

1 **Effect of dung beetle species richness and chemical perturbation on multiple**
2 **ecosystem functions**

3 Paul Manning^{1,2,3}, Eleanor M. Slade^{1,4}, Sarah A. Beynon^{1,2}, Owen T.
4 Lewis¹

5 1. University of Oxford, Department of Zoology, South Parks Road, Oxford OX1 3PS, UK

6 2. Dr Beynon's Bug Farm, Lower Harglodd Farm, St. Davids, Pembrokeshire SA62 6BX, UK

7 3. Dalhousie University, Faculty of Agriculture, 21 Cox Road, Truro, NS, B2N 5E3, CAN

8 4. Lancaster Environment Centre, Lancaster University, Bailrigg, Lancaster LA1 4YQ, UK

9

Abstract

1. The relationship between biodiversity and ecosystem functioning is typically positive but saturating, suggesting widespread functional redundancy within ecological communities. However, theory predicts that apparent redundancy can be reduced or removed when systems are perturbed, or when multifunctionality (the simultaneous delivery of multiple functions) is considered.

2. We used manipulative experiments to test whether higher levels of dung beetle species richness enhanced individual functions and multifunctionality, and whether these relationships were influenced by perturbation (in this case, non-target exposure to the veterinary anthelmintic ivermectin). The four ecosystem functions tested were dung removal, primary productivity, soil faunal feeding activity, and reduction in soil bulk density.

3. For individual functions, perturbation had limited effects on functioning, with only dung removal significantly (negatively) affected. Species richness did not, on its own, explain significant variation in the delivery of individual functions. In the case of primary productivity, we found an interaction between richness and perturbation: species-rich dung beetle assemblages enhanced forage growth in the unperturbed treatment, relative to the perturbed treatment.

4. Using a composite 'multifunctionality index' we found that species-rich dung beetle assemblages delivered marginally higher levels of multifunctionality in unperturbed conditions; however, this benefit was lost under perturbation. Using a relatively new and robust method of assessing diversity-multifunctionality relationships across a range of

thresholds, we found no significant effect of species richness on multifunctionality.

Introduction

Human activities alter the composition of communities by causing extinctions at local, regional, and global scales. These extinctions can have consequences for ecosystem functions: the biological, geochemical, and physical processes occurring within an ecosystem (de Groot *et al.*, 2002). The relationship between biodiversity and ecosystem functioning has been intensively researched. Widespread evidence suggests that more diverse communities deliver enhanced functioning compared to less diverse communities (Hooper *et al.*, 2005). However, the benefits of higher biodiversity are frequently saturating, with maximal functioning often achieved by a small proportion of the overall community (Perkins *et al.*, 2010). This suggests that, from a purely functional perspective, some species within ecosystems can be considered ‘functionally redundant’.

This notion of functional redundancy becomes weaker when systems are perturbed. As species exhibit differing sensitivities and responses to disturbance, the value of higher biodiversity in maintaining functioning may become more apparent under perturbation (Hooper *et al.*, 2005). This has been demonstrated experimentally in a wide variety of contexts, including thermal perturbation of processes mediated by benthic invertebrates (Perkins *et al.*, 2014), and salinity and temperature perturbations of biomass production in algal communities (Steudel *et al.*, 2012).

Functional redundancy is further challenged by the observation that species, or groups of species, typically provide numerous beneficial functions. When biodiversity-

ecosystem functioning studies focus on single functional measures, the true value of biodiversity may be underestimated or even overlooked (Lefcheck *et al.*, 2015). A more complete understanding of the importance of biodiversity must consider the simultaneous delivery of multiple ecosystem functions, a concept known as ecosystem multifunctionality (Hector & Bagchi, 2007).

Although the value of biodiversity may become more evident when systems are perturbed, or when multifunctionality is considered, few studies have examined these questions simultaneously (but see Perkins *et al.*, 2014). Understanding the consequences of species loss and perturbation for ecosystem multifunctionality is important in the context of rising extinction rates (Barnosky *et al.*, 2011), intensifying land use (Foley *et al.*, 2005), and a changing climate (Meehl & Tebaldi, 2004).

Understanding biodiversity-multifunctionality relationships is highly relevant to agricultural production. Agriculture represents approximately half of global land use (Lambin & Meyfroidt, 2011) and widespread intensification during the last century has caused considerable losses of farmland biodiversity (Robinson & Sutherland, 2002). Many organisms in decline, provide beneficial functions which underpin agricultural production. For example, farmland birds (Donald *et al.*, 2001) and wild bees (Ollerton *et al.*, 2014) can support pest control and pollination, respectively. From an ecological perspective, elements of agricultural management (e.g. intensive soil cultivation, application of an insecticide, or the use of a synthetic fertiliser) can be considered as perturbations with the potential to disrupt biodiversity-multifunctionality relationships.

Dung beetles (Coleoptera: Scarabaeoidea) provide an excellent model system for understanding how perturbation influences biodiversity-multifunctionality relationships

78 within agroecosystems. Most dung beetle species feed on animal dung as larvae and
79 adults, and play a key role in mediating dung removal through burying, consuming, and
80 fragmenting livestock dung (Doube, 1990). Alongside the direct benefit of preventing
81 pasture fouling, dung beetle activity has numerous benefits for agroecosystems. These
82 include enhancing nutrient cycling (Yokoyama *et al.*, 1991), preventing the spread of
83 pathogenic bacteria (Jones *et al.*, 2015), stimulating litter decomposition (Tixier *et al.*,
84 2015; Manning *et al.*, 2016), and reducing greenhouse gas emissions (Slade *et al.*,
85 2016).

86 Dung beetles in agricultural landscapes are sensitive to land use intensity (Lobo, 2001;
87 Hutton & Giller, 2003). One example of this sensitivity involves the administration of
88 anthelmintic products to livestock, most notably the macrocyclic lactones (Floate *et al.*,
89 2005). These compounds agonise chloride channels in the parasite's nervous tissue
90 causing hyperpolarisation of nervous cells, flaccid paralysis, and death (Shoop & Soll,
91 2002). Macrocyclic lactones are poorly metabolised, and are excreted in dung and urine
92 (McKellar & Gokbulut, 2012). Through the same mode of action, exposure has lethal
93 and sub-lethal effects on dung beetles exposed to residues (O'Hea *et al.*, 2010b;
94 Beynon *et al.*, 2012; Verdú *et al.*, 2015). In some cases, this may lead to impaired dung
95 decomposition (Wall & Strong, 1987).

96 We investigated how perturbation influences biodiversity-ecosystem functioning
97 relationships using manipulative experiments of dung beetle species richness in
98 combination with a perturbation of ivermectin, a widely used macrocyclic lactone (Boxall
99 *et al.*, 2007). We measured four functions with relevance for pasture production: dung
100 removal, primary productivity, feeding activity of soil fauna, and reduction of soil

compaction. We predicted that increased biodiversity (higher species richness) would enhance individual functions and ecosystem multifunctionality. As ivermectin has well-documented negative effects on dung beetle survival and functioning (Beynon *et al.*, 2012) we predicted that individual functions and multifunctionality would be reduced across all species richness levels in response to perturbation.

