

RESEARCH ARTICLE

Functional Ecology



Dung beetles drive direct and indirect changes in ecosystem multifunctionality

Fevziye Hasan¹ | Kiri J. Wallace² | Simon V. Fowler³ | Louis A. Schipper¹ |
Zac Hemmings⁴ | Jacob D. Berson^{5,6} | Andrew D. Barnes^{1,2}

¹Te Aka Mātutua—School of Science, University of Waikato, Hamilton, New Zealand; ²Te Tumu Whakaora Taiao—Environmental Research Institute, University of Waikato, Hamilton, New Zealand; ³Manaaki Whenua—Landcare Research, Lincoln, New Zealand; ⁴Centre for Behavioural and Physiological Ecology, Zoology, University of New England, Armidale, New South Wales, Australia; ⁵School of Biological Sciences, University of Western Australia, Crawley, Western Australia, Australia and ⁶Centre for Environment and Life Sciences, CSIRO Health & Biosecurity, Floreat, Western Australia, Australia

Correspondence

Fevziye Hasan

Email: fevziyehasan@gmail.com

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Abstract

1. Measuring ecosystem multifunctionality (EMF) could be a valuable method for assessing whether nature-based solutions, such as introducing non-native species to benefit ecosystem functioning, yield net positive outcomes. While EMF metrics may provide a coarse overview of the functioning of a system, such metrics can obscure the interactions among multiple simultaneously occurring ecosystem functions. This could limit the predictive value and overall efficacy of EMF metrics for the management of environmental change impacts on ecosystem functioning.
2. We carried out a mesocosm experiment with dung beetle temporal species richness (diversity) treatments in a pasture ecosystem in Aotearoa New Zealand and quantified cascading biotic and abiotic effects of introduced dung beetles (*Onthophagus binodis* and *Onitis alexis*) on multiple ecosystem functions. We measured nine individual ecosystem functions affected by dung beetles, including dung removal, total soil carbon, nitrogen, phosphorus, soil microbial respiration, nitrate N, ammonium N, anaerobically mineralisable N and pasture biomass production.
3. We first tested the effects of temporal dung beetle diversity on the nine individual ecosystem functions. We then tested how introduced dung beetle temporal diversity affects ecosystem multifunctionality by calculating EMF metrics using the averaging, threshold and the Hill numbers approaches. Finally, to investigate the underlying interactions among individual functions, we tested the direct and indirect effects of introduced dung beetles on the nine ecosystem functions using structural equation modelling (SEM).
4. Only one out of the nine individual ecosystem functions was significantly affected by dung beetle introductions, whereas five of the six calculated EMF metrics increased significantly with temporal diversity of introduced dung beetles. However, when modelled within an SEM framework, dung beetle temporal

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diversity was found to directly affect dung removal, but with cascading effects on total N and anaerobically mineralisable N.

5. Our results suggest that introducing and increasing dung beetle temporal diversity enhances the provisioning of multiple ecosystem functions, but that our ability to detect and explain changes in ecosystem multifunctionality is critically dependent on how EMF is quantified.

KEYWORDS

introduced species, Mesocosm experiment, nature-based solutions, nitrogen cycling, plant productivity, Scarabaeinae, soil processes, temporal diversity

1 | INTRODUCTION

Sustainable management of anthropogenic landscapes, such as agricultural pastures, can be achieved through the implementation of nature-based solutions (Seddon, 2022). An example of such solutions is the introduction of species that are known to facilitate the simultaneous delivery of multiple ecosystem functions and the long-term delivery of ecosystem services (Grelet & Lang, 2021; Keesstra et al., 2018). Quantifying individual ecosystem functions and ecosystem multifunctionality (EMF)—defined as the simultaneous delivery of multiple ecosystem functions (Hector & Bagchi, 2007)—can therefore be a valuable tool for monitoring outcomes of nature-based solutions. Measuring single functions and EMF can also offer valuable evidence to inform decision-making related to management strategies designed to enhance and maintain ecosystem services within production landscapes. While there are well-established metrics to quantify EMF (Garland et al., 2021), there remains a lack of quantitative comparisons with other approaches that can capture the complexities of effects resulting from introduced species, such as dung beetles (Giling et al., 2019).

Dung beetles (Coleoptera: Scarabaeoidea) have been shown to have positive net effects on multiple pasture ecosystem functions through biotic and abiotic pathways, such as dung removal, bioturbation and nutrient cycling (Bertone et al., 2006; Beynon et al., 2012; Doube, 2018), among others (Piccini et al., 2018). Dung burial by dung beetles can lead to increased nutrient availability in soils, such as carbon (C), nitrogen (N) and phosphorus (P), which can lead to cascading direct or indirect effects on primary productivity (Bertone et al., 2006; Hea et al., 2005). They can also influence carbon and microbial activity pathways (Menéndez et al., 2016) and benefit microbial resource availability, such as nitrate N, ammonium N and anaerobically mineralisable N which has cascading effects on other ecological processes, such as plant growth (Yoshitake et al., 2014). While these different stages of cascading effects of dung beetles on multiple ecosystem functions have been identified (Nervo et al., 2017), they have not been modelled together in a single framework that explicitly describes the direct and indirect pathways through which dung beetles affect ecosystem multifunctionality (Figure 1).

Ecosystem multifunctionality can be quantified using EMF metrics (Byrnes, Gamfeldt, et al., 2014; Byrnes, Lefcheck, et al., 2014), which involves measuring multiple individual ecosystem functions and converting them into a standardised unit scale (Garland et al., 2021). For example, the averaging approach calculates an average score across each measured ecosystem function, whereas the single threshold approach calculates the number of functions delivered above a pre-defined threshold percentage (Byrnes, Gamfeldt, et al., 2014; Byrnes, Lefcheck, et al., 2014; Gamfeldt et al., 2008; Oliver et al., 2015). Metrics such as these provide a convenient, simplified overview of ecosystem multifunctionality. However, even simple systems are dynamic and complex, highlighting the potential limitations of EMF metrics, as they are reductionist in nature and can mask changes in specific functions (Hines, 2019). Thus, it can be argued that both the advantages and disadvantages of these different EMF metrics are the same: attempting to capture the complexity of ecosystems in a single value.

