Dung beetle diets, networks, and ecosystem processes in regenerating forests

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Summary

Maintaining biodiversity is important for maintaining ecosystem processes. Dung beetles are a globally distributed group that contributes to two important ecosystem processes, decomposition and seed dispersal, making them potentially important organisms for maintaining ecosystem functionality. However, human disturbances, such as deforestation and agricultural expansion, alter environmental variables in a way that provides less habitat for some species. This can result in ecological networks disintegrating, which causes a loss of ecosystem services and processes. Since the majority of ecosystems are experiencing some form of degradation, there is interest in ecosystem recovery after disturbance. Passive forest recovery is a cost-effective form of conservation. Making it worthwhile to study the efficacy of passive forest recovery on ecosystem processes.

We examined the dung beetle-resource networks and estimated mammal-derived resources across a forest recovery gradient in the Ecuadorian Chocó to assess how dung beetle communities and their resource use respond to forest recovery. For this, we used pitfall traps with different bait types and camera traps to estimate the mammal resources. From these data, we evaluated dung beetle abundance, diversity, and resource specialization. Our results indicate that forest age is positively correlated with dung beetle diversity and network complexity, suggesting that older forests support more functionally diverse communities. In degraded habitats, dung beetle abundance declined, and their networks became more specialized, leading to a reduction in the decomposition efficiency of dung and carrion resources. The camera trap data suggested a high availability of mammal-derived resources in agricultural areas, particularly pastures. Despite high dung availability in pastures, dung beetles remained scarce, likely due to unfavorable abiotic conditions or the absence of nocturnal mammals.

To facilitate an understanding of the resources used by adult dung beetles, we developed methods to more accurately identify the meals of dung beetles based on DNA analysis of gut content. Preliminary analysis of dung beetle gut contents highlighted associations between dung beetles and native frugivores, particularly howler and spider monkeys, which play a critical role in seed dispersal. This suggests that secondary seed dispersal by dung beetles may be a significant factor in the seed dispersal of tree species in the diets of monkeys at our study site. To facilitate an understanding of seed dispersal by dung beetles, we measured seed traits (size, shape, and surface) for seeds included in dung balls relative to those in monkey feces references. We found that seed traits, such as size and surface texture, influenced the likelihood of burial. Secondary seed dispersal by dung beetles has been shown in other studies to increase

seedling establishment and above-ground plant biomass. Given that increased diversity and abundance have been shown to enhance the rate of secondary dispersal in other studies, it can be expected that the rate of dispersal will increase as the beetle community reforms.

Our findings suggest that passive forest regeneration is an effective conservation strategy, as it facilitates the recovery of dung beetle abundance and biodiversity and reduces network specialization. This implies that recovering dung beetle communities become more effective at recycling nutrients and dispersing seeds as they recover.

German Summary

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Die Erhaltung der Artenvielfalt ist wichtig für die Aufrechterhaltung von Ökosystemprozessen. Mistkäfer sind eine weltweit verbreitete Gruppe, die zu zwei wichtigen Ökosystemprozessen beiträgt, nämlich zur Zersetzung und zur Verbreitung von Samen, was sie zu potenziell wichtigen Organismen für die Aufrechterhaltung der Ökosystemfunktionen macht. Störungen durch den Menschen, wie die Abholzung von Wäldern und die Ausweitung der Landwirtschaft, verändern jedoch die Umweltvariablen in einer Weise, die für einige Arten weniger Lebensraum bietet. Dies kann dazu führen, dass sich ökologische Netzwerke auflösen, was einen Verlust von Ökosystemleistungen und -prozessen zur Folge hat. Da die meisten Ökosysteme in irgendeiner Form geschädigt werden, besteht ein Interesse an der Wiederherstellung von Ökosystemen nach einer Störung. Die passive Wiederherstellung von Wäldern ist eine kosteneffiziente Form des Naturschutzes. Daher lohnt es sich, die Wirksamkeit der passiven Walderholung auf Ökosystemprozesse zu untersuchen.

Wir untersuchten die Mistkäfer-Ressourcennetzwerke und schätzten die von Säugetieren stammenden Ressourcen über einen Gradienten der Walderholung im ecuadorianischen Chocó, um zu beurteilen, wie Mistkäfergemeinschaften und ihre Ressourcennutzung auf die Walderholung reagieren. Zu diesem Zweck verwendeten wir Fallgrubenfallen mit verschiedenen Ködern und Kamerafallen zur Schätzung der Säugetierressourcen. Anhand dieser Daten haben wir die Abundanz, Vielfalt und Ressourcenspezialisierung der Mistkäfer untersucht. Unsere Ergebnisse zeigen, dass das Alter des Waldes positiv mit der Vielfalt der Mistkäfer und der Komplexität des Netzwerks korreliert, was darauf hindeutet, dass ältere Wälder funktional vielfältigere Gemeinschaften beherbergen. In geschädigten Lebensräumen nahm die Häufigkeit von Mistkäfern ab, und ihre Netzwerke wurden spezialisierter, was zu einer geringeren Zersetzungseffizienz von Dung- und Aasressourcen führte. Die Daten der Kamerafallen lassen auf eine hohe Verfügbarkeit von Säugetierressourcen in landwirtschaftlichen Gebieten, insbesondere auf Weiden, schließen. Trotz der hohen Verfügbarkeit von Dung auf den Weiden blieben die Dungkäfer rar, was wahrscheinlich auf ungünstige abiotische Bedingungen oder das Fehlen nachtaktiver Säugetiere zurückzuführen ist.

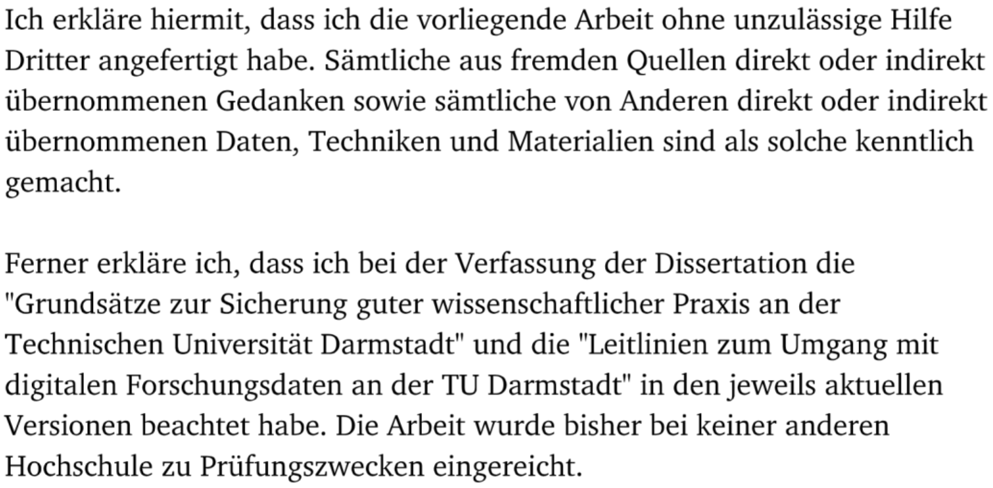
Um die von erwachsenen Mistkäfern genutzten Ressourcen besser verstehen zu können, entwickelten wir Methoden zur genaueren Identifizierung der Mahlzeiten von Mistkäfern auf der Grundlage von DNA-

Analysen des Darminhalts. Vorläufige Analysen des Darminhalts von Mistkäfern zeigten Zusammenhänge zwischen Mistkäfern und einheimischen Frugivoren, insbesondere Brüllaffen und Klammeraffen, die eine wichtige Rolle bei der Verbreitung von Samen spielen. Dies deutet darauf hin, dass die sekundäre Samenverbreitung durch Mistkäfer ein bedeutender Faktor bei der Samenverbreitung von Baumarten sein könnte, die auf dem Speiseplan der Affen an unserem Studienstandort stehen. Um die Verbreitung von Samen durch Mistkäfer besser zu verstehen, haben wir die Samenmerkmale (Größe, Form und Oberfläche) von Samen in Mistkugeln im Vergleich zu denen in Affenkotreferenzen gemessen. Wir fanden heraus, dass Samenmerkmale wie Größe und Oberflächenbeschaffenheit die Wahrscheinlichkeit des Vergrabens beeinflussten. Andere Studien haben gezeigt, dass die sekundäre Samenverbreitung durch Mistkäfer die Etablierung von Sämlingen und die oberirdische Pflanzenbiomasse erhöht. Da sich in anderen Studien gezeigt hat, dass eine größere Vielfalt und Abundanz die Rate der sekundären Ausbreitung erhöht, kann davon ausgegangen werden, dass die Ausbreitungsrate zunimmt, wenn sich die Käfergemeinschaft reformiert.

Unsere Ergebnisse deuten darauf hin, dass die passive Waldverjüngung eine wirksame Erhaltungsstrategie ist, da sie die Wiederherstellung der Abundanz und der Artenvielfalt von Mistkäfern erleichtert und die Spezialisierung der Netzwerke verringert. Dies bedeutet, dass sich erholende Mistkäfergemeinschaften bei der Wiederverwertung von Nährstoffen und der Ausbreitung von Samen effektiver werden, wenn sie sich erholen.

Declaration of Honor

Ehrenwörtliche Erklärung:



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Unterschrift

Introduction

Earth. The planet on which we humans (*Homo sapiens*) complete our life cycle. We share our planet with 8.7 billion other species. Of those billions of species, approximately 5.6 million are insects (Wiens, 2023). Among the insects, Coleoptera (beetles) are among the most diverse, with 1.5 million species (Stork et al., 2015). Charles Darwin is even credited with the quote, "If there is a Creator, he must have an inordinate fondness for beetles, " describing the sheer number and diversity of forms that beetles take. Long before Darwin, the ancient Egyptians observed dung beetles. They saw them emerging from dungballs. They concluded that insects came from nothing. This idea of spontaneous generation persisted for years after. The Egyptians further linked the spontaneous appearance of dung beetles to the sun's daily appearance and disappearance. This is why the sun god Khepri is scarab-faced. Even today, the plethora of scientific studies underscores our collective continued fascination with this group. As of 20 March 2025, 95,200 results were returned from a Google Scholar search with the keyword "dung beetles." Open the Amazon website and search for dung beetles; the results return multiple children's books on the subject. Here, I briefly introduce dung beetles, their diets, and their roles in the ecosystem both in nutrient cycling and in secondary seed dispersal.

Dung beetles are a cosmopolitan group found on all continents except Antarctica. They consist of three phylogenetic groups: Geotrupidae (Family), the subfamilies Scabaeniae and Aphodiinae, with over 6000 species. There are three major **functional groups** of dung beetles: rollers, tunnelers, and dwellers. Rollers are the stereotypical dung beetles that form balls and roll the dung away from the source. Tunnelers, dig into the dirt below the soil surface and pull the dung into the brood chamber below the soil surface. Dwellers complete their life cycle in the dung. As competition for dung resources can be fierce e.g. when observed one kilo of elephant dung can be removed within 2 hours by between 2000 and 16,000 beetles (Krell and Krell-Westerwalbesloh, 2024). This results in conflicts over dung resources, leading beetles to steal balls from each other. Rollers often try to move dung away from the source as fast as possible, which can result in larger dung beetles stealing dungballs from smaller beetles (Hanski and Cambefort, 1991). Morphologically rollers tend to have longer legs and lack horns (Fig 1). Tunnelers stay closer to the dung source, with males and females cooperating to move dung below the soil surface. Typically, tunneling males have two morphs: major males with horns and minor males without horns, which makes them physically similar to females. Large males with horns will compete for mates, and males with larger horns usually win contests due to their longer levers (Emlen and Philips, 2006). Minor males will sneak past major males and mate with females in the brood chamber defended by the major male (Emlen, 1997 and references therein). After mating, the female will prepare the brood ball for her offspring.

It is hypothesized that part of the females' brood ball preparation involves providing the larva with the microbes required to get a nutritionally complete diet from dung. This is due to dung lacking some essential amino acids. The behavior has been primarily studied in one genus *Onthophagus*. In this genus, female dung beetles pass their microbiome to their offspring by creating a small hole in the brood ball in which they place an egg on a pedestal of their own feces. When the larvae emerge from the egg, they first consume their mother's dung before consuming the brood ball. This provides the microbes needed to extract essential nutrients from dung (Estes et al., 2013).

Unlike many other insects with complete metamorphosis, for those species where the diet has been described for both adults and larvae, both life stages feed on the same resources, meaning that if adults feed on dung, they create brood balls of dung similarly for rotten vegetable matter, and carrion. During metamorphosis, as the larvae transform into adult beetles, their chewing mouthparts are replaced by spongier mouthparts. This shift means that larvae can consume all the material in the dung, including undigested plant material, but adults can only consume the liquid and small particles. While the upper limit in particle size is variable, depending on the species, the upper particle size limit for adult dung beetles ranges from 2 µm to 130 µm (Holter and Scholtz, 2007).

Figure 1 Showing examples of a rolling *Canthon delicatalus* and tunnelling major male *Sulcophanaeus noctis* dung beetle (photos Karen Marie Pedersen 2024, with Keyance VHX-S550E).

Dung beetle diets typically consist of material with a rich microbial community, including carrion, dung, and rotten fruit (Hanski and Cambefort, 1991). However, other resources have been documented, including snail mucus (Vaz-de-Mello, 2007), injured millipedes (Bedoussac et al., 2007; Krell et al., 1997; Larsen et al., 2009; Schmitt et al., 2004) and goat hair balls (Fuentes-Jacques et al., 2023). Though mammal dung is a consistent part of dung beetle diets globally, there is some geographic variation in the diet of dung beetles (Hanski and Cambefort, 1991). This variation depends on which resources and resource competitors are present. Dung beetles are most famous for their creation of huge dungballs from the dung of large herbivores, e.g., elephants and rhinos. This stereotype is well earned because the interaction between dung beetles and elephant dung really is impressive, with an estimated 2 million dung beetles supported by one elephant in the African savanna (Krell and Krell-Westerwalbesloh, 2024). In the African savanna, dung beetles are recorded almost exclusively on the dung of large mammals (Hanski and Cambefort, 1991). There is some evidence that adults may also consume rodent dung (Kerley et al., 2018). In many studies where mammal dung is offered along with other resources, mammal dung has the most exclusive community (Correa et al., 2016; Da Silva et al., 2012; Da Silva and Dorneles-Audino, 2011; Davis, 1994; Davis et al., 2010; Ratcliffe, 2013). However, in New Zealand, where mammals were historically absent, dung beetles also consume bird dung, carrion, and invertebrate dung (Stavert et al., 2014). Similarly, reptile dung is a frequently exploited resource on islands with limited mammal populations (Lopes et al., 2023). In the Neotropics, where there is less competition for carrion resources, many species consume carrion in addition to mammal dung, with some species exclusively collected in carrion baited traps (Correa et al., 2023; Ratcliffe, 2013). The use of millipedes varies, some dung beetles are predators on injured millipedes (Krell et al., 1997; Schmitt et al., 2004), and others on millipede corpses (Fuentes-Jacques et al., 2023; Nganhane et al., 2023). More specialized resources, such as snail mucus and the exclusive use of sloth dung, are geographically limited and the exception rather than the rule. When multiple resources have been studied for dung beetles, only a small portion of the dung beetle community specializes in a particular group of resources, e.g., exclusively uses carrion resources (Correa et al., 2023; Da Silva et al., 2012; Davis, 1994; Stavert et al., 2014). Similarly, within dung resources, dung beetles will exploit a wide range of dung types from different mammals (Frank et al., 2018). While mammal dung is broadly accepted as a significant dietary resource for most dung beetles, systematic in-depth studies of the breadth of dung beetle diets are lacking in most ecosystems.

Due to their diet of dung and decomposing matter, dung beetles are important **decomposers** in many ecosystems. By removing nutrients from the soil surface in smaller quantities and relocating them below ground, dung beetles increase the surface area of dung available to microbes, they move nutrients closer to plant roots, and aerate the soil (Nichols et al., 2008b). This process is positively correlated with plant biomass. The increase scales with increased dung beetle abundance (Anderson et al., 2024). Increased plant biomass suggests that dung removal by dung beetles aids in the efficient turnover of nutrients. In addition to supporting increased plant biomass, dung removal decreases breeding habitat for pathogens in natural and agricultural settings. This became apparent in Australia when cattle were introduced. Cattle significantly increased wet dung production, which was too much for the native dung beetle population. Dung produced by native mammals was dry and in smaller quantities; the new inputs of voluminous wet dung built up on pastures, not allowing grass to grow. Due to the absence of dung beetles adapted to large quantities of moist dung, dung built up on the soil surface and washed into the waterways, polluting the freshwater sources. The excess dung also provided breeding grounds for *Musa vetustissima*, a fly that serves as a vector for *Chlamydia trachomatis*, bacteria that infects the eyes. Additionally, cattle gut parasite densities were high on the soil surface, causing increased contact between cattle and parasites.

This increased density of flies and parasites caused a myriad of health issues for both humans and livestock. The introduction of dung beetles native to Africa ameliorated the aforementioned issues of excess dung production by introduced cattle. This illustrates both the role of dung beetles and the consequences of extreme anthropogenic changes to the landscape. The results would be expected to be less extreme when the disturbance to the ecosystem is less intense. However, understanding the degree to which ecosystem services are changed by anthropogenic disturbance becomes more critical with the increasing global human populations. The current age of human influence has touched two thirds of all tropical forests and is only growing (Malhi et al., 2014; Pain et al., 2021a). Humans and livestock produce large amounts of feces with an estimated 425.1 metric tons in 2014, this will only rise with population growth (Berendes et al., 2018).

If dung beetles and other decomposers cannot recycle these nutrients due to dramatic changes in abiotic conditions, this represents a major loss in nutrients. Anthropogenic changes to the landscape, particularly deforestation for cattle ranching, change the abiotic environment dramatically, increase daily temperature fluctuations, and reduce humidity compared to forests. Abiotic factors have been shown at least at a local scale to be more important than mammal communities for dung beetle diversity (Gebert et al., 2020). This suggests that the forest conversion to pasture could reduce the diversity of dung beetles, and possibly result in a reduced efficiency in decomposing dung. However, mammal communities are undoubtedly important for dung beetle communities as the loss of mammals represents the loss of food resources, and thus the ecosystem's carrying capacity for dung beetles (Nichols et al., 2009).

Mammal dung is by far the most documented food source (Chiew et al., 2022; Correa et al., 2016; Da Silva and Dorneles-Audino, 2011; Davis, 1994; Davis et al., 2010; Frank et al., 2018 and references therein; Hanski and Cambefort, 1991; Holter and Scholtz, 2007; Raine and Slade, 2019 and references therein; Simmons and Ridsdill-Smith, 2011). As dung is a patchy and relatively rare resource, specialization on one or a few dung types would be a risky strategy. However, when the dung beetle lives on the dung producer, there are a few exceptions. For example, sloth dung is not attractive when used as bait for pitfall traps, nor fed on in laboratory settings. Despite this apparent lack of palatability, at least four dung beetle species live in sloth fur and use the fresh sloth dung when they defecate about once a week (Ratcliffe, 1980; Young, 1981). One species of dung beetle *Canthon aff. quadriguttatus* has been observed falling to the forest floor with the dung of brown titi monkey*,* *Callicebus brunneus*, and the dung of the bald-faced saki monkey, *Pithecia irrorata irrorata* (Jacobs et al., 2008). This ensures they get to the dung while it is fresh and before any other beetles.

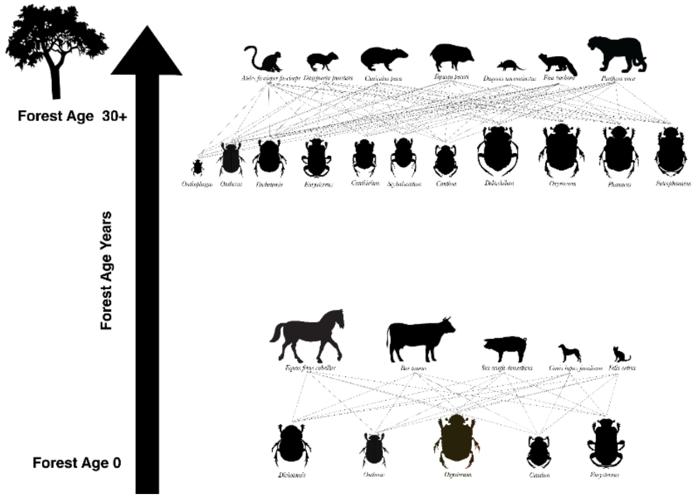

Figure 2 Hypothetical shift in dung beetle and mammal communities in relation to forests with different levels of human intervention. Older forests could be expected to support a greater diversity of beetles and a larger mammal population.

These are extreme examples of the use of mammal dung. Most dung beetles will use various mammal dung resources. This is often measured by studies use dung baited pitfall traps to study dung beetle diets. However, the use of pitfall traps has some limitations when it comes to the study of dung beetles. First, in most areas it is not feasible to collect dung from all the possible dung producers, second, while dung in the natural ecosystems is buried when it is discovered, usually shortly after it is produced. In contrast, dung in baited pitfall traps persists no matter how many dung beetles arrive and is placed in the ecosystem at the whims of the experimenter not necessarily when it would be produced. This means that pitfall traps measure attraction to dung but not necessarily access to dung. While attraction is undeniably an important component of natural dung use, a complementary method examining the gut content DNA or iDNA (invertebrate derived DNA) of dung beetles could provide a more complete picture of dung use by adult dung beetles in the ecosystem (Raine and Slade, 2019). Dung beetle gut contents could also provide information about which dung beetles are consuming and burying dung of fruit-feeding mammals, which has implications for **secondary seed dispersal**

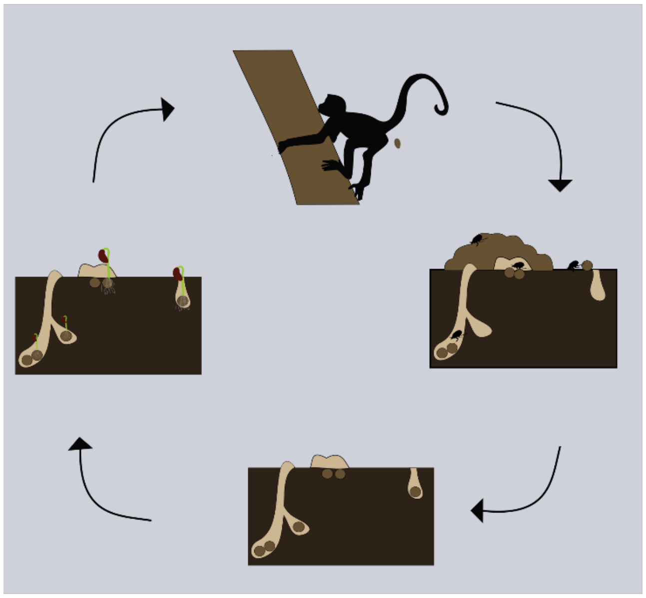

Figure 3 The hypothetical diagram of dung and seed fate after secondary dispersal by dung beetles.

Secondary seed dispersal occurs when seeds are first dispersed by a primary disperser (an animal that eats the fruit and then drops the seed away from the parent tree); the secondary disperser moves the seed a second time. When dung beetles bury dung (from the primary disperser), they move seeds within the dung (secondary dispersal) and the existing seed bank already in the soil (Vespa et al., 2025)(Fig 3). Zoochory (animal-mediated dispersal) is prevalent in tropical forests, with 65% to 100% of woody species dependent on animals for dispersal (Muller-Landau and Hardesty, 2005; Seidler and Plotkin, 2006). This high prevalence suggests that plants benefit from zoochory. One of the hypotheses for both high diversity in the tropics and high dependence on dispersers for moving seeds is the Janzen-Connell hypothesis. The hypothesis posits that increasing space between conspecifics increases survival by decreasing pathogen transmission and decreasing seed predation, which are increased by clumping seeds and seedlings together (Connell, 1970; Janzen, 1970). For example, seedlings that are clumped together under the parent tree can suffer extreme mortality when browsed by large herbivores (Pulido and Díaz, 2005). Thus, dispersal by animals, which creates space between seeds and subsequently seedlings, should be advantageous, as it can put large distances between parent trees and seeds. However, primary dispersal by birds, and large-bodied mammals may also deposit seeds close together (Vander Wall and Longland, 2004). Thus, secondary seed dispersal, which is often at a smaller scale, may further reduce clumping (Fig 4). When burying the dung of fruit-feeding mammals, dung beetles disperse seeds with their brood balls. The dispersal is incidental and does not benefit the beetle as the seeds are not consumed by the larvae (but see Pérez-Ramos et al., 2007). In addition to the reduced clumping, seed burial by dung beetles provides the seeds some protection from seed predators. Rodents are known seed predators, and seed burial even at depths as shallow as 1 cm has been shown to reduce seed predation by rodents from 95% to 98% (Beaune et al., 2012; Shepherd and Chapman, 1998; Vulinec, 2002).

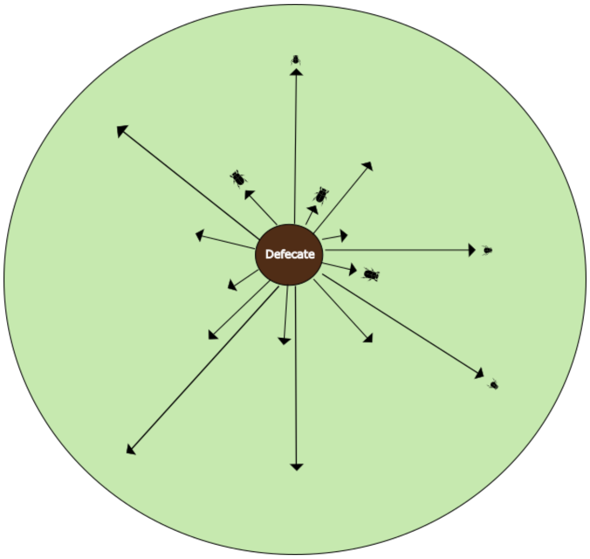

Figure 4 Hypothetical diagram with a defecate in the center and arrows which represent the possible trajectories of dung beetles as they move feces with seeds. The green area represents the area where seeds can be found after secondary dispersal, and the brown circle is the area before secondary dispersal. Even short secondary dispersal distances can significantly decrease seed clumping.

Dung beetles have been shown to improve seedling recruitment (Culot et al., 2018), possibly due to reduced seed clumping and predation. This leads to reduced clumping of seedling distributions (Lawson et al., 2012). The dung beetle community changes the proportion of seeds that are secondarily dispersed, with larger beetles dispersing more seeds (Morales-

Alba et al., 2022), and more diverse dung beetle community also increases the rates of seed dispersal (Milotić et al., 2019). While larger beetles bury more seeds (Morales-Alba et al., 2022), they also create deeper tunnels, which results in deeper burial depths. Deeper burial depth is inversely proportional to seed survival (Andresen and Levey, 2004a; Shepherd and Chapman, 1998). When buried too deep, seeds do not survive, so the relationship between seeds and beetles is not without risk to the seeds, and has been shown to be detrimental in some cases (deCastro-Arrazola et al., 2020). However, burial depths by dung beetles tend to be shallow overall, with most seeds buried in the first 2 cm (Shepherd and Chapman, 1998).

Dung beetle community composition is not the only significant variable in seed dispersal, **seed traits** influence the likelihood of seed dispersal by dung beetles. Such traits include seed size (Andresen, 2002; Andresen and Levey, 2004a; Feer, 1999), seed odor (Midgley et al., 2021, 2015), and seed abundance (Pérez-Ramos et al., 2007). Seed size is an important trait because Dung beetles have an upper limit to the size of seeds they will include in their dungballs. Small seeds are usually not removed, increasing the dispersal rate of small seeds by dung beetles. Seed odor as a seed trait encouraging seed dispersal by dung beetles has been documented for *Ceratocaryum argenteus*. Which has a volatile profile similar to herbivore dung, and dung beetles have been observed rolling these seeds like dungballs in South Africa (Midgley et al., 2021, 2015). Seed abundance has been documented as an important factor in the dispersal of acorns from *Quercus suber* and *Quercus canariensis* by dung beetles in Spain. Acorns are abundant in the fall when consumed and dispersed by *Thorectes lusitanicus*. With approximately 50% of the seeds buried in a viable state (Pérez-Ramos et al., 2007). These exceptions beg the question, are other seed traits overlooked in the study of seed dispersal by dung beetles?

With the current rapid rate of habitat destruction, species abundance and diversity are also declining. Leading to an unraveling of ecological networks and a loss of ecosystem services. Dung beetles are no exception. In addition to their role in ecosystem processes dung beetles are good indicator species of ecosystem health because they are sensitive to abiotic conditions and shifts in animal communities (Raine and Slade, 2019). The current rate of ecosystem loss is astounding with a 47% reduction in natural ecosystems from pre-industrial baselines as of 2019 with a projected increase. This extreme loss of habitat among other anthropogenic disturbances has resulted in 25% of our planets extant species being at risk of extinction ("The global assessment report of the intergovernmental science-policy platform on biodiversity and ecosystem services," 2019). The loss of species results in further degradation of ecosystems and **ecological networks** (Heleno et al., 2020). This translates into a loss of ecosystem services and ecosystem processes.