Methods

Experimental design

To manipulate dung beetle communities we introduced known combinations of dung beetles into enclosures constructed by digging 14 L, 35 cm d, baseless, black, plastic buckets (n = 72) into the ground to a depth of 8 cm as described by Manning *et al.* (2016). Enclosures were separated by 1 m, and arranged into three parallel rows of 24, each row separated by c. 1 m. Enclosures were covered by a fine (2 mm aperture) mesh secured with elastic. The experiment was conducted on a grassy margin in a courtyard at the John Krebs Field Station, Wytham, Oxfordshire, UK.

We collected dung on June 30th 2015 from beef cattle under organic management at Wytham Farm, adjacent to the experimental site. Dung was collected immediately following defecation, before invertebrates could colonise, and was thoroughly homogenised in a large container using a trowel. A standardised quantity of dung (550 g wet mass), equivalent to pats observed in the field, was used to form a pat within each enclosure. As ivermectin administered to livestock undergoes negligible metabolism (González Canga *et al.*, 2009), and we aimed to minimise differences in dung quality among replicates, ivermectin was added directly to dung using a standardised protocol described by Römbke *et al.* (2009). For the perturbation treatment, a 1.00 mL aliquot of

124 a 275 ppm solution of ivermectin (5mg / mL Molemec pour-on, Mole Valley Farmers,
125 South Molton, Devon EX36 3LH, UK) in acetone was added to each dung pat. This
126 resulted in a final ivermectin concentration of 0.5 ppm (wet weight), consistent with peak
127 faecal concentrations following subcutaneous injection, and c. 50% of peak faecal
128 concentrations following use of pour-on formulations (Sommer *et al.*, 1992). In controls,
129 we used 1 mL aliquots of pure acetone in place of ivermectin (Römbke *et al.*, 2009).
130 Each aliquot was mixed thoroughly through the dung, and dung pats were formed using
131 a 12 cm diameter circular mould on a 2 cm aperture wire mesh. This mesh later allowed
132 separation of the dung pat from underlying herbage and soil. The experiment began
133 when beetles were added 24 hours later, after allowing for acetone to evaporate
134 (Römbke *et al.*, 2009).

135 Four native dung beetle species from the genus *Aphodius* were used in the experiment
136 (Table 1). In the United Kingdom, a maximum richness of four species at a patch (dung
137 pat) level is representative of a typical improved agricultural pasture (P. Manning, pers.
138 obs). Beetles were collected by hand from pastures under organic management
139 adjacent to the field site. All species had been observed in the pastures several weeks
140 before collection and were assumed to be sexually mature. Prior to the experiment
141 beetles were stored in a well-ventilated shed, separated into single-species, mixed-sex
142 terraria and were fed cattle dung free from anthelmintics.

143 Beetle communities were standardised based on observations of typical field densities
144 (Beynon *et al.*, 2012), with a target of 102 ± 0.5 mg of beetles added to each dung pat
145 (Table 1). All possible combinations of the four species were included to generate
146 species richness levels (SRL) ranging from 1 to 4 (Table 1). Because our protocol for

standardising communities was based on mass (Beynon *et al.*, 2012), our two heaviest species (*A. fossor* and *A. rufipes*) were each represented by a single individual at SRL3 and SRL4 (Table 1). Since female beetles are likely to contribute more to functioning than males through the activity of their offspring, we used female *A. fossor* and *A. rufipes* in the SRL3 treatment, and males in the SRL4 treatment. This was to ensure that any additional functioning provided by offspring was not attributed to higher species richness levels, making our manipulation of species richness conservative.

Each combination of species was replicated twice, except for the four-species community, which was replicated four times to generate a more balanced number of replicates at each species richness level. For comparison, beetle-free controls (n = 4) were included to determine background functioning by soil fauna. As the experiment was conducted under perturbed (dung containing ivermectin) and control (dung without ivermectin) conditions, there was a total of 72 enclosures, 64 of which contained dung beetles.

To estimate dung mass loss due to evaporation, we formed six additional reference dung pats from which all invertebrates were excluded, three with ivermectin and three without. These were placed on 2 cm aperture wire mesh, in 5 L plastic buckets (with the bottoms intact) filled with 5 cm of sieved soil collected on-site. Buckets were covered with a 2 mm aperture mesh, and placed adjacent to the main experiment.

The species included in our experiment spend varying periods in dung pats as adults (Holter, 1982), so we monitored enclosures multiple times daily, attaching emergence traps when adult beetles were first observed walking or flying within enclosures (Day 14). We fitted emergence traps to each enclosure using a short length of 2 cm clear

170 plastic tubing. Emergence traps consisted of 750 mL translucent plastic containers filled
171 with a 2 cm layer of sieved soil, and 50 g of fresh cattle dung. On Day 18 we added
172 another 100 g of fresh cattle dung to each emergence trap to enhance attractiveness
173 and encourage beetles into the emergence traps. On Day 21, the soil and dung in each
174 emergence trap were hand-searched for beetles, before submerging the trap contents
175 in water to remove additional beetles by flotation. Any beetles that did not emerge were
176 assumed to have died in the enclosure, and we used the number of emigrated beetles
177 as a proxy for adult survival. The experiment continued uninterrupted until Day 42
178 (August 13th, 2015). At this point, beetle larvae were assumed to have pupated,
179 concluding their feeding within the dung pat (Stevenson & Dindal, 1985).

180 ***Measurement of ecosystem functions***

181 To measure feeding activity of soil invertebrates, we used the bait lamina test (Terra
182 Protecta GmbH, Berlin, Germany). Bait lamina are PVC strips (1 mm x 6 mm x 120 mm)
183 with sixteen 1.5 mm diameter perforations at 5 mm intervals. These perforations were
184 filled with a standardised bait of cellulose powder, ground wheat bran, and activated
185 charcoal (70:27:3). Following Manning *et al.* (2016), on Day 42, we inserted five bait
186 lamina through each dung pat to a consistent depth, so that the uppermost hole was
187 located immediately below the soil surface. Test strips were placed alongside the
188 experiment and a subset were checked daily until appreciable (30 - 50 %) feeding
189 activity was observed (Day 49). Bait lamina were removed from the ground and
190 assessed by viewing strips against a light source, classifying each bait as 'consumed' or
191 'intact' depending on whether light penetrated (Kratz, 1998).

192 Remaining functions were measured the following day (21st August 2015, Day 50). We
193 removed dung pats and underlying wire mesh from the enclosures and took soil
194 samples from the area directly under the former location of the dung pat. We measured
195 soil compaction by taking a measure of bulk density. We hammered a segment of 5.5
196 cm diameter PVC pipe into the soil to a depth of 5 cm and carefully removed the intact
197 sample with a trowel. Soil and dung samples were dried for 48 hours at 80°C. Dung
198 removal (dry mass) was measured to the nearest gram. Measurements were corrected
199 by subtracting this figure from the mean dry mass of reference dung pats from which
200 dung beetles and other soil fauna had been excluded. Soil bulk density (g / cm^3) was
201 calculated as the dry mass of soil solids divided by the core volume. We did not sieve
202 small stones or fine roots from the bulk density sample.