Previous research investigating dung beetle effects on ecosystem multifunctionality have reported the relationship between dung beetle species richness and environmental perturbations (such as anthelmintic exposure effects) on continuous threshold metrics (Manning, Beynon, & Lewis, 2017; Manning, Slade, et al., 2017), while other studies test dung beetle effects on individual functions and discuss EMF without quantifying associated EMF metrics (Piccini et al., 2018; Slade et al., 2017; Slade & Roslin, 2016). To our knowledge, however, there have been no studies that explicitly investigate the pathways through which multiple interrelated ecosystem functions respond to the introduction of dung beetles to pasture ecosystems. More generally, research is lacking on how different approaches to quantifying changes in EMF, such as analysing individual functions separately versus metrics of ecosystem multifunctionality, or within a causal inference framework like structural equation modelling, can determine the outcome of studies on ecosystem multifunctionality.

A framework proposed by Giling et al. (2019) emphasises the importance of developing a suite of evidence-based a priori hypotheses to gain deeper insights into the interdependencies that govern multifunctionality within ecosystems. This framework focuses on the quantification of possible direct and indirect cascading effects through the measurement of individual functions and the use of structural equation

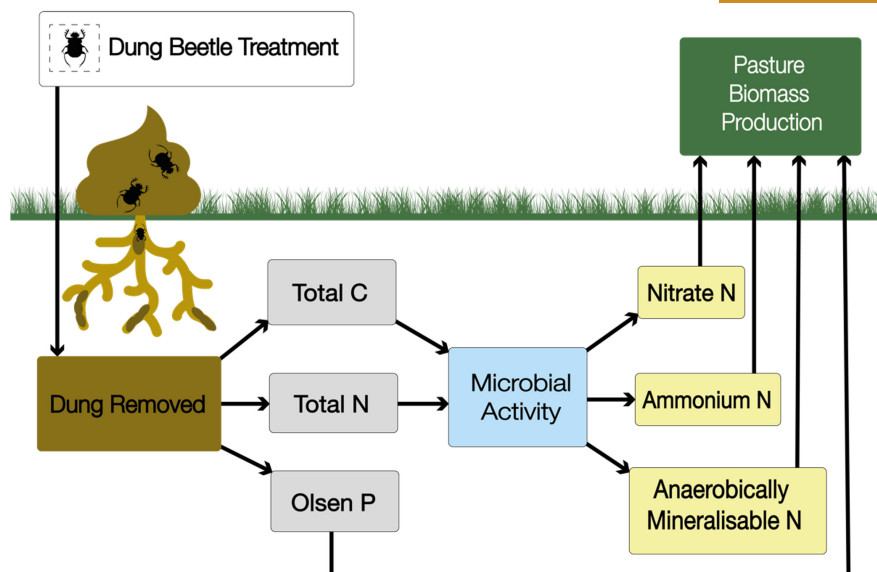


FIGURE 1 Hypothetical model showing how dung beetles can directly and indirectly affect ecosystem multifunctionality in pasture ecosystems. Arrows indicate hypothesised direct effects, all of which can contribute to indirect causal pathways via multiple variables. Dung beetle activity should directly increase dung removal through burial and bioturbation (brown box), leading to indirect increases in a suite of physiochemical soil properties including macro-nutrients such as total carbon (total C), total nitrogen (total N) and Olsen phosphorus (Olsen P) (grey boxes). In turn, we expect Olsen P to directly enhance pasture biomass production (green box), and total C and total N to increase soil microbial activity (blue box), subsequently leading to the formation of three further plant-available forms of inorganic nitrogen (yellow boxes), which then ultimately enhances primary productivity (green box).

modelling (SEM), a causal inference method that can disentangle the individual functions that contribute to overall ecosystem multifunctionality. This approach can therefore provide a mechanistic understanding of shifts in multiple ecosystem functions following environmental changes, such as the introduction of novel dung beetle species.

In this study, we use exotic dung beetle species in Aotearoa New Zealand as a model system to evaluate how the intentional introduction of multiple dung beetle species to agricultural pasture ecosystems influences ecosystem multifunctionality over two consecutive years. We compare single functions, EMF metrics and structural equation modelling to test the hypotheses that (1) the introduction of two dung beetle species over time, regardless of species identity, will improve single ecosystem functions, namely dung removal, associated physicochemical soil processes and pasture growth; (2) these enhanced individual functions will lead to overall increases in EMF (i.e. metrics of EMF); and (3) using structural equation modelling will provide a mechanistic understanding of EMF trends by describing the direct and indirect effects of dung beetles on multiple interconnected soil processes and pasture biomass production via dung removal (Figure 1).

2 | MATERIALS AND METHODS

2.1 | Study site

To test the effects of introduced dung beetles on ecosystem multifunctionality in pasture systems, we carried out a mesocosm experiment on a dry stock farm in Otorohanga, Aotearoa New Zealand.

Permission to conduct fieldwork on the property was granted by the landowners, Rob and Beth Atkinson. The mean annual temperature of the site was 13.2°C, with an average annual precipitation of 1495 mm per year (McCarthy et al., 2021). The soil at the site was classified as allophanic, which is known to have very high soil organic matter stocks (Barrat, 1981).

2.2 | Experimental design

To evaluate the effects of dung beetle species introductions on ecosystem multifunctionality in pasture ecosystems, we conducted a temporally replicated experiment that occurred in two consecutive years, with trials lasting 6 months each: February 2020–September 2020 (year 1) and March 2021–September 2021 (year 2). We added *Onthophagus binodis* to beetle treatments in Year 1 and *Onitis alexis* to beetle treatments in year 2 of the experiment (Figure S1). To test the pathways outlined in our a priori hypotheses (Figure 1) we established treatments including: (1) 'No_beetles' treatments ($n=10$), where only dung was added to the experimental plots in each of Year 1 and Year 2, serving as a control group with no dung beetles added; (2) 'One_beetle' treatments ($n=20$), where one species of dung beetle (and dung) was added at the start of Year 1, and only dung was added in Year 2, or vice versa, where the second species of dung beetle (and dung) was added at the start of Year 2, and only dung was added in Year 1. This allowed for testing the effect of a single species of dung beetle in either Year 1 or Year 2; and (3) 'Two_beetles' treatments ($n=10$) where one species of dung beetle (and dung)

was added at the start of Year 1, and a second species of dung beetle (and dung) was added at the start of Year 2 (separately). This treatment allowed for testing the interannual temporal diversity effects of two different dung beetle species added sequentially in Years 1 and 2 (Table 1).

