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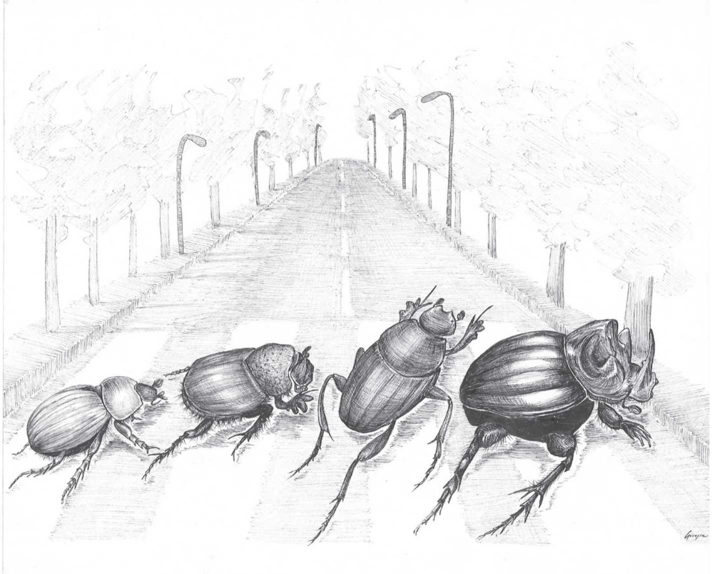

PhD in Biology and Applied Biotechnologies

Dung beetles as drivers of ecosystem multifunctionality

Provisioning of ecological functions in relation to body traits, assemblage features and environmental variables

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Abstract

Rapid biodiversity loss has emphasized the need to understand how biodiversity may affect ecosystem functioning. Instead of focusing on taxonomic identity of organisms, recent research has focused on species' traits, thereby aiming to both simplify and improve predictions and to provide a mechanistic understanding of multiple ecosystem processes. In my thesis, I investigated whether and how species traits (i.e. body mass and nesting strategy), assemblage features (i.e. species richness, abundance and species compositions) and environmental variables (e.g. habitat and temperature) affect the provisioning of ecological functions. In doing this, I focused this research on a functionally important group of insects: dung beetles (Coleoptera: Scarabaeoidea), which play a substantial ecological role in providing multiple ecological functions at the same time.

In a field experiment in Sweden, I compared the ecological role of threatened versus non-threatened dung beetle species. Learning what specific role these threatened species play will be important to understand the future repercussions of dung beetle decline in terms of ecosystem functioning. Through a trait-based approach, I identified which traits – in particular body mass and nesting strategy – make dung beetle species sensitive to environmental perturbations (so called *response traits*) and which traits make species efficient in providing multiple ecological functions (so called *effect traits*). I found a link between effect and response traits. Such a linkage may, in the future, aggravate the consequences of species decline on functioning. In fact, nesting strategy was proven to be both a response and an effect trait. Specifically, tunneler species, which dig galleries beneath dung pats to construct the nest, were the most efficient in ecosystem functioning. At the same time, they proved to be the most prone to extinction.

In two laboratory experiments in Italy, I analysed whether traits (i.e. body mass and nesting strategy) and assemblage features (i.e. density, assemblage and species composition) make dung beetles and/or assemblages efficient in providing multiple ecological functions at the same time. Specifically, I investigated how species identity and assemblage composition (monospecific vs multi-species assemblages) may affect dung removal and greenhouse gas (GHG) emissions from dung pats. I found that different species have an uneven effect on ecosystem functioning, and that multi-species assemblages were proven to be the most effective in reducing CO_2-equivalent emissions. In the second laboratory experiment, I investigated the effect of abundance in the dung pats (i.e. density) and body size (i.e. small-bodied *Onthophagus illyricus* vs large-bodied *Copris lunaris*) on dung removal, seed germination and seed dispersal. I found that densities of the two species had different effect on ecosystem functioning: an increase in the density of the large-bodied species increased efficiency in removing dung, whereas an increase in the density of the small-bodied species decreased efficiency. Moreover, the small species had a limited effect on seed germination and dispersal but, on the other hand, the large species increased the number of germinated seeds and, by increasing its density, the large species enhanced seed dispersal.

In a field experiment conducted in Sweden, I investigated the effect of farming practices (organic vs. conventional) and environmental factors (i.e. macrohabitat, microhabitat and microclimate) on the dung beetle communities colonizing dung pats and the associated ecological functions (i.e. dung removal). These findings confirm that factors other than the farming system may be crucial in shaping agrobiodiversity. Specifically, decisions made by farmers can shape the composition of dung beetle communities and associated ecological functions.

Overall, these findings confirm that optimal ecosystem functioning is context-dependent and that it relies on multiple features of both dung beetle species (such as body mass, nesting strategy, species identity) and assemblages (such as density and assemblage composition), as well as on the ecological functions investigated. Thus, I suggest an approach based on the analysis of multiple functions to better understand, manage and optimize ecosystem functioning. On the other hand, local, farm- and site-specific variations in environmental conditions are important factors influencing dung beetle communities for ecosystem functioning. From an applied point of view, local environmental variables managed by farmers on their own property can shape the local dung beetle community and thus ecosystem functioning.

Keywords: assemblage composition; body size; density; ecosystem functioning; environment variables; farming system; species identity.

1. Introduction

Irene Piccini

1.1 Ecosystem functioning and biodiversity

Ecology can be defined as "*the study of the relationships between structure and function in nature*" (Odum 1962). Indeed, the features of biological communities (e.g. species, biomass...) distribution of resources (e.g. nutrients, water…) and abiotic conditions (e.g temperature, light…) shape matter and energy flows (Odum 1968) and, thus, ecosystem functioning.

Ecological functions refer to the habitat or biological processes of ecosystems. Benefits for humans derived directly or indirectly from these functions are called ecosystem goods (e.g. food) and services (e.g. waste assimilation; Daily 1997; de Groot et al. 2002). For simplicity, the latter are jointly referred to as ecosystem services. Ecological functions are associated with ecosystem services. For example, through their movements between flowers, various insect taxa contribute greatly to pollination (Free 1993). The understanding of trophic dynamics would be useful for enhance biological control (Duffy et al. 2007; Obrycki et al. 2009). Storage, consume, transfer and processing of nutrients contribute to nutrient cycling in freshwater (Vanni 2002) or in terrestrial ecosystems (Lavelle et al. 2006; Metcalfe et al. 2013). Dung removal reduces nutrient waste (Losey and Vaughan 2006; Beynon et al. 2015).

Ecosystem services have often been linked to biodiversity (Cardinale et al. 2012; Mace et al. 2012). The term biodiversity identifies the variability among living organisms (terrestrial, marine, other aquatic organisms and ecological systems of which they are part; Convention on Biological Diversity 1992) and includes the diversity within species (e.g. genetic variability) and between species at several levels (taxa, population, ecosystems, landscape...). Several components of biodiversity may contribute to ecosystem processes, such as the number of species present (species richness), their relative abundance, species composition, biotic interactions and spatiotemporal variation in all these factors (Chapin III et al. 2000). Thus, changes of these biodiversity components may also affect the functioning of ecosystems. Specifically, the abundance of dominant species has been found to be a major driver for crop pollination in several systems (watermelon, blueberry and cranberry systems; Winfree et al. 2015). Likewise, both the abundance and richness of below-ground diversity has been found to enhance functioning in grasslands (Wagg et al. 2014), and the species richness of plants has been linked to ecosystem productivity (Grace et al. 2016). Moreover, spatial-temporal species dynamics and species interaction affect functioning (Valiente-Banuet et al. 2015) in forests (Forrester 2014) and in Artic regions (Schmidt et al. 2016).

At present, biodiversity is changing due to species decline (Didham et al. 2007), extinctions (both locally and globally; Sepkoski 2016; Goncalez et al. 2016), changes in composition (Allan et al. 2015) and community structure (Galiana et al. 2014).

Many of these changes are caused by anthropogenic environmental change (Wood et al. 2000). One of the main concerns in biodiversity conservation specifically relates to species loss. Indeed, even if extinction is a natural process, during the last few decades it is occurring at unnaturally rapid rates (Chapin III et al. 2000; Pimm et al. 2014; Gonzalez et al. 2016). This current species decline rate, higher than would be expected from the fossil records, suggest that a “sixth mass extinction” may be under way (Barnosky et al. 2011)[1].

Terrestrial and aquatic ecosystems have changed by direct or indirect human presence and activities, which influence land-use and habitat composition, altering climate and biogeochemical cycles. Several factors influence terrestrial biodiversity change, specifically the major ones are: land-use changes (including habitat loss, degradation and fragmentation), climate change, nitrogen deposition and alien species habitat-inclusion (Aguilar et al. 2006; Bellard et al. 2012; Newbold et al. 2016). The causes of the decline in species richness vary among taxa and biomes. For instance, the tropics seems more affected by land use changes (specifically habitat loss), whereas in the boreal and Northern temperate forests species invasions had the largest effect on species richness decline (Murphy and Romanuk 2014).

Changes in biodiversity have been proven to be the major factors affecting ecosystem functioning, sustainability and stability (Purvis and Hector 2000; Naeem et al. 2012; Tilman et al. 2014). A meta-analysis of published data has revealed that species loss is one of the main drivers of alteration of ecosystem processes, comparable in magnitude to other global change stressors (Hooper et al. 2012). Moreover, the stability of ecosystems is strongly related to biodiversity, even more than to other anthropogenetic environment drivers, such as nitrogen enrichment, carbon dioxide over-presence, fire, herbivory and water amount (Hautier et al. 2015).

Resilience of ecosystem functioning is determinate by multiple factors at different scales, such as individual phenotypic plasticity, by species sensitivity to environment changes, by community compositions, by functional redundancy and by network structure (Hulvey and Zavaleta 2011; Oliver et al. 2015). Therefore, a decline in species diversity might correspond to a decline in functioning, too. Indeed, a decline of species that may facilitate or complement the functionality of other species affect ecosystem resilience (Zavaleta and Hulvey 2004) and a loss of species diversity might implicate the loss of functional traits that drive ecosystem functioning. Recently, it has been demonstrated that ecosystem functioning is

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[1] Palaeontologists characterize mass extinctions as times when the Earth loses more than three-quarters of its species in a geologically short interval, as has happened only five times in the past 540 million years or so (Novacek et al. 2001, Jablonski 1994).

better predicted by trait composition than by the number or abundance of species per se (Gagic et al. 2015).

Invertebrates account for most of biodiversity on Earth. Insects dominate virtually every ecosystem in terms of species richness, animal biomass, and provisioning of pivotal ecological functions (Weisser and Siemann 2008; Stork 2017). To understand ecosystem functioning, research on invertebrates deserves priority. Some taxa have already been proposed as bioindicators (Gerlach et al. 2013). Many invertebrate groups have been targeted by studies because of their abundance, habitat specialization, response to small-scale habitat heterogeneity and importance in ecosystem function (e.g. Andersen and Mayer 2004). Dung beetles (Coleoptera: Scarabaeoidea) have been repeatedly proposed as invertebrate focal group for research and conservation (Davis et al. 2004; Spector 2006; Nichols and Gardner 2011). Due to their ease of sampling (Larsen and Forsyth 2005), a relatively well-resolved taxonomy, broad geographic distribution (Hanski and Cambefort 2014) and ecological and economic importance (Losey and Vaughan 2006; Nichols et al. 2008; Beynon et al. 2015), dung beetles have been broadly recognized as a focal group for research (Favila and Halffer 1997; Spector 2006). Here, the main interest regards the ecological role of dung beetles in ecosystems. Loss of dung beetle species or changes in beetle community structure due to environmental perturbations can have crucial effects on ecosystem functioning (Larsen et al. 2005; Slade et al. 2011; Beynon et al. 2012).

1.2 Dung beetles

Dung beetles are a globally distributed insect group, with their highest diversity in tropical forests and savannas (Hanski and Cambefort 2014). Their distribution depends on *inter alia* water availability, temperature, dung resource availability (Finn et al. 1999; Hernández et al. 2009), altitude (Escobar et al. 2005) and soil texture (Beiroz et al. 2016). Indeed, soil structure and consistency can directly affect the selection of reproductive site (Vessby and Wiktelius 2003) and the reproductive success of dung beetle species (Lumaret and Kirk 1987; Sowig 1995).

Most present-day dung beetles are coprophagous, but their ancestral food might likely have been detritus (Philips 2011). Adults feed on small particles (2-130 μm; Holter 2000; Holter and Scholtz 2007) in the microorganism-rich liquid component of mammalian dung. The more fibrous material is used to brood their larvae (Halffter and Matthews 1966; Halffter and Edmonds 1982). Considering that nearly anoxic conditions (1-2% O_2 and up to 20% of CO_2) can be found in the fresh cattle dung, dung beetles have an exceptional ability to extract oxygen at low concentrations (Holter and Spangenberg 1997).

Most temperate dung beetles have one generation per year and hibernation may occur at the egg, larval, pupal or adult stages (Hanski and Cambefort 2014) in the soil.

Dung beetles are not alone in the droppings, but part of a complex community comprising other Coleoptera including Hydrophiloidea, Staphylinoidea (comprising also Ptilidae and Silphidae), and Histeroidea. They also share the droppings with eggs of flies in the order of Diptera, and several microorganisms, such as mites, nematodes and an enormous number of bacteria and archaea (Hammer et al. 2016; Slade et al. 2016b). Among other dung-inhabiting beetles, adult Hydrophilidae are also coprophagous. Ptiliidae feed on decaying vegetable material and on fungi. Some other beetle species are predators, such as those belonging to Carabidae, Staphylinidae and Histeridae families (Roslin et al. 2014).

Dung beetles can be roughly classified into three main functional groups based on their breeding strategies (e.g Hanski and Cambefort 2014): tunneler species (paracoprids) dig tunnels beneath the dung pat where they bury brood balls; dweller species (endocoprids) lay eggs inside the pat; roller species (telecoprids) transport dung balls some distance away from the dung pat before burying them into the soil (Fig.1). Some dung beetle species use food resources buried by others, and are thus called kleptoparasites (coined by Paulian 1943).

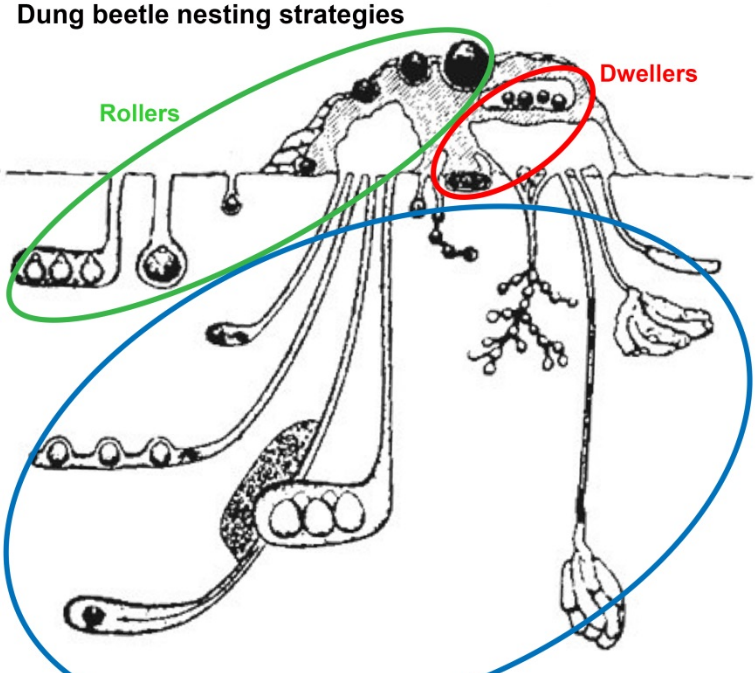

Figure 1: **Dung beetle nesting strategies**. The roller strategy is identified by a green circle; the tunneler strategy by a blue circle; the dweller strategies by a red one. Figure modified from Doube 1990.

Among invertebrates, many dung beetles have recently declined. In the Mediterranean area, 21 out of 150 endemic dung beetle species are threatened (IUCN 2016) and 25 out of 61 dung beetle species are threatened in the Nordic countries (Sweden, Finland, Norway and Denmark; Roslin et al. 2014), respectively 14% and 41%. In Italy, roller species have declined and six species may face a significant probability of extinction (Carpaneto *et al*. 2007). The situation is even more alarming in Sweden, where overall 12 species are already considered locally extinct (Roslin et al. 2014). Overall, large species tend to be more sensitive to environment changes than small ones (McKinney 1997; Pimm et al. 1988), and the same applies to dung beetles (Larsen et al. 2005; Larsen et al. 2008; Roslin et al. 2014). Indeed, in the Mediterranean area, 76% of threatened species belong to the tunnelers and 61% of these threatened tunnelers are also large-bodied.

Overall dung beetle decline is largely due to human-induced habitat alterations, such as, habitat loss (including habitat fragmentation and pastoral practise changes), large-bodied vertebrate hunting, climate change and anthelmintic drug usage. Below, these factors will be examined in turn.

Beetle loss is strongly linked to **forest modification and fragmentation** (Roslin and Koivunen 2001; Nichols et al. 2007), especially in tropical areas (Halffter and Arellano 2002; Arellano et al. 2005; Scheffler 2005). This sensitivity of tropical dung beetle species to habitat alteration might be related to their high specialization (Roslin and Viljanen 2011). Indeed, tropical beetles are highly specialized to particular habitat types. Within forested regions, only few species can typically be caught in open habitats (Klein 1989; Estrada et al. 1998; Quintero & Roslin 2005), whereas primary and secondary forests have been found to support a high diversity of dung beetles (Escobar 2004; Avendaño-Mendoza et al. 2005; Kanda et al. 2005; Gardner et al. 2008). In the long run (i.e. 15 years), dung beetles may recolonize human-disturbed forests, likely due to the regrowth of secondary vegetation between forest fragments. Thus, the preservation of forest fragments and secondary vegetation may provide a key factor to mitigate beetle decline in forest habitats (Quintero and Roslin 2005). On the other hand, dung beetles themselves can contribute to forest regeneration due to their secondary dispersal of seeds and their promotion of seed germination (Shepherd and Chapman 1998; Andresen 2002; Estrada and Coates-Estrada 2002; Andresen and Levey 2004; Andresen and Feer 2005; Audino et al. 2014). In the Italian Alps, it has been found that the structure and distribution of dung beetle communities has changed as a result of increasing pastoral abandonment (Macagno and Palestrini 2009; Negro et al. 2011; Tocco et al. 2013a, 2013b).

The **decline of large-bodied vertebrates** (Peres 2000; Corlett 2007) might disrupt the diversity and abundance of dung beetles in tropical areas (Andresen and Laurance 2007; Nichols et al. 2009). Specifically, larger-bodied dung beetle species may suffer from a reduction of dung pats both in terms of pat size and number/abundance. Conversely, small-bodied beetles are likely less affected by hunting, because they need smaller and more ephemeral resources, and may utilize the droppings of small-bodied vertebrates which are not the targets of hunters (Nichols and Gardner 2011).

Climate change may result in changes in the distribution, habitat associations and biotic interactions of species (e.g. Pearson and Dawson 2003 and Kelly and Goulden 2008). Mountain regions are suitable areas to investigate whether species distribution may change. Dung beetle species have changed their elevation ranges in both the SW Alps (France) and the Sierra Nevada (Spain). The pattern of an expansion of the upper range limit and a contraction of the lower limit suggests that climate change has likely contributed to the changed ranges (Menéndez et al. 2014).

Anthelmintic drugs are substances used to control nematodes that affect livestock. The most common one is invermectin (Campbell et al. 1984). The effect of these drugs on dung beetles is well-documented. The main effects are the loss of the sensorial and mechanical activity (Verdù et al. 2015), disruption of reproductive physiology (Martínez et al. 2017), disruption of diversity (Tonelli et al. 2017; Verdù et al. 2018) and mortality for both larval stages and adults (Wardhaugh and Rodriguez-Menendez 1988; Krüger and Scholtz 1997; Iwasa et al. 2007; Jacobs and Scholtz 2015).

1.3 Dung beetles as ecosystem engineers

Due to their role in shaping habitats by altering system-level flows, availability, or quality of nutrients, food, and physical resources (e.g. living space), dung beetles can be considered ecosystem engineers. Throughout their dung feeding and nesting activity, within ecosystems across the world, dung beetles have a pivotal role in several key processes such as dung removal, nutrient cycling, bioturbation, secondary seed dispersal and parasite suppression (Nichols et al. 2008). Biotic interactions involving dung beetles have also been suggested to affect greenhouse gas (GHG) emissions (Pentillä et al. 2013; Iwasa et al. 2015; Slade et al. 2016a; Piccini et al. 2017). Moreover, dung beetles modify the composition of soil microorganisms (Slade et al. 2016b) and the availability of nutrients (Yamada *et al*. 2007) with significant impacts on plant biomass (Lastro 2006; Nervo et al. 2017) and protein content (Bang et al. 2005).

Many of these ecological functions affect human benefits, through effects on soil fertilization, biological pest control, enhanced vegetation growth, etc. (Nichols *et al*. 2008). Thus, they are ecosystem services. Few studies estimated the economic incomes derived by the provisioning of ecosystem services by dung beetles in natural pastures (but see Losey and Vaughan 2006; Beynon et al. 2015).

Measures of dung beetle-mediated ecological functioning are often focused on dung removal rate (Beynon et al. 2012; Kaartinen et al. 2013), whereas other functions and services have received less attention (Manning et al. 2016). Different species have different roles depending on the ecological function investigated (Slade et al. 2017). For example, *Copris lunaris* was found to be highly effective in terms of dung removal, yet it increased methane emissions from dung (Piccini et al. 2017). For this reason, several recent studies have focused on evaluating the ecological role of dung beetles for several ecological functions at the same time – a so called multifunctionality approach (Manning et al. 2016; Nervo et al. 2017; Piccini et al. 2018). Moreover, for ecosystem functioning it is also important how functions are evaluated (Slade et al. 2017) and the type of enclosures used (Lähteenmäki et al. 2015).

To investigate the multifunctionality driven by dung beetles in relation to body traits, assemblage features and environment variables, I evaluated several functions in my thesis work. Below, these functions are presented in turn.

DUNG REMOVAL – Considering that the livestock avoids grazing in areas that soiled by dung (Andereson et al. 1984), removing dung from pastures is essential for continued livestock grazing. Indeed, of the 33 millions of tons of dung produced per year by Australian livestock (Bornemissza, 1960; Bornemissza, 1976), the introduction of exotic dung beetles at a Camberra site reduced on average 78% of dung on the ground (Hughes 1975). Thus, dung beetles have a relevant role in decomposing dung both at a small local scale (Rosenlew and Roslin 2008) and at a large national scale (Kaartinen et al. 2013).

DUNG-SOIL NUTRIENT TRANSFER – Vertebrate excreta contain a high quantity of nutrients not-assimilated by vertebrates (Steinfeld et al. 2006). The fraction of such nutrients that can return to the cycle in the soil depends greatly on dung beetle activities. Beetles facilitate nutrient transfer from the dung to the soil (Kazuhira et al. 1991; Yamada et al. 2007; Nervo et al. 2017). By altering the microorganism fauna of dung and soil, dung beetles accelerate mineralization rates (Kazuhira et al. 1991). Indeed, beetle activity increases aerobic conditions in dung and C and N amount in the upper soil layers and consequently enhanced bacterial growth, including ammonifier bacteria responsible for continued N-mineralization (Yokoyama and Kai 1993; Nervo et al. 2017). Apart from soil contents of carbon and nitrogen, dung beetles were also found to increase phosphorus and potassium levels (Yamada et al. 2007).

GREENHOUSE GAS (GHG) EMISSIONS FROM DUNG PATS – Grazing animals release high amounts of nitrogen and carbon in pastures, where nutrients generally exceed the immediate demand of plants. This creates a large loss of GHGs that steadily leaks into the atmosphere (Flessa et al. 1996; Dubeux et al. 2007). Thus, dung is a relevant source for atmospheric GHGs such as nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2) (Petersen et al. 1998; Saggar et al. 2004; Lin et al. 2009; Cai et al. 2013). Dung beetles influence the GHG emissions thourgh e.g., aerating the dung and the soil, reducing organic matter, relocating dung, and altering microorganism presence (Holter 1979; Bang et al. 2005; Slade et al. 2016b). GHG emissions are, in fact, primarily and directly dependent on microbiological processes: CO_2 originates from the decomposition of organic material by micro-organisms, CH_4 from the methanogenic archaea thriving in anoxic conditions and N_2O as well from microbial nitrification, denitrification and nitrifier denitrification, i.e. nitrite reduction by ammonia oxidizers (Bunnell et al. 1977; Oremland 1988; Firestone and Davidson 1989; Davidson 1991; Moss et al. 2000; Kool et al. 2010).

SECONDARY SEED DISPERSAL – Some plants use vertebrates as their primary agents of seed dispersal. Seed dispersal can happen through external transport in the animal fur or by the digestion and the dropping of seeds in the dung (Janzen 1984). However, the risk that seeds do not establish in soil suitable for seedling emergence is still high, and predators (e.g. rodents) and pathogens may also prevent germination (Hulme 1994; Chambers and MacMahon 1994; Hulme 2002). Therefore, secondary seed dispersal by dung beetles might play an important role in seedling establishment. Seed relocation both horizontally and vertically along the soil depth by rollers and tunnelers increases seed survival (Estrada and Coates-Estrada 1991; Chambers and MacMahon 1994; Shepherd and Chapman 1998; Andresen 1999; Feer 1999; Andresen and Levey 2004; Amézquita and Favila 2010). Indeed, seeds placed in the dung and transported directly from dung to soil are less prone to be predated (Perez-Ramos et al. 2013) and are likely placed in organic-rich soils favorable for seedling establish. The probability and the burial depth depend on the seed size (Estrada and Coates-Estrada 1991; Feer 1991; Andresen 2002; Slade et al. 2007), on the community composition of dung beetles (Vulinec 2002), on the amount of dung (Andresen 2001) and on dung type (Ponce-Santizo et al. 2006).

SEED GERMINATION – Even after secondary seed dispersal, seed might not germinate because of the unfavorable soil conditions or unfavourable burial depth. Thus, it is ecologically important to understand if, throughout beetle transport to soil, seeds are more prone to germinate. The percentage of germinated seeds is negatively correlated with burial depth (Andresen and Levey 2004), but buried seeds are more prone to germinate than seeds left on the ground (Perez-Ramos et al. 2013). More specifically, seed germination was found to be greater in the first few centimeters of soil depth (up to 5cm) than at the surface or at greater depths (Shepherd and Chapman 1998; Koike et al. 2012). Few studies have investigated the influence of dung beetles on seed dispersal and germination in temperate forests (but see studies from Japan, Koike et al. 2012, and Spain, Perez-Ramos et al. 2012). Most of the studies of seed dispersal and germination have been conducted in tropical areas: Mexico (Estrada and Coates-Estrada, 1991), Peru (Andresen, 1999), Brazil (Vulinec 2002; Andresen 2002; Andresen and Levey 2004), French Guiana (Feer, 1999), Uganda (Shepherd and Chapman, 1998) and Malaysian Borneo (Slade et al. 2007).

1.3.1 Factors affecting ecological provisioning

The effcieincy of dung beetle species in providing ecological functions might depend on species traits, such as nesting strategy and body mass (Slade et al. 2007; Nervo et al. 2014; Tixier et al. 2015).

Indeed, the **functional group** of tunnelers was found to be more efficient in removing dung than dwellers and rollers in the short term (Andresen 1999; Slade et al. 2007; Rosenlew and Roslin 2008; Kaartinen et al. 2013). Although dwellers are less efficient over a short-term period (e.g. Stevenson and Dindal 1985), Nervo and colleagues (2014) suggested that their larvae may play a crucial role at a longer time scale, since they found the residual dung in tunneler and dweller treatments to be roughly equivalent after one year. Tunnelers are also more efficient than rollers (Slade et al. 2007), likely because they have to elaborate and transport spherical brood ball over the ground far away from the pat and thus cannot transport high amounts of dung.

In theory, the magnitude of dung beetle effects may be dependent on the differences in species burrowing activity or **body mass** (Holter et al. 2002; Larsen et al. 2005; Nervo et al. 2014). Body-mass is considered an ecologically relevant trait (La Barbera 1989; Doube 1990; Feer 1999; Andresen 2002; Amézquita and Favila 2010) and, accordingly, large tunnelers usually perform better than small ones in dung removal (Kaartinen et al. 2013; Nervo et al. 2014), in soil nutrient cycling (Nervo et al. 2017) and in seed dispersal (Slade et al. 2007).

Factors other than species traits may also influence functioning. The attributes of the dung beetle assemblages in terms of species richness and abundance in the dung pats may play an important role for ecosystem functioning. Multi-species assemblages have been found to be more efficient in providing ecological functions than monospecific assemblages (Beynon et al. 2012; Nervo et al. 2014; Nervo et al. 2017), even more if species belong to different functional groups (Slade et al. 2007; Gagic et al. 2015). Moreover, species-rich assemblages promote ecosystem functioning under environmental perturbation (Beynon et al. 2012; Manning et al. 2017), such as climate warming (Slade and Roslin 2016).

Dung beetle richness has been found to increase dung removal rate (Nervo et al. 2014) and soil nutrient levels (Yoshihara and Sato 2015; Nervo et al. 2017). Nonetheless, functional richness predicts dung removal and seed dispersal better than does species richness (Gagic et al. 2015; Griffiths et al. 2015). Indeed, functional-complementarity makes dung beetle assemblages more efficient in providing ecological functions, such as carbon cycle enhancement (Menéndez et al. 2016), dung removal and secondary seed dispersal (Slade et al. 2007). For example, interactions between *Geotrupes stercorarius* (Linnaeus, 1758), a large-bodied tunneler, and *Aphodius fossor* (Linnaeus, 1758), a small-bodied dweller, were found to both reduce GHG emissisons and to increase dung decomposition (Lähteenmäke et al. 2015; Slade and Roslin 2016). Moreover, the co-occurrence of species in the same dung pat provides a higher amount of ecological functions, throughout facilitation and/or compensation effect (Tscharntke et al. 2005; Slade et al. 2007).

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 functionally-unrelevant species (Winfree et al. 2015). On the other hand, the functional role of rare species is still debated. In tropical agoecosystems, natural rich-community of ants was found to contribute to higher yield than the manipulated community composed by pest-control species (Wielgoss et al. 2013). Thus, rare species and their interactions may also play an important role in ecosystem productivity and functioning. For dung beetles, a few functionally important species (such as larger-bodied species) can contribute greatly towards ecosystem functioning at higher abundances (Slade et al. 2007; Braga et al. 2013). Only few studies have addressed how dung beetle density influences their provisioning of ecological functions (Yamada et al. 2007; Tixier et al. 2015). One of the main explanatory variable for high levels of ecological functioning is dung beetle abundance (Slade et al. 2007; Braga et al. 2013), even if the efficiency of a higher number of beetles depends on species identity investigated (Tixier et al. 2015). Moreover, higher local abundance implicates higher competition for the resource, and consequently faster dung removal rate – especially in tropical areas (Peck and Forsyth 1982). However, little is known about the influence of beetle size and density on dung removal, seed dispersal and seed germination in the Mediterranean area.

Dung beetle communities and their associate provisioning of ecological functions vary with **environment variables**, such as landscape, macro-, micro-habitat conditions and with the microclimate (Lobo et al. 2006; Slade et al. 2011). Dung beetles are highly responsive to environmental heterogeneities across multiple scales and levels of ecological organization (Hanski and Cambefort 1991).

Dung beetle distribution depends on several environment variables, such as the landscape matrix (Numa et al. 2009; Numa et al. 2012), land-use change (Barragán et al. 2011; Beiroz et al. 2016), habitat fragmentation (Roslin and Koivunen 2001; Braga et al. 2013) and the intensity of grazing (Verdú et al. 2007). To understand how dung beetle assemblage structure changes, fine-scale variables might be important to be considered. Major differences in community composition have been detected across neighbouring habitat types, often over very short distances (i.e. hundreds of metres; Barbero et al. 1999; Davis et al. 2001; Spector and Ayzama 2003). Vegetation structure, soil type, moisture, leaf cover and the subsequent microclimate appear to be consistently stronger determinants of beetle communities and their subsequent ecosystem functioning (Nealis 1977; Roslin et al. 2009; Mehrabi et al. 2014).

Farming management systems have been shown to affect insect communities. Overall, organic systems tend to support higher biodiversity than conventional ones across many different taxa (Fuller et al. 2005; Caprio et al. 2015; Duru et al. 2015). Indeed, organic farms prevent the use of synthetic pesticides and herbicides, which tend to have negative effect on biodiversity (Geiger et al. 2010a). Few studies have addressed whether and how dung beetles might be affected by farming systems. Yet, two studies suggest that dung beetles are more abundant in organic farms than in conventional, intensive and rough grazing farms (Hutton and Giller 2003; Geiger et al. 2010b).

1.3.2 Consequences of dung beetle decline

Dung beetle decline may have worrying consequences for ecosystem functioning. Species are not equally affected by environmental changes (Kopecky et al. 2013; Püttker et al. 2015), but instead they differ in their sensitivity to disturbance. Species traits have been used to predict extinction risk (Davidson et al. 2009; Pearson et al. 2014) and body size is increasingly considered as one of the main response traits which expose species to local extinction in fragmented forests (Klein 1989; Larsen et al. 2005), in changing agriculture (Gardner et al. 2008) and in deforested areas (Scheffler 2005; Slade et al. 2011). This sensitivity might be related to physiological intolerance for thermal stress, to size-dependent responses to declining diversity or abundance of dung resources, or to combined effects of these factors (Nichols et al. 2009). Similarly, functional groups may also differ in their sensitivity to environment changes, with tunnelers (Piccini et al. 2018) and rollers being particularly sensitive (Carpaneto et al. 2007; Lobo et al. 2001). For their different nesting habits, functional groups are affected by different factors: rollers and tunnelers, of which larval period is spent into the soil, suffer from changes in soil texture, temperature and composition; dwellers might suffer from low dung quality and the usage of anthelmintic drugs to cattle.

The loss of large-bodied, tunneler and roller dung beetle species may have significant consequences for community structure and subsequent patterns of ecological functioning (Larsen et al. 2005; Slade et al. 2007; Batilani-Fiho and Hernandez 2017). Indeed larger-bodied *Geotrupes* species account for the 61% of overall dung removal in Finland (Kaartinen et al. 2013). In other cases, a direct link between size and efficiency has been challenged by experiments comparing multi-species dung beetle assemblages, including large tunnelers versus assemblages encompassing dwellers only (Nervo et al. 2014) and rollers only (Slade et al. 2007). In these studies, large tunnelers have been proven to be more efficient, not only in dung removal, but also in the cycling of soil nutrients (Nervo et al. 2017) and seed dispersal (Slade et al. 2007).

Large-bodied species are the most prone to extinction and also the most relevant in terms of ecological function provisioning (Larsen et al. 2005, 2008). Thus, the functional consequences of extinctions will therefore depend on the strength of the link between traits rendering species more extinction-prone (so called *response traits*) and those that determine the functional contribution of a species (so called *effect traits*; Lavorel and Garnier 2002; Naeem and Wright 2003; Hevia et al. 2017). As a consequence of the links between species response and effect traits, the effects of environment change on species compositions may translate into changes in ecosystem functioning due to changes in the distribution of trait values represented in the modified versus original communities (Chapin III et al. 2000).

For dung removal, the loss of some species can be compensated by an increase in abundance and biomass of other species (Amézquita and Favila 2010). This mechanism might be able to maintain decomposition rates and thus not change the ecosystem functioning (Rosenlew and Roslin 2008). However, more research is needed to expose the full consequences of dung beetle loss.

1.4 The species investigated

In this project, I investigated the ecological role of several dung beetle species in monospecific and/or mixed assemblages. The species investigated in this project belong to subfamily Aphodiinae (*Aphodius ater, A. erraticus, A. fimentarius, A. foetens, A. fossor, A. heamorrhoidalis, A. ictericus, A. pedellus, A. puntatosulcatus, A. pusillus, A. rufipes and A. sordidus),* Scarabaeinae subfamily (*Copris lunaris, Onthophagus coenobita, O. fracticornis, O. illyricus, O. nuchicornis and Sisyphus schaefferi)* and Geotrupinae *(Geotrupes spiniger* and *Trypocopris vernalis;* for identification feature of all these species see description in Table A1 in Appendix). Some of the species are dwellers, some tunnelers and one a roller (*S. schaefferi*), and in terms of body mass, the species range from small to large (from 0.001g to 0.22g).

For dwellers, most of which belong to the genus *Aphodius*, the entire egg, larval and pupal development (Fig. 2) typically takes place directly in the dung pats. Some species lay their eggs singly, others lay from one to four eggs in small clutches (Hanski and Cambefort 2014). Among the *Aphodius* species investigated here, *A. erraticus* is the exception because it is a tunneler. Indeed, female *A. erraticus* digs beneath the dung pat both vertical and oblique galleries, on average 3-5 cm deep (Hanski and Cambefort 2014). After the deposition of one egg at the end of the gallery, the tunnel is filled with dung (Hanski and Cambefort 2014). The other *Aphodius* species lay their eggs inside the dung mass or in the interface between dung and soil, e.g. *A. rufipes* lays its eggs in a small chamber in the interface (e.g. Klemperer 1980).

Figure 2: **Larval and pupal development of *Aphodius fossor***. Figures from left to right represents the larvae, pupae (second to fourth image) and adult of *Aphodius fossor*.

Most of the species in subfamily Scarabaeinae are tunnelers, showing male and female parental care. All species belong to subfamily Geotrupinae are large tunnelers and some of them provide parental care. In almost all tunneler species, females excavate the tunnel and prepare the chamber which will contain the brood. The size and the depth of the chamber depend on the species. Small tunneler species lay numerous brood masses in shallow galleries, whereas large tunnelers make deep nests with few brood masses. In tribes Coprini and Dichotomiini, females provide maternal care (Hanski and Camebford 2014).

Males of genus *Onthophagus* exhibit different types of horn that can arise from the front, middle or rear of the head, characterizing the species. Moreover, pre- and post-copulatory processes drive the diversification of horn growth, which may divide *Onthophagus* males in major and minor types (*Onthophagus taurus* major and minor males; e.g. Moczek 1998). *Onthophagus* species provide parental care where horned males and females cooperate to provide food. Here, male assistance increases the number and weight of broods (Hunt and Simmons 2000; Palestrini and Rolando 2001). For some species, it has already been proved that major and minor males adhere to different reproductive tactics, with minor males adopting a sneak strategy (Hanski and Cambefort 2014). Dung beetles can discriminate dung on the basis of nutritional content (Dormont et al. 2004; Dormont et al. 2007), female of *Onthophagus taurus* (close phylogenetically related to *O. illyricus* used in this project) can adaptively adjust the level of investment in response to the quality of the food resource provided in the galleries (Hunt and Simmons 2004) and in response of male parental care (Simmons and Ridsdill-Smith 2011).

In genus *Copris*, males and females cooperate in excavating a nest and supply it with dung (Halffter et al. 1996). Cooperation may have arisen in response to the need to sequester dung quickly in the face of intense intraspecific and interspecific competition for the limited resource (Simmons and Ridsdill-Smith 2011). Females of *Copris lunaris* (Fig 3a) maintain the brood balls and will repair them should they break open during the development of the larvae (Klemperer, 1982). Olfactory communication may play an important role in interactions between females and broods (Klemperer 1982). *C. lunaris* performs peculiar nesting strategy: males and females dig one wide chamber at the end of the gallery where the dung is relocated in 4-7 large pear-shaped brood balls (Fig 3b). Chambers are on average 10.6 cm wide, 5.33 cm high and 7 cm deep and the brood balls weigh an avetrage of 6-7 g dry weight each (17-18 g wet weight; unpublished data).

Figure 3: **Copris lunaris beetles and its nest with brood balls**. Picture a) shows females of the species; note the bifid horn on the head. Picture b) represents a nest with an exceptional number of 7 brood balls, photographed from the bottom of the terrarium.

In the Mediterranean region, there are only three genera of roller beetles (*Sisyphus*, *Gymnopleurus* and *Scarabaeus*) with 18 species (Baraud 1992). Of these, only *Sisyphus schaefferi* was used in this project. Males and females of this species cooperate to transport the brood ball away from the dung mass and to bury it at a suitable site. Thus, for tunnelers and rollers, egg, larval and pupal development take place in the galleries or nests in the soil, with the type of galleries and nests varying with the species (Fig.1).

Trypocopris vernalis and *Geotrupes spiniger* beong to Geotrupinae. Within this subfamily, some species excavate forked tunnels, and the males may assist in taking care of the broods. If there are two branches, the first one is usually the deepest one. *Geotrupes* species have been seen stealing brood balls from roller species (e.g. *Geotrupes* species stealing form *Sisyphus schaefferi* Fig. A1 in Appendix).

1.5 Aims of the project

This PhD project is focused on ecological functions provided by dung beetle species both in Italy and Sweden. The main objectives of this project were to investigate the ecological role of dung beetles under different conditions, building on experiments involving both natural beetle colonization in the field and artificial manipulation of assemblages in lab and and in the field.

Within the context of a global biodiversity crisis, I aimed to identify the **ecological role of threatened species.** This is a crucial task to understand the future repercussions of the dung beetle decline in terms of ecosystem functioning. Species differ in their sensitivity to disturbance and previous studies have already proven that species prone to extinction may be also particularly important for providing ecological functions (Larsen et al. 2005, 2008). A trait-based approach was used to identify which traits make species efficient in providing multiple ecological functions (so called *effect traits*) and which ones make species sensitive to environment perturbations (so called *response traits*; Lavorel and Garnier 2002), and whether the two types of traits are indeed linked to each other (Chapter 2).

To target the level of *multifunctionality*, I analysed whether traits (i.e. body mass and nesting strategy) and assemblage features (i.e. density, assemblage and species composition[2]) make dung beetles and/or assemblages efficient in providing multiple ecological functions at the same time. Specifically, I used two lab experiments to explore the effect of dung beetle **density**, **body size**, **assemblage composition** and **species identity** on several ecological functions occurring at the same time (Chapter 3-4).

To target the effect of environmental change and management, I investigated the effect of farming practices (organic vs conventional) and environment factors (i.e. macrohabitat, microhabitat and microclimate) on the

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[2] In this case, *assemblage composition* identifies the composition in terms of how many species are presented in the assemblage (from monospecific to multi-species assemblages); on the other hand, *species composition* stresses the accent on which species belong to the assemblages, underling that the presence of some specific-species might play a crucial role in assemblages.

natural dung beetle communities and their associated ecological functions (i.e. dung removal) in a field experiment in Sweden (Chapter 5).

Overall, this thesis aims to answer the following specific questions:

- Are threatened species more functionally efficient than non-threatened species? What traits make dung beetle species functionally efficient? What traits make species prone to extinction? Do the traits that make species more vulnerable also affect their functional efficiency? Is a species particularly efficient in promoting one ecological function also efficient in promoting others? (Chapter 2)
- Do different species exhibit different GHG emission patterns? Do mixed species perform differently from single-species assemblages? (Chapter 3)
- Does dung removal increase with an increase in beetle density? Is there a similar pattern for body mass? Is seed germination facilitated by higher densities of dung beetles and by presence of larger-bodied beetles? Does seed germination depend on seed transport along soil profile? Does the removal and transport of seed mimics along the soil profile (i.e. burial depth) depend on dung beetle density and body size? Are the ecological functions investigated correlated with each other? (Chapter 4)
- Do farming system, farm management within each system, macro- and micro-habitats affect dung beetle community composition and associated ecological functions? Do experimentally-elevated temperatures alter the provisioning of ecological functions? (Chapter 5)

References

Aguilar, R., Ashworth, L., Galetto, L. & Aizen, M.A. (2006) Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecology Letters*, **9**, 968–980.

Allan, E., Manning, P., Alt, F., Binkenstein, J., Blaser, S., Blüthgen, N., Böhm, S., Grassein, F., Hölzel, N., Klaus, V.H., Kleinebecker, T., Morris, E.K., Oelmann, Y., Prati, D., Renner, S.C., Rillig, M.C., Schaefer, M., Schloter, M., Schmitt, B., Schöning, I., Schrumpf, M., Solly, E., Sorkau, E., Steckel, J., Steffen-Dewenter, I., Stempfhuber, B., Tschapka, M., Weiner, C.N., Weisser, W.W., Werner, M., Westphal, C., Wilcke, W. & Fischer, M. (2015) Land use intensification alters ecosystem multifunctionality via loss of biodiversity and changes to functional composition. *Ecology Letters*, **18**, 834–843.

Amézquita, S. & Favila, M.E. (2010) Removal rates of native and exotic dung by dung beetles (Scarabaeidae: Scarabaeinae) in a fragmented tropical rain forest. *Environmental entomology*, **39**, 328–336.

Andersen, A.N. & Majer, J.D. (2004) Ants show the way Down Under: invertebrates as bioindicators in land management. *Frontiers in Ecology and the Environment*, **2**, 291–298.

Anderson, J.R., Merritt, R.W. & Loomis, E.C. (1984) The insect-free cattle dropping and its relationship to increased dung fouling of rangeland pastures. *Journal of Economic Entomology*, **77**, 133–141.

Andresen, E. (1999) Seed dispersal by monkeys and the fate of dispersed seeds in a Peruvian rain forest. *Biotropica*, **31**, 145–158.

Andresen, E. (2001) Effects of dung presence, dung amount and secondary dispersal by dung beetles on the fate of Micropholis guyanensis (Sapotaceae) seeds in Central Amazonia. *Journal of Tropical Ecology*, **17**, 61–78.

Andresen, E. (2002) Dung beetles in a Central Amazonian rainforest and their ecological role as secondary seed dispersers. *Ecological Entomology*, **27**, 257–270.

Andresen, E. (2005) Effects of season and vegetation type on community organization of dung beetles in a tropical dry forest. *Biotropica*, **37**, 291–300.

Andresen, E. & Feer, F. (2005) The role of dung beetles as secondary seed dispersers and their effect on plant regeneration in tropical rainforests. *Seed fate: Predation, dispersal and seedling establishment*, 331–349.

Andresen, E. & Laurance, S.G. (2007) Possible indirect effects of mammal hunting on dung beetle assemblages in Panama. *Biotropica*, **39**, 141–146.

Andresen, E. & Levey, D.J. (2004) Effects of dung and seed size on secondary dispersal, seed predation, and seedling establishment of rain forest trees. *Oecologia*, **139**, 45–54.

Arellano, L., Favila, M.E. & Huerta, C. (2005) Diversity of dung and carrion beetles in a disturbed Mexican tropical montane cloud forest and on shade coffee plantations. *Biodiversity and Conservation*, **14**, 601–615.

Audino, L.D., Louzada, J. & Comita, L. (2014) Dung beetles as indicators of tropical forest restoration success: Is it possible to recover species and functional diversity? *Biological Conservation*, **169**, 248–257.