The role of deforestation in climate change has brought concern for vanishing forest ecosystems to the forefront (Busch et al., 2019). Two-thirds of all forests have experienced major human intervention, and are now classified as secondary forests (Pain et al., 2021b). These forests often differ from those that have either experienced limited or no measurable disturbance in terms of community composition, diversity, above ground tree biomass, among others (Escobar et al., 2024; Hanberry et al., 2016; Malhi et al., 2014). For this reason, conservation efforts have centered around the restoration of forests to a state that is similar to pre-disturbance (Pain et al., 2021b; Schmitt et al., 2009). Allowing the ecosystem to reassemble naturally (passive restoration) is becoming increasingly interesting to researchers because it is cost effective (Busch et al., 2024). However, it is important to know if the community will recover after anthropogenic disturbance, and if so, how quickly, or is human intervention a requirement. For this reason, it is crucial to investigate the effectiveness of passive recovery. Dung beetles are a group of organisms that are informative to study in the context of forest recovery because they serve as indicator species for ecosystem health and participate in two important ecological processes 1) decomposition, and 2) seed dispersal, and have been shown to increase plant biomass.

Research Questions and Goals

I have highlighted the need for extended research on dung beetles, their role in ecological processes, and the resilience of communities within recovering ecosystems in a few key areas. 1) The spectrum of resources used by dung beetles for food, 2) the use of iDNA to study adult dung beetle diets to confirm the natural origin of mammal-derived resources, and 3) seed traits that encourage seed dispersal by dung beetles. The subsequent chapters each look at one of these aspects in more detail, questions are enumerated below. All these studies were conducted in the Ecuadorian Chocó. The study area is a patchy mix of cattle pastures, cacao plantations, abandoned agricultural land (passive forest recovery), and historically intact forest. Study sites are within the two neighboring reserves (Tesoro Escondido and Reserva Canandé) and private land. The area's extent was approximately 130 km². The site was a lowland tropical forest on the western side of the Andes with a moderate climate (22 °C - 23 °C with high rainfall,3000 - 6000 mm per year) (Fig 5)(Escobar et al., 2024).

Specific Research Questions

1) How do multi-resource dung beetle networks reform in response to habitat recovery?
2) How do mammal communities shift across the landscape in response to habitat recovery?
3) Can we use iDNA to identify dung beetle meals and create dung beetle–mammal dung networks?
4) For dung beetles using monkey dung as a resource, which seed traits are important in dispersal?

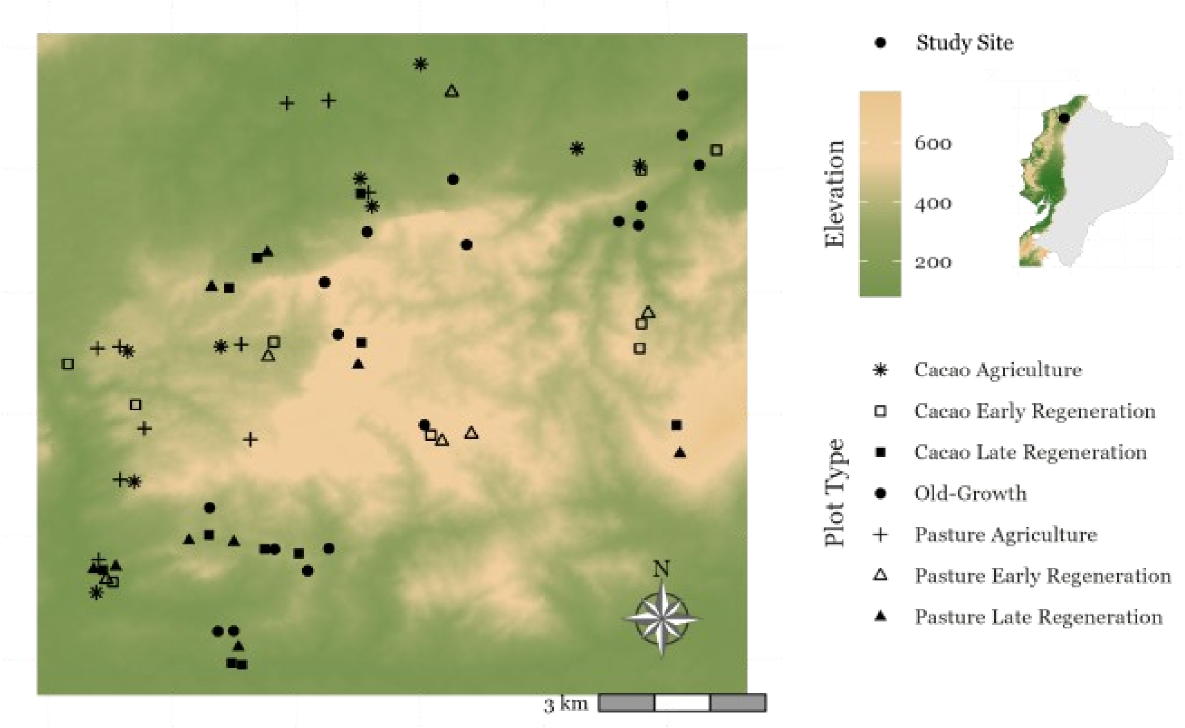

Figure 5 Depiction of Study region and sample sites. Th top right Ecuador is depicted with a circle indicating the study site. Elevation is indicated by color and only the Chocó is filled in. To the left within the larger square, the sample sites are indicated with symbols. The symbols correspond to the type of sample site and is labeled as plot type in the legend (Appendix 1 Johann et al., resubmission).

Chapter:1 Dung beetle diets and land use

Synopsis

Dung beetles consume and recycle resources (Hanski and Cambefort, 1991). Understanding which resources, they will use, which species in the community use those resources, and in which habitat types they use which resources is fundamental to understanding their role in an ecosystem and the ecosystem services dung beetles provide. This is a question of dung beetle-resource networks in response to habitat type, and the implications for decomposition of rotting material (Appendix 1).

Despite the importance of nutrient recycling for ecosystems, few studies have used multiple resources to study diet breadth in dung beetles and fewer in response to habitat (Correa et al., 2023; Da Silva et al., 2012; Davis, 1994). We provide a study aimed at rectifying the knowledge gap by studying dung beetle diet breadths in the Ecuadorian Chocó with respect to forest recovery stage. Specifically, we focus our research on the following 1) quantifying resource use in terms of abundance of beetles and diversity of beetles, 2) niche breadth in response to multiple resources in contrast with published multiple mammal-dung types in terms of network specialization (H_2'), and 3) the change in network metrics including abundance (N), diversity (H'), complexity (H_2) and specialization (H_2') respond to habitat recovery.

Our study area is a musique of pastures, cacao plantations, abandoned agricultural land in various stages of recovery, and uncut forest providing a variety of habitats with little geographic variation. We selected resources with previous literature support including mammal dung, carrion, and fermented fruit, in addition we included rotten millipedes because we observed their use by dung beetles in the field. Over two field seasons of three months each, we sampled 72 sites (50 m x 50 m). Those sites can be broken down into habitat categories: active cacao (n = 8), active pasture (n = 8), cacao regeneration (n = 21), pasture regeneration (n = 17), old-growth forest (n = 18). Regeneration signifies that the study site was either historically a cacao plantation or a pasture and was abandoned and then allowed to regrow naturally. Time since abandonment ranges from 1 – 37 years in the past (full site description in Escobar et al., 2024). Within each site we set pitfall traps with one of four resources as baits including cow dung, carrion, rotten millipede, and fermented fruit. After 48 hours we returned to the site collected the beetles and then transported them back to the lab, where we sorted and identified them to (morpho)species.

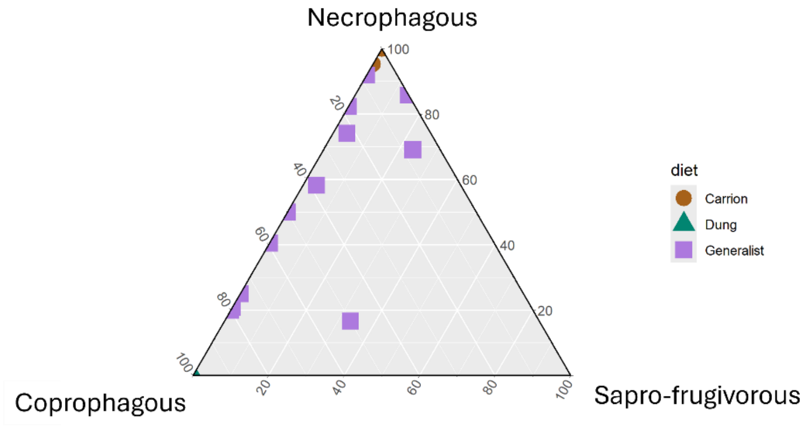

Figure 6 Ternary plot shows the relative makeup of resource categories in the diet of the dung beetle species studied. Each point represents a species. Species with the same resource use are plotted on top of each other. Colors distinguish resource use: dung specialists in green triangles, carrion specialists in brown circles, and generalists in purple squares. To qualify as a specialist 95 % of the captures of the beetle species needed to occur in the pitfall traps with baits of that category.

The dung beetle community in our study region is not resource category specific. Meaning that most species were attracted to multiple bait types. Of the 23 (morpho)species four are coprophagus , seven are necrophagus (vertebrate or arthropod) , and the other 12 feed generally on available resources (Fig 6).

The dung beetle communities in the pasture and in the old growth were different from each other with the recovering forest community an intermedium (Appendix 1 Figure 4). The abundance and diversity of dung beetles increased with forest age. These metrics increase faster in the sites that were previously cacao plantations than in the sites that were previously grazing area for cattle (Appendix 1 Figure 5a and Figure 5b). Dung, carrion and rotten millipede all capture greater beetle abundances and diversities with forest age. The fermented fruit baits did not capture enough beetles to make any conclusions though the fitted line is flat. Low capture rates by fermented fruit in all habitats suggest that dung beetles are not particularly important recyclers of this resource. Dung is the most attractive bait type in pastures and continues to capture beetles into the forest. The abundance of carrion-feeding beetles increased dramatically in the forest. While not previously used in multi-resource studies, rotten millipede was a surprisingly attractive bait type with diversity and abundance similar to that of dung (Appendix 1 Figure 2 and Figure 3). The number and diversity of beetles increased the network complexity (number and diversity of links between resources and beetle species). This is similar to the null model, suggesting that this pattern is abundance-driven. Additionally, network complexity increases with forest age at the same rate as would be predicted by the null model of the networks. However, the networks are more specialized than the null model would predict for the diversity and abundance of beetles sampled (Appendix 1 Figure 5c and Figure 5d). This implies that dung beetle communities will be more effective decomposers in older forests where they have higher diversity and abundance, thus enough individuals and enough different species to recycle available resources. Carrion traps captured very few beetles in agriculture, which implies that dung beetles are unlikely to be effective carrion recyclers in agriculture. Similarly, the low dung beetle abundance in the pasture also suggests the community is ineffective in recycling dung in this habitat. However, the network complexity increased as the forest recovered suggesting that the community can recover with the recovering forest and will reestablish their role as decomposers of carrion and mammal dung. Low abundance and diversity of dung beetles in agriculture particularly pastures could be due to two factors 1) resource limitations with fewer resources available in pastures or 2) due to the relatively short period of more intensive land use (~50 years) they are not yet able to tolerate the harsher abiotic conditions of more open habitats (Appendix 1).

Chapter 2: Camera traps to estimate the relative abundance of mammal resources for dung beetle consumption

Synopsis

Mammal dung is an essential resource in all habitats in our study site (Chapter 1). Understanding the distribution of large and medium-bodied mammals could indicate the dung availability across the chronosequence, in addition to providing important information for biological monitoring to both local reserves. Studies like this can provide information about the use of habitat by species and possibly the relative abundances of mammal dung. Camera traps were place in 57 of the study plots (Appendix 2 Figure1) and collected after three weeks. Vertebrate species were identified, and the number of capture events was quantified independent event determined by the time interval between captures. The cameras captured 24 mammal species two of those mammals species are classified as vulnerable by the IUCN Red list *Tayassu pecari* (White lipped pecari), and *Cebus capucinus* (Colombian white-faced capuchin), two species are near threatened *Leopardus wiedii* (margay), and *Panthera onca* (jaguar). The mammal community shifted from domestic and livestock-dominated agriculture to native animal communities in older forests (Appendix 2, Figure 3, and Figure 4). Similarly, the proportion of biomass from domestic and livestock animals declined as the forest recovered from agriculture. Wild animal biomass showed the opposite trend and increased with forest age (Appendix 2 Figure 3). Wild mammals were not habitat specialists and were found across habitats. Domestic animals and livestock are more spatially

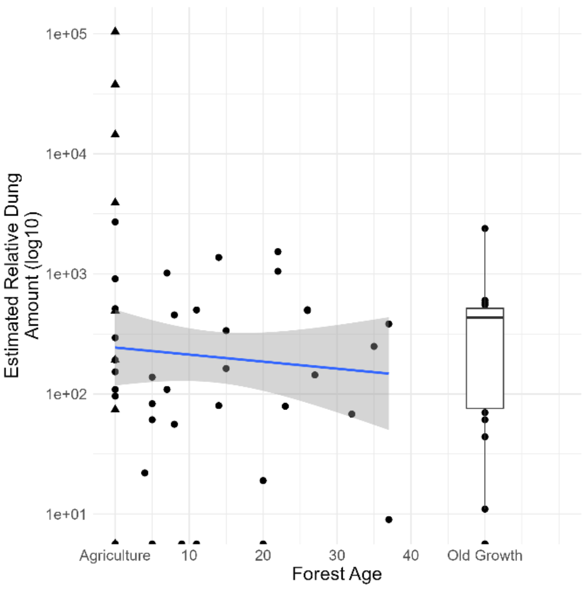

Figure 7 Scatter plot with estimated relative dung availability predicted by forest age. Box plot visualizes the old growth reference sites. A linear model (blue line) was fitted to the data with the 95% CI indicated by gray shading. Each black symbol represents the estimated dung availability of a single plot, pasture plots are indicated by triangles, all other plots are indicated with circles ($R^2 = 0.04$, p-value = 0.06).

restricted. Dung availability may partially explain dung beetle abundance. While not included in Appendix 2, I calculated dung production by mammals from these data and used metabolic rate as a proxy for dung production (formula for metabolic rate from Glazier, 2008).Forest recovery age was not a significate predictor of estimated dung availability ($R^2 = 0.04$, p-value = 0.06). However, estimated dung availability is highest in agriculture where dung beetle abundances are lowest (Figure 7, Chapter 1). This high dung production in agriculture is probably driven by cattle. The biomass of a cow (*Bos taurus*) is 9 times that of the largest wild animal, a jaguar (*Panthera onca*). This could indicate that environmental variables rather than resource availability may be responsible for the low abundance of dung beetles in agriculture. A closer examination of the gut contents of dung beetles could illuminate this further. The gut contents could show if dung beetles are not consuming livestock dung in the same habitat where the livestock is present.

Chapter 3: iDNA from dung beetles

Synopsis

Dung is an important component in dung beetle diets; previously, we estimated dung availability and dung producers with camera traps (Chapter 2). To understand in a bit more detail which dung types are used by dung beetles we worked on a method to identify mammal DNA in the digestive systems of dung beetles caught in pitfall traps. This allows for more detailed study of the dung beetle mammal networks, which advances our understanding of which beetles are recycling the dung of which mammals and if there is any dietary specialization due to lack of access e.g. diurnal beetles are probably the first to access dung from diurnal mammals similarly for corpuscular or nocturnal pairings of beetles and mammals. This would also indicate if some mammal dung types are not being used. In addition to an improved understanding of dung beetle diets the method has potential applications for biological monitoring. The use of baits draws dung beetles from the surrounding area that have likely used the local mammal resources, collecting the DNA locally. This method should be less sensitive to site selection than camera traps, which can produce very different results depending on site selection methods. For example, a camera traps set near a hiking trail captures many more mammals than those set up in random locations (Kolowski and Forrester, 2017).

To test if it was possible to amplify mammal DNA from the gut contents of dung beetles, we collected a list of published mammal-specific primers and then tested them with mammal DNA extracts. We narrowed down the preliminary list of primers to two: 1) vertebrate 12S rDNA primers (Ushio et al., 2017) and mammal-specific primers 16smama1 (forward) and 16smama2 (reverse) (Taylor, 1996). We then tested if we could identify a known mammal dung meal from the digestive tracts of dung beetles. With this objective, we asked the local zoo (Vivarium Darmstadt) for dung from several mammals. They provided the following five dung types: tapir (*Tapirus terrestris*), fennec fox (*Vulpes zerda*), otter (*Aonyx cinerea*), porcupine (*Hystrix cristata*), macaque (*Macaca nigra*), we also collected cow (*Bos taurus*) dung. Dung was then provided to dung beetles. After feeding the dung beetles were euthanized in the freezer. We then washed the outside of the beetles with a chlorine solution to remove DNA from the surface and removed the digestive tract. The digestive tracts were then extracted, the DNA amplified and sequenced, and then, using the MegaBLAST search algorithm, the mammal dung provided was checked to ensure that it matched the mammal sequence. Matches are accurate to the genus level due to the short target sequences. Once proof of concept was established, we began to use dung beetles from pitfall traps.

We extracted, amplified, and sequenced the iDNA from dung beetles captured by pitfall traps in Germany and Ecuador. In about 35 % of the beetle digestive tracks, we were able to identify mammal meals. Success was higher in Germany, where beetles were frozen, not preserved in EtOH. Surprisingly, dung beetles in Germany captured large amounts of DNA from rodents (Appendix 1 Figure 1). This could be explained in two ways: 1) that dung beetle diets, at least that of adults, are shifting to smaller mammals as larger mammals become less abundant, or 2) that the omission of rodent dung from most pitfall trap experiments has provided incomplete information about dung beetle diets. Either way, this indicates that, at least in Germany, dung beetles could provide more complete mammal community data than camera traps, which do not sample rodents with the same efficiency as medium to large-bodied mammals. In Ecuador, we did find a diurnal, nocturnal split in the dung beetle-mammal networks, suggesting that opportunity might be just as important as attraction in dung beetle diets (Appendix 1 Figure 2). In both Germany and Ecuador, network specialization is higher than that measured by global networks of pitfall traps (Frank et al., 2018), suggesting that attraction to dung types may differ from dung use in natural environments. Monkey dung also appears to be an important resource for dung beetles in our site in Ecuador, suggesting that dung beetles could be important secondary seed dispersers.

Chapter 4: Seed traits and secondary seed dispersal

Synopsis

The results of our gut content analysis suggest that diurnal dung beetles might be more important in the secondary dispersal of seeds in primate dung (Chapter 3). Primates are widely accepted as important seed dispersers in tropical forests (Andresen et al., 2018). Primates are primary dispersers of seeds, when dung beetles create dungballs and move them, they act as secondary dispersers. Seed dispersal is thought to be advantageous for the seeds, by creating space between seeds of the same species, thus reducing pathogen transfer and competition for resources. The fate of these seeds potentially influences forest recovery dynamics and seedling recruitment (Nichols et al., 2008a). Not all seeds are equally likely to be dispersed, seed size is a trait known to influence seed dispersal (Andresen and Levey, 2004b). With larger seeds dispersed by larger beetles, and smaller beetles excluding or unable to disperse large seeds (Morales-Alba et al., 2022) Seed burial could potentially improve seed survival by decreasing the chance that a seed is subject to predation (Beaune et al., 2012).

To quantify which seeds are dispersed by which beetles we opportunistically sampled primate dung, collecting intact dung samples to provide a baseline for the number of seeds and the range of seed sizes found in local primate feces. These feces were collected from two of the three local primate species the brown-headed spider monkeys (*Ateles fusciceps fusciceps*) and the mantled howler monkeys (*Alouatta palliata*). Then, to quantify and characterize the seeds in dungballs, we observed primates until they defecated and observed the dung on the forest floor until dung beetles arrived. When a dung beetle formed its ball, the ball was then collected. From these dungballs, we quantified the number of seeds in the dungballs and the seed lengths, widths, and surface characters. We found that seed length and seed surface were good predictors for inclusion in dungballs. Seeds with hairs on their surface were more likely to be dispersed by dung beetles, particularly the larger seeds. We suggest that the hairs make the seed harder to distinguish from the dung, increasing its chances of dispersal (Appendix 4 Figure 1 and Figure 2). We also show that the proportion of large-seeded morphospecies are more likely to have hairs than smaller seeds, which are likely to be included in dungballs regardless of surface characteristics (Appendix 4 Figure 4b).

Discussion

A majority of forest ecosystems have experienced intense recent human disruption(Malhi et al., 2014; “The global assessment report of the intergovernmental science-policy platform on biodiversity and ecosystem services,” 2019) that results in dramatic shifts in ecosystem dynamics (Kaiser-Bunbury et al., 2017; Potapov et al., 2024), it becomes more important for those responsible for the conservation of biodiversity and the maintenance of human wellbeing to understand from what level of intervention ecosystems maintain functionality (“The global assessment report of the intergovernmental science-policy platform on biodiversity and ecosystem services,” 2019) and if the functionality is lost how long does it take to recover if ever(Dashti et al., 2024; Turner et al., 2019). Network metrics provide a tool for the understanding of forest recovery, and the reassembly of ecosystem functions and processes(Chiew et al., 2022; Kaiser-Bunbury et al., 2017, Chapter 1). In the case of dung beetles, they catalyze the process of decomposition specifically of dung, carrion, and other rotting resources (Escobar et al., 2024). They also incidentally change seed dispersal and seedling establishment dynamics.

With the level of human intervention present in forests within our study site in the Ecuadorian Chocó we find that all network metrics indicate recovery, from this information, we can extrapolate that not only does the species community recover to the old growth reference state, but the ecological processes provided by dung beetles due to their resource use also recover. We can also surmise from this that our study site is not yet so degraded that it cannot recover. However, in early stages of forest recovery dung beetles are less abundant, with more specialized networks. Reduced abundance and increased network specialization in degraded habitats indicate that the loss of forest habitat reduces decomposition aided by dung beetles of dung and carrion resources. The reason for the loss of dung beetle functionality could be due to either intolerance of the abiotic conditions or the loss of resources.

While we could not map all resources, we could map large and medium-bodied mammal resources with camera traps (Chapter 2). Large and medium vertebrates represent significant inputs of both carrion and dung resources (Bartel et al., 2023; Berendes et al., 2018), so they could be used as a proxy for the abundance of resources important to dung beetles. The estimated biomass available and dung production of those animals show that dung resources are evenly distributed across habitats, except for pastures where cows and horses provide extremely high densities coupled with high individual biomass and, thus, a high relative abundance of dung resources compared to other habitats (Chapter 2). Carrion resources from livestock may not be directly related to the relative biomass of the vertebrate community because they might be used for meat. Dung beetle abundances are lowest in the pastures, suggesting that livestock dung, while most abundant, is not used or that dung beetles cannot survive the abiotic conditions in the pastures (Chapter 1, Chapter 2). In the south of Ecuador we found, *Dichotomous divergence* exhibits morphological adaptations to harsher abiotic conditions in open habitats (Marín-Armijos et al., 2023), suggesting that with enough time, abundance might increase to match resources. As the study site has only recently been deforested (Escobar et al., 2024), it is possible that dung beetles have not yet had enough generations to adapt to more open habitats.

The gut contents DNA of dung beetles may allow us to more precisely track which mammals serve as the most important sources of nutrients for dung beetles. With enough beetles, we could track which species consume dung from livestock and other domestic mammals. Preliminary results in Ecuador did not show the use of domestic animals however, sampling is too low to draw any conclusions (Chapter 3). Results did show that some dung types like rodent have possibly been overlooked in pitfall trap studies and may be more important previously thought (Chapter 3, Appendix 3 Figure 2). We also see evidence of a diurnal-nocturnal split in the tropical beetles (Chapter 3, Appendix 3 Figure 1). This suggests that there is a need to maintain both diurnal and nocturnal dung beetle communities for the preservation of efficient dung recycling. Conversely, these results may also indicate that the loss of either diurnal or nocturnal mammals could result in a loss of that part of the dung beetle community. Diurnal domestic animals dominate pastures and agricultural systems; this could provide an explanation for the reduced diversity and abundance of dung beetles in those habitats (Chapter 1, Chapter 2).

The distribution of dung beetles and fruit-feeding mammals is also important for forest recovery in degraded landscapes. Native animals consuming forest fruits as a large part of their diet play an important role in seed dispersal (Muller-Landau and Hardesty, 2005; Seidler and Plotkin, 2006). Of the 18 native mammal species from the camera traps (Chapter 2), 12 consume fruit as part of their diet (Wilman et al., 2014), making them potential seed dispersers. The limited relative abundance of native mammals in agriculture suggests that they are likely to disperse fewer seeds in these ecosystems (Chapter 2). This indicates that this seed dispersal, like dung decomposition, also depreciates in disturbed sites. This could be further compounded by the loss of arboreal mammals, particularly primates. Spider and howler monkeys are important seed dispersers (Andresen et al., 2018) but depend on trees (Gregory et al., 2017). Both howler monkey and spider monkey DNA were common in the guts of the dung beetles we sampled (Chapter 3, Appendix 3 Figure 1). This suggests that they are an important dung source and that dung beetles are likely to be secondary dispersers of the seeds in howler and spider monkey feces in our study site.

We were able to observe several instances of the dispersal of seeds in monkey dung by dung beetles. Suggesting that this is indeed a common interaction. Our finding that larger seeds (from several genera) are more likely to have a hairy surface that might make it harder to distinguish them from dung also suggests that this relationship could be important for seed survival and thus maintaining the local ecosystem dynamics (Chapter 4, Appendix 4 Figure 3a, Figure 4b, Figure 5b).

Conclusion

Our findings suggest that despite anthropogenic disturbance, the studied forest ecosystem in the Ecuadorian Chocó retains the potential for recovery to the old-growth reference state. We base this conclusion on the network metrics used to quantify the increased abundance, diversity, network complexity, and decreased network specialization of the dung beetle communities in response to forest recovery. While dung beetles play a critical role in the decomposition of mammal dung in all habitats and carrion in forested habitats, their reduced abundance and increased specialization in degraded habitats highlight the challenges of early-stage forest recovery (Chapter 1). Similarly, this has implications for seed dispersal, which is likely to increase with forest age as the number of dung beetles increases, so should the number of seeds dispersed (Milotić et al., 2019, Chapter 1, Chapter 4). The presence of complete mammal communities, particularly from native frugivores (Chapter 2), is crucial for maintaining ecosystem functions. Given the interactions between dung beetles, vertebrate communities, and our results here (Chapter 3), conservation efforts should prioritize increasing the area devoted to passive recovery and ensure protection of older regenerating forests. This will protect the species of communities that drive ecological processes.

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Appendix 1

The below text is from a resubmission to the Journal for Animal Ecology.

Multi-Resource Dung Beetle Networks within the Context of Forest Recovery

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Conflict of Interest

The authors have no conflicts of interest

Author Contributions

Jan Johann, Karen Marie Pedersen, Nico Blüthgen and Thomas Schmitt conceived the ideas and designed methodology; Jan Johann, Karen Marie Pedersen, Diego Marin-Armijos, and Silvia Canelos Raimann collected the data; Jan Johann, Karen Marie Pedersen, Silvia Canelos Raimann, and Nico Blüthgen analysed the data; Jan Johann, Karen Marie Pedersen, Nico Blüthgen led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication. Statement on inclusion: Our study involved people from both within the country where the study was conducted as well as researchers from abroad.

Data availability statement

Data will be made publicly available on Zenodo upon manuscript acceptance.

Abstract

1. Global climate change and agricultural expansion threaten the functioning of many ecological networks. However, little is known about their ability to recover after disturbances such as agriculture. Here, we describe the dynamics involved in dung beetle food web reassembly in secondary forests after the cession of agricultural practice in the Ecuadorian Chocó.
2. While many studies compared dung beetle preferences for different types of dung, the importance of other resources was often overlooked. We provide multi-resource food webs based on herbivore dung, vertebrate carrion, fermented fruit, and millipede carcass. Samples were collected from four habitat types: agriculture, early regeneration, late regeneration, and old growth. Collections were made with baited pitfall traps over two three-month periods. In addition, we compare our findings with seven other multi-resource dung beetle studies.

3. In agricultural sites, dung captured both the greatest abundance and diversity of dung beetles. Beetle abundance (but not diversity) was highest in secondary forests for vertebrate and invertebrate carrion.
4. Dung beetle abundance, diversity, and food webs in cacao plantations were more similar to old forests and recovered more quickly than in pastures. In contrast, pastures had a smaller portion of the beetle community and simpler webs, resulting in delayed regeneration.
5. The recovery of the food webs mirrored the recovery of dung beetle abundance and diversity. Network complexity was driven by the abundance of beetles. As a result, webs regained pre-disturbance levels as fast as the dung beetle community recovered
6. Food webs in patches of small-scale agriculture in proximity to old-growth forests have a high potential for rapid recovery. In this context, cacao plantations have an advantage over pastures due to increased resistance and resilience.
7. Studying trophic webs or interaction networks allows us to quantitatively predict shifts in ecosystem processes and their recovery. Dung beetles feeding on carrion require forest cover and, thus, are not effective carrion decomposers in grasslands. This may be characteristic of ecosystem processes that depend more strongly on species with narrow habitats or dietary niches.