In treatments containing beetles, 1 g total dry mass equivalent of dung beetles was added to each of the beetle-treatment mesocosms. The average dry weight of a single *O. binodis* beetle was measured at 27 mg and *O. alexis* was 95 mg. To facilitate dung burial success, we ensured that 30 individual *O. binodis* beetles and 10 individual *O. alexis* with an equal sex ratio of approximately 50:50 during Year 1 and Year 2, respectively. We established two controls to test for the effects of the mesocosm structure, including (1) 'mesocosm_control' treatments ($n=3$), which did not receive any dung or dung beetles set up within the mesocosms; and (2) 'open_control' treatments ($n=3$), which also did not receive any dung or dung beetles in an adjacent area of pasture that is equivalent in size to the mesocosms, but without the steel enclosures (see Supplementary material). All dung beetles were sourced from a commercial mass-rearing facility (Dung Beetle Innovations™) (www.dungbeetles.co.nz). Ethical approval was not required for the use of dung beetles in this experiment.

2.3 | Mesocosm design

Experimental mesocosms were installed at the study site on flat land 100 m from tall woody vegetation to avoid shade effects. We built 43 square mesocosms measuring 590 mm length $\times$ 590 mm width $\times$ 220 mm height (Figure S2) from 1.5 mm thick stainless steel to avoid rust and chemical reactions in the soil once placed in the ground. We partially buried each mesocosm in the soil at a depth of 100 mm, leaving 120 mm above the ground and with a 0.5 m gap between each replicate mesocosm or open control. An insect-grade mesh was secured over the mesocosms, preventing added dung beetles from escaping and unwanted insects (e.g. blowflies and other dung beetles) from entering. To prevent cattle from grazing the experimental area and immediate surrounds, we placed a fence around the mesocosm array measuring 60 m long $\times$ 4 m wide $\times$ 1.5 m high, creating a protective buffer zone (Figure S3).

2.4 | Dung source

Anthelmintics are used for internal parasite control of livestock and decreases the emergence of dung beetles from the dung, negatively affecting associated decomposition processes (Manning, Beynon,

& Lewis, 2017; Manning, Slade, et al., 2017; Manning et al., 2018). The use of antibiotics also causes a decrease in microbial communities in cattle dung, eventually affecting the symbiosis between dung beetles and their own gut microbiota, reducing the individual fitness of beetles (Hammer et al., 2016). To ensure that this did not influence our results, cattle at the study site had not received pharmaceutical treatment for over 3 years. Fresh cattle dung was collected and frozen at -20°C to kill any macroinvertebrates present in the dung before the start of each treatment input. Frozen dung was thawed at ambient temperature for 24 h and homogenised ensuring that the dung quality with treatments containing dung were comparable.

2.5 | Measuring ecosystem functions

Following the hypotheses outlined in the a priori model (Figure 1) we measured nine ecosystem functions related to dung beetle activity including (1) proportion of dung removed from pasture surface, (2) total soil C, (3) total soil N, (4) Olsen P, (5) microbial activity, (6) nitrate N, (7) ammonium N, (8) anaerobically mineralisable N and (9) mean pasture biomass production.

2.5.1 | Dung removal

Each mesocosm received 1 kg homogenised dung in each of Years 1 and 2, placed on a 25 cm² square of plastic trellis with mesh size 3 cm² on the pasture surface to ensure the easy removal of the remaining dung from the pasture surface. At the same time, three sub samples of homogenised dung were dried at 70°C for a minimum of 24 h and weighed to calculate the average dry biomass of the dung input into each treatment. All mesocosms were covered with insect-grade mesh for 28 days after the addition of dung beetles to the appropriate treatment mesocosms. After 28 days, the remaining dung was collected by lifting the trellis from the pasture surface and then fully drying samples in an oven at 70°C for a minimum of 24 h or until there was no further weight change due to moisture. The proportion of dung removed from the pasture surface was then calculated by dividing the total dry mass of dung remaining on the pasture surface by the total dry mass of dung input.

2.5.2 | Physiochemical properties

Soil was sampled to 1 cm depth using a 2.5 cm diameter stainless steel soil corer (three mixed subsamples per mesocosm

TABLE 1 Replication statement.

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Mesocosm	Mesocosm	10 $\times$ 'no_beetles' treatment
		20 $\times$ 'one_beetle' treatment
		10 $\times$ 'two_beetles' treatment

sub-quadrat), and samples were kept cool at $\sim 4^{\circ}\text{C}$ to ensure the reliability of tests conducted on field fresh fraction of samples. To characterise background levels of soil properties, we took pre-treatment soil samples immediately prior to the start of Years 1 and 2. Post-treatment soil cores were taken 6 months after treatment additions (Figure S4). In preparation for quantifying total C, total N and Olsen P, samples were air-dried in a forced air convection dryer at 35°C and crushed to pass through a 2 mm sieve. Tests for nitrate N, ammonium N and anaerobically mineralisable N were all conducted on field-fresh samples. Analyses were done at Hill Laboratories (Hamilton, NZ). Total C (%) and total N (%) were determined by a near infra-red spectroscopy (NIRS) calibration based on the Dumas combustion method (Hill Laboratories Technical Note [Nitrogen], 2020). The organic fraction of P that is plant-available (mg/L) was quantified using the Olsen P method, which is a 30-min bicarbonate extraction at pH 8.5 followed by Molybdenum Blue colorimetry (Hill Laboratories Technical Note [Phosphorus], 2020; Olsen & Sommers, 2015). Nitrate N was quantified from a field-fresh fraction of each sample using a 0.1 M potassium chloride (KCl) extraction followed by a Cadmium (Cd) reduction and NED (N-(1-Naphthyl) ethylenediamine) colorimetry and reported as a dry weight (mg/kg) (Hill Laboratories Technical Note [Nitrogen], 2020). Ammonium N was also quantified from a field-fresh fraction of each sample using a 0.1 M KCl extraction using Berthelot's reagent, a solution which turns blue in the presence of ammonia that is then analysed through colorimetry and reported as a dry weight (mg/kg) (Hill Laboratories Technical Note [Nitrogen], 2020). To quantify anaerobically mineralisable N (AMN), soil samples were incubated at 40°C in anaerobic conditions for 7 days followed by a 2 M KCL extraction and Berthelot colorimetry to determine any free ammonium N. The readily mineralisable N fraction is then added to this to produce a $\mu\text{g/g}$ value for each sample (Hill Laboratories Technical Note [Nitrogen], 2020). The analyses of pre-treatment baseline measurements of physicochemical variables showed that there were no significant differences in baseline values between treatments of all but one (nitrate N) of the measurements, before starting the long-term experiment (Table S1).

