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Composition of dung beetle communities in a tropical montane forest alters the rate of dung removal more than species diversity alone

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COMPOSITION OF DUNG BEETLE COMMUNITIES IN A TROPICAL MONTANE FOREST ALTERS THE RATE OF DUNG REMOVAL MORE THAN SPECIES DIVERSITY ALONE

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requirements for the degree of
Master of Science

by

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Elizabeth A. Engle ENTITLED Composition of dung beetle communities in a tropical montane forest alters the rate of dung removal more than species diversity alone BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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ABSTRACT

Engle, Elizabeth A. M.S., Department of Biological Sciences, Wright State University, 2020. Composition of dung beetle communities in a tropical montane forest alters the rate of dung removal more than species diversity alone.

Dung beetles provide key ecological functions by degrading and recycling dung. I used experimentally-assembled communities to examine the role of species richness, community biomass, species diversity, species identity, and community composition in dung removal, using *Ateuchus chrysopyge*, *Copris nubilosis*, *Onothophagus cyanellus,* and *Dichotomius satanas*. I hypothesized: (1) that as species richness, biomass, and diversity increases within a community, dung removal increases; and (2) species are not functionally equivalent, so community composition should influence dung removal rates.

As species richness, biomass, and diversity of experimentally-assembled communities increased, the proportion of dung removed also increased. Also, the four species in this study were not functionally equivalent at dung removal. *Dichotomius satanas* removed the most dung, even when beetle biomass was standardized. Assemblages of *A. chrysopyge, D. satanas,* and *C. nubilosis,* and of *O. cyanellus*, *D. satanas* and *C. nubilosis* removed the most dung. Additionally, communities containing at least one *D. satanas* beetle removed significantly more dung than communities without any *D. satanas* beetles.

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INTRODUCTION

Biodiversity has been described as the variety of life at all levels from genes to ecosystems, often partitioned into three broad classifications: phylogenetic diversity, species diversity, and ecological diversity (Nunes et. al 2016). These classifications, as well as biodiversity as a whole, have commonly been linked with ecosystem functioning (different life activities of animals, plants, and microbes, and the effects of these activities on the physical and chemical conditions of the environment; El Serafy & Leitão, 2020). Conservation management has long focused on utilizing methods to maintain biodiversity, but recently more consideration has been given to the relationship between species diversity and ecosystem function (Loreau et al. 2001; Yoshihara and Sato 2015). There has been growing concern recently about the conservation status of dung beetles on a global scale, mainly due to decreases in both habitat and food availability (Nichols et al. 2007, 2008; Beynon et al. 2012; Braga et al. 2013; Tixier, Bloor, and Lumaret 2015; Yoshihara and Sato 2015).

Dung beetles belong to one of the largest families of beetles with roughly 30,000 documented species worldwide, and these have been shown to provide many ecological functions (Cambefort and Hanski1991; Bang et al. 2005). One key ecological function exhibited by dung beetles is degrading and recycling dung within ecosystems (Yamada et al. 2007). Adult dung beetles have been known to use dung either as a food source, or it is manipulated into larval provisions (Yoshihara and Sato 2015). The effects of losing this key ecological function was demonstrated by the 1788 cattle introduction to Australia (Doube 2018). The appropriate dung beetles were not present in the community, allowing dung to remain on the soil surface and causing the soil to become flooded with nitrogen, leading to both poor plant growth and poor plant productivity, ultimately giving rise to a deteriorated ecosystem (Doube 2018).

Dung beetles have been grouped into guilds, based on their dung removal strategies (Camberfort and Hanski 1991; Yamada et al. 2007). Described guilds include telecoprids (rollers— beetles known to roll balls of dung away from the dung resource and nest elsewhere), paracoprids (tunnelers— beetles known to dig tunnels directly below the dung resource and nest there), and endocoprids (dwellers— beetles known to nest within the dung resource itself; Floate 2011). A small percentage of dung beetles have been described as cleptocoprids (kleptoparasites known to steal dung resources from other beetles; Martín-Piera & Lobo 1993).

Paracoprid species have been shown to alter physiochemical characteristics of soil by incorporating organic matter (Bang et al. 2005) and facilitating nutrient mineralization (Yamada et al. 2007; Yoshihara and Sato 2015). Additionally, dung burial has been known to reduce the abundance of both dung-breeding flies and dung-dispersed protozoa, possibly providing disease prevention to both humans and wildlife (Byford et al. 1992; Nichols et al. 2008). Several laboratory studies have shown that dung burial may also enhance plant growth, but more field work is needed with both multi-species dung beetle communities and multi-species plant assemblages to determine these relationships (Nichols et al. 2008; Yoshihara and Sato 2015). For these reasons, paracoprid species most likely have the strongest effects on terrestrial ecosystem function.

Researchers have also asked how alterations in dung beetle species diversity specifically influences rates of dung burial (O'Hea et al. 2010). Previous studies have examined the role of species diversity in multiple functional processes, including increasing dung removal, increasing soil carbon and nitrogen content, accelerating soil bioturbation (reworking of soil by the beetles), and increasing plant productivity (Nichols et al. 2008). Much of the published species diversity research has focused on the relationship between function and species richness (number of species; O'Hea et al. 2010). However, relative abundance of individuals (number of individuals per species) could be as or more important than species richness for several reasons (O'Hea et al. 2010). First, in communities with an equivalent species richness, the abundance of individuals could range from all individuals of all species occurring equally, to one species being numerically dominant, with potentially important combinations in between (O'Hea et al. 2010). Second, environmental threats such as habitat loss or food availability often have had larger and faster impacts on less abundant species (Chapin et al. 2000; Dangles and Malmqvist 2004; O'Hea et al. 2010). These impacts could shift relative abundance within a community, possibly without changing species richness at all (Chapin et al. 2000; Dangles and Malmqvist 2004; O'Hea et al. 2010). Lastly, no naturally-occurring communities have been shown to be perfectly even, and a small number of species usually comprise most of the individuals in a community, possibly causing one or a few particular species to have disproportionate effects on ecological functions, due to their increased abundance (Schwartz et al. 2000; O'Hea et al. 2010). However, the opposite has been shown to be true as well in some cases, mainly when a keystone species is present in the ecosystem (a species with a small relative abundance but with a disproportionately large effect on its environment).

Significant species-level variation has been shown to exist within a single genera of dung beetle (O'Hea et al. 2010; Bang et al. 2005; Beynon et al. 2012; Tixier, Bloor, and Lumaret 2015), suggesting that biological attributes, such as reproductive strategies or nesting behaviors could account for differences in dung removal rates. For example, examining the life history and nesting biology of *Onthophagus lecontei* and comparing it to other species of the *Onthophagus* genus showed that the weights of brood masses (constructed from dung), the number of eggs in each brood mass, and length of life cycle stages differed considerably (Arellano et al. 2017).

Similarly, in monoculture communities of *Aphodius rufipes* and *Aphodius ater*, it was found that *A. rufipes* has significantly different dung removal abilities than *A. ater* (O'Hea et al. 2010). Species-level variation such as this has revealed the need for more biological research on dung beetles at the species level.

There have been few attempts to experimentally manipulate both species richness and diversity within diversity studies (Yoshihara and Sato 2015). Most dung beetle studies examining diversity have either (1) focused on spatial or temporal patterns of diversity for a particular ecosystem (Salomão et al. 2020), or (2) examined how variables such as land use (Giménez Gómez et al. 2018) or climate events like El Niño (França et al. 2019) influenced diversity within an ecosystem. However, experimental manipulations of richness and diversity allow for more detail on the influences of community composition on dung removal compared to observational approaches. Because there has been considerable species-level variation in dung removal rates (Bang et al. 2005; Beynon et al. 2012; Tixier, Bloor, and Lumaret 2015), this study examined the role of species richness, community biomass, species identity, and community composition in dung removal in Cusuco National Park in northwest Honduras. I asked the following questions:

1. How does species richness influence the rate of dung removal?
2. How does biomass of the beetle community influence the rate of dung removal?
3. How does species diversity influence the rate of dung removal?
4. Are all four dung beetle species functionally equivalent in terms of dung removal?
5. Does community composition influence the rate of dung removal?

To address these questions, four of the most abundant species of paracoprid dung beetles within the park were used: *Ateuchus chrysopyge*, *Copris nubilosis*, *Onothophagus cyanellus,* and

Dichotomius satanas. Experimental communities of dung beetles were constructed varying in both dung beetle species richness (1 – 4 species) and biomass (0.6 g – 24.2 g) to determine how species diversity (proxied in three ways: as species richness exclusively, as biomass exclusively, and as Simpson's diversity index), as well as species identity and community composition, influenced the rate of dung removal.

MATERIALS AND METHODS

Study Site

Cusuco National Park (PNC) is a 23,400 ha nationally protected montane tropical forest located in the Merendón mountains of northwest Honduras, with an elevation gradient from just above sea level to 2,425 m (Field and Long 2007). The park is separated into two zones, a relatively undisturbed core zone (~7,700 ha) and a deteriorating buffer zone vulnerable to coffee production and logging (~15,700 ha; Field and Long 2007; Slater et al. 2011). The park contains 40 species of dung beetle (Creedy 2018), as well as four distinct habitats (semi-arid pine forest, moist pine forest, moist broadleaf forest and dwarf forest; Field and Long 2007). It is identified by the IUCN as a Key Biodiversity Area (Slater et al. 2011).

Operation Wallacea ('Opwall') has been monitoring biodiversity in PNC since 2006 and conducting research on the distribution of species, effects of habitat degradation, and anthropogenic disturbances throughout the park since this time (Slater et al. 2011). Opwall conducts research within a two-month period, from June to August, at seven research camps within Cusuco (Field and Long 2007; Slater et al. 2011). Five occur in the core zone, and two occur in the buffer zone (**Fig. 1**; Field and Long 2007; Slater et al. 2011). Each camp has 3-4 sample routes, and each route has established sampling sites for data collection (Creedy 2018). This study examined the role of species richness, biomass, species diversity, species identity, and community composition in dung removal. Research was conducted solely at Base Camp (located in the core zone). Field experiments were conducted in a plot on the forest edge, and beetles were collected at sampling sites on transects at Base Camp.

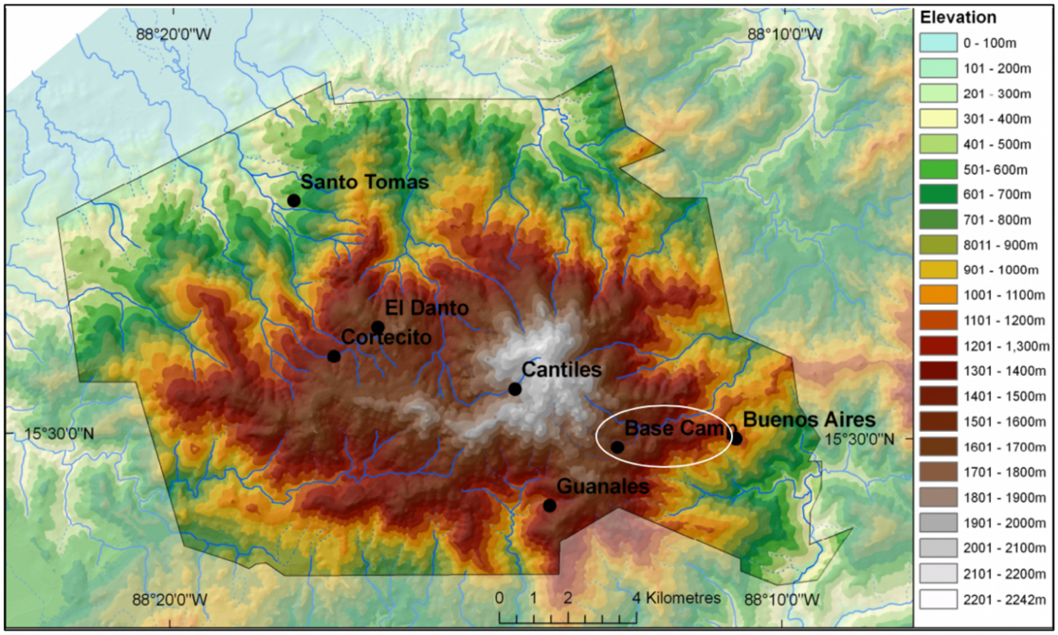

Fig. 1. Cusuco National Park (PNC). Map of elevation and locations of the seven research camps. Base Camp circled in white (Slater et al. 2011).

