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

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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.

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. in press) 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 (Cambefort & Hanski 1991). 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 & 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. Materials and methods

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 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).

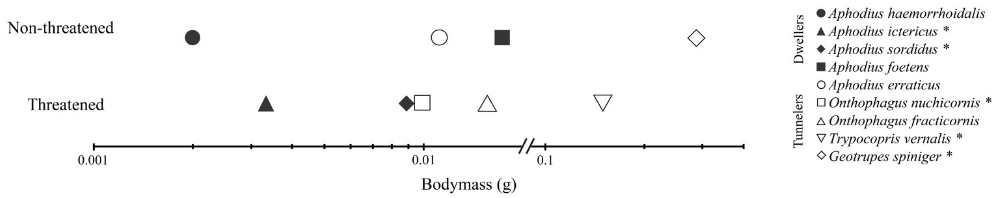

Figure 1: Species used in the experiment. Rows correspond to threat status (non-threatened species versus threatened ones), and a species' position along the x-axis reflects its body mass (note the axis break reflecting a seven-fold jump – 0.07g – in body mass). Nesting strategies are identified by symbols: 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).

2.3 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.

2.4 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 CO_2 and CH_4 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 CO_2 and CH_4 in CO_2 equivalents, by converting compound-specific fluxes of CH_4 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 CO_2 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 NH_4^+ and NO_3^- 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 CO_2 and CH_4 measured from the chamber headspace (in ppm) were converted into mg m^{-2} h^{-1}. Cumulative fluxes of CO_2 and CH_4 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 CO_2 and CH_4 by converting to CO_2 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 NH_4^+ and NO_3^-) 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 NH_4^+ and NO_3^- was determined spectrophotometrically as described in Cucu et al. (2014).

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 (R^2=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, NH_4^+, NO_3^-, and cumulative emissions of CO_2, CH_4 and CO_2-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, CO_2 and CH_4 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 CH_4 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 CO_2, CH_4 and combined emissions of CO_2 and CH_4, in CO_2 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).

3. Results

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.

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 CH_4 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: $F_{1;182.4}$= 5.31, p=0.022; Figure 2; Appendix, Table A5).

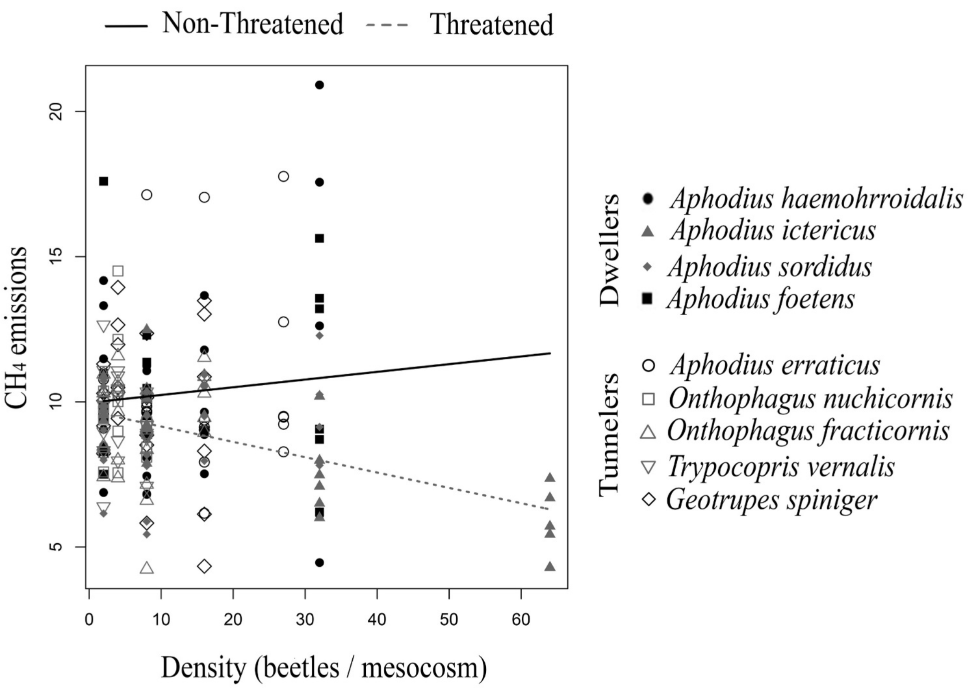

Figure 2: Threat status versus functional efficiency in terms of methane emissions. Shown is the interaction between threat status (black line for non-threatened and grey dashed line for threatened species) and the number of individuals per mesocosm (density) for methane emissions (mg m^{-2} h^{-1}). 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.