203 We measured primary productivity by planting tetraploid perennial ryegrass (*Lolium*
204 *perenne* L.) seeds into soils sampled from experimental enclosures (Slade & Roslin,
205 2016). Two additional 5.5 cm diameter x 5 cm deep soil samples were taken from the
206 area beneath each dung pat, as described above for bulk density. The soil samples
207 were homogenised and sieved. A 185 mL sample of the soil from each enclosure was
208 added to a 250 mL plant pot and 1 mL of tetraploid perennial ryegrass seed (Cotswold
209 Seeds, Moreton-in-Marsh, Gloucestershire, GL56 0JQ, UK) was sown evenly across the
210 pots surface. Seeds were then covered with 15 mL of soil from the same sample, which
211 was spread evenly over the surface. All pots were placed in an unheated greenhouse,
212 and watered regularly. After three weeks, we harvested all aboveground biomass by
213 trimming the grass at the soil surface. We dried samples at 75°C for 48 hours (Zhao *et*
214 *al.*, 2013) and weighed above ground biomass with a balance accurate to 0.01 g.

215 ***Analysis***

216 We used linear models with Gaussian error structures (ANCOVA) to investigate the
217 effects of species richness and perturbation for each ecosystem function. Examination
218 of diagnostic plots demonstrated that residuals were homogenous and normally
219 distributed, and thus all values were left untransformed. We fitted maximal models and
220 dropped interactions until the minimum adequate model was obtained, selecting the
221 model with the smallest Akaike's Information Criterion (AIC) value (Burnham &
222 Anderson, 2003).

223 To explore relationships between species richness and multifunctionality, we first
224 employed the averaging approach to multifunctionality (Hooper & Vitousek, 1998). The
225 averaging approach creates a 'multifunctional index' for each experimental unit by
226 taking a mean estimate of all functions after standardising to a common scale. To
227 standardise functions, the 'desirable' direction of each function must be consistent
228 across measures. We considered higher values of dung removal, soil fauna feeding
229 activity, and primary productivity to be beneficial and hence desirable. As compacted
230 soils are known to limit plant growth (e.g. Bartholomew & Williams, 2010), we
231 considered a lower bulk density to be more desirable. We reflected our values for soil
232 bulk density (multiplied each observation by -1), and added the un-reflected maximum
233 to each observation so that the lowest level of soil compaction was 0. We then
234 standardised our functions to a common scale by expressing each function as a
235 proportion of the maximum. Each maximum was defined using a mean of the three
236 highest observations from all enclosures containing beetles. The multifunctionality index
237 value was calculated by taking the mean of the four standardised functions. We used a

238 linear ANCOVA model to investigate the effects of perturbation and species richness on
239 average multifunctionality.

240 While the averaging approach to multifunctionality is readily interpreted, a limitation of
241 this method is that variability in responses amongst functions is not discernible (Byrnes
242 *et al.*, 2014). For example, a strong negative relationship between biodiversity and
243 functioning for a single process could obscure modest benefits of greater levels of
244 biodiversity in supporting all other functions. Thus, we also used the 'Exceeding
245 Thresholds Approach' (Byrnes *et al.*, 2014) to explore how perturbation influences the
246 relationship between species richness and ecosystem multifunctionality.

247 The Exceeding Thresholds Approach works by estimating a linear relationship between
248 species richness and the number of processes performing at, or above, a given
249 threshold. The value of each threshold is calculated as a proportion of the function's
250 maximum (as above, defined as the mean of the three highest observations). For
251 example, if each function had a maximum value equal to 1 at a given threshold (e.g.
252 65%), we then determined how many of the measured functions (0 – 4) were equal to,
253 or exceeded, the corresponding threshold value (e.g. 0.65) for each replicate. As
254 species richness within enclosures was manipulated experimentally, we could fit a linear
255 model with a quasi-binomial error structure to estimate the relationship between species
256 richness (1 – 4) and the number of functions (0 – 4) exceeding each threshold. For each
257 threshold, the slope estimate represents the number of functions supported by the
258 addition of a single species. This slope was calculated for all possible thresholds (in our
259 case, whole number increments between 1 % and 72 %). Above this upper threshold
260 our models would not converge. To visualise the relationship between species richness

and multifunctionality, these slopes and their 95 % confidence intervals were plotted across the full range of possible thresholds.

Finally, we tested the influence of ivermectin on the emigration of beetles (a proxy for survival) using generalised linear models with binomial errors. Emigration was calculated as the proportion of adult beetles entering the emergence traps. Where data were over-dispersed, a quasi-binomial error structure was used.

The statistical computing environment R 3.1.1 (R Core Team, 2014) was used for all analyses. The package 'multifunc' (Byrnes *et al.*, 2014) was used for analysis of multifunctionality data. Figures were created using the package 'ggplot2' (Wickham, 2009).

Results

Effects of species richness and perturbation on single functions and dung beetle emigration

Perturbation reduced dung removal significantly, by approximately 17 % (Figure 1a, Table 2; Perturbed 27.5 ± 1.8 g, Control 32.1 ± 0.9 g, mean \pm SE). Species richness did not have a significant effect on dung removal (Table 2).

For primary productivity, we found a marginally significant interaction between perturbation and species richness (Figure 1b, Table 2). In the absence of perturbation, primary productivity tended to be higher in more species-rich assemblages. Neither soil fauna feeding activity (Figure 1c), nor soil bulk density (Figure 1d) were influenced significantly by perturbation or by species richness (Table 2).

Ivermectin significantly reduced adult emigration (a proxy for survival) for all four species (Figure 2), lowering the mean emigration of *A. ater* by approximately 15%, *A. erraticus* by 49%, *A. fossor* by 60%, and *A. rufipes* by 13%, relative to unperturbed controls.

Effects of species richness and perturbation on average multifunctionality

We found weak evidence of an interaction between perturbation and species richness (Table 2), indicating that species rich communities tended to provide higher levels of average multifunctionality than species poor communities, but only under unperturbed conditions (Figure 3). Although non-significant at conventional levels of statistical significance, the interaction was retained within our model because the main effects on their own explained little variation in average multifunctionality. At the lowest levels of species richness, perturbation with ivermectin did not affect average multifunctionality (Perturbed 0.57 ± 0.03 , Control 0.57 ± 0.01 , mean \pm SE). Average multifunctionality was c. 30 % higher in the control treatment than in the perturbed treatment at the highest level of species richness (Perturbed = 0.49 ± 0.03 , Control 0.64 ± 0.04). While perturbation significantly affected average multifunctionality (Table 2), effects were small when considering perturbation in isolation: the control treatment had a mean multifunctionality index c. 10 % greater than the perturbed treatment (Perturbed 0.533 ± 0.016 , Control 0.590 ± 0.018 , mean \pm SE). We found no significant main effect of species richness on average multifunctionality (Table 2).