Avendaño-mendoza, C., Morón-ríos, A., Cano, E.B. & León-cortés, J. (2005) Dung beetle community (Coleoptera: Scarabaeidae: Scarabaeinae) in a tropical landscape at the Lachua Region, Guatemala. *Biodiversity and Conservation*, **14**, 801–822.

Bang, H.S., Lee, J.-H., Kwon, O.S., Na, Y.E., Jang, Y.S. & Kim, W.H. (2005) Effects of paracoprid dung beetles (Coleoptera: Scarabaeidae) on the growth of pasture herbage and on the underlying soil. *Applied Soil Ecology*, **29**, 165–171.

Baraud, J. (1992) *Coléoptères Scarabaeoidea d'Europe*. Faune De France.

Barbero, E., Palestrini, C. & Rolando, A. (1999) Dung beetle conservation: effects of habitat and resource selection (Coleoptera: Scarabaeoidea). *Journal of Insect Conservation*, **3**, 75–84.

Barnosky, A.D., Matzke, N., Tomiya, S., Wogan, G.O.U., Swartz, B., Quental, T.B., Marshall, C., McGuire, J.L., Lindsey, E.L., Maguire, K.C., Mersey, B. & Ferrer, E.A. (2011) Has the Earth's sixth mass extinction already arrived? *Nature*, **471**, 51.

Barragán, F., Moreno, C.E., Escobar, F., Halffter, G. & Navarrete, D. (2011) Negative impacts of human land use on dung beetle functional diversity. *PloS one*, **6**, e17976.

Batilani-Filho, M. & Hernandez, M.I.M. (2017) Decline of Ecological Functions Performed by Dung Beetles in Areas of Atlantic Forest and Contribution of Rollers and Tunnellers in Organic Matter Removal. *Environmental Entomology*, nvx091.

Beiroz, W., Slade, E.M., Barlow, J., Silveira, J.M., Louzada, J. & Sayer, E. (2017) Dung beetle community dynamics in undisturbed tropical forests: implications for ecological evaluations of land-use change. *Insect Conservation and Diversity*, **10**, 94–106.

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W. & Courchamp, F. (2012) Impacts of climate change on the future of biodiversity. *Ecology letters*, **15**, 365–377.

Bertone, M.A. (2004) Dung beetles (Coleoptera: Scarabaeidae and Geotrupidae) of North Carolina cattle pastures and their implications for pasture improvement.

Beynon, S.A., Mann, D.J., Slade, E.M. & Lewis, O.T. (2012) Species-rich dung beetle communities buffer ecosystem services in perturbed agro-ecosystems. *Journal of Applied Ecology*, **49**, 1365–1372.

Beynon, S.A., Wainwright, W.A. & Christie, M. (2015) The application of an ecosystem services framework to estimate the economic value of dung beetles to the UK cattle industry. *Ecological entomology*, **40**, 124–135.

Bornemissza, G.F. (1960) Could dung eating insects improve our pastures? *Journal of the Australian Institute of Agricultural Science*, **26**, 54–56.

Bornemissza, G.E. (1976) Australian dung beetle project, 1965-1975. *AMRC Rev Aust Meat Res Comm*.

Braga, R.F., Korasaki, V., Andresen, E. & Louzada, J. (2013) Dung beetle community and functions along a habitat-disturbance gradient in the Amazon: a rapid assessment of ecological functions associated to biodiversity. *PLoS One*, **8**, e57786.

Bunnell, F.L., Tait, D.E.N., Flanagan, P.W. & Van Clever, K. (1977) Microbial respiration and substrate weight loss—I: A general model of the influences of abiotic variables. *Soil Biology and Biochemistry*, **9**, 33–40.

Cai, Y., Wang, X., Ding, W., Tian, L., Zhao, H. & Lu, X. (2013) Potential short-term effects of yak and Tibetan sheep dung on greenhouse gas emissions in two alpine grassland soils under laboratory conditions. *Biology and fertility of soils*, **49**, 1215–1226.

Campbell, W.C. & Benz, G.W. (1984) Ivermectin: a review of efficacy and safety. *Journal of Veterinary Pharmacology and Therapeutics*, **7**, 1–16.

Caprio, E., Nervo, B., Isaia, M., Allegro, G. & Rolando, A. (2015) Organic versus conventional systems in viticulture: Comparative effects on spiders and carabids in vineyards and adjacent forests. *Agricultural Systems*, **136**, 61–69.

Carpaneto, G.M., Mazziotta, A. & Valerio, L. (2007) Inferring species decline from collection records: roller dung beetles in Italy (Coleoptera, Scarabaeidae). *Diversity and Distributions*, **13**, 903–919.

Chambers, J.C. & MacMahon, J.A. (1994) A day in the life of a seed: movements and fates of seeds and their implications for natural and managed systems. *Annual review of ecology and systematics*, **25**, 263–292.

Chapin III, F.S., Zavaleta, E.S., Eviner, V.T., Naylor, R.L., Vitousek, P.M., Reynolds, H.L., Hooper, D.U., Lavorel, S., Sala, O.E., Hobbie, S.E., Mack, M.C. & Díaz, S. (2000) Consequences of changing biodiversity, https://www.nature.com/articles/35012241

Corlett, R.T. (2007) The impact of hunting on the mammalian fauna of tropical Asian forests. *Biotropica*, **39**, 292–303.

Daily, G.C. (1997) *Nature's Services*. Island Press, Washington, DC.

Davidson, E.A., Rogers, J.E., Whitman, W.B. & others. (1991) Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems. *Microbial production and consumption of greenhouse gases: methane, nitrogen oxides, and halomethanes.*, 219–235.

Davis, A.J., Holloway, J.D., Huijbregts, H., Krikken, J., Kirk-Spriggs, A.H. & Sutton, S.L. (2001) Dung beetles as indicators of change in the forests of northern Borneo. *Journal of Applied Ecology*, **38**, 593–616.

Davis, A.L., Scholtz, C.H., Dooley, P.W., Bham, N. & Kryger, U. (2004) Scarabaeine dung beetles as indicators of biodiversity, habitat transformation and pest control chemicals in agro-ecosystems. *South African Journal of Science*, **100**, 415–424.

De Groot, R.S., Wilson, M.A. & Boumans, R.M. (2002) A typology for the classification, description and valuation of ecosystem functions, goods and services. *Ecological economics*, **41**, 393–408.

Didham, R.K., Tylianakis, J.M., Gemmell, N.J., Rand, T.A. & Ewers, R.M. (2007) Interactive effects of habitat modification and species invasion on native species decline. *Trends in ecology & evolution*, **22**, 489–496.

Dormont, L., Epinat, G. & Lumaret, J.-P. (2004) Trophic preferences mediated by olfactory cues in dung beetles colonizing cattle and horse dung. *Environmental Entomology*, **33**, 370–377.

Dormont, L., Rapior, S., McKey, D.B. & Lumaret, J.-P. (2007) Influence of dung volatiles on the process of resource selection by coprophagous beetles. *Chemoecology*, **17**, 23–30.

DOUBE, B.M. (1990) A functional classification for analysis of the structure of dung beetle assemblages. *Ecological Entomology*, **15**, 371–383.

Duffy, J.E., Cardinale, B.J., France, K.E., McIntyre, P.B., Thébault, E. & Loreau, M. (2007) The functional role of biodiversity in ecosystems: incorporating trophic complexity. *Ecology Letters*, **10**, 522–538.

Duru, M., Therond, O., Martin, G., Martin-Clouaire, R., Magne, M.-A., Justes, E., Journet, E.-P., Aubertot, J.-N., Savary, S. & Bergez, J.-E. (2015) How to implement biodiversity-based agriculture to enhance ecosystem services: a review. *Agronomy for sustainable development*, **35**, 0.

Escobar, F., Lobo, J.M. & Halffter, G. (2005) Altitudinal variation of dung beetle (Scarabaeidae: Scarabaeinae) assemblages in the Colombian Andes. *Global Ecology and Biogeography*, **14**, 327–337.

Estrada, A. & Coates-Estrada, R. (2002) Dung beetles in continuous forest, forest fragments and in an agricultural mosaic habitat island at Los Tuxtlas, Mexico. *Biodiversity and conservation*, **11**, 1903–1918.

Estrada, A., Coates-Estrada, R., Dadda, A.A. & Cammarano, P. (1998) Dung and carrion beetles in tropical rain forest fragments and agricultural habitats at Los Tuxtlas, Mexico. *Journal of Tropical Ecology*, **14**, 577–593.

Favila, M.E. & Halffter, G. (1997) The use of indicator groups for measuring biodiversity as related to community structure and function. *Acta Zoológica Mexicana (nueva serie)*, 1–25.

Feer, F. (1999) Effects of dung beetles (Scarabaeidae) on seeds dispersed by howler monkeys (Alouatta seniculus) in the French Guianan rain forest. *Journal of Tropical Ecology*, **15**, 129–142.

Finn, J., Gittings, T. & Giller, P. (1999) Spatial and temporal variation in species composition of dung beetle assemblages in southern Ireland. *Ecological Entomology*, **24**, 24–36.

Firestone, M.K. & Davidson, E.A. (1989) Microbiological basis of NO and N2O production and consumption in soil. *Exchange of trace gases between terrestrial ecosystems and the atmosphere*, **47**, 7–21.

Flessa, H., Ruser, R., Dörsch, P., Kamp, T., Jimenez, M.A., Munch, J.C. & Beese, F. (2002) Integrated evaluation of greenhouse gas emissions (CO 2, CH 4, N 2 O) from two farming systems in southern Germany. *Agriculture, Ecosystems & Environment*, **91**, 175–189.

Forrester, D.I. (2014) The spatial and temporal dynamics of species interactions in mixed-species forests: from pattern to process. *Forest Ecology and Management*, **312**, 282–292.

Free, J.B. (1993) *Insect Pollination of Crops.* Academic press.

Fuller, R.J., Norton, L.R., Feber, R.E., Johnson, P.J., Chamberlain, D.E., Joys, A.C., Mathews, F., Stuart, R.C., Townsend, M.C. & Manley, W.J. (2005) Benefits of organic farming to biodiversity vary among taxa. *Biology letters*, **1**, 431–434.

Gagic, V., Bartomeus, I., Jonsson, T., Taylor, A., Winqvist, C., Fischer, C., Slade, E.M., Steffan-Dewenter, I., Emmerson, M. & Potts, S.G. (2015) Functional identity and diversity of animals predict ecosystem functioning better than species-based indices. *Proceedings of the Royal Society of London B: Biological Sciences*, **282**, 20142620.

Galiana, N., Lurgi, M., Montoya, J.M. & López, B.C. (2014) Invasions cause biodiversity loss and community simplification in vertebrate food webs. *Oikos*, **123**, 721–728.

Gardner, T.A., Hernández, M.I., Barlow, J. & Peres, C.A. (2008) Understanding the biodiversity consequences of habitat change: the value of secondary and plantation forests for neotropical dung beetles. *Journal of applied ecology*, **45**, 883–893.

Geiger, F., Bengtsson, J., Berendse, F., Weisser, W.W., Emmerson, M., Morales, M.B., Ceryngier, P., Liira, J., Tscharntke, T., Winqvist, C., Eggers, S., Bommarco, R., Pärt, T., Bretagnolle, V., Plantegenest, M., Clement, L.W., Dennis, C., Palmer, C., Oñate, J.J., Guerrero, I., Hawro, V., Aavik, T., Thies, C., Flohre, A., Hänke, S., Fischer, C., Goedhart, P.W. & Inchausti, P. (2010a) Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. *Basic and Applied Ecology*, **11**, 97–105.

Geiger, F., Lubbe, S.C.T.M. van der, Brunsting, A.M.H. & Snoo, G.R. de. (2010b) Insect abundance in cow dung pats of different farming systems. *Entomologische Berichten*, **70**, 106–110.

Gerlach, J., Samways, M. & Pryke, J. (2013) Terrestrial invertebrates as bioindicators: an overview of available taxonomic groups. *Journal of insect Conservation*, **17**, 831–850.

Giraldo, C., Escobar, F., Chara, J.D. & Calle, Z. (2011) The adoption of silvopastoral systems promotes the recovery of ecological processes regulated by dung beetles in the Colombian Andes. *Insect Conservation and Diversity*, **4**, 115–122.

Gonzalez, A., Cardinale, B.J., Allington, G.R.H., Byrnes, J., Arthur Endsley, K., Brown, D.G., Hooper, D.U., Isbell, F., O'Connor, M.I. & Loreau, M. (2016) Estimating local biodiversity change: a critique of papers claiming no net loss of local diversity. *Ecology*, **97**, 1949–1960.

Grace, J.B., Anderson, T.M., Seabloom, E.W., Borer, E.T., Adler, P.B., Harpole, W.S., Hautier, Y., Hillebrand, H., Lind, E.M. & Pärtel, M. (2016) Integrative modelling reveals mechanisms linking productivity and plant species richness. *Nature*, **529**, 390–393.

Griffiths, H.M., Louzada, J., Bardgett, R.D., Beiroz, W., França, F., Tregidgo, D. & Barlow, J. (2015) Biodiversity and environmental context predict dung beetle-mediated seed dispersal in a tropical forest field experiment. *Ecology*, **96**, 1607–1619.

Halffter, G. & Arellano, L. (2002) Response of dung beetle diversity to human-induced changes in a tropical landscape. *Biotropica*, **34**, 144–154.

Halffter, G. & Edmonds, W.D. (1982) The nesting behavior of dung beetles (Scarabaeinae). An ecological and evolutive approach. *The nesting behavior of dung beetles (Scarabaeinae). An ecological and evolutive approach.*

Halffter, G., HUERTA, C. & LOPEZ-PORTILLO, J. (1996) Parental care and offspring survival inCopris incertusSay, a sub-social beetle. *Animal Behaviour*, **52**, 133–139.

Halffter, G. & Matthews, E.G. (1966) The natural history of dung beetles of the subfamily Scarabaeinae (Coleoptera, Scarabaeidae).

Hammer, T.J., Fierer, N., Hardwick, B., Simojoki, A., Slade, E., Taponen, J., Viljanen, H. & Roslin, T. (2016) Treating cattle with antibiotics affects greenhouse gas emissions, and microbiota in dung and dung beetles. *Proc. R. Soc. B*, **283**, 20160150.

Hanski, I. & Cambefort, Y. (2014) *Dung Beetle Ecology*. Princeton University Press.

Hautier, Y., Tilman, D., Isbell, F., Seabloom, E.W., Borer, E.T. & Reich, P.B. (2015) Anthropogenic environmental changes affect ecosystem stability via biodiversity. *Science*, **348**, 336–340.

Hernández, M.I.M. & Vaz-de-Mello, F.Z. (2009) Seasonal and spatial species richness variation of dung beetle (Coleoptera, Scarabaeidae s. str.) in the Atlantic Forest of southeastern Brazil. *Revista Brasileira de Entomologia*, **53**, 607–613.

Hevia, V., Martín-López, B., Palomo, S., García-Llorente, M., Bello, F. & González, J.A. (2017) Trait-based approaches to analyze links between the drivers of change and ecosystem services: Synthesizing existing evidence and future challenges. *Ecology and evolution*.

Holter, P. (1979) Effect of Dung-Beetles (Aphodius spp.) and Earthworms on the Disappearance of Cattle Dung. *Oikos*, **32**, 393–402.

Holter, P. (2000) Particle feeding in Aphodius dung beetles (Scarabaeidae): old hypotheses and new experimental evidence. *Functional Ecology*, **14**, 631–637.

Holter, P. & SCHOLTZ, C. (2007) What do dung beetles eat? *Ecological Entomology*, **32**, 690–697.

Holter, P., Scholtz, C.H. & Wardhaugh, K.G. (2002) Dung feeding in adult scarabaeines (tunnellers and endocoprids): even large dung beetles eat small particles. *Ecological Entomology*, **27**, 169–176.

Holter, P. & Spangenberg, A. (1997) Oxygen uptake in coprophilous beetles (Aphodius, Geotrupes, Sphaeridium) at low oxygen and high carbon dioxide concentrations. *Physiological Entomology*, **22**, 339–343.

Hooper, D.U., Adair, E.C., Cardinale, B.J., Byrnes, J.E., Hungate, B.A., Matulich, K.L., Gonzalez, A., Duffy, J.E., Gamfeldt, L. & O'Connor, M.I. (2012) A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature*, **486**, 105–108.

Hughes, R.D. (1975) Assessment of the burial of cattledung by australian dung beetles. *Austral Entomology*, **14**, 129–134.

Hulme, P.E. (1994) Seedling herbivory in grassland: relative impact of vertebrate and invertebrate herbivores. *Journal of Ecology*, 873–880.

Hulme, P.E. (2002) 17 Seed-eaters: Seed Dispersal, Destruction and Demography. *Seed dispersal and frugivory: Ecology, evolution, and conservation*, 257.

Hunt, J. & Simmons, L.W. (2000) Maternal and paternal effects on offspring phenotype in the dung beetle Onthophagus taurus. *Evolution*, **54**, 936–941.

Hunt, J. & Simmons, L.W. (2004) Optimal maternal investment in the dung beetle Onthophagus taurus? *Behavioral ecology and sociobiology*, **55**, 302–312.

Hutton, S.A. & Giller, P.S. (2003) The effects of the intensification of agriculture on northern temperate dung beetle communities. *Journal of Applied Ecology*, **40**, 994–1007.

Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W.S., Reich, P.B., Scherer-Lorenzen, M., Schmid, B., Tilman, D., Ruijven, J. van, Weigelt, A., Wilsey, B.J., Zavaleta, E.S. & Loreau, M. (2011) High plant diversity is needed to maintain ecosystem services. *Nature*, **477**, 199.

Iwasa, M., Maruo, T., Ueda, M. & Yamashita, N. (2007) Adverse effects of ivermectin on the dung beetles, Caccobius jessoensis Harold, and rare species, Copris ochus Motschulsky and Copris acutidens Motschulsky (Coleoptera: Scarabaeidae), in Japan. *Bulletin of entomological research*, **97**, 619–625.

Iwasa, M., Moki, Y. & Takahashi, J. (2015) Effects of the activity of coprophagous insects on greenhouse gas emissions from cattle dung pats and changes in amounts of nitrogen, carbon, and energy. *Environmental entomology*, **44**, 106–113.

Jacobs, C.T. & Scholtz, C.H. (2015) A review on the effect of macrocyclic lactones on dung-dwelling insects: Toxicity of macrocyclic lactones to dung beetles. *Onderstepoort Journal of Veterinary Research*, **82**, 01–08.

Janzen, D.H. (1984) Dispersal of small seeds by big herbivores: foliage is the fruit. *The American Naturalist*, **123**, 338–353.

Kaartinen, R., Hardwick, B. & Roslin, T. (2013) Using citizen scientists to measure an ecosystem service nationwide. *Ecology*, **94**, 2645–2652.

Kazuhira, Y., Hdeaki, K., Takuro, K. & Toshiharu, A. (1991) Nitrogen mineralization and microbial populations in cow dung, dung balls and underlying soil affected by paracoprid dung beetles. *Soil Biology and Biochemistry*, **23**, 649–653.

Kelly, A.E. & Goulden, M.L. (2008) Rapid shifts in plant distribution with recent climate change. *Proceedings of the National Academy of Sciences*, **105**, 11823–11826.

Klein, B.C. (1989) Effects of forest fragmentation on dung and carrion beetle communities in central Amazonia. *Ecology*, **70**, 1715–1725.

Klemperer, H.G. (1982) Parental behaviour in Copris lunaris (Coleoptera, Scarabaeidae): care and defence of brood balls and nest. *Ecological Entomology*, **7**, 155–167.

Koike, S., Morimoto, H., Kozakai, C., Arimoto, I., Soga, M., Yamazaki, K. & Koganezawa, M. (2012) The role of dung beetles as a secondary seed disperser after dispersal by frugivore mammals in a temperate deciduous forest. *Acta oecologica*, **41**, 74–81.

Kool, D.M., Wrage, N., Zechmeister-Boltenstern, S., Pfeffer, M., Brus, D., Oenema, O. & Van Groenigen, J.-W. (2010) Nitrifier denitrification can be a source of N2O from soil: a revised approach to the dual-isotope labelling method. *European Journal of Soil Science*, **61**, 759–772.

Kopecký, M., Hédl, R. & Szabó, P. (2013) Non-random extinctions dominate plant community changes in abandoned coppices. *Journal of Applied Ecology*, **50**, 79–87.

Krüger, K. & Scholtz, C.H. (1997) Lethal and sublethal effects of ivermectin on the dung-breeding bettles Euoniticellus intermedius (Reiche) and Onitis alexis Klug (Coleoptera, Scarabaeidae). *Agriculture, ecosystems & environment*, **61**, 123–131.

LaBarbera, M. (1989) Analyzing body size as a factor in ecology and evolution. *Annual review of ecology and systematics*, **20**, 97–117.

Lähteenmäki, S., Slade, E.M., Hardwick, B., Schiffler, G., Louzada, J., Barlow, J. & Roslin, T. (2015) MESOCLOSURES–increasing realism in mesocosm studies of ecosystem functioning. *Methods in Ecology and Evolution*, **6**, 916–924.

Larsen, T.H. & Forsyth, A. (2005) Trap spacing and transect design for dung beetle biodiversity studies. *Biotropica*, **37**, 322–325.

Larsen, T.H., Lopera, A. & Forsyth, A. (2008) Understanding Trait-Dependent Community Disassembly: Dung Beetles, Density Functions, and Forest Fragmentation. *Conservation Biology*, **22**, 1288–1298.

Larsen, T.H., Williams, N.M. & Kremen, C. (2005) Extinction order and altered community structure rapidly disrupt ecosystem functioning. *Ecology letters*, **8**, 538–547.

Lastro, E. (2006) Dung beetles (Coleoptera: Scarabaeidae and Geotrupidae) in North Carolina pasture ecosystem.

Lavelle, P., Decaëns, T., Aubert, M., Barot, S., Blouin, M., Bureau, F., Margerie, P., Mora, P. & Rossi, J.-P. (2006) Soil invertebrates and ecosystem services. *European Journal of Soil Biology*, **42**, S3–S15.

Lavorel, S. & Garnier, E. (2002) Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology*, **16**, 545–556.

Lin, X.W., Wang, S.P., Ma, X.Z., Xu, G.P., Luo, C.Y., Li, Y.N., Jiang, G.M. & Xie, Z.B. (2009) Fluxes of CO2, CH4, and N2O in an alpine meadow affected by yak excreta during summer grazing periods on the Qinghai-Tibetan plateau. *Soil Biol Biochem*, **41**, 718–725.

Lobo, J.M. (2001) Decline of roller dung beetle (Scarabaeinae) populations in the Iberian peninsula during the 20th century. *Biological Conservation*, **97**, 43–50.

Lobo, J.M., Verdú, J.R. & Numa, C. (2006) Environmental and geographical factors affecting the Iberian distribution of flightless Jekelius species (Coleoptera: Geotrupidae). *Diversity and Distributions*, **12**, 179–188.

Losey, J.E. & Vaughan, M. (2006) The economic value of ecological services provided by insects. *Bioscience*, **56**, 311–323.

Lumaret, J.-P. & Kirk, A.A. (1991) South temperate dung beetles. *Dung beetle ecology*, 97–115.

Macagno, A.L.M. & Palestrini, C. (2009) The maintenance of extensively exploited pastures within the Alpine mountain belt: implications for dung beetle conservation (Coleoptera: Scarabaeoidea). *Biodiversity and conservation*, **18**, 3309.

Mace, G.M., Norris, K. & Fitter, A.H. (2012) Biodiversity and ecosystem services: a multilayered relationship. *Trends in ecology & evolution*, **27**, 19–26.

Manning, P., Lewis, O.T. & Beynon, S.A. (2017) Effects of the veterinary anthelmintic moxidectin on dung beetle survival and ecosystem functioning. *bioRxiv*, 213173.

Manning, P., Slade, E.M., Beynon, S.A. & Lewis, O.T. (2016) Functionally rich dung beetle assemblages are required to provide multiple ecosystem services. *Agriculture, Ecosystems & Environment*, **218**, 87–94.

Martínez, I., Lumaret, J.-P., Zayas, R.O. & Kadiri, N. (2017) The effects of sublethal and lethal doses of ivermectin on the reproductive physiology and larval development of the dung beetle Euoniticellus intermedius (Coleoptera: Scarabaeidae). *The Canadian Entomologist*, 1–12.

Mehrabi, Z., Slade, E.M., Solis, A. & Mann, D.J. (2014) The importance of microhabitat for biodiversity sampling. *PloS one*, **9**, e114015.

Menéndez, R., González-Megías, A., Jay-Robert, P. & Marquéz-Ferrando, R. (2014) Climate change and elevational range shifts: evidence from dung beetles in two European mountain ranges. *Global Ecology and Biogeography*, **23**, 646–657.

Menéndez, R., Webb, P. & Orwin, K.H. (2016) Complementarity of dung beetle species with different functional behaviours influence dung–soil carbon cycling. *Soil Biology and Biochemistry*, **92**, 142–148.

Metcalfe, D.B., Asner, G.P., Martin, R.E., Espejo, S., E, J., Huasco, W.H., Amézquita, F., F, F., Carranza-Jimenez, L., Cabrera, G., F, D., Baca, L.D., Sinca, F., Quispe, H., P, L., Taype, I.A., Mora, L.E., Dávila, A.R., Solórzano, M.M., Vilca, P., L, B., Román, L., M, J., Bustios, G., C, P., Revilla, N.S., Tupayachi, R., Girardin, C.A.J., Doughty, C.E. & Malhi, Y. (2014) Herbivory makes major contributions to ecosystem carbon and nutrient cycling in tropical forests. *Ecology Letters*, **17**, 324–332.

Moczek, A.P. (1998) Horn polyphenism in the beetle Onthophagus taurus: larval diet quality and plasticity in parental investment determine adult body size and male horn morphology. *Behavioral Ecology*, **9**, 636–641.

Moss, A.R., Jouany, J.-P. & Newbold, J. (2000) Methane production by ruminants: its contribution to global warming. *Annales de zootechnie*, pp. 231–253. EDP Sciences.

Murphy, G.E. & Romanuk, T.N. (2014) A meta-analysis of declines in local species richness from human disturbances. *Ecology and evolution*, **4**, 91–103.

Naeem, S., Duffy, J.E. & Zavaleta, E. (2012) The functions of biological diversity in an age of extinction. *Science*, **336**, 1401–1406.

Naeem, S. & Wright, J.P. (2003) Disentangling biodiversity effects on ecosystem functioning: deriving solutions to a seemingly insurmountable problem. *Ecology Letters*, **6**, 567–579.

Nealis, V.G. (1977) Habitat associations and community analysis of south Texas dung beetles (Coleoptera: Scarabaeinae). *Canadian Journal of Zoology*, **55**, 138–147.

Negro, M., Rolando, A. & Palestrini, C. (2011) The impact of overgrazing on dung beetle diversity in the Italian Maritime Alps. *Environmental entomology*, **40**, 1081–1092.

Nervo, B., Caprio, E., Celi, L., Lonati, M., Lombardi, G., Falsone, G., Iussig, G., Palestrini, C., Said-Pullicino, D. & Rolando, A. (2017) Ecological functions provided by dung beetles are interlinked across space and time: evidence from 15N isotope tracing. *Ecology*, **98**, 433–446.

Nervo, B., Tocco, C., Caprio, E., Palestrini, C. & Rolando, A. (2014) The effects of body mass on dung removal efficiency in dung beetles. *PloS one*, **9**, e107699.

Newbold, T., Hudson, L.N., Arnell, A.P., Contu, S., Palma, A.D., Ferrier, S., Hill, S.L.L., Hoskins, A.J., Lysenko, I., Phillips, H.R.P., Burton, V.J., Chng, C.W.T., Emerson, S., Gao, D., Pask-Hale, G., Hutton, J., Jung, M., Sanchez-Ortiz, K., Simmons, B.I., Whitmee, S., Zhang, H., Scharlemann, J.P.W. & Purvis, A. (2016) Has land use pushed terrestrial biodiversity beyond the planetary boundary? A global assessment. *Science*, **353**, 288–291.

Nichols, E.S. & Gardner, T.A. (2011) Dung beetles as a candidate study taxon in applied biodiversity conservation research. *Ecology and evolution of dung beetles*, 267–291.

Nichols, E., Gardner, T.A., Peres, C.A. & Spector, S. (2009) Co-declining mammals and dung beetles: an impending ecological cascade. *Oikos*, **118**, 481–487.

Nichols, E., Larsen, T., Spector, S., Davis, A.L., Escobar, F., Favila, M., Vulinec, K. & Network, T.S.R. (2007) Global dung beetle response to tropical forest modification and fragmentation: a quantitative literature review and meta-analysis. *Biological conservation*, **137**, 1–19.

Nichols, E., Spector, S., Louzada, J., Larsen, T., Amezquita, S., Favila, M.E. & Network, T.S.R. (2008) Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. *Biological conservation*, **141**, 1461–1474.

Numa, C., Verdú, J.R., Rueda, C. & Galante, E. (2012) Comparing dung beetle species assemblages between protected areas and adjacent pasturelands in a Mediterranean savanna landscape. *Rangeland Ecology & Management*, **65**, 137–143.

Numa, C., Verdú, J.R., Sánchez, A. & Galante, E. (2009) Effect of landscape structure on the spatial distribution of Mediterranean dung beetle diversity. *Diversity and Distributions*, **15**, 489–501.

Obrycki, J.J., Harwood, J.D., Kring, T.J. & O'Neil, R.J. (2009) Aphidophagy by Coccinellidae: Application of biological control in agroecosystems. *Biological Control*, **51**, 244–254.

Odum, P.O. (1962) RELATIONSHIPS BETWEEN STRUCTURE AND FUNCTION IN THE ECOSYSTEM. *JAPANESE JOURNAL OF ECOLOGY*, **12**, 108–118.

Odum, E.P. (1968) Energy flow in ecosystems: a historical review. *American Zoologist*, **8**, 11–18.

Oliver, T.H., Heard, M.S., Isaac, N.J., Roy, D.B., Procter, D., Eigenbrod, F., Freckleton, R., Hector, A., Orme, C.D.L. & Petchey, O.L. (2015) Biodiversity and resilience of ecosystem functions. *Trends in Ecology & Evolution*, **30**, 673–684.

Oremland, R.S. (1988) Biogeochemistry of methanogenic bacteria. *Biology of anaerobic microorganisms*, 641–705.

Palestrini, C. & Rolando, A. (2001) Body size and paternal investment in the genus Onthophagus (Coleoptera, Scarabaeoidea). *Journal of Zoology*, **255**, 405–412.

Pearson, R.G. & Dawson, T.P. (2003) Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global ecology and biogeography*, **12**, 361–371.

Pearson, R.G., Stanton, J.C., Shoemaker, K.T., Aiello-Lammens, M.E., Ersts, P.J., Horning, N., Fordham, D.A., Raxworthy, C.J., Ryu, H.Y. & McNees, J. (2014) Life history and spatial traits predict extinction risk due to climate change. *Nature Climate Change*, **4**, 217–221.

Peck, S.B. & Forsyth, A. (1982) Composition, structure, and competitive behaviour in a guild of Ecuadorian rain forest dung beetles (Coleoptera; Scarabaeidae). *Canadian Journal of Zoology*, **60**, 1624–1634.

Penttilä, A., Slade, E.M., Simojoki, A., Riutta, T., Minkkinen, K. & Roslin, T. (2013) Quantifying beetle-mediated effects on gas fluxes from dung pats. *PLoS One*, **8**, e71454.

Peres, C.A. (2000) Effects of subsistence hunting on vertebrate community structure in Amazonian forests. *Conservation Biology*, **14**, 240–253.

Pérez-Ramos, I.M., Volaire, F., Fattet, M., Blanchard, A. & Roumet, C. (2013) Tradeoffs between functional strategies for resource-use and drought-survival in Mediterranean rangeland species. *Environmental and Experimental Botany*, **87**, 126–136.

Petersen, S.O., Sommer, S.G., Aaes, O. & Søegaard, K. (1998) Ammonia losses from urine and dung of grazing cattle: effect of N intake. *Atmospheric Environment*, **32**, 295–300.

Philips, T.K. (2011) The evolutionary history and diversification of dung beetles. *Ecology and evolution of dung beetles*, 21–46.

Piccini, I., Arnieri, F., Caprio, E., Nervo, B., Pelissetti, S., Palestrini, C., Roslin, T. & Rolando, A. (2017) Greenhouse gas emissions from dung pats vary with dung beetle species and with assemblage composition. *PLoS One*, **12**, e0178077.

Piccini, I., Nervo, B., Forshage, M., Celi, L., Palestrini, C., Rolando, A. & Roslin, T. (2018) Dung beetles as drivers of ecosystem multifunctionality: Are response and effect traits interwoven? *Science of The Total Environment*, 616, 1440-1448.

Pimm, S.L., Jenkins, C.N., Abell, R., Brooks, T.M., Gittleman, J.L., Joppa, L.N., Raven, P.H., Roberts, C.M. & Sexton, J.O. (2014) The biodiversity of species and their rates of extinction, distribution, and protection. *Science*, **344**, 1246752.

Pimm, S.L., Jones, H.L. & Diamond, J. (1988) On the risk of extinction. *The American Naturalist*, **132**, 757–785.

Pineda, E., Moreno, C., Escobar, F. & Halffter, G. (2005) Frog, bat, and dung beetle diversity in the cloud forest and coffee agroecosystems of Veracruz, Mexico. *Conservation Biology*, **19**, 400–410.

Ponce-Santizo, G., Andresen, E., Cano, E. & Cuarón, A.D. (2006) Dispersión primaria de semillas por primates y dispersión secundaria por escarabajos coprófagos en Tikal, Guatemala. *Biotropica*, **38**, 390–397.

Purvis, A. & Hector, A. (2000) Getting the measure of biodiversity. *Nature*, **405**, 212–219.

Püttker, T., de Arruda Bueno, A., Prado, P.I. & Pardini, R. (2015) Ecological filtering or random extinction? Beta-diversity patterns and the importance of niche-based and neutral processes following habitat loss. *Oikos*, **124**, 206–215.

Quintero, I. & Roslin, T. (2005) Rapid recovery of dung beetle communities following habitat fragmentation in Central Amazonia. *Ecology*, **86**, 3303–3311.

Rosenlew, H. & Roslin, T. (2008) Habitat fragmentation and the functional efficiency of temperate dung beetles. *Oikos*, **117**, 1659–1666.

Roslin, T. (2014) Nordens dyngbaggar (Dung beetles of Northern Europe) - ENTO SPHINX s.r.o., http://www.entosphinx.cz/en/literatura/1114-roslin-t-et-al-2014-nordens-dyngbaggar-dung-beetles-of-northern-europe.html

Roslin, T., Avomaa, T., Leonard, M., Luoto, M. & Ovaskainen, O. (2009) Some like it hot: microclimatic variation affects the abundance and movements of a critically endangered dung beetle. *Insect Conservation and Diversity*, **2**, 232–241.

Roslin, T. & Koivunen, A. (2001) Distribution and abundance of dung beetles in fragmented landscapes. *Oecologia*, **127**, 69–77.

Roslin, T. & Viljanen, H. (2011) Dung beetle populations: structure and consequences. *Ecology and evolution of dung beetles*, 220–244.

Saggar, S., Bolan, N.S., Bhandral, R., Hedley, C.B. & Luo, J. (2004) A review of emissions of methane, ammonia, and nitrous oxide from animal excreta deposition and farm effluent application in grazed pastures. *New Zealand Journal of Agricultural Research*, **47**, 513–544.

Scheffler, P.Y. (2005) Dung beetle (Coleoptera: Scarabaeidae) diversity and community structure across three disturbance regimes in eastern Amazonia. *Journal of Tropical Ecology*, **21**, 9–19.

Schmidt, N.M., Mosbacher, J.B., Nielsen, P.S., Rasmussen, C., Høye, T.T. & Roslin, T. (2016) An ecological function in crisis? The temporal overlap between plant flowering and pollinator function shrinks as the Arctic warms. *Ecography*, **39**, 1250–1252.

Secretariat, C.B.D. (1992) Convention on biological diversity. *Convention on Biological Diversity*

Sepkoski, D. (2016) Extinction and biodiversity. *The Routledge Handbook of Philosophy of Biodiversity*.

Shepherd, V.E. & Chapman, C.A. (1998) Dung beetles as secondary seed dispersers: impact on seed predation and germination. *Journal of Tropical Ecology*, **14**, 199–215.

Simmons, L.W. & Ridsdill-Smith, T.J. (2011) *Ecology and Evolution of Dung Beetles*. John Wiley & Sons.

Slade, E.M., Kirwan, L., Bell, T., Philipson, C., Lewis, O.T. & Roslin, T. (2017) The importance of species identity and interactions on multifunctionality depends on how ecosystem functions are valued. *Ecology*.

Slade, E.M., Mann, D.J. & Lewis, O.T. (2011) Biodiversity and ecosystem function of tropical forest dung beetles under contrasting logging regimes. *Biological Conservation*, **144**, 166–174.

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

Slade, E.M., Riutta, T., Roslin, T. & Tuomisto, H.L. (2016) The role of dung beetles in reducing greenhouse gas emissions from cattle farming. *Scientific reports*, **6**.

Slade, E.M. & Roslin, T. (2016) Dung beetle species interactions and multifunctionality are affected by an experimentally warmed climate. *Oikos*, **125**, 1607–1616.

Sowig, P. (1995) Habitat selection and offspring survival rate in three paracoprid dung beetles: the influence of soil type and soil moisture. *Ecography*, **18**, 147–154.

Spector, P.E. (2006) Method variance in organizational research: truth or urban legend? *Organizational research methods*, **9**, 221–232.

Spector, S. & Ayzama, S. (2003) Rapid turnover and edge effects in dung beetle assemblages (Scarabaeidae) at a Bolivian Neotropical forest–savanna ecotone. *Biotropica*, **35**, 394–404.

Steinfeld, H., Gerber, P., Wassenaar, T.D., Castel, V. & de Haan, C. (2006) *Livestock's Long Shadow: Environmental Issues and Options*. Food & Agriculture Org.

Stevenson, B.G. & Dindal, D.L. (1985) Growth and development of Aphodius beetles (Scarabaeidae) in laboratory microcosms of cow dung. *The Coleopterists' Bulletin*, 215–220.

Stork, N.E. (2017) How Many Species of Insects and Other Terrestrial Arthropods Are There on Earth? *Annual review of entomology*.

Tilman, D., Isbell, F. & Cowles, J.M. (2014) Biodiversity and ecosystem functioning. *Annual Review of Ecology, Evolution, and Systematics*, **45**.

Tixier, T., Bloor, J.M. & Lumaret, J.-P. (2015) Species-specific effects of dung beetle abundance on dung removal and leaf litter decomposition. *Acta Oecologica*, **69**, 31–34.

Tocco, C., Negro, M., Rolando, A. & Palestrini, C. (2013a) Does natural reforestation represent a potential threat to dung beetle diversity in the Alps? *Journal of insect conservation*, **17**, 207–217.

Tocco, C., Probo, M., Lonati, M., Lombardi, G., Negro, M., Nervo, B., Rolando, A. & Palestrini, C. (2013b) Pastoral practices to reverse shrub encroachment of sub-alpine grasslands: dung beetles (Coleoptera, Scarabaeoidea) respond more quickly than vegetation. *PLoS One*, **8**, e83344.

Tonelli, M., Verdú, J.R. & Zunino, M. Effects of the progressive abandonment of grazing on dung beetle biodiversity: body size matters. *Biodiversity and Conservation*, 1–16.

Tscharntke, T., Klein, A.M., Kruess, A., Steffan-Dewenter, I. & Thies, C. (2005a) Landscape perspectives on agricultural intensification and biodiversity–ecosystem service management. *Ecology letters*, **8**, 857–874.

Tscharntke, T., Klein, A.M., Kruess, A., Steffan-Dewenter, I. & Thies, C. (2005b) Landscape perspectives on agricultural intensification and biodiversity–ecosystem service management. *Ecology letters*, **8**, 857–874.

Valiente-Banuet, A., Aizen, M.A., Alcántara, J.M., Arroyo, J., Cocucci, A., Galetti, M., García, M.B., García, D., Gómez, J.M. & Jordano, P. (2015) Beyond species loss: the extinction of ecological interactions in a changing world. *Functional Ecology*, **29**, 299–307.

Vanni, M.J. (2002) Nutrient Cycling by Animals in Freshwater Ecosystems. *Annual Review of Ecology and Systematics*, **33**, 341–370.

Verdú, J.R., Cortez, V., Ortiz, A.J., González-Rodríguez, E., Martinez-Pinna, J., Lumaret, J.-P., Lobo, J.M., Numa, C. & Sánchez-Piñero, F. (2015) Low doses of ivermectin cause sensory and locomotor disorders in dung beetles. *Scientific reports*, **5**.

Verdú, J.R., Lobo, J.M., Sánchez-Piñero, F., Gallego, B., Numa, C., Lumaret, J.-P., Cortez, V., Ortiz, A.J., Tonelli, M. & García-Teba, J.P. (2018) Ivermectin residues disrupt dung beetle diversity, soil properties and ecosystem functioning: An interdisciplinary field study. *Science of The Total Environment*, **618**, 219–228.

Verdú, J.R., Moreno, C.E., Sánchez-Rojas, G., Numa, C., Galante, E. & Halffter, G. (2007) Grazing promotes dung beetle diversity in the xeric landscape of a Mexican Biosphere Reserve. *Biological Conservation*, **140**, 308–317.

Vessby, K. & Wiktelius, S. (2003) The influence of slope aspect and soil type on immigration and emergence of some northern temperate dung beetles. *Pedobiologia*, **47**, 39–51.

Vulinec, K. (2002) Dung beetle communities and seed dispersal in primary forest and disturbed land in Amazonia. *Biotropica*, **34**, 297–309.

Wagg, C., Bender, S.F., Widmer, F. & van der Heijden, M.G. (2014) Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences*, **111**, 5266–5270.

Wardhaugh, K.G. & Rodriguez-Menendez, H. (1988) The effects of the antiparasitic drug, ivermectin, on the development and survival of the dung-breeding fly, Orthelia cornicina (F.) and the scarabaeine dung beetles, Copris hispanus L., Bubas bubalus (Oliver) and Onitis belial F. *Journal of Applied Entomology*, **106**, 381–389.

Weisser, W.W. & Siemann, E. (2008) The Various Effects of Insects on Ecosystem Functioning. *Insects and Ecosystem Function* Ecological Studies., pp. 3–24. Springer, Berlin, Heidelberg.

Wielgoss, A., Tscharntke, T., Rumede, A., Fiala, B., Seidel, H., Shahabuddin, Y.C., Würzburg, A.H. & AW, Y. (2013) III Interaction complexity matters: Disentangling services and disservices of ant communities driving yield in tropical agroecosystems. " *Services and disservices driven by ant communities in tropical agroforests*, 11.

Winfree, R., W Fox, J., Williams, N.M., Reilly, J.R. & Cariveau, D.P. (2015) Abundance of common species, not species richness, drives delivery of a real-world ecosystem service. *Ecology Letters*, **18**, 626–635.

Wood, A., Stedman-Edwards, P. & Mang, J. (2000) *The Root Causes of Biodiversity Loss*. Earthscan.

Yamada, D., Imura, O., Shi, K. & Shibuya, T. (2007) Effect of tunneler dung beetles on cattle dung decomposition, soil nutrients and herbage growth. *Grassland Science*, **53**, 121–129.

Yokoyama, K. & Kai, H. (1993) Distribution and flow of nitrogen in a cow dung-soil system colonized by paracoprid dung beetles. *Edaphologia (Japan)*.

Yoshihara, Y. & Sato, S. (2015) The relationship between dung beetle species richness and ecosystem functioning. *Applied Soil Ecology*, **88**, 21–25.

Zavaleta, E.S. & Hulvey, K.B. (2004) Realistic species losses disproportionately reduce grassland resistance to biological invaders. *Science*, **306**, 1175–1177.

Appendix

Table A1: **Species features.** Functional group, name of the species, short description of identification features, body mass and body length.

Functional group	Species	Short description	Body mass (g)	Length (mm)
Dwellers	*Aphodius ater* (De Geer, 1774)	It is small size black-colored dweller that has rounded bold shape.	0.004	5
	A. foetens (Fabricius, 1787)	It is a medium size dweller with orange colored elytra and bold shape.	0.017	7.1
	A. fossor (Linnaeus, 1758)	It is a medium size dweller that has a bold, rounded, convex body shape with defined stripes and glossy black-colored body.	0.04	10.75
	A. haemorrhoidalis (Linnaeus, 1758)	It is a small size shining-bodied dweller with red-colored final part of elytra. Head with pronounced cheeks and abdomen with an elongate scutellum.	0.002	4.5
	A. ictericus (Laicharting, 1781)	It is a small size dweller, shining gold-colored elytra.	0.003	4.9
	A. pedellus (De Geer, 1774)/*A. fimetarius* (Linnaeus, 1758)	It is a medium size dweller with red colored elytra and bold shape. It is similar to *A. foetens*	0.009	6.85

		but differently colored.		
	A. puntatosulcatus (Linnaeus, 1758)	It is a small size dweller with rugose striped elytra.	0.003	4.5
	A. pusillus (Herbst, 1789)	It a small dweller without cheeks on the head, with a short scutellum and with a not-glossy body. It does not present a spur on the last metatarsus on legs.	0.002	3.75
	A. rufipes (Linnaeus, 1758)	It is a medium size dweller with red-colored shade legs. Pronotum has some large punctures among the evenly distributed fine puncturation.	0.034	11
	A. sordidus (Fabricius, 1775)	It is a medium size dweller with yellow black-marked elytra.	0.009	6.75
Tunnelers	*A. erraticus*(Linnaeus, 1758)	It is a medium size tunneler with a flattered body-shape, with dark-pale-yellow elytra and an elongate scutellum.	0.011	7.1
	Copris lunaris (Linnaeus, 1758)	It is a large black shining tunneler with deep-striped elytra. Both sexes present a single horn on the head, that for females is bifid.	0.22	18.75
	Onthophagus coenobita (Herbst, 1783)	It is a medium size tunneler with pale-yellow/light-brown	0.009	6.7

		dark-marked elytra and shining metallic-bordeaux pronotum.		
	O. fracticornis (Preyssler, 1790)	It is a medium size tunneler with pale-yellow/light-brown dark-marked elytra and opaque-dark pronotum. The male head presents a single horn.	0.015	7.5
	O. illyricus (Scopoli, 1763)	It is a medium size tunneler with opaque black elytra and shining metallic-black pronotum. It presents striped elytras with dots and numerous setolas, conversely to *O. taurus* that has almost plane striped elytra. The male head presents a double horn.		
	O. nuchicornis (Linnaeus, 1758)	It is a medium size tunneler with yellow/light-brown black-marked elytra and opaque-dark pronotum. It is smaller and shinier than *O. fracticornis.* The male head presents a single horn.	0.01	7
	Geotrupes spiniger (Marsham, 1802)	It is a large size black-opaque colored tunneler with deep stripes on elytra. The ventral-side is blue-	0.29	21

		metallic colored. It is similar to *G. stercorarius* but it is possible to distinguish it by the outer edge of the mandible that has a distinct lobe.		
	Trypocopris vernalis (Linnaeus, 1758)	It is a large size tunneler with shining plane (not striped and not-dotted) green body.	0.15	17
Roller	*Sisyphus schaefferi* (Latreille, 1807)	It is a medium size roller with opaque black body with long hind legs. The abdomen has an elongate edged end.		