3.2 Species traits versus functional efficiency

REMAINING DUNG MASS – Density had a general, significant effect on the amount of remaining dung (Density: $F_{1;19.31}$= 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: $F_{1;19.52}$= 33.80, p<0.001), with large tunneling species proving the most effective in removing dung per individual (Figure 3a; Appendix, Table A3).

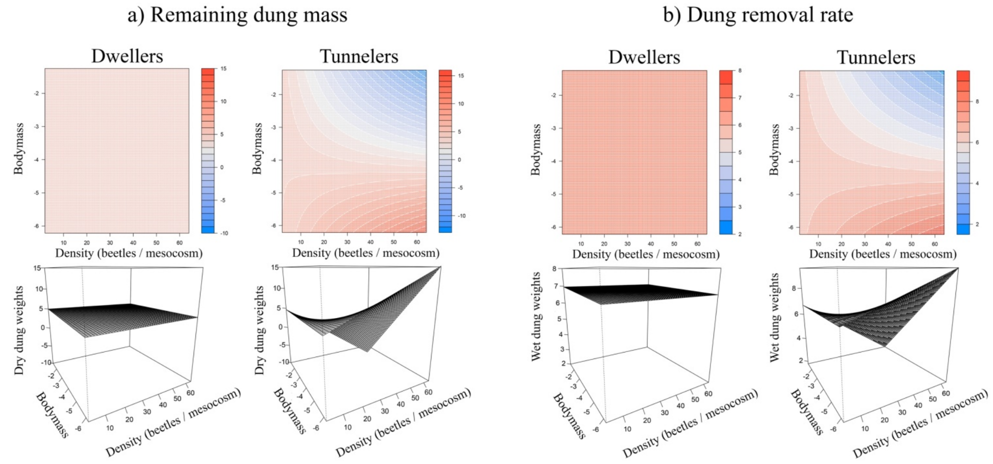

Figure 3: Interaction between body mass and nesting strategy in determining ecological functioning. Shown is the significant interaction between the species body mass, nesting strategy and the number of individuals per mesocosm for dung removal, measured as: (a) remaining dung mass (g) and (b) dung removal 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). 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).

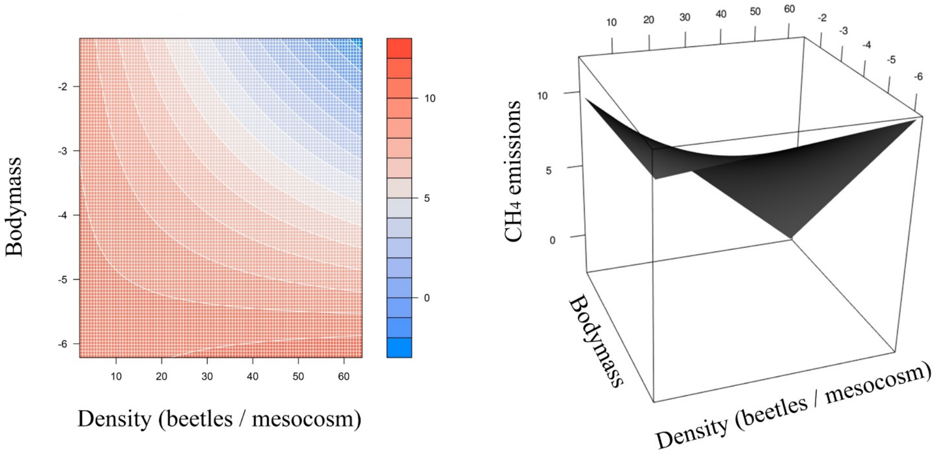

Figure 4: The effect of species body mass on methane emissions. Shown is the significant interaction between species body mass and the number of individuals per mesocosm in determining methane emissions from dung pats (mg m^{-2} h^{-1}). The two graphs represent the same interactions from different perspectives; 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 dung remaining on the surface whereas a bluer color represents less dung remaining.

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.

