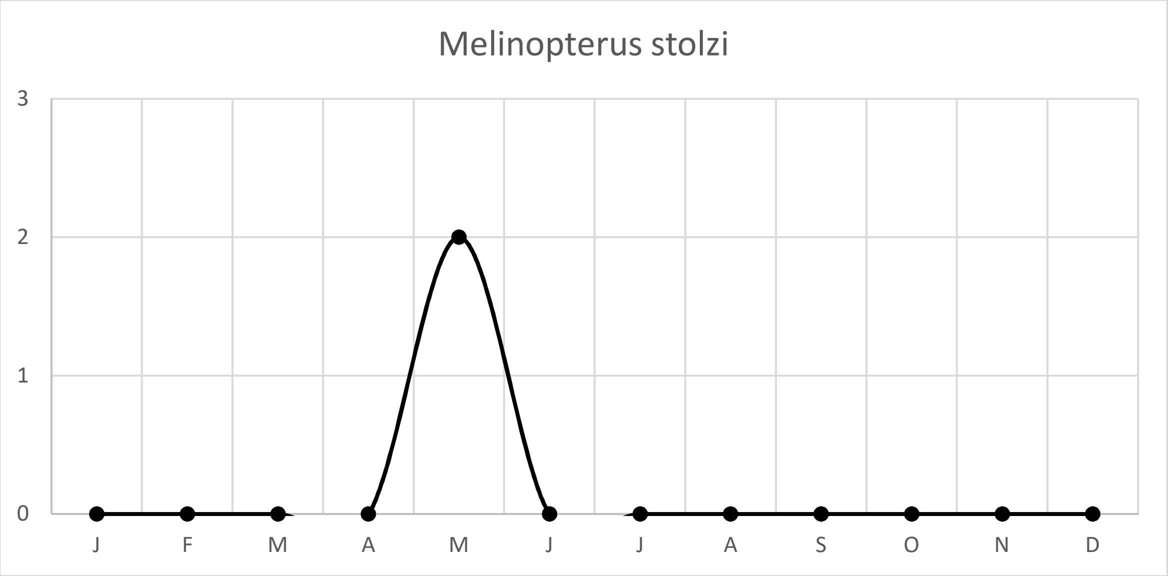
PHENOLOGY: *M. reyi* have two generations per year, one in spring and other in autumn (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006), with its demographical maximum during early spring (March-April) (Lumaret, 1990). The aestivation occurs at adult stage (Veiga, 1998; Dellacasa and Dellacasa, 2006). Contrary to the above cited literature, we found its maximum demographic peak during autumnal months, and only a little activity in spring. However, the start of our sampling period in May can have prevented us to record the springly population peak.



Melinopterus stolzi (Reitter, 1906)

BIOLOGY: it is a stenotopic species, which colonize exclusively open habitat such as exposed xeric pastures (Lumaret, 1990; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006). The literature about its trophic preference is scarce and inconsistent. Indeed, Dellacasa and Dellacasa (2006) and Dellacasa and Kirgiz (2002) found it mainly in horse dung, whereas Lumaret (1990) highlight its preference toward sheep dung. Although the scarcity of the specimens trapped in our study (N=2; Indval 6.7; *P* = 1.000), we found this species only in cow dung (100%).

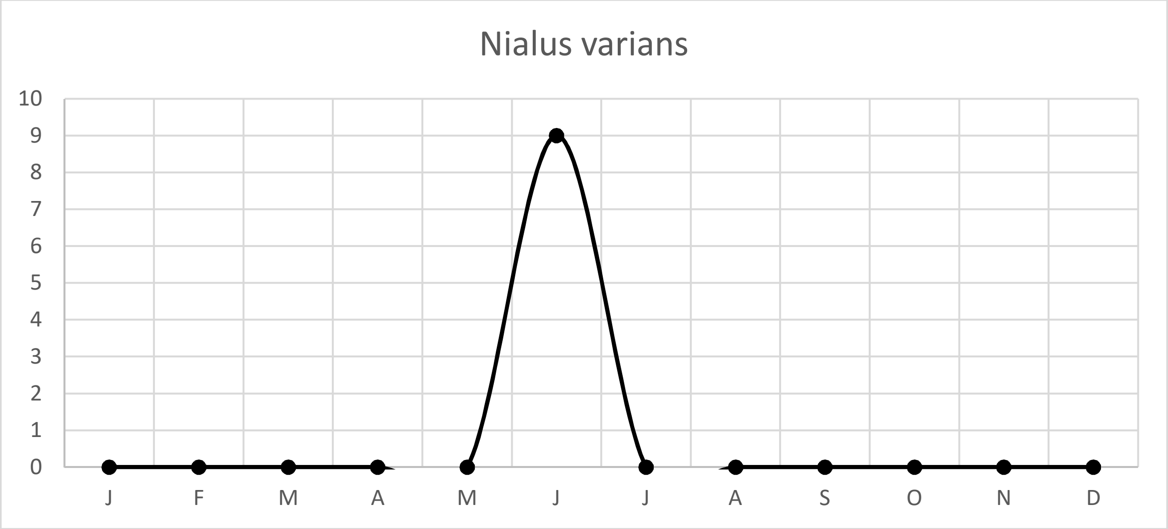
PHENOLOGY: we found the two specimens in spring (May), in accordance with the demographics peak highlight by other authors (Lumaret, 1990; Dellacasa and Dellacasa, 2006).



Nialus varians **(Duftschmid, 1805)**

BIOLOGY: *N. varians* is a oligotopic species which prefer open pastures (Lumaret, 1990; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006) with moist soils rich in organic matter (Lumaret and Kirk, 1987; Lumaret, 1990). However, some authors quoted its preference toward dry soils (Veiga, 1998; Sullivan et al., 2016) and wooded habitats (Barbero et al., 1999). It is a polyphagous species which can be found in several types of dung such as sheep, pig, cow, horse, human, wild boar and deer (Lumaret, 1990; Veiga, 1998; Barbero et al., 1999; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008). It was found also in carrions and rotting vegetables (Horion, 1958; Lumaret, 1990; Dellacasa and Kirgiz, 2002; Agoiz-Bustamante, 2008). *N. varians* seem to have a trophic preference toward horse dung (Barbero et al., 1999; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006), although it was found to prefer also sheep dung (Veiga, 1998). Contrary to this authors, we found a clear preference toward cow dung (100%), although not significant (N=9; IndVal 6.7; P = 1.000).

PHENOLOGY: it is a spring summerly species, which activity peak occur between April and June (Lumaret, 1990; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008). This phenology pattern is reflecting also in our area of study, where we found this species in June.


Nialus varians
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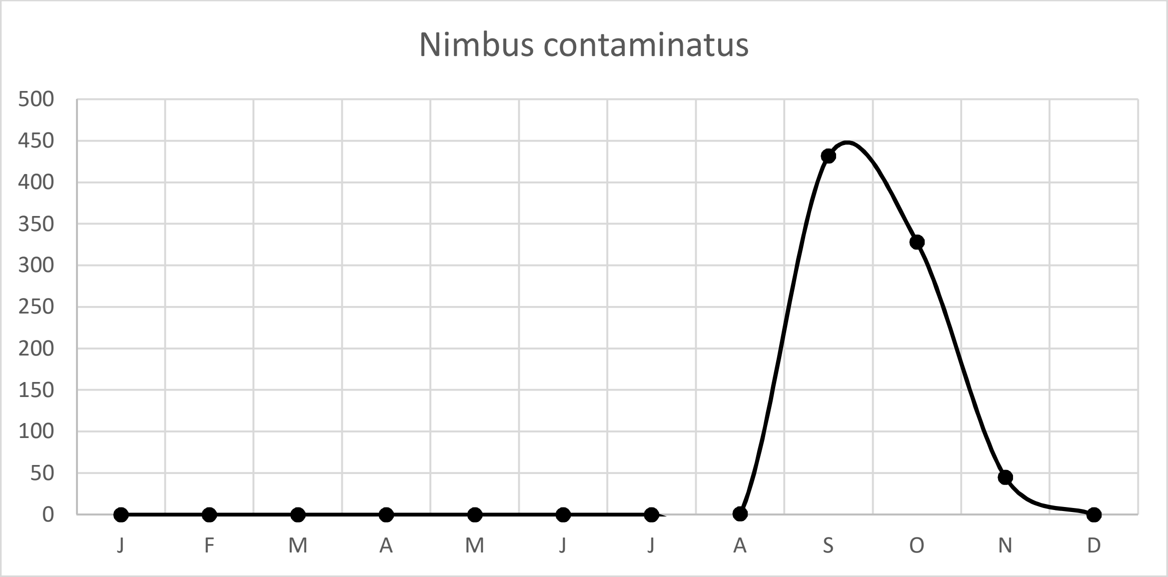
Nimbus contaminatus (Herbst, 1783)

BIOLOGY: *N. contaminatus* is a oligotopic species that mainly occur in open habitats (Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006) on sandy soils (Dellacasa and Dellacasa, 2006; Sullivan et al., 2016). It is a polyphagous species, which may be found in various type of dung such as horse, cow, sheep, human and fox (Lumaret, 1990; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006) and also in rotting vegetables (Landin, 1961; Holter, 2000). Its polyphagy may be explicate by its capacity to ingests even big dung particles (until ~25 µm) (Holter, 2000). However, it seems to have a strong preference toward horse dung (Lobo, 1985; Dellacasa and Kirgiz, 2002; D'hondt et al., 2008; Bebermans et al., 2016; Borowski et al., 2016). We do not found any significant preference (N=806; IndVal 39.6; $P = 0.698$), because only a slight predilection toward horse (54%) dung exists compared with cow dung (46%). *N. contaminatus* seem more attracted by dung contaminated with ivermectin (Römbke et al., 2010).

It is part of the diet of *Sturnus vulgaris*, *Corvus frugilegus*, *Pyrrocorax pyrrochorax* (Young, 2015).

PHENOLOGY: this is a tipical autumnal species, which begin its activity after the first autumnal rains, and show an explosive emergence during September and October (Lobo, 1985; Lumaret, 1990; Veiga, 1998; Verdú, 1998; Roslin, 2000; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Jay-Robert et al., 2008; Borowski et al., 2016; Sullivan et al., 2016). Overwinter as egg and occasionally as adult (Lumaret, 1990; Dellacasa and Dellacasa, 2006). We found this species mainly during autumnal months

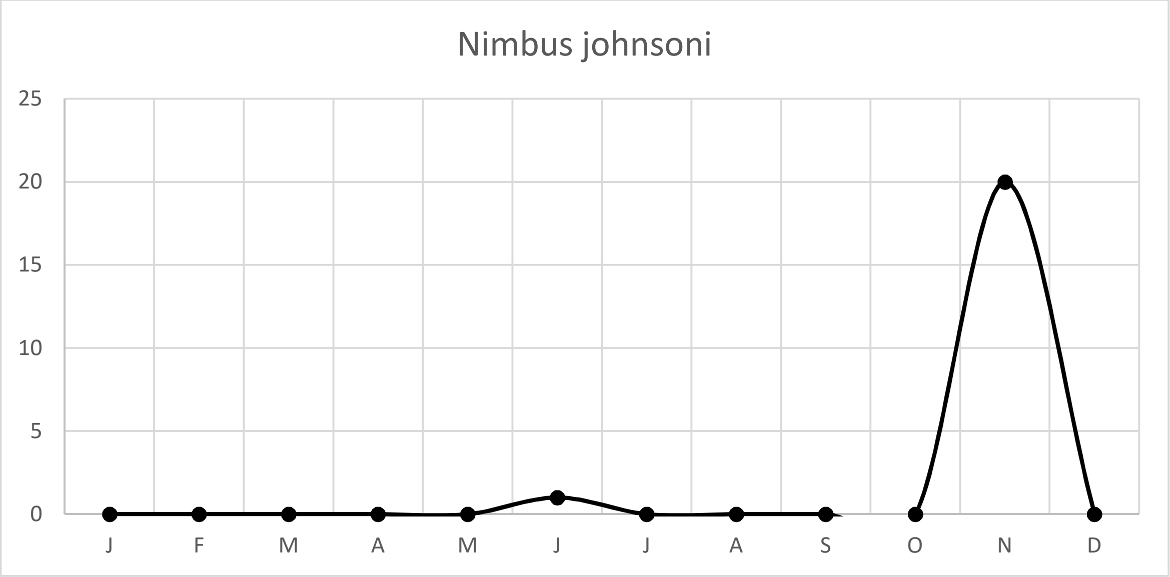
(September-October), in accordance with the demographics peaks recorded by previous cited authors.



Nimbus johnsoni (Baraud, 1976)

BIOLOGY: it is an oligotopic species which prefer open habitat as exposed pastures (Dellacasa and Dellacasa 2006). Polyphagous species that can be found in any type of dung, from horse and cow to dog (Carpaneto et al., 2005; Dellacasa and Dellacasa, 2006). We do not found any significant trophic preference (N=21; IndVal 14.3; *P* = 0.87) toward cow (43%) or horse dung (57%).

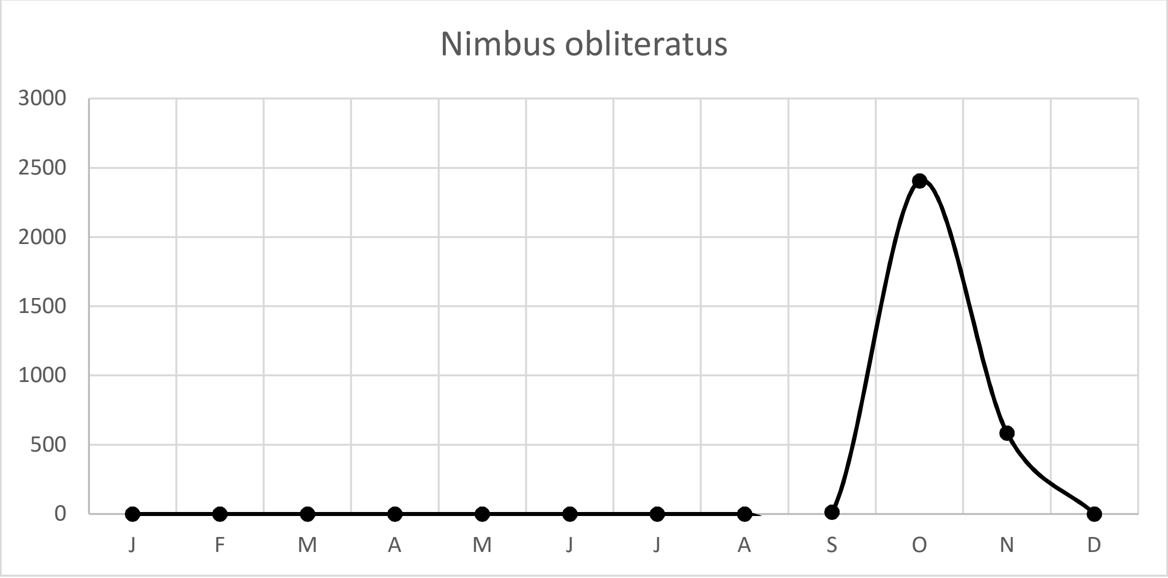
PHENOLOGY: it is a typical autumnal species (Dellacasa and Dellacasa, 2006; Agoglitta et al., 2012).