Keywords

Brown food webs, carrion, different baits, millipedes, resistance, resilience, trophic webs, complexity

1 Introduction

Globally, food webs are at risk of partial collapse due to increasing mean global temperature and drought, habitat conversion and fragmentation, and over-exploitation of resources (Heleno et al. 2020). This is of particular importance for tropical ecosystems because the loss of tree cover is ubiquitous in equatorial regions (Hansen et al. 2013). In Latin America, a substantial decline in tree cover is attributed to an ongoing shift in agricultural practice (Curtis et al. 2018). This could be exacerbated by the expected agricultural expansion (Newbold et al. 2020). An important but unresolved question is whether degraded food webs recover to pre-disturbance levels in regenerating forests.

Ecological functioning at the ecosystem scale is determined by the interactions and trophic relationships between species rather than biodiversity alone (Van Der Putten et al. 2004; Thébault & Loreau 2006; Tylianakis & Morris 2017). Such networks of species interactions are not static and are subject to change for stochastic and environmental reasons. Environmental change, habitat conversion, or its reversal through ecosystem restoration may lead to a shift in interaction frequencies or to one species taking the place of another (Kaiser-Bunbury & Blüthgen 2015; Tylianakis & Morris 2017). For example, habitat restoration by removal of invasive plants has led to more complex pollinator networks and improved fruit sets of native plants (Kaiser-Bunbury et al. 2017). Global comparisons of networks (e.g., Schleuning et al. 2012; Morris et al. 2014) started to unravel variation in specialization but did not consider global change and land use impacts thus far unlike studies on biodiversity (e.g., Outhwaite et al. 2022). The study of global change impacts on trophic interactions is relatively recent (Heleno et al. 2020) in ecology and is still poor in large-scale data to make quantitative global predictions. The resolution of network data is especially low in tropical regions (Poisot et al. 2021). Consequently, forecasting the network responses in these habitats to the environmental changes we face (IPCC 2021) remains a complicated task.

The brown food web (network) could be substantially altered by habitat conversion. Deforestation leads to changes in abiotic variables, including temperature and humidity and their variability. These changes can affect and alter the decomposer communities. Decomposers are important in recycling the nutrients from previously living tissues, including dead plant matter, carrion, and dung. The importance of recycling plant matter is a well-studied aspect of decomposition (Potapov et al. 2024). Additionally, the recycling of dung and carrion has also been shown to be important for ecosystem functioning (Mathison & Ditrich 1999; Nichols et al. 2007; Barton et al. 2013; Arnberg et al. 2024). With these resources projected to increase globally, understanding their role in networks becomes increasingly important.

Worldwide feces production by humans and livestock was estimated to be 425.1 metric tons in 2014 and would only be expected to rise with increasing global populations (Berendes et al. 2018). If this is not decomposed, it would represent a huge loss in nutrients and increase parasite loads (Barton et al. 2013; Doube 2018). Carrion is another resource for which decomposition has ecosystem-wide implications. Domestic animal carrion is estimated at 630 metric tons (Bartel et al. 2023). As deforestation continues to convert forests to agriculture, this number will only increase. Domestic animals and humans account for much more biomass than wild animals (Bar-On et al. 2018), which only contribute an estimated 20 metric tons of carrion per year (Bartel et al. 2023). This could mean rising domestic animal biomass decomposition becomes more important with an increase in anthropogenic land use, and the ability of decomposers to survive and function in the changing habitats becomes essential. All of this applies to dung beetles. They aid in the decomposition of ephemeral resources such as dung, carrion (vertebrate and arthropod), and fermented fruits (Hanski and Cambefort 1991; Halffter & Halffter 2009; Amézquita and Favila 2010).

Changes in land use – including habitat conversion and ecosystem restoration – not only affect the abiotic environment important to dung beetles but also their biotic environment. At large scale, dung beetles have been linked to dung-providing mammal communities, and it is generally accepted that by changing the mammal community, the dung beetle community will follow (Nichols et al. 2009; Raine et al. 2018; Raine and Slade 2019; Bogoni et al. 2019). This is because large and medium bodied mammals are a major source of nutrition for both adult and larval dung beetles (Nichols et al. 2009). As the mammal community recovers, it would be reasonable to expect that the dung beetle community would also recover. Currently, mammal communities are also shifting due to shifts in land use and hunting, and a global loss of predators. Predators change the timing and input of carrion into the ecosystem (Barton et al. 2019). The loss of large mammals could have cascading effects on dung beetles and the ecosystem services that they provide (Nichols et al. 2009). This is because the loss of large mammals leads to a decrease in dietary resources for dung beetles. However, abiotic factors have also been shown to be important for dung beetle diversity and abundance. Along an elevation gradient, dung beetle diversity and abundance were more closely linked to abiotic variables such as temperature and precipitation than to mammal dung availability (Gebert et al. 2020). As agricultural landscapes have both higher temperatures and lower humidity, agricultural landscapes may have a glut of resources but may be inhospitable to dung beetles, potentially leaving a gap in the decomposer community in local habitats because of both the changes to the mammal community and resources and abiotic factors. How these changes to land use affect dung beetle communities and how dung beetle trophic networks respond to forest recovery is not well understood. This is likely to depend upon how much of the original community remains post-disturbance (**resistance**) and how fast that community recovers (**resilience**). Network complexity, or link diversity, should be lower with fewer species and lower abundances, suggesting that habitat regeneration will be linked to increasing network complexity.

Dung is the most studied food resource for dung beetles because it is a dominant food source in many communities and species (Hanski and Cambefort 1991; Davis 1994; Holter and Scholtz 2007; Scholtz et al. 2009; Davis et al. 2010; Da Silva and Dornelse 2011; Correa et al. 2016; Frank et al. 2018a; Raine and Slade 2019; Chiew et al. 2022). The majority of dung beetles consume dung as adults and use it to form brood balls (Holter and Scholtz 2007). Dung is produced in large quantities and contains parasites which, if not removed from the soil surface, could have negative consequences for the local community (Mathison and Ditrich 1999; Doube 2018). In addition, dung beetles are also important for carrion decomposition, particularly in the absence of large herbivores and when there are few other carrion-consuming arthropods (Hanski and Cambefort 1991; Scholtz et al. 2009). The similar nutrient content of omnivore and carnivore dung to carrion likely facilitated this shift (Halffter and Matthews 1966). Dung beetles have also been observed feeding upon dead or injured millipedes (Schmitt et al. 2004; Fuentes-Jacques et al. 2023; Nganhane et al. 2023). This is both in the form of arthropod carrion and due to an attraction to defensive secretions (Schmitt et al. 2004, Karimbumkara and Priyadarsanan 2016). The ancestral lineage of dung beetles is hypothesized to have consumed rotting plant material, and this has reemerged multiple times in the consumption of rotting fruit and seeds by several species of dung beetles (Halffter and Halffter 2009). However, the decomposition of non-dung resources in response to forest recovery is not well studied.

Anthropogenic changes to the landscape have been linked to shifts in dung beetle communities (Klein 1989; Vulinec 2002; Scheffler 2005; Frank et al. 2017). For example, disturbed forests have fewer large tunneling species. This reduces the efficiency with which dung beetle communities recycle decomposing materials, as larger tunnelers process large amounts of dung (Nervo et al. 2014; López-Bedoya et al. 2022). Some dung beetle species are forest-dependent, while others prefer grasslands (Kunz & Krell 2011; Frank et al. 2017; Da Silva et al. 2019). In areas where both communities are present, deforestation could be expected to favor grassland species. However, in areas without historic grasslands, it is unclear how the network interactions might shift as a response to the conversion of native habitats to anthropogenic spaces. One consequence of a shifting community with respect to habitat disturbance could be altered resource exploitation by the dung beetle community. There has been limited research on dung beetle trophic networks in response to disturbance (Chiew et al. 2022).

Globally, dung beetle-dung networks have a relatively low level of specialization (Frank et al. 2018b). However, it could be expected that multi-resource networks are more specialized than dung networks because the resources are more dissimilar. In this study, we assessed to what extent dung beetle multi-resource trophic networks are capable of reassembling after perturbation of the forest habitat by agriculture. We used pitfall traps baited with multiple resources, including herbivore dung, carrion, fermented fruit, and dead millipedes, to unravel the dung beetle trophic network and resource use across a forest disturbance gradient. The community resistance to perturbation was assessed by comparing agricultural sites with old-growth forests as a reference, and secondary forests of different regeneration times were used to quantify the recovery.

1.1 Predictions

Dung beetles primarily use dung as a dietary resource, thus we expect dung to capture the greatest diversity and abundance of dung beetles, followed by carrion, millipede, and fermented fruit to attract the smallest number of beetles. Niche overlap between species is expected to be reduced (high *d'* and H_2') across multi-resource networks than within a resource category, e.g., between different mammal dung types. Dung beetle diversity (*H'*) and abundance (*N*) are expected to be strongly reduced in agricultural landscapes compared to old-growth forests but to increase with forest age. Driven by increasing beetle abundance, network complexity (H_2) is expected to increase with forest recovery, as more beetle individuals and species interact more broadly with more resources. An increase in network complexity is also driven by a decrease in network specialization (H_2') with forest age.

2 Materials and Methods

2.1 Study Site

The data were collected in the Reserva Río Canandé (0.5263 N, 79.2129 W) and in Tesoro Escondido (0.541998 N, 79.1448 W) in northern Ecuador (Esmeraldas Province) and the surrounding agriculture. The area is a lowland tropical forest in the western foothills of the Andes, and elevation ranges from 90 m.a.s.l. to 762 m.a.s.l. Mean annual temperature was moderate at 22 ℃ - 23 ℃ with a lot of rain (3000 - 6000 mm per year) (Escobar et al. 2024). Samples were collected from 72 sites (50 m x 50 m) within the two neighboring Reserves (Figure 1) in an area of about 130 km^2. Within Tesoro there were 14 plots, within Canandé there were 43, and 15 were on private land. Data were collected in two periods. The first round of sampling was conducted from September to November 2021 and the second round from March to May 2022 (representing six months of fieldwork). The study area was characterized by patches of small-scale ongoing agriculture, regenerating secondary forests, and old-growth forests. Agriculture in the region typically consisted of pastures or cacao plantations. Regenerating forest sites were abandoned pastures or cacao plantations. Thus, they were characterized by the type of former agricultural practice (hereafter referred to as land-use legacy) and their years after the cession of land-use (hereafter referred to as regeneration time). The majority of farming started within the past 50 years, and the regeneration time of secondary forests was assessed by interviews, including current and previous landowners (Hoenle et al. 2022; Escobar et al. 2024).

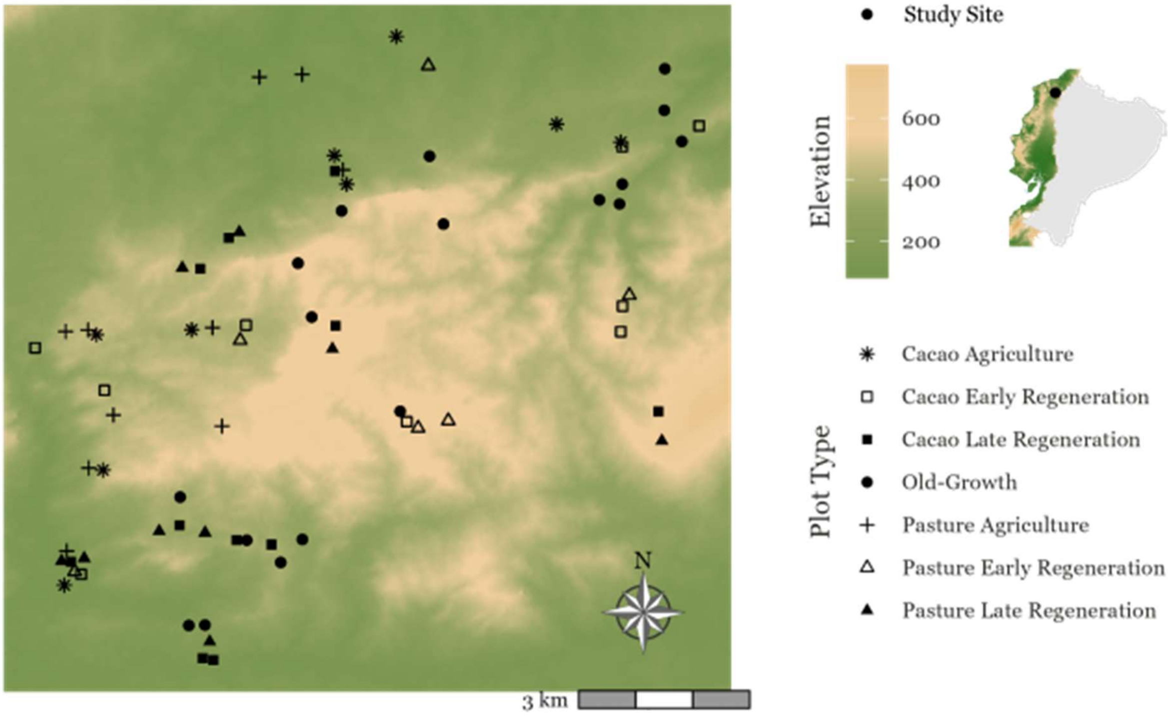

Figure 1. Study region and sample sites. Ecuador is depicted in the top right, with a circle indicating the location of the study site within Ecuador. The color indicates the elevation and only the Chocó is filled in. Within the larger square, the sample sites are indicated with symbols. The symbols correspond to the type of sample site. This is referred to as plot type on the legend.

Dung beetle trophic networks were characterized in different habitats around the reserve. Each site corresponded to one of the following habitat types: active agriculture, regenerating forest after cession of land use, and old-growth forest. The regeneration time of secondary forests ranged from less than a year to 37 years. A total of 72 sites with the following types were sampled: active cacao (n = 8), active pasture (n = 8), cacao regeneration (n = 21), pasture regeneration (n = 17), old-growth forest (n = 18) (see Tables S1 and S2). The mean distance between each site and all other sites was 5.7 km. For a full description of the study site and chronosequence plots, see Escobar et al. 2024.

2.2 Bait Selection

Four baits were selected for inclusion in this study according to the following criteria: first, they needed to represent different groups of resources and second, we needed to procure a large enough amount to make the study possible logistically. Visiting such a large number of sites, some of which take two days to reach by foot, required that baits were prepared in large quantities in advance.

Dung is one of the best-documented resources for dung beetles (Hanski and Cambefort 1991; Davis 1994; Holter and Scholtz 2007; Scholtz et al. 2009; Davis et al. 2010; Da Silva and Dornelse 2011; Correa et al. 2016; Frank et al. 2018a; Raine and Slade 2019; Chiew et al. 2022). A global analysis of dung beetle - dung studies found cow dung to be an attractive bait and it is commonly used globally (Frank et al. 2018b). Although human dung attracts even higher abundances of dung beetles than cow dung, we selected cow dung for its greater availability. Cows were the major livestock on pastures in the study region. Their dung could be collected in large amounts, stored relatively easily, and replaced in large quantities if power outages interrupted freezers. Additionally, there are lower health risks involved than with human dung.

We conducted a pilot study to compare the attractiveness of human dung and cow dung. We set up three transects with ten traps of each bait type per transect with traps spaced 50 m apart. Baits were alternated, and the starting bait was chosen by flipping a coin. Transects were set up along forest trails in January and February 2019. From these 60 pitfall traps, abundance and diversity per trap were higher for human dung baits (21.3 ± 23.1 beetles from 5.2 ± 2.5 species) than for cow dung (6.0 ± 4.3 beetles from 2.6 ± 1.4 species). Three species were captured by human dung but not by cow dung (*Eurysternus caribaeus, Onthophagus* sp 2*.* and *Scybalocanthon trimaculatus*). However, in the multi-resource study (described below) both *Onthophagus* sp 2*.* and *Scybalocanthon trimaculatus* were captured using cow dung.

Carrion has been established as a resource and used as bait, particularly for neotropical dung beetles (Hanski and Cambefort 1991; Scholtz et al. 2009). As a representative of carrion, we procured large amounts of cow muscle (meat). Rotten fruit has also been documented in a few studies (Davis 1994, Halffter and Halffter 2009), for which we selected bananas as the most readily available fruit in large quantities. Living millipedes are another type of dung beetle dietary resource with growing documentation (Schmitt et al. 2004; Fuentes-Jacques et al. 2023; Nganhane et al. 2023). Here, we used dead juliform millipedes (Diplopoda: Juliforma) as a stand-in for arthropod carrion, which has not been commonly studied. They were available in larger quantities in the forest understory. Our field observations suggested that some dung beetles attack injured juliform millipedes and feed on dead specimens locally. Using a single bait type to represent each resource category is a limitation of this methodology. Thus, the resulting communities collected by each bait type may be smaller than a community attracted to several resources within a resource category.

2.3 Sampling Protocol

Baited pitfall traps were set up in accordance with established protocols in the literature (e.g. Frank et al. (2018a)). In each site, we installed four pitfall traps (supplemental figure 1) and covered the traps with two large leaves to protect them from rain. Enough space was left under the leaves to allow beetles to enter and odors to escape. The traps were filled with ethanol to kill and preserve captured beetles.

Each trap contained one of the following bait types: fermented fruit (banana), herbivore dung (cow), carrion (rotten beef), or a rotten millipede (Diplopoda: Juliforma), hanging over the mouth of the cup. Before installation, cow meat was cut into 12.5 g chunks, and overripe bananas were cut into 30 g sections. The prepared bits were then kept in a freezer until one day before the setup to allow for rotting processes to start. Millipedes were collected in the surrounding areas. If they were found already dead but not used by consumers, they were collected and kept in the freezer until the day of installation. We also collected millipedes that we found alive. They were put into the freezer and removed from it the day before the installation, allowing the dead millipedes to decay. We used a whole one if it was small (approximately 5 cm long), and a half one if it was larger (approximately 10 cm long) for the baits. Cow dung was freshly collected *en masse* at the beginning of the study and frozen in small quantities. On the morning of trap installation, small bags of dung were removed from the freezer, and four heaped teaspoons of dung volume were filled into tea bags for bait.

The only exceptions to the above-mentioned bait treatments were two secondary forest plots (1 cacao and 1 pasture legacy) for which the banana and meat had been out of the freezer for two days due to infrastructural necessities. The dung we used for these same plots was collected freshly on the day of trap setup.

Traps were installed at least 50 m apart to sample the diversity of dung beetle species exploiting each resource within the habitat instead of sampling dietary preference for one resource over another (Larsen and Forsyth 2005). We sampled many sites instead of sampling the same sites multiple times to increase the generalizability of our results by incorporating more environmental and temporal variation. Some sites did not allow for distances more than 50 m. Traps were placed around the corners of the plots or, if space allowed, along the trail for the target habitat (Figure S1). We collected the traps after 48 hours. We identified the dung beetle samples to the lowest level of taxonomic resolution possible. Identifications were made using taxonomic keys (Génier 1996, Cook 2000, Edmonds 2000, Kohlmann and Solís 2001, Solis and Kohlmann 2002, Génier and Kohlmann 2003, Solís and Kohlmann 2004, Edmonds and Zídek 2004, Vaz-De-Mello 2008, Génier 2009, Gonzáles-A et al. 2009, Edmonds and Zídek 2010, Rubio 2010, Edmonds and Zídek 2012, Solís and Kohlmann 2013, Delgado and Curoe 2014, González-Alvarado 2014, Sarmiento-Garcés and Amat-García 2014, Darling and Génier 2018, Silva and Valois 2019, Moctezuma et al. 2020, González-Alvarado and Vaz-de-Mello 2021, Arias-Buriticá and Vaz-de-Mello 2023), and by CCC (to be replaced upon acceptance following double blind review process).

2.4 Literature Comparison

We compared the multi-resource dung beetle networks from this and seven other studies (Davis 1994; Davis et al. 2010; Da Silva et al. 2012; Ratcliffe 2013; Da Silva and Dorneles 2011; Correa et al. 2016; Salomão et al. 2018) to more traditionally studied dung beetle–dung networks in terms of network specialization. For this, we used data from the meta-study by Frank et al (2018b), as well as data that was previously collected in the same study region using human dung and cow dung as bait in 2019 (pilot study). Across the literature, multi-resource dung beetle networks were not generally more specialized than dung-only networks. For details, see the supplement section, Literature Comparison.

2.4 Statistical Analysis

We used linear models to describe the recovery of dung beetle network metrics over time. The regeneration time in years of recovery was square-root transformed to linearize the trends. Plots in ongoing agriculture were assigned a regeneration time of 0. Old-growth forests were only used as a reference and excluded from the models.

The following metrics were calculated for each plot:

- Total abundance N of dung beetles
- Raw Shannon diversity of dung beetle species (H') (Frank et al. 2018b).
- The exponential form of the two-dimensional Shannon entropy of links ($\exp(H_2)$) within a network as a metric for network complexity (Blüthgen 2010).
- A weighted network specialization metric (H_2') that standardizes H_2 between the minimum and maximum possible for all configurations of the same marginal totals (Blüthgen et al. 2006): $H_2' = 0$ for the minimum possible specialization for a given network, and $H_2' = 1$ for the maximum specialization.

For each metric, a multiple linear regression model was fitted with the regeneration time, plot elevation, and land-use legacy as predictors. We also included the interaction term between legacy and regeneration time to allow different slopes for the recovery of cacao plantations and pastures. The abundance N was log-transformed ($N' = \ln(N+1)$) for linearization. Models for all metrics were significant (Table S4; further details in Supporting Information).

Moreover, 10^5 random networks were simulated for each sampled plot, based on the observed dung beetle abundance and diversity of the plot (Patefield's algorithm: Patefield, 1981; details in Supporting Information). This way, we obtained a measure of what the network complexity and network specialization metrics could be expected to be at a given regeneration time if there were no species-specific preferences for resources. With these data, we fitted further linear models as baselines of network metric recovery that can be attributed to the recovery of dung beetle abundance and diversity. We compared the slopes of the linear regression for observed networks with the slopes of the linear regression for the null models. In addition, we compared the means for the metrics in the observed networks with the means for the metrics in the null models.

Based on the linear models of observed values, we estimated the resistance and the expected recovery time for the network metrics to reach the old-growth level after disturbance. The resistance was calculated as the proportion of the respective network metric remaining in agriculture compared to the old-growth forest mean. The expected recovery time was calculated per metric by extrapolating the linear models for each land-use legacy until the metric values reached the old-growth median.

The exclusiveness of dung beetles attracted to each bait type and the exclusiveness of the resource types to each beetle species were evaluated using the standardized Kullback–Leibler distance *d'* (Blüthgen et al. 2006). Like H_2' for the network level, this species-level specialization metric ranges from 0 for the least exclusive to 1 for the most exclusive resource in a given network. Niche exclusiveness was also calculated for each dung beetle species using *d'* values.

Two additional network metrics were computed and presented in the Supporting Information section Additional Network Metrics: (1) Modularity, an index that increases with specialization H2' but describes the extent to which species are clustered to similar interaction partners (Schleuning et al. 2014). (2) Algebraic Connectivity, a metric of how many breaks would be needed to disconnect the species pools using particular resources. Numbers closer to 0 indicate lower connectedness and fewer needed breaks, numbers closer to 1 indicate greater connectivity and a larger number of needed breaks to disconnect species pools (Abreu 2006).

To assess the differences in species composition between communities, we conducted permutational multivariate analyses of variance (PERMANOVA, Anderson 2001). For this, community abundance data was standardized to proportions (total per community = 1.0) and translated into Bray-Curtis distances. The effect of the square-rooted regeneration time was assessed in a linear model, excluding old-growth plots, after accounting for plot elevation, sampling period, and legacy. In further analysis, we assigned each plot one of 4 habitat categories to substitute regeneration time: agriculture, early and late regeneration stage, and old-growth forest. The cutoff between the early and late regeneration stages was set to 10 years, where secondary forests have a relatively closed canopy. Plots in each of the first three habitat categories were compared to the old-growth references sequentially. For visualization, plot-wise community dissimilarities were ordinated in a Principal Coordinate Analysis (PCoA), and a vector of the regeneration time ranks fitted to the axes. All agriculture plots shared the first rank, and all old-growth plots shared the last rank. To account for negative eigenvalues in the analyses, the 'Lingoes' correction method was applied, adding a constant to the squared dissimilarities. For details and model formulas, see the Supporting Information section Statistical Analysis.

All analyses were conducted with the statistical software 'R' (version 4.1.2, R Core Team 2020). H_2', H_2, and modularity were computed using the 'bipartite' package (Dormann et al. 2008), *H'* was calculated with the 'vegan' package (Oksanen et al. 2020), and algebraic connectivity was calculated with the 'sna' package (Butts 2023). There was a mentionable correlation between the independent variables of regeneration time (square root-transformed) and elevation both in the continuous scale, including agriculture and secondary forest (Pearson's $r = 0.23$) and in the ranked scale that additionally includes old-growth plots (Spearman's ρ = 0.18) (Figure S11; see Supporting Information section Multicollinearity for the analysis).

3 Results

During the two rounds of sampling, we sampled 72 sites over 144 trap nights. We collected and identified the lowest possible taxonomic level, 844 beetles from 23 (morpho-)species. From the total number of captured dung beetles, 20 beetles from 5 species were captured with the fermented banana, and 209 beetles from 16 species were captured with the herbivore dung bait. 18 species of dung beetles from 276 beetles were captured with rotten millipede, and carrion captured the highest abundance, with 339 beetles, but from only 14 species.

At the site level, the average dung beetle abundance per plot had a 3.2-fold increase from agriculture to the late succession and a 3.9-fold increase from agriculture to the old-growth forest (Figure 2a). The average species richness per plot had a 2.2-fold increase from agriculture to late regeneration, approximately the same as to the old growth (Figure 2b). Herbivore dung was the most prominently used resource in agriculture. However, the proportion of species and individuals captured with vertebrate and invertebrate carrion increased with forest age, outnumbering dung-baited traps in the late regeneration and old-growth (Figures 2, 3, S12, and S13; Table S4). Species richness on dung and fruit remained fairly constant, while an increase was observed with both vertebrate and invertebrate carrion (Figure 2b). Similarly, the proportion of beetles captured by vertebrate carrion increased, and the proportion of Necrophagous species increased with forest age (Supplemental Figures S12, and S13).

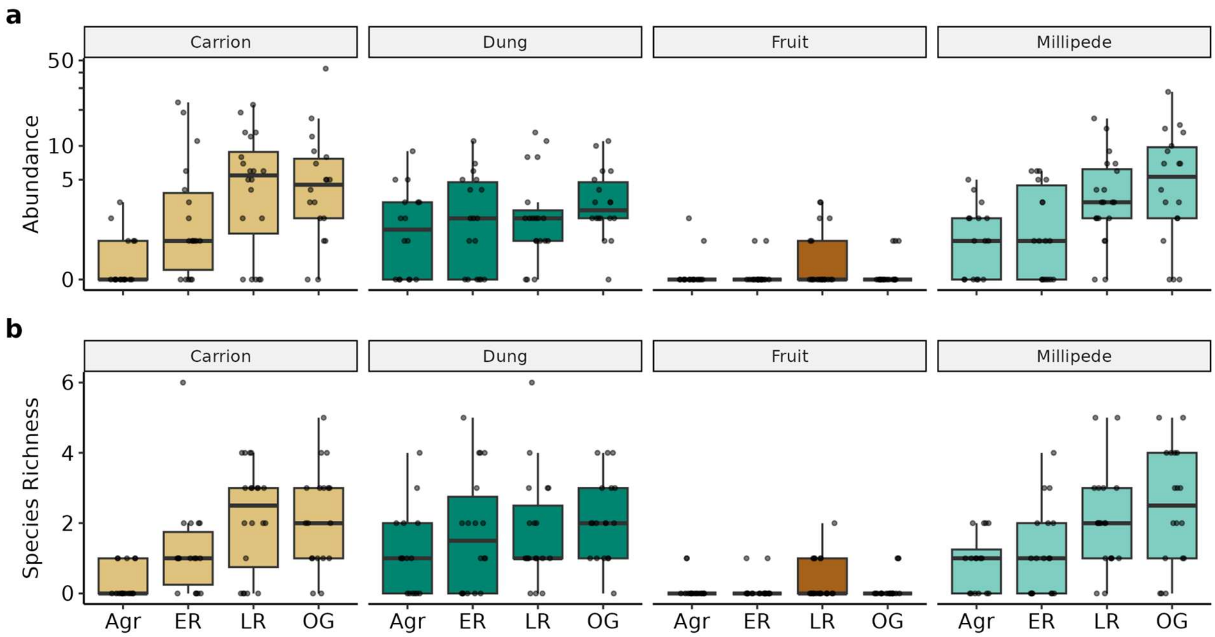

Figure 2. Dung beetle abundance caught per trap (a) and species richness (b) in the different

regeneration stages. Agr = agriculture, ER = early regeneration, LR = late regeneration, OG = old-growth.