Exceeding thresholds approach

Testing the effect of species richness in supporting multifunctionality across a range of thresholds we found that, in the controls, species rich communities generally supported higher levels of functioning than species-poor communities. However, these trends were not statistically significant (Figure 4a). Conversely, we found a generally negative (but never significantly so) relationship between species richness and ecosystem multifunctionality in perturbed communities when moving from lower to higher thresholds (Figure 4b). At no point were our model estimates significantly different from zero.

Discussion

Species-rich dung beetle assemblages supported slightly higher levels of ecosystem multifunctionality than species-poor assemblages (Figures 3, 4a). However, these modest benefits were lost when the system was perturbed with ivermectin. In the absence of perturbation, a positive correlation between ecosystem multifunctionality and species richness is expected in theory (Hooper *et al.*, 2005) and has been shown experimentally (Maestre *et al.*, 2012; Perkins *et al.*, 2014). As feeding traits of dung beetles seem closely affiliated with the delivery of particular ecosystem functions (Manning *et al.*, 2016), variation in feeding traits (Table 1) is likely driving increases in multifunctionality at higher species richness levels in the absence of chemical perturbation.

Limited effects of species richness on multifunctionality

The benefit of maintaining high species richness in unperturbed conditions may have been obscured by the fully factorial nature of this experiment. With this design, if a single species showed a disproportionately large role in supporting multiple functions, this could mask any positive effects of species richness on multifunctionality. Functional dominance by certain dung beetle species has been shown previously (Nervo *et al.*, 2014), although the species involved were larger-bodied tunnellers, which are not common in intensively managed pastures (Hutton & Giller, 2003). Previous results documenting a significant positive association between dung beetle species richness and dung removal (Beynon *et al.*, 2012) could be explained by sampling effects, where functionally dominant species are more likely to be included in more species-rich assemblages (Wardle, 1999). In the current experiment, low replication of single-species assemblages makes it difficult to quantify the likely influence of species identity.

A second possible explanation why higher species richness provided only modest benefits to multifunctionality is that the functional trait diversity included within our experiment was relatively limited. While the assemblages used represent a typical dung beetle community in our study area (P. Manning, pers. obs), traits may be more varied in less simplified landscapes (Slade *et al.*, 2011). Where greater variety in feeding trait diversity is observed (e.g. Palestirini *et al.*, 1998), and the available species pool is larger (Nervo *et al.*, 2014), a stronger relationship between dung beetle species richness and multifunctionality is more likely to be observed.

Effects of perturbation from ivermectin

We found the multifunctional benefits of increased species richness seemed to be lost under perturbation (Figures 3, 4b). The most parsimonious explanation is that the strength of the chemical perturbation pushed the system beyond the buffering capacity provided by higher species richness levels (Steudel *et al.*, 2012; Hofer *et al.*, 2016). Ivermectin exposure increased mortality for all species included in the experiment. Dung beetles in our experiment were exposed to an ivermectin concentration of 500 ppb (wet weight). Sub-lethal effects of ivermectin exposure have been detected at concentrations as low as 1 ppb (Verdú *et al.*, 2015). A combination of lethal and sub-lethal effects resulting from ivermectin exposure is the simplest explanation for observed losses of multifunctionality.

Individual ecosystem functions

While dung removal was significantly reduced by ivermectin, the size of this effect was small. Our results suggest that, despite its prevalence as a proxy for overall functioning (Rosenlew & Roslin, 2008; Beynon *et al.*, 2012; Kaartinen *et al.*, 2013), this measure may not be sufficiently sensitive to detect subtler changes in ecosystem functioning. However, as earthworms and dung beetles may introduce soil into dung (Owen *et al.*, 2006), removal rates may have been underestimated. Measuring organic matter loss (Holter, 1979) may provide a better understanding of how species richness and perturbation influence dung removal.

Primary productivity was best explained by the interaction between species richness and perturbation. Under unperturbed conditions, species-rich dung beetle assemblages

increased ryegrass biomass compared with assemblages with low richness. A similar benefit of higher species richness was found by Slade and Roslin (2016) who reported that higher levels of species richness supported enhanced primary productivity in experimentally warmed enclosures.

Neither perturbation nor species richness affected feeding activity in soil underlying dung pats significantly. The influence of dung beetles on feeding activity or plant litter decomposition might be more strongly affected by tunnelling species (Tixier *et al.*, 2015). While our experiment included two tunnelling (paracoprid) species (*A. erraticus*, and *A. rufipes*), these species excavate relatively shallow burrows and, in the case of *A. rufipes*, show plasticity in reproductive strategies (Klemperer, 1980). Therefore, the activity of these dung beetles might not stimulate soil processes to the same extent as other larger-bodied (Tixier *et al.*, 2015), or deeper tunnelling (Manning, 2016) species. While we did not find any significant relationships between feeding activity and either species richness or perturbation, the presence of dung beetles appeared to enhance feeding activity relative to controls without beetles (Figure 1c). This may have been caused by dung beetles promoting rapid aggregation of earthworms beneath dung pats as reported by Holter (1979), or by dung beetles inducing changes in the soil microbial community (Slade *et al.*, 2015).

We found no significant effect of species richness or perturbation on soil bulk density. Soils at our site had low levels of compaction, and the contribution of dung beetles to improving soil structure may be greater where soils are more compacted. Few studies

have explored the benefits of dung beetles in improving the physical properties of soils, but available data suggest that tunnelling activity can improve soil porosity (Bang *et al.*, 2005; Brown *et al.*, 2010). The dung beetles included in our experiment do not tunnel to the same extent as many larger-bodied species, and thus may be less likely to influence physical parameters of soil. Nevertheless, *A. fossor* has been shown to improve penetrability at the soil surface (Manning *et al.*, 2016).

Limitations of the experimental design and directions for future work

While adults of each species included in this experiment were all affected by ivermectin exposure, we were unable to determine when beetles died, or to measure how our experimental perturbation influenced the development and survival of larvae. Future work would benefit from a longer-term, multi-generational perspective to capture the functional contribution of dung beetle larvae, which can contribute significantly to dung removal (Holter, 2016) and are typically more sensitive to perturbation than adult beetles (Beynon *et al.*, 2012). Thus, our estimates of beetle survival will not have fully captured the magnitude of the perturbation. Because survival in unperturbed conditions varied among species, assemblages dominated by species that perform poorly in experimental enclosures (*A. ater*, *A. rufipes*: Figure 2) likely added further noise to our data. With higher replication of monocultures, it may have been possible to account for these species-specific responses in our models.

While dung beetles have been described as key providers of ecosystem services (Nichols *et al.*, 2008), other soil invertebrates have a functional role comparable to dung

beetles in supporting dung removal (O’Hea *et al.*, 2010a; Kaartinen *et al.*, 2013). The role of soil fauna in supporting other functions related to dung removal are poorly understood and likely contributed additional noise to our data. Nevertheless, through allowing soil fauna to colonise, the effects of dung beetle diversity and chemical perturbation on ecosystem functioning were considered within a context that more closely represents field conditions.