2.5.3 | Microbial activity

Soil samples were processed for microbial respiration at the University of Waikato soil lab. To prevent non-soil-related respiration, we removed any obvious vegetation from field-fresh soil samples during sieving soil through a 4 mm soil sieve. A 10 g subsample of homogenised soil was then placed in a 350 mL preserving jar and sealed. We also prepared three empty 350 mL jars as blanks. Samples were incubated for 7 days at 18°C , after which we extracted 1.0 mL gas from the headspace and evolved CO_2 analysed using a LI-7000 infrared gas analyser. Microbial respiration was expressed as $\mu\text{g CO}_2 \text{ g}^{-1} \text{ soil h}^{-1}$ (Robinson et al., 2017).

2.5.4 | Pasture biomass

We measured biomass production in each mesocosm by cutting pasture $>5 \text{ cm}$ above-ground every 28 days after treatment addition totalling six pasture samples. To obtain biomass (g), we dried samples in an oven at 60°C for 48 h. We then calculated average biomass production across 6 months per mesocosm for Years 1 and 2.

2.6 | Data preparation

To ensure the data were fully able to be interpreted in an SEM framework, we developed the temporal diversity treatment, which allowed us to condense the experimental design to the 0, 1 and 2 beetle temporal diversity. Temporal diversity, in this case, refers to the interannual variation in dung beetle species that were present in each mesocosm. To test for the effects of introducing one and two species of dung beetle on individual ecosystem functions and EMF metrics, we calculated aggregate values for all ecosystem functions. First, the total accumulated dung removed across both Years 1 and 2 was calculated as a measure of dung removal. Second, the change in physicochemical variables from baseline values was determined and averaged across Years 1 and 2. Third, the average microbial respiration across Years 1 and 2 was calculated as a measure of microbial activity. Finally, the accumulative pasture growth summed across both years was quantified as a measure of pasture growth.

2.6.1 | Calculating ecosystem multifunctionality metrics

Quantifying EMF metrics can be employed to characterise the simultaneous provisioning of multiple ecosystem functions and services (Byrnes, Gamfeldt, et al., 2014; Byrnes, Lefcheck, et al., 2014; Hector & Bagchi, 2007; Lefcheck et al., 2015). We calculated three different types of EMF metrics using the averaging approach, the single threshold approach and the Hill numbers approach. These metrics were calculated using all nine individual ecosystem functions measured. We used the 'multifunc' package in R (Byrnes, Gamfeldt, et al., 2014; Byrnes, Lefcheck, et al., 2014) to standardise the values of the nine ecosystem functions in each mesocosm and calculated the 'meanFunction', which produced the metric for the averaging approach. For the single threshold approach, we calculated the number of ecosystem functions that were provided versus the number of ecosystem functions that were not provided at four different thresholds of 20%, 40%, 60%, and 80% using the 'getFuncMaxed' function with 'proportion = FALSE' (Byrnes, Gamfeldt, et al., 2014; Byrnes, Lefcheck, et al., 2014). For the Hill numbers approach, we used the 'getMF_eff()' function with the Shannon case setting, with the order $q=1$ and the argument 'standardized = TRUE' to return a value for the product of the average standardised function and

the Hill numbers of functions for each mesocosm replicate (Byrnes et al., 2022).

2.7 | Statistical analysis

To determine whether treatments with no, one and two dung beetle species affected individual ecosystem functions, we first compared the means of each of the nine measured ecosystem functions using ANOVA models. We then tested the effects of treatments on EMF metrics based on (1) the averaging approach, (2) the single threshold approach and (3) the Hill numbers approach. For the averaging and the Hill numbers approach, we used ANOVA models and for the individual thresholds approach we used generalised linear models (GLM) to examine the binomial outcome of 'successes' versus 'fails' of ecosystem functions provided at thresholds of 20%, 40%, 60% and 80%. Post hoc pairwise comparisons of the addition of no beetles and one beetle species, no beetles and two beetle species, and one beetle species and two beetle species were conducted using the Tukey method in the 'emmeans' package (V. 1.8.3).

We used SEM to investigate the effects of dung beetle treatments on the direct and indirect pathways of multiple ecosystem functions using the 'piecewiseSEM' package in R (Lefcheck, 2016). We treated dung beetle treatments (no beetles, one beetle species and two beetle species) as a continuous predictor variable, using a coding of 0, 1 and 2, to facilitate the construction and presentation of the SEM. We logit-transformed dung removed (a continuous proportion), and log-transformed total C, total N, Olsen P, nitrate N and ammonium N to ensure that models met the assumptions of normally distributed residuals and homogeneity of variance. The remaining variables remained untransformed.

We created nine linear models representing each pathway in the hypothetical a priori model (Figure 1) with mesocosm code as a random effect to account for repeated sampling across year 1 and 2. A directed separation test (the d-sep test; Shipley, 2009) was then used to test for any missing paths in the model, based on Fisher's C-test statistic calculated as:

$$C = \sum_{i=1}^k \ln(p_i),$$

where p_i is the probability that a given response and predictor variable pair in the model are conditionally independent (Shipley, 2009). C is then compared to a Chi square (χ^2) distribution with $2K$ degrees of freedom (where K is the number of independence claims in the model). A global p -value <0.05 from the χ^2 distribution suggests that the data are significantly different from what would be expected under the specified causal model. A p -value >0.05 indicates that the hypothesised causal model adequately describes the data. We created two global models (SEMs) and compared them using the Akaike information criterion (AIC) using the formula:

$$AIC = C + 2K,$$

where C is the Fisher's C test statistic and K is the total number of parameters in the global model. The maximal model included all possible paths in the hypothetical model that still yielded $p > 0.05$ (Figure S5; Table S2; AIC = 1761.05, $p = 0.727$), which was compared to a simplified model where non-significant paths were removed from the structural equations until there was no further improvement in the global model's AIC score. The maximal SEM model was refined to produce a minimum adequate SEM model (AIC = 1752.65, $p = 0.711$) that better fit the data. All analyses were conducted in R (R Core Team, 2022).

