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Ecosystem functioning in relation to species identity, density and biomass in two tunneler dung beetles

Short title: Functioning in relation to assemblage attributes

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Abstract

1. Species abundance, biomass and identity are main factors that influence ecosystem functioning. Previous studies have shown that community attributes and species identity help to maintain natural ecosystem functioning.

2. In this study, we examined how species identity, biomass and abundance in dung pats (i.e. density) of dung beetles affect multiple ecological functions: dung removal, seed dispersal and germination. Specifically, we targeted two species of tunnelers: *Onthophagus illyricus* (Scopoli, 1763) and *Copris lunaris* (Linnaeus, 1758). In accordance with their natural abundance, we considered densities ranging from 10 to 80 individuals for *O. illyricus*, and from 2 to 8 for *C. lunaris,* spanning the total biomass per treatment from 0.22 to 1.76 g.

3. Results showed that, even at higher abundance, *O. illyricus* is not likewise efficient as *C. lunaris*. Species identity, biomass and density are crucial factors for maintaining ecosystem functioning. The combined effect of species identity and density/biomass facilitated dung removal and seed dispersal. Conversely, ~~we found that~~ species identity is the only relevant factor for germination. Moreover, relationships among functions depend on the species

investigated; *C. lunaris* showed a positive correlation between dung removal and seed dispersal, whereas *O. illyricus* showed a positive correlation between germination and dung removal.

4. In conclusion, several optimal ecosystem functioning depends on multiple factors such as density and species identity, thus also on body size, nesting strategies and ecological functions investigated. Moreover, the loss of larger and efficient species cannot be compensated by higher abundances of small species.

Keywords: ecological functions, species identity, ecosystem functioning, density, abundance, biomass.

Introduction

Over the last few decades, the loss of biological diversity has accentuated the need to understand how community attributes (such as biomass, abundance and species presence) affect ecological processes (Purvis and Hector 2000; Naeem et al. 2012; Gagic et al. 2015). Loss of ecosystem functioning can be related to several factors, such as the reduction in the number of species that are more functionally important (Kremen 2005; Larsen et al. 2008), the loss of species that facilitate or complement the functionality of other species (Zavaleta and Hulvey 2004), or the massive reduction in species abundance (Estes and Palmisano 1974; Jackson et al. 2001). It was recently shown that the abundance of a few common species can drive ecosystem functioning, even more than species composition and species richness that is often dominated by many rare but functionally unimportant species (Winfree et al. 2015). Species identity have been found to play a crucial role in ecosystem functioning (O’Connor and Crowe 2005) and its role depends on which functions are investigated (Slade et al. 2017).

Dung beetles (Coleoptera: Scarabaeoidea) contribute to a full suite of ecosystem services including dung removal, nutrient cycling, and greenhouse gas reduction (e.g. Nichols et al. 2008; Beynon et al. 2012; Nervo et al. 2017; Slade et al. 2016). Dung beetles are frequently classified according to their nesting habits (Hanski and Camberfort 1991). Tunneler dung beetles dig galleries below dung pats and bury dung for feeding and breeding activities. By transporting dung into soil, tunnelers contribute to seed dispersal and facilitate seed germination (Estrada and Coates-Estrada 1991; Feer 1999; Andresen 2001; Amézquita and Favila 2010). Different species have different effects in relation to the ecological functions investigated. *Geotrupes* spp have been found to be very efficient in dung removal (Rosenlew and Roslin 2008; Kaartinen et al. 2013; Nervo et al. 2014), while *Catharsius* and *Copris* spp in seed dispersal (Slade et al. 2007).

Previous research has shown that provisioning of ecological functions by dung beetles may vary depending on species assemblage attributes and species identity (Bang et al., 2005; O'Hea et al., 2010; Beynon et al., 2012; Nervo et al. 2016; Piccini et al. 2017). Abundance and biomass of dung beetle communities are pivotal factors that have shown to be relevant for ecosystem functioning (Tixier et al. 2015; Griffiths et al. 2016). Large-bodied species have been found to provide a greater amount of ecological functions (Kaartinen et al. 2013; Nervo et al. 2014), even more at higher abundance (Braga et al. 2013; Ortega-Martínez et al. 2016), but at the same time they are sensitive to ecological stressors (Larsen et al. 2005). Moreover, few functionally important species can contribute greatly towards ecosystem multifunctioning at high abundance (Slade et al. 2007; Braga et al. 2013; Manning and Cutler 2018).

Few studies have been published on how dung beetle density influences provisioning of ecological functions (Yamada et al. 2007; Tixier et al. 2015). The magnitude of dung beetle effects may be dependent on the differences in species' burrowing activity (Holter et al., 2002; Larsen et al., 2005; Piccini et al. 2017). However, little is known about the influence of density on dung removal, seed dispersal and seed germination. Giller and Doube (1989) have proof that, at high density, the intraspecific competition in two species, large (Coprinae) and small beetles (*Onitis alexis*), reduced dung removal. Moreover, when the competition is high, the percentage of dung buried is high but lower than expected (Giller and Doube 1989).