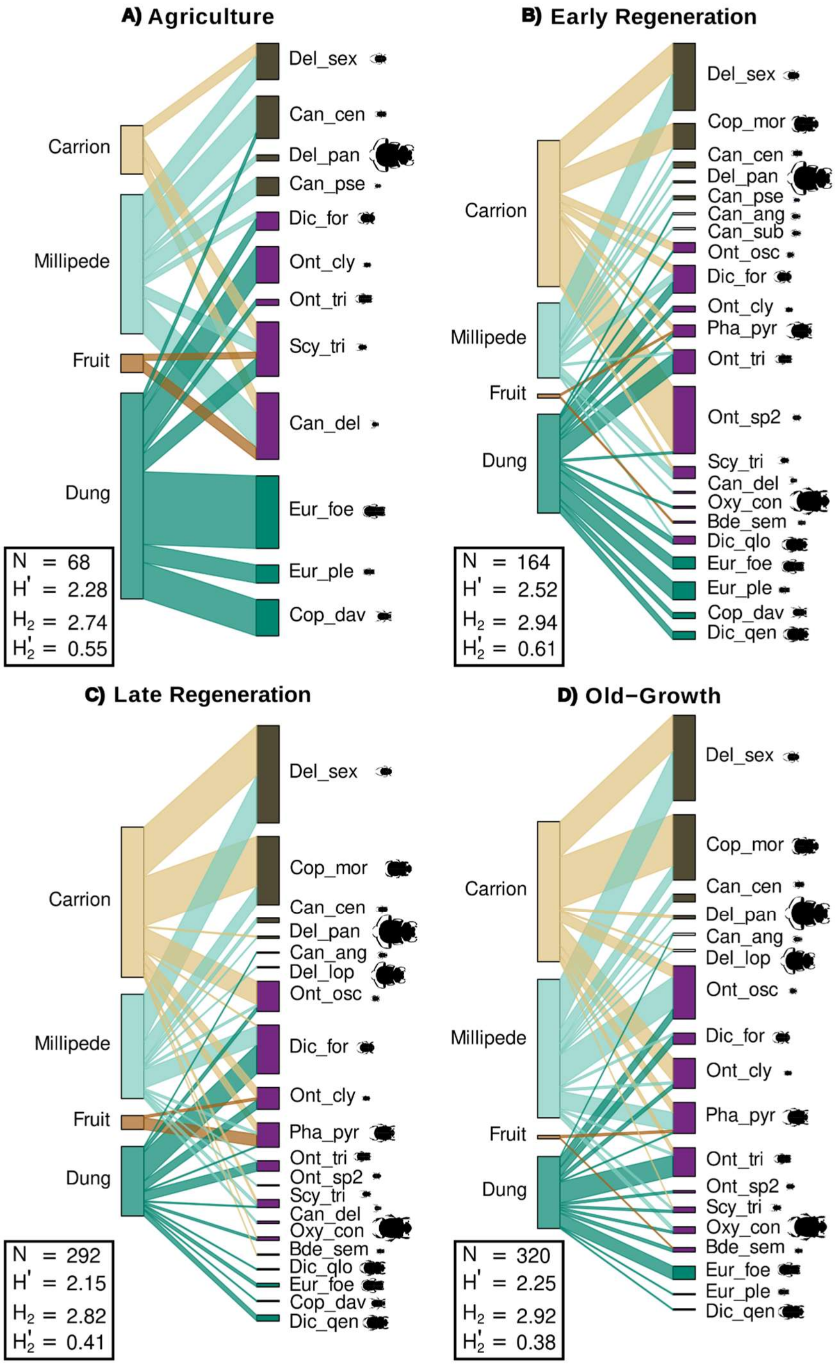

Figure 3. Ecological networks from four habitat types: agriculture (a), early regeneration (b), late regeneration (> 10 y); c), and old-growth (d). The colors of the dung beetle boxes represent their feeding guild: necrophagous (black), generalist (purple), or coprophagous (green). Dung beetles were defined as necrophagous if at least 95 % of individuals of a species were captured with vertebrate or invertebrate carrion. Coprophagous was defined as at least 95 % of a species

on dung-baited traps. Boxes for beetles with less than 5 total observations were not colored. Dung beetle abundance (N) and diversity (*H'*), and network complexity (H_2) and specialization (H_2') of the pooled networks are shown for each web. Beetle silhouette relative sizes are to scale.

The niche specialization of different baits had varying levels of specialization. Arthropod carrion (dead millipede, *d'* = 0.25), and carrion (*d'* = 0.30) were attractive for relatively generalist species, whereas fermented fruit (*d'* = 0.52) and herbivore dung (*d'* = 0.61) captured a more exclusive spectrum of beetles. Twenty of the 22 dung beetle species were attracted by two or more resources. The specialization measured by *d'* values of beetle species ranged from *d'* < 0.01 in *Scybalocanthon trimaculatus* (attracted to all four bait types), to *d'* = 0.43 in *Eurysternus foedus*, which was captured only with dung (Table 1). Two other species *Copris davidi* and *Eurysternus plebejus* were also captured exclusively with dung. Additionally, all three *Deltochilum* species and *Coprophanaeus morenoi* were captured exclusively with carrion or rotten millipede baits. *Canthidium centrale* was also captured largely by traps baited with rotten millipede. Individuals of this species have also been collected feeding on injured or dead millipedes in the field site by a couple of the authors XXX and YY.

Table1. Species abbreviations, total abundance, abundance on each bait type, and niche exclusiveness *d'* of each dung beetle species. Species are ordered from least exclusive to most exclusive in terms of resource use (low *d'* – high *d'*).

		N Beetles					
Beetle Species	**Abbreviation**	**Total**	**Carrion**	**Dung**	**Fruit**	**Millipede**	***d'***
Scybalocanthon trimaculatus	Scy_tri	27	9	6	1	11	<0.01
Onthophagus osculatii	Ont_osc	79	34	14	0	31	0.02
Canthon subhyallynoides	Can_sub	1	0	0	0	1	0.04
Oxysternon conspicillatum	Oxy_con	10	1	5	0	4	0.06

Deltochilum loperae	Del_lop	3	1	0	0	2	0.07
Deltochilum panamensis	Del_pan	7	3	0	0	4	0.07
Onthophagus aff. *clypeatus*	Ont_cly	55	30	21	2	2	0.1
Canthon delicatulus	Can_del	14	3	0	2	9	0.13
Canthon angustatus	Can_ang	4	0	3	0	1	0.14
Phanaeus pyrois	Pha_pyr	55	20	4	13	18	0.15
Dichotomius quinquelobatus	Dic_qlo	5	0	4	0	1	0.16
Dichotomius fortepunctatus	Dic_for	69	5	41	0	23	0.16
Onthophagus sp. 2	Ont_sp2	37	33	3	0	1	0.18
Canthidium pseudaurifex	Can_pse	5	0	0	0	5	0.21
Bdelyrus seminudus	Bde_sem	6	1	3	2	0	0.21
Coprophanaeus morenoi	Cop_mor	131	101	0	0	30	0.23
Ontherus trituberculatus	Ont_tri	48	6	38	0	4	0.23

Deltochilum (sextuberculatum group)	Del_sex	201	92	0	0	109	0.23
Canthidium centrale	Can_cen	21	0	1	0	20	0.25
Copris davidi	Cop_dav	10	0	10	0	0	0.31
Dichotomius quinquedens	Dic_qen	10	0	10	0	0	0.31
Eurysternus plebejus	Eur_ple	13	0	13	0	0	0.33
Whole Network		844	339	209	20	276	-

Dung beetle communities shifted with forest regeneration, as did bait use. A significant proportion of the variation in communities was explained by regeneration time ($R^2_{part} = 0.07$, pseudo $F_{1,35} = 3.1$, $p < 0.001$; Figure 4). Elevation and sampling period also affected communities (elevation: $R^2_{part} = 0.04$, pseudo $F_{1,35} = 1.9$, $p < 0.01$; period: $R^2_{part} = 0.06$, pseudo $F_{1,35} = 2.9$, $p < 0.001$). The dung beetle community in old-growth forests was distinct from agricultural communities ($R^2_{part} = 0.04$, pseudo $F_{1,54} = 2.4$, $p < 0.001$), but not from those in early secondary forests ($R^2_{part} = 0.01$, pseudo $F_{1,54} = 0.94$, $p = 0.55$). A marginally significant difference between late regeneration and old-growth forest communities was indicated ($R^2_{part} = 0.02$, pseudo $F_{1,54} = 1.4$, $p = 0.06$).

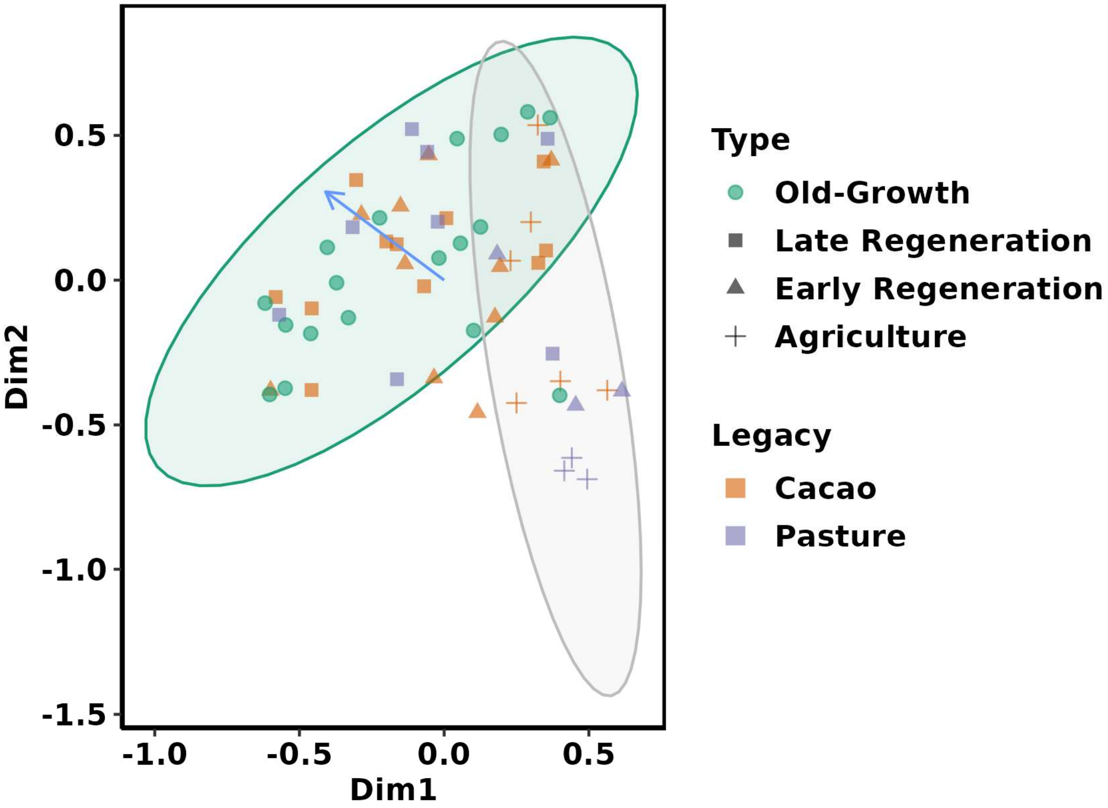

Figure 4. Principal Coordinate Analysis (PCoA) plot of Bray-Curtis distances between dung beetle communities. The blue arrow represents the fitted vector of the rank of regeneration time of each plot ($r^2 = 0.26$). The green ellipse depicts the old-growth cluster at 95 % CI. The agriculture cluster is shown as a gray ellipse at 95 % CI. An additive constant of 0.61 was added to the squared dissimilarities. Dimensions = 2.

Also dung beetle diversity shifted with forest regeneration. Regeneration time was a significant predictor for diversity ($F_{1,49} = 6.8$, $p = 0.01$), but was not significant at the 5 % level for abundance ($F_{1,49} = 3.3$, $p = 0.07$). Both beetle abundance and diversity were lower in pasture legacy sites (i.e. active pastures and secondary forests from previous pastures) than in cacao legacy sites at least during early stages of forest recovery. This made land-use legacy the best predictor for abundance and diversity ($F_{1,49} = 5.8$, $p = 0.02$, and $F_{1,49} = 6.8$, $p = 0.002$, respectively; Figure 5a). This difference also resulted in higher resistance values for the cacao legacy in abundance and species diversity (Table 2).

Table 2. Expected recovery time and resistance of network parameters in the chronosequence.

Network Metric	**Legacy**	**Resilience** **Recovery time** **[years]**	**Resistance** **[Proportion of old-growth level]**
Abundance (N)	Cacao	33	0.61
	Pasture	41	0.50
Species Diversity (H')	Cacao	21	0.65
	Pasture	47	0.19
Network Complexity (H_2)	Cacao	15	0.57
	Pasture	52	0.34
Network Specialization (H_2')	Cacao	12	0.70
	Pasture	17	0.70

The network-level metrics H_2 and H_2' of observed networks showed the same trends as the corresponding null model simulations. Network complexity (H_2) followed the increase of dung beetle abundance and diversity, and significantly increased with forest recovery ($t_{46} = 3.0$, $p < 0.01$, Figure 5b). Likewise, the trajectory of network complexity in pasture legacy sites started at lower levels than in cacao legacy sites ($t_{46} = -1.9$, $p = 0.07$, Figure 5b). To test whether the overall level and trends of the network metrics H_2 and H_2' mirrored the variation in abundance and diversity with forest recovery, we applied null model simulations based on dung beetle abundances and diversity. Network complexity H_2 in both land-use legacies was smaller than in the respective null models (cacao: $z = -0.53$, $p = 0.001$; pasture: $z = -0.29$, $p = 0.05$). Network specialization H_2' was higher than in null models (cacao: $z = 1.32$, $p < 0.001$; pasture: $z = 1.00$, $p < 0.001$) and not different between the two land-use legacies ($t_{37} = 0.3$, $p = 0.7$). For both trajectories, the slopes of observed network complexity H_2 were similar to the slopes of the respective null model simulations (cacao: $z = -0.07$, $p = 0.9$; pasture: $z = -0.62$, $p = 0.3$; Table S5). The same was true for H_2' (cacao: $z = -0.13$, $p = 0.8$; pasture: $z = 0.83$, $p = 0.2$). Hence, while the level of H_2 and H_2' were significantly different from null models, their trajectories were not independent of changes in abundance and diversity of beetle species. Both complexity H_2 and specialization H_2' recover faster in the cacao legacy than in the pasture legacy (Figure 5b, Table 2).

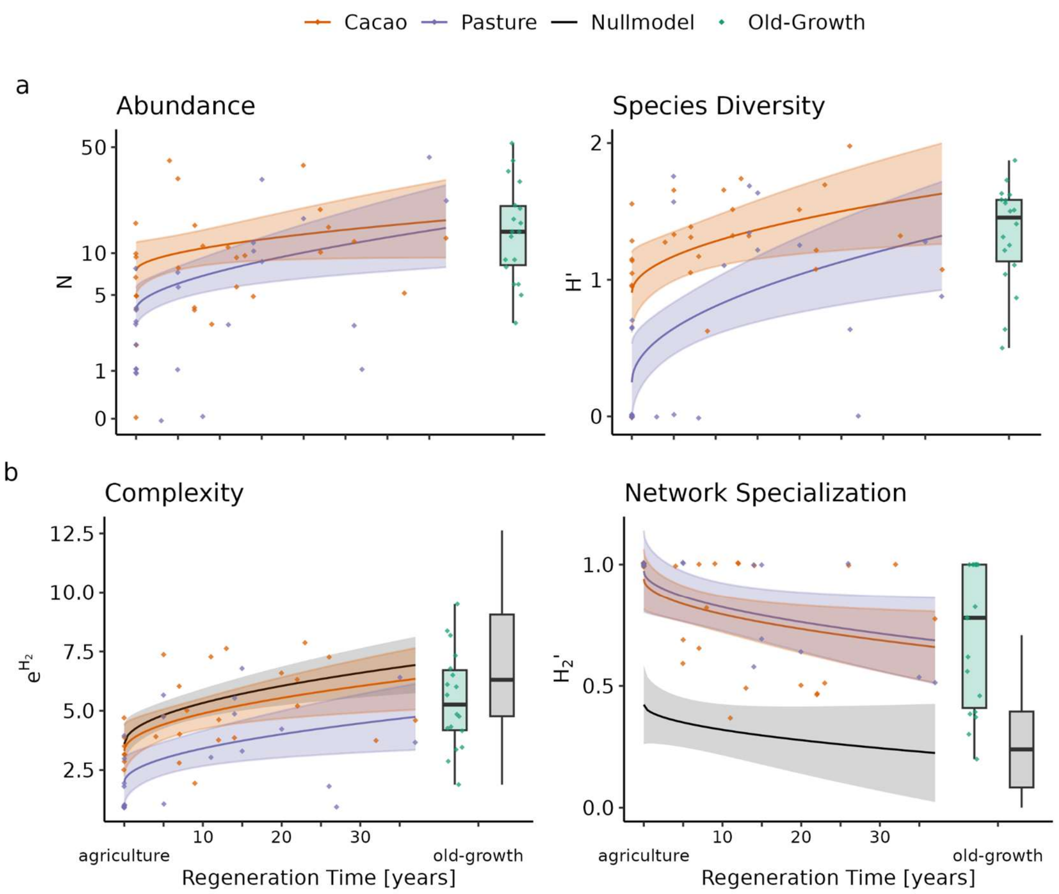

Figure 5. Regeneration trends at a per plot level for a) abundance and diversity, and b) network complexity and network specialization over the forest recovery gradient. Data from old-growth plots are shown in a green box plot as a reference. Cacao agriculture and regeneration are represented in orange, and pasture agriculture and regeneration are represented in blue. The colored lines represent the linear models for the respective legacy at mean elevation with 95 % CI. For b), the average of the null model simulations for both legacy lines is shown as a black line and the null model simulations for old-growth plots are shown as a gray boxplot.

4 Discussion

Trophic networks represent important ecosystem processes. It is often assumed, but rarely shown, that loss of network complexity or increasing network specialization (i.e., lower functional redundancy) may result in more fragile networks and decreased level and stability of processes. As each network or process depends on other processes or networks in an ecosystem, it is important to understand several trophic networks and their responses to environmental changes such as disturbance and recovery (Malhi et al 2022,Potapov et al. 2022).

In our study, dung beetle communities and trophic networks recovered over time from agriculture to late regenerated forests where networks resembled those in old-growth forest references. Most network metrics included here showed strong impacts of disturbance (i.e., levels in agriculture differed from old-growth forests) as well as successional recovery towards old-growth forest levels. This change in communities and trophic relationships can be viewed as resistance–resilience dynamics of ecosystems (Pimm et al. 2019). Recovery of high network complexity (H_2) was driven by an increasing abundance and diversity of beetles, but also by an increasing generalization (reduction in H_2') of trophic links for each species. In addition, connectivity increased, whereas modularity was relatively consistent along the chronosequence (Figures S8 and S9). Whereas a few species were more specialized on dung or carrion, most dung beetle species were relatively generalized across resources. This likely contributed to the rapid recovery of trophic interactions.

Whereas several studies compared networks along environmental gradients, studies on network responses to land use or ecosystem recovery are scarce. For example, Tylianakis et al. 2007 showed land use effects on bee – parasitoid networks in the Ecuadorian Andes. Kaiser-Bunbury et al. (2017) showed the effect of restoration on plant – pollinator networks on the Seychelles. In both studies, networks were more complex (i.e. had a higher diversity of links) in natural or restored habitats than in disturbed habitats, including an improved abundance and diversity of species, but also an increased generalization (Kaiser-Bunbury et al. 2017) like in our study. In contrast, network specialization in dung beetle – dung networks across a land use gradient in Borneo was found to increase with decreasing anthropogenic disturbance but decreased with increasing dung beetle diversity (Chiew et al. 2022).

There have been very few multi-resource network studies for which land use was considered in the design. For seven studies that provided suitable data tables and used multiple resources, we calculated H_2' values for the multi-resource dung beetle networks. There was only one other network with a remarkably high specialization in the pristine Brazilian Amazon with $H_2' = 0.57$ (Ratcliffe 2013). The networks from the other studies in disturbed grasslands (Davis 1994; Davis et al. 2010, Da Silva & Dorneles 2011; Da Silva et al 2012 ; Correa et al. 2016) or Brazilian dry forest (Salomão et al. 2018) had H_2' values comparable to the ones in the dung beetle-dung networks from Frank et al. (2018b) (see Figure S2). This suggests that multi-resource dung beetle networks are not necessarily more specialized than dung beetle-dung networks.

In six comparable studies, dung was the preferred resource with the greatest abundance of dung beetles (Davis 1994; Davis et al. 2010; Ratcliffe 2013; Da Silva & Dorneles 2011;Da Silva et al 2012; Correa et al. 2016). However, as in our study, carrion attracted a larger abundance of dung beetles (though not diversity) similar to a study in a Brazilian dry forest (Salomão et al. 2018). We additionally found that the relative resource use of carrion was connected with forest regeneration (Figures 2, 3, and S12). This effect was visible in the community structure (Figure 3) and affected the proportions of feeding guilds in the community (Figure S13). This indicates that species specialized on carrion are more narrowly bound to forest habitats than generalists or dung specialists.

Cow dung generally performed well as bait globally (Frank et al. 2018b) which is consistent with our study where it captured a large proportion of the dung beetle diversity in all habitat types, including dung specialists. However, our previously collected data showed that cow dung is less attractive than omnivore dung in the region. Using omnivore dung could have increased the relative dung use across the chronosequence (Figure S12). However, the proportion of species captured by dung in all habitats is high and increases in the old growth (Table S8).

While rotten millipede has been used in previous studies it has rarely been used in a multi-resource study (Karimbumkara & Priyadarsanan 2016; Fuentes-Jacques et al. 2023). As a bait, it performed surprisingly well in comparison to carrion (with more species) and herbivore dung (with greater abundance in older forests), both more commonly studied baits (Hanski & Cambefort 1991; Frank et al. 2018a; Ratcliffe 2013). This suggests it might be a valuable bait for future dung beetle pitfall trapping studies. Compared to herbivore dung, it captured a similar diversity of dung beetles, though it is worth noting that using omnivore dung may have captured a higher abundance. In addition, invertebrate carrion is an abundant resource in many ecosystems. As rotten millipedes could be considered a stand-in for arthropod carrion, our results indicate that dung beetles are important in the decomposition of invertebrates. It has been suggested that arthropod carrion is an overlooked resource in many studies (Bartel et al., 2023). Moreover, only a few studies of dung beetles used fermented fruit as bait (Halffter & Halffter, 2009; Fuentes-Jacques et al. 2023). In our study, fermented bananas attracted a less exclusive community than dung. Although three of the five species captured with the fruit were relatively rare, none was exclusively found in fruit-baited traps. However, using fermented fruit as bait might help to capture rare species in the dung beetle community.

Possibly due to the maintenance of tree cover in cacao plantations, the networks in cacao plantations were more resistant to disturbance than pasture, although there were no apparent differences in the community compositions between the two land-use legacies cacao and pasture. The shade-providing cacao trees construct microclimates more similar to the original habitat of the native dung beetles. The recovery of trophic networks suggests that dung beetles could quickly resume their role as decomposers after the biotic or abiotic conditions are re-established. The key role of abiotic conditions in this process is consistent with Gebert et al. (2020), who showed that dung beetle communities were more dependent on abiotic variables than on mammal dung. This is consistent with our findings that many species use at least two resources. Our study region has been dominated by forests until recently, and thus our findings might not apply to other regions with a longer history of natural savannas. For instance, the decomposition of dung and carrion by dung beetles may not be reduced if an existing community in open vegetation can fulfill the functional roles of forest species.

Our predicted recovery times are in line with other studies in the Chocó ecoregion. Ant communities in the area recovered after about 30 years (Hoenle et al. 2022). Moreover, for the Colombian Chocó-Andes dung beetle communities, a similar recovery time was predicted (Davies et al. 2020). For all of these studies, recovery is likely to be associated with the mosaic-like distribution of agricultural patches in an area with mostly connected forest habitats. All plots in our study system were relatively close to old-growth forests. The individual distance to the nearest old-growth forest as a potential source of biodiversity did not influence dung beetle abundance or diversity (Figures S14 and S15).

While the previously mentioned studies were limited to community-level metrics, we additionally report the recovery of trophic webs. Network-level metrics do not necessarily mirror community metrics like species diversity or abundance (Tylianakis et al. 2007). However, in our study, the observed recovery of network complexity and specialization was similar to the change in average null models based on the species abundances and diversity alone. This means that the increase in complexity and the decrease in specialization can be attributed to the recovering dung beetle abundance and diversity. Our approach is consistent with the suggestion and criticism that variation in networks needs to be viewed in the light of sampling, abundance, and diversity, in order to better understand the underlying drivers (Blüthgen & Staab 2024 and references therein). Hence, trophic interactions reassembled in parallel with the underlying abundance of dung beetles – and were thus determined by the same changes in environmental conditions that apply to the beetle community, e.g. changes in canopy closure and microclimate, or increased availability of resources. The simplified dung beetle networks in agriculture indicate the importance of environmental conditions over the availability of resources.

4.1 Conclusions

Most ecosystems and land cover experienced some form of anthropogenic disturbance (Malhi et al 2014). While the degree of ecosystem disturbance or its recovery potential is often characterized based on species diversity, important aspects of the performance and stability of tropic networks were rarely included in previous studies. Trophic webs or interaction networks are the backbone of important ecosystem processes and energy fluxes, including decomposition, herbivory, pollination, seed dispersal, predation, and parasitism. With this premise, it becomes important to both measure and understand network recovery for several types of ecosystem processes in response to disturbance and their resilience. There is evidence that active restoration such as removal of invasive plants can restore pollinator-plant networks and fruit production of native plants (Kaiser-Bunbury et al 2017). The energetics on a larger scale were recently estimated for ecosystems using biodiversity surveys, literature based feeding preferences, estimated biomass, and some calculated energy flux for birds and mammals (Malhi et al 2022) and birds and arthropods (Potapov et al. 2022). However, in both cases, feeding preferences were assumed to remain static in different habitats. Here, we provide one example where resource use was measured across habitats. Feeding preferences of functional groups could shift strongly in groups with more restrictive diets, e.g. insect herbivores (Forister et al. 2014). Further, there may be proportional losses of higher-level trophic interactions: Energy may shift from high trophic levels in less disturbed ecosystems to lower trophic levels in highly disrupted ecosystems (Malhi et al 2022). As ecosystem processes are highly interdependent, and disturbance could retard the recovery of some processes more than others, this could result in an imbalance in the recovery. For these reasons, studies of diet preferences in conjunction with disturbance and land use gradients can enhance the understanding of ecosystem functioning in a broader sense. This could also provide valuable information for conservation planning. For instance, ecosystem processes or networks that recover slowly or not at all could be accelerated or catalyzed with intervention practices.

Our results suggest that trophic networks can recover rapidly after the cession of agricultural practice, driven by the activity of mobile species and their functions. Hence, the connection to habitats that accommodate a sufficiently diverse pool of species is crucial for this recovery potential. In this case, forest habitats, including secondary forest, can provide a refuge and source for dung beetles that maintain trophic interactions in disturbed sites or early recovery stages.

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Multi-Resource Dung Beetle Networks within the Context of Forest Recovery

Supplementary Information for Methods and Results

Distribution of Covariates

The study on trophic web focuses on land-use legacy (active and former cacao plantations or pastures) and regeneration time (in the case of secondary forests) as main predictor variables. Elevation and sampling period may play an additional role as covariates. Here we summarize the distribution of these variables.

Table S1. Distribution of regeneration time and elevation of the respective sites. n = number of plots sampled.

Legacy	Type2	n	Regeneration Time [y]	Elevation [m.a.s.l]
Old-Growth	Old-Growth	18		365 ± 117
Cacao	Late Regeneration	11	21.2 (12 – 37)	322 ± 140
Pasture	Late Regeneration	9	22.6 (14 – 37)	346 ± 156
Cacao	Early Regeneration	10	6.3 (0 – 11)	373 ± 112
Pasture	Early Regeneration	8	4.6 (0 – 11)	368 ± 143
Cacao	Agriculture	8	0	271 ± 104
Pasture	Agriculture	8	0	293 ± 158

Table S2. Distribution of plots by sampling period and habitat type

Type	Period	n
Agriculture	1	11
Agriculture	2	5
Regeneration	1	21
Regeneration	2	17
Old-Growth	1	10
Old-Growth	2	8

Experimental Setup

As far as was possible our pitfall traps were set up in the manner shown in Figure S1. There are a few exceptions.