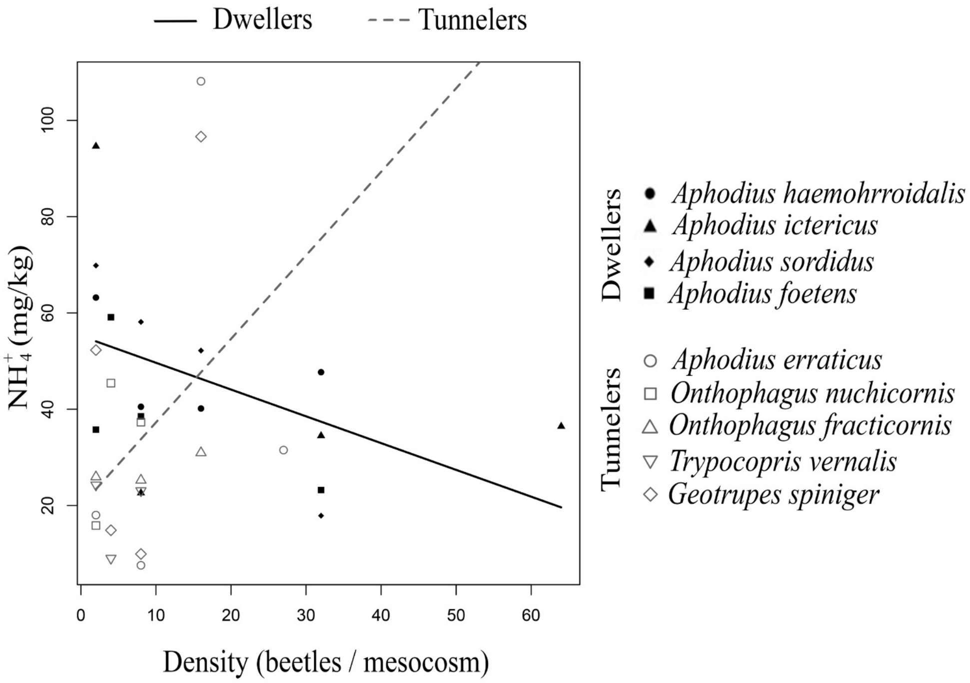

Figure 5: The effect of nesting strategy on nutrient cycling. Shown is the marginally significant interaction between density and nesting strategy (black line for dwellers and grey dashed line for tunnelers) in determining ammonium concentration in the soil (mg kg^{-1}). The data points show partial 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.

3.3. 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).

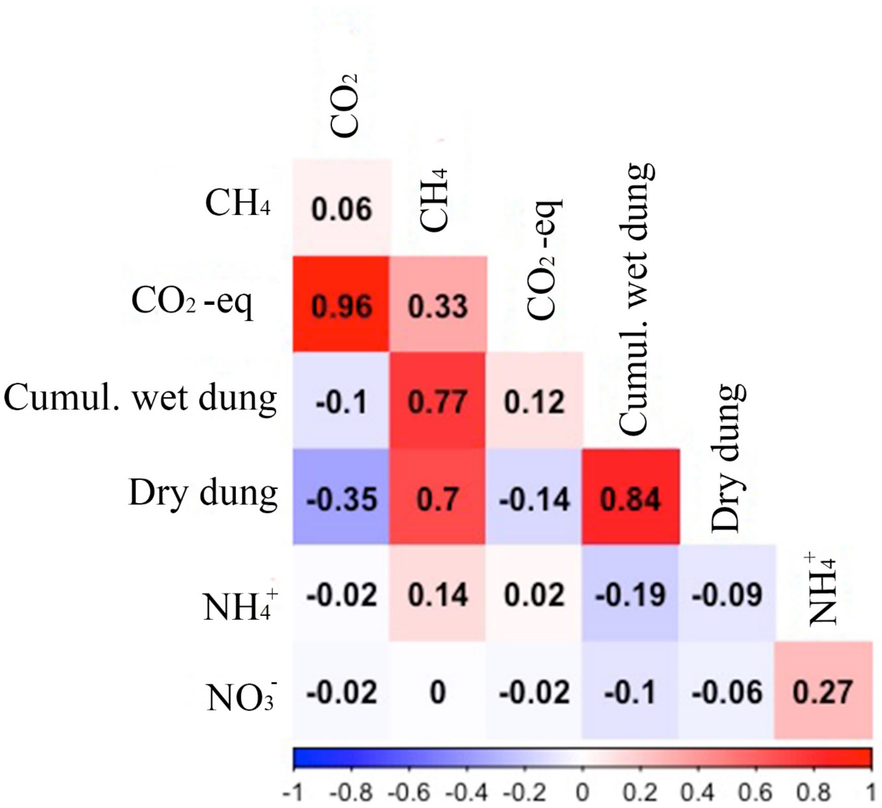

Figure 6. Associations between pairs of ecological functions. Shown are Pearson's correlation coefficients between different aspects of ecosystem functioning (cumulative fluxes of carbon dioxide (CO_2), methane (CH_4) and both gases (CO_2+CH_4) combined into CO_2 equivalents, cumulative weight of wet 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.

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.

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).

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.

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).

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).

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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