Experimental Setup:

Dung Beetle Collection

To collect live dung beetles, dry pitfall traps were baited with horse dung as this is the standard procedure of Opwall for pitfall trap setup (Slater et al. 2011). Pitfall traps consisted of two 16 oz plastic cups, each with rim diameters of 9.5 cm. Cups were buried just below the soil surface, one inside the other, for easy collection. Leaf litter was added to the bottom of the traps to provide shelter and moisture for the beetles. Traps were baited with dung that was hung over the trap. Dung was rolled into golf-ball-sized amounts (~ 5 cm in diameter), placed in a cheesecloth, and tied it to a stick (~ 20 cm long). The stick was then placed in the ground, allowing the dung to hover above the pitfall trap, inaccessible to the beetles (**Fig. 2A**). A disposable plastic plate (~ 26 cm in diameter), was set diagonally against the dung stick, to shield the trap from excess rain (**Fig. 2B**). Pitfall trapping was conducted each night, on one of four transects at Base Camp. Trapping began 15 June 2019 and continued throughout the eight-week field season. The traps were collected each morning and reset concurrently or moved to a different transect, for collection. Beetles from all traps were pooled into a small, 24 oz, plastic container with a lid. Back at Base Camp, each beetle was identified based on easily identifiable morphological characteristics and separated into species-specific terrariums.

Fig. 2. Pitfall Trap Setup. The pitfall trap and dung bait setup can be seen on the left (A) and the rain shield cover can be seen on the right (B).

Dung Beetle Focal Species

Four dung beetle species were collected, ranging in size from the small-bodied, *Ateuchus chrysopyge* (7.0 - 8.5mm) and *Onthophagus cyanellus* (7.5 - 10.5mm), to medium-bodied, *Copris nubilosus* (13.8 - 16.9mm), to large-bodied, *Dichotomius satanas* (17.0 - 23.0mm) (**Fig. 3**; Creedy and Mann 2011).

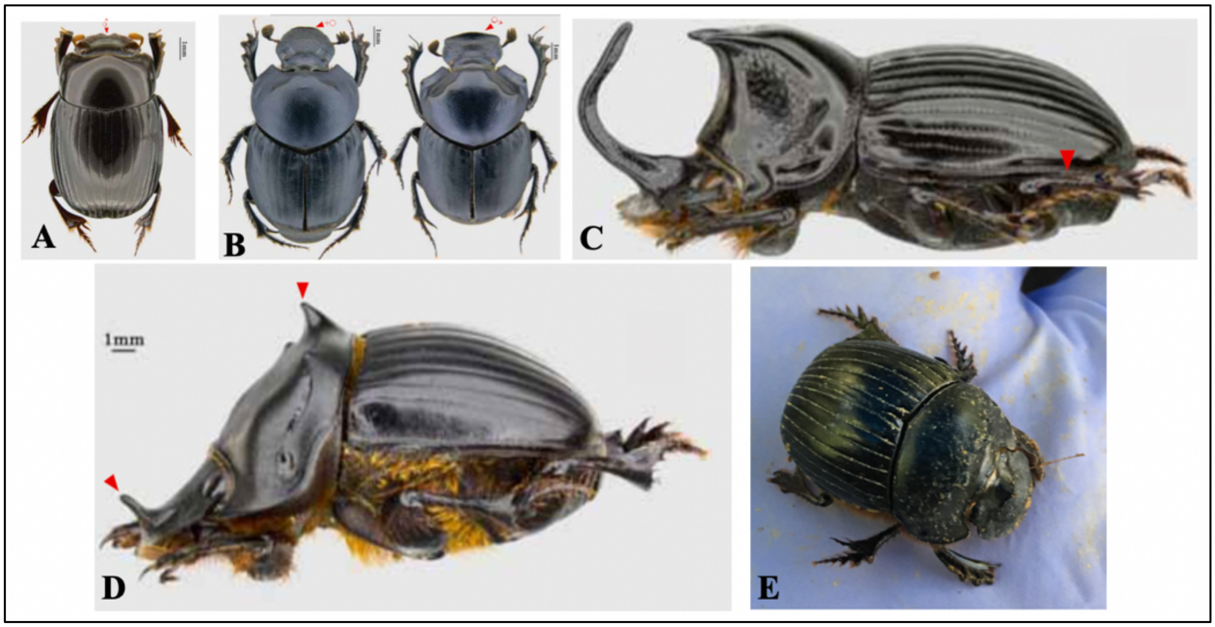

Fig. 3. Dung Beetle Focal Species. (A) *Ateuchus chrysopyge* (distinguishing characteristics: small size, black), (B) *Onthophagus cyanellus* (distinguishing characteristics: small, matte green or teal in sunlight), (C) *Copris nubilosus* (distinguishing characteristic: head horn), and side view (D) and top view (E) of *Dichotomius satanas* (distinguishing characteristics: large size and round body shape). Figure modified from Creedy and Mann (2011).

Average beetle mass per species was determined by weighing 10 beetles for each species (3 replicates per species). Mean values for each species were 0.060 ± 0.004 g (*A.chrysopyge*), 0.127 ± 0.004 g (*O. cyanellus*), 0.298 ± 0.002 g (*C. nubilosus*), and 1.111 ± 0.031 g (*D. satanas*). These values were used to calculate biomass of each species within a community and total community biomass (Eqn. 1).

Dung Removal Trials

Dung beetles were maintained in four terrariums, each containing a single species of dung beetle, and new beetles were added daily after pitfall trap collection. Each terrarium consisted of a plastic, rectangular container (30 x 20 x 6 cm), filled with ~ 4 cm of soil and a water-soaked sponge. Loose soil was collected from a previously excavated area at Base Camp. Water was collected from a tapped water source at Base Camp. Once per week, horse dung was added in each terrarium (~100 g). The terrariums were covered with a rectangular piece of mesh attached with a large rubber band to prevent both the beetles from escaping and other insects from entering.

Experimental Beetle Community Composition

Dung removal was determined from experimentally-assembled communities of the four focal species, with communities ranging in species richness and biomass (**Table 1**). Biomass was determined for multispecies communities where N_i is the number of individuals in the i^{th} species and m_i is the average mass per beetle of the i^{th} species (**Eqn. 1**).

$$\textbf{Total community Biomass} = \sum N_i * m_i \qquad \textbf{[Eqn 1.]}$$

Table 1. Experimental beetle community compositions. Experimental communities differing in species richness, total biomass, and diversity (measured with both individuals and total community biomass). Abbreviations are as follows *A. chrysopyge* (chry), *D. satanas* (sat), *C. nubilosis* (nub), and *O. cyanellus* (cyan). Numbers within community composition column indicate number of individual beetles for each species. Diversity columns calculated from Simpson's diversity index (D) using both number of individuals and biomass of species, both were negative natural log transformed (Eqns. 4 and 5).

Species Richness	Community Composition	Total Community Biomass (g)	Diversity (D) (individuals)	Diversity (D) (biomass)
One	100_chry	6.00	0.00	0.00
	74_chry	4.44	0.00	0.00
	50_chry	3.00	0.00	0.00
	37_chry	2.22	0.00	0.00
	25_chry	1.50	0.00	0.00
	20_chry	1.20	0.00	0.00
	10_chry	0.60	0.00	0.00
	25_nub	7.45	0.00	0.00
	10_nub	2.98	0.00	0.00
	7_nub	2.09	0.00	0.00
	25_cyan	3.18	0.00	0.00
	18_cyan	2.29	0.00	0.00
	6_sat	6.67	0.00	0.00
	5_sat	5.56	0.00	0.00
	3_sat	3.33	0.00	0.00
	2_sat	2.22	0.00	0.00
	1_sat	1.11	0.00	0.00

Two	Chry21_cyan3	1.64	0.26	0.44
	Chry12_cyan12	2.24	0.74	0.57
	Chry3_cyan21	2.85	0.26	0.13
	Chry21_nub3	2.15	0.26	0.67
	Chry12_nub12	4.30	0.74	0.33
	Chry3_nub21	6.44	0.26	0.06
	Chry21_sat3	4.60	0.26	0.51
	Chry12_sat12	14.05	0.74	0.10
	Nub21_cyan3	6.64	0.26	0.11
	Nub12_cyan12	5.10	0.74	0.54
	Nub3_cyan21	3.56	0.26	0.47
	Sat21_nub3	24.23	0.26	0.07
	Sat3_nub21	9.60	0.26	0.60
	Sat3_cyan21	6.00	0.26	0.68
Three	Chry18_nub3_cyan3	2.36	0.55	0.97
	Chry8_nub8_cyan8	3.88	1.19	0.77
	Chry3_nub18_cyan3	5.93	0.55	0.19
	Chry3_nub3_cyan18	3.36	0.55	0.62
	Chry18_sat3_nub3	5.31	0.55	0.77
	Chry8_sat8_nub8	11.75	1.19	0.49
	Chry3_sat18_nub3	21.07	0.55	0.10
	Chry3_sat3_nub18	8.88	0.55	0.68
	Chry18_sat3_cyan3	4.79	0.55	0.62
	Chry8_sat8_cyan8	10.38	1.19	0.30
	Chry3_sat3_cyan18	5.80	0.55	0.72
	Sat18_nub3_cyan3	21.27	0.55	0.12
	Sat8_nub8_cyan8	12.29	1.19	0.57
	Sat3_nub18_cyan3	9.08	0.55	0.72
	Sat3_nub3_cyan18	6.51	0.55	0.91
	Chry18_sat2_nub2_cyan2	4.15	0.57	0.97
	Chry16_sat1_nub6_cyan1	3.99	0.72	1.09
	Chry16_sat1_nub1_cyan6	3.13	0.72	1.24
	Chry11_sat2_nub10_cyan1	5.99	1.01	0.92
	Chry11_sat2_nub1_cyan10	4.45	1.01	1.03
	Chry10_sat10_nub2_cyan2	12.56	1.10	0.24
	Chry10_sat2_nub11_cyan1	6.23	1.01	0.88
	Chry10_sat2_nub10_cyan2	6.06	1.10	0.95
	Chry10_sat2_nub7_cyan5	5.54	1.28	1.12
	Chry10_sat2_nub5_cyan7	5.20	1.28	1.18

Four	Chry10_sat2_nub2_cyan10	4.69	1.10	1.11
	Chry10_sat2_nub1_cyan11	4.52	1.01	1.02
	Chry9_sat3_nub7_cyan5	6.59	1.37	0.99
	Chry9_sat3_nub5_cyan7	6.25	1.37	1.00
	Chry9_sat2_nub9_cyan4	5.95	1.25	1.03
	Chry9_sat2_nub4_cyan9	5.10	1.25	1.18
	Chry7_sat3_nub9_cyan5	7.07	1.37	0.97
	Chry7_sat3_nub5_cyan9	6.39	1.37	1.01
	Chry7_sat2_nub10_cyan5	6.26	1.28	1.00
	Chry7_sat2_nub5_cyan10	5.40	1.28	1.18
	Chry6_sat6_nub6_cyan6	9.58	1.53	0.64
	Chry2_sat10_nub10_cyan2	14.46	1.10	0.46
	Chry2_sat10_nub2_cyan10	13.10	1.10	0.31
	Chry2_sat2_nub18_cyan2	7.96	0.57	0.63
	Chry2_sat2_nub10_cyan10	6.59	1.10	1.03
	Chry2_sat2_nub2_cyan18	5.22	0.57	0.95

Nightly Trials

For all trials, communities were chosen randomly and given a random location within the field plot, prior to set up. Individual beetles were also randomly selected, from respective terrariums. Eight experiments were ran within the field plot each night (**Fig. 4**), for approximately seven weeks, when there were sufficient numbers of beetles. Along with one to seven experimental treatments of different community compositions, each nightly trial included a control enclosure containing only dung, to account for evaporation and/or hydration of the dung (Slade et al. 2007). Experiments were run concurrently and all enclosures were within 6 m of each other to minimize environmental differences (Amore et al. 2018).