3 | RESULTS

3.1 | Effects of dung beetles on individual ecosystem functions

We found significantly higher rates of dung removal with the addition and increasing temporal diversity of dung beetles ($F = 15.508_{2,37}$, $p < 0.001$; see Supplementary material) and post-hoc pairwise comparisons of dung removal against the dung-only treatment showed the greatest increase in dung removal in treatments that received two species over the course of the experiment (estimate = -0.117 , $p < 0.01$) compared with treatments containing only one species in either year one or year two (estimate = -0.302 , $p < 0.05$; Figure 2a). However, we did not detect any significant effects of temporal dung beetle diversity on all remaining ecosystem functions when analysed individually (Figure 2b–i; Table 2).

3.2 | Dung beetles improve ecosystem multifunctionality

When testing the effects of dung beetle temporal diversity on ecosystem multifunctionality from six different metrics, we found that the presence of dung beetles generally enhanced EMF (Figure 3). Specifically, we found a general increasing EMF value with increasing dung beetle temporal diversity on all but one of the quantified EMF metrics (Table 3). The only exception was the metric calculated using the threshold approach, where the threshold was set to 60% (for minimum rates of ecosystem functions to be considered as present in the ecosystem).

3.3 | Ecosystem multifunctionality through direct and indirect pathways

Our structural equation model revealed that, while dung beetle temporal diversity directly enhanced only dung removal, this resulted in cascading effects on other related ecosystem functions (Figure 4). In particular, the model revealed that dung beetles had a significant effect on ecosystem multifunctionality through three direct and indirect positive effects, with one isolated

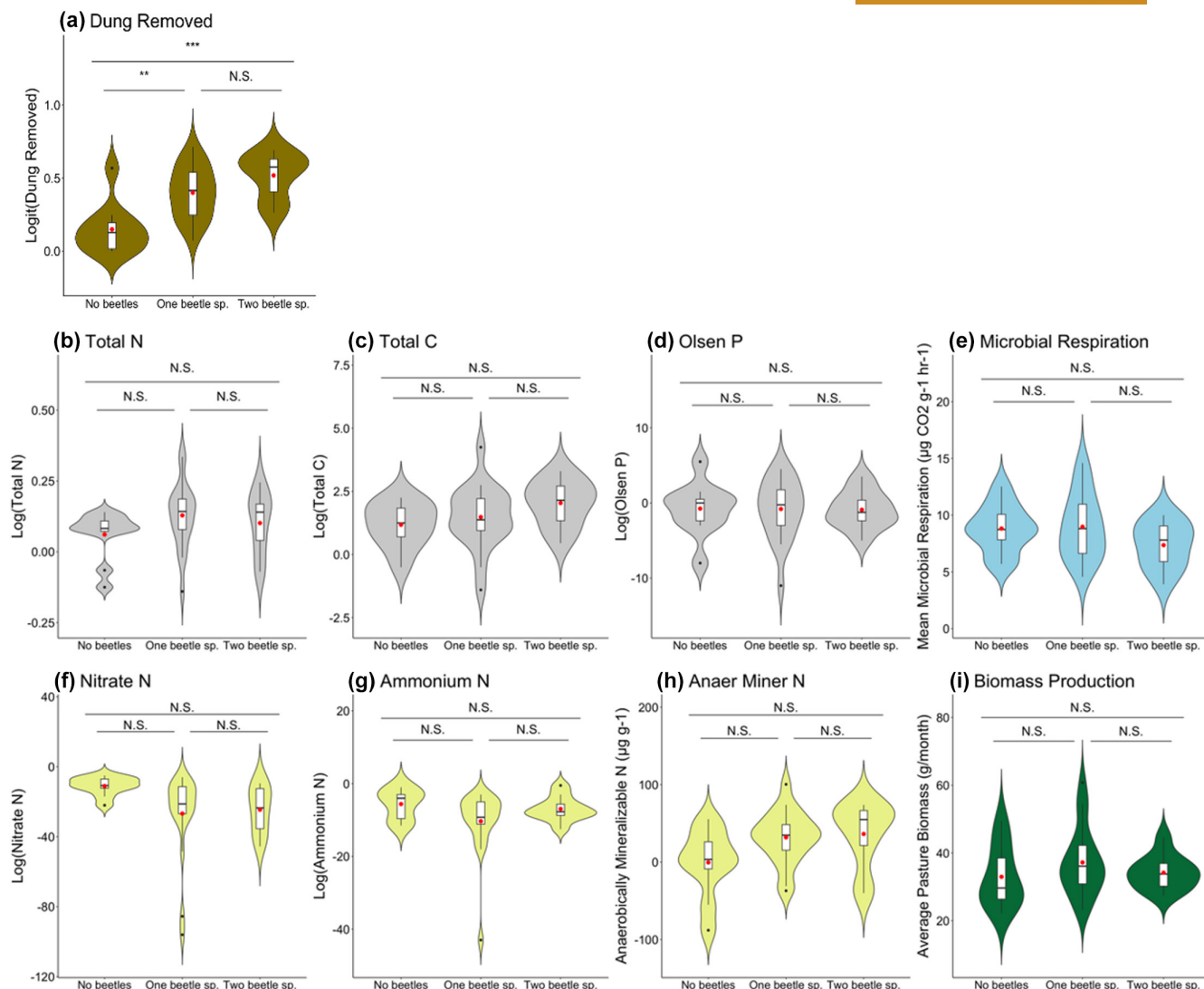


FIGURE 2 The influence of introduced dung beetles (with one or two species added over time) on (a) accumulative proportion of dung removed, (b) total N, (c) total C, (d) Olsen P, (e) microbial respiration, (f) nitrate N, (g) ammonium N, (h) anaerobically mineralisable N, and (i) pasture biomass production. Box plots show the median and quartiles with whiskers, and outliers are shown by black points. The shaded area around the box plots shows the density data (estimated by the kernel probability density of the data). The red point shows the average of each ecosystem function. Tukey pairwise comparisons indicate significant treatment differences in ecosystem functions delivered. $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*) and non-significant (N.S.).

relationship between microbial activity on ammonium N that was not significantly associated with the dung beetle treatments (Figure 4; Table 4). For example, there was a direct positive effect of dung beetles on dung removal ($p < 0.001$), and dung removal subsequently had a significant flow-on effect on total N ($p < 0.05$) which enhanced anaerobically mineralisable N ($p < 0.001$). While there was a direct positive effect of microbial activity on ammonium N ($p < 0.001$), this was not directly detected through a significant flow-on effect of dung beetle treatment (Figure 4; Table 4). Contrary to expectations, there was no indirect effects of dung beetle temporal diversity on pasture biomass production (Figure 4; Table 4).