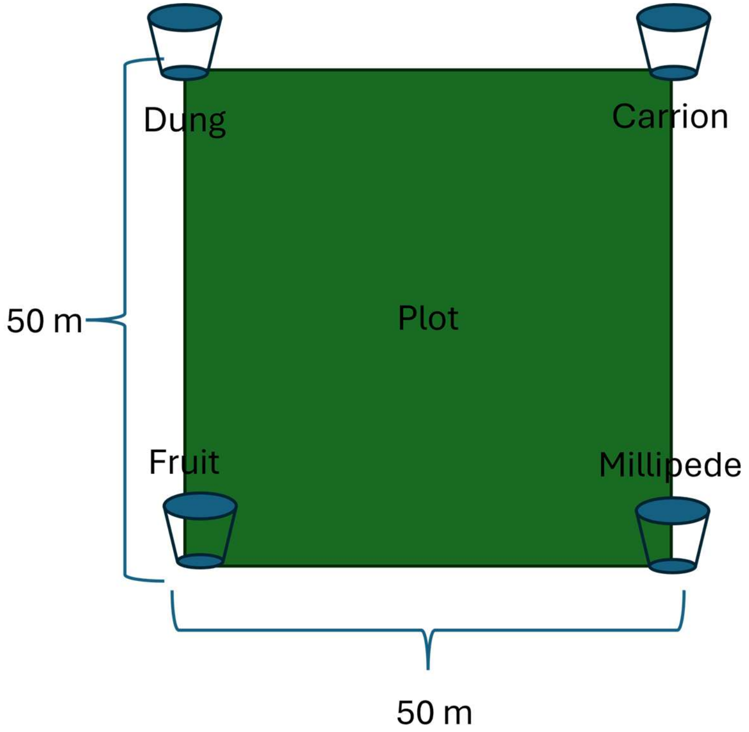

Figure S1. Trap set up within a typical 50 m x 50 m plot. The marking of the 50 m x 50 m plot edges was completed in 2022, in 2021 some traps were set up in a transect when the habitat patch was large enough and the terrain did not allow for a square near the GPS point with that particular plot ID.

Literature Comparison

The multitrophic networks in our study were relatively specialized (Figures 3 and 5). The pooled overall network in our study had a higher network specialization (H_2' = 0.39) than 87 % of the global dung networks in Frank et al. (2018b) (Table S3). In contrast, the H_2' value of the network in the pilot study (human and cow dung, H_2' = 0.08) that was sampled in the same study area was only higher than 22 % of the networks in Frank et al. (2018b). In both cases, we pooled the data over all habitats because H_2' values of dung beetle networks were influenced by small network sizes (t_{114} = -3.4, p = 0.001; Figure S2). Although the multi-resource networks had higher H_2' values than the dung networks in our study, eight other published multi-resource networks (Davis 1994; Davis et al. 2010; Ratcliffe 2013; Da Silva & Dorneles 2011; Da Silva et al 2012; Correa et al. 2016; Salomão et al. 2018) did not have a higher H_2' value than dung-only networks from the literature ($t_{7.9}$ = -0.02, p > 0.9; Figure S2).

Table S3. By-habitat comparison of the H_2' values of this multi-resource study and a previous dung beetle - dung study in the same region with dung beetle - dung networks from Frank et al (2018b), Da Silva et al, 2012, and Ratcliffe 2013. The percentile is the proportion of networks in the meta study that had a lower H_2' value than the respective network.

Study	Study Category	Habitat Type	Legacy	N Beetles	N Species	H_2'	Literature Percentile

Author Data	this	Agriculture	Cacao	42	12	0.65	0.97
Author Data	this	Agriculture	Pasture	26	5	0.49	0.92
Author Data	this	Regeneration	Cacao	288	23	0.50	0.92
Author Data	this	Regeneration	Pasture	168	16	0.46	0.91
Author Data	this	Old-Growth	Old-Growth	320	18	0.38	0.84
Author Data	this	Whole Network	NA	844	23	0.39	0.87
Author Data	Human vs. Cow	Old-Growth	NA	472	17	0.10	0.22
Author Data	Human vs. Cow	Regeneration	NA	284	17	0.18	0.47
Author Data	Human vs. Cow	Whole Network	NA	756	17	0.08	0.22
Davis et al 2010	Multires. Literature	Whole Network	NA	64800	47	0.07	0.17
Correa et al 2016	Multires. Literature	Whole Network	Savanna	4619	43	0.17	0.46
Correa et al 2016	Multires. Literature	Whole Network	Pasture	2925	43	0.05	0.09
Da Silva & Dorneles-Audino 2011	Multires. Literature	Whole Network	NA	154	10	0.32	0.78
Salomão et al 2018	Multires. Literature	Whole Network	NA	297	9	0.08	0.17
Ratcliffe 2013	Multires. Literature	Whole Network	NA	19416	66	0.57	0.93
Davis 1994	Multires. Literature	Whole Network	NA	14994	52	0.34	0.81
Da Silva et al 2012	Multires. Literature	Whole Network	NA	19699	33	0.21	0.58

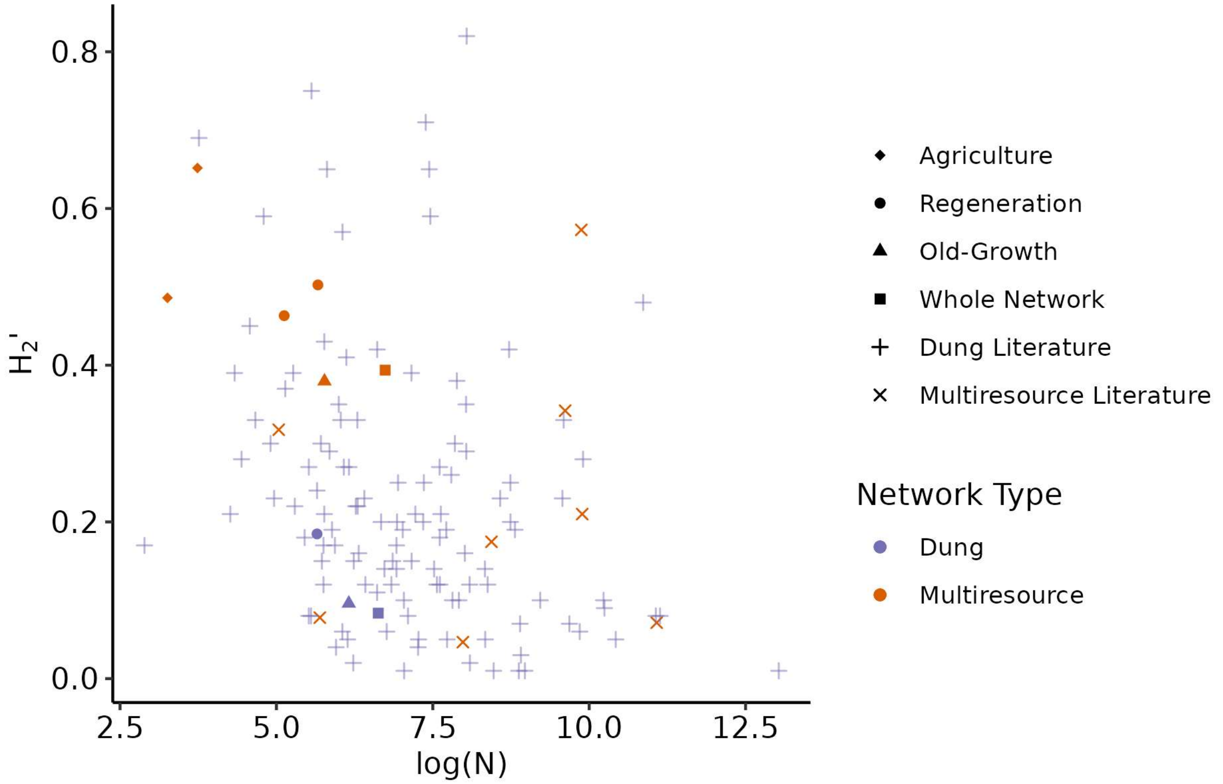

Figure S2. H_2'-log(N) ratios of dung beetle-dung networks (purple) and multiresource dung beetle networks (red). The networks from this study are shown as red diamonds, circles, triangles, and squares, depending on habitat type. The dung networks from this study come from previously collected data with cow dung and human feces in the same study region. The dung networks from the meta-study by Frank et al. (2018b) are shown as crosses. The multiresource networks from the literature for which we calculated H_2' values are shown as red diagonal crosses.

Statistical Analysis

- Linear models: Statistical significance of models and predictor terms were assessed with the Anova ('car,' Fox, Weisberg, and Price 2023) function with type III sums of squares.
- Null model: Based on Patefield's algorithm (Patefield, 1981; package 'vegan,' method = "r2dtable", Oksanen et al. 2022)
- PERMANOVA:
  - Regeneration time and covariates: With adonis2 ('vegan', Oksanen et al. 2022) and the by = "term" option, with the model
    - Formula: 'D ~ Elevation + Period + Legacy * sqrt(Regeneration Time)'.
  - Habitat categories: The communities were compared to old-growth communities with the by = "onedf" option of the adonis2 (Oksanen et al. 2022) function.
    - Formula: 'D ~ Period + Type2' (Type2 distinguishes early and late regeneration)
  - PCoA: cmdscale function ('stats,' R Core Team 2021)

- for PERMANOVAs and PCoA, the 'lingoes' correction (adding a constant to the squared dissimilarities) was applied to deal with negative eigenvalues of significant magnitude

Linear Models

All MLR (multiple linear regression) models were significant (Table S4). The square-root transformed regeneration time explained a significant amount of variance in the models with H, $\exp(H_2)$, and H_2' as response ($p = 0.01$, $p < 0.01$, $p = 0.02$, respectively; Figure 5). Land-use legacy had a significant effect on N and H ($p = 0.02$, $p < 0.01$, respectively; Figure 5), while the interaction term with regeneration time was never significant at the 5 % level.

Table S4. Fitness parameters of MLR models for each network metric. Models included the terms square-root transformed regeneration time, plot elevation, land-use legacy, and the interaction between square-root transformed regeneration time and land-use legacy.

Response	Df	F	*p*	R^2
N	4, 49	5.5	<0.001	0.25
H	4, 49	10.4	<0.001	0.41
$\exp(H_2)$	4, 49	7.7	<0.001	0.35
H_2'	4, 37	5.4	<0.001	0.30

Network metrics in late regeneration are similar to old-growth metrics. This means that the old-growth level might be reached before the model predicts, since the model assumes a slope for the whole chronosequence.

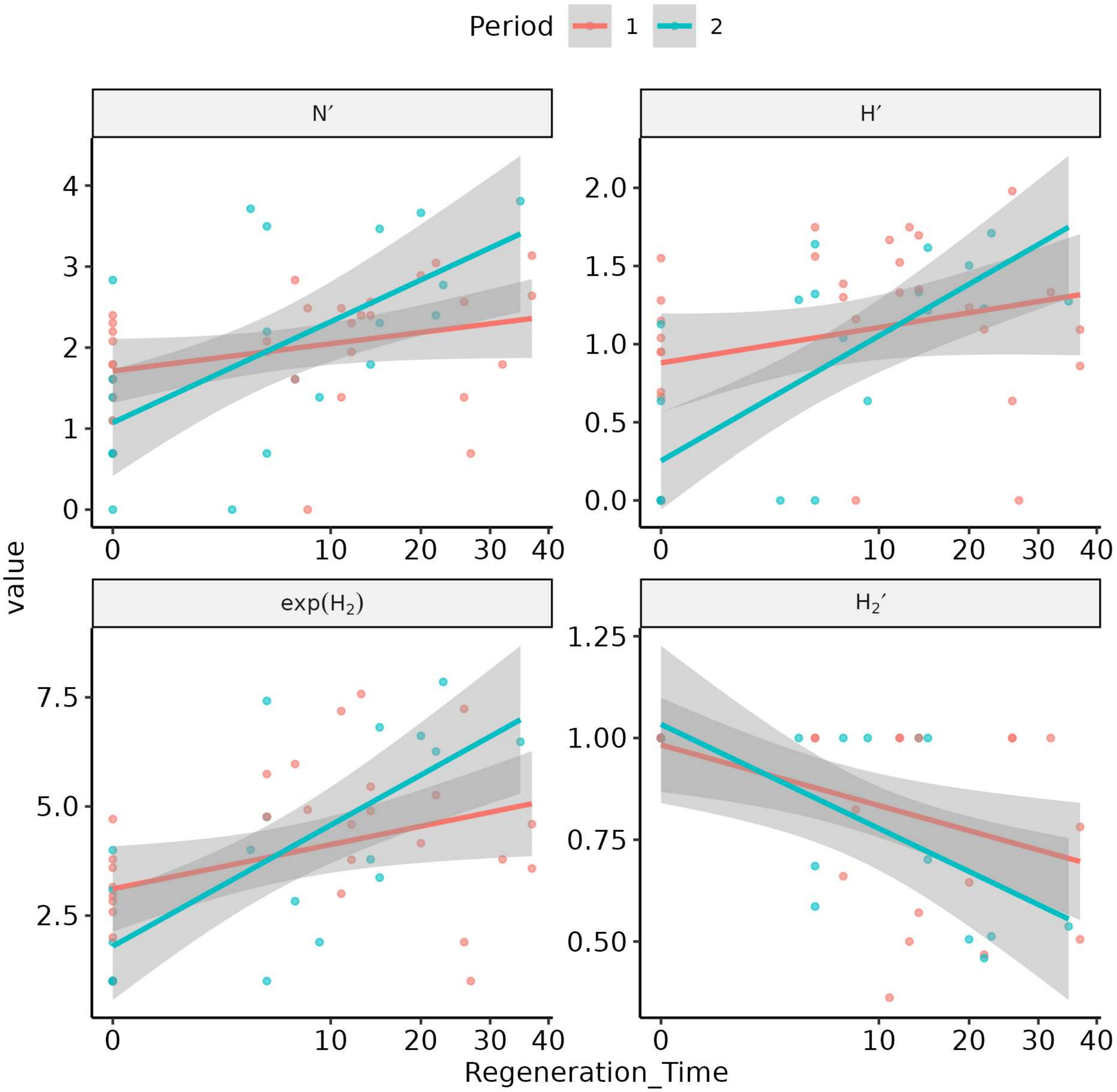

Figure S3. Network metrics by sampling period. The x-axis is square-root transformed as is regeneration time in the linear regressions. N’ = log(N+1), this transformation is also used in the linear models in the main text. The regressions for both sampling periods largely overlap. Thus, including the sampling period as a random intercept was not required.

Nullmodel Comparison

Table S5. Comparison between observed networks and null models. The z value for the slope comparison is calculated as y of the linear model of observed networks minus y of the linear model for the null models (based on Patefield’s algorithm), divided by the standard deviation of residuals in the null model regression (SD NM) for a given metric. The p-value for the slope comparison comes from a linear interaction model with both observed networks and null models (calculated from model parameter t-value). The z-value for the mean comparison is calculated as observed mean minus null model mean, divided by the standard deviation of nullmodels (SD NM). The p-value for the mean comparison is the result of a two-sample Welch t-test of null model values and observed values.

Metric	Legacy	Parameter	Observed	Nullmodel	SD NM	z	*p*
exp(H_2)	Cacao	Slope	3.08	3.26	2.12	-0.088	0.88
exp(H_2)	Cacao	Means	4.61	5.86	2.36	-0.530	<0.001
exp(H_2)	Pasture	Slope	2.81	4.33	2.52	-0.604	0.29
exp(H_2)	Pasture	Means	3.00	3.85	2.97	-0.288	0.05
H_2’	Cacao	Slope	-0.33	-0.28	0.36	-0.134	0.82
H_2’	Cacao	Means	0.83	0.34	0.37	1.319	<0.001
H_2’	Pasture	Slope	-0.38	-0.67	0.35	0.832	0.24
H_2’	Pasture	Means	0.86	0.43	0.43	1.005	<0.001

Additional Network Metrics

In addition to the metrics of abundance, diversity, network complexity, and network specialization, we also calculated the modularity and algebraic connectivity (not connectance) of the network both at the habitat level and at the sample site (plot) level. We show the modules for each habitat and the modularity for each habitat. Additionally, we show the relationship between site-level modularity and forest age below.

Network modularity was stable across the chronosequence, (r = -0.194), p = 0.23). Using the larger networks at the habitat level (agriculture, early regeneration, late regeneration, and old growth) had similar results (Table S6; Figures S4 - S8). In contrast, network connectivitiy increased with forest age R = 0.47, p-value = 0.003 at the site level (Figure S9) but remained stable and extremely interconnected at the habitat level with larger networks with higher abundance (Table S6).

Modularity

Habitat Level Modularity

Table S6 Modularity values for each habitat type.

Habitats	Habitat_modularity

Agriculture	0.484
Early Regeneration	0.441
Late Regeneration	0.324
Old Growth	0.333

The modularity is slightly higher in younger sites and it also decreases over time, this suggests that our network becomes slightly more interconnected with time.

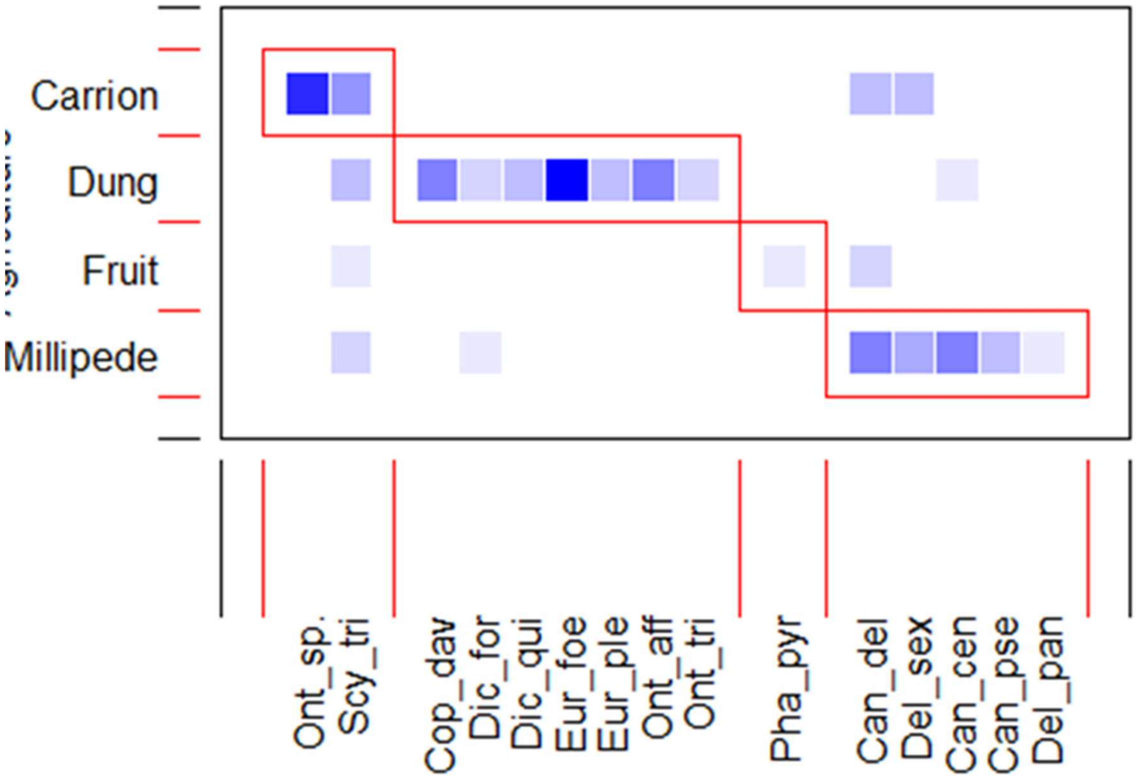

Figure S4. Module plot for agriculture.

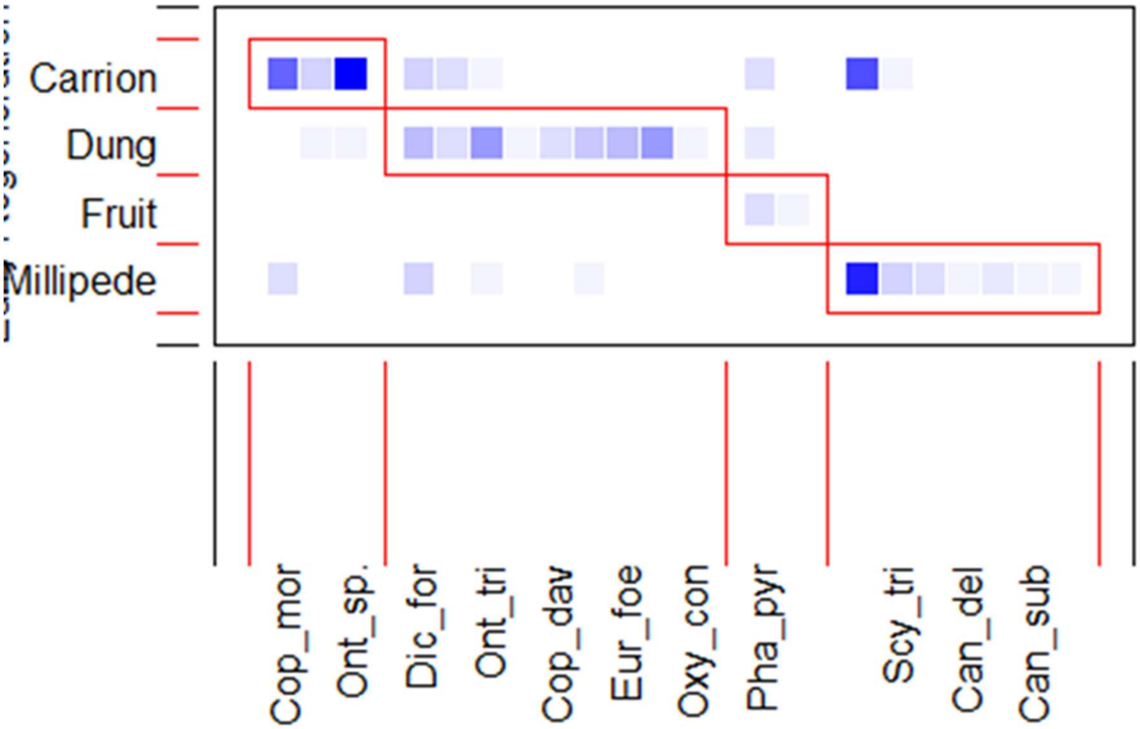

Figure S5. Module plot for early regeneration.

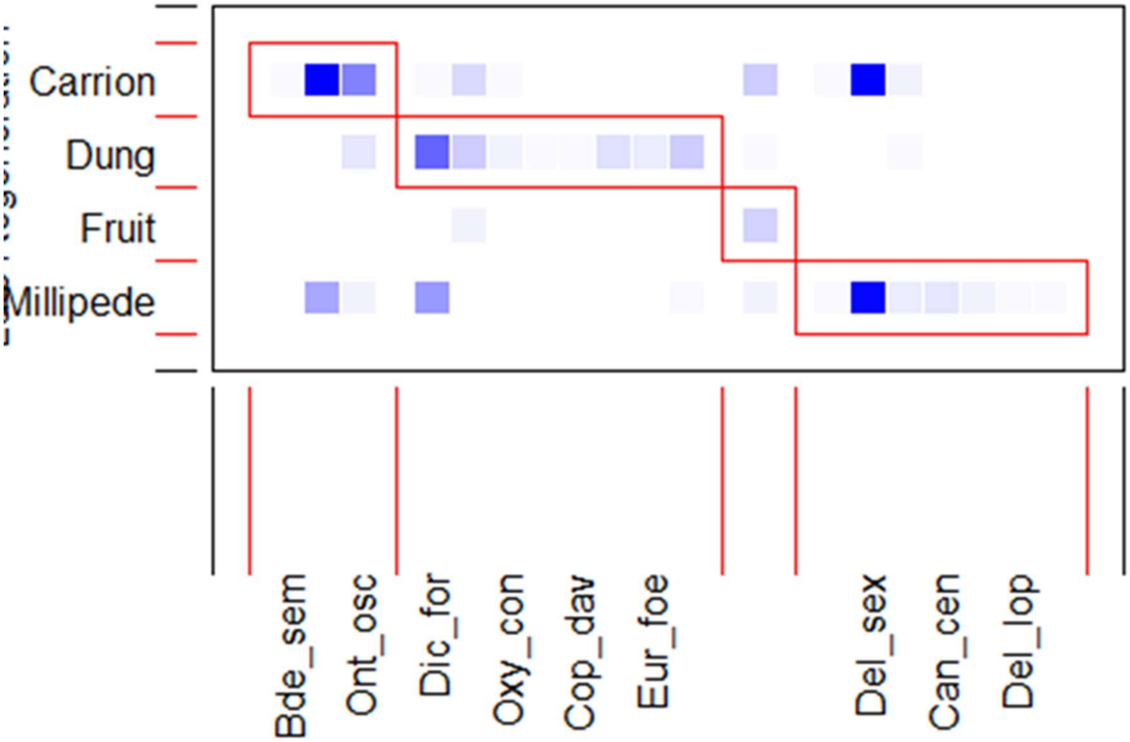

Figure S6. Module plot for modules in late regeneration.

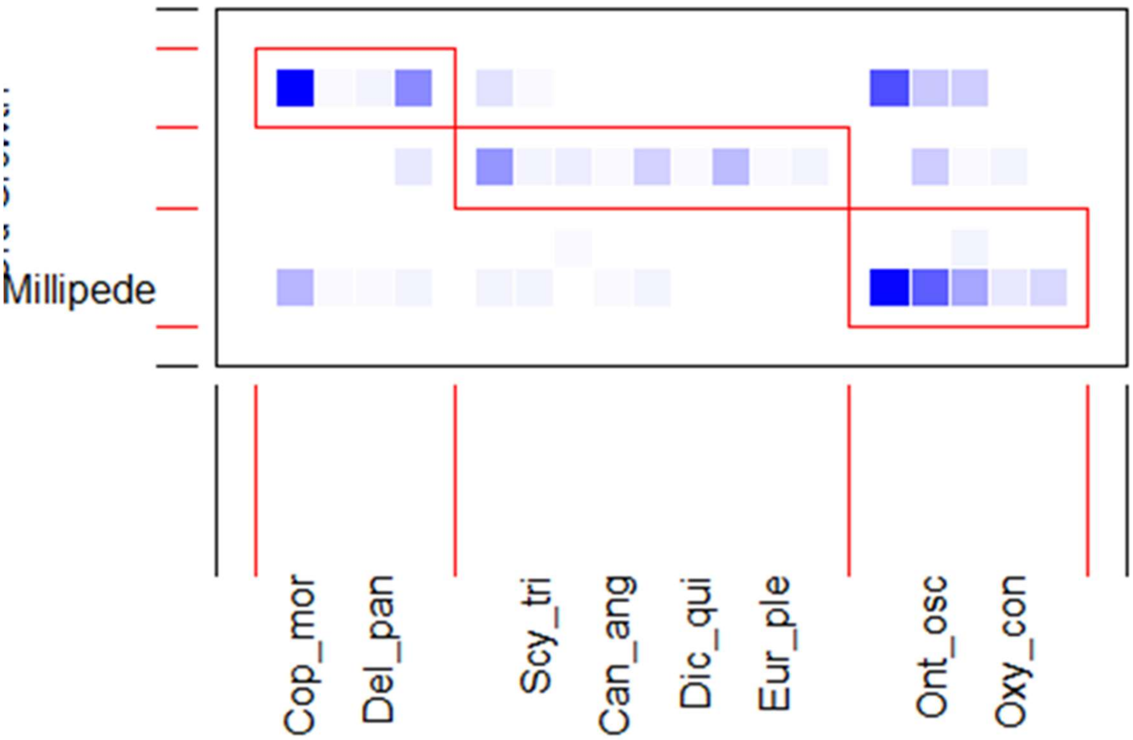

Figure S7. Module plot for modules in the old growth.

The dung and millipede modules have the largest number of members, across all habitat types. Dung is consistently the largest module. Fruit loses its status as a module in the old growth. However, in all cases, there are clearly many interactions taking place outside the modules, which suggests they are artificial. Species also jump modules, for example *Oxysternon conspicillatum* jumps from the dung module to the carrion module and *Scybalocanthon trimaculatus* moves from the carrion module to millipede and then to the dung module.