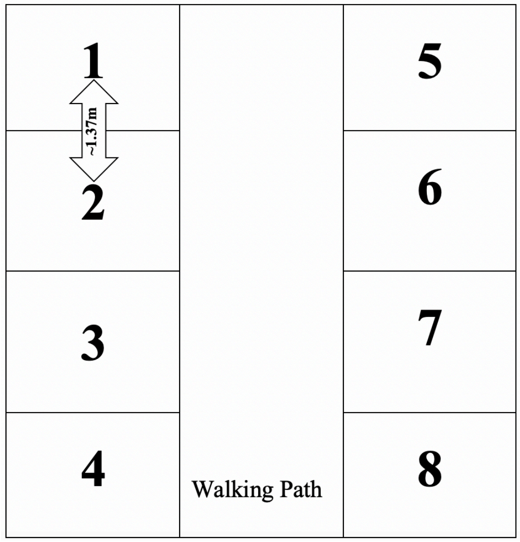

Fig. 4. Schematic of field plot. Numbers represent experimental enclosures (~1.37m from the next enclosure). Zippers faced the walking path for easy access.

Enclosures consisted of a fine nylon netting (30 x 30 x 30 cm) with a zipper in the front. The top of the enclosure was covered with a square plastic rain shield (~60 x 40 cm), secured with a piece of twine (**Fig. 5**). The bottom of the enclosure was cut out, with a square wire sheet (~ 61 x 61 cm) replacing the bottom panel. The wire sheet sagged in the middle, allowing extra room for the beetles to tunnel.

Fig. 5. Enclosure Setup. One of eight nylon netting enclosures (30 x 30 x 30 cm) with zipper in the front and a rain shield held to the top with twine.

Eight holes (~30.5 x 30.5 x 30.5 cm) were dug into the ground in an experimental plot of two rows of four holes, one for each enclosure used (**Fig. 6**). The square wire sheet was placed in the hole and the enclosure was placed on top. Approximately $0.02m^3$ of soil from the same previously excavated site at Base Camp as the terrariums was added to each enclosure, one at a time, and compacted. Eight sticks (~10 cm long) were placed vertically in the soil, on the corners and on the sides of the enclosure to hold the wire flush to the enclosure, detaining the beetles. Four sticks (~10 cm long) were placed horizontally on top of the soil, centered in the enclosure to create a platform for the dung. Then, the enclosures were zipped up to inhibit contamination.

Fig. 6. Field Plot. All eight enclosures with walking path down the middle. Covered workstation in the back housed terrariums under a rain shield.

Next, communities for that night's experiments were assembled (**Table 2**). Individuals were chosen randomly from terrariums and placed into temporary, 24 oz, plastic containers with lids. Temporary containers were placed next to the appropriate assigned enclosure. The beetles were never left in the temporary containers for longer than 30 minutes. Approximately 100 g of homogenized cow dung was placed in each enclosure. Cow dung is widely recognized as an appropriate dung source for tropical dung beetle experiments, and preliminary field experiments of dung type (horse, cow, and human) showed cow dung as a suitable choice for enclosure experiments. Homogenized cow dung (~ 100 g) was placed on a piece of foil and the weight was recorded. The dung was then placed on the dung platform in the center of the enclosure and the foil was re-weighed to calculate the exact amount of dung added to each enclosure. Beetles were added from the appropriate temporary container, the time was noted, and the enclosure was zipped up. After adding both dung and beetles to an enclosure, it was left overnight (~15 hours).

Table 2. Nightly Trials. Experimental trials for each night with corresponding community compositions. Abbreviations are as follows *A. chrysopyge* (chry), *D. satanas* (sat), *C. nubilosis* (nub), and *O. cyanellus* (cyan). Numbers within community composition column indicate number of individual beetles for each species. One control enclosure ran each night, but it is not listed below.

Nightly Trials	Setup Date	Community Composition
1	06.25.19	18 cyan
		3 sat
		2 sat
		7 nub
2	06.27.19	25 chry
		10 chry
		Chry 3 / sat 3 / nub 18
		Chry 3 / cyan 21
		Chry 18 / sat 3 / cyan 3
3	06.28.19	Chry 18 / sat 3 / nub 3
		Chry 10 / sat 2 / nub 2 / cyan 10
		Chry 12 / nub 12
		Chry 21 / cyan 3
		Chry 18 / nub 3 / cyan 3
		Sat 8 / nub 8 / cyan 8
4	06.29.19	Chry 8 / sat 8 / nub 8
		1 sat
		Chry 21 / nub 3
		Chry 2 /sat 2 /nub 18 / cyan 2
		Chry 10 / sat 2 / nub 10 / cyan 2
		Nub 12 / cyan 12
		Chry 12 / cyan 12
		Chry 18 / sat 2 / nub 2 / cyan 2
		5 sat

5	06.30.19	Chry 2 /sat 2 /nub 2/ cyan 18
		Chry 6 / sat 6 / nub 6 / cyan 6
		Chry 3 / sat 3 / cyan 18
		25 nub
		Chry 3 / nub 21
6	07.01.19	Chry 2 / sat 2 / nub 10 / cyan 10
		Sat 3 / nub 21
		Chry 8 / sat 8 / cyan 8
		3 sat
		5 sat
		Chry 3 / nub 3 / cyan 18
		Sat 3 / nub 18 / cyan 3
7	07.02.19	50 chry
		Sat 3 / nub 3 / cyan 18
		Chry 2 / sat 10 / nub 2 / cyan 10
		Chry 8 / nub 8 / cyan 8
		Nub 21 / cyan 3
		Chry 3 / nub 18 / cyan 3
		Chry 2 / sat 10 / nub 10 / cyan 2
8	07.04.19	Sat 3 / cyan 21
		Chry 3 / nub 18 / cyan 3
		Chry 12 / sat 12
		2 sat
		Chry 10 / sat 10 / nub 2 / cyan 2
		25 nub
		Chry 3 / nub 21
9	07.05.19	Chry 9 / sat 2 / nub 4 / cyan 9
		Chry 9 / sat 3 / nub 5 / cyan 7
		Chry 9 / sat 2 / nub 9 / cyan 4
		Chry 11 / sat 2 / nub 10 / cyan 1
		Chry 16 / sat 1 / nub 6 / cyan 1
		Chry 9 / sat 3 / nub 7 / cyan 5
		Chry 10 / sat 2 / nub 7 / cyan 5
10	07.06.19	Chry 11 / sat 2 / nub 1 / cyan 10
		Chry 3 / sat 18 / nub 3
		25 nub
		Chry 10 / sat 2 / nub 11 / cyan 1
		Chry 10 / sat 2 / nub 5 / cyan 7
		Chry 16 / sat 1 / nub 1 / cyan 6

		Chry 3 / nub 3 / cyan 18
11	07.07.19	Chry 7 / sat 3 / nub 9 / cyan 5
		10 nub
		Sat 12 / nub 12
		Chry 7 / sat 3 / nub 5 / cyan 9
12	07.08.19	2 sat
		Chry 7 / sat 2 / nub 5 / cyan 10
		7 nub
		100 chry
		Sat 18 / nub 3 / cyan 3
		Nub 3 / cyan 21
		Chry 7 / sat 2 / nub 10 / cyan 5
13	07.09.19	Sat 21 / nub 3
14	07.13.19	Chry 21 / cyan 3
		Chry 9 / sat 3 / nub 5 / cyan 7
		Chry 3 / nub 18 / cyan 3
		Nub 12 / cyan 12
		Chry 10 / sat 2 / nub 10 / cyan 2
		10 nub
		50 chry
15	07.14.19	Chry 21 / cyan 3
		Chry 18 / nub 3 / cyan 3
		5 sat
		7 nub
		Nub 3 / cyan 21
		Chry 8 / nub 8 / cyan 8
		Chry 12 / nub 12
16	07.15.19	Chry 7 / sat 2 / nub 10 / cyan 5
		Chry 21 / nub 3
		Chry 8 / nub 8 / cyan 8
17	07.16.19	37 chry
		10 chry
		25 chry
		20 chry
		Chry 11 / sat 2 / nub 1 / cyan 10
		74 chry
		Chry 10 / sat 2 / nub 5 / cyan 7
		100 chry
		20 chry

18	07.17.19	Chry 16 / sat 1 / nub 6 / cyan 1
		Chry 18 / nub 3 / cyan 3
		Chry 16 / sat 1 / nub 1 / cyan 6
19	07.18.19	Chry 3 / nub 21
		25 chry
		Chry 9 / sat 2 / nub 4 / cyan 9
		Chry 11 / sat 2 / nub 10 / cyan 1
		37 chry
		10 chry
20	07.19.19	74 chry
		Chry 9 / sat 3 / nub 7 / cyan 5
		Nub 3 / cyan 21
		Chry 10 / sat 2 / nub 11 / cyan 1
21	07.20.19	Chry 3 / cyan 21
		Chry 9 / sat 2 / nub 9 / cyan 4
		Chry 21 / sat 3
22	07.21.19	Chry 3 / sat 3 / cyan 18
		Chry 2 /sat 2 /nub 18 / cyan 2
		18 cyan
23	07.22.19	Chry 18 / sat 2 / nub 2 / cyan 2
		Chry 10 / sat 2 / nub 1 / cyan 11
		Chry 10 / sat 2 / nub 2 / cyan 10
24	07.23.19	Chry 2 / sat 2 / nub 10 / cyan 10
		Chry 21 / sat 3
		Chry 7 / sat 2 / nub 5 / cyan 10
		Chry 7 / sat 2 / nub 10 / cyan 5
25	07.26.19	Chry 3 / sat 3 / nub 18
		Sat 3 / nub 21
26	07.27.19	Chry 7 / sat 3 / nub 5 / cyan 9
		Chry 7 / sat 3 / nub 9 / cyan 5
		Chry 3 / nub 3 / cyan 18
27	07.28.19	25 cyan
		Chry 18 / sat 3 / cyan 3
		Chry 18 / sat 3 / nub 3
28	07.29.19	6 sat
29	07.30.19	Sat 3 / nub 18 / cyan 3
		Chry 2 /sat 2 /nub 2/ cyan 18
		Chry 9 / sat 3 / nub 7 / cyan 5
		Chry 11 / sat 2 / nub 1 / cyan 10

30	07.31.19	Sat 3 / nub 3 / cyan 18
		Chry 3 / sat 3 / nub 18
31	08.01.19	Chry 2 /sat 2 /nub 18 / cyan 2
		Sat 3 / cyan 21
		Sat 3 / nub 21
32	08.02.19	Chry 6 / sat 6 / nub 6 / cyan 6
		Chry 11 / sat 2 / nub 10 / cyan 1
33	08.03.19	Chry 8 / sat 8 / nub 8
		18 cyan
34	08.04.19	Sat 8 / nub 8 / cyan 8

Dung and Beetle Collection

The following morning, dung removal was measured by weighing the remaining dung. This was done one enclosure at a time, in the same order that they were set up and time was recorded simultaneously. Measuring dung removal from enclosures in the same order as they were set up allowed the beetles to be in the enclosure for approximately the same amount of time. After recording remaining dung weight for all enclosures, the soil of each enclosure was sifted to record, collect, and return dung beetles to the appropriate terrariums. After all enclosures were processed, the soil was discarded and then each enclosure was reconstructed for the next night's trials.

Proportion of Dung Removed

The proportion of dung removed nightly from each experimental enclosure (**Eqn. 2**) was calculated based on the dung starting mass (S_E) and the dung final mass (F_E) as well as an evaporation rate (R, **Eqn. 3**). Nightly evaporation rate (R) was calculated using the control enclosure dung starting mass (S_C) and dung final mass (F_C). The evaporation rate accounted for the gain or loss of mass in dung due to rehydration or dehydration during the night.

$$\textit{\textbf{Proportion of Dung Removed}} = \frac{[(1- R\ )*S_E - F_E]}{S_E} \qquad \textbf{[Eqn. 2]}$$

$$\boldsymbol{R} = \frac{(S_C - F_C)}{S_C} \qquad \textbf{[Eqn. 3]}$$

Over the eight-week field season, experimental communities had one to four replicates depending on availability of beetles. The mean proportion of dung removed across replicates for each experimental community was used for statistical analyses to standardize data.