Nimbus obliteratus (Panzer, 1823)

BIOLOGY: oligotopic species which prefer open pastures on sandy soils (Veiga, 1998; Dellacasa and Dellacasa, 2006). It is a plyphagous species which can be found in every type of dung such as cow, horse, sheep, human, and also in rotting vegetables or carrions (Lumaret, 1990; Král and Malý, 1993; Veiga, 1998; Dellacasa and Dellacasa, 2006). Although the trophic preference was not significant (N=3004; IndVal 67.6; P = 0.752), we found a strong predilection toward horse dung (72%) compared with cow dung (28%).

PHENOLOGY: *N. obliteratus* is tipical autumnal species, which demographic peak occur from September to November (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006). In accordance with the literature, we found its activity maximum in October.



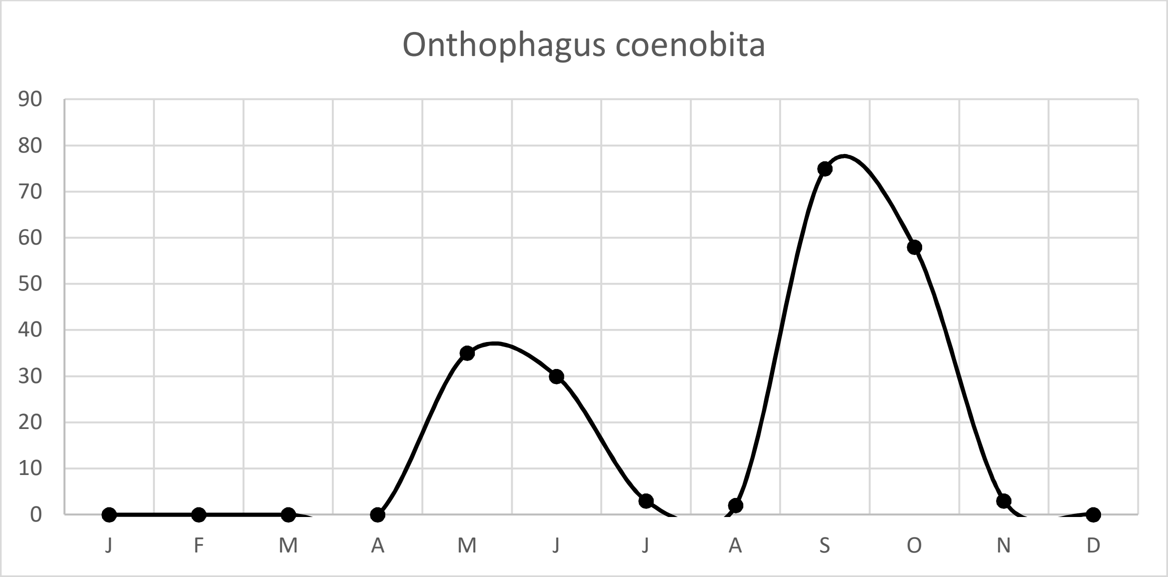
Onthophagus coenobita (Herbst, 1783)

BIOLOGY: *O. coenobita* is a eurytopic species with a high ecological plasticity that can colonize every type of habitats (Zunino, 1982; Lumaret and Kirk, 1987; Lumaret, 1990; Barbero et al., 1999; Plexida et al., 2014), although some preference toward sheltered habitat with moist soil was highlight (Lumaret and Kirk, 1987; Sowig, 1995). Its ecological plasticity is reflected also in its trophics needs, being a very generalist species that can be found in every type of dung such as cow, horse, dog, fox, pig, deer, wild boar, human, sheep and badger (Lumaret, 1990; Wassmer, 1995; Barbero et al., 1999; Martín Piera and López-Colón, 2000; Carpaneto et al., 2005) even in mushrooms, rotting vegetables and carrions (Peschke et al., 1987; Lumaret, 1990; van Wielink, 2004; Matuszewski et al., 2008; Schlechter, 2008). In accordance with above cited authors, we do not found any trophic preference (N=206; IndVal 48.9; P = 0.603), with only a slight trend of preferably colonizes cow dung (61%) compared with horse dung (39%).

It is a tunneler species which nesting below dung pat. There is not accordance in literature about the morphology of *O. coenobita* nests: Burmeister (1930, 1936) stay that they are simples nests with all brood masses located into a singles gallery; whereas Halffter and Edmonds (1982) categorized it into composed nests.

A previsional model highlights the possibilites that it can lost until 65% of its distributional area before 2080 under climate change scenarios (Dortel et al., 2013). *O. coenobita* form part of the diet of *Glareola pratincola* (Young, 2015).

PHENOLOGY: this species shows a wide phenological activity which, generally, have a bimodal pattern with two peak, in late spring-early summer and other in autumn (Zunino, 1982; Lumaret and Kirk, 1987; Lumaret, 1990; Jay-Robert et al., 2008) when adult emergence occurs (Lumaret, 1990). It probably overwinters as adult (Wassmer, 1994).

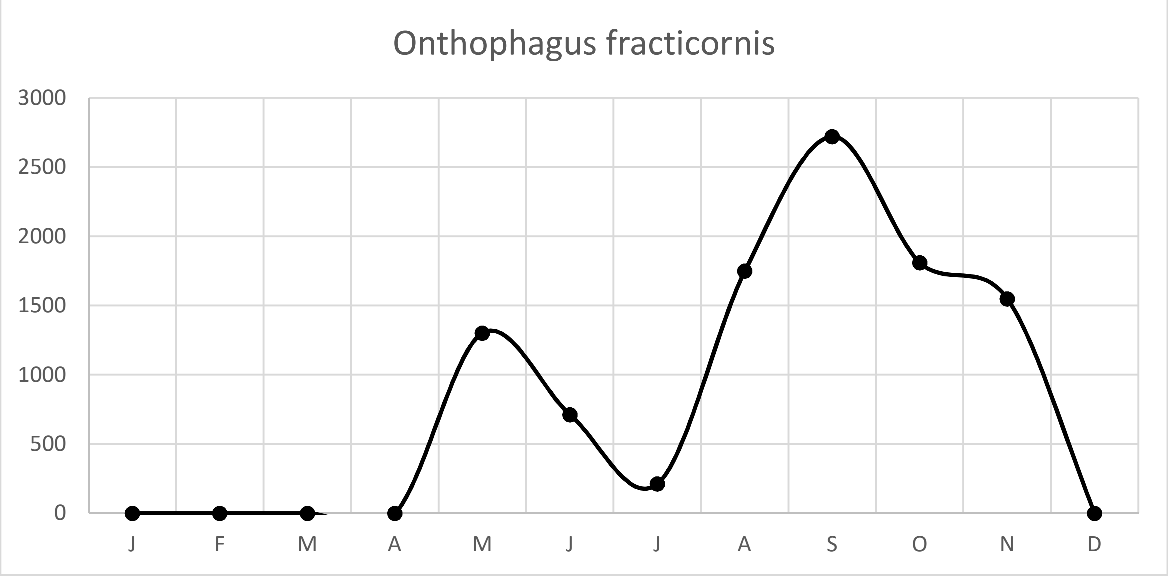


Onthophagus fracticornis (Preyssler, 1790)

BIOLOGY: *O. fracticornis* is a diurnal species (Psarev, 2001b), which prefer open habitat as pastures (Wassmer, 1995; Martín Piera and López-Colón, 2000; Borghesio et al., 2001; Dormont et al., 2007; Macagno and Palestrini, 2009; Tocco et al., 2013; Kamiński et al., 2015), although in some areas it is related to forested habitats (Negro et al., 2011; Verdú et al., 2011; Numa et al., 2012), probably as function of its preference toward moist soils (Sowig, 1995). It seems to prefer dung from large domestic herbivores as cow (Lumaret, 1990; Martín Piera and López-Colón, 2000; Errouissi et al., 2004) and horse (Lobo, 1985; Dormont et al., 2007), even if it can be found regularly in sheep dung (Galante, 1983; Lumaret, 1990; Wassmer, 1995) and occasionally in wild boar, human, goat, dog and fox (Lumaret, 1990; Martín Piera and López-Colón, 2000; Borghesio et al., 2001). Ziani and Moradi Gharakhloo (2011) found it into rodent burrows in Iran. *O. fracticornis* is among the earliest successional species in dung colonization process, and seem to avoid dung contaminated with ivermectin (Psarev, 2001a; Errouissi and Lumaret, 2010). We do not found significant trophic preference (N=10059; IndVal 60.9; $P = 0.488$), even if it seem to predilect to colonize cow dung (61%) compared with horse dung (39%).

A previsional model highlight the possibility of a lost until -75% of its actual distributional range undr a scenario of climate change by the 2080 (Dortel et al., 2013)

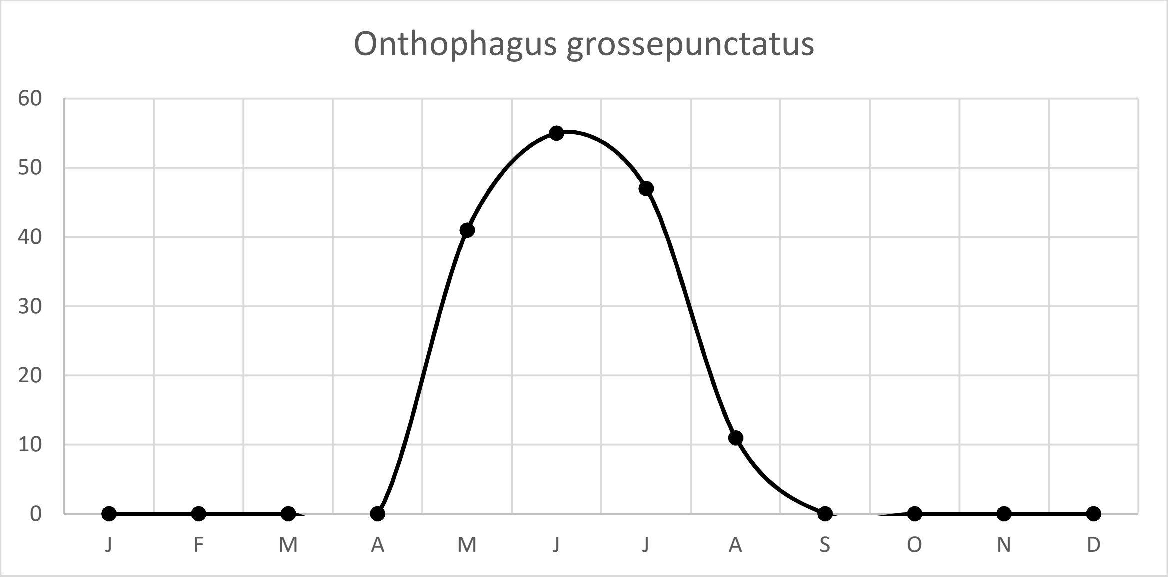
PHENOLOGY: this species seems to have a bimodal phenology deriving from two generations, one in spring and other in late summer-early autumn (Galante, 1979; Lumaret, 1990; Wassmer, 1994; Martín Piera and López-Colón, 2000; Borghesio et al., 2001; Jay-Robert et al., 2008). The eggs are laid in June-July, the nymphosis lasts 3 weeks and the adults of new generation emerges in August and September (Lumaret, 1978, 1990). Overwinter as adult or larvae (Galante, 1979; Wassmer, 1994; Borghesio et al., 2001). Our data are in accordance with above cited authors and reflect the biological cycle of this species. The spring demographical peak derive from overwintering adults or larvae, whereas the late summer-early autumnal peak originate from the adults of new generation which eggs were laid in spring.



Onthophagus grossepunctatus Reitter, 1905

BIOLOGY: *O. grossepunctatus* is a eurytopic species that can be found indifferently in open and closed habitats with some preference toward arid soils (Zunino, 1982; Lumaret and Kirk, 1991). It is a polyphagous species which can be found indiscriminately in a wide range of dung types such as cow, horse, sheep, goat, human, fox, wild boar, badger (Zunino, 1982; Lumaret, 1990; Martín Piera and López-Colón, 2000; Dormont et al., 2004; Carpaneto et al., 2005) and also in carrions (Grosso-Silva and Soares-Vieira 2009). In Macedonia, Pittino (2001) found it at the entrance of the burrow of *Spermophilus citellus macedonicus*. It seems to prefer dung contaminated with ivermectin (Errouissi and Lumaret, 2010). In accordance with previous cited authors, we do not found any trophic preference (N=154; IndVal 28.1; $P = 0.517$), even if there seem to be a tendency toward cow dung predilection (70%) compared with horse dung (30%).

PHENOLOGY: *O. grossepunctatus* is a species active in spring and summer months, from March to August, with a demographical peak during May-August period (Lumaret, 1990; Martín Piera and López-Colón, 2000; Jay-Robert et al., 2008; Agoglitta et al., 2012). Overwinter as adult (Lumaret and Kirk, 1991). The oviposition occur in May-June (Lumaret, 1990; Lumaret and Kirk, 1991) and the ontogenetic development occur in 40 days (Lumaret, 1978). We are in accordance with previous literature, being the peak of activity in our area of study coincident with other studies.