Dung beetle activity can favor seed dispersal in different ways: seeds can be transported in a more suitable microclimate (Shepherd and Chapman 1998; Andresen and Levey 2004; Griffiths et al. 2015, 2016), they can be protected by predators and pathogens (Shepherd and Chapman 1998; Feer 1999) and they can benefit of a reduction in clumping with lower competition and density-dependent mortality (Andresen and Feer 2005; Lawson et al. 2012). Affecting seed survival in these ways, dung beetle activity could have important impacts upon plants regeneration and community composition (Griffith et al. 2016). Seed burial depth mediated by dung beetles varies according to the size of the seed, with the smallest seeds more likely to be buried and the bigger ones that remain on the soil surface (Braga et al 2017). Seed burial also influence seed viability: seeds found in the first 1-5 cm of soil have higher probability to germinate than those at higher depths (Andresen & Levey 2004).

Endozoochory may be one of the main drivers shaping temperate grassland communities. However, few studies have investigated the graminoid-seed germination in relation to dung beetle activity (Wicklow et al. 1984), even though many seeds of grassland species have been found digested and highly concentrated in dung (Pakeman et al. 2002; Cosyns et al. 2005; Couvreur et al. 2005). The activity of dung inhabiting fauna which remove and manipulate dung may kill or harm vulnerable seedlings (Janzen 1984). On the other hand, dung might be a beneficial microhabitat for grass seed germination because of the reduced competition with the already developed vegetation (Traveset 1998).

Here, we investigated the effects of dung beetle density, biomass and species identity on three main ecological functions provided by two tunneler species: dung removal, dispersal of seed mimics (beads), and graminoid-seed germination in the short term. The percentage of seeds found in dung that are still viable is species-dependent (Milotic and Hoffmann 2016a, 2016b, 2016c). There are no data on *Lolium multiflorum* survival through the cow digestive duct, but it is known that only 12% of *Lolium rigidum* seeds ingested remained viable once it ends in cattle dung (Stanton et al. 2002). Considering the low rate of *L. rigidum* seed survival along the digestive tract of cows, we preferred to do not place seeds in dung pats (i.e. assuming they had been ingested by cows) to test seed germination. Instead, we placed the seeds of *L. multiflorum* (Lam., 1799) directly on the surface of the ground and covered them with a dung pat. Thus, we simulated a situation in which seeds were covered by dung pats dropped by grazing cattle. Indeed, considering *L. multiflorum* can produce 100000 seeds/m^2 in a pasture (Young et al. 1996), it is likely that many seeds would be covered by dung.

In order to test density and biomass effects in dung-system functioning, we selected the two most abundant and widespread tunneler species in our study area in north-western Italy: *Onthophagus illyricus* and *Copris lunaris*. These species are both tunnelers, they present different bodymass (the larger species 10 times bigger than the small one) and nesting strategies: *O. illyricus,* 0.022g±0.009 of bodymass (personal data), lays eggs directly in the tunnels below dung pats (Macagno et al. 2016), while *C. lunaris,* 0.22g±0.07 of bodymass (personal data)*,* constructs a proper large nest at the end of the tunnels where it takes care of the brood (Klemperer 1982). Considering that species identity and abundance in dung pats (i.e. density and biomass) are crucial factors for dung removal (Slade et al. 2007; Nervo et al. 2014; Tixier et al. 2015), we hypothesized that an increase of beetle density would lead to a logarithmic increase of ecological function curve with an asymptotic tail when the intraspecific competition would be strong enough to stop provisioning increase. Moreover, in accordance with natural abundance of each species, we organized experimental mesocosms that have comparable biomass between the species treatments even though they present different density. This experimental design leads to test if (1) a higher abundance of the small species, *O. illyricus*, can functionally compensate a loss of the large species, *C. lunaris*, that is more prone to extinction (as large species: Larsen et al. 2005; Roslin et al. 2014). In accordance with Giller and Doube (1989), different species have different effects on ecosystem functioning, in relation to their density and biomass. Moreover, we expected that species identity, density and biomass would affect: (2) the amount of dung remaining on the ground; (3) seed dispersal and (4) short-term seed germination (through differential removal of dung mass over the seeds). We investigated (5) the correlations among these functions to understand their interconnections for both species.

Experimental design

To examine the functional consequences of different densities of *O. illyricus* and *C. lunaris* on multiple ecological functions, we established monospecific experimental replicates of laboratory terraria with 4 different densities.

Dung beetles were collected from La Mandria Natural Park (45° 08' 48.83" N, 7° 36' 02.53" E), from IPLA fields (Istituto per le Piante da Legno e l'Ambiente, 45° 05' 18.5" N, 7° 44' 28.5" E) in north-western Italy. The two species are neither endangered nor protected and the collection was authorized by the "Ente di Gestione delle Aree Protette dei Parchi Reali" (Venaria, Piedmont, Italy) and by the IPLA operative unit. Beetles were collected in May 2015, using 20 standard cattle-dung-baited pitfall traps separated by distances of at least 10 m, each emptied after 48 hours. We collected 800 *O. illyricus* (Scopoli, 1763) at IPLA fields and 75 *C. lunaris* (Linnaeus, 1758) at La Mandria Natural Park. During the 2015 field season, both were the most abundant species in Turin (Piedmont, Italy) and the surrounding area (North-West Italy).