Simpson's Diversity Index (D)

Simpson's diversity index was utilized for diversity because it provides a good estimation of diversity at relatively small sample sizes (such as a finite dung beetle community) and it ranks assemblages consistently (Magurran 2004). It was calculated in two different ways for all communities: 1) using the number of individuals of each species within each community, and 2) using the biomass of each species within each community. The diversity index using individuals (D_I) was calculated such that n_i is the number of individuals in the i^{th} species and N is the total number of individuals, utilizing the finite correction factor which is necessary for the beetle communities within this experiment (**Eqn. 4**; Magurran 2004). The diversity index using biomass (D_B) was calculated such that m_i is the biomass (g) of the i^{th} species within the community and M is the total biomass (g) of the community (**Eqn. 5**; Magurran 2004). After calculation of both diversity indices, they were transformed using the negative natural log (-ln(D)) to reflect underlying diversity, independently of sample size, and for easier interpretation (the transformation allows higher values to indicate higher diversity; Magurran 2004).

$$\boldsymbol{D_I} = \sum \left[\frac{n_i * (n_i - 1)}{N * (N - 1)} \right] \qquad \textbf{[Eqn. 4]}$$

$$\boldsymbol{D_B} = \sum \left(\frac{m_i}{M} \right)^2 \qquad \textbf{[Eqn. 5]}$$

Statistical Methods:

All analyses were conducted with the statistical programming platform R, version 4.0.2 (R Core Team 2020).

Species Richness

To determine the relationship between species richness and proportion of dung removed, the lm function from the car package (Fox and Weisberg 2019) was used to create a linear model with proportion of dung removed as a function of species richness. A one-way ANOVA was performed using the anova function from the car package, followed by Tukey's HSD post hoc analysis on significant effects using the aov function from the car package to identify groups that were different at the α=0.05 level. This analysis tested the influence of the number of species present (species richness) on proportion of dung removed. Then, to determine if the relationship between species richness and proportion of dung removed was independent of biomass, proportion of dung removed was normalized using total community biomass (i.e., proportion of dung removed/total community biomass). Again, the lm function from the car package (Fox and Weisberg 2019) was used to create a linear model with normalized proportion of dung removed as a function of species richness. A one-way ANOVA was performed using the anova function from the car package. This second analysis tested the influence of species richness on proportion of dung removed normalized for biomass. Figures were produced using functions from ggplot2 (Wickham 2016) with color schemes from RColorBrewer (Neuwirth 2014).

Total Community Biomass

To determine the relationship between total beetle community biomass and proportion of dung removed, the cor.test function from the car package (Fox and Weisberg 2019) was used to run a non-parametric Spearman's rank-order correlation. This analysis tested for a rank correlation between total community biomass and proportion of dung removed, regardless of species richness or community composition. Figures were produced using functions from ggplot2 (Wickham 2016).

Simpson's Diversity Index

The relationship between diversity and proportion of dung removed was explored using both the count-based and mass-based estimates of Simpson's Diversity (Eqns. 4 and 5 with negative natural log transformation). Both calculations were used to determine the relationship between diversity and proportion of dung removed. For both calculations, the lm function from the car package (Fox and Weisberg 2019) was used to test linear models with proportion of dung removed as a function of diversity. After setting up linear models, a Pearson's correlation was performed on both diversity calculations using the cor.test function from the car package to determine the relationship between diversity of the community and the proportion of dung removed. Figures were produced using functions from ggplot2 (Wickham 2016).

Single Species Community Variation

Communities containing only one species were analyzed in terms of grams of dung removed per grams of beetle, which standardized the differences in mass between each beetle species. This determined the relationship between each individual species and dung removal. The lm function from the car package (Fox and Weisberg 2019) was used to set up a linear model of grams of dung removed per gram of beetle as a function of species identity. A one-way ANOVA was performed using the anova function from the car package, followed by a Tukey's HSD post hoc analysis using the aov function from the car package to identify groups that were significantly different at the $\alpha=0.05$ level. Figures were produced using functions from ggplot2 (Wickham 2016) with a color scheme from RColorBrewer (Neuwirth 2014).

Community Composition

To determine the influence of community composition on the proportion of dung removed, the lm function from the car package (Fox and Weisberg 2019) was used to create four linear models, one for each of the four species: *A. chrysopyge, C. nubilosis*, *O. cyanellus*, and *D. satanas*. Each model contained the single species community of the focal species, and all other experimental communities that contained at least one individual from that focal species (**Table 2**). A one-way ANOVA was performed for all models using the anova function from the car package, followed by Tukey's HSD post hoc analyses using the aov function from the car package to identify experimental communities that were significantly different at the α=0.05 level. This analysis tested the influence of community composition on proportion of dung removed. Figures were produced using functions from ggplot2 (Wickham 2016) with color schemes from RColorBrewer (Neuwirth 2014).

Communities varying in number of *D. satanas*

To determine the influence of varying numbers of *D. satanas* individuals within a community on the proportion of dung removed, the lm function from the car package (Fox and Weisberg 2019) was used to create three linear models, communities with a species richness of one, two, and three. Each model contained communities with zero *D. satanas* individuals and communities with one or more *D. satanas* individuals. A one-way ANOVA was performed for all models using the anova function from the car package, followed by Tukey's HSD post hoc analyses using the aov function from the car package to identify experimental communities that were significantly different at the α=0.05 level. This analysis tested the influence of the number of *D. satanas* individuals within a community on proportion of dung removed. Figures were produced using functions from ggplot2 (Wickham 2016) with color schemes from RColorBrewer (Neuwirth 2014).

RESULTS

Species Richness

Species richness significantly predicted proportion of dung removed ($F_{3,68} = 6.00$, $p = 0.001$, **Fig. 7**). Communities containing only one species had 50% less dung removed than the communities containing three or four species.

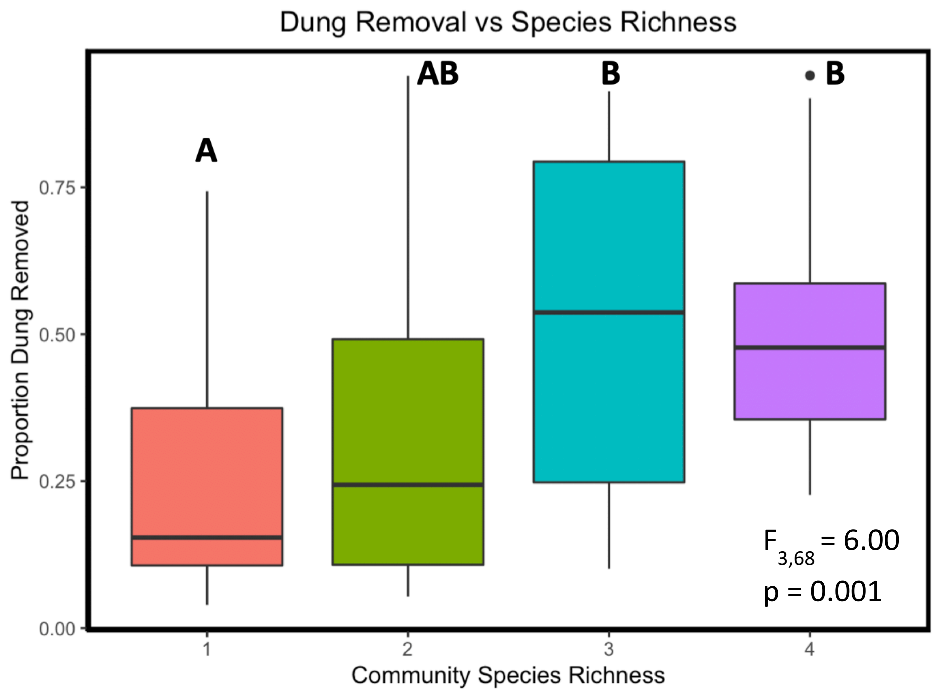

Fig. 7. Species richness influences dung removal. Species richness significantly determined proportion of dung removed ($F_{3,68} = 6.00$, $p = 0.001$). Colored boxes represent communities with different numbers of species: one species (red), two species (green), three species (blue), and communities containing all four species (purple). Horizontal lines within each box indicate mean and vertical lines indicate minimum and maximum values. Letters indicate significant differences based on Tukey HSD.

Species richness did not significantly predict proportion of dung removed when dung removal was normalized with total community biomass. ($F_{3,68} = 2.31$, $p = 0.08$, **Fig. 8**). When dung removal was normalized, communities differing in species richness removed the same proportion of dung. This indicates that the positive relationship found between species richness and proportion of dung removed (**Fig. 7**) is a statistical artifact, masking the role of the total dung beetle community biomass.

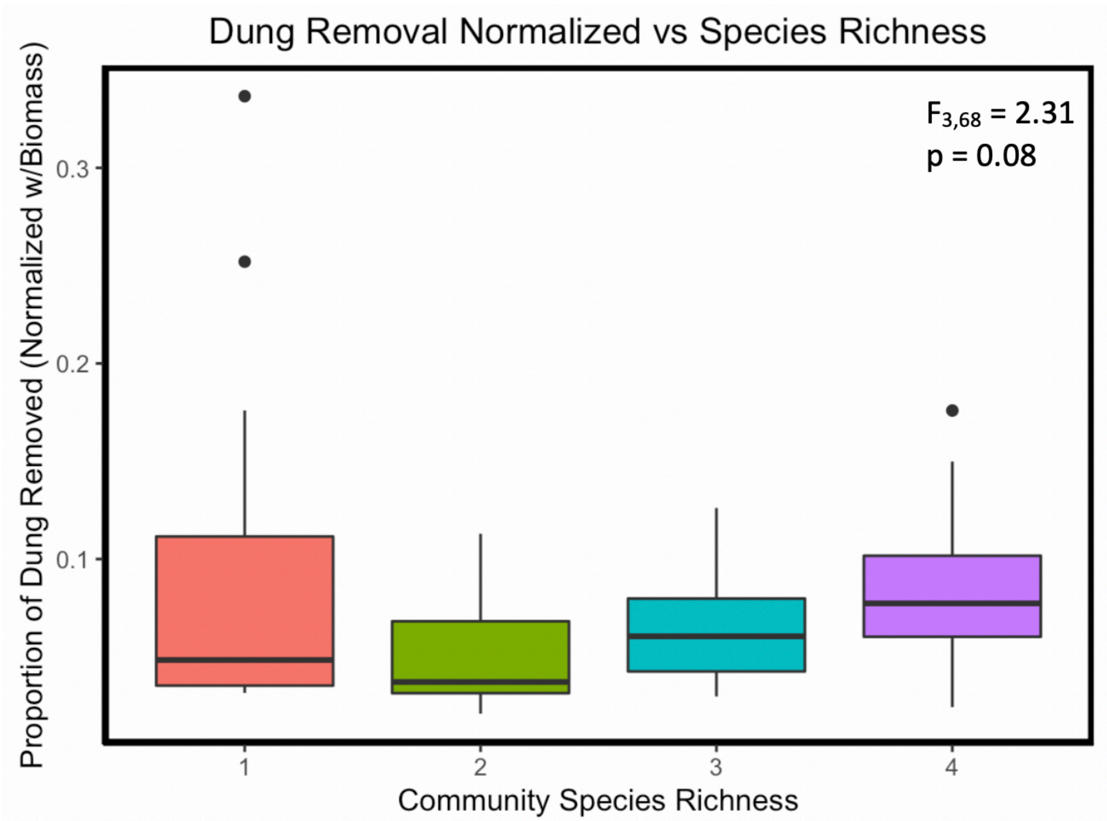

Fig. 8. Species richness does not influence normalized dung removal. Species richness does not influence proportion of dung removed when normalized with total community biomass ($F_{3,68} = 2.31$, $p = 0.08$). Colored boxes represent communities with different numbers of species: one species (red), two species (green), three species (blue), and communities containing all four species (purple). Horizontal lines within each box indicate mean and vertical lines indicate minimum and maximum values.

Total Community Biomass

Total community biomass significantly predicted proportion of dung removed with a positive rank correlation between total biomass and proportion of dung removed ($r_s = 0.69$, $p < 0.001$, **Fig. 9**). As the total biomass increases, proportion of dung removed also increases.