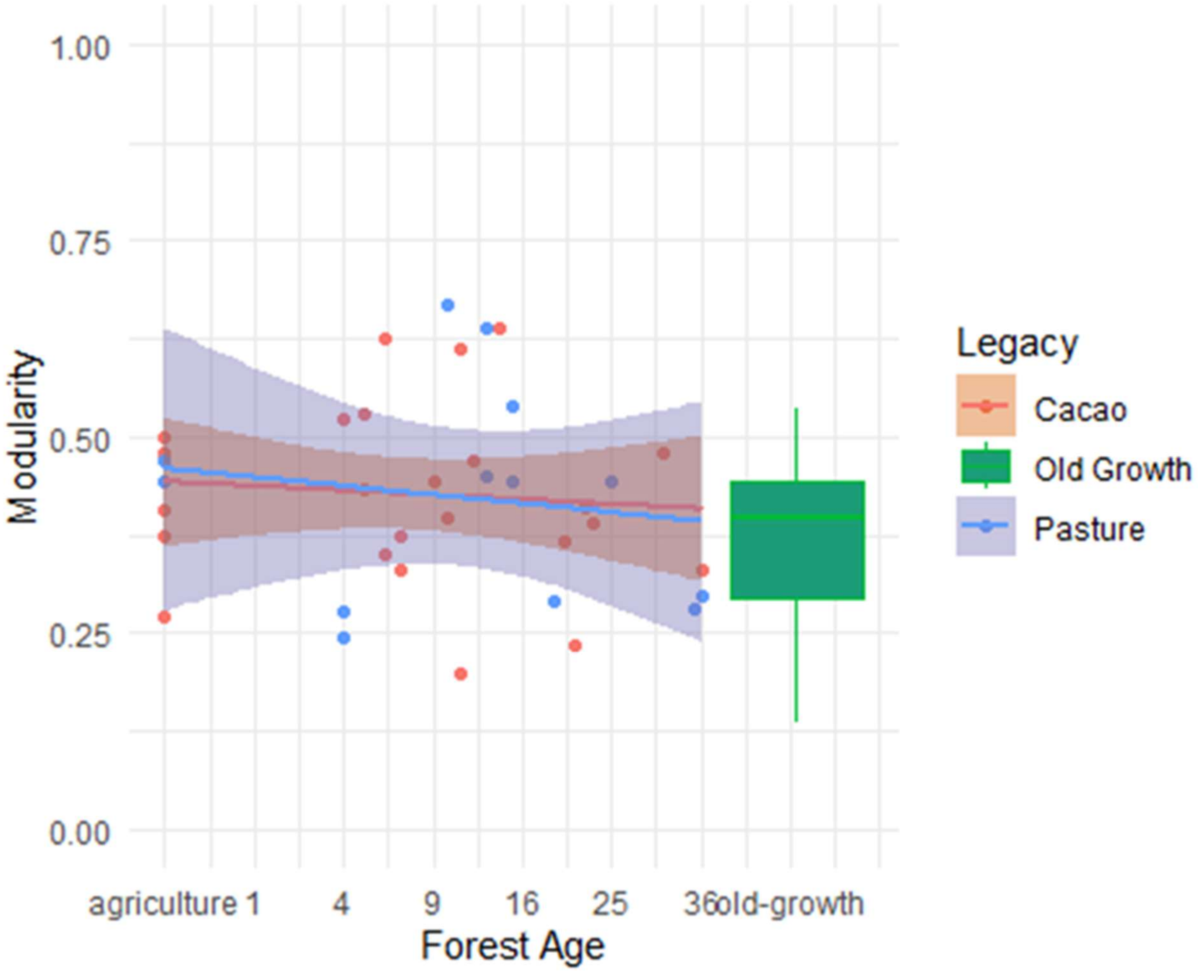

Figure S8. Plot of site level modularity across the chronosequence. The slope is very weakly negative with R = -0.194 but not significant p = 0.2354. This suggests no real relationship between modularity and forest age, though site level networks are very small.

Connectivity

Table S6. Habitat Level Algebraic Connectivity

Algebraic connectivity values for each habitat type.

Habitats	connectivity_values
Agriculture	1
Early Regeneration	1

Late Regeneration	1
Old Growth	1

Plot Level Algebraic Connectivity

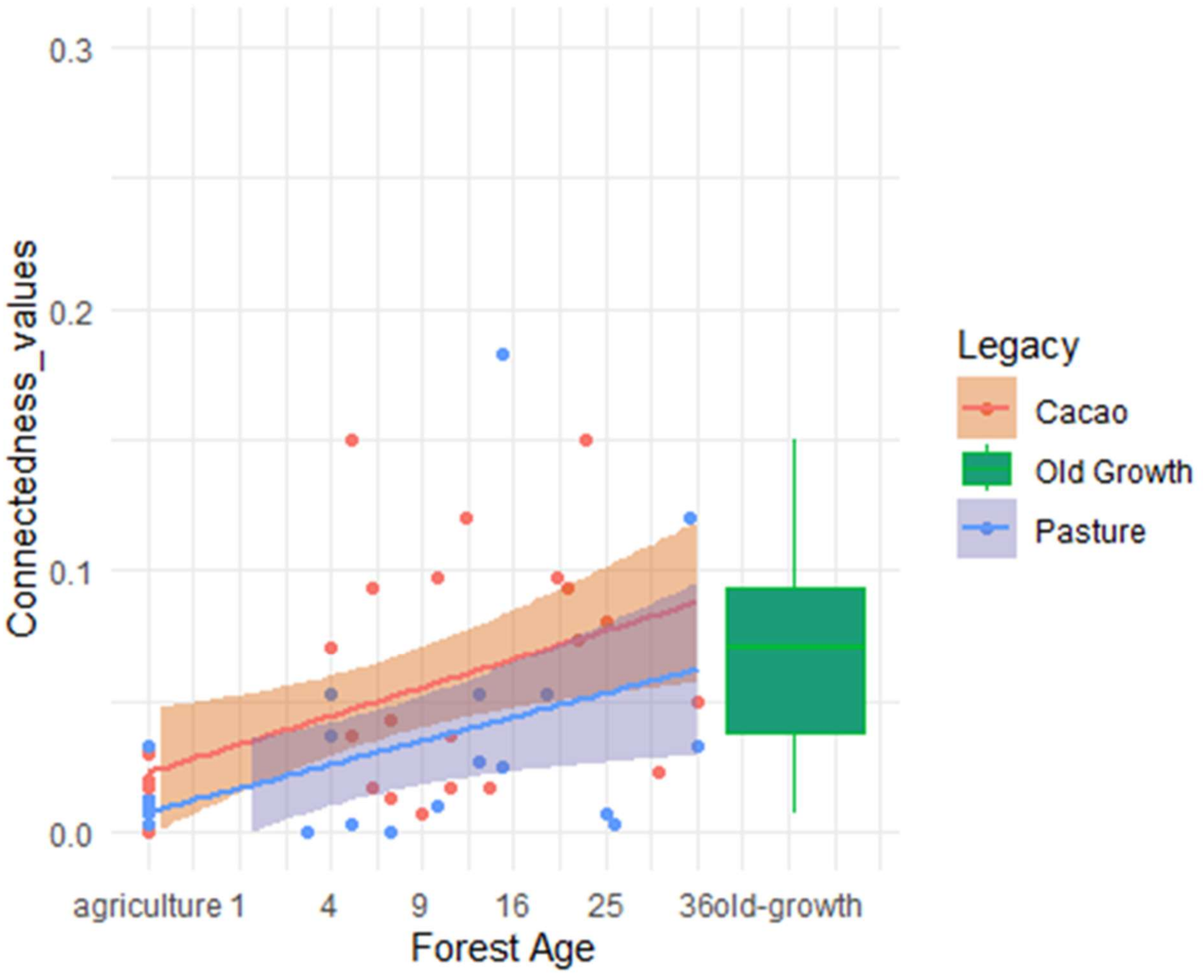

Figure S9. A scatter plot with the connectivity calculated for each sample site (plot). A box plot represents the old growth. The connectivity increases with forest age (R = 0.47, p-value = 0.003). This is probably abundance and diversity-driven, as the networks are larger in older forests, thus allowing for more links per plot and more connected networks.

Multicollinearity

Multicollinearity of the categorical variables land-use legacy and sampling period with regard to the distribution of regeneration ages of secondary forests was assessed visually and via ANOVA (Type II SS). Moreover, we conducted statistical correlation tests for the continuous variables of regeneration age and elevation. To assess multicollinearity of these variables for agricultural plots and secondary forest plots, we used continuous scales (Pearson's product moment correlation). For the analysis of old-growth plots in this context, we used ranked scales (Spearman's ρ). Pearson's correlation was computed with the square-root transformed regeneration time, as this is the transformation used in the model.

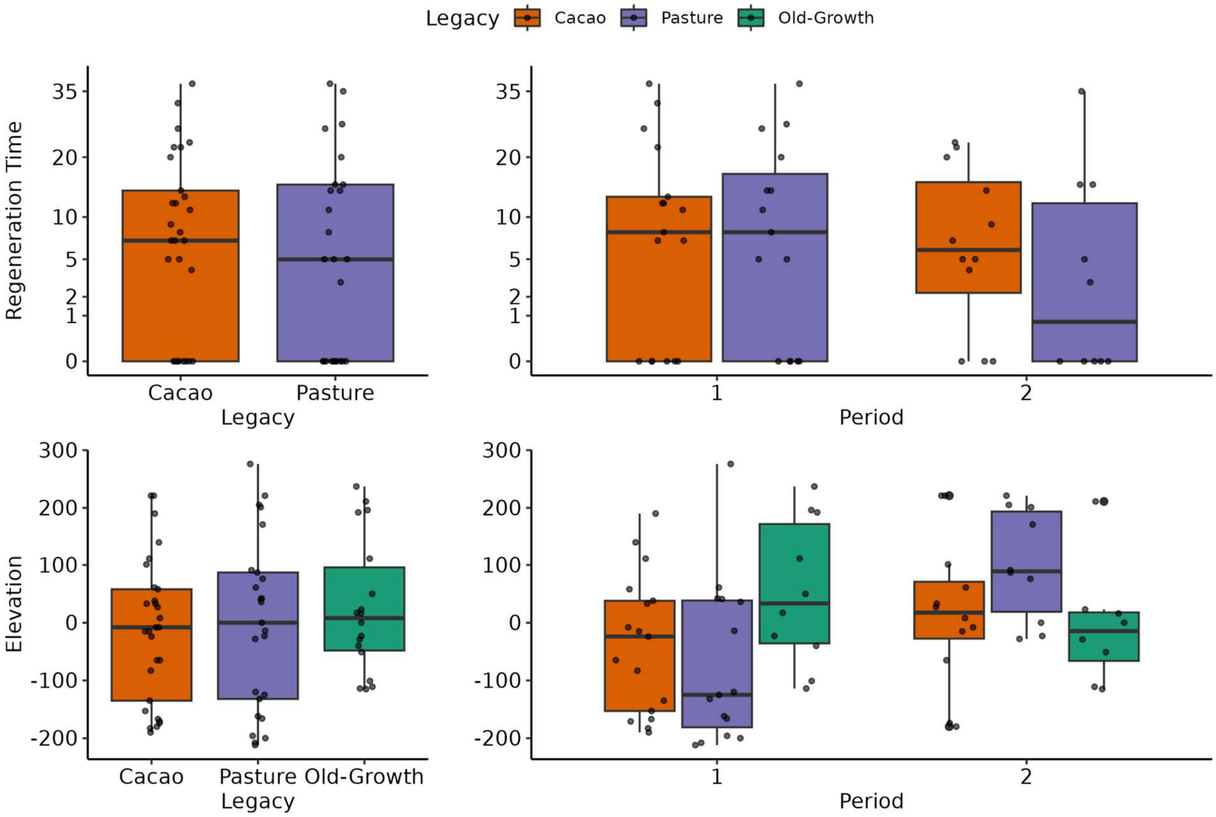

Figure S10. Box plots with distributions of the independent variables regeneration time and elevation by sampling periods and land-use legacy. Left: both sampling periods combined. Right: Split into the sampling periods.

Table S7. Anova results for categorical bias in continuous variables. The continuous variables plot elevation and regeneration time of secondary forest plots are evenly distributed on the different categories.

Response	IV	df	F	p
Elevation	Sampling Period	68, 1	4.7	0.03
Elevation	Legacy	68, 2	0.5	0.62

Regeneration Time	Sampling Period	51, 1	0.8	0.36
Regeneration Time	Legacy	51, 1	<0.1	0.83

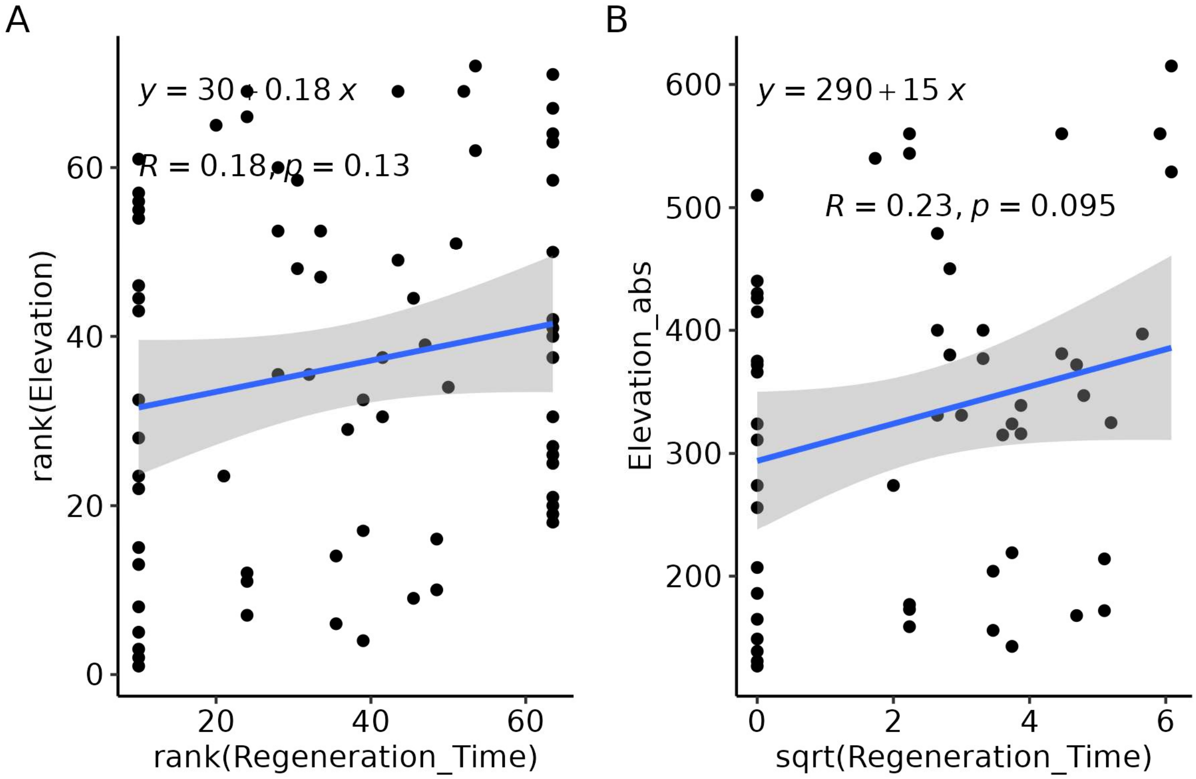

Figure S11. (A) Correlation of ranks of plot elevation and ranks of regeneration time. Due to ranked regeneration time, old-growth plots are included. (B) Correlation of model predictors elevation and sqrt(regeneration time) for agriculture and secondary forest.

We did not detect significant skews in the distribution of plot elevation or regeneration times of secondary forest among the categorical plot variables of land-use legacy and sampling period (Figure S10). Thus, the effects of sampling period and land-use legacy on plot elevation or regeneration age were statistically insignificant (Table S7). Moreover, the sampling period did not have a significant effect on the measured network metrics (Figure S3). The covariates regeneration time of secondary forests and plot elevation are slightly positively correlated (Pearson’s product-moment correlation: $t_{52} = 1.7$, $p = 0.1$, $r = 0.23$), majorly driven by the three oldest regenerating forest plots (Figure S11). When using ranked scales and thus including old-growth plots as the last rank, there is a similar correlation in the data ($S = 5.1 \cdot 10^5$, $p = 0.13$, $\rho = 0.18$).

Resource Use

Below we show the proportion of beetles captured on each resource within different habitats and what proportion of species used each resource within those habitat types. We also show the proportion of beetles captured by each resource at each sample site (plot level) and the proportion of beetles from each feeding guild captured at each site. Feeding guilds were classified as dung feeding, carrion feeding, or generalist no species was specialized on fermented fruit.

Table S8. Relative attractiveness and proportion of species captured with the different bait types per habitat. Relative abundance is calculated as abundance at bait divided by total abundance per habitat. The proportion of species is the proportion of species in a habitat that were captured with the specific bait type.

Habitat	Resource	relative Abundance	Proportion of Species
Old-Growth	Carrion	0.40	0.53
Old-Growth	Dung	0.20	0.74
Old-Growth	Fruit	0.01	0.16
Old-Growth	Millipede	0.39	0.74
Late Regeneration	Carrion	0.45	0.52
Late Regeneration	Dung	0.21	0.62
Late Regeneration	Fruit	0.04	0.14
Late Regeneration	Millipede	0.31	0.62
Early Regeneration	Carrion	0.45	0.39
Early Regeneration	Dung	0.30	0.61
Early Regeneration	Fruit	0.01	0.13
Early Regeneration	Millipede	0.23	0.52
Agriculture	Carrion	0.12	0.31
Agriculture	Dung	0.50	0.69
Agriculture	Fruit	0.04	0.23
Agriculture	Millipede	0.34	0.62

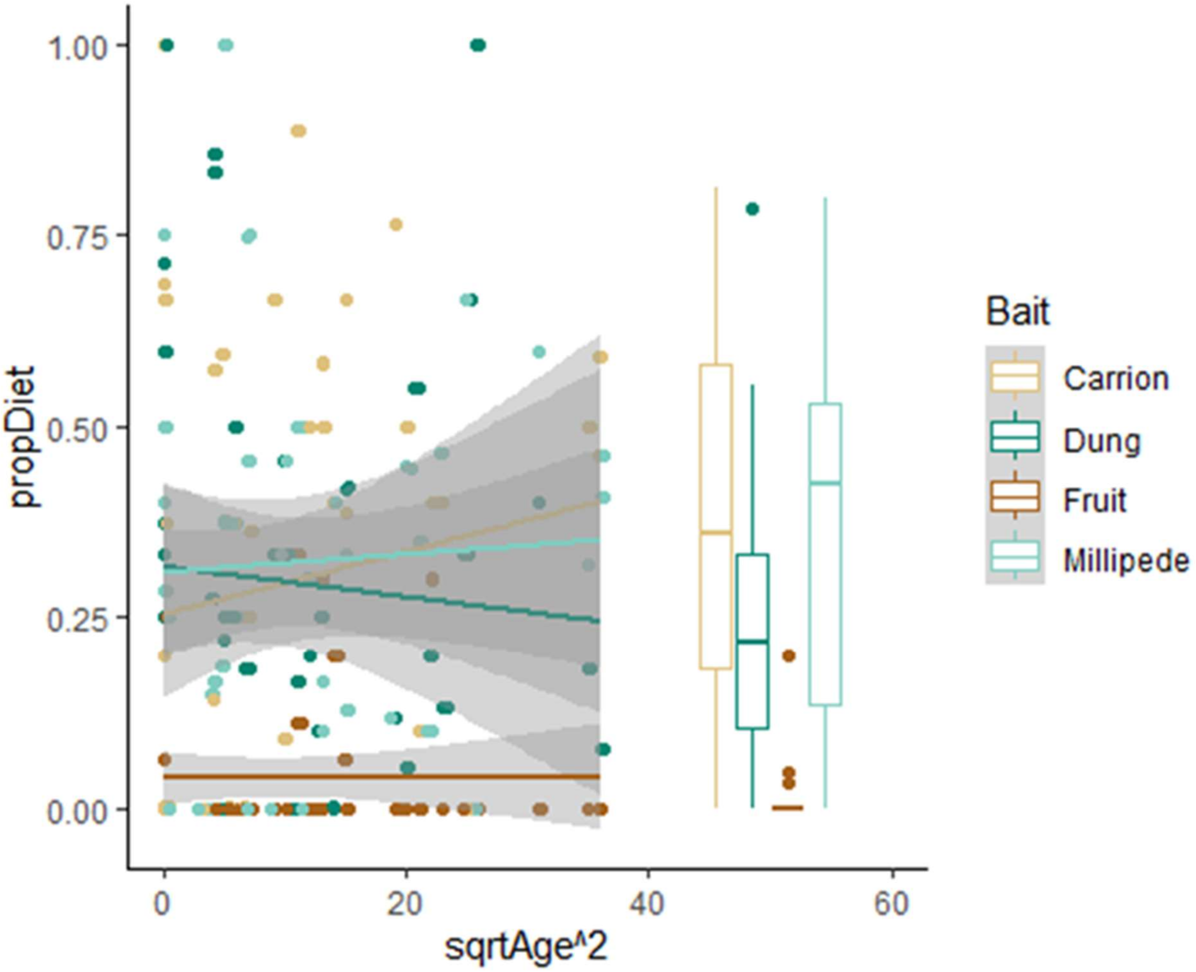

Figure S12. Scatter plot of the proportion of beetles caught in each trap, by each bait type. Fermented fruit (brown) consistently captures a small proportion of the beetles caught at a sample site (plot), the slope for the proportion of beetles captured on dung declines with forest age, and increases on carrion. Millipede shows no real trend.

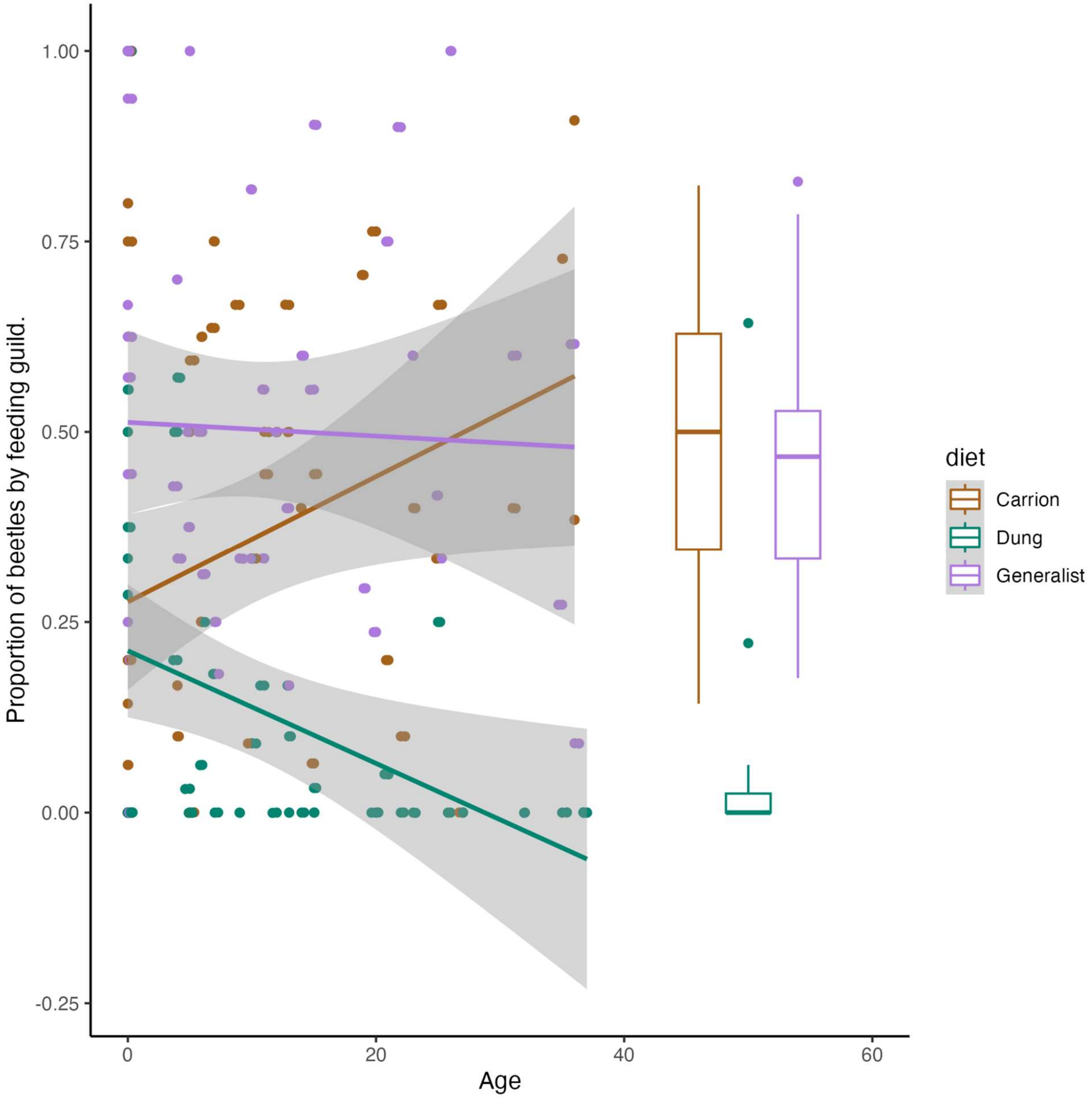

Figure S13. Scatter Plot of the change in the proportion of beetles belonging to different feeding guilds. To belong to a feeding guild a species was caught in a trap for a particular bait type 95% of the time or more.

Distance to the Nearest Old Growth Forest

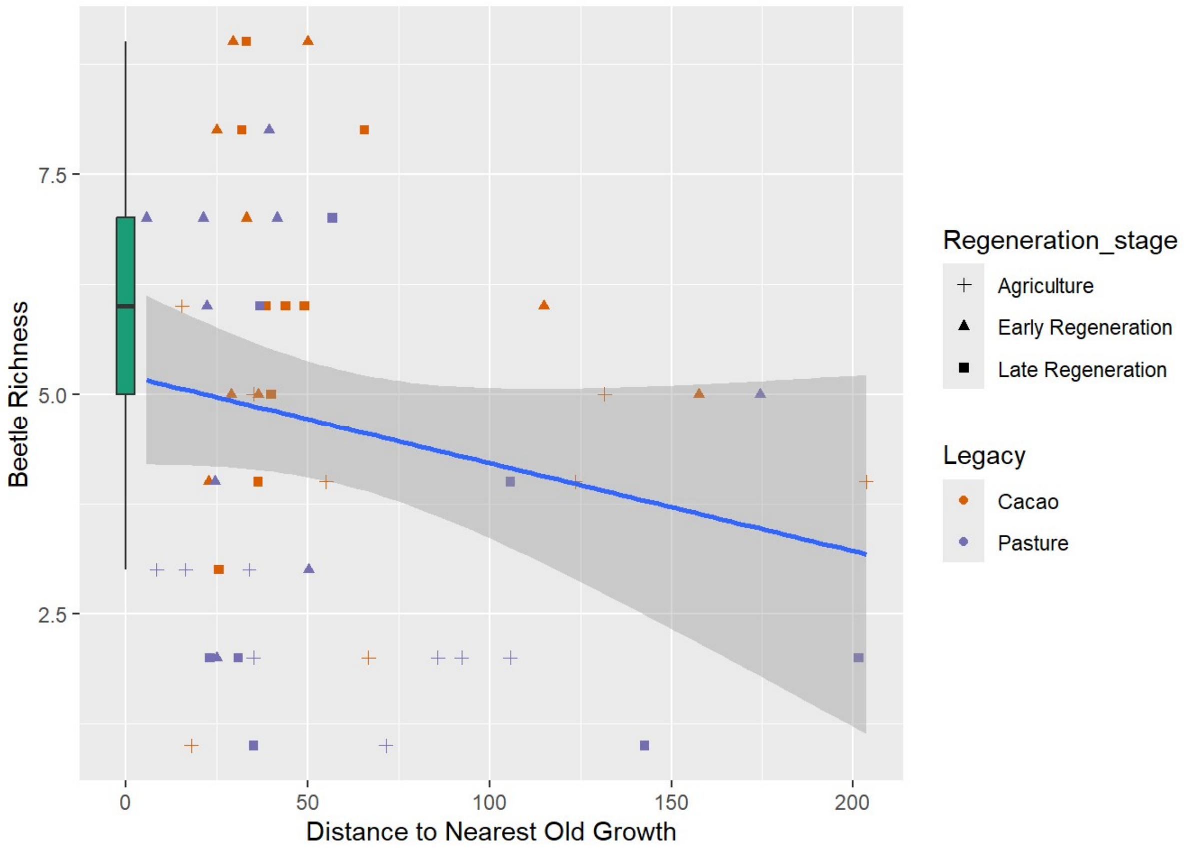

Figure S14. Scatter plot showing species richness and distance to nearest old growth forest. Old growth reference is plotted in a box plot. Different habitat types are represented with different symbols, agriculture is represented by a plus sign, early regeneration is represented by triangles, and late regeneration is represented by squares. Legacy is represented with colors: cacao is in orange, and pasture in purple. The relationship is weakly negative (R = -0.13), but not significant (p.value = 0.36).