4 | DISCUSSION

Our results suggest that dung beetle additions of varying temporal diversity significantly increase ecosystem multifunctionality (EMF). However, this was not detectable by analysing multiple functions individually, while EMF metrics overstated the influence of dung beetles on multiple functions, which was only revealed by structural equation modelling. Species temporal diversity (whether there was one or two species added over the course of the experiment) played a significant role in determining the provisioning of multiple functions. In this experiment, we did not have any treatments where multiple dung beetle species were present simultaneously in each mesocosm.

Rather, we observed that dung removal was greatest when two species were sequentially added over the 1.5 year experimental duration, compared to when only one species was added. In doing so, our results demonstrate the importance of dung beetle interannual temporal diversity for maintaining ecosystem multifunctionality, while simultaneously highlighting the need to explicitly model interactions among measured ecosystem functions to understand real trends in ecosystem multifunctionality.

The results from the analysis of individual functions across treatments only detected an effect of dung beetles on the one function that is known to be directly influenced by their activity: that is dung removal, which was found to be highest in the two-species treatment. This suggests that analysing individual ecosystem functions in isolation does not accurately detect significant changes in multiple ecosystem functions in response to dung beetle species introductions (Figure 2). This is likely because dung beetle addition

TABLE 2 ANOVA outputs from the linear models testing for differences in each of the nine individual ecosystem functions among the three dung beetle treatments (no beetles, one beetle species and two beetle species introduced consecutively over the 2-year experiment).

Ecosystem function	nDf	dDf	F	p-value
Dung removed	2	37	15.508	<0.001
Total N	2	37	2.412	0.104
Total C	2	37	0.667	0.519
Olsen P	2	37	0.118	0.889
Microbial respiration	2	37	1.470	0.243
Nitrate N	2	37	1.818	0.177
Ammonium N	2	37	1.123	0.336
Anaerobically mineralisable N	2	37	2.968	0.064
Pasture biomass production	2	37	0.900	0.415

Note: Bold values denote statistically significant effects.

and temporal diversity, alone, cannot explain sufficient variation seen in the ecosystem functions that are not directly altered by dung beetle activity (unlike dung burial). In a sub-analysis of dung beetle presence versus absence, we did find that the presence of dung beetles significantly enhanced three ecosystem functions out of nine, including dung removal ($p < 0.001$), total N ($p = 0.036$) and anaerobically mineralisable N ($p = 0.019$) (Figure S6; Table S3), though effects on total N and anaerobically mineralisable N were only barely detectable. This further indicates that, while dung beetles play a significant role in enhancing certain ecosystem functions, their impacts could vary with changing diversity or species identity, so it is important to consider temporal turnover in ecosystem function studies. Furthermore, modelling ecosystem functions within a framework that accounts for interrelatedness among environmental processes can increase the accuracy of the estimates of treatment effects on individual functions, as demonstrated in our SEM. For example, Grace et al. (2016) used SEM to uncover the mechanisms linking productivity and plant species richness, which revealed substantially higher explanatory power than traditional bivariate analyses. Ecological studies assessing dung beetle effects on ecosystem functioning often use dung removal as a proxy indicator for other ecosystem processes (Carvalho et al., 2020). However, our results

TABLE 3 ANOVA tables from the models testing for effects of the dung beetle treatments on ecosystem multifunctionality (EMF) metrics.

EMF metrics	Estimate	Standard error	p-value
Averaging	0.553	0.026	<0.001
Hill numbers	0.496	0.031	<0.001
20% threshold	2.197	0.351	<0.001
40% threshold	1.318	0.258	<0.001
60% threshold	-0.406	0.215	0.059
80% threshold	-1.386	0.264	<0.001

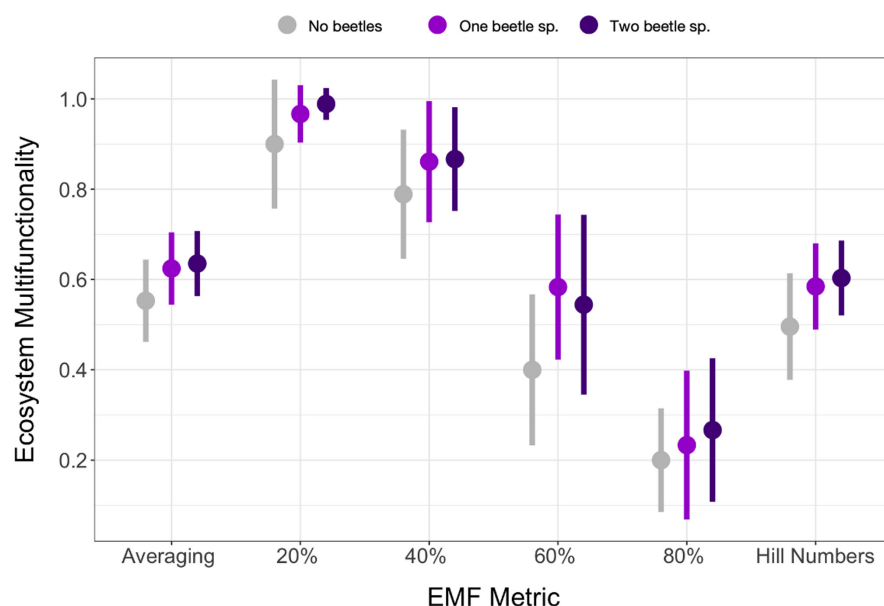


FIGURE 3 The mean (± 1 SD) values of ecosystem multifunctionality for treatments with no beetles, one beetle species (either in year one or year two) and two beetle species (across year one and two), based on six ecosystem multifunctionality (EMF) metrics from the averaging approach, threshold approach (set to: 20%, 40%, 60%, 80% thresholds) and Hill numbers approach.