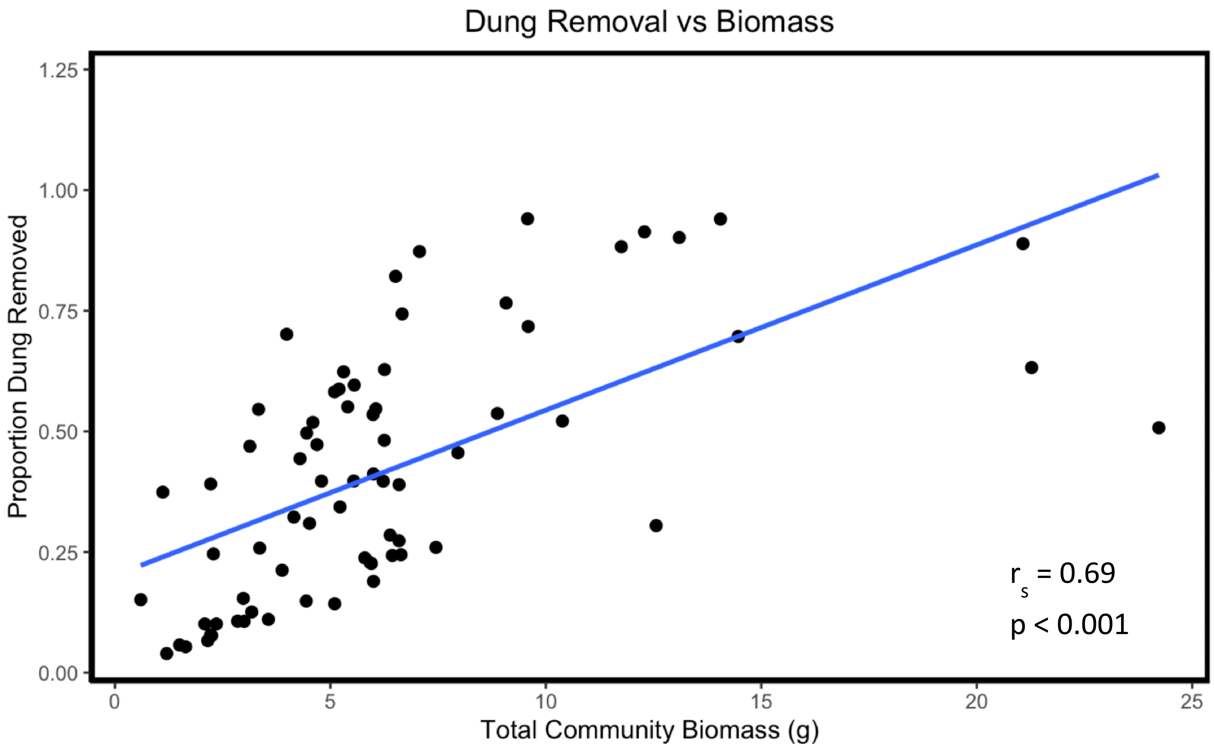

Fig. 9. Positive rank correlation between total community biomass and proportion of dung removed. Total community biomass significantly influences proportion of dung removed ($r_s = 0.69$, $p < 0.001$). Community biomass calculated using **Eqn. 1**. Line represents significant positive correlation.

Simpson's Diversity Index

To determine how diversity influences proportion of dung removed, Simpson's diversity index was calculated in two ways: (1) using the number of individuals of each species within the community and (2) using biomass of each species within the community. Both calculations resulted in a significant, positive correlation of diversity on the proportion of dung removed ($r_{70} = 0.45$, $p < 0.001$, **Fig. 10A**; $r_{70} = 0.24$, $p = 0.041$, **Fig. 10B**), but the calculation with individuals showed a stronger relationship as determined by the higher correlation coefficient (r). As diversity increases, proportion of dung removed also increases.

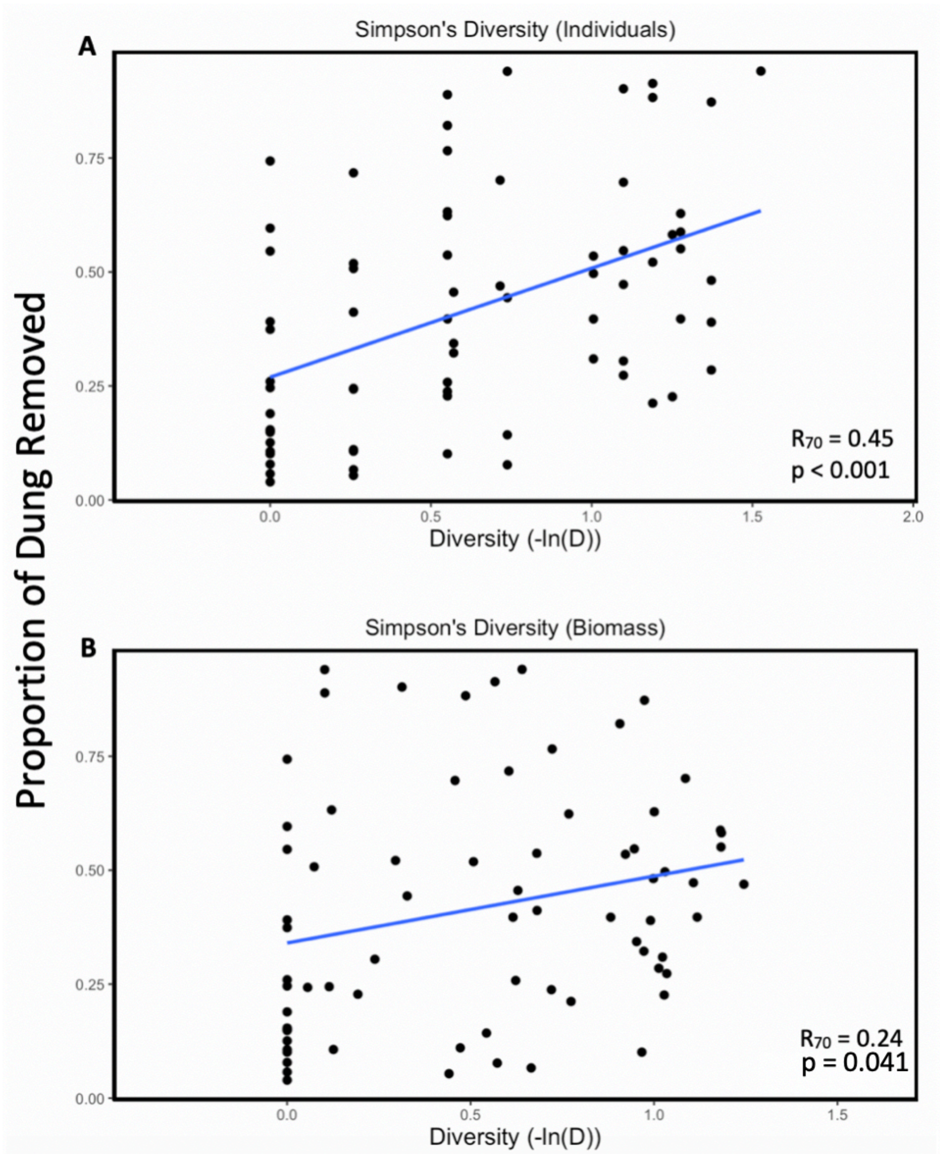

Fig. 10. Diversity positively influences dung removal. Simpson's diversity index, calculated with both number of individuals (A: $r_{70} = 0.45$, $p < 0.001$, $r^2 = 0.20$) and biomass of individuals (B: $r_{70} = 0.24$, $p = 0.041$, $r^2 = 0.06$), positively influences proportion of dung removed. Diversity was calculated using equation 5. Line represents significant positive correlation.

Species Identity

Communities containing only a single species (either *A. chrysopyge, O. cyanellus, C. nubilosis,* or *D. satanas*) were analyzed to determine how species identity influences dung removal. The influence of single species community variation was evaluated by determining grams of dung removed per gram of beetle, to standardize for mass of the beetle species.

Species identity significantly predicted proportion of dung removed when biomass was standardized for each species ($F_{3,12} = 6.97$, $p = 0.006$, **Fig. 11**). *D. satanas* removed ~4x more grams of dung per gram of beetle than *A. chrysopyge* and *C. nubilosis*.

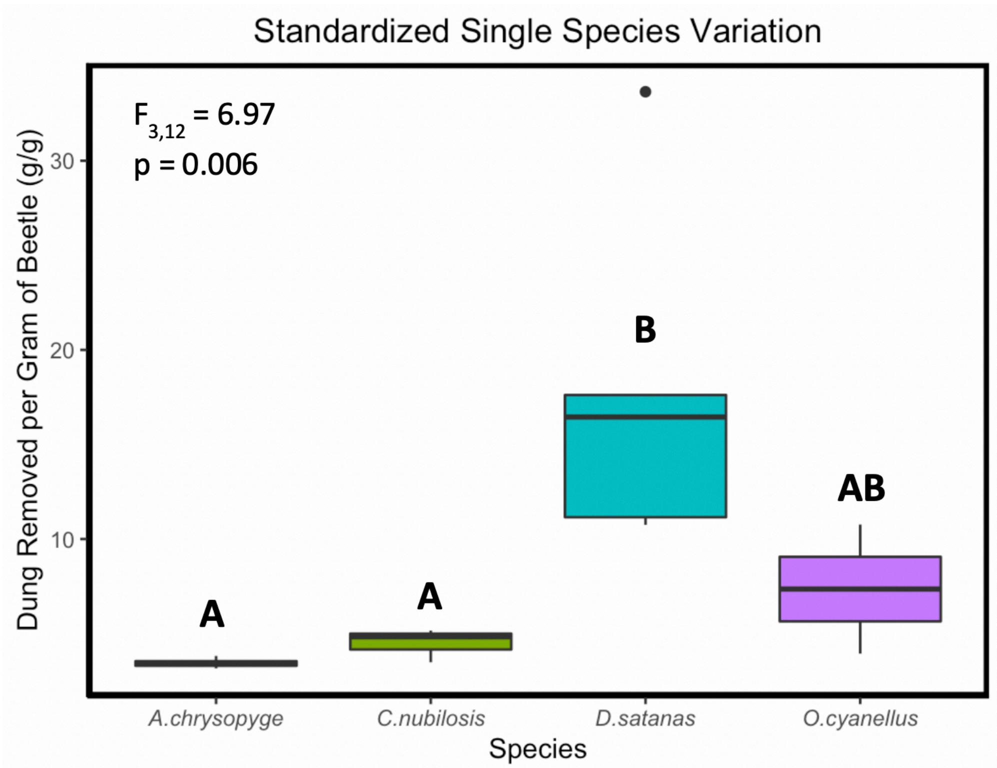

Fig. 11. Standardized for biomass, *D. satanas* removed more dung than *A. chrysopyge* and *C. nubilosis.* Species identity significantly influences proportion of removed, with *D. satanas* removing the most dung ($F_{3,12} = 6.97$, $p = 0.006$). Colored boxes represent communities containing only *A. chrysopyge* (red)*, O. cyanellus* (purple)*, C. nubilosis* (green), *or D. satanas* (blue). Horizontal lines within each box indicate mean and vertical lines indicate minimum and maximum values. Letters indicate significant differences based on Tukey HSD.

Community Composition

To determine how community composition influences dung removal, each of the four species were analyzed individually, *A. chrysopyge, O. cyanellus, C. nubilosis, or D. satanas*, along with all communities that contained them.

Examining communities containing *A. chrysopyge*, community composition influenced proportion of dung removed ($F_{7,44} = 10.18$, $p < 0.001$, **Fig. 12**). The community of *A. chrysopyge,* *D. satanas,* and *C. nubilosis* had more dung removed than four of the eight other communities, removing ~3x more dung than the communities including: *A. chrysopyge*; *A. chrysopyge* and *C. nubilosis*; *A. chrysopyge, C. nubilosis* and *O. cyanellus*; and *A. chrysopyge* and *O. cyanellus*. Also, the communities that contain *D. satanas* removed more dung than the other communities, perhaps suggesting a disproportionate influence from *D. satanas* beetles.