Figure A1: ***Anoplotrupes stercorosus species* trying to steal a dung ball from *Sysiphus schaefferi***. Pictures A and B represent two consecutive moment of the same competition between *A. stercorosus* and *S. schaefferi* to obtain the brood ball in the picture.

2. Dung beetles as drivers of ecosystem multifunctionality: are response and effect traits interwoven?

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Abstract

Rapid biodiversity loss has emphasized the need to understand how biodiversity affects the provisioning of ecological functions. Of particular interest are species and communities with versatile impacts on multiple parts of the environment, linking processes in the biosphere, lithosphere, and atmosphere to human interests in the anthroposphere (in this case, cattle farming). In this study, we examine the role of a specific group of insects – beetles feeding on cattle dung – on multiple ecological functions spanning these spheres (dung removal, soil nutrient content and greenhouse gas emissions). We ask whether the same traits which make species prone to extinction (i.e. *response traits*) may also affect their functional efficiency (as *effect traits*). To establish the link between response and effect traits, we first evaluated whether two traits (body mass and nesting strategy, the latter categorized as tunnelers or dwellers) affected the probability of a species being threatened. We then tested for a relationship between these traits and ecosystem functioning. Across Scandinavian dung beetle species, 75% of tunnelers and 30% of dwellers are classified as threatened. Hence, nesting strategy significantly affects the probability of a species being threatened, and constitutes a response trait. Effect traits varied with the ecological function investigated: density-specific dung removal was influenced by both nesting strategy and body mass, whereas methane emissions varied with body mass and nutrient recycling with nesting strategy. Our findings suggest that among Scandinavian dung beetles, nesting strategy is both a response and an effect trait, with tunnelers being more efficient in providing several ecological functions and also being more sensitive to extinction. Consequently, functionally important tunneler species have suffered disproportionate declines, and species not threatened today may be at risk of becoming so in the near future. This linkage between effect and response traits aggravates the consequences of ongoing biodiversity loss.

Keywords: body mass, dung removal, endangered species; GHG emissions; nesting strategy; soil nutrient content.

2.1 Introduction

During the last few decades, the accelerating rate of species extinction has intensified the need to understand how biodiversity loss might alter the provisioning of ecological processes (Purvis and Hector 2000; Naeem et al. 2012). Previous studies have shown that species are not equally affected by environmental changes (Kopecky et al. 2013; Püttker et al. 2015), but instead differ in their sensitivity to disturbance. Quite worryingly, the very same species which are particularly sensitive to disturbance may also be especially important to ecosystem functions (Larsen et al. 2005). The functional consequences of extinctions will therefore depend on the strength of the link between traits rendering species more extinction-prone (so called *response traits*) and those that determine the functional contribution of a species (so called *effect traits*) (Naeem and Wright 2003; Solan et al. 2004). As a consequence of the links between species response and effect traits, the effects of environment change on species compositions may translate into changes in ecosystem functioning due to changes in the distribution of trait values represented in the modified versus original communities (Chapin et al. 2000).

Focusing on plants, Lavorel and Garnier (2002) proposed a general framework to account for relationships between effect and response traits. In brief, these authors proposed that ecosystem functioning is the end result of environmental filters operating at a hierarchy of scales which, by selecting individuals with appropriate responses, result in assemblages with varying trait composition. Functional linkages and trade-offs among traits, each of which relates to one or several processes, will then determine whether or not an individual will pass a given filter, and whether ecosystem-level effects can be easily deduced from the knowledge of the individual filters active in a given environment. The last two decades have seen a plethora of studies addressing relationships between response and effect traits (reviewed in Hevia et al. 2017). Of these, several have highlighted body mass as a response trait which may make species prone to extinction (McKinney 1997; Pimm et al. 1988) – and also affect functional efficiency, thereby rendering it an effect trait (Larsen et al. 2005).

Dung beetles (Coleoptera: Scarabaeoidea) contribute to a wide range of ecosystem services, including dung removal rates, nutrient cycling and seed dispersal (e.g. Nichols et al. 2008; Beynon et al. 2012). Biotic interactions involving dung beetles have also been suggested to affect greenhouse gas (GHG) emissions (Pentillä et al. 2013; Iwasa et al. 2015; Slade et al. 2016a; Piccini et al. 2017) and nutrient transfer from the dung to the soil (Kazuhira et al. 1991; Yamada et al. 2007; Nervo et al. 2017). Through their activity, dung beetles change the soil composition of microorganisms (Slade et al. 2016b) and the availability of nutrients with significant impacts on plant biomass (Nervo et al. 2017) and protein content (Bang et al. 2005). Thus, the functional activity of dung beetles ties together key parts of the total environment: being part of the biosphere, they rely on and directly promote the functioning of the anthroposphere (by feeding on and decomposing the dung of domestic cattle), and mitigate processes between the anthroposphere and the atmosphere (by affecting emissions of greenhouse gases from cattle dung) and the lithosphere (by affecting the release of nutrients from the dung to the soil).

Dung beetles are frequently classified according to their nesting habits: dwellers (syn. endocoprids) are species that spend their full life cycle within the dung pats, and tunnelers (syn. paracoprids) are species that reproduce in dung which they remove and bury (Hanski and Cambefort 2014). Globally, dung beetle diversity is facing many pressures (e.g. global warming, pastoral abandonment, habitat loss, modification and fragmentation, chemicals), leading to potential population declines, range contractions and species extinctions (Lobo et al. 2001; Carpaneto et al. 2007; Rosenlew and Roslin, 2008). These declines have negative impacts on ecosystem functioning (Nichols et al. 2008). Current concerns about the decline of dung beetles are particularly focused on large-bodied species, which have been suggested to be both the most extinction-prone (Larsen et al. 2008) and the most functionally efficient (Larsen et al. 2005; Lähteenmäki et al. 2015). In some cases, large-bodied species are also functionally important for ecosystems (Solan et al. 2004; Larsen et al. 2005; Slade et al. 2007). For example, larger-bodied beetles (i.e. *Geotrupes*) account for the 61% of overall dung removal in Finland (Kaartinen et al. 2013). In other cases, a direct link between size and efficiency has been challenged by experiments comparing multi-species dung beetle assemblages, including large tunnelers versus assemblages encompassing dwellers only (Nervo et al. 2014) and rollers only (Slade et al. 2007). In these studies, large tunnelers have been proven to be more efficient, not only in dung removal, but also in soil nutrient cycling (Nervo et al. 2017) and seed dispersal (Slade et al. 2007).

In this study, we target the dung beetle fauna of Sweden. We ask what traits make species particularly effective in sustaining a given function. We then examine whether the dung beetle species most prone to extinction are also the functionally most important, thereby aggravating the consequences of biodiversity loss. More specifically we ask: 1) What traits make species prone to extinction, as reflected by their current threat status; 2) Are threatened species more functionally efficient than non-threatened species; 3) What traits make dung beetle species functionally efficient; 4) Do the traits that make species more vulnerable also affect their functional efficiency; and 5) Is a species particularly efficient in promoting one ecological function also efficient in promoting others?

2.2 Materials and methods

2.2.1 Study area and mesocosm design

Many dung beetle species have suffered extensive population declines in Scandinavia (Roslin et al. 2014). Our experiment was conducted on the island of Öland (S-E Sweden), an area supporting substantial dung beetle diversity. Preliminary studies have shown that several dung beetle species which are rare or declining elsewhere are locally common in Öland (Roslin et al. 2014).

To evaluate the functional efficiency of beetle species, we used a mesocosm design. Each mesocosm was constructed as a metallic square enclosure of 1m width, 1m length and 25cm height, of which 5 cm was inserted into the soil. The mesocosms were constructed on a uniform grass sward at Station Linné (56° 37' 07'' N, 16° 29' 57'' E). Within the experimental unit, the spatial distribution of specific assemblages (Appendix, Table A1) was randomized among a set of 36 mesocosms (33 dung pats with dung beetles and 3 controls).

Dung beetles were collected manually and using pitfall traps baited with cattle dung. The collections were made in August 2016 at five localities within an overall area of ca 55 km^2. We placed 15 pitfall traps for 24 hours at the localities identified in the Appendix (Table A2).

The methods of beetle collection conformed with all applicable laws, and explicit permission for sampling in those areas was obtained from the farmers and from the responsible authority of Gårdby Natural Reserve (Länsstyrelsen i Kalmar län). After capture, individuals were stored in moist paper under cool conditions until used in the experiment.

2.2.2 Traits evaluated

Species were classified according to their threat status, individual body mass and nesting strategy. We defined a species as threatened in Scandinavia if it was classified as “Near Threatened” (NT), VUlnerable (VU), ENdangered (EN), CRitically endangered (CR) or Regionally Extinct (RE) in Sweden and/or in at least three Nordic countries (Sweden, Norway, Denmark or Finland; Roslin et al. 2014). Five species collected fitted this description: *Aphodius sordidus* (Fabricius, 1775)*, Aphodius ictericus* (Laicharting, 1781)*, Onthophagus fracticornis* (Preyssler, 1790), *Onthophagus nuchicornis* (Linnaeus, 1758) and *Trypocopris vernalis* (Linnaeus, 1758). As a reference group, we selected four non-threatened widespread species with a similar body mass to the selected 5 threatened species: *Aphodius foetens* (Fabricius, 1787), *Aphodius haemorrhoidalis* (Linnaeus, 1758), *Aphodius erraticus* (Linnaeus, 1758) and *Geotrupes spiniger* (Marsham, 1802) (Roslin et al. 2014). The species included were further classified into two nesting strategies: tunnelers (5 species) and dwellers (4 species). Together, they were chosen to represent a broad range of individual body size, from *A. haemorrhoidalis* (0.002 g) to *G. spiniger* (0.28 g), with size roughly matched between threatened and non-threatened species (Figure 1).

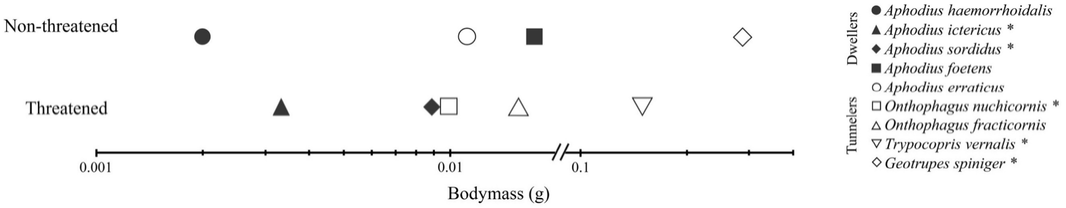

Figure 4: Species used in the experiment. Rows correspond to threat status (non-threatened species versus threatened ones), and a species' position along the x-axis reflects its body mass (note the axis break reflecting a seven-fold jump – 0.07g – in body mass). Nesting strategies are identified by symbols: filled symbols point to dwellers and open ones to tunnelers (the same symbols are used to identify the same species in Figs 2 and 5). An asterisk (*) identifies species for which the body mass was estimated by the method described in Materials and Methods. For the other species, body mass was derived from Piccini (unpublished data) and Nervo et al. (2014).

2.2.3 Experimental design

To compare the functional efficiency of threatened versus non-threatened dung beetle species, and of species with different traits (nesting strategy and body mass), we established monocultures of 9 species: 3 threatened and 2 non-threatened tunnelers, and 2 threatened and 2 non-threatened dwellers (Figure 1). For each species, we set up a series of increasing densities, where the number of individuals varied from 2 to a maximum of 64 individuals, the highest density being set by the local seasonal abundance of the species (for exact densities used, see Appendix, Table A1.

The experiment ran from 14th of August to 4th of October. Fresh dung was collected from a single herd of Swedish Red cattle grazing on grasslands dominated by graminoids. In order to avoid any previous colonization of dung pats by insects, we collected fresh dung from inside the milking barn of the farm. No cow in the herd had been treated with antibiotics or antihelmintics. The dung was homogenized before being divided into experimental pats of 1kg per mesocosm. Once the dung and beetles had been added to an enclosure, it was covered with nylon mesh cloth in order to prevent the beetles from escaping and others from entering.

2.2.4 Ecological functions investigated

To evaluate the functional efficiency of dung beetles, we focused on three types of ecological functions interconnected in the pasture ecosystem. Of these, the first one reflected impacts on cattle farming as a part of the anthroposphere (dung removal from cattle pasture), the second reflected impacts on greenhouse gas emissions, which are part of the atmosphere, and the third reflected impacts on soil nutrient contents which are part of the lithosphere. Overall, this approach resulted in 7 different response measures as mutually complementary aspects of the three main ecological functions:

1) As metrics of dung removal, we took repetitive weights of wet dung (g) over the course of the experiment. Changes in this metric are henceforth referred to as dung removal rate. We also recorded the weight of dry dung (g) remaining on the surface at the end of the experiment (henceforth remaining dung mass). By using dry weight, we controlled for any difference in evaporation, thereby isolating the contribution of the insects themselves to dung removal. Importantly, dung removal rate and final remaining dung mass are complementary aspects of the removal function, since one may arrive at the same final weight through steeper or shallower removal trajectories, with a slower removal rate resulting in undecomposed dung remaining on the pasture and retaining nutrients for a longer period. As a combination of dung removal rate and final remaining dung mass, we characterized cumulative wet mass of dung pats by calculating the area under the curve of dung wet weights over time, expressed in gram-days. Quick dung decay will be reflected by low cumulative mass, and slow decay by a high value (cf. Slade et al. 2017). Since cumulative wet mass will per necessity be closely related to other metrics of dung removal, we refrained from analyzing this metric as a separate response (see section Effects of variables on functional efficiency) and only evaluated the extent to which it covaried with other metrics (see section Correlations between functions).

2) As measures of impacts on greenhouse gas emissions from dung pats, we measured fluxes (mg m-2 h-1) of CO2 and CH4 which give an estimate of the GHG emissions per day. Moreover, we evaluated cumulative emissions (mg m-2) that give an estimate of total amount of gas emitted at the end of the experiment. Thus, we combined these cumulative emissions of CO2 and CH4 in CO2 equivalents, by converting compound-specific fluxes of CH4 by using the IPCC 2013 global warming potential (GWP). These metrics are henceforth referred to as GHG emissions. As for the other main functions addressed (above and below), the individual responses targeted reflect complementary aspects of the overall function: Daily compound-specific fluxes may hypothetically combine in multiple ways into the same total (i.e.

cumulative emissions of CO2 equivalents), and analyzing compound-specific patterns will help elucidate overall effects of total GHG fluxes.

3) As measures of impacts on nutrient transfer to the soil, we measured NH4+ and NO3- concentrations (mg kg-1) underneath the pats at the end of the experiment. These metrics are referred to as soil nutrient content, with compound-specific responses revealing different, complementary aspects of nutrients actually available to plant growth.

Individual ecological functions were measured by the following methods and techniques:

To measure dung removal rates, we recorded the mass of the dung pats (wet mass) on 6 occasions from 14 to 26 of August (i.e. 1, 2, 5, 7, 9, and 12 days from the start of the experiment). To facilitate these measures, we placed a piece of chicken wire (mesh size 2 cm) under each dung pat. At the end of the experiment (4th of October 2016), we dried the remaining dung at 70°C and recorded this final dry mass (i.e. remaining dung mass).

To evaluate gas emissions from the mesocosms, we used a non-steady-state closed chamber technique (Aim et al. 2007; Livingston and Hutchinson 1995). Gas fluxes were measured using a portable gas analyser Gasmet™ DX4015 (Gasmet Technologies Oy, Helsinki, Finland) in a closed-loop system in which the gas sample was circulated through the analyzer and back to the chamber. During the measurements, the chamber was fitted in a slit cut in the ground around the dung pat, and the chamber headspace was constantly mixed with a fan. (Since the volume of the dung pat accounted for less than 1/20 of the chamber head space, this volume was not considered in further calculations.) Once the chamber was placed into the soil, the concentration of gases per chamber was measured over a minimum period of 5 minutes. The temperature inside the chamber was recorded during each measurement and later used to correct flux estimates (for details see Appendix, Text 1). Between measurements, the chamber was removed and the mesh replaced. Gas emissions were measured on 6 occasions between 15th and 26th of August (i.e. 15th, 16th, 19th, 21st, 23rd, and 26th of August). Gas contents of CO2 and CH4 measured from the chamber headspace (in ppm) were converted into mg m−2 h−1. Cumulative fluxes of CO2 and CH4 over the course of the experiment were calculated for each enclosure and expressed as areas under the curve of the gas flux over time (for equations, see Appendix, Text 2). Two data points on methane fluxes from mesocosms with Geotrupes spiniger (with 2 and 8 individuals, respectively, as recorded on 19th and 16th August) proved completely out of range compared to all other data. These outliers were assumed to be technical errors and were omitted from all GHG analyses. To evaluate the warming potential of GHGs from dung pats, we combined the cumulative fluxes of CO2 and

CH4 by converting to CO2 equivalents, based on 100 year warming potential (IPCC 2013).

To estimate the effect of dung beetles on nutrient transfer from the dung into the soil, we collected 5 cm soil cores beneath the center of each dung pat. After removing the remaining visible plant parts, fresh soil samples were sieved at 2 mm. Inorganic N (total NH4+ and NO3-) was extracted from the moist soil samples (10 g) with 1 mol/L KCl (1:5 soil: solution ratio), suspensions were centrifuged at 1,200×g for 10 min, and the supernatant filtered first through Whatman No. 42 filter paper and subsequently through a 0.45-µm membrane filter. Moisture content was also determined on a separate aliquot of soil to allow all concentrations to be expressed as dry soil weight. The concentration of total NH4+ and NO3- was determined spectrophotometrically as described in Cucu et al. (2014).

2.2.5 Statistical analysis

Species-specific body mass – Since we used several endangered species in the experiment, we refrained from estimating the species-specific body mass from any extensive, purpose-collected and dried material. Instead, we estimated the species dry weight from a regression model described below. Thus, to estimate the body mass of all dung beetle species present in Scandinavia, we used size data (i.e. body-mass and length) available for Aphodius haemorrhoidalis, Aphodius erraticus, Onthophagus fracticornis, Anoplotrupes stercorosus and Geotrupes stercorarius. For these species, $\log_e$-transformed dry body mass (Nervo et al. 2014, and Piccini unpublished data) was regressed against the $\log_e$-transformed mean length of the species (R2=0.98, with length data extracted from Roslin et al. 2014). Following Radtke and Williamson (2005), we then interpolated the body mass of remaining species using the resultant regression line and the typical length of species (as adopted from Roslin et al. 2014).

Identification of response traits – In order to identify which traits make species more sensitive to extinction, we examined whether species threatened in Scandinavia shared some specific traits. More specifically, to evaluate the effect of traits (nesting strategy and body mass) on species' response, we built a generalized linear model (GLM) threat status of all Scandinavian species (threatened versus non-threatened; 0/1), as a function of nesting strategy and body mass, assuming a logit link function and binomially distributed errors. This model was fitted using the 'stats' package in the R (v3.2.1) statistical and programming environment (R Development Core Team 2005). To check for overdispersion of residuals from the final model, we divided the residual deviance by the degrees of freedom. Since the result was 1.2 and thus close to unity, and closely adhered to both binomial and quasi-binomial distribution families (P>0.32), we inferred no signs of overdispersion.

Effects of variables on functional efficiency – To identify how body mass and/or nesting strategy affect the functional efficiency of the species, we applied the framework of generalized linear mixed effects models to our hierarchical, partly repeated data structure (e.g. Zuur et al. 2009). Remaining dung mass (pat-specific dry weight), dung removal rates (pat-specific wet weight trends over time), nutrient content in the soil and GHG emissions, were first adopted as metrics of ecological functioning. Since plots of dung mass over time were generally indicative of exponential decline, we linearized the relation by applying a natural logarithmic transformation ($\log_e$). Each response (dry dung weight, NH4+, NO3-, and cumulative emissions of CO2, CH4 and CO2-equivalents) was then modelled as a separate linear function of $\log_e$-transformed species body mass (Body_mass) and densities (Density) as continuous variables, and nesting strategy (Nesting_Strategy) and threat status (Threat_Status) as categorical variables. Here, the density will capture the change in functioning with the addition of individuals of the focal species, and the effect of body mass will reflect the change in functioning with increasing species size. Thus, our focal interest concerns the interactions Body_mass × Density; Nesting_Strategy × Density; Threat_Status × Density and Body_mass × Nesting_Strategy × Density, which capture the extent to which these effects vary with the body mass and nesting strategy of the species. To adjust for variation between species, species identity was included as a random intercept. Data from beetle-free control mesocosms were excluded from these analyses.

For dung removal rates and GHG emissions, our data consisted of repetitive measures over time (of wet weight, CO2 and CH4 fluxes, respectively). Hence, to adjust for multiple observations of the same units (i.e. for the separate variation arising from the fact that we repeatedly inspected particular mesocosms and particular species, rather than repeatedly randomized units), we included species and mesocosms as a random intercept and measurement day as a random slope term. For dung removal rates and CH4 emissions, where daily emissions varied in a highly non-linear pattern, we modelled measurement day as a fixed categorical effect.

Following the principle of model reduction, we removed the three-way interaction from models when non-significant (i.e. from the models of GHG emissions and soil nutrient content). Each model was fitted using the 'lmerTest' package in the R (v3.2.1) statistical and programming environment (R Development Core Team 2005), assuming an identity link and normally distributed errors. P-values were estimated using type III F-tests with the Satterthwaite approximation of the relevant degrees of freedom (Satterthwaite 1946).

Correlations between functions –To establish whether a species that was particularly efficient in promoting one ecological function was also efficient in promoting others, we analyzed the pairwise relationships between the mesocosm-specific rates of each ecological function (n=36 per function). For gas fluxes, we used the cumulative emissions (mg m-2) observed during the course of the experiment (of CO2, CH4 and combined emissions of CO2 and CH4, in CO2 equivalents, respectively). In a logically equivalent vein, we characterized dung decomposition rates by the cumulative wet mass of dung pats (called 'Cumulative wet dung' in Figure 6).

2.3 Results

2.3.1 Identification of response traits

Among 61 dung beetle species encountered in Sweden, 45 are dwellers and 16 are tunnelers. Of these, 13 out of 45 dweller species and 12 out of 16 tunneler species are currently regarded as threatened in Sweden or in at least three Scandinavian countries (Table 3 from Roslin et al. 2014). Indeed, nesting strategy is significantly related to threat status (Z=2.19, p=0.03), whereas neither biomass (Z=-0.47; p=0.64), nor the interaction between nesting strategy and biomass (Z=0.42, p=0.67), has a detectable impact on threat status.

2.3.2 Species threat status versus functional efficiency

Overall, threat status influenced neither dung removal nor nutrient content in the soil. However, threat status had a significant impact on GHG emissions from dung pats – more specifically, on CH4 emissions. Significantly less methane was released into the atmosphere with an increase in the density of threatened species than with an equivalent increase of non-threatened species (interaction Density × Threat_Status: F1;182.4= 5.31, p=0.022; Figure 2; Appendix, Table A5).

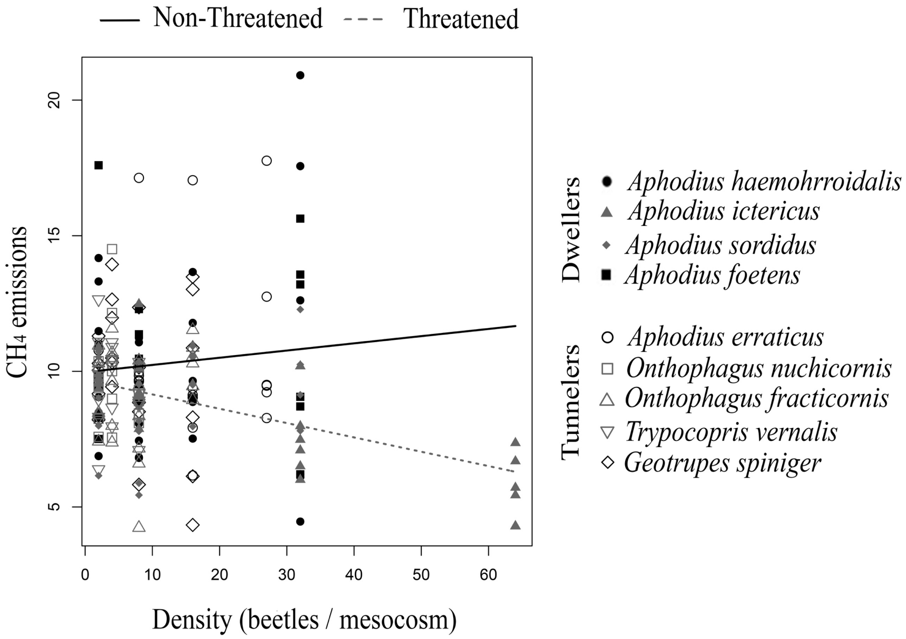

Figure 2: Threat status versus functional efficiency in terms of methane emissions. Shown is the interaction between threat status (black line for non-threatened and grey dashed line for threatened species) and the number of individuals per mesocosm (density) for methane emissions (mg m-2 h-1). The data points show partial residuals, with the lines derived from the linear regression described in Materials and Methods. Symbols identify species, where black corresponds to non-threatened species and grey to threatened species; filled symbols identify dwellers and open symbols tunnelers.

2.3.3 Species traits versus functional efficiency

Remaining dung mass – Density had a general, significant effect on the amount of remaining dung (Density: F1;19.31= 34.36, p<0.001; Appendix, Table A3). Nonetheless, there was a strong interaction between density, body mass and nesting strategy (interaction Density × Body_mass × Nesting_Strategy: F1;19.52= 33.80, p<0.001), with large tunneling species proving the most effective in removing dung per individual (Figure 3a; Appendix, Table A3).

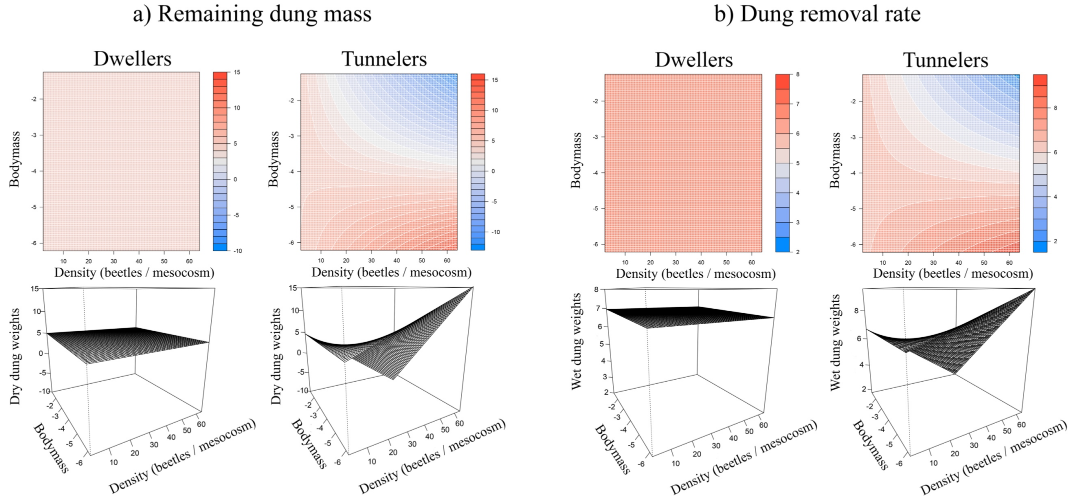

Figure 3: Interaction between body mass and nesting strategy in determining ecological functioning. Shown is the significant interaction between the species body mass, nesting strategy and the number of individuals per mesocosm for dung removal, measured as: (a) remaining dung mass (g) and (b) dung removal rate (g). The two rows of graphs represent the same interactions from different perspectives; the top row shows two-dimensional heat maps of the three-dimensional surfaces represented on the lower row. In the heat maps, a redder color represents more dung remaining on the surface whereas a bluer color represents less dung remaining.

DUNG REMOVAL RATE – Dung wet weight progressively decreased over the course of the experiment (Measurement days: $F_{1;183.21}$=125.83, p<0.001; Appendix, Table A4), with a significant effect of density ($F_{1;186.84}$=27.86, p<0.001; Appendix, Table A4). However, both nesting strategy and body mass affected the density-specific dung removal rates per individual (interaction Density × Nesting_Strategy × Body_mass: $F_{1;186.16}$ =26.93, p<0.001), with larger tunnelers removing more dung per individual than small dwellers (Figure 3b; Appendix, Table A4).

GHG EMISSIONS – Overall, the amount of GHG emissions decreased with time (Measurement days: $F_{1;177.97}$=43.22, p<0.001; Appendix, Table A5) and density (Density: $F_{1;181.93}$=5.34, p=0.02; Appendix, Table A5). However, the per capita effect of dung beetles on CH_4 emissions drastically increased with an increase in individual body mass (interaction Density × Body_mass: $F_{1;181.74}$=65.20, p=0.024; Figure 4; Appendix, Table A5). At high density emissions of CH_4 are higher when body mass is smaller, conversely emissions are lower when body mass is higher (right-hand panel in Figure 4). Neither nesting strategy nor body mass had any detectable effect on fluxes of CO_2 or CO_2-equivalents, or on cumulative emissions of any of the three responses (CO_2, CH_4 and CO_2-equivalents).

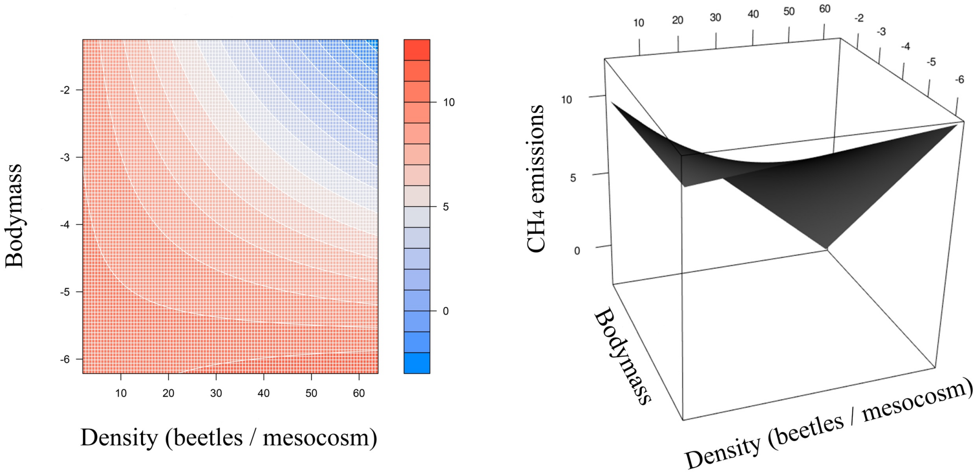

Figure 4: The effect of species body mass on methane emissions. Shown is the significant interaction between species body mass and the number of individuals per mesocosm in determining methane emissions from dung pats (mg m-2 h-1). The two graphs represent the same interactions from different perspectives; the left-hand panel shows a two-dimensional heat map of the three-dimensional surface represented on the right. In the heat maps, a redder color represents more methane emissions whereas a bluer color represents less methane emissions.

NUTRIENT CONTENT OF THE SOIL – Dwellers affected ammonium concentration in soil more than tunnelers (Nesting strategy: $F_{1;24.99}$= 4.11, p=0.053; Appendix, Table A6). However, NH_4^+ concentration in the soil underneath dung pats showed a tendency towards higher values with higher densities of tunnelers in the pat, whereas there was a decrease in NH_4^+ concentration with increasing dweller density (interaction Density × Body_mass: $F_{1;24.99}$=3.28, p=0.082; Figure 5; Appendix, Table A6). NO_3^- concentration showed no detectable change with either nesting strategy or biomass.

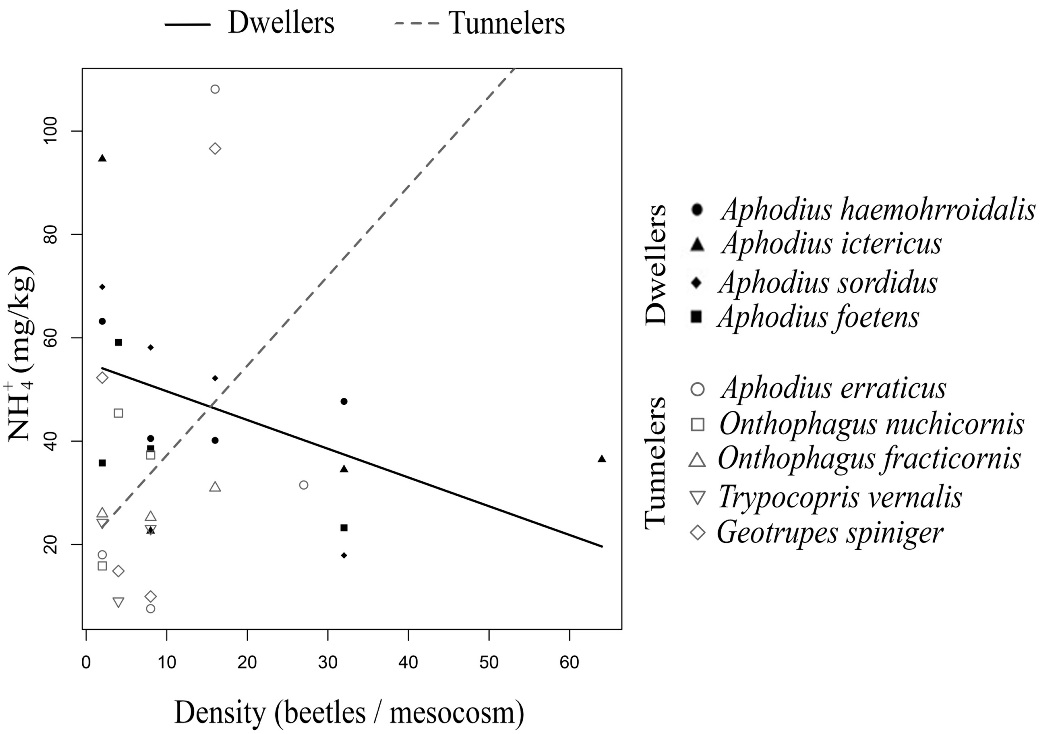

Figure 5: The effect of nesting strategy on nutrient cycling. Shown is the marginally significant interaction between density and nesting strategy (black line for dwellers and grey dashed line for tunnelers) in determining ammonium concentration in the soil (mg kg^{-1}). The data points show partial residuals, with the lines derived from the linear regression described in Materials and Methods. Symbols identify species, filled symbols identify dwellers and open symbols tunnelers.

2.3.4 Comparison of different functions

In terms of the multiple ecological functions investigated in this experiment, we found highly variable relationships between individual pairs of functions (Figure 6). Overall, several functions were only weakly related to each other (as illustrated by light colors in Fig. 6). In terms of the stronger associations observed, some were self-evident. For example, since more wet dung at an early stage will result in more dry dung at the end of the experiment, these two metrics were naturally highly correlated (Figure 6). Likewise, since overall CO2 equivalents are composed of CO_2 and CH_4, all three quantities were correlated, but this correlation was stronger for CO_2 as it accounts for the main part of the combined fluxes (Fig. 6). However, a higher dung mass was primarily correlated with higher CH_4 fluxes, whereas higher emissions of CO_2 were associated with a lower mass of remaining dung. Higher transfer of NH_4^+ to the soil was also associated with higher transfer NO_3, and with less dung remaining at the soil surface (Figure 6).

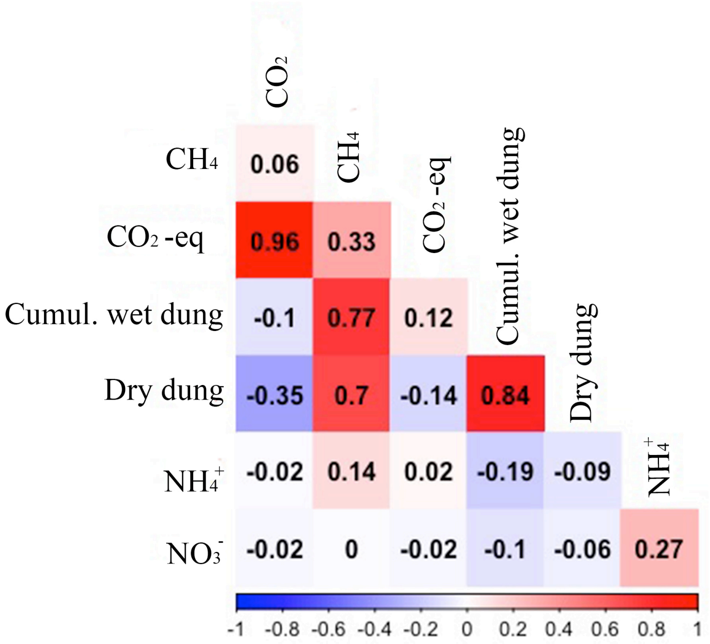

Figure 6. Associations between pairs of ecological functions. Shown are Pearson's correlation coefficients between different aspects of ecosystem functioning (cumulative fluxes of carbon dioxide (CO_2), methane (CH_4) and both gases (CO_2+CH_4) combined into CO_2 equivalents, cumulative weight of wet dung remaining on the ground over time (Cumul. wet dung), total dry dung remaining at the end of the experiment, ammonium and nitrate concentration found in soil beneath dung pats). Red reflects positive correlations, blue corresponds to negative correlations, with the darkness of the color reflecting the strength of association.

2.4 Discussion

Species extinction is a non-random process (Purvis et al. 2000a, b; Ives and Cardinale 2004; Vamosi and Wilson et al. 2008), with some species being more sensitive than others to joint environmental stressors. Of particular concern is the extinction of functionally important species linking multiple parts of the environment. The strength of the link between traits rendering species more extinction-prone and those that determine the functional contribution of a species will depend on how they are jointly filtered by the environment (Lavorel and Garnier 2002). In our study, we sought for traits affecting the efficiency of dung beetle species in determining the functioning of different parts of the pasture environment. We also compared the functional efficiency of non-threatened versus threatened dung beetle species across this range of ecological functions. In doing so, we asked whether traits which make species sensitive to disturbance also make these species functionally efficient. Overall, we found that nesting strategy has a strong impact on the probability with which a species is threatened. In terms of functional efficiency, the impact of specific traits depends on the exact ecological functions investigated. However, the key trait associated with threat status (nesting strategy) was also associated with functional efficiency across a range of functions, as was body mass. Within threat categories, non-threatened species differed from threatened ones in terms of functional efficiency only for methane emissions. Below, we will discuss each of these findings in turn.

2.4.1 Nesting strategy is a response trait

Among dung beetles, large species have been proposed as being more prone to extinction than small species (Larsen et al. 2005, Larsen et al. 2008). Yet, in the set of Scandinavian dung beetle species, individual size appeared to have little effect, whereas nesting strategy left a consistent imprint on threat status. This feature in the life history of species is closely associated with phylogeny. Tunnelers belonging to the subfamily Scarabaeinae (of which 100% are threatened) seem more sensitive to environment change than species in Geotrupinae (of which 40% are threatened) and Aphodiinae (of which 30% are threatened). In fact, all Scandinavian species of Scarabaeinae are either currently classified as threatened, or have been so in previous, national red lists (Roslin et al. 2014). As a consequence, a much larger proportion of tunnelers (75%) than dwellers are currently threatened (30%), with taxonomy as good a predictor of threat status as nesting habit *per se*, since the two are intimately related.

As a potential explanation for this *status quo*, Roslin et al. (2014) have proposed that tunnelers have suffered disproportionately from the decline in cattle farming and thus grazing pressure across Scandinavia. Being characterized by a long larval period spent under the soil surface, these taxa may be particularly sensitive to the amount of sunlight reaching the soil surface, and thus prone to the adverse effect of decreased grazing pressure coupled with increased vegetation height and decreased insolation. This is particularly valid for Scarabaeinae, since species belonging to this subfamily have limited fecundity (a single ovary) and a general lack of adaptation to cool climates. Contrary to the patterns found by Larsen et al. (2005), extinction sensitivity for Swedish species is not detectably related to the biomass of the species. Thus, biomass itself does not explain a species' vulnerability to extinction (an observation supported by Foufopoulos and Ives 1999). Naturally, threat status could also be related to some other traits not investigated here, making the species more resistant to stressors (e.g. physiological traits such as temperature tolerance and metabolic capacity; Moretti et al. 2016).

2.4.2 Nesting strategy and body mass are sometimes effect traits

The trait that makes a species functionally efficient depends on the ecological function investigated. More specifically, both body mass and nesting strategy affect dung removal rates, as equally reflected by the decline in wet weight over time and the dung mass remaining at the end of the experiment. Large tunnelers have been previously shown to have the largest effect on dung removal (Slade et al. 2007; Amézquita and Favila 2010; Kaartinen et al. 2013; Nervo et al. 2014, 2017). Moreover, we identified body mass as a pivotal trait affecting methane fluxes from dung pats, with larger species reducing methane emissions more per individual than smaller ones. This finding is likely due to the fact that large beetles excavate larger holes and galleries inside and below the dung mass, consequently aerating the dung and reducing methane formation by anaerobic archaea (cf. Piccini et al. 2017).

Nesting strategy also affected the nutrient content of the soil. Tunneling species transport more dung into the soil, and are therefore more efficient than dwellers in soil fertilization (Yamada et al. 2007). Indeed, the concentration of ammonium in the soil increased with an increase in tunneler density. On the other hand, soil ammonium concentration decreased when dweller density increased. This pattern was probably related to a greater consumption of nutrients in the dung by dweller adults and larvae, thus tying up nitrogen as protein rather than releasing it. Overall, increasing the number of small-bodied dwellers in the mesocosms did not markedly change the provisioning of ecological functions. In contrast, when large-bodied tunnelers were added, increased functioning was generally observed. In Sweden, small-sized dwellers typically make up the main part of the dung-inhabiting beetle community, whereas large tunnelers are scarcer but have proven to be the most functionally efficient (Kaartinen et al. 2013). This suggests that the loss of such larger species may have particularly important consequences for the functioning of the overall ecosystem.

2.4.3 Effect and response traits are linked

Among dung beetles, large species have been proposed to be both more prone to extinction and more functionally efficient than small ones (Larsen et al. 2005). In our experiment, non-threatened species did not detectably differ from threatened ones in terms of efficiency, except for methane emissions from dung pats, where threatened species proved more efficient than non-threatened species.

This difference could not be specifically attributed to any of the traits that we measured.

Overall, our study identifies nesting strategy as a key response trait, with a major impact on species' threat status in Scandinavia. At the same time, the sensitive, tunneling species are also efficient providers of ecosystem functioning, especially in terms of dung removal and increasing nutrient content in the soil. Thus, our study identifies nesting strategy as both a response and an effect trait. Links between response and effect traits have also been observed in several other studies (reviewed in Hevia et al. 2017), including some plants (Lavorel and Garnier 2002; Violle et al. 2007; Suding et al. 2008) and among plants and grasshoppers (Moretti et al. 2013). Among the current set of dung beetles, the link can be attributed to the relocation of dung into the soil for breeding, which simultaneously makes tunnelers more functionally efficient (by increasing dung removal rates, promoting nutrient transfer and decreasing dung mass remaining on the surface) and more sensitive to environmental stressor. By breeding in the soil, they become sensitive to current changes in the Scandinavian landscape, including increased vegetation height, less insolation and thus lower soil temperatures. These effects may prolong critically larval development in the cool climate of higher latitudes (Roslin et al. 2014).

2.4.4 Dung beetle traits influence the overall pasture environment

Recent research has focused on the complex relations between species richness and ecosystem multi-functionality (Wagg et al. 2014; Maestre et al. 2012; Hector and Bagchi 2007). Instead of using a single function as a proxy for overall ecosystem functioning (cf. the critique by Rosenfeld 2002, Nervo et al. 2017), we estimated the influence of dung beetles on a range of functions linking the different spheres of the pasture ecosystem: the amount of soil nutrients (lithosphere) and GHG emissions (atmosphere) through transfer and decomposition of dung (i.e. dung removal rate and remaining dung mass). While we were only able to address a few selected responses per sphere, we found different functions to vary in discord rather than in concert: beyond obvious relations between wholes and their parts (see Results), individual functions were mostly weakly correlated (Fig. 6). Fluxes of different GHGs were, in fact, negatively related: greater dung mass on the soil surface was associated with higher CH_4 fluxes, but lower emissions of CO_2. This difference is likely related to their different modes of emergence. CO_2 is mostly a product of aerobic respiration, resulting in higher dung decomposition. CH_4 is primarily generated under anaerobic conditions (Penttilä et al. 2011), as favored by higher and wetter dung mass. Overall, different dung beetle species with different impacts on dung aeration and dung removal will thus have different impacts on GHG fluxes (Piccini et al. 2017). These findings illustrate the general importance of maintaining diverse dung beetle communities to provide multiple ecosystem services (Benyon et al. 2012; Manning et al. 2016; Piccini et al. 2017; Slade et al. 2017).

2.5 Conclusions

The results presented here identify dung beetles as key engineers of the pasture environment, influencing GHG emissions to the atmosphere, nutrient cycling between dung and soil and the amount of dung and the time it remains on the surface of pastures. Our findings suggest that some of the same traits which make Scandinavian dung beetle species sensitive to environmental change will also render them functionally efficient, and thus they fit squarely with the paradigm that functional and response traits are linked. This is a worrying scenario, since it implies both that some of the most functionally important species are already threatened, and that further species, although not threatened to date, are likely to become so in the near future. To conserve the functional integrity of pasture ecosystems, we should therefore conserve species of the highest functional efficiency, with the highest risk of suffering decline. Among dung beetles, large tunnelers should be the first priority. Yet, two observations add nuances to this simplistic rule of thumb: first, the observation that different ecosystem functions vary in discord rather than unison, and second, that several functions were only weakly related to any of the traits examined here. Both considerations highlight the importance of maintaining diverse communities rather than dung beetles of a single type. Given the paramount impact of pasture processes on global change (Slade et al. 2016a), maintaining their functional integrity emerges as a key concern – a task which we can only achieve by understanding how their functioning hinges on the composition of local communities.

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References

Aim, J., N. J. Shurpali, E. Tuittiia, T. Lauriia, M. Maljanen, S. Saarnio, and K. Minkkinen. 2007. Methods for determining emission factors for the use of peat and peatlands—flux measurements and modelling. Boreal Environment Research 12.