Onthophagus grossepunctatus
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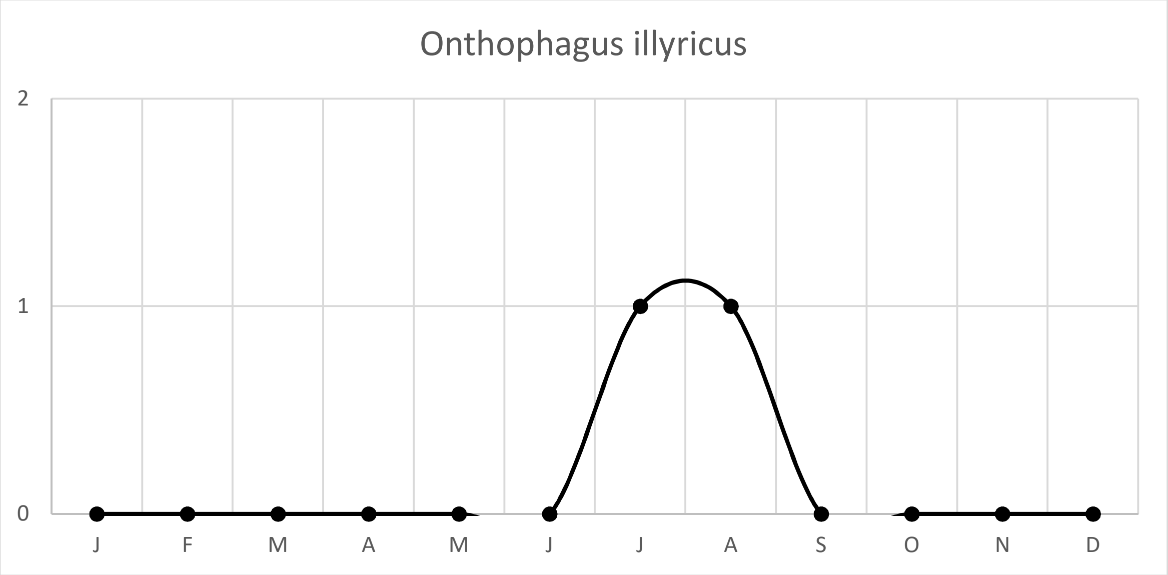
Onthophagus illyricus (Scopoli, 1763)

BIOLOGY: it is a species that colonize preferably open habitat as pastures, although avoid too dry areas (Zunino, 1982; Barbero et al., 1999). It prefers dung with high moisture content such as cow dung (Zunino, 1982; Barbero et al., 1999; Borghesio et al., 2001), even if it can be found in horse, deer, wild boar, and human dung (Zunino, 1982; Lumaret, 1990; Barbero et al., 1999; Martín Piera and López-Colón, 2000). We only found two specimens (1 in cow and 1 in horse dung), which prevent us to highlight any trophic preference (IndVal 3.3; *P* = 1.000).

PHENOLOGY: it a spring-summerly species whit a demographic peak from May to August (Galante, 1979; Lumaret, 1990; Martín Piera and López-Colón, 2000; Agoglitta et al., 2012). The eggs are laid in June and the nymphosis occur in July-August (Lumaret, 1990). It probably overwinters as adult (Galante, 1979). The scarcity of data for our area of study prevent us to define its phenological pattern, but the 2 specimens are trapped during its generational maximum in accord with above cited authors.

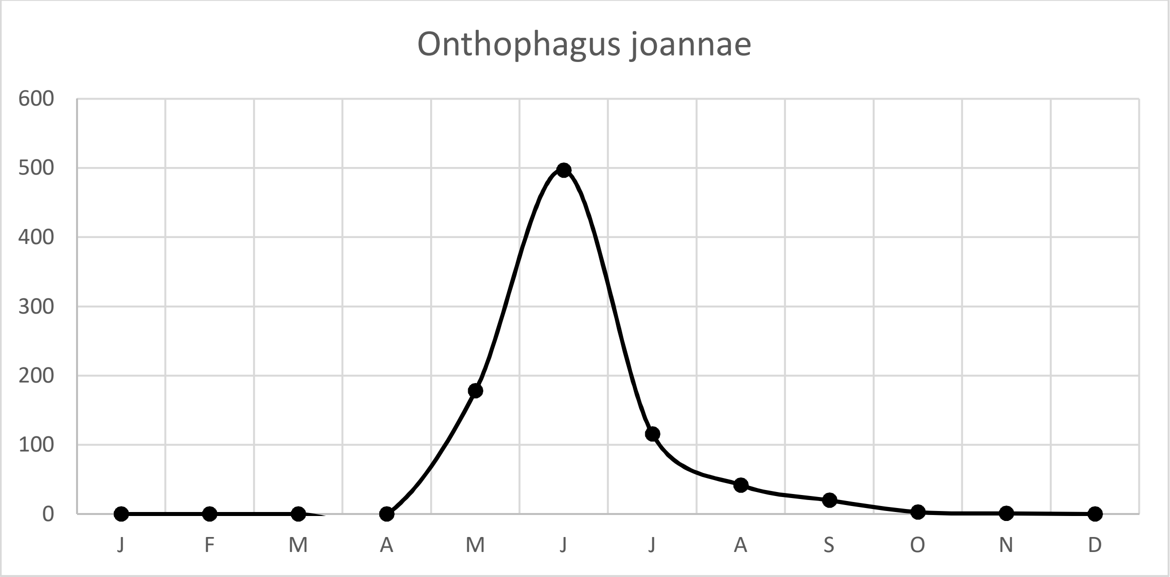


Onthophagus joannae Goljan, 1953

BIOLOGY: It a species that can colonize a wide range of habitat from pasture (Galante, 1983; Borghesio et al., 2001; Macagno and Palestrini, 2009; Tocco et al., 2013) to forested areas (Lumaret and kirk 1987, 1991). *O. joannae* may be found in different dung types as cow, horse, sheep, pig, human, fox, rabbit, wild boar, goat (Galante, 1983; Martín Piera and López-Colón, 2000; Borghesio et al., 2001; Errouissi et al., 2004) and also in carrions (Grosso-Silva and Soares-Vieira, 2009), even if some authors highlight its preference toward horse dung (Martín Piera, 1980; Dormont et al., 2004). It colonizes the dung in the first 2-5 days after deposition (Lobo, 1992), and seems more attracted by dung contaminated with ivermectin (Errouissi and Lumaret, 2010). Although the analysys about its trophic preference was not significant (N=857; IndVal 34.9; $P = 0.377$), our data suggest a tendency toward cow dung preference (65%) if compared with horse dung (35%).

A previsional model highlight the possibility of a decrease in its distributional range until 57% in a scenario of climate change by the 2080 (Dortel et al., 2013).

PHENOLOGY: it is a tipical species of spring and summer months, which maximum activity occur from April to August, with small shifts in function of the considered area (Martín Piera, 1980; Lumaret, 1990; Martín Piera and López-Colón, 2000; Jay-Robert et al., 2008). The eggs are laid in May and the ontogenetic development occur in 40 days (Lumaret, 1990). We encounterd its demographic peak during June with subsequently slight decreases toward midsummer and autumn months.


Onthophagus joannae
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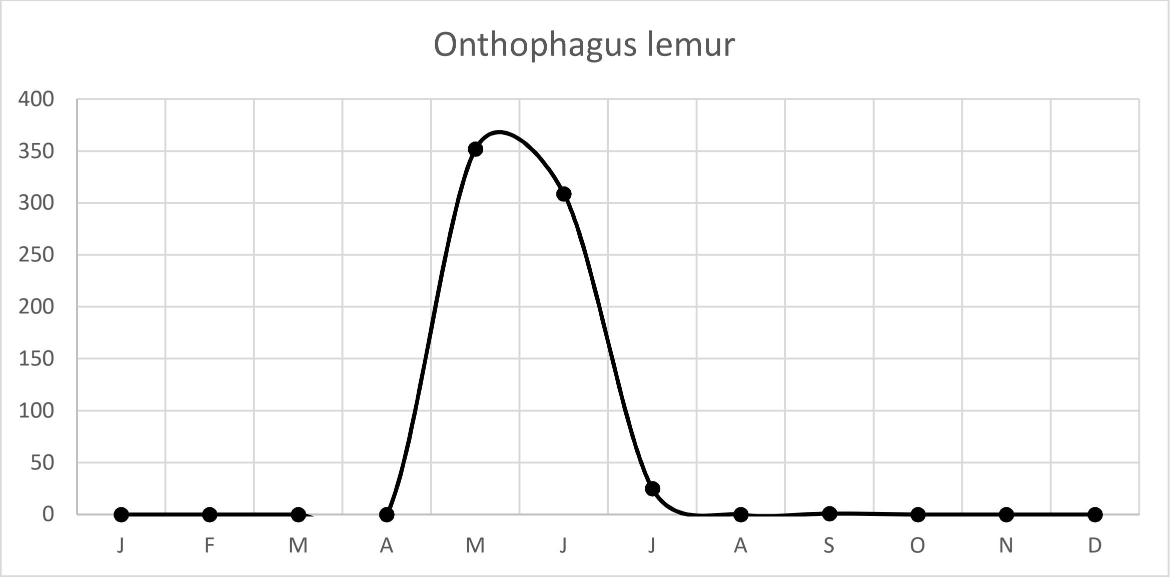
Onthophagus lemur (Fabricius, 1781)

BIOLOGY: *O. lemur* is a diurnal species that colonize preferably open habitat as pastures (Zunino, 1982; Lumaret, 1990; Lumaret and Kirk, 1987, 1991; Verdú, 1998), even if it can found also in forested areas with clear underbrush (Lumaret, 1990; Lumaret and Kirk, 1987, 1991). It has a wide trophic niche being found into several dung type such as cow, horse, sheep, goat, dog, wild boar, fox, human and weasel (Zunino, 1982; Lumaret, 1990; Verdú, 1998; Martín Piera and López-Colón, 2000; Dormont et al., 2004; Errouissi et al., 2004), and it was found also under carrions (Martín Piera and López-Colón, 2000). It prefers to colonize fresh dung during the first 2 days after its deposition (Lobo, 1992). We do not found any trophic preference (N=687; IndVal 71.8; P = 0.167), even if a tendency toward predilect cow dung (72%) compared with horse (28%) dung was highlight. This is in accordance with previous literature that is incongruent about its trophic preference that vary from cow (Dormont et al., 2004; Errouissi et al., 2004), to horse (Martín Piera and López-Colón, 2000; Dormont et al., 2004) and sheep (Lumaret, 1990). However, Errouissi and Lumaret (2010) showed that it was strongly attracted by dung contaminated with ivermectin.

A previsional model highlight the possibilities that this species lost until -56% of its distributional range by the 2080, in a scenario of climate change (Dortel et al., 2013).

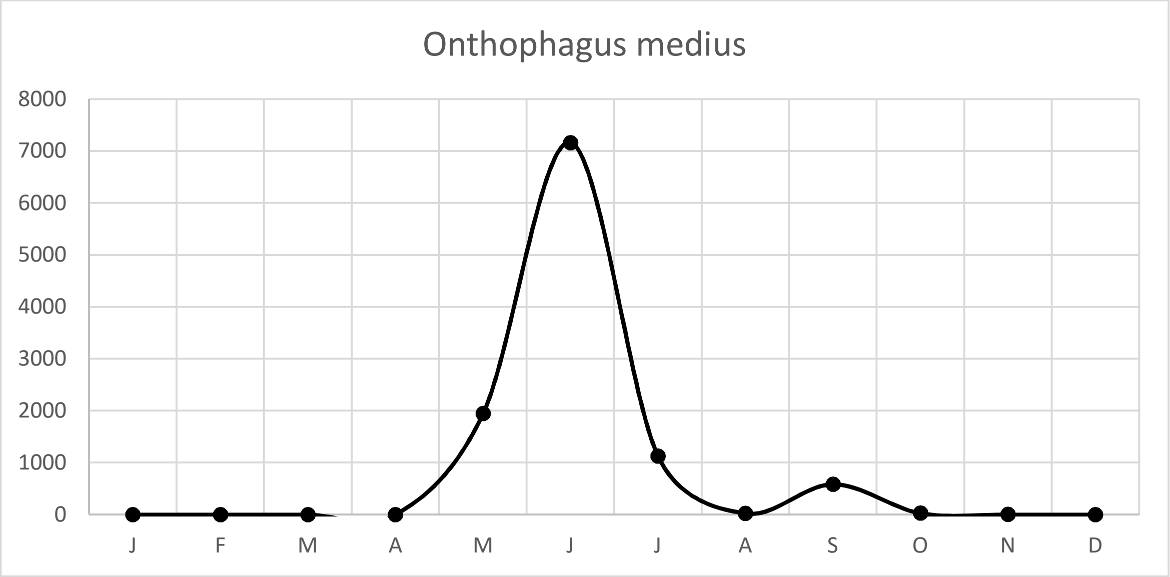
PHENOLOGY: this species is active during spring and early summer, with a demographic maximum during April, May and June (Galante, 1979; Lumaret, 1990; Verdú, 1998; Martín Piera and López-Colón, 2000). It has a short biological cycle (37-40 days) that allows the existence of two generations per year (Lumaret, 1978; Lumaret, 1990; Martín Piera and López-Colón, 2000), although normally it shows a univoltine cycle, which lasts from 3-7 to 7-11 months (Lumaret and Kirk, 1991). Overwinter as adult, larvae or pupae (Lumaret and Kirk, 1991). In our area of study, we found only one generation during the year, which reaches its peak during May and June with a rapid decrease toward midsummer.



Onthophagus medius (Kugelann, 1792)

BIOLOGY: The *Onthophagus vacca* species complex was recently splitted in two species, *O. vacca* and *O. medius*, based on morphological and molecular data (Rössner et al., 2010). The majority of biological data in the past litterature are about *O. vacca* and little is known about *O. medius*. Then more investigations are nedded to assess any differences in the bionomy of the two species. We report the ecological difference described by Rössner et al. (2010), which is the only work that allow to highlights any difference in comparative terms. They have stated: ”The ranges of *O. vacca* and *O. medius* overlap, both being widely distributed in the western Palaearctic. Beyond their wide sympatry, there are some significant differences: *O. vacca* is mainly concentrated in southern and south-central Europe expanding its range towards northern Africa (Morocco and Algeria). In central Europe, *O. vacca* seems to be absent north of 50° latitudes. It seems to be more thermophilous than *O. medius* as it is found in the Apennine Peninsula and in south-central Europe mainly at lower altitudes, lacking completely on the Alps, while it occurs also in higher mountains in southern Europe. Many records from central Europe originate from last century and their current distribution there seems to be limited to the Pannonic plain. *Onthophagus medius* has a much wider distribution in the humid and temperate climate and occurs much further north than *O. vacca* while it is absent from areas with Mediterranean climate including several larger and more distant Mediterranean islands such as Corsica, Sardinia, Crete, and Aegean Islands. In southern Europe it occurs exclusively in medium and higher altitudes. This distributional and the ecological differentiation of both taxa is linked to the seasonal occurrence of the both species. *Onthophagus vacca* seem to overwinter as adults and occur quite early in the year with peak activity during April and May. According to their more northern and mountainous distribution, adults of *O. medius* emerge later in the year compared to *O. vacca*. They have a generally shorter period of activity that peaks during May" (Rössner et al., 2010). We do not found any trophic preference (N= 10874; IndVal 51.5; *P* = 0.939) between cow (52%) and horse dung (48%).