Terraria were filled with artificial soil made of commercial gardening humus, homogenized through a 1cm-mesh, and mixed with sand and water compressed into the terrarium to obtain a hardness similar to natural soils (hereafter called soil; for supplier information see Piccini et al. 2017). We set 8 cm of soil for *O. illyricus* and 15 cm for *C. lunaris*, reflecting the differential digging capacity of these species (Macagno et al. 2016 and Piccini, pers. obs.). We ran 8 monospecific treatments with 4 different densities and 4 controls without beetles. In accord with the natural species abundance found in dung pats (Piccini pers. obs., see details in Appendix), the densities were 10, 50, 60 and 80 individuals for *O. illyricus* (O10, O50, O60 and O80, respectively); and 2, 4, 6 and 8 individuals for *C. lunaris* (Co2, Co4, Co6 and Co8, respectively). For *C. lunaris,* the sex ratio in each treatment was 1:1. For *O. illyricus*, considering the high number of individuals was not possible to identify all of them without stressing them. Thus we extract 20 individuals from the collection and we sexed them. We counted a sex ratio that was slightly higher for females (1:1.86). We also ran three types of control: terraria with dung without beetles (Cntr) and terraria with neither dung nor beetles, with seeds placed either on the surface (Csur), or inside the first 5cm of soil (C5cm). Four replicates were established for treatment and control types. However, for the highest density treatment of *C. lunaris* we ran only 3 replicates due to the scarcity of individuals collected in the field. This yielded a total of 43 terraria (7 treatments x 4 replicates + Co8 treatment x 3 replicates + 3 controls x 4 replicates = 43).

Fresh dung was collected from a herd of 12 Aberdeen Angus cattle grazing on natural grasslands dominated by graminoids (genera *Dactylis*, *Festuca*, *Poa*, *Lolium* and *Setaria*) at IPLA. The dung was frozen for at least two weeks at -8°C to kill potential predators and other insects (O'Hea et al 2010). Cows were not treated with antibiotics or anti-helmintics. The dung was defrosted for 96 hours, and manually homogenized before being partitioned into 500 g, 16 cm in diameter standard-sized pats (the typical pat weight found in the field) to each treatment and to the control Cntr. The dung was located in the center of the terrarium to leave an uncovered strip of ground (around 5cm width) surrounding the pat.

The experiment lasted for 96 hours, which is the time needed for *C. lunaris* to remove the dung to construct its nest (Klemperer 1982). It can be a sufficient amount of time also for *Onthophagus* spieces. Indeed, it is known that *O. fracticornis* removes 80% of the dung in 80 hours (Nervo et al. 2014). Throughout the experiment, the laboratory was kept at a constant temperature around 25°C with 60 % humidity.

Ecological functions investigated

To evaluate the functional efficiency of dung beetles, we focused on three types of ecological functions: dung removal, seed germination and seed dispersal.

Dung removal was measured by weighing the dry dung (g) remaining on the surface of the soil at the end of the experiment.

In order to compare the species identity and assemblage attribute (i.e. density and biomass) effects of tunnelers on short term **seed germination,** we used seeds of *L. multiflorum,* one of the most widespread and common species of the local pastures. We sowed *L. multiflorum* (35 kg seed ha−1) on the soil surface below the dung pat (0.07g, i.e. 15 seeds; Figure A1 in Appendix) and measured short term germination by counting the total number of stems visible at the end of the experiment (after 4 days). As a caveat, we did not exclude that those seeds that did not germinate in the short term could still potentially germinate in longer time.

To investigate the **seed dispersal,** we used colored beads as seed mimics, considering the difficulties of finding very small non-germinated seeds in the soil. We evaluated dispersal of beads placed below and inside the dung pats, in this latter case simulating the dispersal of particles presented inside the dung and bioturbation. Thus, we placed 15 blue beads (2mm diameter) on the surface of the soil, below the dung pats (together with the seeds) and 30 red beads (2mm diameter) inside the dung. We considered two aspects of bead dispersal: *the bead removal* from their original position (i.e. surface of the soil or inside the dung) and *the bead dispersal in the soil* of all beads placed on the surface of the soil and inside the dung. In order to evaluate burial depth, we divided the soil into different layers. For *O. illyricus,* we divided the 8-cm soil into two layers of 4 cm each (upper and lower layers) and for *C. lunaris*, we divided the 15-cm soil into three layers of 5-cm each (upper, middle and lower layers).

Statistical analysis

DUNG REMOVAL AND SEED GERMINATION – To investigate which factors affected dung removal, we modelled dry dung mass removed (evaluated by average of dung remained in controls Cntr minus dung remained per terrarium) in a linear model where species identity, as categorical variable, and density, as continuous variable, nested within species identity were used as explanatory variables (Species_identity + Species_identity/Density). Density was nested within species identity, since the range of density values are very different among species. We also tested whether dung removal was affected by total biomass of dung beetles, in a linear model where species identity, as categorical variable, and biomass, as continuous variable, were used as explanatory variables. We also tested for the interaction term, since the range of values are the same for both species (Species_identity × Biomass).

To evaluate which factors affected seed germination, we modelled the number of stems in a generalized linear model where species identity, as categorical variable, and density as continuous variable, nested in species identity were used as explanatory variables (Species_identity + Species_identity/Density). We also tested whether seed germination was affected by total biomass of dung beetles, in a generalized linear model where species identity, as categorical variable, and biomass as continuous variable were used as explanatory variables (Species_identity × Biomass). In both models, we specified a Poisson distribution of errors and we excluded controls without beetles (Cntr).

Considering that we performed several different controls with seeds at different depths, we decided to investigate which treatment affected seed germination comparing treatments and controls. Hence, we modelled the number of visible stems as a generalized linear function of treatments and controls (Csur and C5cm) as a categorical variable (O10, O50, O60, O80, Co2, Co4, Co6 and Co8), setting controls without beetles with seed placed under the dung (Cntr) as a reference category and specifying a Poisson distribution of errors. All models were checked for overdispersion via the ratio between Pearson residuals of the model and the degrees of freedom. To identify which treatment differed from others, we applied a Tukey post hoc test on the number of stems.