Amézquita, S., and M. E. Favila. 2010. Removal Rates of Native and Exotic Dung by Dung Beetles (Scarabaeidae: Scarabaeinae) in a Fragmented Tropical Rain Forest. Environmental Entomology 39:328–336.

Bang, H. S., J.-H. Lee, O. S. Kwon, Y. E. Na, Y. S. Jang, and W. H. Kim. 2005. Effects of paracoprid dung beetles (Coleoptera: Scarabaeidae) on the growth of pasture herbage and on the underlying soil. Applied Soil Ecology 29:165–171.

Beynon, S. A., D. J. Mann, E. M. Slade, and O. T. Lewis. 2012. Species-rich dung beetle communities buffer ecosystem services in perturbed agro-ecosystems. Journal of Applied Ecology 49:1365–1372.

Carpaneto, G. M., Mazziotta, A. and Valerio, L. 2007. Inferring species decline from collection records: roller dung beetles in Italy (Coleoptera, Scarabaeidae). Diversity and Distributions, 13:903-919.

Chapin, F. S. III, Zavaleta, E. S., Eviner, V. T. and Naylor, R. L. (2000). Consequences of changing biodiversity. Nature, 405(6783), 234.

Cucu, M. A., Said-Pullicino, D., Maurino, V., Bonifacio, E., Romani, M. and Celi, L. 2014. Influence of redox conditions and rice straw incorporation on nitrogen availability in fertilized paddy soils. Biology and fertility of soils 50:755-764.

Foufopoulos, J., and A. R. Ives. 1999. Reptile Extinctions on Land-Bridge Islands: Life-History Attributes and Vulnerability to Extinction. The American Naturalist 153:1–25.

Hanski, I., and Y. Cambefort. 2014. Dung Beetle Ecology. Princeton University Press.

Hector A. and Bagchi R. 2007. Biodiversity and ecosystem multifunctionality. Nature 448:188–190.

Hevia, V., B. Martín-López, S. Palomo, M. García-Llorente, F. Bello, and J. A. González. 2017. Trait-based approaches to analyze links between the drivers of change and ecosystem services: Synthesizing existing evidence and future challenges. Ecology and Evolution 7:831–844. doi:10.1002/ece3.2692.

Ives, A. R., and B. J. Cardinale. 2004. Food-web interactions govern the resistance of communities after non-random extinctions. Nature 429:174–177.

Iwasa, M., Y. Moki, and J. Takahashi. 2015. Effects of the Activity of Coprophagous Insects on Greenhouse Gas Emissions from Cattle Dung Pats and Changes in Amounts of Nitrogen, Carbon, and Energy. Environmental Entomology 44:106–113.

Kaartinen, R., B. Hardwick, and T. Roslin. 2013. Using citizen scientists to measure an ecosystem service nationwide. Ecology 94:2645–2652.

Kazuhira, Y., K. Hdeaki, K. Takuro, and A. Toshiharu. 1991. Nitrogen mineralization and microbial populations in cow dung, dung balls and underlying soil affected by paracoprid dung beetles. Soil Biology and Biochemistry 23:649–653.

Kopecký, M., R. Hédl, and P. Szabó. 2013. Non-random extinctions dominate plant community changes in abandoned coppices. Journal of Applied Ecology 50:79–87.

Lähteenmäki, S., E. M. Slade, B. Hardwick, G. Schiffler, J. Louzada, J. Barlow, and T. Roslin. 2015. MESOCLOSURES – increasing realism in mesocosm studies of ecosystem functioning. Methods in Ecology and Evolution 6:916–924.

Larsen, T. H., Lopera, A. and Forsyth, A. 2008. Understanding Trait-Dependent Community Disassembly: Dung Beetles, Density Functions, and Forest Fragmentation. Conservation Biology 22:1288-1298.

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

Lavorel, S., and E. Garnier. 2002. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. Functional Ecology 16:545–556.

Livingston, G. P., and G. L. Hutchinson. 1995. Enclosure-based measurement of trace gas exchange: applications and sources of error. Biogenic trace gases: measuring emissions from soil and water:14–51.

Lobo, J. M. 2001. Decline of roller dung beetle (Scarabaeinae) populations in the Iberian peninsula during the 20th century. Biological Conservation 97:43–50.

Maestre F.T., Quero J.L., Gotelli N.J., Escudero A., Ochoa V., Delgado-Baquerizo M., García-Gómez M., Bowker M.A., Soliveres S., Escolar C., García-Palacios P., Berdugo M., Valencia E., Gozalo B., Gallardo A., Aguilera L., Arredondo T., Blones J., Boeken B., Bran D., Conceição A.A., Cabrera O., Chaieb M., Derak M., Eldridge D.J., Espinosa C.I., Florentino A., Gaitán J., Gatica M.G., Ghiloufi W., Gómez-González S., Gutiérrez J.R., Hernández R.M., Huang X., Huber-Sannwald E., Jankju M., Miriti M., Monerris J., Mau R.L., Morici E., Naseri K., Ospina A., Polo V., Prina A., Pucheta E., Ramírez-Collantes D.A., Romão R., Tighe M., Torres-Díaz C., Val J., Veiga J.P., Wang D. and Zaady E. 2012. Plant Species Richness and Ecosystem Multifunctionality in Global Drylands. Science 335:214–218.

Manning, P., Slade, E. M., Beynon, S. A. and Lewis, O. T. 2016. Functionally rich dung beetle assemblages are required to provide multiple ecosystem services. Agriculture, Ecosystems & Environment, 218:87-94.

McKinney, M. L. 1997. Extinction vulnerability and selectivity: combining ecological and paleontological views. Annual Review of Ecology and Systematics, 28:495-516.

Moretti, M., Bello, F., Ibanez, S., Fontana, S., Pezzatti, G. B., Dziock, F., Rixen, C., Lavorel, S. 2013. Linking traits between plants and invertebrate herbivores to track functional effects of land-use changes. Journal of Vegetation Science, 24:949-962.

Moretti, M., A. T. C. Dias, F. de Bello, F. Altermatt, S. L. Chown, F. M. Azcárate, J. R. Bell, B. Fournier, M. Hedde, J. Hortal, S. Ibanez, E. Öckinger, J. P. Sousa, J. Ellers, and M. P. Berg. 2017. Handbook of protocols for standardized measurement of terrestrial invertebrate functional traits. Functional Ecology 31:558–567.

Naeem, S., J. E. Duffy, and E. Zavaleta. 2012. The Functions of Biological Diversity in an Age of Extinction. Science 336:1401–1406.

Naeem, S., and J. P. Wright. 2003. Disentangling biodiversity effects on ecosystem functioning: deriving solutions to a seemingly insurmountable problem. Ecology Letters 6:567–579.

Nervo, B., E. Caprio, L. Celi, M. Lonati, G. Lombardi, G. Falsone, G. Iussig, C. Palestrini, D. Said-Pullicino, and A. Rolando. 2017. Ecological functions provided by dung beetles are interlinked across space and time: evidence from 15N isotope tracing. Ecology 98:433–446.

Nervo, B., C. Tocco, E. Caprio, C. Palestrini, and A. Rolando. 2014. The Effects of Body Mass on Dung Removal Efficiency in Dung Beetles. PLOS ONE 9:e107699.

Nichols, E., S. Spector, J. Louzada, T. Larsen, S. Amézquita, and M. E. Favila. 2008. Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. Biological Conservation 141:1461–1474.

Penttilä, A., E. M. Slade, A. Simojoki, T. Riutta, K. Minkkinen, and T. Roslin. 2013. Quantifying Beetle-Mediated Effects on Gas Fluxes from Dung Pats. PLOS ONE 8:e71454.

Piccini, I., F. Arnieri, E. Caprio, B. Nervo, S. Pelissetti, C. Palestrini, T. Roslin, A. Rolando. 2017. Greenhouse gas emissions from dung pats vary with dung beetle species and with assemblage composition. PLOS ONE 12:e0178077.

Pimm, S. L., H. L. Jones, and J. Diamond. 1988. On the Risk of Extinction. The American Naturalist 132:757–785.

Purvis, A., P.-M. Agapow, J. L. Gittleman, and G. M. Mace. 2000a. Nonrandom Extinction and the Loss of Evolutionary History. Science 288:328–330.

Purvis, A., J. L. Gittleman, G. Cowlishaw, and G. M. Mace. 2000b. Predicting extinction risk in declining species. Proceedings of the Royal Society of London B: Biological Sciences 267:1947–1952.

Purvis, A., and A. Hector. 2000. Getting the measure of biodiversity. Nature 405:212–219.

Püttker, T., A. de Arruda Bueno, P. I. Prado, and R. Pardini. 2015. Ecological filtering or random extinction? Beta-diversity patterns and the importance of niche-based and neutral processes following habitat loss. Oikos 124:206–215.

Radtke, M. G., and G. B. Williamson. 2005. Volume and Linear Measurements as Predictors of Dung Beetle (Coleoptera: Scarabaeidae) Biomass. Annals of the Entomological Society of America 98:548–551.

Rosenfeld J.S. 2002. Functional redundancy in ecology and conservation. Oikos 98:156–162.

Rosenlew, H., and T. Roslin. 2008. Habitat fragmentation and the functional efficiency of temperate dung beetles. Oikos 117:1659–1666.

Roslin T., M. Forshage, F. Ødegaard, C. Ekblad, G. Liljeberg. 2014. Nordens dyngbaggar (Dung beetles of Northern Europe) - Tibiale, Helsinki

Satterthwaite, F. E. 1946. An approximate distribution of estimates of variance components. Biometrics bulletin: 2:110-114.

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

Slade, E. M., T. Riutta, T. Roslin, and H. L. Tuomisto. 2016a. The role of dung beetles in reducing greenhouse gas emissions from cattle farming. Scientific reports 6.

Slade, E. M., T. Roslin, M. Santalahti, and T. Bell. 2016b. Disentangling the "brown world" faecal–detritus interaction web: dung beetle effects on soil microbial properties. Oikos 125:629–635.

Slade, E. M., Kirwan, L., Bell, T., Philipson, C., Lewis, O., Roslin, T. 2017. The importance of species identity and interactions on multifunctionality depends on how ecosystem functions are valued. Ecology.

Solan, M., B. J. Cardinale, A. L. Downing, K. A. M. Engelhardt, J. L. Ruesink, and D. S. Srivastava. 2004. Extinction and Ecosystem Function in the Marine Benthos. Science 306:1177–1180.

Suding, K. N., S. Lavorel, F. S. Chapin, J. H. C. Cornelissen, S. Díaz, E. Garnier, D. Goldberg, D. U. Hooper, S. T. Jackson, and M.-L. Navas. 2008. Scaling environmental change through the community-level: a trait-based response-and-effect framework for plants. Global Change Biology 14:1125–1140.

Vamosi, J. C., and J. R. U. Wilson. 2008. Nonrandom extinction leads to elevated loss of angiosperm evolutionary history. Ecology Letters 11:1047–1053.

Violle, C., M.-L. Navas, D. Vile, E. Kazakou, C. Fortunel, I. Hummel, and E. Garnier. 2007. Let the concept of trait be functional! Oikos 116:882–892.

Wagg C., Bender S.F., Widmer F. and Van der Heijden M.G.A. 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences, 111:5266–5270.

Yamada, D., O. Imura, K. Shi, and T. Shibuya. 2007. Effect of tunneler dung beetles on cattle dung decomposition, soil nutrients and herbage growth. Grassland Science 53:121–129.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Gail M, Krickeberg K, Samet JM, Tsiatis A, Wong W, editors. New York, NY: Spring Science and Business Media.

Appendix

Text 1. Calculation of GHG flues

In order to estimate the flux for CO_2 and CH_4, we converted the ppm measured by portable gas analyser Gasmet™ DX4015 (Gasmet Technologies Oy, Helsinki, Finland) into mg m−2 h−1.
To do so, we transformed ppm into mg/m3 per each gas measure: ppm → mg/m3.
In order to do this, we reversed the ideal gas law and we obtained: N (quantity of compound) / V (volume) =p (pressure) / R (constant) T (temperature).
We applied this formula to our data:

N [mg] / V [m3] = (ppm/10^6 × (molar Weight ×1000) [**mg**]/[mol] × p [atm]) / (R [**m3** atm / K mol] × T [K].

T was the temperature recorded during the measurements inside the chambers. The p is the environmental pressure. To extrapolate only parts from parts per million, ppm are divided per 10^6.
Simplifying the units, mg/m3 remained, as intended.

In order to obtain the mg, we multiplied by the total volume of the chamber:

C [mg] = n [mg] / V [m3] × Volume_chamber [m3]

We calculated **C** for each estimate recorded by GASMET.

We considered that the emissions from each dung pat were linear over time, and consequently we evaluated the linear regression for each pat each day over the measurement time, which was at least 5 minutes (we recorded 36 measurements, i.e. the total number of chambers, per each recording day).
To evaluate the flux, we divided the slope obtained from the regression model for the total area of the chamber [m^2] and multiplied by 3600 seconds (i.e. the no. seconds in an hour):

Flux [mg/m^2×h] = slope / (Area_chamber) × 3600.

Text 2. Calculation of area under the flux curve

To calculate the cumulative emissions for both CO_2 and CH_4 analysed, we estimated the area under the interpolation line of the measurement points. Each measurement point (i) has measurement day as X_i and GHG emission as Y_i. The formula is: $(x_{i+1} - x_i)×[(\max(y_{i+1};y_i) – \min(y_{i+1};y_i)) / 2 + \min(y_{i+1};y_i)]$.

Table A1: ***Experimental design.*** *Shown is the number of individuals included in each mesocosm. Tunnelers are identified by the upper black frame, dwellers by the lower dark grey frame. Grey rows identify non-threatened species, whereas white rows identify threatened species. * For the highest density of Aphodius erraticus, we placed 27 individuals instead of 32, due to limited availability.*

Species	Number of individuals per species					
	2	**4**	**8**	**16**	**32**	**64**
Tunnelers	*G. spiniger*	*G. spiniger*	*G. spiniger*	*G. spiniger*		
	T. vernalis	*T. vernalis*	*T. vernalis*			
	A. erraticus		*A. erraticus*	*A. erraticus*	*A. erraticus**	
	O. nuchicornis	*O. nuchicornis*	*O. nuchicornis*			
	O. fracticornis	*O. fracticornis*	*O. fracticornis*	*O. fracticornis*		
Dwellers	*A. foetens*		*A. foetens*		*A. foetens*	
	A. sordidus		*A. sordidus*	*A. sordidus*	*A. sordidus*	
	A.haemorrhoidalis		*A.haemorrhoidalis*	*A.haemorrhoidalis*	*A.haemorrhoidalis*	
	A. icterıcus		*A. icterıcus*		*A. icterıcus*	*A. icterıcus*

Table A2: ***Details of dung beetle collection****. Shown are species name, collection localities, dates and collecting method. Only species used in the experiment are included in the table, whereas added species were encountered in the field (Aphodius fossor, A. rufipes, A. pedellus, A. rufus, A. pusillus, A. ater, A. borealis, A. zenkeri and Copris lunaris). Note that individuals of different origin were randomly mixed before being distributed among the mesocosms. Beetle collection was carried out by Mattias Forshage, Beatrice Nervo and Irene Piccini.*

Species	**Number**	**Locality**	**Nearest village**	**Dung**	**Method**
Aphodius erraticus	28	Degerrörsbacken NR	Kåtorp	horse	hand-picked
Aphodius erraticus	13	Degerrörsbacken NR	Kåtorp	cow	pitfall trap
Aphodius foetens	23	Degerrörsbacken NR	Kåtorp	horse	hand-picked
Aphodius foetens	1	Degerrörsbacken NR	Kåtorp	cow	pitfall trap

Aphodius haemorrhoidalis	13	Degerrörsbacken NR	Kåtorp	horse	hand-picked
Aphodius ictericus	1	Degerrörsbacken NR	Kåtorp	horse	hand-picked
Aphodius ictericus	3	Degerrörsbacken NR	Kåtorp	cow	pitfall trap
Aphodius sordidus	2	Degerrörsbacken NR	Kåtorp	horse	hand-picked
Aphodius sordidus	2	Degerrörsbacken NR	Kåtorp	cow	pitfall trap
Geotrupes spiniger	3	Degerrörsbacken NR	Kåtorp	horse	hand-picked
Onthophagus fracticornis	1	Degerrörsbacken NR	Kåtorp	horse	hand-picked
Aphodius foetens	1	E Skarpa Alby	Skarpa Alby	cow	pitfall trap
Geotrupes spiniger	3	E Skarpa Alby	Skarpa Alby	cow	pitfall trap
Aphodius ictericus	few	Gårdby	Gårdby	cow	hand-picked
Aphodius sordidus	49	Gårdby	Gårdby	cow	hand-picked
Geotrupes spiniger	1	Gårdby	Gårdby	cow	hand-picked
Onthophagus fracticornis	1	Gårdby	Gårdby	cow	hand-picked
Onthophagus nuchicornis	10	Gårdby	Gårdby	cow	hand-picked
Aphodius ictericus	few	Gråborg	Borg	sheep	hand-picked
Aphodius erraticus	3	Höge ås	Jordtorp	cow	hand-picked
Aphodius erraticus	3	Höge ås	Jordtorp	cow	hand-picked
Aphodius foetens	1	Höge ås	Jordtorp	cow	hand-picked
Aphodius foetens	2	Höge ås	Jordtorp	cow	hand-picked
Aphodius haemorrhoidalis	67	Höge ås	Jordtorp	cow	hand-picked
Aphodius haemorrhoidalis	>5	Höge ås	Jordtorp	cow	hand-picked
Aphodius ictericus	82	Höge ås	Jordtorp	cow	hand-picked
Aphodius ictericus	31	Höge ås	Jordtorp	cow	hand-picked
Aphodius sordidus	1	Höge ås	Jordtorp	cow	hand-picked
Geotrupes spiniger	1	Höge ås	Jordtorp	cow	hand-picked
Geotrupes spiniger	6	Höge ås	Jordtorp	cow	hand-picked
Onthophagus nuchicornis	17	Höge ås	Jordtorp	cow	hand-picked
Aphodius foetens	7	Källheden	Glömminge	horse	hand-picked
Geotrupes spiniger	1	Källheden	Glömminge	horse	hand-picked
Aphodius erraticus	3	Kvarnbackarna	Jordtorp	cow	hand-picked
Aphodius foetens	2	Kvarnbackarna	Jordtorp	cow	hand-picked

Aphodius haemorrhoidalis	>3	Kvarnbackarna	Jordtorp	cow	hand-picked
Geotrupes spiniger	12	Kvarnbackarna	Jordtorp	cow	hand-picked
Aphodius erraticus	2	Lilla Hult (E)	Algutsrum	cow	pitfall trap
Geotrupes spiniger	2	Lilla Hult (E)	Algutsrum	cow	pitfall trap
Aphodius erraticus	1	Skarpa Alby	Skarpa Alby	cow	pitfall trap
Aphodius foetens	2	Skarpa Alby	Skarpa Alby	cow	pitfall trap
Aphodius foetens	3	Skarpa Alby	Skarpa Alby	cow	pitfall trap
Aphodius haemorrhoidalis	1	Skarpa Alby	Skarpa Alby	cow	pitfall trap
Aphodius haemorrhoidalis	3	Skarpa Alby	Skarpa Alby	cow	pitfall trap
Aphodius ictericus	6	Skarpa Alby	Skarpa Alby	cow	pitfall trap
Aphodius ictericus	2	Skarpa Alby	Skarpa Alby	cow	pitfall trap
Aphodius sordidus	4	Skarpa Alby	Skarpa Alby	cow	pitfall trap
Geotrupes spiniger	4	Skarpa Alby	Skarpa Alby	cow	pitfall trap
Onthophagus fracticornis	24	Skarpa Alby	Skarpa Alby	cow	pitfall trap
Onthophagus fracticornis	6	Skarpa Alby	Skarpa Alby	cow	hand-picked
Trypocopris vernalis	15	Skarpa Alby	Skarpa Alby	cow	pitfall trap
Geotrupes spiniger	few	W Kåtorp	Kåtorp	cow	pitfall trap

Text 3. Supplementary details on results

To measure the functional efficiency in relation to the traits, we modeled response variables versus several variables (density in the mesocosm, nesting strategy, bodymass, threat status). Below, we present the results per each function investigated.

REMAINING DUNG – Both nesting strategy and bodymass per individual unit affected the total remaining dung (Table A3).

Table A3: ***Results for remaining dung mass.*** *Linear model on remained dry dung (g) as a function of species densities in the mesocosms, nesting strategy, species bodymass and conservation status. Shown are estimated coefficients: mean squared, F-value with degrees of freedom (for both numerator and denominator), and statistical significance.*

Total remaining dung mass	Mean Sq	NumDF	DenDF	F-value	p-value
Density	4.6738	1	19.3183	34.357	**< 0.001**
Nesting_Strategy	0.0048	1	6.509	0.035	0.8569
Bodymass	0.0206	1	6.4514	0.151	0.7097
Threat_Status	0.046	1	5.814	0.336	0.5837
Bodymass × Nesting_Strategy	0.007	1	6.451	0.054	0.8234
Density × Nesting_Strategy	4.076	1	19.161	29.965	**< 0.001**
Density × Bodymass	5.120	1	19.605	37.635	**< 0.001**
Density × Threat_Status	0.0063	1	20.559	0.047	0.8314
Density × Nesting_Strategy × Bodymass	4.598	1	19.526	33.799	**< 0.001**

DUNG REMOVAL RATE – Both nesting strategy and bodymass per individual unit affected the dung removal rates (Table A4).

Table A4: ***Results for dung removal rates.*** *Linear model on remaining wet dung removal rates (g) as a function of species densities in the mesocosms, nesting strategy, species bodymass, conservation status and measurement days. Shown are estimated coefficients: mean squared, F-value with degrees of freedom (for both numerator and denominator), and statistical significance.*

Dung removal rates	Mean Sq	NumDF	DenDF	F-value	p-value
Density	2.366	1	186.84	27.866	**< 0.001**
Nesting_Strategy	0.005	1	9.952	0.063	0.8071
Bodymass	0.0343	1	9.741	0.404	0.5395
Threat_Status	0.002	1	8.117	0.023	0.8840
Measurement days	10.686	1	183.21	125.836	**< 0.001**
Bodymass × Nesting_Strategy	0.004	1	9.776	0.047	0.8336
Density × Nesting_Strategy	2.1639	1	186.705	25.481	**< 0.001**
Density × Bodymass	2.308	1	185.42	27.173	**< 0.001**
Density× Threat_Status	0.0075	1	163.078	0.089	0.7660
Density × Nesting_Strategy × Bodymass	2.287	1	186.163	26.932	**< 0.001**

GHG EMISSIONS – The per capita effect of dung beetles on methane emission reduction drastically increased with an increase in individual bodymass; non-threatened species per individual are more effective reducing methane emissions than threatened ones (Table A5).

Table A5: ***CH_4 emission results.*** *Linear model on methane fluxes (mg/m^2×h) as a function of species densities in the mesocosms, nesting strategy, species bodymass, conservation status and measurement days. Shown are estimated coefficients: mean squared, F-value with degrees of freedom (for both numerator and denominator), and statistical significance.*

CH$_4$ emissions	Mean Sq	NumDF	DenDF	F-value	p-value
Density	32.68	1	181.93	5.34	**0.021**
Nesting_Strategy	0.85	1	7.50	0.139	0.719
Measurement days	265.25	1	177.97	43.35	**< 0.001**
Bodymass	0.25	1	7.27	0.04	0.846
Threat_Status	0.57	1	6.90	0.09	0.77
Density × Nesting_Strategy	0.003	1	182.91	0.000	0.98
Density × Bodymass	31.81	1	181.74	65.197	**0.024**
Density × Threat_Status	32.47	1	182.40	5.31	**0.022**

NUTRIENT CONTENT OF THE SOIL – NH$_4^+$ concentration in the soil underneath dung pats showed a tendency towards higher values with tunnelers in the pat than with dwellers (Table A6).

*Table A6: **Results for soil NH$_4$+ content.** Linear model on ammonium concentration into soil (mg/kg) as a function of species densities in the mesocosms, nesting strategy, species bodymass, conservation status and measurement days. Shown are estimated coefficients: mean squared, F-value with degrees of freedom (for both numerator and denominator), and statistical significance.*

Soil NH$_4$+ content	Mean Sq	NumDF	DenDF	F-value	p-value
Density	63.25		24.999	0.1036	0.750
Nesting_Strategy	2509.40		24.999	4.111	**0.053**
Bodymass	371.47		24.999	0.609	0.443
Threat_Status	636.41		24.999	1.043	0.31699
Density × Nesting_Strategy	2002.58		24.999	3.281	**0.082**
Density × Bodymass	6.86		24.999	0.011	0.916
Density × Threat_Status	65.99		24.999	0.108	0.745

3. Greenhouse gas emissions from dung pats vary with dung beetle species and with assemblage composition

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Abstract

Cattle farming is a major source of greenhouse gases (GHGs). Recent research suggests that GHG fluxes from dung pats could be affected by biotic interactions involving dung beetles. Whether and how these effects vary among beetle species and with assemblage composition, is yet to be established. To examine the link between GHGs and different dung beetle species assemblages, we used a closed chamber system to measure fluxes of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) from cattle dung pats. Targeting a total of four dung beetle species (a pat-dwelling species, a roller of dung balls, a large and a small tunnelling species), we ran six experimental treatments (four monospecific and two mixed) and two controls (one with dung but without beetles, and one with neither dung nor beetles). In this setting, the overall presence of beetles significantly affected the gas fluxes, but different species contributed unequally to GHG emissions. When compared to the control with dung, we detected an overall reduction in the total cumulative CO_2 flux from all treatments with beetles and a reduction in N_2O flux from the treatments with the three most abundant dung beetle species. These reductions can be seen as beneficial ecosystem services. Nonetheless, we also observed a disservice provided by the large tunneler, *Copris lunaris,* which significantly increased the CH_4 flux – an effect potentially traceable to the species' nesting strategy involving the construction of large brood balls. When fluxes were summed into CO_2-equivalents across individual GHG compounds, dung with beetles proved to emit less GHGs than did beetle-free dung, with the mix of the three most abundant species providing the highest reduction (-32%). As the mix of multiple species proved the most effective in reducing CO_2-equivalents, the conservation of diverse assemblages of dung beetles emerges as a priority in agro-pastoral ecosystems.

Key-words: biotic interactions; GHG emissions; dung removal; CO_2; CH_4; N_2O; ecosystem services; ecosystem disservices.

3.1 Introduction

Grazing animals release large amounts of nitrogen and carbon through their excreta in pastures. The excess of nutrients creates a release of Green House Gases (GHGs) which steadily leaks into the atmosphere [1,2]. The dung produced by livestock, in particular, is a significant source of GHGs such as nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2) [3,4,5,6,7]. GHG emissions from dung are primarily and directly dependent on microbiological processes. CO_2 originates from the decomposition of organic material by micro-organisms, CH_4 from methanogenic bacteria thriving in anoxic conditions and N_2O from microbial nitrification, denitrification and nitrifier denitrification, i.e. nitrite reduction by ammonia oxidizers [8,9,10,11,12,13].

Yet, GHG fluxes are also affected by the macroscopic fauna. Recent studies reveal that dung beetles (Coleoptera: Scarabaeoidea) may influence the GHG emissions by aerating the dung and soil, by reducing organic matter, by relocating dung and by altering microbe communities [14,15,16]. Importantly, studies of beetle-mediated effects on GHG emissions have so far been focused on the general effect of either including or excluding dung beetles [17,18,19], or on the effects of single species [20]. In contrast, the effects of variation in species identity and community composition has received little or no attention. This status quo clashes with a general interest in the functional correlates of overall species diversity (from e.g. 21), and of nesting strategies within species assemblages [22, with a general review in 23]. What these studies reveal is that even within larger assemblages, the level of ecosystem functioning may often be hinged on the presence of specific species [24]. Thus, to understand overall fluxes of GHGs from dung, we need to dissect the functional contributions of different dung beetle assemblages, and of individual taxa within such assemblages.

Importantly, different dung beetle taxa can be expected to modify gas fluxes to different extents. Dung beetle taxa vary in their nesting strategies, and can be divided in dwellers, tunnelers and rollers [25]. Of these, the 'dwellers' do not translocate dung but utilise dung pats by living inside them. The 'tunnelers' translocate dung to tunnels dug underneath the dung pat, whereas the 'rollers' first translocate pieces of dung horizontally, then bury them vertically. *A priori*, these different nesting strategies and/or the body mass of the species may significantly affect ecological function, such as dung removal efficiency [22,26,27,28,29]. As they result in *inter alia* holes of different diameter in different parts of the dung pat, and in different sizes of brood balls being translocated to different micro-environments, we hypothesized that they may also affect GHG fluxes differently. To test this hypothesis, we quantified GHG emissions from dung pats as a function of the identity and assemblage structure of dung beetles within them. The specific aims of this study were thus to test: *i)* whether different species displayed different GHG emission patterns; and, *ii)* whether mixed species performed differently from single-species assemblages.

3.2 Materials and Methods

To examine the functional consequences of variation in dung beetle assemblage composition, we established replicate laboratory terraria with four monospecific and two mixed assemblages, then recorded the outcome in terms of dung removal and on GHG emissions.

3.2.1 Experimental design

Dung beetles were collected from La Mandria Natural Park (45° 08' 48.83'' N, 7° 36' 02.53'' E, 290 m above sea level), north-western Italy (using the same locality as [30]). This collection was authorized by the supervisory official of the "Ente di Gestione delle Aree Protette dei Parchi Reali" (Venaria, Italy). Species collected were neither endangered nor protected. Beetles were collected in May 2015, using standard cattle-dung-baited pitfall traps located in the broadleaf forest (dominated by *Quercus robur* and *Carpinus betulus*). Following [31], a total of 30 traps were interspersed by distances of at least 50 m, and the beetles collected after 48 hours. The design of our experiment was subsequently based on the snapshot of the local dung beetle fauna thus derived. Thus, the four species most abundantly caught were used in the experiment (Table 1): *Aphodius fimetarius* (Linnaeus, 1758) a small dweller; *Onthophagus coenobita* (Herbst, 1783), a small tunneler; *Sisyphus schaefferi* (Linnaeus, 1758), a small roller; and *Copris lunaris* (Linnaeus, 1758), a large tunneler (Fig 1) (with the classification into "small" and "large" species based on body mass, following [32]).

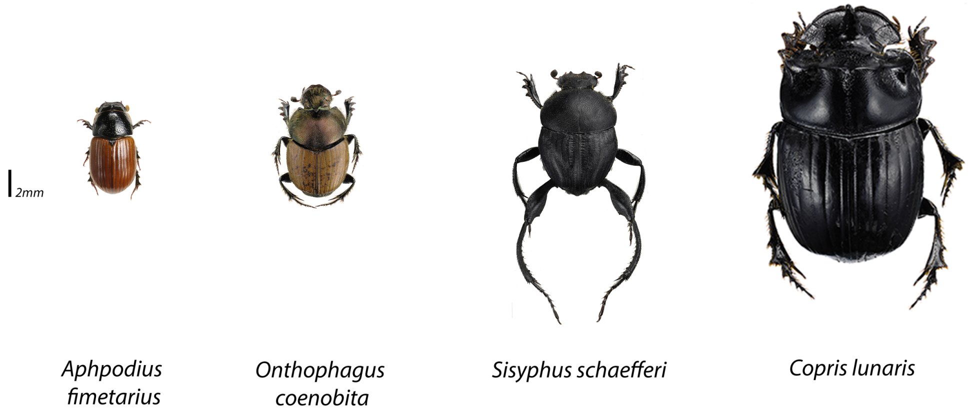

Fig 1. **Species used in the experiment.** The same pictograms are used to identify treatments in Figs 2-5. The length of each pictogram is proportional to the average body size of beetles, as adopted from [33]. Photographs by Göran Liljeberg.

To keep the total biomass of beetles at roughly 0.30 g per assemblage, species-specific numbers of individuals were varied between two and 31, with a minimum of two individuals per species (Table 1). This total biomass was chosen based on the mean total dung beetle biomass found in dung pats of 300g each in a previous pilot field study (mean value= 0.33g, SD.= 0.20g) (with more details in S1 Appendix). Since the bigger species were of vastly larger size and biomass than the smaller ones, we chose to omit them from the mixed assemblages to maintain control over total biomass per treatment.

We ran six treatments (see Table 1): four monospecific treatments (T1-T4, each with one species only), and two mixed treatments (T5, T6) where the two versus three most abundant species, respectively, were included in proportions representative of field densities (Table 1). We also ran two controls: C1 dung without beetles, and C2 with neither dung nor beetles. Seven replicates were established for each treatment and control, thus yielding a total of 56 terraria (6 treatments x 7 replicates + 2 controls x 7 replicates = 56). We used terraria that consisted of a 16.5 litre plastic bucket (diameter 28 cm, height 27 cm). Since our experiment required a total of 576 litres of soil, we decided to use an artificial synthetic substrate rather than the natural soil from the site of origin. For this purpose, we used humus for gardening (0.5 Kg NPK 12-14-24 + 2MgO), homogenizing it through a 1cm-mesh. To reduce the organic content and to arrive at a composition similar to the natural substrate, we then mixed it with sand in a ratio of 1:2, following [34].

Fresh dung was collected from a herd of twelve Aberdeen Angus cattle grazing on natural grasslands dominated by graminoids (genera *Dactylis*, *Festuca*, *Poa*, *Lolium* and *Setaria*). No cow in the herd was treated with antibiotics or anthelmintics. The dung was manually homogenized before partitioning 300g to each treatment T1-T6 and to the control C1. This pat size was chosen from the range of typical pat sizes encountered in nature, selected to leave an uncovered strip of ground surrounding the pat.

The experiment lasted for 32 days, during which time the laboratory was kept at a constant temperature of 25° [35] and 60 % humidity [36]. To simulate rain, we added 100 ml of deionized water to each terrarium after 8, 14, 19 and 24 days.

At the end of the experiment we weighed dry residual dung to evaluate the efficiency of dung removal for each treatment. By using dry weight, we controlled for any difference in evaporation, thereby isolating the contribution of the insects themselves in dung removal.

*Table 1: **Dung beetle species used in the experiment**. The table identifies the nesting strategies, species, total number of individuals, mean individual dry body mass and number of individuals used in each experimental treatment.*

Nesting strategies and beetle size		Species	Total number of individuals	Mean individual body mass [g]	Number of individuals in each treatment					
					Monospecific treatments				Mixed treatments	
					T1	**T2**	**T3**	**T4**	**T5**	**T6**
Small dweller		*Aphodius fimetarius*	413	0.01	31				17	11
Tunneler	*Small tunneler*	*Onthophagus coenobita*	161	0.02		13			6	4
	Large tunneler	*Copris lunaris*	14	0.20				2		
Small roller		*Sisyphus schaefferi*	56	0.05			6			2

3.2.2 Chamber and gas flux measurement

To evaluate gas fluxes from the terraria, we used a non-steady-state closed chamber technique [37, 38] (overall approach adopted from [17], with additional details offered in S1 Appendix). To close the terraria, we used lids organized with a vent tube and a sampling port following the USDA-ARS GRACEnet Chambers-base trace gas flux measurement protocol [39]. Between measurements, buckets were closed with a plastic mesh to avoid the escape of any dung beetles.

Gas fluxes were measured between 09:00am and 2:00pm on eight occasions between June 5th and July 6th, with the timing of measurement (i.e. 1, 4, 7, 11, 15, 20, 26 and 32 days from the start of the experiment) following that of [17]. On each specific day, gas fluxes were measured in seven consecutive rounds, with each round encompassing one replicate of each treatment (T1, T2, T3, T4, T5, T6) and control (C1 and C2). The first round was initiated at 9:00 am, the last one at 1:30 pm. Gas fluxes measured during different rounds did not detectably differ from each other (see S2 Appendix: S2.1 Table).

Samples were taken with a syringe (50 ml) after 0, 8, and 16 minutes of the chamber being sealed, and injected into glass vials (12 ml). The contents of CO_2, CH_4 and N_2O were then quantified in parts per million (ppm by volume) by a gas chromatograph (Agilent mod. 7890A) equipped with thermal conductivity, flame ionization and electron capture detectors.

Fluxes were calculated from the linear or nonlinear [40] increase over time (per hour) in concentration (selected according to the emission pattern) in the chamber headspace, as suggested by [38].

To evaluate the overall warming effect of GHG emissions from dung pats, compound-specific emissions should be combined. To weigh the fluxes together, we converted compound-specific fluxes of N_2O and CH_4 to CO_2-equivalents by using the IPCC 2013 global warming potential (GWP) impact factors for 100 years' time, i.e. 298 for N_2O and 34 for CH_4. These fluxes were then summed with the fluxes of CO_2.

Following [17] and [20], cumulative fluxes of CO_2, N_2O, CH_4, and CO_2-equivalents were calculated for each chamber and expressed as areas under the curve (AUC) of the gas flux over time. For the period from the start of the experiment to day *i*, the cumulative area under the curve A_i was calculated as: $A_i = \Sigma_{k=1}^{i} A_k$. Accumulation rates (as trends in cumulative areas under the curves) and total cumulative fluxes (i.e. sums up to *i*=32 days) were used as separate responses in subsequent analyses.

3.2.3 Statistical Analysis

Generalized Least Squares (GLS) models were used to analyse dung removal efficiency and gas fluxes, which allow the incorporation of autocorrelation structures (i.e. temporal dependencies between observations). To account for the heterogeneity of variance between treatments, we included a separate variance structure for each treatment where necessary. The most appropriate structure of random components was determined using a REstricted Maximum Likelihood (REML) estimation. The optimal random structure was determined by starting with a model without any variance–covariate terms (equivalent to linear regression) and comparing this model with subsequent GLS models that contained specific variance structures [41]. Comparisons of these models were made using Akaike Information Criteria (AIC) (S1.2 Table in S1 Appendix) and plots of residuals versus fitted values. Individual responses were modelled as follows.

To analyse how dung removal varied with dung beetle assemblage composition, residual dung weight was modelled as a function of treatment, while including a separate variance structure for each treatment.

To analyse how the fluxes (both hourly and cumulative) of different gases varied over time and treatments, a separate model was derived for each compound (CO_2, CH_4, N_2O and CO_2-equivalents, respectively). We run models that took into account the high variability within treatments and the temporal non-independence of consecutive measurements. Thus, each compound was modelled as function of measurement day and treatments, using terrarium as a random effect and including a separate variance structure for each treatment.

To analyse total cumulative fluxes at the end of the experiment, we applied a separate GLS to each compound, including a separate variance structure for each treatment. Consequently, each compound was modelled as function of treatments, including a separate variance structure for each treatment.

To adjust for multiple comparisons in all GLS models and post hoc test, we recalculated the p-value with a Holm correction (equal to a sequential Bonferroni correction; [42]). In other words, we multiplied the lowest p-value observed by the number (n) of independent tests conducted or by the number of independent variables, the next-lowest with n-1 etc. Both the adjusted and non-adjusted p-value are presented in the Tables in Appendix S2.

All analyses were performed using the ‘nlme’ package (v3.1-124; [43]) in the R (v3.2.1) statistical and programming environment (R Development Core Team 2005, for the R-scripts see S1.1 Table in S1 Appendix).

Data exploration of GHG fluxes per treatment and day highlighted the presence of an outlier from methane emissions of treatment T1 (day 3). This value was completely out of range of all other data, suggesting that it may come from an error during the gas extraction. For this reason, this data had not been taken into account in the analysis.

3.3 Results

3.3.1 Dung removal

At the end of the experiment the dry mass of dung remaining did not significantly differs between the treatments and the beetle-free control C1. The treatment T4 with *Copris lunaris* offered a notable exception, as this species removed much more dung than the others (Fig 2 and S2 Appendix: S2.2 Table).

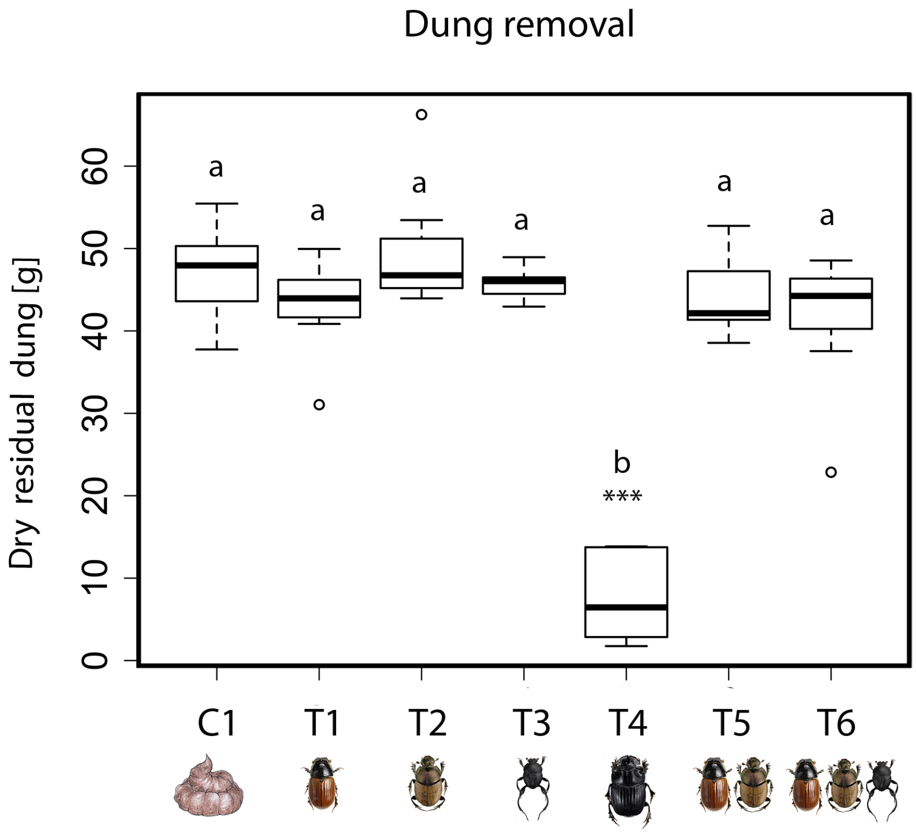

Fig 2. **Dung removal in different treatments.** Shown are box plots of the dry weight of dung (in grams) left at the end of the experiment. Letters above boxes identify differences among means as revealed by post-hoc analyses of GLS models. Boxes not sharing a letter were significantly different from each other, with significance levels derived from post-hoc analysis of the GLS model: '***' = p<0.001.

3.3.2 GHG emissions

GHG fluxes from soil (i.e. from control C2, containing neither dung nor beetles) were much lower than fluxes from terraria with dung (Figs 3-5). Fluxes from dung pats decreased over time and showed different patterns among the compounds considered: while fluxes of all compounds were highest from fresh dung, this pattern was most pronounced for methane and nitrous oxide, which emissions essentially stopped within a week. By comparison, carbon dioxide fluxes continued – albeit at a reduced rate – throughout the duration of the experiment (Fig 3).

When focusing on the seven terraria types with dung pats (i.e. the set of six treatments with dung beetles and the control C1 containing dung only), the presence of dung beetles significantly reduced GHG emissions as compared to the control C1 containing dung only (Tables S2.3 and S2.4 in S2 Appendix).

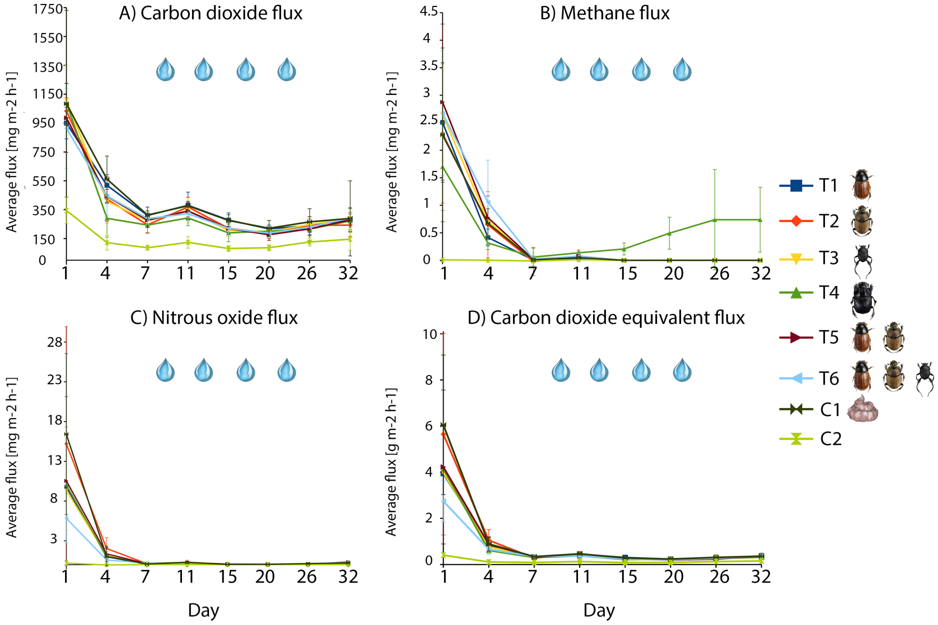

Fig 3. **Compound-specific gas fluxes observed over time**. Individual panels show fluxes of CO_2 (a), CH_4 (b), N_2O (c) and CO_2-equivalents (d), with each treatment represented by day-specific averages and standard deviations from empirical data. The water drops symbolize the addition of 100 ml of deionized water to each terrarium. Error bars show standard deviations.

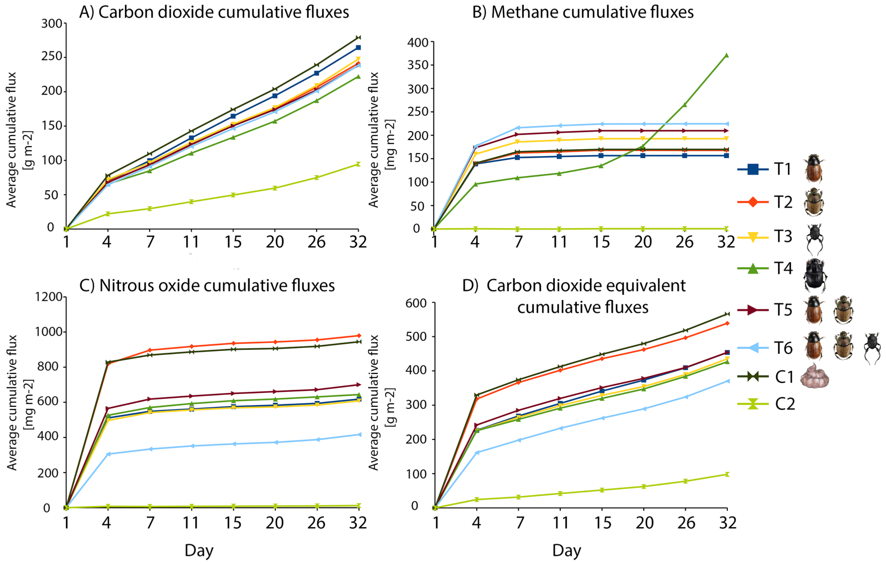

Fig. 4. **Compound-specific cumulative fluxes observed over time**. Individual panels show cumulative fluxes of CO_2 (a), CH_4 (b), N_2O (c) and CO_2-equivalents (d) in different treatments and controls (see details in S1 Appendix and GLS result in S2 Appendix).