PHENOLOGY: we found the peak of activity in June and a second lower demographical peak in September that may indicate the presence of a second generation, but more studies are need.

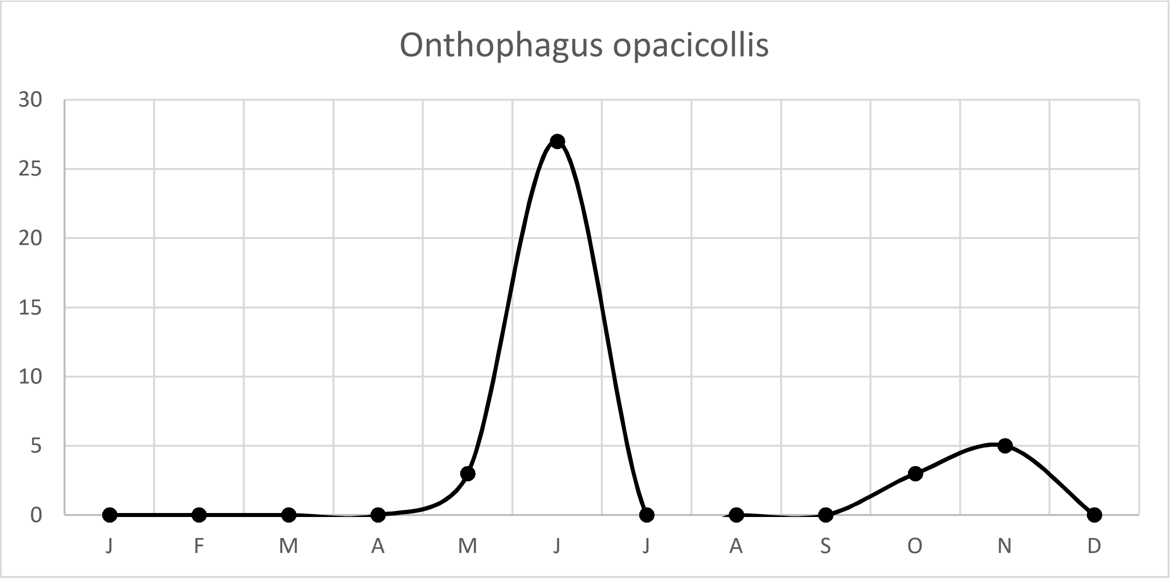


Onthophagus opacicollis (Reitter, 1892)

BIOLOGY: *O. opacicollis* is a diurnal species (Mena et al., 1989) that can be found is several habitats from open areas as pastures, to shrublands and forest (Lumaret, 1990; Galante et al., 1995; Micó et al., 1998; Verdú, 1998; Martín Piera and López-Colón, 2000; Verdú et al., 2000; Zamora et al., 2007; Numa et al., 2012) on clayey and silty-sandy soils (Lumaret, 1990; Sullivan et al., 2016). It can exploit several dung type such as cow, horse (without preference between each one), sheep, deer, fallow deer, wild boar, human, dog and occasionally rabbit (Lumaret, 1990; Král and Malý, 1993; Martín Piera and Lobo, 1996; Verdú, 1998; Martín Piera and López-Colón, 2000; Carpaneto et al., 2005). We do not found any significant trophic preference (N=38; IndVal 33.2; $P = 0.257$) between cow (71%) and horse dung (29%).

A previsional model highlighted the possibilities of a decrease of its distributional range until 70% by the 2080 (Dortel et al., 2013).

PHENOLOGY: this species shows two activity peaks, during spring and autumn, with a minimum during midsummer months (Lumaret, 1990; Galante et al., 1995; Verdú, 1998; Martín Piera and López-Colón, 2000; Errouissi et al., 2009). The eggs are laid from April to June (Lumaret, 1990; Verdú, 1998). We found its tipical phenological pattern, as described by previous authors, with a maximum during reproduction in June, and a second in autumn.


Onthophagus opacicollis
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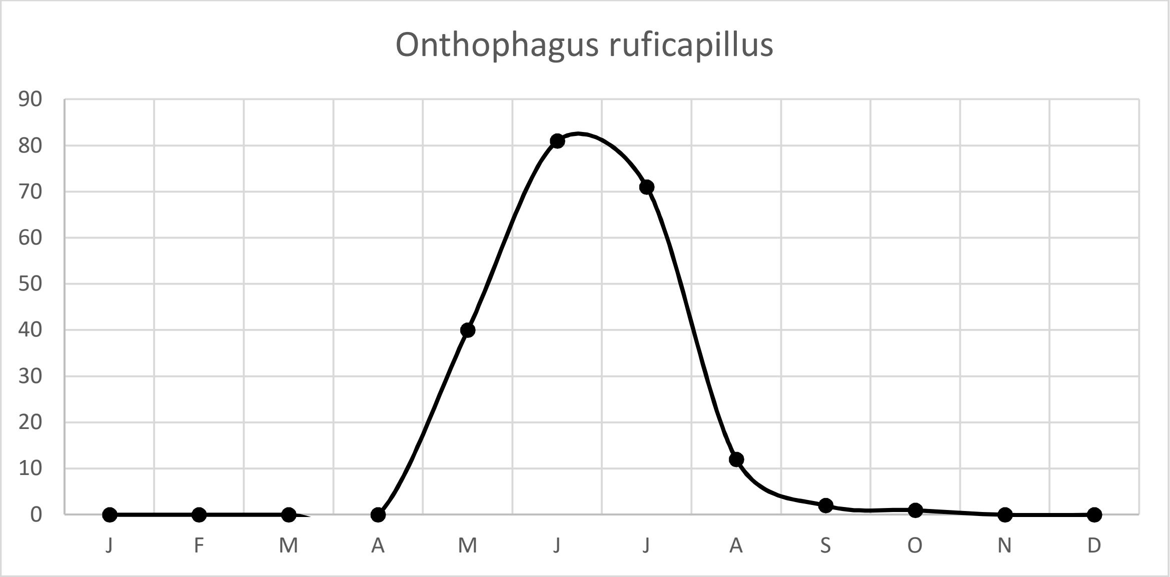
Onthophagus ruficapillus Brullé, 1832

BIOLOGY: *O. ruficapillus* is a diurnal species (Lumaret and Kirk, 1987; Mena et al., 1989) which colonize open habitats as pastures (Lumaret, 1990; Galante et al., 1991; Martín Piera and López-Colón, 2000) on moist clayey and silty soils (Lumaret, 1990; Verdú, 1998). It is a polyphagous species that can be found in several dung types such as cow, sheep, horse, human, dog, pig, fox and rabbit (Galante, 1979; Lumaret, 1990; Verdú, 1998; Martín Piera and López-Colón, 2000; Carpaneto et al., 2005), and also into rodent burrows (Pittino, 2001; Ziani and Moradi Gharakhloo, 2011). Hidalgo and Cárdenas (1994, 1996) and Hidalgo et al. (1998) highlight its preference toward horse dung and its presence in carrions. We do not found any significant trophic preference (N=207; IndVal 53.5; *P* = 0.144), even if a trend toward cow dung (80%) predilection compared with horse dung (20%) was recorded, contrarily to Hidalgo and Cárdenas (1994, 1996) and Hidalgo et al. (1998). It is a tunneler species that nest at about 10 cm of depth and each nest contain about 2 brood masses (González-Megías and Sánchez-Piñero, 2003).

A previsional model shows that it may increase its distributional range of about 43% by 2080 in a climate change scenario (Dortel et al., 2013).

PHENOLOGY: the adult emergence occurs in the late winter, but its demographical peak happens in spring and summer months (Galante, 1979; Lumaret and Kirk, 1987; Galante et al., 1991; Galante et al., 1995; Verdú, 1998; Martín Piera and López-Colón, 2000; Anlaş et al., 2011; Agoglitta et al., 2012; Sullivan et al., 2016), when eggs are laids (Lumaret, 1990; Lumaret and Kirk, 1991; Verdú, 1998). The ontogenetical cycle occur in 40 days and probably overwinter as larvae (Galante, 1979; Martín Piera and López-Colón, 2000). We found strong accordance with above cited literature, recording its populational maximum during spring-summer months. However, our sampling period prevent us to recorded the adult emergence phase during late winter months.



Onthophagus taurus (Schreber, 1759)

BIOLOGY: *O. taurus* is a diurnal species (Lumaret and Kirk, 1987; Mena et al., 1989) which colonize open grassland habitat as exposed pastures (Lumaret and Kirk, 1987; Lumaret, 1990; Wassmer, 1995; Barbero et al., 1999; Martín Piera and López-Colón, 2000; Lobo et al., 2001; Zamora et al., 2007; Macagno and Palestrini, 2009; Verdú et al., 2011) silty, clayey and sandy soils (Lumaret, 1990). It is a coprophagous species that colonize preferably cow dung (Lumaret, 1990; Wassmer, 1995; Martín Piera and Lobo, 1996; Verdú, 1998; Barbero et al., 1999; Martín Piera and López-Colón, 2000; Borghesio et al., 2001), although it can be found also in horse, sheep, goat, deer, fallow deer, wild boar, pig, human, dog and rabbit (Lumaret, 1990; Martín Piera and Lobo, 1996; Verdú, 1998; Barbero et al., 1999; Martín Piera and López-Colón, 2000; Borghesio et al., 2001; Carpaneto et al., 2005). Although we do not found significant trophic preference (N=871; IndVal 50.3; P = 0.697), we are in accordance with previous authors highlighting the tendency toward predilect cow dung (58%) compared with horse dung (42%).

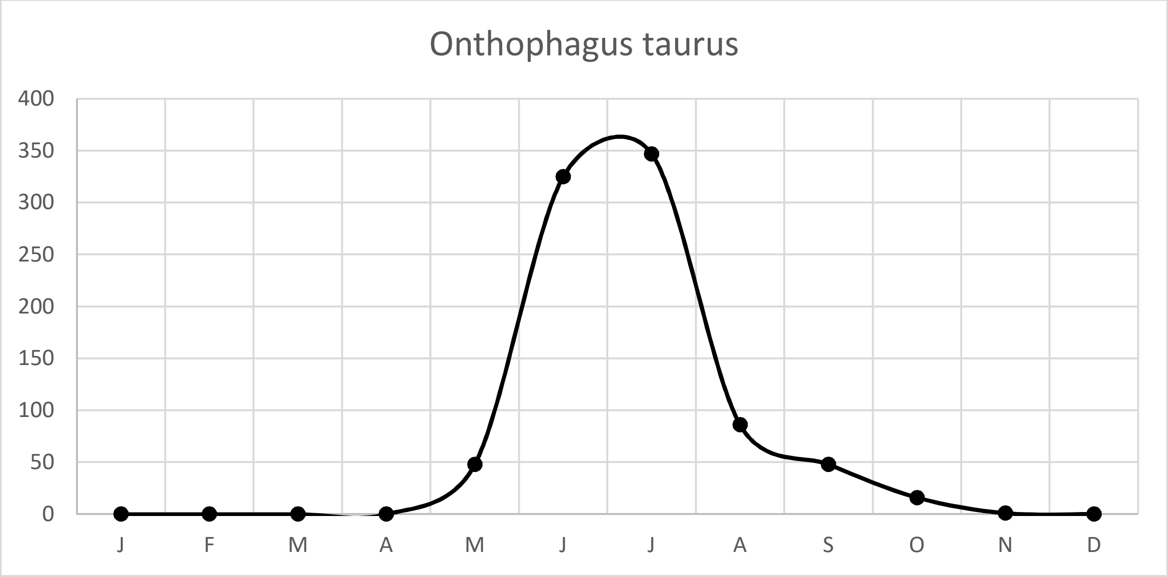
O. taurus is tunneler species which make compound nest with several brood masses buried at the bottom of a gallery (about 10 cm depth) where in each brood mass the female laid one egg (Goidanich and Malan, 1964; Halffter and Edmonds, 1982).

It has been demonstrated that endectocides can have lethal and sub-lethal effects on this species. For example, ivermectin can kill and delayed sexual maturation of newly emerged adults although had no effect on the survival of sexually mature beetles, but reduce its fecundity (Wardhaugh et al., 2001b; Lumaret and Errouissi, 2002). Moreover, faeces voided by cattle treated with a pour-on formulation of eprinomectin were associated with high juvenile mortality during first 1-2 weeks after treatment. Increased mortality also occurred among newly emerged beetles fed on faeces collected 3 days after eprinomectin treatment and there was evidence of suppressed brood production among those that survived (Wardhaugh et al., 2001b).

A previsional model showed the possibilities of an increase of its distributional range until 34% by the 2080 in a scenario of climate change (Dortel et al., 2013).

It has been documented that *O. taurus* form part of the diet of *Glareola pratincola* and *Merops apiaster* (Young, 2015)

PHENOLOGY: it is a spring-summerly species, that can be found from March to October with a demographical peak during midsummer (June, July and August) (Galante, 1979; Galante et al., 1991, 1995; Lumaret and Kirk, 1987; Lumaret, 1990; Palestrini et al., 1995; Verdú, 1998; Martín Piera and López-Colón, 2000; Borghesio et al., 2001; Errouissi et al., 2009; Agoglitta et al., 2012). The activity of *O. taurus* start in spring that correspond to the emergence of overwinter adults. These individuals begin nesting and egg laying, from which the new adults emerge in midsummer (Galante, 1979; Lumaret and Kirk, 1991). It may overwinter as adult or larvae (Galante, 1979; Lumaret and Kirk, 1991). Our data are in accordance with above cited authors, highlight the demographical peak during summer months (June and July) probably coincident with the emergence of new generation adults. The activity persists until November with a small number of specimens.