Overall, our results suggest that, in systems with relatively low total dung beetle species diversity (typical of moderate to intensively farmed pastures in the UK), any positive effect of dung beetle species richness on the delivery of single ecosystem functions is limited. The functional benefits of higher species richness appear to be greater when multifunctionality is considered. Furthermore, perturbation with the anthelmintic ivermectin has the potential to reduce functional benefits by inducing lethal and sublethal effects on dung beetles. We recommend that future research explores functions and multifunctionality in more species-rich systems, and with greater replication to better understand the role of species identity.

Acknowledgements

We thank Phil Smith at Wytham Field Station for his help with logistics, and Jo Copping and the Farm Animal Initiative staff for logistical help and allowing us to collect beetles and dung for the experiment. We thank many members of the CERO group for providing helpful comments on early drafts. We thank Sarah Jane Fanning

443 and Chris McCloskey for their assistance in the field. John Finn, and three
444 anonymous reviewers provided valuable feedback on earlier versions of this
445 manuscript. P.M. was funded through a Rhodes Scholarship, and a Natural
446 Sciences and Engineering Research Council postgraduate scholarship (Canada).

447 **References**

- 448 Bang, H.S., Lee, J.-H., Kwon, O.S., Na, Y.E., Jang, Y.S. & Kim, W.H. (2005) Effects of
449 paracoprid dung beetles (Coleoptera: Scarabaeidae) on the growth of pasture herbage
450 and on the underlying soil. *Applied Soil Ecology*, **29**, 165–171.
- 451 Barnosky, A.D., Matzke, N., Tomiya, S., Wogan, G.O.U., Swartz, B., Quental, T.B., *et al.*
452 (2011) Has the Earth's sixth mass extinction already arrived? *Nature*, **471**, 51–57.
- 453 Bartholomew, P.W. & Williams, R.D. (2010) Effects of soil bulk density and strength on
454 seedling growth of annual ryegrass and tall fescue in controlled environment. *Grass and*
455 *Forage Science*, **65**, 348–357.
- 456 Beynon, S.A., Mann, D.J., Slade, E.M. & Lewis, O. (2012) Species-rich dung beetle
457 communities buffer ecosystem services in perturbed agro-ecosystems. *Journal of*
458 *Applied Ecology*, **49**, 1365–1372.
- 459 Boxall, A.B.A., Sherratt, T.N., Pudner, V. & Pope, L.J. (2007) A screening level index for
460 assessing the impacts of veterinary medicines on dung flies. *Environmental Science &*
461 *Technology*, **41**, 2630–2635.
- 462 Brown, J., Scholtz, C.H., Janeau, J.-L., Grellier, S. & Podwojewski, P. (2010) Dung
463 beetles (Coleoptera: Scarabaeidae) can improve soil hydrological properties. *Applied*
464 *Soil Ecology*, **46**, 9–16.
- 465 Burnham, K.P. & Anderson, D.R. (2003) *Model selection and multimodel inference: a*

466 *practical information-theoretic approach*. Springer Science & Business Media.

467 Byrnes, J.E.K., Gamfeldt, L., Isbell, F., Lefcheck, J.S., Griffin, J.N., Hector, A., *et al.*

468 (2014) Investigating the relationship between biodiversity and ecosystem

469 multifunctionality: Challenges and solutions. *Methods in Ecology and Evolution*, **5**, 111–

470 124.

471 Donald, P.F., Green, R.E. & Heath, M.F. (2001) Agricultural intensification and the

472 collapse of Europe's farmland bird populations. *Proceedings of the Royal Society of*

473 *London B: Biological Sciences*, **268**, 25–9.

474 Doube, B.M. (1990) A functional classification for analysis of the structure of dung beetle

475 assemblages. *Ecological Entomology*, **15**, 371–383.

476 Floate, K.D., Wardhaugh, K.G., Boxall, A.B.A. & Sherratt, T.N. (2005) Fecal residues of

477 veterinary parasiticides: nontarget effects in the pasture environment. *Annual Review of*

478 *Entomology*, **50**, 153–79.

479 Foley, J.A., Defries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., *et al.*

480 (2005) Global consequences of land use. *Science*, **309**, 570–574.

481 González Canga, A., Sahagún Prieto, A.M., José Díez Liébana, M., Martínez, N.F.,

482 Vega, M.S. & Vieitez, J.J.G. (2009) The pharmacokinetics and metabolism of ivermectin

483 in domestic animal species. *The Veterinary Journal*, **179**, 25–37.

484 Klemperer, H.G. (1980) Kleptoparasitic behaviour of *Aphodius rufipes* (L.) larvae in

485 nests of *Geotrupes spiniger* Marsh. (Coleoptera, Scarabaeidae), **5**, 143–151.

486 Groot, R.S. de, Wilson, M.A. & Boumans, R.M.. (2002) A typology for the classification,

487 description and valuation of ecosystem functions, goods and services. *Ecological*

488 *Economics*, **41**, 393–408.

489 Hector, A. & Bagchi, R. (2007) Biodiversity and ecosystem multifunctionality. *Nature*,
490 **448**, 188–190.

491 Hofer, D., Suter, M., Haughey, E., Finn, J.A., Hoekstra, N.J., Buchmann, N., *et al.* (2016)
492 Yield of temperate forage grassland species is either largely resistant or resilient to
493 experimental summer drought. *Journal of Applied Ecology*, **53**, 1023–1034.

494 Holter, P. (1979) Effect of dung-beetles (*Aphodius* spp.) and earthworms on the
495 disappearance of cattle dung. *Oikos*, **32**, 393–402.

496 Holter, P. (1982) Resource Utilization and Local Coexistence in a Guild of Scarabaeid
497 Dung Beetles (*Aphodius* spp.). *Oikos*, **39**, 213–227.

498 Holter, P. (2016) Herbivore dung as food for dung beetles: elementary coprology for
499 entomologists. *Ecological Entomology*, **41**, 367–377.

500 Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., *et al.* (2005)
501 Effects of biodiversity on ecosystem functioning: a consensus of current knowledge.
502 *Ecological Monographs*, **75**, 3–35.

503 Hooper, D.U. & Vitousek, P.M. (1998) Effects of plant composition and diversity on
504 nutrient cycling. *Ecological Monographs*, **68**, 121–149.

505 Hutton, S.A. & Giller, P.S. (2003) The effects of the intensification of agriculture on
506 northern temperate dung beetle communities. *Journal of Applied Ecology*, **40**, 994–
507 1007.

508 Jones, M.S., Tadepalli, S., Bridges, D.F., Wu, V.C.H. & Drummond, F. (2015)
509 Suppression of *Escherichia coli* O157:H7 by dung beetles (Coleoptera: Scarabaeidae)
510 using the lowbush blueberry agroecosystem as a model system. *Plos One*, **10**,
511 e0120904.