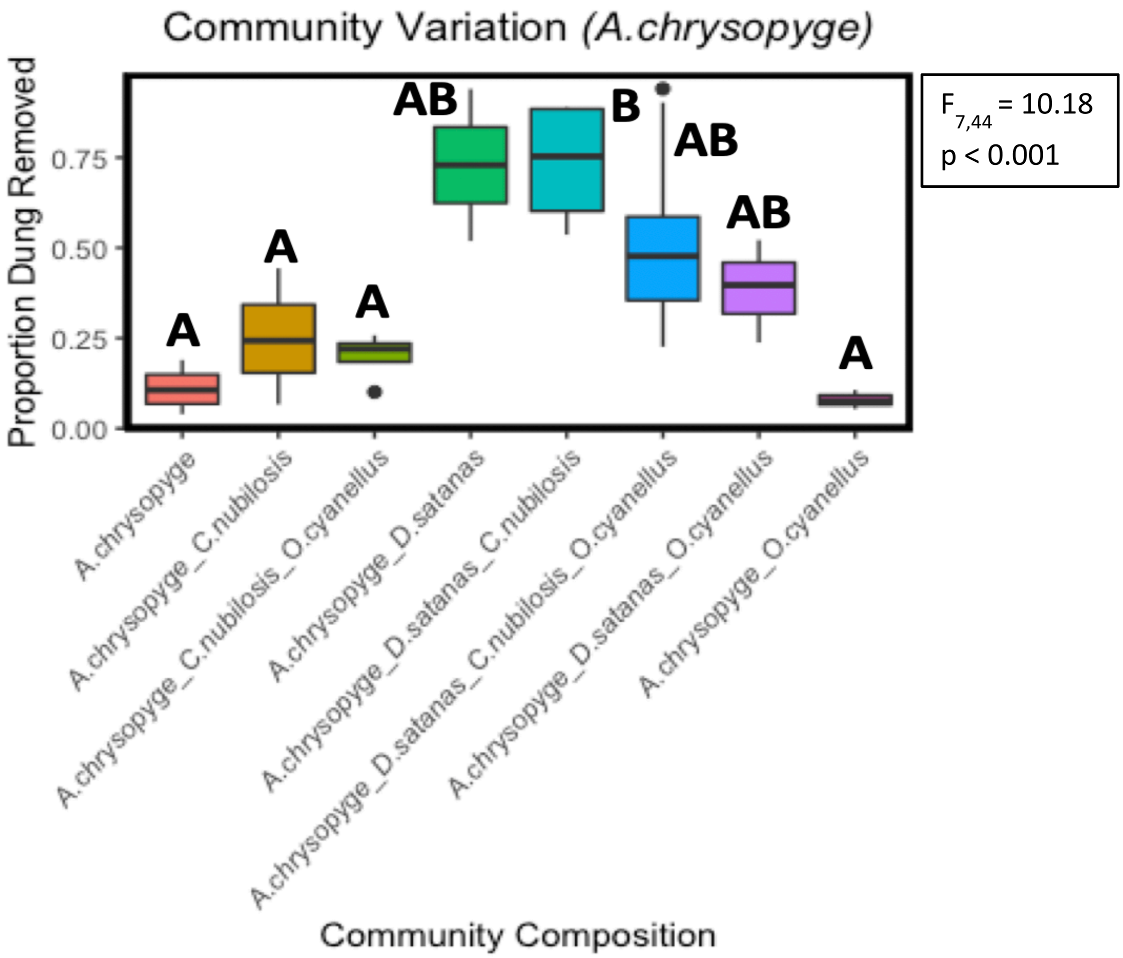

Fig. 12. Communities comprised of *A. chrysopyge, D. satanas,* and *C. nubilosis* remove the largest proportion of dung. Community composition significantly influences proportion of dung removed with *A. chrysopyge, D. satanas,* and *C. nubilosis* removing the most dung ($F_{7,44} = 10.18$, $p < 0.001$). Colored boxes represent different community compositions containing at least one *A. chrysopyge.* Horizontal lines within each box indicate mean and vertical lines indicate minimum and maximum values. Letters indicate significant differences based on Tukey HSD.

Examining communities containing *C. nubilosis*, community composition influenced proportion of dung removed ($F_{7,53} = 7.19$, $p < 0.001$, **Fig. 13**). The community of *C. nubilosis, A. chrysopyge* and *D. satanas* removed more dung than four of the eight other communities, removing ~3x more dung than the communities of: *C. nubilosis*; *C. nubilosis* and *A. chrysopyge*; *C. nubilosis*, *A. chrysopyge* and *O.cyanellus*; and *C. nubilosis* and *O. cyanellus*. Also, the community of *C. nubilosis, D. satanas* and *O. cyanellus* removed more dung than the same four communities, removing ~3x more dung than: *C. nubilosis*; *C. nubilosis* and *A. chrysopyge*; *C. nubilosis*, *A. chrysopyge* and *O.cyanellus*; and *C. nubilosis* and *O. cyanellu*s. The communities that contain *D. satanas* removed more dung than the other communities, supporting the possibility of disproportionate influences of *D. satanas*.

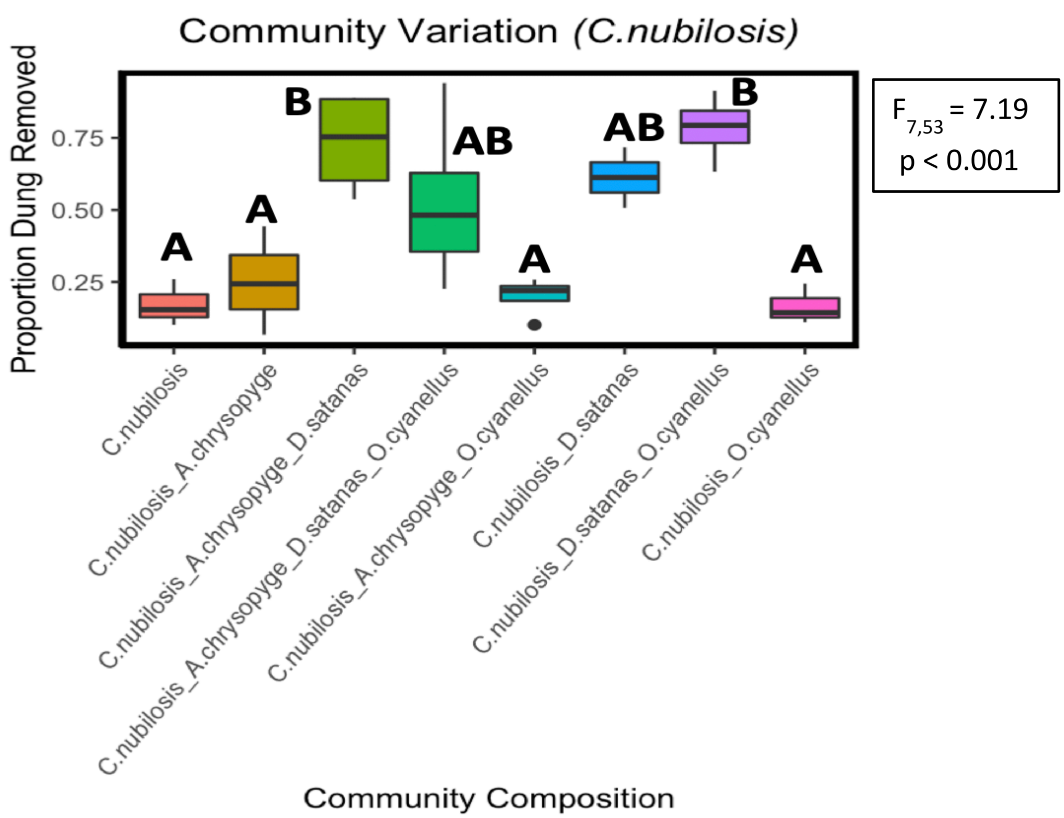

Fig. 13. Communities comprised of *C. nubilosis, A. chrysopyge,* and *D. satanas* and *C. nubilosis, D. satanas,* and *O. cyanellus* remove the largest proportion of dung. Community composition significantly influences proportion of dung removed ($F_{7,53} = 7.19$, $p < 0.001$). Colored boxes represent different community compositions containing at least one *C. nubilosis.* Horizontal lines within each box

indicate mean and vertical lines indicate minimum and maximum values. Letters indicate significant differences based on Tukey HSD.

Examining communities containing *O. cyanellus*, community composition influenced proportion of dung removed ($F_{7,38} = 7.99$, $p < 0.001$, **Fig. 14**). The community of *O. cyanellus*, *D. satanas* and *C. nubilosis* removed more dung than four of the eight other communities, removing ~3.5x more dung than the communities of: *O. cyanellus*; *O. cyanellus* and *A. chrysopyge*; *O. cyanellus, A. chrysopyge* and *C. nubilosis*; and *O. cyanellus* and *C. nubilosis*. The communities containing *D. satanas* removed more dung than the other communities, providing further support for the disproportionate influences of *D. satanas*.

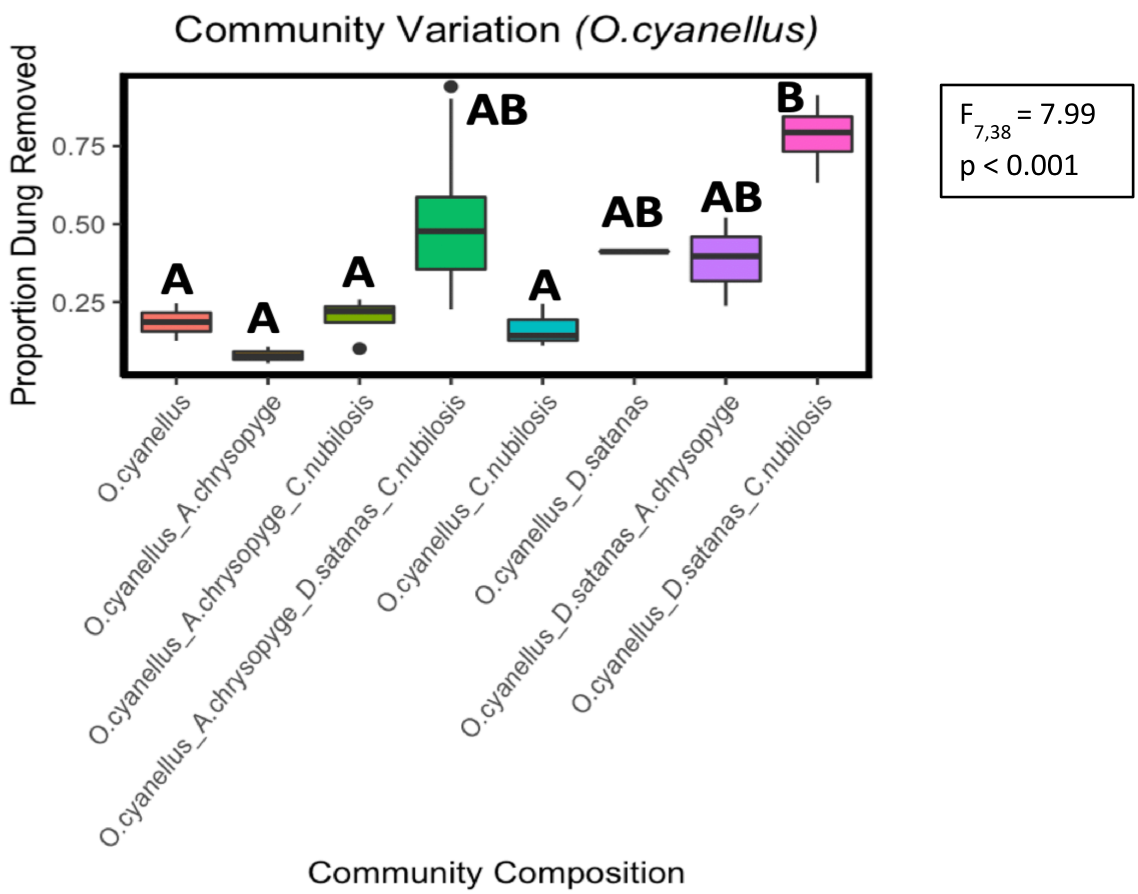

Fig. 14. Communities comprised of *O. cyanellus*, *D. satanas* and *C. nubilosis* remove the largest proportion of dung. Community composition significantly influences proportion of dung removed ($F_{7,38} = 7.99$, $p < 0.001$). Colored boxes represent different community compositions containing at least one *O. cyanellus.* Horizontal lines within each box indicate mean and vertical lines indicate minimum and maximum values. Letters indicate significant differences based on Tukey HSD.

Examining communities containing *D. satanas*, community composition influenced proportion of dung removed ($F_{7,39} = 2.40$, $p = 0.039$, **Fig. 15**); however, there no significant differences among the communities containing *D. satanas.* The disproportionate effects of *D. satanas* seen in the previous communities (Fig. 12-14) and the similarity in dung removal between communities that contain *D. satanas* (Fig. 15) presents the possibility that having just one *D. satanas* individual in a community may significantly influence dung removal.