Onthophagus taurus
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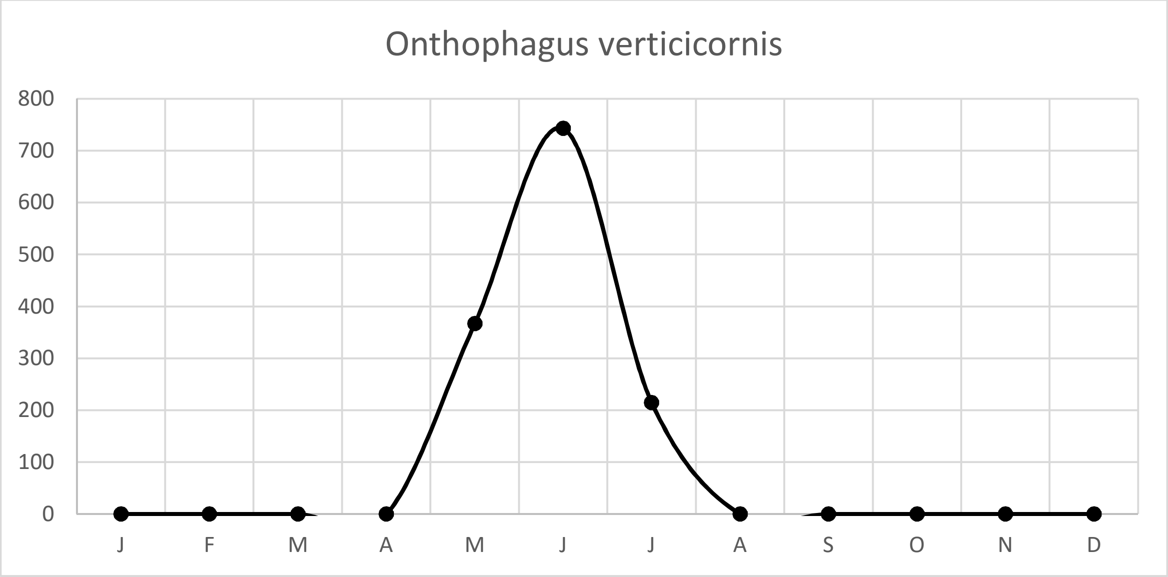
Onthophagus verticicornis **(Laicharting, 1781)**

BIOLOGY: it is a species that prefer habitats with a certain degree of tree cover (Lumaret and Kirk, 1987; Lumaret, 1990; Martín Piera et al., 1992), though it can be found also in open pastures (Lumaret and Kirk, 1987; Lumaret, 1990; Wassmer, 1995; Martín Piera and López-Colón, 2000) on clayey, sandy and stony soils (Lumaret, 1990). *O. verticicornis* shows a wide trophic niche and can be found in several dung types such as cow, horse, sheep, goat, human, dog, fox, badger and pig (Lumaret, 1990; Martín Piera and López-Colón, 2000; Dormont et al., 2004; Errouissi et al., 2004). Moreover, this species seems more attracted by dung contaminated with ivermectin (Errouissi and Lumaret, 2010). Although we do not found any significant trophic preference (N=1325; IndVal 57.4; $P = 0.543$), a silght tendency toward predilect cow dung (57%) compared with horse dung (43%) exist, in accordance with Dormont et al. (2004).

Its distributional range could decrease until -61% by the 2080 in a scenario of climate change (Dortel et al., 2013).

PHENOLOGY: *O. verticicornis* is active from spring to summer, mainly from April to July (Lumaret and Kirk, 1987; Lumaret, 1990; Wassmer, 1994; Martín Piera and López-Colón, 2000; Jay-Robert et al., 2008). The eggs are laid during May and June, whereas the nymphosis occur in July and August (Lumaret, 1990). In our area of study, the phenological pattern of this species reflect totally that encountered in other areas, showing a begin of activity in May, a demographical peak during June and a strong decrease of activity toward midsummer.


Onthophagus verticicornis
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Otophorus haemorrhoidalis (Linnaeus, 1758)

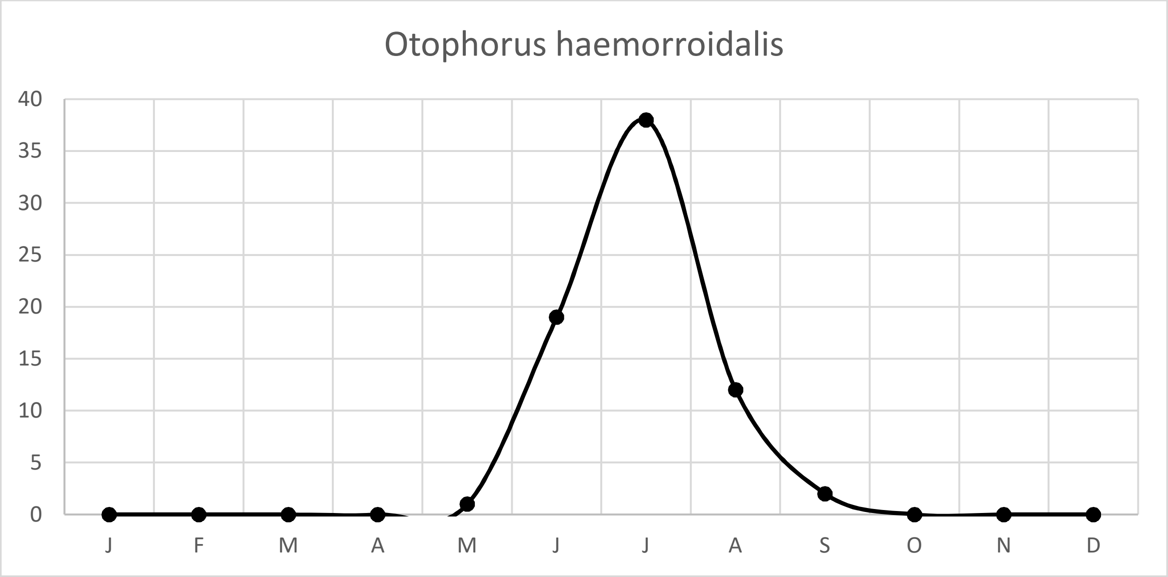
BIOLOGY: *O. haemorrhoidalis* is a diurnal (Koskela, 1979) eurytopic species that colonize open areas such as pastures (Galante, 1983; Lumaret, 1990; Wassmer, 1995; Lobo et al., 2001; Dormont et al., 2007; Romero-Samper and Lobo, 2009). In some areas of Spain, the scarcity of rain seems to has a limiting factor to its distribution (Galante, 1983; Veiga, 1998). It can be found in differet types of dung such as cow, horse, sheep, deer, human, pig, bear (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006; Dormont et al., 2007; Agoiz-Bustamante, 2008) and even below carrions (Lumaret, 1990). However, there is a large accordance in the literature about its preference toward cow dung (Galante, 1983; Lobo, 1985; Lumaret, 1990; Wassmer, 1994, 1995; Veiga, 1998; Borghesio et al., 2001; Dormont et al., 2004; Errouissi et al., 2004; Dellacasa and Dellacasa, 2006), probably due to its mouthparts morphology (Verdú and Galante, 2004). In accordance to above cited authors, we found a marginal significant preference (N=72; IndVal 52.5; P = 0.058) toward cow dung (88%), compared with horse dung (12%). Ivermectin seem to alter the dung attraction toward *O. haemorrhoidalis*, but the direction of this effect is unclear being able to attract (Errouissi and Lumaret, 2010) or repel (Floate, 1998). However, Ivermectin and Moxidectin may affect adult emergence from larvae that feeded into contaminated dung (Kadiri et al., 1999).

It is a dweller species that laid eggs into dung pat where all the ontogenetical cycle occur (Yoshida and Katakura, 1992).

It was found into stomach contents of *Riparia riparia* (Young, 2015).

PHENOLOGY: *O. haemorrhoidalis* have only one generation per year, which reaches its maximum during summer months (June-July) (Lumaret and Kirk, 1987; Lumaret, 1990;

Wassmer, 1994; Veiga, 1998; Palestrini et al., 1995; Borghesio et al., 2001; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008; Jay-Robert et al., 2008; Agoglitta et al., 2012). It may overwinter as third instar larvae or adult (Landin, 1961; Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006). We found the start of the activity in May, the demographical peak in July followed by its slow decrease toward autumn.

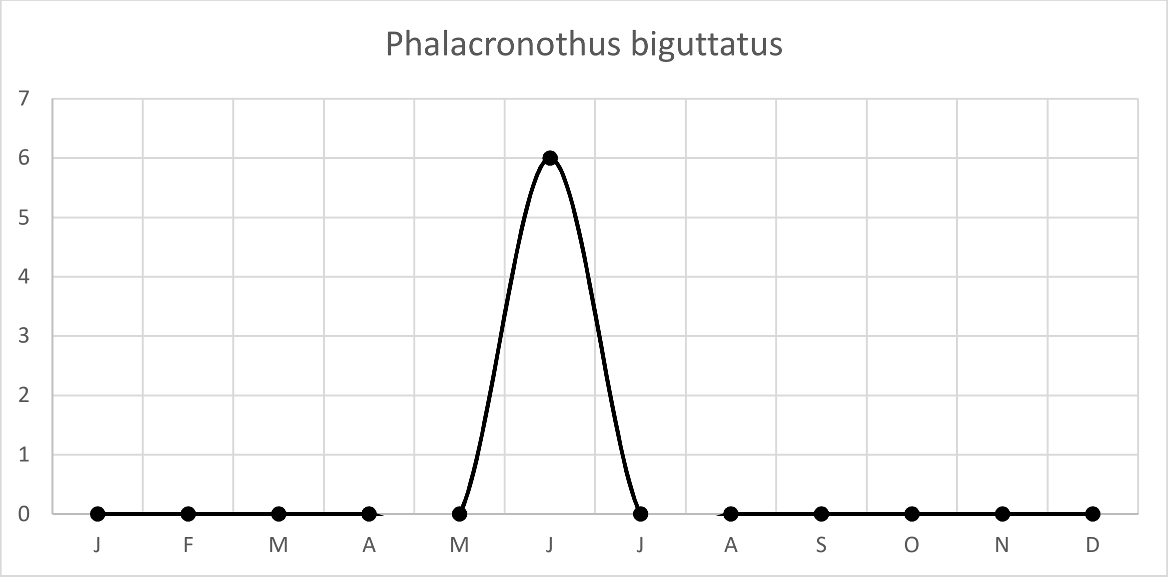


Phalacronothus biguttatus (Germar, 1824)

BIOLOGY: *P. biguttatus* is a oligotopic species which prefer open habitat such as xeric grasslands (Zunino, 1982; Lumaret and Kirk, 1987; Lumaret, 1990; Král and Malý, 1993; Wassmer, 1995; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006). It seems to prefer dung with low moisture contents such as sheep (Zunino, 1982; Lumaret, 1990; Král and Malý, 1993; Wassmer, 1995; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006) and horse (Dormont et al., 2004), although it can be found even into goat, lagomorphs, cow, dog, human and into rodent burrows (Falcoz, 1915; Horion, 1958; Lumaret, 1990; Král and Malý, 1993; Dellacasa and Dellacasa, 2006).

We only found 6 specimes that prevent us to highlight any preference (Indval 13.3; $P = 0.744$) toward cow (67%) or horse dung (33%).

PHENOLOGY: it is a species that reach its population peak during late spring early summer months (May-June) (Lumaret and Kirk, 1987; Lumaret, 1990; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Jay-Robert et al., 2008). Although we trapped a low number of specimens, they were captured during its demographic peak highlight by above cited authors.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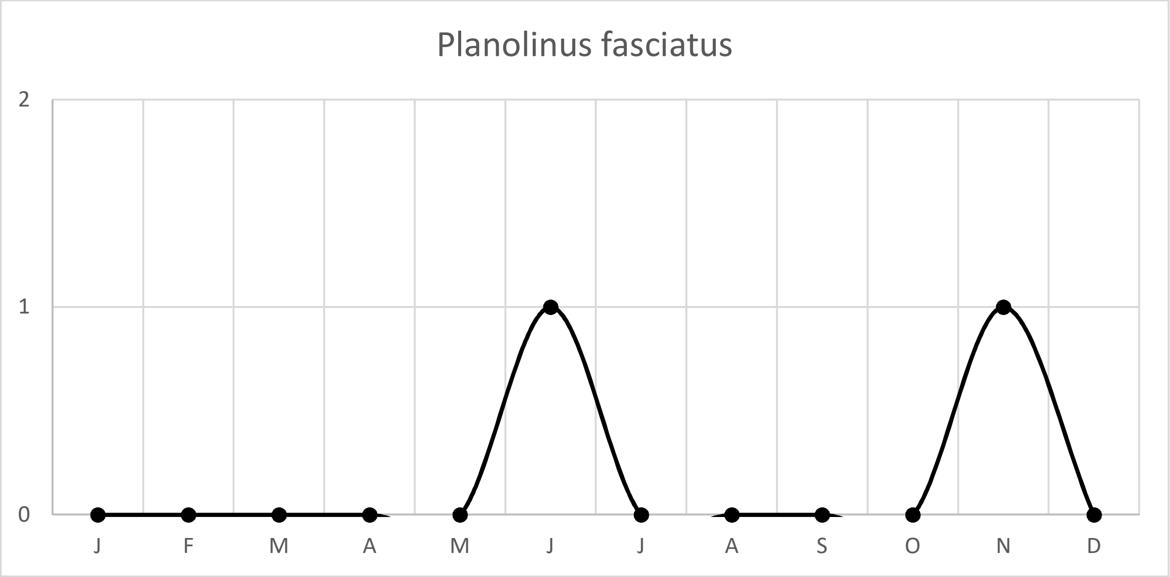
Planolinus fasciatus (Olivier, 1789)

BIOLOGY: it is an oligotopic species which colonizes haltered habitats such as forest or grasslands near forests (Lumaret, 1990; Dellacasa and Dellacasa, 2006; Negro et al., 2011; Borowski et al., 2016). It can be found indiscriminatle in several dung type such as cow, sheep, horse, human, cervids, lagomorphs, wild boar, bear (Carpaneto and Fabbri, 1984; Lumaret, 1990; Dellacasa and Dellacasa 2006) and even in carrions (Landin, 1961). The scarcity of trapped specimens (2 in cow dung) prevents us to highlight any trophic preference (IndVal 13.3; *P* = 0.472).