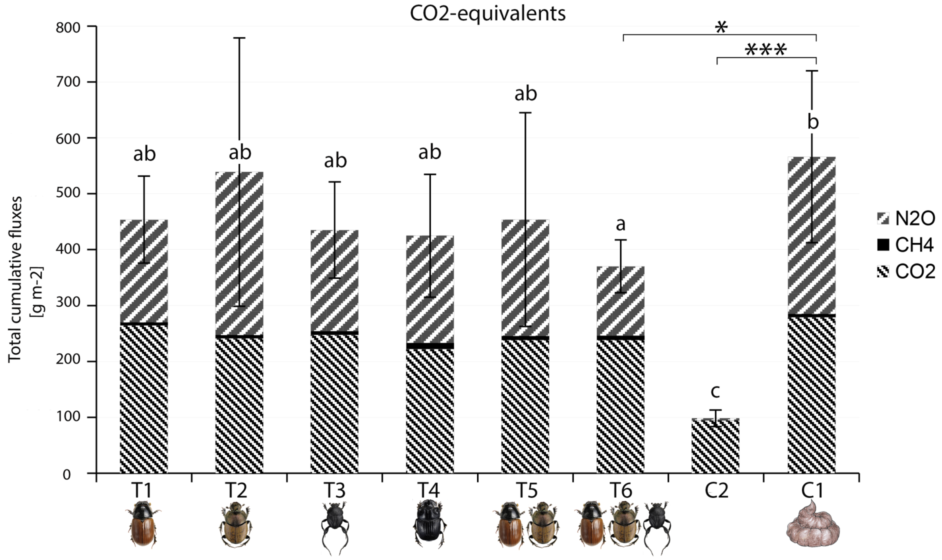

Fig. 5. **Total CO_2-equivalents of greenhouse gases emitted in different treatments.** To weigh together individual GHG compounds, we used compound-specific multipliers derived from IPCC (2013). Letters above bars identify differences among means revealed by post-hoc analyses of GLS models (more details in Table S2.7 in Appendix S2). Bars not sharing the same letter were significantly different from each other. Stars define significant differences between terraria (treatment T6 and control C2 without dung) and reference category (control C1 with dung), revealed by GLS models. Error bars show standard deviations. '***' = $p<0.001$ and '*' = 0.05.

3.3.2.1 Carbon dioxide

Emissions of CO_2 varied between a maximum of 2421.15 mg C m^{-2} h^{-1} and a minimum of 23.96 mg C m^{-2} h^{-1} among the terraria with dung pats (treatments T1-T6 and C1; Fig 3a). CO_2 fluxes differed among terraria ($F_{6,336}$=2.57, p=0.02, adjusted p=0.057; with T1/T6 differing from C1) and time periods ($F_{7,336}$=408.32, p<0.001, adj. p<0.001), with the size of the difference varying between time periods (Interaction treatments × days: $F_{42,336}$=1.54, p= 0.02, adj. p=0.04; for more details see Table S2.5 in Appendix S2) (Figs 3a and 4a).

The cumulative CO_2 flux was lower in all treatments than that of the control C1 (as containing dung only; T2: t_{56}=-2.25, p=0.03, adjusted p=0.12; T3: t_{56}=-1.91, p=0.06, adjusted p=0.12, T5: t_{56}=-2.50, p=0.02, adjusted p=0.08, T6: t_{56}=-2.18, p=0.03, adjusted p=0.12), but this difference was strong in the presence of *C. lunaris* (T4: t_{56}=-3.67, p<0.001, adj. p=0.001; for more details see Table S2.6 in Appendix S2) (Fig 4a).

3.3.2.2 Methane

Fluxes of CH_4 ranged from a maximum of 5.73 mg C m^{-2} h^{-1} to a minimum of -0.55 mg C m^{-2} h^{-1} (treatments T1-T6 and control C1). For this compound, fluxes did not differ significantly among terraria ($F_{6,335}$=1.03, p=0.40, adj. p=0.81), but only between time periods ($F_{7,335}$=182.15, p<0.001, adj. p<0.001; interaction treatments × days: $F_{42,335}$=1.58, p=0.02, adj. p=0.048; more details in Tables S2.4 and S2.5 in Appendix S2) (Fig 3b). The same patterns were evident in cumulative CH_4 fluxes (Fig 4b).

Total cumulative fluxes of CH_4 from the beetle-free control C1 were significantly lower than those from the treatment with the big tunneler *C. lunaris* (treatment T4: t_{56}=2.91, p=0.00, adj. p=0.037; more details in Table S2.5 in Appendix S2). The emission pattern from this treatment (T4) changed over time, with CH_4 emissions decreasing until the 7th day, when they started to increase. As a result of this trend, cumulative emissions were lower than those from control C1 with dung at the beginning of the experimental period and higher at the end (Figs 3b and 4b).

3.3.2.3 Nitrous oxide

Fluxes of N_2O varied between a maximum of 43.31 mg N m^{-2} h^{-1} and a minimum of -0.62 mg N m^{-2} h^{-1} among the terraria with dung pats (treatments T1-T6 and C1). The specific flux rates differed significantly among treatments over time ($F_{6,336}$=2.27, p=0.04, adj. p=0.04) and over time ($F_{7,336}$=95.64, p<0.001, adj. p <0.001; interaction treatment × days: $F_{42,336}$=1.95, p<0.001, adj. P=0.001; with more details in Tables S2.4 and S2.5 in Appendix S2) (Figs 3c and 4c).

Cumulative N_2O fluxes accumulated slower over time in treatments with beetles than in the control C1 with dung only (with the notable exception of treatment T2 containing the small tunneller, *Onthophagus coenobita*; Fig 4c). However, these differences were significant only between treatment T6 (with all three dung beetle species present) and the beetle-free control with dung, C1 (Treatment T6: t_{56}=-2.65, p=0.01, adj. p=0.07; more details in Table S2.6 in Appendix S2).

3.3.2.4 CO_2-equivalents

To the total fluxes of CO_2-equivalents, CO_2 and N_2O contributed the majority, with a substantially smaller contribution from CH_4 (Fig 5). Among the terraria with dung pats (treatments T1-T6 and control C1), emissions of CO_2-equivalents differed significantly among terraria ($F_{6,336}$=*2.68*, p=0.02, adj. p=0.02) and over time

($F_{7,336}$=162.10, p<0.001, adj. p <0.001; interaction treatment × days: $F_{42,336}$=2.14, p<0.001, adj. P=0.001; with more details in Tables S2.4 and S2.5 in Appendix S2) (Figs 3d and 4d).

Cumulative fluxes of CO_2-equivalents accumulated slower in the presence (T1-T6) than in the absence (control C1) of beetles, with an average reduction of -21,33% [calculated from the data shown in Fig 5 as (T_i-C1)/C1*100; see [17]). The largest reduction was provided by the blend of three species (treatment T6), which was also significantly lower than that from the control C1 (Treatment T6: t_{56}= -3.22, p=0.00, adj. p-value 0.02; more details in Appendix S2: Table S2.6; for post hoc analysis details in Appendix S2: Table S2.7) (Fig 5).

3.4 Discussion

Where previous studies have revealed a general impact of dung beetles on GHG fluxes from cow pats [17,18,20], the current study reveals a new pattern: that the specific reduction in GHG emissions depends on the composition of the dung beetle assemblage. Quite surprisingly, we found the very same species to maximize the ecosystem service of dung removal and of carbon dioxide reduction and the ecosystem disservice of methane emissions from dung pats. These patterns come with two main implications: first, they support our *a priori* hypothesis that different dung beetle species, and different dung beetle assemblages, do indeed affect GHG fluxes differently. Second, they suggest that different ecosystem services may trade off against each other, and that functionally efficient organisms may simultaneously increase both desirable and undesirable ecosystem processes. Below, we will address each one in turn.

3.4.1 Different dung beetle assemblages affect GHG emission differently

While previous studies have mainly targeted the overall effect of dung beetles on GHG emissions from dung pats [17,18], not all dung beetles are equal. Variation in nesting strategies [25] and in the body mass of species may significantly affect their functional efficiency [22,27,28,29,44]. Thus, we expected different beetles to affect GHG fluxes differently – a hypothesis for which we found direct support.

Even though our experimental design was explicitly based on the same total biomass of dung beetles in each experimental unit, assemblages of the large tunneler, *Copris lunaris,* released more total methane per unit beetle mass than did other beetle assemblages – and in fact, even more methane than did the control with dung only and no beetles. The exact patterns differed not only with the assemblage structure but with the GHG compound considered. When emissions of all compounds (CH_4, N_2O and CO_2) were combined into the common currency of

CO_2-equivalents, dung beetle assemblages consisting of three species proved to release a full third (32%) less of GHGs than did beetle-free controls.

Exactly what processes are behind the patterns detected is yet to be clarified. For assemblages with *C. lunaris*, fluxes first decreased until day 7 of the experiment, then increased again. These patterns may reflect the nesting behaviour of this large tunneler, with decreasing CH_4 fluxes during the first week corresponding to dung relocation into chambers before the brood ball formation starts [45]. During this period, *C. lunaris* manipulated and transported the dung into the soil, this may have enhanced its drying and increased the availability of oxygen. This may have decreased anaerobic decomposition and reduced methanogenesis (cf. [17]). Once in the larval chamber, brood balls will be kept moist by parental care, and may therefore continue to emit CH_4 until the end of the experiment.

That the different activities of different dung beetle species may interact in determining the net functional outcome is suggested by the functional patterns emerging from monospecific versus mixed species assemblages.

Overall, the total emission of CO_2 equivalents – i.e. the pooled climatic impact – was lower in the presence than in the absence of beetles and with the mix of *three species providing the highest significant reduction*. Yet, the exact mechanisms behind this desirable pattern of attenuation of GHG emissions in terms of CO_2 equivalents are again to be targeted by further work. As our current experimental design was explicitly devised to resolve differences among species, and constrained by limitations on resources and overall terraria numbers, it falls short of resolving complementarity from facilitation effects (sensu [21]) – or indeed any other specific mechanisms behind the patterns detected. Yet, it does suffice to generate the data-driven hypothesis that more diverse dung beetle communities may release less GHGs – an explicit hypothesis to be targeted by future experiments.

3.4.2 Functionally efficient taxa may provide both ecosystem services and disservices

While the large tunneler *C. lunaris* was associated with unexpectedly high methane emissions, it was also the most efficient species in removing dung and reducing CO_2 emissions, even more efficient than mixed assemblages. Thus, functionally efficient organisms may simultaneously increase both desirable and undesirable ecosystem processes [46,47] and different ecosystem services may trade off against each other [48,49]. Across different taxa, many species provide both ecosystem services and disservices. Important pollinators, as hawkmoth species (Lepidoptera: Sphingidae), have voracious herbivore larval-stages that, damaging the plants, have an effective fitness cost [50, 51] and incur an indirect disservice for the crop. Ants provide several ecosystem services (reducing leaf herbivory, fruit pest damage and indirect pollination facilitation), but also disservices, increased mealybug density, phytopathogen dissemination and indirect pest damage enhancement [47]. Earthworms are also important as soil ecosystem engineers, they modify soil structure and interact with microbes through their feeding, burrowing and casting activities (ecosystem services) but it was proven that they also increase GHG emissions from soil (ecosystem disservice) [46].

Since dung beetles as a group are known to sustain a whole range of ecosystem services ranging from seed dispersal to parasite control [52], this points to a need for quantifying the relative role of species identity, functional diversity and overall species diversity for multiple functions at the same time. Dung removal *per se* seems not to reflect all other functions and services (current study; see also [53]), and a species excelling at producing one service may be either inefficient in producing another, or distressingly efficient in simultaneously sustaining a disservice.

3.5 Conclusions

Overall, our study demonstrates that different dung beetle species contribute differently to dung removal and to GHG emissions from dung pats – and that one and the same species (*C. lunaris*) may contribute to both ecosystem services (dung removal and CO_2 reduction) and disservices (increasing methane emissions). As different species may perform differently under different conditions [54], the best approach to safeguarding ecological functioning will be conserving diverse dung beetle communities [53].

In many countries worldwide, dung beetles are currently threatened by changes in pastoral practices and chemicalization of cattle farming [28,55,56,57]. As a case in point, the large tunneler, *C. lunaris*, which we identified as so functionally important in our study, has been declining in many parts of Northern Europe [33]. This and similar changes may incur unpredictable changes in the functioning of pasture ecosystems.

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References

1. Flessa H, Dörsch P, Beese F, König H, Bouwman AF. Influence of cattle wastes on nitrous oxide and methane fluxes in pasture land. J Environ Qual. 1996; 25: 1366–70.
2. Dubeux JCB, Sollenberger LE, Mathews BW, Scholberg JM, Santos HQ. Nutrient cycling in warm-climate grasslands. Crop Sci. 2007; 47(3): 915–28.
3. Petersen SO. Ammonia losses from urine and dung of grazing cattle: effect of N intake. Atmos Environ. 1998; 32: 295–300.
4. Saggar S, Bolan NS, Bhandral R, Hedley CB, Luo J. A review of emissions of methane, ammonia, and nitrous oxide from animal excreta deposition and farm effluent application in grazed pastures. New Zeal J Agr Res 2004; 47(4): 513–44.
5. Lin XW, Wang SP, Ma XZ, Xu GP, Luo CY, Li YN, et al. Fluxes of CO2, CH4, and N2O in an alpine meadow affected by yak excreta during summer grazing periods on the Qinghai-Tibetan plateau. Soil Biol Biochem 2009; 41: 718–25.
6. Virkajärvi P, Maljanen M, Saarijärvi K, Haapala J, Martikainen PJ. N 2 O emissions from boreal grass and grass-clover pasture soils. Agr Ecosyst Environ. 2010; 137: 59–67.
7. Cai Y, Wang X, Ding W, Tian L, Zhao H, Lu X. Potential short-term effects of yak and Tibetan sheep dung on greenhouse gas emissions in two alpine grassland soils under laboratory conditions. Biol Fertil Soils 2013; 49: 1215–26.
8. Bunnell FL, Tait DEN, Flanagan PW, Van Clever K. Microbial respiration and substrate weight loss—I: A general model of the influences of abiotic variables. Soil Biol Biochem. 1977; 9: 33–40.
9. Oremland RS. Biogeochemistry of methanogenic bacteria. In: Zehnder AJB editor. Biology of anaerobic microorganisms, New York: J Wiley & Sons; 1988: 641–705.
10. Firestone MK, Davidson EA. Microbiological basis of NO and N2O production and consumption in soil. In: Andreae MO, Schimel DS, editors. Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere. Chichester: J Wiley & Sons; 1989; 47: 7–21.
11. Davidson EA, Rogers JE, Whitman WB, others. Fluxes of nitrous oxide and

nitric oxide from terrestrial ecosystems. In: Rogers JE, Whitman WB, editors. Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides, and Halomethanes 1Washington: American Society of Microbiology; 1991: 219–35.

12. Moss AR, Jouany J-P, Newbold J. Methane production by ruminants: its contribution to global warming. Ann zootech, vol. 49, EDP Sciences; 2000: 231–53.
13. Kool DM, Wrage N, Zechmeister-Boltenstern S, Pfeffer M, Brus D, Oenema O, et al. Nitrifier denitrification can be a source of N2O from soil: a revised approach to the dual-isotope labelling method. Eur J Soil Sci 2010; 61: 759–72.
14. Holter P. Effect of Dung-Beetles (Aphodius spp.) and Earthworms on the Disappearance of Cattle Dung. Oikos. 1979; 32: 393–402. doi:10.2307/3544751.
15. Bang HS, Lee J-H, Kwon OS, Na YE, Jang YS, Kim WH. Effects of paracoprid dung beetles (Coleoptera: Scarabaeidae) on the growth of pasture herbage and on the underlying soil. Appl Soil Ecol. 2005; 29: 165–71.
16. Slade EM, Roslin T, Santalahti M, Bell T. Disentangling the "brown world" faecal–detritus interaction web: dung beetle effects on soil microbial properties. Oikos. 2015. DOI: 10.1111/oik.02640.
17. Penttilä A, Slade EM, Simojoki A, Riutta T, Minkkinen K, Roslin T. Quantifying beetle-mediated effects on gas fluxes from dung pats. PLoS One. 2013; 8: e71454.
18. Slade EM, Riutta T, Roslin T, Tuomisto HL. The role of dung beetles in reducing greenhouse gas emissions from cattle farming. Sci Rep. 2016; 6. 18140.
19. Hammer TJ, Fierer N, Hardwick B, Simojoki A, Slade E, Taponen J, et al. Treating cattle with antibiotics affects greenhouse gas emissions, and microbiota in dung and dung beetles. Proc R Soc Lond, B, Biol Sci. 2016; 283. DOI 10.1098/rspb.2016.0150.
20. Iwasa M, Moki Y, Takahashi J. Effects of the Activity of Coprophagous Insects on Greenhouse Gas Emissions from Cattle Dung Pats and Changes in Amounts of Nitrogen, Carbon, and Energy. Environ Entom. 2015; 44: 106–113.
21. Loreau M, Naeem S, Inchausti P, Bengtsson J, Grime JP, Hector A, et al. Biodiversity and ecosystem functioning: current knowledge and future

challenges. Science. 2001; 294: 804–808.

22. Slade EM, Mann DJ, Villanueva JF, Lewis OT. Experimental evidence for the effects of dung beetle functional group richness and composition on ecosystem function in a tropical forest. J Anim Ecol 2007; 76: 1094–104.
23. Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail P, et al. Biodiversity loss and its impact on humanity. Nature. 2012; 486: 59–67. doi:10.1038/nature11148.
24. Cardinale BJ, Srivastava DS, Duffy JE, Wright JP, Downing AL, Sankaran M, et al. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature 2006; 443: 989–92.
25. Halffter, G. and Edmonds, W.D. The nesting behavior of dung beetles (Scarabaeinae). An ecological and evolutive approach. Mexico: Instituto de Biologia, 1982.
26. LaBarbera M. Analyzing body size as a factor in ecology and evolution. Ann Rev Ecol Syst. 1989: 97–117.
27. Rosenlew H, Roslin T. Habitat fragmentation and the functional efficiency of temperate dung beetles. Oikos. 2008; 117: 1659–66.
28. Beynon SA, Mann DJ, Slade EM, Lewis OT. Species-rich dung beetle communities buffer ecosystem services in perturbed agro-ecosystems. J Appl Ecol. 2012; 49: 1365–72.
29. Kaartinen R, Hardwick B, Roslin T. Using citizen scientists to measure an ecosystem service nationwide. Ecology. 2013; 94: 2645–52. doi:10.1890/12-1165.1.
30. Barbero E, Palestrini C, Rolando A. Dung beetle conservation: effects of habitat and resource selection (Coleoptera: Scarabaeoidea). J Ins Conserv. 1999; 3: 75–84.
31. Larsen TH, Forsyth A. Trap Spacing and Transect Design for Dung Beetle Biodiversity Studies. Biotropica. 2005; 37: 322–5.
32. Davis ALV. Community organization of dung beetles (Coleoptera: Scarabaeidae): differences in body size and functional group structure between habitats. Afr J Ecol. 1996; 34: 258–75.
33. Roslin T, Forshage M, Ødegaard F, Ekblad C, Liljeberg G. Nordens dyngbaggar. TIBIALE Ltd. 2014; 360. ISBN: 978-952-67544-4-4.
34. Macagno AL, Beckers OM, Moczek AP. Differentiation of ovarian development and the evolution of fecundity in rapidly diverging exotic

beetle populations. J Exper Zool Part A: Ecol Gen Physiol. 2015; 323: 679–88.

35. Yokoyama K., Kai, Tsuchiyama. Paracoprid dung beetles and gaseous loss of nitrogen from cow dung. Soil Biol Bioch. 1991; 23; 643–647.
36. Shafiei M, Moczek AP, Nijhout HF. Food availability controls the onset of metamorphosis in the dung beetle Onthophagus taurus (Coleoptera: Scarabaeidae). Physiol Entom. 2001; 26: 173–80.
37. Aim J, Shurpali NJ, Tuittiia E, Lauriia T, Maljanen M, Saarnio S, et al. Methods for determining emission factors for the use of peat and peatlands—flux measurements and modelling. Bor Env Res. 2007; 12.
38. Livingston GP, Hutchinson GL. Enclosure-based measurement of trace gas exchange: applications and sources of error. In Matson PA, Harriss RC, editors. Biogenic trace gases: measuring emissions from soil and water. USA: John Wiley & Sons; 1995; 14–51.
39. Parkin TB, Venterea RT. USDA-ARS GRACEnet project protocols, chapter 3. Chamber-based trace gas flux measurements. Sampling Protocols USDA-ARS, Fort Collins, CO 2010:3–1.
40. Hutchinson GL, Mosier AR. Improved soil cover method for field measurement of nitrous oxide fluxes. Soil Sci Soc Amer J 1981; 45: 311–6.
41. Holm, S. A simple sequentially rejective multiple test procedure. Scand J Stat 1979; 65-70.
42. Pinheiro JC, Bates DM. Linear mixed-effects models: basic concepts and examples. Mixed-Effects Models in S and S-Plus 2000; 3–56.
43. Pinheiro J, Bates D, DebRoy S, Sarkar D. nlme: an R package for fitting and comparing Gaussian linear and nonlinear mixed-effects models. See Http://www Stats Bris Ac uk/R 2006.
44. Nervo B**,** Tocco C, Caprio E, Palestrini C, Rolando A. The Effects of Body Mass on Dung Removal Efficiency in Dung Beetles. PLoS One 2014; 9: e107699.
45. Klemperer HG. Normal and atypical nesting behaviour of Copris lunaris (L.): comparison with related species (Coleoptera, Scarabaeidae). Ecol Ent 1982; 7:69–83.
46. Lubbers IM, van Groenigen KJ, Fonte SJ, Six J, Brussaard L, van Groenigen JW. Greenhouse-gas emissions from soils increased by earthworms. Nat. Clim. Change 2013; 3: 187–94.
47. Wielgoss A, Tscharntke T, Rumede A, Fiala B, Seidel H, Shahabuddin S, et al.

Interaction complexity matters: disentangling services and disservices of ant communities driving yield in tropical agroecosystems. Proc R Soc Lond B, Biol Sci 2014; 281: 20132144.doi: 10.1098 /rspb.2013.2144.

48. Sletvold N, Moritz KK, \AAgren J. Additive effects of pollinators and herbivores result in both conflicting and reinforcing selection on floral traits. Ecology 2015; 96: 214–21.
49. Lucas-Barbosa D. Integrating studies on plant–pollinator and plant–herbivore interactions. Trends Plant Sci 2016; 21: 125–33.
50. Adler LS, Bronstein JL. Attracting antagonists: does floral nectar increase leaf herbivory? Ecology 2004; 85: 1519–26.
51. Kessler D, Kallenbach M, Diezel C, Rothe E, Murdock M, Baldwin IT. How scent and nectar influence floral antagonists and mutualists. Elife 2015; 4:e07641.
52. Nichols E, Spector S, Louzada J, Larsen T, Amezquita S, Favila ME, et al. Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. Biol Cons 2008; 141: 1461–74.
53. Manning P, Slade EM, Beynon SA, Lewis OT. Functionally rich dung beetle assemblages are required to provide multiple ecosystem services. Agr Ecosyst Environ 2016; 218: 87–94.
54. Nervo B, Caprio E, Celi L, Lonati M, Lombardi G, Falsone G, Iussig G, Palestrini C, Said-Pullicino D, Rolando A. Ecological functions provided by dung beetles are interlinked across space and time: evidence from 15N isotope tracing. Ecology 2017; 98: 433-446.
55. Negro M, Rolando A, Palestrini C. The impact of overgrazing on dung beetle diversity in the Italian Maritime Alps. Environ Ent 2011; 40: 1081–92.
56. Tocco C, Negro M, Rolando A, Palestrini C. Does natural reforestation represent a potential threat to dung beetle diversity in the Alps? J Insect Conserv 2013; 17: 207–17.
57. Tocco C, Probo M, Lonati M, Lombardi G, Negro M, Nervo B, et al. Pastoral practices to reverse shrub encroachment of sub-alpine grasslands: dung beetles (Coleoptera, Scarabaeoidea) respond more quickly than vegetation. PLoS One 2013;8:e83344.

Appendix

1. Supplementary methods

Our experimental design was based on a constant biomass of 0.30 g among dung beetle assemblages (see main text). To determine the number of individuals to insert in the treatments, we weighted a minimum of ten dried individuals of each species with analytical balance (0.0001 mg), with the exact number weighted varying with the availability of specimens in the collection of University of Turin.

In order to establish the total biomass per each dung pat, we set up a pilot experiment in May 2015. We set up 10 300g-dung pats in a linear transect in an open pasture at IPLA (N45°05'20.9" E7°44'24.4"). Dung beetles were extracted after 48 hours of pat exposure. All species were counted and identified.

To evaluate gas fluxes from the dung pats, we used a closed chamber method. Once the lid was installed on the respective terrarium, the volume of the chamber without the dung pat was 3.077 litres. To avoid the stratification of the gases, we mixed the air inside the chamber (extracting it by syringe and re-expelling it again inside the chamber). The lid was gently replaced so as not to alter the pressure in the chamber.

Gas samples (35 ml) were drawn into 50 ml polypropylene syringes through a 2-way stopcock, 20 ml of gas was expelled to clean the needle and the remaining 30 ml gas was injected directly into 12-ml soda glass vials (Exetainer®, Labco Ltd., Buckinghamshire, UK). Each vial had been evacuated with a vacuum pump before use.

Gas fluxes were measured between 09:00am and 2:00pm on eight occasions between June 5th and July 6th. Specifically, the dates were 5th, 8th, 11th , 15th , 19th , 24th , and 30th June and the 6th July 2015, corresponding to days 1, 4, 7, 11, 15, 20, 26 and 32 of the experiment (following [1]).

Gas samples were taken after 0, 8, and 16 minutes of the chamber being sealed. The gas within the syringe was injected into a 12ml vial.

In order to minimize sample contamination in case of leakage, and to allow multiple injections if needed, this procedure created an overpressure in the vial.

All three gases were analysed with gas chromatography, and the analysis were carried out within 5 days of extraction.

Instrument calibration was performed several times a day to avoid changes in atmospheric conditions during the analysis, with a three-point external calibration carried out with certified multi-standard gas samples (for CO_2, CH_4, N_2O; certified standard mixtures are provided by SIAD spa) at three different concentration levels. The calibration curve was recalculated after around 50 vials analysed. The nonlinearity of ECD response to N_2O concentration was corrected by a non-linear empirical function of the measured concentration.

The system was an Agilent mod. 7890A gas-chromatograph, equipped with a Gerstel Maestro MPS2 autosampler. After the injection (injector temperature: 70°C) the sample was split into two lines for gas detection; line 1 was equipped with two packed columns (Supelco Sigma Aldrich Porapack Q and Porapack QS) kept at 80°C and with a TCD for CO_2 detection and a FID for methane detection, placed in-line. On this line, it was used as carrier at 30.00 ml/min flow. Operating temperatures were 200°C for TCD and 250°C for FID. Line 2 was equipped with 2 packed columns (Sigma Aldrich Porapack Q) and with an ECD for N_2O detection; on this line, a 5% Argon-Methane mix was used both as carrier and makeup (30.00 ml/min). ECD operating temperature was 350°C. All detectors were manufactured by Agilent Technologies. Each line was preceded by a 500 µL loop for sample volume determination; the system allowed sample edge and tail cutting by a two-valve system, in order to limit time analysis to nearly 6 minutes.

Minimum Detectable Concentrations for each gas were: 110 ppb for CH_4, 16.5 ppm for $CO_{2,}$ 10 ppb for N_2O. MDF. Converted to fluxes (as based on MDC and chamber space), this corresponds to 0.02 mg m^{-2} h^{-1} for CH_4, 2.43 mg m^{-2} h^{-1} for CO_2, 0.0033 mg m^{-2} h^{-1} for N_2O. Fluxes that lay below detection limits were set to zero.

Table S1.1: Formula applied to each model. Alternative models fitted to flux data, with the resultant AIC values offered in Table S1.2.

Model	Formula	Correlation	Weights
GLMM	gas ~ Treatment*Day + (1|Terrarium).		
GAMM	gas ~ s(Day, k=5) + Treatment, random = list(Terrarium=~1), method = "REML".	Correlation=corAR1(0.8, form = ~ 1 | Treatment/Terrarium)	
LM	gas ~Treatment*Day , random=~1|Terrarium.	Correlation=corAR1(0.8, form = ~ 1 | Terrarium)	VarIdent(form= ~1|Treatment))
GLS	gas ~ Treatment*Day.	Correlation=corAR1(0.8, form = ~ 1 | Terrarium)	VarIdent(form= ~1|Treatment.

Table S1.2: AIC results for each model applied. AIC values for each of the models outlined in Table S1.1, as fitted to compound-specific gas fluxes.

	Model	AIC
CO_2	GLMM	568.4
	GAMM	786.3
	LME	490.6
	GLS	**491.8**
CH_4	GLMM	849.4
	GAMM	890.3
	LME	819.1
	GLS	**582.0**
N_2O	GLMM	959.1
	LME	648.6
	GAMM	976.6
	GLS	**646.6**
CO_2-equivalents	GLMM	836.4
	GAMM	872.1
	LME	570
	GLS	**570**

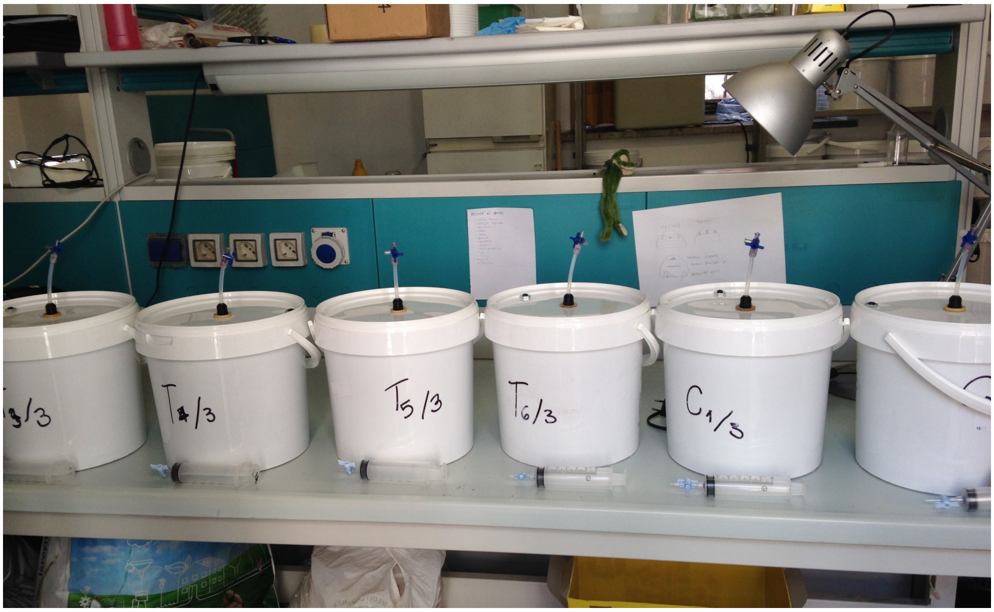

Figure A1: Terraria. Buckets with lids organized with the vent port and the syringes for the gas extractions

2. Supplementary results

Proportion of CO_2 fluxes emanating from beetle respiration

Part of the CO_2 fluxes observed in our terraria with beetles will have derived from respiration buy the beetles themselves. In order to have a rough estimation of this quantity, we derived a general relationship between dung beetle biomass and CO_2 emission, as based on data available from [2]. Our regression included the following species: *Sisyphus fasciculatus, Scarabaeus rusticus, Anachalcos convexus, Scarabeus flavicornis and Circellium bacchus*. More specifically, body mass accounted for 98% of variation in CO_2 emissions in respirometry system measurements (R^2=0.98, [2]). Applying the parameterised regression model to *Aphodius fimetarius*, *Onthophagus coenobita, Sisyphus schaefferi* and *Copris lunaris* suggests that a single individual of these species will emit: 0.000103, 0.000104, 0.000108 and 0.00012 g of CO_2 per hour. Converted to the number of individuals used per experiment, this suggests the assemblage-wide fluxes from the beetles themselves presented in S2.1 Table. These estiamtions are based on data recorded when beetles were in resting period [2], consequently the respiration rates might be higher during beetle moving activities (i.e. flying, digging, etc).

Table1 S2.1: Respiration rates per mesocosm. The respiration rate per each species was estimated using data available from [2]. To evaluate the beetle respiration per each mesocosm, the species respiration rate was multiplied by the number of individuals in each treatment. In order to compare the respiration rates with the data recorded in this experiment, the means of the CO_2 fluxes recorded in the experiment were presented in the second column of the table.

Mesocoms	Beetle respiration per mesocosm (g/h)	Mean CO_2 fluxes (g/h) observed during the first day of the experiment
T1	0.00321	0.105
T2	0.00136	0.106
T3	0.00064	0.097
T4	0.0002483	0.096
T5	0.00238	0.101
T6	0.00177	0.111

Overall it is thus clear that respiration by the dung beetles themselves made an only weak contribution to overall CO_2 emissions observed.

Table S2.2: Generalized Least Square models of GHG fluxes over measurements series (i.e. gas fluxes were measured in different 7 rounds -series - from 9 to 13:30). Shown are estimates of GLS model gas fluxes over time series with standard errors and statistical significance. Reference level: Series 1. Models were fitted assuming a Gaussian error distribution.

GLS	**CO2**				**CH4**				**N2O**			
	Estimate	Std. Error	z value	p-value	Estimate	Std. Error	z value	p-value	Estimate	Std. Error	z value	p-value
Intercept	-0.14	0.12	-1.19	0.23	-0.39	0.01	-64.30	<0.001 ***	-0.31	0.01	-27.40	<0.001 ***
Series 2	0.08	0.17	0.45	0.65	-0.01	0.01	-1.33	0.18	-0.00	0.01	-0.02	0.98
Series 3	0.04	0.17	0.23	0.82	0.00	0.01	0.49	0.62	-0.00	0.01	-0.17	0.87
Series 4	0.15	0.17	0.91	0.36	0.00	0.01	0.26	0.79	-0.00	0.01	-0.00	0.99
Series 5	0.23	0.17	1.35	0.18	0.00	0.01	0.01	0.99	0.001	0.01	0.09	0.93
Series 6	0.09	0.17	0.55	0.58	0.01	0.01	1.01	0.31	-0.00	0.01	-0.03	0.98
Series 7	-0.06	0.17	-0.33	0.72	0.01	0.01	0.73	0.46	0.017	0.01	1.05	0.29

Table S2.3: GLS models of dung removal. Generalized least squares (GLS) models on residual dry dung (g) as a function of treatment. Shown are estimated coefficients with standard errors, t-value and statistical significance. Here, control probabilities derived from an t-distribution with the appropriate degrees of freedom, whereas column "Adjusted p-value" refers to probabilities after Holm-Bonferroni correction. For the latter, we multiplied the lowest p-value observed with the number (n) of independent variables, the next-lowest p-value with n-1 etc. (here: n=7 independent variables).

	Dry residual dung				
	Estimate	Std. Error	t value	p-value	Adjusted p value
Int.	46.99	2.20	21.31	<0.001 ***	<0.001 ***
T1	-4.04	3.17	-1.28	0.21	0.83
T2	2.97	3.70	0.80	0.43	1
T3	-1.29	2.32	-0.55	0.58	1
T4	-39.10	3.08	-12.68	<0.001 ***	<0.001 ***
T5	-2.61	2.89	-0.90	0.37	1
T6	-5.73	4.01	-1.43	0.16	0.80

Table S2.4: GLS models of cumulative flux trend. Generalized Least Squares models of the cumulative fluxes of CO_2, CH_4, N_2O and CO_2-equivalents among treatments (T1-T6) over time. Fluxes of CO_2, CH_4, N_2O and CO_2-equivalents, respectively, were modelled as a function of treatments and measurement time, i.e. days since the start of the experiment, used as a categorical variable. For further details, see Materials and methods. To estimate the specific effect of variation in the beetle assemblage on GHG emissions over time, we removed the control without dung (C2). Here, control C1 was used as reference category. Column “p-value” refers to unadjusted probabilities derived from an F-distribution with the appropriate degrees of freedom, whereas column “Adjusted p-value” refers to probabilities after Holm-Bonferroni correction. For the latter, we multiplied the lowest p-value observed with the number (n) of independent tests conducted, the next-lowest p-value with n-1 etc. (here: n=4 separate compounds).

Trend of cumulative flux over time					
Variables		Df	F value	p-value	Adjusted p-value
CO_2	Intercept	1	18.38	< 0.001 ***	< 0.001 ***
	Treatment	6	1.68	0.13	0.13
	Days	7	1709.19	< 0.001 ***	< 0.001 ***
	Treatment * Days	42	1.39	0.06	0.12
CH_4	Intercept	1	25.88	< 0.001 ***	< 0.001 ***
	Treatment	6	0.86	0.52	0.52
	Days	7	215.23	< 0.001 ***	< 0.001 ***
	Treatment * Days	42	2.71	< 0.001 ***	< 0.001 ***
N_2O	Intercept	1	74.12	< 0.001 ***	< 0.001 ***
	Treatment	6	1.63	0.14	0.14
	Days	7	120.54	< 0.001 ***	< 0.001 ***
	Treatment * Days	42	2.35	< 0.001 ***	< 0.001 ***
CO_2-eq	Intercept	1	94.78	< 0.001 ***	< 0.001 ***

Table S2.5: GLS models of hourly GHG fluxes over time. Fluxes of CO_2, CH_4, N_2O and CO_2-equivalents, respectively, were modelled as a function of treatments and measurement time, i.e. days since the start of the experiment, used as a categorical variable. For further details, see Materials and methods. To estimate the specific effect of variation in the beetle assemblage on GHG emissions over time, we removed the control without dung (C2). Here, control C1 was used as reference category. Column "p-value" refers to unadjusted probabilities derived from an F-distribution with the appropriate degrees of freedom, whereas column "Adjusted p-value" refers to probabilities after Holm-Bonferroni correction. For the latter, we multiplied the lowest p-value observed with the number (n) of independent tests conducted, the next-lowest p-value with n-1 etc. (here: n=4 separate compounds).

Analysis of hourly flux over time					
Variables	Df	F value	p-value	p-value	Adjusted p-value
CO_2	Intercept	1	0.91	0.34	0.34
	Treatment	6	2.57	0.02 *	0.057
	Days	7	408.32	< 0.001***	< 0.001***
	Treatment * Days	42	1.54	0.02 *	0.04 *
CH_4	Intercept	1	0.02	0.88	0.88
	Treatment	6	1.03	0.40	0.81
	Days	7	182.15	< 0.001 ***	< 0.001 ***
	Treatment * Days	42	1.58	0.02 *	0.048 *
N_2O	Intercept	1	14.13	< 0.001 ***	< 0.001 ***
	Treatment	6	2.27	0.04 *	0.04 *
	Days	7	95.64	< 0.001 ***	< 0.001 ***
	Treatment * Days	42	1.95	< 0.001 ***	< 0.001 ***
CO_2-eq	Intercept	1	14.61	< 0.001***	< 0.001***
	Treatment	6	2.68	0.02 *	0.02 *
	Days	7	162.10	< 0.001 ***	< 0.001 ***
	Treatment * Days	42	2.14	< 0.001 ***	< 0.001 ***

Table S2.6: GLS models of cumulative GHG fluxes. Total fluxes of CO_2, CH_4, N_2O and CO_2-equivalents, respectively, accumulated by the end of the experiment, were modelled as a function of treatment. The table shows compound-specific differences (columns) between treatments (as rows; see Table 1 for summary) control C2 (without beetles and dung) versus the control C1 (which include dung but no beetles) as reference category. Column “p-value” refers to unadjusted probabilities derived from an t-distribution with the appropriate degrees of freedom, whereas column “Adj. p-value” refers to probabilities after Holm-Bonferroni correction. For the latter, we multiplied the lowest p-value observed with the number (n) of independent variables, the next-lowest p-value with n-1 etc. (here: n=8 independent variables).

Treat.	**CO_2**					**CH_4**					**N_2O**					**CO_2-eq**				
	Estimate	Std. Error	t value	p-value	Adj. p-value	Estimate	Std. Error	t value	p-value	Adj. p-value	Estimate	Std. Error	t value	p-value	Adj. p-value	Estimate	Std. Error	t value	p-value	Adj. p-value
Intercept	0.85	0.21	3.98	0.00 ***	0.00 ***	-0.13	0.26	-0.47	0.64	1	0.64	0.37	1.71	0.09	0.55	0.80	0.31	2.55	0.01 *	0.084
C2	-3.1	0.23	-13.2	0.00 ***	0.00 ***	-1.26	0.26	-4.77	0.00 ***	0.00 ***	-1.82	0.37	-4.86	0.00 ***	0.00 ***	-2.53	0.31	-8.02	0.00 ***	0.00 ***
T1	-0.24	0.28	-0.87	0.39	0.39	-0.10	0.33	-0.30	0.76	1	-0.64	0.44	-1.44	0.16	0.65	-0.61	0.35	-1.73	0.09	0.28
T2	-0.63	0.28	-2.25	0.03 *	0.12	-0.01	0.42	-0.03	0.97	1	0.07	0.72	0.09	0.92	0.92	-0.15	0.58	-0.25	0.80	0.80
T3	-0.53	0.27	-1.91	0.06	0.12	0.17	0.32	0.53	0.60	1	-0.66	0.43	-1.54	0.13	0.65	-0.71	0.36	-1.97	0.06	0.28
T4	-0.95	0.26	-3.67	0.00 ***	0.00 ***	1.50	0.52	2.91	0.00 **	0.037 *	-0.59	0.48	-1.23	0.23	0.68	-0.75	0.39	-1.95	0.06	0.28
T5	-0.68	0.27	-2.50	0.02 *	0.08	0.30	0.39	0.77	0.44	1	-0.48	0.57	-0.83	0.41	0.82	-0.61	0.50	-1.21	0.23	0.47
T6	-0.68	0.31	-2.18	0.03 *	0.1	0.41	0.43	0.95	0.35	1	-1.03	0.39	-2.65	0.01 *	0.07	-1.06	0.33	-3.22	0.00 **	0.02 *

Table S2.7: Post hoc analysis of cumulative CO_2-equivalents. Cumulative emissions of CO_2-equivalents, accumulated by the end of the experiment, were modelled as a function of treatment. Column "Adjusted p-value" refers to probabilities after Holm-Bonferroni correction. For the latter, we multiplied the lowest p-value observed with the number (n) of independent variables, the next-lowest p-value with n-1 etc. (here: n=28 total number of contrasts).

Contrast	Estimate	SD	DF	t ratio	Adjusted p-value
C2 – C1	2.53	0.32	48	8.02	< 0.001 ***
C2 – T1	-1.92	0.16	48	-11.91	< 0.001 ***
C2 – T2	-2.38	0.49	48	-4.85	< 0.001 ***
C2 – T3	-1.82	0.18	48	-10.22	< 0.001 ***
C2 – T4	-1.78	0.23	48	-7.82	< 0.001 ***
C2 – T5	-1.92	0.39	48	-4.91	< 0.001 ***
C2 – T6	-1.47	0.10	48	-14.51	< 0.001 ***
C1 – T6	1.06	0.33	48	3.22	0.048

Reference

1. Penttilä A, Slade EM, Simojoki A, Riutta T, Minkkinen K, Roslin T. Quantifying beetle-mediated effects on gas fluxes from dung pats. PLoS One. 2013; 8: e71454.

2. Duncan F D, Byrne M. J. Discontinuous gas exchange in dung beetles: patterns and ecological implications. Oecol. 2000; 122(4), 452-458.

4. Density and body size of tunneler dung beetles affect multiple ecological functions

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[•] Considering the borad topic of the last paragraph "Conservation implications" of this manuscript, it is presented in "Discussion and Conclusions" chapter.

Abstract

Species abundance and body size are two main factors that influence ecosystem functioning. Previous studies have shown that species abundance helps to maintain natural ecosystem functioning, and large-bodied species provide higher amount of ecological functions. In this study, we examined how body size and abundance in dung pats (i.e. density) of a specific insect group – dung beetles – affect multiple ecological functions: dung removal, seed dispersal and germination. Specifically, we targeted two species as representatives of small and large tunnelers: *Onthophagus illyricus* and *Copris lunaris*. In accordance with natural abundance, we considered densities ranging from 10 to 80 individuals for the small tunneler, and from 2 to 8 for the large one. We found that both density, body size and/or species identity are crucial factors for maintaining ecosystem functioning. Indeed, the combined effect of body size and density facilitated dung removal and seed dispersal. Moreover, we found that body size is a pivotal factor for seed germination, where only the large species facilitated germination. Relationships among functions depend on the species investigated; the large species showed a correlation between dung removal and seed dispersal, whereas the small one showed a correlation between seed germination and dung removal. In conclusion, optimal ecosystem functioning depends on multiple factors such as density, body size and species identity, with large species at higher densities performing better than small ones. Given that, in tunneling dung beetles, large species are more prone to extinction than smaller ones, our results suggest that management measures for the conservation of large dung beetle species should gain priority.

Keywords: body size, density, dung removal, ecosystem functioning, seed dispersal, seed germination.

4.1 Introduction

Over the last few decades, the loss of biological diversity has accentuated the need to understand how species abundance and diversity affect ecological processes (Purvis & Hector 2000; Naeem et al. 2012). 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), loss of species that facilitate or complement the functionality of other species (Zavaleta and Hulvey 2004), or massive reduction in species abundance (Estes and Palmisano 1974; Jackson et al. 2001). Without knowing the functional role of each species in the community, we cannot distinguish whether species richness itself or few abundant functional species within the community may drive ecosystem functioning (Winfree et al. 2015). Large-bodied species have been found to provide a greater amount of ecological functions, but at the same time they are sensitive to ecological stressors (Larsen et al. 2005). Indeed, traits that are responsible for the provisioning of ecological functions ("effect traits"), might also be the same traits that make species prone to extinction ("response traits"; Piccini et al. 2018). Consequently, few functionally important species such as larger bodied species can contribute greatly towards ecosystem functioning at higher abundances (Slade et al. 2007; Braga et al. 2013).