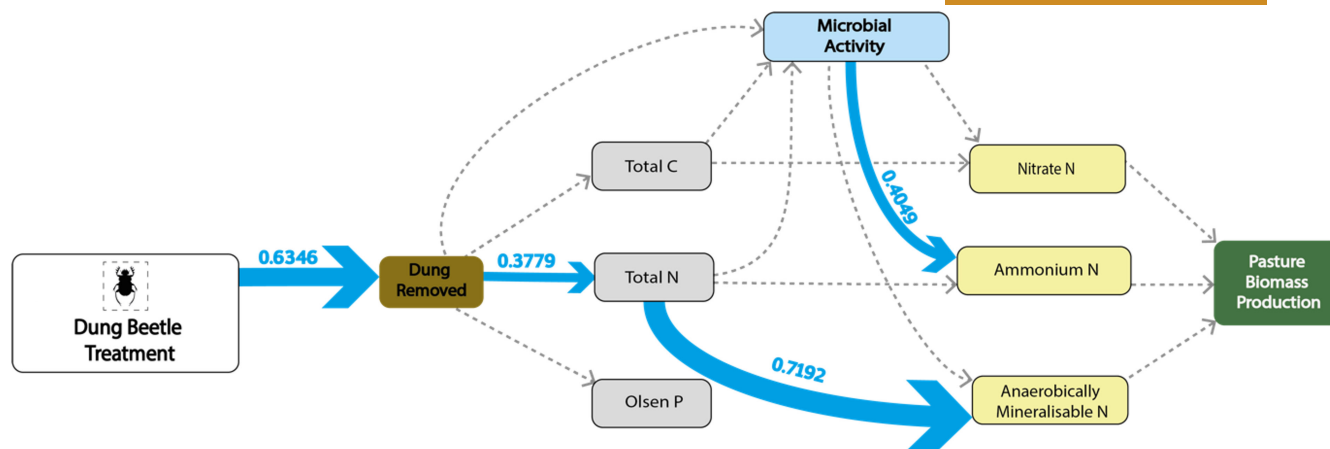


FIGURE 4 Final structural equation modelling (minimum adequate model; Fisher's $C = 30.872$, $p = 0.711$, $df = 36$) showing significant positive effects with solid blue arrows, significant negative effects with solid red arrows and dashed arrows indicating non-significant effects. The width of the arrows are proportional to their standardised effect sizes (only shown for significant effects; see Table 4; for details).

TABLE 4 Results from the minimum adequate structural equation modelling.

Response	Predictor	Estimate	Standard error	<i>p</i> -value	Standardised estimate	Marginal R^2
Dung removed	Dung beetle treatment	1.062	0.210	0.000	0.635	0.40
Total N	Dung removed	0.029	0.011	0.016	0.378	0.14
Total C	Dung removed	0.259	0.163	0.121	0.249	0.06
Olsen P	Dung removed	0.132	0.325	0.686	0.066	0.00
Microbial respiration	Total N	-0.519	5.411	0.924	-0.019	0.02
	Total C	0.059	0.379	0.877	0.029	
	Dung removed	-0.277	0.381	0.472	-1.302	
Nitrate N	Microbial respiration	-0.088	0.676	0.898	-0.021	0.08
	Total N	-32.832	18.865	0.090	-0.276	
Ammonium N	Microbial respiration	0.549	0.204	0.011	0.405	0.16
	Total N	0.279	5.691	0.961	0.007	
Anaerobically mineralisable N	Microbial respiration	2.404	1.787	0.187	0.152	0.53
	Total N	317.083	49.848	<0.001	0.719	
Pasture biomass	Nitrate N	-0.161	0.140	0.259	-0.199	0.09
	Ammonium N	0.473	0.418	0.266	0.186	
	Anaerobically mineralisable N	-0.033	0.035	0.362	-0.149	
	Olsen P	0.710	0.621	0.261	0.194	

Note: The marginal R^2 values indicate the total proportion of variance explained by all significant predictors and their conditional variables in the mixed-effects models. Significant *p*-values are indicated in bold.

demonstrate that dung removal can often be a poor proxy of a broader suite of ecosystem functions and there is a need to explicitly model interactions among functions to capture the underlying mechanisms leading to ecosystem multifunctionality.

Our results showed that dung beetle temporal diversity enhanced ecosystem multifunctionality metrics, as evidenced by the significant increase in ecosystem multifunctionality across all metrics in treatments where dung beetles were present in both years

compared to no beetles present (Figure 3). This raises the question of whether calculating EMF metrics can overinflate the importance of introduced dung beetle activity for the provisioning of ecosystem multifunctionality. We found that even when only a subset of functions, (dung removal, total N and anaerobically mineralisable N), were significantly influenced by the dung beetle treatments (as seen in the SEM), five out of the six calculated EMF metrics were significantly enhanced. Furthermore, certain functions that were

more responsive to the treatments (such as dung removal) led to a disproportionate influence on the overall EMF metric responses despite other functions, such as pasture biomass production, being unaffected by the treatments (Figure 4). Our findings are in line with previous studies, which suggest that the interconnected nature of the ecosystem functions, such as those measured in our study, can strongly influence variation in quantified EMF metrics (Garland et al., 2021). Therefore, our results suggest that, regardless of the metric used, reporting EMF metrics alone may not be suitable for understanding dung beetle effects on ecosystem multifunctionality. Furthermore, it is important to interpret results from EMF metrics with great caution. This is because the contributions of individual functions can strongly influence the overall outcome of the EMF metric (Gamfeldt & Roger, 2017), whether using averaging, threshold or the more recently developed Hill numbers approaches.