PHENOLOGY: this species is present all year round, but mainly during summer and early autumn months (Lumaret, 1990; Dellacasa and Dellacasa, 2006; Agoglitta et al., 2012; Borowski et al., 2016). It can overwinter as third instar larvae, adult and even egg (Landin, 1961; Lumaret, 1990; Dellacasa and Dellacasa, 2006). The scarcity of records prevents us to define its phenological pattern.

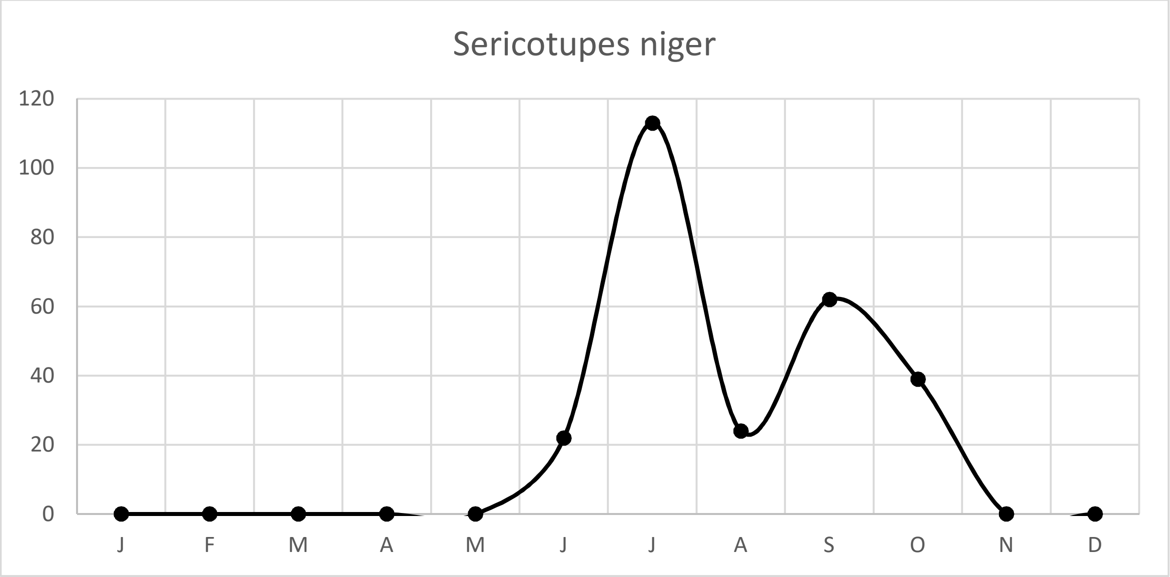


Sericotrupes niger (Marsham, 1802)

BIOLOGY: *S. niger* is a generalist species (Lumaret, 1990; Verdú, 1998) which can be found in any kind of habitat, from pastures (Zunino, 1982; Lumaret and Kirk, 1987; Zamora et al., 2007), to shrublands (Lumaret and Kirk, 1987; Zamora et al., 2007) and forests (Zamora et al., 2007; Verdú et al., 2011; Numa et al., 2012). Even the soil texture seems to be indifferent to this species, even if it seems to prefer sandy soils (Zunino, 1982; Verdú, 1998; Martín Piera and López-Colón, 2000). It is characterized by a wide trophic spectrum, although it prefers dung with a medium/high moisture content (Martín Piera and López-Colón, 2000) that colonize in the firsts days after deposition (Lobo, 1992). It can be found in cow, horse, human, rabbit, sheep, dog, fox and wild boar (Zunino, 1982; Lumaret, 1990; Verdú, 1998; Martín Piera and López-Colón, 2000). We do not found any trophic preference (N=260; IndVal 48.3; $P = 0.392$) between cow (56%) and horse dung (44%).

It has been documented tha *S. niger* form part of the diet of *Athene noctua* and *Meles meles* (Pigozzi, 1991; Fattorini et al., 1999, 2001).

PHENOLOGY: *S. niger* have summer-autumnal phenology, which activity reach the maximum from July to October (Lumaret and Kirk, 1987; Lumaret, 1990; Verdú, 1998; Martín Piera and López-Colón, 2000; Jay-Robert et al., 2008). The nesting occurs in autumn, the larval development need about two months and the nymphosis occur the subsequent spring (Lumaret, 1990).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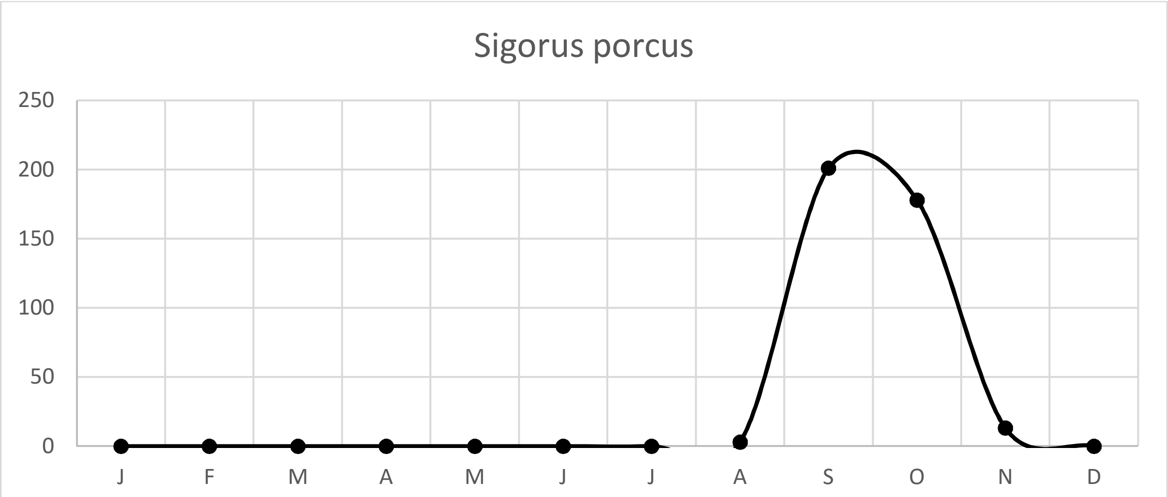
Sigorus porcus (Fabricius, 1792)

BIOLOGY: it is an oligotopic species which prefer open habitat as exposed pastures with scarce tree cover (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006; Macagno and Palestrini, 2009). It can be found in several kind of dung such as sheep and human (Lumaret, 1990; Dellacasa and Dellacasa, 2006), although it prefers cow and horse dung (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006). It was quoted as kleptoparites of *Geotrupes* brood masses (Chapman, 1869, 1870). We do not found any trophic preference (N=395; IndVal 54.7; *P* = 0.679) between cow (45%) and horse dung (55%).

PHENOLOGY: it is an autumnal species that reach the activity peak during September and October (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006; Jay-Robert et al., 2008; Agoglitta et al., 2012; Borowski et al., 2016) after the firsts autumnal rains (Lumaret, 1990). Overwinter as egg (Landin, 1961). We are in accordance with above cited literature, because we recorded the start of the activity in August with few specimens, the demographical peak during September and October, and a quick decrease toward winter months.

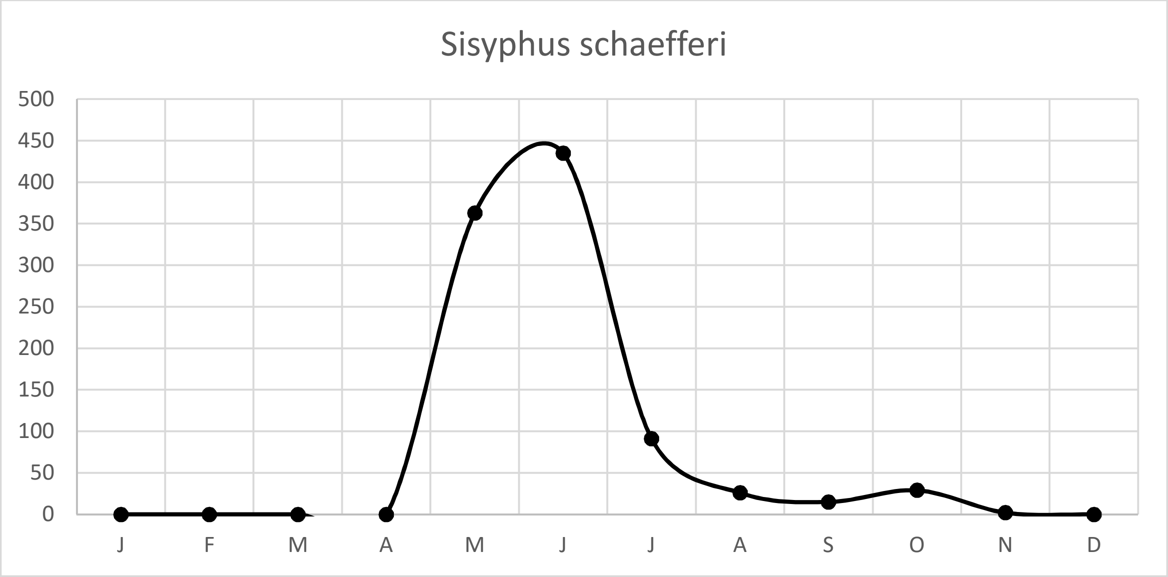


Sisyphus schaefferi (Linnaeus, 1758)

BIOLOGY: it is a diurnal species (Galante, 1979; Lumaret, 1990; Martín Piera et al., 1994) that can colonize every type of habitat from pastures to shrubland and forest (Lumaret and Kirk, 1987; Lumaret, 1990; Martín Piera et al., 1992; Martín Piera and López-Colón, 2000), although seem to prefer areas with a certain degree of arboreal cover (Verdú et al., 2011; Numa et al., 2012). It is an euryphagous species that can exploite several dung types such as cow, horse, sheep, goat, fox, human and badger (Lumaret, 1990; Martín Piera and López-Colón, 2000; Errouissi et al., 2004), moreover it was recorded its capacity to use mushrooms for feeding (Zunino, M., pers. comm. 2016). Dormont et al., (2004) quoted its preference toward cow dung. We do not record any significant trophic preference (N=961; IndVal 43.3; $P = 0.224$), but a strong tendency of predilection toward cow dung (81%) compared with horse dung (19%) was found, in accordance with Dormont et al., (2004). Probably this species is more attracted by dung contaminated with ivermectin (Errouissi and Lumaret, 2010).

Although the number of records, individuals and UTM cells where this species was found, increased after 1950 (Lobo, 2001; Carpaneto et al., 2007), a provisional model highlight the possibilities of a strong decrease (until -86%) in its distributional area by the 2080 in a scenario of climate change (Dortel et al., 2013).

PHENOLOGY: this is a late spring-early summer species, which activity peak occur during May-July (Galante, 1979; Lumaret and Kirk, 1987; Lumaret, 1990; Martín Piera and López-Colón, 2000; Jay-Robert et al., 2008; Agoglitta et al., 2012). The eggs are laid in June-July and the nymphosis occur in July-August (Paulian and Lumaret, 1975; Lumaret, 1978). The specimens active during August-November period are occasional and coincide with the emergence of the new generation adults (Lumaret, 1978; Lumaret and Kirk, 1987; Jay-Robert et al., 2008). The phenological pattern that we record in our area of study coincide with above cited authors. We found the peak of activity during May-June with a strong reduction from July. However, the activity is maintained up to November probably due the emergence of the new generation adults.



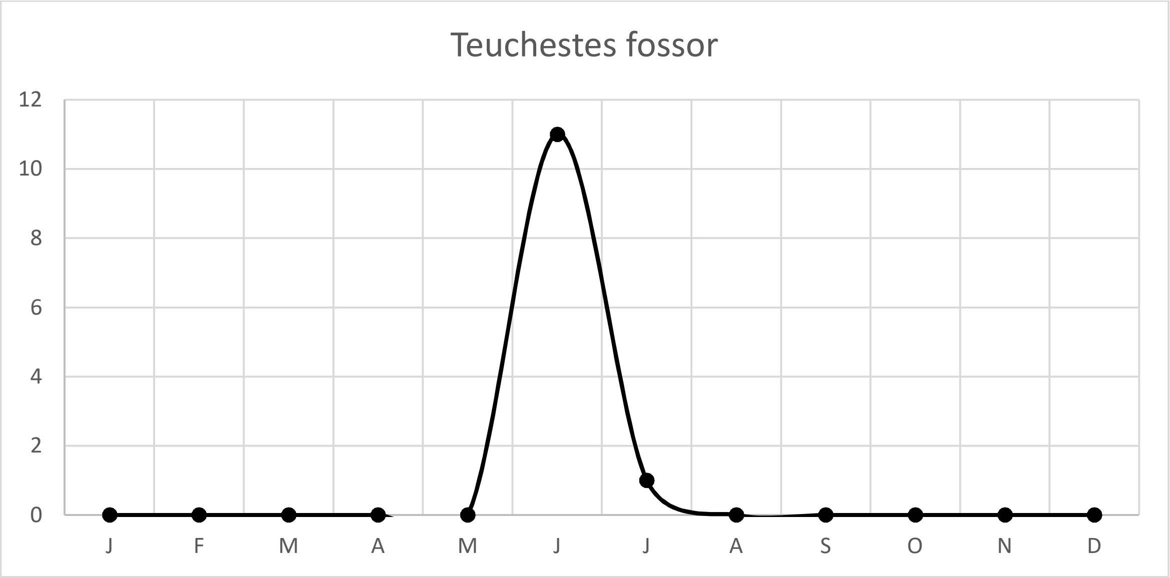
Teuchestes fossor (Linnaeus, 1758)

BIOLOGY: *T. fossor* is a oligotopic species that prefer open habitat as exposed pastures on moist soils (Galante, 1983; Lumaret, 1990; Wassmer, 1995; Gittings and Giller, 1998; Veiga, 1998; Borghesio et al., 2001; Dellacasa and Dellacasa, 2006; Dormont et al., 2007; Macagno and Palestrini, 2009; Tocco et al., 2013). Humidity and exposition of the habitat strongly affect reproduction and size of this species which show more eggs and larvae in shaded and humid areas and more adult emergence in exposed and humid habitats. Moreover, the adults born in shaded and humid areas are larger (Vessby, 2001). Moreover, the adult size seems to be density dependent (Stevenson and Dindal, 1985). It shows a strong preference toward cow dung (Galante, 1983; Lumaret, 1990; Wassmer, 1995; Gittings and Giller, 1998; Veiga, 1998; Borghesio et al., 2001; Dellacasa and Dellacasa, 2006), though it can be found in sheep, horse, deer, human, rabbit (Galante, 1983; Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006; Dormont et al., 2007) and carrions (van Wielink, 2004). Ivermectin and doramectin seem to increase dung actraction toward this species (Floate, 2007). Its high moisture requirement (Gittings and Giller, 1998) is reflected in its early successional colonization, that occur in the firsts days after dung deposition (Psarev, 2001a; Lee and Wall, 2006). The maximum diameter of dung particles that it can eat is about 25µm (Holter, 2000). Although we do not found any significant trophic preference (N=12; IndVal 32.7; *P* = 0.336), probably due to the low number of specimens recorded, a strong predilection toward cow dung (92%) compared with horse dung (8%) was found, in accordance with above cited literature.