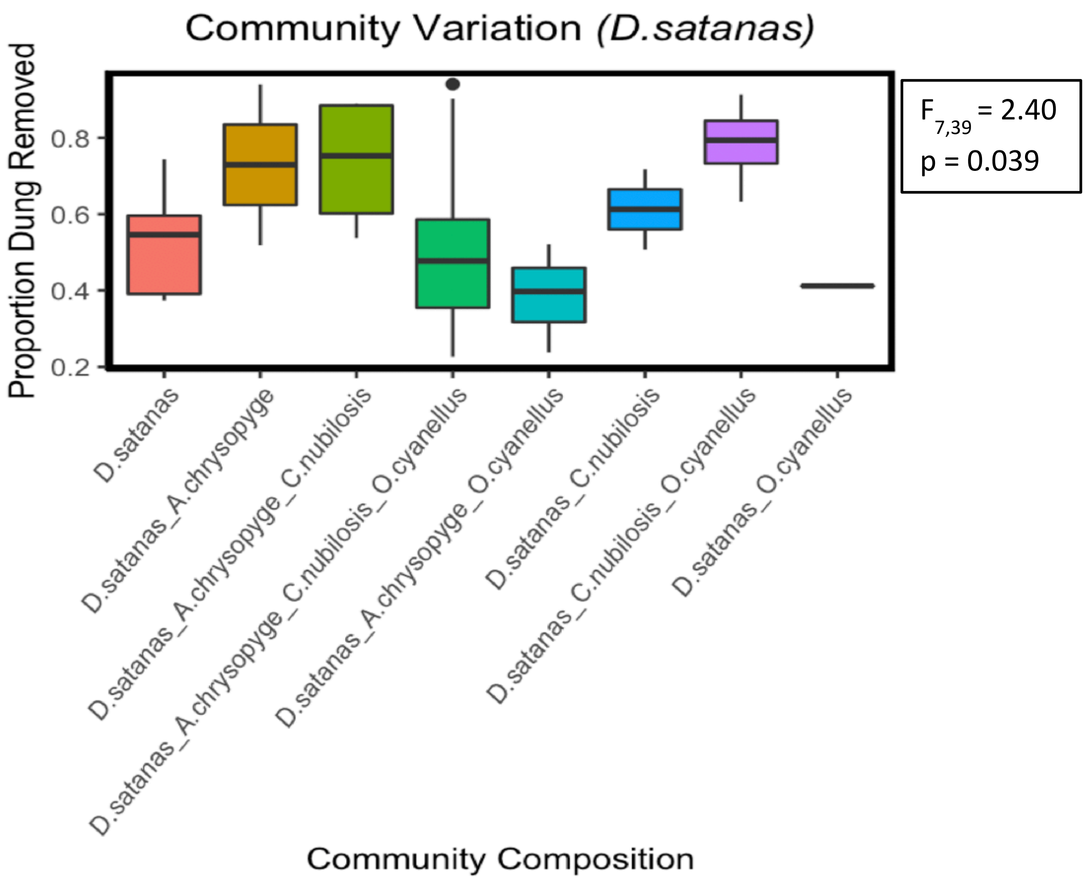

Fig. 15. No significant differences among communities containing *D. satanas.* Community composition influenced proportion of dung removed ($F_{7,39} = 2.40$, $p = 0.039$); however, there were no differences among communities. Colored boxes represent different community compositions containing at least one *D. satanas.* Horizontal lines within each box indicate mean and vertical lines indicate minimum and maximum values.

Lastly, communities containing one or more *D. satanas* individual were compared to communities that contained zero *D. satanas* individuals within communities of the same species richness. This analysis shows if the presence of one or more *D. satanas* individuals in a community greatly increases dung removal.

Examining communities with a species richness of one, number of *D. satanas* individuals significantly influenced proportion of dung removed ($F_{5,11} = 26.96$, $p < 0.001$ **Fig. 16**). Communities containing two, three, five, and six *D. satanas* individuals removed ~2.5-4.5x more dung that communities containing zero *D. satanas* individuals.

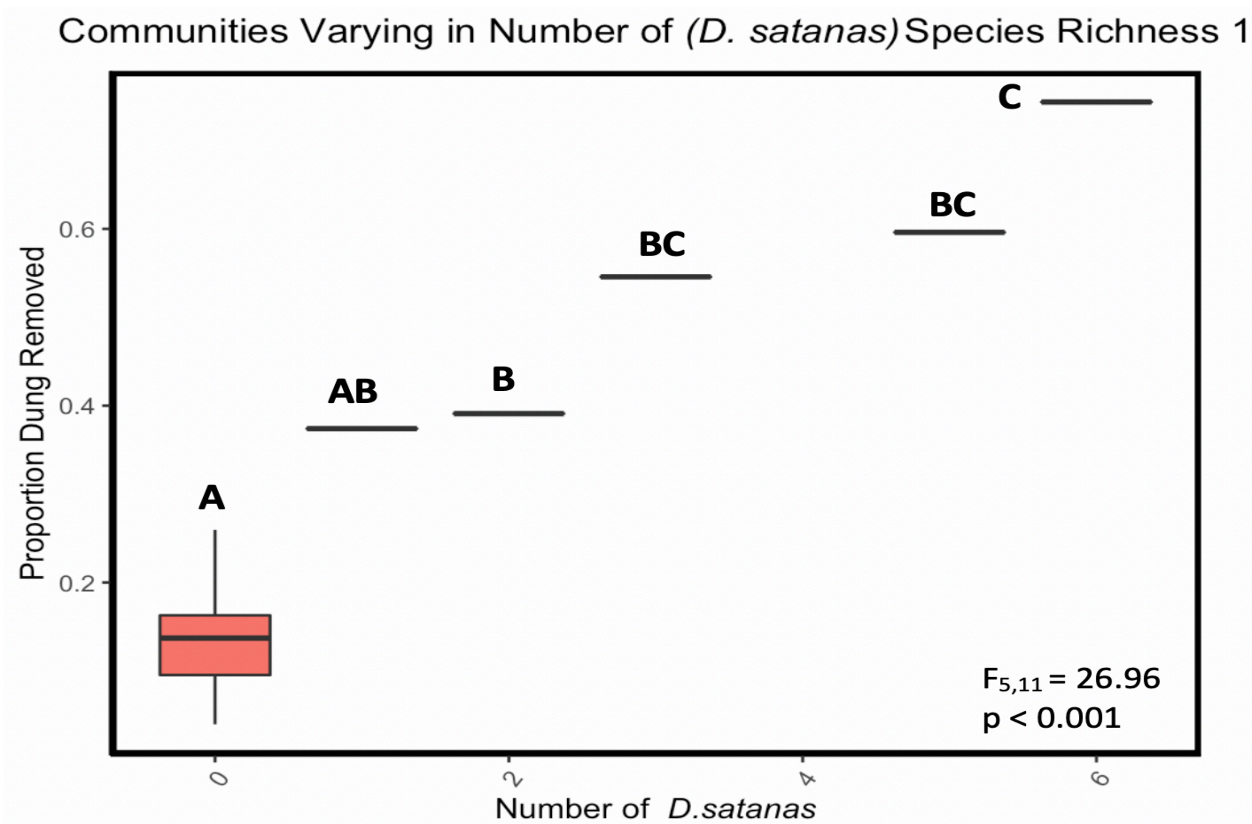

Fig. 16. Communities with a species richness of one that don't contain *D. satanas* remove the lowest proportion of dung. Presence or absence of *D. satanas* in single species communities influenced proportion of dung removed ($F_{5,11} = 26.96$, $p < 0.001$). Boxes represent communities varying in the number of *D. satanas* individuals (0-6 individuals). Horizontal lines within each box indicate mean and vertical lines indicate minimum and maximum values. Letters indicate significant differences based on Tukey HSD.

Examining communities with a species richness of two, number of *D. satanas* individuals influenced proportion of dung removed ($F_{3,10} = 15.10$, $p < 0.001$ **Fig. 17**). Communities containing three and twelve *D. satanas* individuals removed ~4-7x more dung that communities containing zero *D. satanas* individuals.

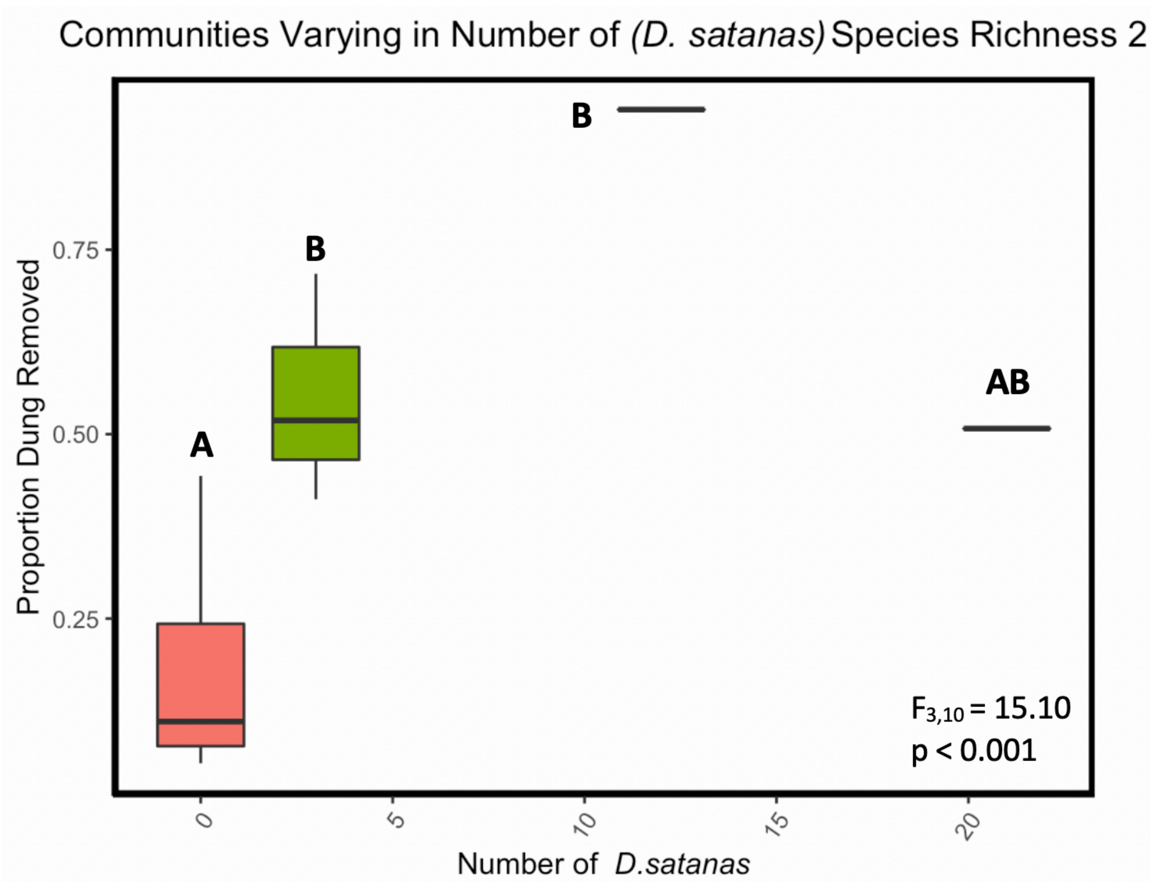

Fig. 17. Communities with a species richness of two that don't contain *D. satanas* remove the lowest proportion of dung. Presence or absence of *D. satanas* in communities with a species richness of two influenced proportion of dung removed ($F_{3,10} = 15.10$, $p < 0.001$). Boxes represent communities varying in the number of *D. satanas* individuals (0-21 individuals). Horizontal lines within each box indicate mean and vertical lines indicate minimum and maximum values. Letters indicate significant differences based on Tukey HSD.

Examining communities with a species richness of three, number of *D. satanas* individuals influenced proportion of dung removed ($F_{3,11} = 6.86$, $p = 0.007$; **Fig. 18**). Communities containing eight and eighteen *D. satanas* individuals removed ~3.5-4.5x more dung that communities containing zero *D. satanas* individuals.