512 Kaartinen, R., Hardwick, B. & Roslin, T. (2013) Using citizen scientists to measure an
 513 ecosystem service nationwide. *Ecology*, **94**, 2645–52.

514 Kratz, W. (1998) The bait-lamina test: General aspects, applications and perspectives.
 515 *Environmental Science and Pollution Research International*, **5**, 94–6.

516 Lambin, E.F. & Meyfroidt, P. (2011) Global land use change, economic globalization,
 517 and the looming land scarcity. *Proceedings of the National Academy of Sciences*, **108**,
 518 3465–3472.

519 Lefcheck, J.S., Byrnes, J.E.K., Isbell, F., Gamfeldt, L., Griffin, J.N., Eisenhauer, N., *et al.*
 520 (2015) Biodiversity enhances ecosystem multifunctionality across trophic levels and
 521 habitats. *Nature Communications*, **6**, 6936.

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

524 Maestre, F.T., Quero, J.L., Gotelli, N.J., Escudero, A., Ochoa, V., Delgado-Baquerizo,
 525 M., *et al.* (2012) Plant species richness and ecosystem multifunctionality in global
 526 drylands. *Science*, **335**, 214–8.

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

530 McKellar, Q. & Gokbulut, C. (2012) Pharmacokinetic features of the antiparasitic
 531 macrocyclic lactones. *Current Pharmaceutical Biotechnology*, **13**, 888–911.

532 Meehl, G.A. & Tebaldi, C. (2004) More intense, more frequent, and longer lasting heat
 533 waves in the 21st century. *Science*, **305**, 994–997.

534 Nervo, B., Tocco, C., Caprio, E., Palestini, C. & Rolando, A. (2014) The effects of body

535 mass on dung removal efficiency in dung beetles. *PloS one*, **9**, e107699.

536 Nichols, E., Spector, S., Louzada, J., Larsen, T., Amezquita, S. & Favila, M.E. (2008)

537 Ecological functions and ecosystem services provided by Scarabaeinae dung beetles.

538 *Biological Conservation*, **141**, 1461–1474.

539 O’Hea, N.M., Kirwan, L. & Finn, J. a. (2010a) Experimental mixtures of dung fauna

540 affect dung decomposition through complex effects of species interactions. *Oikos*, **119**,

541 1081–1088.

542 O’Hea, N.M., Kirwan, L., Giller, P.S. & Finn, J.A. (2010b) Lethal and sub-lethal effects of

543 ivermectin on north temperate dung beetles, *Aphodius ater* and *Aphodius rufipes*

544 (Coleoptera: Scarabaeidae). *Insect Conservation and Diversity*, **3**, 24–33.

545 Ollerton, J., Erenler, H., Edwards, M. & Crockett, R. (2014) Pollinator declines.

546 Extinctions of aculeate pollinators in Britain and the role of large-scale agricultural

547 changes. *Science*, **346**, 1360–1362.

548 Owen, W.K., Lloyd, J.E., Legg, D.E. & Kumar, R. (2006) Endocoprid activity of *Aphodius*

549 fossor (Coleoptera: Scarabaeidae) related to bovine dung decomposition in a mixed

550 grass prairie. *Journal of Economic Entomology*, **99**, 2210–2215.

551 Palestrini, C., Barbero, E. & Ronaldo, A. (1998) Intra- and interspecific aggregation

552 among dung beetles (Coleoptera: Scarabaeoidea) in an Alpine pasture. *Journal of*

553 *Zoology*, **245**, 101–109.

554 Perkins, D.M., Bailey, R. a., Dossena, M., Gamfeldt, L., Reiss, J., Trimmer, M., *et al.*

555 (2014) Higher biodiversity is required to sustain multiple ecosystem processes across

556 temperature regimes. *Global Change Biology*, **21**, 396–406.

557 Perkins, D.M., McKie, B.G., Malmqvist, B., Gilmour, S.G., Reiss, J. & Woodward, G.

558 (2010) Environmental warming and biodiversity-ecosystem functioning in freshwater
 559 microcosms: partitioning the effects of species identity, richness and metabolism. In
 560 *Integrative Ecology: From Molecules to Ecosystems*. Elsevier Science, pp. 177–205.
 561 R Core Team. (2014) R: A Language and Environment for Statistical Computing.
 562 Robinson, R.A. & Sutherland, W.J. (2002) Post-war changes in arable farming and
 563 biodiversity in Great Britain. *Journal of Applied Ecology*, **39**, 157–176.
 564 Römbke, J., Floate, K.D., Jochmann, R., Schäfer, M.A., Puniamoorthy, N., Knäbe, S., et
 565 al. (2009) Lethal and sublethal toxic effects of a test chemical (ivermectin) on the yellow
 566 dung fly (*Scathophaga stercoraria*) based on a standardized international ring test.
 567 *Environmental Toxicology and Chemistry*, **28**, 2117–2124.
 568 Rosenlew, H. & Roslin, T. (2008) Habitat fragmentation and the functional efficiency of
 569 temperate dung beetles. *Oikos*, **117**, 1659–1666.
 570 Shoop, W. & Soll, M. (2002) Ivermectin, abamectin and eprinomectin. In *Macrocyclic*
 571 *Lactones in Antiparasitic Therapy*. CABI Publishing Oxon, UK, pp. 1–29.
 572 Slade, E.M., Mann, D.J. & Lewis, O.T. (2011) Biodiversity and ecosystem function of
 573 tropical forest dung beetles under contrasting logging regimes. *Biological Conservation*,
 574 **144**, 166–174.
 575 Slade, E.M., Riutta, T., Roslin, T. & Tuomisto, H.L. (2016) The role of dung beetles in
 576 reducing greenhouse gas emissions from cattle farming. *Scientific Reports*, **6**, 18140.
 577 Slade, E.M. & Roslin, T. (2016) Dung beetle species interactions and multifunctionality
 578 are affected by an experimentally warmed climate. *Oikos*, 10.1111/oik.03207.
 579 Slade, E.M., Roslin, T., Santalahti, M. & Bell, T. (2015) Disentangling the “brown world”
 580 faecal–detritus interaction web: dung beetle effects on soil microbial properties. *Oikos*.

581 Steudel, B., Hector, A., Friedl, T., Löffke, C., Lorenz, M., Wesche, M., *et al.* (2012)
582 Biodiversity effects on ecosystem functioning change along environmental stress
583 gradients. *Ecology Letters*, **15**, 1397–1405.

584 Stevenson, B.. & Dindal, D.. (1985) Growth and development of *Aphodius* beetles
585 (Scarabaeidae) in laboratory microcosms of cow dung. *The Coleopterists Bulletin*, **39**,
586 215–220.

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

589 Verdú, J.R., Cortez, V., Ortiz, A.J., González-Rodríguez, E., Martínez-Pinna, J.,
590 Lumaret, J.-P., *et al.* (2015) Low doses of ivermectin cause sensory and locomotor
591 disorders in dung beetles. *Scientific Reports*, **5**, 13912.