SEED DISPERSAL – To investigate whether species identity, density and biomass affected seed dispersal, we modelled the proportion of beads removed either from dung and from the ground surface in a generalized linear model where species identity was a categorical variable, and density nested into species as continuous variable (Species_identity + Species_identity/Density), specifying a binomial distribution of error and a logit link function. To investigate the effect of density and biomass on beads dispersal in the soil, we modelled, for each species, the proportion of beads that were placed either in the dung and on the surface in relation to the layer where they were found (i.e. dung, soil surface, upper, middle and lower layer as categorical variable) and density (or biomass) as continuous variable (and their interaction term) with a generalized linear model, specifying a binomial distribution of error and a logit link function (Layer × Density or Layer × Biomass). Since the layers are spatially autocorrelated, we decided to perform a model comparing layers pairwise (i.e. Dung vs Soil Surface, Soil surface vs Upper layer, etc.).

CORRELATION BETWEEN FUNCTIONS – To establish the relationship among the three ecological functions investigated, we analyzed the Pearson pairwise correlations of the following measures: dung removal, evaluated as dung removed, seed germination, bead removal from the soil surface and bead removal from inside the dung. In order to make a comparison between the two species, we excluded the controls. This resulted in 6 comparisons per species.

Each model was fitted using the 'lmerTest' package in R (v3.2.1) statistical and programming environment (R Development Core Team 2005). For post hoc analysis, we used 'multcomp' package (Hothorn et al. 2008). For each model, we evaluated the omega squared (Ω^2) that is a measure of effect size or the degree of association for a population. It is an estimate of how much variance in the response variables are accounted for by the explanatory variables (Xu 2003). We found that it was higher than 50 in each model, meaning that high percentage of variance was estimated in the response variables.

Results

DUNG REMOVAL EFFICIENCY – Statistical analyses suggested that density and species identity both affect dung removal (GLM: Species_identity/Density: $F_{2;30}$=10.23, p<0.001; Species_identity: $F_{1;30}$=46.83, p<0.001). Similarly, both species identity and biomass affect dung removal (Species_identity × Biomass: $F_{1;30}$=19.68, p<0.001; Species_identity: $F_{1;30}$=46.83, p<0.001). The amount of dung remaining on the ground decreased with increasing biomass in *C. lunaris*, whilst remained constant or slightly decreased in *O. illyricus* (Figure 1A).

SEED GERMINATION – We did not find any significant effect of density (GLM: Species_identity (*O. illyricus*)/Density: DF: 27, z=-0.86, p=0.38; Species_identity (*C. lunaris*)/Density: DF: 27, z=-0.41, p=0.68) or biomass (GLM: Species_identity × Biomass: DF: 27, z=-0.65, p=0.51) on seed germination for any of the dung beetle. However, the analyses indicated that *C. lunaris* significantly increased the amount of seeds germinated compared to *O. illyricus* (GLM: Species_identity: DF 30, z=-3.45, p<0.001).

Results of Tukey posthoc test showed that treatments with *C. lunaris* facilitated seed germination compared to Cntr controls with only dung (GLM: Co2: DF 32, t-value =3.089, p=0.004; Co4: DF 32, t-value =3.346, p=0.002; Co6: DF 32, t-value=1.93, p=0.06; Co8: DF 32, t-value=2.979, p=0.005), whereas treatments with *Onthophagus illyricus* did not (GLM: O10: DF 32, t-value =-0.90, p=0.37; O50: DF 32, t-value =-1.03, p=0.31; O60: DF 32, t-value =-0.77, p=0.44; O80: DF 32, t-value =-1.29, p=0.21). Moreover, the presence of dung pats (with or without dung beetles) obstructed seed germination because controls without dung

(Csur and C5cm) showed significantly higher number of stems than all the other treatments and controls (Figure 1B; Csur: t_{42}=6.564, p<0.001; C5cm: t_{42}=7.078, p<0.001; Table A1 in Appendix).

SEED DISPERSAL – *The bead removal*

Statistical analyses showed a different pattern of the species and an effect of density influencing the transport of beads **placed on the surface of the soil** (GLM: Species_identity /Density: $F_{2;30}$ =9.38, p<0.001; Species_identity: $F_{1;30}$ =14.56, p<0.00; Fig. 2, Fig. A2 in Appendix) and of beads **placed inside the dung** (GLM: Species_identity /Density: $F_{2;30}$ =18.59, p<0.001; Species_identity: $F_{1;30}$ =80.49, p<0.001; Figure 2). Similarly, we found a different pattern of the species along the increasing biomass influencing the transport of beads **placed on the surface of the soil** (GLM: Species_identity× Biomass: $F_{2;30}$ =7.61, p=0.011; Biomass: $F_{1;30}$ =11.16, p=0.002; Species_identity: $F_{1;30}$ =14.56, p<0.001; Figure 2) and of beads **placed inside the dung** (GLM: Species_identity× Biomass: $F_{2;30}$ =17.86, p<0.001; Biomass: $F_{1;30}$ =19.31, p<0.001; Species_identity: $F_{1;30}$ =80.49, p<0.001; Figure 2). The proportion of beads remaining in the dung decreased with increasing *Copris lunaris* density (from an average of 72% of beads remained in the dung at the end of the experiment for treatments with 2 individuals to an average of 28% of beads remained for treatments with 8 individuals). On average, 0.01% of beads were left on the surface, but most of them were transported into the soil (from a mean of 26% of beads for treatments with 2 *individuals* to a mean of 70% for treatments with 8 *individuals*; Figure A2 in Appendix A). Conversely, the increase in *Onthophagus illyricus* density did not change seed transport into the soil (on average, for all treatments, 90% of beads were still present inside the dung at the end of the experiment), but the few beads transported were found on the surface (on average, 0.06% of beads on the surface; Figure A2 in Appendix A).