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 2014). 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). Body size plays a pivotal role in ecological functions related to dung transport to the soil. Indeed, large-bodied tunneler species have been proven to be the most efficient in dung removal (Kaartinen et al. 2013; Nervo et al. 2014), in soil nutrient cycling (Nervo et al. 2017) and in seed dispersal (Slade et al. 2007). On the other hand, larger tunneler species have been proven to be more susceptible to local extinctions (Larsen et al. 2005; Larsen et al. 2008; Tonelli et al. 2017).

Previous research has shown that provisioning of ecological functions by dung beetles may vary depending on species assemblage composition, functional group richness and species identity (Bang et al., 2005; Slade et al., 2007; O'Hea et al., 2010; Beynon et al., 2012; Nervo et al. 2016; Piccini et al. 2017). In theory, the magnitude of dung beetle effects may be dependent on the differences in species' burrowing activity or body mass (Holter et al., 2002; Larsen et al., 2005; Nervo et al., 2014). Few studies have been published on how dung beetle density influences provisioning of ecological functions (Yamada et al. 2007; Tixier et al. 2015). However, little is known about the influence of beetle density on dung removal, seed dispersal and seed germination. Some studies have investigated dung beetle influence on seed dispersal and germination in temperate forests, examples being from Japan (Koike et al. 2012) and Spain (Perez-Ramos et al. 2007; Verdù et al. 2007; Perez-Ramos et al. 2013). Most of the studies on seed dispersal and germination have been conducted in tropical areas: Mexico (Estrada and Coates-Estrada, 1991), Peru (Andresen, 1999), Brazil (Vulinec 2002; Andresen 2002; Andresen and Levey 2004), French Guiana (Feer, 1999), Uganda (Shepherd and Chapman, 1998) and Malaysian Borneo (Slade et al. 2007).

Many seeds of grassland species have been found digested, and highly concentrated in dung, suggesting that endozoochory may be one of the main drivers shaping temperate grassland communities (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). However, dung might be a beneficial microhabitat for seed germination because of the reduced competition with the already developed vegetation (Traveset 1998). However, few studies have investigated graminoid seed dispersal by dung beetles (Wicklaw et al. 1984). Here, we investigated the effects of dung beetle density and body size on three main ecological functions provided by two tunneler species: dung removal, dispersal of graminoid seed mimics and graminoid-seed germination. The percentage of seeds found in dung that are still viable is species-dependent (Milotic and Hoffmann 2016a, 2016b, 2016c). In *Lolium* genera is known that only 12% of *Lolium rigidum* seeds ingested, remained viable once it ends in cattle dung (Stanton et al. 2002). Given the low rate of *Lolium* seed survival along the digestive tract of cows, we did not place seeds in dung pats to test seed germination. Instead, we placed *L. multiflorum* seeds directly on the surface and covered them with the dung, simulating a situation in which seeds were covered by dung pats dropped by grazing cattle. Indeed, considering *L. multiflorum* can produce 100000 seeds/m^2 (Young et al. 1996) in a pasture it is likely that many seeds would be covered by dung.

In order to determine the role of body size and density in dung-system functioning, we selected the two most abundant and widespread species in the study area: one small-sized *Onthophagus illyricus* (Scopoli, 1763) and one large-sized *Copris lunaris* (Linnaeus, 1758) species. Considering that body size and abundance in dung pats (i.e. density) are pivotal factors for dung removal (Slade et al. 2007; Nervo et al. 2014; Tixier et al. 2015), we hypothesized that remaining dung on the surface would decrease with an increase in beetle density as well as body size. Moreover, we hypothesized that through increasing transportation of dung, seed dispersal would also increase with increase of density and body size. Seed germination would be facilitated through higher removal of dung mass over the seeds due to high densities of dung beetles and to the presence of larger-bodied beetles. We investigated the correlations among these functions to understand their interconnections for both species.

4.2 Materials and methods

To examine the functional consequences of dung beetle densities and body size on multiple ecological functions, we established experimental replicates of laboratory terraria with 4 different densities for two species: *Onthophagus illyricus* and *Copris lunaris*, representatives of small and large tunneler species, respectively. Both are commonly distributed in the Mediterranean area.

4.2.1 Experimental design

Dung beetles were collected from La Mandria Natural Park (45° 08' 48.83'' N, 7° 36' 02.53'' E, 290 m above sea level), and at 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, 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 *Onthophagus illyricus* (Scopoli, 1763) at IPLA and 75 *Copris lunaris* (Linnaeus, 1758) in 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). To calculate the average weight for each species, we weighed 83 and 13 dry beetles of *O. illyricus* and *C. lunaris* that were present in the entomological collection of the University of Turin. For *C. lunaris* the average weight was 0.22g ± SD $6.0 \cdot 10^{-3}$ and for *O. illyricus* it was 0.022g ± SD $8.0 \cdot 10^{-5}$.

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). Terraria were filled with 8 cm of soil for *O. illyricus* and 15 cm for *C. lunaris*, reflecting the digging capacity of these species (Macagno et al. 2016 and Piccini, pers. Obs.). We ran 8 monospecific treatments with 4 different densities per species and 4 controls without beetles. In accord with the natural species abundance found in dung pats (Piccini pers. Obs., details in Appendix), the densities were respectively 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). We also ran three types of control: terraria with dung without beetles (Cntr) and terraria with neither dung nor beetles, but with seeds either placed on the surface or in the first 5cm of soil (Csur and C5cm, respectively). Four replicates were established for treatment and control types except for the highest density of *C. lunaris*, where we ran only 3 replicates due to a lack of individuals captured. This yielded a total of 43 experimental terraria in our study (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*). The dung was frozen for at least two weeks at -8°C to kill potential predators and other insects. 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 standard-sized pats (the typical pat weight found in the field) to each treatment and to the control Cntr. The dung was manipulated to form pats of about 16 cm in diameter, and 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 average time needed for species to remove the dung to construct its nest (Klemperer 1982; Sowig 1996). Throughout the experiment, the laboratory was kept at a constant temperature around 25°C with 60 % humidity (Piccini et al. 2017).

4.2.2 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 at the end of the experiment.

In order to compare the density and body size effect of tunnelers on **seed germination,** we used seeds of *Lolium multiflorum* (Lam., 1799), one of the most widespread and common species of the local pastures. We sowed *L. multiflorum* (35 kg seed ha−1) on the surface below the dung pat (0.07g, i.e. 15 seeds; Figure A1 in Appendix). During the experiment (4 days), seeds could germinate at any time. We measured seed germination by counting the total number of stems at the end of the experiment.

To investigate the **seed dispersal,** we used colored beads as seed mimics, considering the difficulties of finding small non-germinated seeds in the soil. We considered two aspects of bead dispersal: the removal of beads from their original place (i.e. surface or dung) and the burial depth (i.e. bead dispersal in the soil). In order to evaluate burial depth, we divided the soil into different layers. For *O. illyricus,* we divided the total 8-cm of soil in two layers of 4 cm each (upper and lower layers) and for *C. lunaris* we divided the 15-cm soil in three layers of 5-cm each (upper, middle and lower layers). We evaluated dispersal of beads placed either below the dung pats and those placed in the dung, simulating the dispersal of seeds by ingestion of cattle (in accordance with Janzen et al. 1984). Thus, we placed 15 blue beads (2mm diameter) below the dung pats (together with the seeds) and 30 (2mm diameter) red beads in the dung.

4.2.3 Statistical analysis

DUNG REMOVAL AND SEED GERMINATION – To investigate which factors affected dung removal, we modelled dung mass removed (evaluated by average of dung remained in controls Cntr minus dung remained per terrarium) as a generalized linear function of density as a continuous variable, and body size as categorical variable, testing for their interaction (Density × Body_size). To evaluate which factors affected seed germination, we modelled the number of stems as a generalized linear function of density as a continuous variable, and body size as a categorical variable, specifying a Poisson distribution of errors. In both models, we excluded controls without beetles (Cntr). To identify which treatment affected seed germination, we modelled the number of visible stems as a generalized linear function of treatments as a categorical variable (O10, O50, O60, O80, Co2, Co4, Co6 and Co8), setting controls without beetles 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 numbers of stems.

SEED DISPERSAL – To investigate whether body size and density affected seed dispersal, we excluded controls without beetles from the analysis and we modelled percentage of beads removed either from dung and from the ground surface as a generalized linear model of body size as categorical variable, and density as continuous variable. We checked that errors followed the normal distribution. To show the burial depth of beads placed either in the dung and on the surface, we present raw data per layer using a boxplot graph for each species.

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, seed germination, bead

removal from surface and bead removal from dung. This resulted in 6 comparisons per species.
Each model was fitted using the 'lmerTest' package in the 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 for each model the overall 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. We evaluated it as suggested in Xu (2003).

4.3 Results

DUNG REMOVAL EFFICIENCY – We found that density and body size together affected dung removal (LM: Density x Body_size: $F_{1;30}$=17.23, p<0.001). We observed that the higher density of *C. lunaris* increased the amount of dung removed from the surface while the opposite trend was detected for *O. illyricus* that slightly decrease the amount of dung removed (Figure 1A). The effect size for this model is Ω^2=0.71.

SEED GERMINATION – We found that the interaction between density and body size did not affect seed germination (GLM: Density x Body_size: DF 30, z-value=0.19, p=0.85), indeed there were no significant trends in relation to density. We found that the higher body size in the large species significantly increased the amount of seeds germinated (GLM: Body_size: DF 30, z=-3.45, p<0.001). The effect size for this model is Ω^2=0.92.

Overall, all treatments with *C. lunaris* facilitated seed germination compared to Cntr controls with only dung (LM: 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). Even if large species facilitated seed germination below dung pats, increasing the density of *C. lunaris* did not increase seed germination (Figure 1B; Table A1 in Appendix). Treatments with *Onthophagus illyricus* did not differ from Cntr controls with only dung (LM: 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, we found that the presence of dung pats obstructed seed germination compared to controls without dung (Csur and C5cm) which showed significantly higher number of stems (Figure 1B; Csur: t_{42}=6.564, p<0.001; C5cm: t_{42}=7.078, p<0.001; Table A1 in Appendix).

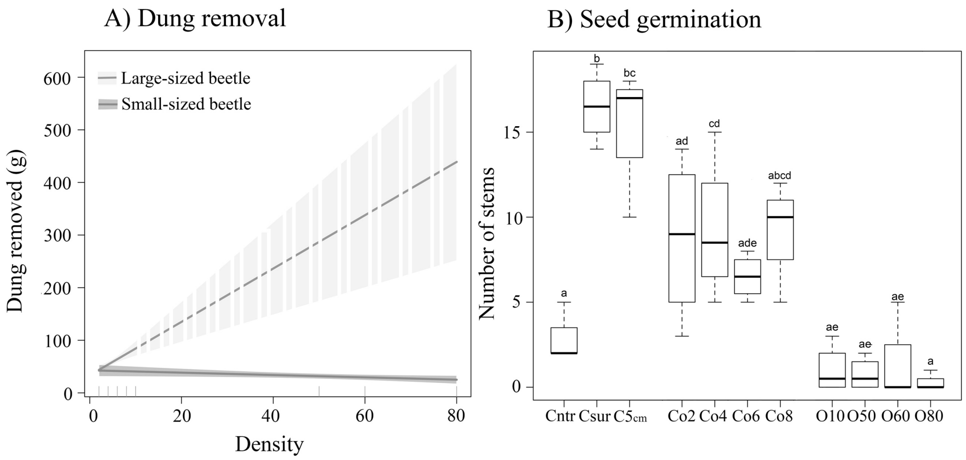

Figure 1: ***Dung removal efficiency and seed germination.*** *A) The graph illustrates the significant interaction between beetle size (light line for the large-sized beetle and dark line for the small-sized beetle) and the number of individuals per terrarium (density) in dung removal. The lines were derived from the linear regression, described in Materials and Methods; the dashed line (starting from 8 individuals up to 80) represents estimated values for Copris lunaris. B) Boxplots illustrate raw data of the number of stems derived from germinated seeds. Letters above boxes identify differences among means as revealed by Tukey post-hoc analyses of linear models (data presented in Table A1). Boxes not sharing a letter were significantly different from each other (for significance see Table A1 in Appendix). Controls with dung and without beetles were indicated by "Cntr"; controls wihout neither beetles or dung but with seeds placed in 5 cm of soil were indicated by "C5cm" and with seeds placed on the surface by "Csur"; treatments with Onthophagus illyricus by "O_x" and those with Copris lunaris by "Co_x", where x is the number of individuals per treatment.*

SEED DISPERSAL – We found that both body size and density affected transport of beads placed on the surface (LM: Density × Body_size: DF 30, t-value =-4.19, p<0.001; Figure A2A in Appendix), of beads placed in the dung (Density × Body_size: DF 30, t-value =-5.98, p<0.001; Figure A2B in Appendix). The effect sizes for these models are: Ω^2=0.55 and Ω^2=0.81, respectively.

The two species showed dissimilar behavior in relation to density for transport of beads placed in the dung (Figure A3 in Appendix). The proportion of beads remaining in the dung decreased with an increase of *Copris lunaris* density, from around 72% of beads still present in the dung at the end of the experiment for treatments with 2 *C. lunaris* to around 28% of beads in the dung for treatments with 8 *C. lunaris*. On average, 0.01% of beads were left on the surface, but most of them were transported into the soil (from around 26% of beads for treatments with 2 *C. lunaris* to around 70% for treatments with 8 *C. lunaris*; Figure A3 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 in 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 A3 in Appendix A).

Investigating the burial depth of beads transported from the dung to different soil layers, we found that the two species have different effect on bead transport along the soil depth (Figure 2). *O. illyricus* transported few beads to the upper layer of terrarium (Figure 2A). Conversely, *C. lunaris* transported few beads on the surface of soil and most of them to all the soil layers (Figure 2B).

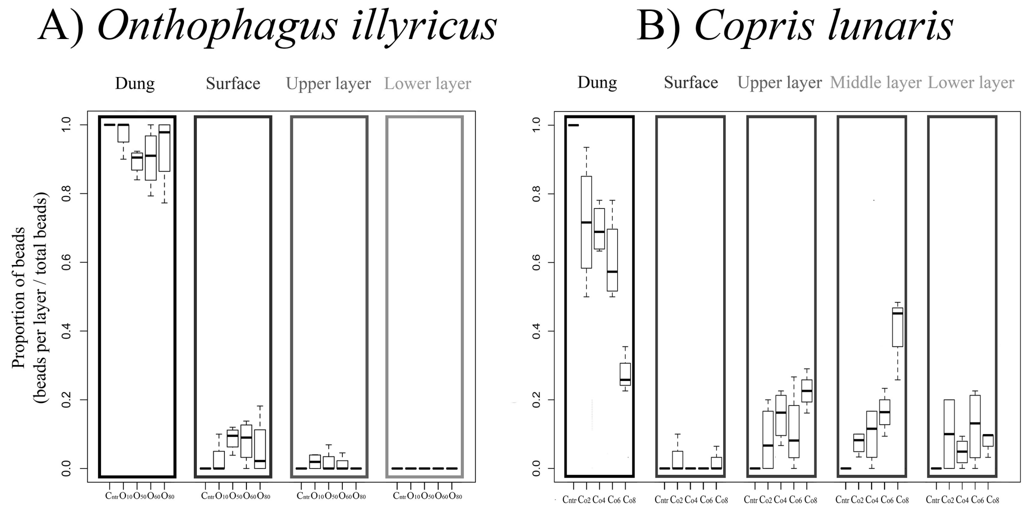

Figure 2: ***Burial depth of beads placed in the dung.*** *Box plots illustrating the proportion of beads (beads per layer / total beads) transported to the soil at different dung beetle densities. Cntr: Control group,* O_x*: x number of Onthophagus illyricus individuals,* Co_x*: x number of Copris lunaris individuals.*

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 negative correlation between dung and bead removal from dung (r = -0.84; DF 13, t=5.57, p<0.001), and from the surface (r = -0.62, DF 13, t=2.84, p=0.014). A positive correlation was also found between beads placed on the surface and beads placed in the dung (r = 0.69, DF 13, t=3.45, p=0.004; Figure 3b). No significant correlations were found between seed germination and all the other measures for *C. lunaris* (Figure 3b).

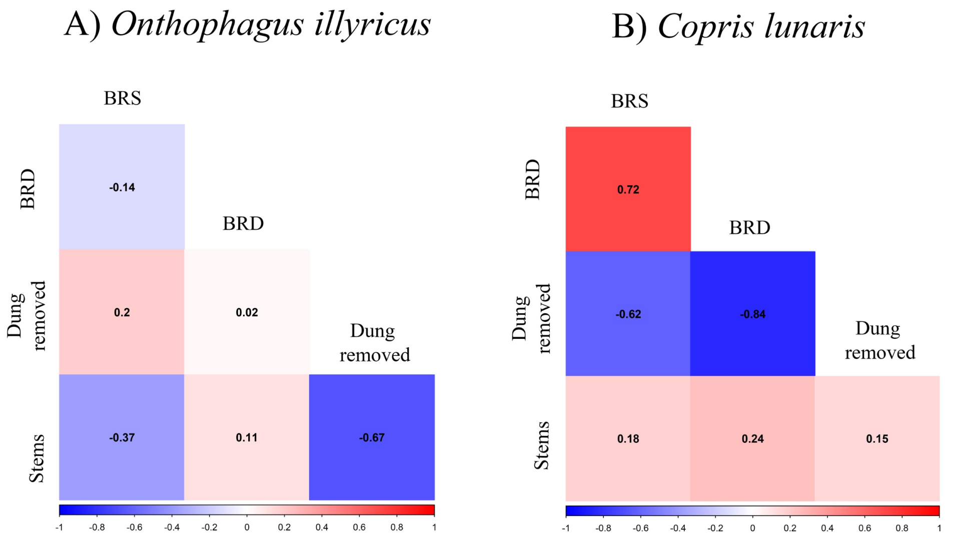

Figure 3: ***Relationships between pairs of measures of ecological functions.*** *Boxes illustrate Pearson's correlation coefficients between different aspects of ecosystem functioning: removal of beads from dung (bead removal from dung called "BRD"); bead removal from surface (called "BRS"), dung removal (evaluated by "Dung removed") and seed germination (called "Stems"). Red reflects positive correlation, blue corresponds to negative correlations, with the darkness of the color reflecting the strength of association.*

4.4 Discussion

Our results confirm that body size and abundance in dung pats (i.e. density) might be pivotal factors for high provisioning of ecological functions. This is in the light of previous studies that showed that both species diversity and abundance are necessary for the maintenance of natural ecosystem functioning (Schwartz et al. 2000; Naeem and Wright 2003). Moreover, based on the results from previous work on dung decomposition by monospecific (Tixier et al. 2015) and mixed (Yamada et al., 2007) assemblages of dung beetle species at high densities, we hypothesized that both beetle abundance, beetle size and/or species identity would have a positive effect on dung removal across treatments. We found that the two species have completely different effects on ecological functions investigated and their correlations (Figure 3). In fact, the dung removal pattern across densities was consistent with previous findings for the large species, *Copris lunaris,* but not for the small species *Onthophagus illyricus* for which the increase of density corresponds to a decrease of dung removal on the surface. For both species, increasing density did not enhance the number of stems, but the large-bodied 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 body size; only the large-bodied beetle transported beads and in this case density also affected transport (Figure 2).

4.4.1 Dung removal and seed dispersal - the importance of combined body size and abundance

The large beetle species was the one most responsible for all the ecological functions investigated, whereas the small one was not as efficient (in accordance with Kaartinen et al. 2013; Braga et al. 2013; Slade et al. 2007). Beyond body size, we found that the combination of body size and beetle abundance in dung pats plays a crucial role in dung system functioning, specifically in dung and bead removal. The higher abundance of large beetles increases dung removal efficiency and the amount of seed dispersal (Feer 1999; Yamada et al., 2007). In accord with this, we found that the amount of dung removal and bead dispersal increased with C. lunaris density. High densities of this large species (up to 8 beetles per dung pat) do 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, this species constructs a wide nest with a large amount of dung allocated in several (up to 7) brood balls (Figure A4 in Appendix). The high efficiency in dung and seed removal of nocturnal large-bodied tunnelers has already been demonstrated by Slade et al. (2007), where Catharsius dayacus was found to have probably been responsible for the high levels of dung and seed removal.

Conversely, when the density of O. illyricus was high (50, 60 and 80 individuals), the interference and/or the competition for the resource or the space was too high and tended to obstruct dung removal. Instead of transporting dung into the soil to construct the nest, O. illyricus spread the dung all over the surface of the terrarium (Figure A5 in Appendix). We found that the lowest average of dung remaining was recorded for assemblages with 10 O. illyricus, which might be the optimal abundance in dung pats. Similarly, Tixier et al. (2015) found that assemblages with 8 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, unlike the large beetle species that actively transported beads in brood balls, the small tunneler species tried to avoid bead transport to the soil. Indeed, dung beetles use dung for feeding and laying eggs, hence they often exclude seeds from the dung that they bury. Thus, large seeds, which are larger contaminants and more easily removed than small seeds, are often discarded from dung during relocation by beetles (Slade et al. 2007).

4.4.2 Seed germination - body size matters

We found that assemblages with the large-bodied species facilitated seed germination, but density did not affect the number of stems across the dung pats. In the assemblages with *C. lunaris*, the low amount of dung remaining on the surface may not obstruct seed germination, conversely to what we found in controls with higher amount of dung remaining.

In their natural environment, the percentage of germinated seeds was negatively correlated with burial depth (Andresen and Levey 2004). Even though this, buried seeds were less susceptible to predation and more likely to germinate than ones left on the ground (Perez-Ramos et al. 2012). More specifically, seed germination was proven to be greater in the first few centimeters of soil depth (up to 5cm) than on the surface - where predetion may occur - 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. We found that beads were transported by *C. lunaris* from the surface to the first layer of soil (5 cm depth; Figure A6 in Appendix), 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, 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 have shown that *O. illyricus* did not facilitate seed germination compared to dung pat controls. This might be related to high manipulation of dung that may have inhibit seed germination, even slightly less germination than in control pats. Moreover, we found that all assemblages with *O. illyricus* did not move most of the beads placed on the surface.

In conclusion, we found that beetle body size, but not density, is a decisive factor that affects the number of seeds that successfully germinated. Furthermore, as opposed to that observed for *C. lunaris*, the small species does not bury seeds placed on the surface and thus it might not prevent seed predation in a natural environment.

4.4.3 Correlation among functions

Species identity and/or body size greatly influenced the provisioning and the relationships among ecological functions (Gagic et al. 2015; Slade et al. 2017). We found different correlations between functions when we investigated the large-sized and small-sized 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, we found strong evidence for an interconnection between dung and bead removal for *C. lunaris.* In other words, higher bead removal from the surface and from dung corresponds to higher dung removal efficiency. Due to its particular nesting behavior, *C. lunaris* removed high amounts of dung, transporting more beads in this process. Indeed, we found most of the beads in the brood balls in the last two layers of the soil. Conversely, we did not detect the same pattern for *O. illyricus*. 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 A5). 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 found due to differences in dung beetle communities: a low percentage of seed removal (around 12%) was found in those assemblages where only few species were present, and dominated by small-sized species (Andresen 1999; Estrada and Coates-Estrada 1991; Slade et al. 2007).

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References

Amézquita S, Favila ME (2010) Removal rates of native and exotic dung by dung beetles (Scarabaeidae: Scarabaeinae) in a fragmented tropical rain forest. Environmental entomology 39:328–336

Andresen E (1999) Seed Dispersal by Monkeys and the Fate of Dispersed Seeds in a Peruvian Rain Forest1. Biotropica 31:145–158

Andresen E (2001) Effects of dung presence, dung amount and secondary dispersal by dung beetles on the fate of Micropholis guyanensis (Sapotaceae) seeds in Central Amazonia. Journal of Tropical Ecology 17:61–78

Andresen E (2002) Dung beetles in a Central Amazonian rainforest and their ecological role as secondary seed dispersers. Ecological Entomology 27:257–270

Andresen E, Levey DJ (2004) Effects of dung and seed size on secondary dispersal, seed predation, and seedling establishment of rain forest trees. Oecologia 139:45–54

Bang HS, Lee J-H, Kwon OS, et al (2005) Effects of paracoprid dung beetles (Coleoptera: Scarabaeidae) on the growth of pasture herbage and on the underlying soil. Appl Soil Ecol 29:165–171

Beynon SA, Mann DJ, Slade EM, Lewis OT (2012) Species-rich dung beetle communities buffer ecosystem services in perturbed agro-ecosystems. Journal of Applied Ecology 49:1365–1372

Braga RF, Korasaki V, Andresen E, Louzada J (2013) Dung Beetle Community and Functions along a Habitat-Disturbance Gradient in the Amazon: A Rapid Assessment of Ecological Functions Associated to Biodiversity. PLOS ONE 8:e57786 . doi: 10.1371/journal.pone.0057786

Cosyns E, Delporte A, Lens L, Hoffmann M (2005) Germination success of temperate grassland species after passage through ungulate and rabbit guts. Journal of Ecology 93:353–361

Couvreur M, Cosyns E, Hermy M, Hoffmann M (2005) Complementarity of epi-and endozoochory of plant seeds by free ranging donkeys. Ecography 28:37–48

Estes JA, Palmisano JF (1974) Sea otters: their role in structuring nearshore communities. Science 185:1058–1060

Estrada A, Coates-Estrada R (1991) Howler monkeys (Alouatta palliata), dung beetles (Scarabaeidae) and seed dispersal: ecological interactions in the tropical rain forest of Los Tuxtlas, Mexico. Journal of Tropical Ecology 7:459–474

Feer F (1999) Effects of dung beetles (Scarabaeidae) on seeds dispersed by howler monkeys (Alouatta seniculus) in the French Guianan rain forest. Journal of Tropical Ecology 15:129–142

Gagic V, Bartomeus I, Jonsson T, et al (2015) Functional identity and diversity of animals predict ecosystem functioning better than species-based indices. Proceedings of the Royal Society of London B: Biological Sciences 282:20142620 . doi: 10.1098/rspb.2014.2620

Hanski I, Cambefort Y (1991) Dung beetle ecology. Princeton University Press New Jersey

Holter P, Scholtz CH, Wardhaugh KG (2002) Dung feeding in adult scarabaeines (tunnellers and endocoprids): even large dung beetles eat small particles. Ecological Entomology 27:169–176 . doi: 10.1046/j.1365-2311.2002.00399.x

Hothorn T, Bretz F, Westfall P (2008) Simultaneous Inference in General Parametric Models. Biom J 50:346–363 . doi: 10.1002/bimj.200810425

Jackson JBC, Kirby MX, Berger WH, et al (2001) Historical Overfishing and the Recent Collapse of Coastal Ecosystems. Science 293:629–637 . doi: 10.1126/science.1059199

Janzen DH (1984) Dispersal of small seeds by big herbivores: foliage is the fruit. The American Naturalist 123:338–353

Kaartinen R, Hardwick B, Roslin T (2013) Using citizen scientists to measure an ecosystem service nationwide. Ecology 94:2645–2652 . doi: 10.1890/12-1165.1

Klemperer HG (1982) Normal and atypical nesting behaviour of Copris lunaris (L.): comparison with related species (Coleoptera, Scarabaeidae). Ecological Entomology 7:69–83

Koike S, Morimoto H, Kozakai C, et al (2012) The role of dung beetles as a secondary seed disperser after dispersal by frugivore mammals in a temperate deciduous forest. Acta Oecologica 41:74–81 . doi: 10.1016/j.actao.2012.04.009

Kremen C (2005) Managing ecosystem services: what do we need to know about their ecology? Ecology Letters 8:468–479 . doi: 10.1111/j.1461-0248.2005.00751.x

Larsen TH, Lopera A, Forsyth A (2008) Understanding Trait-Dependent Community Disassembly: Dung Beetles, Density Functions, and Forest Fragmentation. Conservation Biology 22:1288–1298 . doi: 10.1111/j.1523-1739.2008.00969.x

Larsen TH, Williams NM, Kremen C (2005) Extinction order and altered community structure rapidly disrupt ecosystem functioning. Ecology letters 8:538–547

Macagno ALM, Moczek AP, Pizzo A (2016) Rapid Divergence of Nesting Depth and Digging Appendages among Tunneling Dung Beetle Populations and Species. The American Naturalist 187:E143–E151 . doi: 10.1086/685776

Milotić T, Hoffmann M (2016a) Cost or benefit for growth and flowering of seedlings and juvenile grassland plants in a dung environment. Plant ecology 217:1025–1042

Milotić T, Hoffmann M (2016b) How does gut passage impact endozoochorous seed dispersal success? Evidence from a gut environment simulation experiment. Basic and Applied Ecology 17:165–176

Milotić T, Hoffmann M (2016c) Reduced germination success of temperate grassland seeds sown in dung: consequences for post-dispersal seed fate. Plant Biology 18:1038–1047

Naeem S, Duffy JE, Zavaleta E (2012) The Functions of Biological Diversity in an Age of Extinction. Science 336:1401–1406 . doi: 10.1126/science.1215855

Naeem S, Wright JP (2003) Disentangling biodiversity effects on ecosystem functioning: deriving solutions to a seemingly insurmountable problem. Ecology Letters 6:567–579 . doi: 10.1046/j.1461-0248.2003.00471.x

Nervo B, Caprio E, Celi L, et al (2017) Ecological functions provided by dung beetles are interlinked across space and time: evidence from 15N isotope tracing. Ecology 98:433–446 . doi: 10.1002/ecy.1653

Nervo B, Tocco C, Caprio E, et al (2014) The Effects of Body Mass on Dung Removal Efficiency in Dung Beetles. PLOS ONE 9:e107699 . doi: 10.1371/journal.pone.0107699

Nichols E, Spector S, Louzada J, et al (2008) Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. Biological conservation 141:1461–1474

O’Hea NM, Kirwan L, Finn JA (2010) Experimental mixtures of dung fauna affect dung decomposition through complex effects of species interactions. Oikos 119:1081–1088

Pakeman RJ, Digneffe G, Small JL (2002) Ecological correlates of endozoochory by herbivores. Functional Ecology 16:296–304

Pérez-Ramos IM, Marañón T, Lobo JM, Verdú JR (2007) Acorn removal and dispersal by the dung beetle Thorectes lusitanicus: ecological implications. Ecological Entomology 32:349–356 . doi: 10.1111/j.1365-2311.2007.00874.x

Pérez-Ramos IM, Verdú JR, Numa C, et al (2013) The comparative effectiveness of rodents and dung beetles as local seed dispersers in Mediterranean oak forests. PLoS One 8:e77197

Piccini I, Arnieri F, Caprio E, et al (2017a) Greenhouse gas emissions from dung pats vary with dung beetle species and with assemblage composition. PLOS ONE 12:e0178077 . doi: 10.1371/journal.pone.0178077

Piccini I, Nervo B, Forshage M, et al (2017b) Dung beetles as drivers of ecosystem multifunctionality: Are response and effect traits interwoven? Science of The Total Environment, 616, 1440-1448. doi:10.1016/j.scitotenv.2017.10.171

Purvis A, Hector A (2000) Getting the measure of biodiversity. Nature 405:212–219

Samways MJ (1994) Insect Conservation Biology (Conservation Biology, No 2). Springer Science & Business Media

Schwartz MW, Brigham CA, Hoeksema JD, et al (2000) Linking biodiversity to ecosystem function: implications for conservation ecology. Oecologia 122:297–305 . doi: 10.1007/s004420050035

Shepherd VE, Chapman CA (1998) Dung beetles as secondary seed dispersers: impact on seed predation and germination. Journal of Tropical Ecology 14:199–215

Slade EM, Kirwan L, Bell T, et al The importance of species identity and interactions on multifunctionality depends on how ecosystem functions are valued. Ecology n/a–n/a . doi: 10.1002/ecy.1954

Slade EM, Kirwan L, Bell T, et al (2017) The importance of species identity and interactions for multifunctionality depends on how ecosystem functions are valued. Ecology 98:2626–2639 . doi: 10.1002/ecy.1954

Slade EM, Riutta T, Roslin T, Tuomisto HL (2016) Erratum: The role of dung beetles in reducing greenhouse gas emissions from cattle farming. Sci Rep 6: . doi: 10.1038/srep22683

Sowig, P. (1996). Brood care in the dung beetle Onthophagus vacca (Coleoptera: Scarabaeidae): the effect of soil moisture on time budget, nest structure, and reproductive success. Ecography, 19:254-258.

Stanton R, Piltz J, Pratley J, et al (2002) Annual ryegrass (Lolium rigidum) seed survival and digestibility in cattle and sheep. Australian journal of experimental agriculture 42:111–115

Tixier T, Bloor JMG, Lumaret J-P (2015) Species-specific effects of dung beetle abundance on dung removal and leaf litter decomposition. Acta Oecologica 69:31–34 . doi: 10.1016/j.actao.2015.08.003

Traveset A (1998) Effect of seed passage through vertebrate frugivores' guts on germination: a review. Perspectives in Plant ecology, evolution and systematics 1:151–190

Verdú JR, Lobo JM, Numa C, et al (2007) Acorn preference by the dung beetle, Thorectes lusitanicus, under laboratory and field conditions. Animal behaviour 74:1697–1704

Vulinec K (2002) Dung beetle communities and seed dispersal in primary forest and disturbed land in Amazonia. Biotropica 34:297–309

Wicklow DT, Kumar R, Lloyd JE (1984) Germination of blue grama seeds buried by dung beetles (Coleoptera: Scarabaeidae). Environmental entomology 13:878–881

Winfree R, W. Fox J, Williams NM, et al (2015) Abundance of common species, not species richness, drives delivery of a real-world ecosystem service. Ecol Lett 18:626–635 . doi: 10.1111/ele.12424

Xu R (2003) Measuring explained variation in linear mixed effects models. Statist Med 22:3527–3541 . doi: 10.1002/sim.1572

Yamada D, Imura O, Shi K, Shibuya T (2007) Effect of tunneler dung beetles on cattle dung decomposition, soil nutrients and herbage growth. Grassland Science 53:121–129 . doi: 10.1111/j.1744-697X.2007.00082.x

Young WC, Chilcote DO, Youngberg HW (1996) Annual ryegrass seed yield response to grazing during early stem elongation. Agronomy journal 88:211–215

Zavaleta ES, Hulvey KB (2004) Realistic species losses disproportionately reduce grassland resistance to biological invaders. Science 306:1175–1177

Appendix

Details on beetle density in dung pats

Dung beetle density in dung pats depends on weather, temperature, habitat and resource availability (Hanski and Cambefort 2014). We have found dung pats densely colonized and manipulated with several *Onthophagus* species at IPLA (Istituto delle Piante da Legno, Turin). We have placed 20 pitfall traps and we caught more than 800 individuals of *Onthophagus illyricus* (average of 40 *O. illyricus* per pitfall). Similarly, at La Mandria Natural Park we have found several *Copris lunaris* in the same dung pat and we caught more than 90 individuals of the species in April 2015.

Table A1: Post hoc analysis for seed germination. The number of stems found at the end of the experiment was modelled as a function of treatment

Germination	Estimate	Std. Error	z value	p-value
Cntr – Csur	13.750	1.942	7.078	<0.01 ***
Cntr – C5cm	12.750	1.942	6.564	<0.01 ***
Csur – C5cm	-1.00	1.942	-0.515	1.0000
Co2 - Cntr	6.00	1.942	3.089	0.0729
Co4 - Cntr	6.50	1.942	3.346	0.0342 *
Co6 - Cntr	3.75	1.942	1.93	0.6969
Co8 - Cntr	6.25	2.098	2.979	0.0988
O10 - Cntr	-1.75	1.942	-0.901	0.9982
O50 - Cntr	-2.00	1.942	-1.03	0.9947
O60 – Cntr	-1.50	1.942	-0.772	0.9995
O80 - Cntr	-2.500	1.942	-1.287	0.9712
Co2 - Csur	-7.750	1.942	-3.99	<0.01 **
Co4 - Csur	-7.25	1.942	-3.732	<0.01 **
Co6 - Csur	-10.000	1.942	-5.148	<0.01 ***
Co8 - Csur	-7.500	2.098	-3.575	0.0159 *
O10 - Csur	-15.500	1.942	-7.979	<0.01 ***
O50 - Csur	-15.750	1.942	-8.108	<0.01 ***
O60 – Csur	-15.250	1.942	-7.851	<0.01 ***
O80 - Csur	-16.250	1.942	-8.365	<0.01 ***
Co2 – C5cm	-6.750	1.942	-3.475	0.0219 *
Co4 – C5cm	-6.250	1.942	-3.217	0.0505
Co6 – C5cm	-9.000	1.942	-4.633	<0.01 ***
Co8 – C5cm	-6.500	2.098	-3.098	0.0609

O10 – C5cm	-14.500	1.942	-7.464	<0.01 ***
O50 – C5cm	-14.750	1.942	-7.593	<0.01 ***
O60 – C5cm	-14.250	1.942	-7.336	<0.01 ***
O80 – C5cm	-15.250	1.942	-7.85	<0.01 ***
Co4 – Co2	0.50	1.942	0.257	1.000
Co6 – Co2	-2.25	1.942	-1.158	0.9867
Co8 – Co2	0.25	2.098	0.119	1.00
O10 – Co2	-7.75	1.942	-3.99	<0.01 **
O50 – Co2	-8.00	1.942	-4.118	<0.01 **
O60 – Co2	-7.50	1.942	-3.861	<0.01 **
O80 – Co2	-8.50	1.942	-4.376	<0.01 ***
Co6 – Co4	-2.75	1.942	-1.416	0.945
CO8 – Co4	-0.25	2.098	-0.119	1.000
O10 – Co4	-8.25	1.942	-4.247	<0.01 **
O50 – Co4	-8.50	1.942	-4.376	<0.01 ***
O60 – Co4	-8.00	1.942	-4.118	<0.01 **
O80 – Co4	-9.00	1.942	-4.633	<0.01 ***
Co8 - Co6	2.50	2.098	1.192	0.9836
O10 - Co6	-5.50	1.942	-2.831	0.1449
O50 - Co6	-5.75	1.942	-2.96	0.1042
O60 - Co6	-5.25	1.942	-2.703	0.1975
O80 - Co6	-6.25	1.942	-3.22	0.0499 *
O10 - Co8	-8.00	2.098	-3.81	<0.01 **
O50 - Co8	-8.25	2.098	-3.932	<0.01 **
O60 - Co8	-7.75	2.098	-3.694	0.0104 *
O80 - Co8	-8.75	2.098	-4.17	<0.01 **
O50 - O10	-0.25	1.942	-0.129	1.00
O60 - O10	0.25	1.942	0.129	1.00
O80 - O10	-0.75	1.942	-0.386	1.00
O60 - O50	0.50	1.942	0.257	1.00
O80 - O50	-0.50	1.942	-0.257	1.00
O80 - O60	-1.00	1.942	-0.515	1.00

Figure A1: **Lolium multiflorum seeds and beads.** Seeds are elongated, tiny and and light. 0.07g of *L. multiflorum* seeds (average of 15 seeds) were placed on the surface under the dung pats. Beads were in plastic, rounded and with a hole in the middle. 15 blue beads of 2mm size were placed under the dung pats together with seeds. 30 small – same size of blue ones – plastic and rounded beads were placed in the dung.

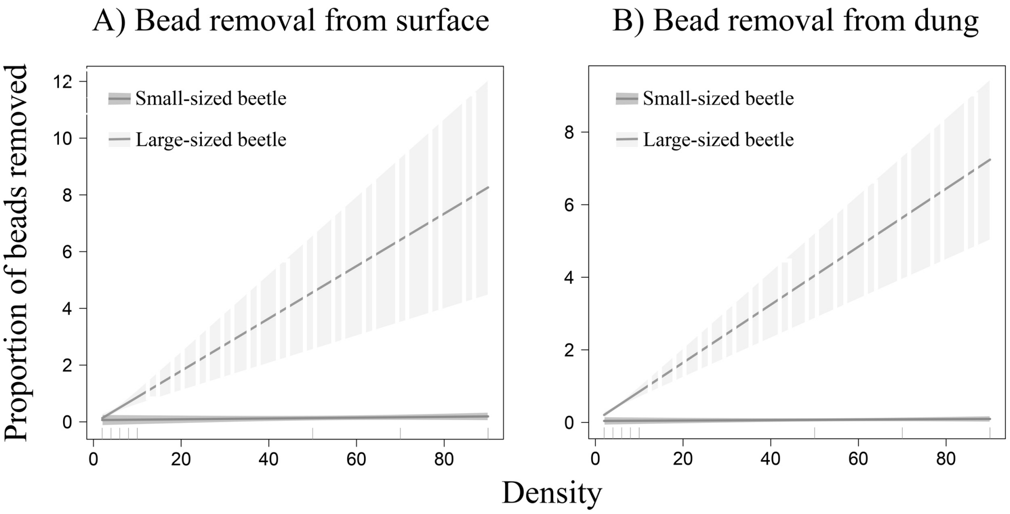

Figure A2: **Bead removal**. Shown is the interaction between beetle size (light line for the large-sized beetle and dark line for the small-sized beetle) and the number of individuals per terrarium (density) for: A) bead removal from the surface, B) bead removal from dung, and. The lines derived from the linear regression described in Materials and Methods and the dashed line (starting from 8 individuals up to 80) represents estimated values for *Copris lunaris.*

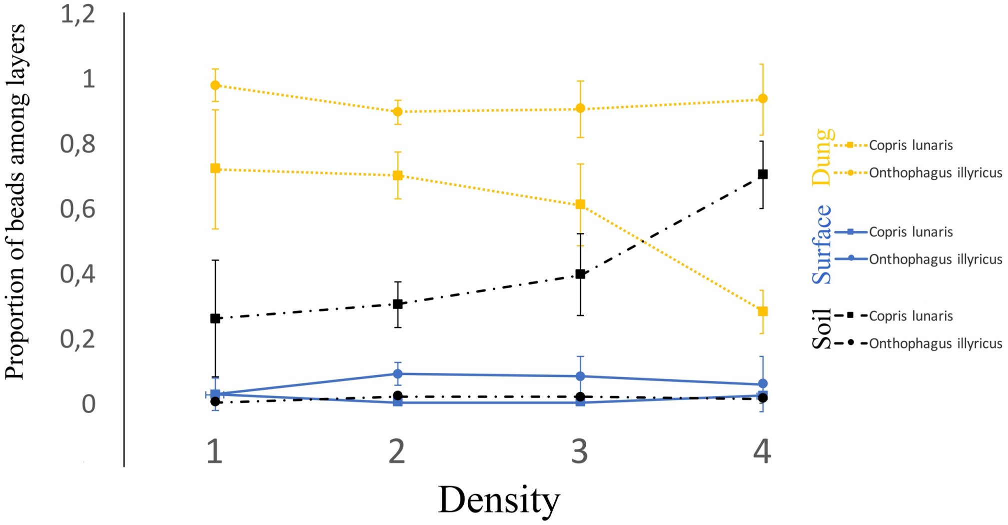

Figure A3: **Removal of beads placed in the dung.** The mean and standard deviation of the proportion of beads found in the dung (dotted light lines), on the surface (continous lines) and in the soil (dashed dark lines) for different densities of *Onthophagus illyricus* (rounded symbols) and *Copris lunaris* (square symbols). Densities are presented as categorical variables to show the general trend for both species. Density (1): 2 individuals for *C. lunaris*; 10 individuals for *O. illyricus*. Density (2): 4 individuals for *C. lunaris*; 50 individuals for *O. illyricus*. Density (3): 6 individuals for *C. lunaris*; 60 individuals for *O. illyricus*. Density (4): 8 individuals for *C. lunaris*, 80 individuals for *O. illyricus*.

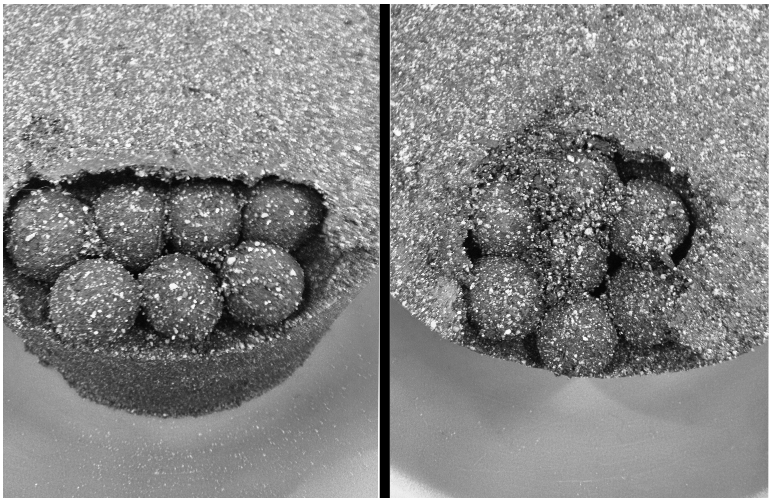

Figure A4: **Brood balls in the nest.** Examples of two nests constructed by *Copris lunaris.* We placed the terraria upside down to take pictures.

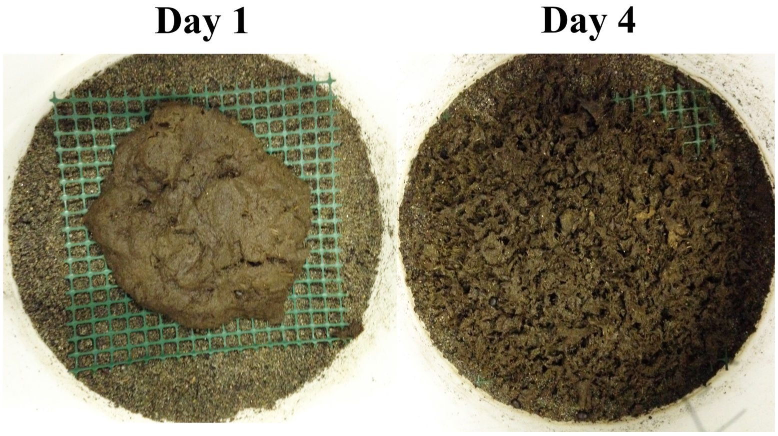

Figure A5: **Dung pat elaboration.** An example of a dung pat at the beginning and at the end of the experiment, showing the elaboration and spreading due to a high density of *Onthophagus illyricus*.

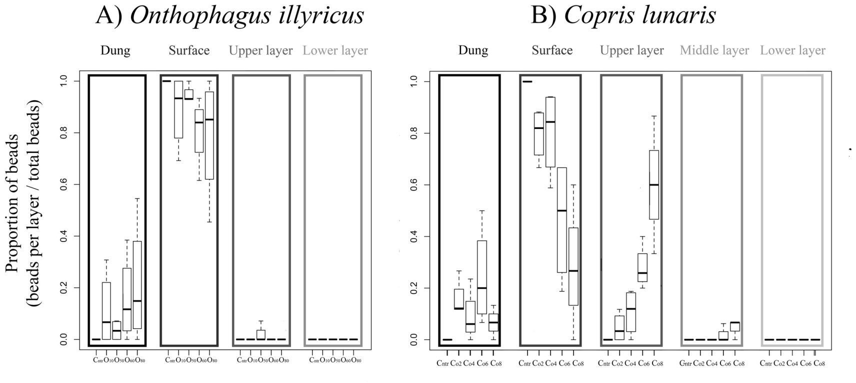

Figure A6: **Beat dispersal from surface to soil.** Box plots illustrate proportion of beads placed on the surface below the dung pats (beads per layer / total beads) transported to different layers of soil by different densities of: a) Onthophagus illyricus(“O”) and b) Copris lunaris (“Co”).