592 Wall, R. & Strong, L. (1987) Environmental consequences of treating cattle with the
593 antiparasitic drug ivermectin. *Nature*, **327**, 418–421.

594 Wardle, D.A. (1999) Is “sampling effect” a problem for experiments investigating
595 biodiversity-ecosystem function relationships? *Oikos*, **87**, 403–407.

596 Wickham, H. (2009) *ggplot2: elegant graphics for data analysis*. Springer New York.

597 Yokoyama, K., Hideaki, K. & Hirofumi, T. (1991) Paracoprid dung beetles and gaseous
598 loss of nitrogen from cow dung. *Soil Biology and Biochemistry*, **23**, 643–647.

599 Zhao, C., Griffin, J.N., Wu, X. & Sun, S. (2013) Predatory beetles facilitate plant growth
600 by driving earthworms to lower soil layers. *Journal of Animal Ecology*, **82**, 749–58.

601

602 **Figures**

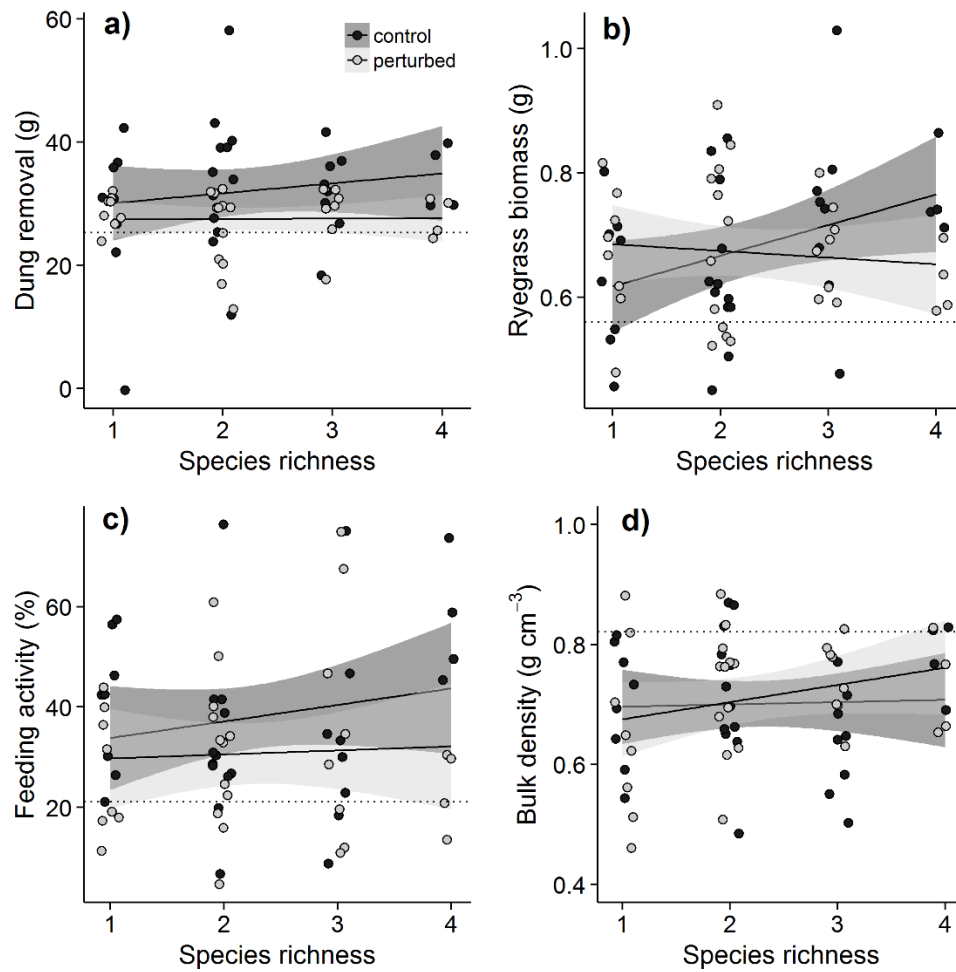


Figure 1. Changes in individual ecosystem functions in response to perturbation and species richness levels. Functions are dung removal (a), forage growth (b), soil fauna feeding activity (c), and soil bulk density (d). Shaded areas indicate 95% confidence intervals for the slopes. Horizontal dotted lines represent the mean for controls enclosures without dung beetles.

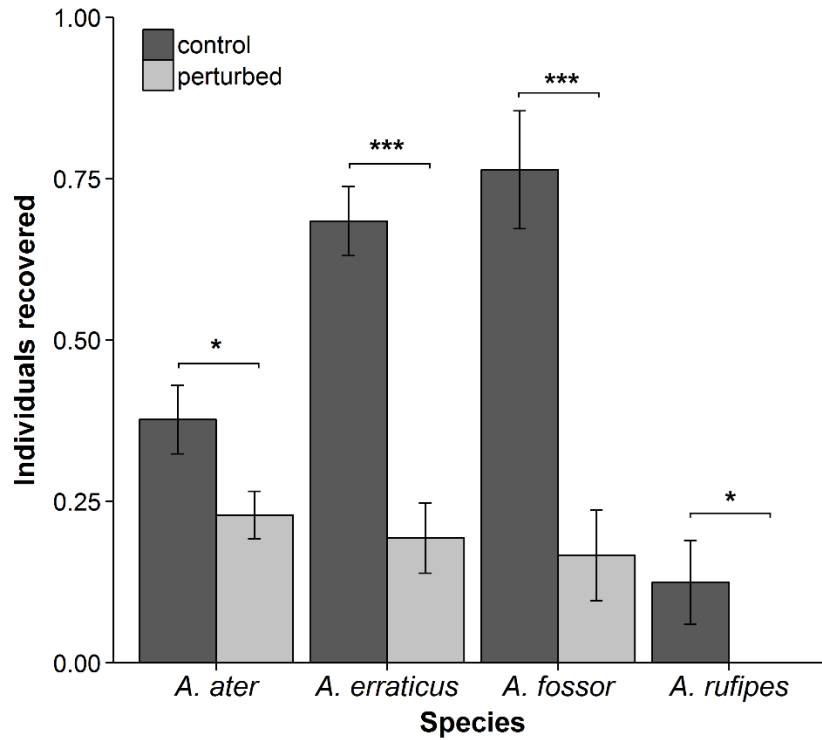


Figure 2. Estimated survival of different species in response to perturbation by ivermectin, based on the recovery of adult beetles in emergence traps. Bars show means with their associated standard errors. No *A. rufipes* were recovered in ivermectin treatment. Asterisks show significant differences in the recovery of beetles for each species between perturbation levels (* $P \leq 0.05$, *** $P \leq 0.001$)