Adults feed directly inside the food mass or at least just below it. However, oviposition does not take place inside the dropping: each egg is layed near the centre of a spheric brood mass, which the female prepares by filling a cavity (diam ±10mm) previously dug below the food source at an average depth of 1 cm. The female may builts a variable number (5 to 12) of single brood masses. A thick layer of soil separates the brood mass from the dung pat; the first and very probably also the second larval stage, develops inside the brood mass; the third larval stage leaves the brood mass and reaches the dung pad, where it develops during a longer period; pupation takes place inside the soil (Zunino and Barbero, 1990; Zunino, 1991). At the prepupal stage, the third instar larvae can repair the pupal chamber by its one excreta (Klemperer, 1978). Some experimental results suggest that the male produce signal that attract females, and that female produces signals that discourage colonisation by additional females (Manning and Ford, 2016). The larvae can acts as kleptoparasite toward *Geotrupes* brood masses (Klemperer, 1980).

It has been documented that *T. fossor* form part of the diet of *Tyrannus tyrannus*, *Corvus brachyrhynchos*, *Turdus migratorius*, *Sialia sialis*, *Anthus spinoletta*, *Sturnus vulgaris*, *Corvus frugilegus* and *Corvus corone* (Horgan and Berrow, 2004; Young, 2015).

PHENOLOGY: it is a springly-summerly species, which activity start in late spring and conclude in late summer, with a demographical peak during May-July (Lumaret, 1990; Palestrini et al., 1995; Gittings and Giller, 1997; Veiga, 1998; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008). The eggs are laid in summer, the larval development continue for about one year and then overwinter as larvae, and occasionally as adult (Landin, 1961; Lumaret, 1990; Gittings and Giller, 1997; Veiga, 1998; Dellacasa and Dellacasa, 2006). In accordance with above cited authors, we found a narrow phenological activity with a peak during June and only one specimen in July.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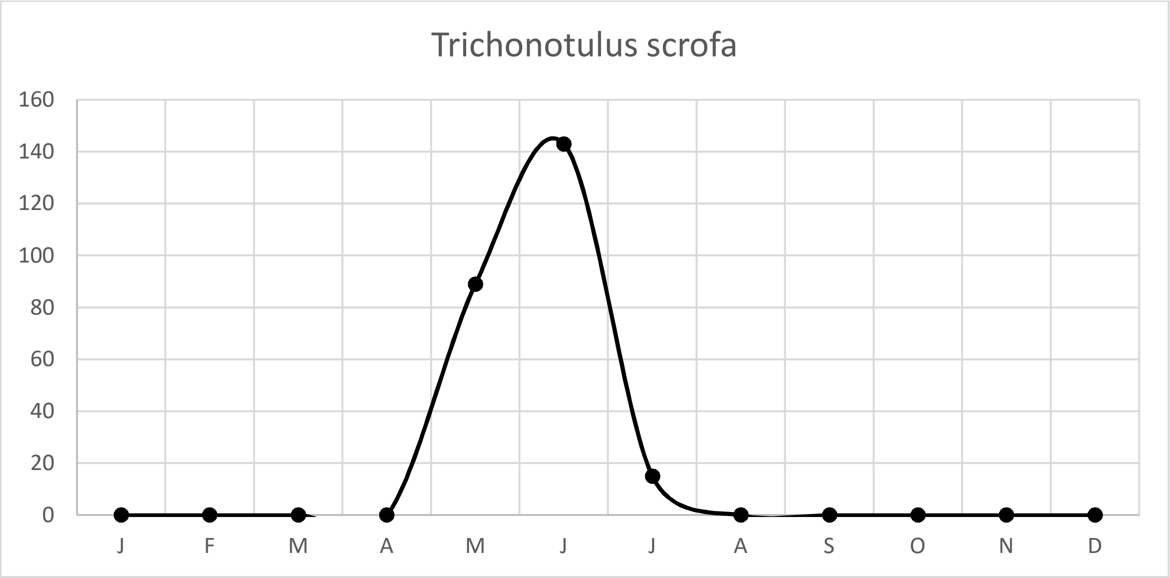
Trichonotulus scrofa (Fabricius, 1787)

BIOLOGY: *T. scrofa* is an oligotopic species which prefer open xeric habitats as exposed pastures on sandy and rocky soils with a good drainage capacity (Lumaret, 1990; Král and Malý, 1993; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006). It can be found in several kind of dung such as cow, horse, human and sheep (Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006; Agoiz-Bustamante, 2008) although seem to prefer semi-dry and fibrous excrements as sheep (Lumaret, 1990; Král and Malý, 1993; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006). Falcoz (1915) signaled this species into rodent burrows. We do not found any significant trophic preference (N=247; Indval 32.7; P = 0.313), however a strong tendency of predilection toward cow dung (82%) compared with horse dung (18%) was recorded, contrary to the literature cited above.

PHENOLOGY: the activity of this species is restricted to late spring-early summer months, with a demographical peak during April-May (Lumaret, 1990; Veiga, 1998; Dellacasa and Kirgiz, 2002; Dellacasa and Dellacasa, 2006; Jay-Robert et al., 2008). It has only one generation per year and overwinter as adult (Landin, 1961; Lumaret, 1990; Veiga, 1998; Dellacasa and Dellacasa, 2006). In accordance with above cited authors, we found a narrow activity period, but the demographical peak is shift toward early summer (June).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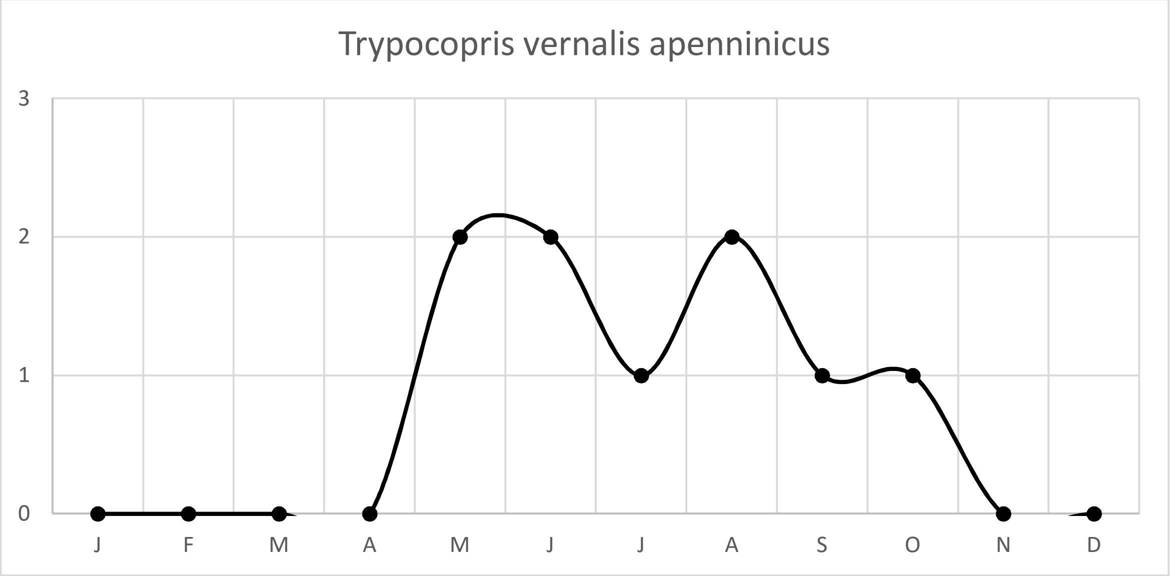
Trypocopris vernalis (Linnaeus, 1758) *apenninicus* Mariani, 1958

BIOLOGY: this is a generalist diurnal (Kamiński et al., 2015) species that can be found in several habitats from open pastures to closed forests, although seem to prefer sandy soils with a good drainage capacity (Lumaret, 1990; Martín Piera and López-Colón, 2000; Byk, 2011; Kamiński et al., 2015). It is a polyphagous species that can feed on cow, horse, sheep, human, rabbit, dog and fox (Lumaret, 1990), even it was found in carrions (Matuszewski et al., 2008; Jarmusz and Bajerlein, 2015). Probably due to the low number of specimens (N=9) we do not found any significant trophic preference (IndVal 13.3; *P* = 0.712) toward cow (67%) or horse dung (33%).

It is a tunneler species that make compound nest underground at 12-68 cm depth where each brood mass (5-10) of the nest receives only one egg which development cycle require 9-10 months (Brussaard, 1985; Kühne, 1995, 1996). However, it can behave as telephagic species, i.e. drawing of a piece of dung from the dung pat, and carries it away for bury it in a previously dug tunnel (Zunino and Palestrini, 1986).

It has been documented that this species form part of the diet of *Falco naumanni*, *Neotis ludwigii*, *Numenius arquata*, *Coprimulgus europaeus*, *Coracias garrulus*, *Lanius collurio*, *Corvus frugilegus* and *Athene noctua* (Kitowski and Pawlega, 2010; Young, 2015).

PHENOLOGY: this species is active from late spring to late summer, mainly from May to September (Lumaret, 1990; Kühne, 1995, 1996; Jay-Robert et al., 2008). The reproductive activity begins in August and reach its peak in September when eggs are laid, which development cycle require 9-10 months (Kühne, 1995, 1996). Even if the scarcity of collected specimens prevents us to define its phenological pattern, the continuous activity from May to October is in accordance with above cited authors.



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ANNEX 2

Selected and measured Functional Traits

All the sampled species was characterized by means of 23 functional traits that are: fresh body mass, six morphological measures of body, seven mouthparts morphological traits and nine bionomical traits. Below we list the functional traits used, their functional significance, the measurement or collection methods used to obtain the data, and the amounts of specimens used to get the average value of the trait. The morphological traits are measured by means of the software Leica Application Suite coupled to the stereo microscope Leica M205 C. In order to obtain the average species value for each morphological trait, ten individuals of each species (when available) were measured. To avoid any bias due to sexual dimorphism, only females were used (when available). Fewer than ten specimens of the following species were measured (the figure in brackets indicate 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); *Nialus varian*s (Duftschmid, 1805) (4); and *Amidorus thermicola* (Sturm, 1800) (7).

The bionomical traits were obtained from literature, expert communications and personal observations.

1) Fresh body mass

Fresh body mass is one of the most important functional traits. It is related to the quantity of buried dung (Nervo et al., 2014), metabolic rate (Davis et al., 1999), thermoregulatory pattern (Verdú et al., 2006) and competition (Horgan and Fuentes, 2005). Its relation with all these parameters defines the functional niche of the species with a strong potential influence toward functional diversity. Fresh body biomass was measured by weighted live dung beetles with a high-precision balance with 0.1 mg accuracy. We weighted ten specimens for each species (when possible), without separating male and female. For the following species, less than 10 specimens was weighted: *Agrilinus constans* (2), *Agrilinus convexus* (4), *Aphodius coniugatus* (2), *Caccobius schreberi* (6), *Copris lunaris* (9), *Esymus merdarius* (7), *Esymus pusillus* (8), *Euorodalus paracoenosus* (1), *Loraphodius suarius* (1), *Nialus varians* (1), *Nimbus obliteratus* (5), *Onthophagus coenobita* (5), *Onthophagus grossepunctatus* (4), *Onthophagus opacicollis* (1), *Phalacronothus biguttatus* (2), *Sigorus porcus* (4), *Teuchestes fossor* (9), *Trichonotulus scrofa* (6), *Trypocopris vernalis* (4). For the species that could not find live specimens (*Acanthobodilus immundus*, *Acrossus rufipes*, *Amidorus thermicola*, *Biralus maunkhaurum*, *Bodiloides ictericus*, *Calamosternus mayeri*, *Chilothorax lineolatus*, *Chilothorax paykulli*, *Labarrus lividus*, *Limarus zenkeri*, *Melinopterus reyi*, *Melinopterus stolzi*, *Nimbus johnsoni*, *Onthophagus illyricus*, *Planolinus fasciatus*), we estimated the fresh body mass by the following polynomial regression of order 2: $1.801x^2 - 11.77x + 21.51$; which is based on the regression developed between beetles fresh mass and body length of beetles.

BODY'S MORPHOLOGICAL TRAITS

2) Sphericity

It is a proxy of dung beetle shape. Shape is an important factor in determining the functional niche of dung beetles by means of the resource partitioning. (Hernández et al., 2011). This trait was calculated by the formula of Sneed and Folk (1958): $\sqrt[3]{\left(\frac{b}{a}\right)\left(\frac{c}{b}\right)^{3}}$; where a= maximum length, b=maximum width, c=maximum depth of profile.

3) Head area/Total area Ratio

This trait can have a functional implication due to the use of head during burying behavior or dung disruption.

4) Hind tibiae length

It is an important functional trait because it seems a roller characteristic, which shows a longer hind tibia for modelling and rolling the dung ball (Inward et al., 2011). This trait was measured as linear length from femur-tibiae articulation to the distal part of tibia.

5) Metamesosternal area

It is an indirect trait of the capacity of the species fly capacity because it is linked to the insertion of the flight muscles.

6) Abdomen length

This trait is an indirect measure of digestive system length (midgut + hindgut), which may be related to the trophic niche of the species and its digestive capacity (Holter and Scholtz, 2013).

7) Wing load

This trait was measured as the ratio of fresh body mass to total wing area (mg/mm^2). This trait is strongly linked to the dispersal capacity of each species, disentangling the foraging strategy (cruise flight vs. perching) (Peck and Forsyth, 1982; Howden and Nealis, 1975, 1978; Larsen et al., 2008; Silva and Hernández, 2015) and the habitat colonization capacity of the species (Barnes et al., 2014). Moreover, this trait is strongly linked to dung beetle thermoregulatory performances (Merrick and Smith, 2004).