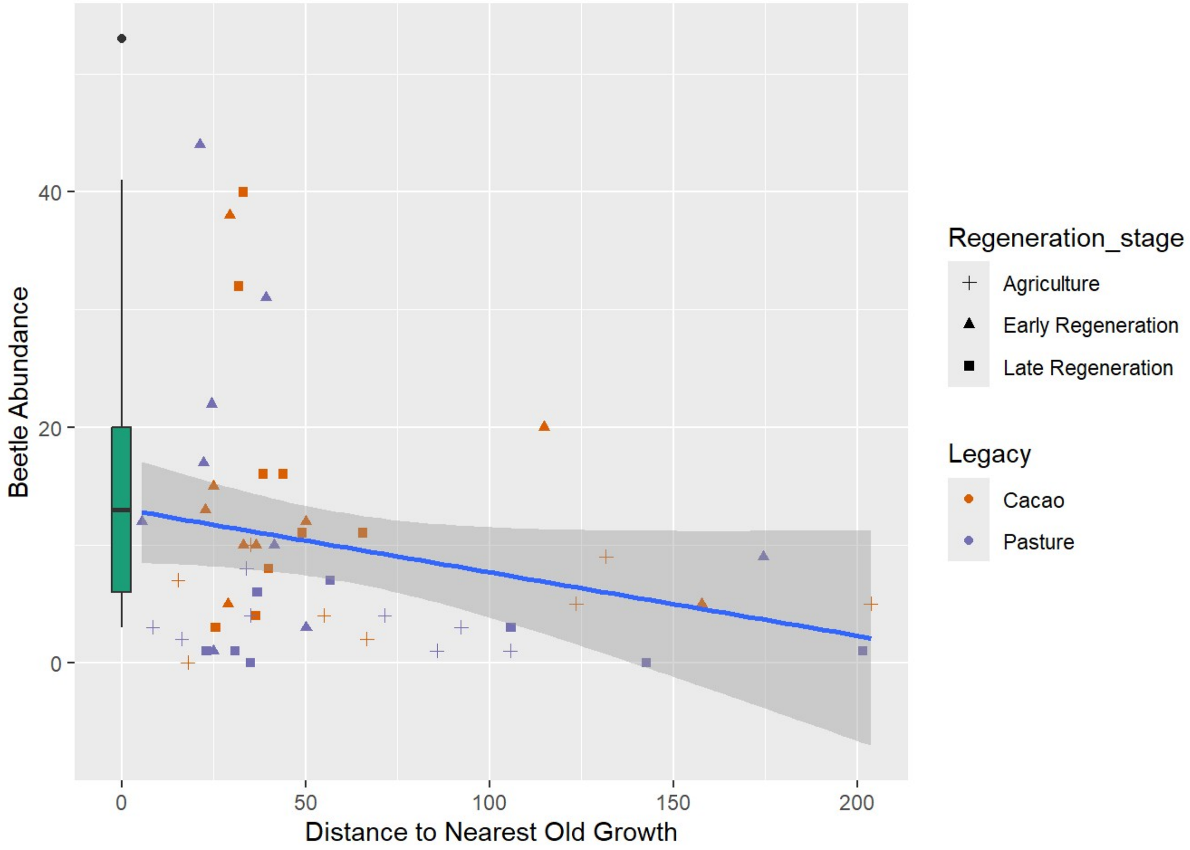

Figure S15. Scatter plot showing beetle abundance and distance to nearest old growth forest. Old growth reference is plotted in a box plot. Different habitat types are represented with different symbols, agriculture is represented by a plus sign, early regeneration is represented by triangles, and late regeneration is represented by squares. Legacy is represented with colors: cacao is in orange, and pasture in purple. The correlation between distance to forest and abundance is weakly negative (R = -0.18) and not significantly so (p.value = 0.198).

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ORIGINAL ARTICLE

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Vertebrate diversity and biomass along a recovery gradient in a lowland tropical forest

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Abstract

Deforestation of tropical forests have resulted in extensive areas of secondary forests with the potential to restore biodiversity to former old-growth forest levels. The recovery of vertebrate communities is an essential component of biodiversity and ecosystem restoration, as vertebrates provide key ecosystem functions. However, little is known about the recovery trajectories and habitat preferences of vertebrates in tropical landscapes with differing land-use legacies. We used camera traps covering 3 weeks to study the activity of ground-based mammals and birds in the understory of 57 sites along a forest recovery gradient, ranging from active agriculture, such as pastures and cacao plantations, to naturally recovering forests and old-growth forests in the Chocó rainforest in north-western Ecuador. Our results show that diversity and biomass of wild vertebrates are highest in old-growth forests and late recovery stages, while for domestic vertebrates, these indices are highest in agricultural

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land. Additionally, while species-habitat networks showed low habitat specificity for vertebrate species, an indicator species analysis found no species to indicate old-growth forests, *Dasyprocta punctata* and *Tayassu pecari* to indicate all forest types, and *Aramides wolfi* and *Pecari tajacu* to indicate late regeneration forests. We suggest that these patterns are caused by a high habitat connectivity and large amounts of remaining old-growth forest in our study area. Our findings indicate that secondary forests have a high potential for the recovery of vertebrate species diversity and biomass to old-growth level in lowland tropical forests with short regeneration times.

Abstract in Spanish is available with online material.

KEYWORDS

biodiversity, forest recovery, indicator species, land-use, legacy effects, networks, rainforests

1 | INTRODUCTION

Tropical forests face many threats, such as deforestation, hunting, and climate change (Wright, 2010). Globally, tropical forest cover decreased with an annual rate of 0.49% since 1990 (Achard et al., 2014). However, forests can recover, and regenerating forest ecosystems account for a sizable amount of forest habitat (Global Forest Resources Assessment, 2020). For instance, among Amazonian countries, secondary forests make up more than half of the forested area in Ecuador, Guyana, and Peru (Smith et al., 2021). Due to the crucial importance of tropical forest recovery (Watson et al., 2018), the United Nations General Assembly (UN) declared 2021–2030 the “UN Decade on Ecosystem Restoration” to reverse deforestation and defaunation of the past decades (UN General Assembly, 2019). At large scales, natural forest regeneration is the most cost-effective strategy for forest restoration (Chazdon & Guariguata, 2016; Crouzeilles et al., 2017; Meli et al., 2017), and it is usually chosen over assisted restoration programs.

Compared to other biomes, vertebrate species have experienced the strongest population declines in tropical forests (Dirzo et al., 2014; Jenkins et al., 2013), which harbor 62% of global terrestrial vertebrate species (Pillay et al., 2022), making them a particularly vulnerable group (WWF, Living Planet Report 2022, Almond, 2022). In addition to habitat loss by deforestation, vertebrates are threatened by habitat modification, hunting, pollution, climate change, and the introduction of diseases and invasive species (Redford, 1992; Young et al., 2016). Besides the continuing decrease of old-growth forests and the accompanied threat to forest dwelling vertebrates, it is argued that second-growth forests could mitigate the losses of old-growth forests, giving vertebrate species the chance to recover (Chazdon et al., 2009). A meta-analysis on vertebrates in regenerating tropical forests has shown that diversity recovers after approximately 40 years of succession, whereas recovery of species composition and some functional groups took longer (Acevedo-Charry & Aide, 2019).

However, processes of forest regeneration, including the recovery of different animal taxa, are not fully understood yet and the strategies for successful biodiversity restoration and conservation are still being debated, for example, whether landscapes should be actively restored or be set aside for natural regeneration (Crouzeilles et al., 2017; Langhammer et al., 2024). To assess the restoration success and conservation value of regenerating forests, biodiversity assessments are needed. These provide quantitative metrics that serve as first step for successful conservation planning and the selection of protected areas (Margules & Pressey, 2000).

This study aims to contribute to the assessment of the current status of Ecuador's secondary forests. We investigated the occurrence of vertebrates along a natural forest recovery gradient in the highly threatened Chocó tropical lowland rainforest in northwestern Ecuador. The Chocó is a global biodiversity hotspot (Orme et al., 2005), with less than 11% remaining old-growth forests left (Fagua et al., 2019). Using a camera trap approach, we captured ground-based vertebrates along a chronosequence selected in the framework of the Reassembly research unit (www.reassembly.de). In our analyses we addressed the following questions: (1) Do wild vertebrate species diversity and biomass recover to old-growth level in regeneration forests? As previous studies identified a fast recovery of vertebrates (Acevedo-Charry & Aide, 2019), we expect similar levels of recovery in older regenerating forests and old-growth forests. (2) Are there differences in the recovery patterns and habitat specializations of wild mammals and wild birds? As meta-analyses have shown that globally mammals are less impacted by human disturbance than birds (Gibson et al., 2011), we hypothesize a faster recovery of mammals than birds. (3) Are there indicator species specific to old-growth forest? As species composition and certain functional vertebrate groups often take longer to recover (Acevedo-Charry & Aide, 2019), we test the specificity of old-growth forest with the aim to identify indicator species that could be of importance in conservation efforts. Based on this, we expect that some habitat specialist species in our study area could be specific to old-growth forests. (4) To which extent do domestic vertebrates use forests as habitat in our study area? Domestics are frequently reported in camera trap studies in South America (Antunes et al., 2022; Lima et al., 2017), but the forest ages in which they occur is rarely considered. The role of domestics in forests is largely understudied, although it could be significant given the substantial biomass of species such as cattle and horses and possible consequent ecological impacts. In our study, we expect to find domestics in forests with different regeneration ages along the chronosequence.

2 | METHODS

2.1 | Study site and plot design

The study was conducted in a lowland rainforest located in northwest Ecuador (Esmeraldas Province) within the Reserva Río Canandé and Reserva Tesoro Escondido (Figure 1). Fieldwork was performed during the dry seasons (October–November) of 2021 and 2022 as dry seasons are the standard for camera trapping in the tropics (Jansen et al., 2014). The landscape is characterized by a patchy distribution of small-scale agriculture (pastures and cacao plantations), human settlements, and regenerating and old-growth forests (Figure 2a). Forest cover within a 1-km radius of each plot averaged 74% (SD ± 11; CI 68–79; ranging from 3% to 99%), whereas mean distance to the nearest old growth forest from each plot was on average 59 m (SD ± 46; CI 48–70; ranging from 0 to 202 m) based on analysis of current and historic land cover (Escobar et al., 2024).

We assessed vertebrate occurrences on a total of 65 plots. Plots in actively used agricultural land measured 16 × 16 m, while all other plots were 50 × 50 m. From these, we excluded eight plots due to technical issues with camera trapping. These issues comprised cases in which cameras did not function correctly or vision was reduced by vegetation, such as dropping leaves that blocked the vision. In the following we describe and analyze the data of the remaining 57 plots.

Our plot selection represents a recovery gradient ranging from agricultural land ($n = 14$) over regenerating forests ($n = 28$) to old-growth forests ($n = 15$). We categorized the sites into four land-use categories based on land-use legacy and regeneration ages [Agriculture: 7 pastures; 7 cacao plots; Recovery I: forests with regeneration times between 1 and 20 years (6 former pastures; 9 former cacao plantations); Recovery II: forests with regeneration times between 20 and 38 years (7 former pastures; 6 former cacao plantations); and, Old-growth forest plots (15)]. Agricultural land was still actively managed while recovering pastures and cacao plantations experienced no or only little anthropogenic disturbance similar to old-growth forests. Depending on the analysis, we used these four categories based on the regeneration times or sorted into seven subcategories when including the legacies of active or regenerating pastures and cocoa plantations (PA: Pasture Active; CA: Cacao Active; PRI: Pasture Regeneration 1; PRII: Pasture Regeneration 2; CRI: Cacao Regeneration 1; CRII: Cacao Regeneration 2; OG: Old-Growth Forests). The dates of land purchased by the nongovernmental organization Fundación Jocotoco as well as interviews with park rangers and farmers, revealed the regeneration ages and former land-use legacy (pasture or cacao plantation) of the forests. Further plot details, such as location and regeneration age, are provided in the Table S4.

2.2 | Camera trapping

On each plot, we installed one camera trap (Reconyx Hyperfire). The cameras were deployed over a course of 3 weeks and retrieved in the same order. In doing so, each camera operated over a period of 3 weeks. Plots were selected by the Reassembly research unit (www.reassembly.de) to investigate different reassembly processes along a forest recovery gradient from agriculture to old growth forests. We placed cameras within the plot on a tree at 30–80 cm height to face open areas or animal trails with the aim to increase detection probability. Camera traps were set to take three images per trigger event with a delay of 1 s between triggers. Vertebrates (birds and mammals) on the single images were then identified to species level (Billerman et al., 2022; Ridgely & Greenfield, 2006; Tirira et al., 2023) using the camera-trap management software TRAPPER (Bubnicki et al., 2016). Species identification was conducted by Jörg Müller and his team, which is experienced in identifying species based on images derived by camera traps. In a few ambiguous cases local experts were consulted. For assessing the number of species occurrences, we defined an event for a species as a single image or a consecutive sequence of images of one or several individuals of the same species at the same camera trap location, with a minimum interval of 5 min from the last image or image sequence (Henrich et al., 2022; Rovero & Marshall, 2009). When several individuals of the same species occurred on an image or an image sequence, we counted each individual as an individual event. For most of our captured species, the identification and differentiation of individuals was not possible and single individuals might have been counted several times. Hence, the number of species events in our study represents the habitat use of a specific plot and thus their functional role rather than the species abundance in the area.

Besides wild vertebrates, we also counted individual events of domestic animals as they are functionally present on the plots, for example, as a source of dung, as herbivores, or as prey. To evaluate the use of the various land-use categories, we only counted domestic animals when roaming freely on the plot, not when they accompanied humans (such as horses and donkeys).

We summarized all observed events, but included only ground-based vertebrates in our analysis as these species are the focal group of our investigation. We categorized vertebrates as ground-based when they spend the majority of their time foraging on the ground. The classification was based on information published in the Elton Traits database (Wilman et al., 2014), Birds of the World (Billerman et al., 2022) and expert opinion.

Additionally, we excluded local people from analyses as we assumed humans were not functionally present as described above for the domestic vertebrates.

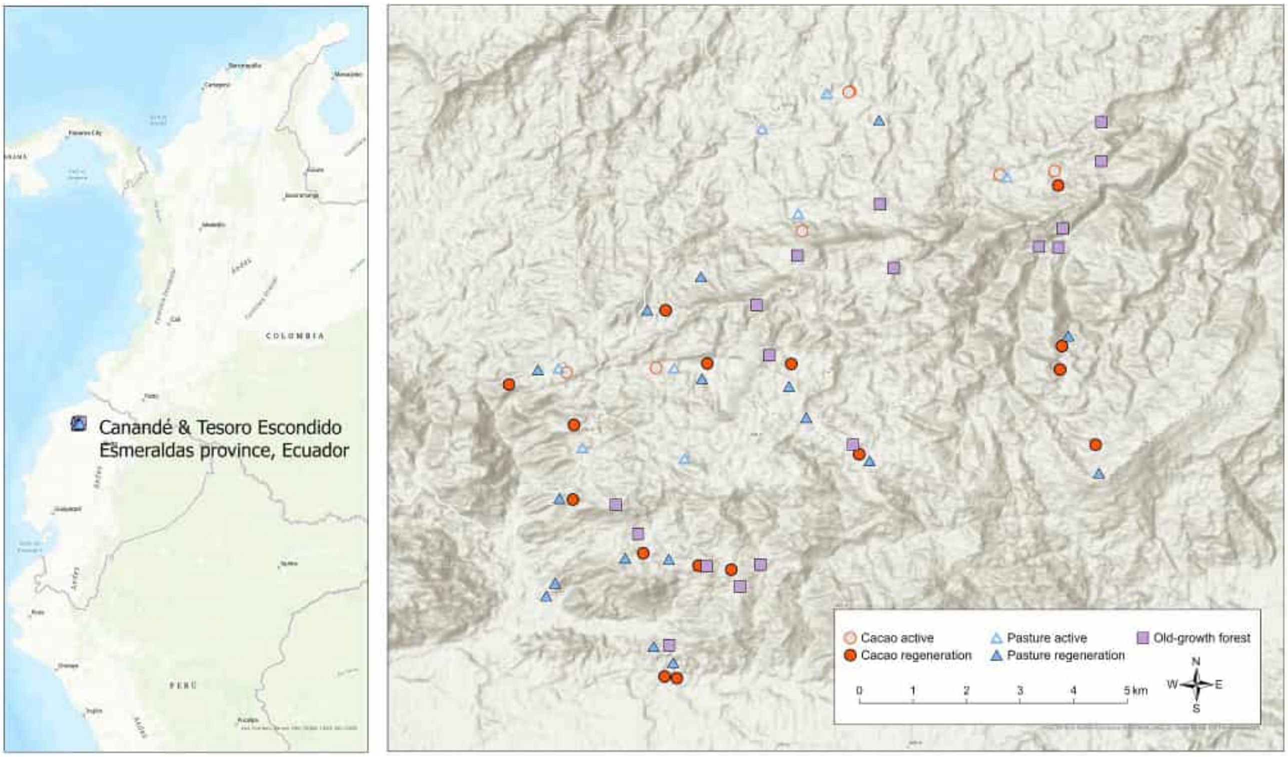

FIGURE 1 Distribution of the 65 study plots of the REASSEMBLY project in the reserves Río Canandé and Tesoro Escondido in the Ecuadorian Chocó rainforest. Symbols indicate different habitats and land-use legacies.

2.3 | Statistical analyses

All analyses were conducted with R version 4.2.0 (R Core Team, 2022). We calculated species diversity and biomass of vertebrates sorted into three groups: wild mammals, wild birds, and domestic vertebrates (comprising both domestic mammals and birds) separately for seven land-use categories described above. Diversity is often expressed with indices such as species richness, Shannon index, Shannon-Weaver index or Simpson index. However, the use of the term species richness has been criticized as it does often not consider the effects of abundance and sampling effort (see Gotelli & Colwell, 2001). As a standardization of our data is not possible due to low numbers of observations in many plots (e.g., for the calculation of species richness), we used the Hill numbers q0 and q1 (Hill, 1973) to quantify species diversity as follows: as the first Hill number (q0) we used the number of observed species. It was calculated using the "specnumber" function from the *vegan* package (Oksanen et al., 2022). As the second Hill number (q1) we calculated in a first step the Shannon indices for our plots with the "diversity" function from the *vegan* package. In a second step, we calculated the exponential function of the Shannon index (exp(Shannon)). This gives weight to abundance as well and as effective number of species it provides a number of statistical advantages (see Jost, 2006).

Biomass data for vertebrates was gathered from published sources (*Sporophila nigricollis* (yellow-bellied seedeater): Dunning Jr., 2007; *Equus caballus* (domestic horse): Carroll & Huntington, 1988; *Equus africanus* (domestic ass): de Aluja et al., 2005; all other vertebrates: Wilman et al., 2014) and then multiplied with the frequency of events per plot for each species to calculate the biomass per plot (Table S7). Similar to our definition of events, in our study, the biomass based on camera trap captures does not represent the plot's actual biomass as individuals could be captured several times. However, we assume that multiple captures of the same individuals indicate a high usage and hence a high impact of the captured species on the plots. Thus, although we do not display actual biomass on the plots, our calculated biomass represents the functional biomass of vertebrates on the plots.

We analyzed species diversity and biomass differences between the different land-use categories using generalized linear models. Land-use categories were set as ordered categories to reflect the order in which the temporal progression of the land-use gradient occurs from active agriculture to regenerating forests to old-growth forests. Pasture was ranked first before cacao, because of its open, grass-dominated habitat. In our generalized linear models, we chose a Gaussian distribution for continuous logarithmic transformed data (species diversity (q1) and biomass+1), to reach normal distribution, and a negative binomial distribution for count data (species diversity (q0)). We tested for spatial independence of the model residuals with cross-correlograms using the "spline.correlog" function of the *nfc* package.

We predicted estimated species diversity (q0), Shannon diversity, and biomass values for each vertebrate group in all 65 plots (including plots with failed cameras), in a generalized additive model (Table S5). We included land-use category, space (latitude,

FIGURE 2 The landscape of Reserva Rio Canandé (Ecuador) consists of forests of different ages intermingled with human settlements and agriculture (a). Habitats can be used by different vertebrate groups. One pasture plot, for example, was used by humans (b), domestic animals like cows, chicken, and horses (c, d), and wild mammals like pumas (e) within 48 h.

longitude), and elevation as input variables as well as forest cover within a radius of 1 km, distance to the nearest forest, and distance to the nearest forest edge. Spatial independence of the model residuals with cross-correlograms was performed as described above (Figure S4). The predicted estimations for all plots can be used in future investigations of our study area, that take into account these landscape variables.

For analyzing habitat specialization, we generated networks of the three vertebrate groups across our four land-use categories using the *bipartite* package (Dormann et al., 2008). As input we used events per species and plot as surrogates for abundance, hence the width of the network links represents the frequency of a species in the respective land-use category. The complementary specialization index (H2'), an indicator for specialization (Blüthgen et al., 2006), was calculated using the function "H2fun". We tested it against a null model with 1000 networks generated with the method "r2dtable" based on the Patefield algorithm. Networks were visualized using the "plotweb" function.

We conducted an indicator species analysis for our four land-use categories using the package *indicspecies* (Cáceres et al., 2022). Input for the community data matrix were species events in each land-use category (Table S8). Using the "multipatt" function, we calculated an indicator value (IndVal) of each species based on Dufrêne and Legendre (1997) with the land-use categories as groups, and we allowed the combination of site groups (as explained in Cáceres et al., 2022). We allowed either single land-use categories or neighboring land-use categories along the recovery gradient as combinations. The statistical significance of the calculated values was assessed with a permutation test with 1000 permutations.

Lastly, we compared our species community with the community derived from an assessment based on sound recorders that were placed on the same plots within the same time (Müller et al., 2023). For this, sound recordings were identified by two specialists (for details see Müller et al., 2023). Here, we identified species that were captured with both methods and counted the presence of each species in each land-use category. As the number of analyzed plots differed between studies (camera traps: $n = 57$, sound recorders: $n = 43$) and definitions for capture frequencies were different between both methods, we calculated the number of plots on which the species occurred (presence-absence) for better comparison.

3 | RESULTS

3.1 | General summary of collected data

In total, we recorded 40 species (24 mammal species and 16 bird species) in 1197 camera days in 1487 events across all land-use categories (Table 1, Table S6). According to the IUCN Red List of Threatened Species (IUCN, 2023), three species are classified as vulnerable (*Aramides wolfi* (brown wood rail), *Tayassu pecari* (white-lipped peccary), and *Cebus capucinus* (Colombian white-faced capuchin)) and one species as endangered (*Neomorphus radiolosus* (banded ground cuckoo)). Three species were classified as near threatened (*Penelope purpurascens* (crested guan), *Leopardus wiedii* (margay), and *Panthera onca* (jaguar)), whereas all other species were classified as least concern or did not have an assessment due to deficient data.

Carnivora was the most species-rich group, with nine species, followed by Rodentia with four species and Galliformes and Columbiformes with three species each. Among the 40 species, we identified 34 wild and six domestic vertebrates. Species with the most events were domestic chicken ($n = 343$), Central American agouti ($n = 258$), cattle ($n = 196$), and lowland paca ($n = 123$).

After assessing the species' prevalent foraging stratum, we excluded eight non-ground-based species (*Cebus capucinus*, *Buteogallus anthracinus*, *Phaethornis striigularis*, *Coragyps atratus*, *Penelope purpurascens*, *Aramus guarauna*, *Furnarius leucopus*, and *Sporophila nigricollis*) from our data set and continued analysis with the remaining 32 ground-based species.

3.2 | Comparison with sound recorders

A comparison of our results with published data of vertebrate communities derived from sound recorders, that were placed on the same plots at the same time, showed that there is little overlap in species detection using both methods (Table S1). From 32 wild vertebrate species identified by camera traps and 316 by sound recorders, only 11 species were detected by both methods.

3.3 | Patterns of species diversity and biomass along the forest recovery gradient

Species diversity (q0 and q1) and biomass of wild mammals each increased along the forest regeneration gradient (Figure 3a,d,g; Table S2). Wild birds showed highest values for these metrics in older regenerating forests (PRII and CRII) and old-growth forest (Figure 3b,e,h), but only a significant trend along the recovery gradient for biomass (Table S2). Domestic vertebrates showed a significant decrease from agriculture towards old-growth forests in diversity (q0 and q1) and biomass (Figure 3c,f,i; Table S2). High biomasses of domestics in agriculture were quantified due to a high number of events by domestic cattle in pastures ($n = 196$) and their high body mass.

The analysis of spatial independence of our selected plots revealed that the residuals of all nine linear models are spatially independent (Figure S3), indicating that vertebrate distributions are not explained by the longitudinal, latitudinal, or altitudinal parameters of the location of our selected plots. Our predicted values for vertebrate species diversity (q0), Shannon diversity, and biomass for all plots based on a generalized additive model are provided in Table S5. They can be used in future investigations of our study area, which consider land-use category, space (latitude, longitude), elevation, forest cover within a radius of 1 km, distance to the nearest forest and distance to the nearest forest edge.

3.4 | Species networks across land-use

Our network analysis examined the habitat preferences and specialization of vertebrate groups (Figure 4). The results showed that wild mammals are not specialized in their habitat use regarding the four land-use categories, which is reflected in a low degree of specialization not significantly different from the null model ($H_2' = 0.12$; $p > .05$). The network for wild mammals showed that most species were recorded in several land-use categories (Figure 4a). Large vertebrates (defined as >3 kg based on Cardillo et al., 2005) were found across all land-use categories. For example, *Cuniculus paca* (lowland paca) and *Dasyprocta punctata* (Central American agouti) were recorded in plots of all four land-use categories. From the two peccari species *Tayassu pecari* (white-lipped peccari) was present in all three forested categories (Recovery I, Recovery II, and Old-growth forest) and *Dicotyles tajacu* (collared peccary) was present in regenerating

TABLE 1 Independent events per species on the different land-use categories along a forest recovery gradient (PA: Pasture, CA: Cacao plantation, PR: Pasture recovery, CR: Cacao recovery, RI: 0–20 years recovery, RII: 20–38 years recovery, OG: Old-growth forest) as well as the total number of events per species, and the number of plots (*n* plots) a species was captured on.

Subcategories		PA	CA	PRI	CRI	PRII	CRII	OG	Total	*n* plots
Wild mammals										
Artiodactyla										
Mazama gualea	South American Red Brocket	0	0	1	1	0	0	3	5	4
Carnivora - Felidae										
Leopardus pardalis	Ocelot	0	1	1	0	2	1	4	9	8
Leopardus wiedii	Margay	0	0	2	1	0	0	2	5	4
Panthera onca	Jaguar	0	0	1	0	2	0	3	6	6
Puma concolor	Cougar	1	0	0	0	2	10	7	20	6
Herpailurus yagouaroundi	Jaguarundi	0	0	0	0	0	0	1	1	1
Carnivora - Mustelidae										
Eira barbara	Tayra	1	0	1	4	1	3	8	18	10
Neogale spec.		0	0	0	0	1	0	0	1	1
Carnivora - Procyonidae										
Procyon cancrivorus	Crab-eating raccoon	0	0	0	2	1	0	1	4	4
Cetartiodactyla										
Dicotyles tajacu	Collared peccary	0	0	0	1	6	17	0	24	4
Tayassu pecari	White-lipped peccary	0	0	4	2	14	6	19	45	15
Cingulata										
Dasypus novemcinctus	Nine-banded armadillo	4	6	9	12	7	7	14	59	20
Didelphimorphia										
Didelphis marsupialis	Common opossum	1	6	0	0	6	2	4	19	12
Pilosa										
Tamandua mexicana	Northern tamandua	0	0	0	0	0	1	1	2	2
Primates										
*Cebus capucinus**	Colombian white-faced capuchin	0	0	0	0	1	0	0	1	1
Rodentia										
Cuniculus paca	Lowland paca	0	8	0	61	5	12	37	123	22
Dasyprocta punctata	Central American agouti	0	7	1	92	19	15	124	258	27
Oecomys spec.		0	17	0	1	0	0	5	23	7
Sciurus granatensis	Red-tailed squirrel	0	0	0	1	8	0	10	19	7
Wild birds										
Accipitriformes										
*Buteogallus anthracinus**	Common black hawk	0	0	0	0	0	0	1	1	1
Caprimulgiformes										
*Phaethornis striigularis**	Stripe-throated hermit	0	0	0	1	0	0	0	1	1
Cathartiformes										
*Coragyps atratus**	Black vulture	14	0	0	0	0	0	0	14	1
Columbiformes										
Geotrygon montana	Ruddy quail-dove	0	0	1	0	5	0	0	6	2
Leptotila pallida	Pallid dove	0	11	0	0	4	1	0	16	4
Leptotrygon veraguensis	Olive-backed quail-dove	0	0	0	0	1	0	3	4	2
Cuculiformes										
Neomorphus radiolosus	Banded ground cuckoo	0	0	0	0	1	0	0	1	1

(Continues)

TABLE 1 (Continued)

Subcategories		PA	CA	PRI	CRI	PRII	CRII	OG	Total	*n* plots
Galliformes										
Odontophorus erythrops	Rufous-fronted wood-quail	0	0	0	0	0	0	2	2	2
*Penelope purpurascens**	Crested guan	0	0	0	0	0	1	0	1	1
Gruiformes										
Aramides wolfi	Brown wood rail	0	0	0	0	2	1	0	3	3
*Aramus guarauna**	Limpkin	0	1	0	0	0	0	0	1	1
Passeriformes										
*Furnarius leucopus**	Pale-legged hornero	1	0	0	0	0	0	0	1	1
*Sporophila nigricollis**	Yellow-bellied seedeater	0	8	0	0	0	0	0	8	1
Struthioniformes										
Crypturellus soui	Little tinamou	0	0	0	0	0	1	0	1	1
Tinamus major	Great tinamou	0	0	0	2	9	0	6	17	8
Domestics										
Bos taurus	Domestic cattle	194	0	2	0	0	0	0	196	5
Canis lupus familiaris	Domestic dog	12	13	0	1	0	5	0	31	10
Equus africanus	Domestic ass	0	0	0	1	1	0	2	4	3
Equus caballus	Domestic horse	69	1	1	3	0	0	6	80	8
Gallus gallus domesticus	Domestic chicken	337	6	0	0	0	0	0	343	6
Sus domesticus	Domestic pig	4	0	0	0	0	0	0	4	2
Homo sapiens		36	46	12	4	0	7	5	110	21
Total events per land-use category		674	131	36	186	102	90	268	1487	

Note: Species marked with asterisk were not classified as ground-based and excluded from analysis together with humans.