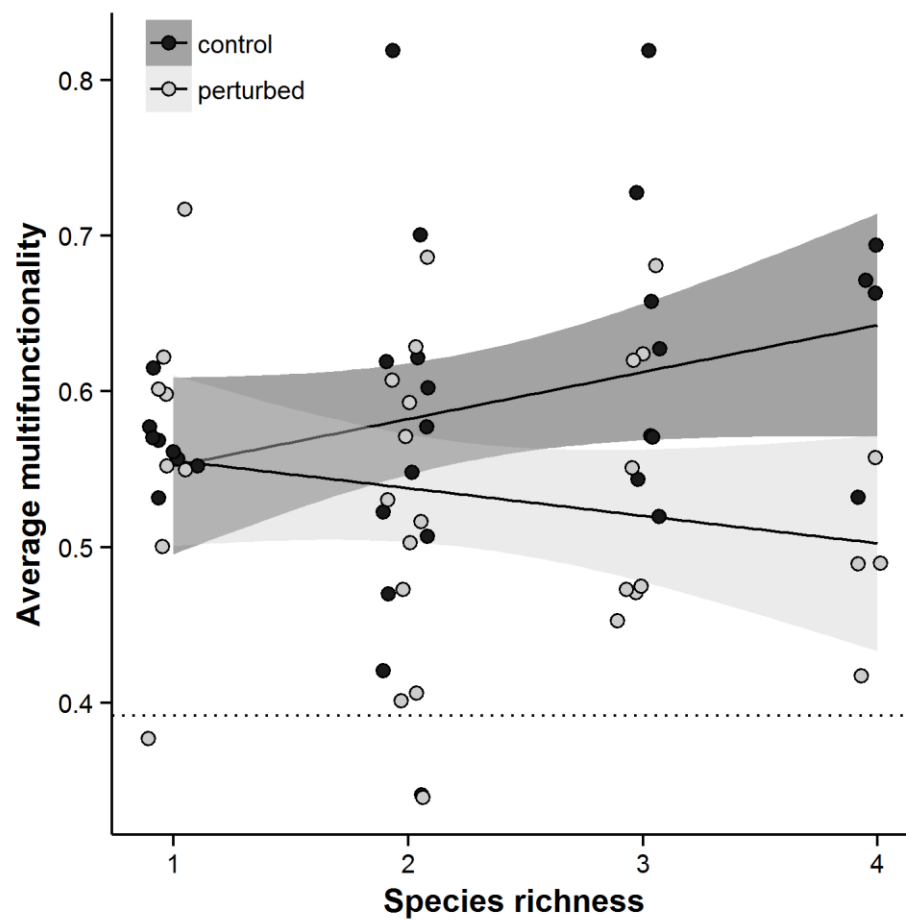


Figure 3. Average multifunctionality as a function of species richness and perturbation level. Shaded areas indicate 95% confidence intervals for the slopes. The horizontal dotted line is the mean of controls without dung beetles.

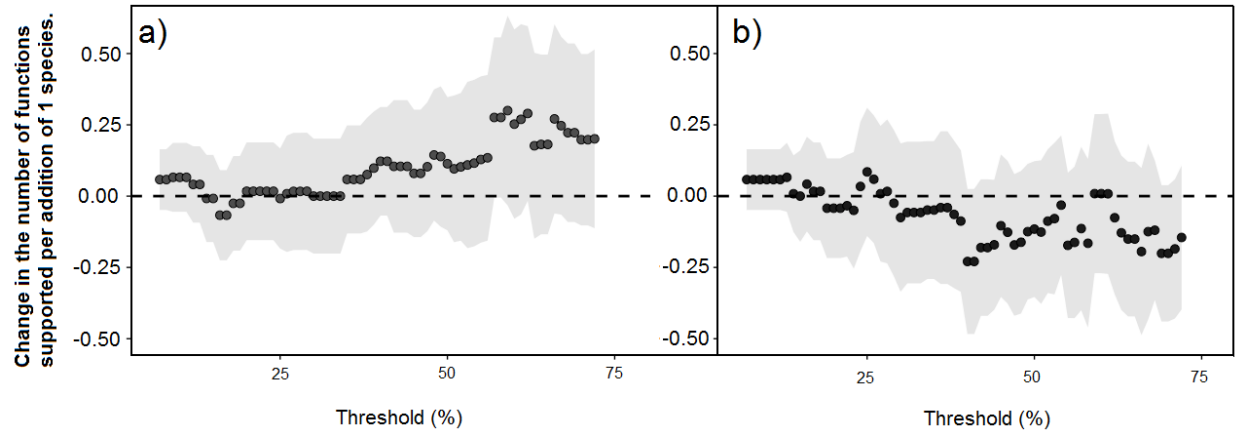


Figure 4. Relationships between species richness and multifunctionality in control (a) and perturbed (b) systems at thresholds from 1-72% of observed maxima. Confidence intervals (95%) around the estimated slopes (filled data points) indicate whether the intervals contain zero, providing a test of the threshold values at which diversity has no effect on multifunctionality.

629

630 **Tables**

631 **Table 1.** Composition of beetle communities used within the experiment. All possible
 632 combinations of four species were used. Integer table values are the abundance of individuals
 633 of the corresponding species included within each replicate. Beetle communities are
 634 standardised based on mass. Shaded columns are used to separate the different assemblages
 635 based on their Species Richness Levels (SRL), which range from 1 to 4. Composition of
 636 individual assemblages can be determined by taking the sum of a column.

Species	Functional Group	Individual dry mass (mg)	SRL1				SRL2				SRL3				SRL4		
<i>Aphodius ater</i> (De Geer)	Dung-ovipositing endocoprid	5.4	18	-	-	-	9	9	10	-	-	-	8	7	6	-	4
<i>Aphodius fossor</i> (L.)	Soil ovipositing endocoprid	26.1	-	4	-	-	2	-	-	2	2	-	1	1	-	1	1
<i>Aphodius rufipes</i> (L.)	Soil-ovipositing endocoprid /Paracoprid	29.9	-	-	4	-	-	2	-	2	-	2	1	-	1	1	1
<i>Aphodius erraticus</i> (L.)	Paracoprid	9.0	-	-	-	11	-	-	5	-	5	5	-	4	5	5	3

637

638

639

640

641

Table 2. Effects of perturbation and species richness on individual functions and multifunctionality.

Term	F	P
a) Dung removal		
Perturbation	$F_{1,61}=5.161$	0.027
Species richness	$F_{1,61}=0.643$	0.426
b) Soil fauna activity		
Perturbation	$F_{1,61}=2.857$	0.357
Species richness	$F_{1,61}=0.860$	0.096
c) Bulk density		
Perturbation	$F_{1,61}=1.430$	0.237
Species richness	$F_{1,61}=0.152$	0.698
d) Primary productivity		
Perturbation	$F_{1,60}=0.063$	0.802
Species richness	$F_{1,60}=1.649$	0.204
Perturbation*Species richness	$F_{1,60}=4.008$	0.050
e) Multifunctionality		
Perturbation	$F_{1,60}=5.688$	0.021
Species richness	$F_{1,60}=0.259$	0.613
Perturbation*Species richness	$F_{1,60}=3.849$	0.054