MOUTHPARTS MORPHOLOGICAL TRAITS[1]

8) Mandible profile

We characterize the mandible profile according to the presence/absence of sclerotized areas in the distal lobe (the incisor lobe or apicalis). This trait is an indication of the capacity of dung beetle to feed on hard trophic resources (Figure 1).

9) Number of teeth in the mandibles profile

When a sclerotized area in the distal lobe of mandible was found, we further characterized this trait by counting the number of teeth that forming this area. This trait may be an indication of the hard resource exploitation performance (Figure 1).

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[1] Mouthparts morphology is an important trait that can divide dung beetle trophic niche by permitting, or avoiding, to feed on particulars trophic resources. Some studies highlight that the different morphology of denticles of mandibles, molar areas, paraglossae, setae of zygum, setae of acropariae, and zygum, may differenciate between "soft-diet consumers" and "hard-diet consumers" (for more details on mouthparts morphology, their ecological significance and dung beetles feeding behavior see: Madle, 1934, Halffter, 1961; Halffter and Matthews, 1966; Bürgis, 1982a, 1982b, 1984a, 1984b; Verdú and Galante, 2004; Dellacasa et al., 2010; Miller, 1961; Hata and Edmonds, 1983; Nel and De Villiers, 1988; Browne and Scholtz, 1999; Nel and Scholtz, 1990; Bai et al., 2015; Holter, 2000, 2004; Holter et al., 2002; Holter and Scholtz, 2011; López-Guerrero and Zunino, 2007).

10) Conjunctive/total mandibles area ratio

Madle (1934) asserts that conjunctive is a system of salivary channels, while Miller (1961) hypothesized that the “flexible area of the mandible” i.e. the conjunctive, “cushions” the grinding action and permits independent movements of the molar lobes “while the mandibles are in the closed position”. However, even though the functional significance of conjunctive is debated and needs further research (Holter, 2004), the presence of conjunctives in all coprophagous taxa, and their absence practically in all other scarabeids (Nel and Scholtz, 1990; Holter, 2004), makes this trait of great interest at least from a heuristic standpoint. Indeed, Holter and Scholtz (2011), demonstrated a strong reduction of conjunctive in pellet feeders compared with the wet-dung feeders (Figure 1).

11) The percentage of filtering/masticator area of mandibular molars

We differentiate the area of mandibular molars area into filtering and masticator area based on the degree of sclerotization and the directionality of transverse ridges. Although Holter (2000) and Holter et al., (2002) assert that molars of dung beetle do not achieve any grinding, Verdú and Galante, (2004) found a strongly developed masticator area in the mandibular molars of hard-feeding dung beetles (Figure 1).

12) Hypopharynx

We differentiate the hypopharynx as filtering or masticator based on the structure and sclerotization of glossa and paraglossa (Figure 1).

13) Acropariae curvature

The acropariae are setae presented in the apical part of epipharynx. We differentiate into curved acropariae and linear acropariae due to their relationship with trophic resource hardness (Verdú and Galante, 2004) (Figure 1).

14) Zygum

The zygum is the central apical part of epipharynx and it is formed by setae which were categorized depending on their adaptation toward hard-feeding diet: underdeveloped, developed and strong prolongation of spatula shaped epizygum (Verdú and Galante, 2004) (Figure 1).

15) Trophic diversity

Due to the high complexity in the trophic preference of dung beetle (Barbero et al., 1999; Dormont et al., 2004, 2007, 2010; Errouissi et al., 2004), and to their capacity to exploit several resources even the ones that are very different from each other (Palestrini and Zunino, 1985), we used an index that represents both the number of aliments that one species may exploit and the qualitative divergence among these aliments. To do this, we developed a hierarchical classification of dung beetle aliments mainly based on their origin and physical conformation (Table 1). For each species we determined whether or not the trophic resource is used (1,0) basing on bibliographical and expert information. Then we calculated the trophic diversity of each species using an index of taxonomic diversity: the average taxonomic distinctness ($\Delta+$) (Clarke and

Warwick, 1998a, 1998b, 2001; Warwick and Clarke, 1995, 1998). This measure takes into account the trophic level to which any two species are related and it can be thought of as the average length between any two randomly chosen species present in the sample. Hence, each species was characterized by a measure that takes into account the quantity of trophic resources exploiedt and their divergence into the hierarchical classification.

Figure 1: Mouthparts morphological traits. Example from *Geotrupes spiniger* (Marsham, 1802). A) Mandible and molar area: 1) sclerotized area of the mandible profile (incisor lobo with one teeth); 2) total mandible area; 3) conjunctive; 4) masticator area of the mandibular molar; 5) filtrator area of the mandibular molar. B) Hypopharinx; C) Epipharynx: 1) Zygum; 2) Acropariae

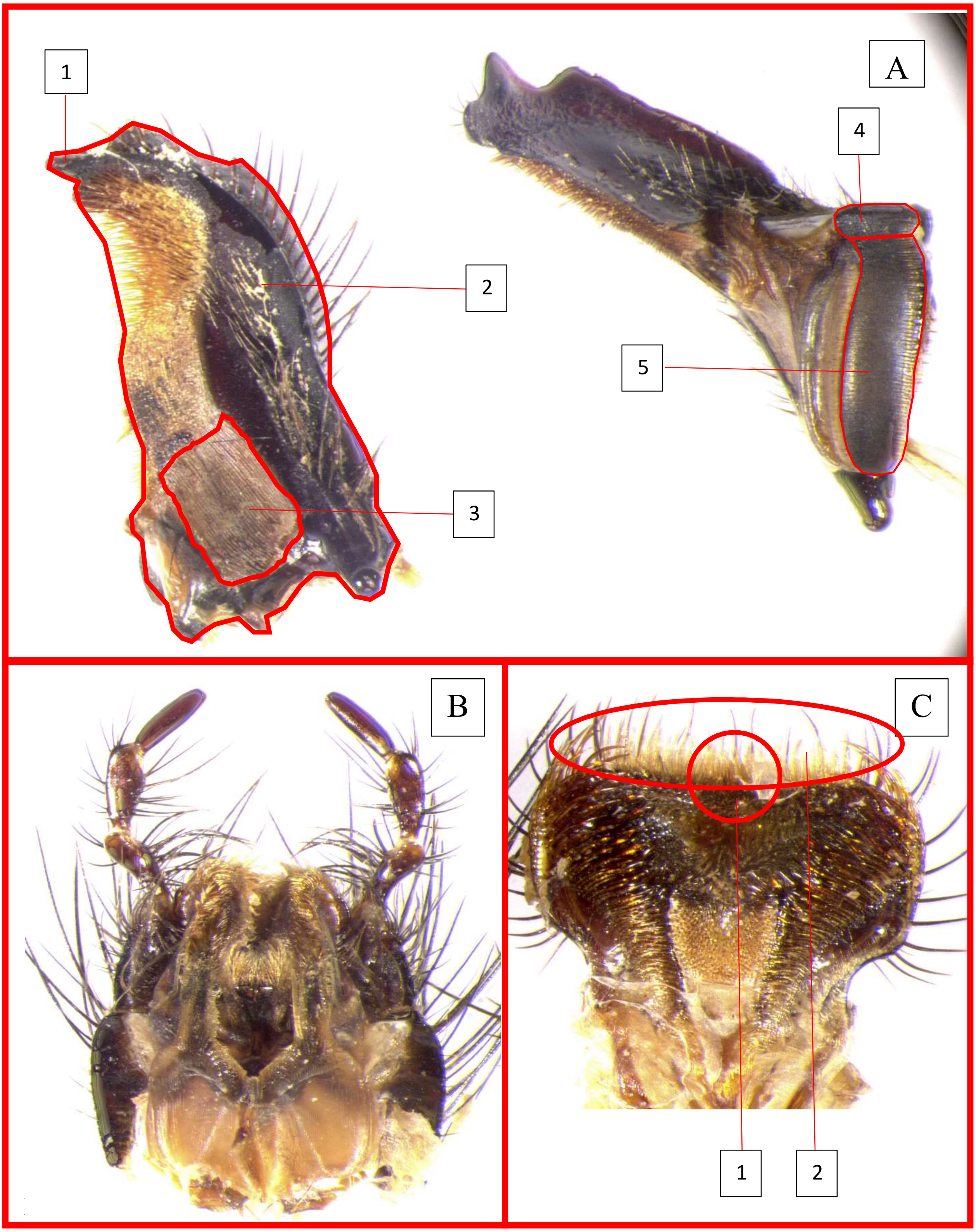

Table 1: Hierarchical classification of the dung beetle trophic resources, used to calculate the trophic diversity index.

Hierarchical level							**Species (0,1)**
Food	No animal origins	Live	Fresh resource	Fruits			
				Flowers			
				Mushrooms			
		Death	Not ingested	Decomposed	Decayed plants and mushrooms		
					Decayed fruits		
			Ingested	Ruminants	Mass	Bovine dung	
					Pellets	Ovine, caprine and cervids dung	
				Semi-ruminants	Mass	Camelids and giraffids dung	
					Pellets	Camelids and giraffids dung	
				Monogastrics	Mass	Equine dung	
					Pellets	Rodents and Lagomorphs dung	
	Animal origins			Carnivorous and omnivorous	Carnivorous and omnivorous dung		
			Not ingested	Vertebrates	Big size	Big vertebrates carrions	
					Small size	Small vertebrates carrions	
				Invertebrates	Arthropods carrions		
		Live	Fresh resource	Invertebrates	Arthropods predation		

16) Hydric content of trophic resource

Due to the fact that even the species with the same trophic preferences can colonize the same resource in different time after its deposition (Koskela and Hanski, 1977; Hanski, 1980; Lobo, 1992; Gittings and Giller, 1998; Menéndez and Gutiérrez, 1999; Psarev, 2001; Sabu et al., 2006, 2007; Sladecek et al., 2013), we characterize each species depending on the trophic resource hydric content: low, medium and high.

17) Nesting behavior

Based on the bibliographic references, personal observations and expert communication, we categorized each species as nester or no nester.

18) Nest type

Due to the high variability of nests (Chapman, 1869, 1870; Halffter and Matthews, 1966; Halffter and Edmonds, 1982; Bornemissza, 1969, 1971; Borghesio and Palestrini, 2002; Brussaard, 1985, 1987; Kirk, 1983; Klemperer, 1978, 1979, 1980, 1981, 1982a. 1982b; Kühne, 1995, 1996; Lumaret, 1975, 1983; Palestrini and Barbero, 1994; Rojewski, 1983; Romero-Samper and Martín-Piera, 2007; Yoshida and Katakura, 1992; Zunino and Barbero, 1990; Goidanich and Malan, 1964), we differentiate the nester species basing on the dung manipulation (masses or balls), nest location (within dung or underground) and nest complexity (Simple or compound). The following categories were identified:

a) Nest composed by single brood mass located within the excrement;

b) Nest composed by several brood masses located within the excrement;
c) Nest composed by single brood mass located underground in a simple nest;
d) Nest composed by several brood masses located underground in a simple nest;
e) Nest composed by several isolated brood masses located underground in a compound nest;
f) Nest composed by several brood masses per chamber, located underground in a compound nest;
g) Nest composed by single brood ball located underground in a simple nest;
h) Nest composed by several isolated brood balls located underground in a simple nest;
i) Nest composed by several brood balls per chamber located underground in a simple nest;
j) Nest composed by several isolated brood balls located underground in a compound nest;
k) Nest composed by several brood balls per chamber located underground in a compound nest.

19) Nest depth

Due to the great variability in the nest depth even in the species with the same nesting pattern, we differentiate the species basing on the nest depth into: within excrement, dung-soil interphase, little depth, great depth.

20) Horizontal nest distance

Basing on the horizontal distance of nest relative to the food source, we define four categories: within food source, starting within food source but with a horizontal extension, out of food source on a short distance, out of food source at a great distance.

21) Nesting behavior

Following the classification of Doube (1990), with some modifications, we identified the following categories based on the nesting behavior and the beetle-resource interaction and spatial relationships:

a) Telecoprid 1: great size beetle which produces brood balls and shows a high interaction with the excrement (i.e. *Scarabaeus*, *Kheper*, *Malagoniella*, *Megathopa* etc.)
b) Telecoprid 2: medium-little size beetle which produces brood balls and shows a high interaction with the excrement (i.e. *Gymnopleurus*, *Sisyphus*, *Canthon* etc.)
c) Telecoprid 3: species not producing brood balls but relocating small size dung (rabbit, goat, llama, maras, etc.) without dung molding (i.e. *Eucraniina*, *Thorectes*, *Jekelius*, etc.)
d) Telecoprid 4: species not producing brood balls but relocating small pieces of big dung pats (cow, horse, etc.) without dung molding (i.e. *Bolbites*, *Chalcocopris*, *Trypocopris*, etc.)
e) Paracoprid 1: big body size species burying dung rapidly and at great depth (≥ 50 cm) (i.e. *Copris*, *Bubas*, etc)
f) Paracoprid 2: big body size species burying dung slowly and at great depth (≥ 50 cm) (i.e. *Onitis*, etc)

g) Paracoprid 3: small body size species burying slowly and at small depth (≤ 30 cm) with well-developed brood mass (i.e. *Onthophagus*)

h) Paracoprid 4: small body size species burying slowly and at small depth (≤ 10 cm) without well-developed brood mass (i.e. *Aphodius*)

i) Endocoprid 1: brood balls developed within dung pat (i.e. *Eurysternus*, *Canthon*, *Oniticellus*, etc.)

j) Endocoprid 2: eggs are laid within dung pat without brood balls construction (i.e. *Aphodius*, *Trichillum*, *Pedaridium*, etc.)

k) Cleptocoprid: use of brood masses/balls of other species (i.e. *Aphodius*, *Onthophagus*, *Caccobius*, etc.)

22) Daily activity

Basing on the species daily activity pattern we have categorized the species as diurnal or crepuscular/nocturnal.

23) Phenology

Because of the strong seasonality of the dung beetle species, we identify the following phenological patterns basing on the species activity:

a) Autumn, winter and spring
b) Winter and spring
c) Spring
d) Winter, spring and summer
e) Spring and summer
f) Summer

g) Spring, summer and autumn
h) Summer and autumn
i) Summer, autumn and winter
j) Spring and autumn
k) Autumn
l) Autumn and winter
m) Winter
n) All the year

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