SEED DISPERSAL – *Bead dispersal in the soil*

The results of GLM model showed a different performance of bead dispersal along soil layers in the two species.

Models with *Copris lunaris* showed an interaction between beetle density (and biomass) and proportion of beads found in each layer, for both beads placed in dung and over soil surface. In particular, in the upper layer proportion of beads placed on the surface below dung increased with increasing beetle density (and biomass), while those found in the soil surface decrease and those found in dung remained constant. Beads placed inside dung were found mainly in the upper and middle layer in higher proportion with increasing density (and biomass), compared to those found in the above layers (see Table A2 and Fig A3a and A3b).

Regarding *Onthophagus illyricus*, in general models show no density (and biomass) effect and show no active transportation, since no beads where found in the soil layers. Only comparing dung vs soil surface, our results show that an increase of beetle density (and biomass) increased the proportion of beads found in dung compared to those found in the surface. (see Table A2 and Fig A4a and A4b for detailed results).

Thus, the two species had different effects on the transportation of beads either placed in the dung and on the surface along the soil depth profile. *O. illyricus* transported few beads from the dung to the upper layer of the terrarium (first 5cm of soil). Conversely, *C. lunaris* transported most of the beads to the soil layers (except for the lowest layer). For beads placed on the surface, *O. illyricus* did not transport beads actively, indeed most of the beads were found where they have been placed or in the layers of the dung that were in contact with the surface. *C. lunaris* transported few beads from surface only to the first layer of soil.

CORRELATION BETWEEN FUNCTIONS – For *O. illyricus* we found a negative correlation between dung removal and seed germination (r = - 0.67, DF 14, t=-3.33, p=0.005; Figure 3a). For *C. lunaris*, we found a positive correlation between dung removed and bead removal from the dung (r = 0.84; DF 13, t=5.57, p<0.001), and bead removal from the surface of the soil (r = 0.62, DF 13, t=2.84, p=0.014). Our results showed a positive correlation between beads removed from the dung and those removed from the surface of the soil (r = 0.72, DF 13, t=3.45, p=0.004; Figure 3b). No significant correlations were found between seed germination and other ecological functions for *C. lunaris* (Figure 3b).

Discussion

Our results confirm that both species identity, biomass, the abundance of individuals in dung pats (i.e. density) and their interactions may be pivotal factors for high provisioning of ecological functions, which also depends on the functions being investigated. Even though the total biomass in the treatments between the two species was comparable, the results in terms of ecosystem functioning is strongly different: higher efficiency of *C. lunaris* species in comparison with *O. illyricus*. This is in accordance with previous studies that have shown that, even at the same total biomass, larger beetles are more effective than smaller ones (Kaartinen et al. 2013; Nervo et al. 2014; Piccini et al. 2017). Moreover, based on the results from previous works on dung removal by monospecific (Tixier et al. 2015) and mixed (Yamada et al., 2007) assemblages of dung beetle species at high densities (Giller and Doube 1989), we hypothesized that both beetle abundance, biomass and species identity would have a positive effect on dung removal across treatments. Our results showed that the two species have completely different effects on the ecological functions that were investigated in this study and how these functions correlate with one another within each species (Figure 3). In fact, the dung removal pattern across densities was consistent with previous findings for *Copris lunaris,* but not for *Onthophagus illyricus*, for which the increase of density corresponds to a slight increase of dung remained on the surface. For both species, increasing density did not enhance the number of stems, but *C. lunaris* species facilitated seed germination compared to dung pats without beetles. On the other hand, the amount of bead transport depended on bead position (on the surface beneath dung pats or inside dung) and species present in the dung pat; only *C. lunaris* transported beads and in this case density also affected transport (Figure 2).

Dung removal and seed dispersal - the importance of combined species identity, biomass/abundance

Copris lunaris was the species most effective in all the ecological functions investigated, whereas *Onthophagus illyricus* was not as efficient. ~~Beyond this, we found that the abundance of *C. lunaris* in dung pats plays a crucial role in dung system functioning, specifically in dung and bead removal.~~

The higher abundance of large species increases dung removal efficiency and the amount of seed dispersal (Feer 1999; Yamada et al., 2007). High densities of *C. lunaris* (up to 8 beetles per dung pat), equal to 1,76 g of total biomass, did not obstruct dung transport to the soil, i.e. the more beetles that were present, the more dung was transported. The high dung removal efficiency of this species might be related to its particular nesting behavior. Indeed, *C. lunaris* constructs a wide nest with a large amount of dung allocated in several (up to 7) brood balls (Figure A5 in Appendix). The high efficiency in dung and seed removal of nocturnal large-bodied tunnelers, as *C. lunaris*, has been demonstrated by Slade et al. (2007). Specifically, they found that *Catharsius dayacus* was probably responsible for the high levels of dung and seed removal.