5. Local management actions by farmers override the impact of farming systems in determining dung beetle community composition and associated ecosystem services

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Abstract

Biodiversity sustains the functioning of agroecosystem. Organic farming systems have been proposed to support higher biodiversity than conventional ones, which could buffer systems against environmental change. We investigated how dung beetle communities and their provisioning of ecological functions (here: dung removal) vary at multiple hierarchical levels among Swedish dairy farms: between farming systems (organic vs conventional), among farms within farming systems, and between macro- and microhabitats within a single organic farm. To evaluate the role of local variation in community composition as a buffer against environmental change, we compared ecological functioning at experimentally warmed-up versus ambient temperatures among sites within macro- and microhabitats. At the level of farming systems, we detected no differences in dung beetle species richness, abundance or diversity. The total biomass of beetles was slightly higher on conventional farms than on organic ones, but this difference was not reflected in different provisioning of beetle-associated functions. At the farm level, we found differences in species richness, abundance and total biomass, and these differences were indeed reflected in different provisioning of ecological functions among farms. Within the focal organic farm, we found no differences in community composition between macrohabitats, and no functional differences between macrohabitats or microclimates. Slight differences in beetle abundance and total biomass between microhabitats did not translate into detectable differences in functioning. Our results identify the importance of local, farm- and site-specific variation in environmental conditions and dung beetle assemblages for ecosystem functioning. These findings suggest that local decisions made by farmers at the level of their own farm override the impact of general differences among farming systems. Farm-level management practices reflect into local variation in species composition and in associated ecosystem functions. The current results are likely context dependent, as based on efficient uptake and substantial environmental awareness among Swedish dairy farmers. From an applied perspective, our findings suggest that local decisions made by farmers shape the composition of dung beetle communities and associated ecological functions. Thus, policies aimed at promoting fine-scale management actions are an impotent supplement to general rules for certification under different farming systems.

Keywords: dung beetle community; ecological functions; farming system; organic and conventional farms; dung removal; macrohabitats; microclimates; microhabitats.

5.1 Introduction

Biodiversity has been identified as a major driver of ecosystem structure, dynamics and functioning (Hooper et al. 2012). Indeed, higher diversity may result in more complete utilization of resources, and thus in increased ecosystem functioning (Tilman et al. 2014; Harrison et al. 2014). Higher diversity has also been proposed to buffer ecosystems against environmental change (Brittain et al. 2012).

In this context, organic farming systems have been shown to support higher biodiversity than conventional ones (Fuller et al. 2005; Duru et al. 2015). In agroecosystems, biodiversity has been shown to enhance a variety of ecological services including the production of food, the recycling of nutrients, and the suppression of undesirable organisms (Altieri 1999). However, the blanket reduction of nutrient and pesticide usage forming the basis of European agri-environment schemes may in itself be insufficient to enhance biodiversity, and might need the support of better-targeted measures (Kleijn and Sutherland 2003; Kleijn et al. 2006).

Among the taxa benefitting from organic farming, dung beetles (Coleoptera: Scarabaeoidea) have been suggested to be more abundant in organic farms than in conventional, intensive and rough-grazing farms (Hutton and Giller 2003; Geiger et al. 2010). Dung beetles contribute to a wide range of ecosystem services, including dung removal, nutrient cycling, seed dispersal and the reduction of Greenhouse Gas (GHG) emissions (e.g. Nichols et al. 2008; Slade et al. 2016; Piccini et al. 2017; Nervo et al. 2017). Thus, dung beetle diversity has been found to promote ecosystem functioning (Slade et al. 2017), with accentuated effects under environmental perturbation (Manning et al. 2017) such as climate warming (Slade and Roslin 2016).

Beyond effects of farming system, dung beetle diversity and associated services may be affected by multiple features of the local and regional environment (e.g., Rosenlew and Roslin 2008). Local dung beetle communities can be shaped by *inter alia* the local habitat (Barbero et al. 1999; Larsen et al. 2006), the landscape matrix (Numa et al. 2012), and the intensity of grazing (Verdú et al. 2007). In an agricultural setting, the most important factor shaping the local environment is typically the farmer. Not only will he or she decide on what farming system (conventional or organic) to adopt, but also on the specific management regime. In this context, it has been proposed that management decisions struck by the individual farmer may equal or override the impact of farming system per se (Bengtsson et al. 2005).

To expose the effect of environmental management on biodiversity, we need to examine patterns at multiple scales (Gabriel et al. 2010). Thus, we studied variation in the dung beetle fauna and associated ecological functions at four hierarchical levels within the Swedish agricultural landscape (Fig. 1): the farming system (conventional or organic), farms within farming systems, and macrohabitats and microhabitats within farms. As a further level within farms, we investigate whether microclimate affects the provisioning of ecological functions by dung beetles. Overall, we hypothesized that organic farms will sustain higher dung beetle diversity than conventional farms, and that this will reflect into increased functioning (*sensu* dung decomposition). As an added level of variation, we expected variation in macro- and microhabitats created by local management actions within farms to affect local dung beetle assemblages, reflecting into local variation in ecosystem functioning.

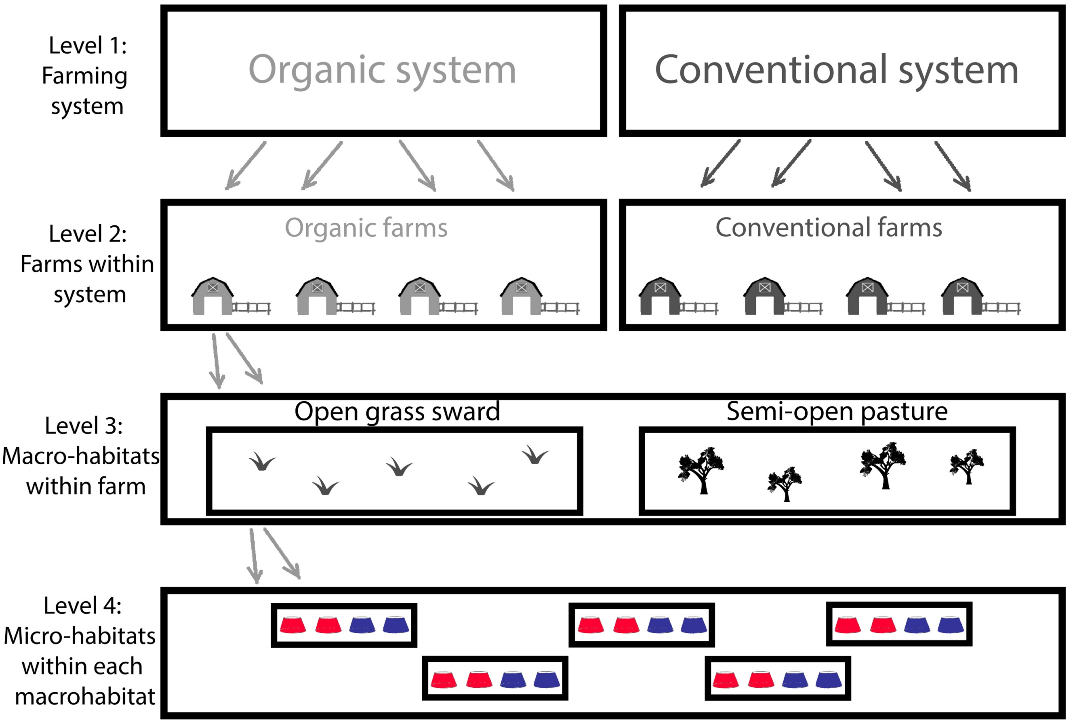

Figure 1: **We studied variation in dung beetle community composition and associated ecological functions at four hierarchical levels**: the farming system (conventional vs organic), farms within farming systems, macrohabitats within an organic farm (open grass sward and semi-open pasture) and microhabitats (sites within macrohabitats). Moreover, to establish whether local variation within farms will buffer the system against environmental change, we experimentally exposed local dung beetle communities to different microclimates (experimentally altered

by open top chambers) within each microhabitats. Microclimates are represented by experimentally warmed (red) versus control (blue) chambers. In the bottom panel, microhabitats are represented by squares.

5.2 Materials and methods

5.2.1 Study area

We selected randomly 8 farms (4 organic and 4 conventional) in the vicinity of Uppsala, south-central Sweden (59.8°N, 17.6 E). Organic farms conformed to the KRAV (2016) system, the main criteria for entering this scheme may all affect dung beetle resources and habitats (see Supplementary material, Text1). The mean distance between organic and conventional fields was 31.1 km, ranging from 2.6 to 50.6 km. All field work was carried out from 31th of May to 12th of July in 2016. During each round of measurements, visits to individual farms were spread over two consecutive days. To avoid any confounding effects of weather, farms were visited in pairs consisting of an organic and a conventional farm.

5.2.2 Experimental design

5.2.2.1 Effects of farming system (Organic vs conventional)

Within the grass sward of each farm, we set up 5 blocks each encompassing 3 treatments: a dung pat freely colonized by beetles, a control pat from which all beetles were excluded by a mesh, and a sentinel sample pat used to sample the dung beetle community (Fig. 2).

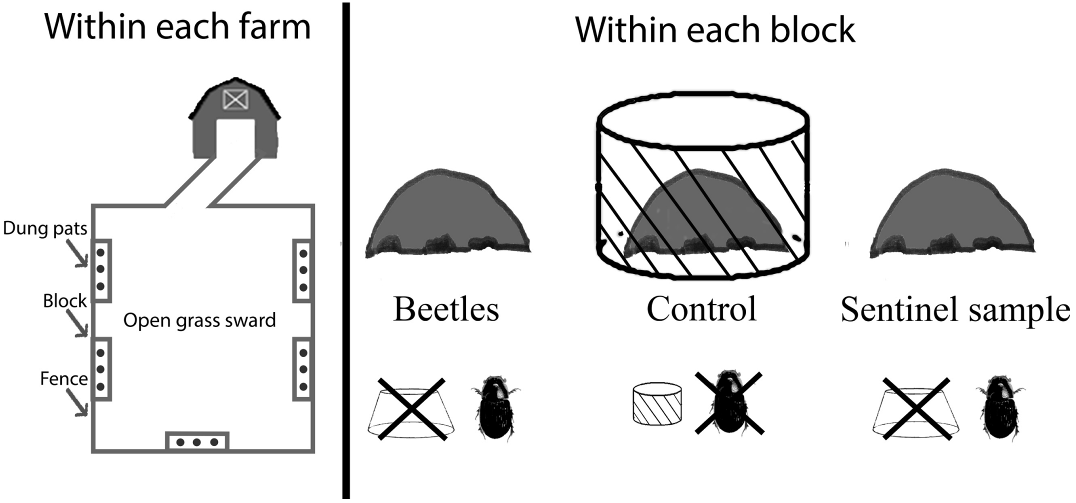

Figure 2: **Experimental design for the comparison of conventional and organic farms**. The left-hand part of the figure shows the approximate positions of the

blocks inside the grass sward of each farm (n=4 farms ×2 farming systems), whereas the right-hand part describes the treatments within each block (n=5 blocks per farm): a dung pat freely colonized by beetles, a control pat from which all beetles were excluded by a mesh, and a sentinel pat used to sample the dung beetle community.

To avoid any previous colonization by insects, we collected the dung inside the milking building of the respective farm. No cow in the herd was treated with antibiotics or anthelmintics. The dung was then homogenized before being divided into experimental pats of 1kg each. Dung pats were created on two consecutive days (31th of May and 1st of June) and the beetle community of each sentinel pat (n=5 per farm) was checked 5 days after the beginning of the experiment).

5.2.2.2 Effects of macrohabitat, microhabitat and microclimate within farm

To evaluate the influence of macro- and microhabitat on the functioning of dung-associated communities, we selected one of the four organic farms (Fig. 1). Here, we focused on the two dominant macrohabitats: open grass sward and semi-open pasture rich in trees, henceforth called ‘open grass sward’ vs ‘semi-open pasture’, respectively. Within each of these macrohabitats, we distributed five blocks, each encompassing seven treatments (i.e. a total of 70 dung pats; Fig. 3). Treatments were named according to the presence or absence of Beetles (B+ and B-), to whether the pats were surrounded by open-top chambers (OTCs; described in Slade and Roslin 2016) or not (C+, C-), which were designed to either experimentally elevate Temperature or to allow ambient, control temperatures (T+, T-, respectively). For an evaluation of the heating effect achieved, see Supplementary material, Text 2; for full details on all treatments implemented, see Supplementary material, Text 3.

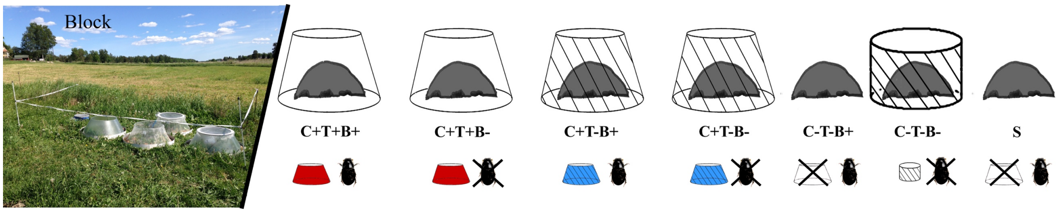

Figure 3: **Experimental design for examining the effects of microhabitat within a focal organic farm**. The left-hand part of the figure shows an example of a block in an open microhabitat. The right-hand part shows the 7 treatments included in each

block, with 10 blocks spread across two macrohabitats (5 blocks in each) and multiple different microhabitats (one per block). Treatments were named according to the presence or absence of Beetles (B+ and B-, respectively; indicated by beetle or crossed-over beetle underneath the figure), to whether the pats were surrounded by Chambers or not (C+, C-), which were designed to either experimentally elevate the Temperature or to allow ambient, control temperatures (T+, T-, respectively; red versus blue cones). From treatment C-T-B-, beetles were excluded by a separate type of enclosures (see Appendix for detail, here symbolized by a cross-hatched white cylinder). Treatment C-T-B+ corresponds to treatment *Beetles* in Fig.2, C-T-B- corresponds to *Control* in Fig.2, and treatment S to treatment *Sentinel sample* in Fig.2.

To prevent any influence of heating devices interfering with colonization by beetles, all pats (except the ones from which beetle colonization was excluded by a mesh) were first left fully exposed. After 5 days, when we assumed that colonization had occurred (see Koskela and Hanski 1977; Roslin 2000), we erected the heating structures and started the measures of functions provided by the beetle community (i.e. dung removal), by weighting each wet dung pat (see below). At the same time, the beetles colonizing the sentinel samples of Treatment 7 were extracted, identified and counted.

5.2.3 Measuring ecological functions

As metrics of ecosystem functioning, we measured dung removal rates (changes in the wet mass of the dung pats; see Supplement, Text 3) and the dry mass of dung remaining at the end of the experiment. Wet mass was recorded at increasing time intervals: on day 1, 7, 14, 21, 28 and 42, corresponding to 31st of May, 6th, 13th, 20th, 27th of June and 11th of July, respectively. To facilitate these measures, we placed a standard piece of metallic mesh under all dung pats (as in Slade and Roslin 2016 and in Piccini et al. 2018), with a mesh size large enough (3 cm) not to interfere with the behavior of tunneling dung beetles.

5.2.4 Statistical analysis

To model our observations of dung weights, we used generalized linear mixed effects models. Each model was fitted using the 'lmerTest' package in R (v3.2.1; R Development Core Team 2005), assuming an identity link and normally distributed errors. P-values were estimated using type III *F*-tests with the Satterthwaite (1946) approximation of the relevant degrees of freedom.

5.2.4.1 Community composition

Does dung beetle community composition differ between farming systems and among farms?

To compare community composition between farming systems (organic vs conventional), we created separate, univariate models of pat-level species richness (*sensu* the observed number of species), species abundances and total biomass as linear functions of farming system and farm identity, both treated as categorical variables. We assumed the number of species to be Poisson distributed, and all other responses to adhere to a normal distribution.

Does dung beetle community composition differ between macrohabitats within farms?

To evaluate whether natural dung beetle communities vary between macrohabitats (open grass sward vs semi-open pasture) within the same farm, we modelled species richness, overall abundance and total biomass as functions of macrohabitats, specifying a Poisson distribution of errors. To test for overdispersion among microhabitats, we compared the ratio between Pearson residuals of the model and the degrees of freedom to a χ^2 distribution with df=1. Here, we had one observation (from the sentinel pat; Fig. 3) from each of five blocks in each of the two macrohabitats. Significant overdispersion would thus imply that microhabitat influences the community structure of dung beetles.

5.2.4.2 Ecosystem functioning

Are there differences in ecosystem functioning at the levels of farming systems and farms?

DUNG REMOVAL RATES – To estimate the functional efficiency of different dung beetle communities at the level of **farming systems**, the wet mass of dung pats was modelled as a linear function of day (a continuous variable), beetle presence (a discrete, binomial variable: dung pats with vs without beetles) and farming system (a discrete, binomial variable: organic vs conventional), and the interactions between them (Beetles × System, Beetles × Day, System × Day and Beetles × Day × System), all of them treated as fixed effects. To adjust for block-level effects, we assumed a hierarchical random structure with dung pats nested under blocks (corresponding to the 5 areas within each pasture; see Fig 2) nested under farms (Farms|Blocks|Pats) within Systems. Here, our main interest is in the interaction Beetles × Day × System, as describing how the effect of beetles on dung pat weight differed between farming systems over time.

Since we did not find any detectable differences between farming systems (Beetles × Day × System: $F_{1,316.3}$=0.15, p=0.70), we next tested for differences in dung removal rates at the level of **individual farms**, modelling dung wet weights as a linear function of day, presence/absence of beetles and their interactions (Beetles × Farm, Beetles × Day, Farm × Day and Beetles × Day × Farm). Here, our main interest is in the interaction Beetles × Day × Farm, as describing how the presence of beetles differed among farms over time. To adjust for block-level effects, we used a hierarchical random structure with dung pats nested under blocks (Blocks|Pats).

REMAINING DUNG MASS – To test for differences among **farming systems** in overall dung removal, we modelled the dry weight of dung pats remaining on the soil surface as a linear function of beetle presence/absence (dung pats with vs without beetles), farming system (organic vs conventional) and the interactions between them (Beetles × System), as categorical variables. Farm identity was included as a random intercept in the model. Here, our main interest is in the interaction Beetles × System, as describing how the effect of beetle presence on dung weight differed between farming systems.

Since we did not detect any significant differences between farming system over days (Beetles × Day × Farm: $F_{1,304.25}$=1.91, p=0.07), we tested for differences in remaining dung mass at the level of **individual farms**. Dry dung was modelled as a linear function of presence/absence of beetles and farms and their interactions (Beetles × Farm), as categorical variables. Note that in this case, we had only one pat per treatment and block, hence block was not included in the analysis.

Does ecosystem functioning vary among macro-, microhabitats and microclimate over time?

DUNG REMOVAL RATES – To evaluate whether ecosystem functioning differed in relation to **macrohabitats** and **temperature** within the focal organic farm, wet dung weights were modelled as a linear function of beetle presence/absence, temperature (experimentally warmed versus ambient) and macrohabitats (open grass sward vs semi-open pasture) and their interactions. Measurement day was included as a fixed, continuous effect capturing gradual loss of mean weight over time. We also included all two- and three-way interactions: Macrohabitat × Beetles × Day, Temperature × Beetles × Day, Temperature × Beetles, Temperature × Day, Beetles × Day and Macrohabitat × Day. Variation at the level of pats nested under blocks (different parts of the macrohabitat) was included as a random effect. Here, our main interest is in the interaction terms Macrohabitat × Beetles × Day and Temperature × Beetles × Day, as capturing how macrohabitat and temperature affect dung mass decay rates over days in presence/absence of beetles. Since interaction Macrohabitat × Beetles × Day proved non-significant, ($F_{1,264.5}$=0.05, p=0.83), as did Temperature × Beetles × Day ($F_{1,267.5}$=0.21, p=0.64), we investigated the differences between **microhabitats**. To evaluate whether ecosystem functioning differed at this level, wet dung weights were modelled as a linear function of microhabitats (Blocks), beetle presence/absence and days, and their interactions (Blocks × Beetles × Day). Variation at the level of the dung pat and the macrohabitat was included as random factors. Here, our main interest is in the interaction terms Block × Day and Block × Day × Beetles, as capturing how microhabitat affects dung mass decay rates over days.

REMAINING DUNG MASS – To estimate functional consequences of **macrohabitat and temperature** variation, dry dung weights recorded at the end of the experiment were modelled as a linear function of beetle presence/absence, temperature (experimentally warmed versus ambient), macrohabitats, and their two- and three-way interactions (Beetles × Temperature × Macrohabitat) as fixed categorical effects, and Blocks as a random effect. Here, our main interest is in the terms Beetles × Macrohabitat, Beetles × Temperature and Beetles × Temperature × Macrohabitat, as capturing how macrohabitat and temperature affect remaining dung mass in presence/absence of beetles. Since interaction Beetle × Temperature × Macrohabitat proved non-significant ($F_{1,43.99}$=1.027, p=0.32), as did Beetle × Macrohabitat ($F_{1,43.99}$=0.73, p=0.39) and Beetle × Macrohabitat ($F_{1,43.99}$=0.02, p=0.88), we investigated the differences between **microhabitats**. To evaluate whether ecosystem functioning differed at this level, wet dung weights were modelled as linear function of microhabitats (Blocks), beetle presence/absence and their interactions (Blocks × Beetles). Here, our main interest is in the interaction term Block × Beetles, as capturing how block-level variation in the dung beetle community interact with microhabitat in affecting remaining dung mass.

5.2.4.3 Relationships between community composition and ecosystem functioning

To establish whether variation in community composition at the level of farming system, macro- and microhabitat translate into differences in functioning, we correlated remaining dung mass with metrics of the dung beetle communities. More specifically, we compared the dry mass remaining of each dung pat to dung beetle species richness, total beetle biomass, and beetle abundance for the overall experiment (45 observation), at the level of the farming system (with 25 observations within the organic system; 20 observations within the conventional system), within the focal organic farm (with 30 observations) and within each macrohabitat within this farm (with 15 observations per macrohabitat).

5.3 Results

5.3.1 Community composition

Does dung beetle community composition differ between farming systems and among farms?

A total of 8 species (*Aphodius ater*, *A. erraticus, A. fossor*, *A. heamorrhoidalis*, *A. pedellus*, *A. puntatosulcatus*, *A. pusillus*, *A. rufipes*) and 791 individual dung beetles were collected in the 8 farms. Overall abundances were highly similar, with 355 individuals recorded in organic farms and 364 individuals in conventional farms. There were no detectable differences in species richness or diversity at the level of **farming systems** (Species richness: $F_{1,12}$=0.12, p=0.73; Overall dung beetle abundance: $F_{1,12}$=0.02, p=0.90). Yet, the total biomass of dung beetles per dung pat was higher on conventional farms than on organic farms (total biomass: mean 0.26g± SD 0.29 and 0.15g± SD 0.15, respectively, $F_{1,12}$=4.18, p=0.049). At the level of **individual farms**, we found significant differences in all aspects of community attributes: species richness ($F_{6,12}$=6.54, p<0.001; Fig. S2A in Appendix), overall dung beetle abundance ($F_{6,12}$=8.33, p<0.001; Fig. S2B in Appendix) and total biomass ($F_{6,12}$=6.51, p<0.001; Fig. S2C in Appendix).

Do dung beetle communities vary with macro- and microhabitats within farms?

Dung beetle communities did not differ between **macrohabitats** (open grass sward vs semi-open pasture) within the same farm (Species richness: Z_9=-0.43, p=0.67; Overall dung beetle abundance: Z_9=-1.19, p=0.23; Total Biomass: $F_{1,9}$=0.582, p=0.47).

At the level of **microhabitats,** we found underdispersion in terms of species richness (χ^2=2.33, dispersion=0.291), and overdispersion in terms of overall abundance (Abundance: χ^2=16.21, dispersion=2.03). Thus, species richness seems no more clumped among blocks than assumed by a Poisson process, whereas abundance is indeed clumped by microhabitats.

5.3.2 Ecosystem functioning

Are there differences in ecosystem functioning at farming system and farm levels?

DUNG REMOVAL RATE – Overall, dung removal rates decreased over time (Time: $F_{1,259.5}$=90.15, p<0.001) and treatments with beetles removed more dung than beetle-free controls (Beetles: $F_{1,308.15}$=74.72, p<0.001). However, pats in different **farming systems** exhibited different dung removal rates (Day × System: $F_{1,316.3}$=6.81, p=0.009, Fig.4A), with dung mass decreasing quicker in conventional farms – irrespective of beetle presence. The wet weight of pats with vs without beetles decayed at different rates in both systems (Beetles × Day: $F_{1,316.3}$=15.47, p<0.001, Fig.4B), so that beetle presence accelerated dung removal rates. In terms of the effects deemed most interesting *a priori* – i.e. whether the beetle community characteristics of conventional farms were associated with removal rates different from those of organic farms (captured by interactions Beetles × System and Beetles × Day × System) – we found no detectable imprint (Beetles × System: $F_{1,259.6.15}$=0.98, p=0.32; Beetles × Day × System: $F_{1,316.3}$=0.153, p=0.69).

At the level of **individual farms**, dung wet weights differed among farms (Farm: $F_{1,139.26}$=26.92, p<0.001), with the presence/absence of beetles (Beetles: $F_{1,274.8}$=126.22, p<0.001) and over time (Time: $F_{1,304.25}$=302.27, p<0.001). Overall, beetle presence had a larger effect on dung weights on some farms than on others (Beetles × Farm: $F_{1,274.8}$=4.09, p<0.001, Fig. 4D), and the effect of beetle presence varied in time (Beetles × Day: $F_{1,304.25}$=19.95, p<0.001). Overall decay rates likewise differed between farms (Farm × Day: $F_{1,304.25}$=14.18, p<0.001; Fig. 4C), but variation among farms in decay rates with beetle presence was only marginally significant (Beetles × Day × Farm: $F_{1,304.25}$=1.91, p=0.07).

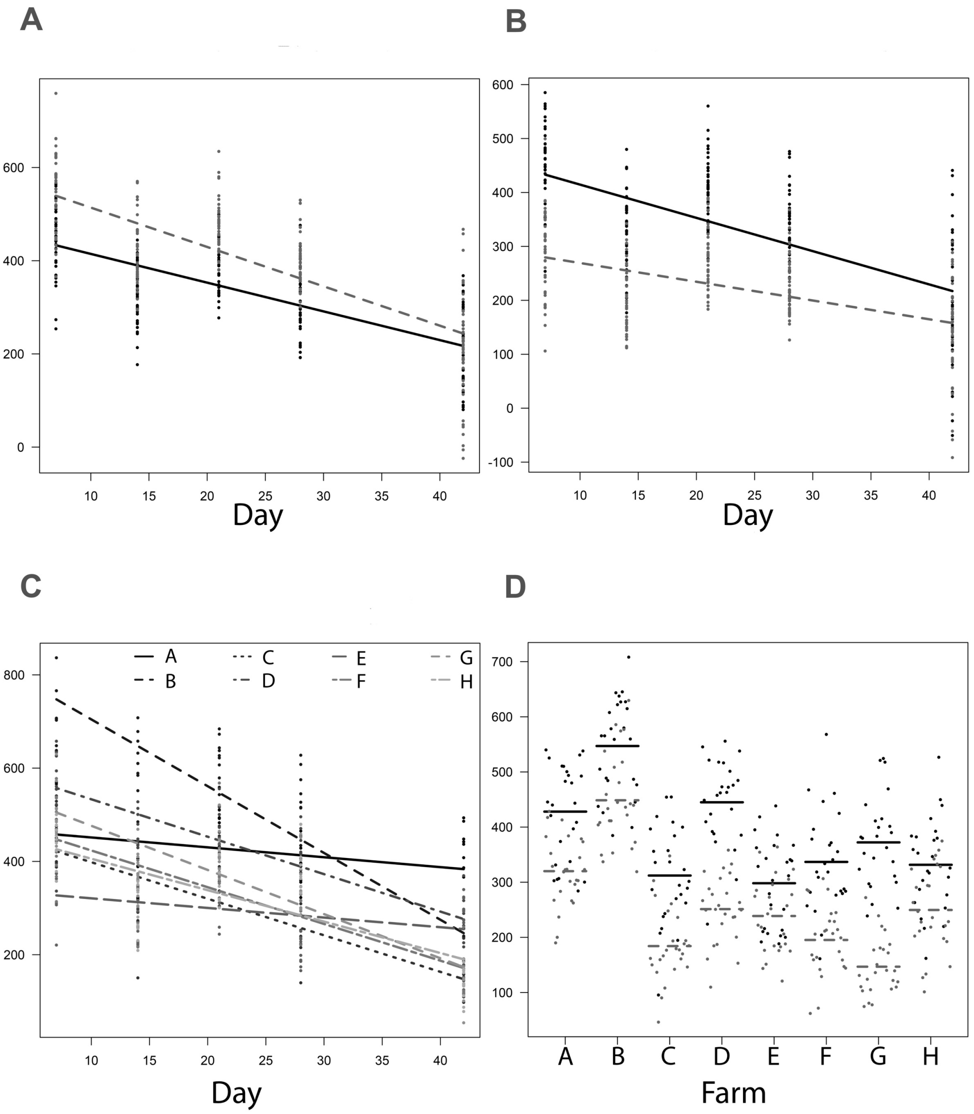

Figure 4: **Differences in dung wet weights** among A) farming systems, B) pats with versus without beetles, C) individual farms over time, and D) pats with versus without beetles on individual farms. In A), solid lines refer to conventional farms, dashed lines to organic farms; in panels B) and D) solid lines refer to treatments with beetle present, dashed lines to treatments from which beetles were absent. Line styles in C) identify individual farms, with the same styles used in D). The data

points show partial residuals, with the lines derived from the generalized linear mixed effects models described in 2. Materials and methods.

REMAINING DUNG MASS – There were no detectable differences in the effect of dung beetle presence/absence among **farming system** on the dry weight of dung remaining at the end of the experiment (Beetles × System: $F_{1,69.9}$=0.01, p=0.92). Overall, less dung remained in the presence than absence of dung beetles (Beetles: $F_{1,69.9}$=26.98, p<0.001). Moreover, the total amount of dry dung remaining at the end of the experiment did not differ between farming system (System: $F_{1,6}$=2.81, p=0.14). At the **farm level**, again less dung remained in the presence of beetles than in the absence of them (Beetles: $F_{1,64}$=28.12, p<0.001, Fig 5a), and the mass of remaining dung differed significantly among individual farms (Farm: $F_{7,64}$=7.13, p<0.001, Fig 5b). In this context, the impact of beetle presence on remaining dung mass was similar across farms (Beetles × Farms: $F_{7,64}$=1.28, p=0.27).

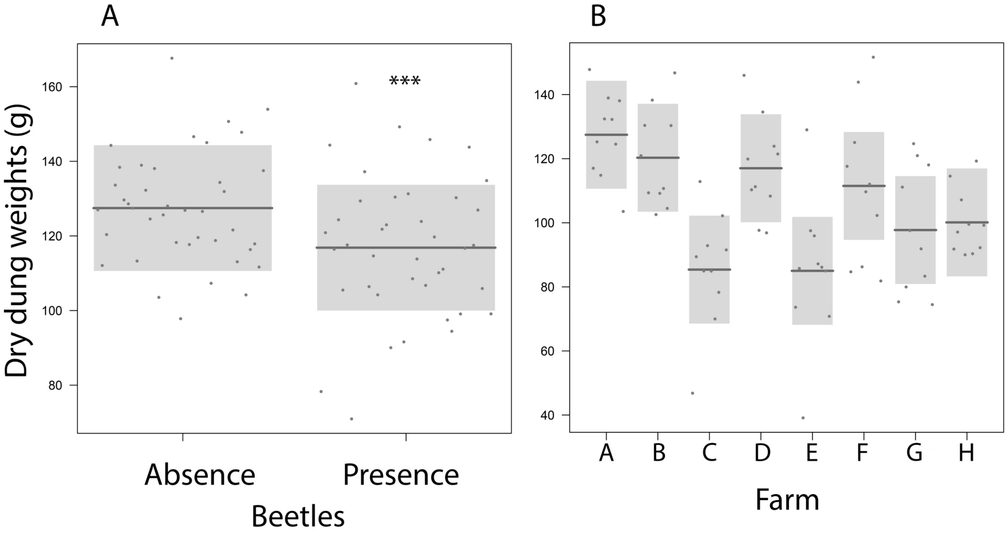

Figure 5: **Differences in remaining dung mass (dry weight)** among a) dung pats with versus without access by beetles and b) individual farms. Figure a) shows estimates (dark grey line) and confidence intervals (light grey intervals) for farms in the presence vs absence of beetles. Figure b) shows estimates per farm. All data points show partial residuals, with estimates derived from the GLMMs described in Materials and methods.

Does ecosystem functioning vary among macro- and microhabitats over time?

DUNG REMOVAL RATES – Overall, dung wet weight decreased over time ($F_{1,231.6}$=687.44, p<0.001) but neither **macrohabitats** ($F_{1,15.8}$=0.23, p=0.64) nor **temperature** (experimentally warmed vs ambient temperature; $F_{1,243.92}$=0.06, p=0.81) affected average dung wet weight. The decay of dung weight over time differed marginally between macrohabitats (Macrohabitat × Day: $F_{1,231.6}$=2.90, p=0.09), and the presence/absence of beetles did not change this pattern (Macrohabitat × Beetles: $F_{1,257.8}$=0.06, p=0.80). Similarly, the decay of wet dung weight did not differ detectably between temperatures (Temperature × Day: $F_{1,231,6}$=0.00, p=0.96), and the presence/absence of beetles did not affect this pattern (Temperature × Beetles: $F_{1,261.1}$=0.14, p=0.71).

Overall, the average wet weight of dung differed among **microhabitats** ($F_{9,223.8}$=3.94, p<0.001), with dung weight decreasing significantly over time ($F_{1,210.4}$=752.27, p<0.001) but not being affected by beetle presence ($F_{1,232.5}$=0.89, p=0.35). The decay rate of dung weight differed between microhabitats (Microhabitat × Day: $F_{9,209.8}$=1.95, p=0.046), but was unmodified by the presence/absence of beetles (Microhabitat × Beetles: $F_{9,229.8}$=1.20, p=0.29). Overall, the presence/absence of beetles left no detectable imprint on the specific rate of dung weight decay in this experiment (Day × Beetles: $F_{1,238.0}$=0.89, p=0.35).

REMAINING DUNG MASS – The amount of dung remaining at the end of the experiment differed in the presence versus absence of beetles ($F_{1,43.99}$=5.01, p=0.03, with a lower amount in the presence of beetles than in their absence) but not between **macrohabitats** (Macrohabitat: $F_{1,8.5}$=0.30, p=0.60) or **temperatures** (experimentally warmed vs ambient temperature; $F_{1,43.99}$=0.99, p=0.33). The presence/absence of beetles did not modify the impact of macrohabitat (Macrohabitat × Beetles: $F_{1,43.99}$=1.03, p=0.40) or temperatures (Temperature × Beetles: $F_{1,43.99}$=0.02, p=0.88). At the level of **microhabitats**, the amount of dry dung did differ among blocks ($F_{9,40}$=3.58, p=0.002), with a lower amount of dung remaining in the presence than absence of beetles (mean 104±20SD and 115±20SD, respectively; $F_{1,40}$=5.08, p=0.03). Dung beetle presence did not modify the impact of microhabitat (Microhabitat × Beetles: $F_{1,40}$=0.43, p=0.91).

5.3.3 Relationships between community composition and ecosystem functioning

Overall, we found no significant correlation between remaining dung mass and the total biomass of dung beetles present in the dung pats (scored at the time when the sentinel dung pats were sampled; r=-0.18, df=38, p=0.26). Neither did we find any significant correlation with species richness (r=-0.07, df=38, p=64) or with beetle abundance (r=-0.25, df=38, p=0.12).

Within the organic system, remaining dung mass was positively correlated with beetle abundance (r=0.44, df=18, p=0.05) but not with species richness (r=0.20, df=18, p=0.39) or with total beetle biomass (r=-0.03, df=18, p=0.89); **within the conventional system**, remaining dung mass was not correlated with any of the variables (total beetle biomass: r=-0.18, df=18, p=0.44; species richness: r=-0.31, df=18, p=0.18; beetle abundance: r=0.08, df=18, p=0.72).

Among the two macrohabitats examined within the focal organic farm, we did not find any significant correlation between remaining dung mass and total beetle biomass (r=-0.04, df=28, p=0.83), beetle abundance (r=0.09, df=28, p=0.65) or species richness (r=0.27, df=28, p=0.15). By contrast, within habitats, we found habitat-specific patterns: **within the open grass sward**, there was no detectable correlation between remaining dung mass and metrics of beetle communities (total beetle biomass: r=-0.40, df=13, p=0.14; beetle abundance: r=-0.27, df=13, p=0.34; species richness: r=0.35, df=13, p=0.19), but **within the semi-open pasture**, remaining dung mass was positively correlated with beetle abundance (r=0.56, df=13, p=0.03), marginally correlated with total biomass (r=0.45, df=13, p=0.08) and not correlated species richness (r=0.13, df=13, p=0.66).

5.4 Discussion

That biodiversity promotes ecosystem functioning is currently well established (Hooper et al. 2012; Tilman et al. 2014). In the context of agriculture, organic farming systems seem to generally support higher levels of biodiversity than conventional systems (Fuller et al. 2005; Bengtsson et al. 2005; Duru et al. 2015). This may convey higher rates of functioning (Altieri 1999; Batáry et al. 2013) such as augmented biological control (Puech et al. 2014). Yet, in the present study, we observed no higher dung beetle diversity on organic than conventional farms. This result contrasts with earlier findings by Hutton and Giller (2003), who reported higher species richness and abundances of dung beetles on organic than conventional cattle farms. Overall, we found slightly higher total biomass of dung beetles on conventional farms than on organic ones, but this difference did not translate into differential provisioning of ecological functions. At the level of farms within farming systems, dung beetle communities did differ among farms, as did ecological functions (i.e. dung removal). Within farms, we found less variation among macro- and micro-habitats, and among experimentally altered microclimates. Below, we will examine each of these findings in turn.

5.4.1 Ecosystem functioning does not differ between farming systems

In previous studies, organic farming has been found to reflect into an increased species richness and abundance of plants, predatory invertebrates, bats and birds (Fuller et al. 2005; Bengtsson et al. 2005). Different organisms react in different ways to organic farming, with non-predatory insects and pests being identified as less responsive to farming system than other guilds (Bengtsson et al. 2005). Within this range of responses, several studies (Hutton and Giller 2003; Geiger et al. 2010) have suggested that dung beetle diversity may be promoted by organic farming, both in terms of species richness and abundance. Other studies have proposed that general differences in community structure will reflect into differences in functioning. Thus, O'Hea, Kirwan and Finn (2010) found a higher total biomass of decomposers in general (dung beetles, flies and earthworms) to reflect into greater dung decomposition, and higher dung beetle species richness has been observed to translate into augmented ecological functioning (Manning et al. 2017).

Contrasting with these previous studies, we detected no differences among farming systems in terms of dung beetle abundance or species richness. Total biomass was slightly higher in conventional system than in organic system, likely due to a slightly higher abundance of *Aphodius fossor* on conventional farms. The lack of a difference in community metrics was matched by a lack of a difference in functioning: overall, we found no difference between farming systems in terms of dung removal rate or in the amount of dung mass remaining at the end of the season.

Our findings match with those of another study conducted in the same region: Roubinet et al. (2017) detected no differences in the densities of aphids, earthworms, or spiders between the farming systems of Central Sweden. This general lack of differences is probably due to the fact that in this region, the differences between the two farming systems are less pronounced than in some other areas of Europe (Jordbruksverket and SCB 2014), with the local farmers showing a high degree of environmental consciousness. Thus, in interviews with local farmers, Andersson et al. (2015) found relatively high use of pesticides in some intensively-cultivated farms, but most farmers declared themselves uncomfortable with using pesticides and preferred to use them in low amounts. Agrochemicals in general are used with care in Central Sweden, and are considered a waste of time and money by farmers when not acutely needed to control pest problems (Weibull et al. 2003). Most conventional farmers in the study area use inorganic fertilisers and herbicides, whereas insecticides and fungicides are used more seldom (e.g. Östman et al. 2001). Across Sweden, a general attention to conservation issues and environment sustainability is reflected by the country achieving the highest environmental sustainability index among the twelve countries investigated by

Siche et al. (2006). Thus, most farms in our study region are extensively rather than intensively managed, regardless of farming system – thereby explaining the lack of major differences in dung beetle communities.

5.4.2 Farm management matters

In an agroecological setting, many factors may enhance the abundance of some taxa. Of these, only some are related to the farming system – and most importantly, variation within farming systems is substantial (Schneider et al. 2013; Puech et al. 2014). Individual farmers can manage their field to increase the abundance of different organism groups. For example, biodiversity may benefit from an increase of heterogeneity within farms (Belfrage et al. 2015; Fahrig et al. 2015). In terms of dung beetles, the maintenance of a local mosaic of different habitats (e.g., open pasture, natural or semi-natural micro-habitats) by the rotation of cattle grazing areas has been found to promote diversity (Verdú et al. 2007; Numa et al. 2012). Thus, the most important factor shaping the local environment is the farmer, through his or her management decisions. Not only will the farmer decide on what farming system (conventional or organic) to adopt, but also on the specific management regime to implement. In this context, it has been proposed that individual variation among farmers, and management decisions struck by him/her may equal or override the impact of farming system *per se* (Bengtsson et al. 2005). In the present study, we found large differences in dung beetle abundances among farms. This variation among farms within the same farming systems suggests that local decisions made by farmers can be crucial in shaping the local habitats and their dung beetle communities. Indeed, in the central part of Sweden, the effect of heterogeneity within farms has been shown to be more effective in promoting the diversity of butterflies, predatory insects and the plants of field margins than the mere conversion to organic farming (Weibull et al. 2000; Weibull et al. 2003; Belfrage et al. 2015).

In terms of dung beetles, the differences in species richness, abundance and total biomass observed among farms will likely reflect farm- and farmer-specific choices with respect to cattle fodder, grazing regimes and/or field management. The resulting differences in dung beetle community composition are also reflected in ecosystem functioning. Indeed, the presence of dung beetles in dung pats significantly reduced the wet weight of dung left on the surface (i.e. dung removal rate) among farms (Fig 4d) and, overall, the mass of dung remaining at the end of the experiments differed significantly among farms (Fig. 5b). Thus, farm-specific management decisions seem to affect both the community attributes of local dung beetle communities and associated ecological functioning.

5.4.3 The importance of environmental variation

The imprint of environmental variation on dung beetle communities seemed most pronounced at the farm level, whereas within a single focal farm, we detected no differences in community composition between either macro- or microhabitats. Likewise, the provisioning of ecological functions varied little at the within-farm level. Only within one of the macrohabitats (semi-open pasture) did we find a positive correlation between dung beetle abundance and dung removal, whereas in other environments and at other levels, such relationships were blurred or missing. This lack of general diversity-functioning relations may be related to the fact that most of the variation in dung beetle abundances and species richness concerned to small-bodied species, which may be less efficient in removing dung in the short term than larger species (Rosenlew and Roslin 2008; Nervo et al. 2014). Within macro-habitats, we found slight differences in dung beetle abundance and species richness among microhabitats. Such differences may be related to different biotic and abiotic features of the microhabitat, such as microclimate (Roslin et al. 2009), soil type, moisture, leaf and vegetation cover (Mehrabi et al. 2014). Even if we detected significant differences in community composition among microhabitats, the provisioning of ecological functions did not differ among microhabitats.

In terms of experimentally-warmed versus control mesocosms, we found no differences in the provisioning of ecological functions. Thus, slight variation in dung beetle community composition among macro- and microhabitats is too small to translate into differences in functional resilience with environmental modification. These patterns agree with those reported by Slade and Roslin (2016), who found no effects of experimentally-manipulated microclimate on dung removal rates. However, functional resilience may depend on the functions investigated, since the same study highlighted that interactions between species (specifically *Geotrupes stercorarius* and *A. fossor*) interact with temperature in determining plant productivity (Slade and Roslin 2016). In the current study, we failed to establish whether other functions might have been affected by microclimatic manipulations.

5.5 Conclusions

Our findings confirm that factors other than the farming system may be crucial in dictating agrobiodiversity – and that local decisions made by farmers can thereby shape the composition of dung beetle communities and associated ecological functions. Thus, the maintenance of biodiversity and ecosystem functioning requires closer collaboration with farmers and foresters (Jackson 2002), scientists (Bengtsson et al. 2005) and policy makers. In the present Swedish system, policies aimed at emphasizing the impact of fine-scale management actions are an impotent supplement to general rules for certification under different farming systems. In cultivated habitats, conservation strategies are *de facto* implemented by farmers, and a close dialogue between academia, policy makers and farmers is thus of utmost importance. Thus, the maintenance of biodiversity and ecosystem functioning requires closer collaboration with farmers, foresters (Jackson 2002), scientists (Bengtsson et al. 2005) and policy makers. In the present Swedish system, policies aimed at emphasizing the impact of fine-scale management actions are an impotent supplement to general rules for certification under different farming systems. In cultivated habitats, conservation strategies are de facto implemented by farmers, and a close dialogue between academia, policy makers and farmers is thus of utmost importance.

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References

Altieri, M.A., 1999. The ecological role of biodiversity in agroecosystems. Agriculture, Ecosystems & Environment 74, 19–31. https://doi.org/10.1016/S0167-8809(99)00028-6

Andersson, E., Nykvist, B., Malinga, R., Jaramillo, F., Lindborg, R., 2015. A social–ecological analysis of ecosystem services in two different farming systems. AMBIO 44, 102–112. https://doi.org/10.1007/s13280-014-0603-y

Barbero, E., Palestrini, C., Rolando, A., 1999. Dung beetle conservation: effects of habitat and resource selection (Coleoptera: Scarabaeoidea). Journal of Insect Conservation 3, 75–84.

Batáry, P., Sutcliffe, L., Dormann, C.F., Tscharntke, T., 2013. Organic Farming Favours Insect-Pollinated over Non-Insect Pollinated Forbs in Meadows and Wheat Fields. PLOS ONE 8, e54818. https://doi.org/10.1371/journal.pone.0054818

Belfrage, K., Björklund, J., Salomonsson, L., 2015. Effects of farm size and on-farm landscape heterogeneity on biodiversity—Case study of twelve farms in a Swedish landscape. Agroecology and Sustainable Food Systems 39, 170–188.