Dung beetles are known to influence a range of ecosystem functions (Nichols et al., 2008; Penttilä et al., 2013; Piccini et al., 2018), but whether they directly or indirectly drive changes in ecosystem functions (such as enhancing nutrient cycling and plant growth) has remained largely unexplored. The results of our SEM revealed the interconnections among ecosystem functions in response to dung beetle introductions, demonstrating that dung beetles have both direct and cascading effects on multiple ecosystem functions that concurrently determine patterns in ecosystem multifunctionality. For example, dung beetles had a direct positive effect on dung removal and (in contrast to results from our bivariate analyses) there was a significant flow-on effect on total nitrogen, which in turn enhanced anaerobically mineralisable nitrogen. These results indicate there could ultimately be a positive effect of dung beetle activity on pasture growth as a result of increasing plant-available N. However, we were unable to detect this in our experiment, where we did not find any significant change in plant biomass production in response to the dung beetle temporal diversity treatment. We also found a direct positive effect of microbial activity on ammonium nitrogen, although this was not mediated through any cascading effects of the dung beetle treatments. Previous research has found that certain beetle-mediated soil processes, such as ammonification, may occur within a few weeks of dung beetles accessing dung (Kazuhira et al., 1991). Therefore, it is possible that we did not capture all of the possible changes in measured functions (e.g. total C and P, nitrate and plant production) because the intervals between treatment additions and the final soil measurements (6 months) may have been too long. Despite potentially missing some of these intermediary relationships that could have allowed us to detect cascading dung beetle effects on pasture biomass production, our results nevertheless shed new light on how dung beetle activity can influence ecosystem multifunctionality.

Biotic factors, such as species traits could also play an important role beyond what we have shown here. Traits are features, including morphological, physiological and behavioural attributes, which can collectively influence dung beetles' roles in their environment (deCastro-Arrazola et al., 2022). Here, our first treatment addition

included *Onthophagus binodis*, which has a smaller body size than *Onitis alexis*. Though our study does not focus on species identity effects, a sub-analysis revealed that *O. alexis* removed a significantly greater amount of dung compared to *O. binodis* (Figure S7; Table S4), though there were no differences found in the other eight functions measured. This is likely a function of its body size, as larger dung beetles have been shown to remove more dung (Gebert et al., 2022; Nervo et al., 2014; Palestini et al., 2001; Piccini et al., 2020). To explore the biotic mechanisms by which dung beetles affect ecosystem multifunctionality, future studies could include dung beetle traits within a SEM framework to further shed light on the cascading effects of dung beetles on multiple ecosystem functions.

5 | CONCLUSIONS

We hypothesised that dung beetles would positively influence the simultaneous delivery of multiple ecosystem functions over time through the cascading effects of physically removing dung from the pasture surface. By comparing individual functions with established EMF metrics and structural equation modelling, our study highlights how multiple approaches lead to different inferences and the importance of using appropriate methods to understand the effects of dung beetle introductions on ecosystem multifunctionality. Our findings indicate that both the bivariate tests of single functions and EMF metrics fail to capture the full picture. They appear to either underestimate (Figure 2) or overestimate (Figure 3) effects of dung beetles on ecosystem multifunctionality, as is revealed by the SEM (Figure 4). Although EMF metrics can serve as a quick tool to identify potential drivers of change in EMF, causal inference methods like SEM provide a more powerful approach for the assessment of ecosystem multifunctionality. Therefore, by detecting effects that simple tests of single functions cannot, there can be a more comprehensive and integrated understanding of the interplay among multiple functions in response to environmental change. This is important for the implementation of nature-based management, as our results highlight the risks of under- or overestimating ecological outcomes of dung beetle introductions for ecosystem multifunctionality, depending the analytical approach that is implemented.

AUTHOR CONTRIBUTIONS

FH, SVF and AB designed the study. FH, SVF, KJW and AB carried out the fieldwork. FH conducted the laboratory work and prepared and analysed the data. All authors interpreted the results. FH drafted the manuscript and all authors contributed to the writing.

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CONFLICT OF INTEREST STATEMENT

The authors have declared that there are no competing interests.

DATA AVAILABILITY STATEMENT

Data are available via Zenodo: <https://doi.org/10.5281/zenodo.10308565> (Hasan et al., 2023).

ORCID

Fevziye Hasan  <https://orcid.org/0000-0001-9670-9775>

Kiri J. Wallace  <https://orcid.org/0000-0003-1236-6846>

Simon V. Fowler  <https://orcid.org/0000-0001-8356-0836>

Louis A. Schipper  <https://orcid.org/0000-0001-9899-1276>

Zac Hemmings  <https://orcid.org/0000-0003-1116-736X>

Jacob D. Berson  <https://orcid.org/0000-0002-7059-2131>

Andrew D. Barnes  <https://orcid.org/0000-0002-6499-381X>

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1: The two species of introduced dung beetle used in the mesocosm experiment (a) *Onthophagus binodis* was added to treatments during year 1 and (b) *Onitis alexis* was added to treatments during year 2.

Figure S2: Diagram of dimensions of a stainless steel mesocosm.

Figure S3: Experimental mesocosms and an open control treatment (red stakes) installed along a 100 m array, with a fenced buffer zone to prevent grazing.

Figure S4: Sampling locations within the mesocosm quadrat during year 1 (left) and year 2 (right).

Figure S5: Initial maximal SEM (Fisher's $C = 29.122$, $p = 0.511$, $df = 30$) showing significant positive effects with solid blue arrows and dashed arrows indicating non-significant effects.

Figure S6: The influence of presence versus absence of dung beetles on (a) accumulative proportion of dung removed; (b) total N; (c) total C; (d) Olsen P; (e) microbial respiration; (f) nitrate N; (g) ammonium N; (h) anaerobically mineralisable N; and (i) pasture biomass production.

Figure S7: The influence of *Onthophagus binodis* versus *Onitis alexis* dung beetles on (a) accumulative proportion of dung removed; (b) total N; (c) total C; (d) Olsen P; (e) microbial respiration; (f) nitrate N; (g) ammonium N; (h) anaerobically mineralisable N; and (i) pasture biomass production.

Table S1: Outputs (ANOVA tables) from models testing for differences in baseline soil parameters between mesocosm treatments (before treatments were applied).

Table S2: Output from the initial maximal model.

Table S3: ANOVA table outputs from the linear models testing for differences in each of the nine individual ecosystem functions in the presence/absence of dung beetle treatments.

Table S4: ANOVA table outputs from the linear models testing for differences in each of the nine individual ecosystem functions between *Onthophagus binodis* versus *Onitis alexis*.

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