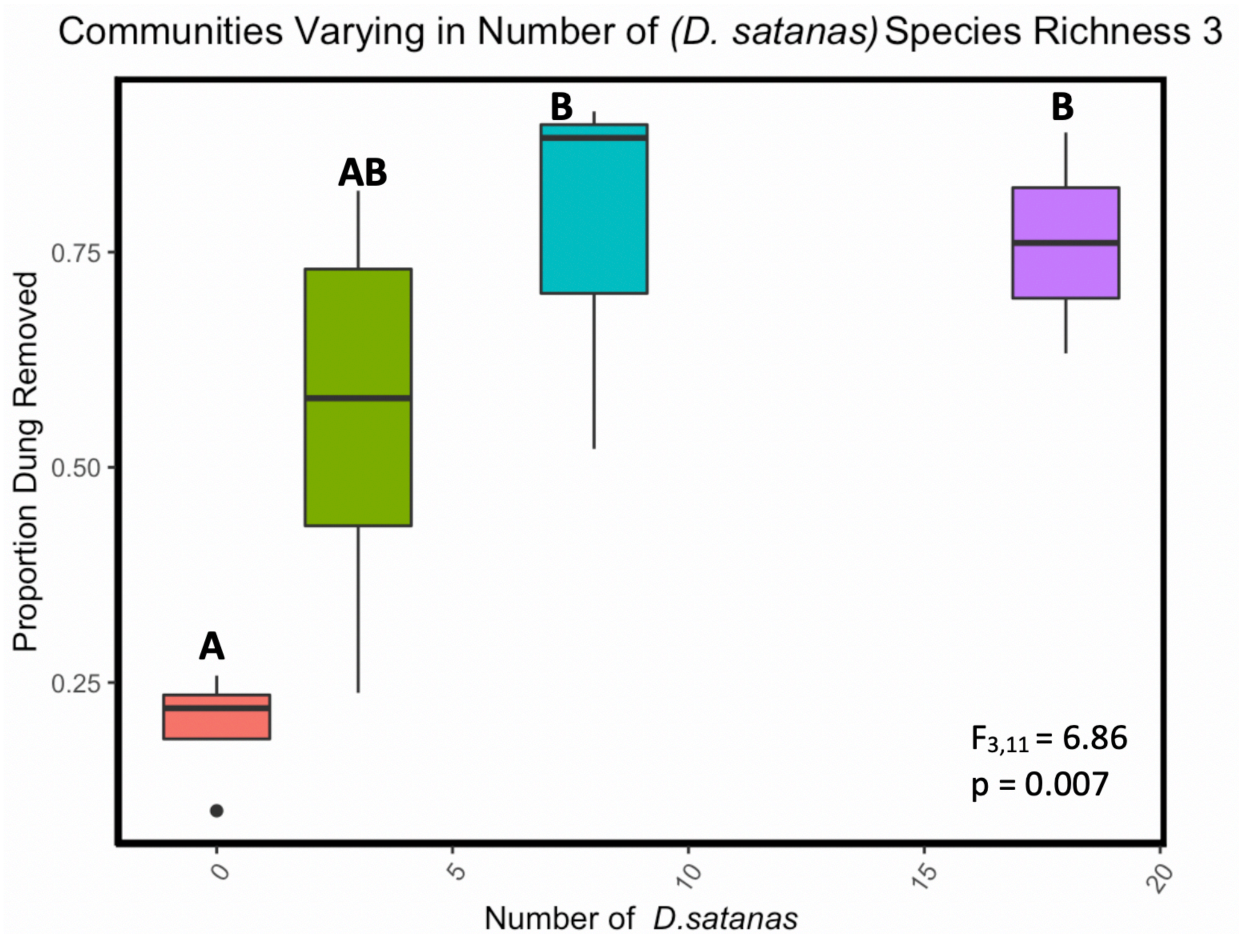

Fig. 18. Communities with a species richness of three that don't contain *D. satanas* remove the lowest proportion of dung. Presence or absence of *D. satanas* in communities with a species richness of two influenced proportion of dung removed ($F_{3,11} = 6.86$, $p = 0.007$). Boxes represent communities varying in the number of *D. satanas* individuals (0-18 individuals). Horizontal lines within each box indicate mean and vertical lines indicate minimum and maximum values. Letters indicate significant differences based on Tukey HSD.

DISCUSSION

Concerns about declining dung beetle abundances due to habitat loss and decreased food availability have led to a recent interest in determining how species identity influences ecological functions, such as dung removal (Larsen and Forsyth 2005; Slade et al. 2007; Nichols et al. 2008; O'Hea et al. 2010; Tixier, Bloor, and Lumaret 2015). Very little is known about the relationships between species identities and dung removal (Tixier, Bloor, and Lumaret 2015) and this is particularly true for the 40 species of dung beetle found in Cusuco National Park. To address this concern, my research examined how species richness, biomass as a proxy for beetle abundance, species diversity, species identity, and community composition of four dung beetle species, altered dung removal.

As species richness within experimental communities increased, the proportion of dung removed also increased (Fig. 7). Communities with three or four species removed approximately two times more dung than communities with only one species, regardless of species identity. However, when the proportion of dung removed was normalized with total beetle community biomass, beetle species richness no longer significantly influenced dung removal (Fig. 8). This confirmed that the positive relationship found between species richness and proportion of dung removed was ultimately dependent on community biomass. Other studies have provided mechanistic support for a link between species richness and dung removal. For example, studies have shown that in the presence of other beetle species, paracoprids transfer dung into their tunnels more quickly (Yoshihara and Sato 2015). Dung beetles may also change their behavior from consuming dung in the absence of competitors to rolling dung balls if the number of competitors promptly increases (Yoshihara and Sato 2015). Other studies suggest mechanisms such as resource partitioning or facilitation could result in increased dung removal with increased species richness (Hooper et al. 2005; O'Hea et al. 2010). In this study however, the observed relationship between species richness and dung removal appeared to be driven by biomass.

The importance of biomass was also supported by the observed increase in proportion of dung removed as total community biomass increased (Fig. 9). This result is reflected in work from others which also found a positive relationship between dung beetle biomass and dung removal (Tixier, Bloor, and Lumaret 2015). Additional investigation is needed to examine the relationship between biomass and dung removal further, but likely the positive relationship conceals interactive effects between biomass and species identity (Tixier, Bloor, and Lumaret 2015).

There was also a significant positive correlation between species diversity and the proportion of dung removed. These results are consistent with the diversity-function theory hypothesis, which states that as diversity increases, it will have positive effects on ecosystem function, due to complementarity between species (O'Hea et al. 2010). Most dung beetle diversity studies imply that as diversity increases interspecific interactions also increase, due to complementary resource use and facilitation (Hooper et al. 2005; O'Hea et al. 2010). Due to the different size classes within this study, complementarity seems likely in this case. An alternative hypothesis exists, called the dominance hypothesis, which states that the trait values of the dominant species (the species with the highest relative abundance in the community) has a proportionally larger effect on ecosystem function (Grime, 1998; Wasof et al. 2018), but this neglects other possibilities such as a the presence of a keystone species.

The results presented here suggest that while some beetle species removed similar amounts of dung, others removed very different amounts of dung, suggesting they are not functionally equivalent in terms of dung removal. Once standardized for biomass, *D. satanas* removed ~14-16 more grams of dung per gram of beetle than two of the other species, *C. nubilosis* and *A. chrysopyge,* respectively (Fig. 11). *D. satanas* and *O. Cyanellus* were not significantly different in terms of dung removal, although *D. satanas* tended to remove more dung (Fig. 11). This increased dung removal capability may qualify *D. satanas* as a keystone species within Cusuco National Park. Interpretation of these results would be greatly improved with greater in-depth biological information regarding the species used in this study. Particularly, information on reproductive strategies or nesting behavior would improve future hypotheses on dung removal characteristics of each species.

Community composition also greatly influenced the rate of dung removal. In communities containing *A. chrysopyge,* the community of *A. chrysopyge, D. satanas,* and *C. nubilosis* removed approximately three times more dung than four of the eight other communities (Fig. 12). For communities containing *C. nubilosis*, two communities removed the most dung: the community of *C. nubilosis, A. chrysopyge* and *D. satanas* removed approximately three times more dung than four of the eight other communities and the community of *C. nubilosis, D. satanas* and *O. cyanellus* removed approximately three times more dung than the same four communities (Fig. 13). For communities containing *O. cyanellus*, the community of *O. cyanellus*, *D. satanas* and *C. nubilosis* removed approximately three and a half times more dung than four of the eight other communities (Fig. 14). For the last species, *D. satanas*, there were no differences in dung removal between the communities (Fig. 15). Communities were analyzed from a species perspective, generating repetition in some community compositions in the previous results (Fig. 12 – Fig. 14) and when looked at collectively, two communities emerged as the best at removing dung. These included communities of *A. chrysopyge, D. satanas,* and *C. nubilosis* and *O. cyanellus*, *D. satanas* and *C.*

nubilosis. This suggests that conservation priority at Cusuco National Park should be given to natural dung beetle community compositions of either *A. chrysopyge, D. satanas,* and *C. nubilosis* or *O. cyanellus*, *D. satanas* and *C. nubilosis.*

The number of *D. satanas* individuals within a community influenced proportion of dung removed. For communities with a species richness of one, the communities containing two, three, five, and six *D. satanas* individuals removed approximately two and a half to four and a half times more dung than communities containing zero *D. satanas* individuals. For communities with a species richness of two, the communities containing three and twelve *D. satanas* individuals removed approximately four to seven times more dung that communities containing zero *D. satanas* individuals. For communities with a species richness of three, the communities containing eight and eighteen *D. satanas* individuals removed approximately three and a half to four and a half times more dung that communities containing zero *D. satanas* individuals. This finding suggests that having just one *D. satanas* beetle in a community can greatly increase dung removal; *D. satanas* may be worth consideration as a keystone species within Cusuco National Park.

Conclusions and Future Directions

This study aimed to answer five research questions: 1) How does species richness influence the rate of dung removal? 2) How does biomass of the community influence the rate of dung removal? 3) How does species diversity influence the rate of dung removal? 4) Are all four dung beetle species functionally equivalent in terms of dung removal? and 5) Does community composition influence the rate of dung removal? The results of this study show: 1) As species richness increases, proportion of dung removed appears to increases, but this increase is driven by beetle community biomass 2) As total community biomass increases, proportion of dung removed also increases 3) As species diversity increases, the proportion of dung removed also increases 4) The four species used in this study were not functionally equivalent in terms of dung removal and *D. satanas* tended to remove the most dung 5) Community composition does influence dung removal and the two communities that removed the most dung include *A. chrysopyge*, *D. satanas*, and *C. nubilosis* and *O. cyanellus*, *D. satanas* and *C. nubilosis*. Further, having *D.satanas* beetles present in a community greatly increases dung removal in communities with a species richness of one, two, and three, suggesting that *D. satanas* is very effective at removing dung.

Studies show that changes in both land-use and climate may threaten the diversity and abundance of dung beetles (Nichols et al. 2008; Nervo et al. 2014; Tixier, Bloor, and Lumaret 2015). There are current threats of deforestation within Cusuco mainly due to illegal logging for coffee plantations, which leads to decreased habitat for all forest animals including dung beetles. The four species within my study, *Ateuchus chrysopyge, Onthophagus cyanellus, Copris nubilosus,* and *Dichotomius satanas,* make up the majority of the dung beetle community within the park, and the remaining 36 species are found at much lower abundances. There may be specialists within these rarer species, although a lack of biological data makes this unclear. Almost all known biological information on dung beetle species within Cusuco National Park is surmised from taxonomic research based on morphology (e.g., long, wide legs presumably make a species a telecoprid, etc.).

Future research which determines biological information on both reproductive strategies (length of pre-nesting period, number of eggs per brood mass, etc.) and nesting behaviors (timing of instars, nest complexity, etc.) for each dung beetle species within Cusuco National Park will greatly improve scientific knowledge of dung beetles and this information can be utilized for future ecological studies on dung beetles. For the four species within this study, future attention should also involve the consideration of a kleptoparasitic relationship between *O. cyanellus* and *D. satanas,* as field observations were made of *O. cyanellus* beetles occurring within the buried dung tunnels of *D. satanas* beetles. The literature supports this possibility as *O. acuminatus* has been recorded as a facultative kleptoparasite of *D. satanas* in Panama, acting as a paracoprid at small dung patches, and a cleptocoprid at larger dung patches (Gill 1991). While species richness and diversity are important measures that need to be accounted for in future management plans, species identity needs to be considered as well, as it may be more important than diversity when it comes to ecological processes like dung removal, as seen in this study with *Dichotomius satanas*.

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