Bengtsson, J., Ahnström, J., WEIBULL, A.-C., 2005. The effects of organic agriculture on biodiversity and abundance: a meta-analysis. Journal of applied ecology 42, 261–269.

Brittain, C., Kremen, C., Klein, A.-M., 2013. Biodiversity buffers pollination from changes in environmental conditions. Global Change Biology 19, 540–547. https://doi.org/10.1111/gcb.12043

Duru, M., Therond, O., Martin, G., Martin-Clouaire, R., Magne, M.-A., Justes, E., Journet, E.-P., Aubertot, J.-N., Savary, S., Bergez, J.-E., Sarthou, J.P., 2015. How to implement biodiversity-based agriculture to enhance ecosystem services: a review. Agron. Sustain. Dev. 35, 1259–1281. https://doi.org/10.1007/s13593-015-0306-1

Fahrig, L., Girard, J., Duro, D., Pasher, J., Smith, A., Javorek, S., King, D., Lindsay, K.F., Mitchell, S., Tischendorf, L., 2015. Farmlands with smaller crop fields have higher within-field biodiversity. Agriculture, Ecosystems & Environment 200, 219–234.

Fuller, R.J., Norton, L.R., Feber, R.E., Johnson, P.J., Chamberlain, D.E., Joys, A.C., Mathews, F., Stuart, R.C., Townsend, M.C., Manley, W.J., Wolfe, M.S., Macdonald, D.W., Firbank, L.G., 2005. Benefits of organic farming to biodiversity vary among taxa. Biology Letters 1, 431–434. https://doi.org/10.1098/rsbl.2005.0357

Gabriel, D., Sait, S.M., Hodgson, J.A., Schmutz, U., Kunin, W.E., Benton, T.G., 2010. Scale matters: the impact of organic farming on biodiversity at different spatial scales. Ecology letters 13, 858–869.

Geiger, F., Lubbe, S.C.T.M. van der, Brunsting, A.M.H., Snoo, G.R. de, 2010. Insect abundance in cow dung pats of different farming systems. Entomologische Berichten 70, 106–110.

Harrison, P.A., Berry, P.M., Simpson, G., Haslett, J.R., Blicharska, M., Bucur, M., Dunford, R., Egoh, B., Garcia-Llorente, M., Geamănă, N., Geertsema, W., Lommelen, E., Meiresonne, L., Turkelboom, F., 2014. Linkages between biodiversity attributes and ecosystem services: A systematic review. Ecosystem Services 9, 191–203. https://doi.org/10.1016/j.ecoser.2014.05.006

Hooper, D.U., Adair, E.C., Cardinale, B.J., Byrnes, J.E.K., Hungate, B.A., Matulich, K.L., Gonzalez, A., Duffy, J.E., Gamfeldt, L., O'Connor, M.I., 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature 486, 105. https://doi.org/10.1038/nature11118

Hutton, S.A., Giller, P.S., 2003. The effects of the intensification of agriculture on northern temperate dung beetle communities. Journal of Applied Ecology 40, 994–1007. https://doi.org/10.1111/j.1365-2664.2003.00863.x

Jackson, W., 2002. Natural systems agriculture: a truly radical alternative. Agriculture, Ecosystems & Environment, Soil Health as an Indicator of Sustainable Management 88, 111–117. https://doi.org/10.1016/S0167-8809(01)00247-X

Jordbruksverket, SCB, 2011. Köttkonsumtionen i siffror [WWW Document]. URL https://www.jordbruksverket.se/download/18.39da9f0113cb389bda880001123/Ra+2013_2.pdf.

Kleijn, D., Baquero, R.A., Clough, Y., Díaz, M., De Esteban, J., Fernández, F., Gabriel, D., Herzog, F., Holzschuh, A., Jöhl, R., Knop, E., Kruess, A., Marshall, E.J.P., Steffan-Dewenter, I., Tscharntke, T., Verhulst, J., West, T.M., Yela, J.L., 2006. Mixed biodiversity benefits of agri-environment schemes in five European countries. Ecol. Lett. 9, 243–254; discussion 254–257. https://doi.org/10.1111/j.1461-0248.2005.00869.x

Kleijn, D., Sutherland, J, W., 2003. How effective are European agri-environment schemes in conserving and promoting biodiversity? Journal of Applied Ecology 40, 947–969. https://doi.org/10.1111/j.1365-2664.2003.00868.x

Koskela, H., Hanski, I., 1977. Structure and succession in a beetle community inhabiting cow dung, in: Annales Zoologici Fennici. JSTOR, pp. 204–223.

KRAV, 2016. Marknadsrapport http://www.krav.se/sites/default/files/krav_marknadsrapport_2016_webb.pdf.

Larsen, T.H., Lopera, A., Forsyth, A., 2006. Extreme trophic and habitat specialization by Peruvian dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae). The Coleopterists Bulletin 60, 315–324.

Mehrabi, Z., Slade, E.M., Solis, A., Mann, D.J., 2014. The importance of microhabitat for biodiversity sampling. PloS one 9, e114015.

Nervo, B., Caprio, E., Celi, L., Lonati, M., Lombardi, G., Falsone, G., Iussig, G., Palestrini, C., Said-Pullicino, D., Rolando, A., 2017. Ecological functions provided by dung beetles are interlinked across space and time: evidence from 15N isotope tracing. Ecology 98, 433–446.

Nervo, B., Tocco, C., Caprio, E., Palestrini, C., Rolando, A., 2014. The Effects of Body Mass on Dung Removal Efficiency in Dung Beetles. PLOS ONE 9, e107699. https://doi.org/10.1371/journal.pone.0107699

Nichols, E., Spector, S., Louzada, J., Larsen, T., Amezquita, S., Favila, M.E., 2008. Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. Biological Conservation 141, 1461–1474. https://doi.org/10.1016/j.biocon.2008.04.011

Numa, C., Verdú, J.R., Sánchez, A., Galante, E., 2009. Effect of landscape structure on the spatial distribution of Mediterranean dung beetle diversity. Diversity and Distributions 15, 489–501.

O'hea, N.M., Kirwan, L., Finn, J.A., 2010. Experimental mixtures of dung fauna affect dung decomposition through complex effects of species interactions. Oikos 119, 1081–1088.

Östman, Ö., Ekbom, B., Bengtsson, J., Weibull, A.-C., 2001. Landscape Complexity and Farming Practice Influence the Condition of Polyphagous Carabid Beetles. Ecological Applications 11, 480–488. https://doi.org/10.2307/3060903

Piccini, I., Arnieri, F., Caprio, E., Nervo, B., Pelissetti, S., Palestrini, C., Roslin, T., Rolando, A., 2017. Greenhouse gas emissions from dung pats vary with dung beetle species and with assemblage composition. PLoS One 12, e0178077.

Piccini, I., Nervo, B., Forshage, M., Celi, L., Palestrini, C., Rolando, A., Roslin, T., 2018. Dung beetles as drivers of ecosystem multifunctionality: Are response and effect traits interwoven? Science of The Total Environment, 616, 1440-1448.

Puech, C., Baudry, J., Joannon, A., Poggi, S., Aviron, S., 2014. Organic vs. conventional farming dichotomy: Does it make sense for natural enemies? Agriculture, Ecosystems & Environment 194, 48–57. https://doi.org/10.1016/j.agee.2014.05.002

Rosenlew, H., Roslin, T., 2008. Habitat fragmentation and the functional efficiency of temperate dung beetles. Oikos 117, 1659–1666. https://doi.org/10.1111/j.1600-0706.2008.16904.x

Roslin, T., 2000. Dung beetle movements at two spatial scales. Oikos 91, 323–335.

Roubinet, E., Birkhofer, K., Malsher, G., Staudacher, K., Ekbom, B., Traugott, M., Jonsson, M., 2017. Diet of generalist predators reflects effects of cropping period and farming system on extra-and intraguild prey. Ecological Applications 27, 1167–1177.

Satterthwaite, F.E., 1946. An approximate distribution of estimates of variance components. Biometrics bulletin 2, 110–114.

Schneider, M.K., Lüscher, G., Jeanneret, P., Arndorfer, M., Ammari, Y., Bailey, D., Balázs, K., Báldi, A., Choisis, J.-P., Dennis, P., Eiter, S., Fjellstad, W., Fraser, M.D., Frank, T., Friedel, J.K., Garchi, S., Geijzendorffer, I.R., Gomiero, T., Gonzalez-Bornay, G., Hector, A., Jerkovich, G., Jongman, R.H.G., Kakudidi, E., Kainz, M., Kovács-Hostyánszki, A., Moreno, G., Nkwiine, C., Opio, J., Oschatz, M.-L., Paoletti, M.G., Pointereau, P., Pulido, F.J., Sarthou, J.-P., Siebrecht, N., Sommaggio, D., Turnbull, L.A., Wolfrum, S., Herzog, F., 2014. Gains to species diversity in organically farmed fields are not propagated at the farm level. Nature Communications 5, 4151. https://doi.org/10.1038/ncomms5151

Siche, J.R., Agostinho, F., Ortega, E., Romeiro, A., 2008. Sustainability of nations by indices: Comparative study between environmental sustainability index, ecological footprint and the emergy performance indices. Ecological Economics 66, 628–637. https://doi.org/10.1016/j.ecolecon.2007.10.023

Slade, E.M., Kirwan, L., Bell, T., Philipson, C.D., Lewis, O.T., Roslin, T., 2017. The importance of species identity and interactions for multifunctionality depends on how ecosystem functions are valued. Ecology 98, 2626–2639. https://doi.org/10.1002/ecy.1954

Slade, E.M., Riutta, T., Roslin, T., Tuomisto, H.L., 2016. The role of dung beetles in reducing greenhouse gas emissions from cattle farming. Scientific reports 6. doi: 10.1038/srep18140

Slade, E.M., Roslin, T., 2016. Dung beetle species interactions and multifunctionality are affected by an experimentally warmed climate. Oikos 125, 1607–1616.

Tilman, D., Isbell, F., Cowles, J.M., 2014. Biodiversity and Ecosystem Functioning. Annual Review of Ecology, Evolution, and Systematics 45, 471–493. https://doi.org/10.1146/annurev-ecolsys-120213-091917

Verdú, J.R., Moreno, C.E., Sánchez-Rojas, G., Numa, C., Galante, E., Halffter, G., 2007. Grazing promotes dung beetle diversity in the xeric landscape of a Mexican Biosphere Reserve. Biological Conservation 140, 308–317.

Weibull, A.-C., Bengtsson, J., Nohlgren, E., 2000. Diversity of butterflies in the agricultural landscape: the role of farming system and landscape heterogeneity. Ecography 23, 743–750.

Weibull, A.-C., Östman, Ö., Granqvist, \AAsa, 2003. Species richness in agroecosystems: the effect of landscape, habitat and farm management. Biodiversity and conservation 12, 1335–1355.

Appendix

Text 1. Criteria for organic milk production in Sweden (KRAV)

Sweden maintains a single environment labelling system for organic milk production: KRAV (2016). The main criteria for entering this scheme encompass restrictions on the use of conventionally-produced fodder (maximum 5%) and concentrated feed (maximum 40%), requirements regarding feed composition (stating that all animals must have free access to good quality of roughage and grazing hay and silage must be included in animals' ration; usage of straw is only temporary permitted) and self-sufficiency in terms of feed production (of which 75% has be produced on the farm itself), and rules requiring access to outdoor grazing (stating that the razing period has to span at least 2 months per year; during the grazing period the cows should spent at least 12 hours outdoors). No synthetic fertilisers or pesticides are allowed (KRAV 2016).

Text 2. Evaluation of experimental differences in microclimates

To record the impact of the heating chambers on the local microclimate, the temperature (above ground) of each chamber was monitored by data-loggers (EL-USB-#-LCD, Lascar electronics, Salisbury SP5 2SJ, UK). To compare the temperatures inside of the heating chamber to natural temperatures, we placed at least two data-loggers in each block: one in a non-warmed chamber and one in a heating chamber. We then fitted generalized linear mixed-effect model of hourly temperatures (available for 25 out of 50 chambers) as a function of measurement time and days (both treated as a class-level variable to allow for any changes in temporal patterns), of warming treatment (warmed versus ambient chambers), and of macrohabitat (open grass sward vs semi-open pasture), with chamber identity as a random effect. For this, we used data from the full duration of the experiment.

As intended, the experimental manipulations significantly increased the temperature in the open top chambers as compared to the control mesh cages ($F_{1,7}$=48.02, p<0.001; Fig. S1). Temperatures also differed significantly between days ($F_{37,7}$=144.74, p<0.001) and with the time of the day ($F_{23,7}$=671.71, p<0.001), but not between the two macrohabitats (open grass sward vs semi-open pasture; $F_{1,7}$=2.86, p=0.09).

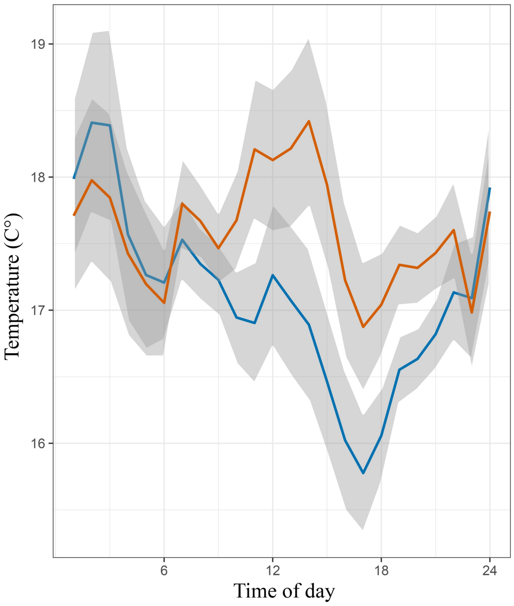

Figure S1: **Average temperature in heated (red line) versus ambient-temperature (blue line) mesocosms during different times of the day.** Lines show the hourly average of raw temperatures recorded for the full duration of the experiments. The shaded areas show standard deviations around these means.

Text 3. Full details on the treatments implemented

To evaluate the influence of macro- and microhabitat on the functioning of dung-associated communities, and the potential buffering effect of variation in dung beetle diversity, we selected one of the four organic farms (Fig. S1). Here, we focused on the two dominant macrohabitats: open grass sward and semi-open pasture rich in trees including Scots pine (*Pinus sylvestris*), birch species (*Betula pendula* and *B. pubescens*) and Norway spruce (*Picea abies*), henceforth called 'open grass sward' vs 'semi-open pasture', respectively. Within each of these macrohabitats, we distributed five blocks, each encompassing seven treatments (i.e. a total of 70 dung pats). Treatments were named according to the presence or absence of Beetles (B+ and B-), to whether the pats were surrounded by open-top chambers (OTCs; described in Slade and Roslin 2016) or not (C+, C-), which were designed to either experimentally elevate Temperature or not (T+, T-, respectively; for an evaluation of the heating effects achieved, see Text 1, above). Thus, we arrived at a total of seven treatments: 1) **C+T+B+:** an experimentally warmed treatment consisting of dung pats covered by chambers and left exposed to beetle colonization; 2) **C+T+B-:** an experimentally warmed mesocosm consisting of dung pats covered by OTC chambers and excluded from beetle colonization; 3) **C+T-B+**: an ambient temperature treatment consisting of dung pats left exposed beetle colonization and then covered by a similar-sized chamber composed with mesh without lid; 4) **C+T-B-**: an ambient temperature mesocosm consisting of dung pats excluded from beetle colonization and covered by a similar-sized chamber of mesh and covered with a lid that excluded colonization; 5) **C-T-B+**: an ambient temperature treatment consisting of dung pats left open to beetle colonization (without any chambers or cages; thus, this treatment corresponds to the **Beetles** treatment represented in Fig. 2 in the main text); 6) **C-T-B-**: a control pat surrounded by a small cage, yielding an estimate of dung decomposition under in the absence of beetles (note that this treatment equals the **Control** mesocosm represented in Fig.2 in the main text); 7) **S**: an ambient temperature treatment consisting of dung pats left open to beetle colonization and used as a sentinel sample of local dung beetle community structure, yielding an estimate of species composition and abundance for each block (thus equaling the **Sentinel sample** represented in Fig.2 in the main text).

As a metric of ecosystem functioning in the different treatments, we measured changes in the wet mass of the dung pats (see main text, Measuring ecological functions). Such changes in wet weight will reflect both desiccation and actual dung removal and/or respiratory loss of mass by pat-dwelling species (see, e.g., Rosenlew and Roslin 2008; Kaartinen et al. 2013). As our primary interest was in the relative contributions of dung beetles to decomposition rates, we used the Control treatment (see above) as a point of comparison: in this treatment, all changes in mass will reflect desiccation and microbial decomposition, with no contribution by macroscopic invertebrates. The physical structure of the mesh used here has been shown to have no detectable effect on desiccation rates (Kaartinen et al. 2013). At the end of the experiment, we dried the remaining dung and weighted it as dry remained mass. By using dry weights as end weights, we controlled for any difference in evaporation, thereby isolating the contribution of the insect themselves to dung removal.

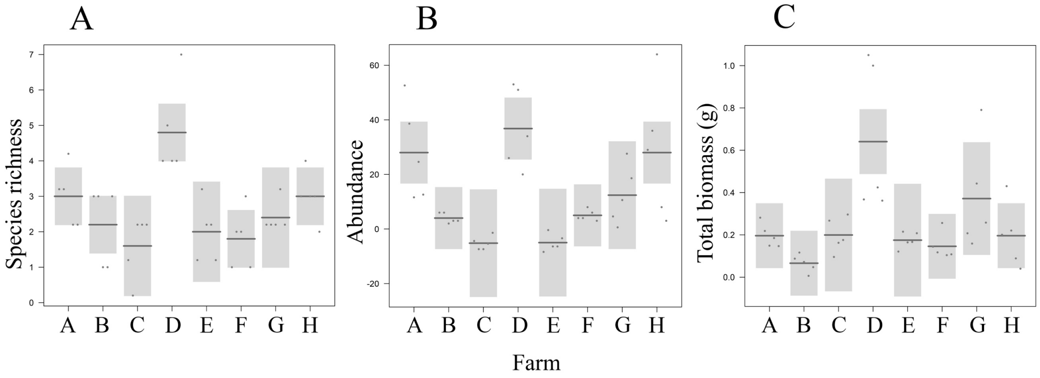

Figure S2: **Differences among farms for community attributes**: A) species richness; B) abundance and C) total biomass. Panels show estimates (dark grey line) and confidence intervals (light grey interval) for farms. All data points show partial residuals, with estimates derived from the GLMMs described in Materials and methods.

References

Kaartinen, R., Hardwick, B. and Roslin, T. (2013) Using citizen scientists to measure an ecosystem service nationwide. Ecology, 94, 2645–2652.

KRAV. (2016) Marknadsrapport, http://www.krav.se/sites/default/files/krav_marknadsrapport_2016_webb.pdf

Rosenlew, H. and Roslin, T. (2008) Habitat fragmentation and the functional efficiency of temperate dung beetles. Oikos, 117, 1659–1666.

6. Discussion and conclusions

Irene Piccini

6.1 Discussion

Through an approach based on species and assemblage characteristics, it is possible to generalize results related to specific communities and ecosystems to other similar contexts (Suding et al. 2008; Moretti et al. 2017). In addition, considering different ecological functions at the same time (i.e. multifunctionality), it is possible to obtain an overview of whether and how different variables influence different aspects of systems. Specifically, I assessed how dung-system multifunctionality is affected by species traits (i.e. body mass and nesting strategy), assemblage features (species-, assemblage composition, density), environment variables (macro-, micro-habitats and microclimates) and their interactions. The findings of this thesis confirmed that ecosystem functioning is context-dependent and it relied on multiple factors - such as density, body mass, nesting strategy, species identity and assemblage composition - and on the ecological functions investigated. Thus, I suggest an approach based on the analysis of multiple functions to better understand, manage and optimize the ecosystem functioning of systems. For istance we found that local, farm- and site-specific variations in environmental conditions are important factors influencing dung beetle communities for ecosystem functioning. Thereby, from an applied point of view, local environment variables managed by farmers in their own property can shape the local dung beetle community and thus ecosystem functioning.

6.1.1 Effects of species traits and assemblage features

Body mass was found to be a crucial factor for several ecological functions (but not all) in different experiments in this project (Table 1): I) in Chapter 3, despite the constant total biomass in each treatment, the assemblage with the largest dung beetle *C. lunaris* had the highest effect on dung removal (in accordance with Kaartinen et al. 2013; Nervo et al. 2014; Lähteenmäke et al. 2015), and was even more efficient than the mixed-species assemblage; II) in Chapter 4, between two species of tunnelers investigated, the larger-bodied one was found to increase seed germination while small-bodied species did not have any effect on germination; III) in Chapter 2 monospecific assemblages with large-bodied beetles reduced CO_2 emissions from dung pats.

Even if species sensitivity to disturbance was found to be often related to species body mass (Larsen et al. 2005), in Chapter 2, I found that between body mass and **nesting strategy** only the latter can be considered a response trait for dung beetle species in Sweden (Table 1). Specifically, tunneler species were shown to be more extinction prone than dwellers, likely due to their long larval period inside the soil that makes them more sensitive to environmental changes that affect soil properties (e.g. temperature and humidity). Indeed, increasing drought conditions and the reduction of grazing pressure all over Scandinavia might play a crucial role in changes to soil conditions, especially water availability and temperatures. On the other hand, both nesting strategy and body mass were identified as effect traits (Table 1): the interaction between these traits influenced dung removal; separately, body mass affected reduction of GHG emissions from dung pats and nesting strategy affected soil nutrient content.

Dung beetle abundance in dung pats (i.e **density**) was not found to be an effect trait *per se*; in fact the effect of abundance varies according to the species investigated (in accordance with Tixier et al. 2015). Assemblages with high density of small-bodied *Onthophagus illyricus* were found to be not as efficient as those with the large-bodied *Copris lunaris*. Indeed, the increase of *Copris lunaris* density increases the provisioning of ecological functions (i.e. dung removal and secondary seed dispersal). This difference in the density effect of the small-bodied and large-bodied beetles was even more evident for dung removal, in which remaining dung amount increased with increasing density of the small-bodied beetle and, conversely, decreased with increasing density of the large-bodied beetles. Thus, in Chapter 4 the interaction between body size and abundance was found to affect differently dung-system functioning. In Chapter 2 I showed that overall the efficiency in ecosystem functioning increased along with the increase in body mass and density.

Assemblage composition is a pivotal factor that influences ecosystem functioning for several taxa (Alvarez-Filip et al. 2013; Wagg et al. 2013). In Chapter 3, the assemblage composed of three species together was more efficient in reducing GHG emissions from dung pats than all the other monospecific assemblages. This is in accordance with previous studies that have shown that multi-species assemblages were more efficient in providing ecological functions than monospecific assemblages (Beynon et al. 2012; Nervo et al. 2014; Nervo et al. 2017). This is even more evident if species belong to different functional groups (Slade et al. 2007; Gagic et al. 2015), similarly to the mixed assemblages of this study in Chapter 3. This recognized pattern for multi-species assemblages might be related to facilitation and/or complementarity effect, species can mutually benefit from each other's presence in the dung through biophysical interactions and manipulation of dung. For instance, in Chapter 3, the additive effect of monospecific assemblages of *Aphodius fimetarius*, *Onthophagus coenobita* and *Sisyphus schaefferi* on GHG reduction was lower than the functional effect of all species combined in the same assemblages (more details in Appendix).

However, the efficiency of assemblages depends on which species is presented in the assemblage and on which function is investigated (Slade et al. 2017). In accordance with this, I found that **species identity** is a pivotal factor for multifunctionality; in fact different species have different effects on the ecological functions investigated. For example, in Chapter 3 *C. Lunaris* was found to be the most efficient in dung removal but at the same time it increased methane emissions over time.

Table 1: **Trait and assemblage feature effect**. Shown is the effect of traits (nesting strategy and body mass) and assemblage features (density and composition) on ecological functions: dung removal, reduction of greenhouse gas emissions, soil nutrient content, seed dispersal and germination. Combined effect of more than one trait or feature is represented by a shared box between different lines. "+" identifies positive effect of traits placed in the rows on the ecological functions placed in the columns; "-" a negative effect; "0" no-effect and "na" not-analyzed effect. Pale grey identifies results presented in Chapter 2, pale-dark grey those presented in Chapter 4 and dark grey those in Chapter 3. Boxes presented results that belong to different chapters show colored circles that indicate which colors should be also there.

<table>
<tr><th></th><th>Dung removal</th><th>GHG reduction</th><th>Nutrient cycle</th><th>Seed dispersal</th><th>Seed germination</th></tr>
<tr><td rowspan="2">Nesting strategy</td><td>+</td><td>+</td><td>+</td><td>na</td><td>na</td></tr>
<tr><td rowspan="2">+</td><td rowspan="2">0</td><td rowspan="2">0</td><td rowspan="2">na</td><td rowspan="2">na</td></tr>
<tr><td rowspan="3">Body mass</td></tr>
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<tr><td rowspan="2">+/-</td><td rowspan="2">na</td><td rowspan="2">na</td><td rowspan="2">+</td><td rowspan="2">0</td></tr>
<tr><td>Density</td></tr>
<tr><td>Assemblage composition</td><td>0</td><td>+</td><td>na</td><td>na</td><td>na</td></tr>
</table>

6.1.2 Effects of farming practices and environment variables

Recently some studies have highlighted the importance of understanding the real complexity of species interactions (Bronstein et al. 2014; Lurgi et al. 2014; Roxburgh 2015; Mayfield et al. 2017). In accordance with this concept, I developed a field experiment where dung beetle species were able to naturally colonize the dung pats. Therefore, I evaluated whether and how this natural community provides ecological functions (i.e. dung removal) in relation to farming practices and environmental variables (macro-, micro-habitats and microclimates).

Farming practices have been demonstrated to be one of the main factors for shaping biodiversity, as the use of pesticides, such as insecticides, and synthetic fertilizers affects non-target fauna, such as dung beetles in agroecosystems (Geiger et al. 2010). Thus, organic farming systems in which the use of these products is not allowed have been shown to support higher biodiversity than conventional ones across many different taxa (Hutton and Giller 2003; Fuller et al. 2005; Duru et al. 2015). Conversely to what expected, in Sweden I found that abundance was higher in conventional system than in organic one but this difference was not reflected in ecosystem functioning. This result might be related to the general attention to ecology and conservation issues of Swedes. Indeed, Sweden achieved the highest environmental sustainability index among twelve countries (Siche et al. 2006). This overall pattern is reflected in agriculture, where farmers declared feeling uncomfortable with using pesticides (Andersson et al. 2015). Specifically, in the Uppsala area most of the conventional farmers use inorganic fertilizers and herbicides but avoid insecticides and fungicides (Bommarco 1998; Östman et al. 2001; Weibull et al. 2003).

I found that farms showed significant differences both in terms of dung beetle community (species richness, abundance and total biomass) and ecological functions associated. Thus, others factors than farming system affected dung beetle communities and their associated ecological functions (in accordance with Bengtsson et al. 2005). Specifically, farmer-specific local choices with respect to cattle fodder, grazing regimes and overall field management likely have a stronger impact on shaping communities than farming systems *per se* and local macro-habitats (e.g. forest presence). Moreover, local micro-habitat conditions, such as soil type, moisture, leaf and vegetation cover, may have played a role in determining dung beetle communities (in accordance with Nealis 1977; Mehrabi et al. 2014). However, the slight differences found among microhabitats are not reflected in different functioning.

These findings confirm that local decisions made by farmers can shape the composition of dung beetle communities and associated ecological functions, more than farming system *per se*. Indeed, even if farms belong to a system (organic or conventional), farmers have several management options that can modify local environment variables (such as vegetation cover, pasture rotation…) and consequently modify insect community (Bengtsson et al. 2005).

6.1.3 Conservation implications

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). 21 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. A general pattern of greater sensitivity to environment change in large tunneler species has been recognized (Larsen et al. 2005; Larsen et al. 2008; Roslin et al. 2014). Large tunneler sensitivity might be attributed either to physical factors (e.g. metabolic and/or physiological factors) related to their body size, or to current changes in the landscape such as increased vegetation height, and thus lower soil temperatures, since tunnelers breed in soil (Roslin et al. 2014). As a result, large tunnelers are not only functionally important, but they are also prone to extinction (Larsen et al. 2005). We found that the high abundance of large species in dung pats resulted in an increase in dung removed, seed dispersal and germination. Thus, the decline of large dung beetle species can negatively impact ecosystem functioning.

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.). We agree that the optimal provisioning of ecological functions by dung beetles depends on species identity, body size and abundance in dung pats. 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 for high densities of the small species. Conversely, an increase in the abundance of large species in dung pats might provide more ecological functions. As a caveat, we recognize that the evaluation of ecosystem functioning is context-dependent and varies with the ecological functions investigated (Slade et al. 2017). Thus, species that are crucial for the provisioning of particular functions might be unimportant for other ecologically relevant functions and/or have different effects due to the co-presence of other species in dung pats (Piccini et al. 2017; Slade et al. 2017).

Moreover, maintenance of grazing areas with locally low vegetation can improve soil properties (such as density, temperature and moisture) that facilitate tunneler survival and reproduction during their long larval period (Roslin et al. 2014). Thus, tunnelers presence would facilitate ecosystem functioning in short term (Nervo et al. 2017)

6.2 Conclusions

Through a trait-based approach, I obtained a deep understanding of dynamics and mechanisms in dung beetle assemblages, thus enhancing predictive capabilities for future perspectives. Trait-based approaches reduce context dependency and allow the generalization of results related to specific community and ecosystems to other similar contexts (Suding et al. 2008; Moretti et al. 2017). Both traits investigated (i.e. body mass and nesting strategy) contributed to ecosystem functioning: together body mass and nesting strategy influence dung removal and separately GHG reduction and nutrient increase into soil, respectively. Thus, I found that also the interaction between traits is one of the major drivers for ecosystem functioning. The combination of efficient effect traits (such as body mass and nesting strategy) and the diversity of different traits seems to be a good predictor for ecosystem functioning (Lavorel and Garnier 2002; Gagic et al. 2015). However, there are other factors that were proven to be important, like the period of time considered (dwellers are as efficient as tunnelers over a long period; Nervo et al. 2014; Nervo et al. 2017) and the ecological functions investigated (Slade et al. 2017). Moreover, I have shown that the link between effect and response traits exists, as nesting strategy was found both as effect and response trait. In Sweden, I found that tunneler species have been proven to be both efficient in dung removal and in enhancing soil nutrient content but at the same time they were the most prone to extinction. As a caveat, I recognize that also traits other than nesting strategy, not investigated in this project such as temperature tolerance and desiccation resistance, might play a crucial role as response traits and consequently change the strength of the response-effect trait link.

I investigated whether and how assemblage features (assemblage, species composition and density) can be a crucial factor for ecosystem functioning (Table 1). I found that species identity, assemblage composition and abundance in dung pats (i.e. density) influence provisioning of ecological functions and the result depends on which ecological function was investigated (in accordance with Slade et al. 2017). Indeed, multi-species assemblages were found as the most effective in reducing CO_2-equivalent emissions from dung pats, but were not similarly efficient in removing dung, for which function the monospecific assemblage with *Copris lunaris* was found the most effective (Chapter 3). Density effects might depend on species-size and/or species identity, as I obtained different results for the two species investigated. I found that the interaction among these factors may bring unexpected results, high density of the small-bodied tunneler *Onthophagus illyricus* that did not remove dung from surface. Conversely, through their movements beetles spread dung and slightly increased its weight by incorporating particles of soil into dung. Thus, a valuable approach is to investigate not only the effect of single factors on ecosystem functioning but also their interactions, as I did by evaluating the combined effect of body mass and nesting strategy and body mass and density.

The multifunctional perspective seems to be a fundamental requisite to increase our understanding of factor-ecosystem function relationships (Manning et al. 2016; Slade et al 2017). Overall, some traits (e.g. body mass) may appear more efficient than other factors (e.g. density) for specific functions (e.g. GHG reduction, seed germination), but this is not correct if the analysis is extended to other functions (nutrient soil content, dung removal). Thus, this study highlighted the importance of a multifunctional approach to better understand dynamics within ecosystems.

Through these findings regarding the effect of farming practices and environmental variables on dung beetle community and associated ecological functions, I suggest that a multi-level investigation is needed for a deep understanding of landscape and local dynamics. In fact, an approach based purely on farming systems (organic vs conventional) may not explain all the differences found among farms (Bengtsson et al. 2005). Specifically, in experiment described in Chapter V local decisions made by farmers were found even more important than farming systems in shaping dung beetle community. Through their local management plans, farmers can modify environmental variables (such as vegetation cover, grazing areas within the farm…) that can modify presence and abundance of dung beetle species and their associated ecological functions. Thus, a multi-level approach was essential to understand that farmers can make the difference for the insect community within their farms, even more than farming systems.

These findings confirm that ecosystem functioning is context-dependent and it relies on multiple factors (such as trait and assemblage features), on the ecological functions investigated and on environmental variables (that can be shaped by farmers throughout managing practices such as grazing regimes, cattle fodder…). Thus, I suggest an approach based on the analysis of multiple functions at multi-levels (from system to local environment) to better understand, manage and optimize the ecosystem functioning of a system.

6.2.1 Future prospects

Dung beetles have been studied since the beginning of the XX century. More recently these taxa have been investigated as drivers of ecosystem multi-functionality. Even if several research areas have been developed on dung beetles, there are some interesting future lines emerging from this thesis.

A first aspect that could be developed regards the investigation on whether and how the ecological role of certain species or of species assemblages changes in different regions in relation to different environmental variables. Even when a certain animal group (e.g. dung beetles) and a given function (e.g. dung removal) are investigated, ecosystem functioning may change in relation to resource type, temperature, altitude, longitude, soil type vegetation cover, and so on. Thus, does a given species provide the same amount of ecological functions in different areas (for example in Sweden and in Italy)? Is provisioning of ecological functions related to species' intrinsic features and/or environment variables? Do species that are locally threatened in Sweden, such as *Onthophagus* spp, provide the same amount of ecological functions also in other countries, such as Italy, where they are not threatened?

Following the idea of investigation of how environment variables affect provisioning of ecological functions, a second interesting aspect regards the effect of spatial variation on the composition and structure of dung beetle communities and on provisioning of ecosystem processes in agro-pastoral ecosystems. Considering that dung beetles can fly and move to colonize pastures 1km away (Roslin 2000), the landscape surrounding the farms matters. How do landscape heterogeneity, pasture presence (at high or low intensity) and grazing period influence dung beetle communities and associated provisioning of ecological functions?

Reference

Alvarez-Filip, L., Carricart-Ganivet, J.P., Horta-Puga, G. & Iglesias-Prieto, R. (2013) Shifts in coral-assemblage composition do not ensure persistence of reef functionality. Scientific Reports, 3.

Andersson, E., Nykvist, B., Malinga, R., Jaramillo, F. & Lindborg, R. (2015) A social–ecological analysis of ecosystem services in two different farming systems. AMBIO, 44, 102–112.

Bengtsson, J., Ahnström, J. & WEIBULL, A.-C. (2005) The effects of organic agriculture on biodiversity and abundance: a meta-analysis. Journal of applied ecology, 42, 261–269.

Bommarco, R. (1998) Reproduction and Energy Reserves of a Predatory Carabid Beetle Relative to Agroecosystem Complexity. Ecological Applications, 8, 846–853.

Braga RF, Korasaki V, Andresen E, Louzada J (2013) Dung Beetle Community and Functions along a Habitat-Disturbance Gradient in the Amazon: A Rapid Assessment of Ecological Functions Associated to Biodiversity. PLOS ONE 8:e57786 . doi: 10.1371/journal.pone.0057786

Bronstein, J.L., Armbruster, W.S. & Thompson, J.N. (2014) Understanding evolution and the complexity of species interactions using orchids as a model system. New Phytologist, 202, 373–375.

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., MacE, G.M., Tilman, D., Wardle, D.A., Kinzig, A.P., Daily, G.C., Loreau, M., Grace, J.B., Larigauderie, A., Srivastava, D.S. & Naeem, S. (2012) Biodiversity loss and its impact on humanity. Nature, 486, 59–67.

Duncan, C., Thompson, J.R. & Pettorelli, N. (2015) The quest for a mechanistic understanding of biodiversity–ecosystem services relationships. Proc. R. Soc. B, p. 20151348. The Royal Society.

Duru, M., Therond, O., Martin, G., Martin-Clouaire, R., Magne, M.-A., Justes, E., Journet, E.-P., Aubertot, J.-N., Savary, S., Bergez, J.-E. & Sarthou, J.P. (2015) How to implement biodiversity-based agriculture to enhance ecosystem services: a review. Agronomy for Sustainable Development, 35, 1259–1281.

Fuller, R.J., Norton, L.R., Feber, R.E., Johnson, P.J., Chamberlain, D.E., Joys, A.C., Mathews, F., Stuart, R.C., Townsend, M.C., Manley, W.J., Wolfe, M.S., Macdonald, D.W. & Firbank, L.G. (2005) Benefits of organic farming to biodiversity vary among taxa. Biology Letters, 1, 431–434.

Gagic, V., Bartomeus, I., Jonsson, T., Taylor, A., Winqvist, C., Fischer, C., Slade, E.M., Steffan-Dewenter, I., Emmerson, M. & Potts, S.G. (2015) Functional identity and diversity of animals predict ecosystem functioning better than species-based indices. Proceedings of the Royal Society of London B: Biological Sciences, 282, 20142620.

Geiger, F., Lubbe, S.C.T.M. van der, Brunsting, A.M.H. & Snoo, G.R. de. (2010) Insect abundance in cow dung pats of different farming systems. Entomologische Berichten, 70, 106–110.

Hutton, S.A. & Giller, P.S. (2003) The effects of the intensification of agriculture on northern temperate dung beetle communities. Journal of Applied Ecology, 40, 994–1007.

Kaartinen, R., Hardwick, B. & Roslin, T. (2013) Using citizen scientists to measure an ecosystem service nationwide. Ecology, 94, 2645–2652.

Krause, S., Le Roux, X., Niklaus, P.A., Van Bodegom, P.M., Lennon, J.T., Bertilsson, S., Grossart, H.-P., Philippot, L. & Bodelier, P.L. (2014a) Trait-based approaches for understanding microbial biodiversity and ecosystem functioning. Frontiers in Microbiology, 5.

Krause, S., Le Roux, X., Niklaus, P.A., Van Bodegom, P.M., Lennon, J.T., Bertilsson, S., Grossart, H.-P., Philippot, L. & Bodelier, P.L. (2014b) Trait-based approaches for understanding microbial biodiversity and ecosystem functioning. Frontiers in Microbiology, 5.

Lähteenmäki, S., Slade, E.M., Hardwick, B., Schiffler, G., Louzada, J., Barlow, J. & Roslin, T. (2015) MESOCLOSURES – increasing realism in mesocosm studies of ecosystem functioning. Methods in Ecology and Evolution, 6, 916–924.

Larsen TH, Lopera A, Forsyth A (2008) Understanding Trait-Dependent Community Disassembly: Dung Beetles, Density Functions, and Forest Fragmentation. Conservation Biology 22:1288–1298 . doi: 10.1111/j.1523-1739.2008.00969.x

Larsen, T.H., Williams, N.M. & Kremen, C. (2005) Extinction order and altered community structure rapidly disrupt ecosystem functioning. Ecology Letters, 8, 538–547.

Lavorel, S. & Garnier, E. (2002) Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. Functional Ecology, 16, 545–556.

Lurgi, M., Galiana, N., López, B.C., Joppa, L.N. & Montoya, J.M. (2014) Network complexity and species traits mediate the effects of biological invasions on dynamic food webs. Frontiers in Ecology and Evolution, 2, 36.

Manning, P., Slade, E.M., Beynon, S.A. & Lewis, O.T. (2016) Functionally rich dung beetle assemblages are required to provide multiple ecosystem services. Agriculture, Ecosystems & Environment, 218, 87–94.

Mayfield, M.M. & Stouffer, D.B. (2017) Higher-order interactions capture unexplained complexity in diverse communities. Nature Ecology & Evolution, 1, 0062.

Mehrabi, Z., Slade, E.M., Solis, A. & Mann, D.J. (2014) The importance of microhabitat for biodiversity sampling. PloS one, 9, e114015.

Moretti, M., Dias, A.T.C., de Bello, F., Altermatt, F., Chown, S.L., Azcárate, F.M., Bell, J.R., Fournier, B., Hedde, M., Hortal, J., Ibanez, S., Öckinger, E., Sousa, J.P., Ellers, J. & Berg, M.P. (2017) Handbook of protocols for standardized measurement of terrestrial invertebrate functional traits. Functional Ecology, 31, 558–567.

Nealis, V.G. (1977) Habitat associations and community analysis of south Texas dung beetles (Coleoptera: Scarabaeinae). Canadian Journal of Zoology, 55, 138–147.

Negro M, Rolando A, Palestrini C (2011) The impact of overgrazing on dung beetle diversity in the Italian Maritime Alps. Environmental entomology 40:1081–1092

Nervo, B., Caprio, E., Celi, L., Lonati, M., Lombardi, G., Falsone, G., Iussig, G., Palestrini, C., Said-Pullicino, D. & Rolando, A. (2017) Ecological functions provided by dung beetles are interlinked across space and time: evidence from 15N isotope tracing. Ecology, 98, 433–446.

Nervo, B., Tocco, C., Caprio, E., Palestrini, C. & Rolando, A. (2014) The Effects of Body Mass on Dung Removal Efficiency in Dung Beetles. PLOS ONE, 9, e107699.

Östman, Ö., Ekbom, B., Bengtsson, J. & Weibull, A.-C. (2001) Landscape Complexity and Farming Practice Influence the Condition of Polyphagous Carabid Beetles. Ecological Applications, 11, 480–488.

Piccini I, Arnieri F, Caprio E, et al (2017a) Greenhouse gas emissions from dung pats vary with dung beetle species and with assemblage composition. PLOS ONE 12:e0178077 . doi: 10.1371/journal.pone.0178077

Roslin, T. (2000) Dung beetle movements at two spatial scales. Oikos, 91, 323–335.

Roslin T., M. Forshage, F. Ødegaard, C. Ekblad, G. Liljeberg. 2014. Nordens dyngbaggar (Dung beetles of Northern Europe) - Tibiale, Helsinki

Roxburgh, S.H. (2015) The hidden complexity of multi-species interactions. Journal of vegetation science, 26, 205–206.

Siche, J.R., Agostinho, F., Ortega, E. & Romeiro, A. (2008) Sustainability of nations by indices: Comparative study between environmental sustainability index, ecological footprint and the emergy performance indices. Ecological Economics, 66, 628–637.

Slade, E.M., Kirwan, L., Bell, T., Philipson, C., Lewis, O.T. & Roslin, T. (2017) The importance of species identity and interactions on multifunctionality depends on how ecosystem functions are valued. Ecology.

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

Suding, K.N., Lavorel, S., Chapin, F.S., Cornelissen, J.H., Diaz, S., Garnier, E., Goldberg, D., Hooper, D.U., Jackson, S.T. & NAVAS, M.-L. (2008) Scaling environmental change through the community-level: a trait-based response-and-effect framework for plants. Global Change Biology, 14, 1125–1140.

Tixier, T., Bloor, J.M.G. & Lumaret, J.-P. (2015) Species-specific effects of dung beetle abundance on dung removal and leaf litter decomposition. Acta Oecologica, 69, 31–34.

Tocco C, Negro M, Rolando A, Palestrini C (2013a) Does natural reforestation represent a potential threat to dung beetle diversity in the Alps? Journal of insect conservation 17:207–217

Tocco C, Probo M, Lonati M, et al (2013b) Pastoral practices to reverse shrub encroachment of sub-alpine grasslands: dung beetles (Coleoptera, Scarabaeoidea) respond more quickly than vegetation. PLoS One 8:e83344

Wagg, C., Bender, S.F., Widmer, F. & Heijden, M.G.A. van der. (2014) Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences, 111, 5266–5270.

Weibull, A.-C., Östman, Ö. & Granqvist, \AAsa. (2003) Species richness in agroecosystems: the effect of landscape, habitat and farm management. Biodiversity and conservation, 12, 1335–1355.

Appendix

1. Facilitation effect of species coexistence in dung pats

To prove the facilitation effect of species coexistence in dung pats, I used data on GHG reduction from dung pats published by Piccini et al. (2017). I investigated whether the additive effect of each species (*Aphodius fimetarius* in treatment T1, *Onthophagus coenobita* in treatment T2 and *Sysiphus schaefferi* in treatment T3) is comparable to the effect of the coexistence of the same species together (treatment T6).

First of all, I evaluated the CO_2eq reduction per each treatment. Thus, I calculated the mean of the cumulative CO_2eq emissions per each typology of terrarium (Control C1, monospecific treatments T1, T2, T3 and mixed-species treatment T6) and I subtracted means for treatments (T1, T2, T3 and T6) from the mean of controls C1 with dung and without beetles. Thus, I obtained the CO_2eq mean reduction per each treatment.

Table A1: Data elaboration of CO_2eq emissions derived by treatments described in Piccini et al. 2017.

Treatment	Species	Beetle number	CO_2eq reduction per treatment
T1	*A. fimetarius*	31	113.3 g/m^2
T2	*O. coenobita*	13	787.02 g/m^2
T3	*S. schaefferi*	6	4315.8 g/m^2
T6	*A. fimetarius*, *O. coenobita*, *S. schaefferi*	11, 4, 2	6405.88 g/m^2

To estimate the contribution of each beetle to the reduction of CO_2eq emissions to atmosphere, I divided the mean obtained from each treatment (T1, T2 and T3) by the number of beetles presented in each treatment (for treatment T1: 31 *A. fimetarius*; T2: 13 *O. coenobita*; T3: 6 *S. schaefferi*). Thus, I obtained on average:

- Each beetle of *A. fimetarius* species reduced 3.65 g/$m^2$of CO_2eq;
- Each beetle of *O. coenobita* species reduced 60. 5 g/$m^2$of CO_2eq;
- Each beetle of *S. schaefferi* species reduced 719.3 g/$m^2$of CO_2eq.

In order to compare the additive effect of these species and the effect of the coexistence of the same species, I summed the CO_2eq reduction per each species, multiplied by the number of individuals presented in treatment T6 (11 beetles per

A. fimetarius, 4 for *O. coenobita* and 2 for S. schaefferi), and I compared the result with the actual CO_2eq reduction for T6. Thus, I obtained that the additive effect of beetles would be of 1720.8 g/m^2 and the effect of coexistence of species in the same terrarium is in mean of 6405.88 g/m^2. Indeed, the reduction effect of 11 *A. fimetarius* + 4 *O. coenobita* + 2 *S. schaefferi* is lower than the one derived from treatment with the same species and the same number of individuals together: 1720.8 $g/m^2 < 6405.88$ g/m^2.

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