Conversely, when the density of *O. illyricus* was high (50, 60 and 80 individuals per pat), the interference and/or the competition for the resource or the space was likely too high, and tended to obstruct dung removal. Instead of transporting dung into the soil to after digging tunnels, *O. illyricus* spread the dung all over the surface of the terrarium (Figure A6 in Appendix). Thus, in accordance with Giller and Doube 1989, when the intraspecific competition is high, the dung is highly manipulated but only a small amount of it can be transported to soil. Results showed that the lowest average amount of dung remaining was recorded for assemblages with 10 individuals, which might be the optimal *O. illyricus* abundance in dung pats. Similarly, Tixier et al. (2015) found that assemblages with 8 individuals of *Onthophagus vacca* were more efficient in removing dung than those with 12 beetles. Most of the beads transported from the dung by *O. illyricus* were found on the surface. This finding may suggest that, some large beetle species may actively transport beads in brood balls, while small tunneler species try to avoid bead transportation into the soil. Indeed, dung beetles use dung for feeding and laying eggs, hence they often exclude seeds from the dung that they bury (Slade et al. 2007).

Seed germination - species identity matters

The presence of dung pats obstructs germination and, in fact, controls without dung (Csur and C5cm) showed significantly higher number of stems than all the other treatments or controls with dung. However, when taking into account the effect of species identity, ~~we found that~~ assemblages with *C. lunaris* facilitated short term seed germination compared to Cntr controls (with only dung) independently from individual density (i.e. the number of stems across the dung pats did not change according to the number of individuals per pat). In the assemblages with *C. lunaris*, the low amount of dung remaining on the surface did not obstruct seed germination.

In their natural environment, the percentage of germinated seeds was negatively correlated with burial depth (Andresen and Levey 2004), and buried seeds were less susceptible to predation and more likely to germinate than ones left on the ground (Pérez-Ramos et al. 2013). More specifically, seed germination was proven to be greater in the first few centimeters of soil depth (up to 5cm) than on the surface or at greater depths (Shepherd and Chapman 1998; Koike et al. 2012). Thus, to understand how bead removal from the surface may influence graminoid seed germination, we investigated to which layers beads placed below the dung pats were transported (i.e. bead dispersal in the soil). Beads were transported by *C. lunaris* from the surface to the first layer of soil (5 cm depth), where we proved that germination of *L. multiflorum* was still possible (Figure 1b). Greater burial depth reduced the probability of *L. multiflorum* seedling emergence (Piccini pers. obs., Andresen and Levey 2004). As a caveat, we recognize that graminoid seeds do not usually have a spherical shape like our beads, rather they have an elongated shape that might better facilitate soil penetration. Consequently, it is possible that our transport evaluation might be an underestimation of seed dispersal.

Conversely, we showed that *O. illyricus* did not facilitate seed germination compared to controls (Cntr). This might be related to high manipulation of dung that may have inhibit seed germination. This experiment showed that all assemblages with *O. illyricus* did not move most of the beads placed on the surface, thus germination would not be affected by seed transportation.

In conclusion, species identity, but not density and total biomass in the terrarium, is a decisive factor that affects the number of seeds that successfully germinated in the short term (4 days). Furthermore, as opposed to that observed for *C. lunaris*, *O. illyricus* does not bury beads placed on the surface and thus it might not prevent seed predation in a natural environment.

Correlation among functions

Species identity greatly influence the provisioning and the relationships among ecological functions (Gagic et al. 2015; Slade et al. 2017). Different correlations between functions were found when we investigated the two species. This might be related to differences in nesting behavior of these species, as *C. lunaris* constructs wide nests filled by several brood balls, whereas *O. illyricus* digs galleries and lays smaller brood balls directly into them.

Andresen and Levey (2004) found that the probability that dung beetles buried seeds was higher when surrounded by larger amounts of dung, providing a case for a relationship between dung removal and seed dispersal. Similarly, our study suggests a strong evidence for an interconnection between dung and bead removal for *C. lunaris.* In other words, higher bead removal from the surface of the soil and from inside the dung corresponds to higher dung removal efficiency. Due to its nesting behavior, *C. lunaris* removed high amounts of dung, transporting more beads in this process. In the last two layers of the soil, beads were found inside brood balls, meaning that this species transport beads actively trough soil layers. Conversely, we did not detect the same pattern for *O. illyricus,* but an increase in the amount of dung remaining on the surface corresponds to an increase in seed germination. Indeed, through the spreading of dung over the ground surface, *O. illyricus* may facilitate stem penetration of dung pats that are no longer compact and defined (Figure A6 in Appendix A). Therefore, we conclude that the relationship between different ecological functions depends on the species investigated. This is in accord with previous studies where differences in the percentage of seeds buried were likely due to differences in dung beetle communities: a low percentage of seed removal (around 12%) was found in those assemblages where only a few species were present and dominated by small-sized species (Andresen 1999; Estrada and Coates-Estrada 1991; Slade et al. 2007).

Conclusions

Dung beetles are threatened by environmental factors such as unsustainable pastoral practises, changes in land management strategies, and implementation of veterinary drugs in the cattle industry (Negro et al. 2011; Tocco et al. 2012; Tocco et al. 2013). Twenty-one dung beetle species are threatened in the Mediterranean area (IUCN 2016), 76% of which belong to the tunneler functional group and 61% of these tunnelers are also large-bodied. Braga et al. (2013) suggested that the natural ecosystem functioning may reflect a balance between beetle abundance and presence of species with specific attributes (body size, nesting strategy, etc.). Our results re-inforce the idea that optimal provisioning of ecological functions by dung beetles depends on species identity and individual abundance in dung pats. Indeed, even though the total biomass was comparable between treatments of the two different species, *C. lunaris* was found more efficient in provisioning of ecological functions than *O. illyricus*.

A decline of insect abundance in several habitats have been recently recorded (Geslin et al. 2016; Hallmann et al. 2016) but on the other side abundance have been found as one of the main factors to maintain ecosystem functioning (Winfree et al. 2015; Gaston et al. 2018). Indeed, an increase in beetle relative abundance in dung pats might bring unexpected results in terms of ecological functions provided, as was the case for the dung removal at high densities of *O. illyricus*. On the other side, even at higher abundance, *O. illyricus* was not likewise efficient. Thus, the loss of large beetles, that are more prone to extinction than small ones (Larsen et al. 2005), cannot be compensated in terms of ecosystem functioning by a higher abundance of small common species.

Here we evaluated the effect of abundance and biomass in provisioning of ecological functions in monospecific mesocosms. Our study supports the idea that some species (usually the large ones) have a strong functional role in ecosystems and they are threatened throughout Europe (Larsen et al. 2008). Small species are generally more abundant than large ones but they seem to have a weaker functional role.

Further investigation should compare results obtained by monospecific experiments with mixed assemblages, to identify possible competition that reduces the efficiency of dung removal or the synergy effect that could enhance the studied functions.

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Contribution of authors

Experiment design: IP, EC, AR, CP; Data collection: IP; Statistical analysis: IP, EC; Supervision: AR, CP; Writing: IP, EC, AR.

STATEMENTS

- The authors declare that they have no conflict of interest.
- **HUMAN AND ANIMAL RIGHTS:** All applicable institutional and/or national guidelines for the care and use of animals were followed.

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Figure 1: Boxplots of the variation of the number of stems derived from germinated seeds. Letters above boxes identify significant as revealed by Tukey post-hoc analyses of linear models (for significance see Table A1 in Appendix). Controls with dung and without beetles were indicated by "Cntr"; treatments with O. illyricus by "O_x*" and those with C. lunaris by "*Co_x*", where x is the number of individuals per treatment.*

Figure 2: Relationship between dung beetle biomass and dung removal (A), bead removal from the soil surface (B) and bead removal from the dung (C) for two different dung beetle species: C. lunaris (light grey) and O. illyricus (dark grey). Lines represent best fit models and shadows 95% CI.

Figure 3: Pearson's correlation coefficients among different ecosystem functions: dung removed, beads removed from dung (BRD), bead removed from soil surface (BRS) and seed germination (Stems).

Figure 1

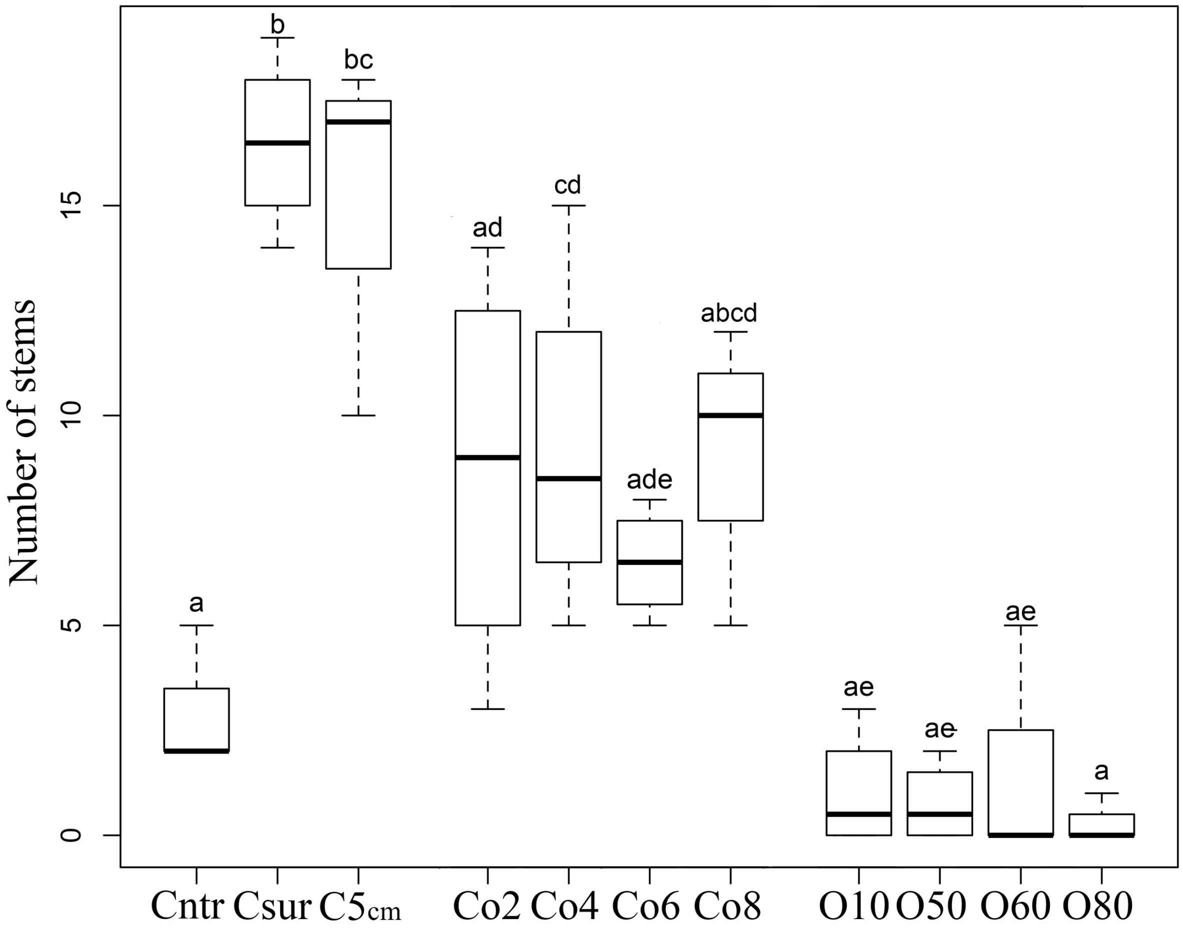

Number of stems
0
5
10
15
b
bc
cd
ad
abcd
ade
a
ae
ae
ae
a
Cntr
Csur
C5cm
Co2
Co4
Co6
Co8
O10
O50
O60
O80

Figure 2

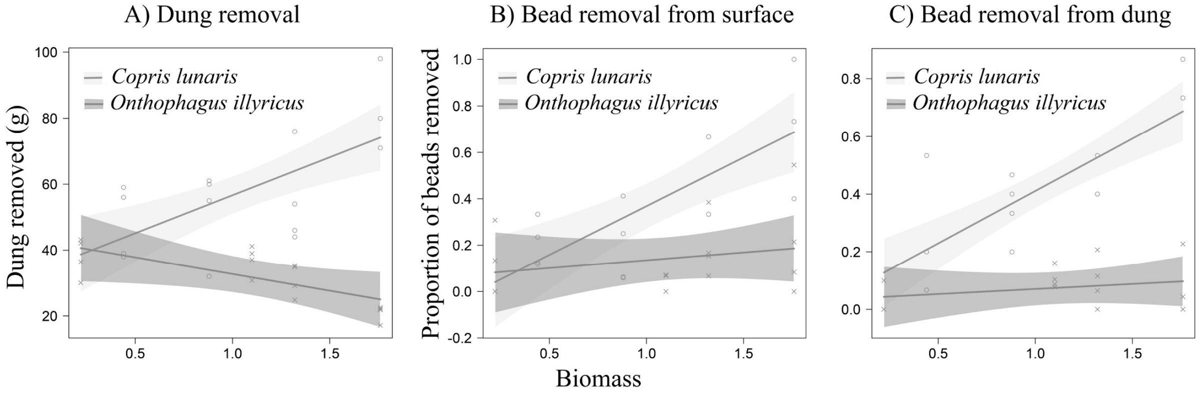
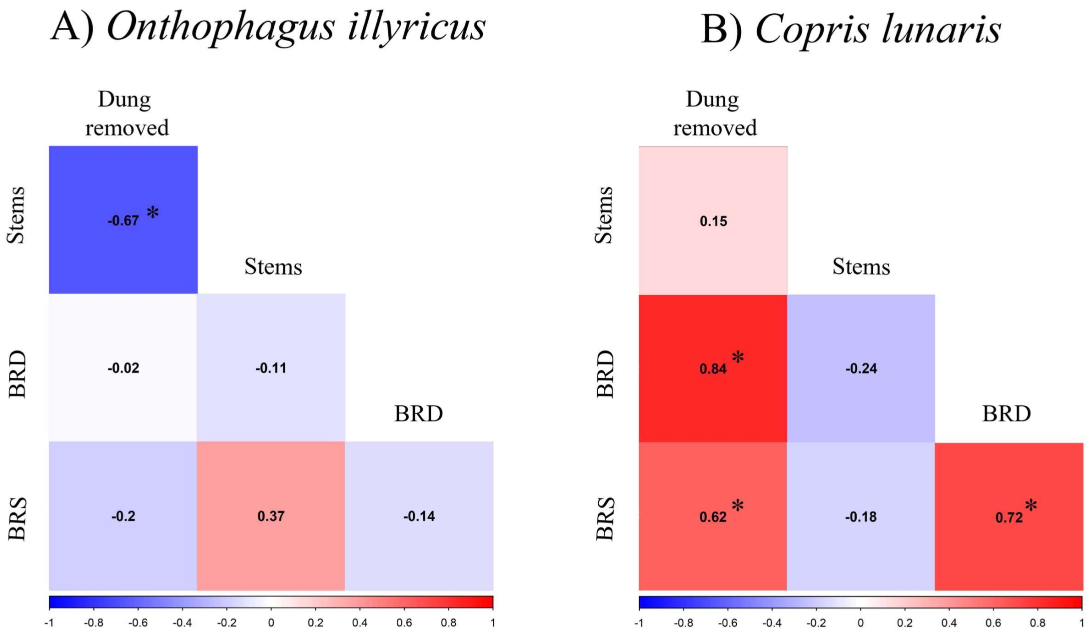

A) Dung removal
B) Bead removal from surface
C) Bead removal from dung
Dung removed (g)
Proportion of beads removed
Biomass
Copris lunaris
Onthophagus illyricus
100
80
60
40
20
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0.5
1.0
1.5

Figure 3


A) *Onthophagus illyricus*

B) *Copris lunaris*