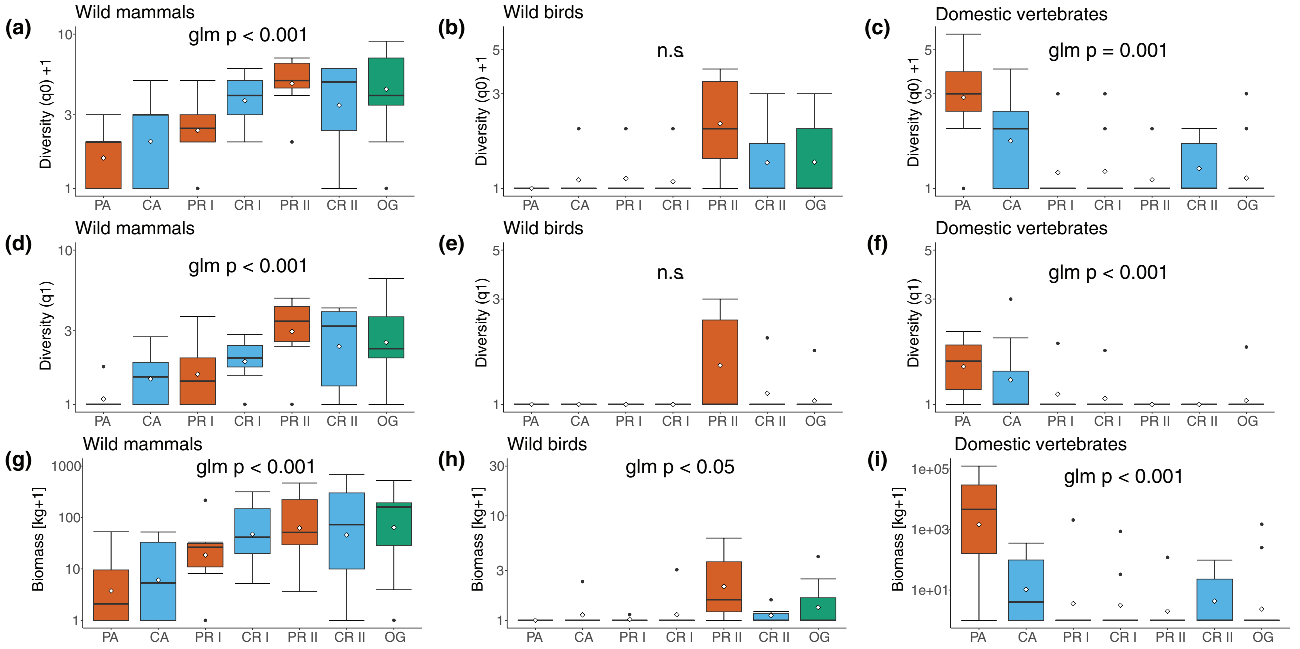

FIGURE 3 Number of observed species (diversity (q0)) (a–c), effective number of species (diversity q1) (d–f), and biomass (g–i) of wild mammals (a, d, g), wild birds (b, e, h), and domestic vertebrates (c, f, i) into seven land-use subcategories (PA: Pastures, CA: Cacao plantations), regenerating forests with different legacies and times since abandonment, and old-growth forests (OG). Recovery I (PR I, CR I) ranges from 0 to 20 years, and recovery II (PR II, CR II) from 20 to 38 years. Red color indicates active or former pastures, blue color indicates active or former cacao plantations, and green color indicates old-growth forest. Means are shown as white diamonds. The axis of the dependent variable was log-transformed for visualization. *p* values were extracted from generalized linear models (lm) using ordered categories in the order displayed. Generalized linear model results are provided in the Appendix S1.

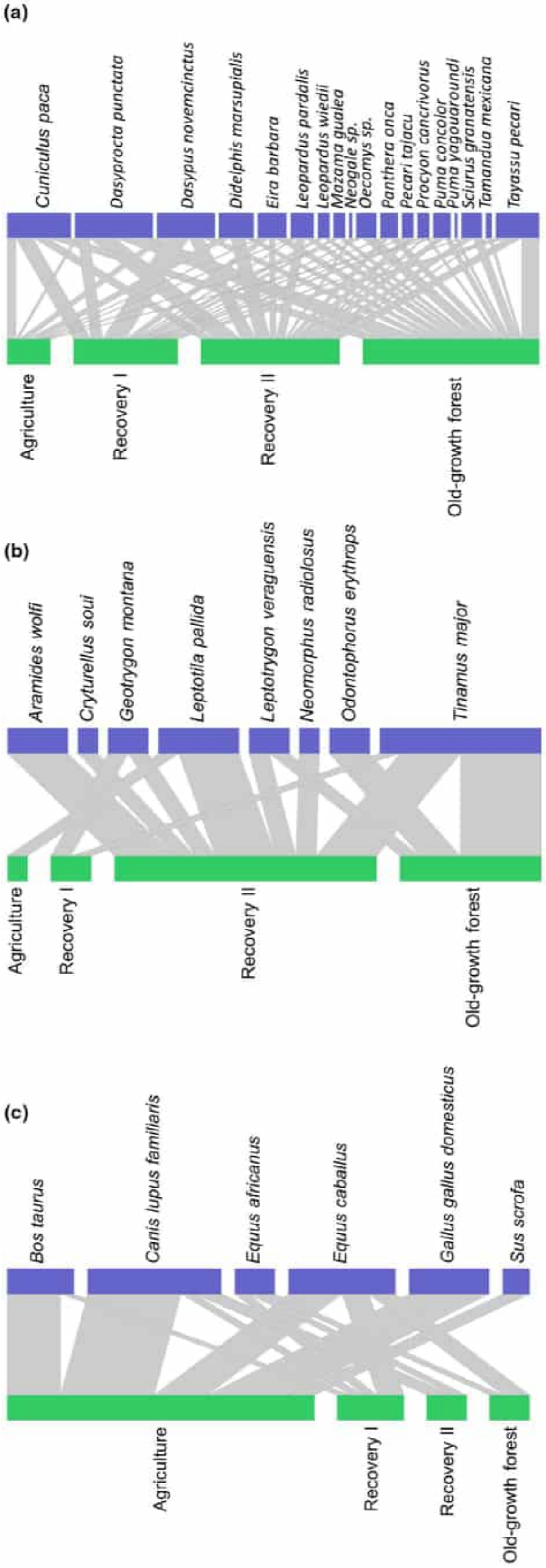

FIGURE 4 Habitat use networks of (a) wild mammals, (b) wild birds, and (c) domestic animal communities in four land-use categories. The recovery categories consist of regenerating forests with different ages since abandonment. Recovery I ranges from 0 to 20 years, and recovery II from 20 to 38 years. Agriculture consisted of pastures and cacao plantations. The thickness of the links represents the frequency of the species in the respective land-use category.

forests (Recovery I and Recovery II). Large predators like *Panthera onca* (jaguar) and *Puma concolor* (cougar) were also present in several land-use categories including forests and agricultural land (*P. onca*: Recovery I, Recovery II, Old-growth forest; *P. concolor*: Agriculture, Recovery II, Old-growth forest).

Wild birds showed a higher degree of specialization than wild mammals but also did not differ significantly from the null model ($H_2'=0.36$; $p>.05$). While most species were only found in a single land-use category, *Tinamus major* (great tinamou) was the only species being recorded in more than two categories (Recovery I, Recovery II, Old-growth forest) (Figure 4b).

Similar to wild vertebrates (birds and mammals), the specialization degree of domestic vertebrate species did not differ from the null model ($H_2'=0.28$; $p>.05$). Here, two-thirds of domestic species (cattle, domestic dogs, domestic horses, and domestic donkeys) were recorded in forests, whereas chicken and domestic pigs were found exclusively in agricultural land (Figure 4c).

3.5 | Indicator species for different land-use categories

We identified eight indicator species for different land-use categories or their combinations (Figure 5). *Bos taurus* (cattle), *Gallus gallus domesticus* (chicken), and *Canis lupus familiaris* (domestic dog) were identified as indicator species for agricultural land. *Aramides wolfi* (brown wood rail) and *D. tajacu* (collared peccary) were identified as indicator species for Recovery II. *T. pecari* and *D. punctata* were identified as indicator species for all three forested categories but not for active agricultural land. We did not identify any species that only serve as indicator species for old-growth forests.

4 | DISCUSSION

4.1 | Recovery of wild mammal diversity

Our analyses showed that wild mammalian species diversity (q0 and q1) in regenerating forests can recover to old-growth forest levels within a short recovery time of 20–38 years. This is consistent with other studies, for example, a review of faunal recovery in the tropics

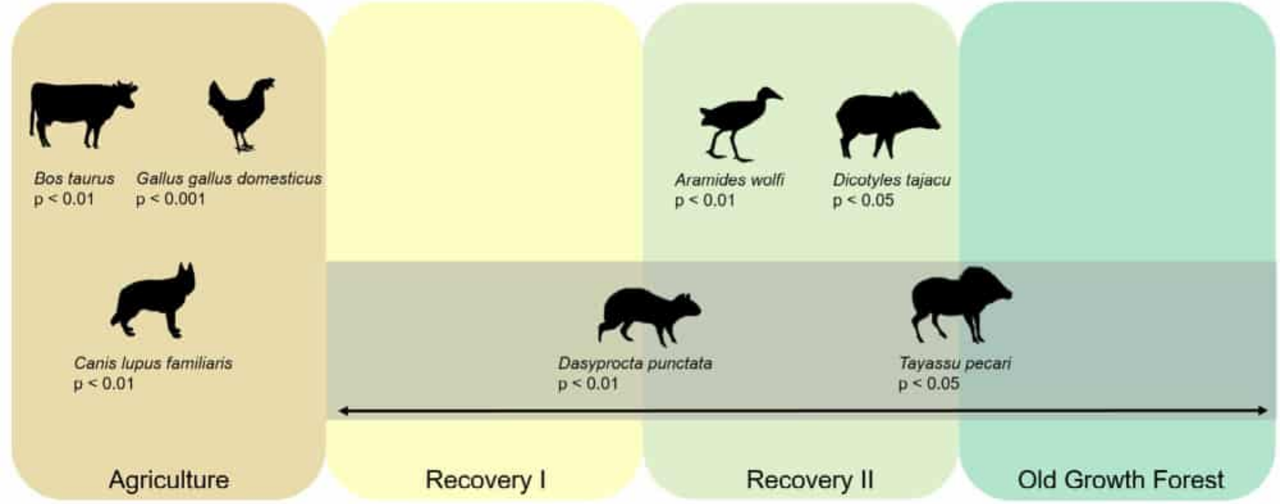

FIGURE 5 Indicator species in four land-use categories. The recovery categories consist of regenerating forests with different ages since abandonment. Recovery I ranges from 0 to 20 years, and recovery II from 20 to 38 years. Agriculture consisted of pastures and cacao plantations. In the analysis, we allowed either single land-use categories or neighboring land-use categories along the recovery gradient as a combination of site groups. Gray background indicates that the animals were identified as indicator species for several land-use categories. We determined species as indicator species when the indicator value (IndVal) had a significance level lower than .05.

has shown that animals species richness across many taxa of vertebrates and invertebrates are predicted to reach old-growth levels after 20–40 years of recovery time (Dunn, 2004), while a more recent meta-analysis has shown a recovery of tropical vertebrate species richness to old-growth levels after 40 years (Acevedo-Charry & Aide, 2019).

The mechanisms and main drivers of restoration success, that cause a return to old-growth forests conditions, are not fully understood yet. A meta-analysis by Crouzeilles et al. (2016) for example did not identify a main driver for mammal restoration. In our study area, we propose that the rapid recovery could be explained by the mosaic landscape consisting of forest fragments and agricultural patches. Our study sites are characterized by short distances to the nearest old-growth forest from each plot (mean: 59 m, ranging from 0 to 202 m), and a high old-growth forest cover (mean: 74% within a 1-km radius of each plot, CI: 68–79) (Escobar et al., 2024). The close proximity of forest fragments to one another might allow for easy access of vertebrates between regenerating forests, while the large amount of remaining old-growth forest could serve as a reservoir for vertebrate species.

Although our method does not allow to assess the time that the animals spend in the different habitats or the activities carried out, we argue that the presence of captured vertebrates represents recovery, as it indicates volitional habitat occupancy by the species. Following this, our study supports the theory that regenerating forests can mitigate the effects of deforestation in areas with large amounts of remaining old-growth and should thus be considered for conservation efforts (Chazdon et al., 2009).

4.2 | Recovery of wild bird diversity

Our analysis of ground-based wild bird species diversity (q0 and q1) did not detect significant trends along the forest recovery gradient. We explain this result by the combination of methods, that we used. Generalized linear models using ordered categories are well suited to explore trends along a trajectory of ordered categories. Using camera traps, we did not find many ground based bird species and captured species showed low frequencies of events due to methodological limitations (see below). These metrics were especially low in active agricultural land and early regenerating forests, which explains the lack of a significant trend. Other methods such as surveys by sight or vocalization might be better suited for detecting bird species and individuals (Falconí-López et al., 2024; Lennox et al., 2018; Reid et al., 2012). Although we did not detect a trend along the regeneration gradient, we showed that the measured metrics are highest in older regenerating and old-growth forests, which is consistent with other studies. Globally, bird species richness has been shown to reach old-growth levels after 40 years, similar to mammals (Acevedo-Charry & Aide, 2019). A study in Costa Rica comparing restoration methods showed that there is no difference in avian communities between active and passive restoration even within short regeneration times of 6–9 years (Reid et al., 2012) when there is enough surrounding old-growth forest. For our study we conclude, that we might not have captured the whole community of ground-based birds, but our results of the birds, that have been captured, support our hypothesis that ground-based birds can recover in older secondary forests, especially when they have a legacy as a former pasture.

4.3 | Recovery of wild mammal and bird biomass

The number of observed species does not need necessarily correlate with biomass, given the significant interspecific variation in size and biomass, that mammal and bird taxa display. In our study, the examined vertebrate groups showed different trajectories of biomass along the recovery gradient. Studies on biomass recovery of tropical forests are rare and focus mostly on plant biomass (Poorter et al., 2016; Staples et al., 2020; Wang et al., 2017). However, the amount of vertebrate biomass can give important information in addition the number of observed vertebrate species (Potapov et al., 2024; Sobral et al., 2017). It can be assumed that high biomasses have the potential for a greater physical impact on the ecosystem due to the occurrence of larger animals with greater energy requirements or high abundances. For example, similar to the influence of high vertebrate abundances on many ecosystem processes (Dirzo et al., 2014), high vertebrate biomasses could also alter processes like seed dispersal, grazing pressure, soil compaction due to trampling and nutrient cycling due to the amount of feces and carcasses. Our results showed that biomass of wild mammals and birds was highest in older regenerating forests and old-growth forests, suggesting that the biomass of wild vertebrates has the highest impact on old-growth and secondary forests with longer regeneration time.

4.4 | Land-use and biomass of domestic vertebrates

In our study area, we observed husbandry of domestics ranging from fenced and semiopen pastures to free-ranging livestock in the villages close to our study sites. Given that domestic animals are usually managed by humans, our focus regarding domestics centres on their habitat selection patterns and ecosystem impacts when they are able to roam into the forest, rather than on their recovery dynamics. So far, knowledge about the habitat preferences of domestic animals are scarce and studies focus more on pastoralist settings where animals are more mobile and free in selecting their habitat (Butt, 2010; Feldt & Schlecht, 2016; Schlecht et al., 2006, 2009). As expected, in our study, agriculture was the most frequently exploited habitat by domesticated vertebrates. However, we showed that free-ranging domestic vertebrates have access to forest environments and also choose to spend time there. This could be of importance considering their ecological impact on tropical forests by their foraging behavior, trampling on vegetation, or depositing nutrients with dung (Aarons et al., 2009; Bloor, 2015; Williams & Haynes, 1995) or urine (Clay et al., 2015). Biomass of domestic vertebrates followed a pattern opposite to wild vertebrates with the highest values in agriculture and fell into much higher range sizes. Globally, the estimated biomass of domesticated mammals is more than 30 times higher than that of terrestrial mammals, with cattle contributing most to total mammal biomass (Greenspoon et al., 2023), which is also the case in our study area with substantially higher biomasses of domestics than of wild vertebrates.

4.5 | Habitat specialization and indicator species

The species-habitat networks revealed a low degree of specialization for all three vertebrate groups which hints to a low specificity of old-growth forests along with the lack of indicator species for these. These findings support the assumption that regenerating forests may be similar habitats for vertebrates after 20–38 years. We did not identify individuals based on camera trap photos since it was not possible for all captured species, but we assume that the individuals move between the different land-use categories and use them concurrently suggesting a high habitat connectivity in our study area. The high habitat connectivity is demonstrated, for example, in detecting *Panthera onca* (Jaguar) in both forest regeneration categories and old-growth forests as well as of *Puma concolor* (Cougar) in agriculture, late recovery forest, and old-growth. Both cats have large home ranges (Nuñez-Perez & Miller, 2019) that far exceed the sizes of patches in the different land-use categories in our study area or even the whole study area. Our results show that apex predators are still present in our study area, which bears the risk of human/wildlife conflicts. *P. concolor*, for example, was captured at night on a pasture plot that has been used by humans and domestic animals the day before (Figure 2c–f). Main sources of conflicts between humans and felids are predation on livestock and attacks on people which can cause retaliatory killing of felids by humans (Inskip & Zimmermann, 2009). For example, a study based on interviews of local people in our study area about the perceived harm by *P. onca* for livestock or humans revealed a general low perception of harm (Álvarez & Zapata-Ríos, 2022). However, when attacks on livestock by *P. onca* occurred, people killed the animal in more than half of the cases.

4.6 | Limitations and outlook

While our research delivered further insights about recovery patterns and habitat selection, several methodological limitations remain that were beyond the scope of our methodologies. For example, we found a much lower number of species and little species overlap when comparing our results with data derived from sound recorders, that were placed on the same plots at the same time (Müller et al., 2023). This can be explained by methodical reasons, as our camera trap approach is suitable only for capturing animals that live or forage on the ground, explaining the absence or low frequency of flying or canopy-dwelling animals. For example, flying birds such as toucans were frequently reported in the sound data, but are underrepresented in our study as they forage in the canopy and rarely move to lower strata. This also applies to mammals frequently observed in our study area, such as canopy-dwelling primates (only one recording in this study). In addition to the limitations of camera traps, this study only covers one season neglecting intra-annual variation and our chronosequence comprises comparatively short regeneration times. Future studies in our study area covering more seasons could give better insights into the development of secondary forests when regeneration ages increase. Another remaining question is the impact of vertebrate biomasses on forests with different ages. Here, we delivered first insights into the distribution of biomass along a recovery gradient, but the consequences and possible negative implications need further investigations.

5 | CONCLUSION

The results of our analyses delivered evidence to answer our initial hypotheses. (1) We showed that wild mammals, primarily expected in old-growth forests, also use regenerating forests and agricultural land reaching old-growth levels of species diversity (q0 and q1) in late regeneration forests. The comparable short regeneration times of 20–38 years in our study area appears to be sufficient to restore habitat for many vertebrate species. Our results can be mainly explained by the high connectivity between the habitats with different restoration times and close distances to the next old-growth forest. This suggests a high regeneration potential for landscapes with comparatively low anthropogenic disturbances by patchily distributed small-scale agriculture. (2) Our results showed that the differentiation of wild mammals and birds can be useful when analyzing vertebrate communities, as demonstrated by different patterns of species diversity (q0 and q1) and biomass. (3) We did not detect indicator species for old-growth forests which indicates a low specificity of old-growth. (4) Our results delivered insights into the habitat preferences of domestic vertebrates, showing that they are not only dominant in agricultural land, but that they have access to forests, which could potentially impact these ecosystems due to their presence and often high biomasses.

We conclude that our study delivers evidence for the importance of remaining old-growth forests but also for recovering forests for the preservation and restoration of biodiversity, which should be considered in conservation efforts, for example, when prioritizing areas for the establishment of protected areas.

AUTHOR CONTRIBUTIONS

Nina Grella was involved in formal analysis, investigation, data curation, writing—original draft, and visualization. Heike Feldhaar was involved in writing—review and editing, supervision. Jörg Müller was involved in conceptualization, formal analysis, writing—review and editing, and supervision. Christian Fiederer, Marco Heurich, Felicity L. Newell, and Karen Pederson were involved in writing—review and editing and formal analysis. Nico Blüthgen, Annika Busse, Ana Falconí-López, Maria de la Hoz, Peter Kriegel, Marcel, Pühls, Dominik Rabl, Martin Schäfer, Sebastian Seibold, and Constance J. Tremlett were involved in fieldwork, writing—review and editing. David A. Donoso was involved in writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

No potential conflict of interest was reported by the authors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the Dryad Digital Repository: 10.5061/dryad.bnzs7h4mj

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SUPPORTING INFORMATION

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Appendix 3

Mammal dung–dung beetle trophic networks: an improved method based on gut-content DNA

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ABSTRACT

Background. Dung beetles provide many important ecosystem services, including dung decomposition, pathogen control, soil aeration, and secondary seed dispersal. Yet, the biology of most dung beetles remains unknown. Natural diets are poorly studied, partly because previous research has focused on choice or attraction experiments using few, easily accessible dung types from zoo animals, farm animals, or humans. This way, many links within natural food webs have certainly been missed. In this work, we aimed to establish a protocol to analyze the natural diets of dung beetles using DNA gut barcoding.

Methods. First, the feasibility of gut-content DNA extraction and amplification of 12s rDNA from six different mammal dung types was tested in the laboratory. We then applied the method to beetles caught in pitfall traps in Ecuador and Germany by using 12s rDNA primers. For a subset of the dung beetles caught in the Ecuador sampling, we also used 16s rDNA primers to see if these would improve the number of species we could identify. We predicted the likelihood of amplifying DNA using gut fullness, DNA concentration, PCR primer, collection method, and beetle species as predictor variables in a dominance analysis. Based on the gut barcodes, we generated a dung beetle-mammal network for both field sites (Ecuador and Germany) and analyzed the levels of network specificity.

Results. We successfully amplified mammal DNA from dung beetle gut contents for 128 specimens, which included such prominent species as *Panthera onca* (jaguar) and *Puma concolor* (puma). The overall success rate of DNA amplification was 53%. The best predictors for amplification success were gut fullness and DNA concentration, suggesting the success rate can be increased by focusing on beetles with a full gut. The mammal dung–dung beetle networks differed from purely random network models and showed a moderate degree of network specialization (H_2': Ecuador = 0.49; Germany = 0.41).

Conclusion. We here present a reliable method of extracting and amplifying gut-content DNA from dung beetles. Identifying mammal dung *via* DNA reference libraries, we created mammal dung-dung beetle trophic networks. This has benefits over previous methods because we inventoried the natural mammal dung resources of dung beetles instead of using artificial mammal baits. Our results revealed higher levels of specialization than expected and more rodent DNA than expected in Germany, suggesting that the presented method provides more detailed insights into mammal dung–dung beetle networks. In addition, the method could have applications for mammal monitoring in many ecosystems.

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INTRODUCTION

Dung beetles have been used as indicator species of habitat quality because of their sensitivity to habitat degradation, both in terms of deforestation and defaunation and their ecological importance (*Nichols et al., 2007*; *Korasaki et al., 2013*; *Bicknell et al., 2014*; *Ong, Slade & Lim, 2020*). The primary source of nutrition for most dung beetles is thought to be moist dung from large mammals (*Hanski & Cambefort, 1991*; *Holter & Scholtz Clarke, 2007*; *Raine et al., 2018*; *Raine & Slade, 2019*). However, some species have been documented consuming rodent dung, carrion, bird dung, millipedes, or rotten fruit (*Schmitt, Krell & Linsenmair, 2004*; *Larsen, Williams & Kremen, 2005*; *Halffter & Halffter, 2009*; *Kerley et al., 2018*; *Silva, Vaz-de Mello & Barclay, 2018*). Adult dung beetles have soft mouthparts that do not allow them to chew hard parts like bits of grass, often present in large herbivores' dung. The larva, however, possess chewing mouthparts and are hypothesized to be able to exploit these solid parts within the dung (*Halffter & Edmonds, 1982*).

By feeding on mammal dung, rotting carrion, and fruits, dung beetles provide essential ecosystem services such as soil nutrient recycling, soil aeration, pathogen control, and secondary seed dispersal (*Hanski & Cambefort, 1991*; *Nichols et al., 2007*). However, the dietary niches of most dung beetles remain unknown, and those that have been described are primarily based on compilations of observations rather than quantitative data (*Young, 1981*; *Hanski & Cambefort, 1991*; *Nichols et al., 2007*; *Edmonds & Zidek, 2010*). For instance, there are some direct feeding observations at dung piles, but this is likely biased towards larger, more obvious dung, such as that from elephants, cows, and humans (*Young, 1981*; *Hanski & Cambefort, 1991*; *Scholtz, Davis & Kryger, 2009*). Further, experimentally deployed dung often represents common or readily available species, even using non-native species from zoos (*Hanski & Cambefort, 1991*; *Frank et al., 2018a*; *Raine & Slade, 2019*; *Chiew et al., 2022*). Overall, this has often led to the assumption that dung beetles primarily consume dung from large- and medium-sized mammals (*Hanski & Cambefort, 1991*; *Scholtz, Davis & Kryger, 2009*; *Simmons & Ridsdill-Smith, 2011*; *Bogoni & Hernández, 2014*; *Frank et al., 2018a*; *Frank et al., 2018b*; *Raine et al., 2018*; *Raine & Slade, 2019*; *Bogoni, Da Silva & Peres, 2019*).

This approach has then been passed on to the realm of mammal dung-dung beetle networks (*Frank et al., 2018a*; *Raine & Slade, 2019*; *Chiew et al., 2022*; *Pryke, Roets & Samways, 2022*). For example, rodent dung is often excluded from cafeteria-style experiments or other dung attraction experiments designed to study mammal dung-dung beetle networks (*Bogoni & Hernández, 2014*; *Frank et al., 2018a*; *Raine & Slade, 2019*; *Ong, Slade & Lim, 2020*; *Chiew et al., 2022*; *Pryke, Roets & Samways, 2022*). Traditional methods of creating mammal dung–dung beetle networks might thus be biased. The use of molecular techniques such as DNA gut barcoding and metabarcoding promises to uncover otherwise hidden trophic interactions (*Wallinger et al., 2015*; *Hoenle et al., 2019*; *Avanesyan, Sutton &*

Lamp, 2021). Extracting mammal DNA from the beetles' digestive tracts provides snapshots of the beetles' last meals, thus allowing natural diets to be uncovered. A proof of concept was provided by *Gómez & Kolokotronis (2017)*, who detected horse DNA in the guts of dung beetles collected directly on horse dung. Furthermore, the excrements of the dung beetle *Circellium bacchus* were used to identify its diet based on DNA metabarcodes (*Kerley et al., 2018*). Recently, a broader assessment of gut content DNA from 31 dung beetles in Borneo suggested that gut barcoding of dung beetles could be used to monitor mammals (*Drinkwater et al., 2021*).

The herein presented method shows similarities to the method presented by *Drinkwater et al. (2021)*, with a few key differences. We used a second primer pair, Sanger sequencing, and a more aggressive washing protocol to minimize contaminations. The present work aims to develop a reliable, broadly applicable, cost-effective method to identify mammal species in dung beetle guts and better understand dung beetle diets. As a test case, we studied two mammal dung–dung beetle communities, one in a German temperate forest and another in an Ecuadorian tropical rainforest. By combining mammal identification *via* DNA barcoding with ecological network analysis, we unveiled the dung beetles' diets and their levels of dietary specialization for both communities.

MATERIALS & METHODS

Beetle collection

We collected dung beetles from a temperate forest in Germany (49°51′54.19″N, 8°41′28.50″E) and a lowland tropical rainforest, as well as five pastures in Ecuador (0°30′20.52″N, 79°10′31.95″W Ecuador). Collections were made in September 2019 (Germany) and from January 2019 to June 2019 (Ecuador). Beetle collections were made under the Escuela Politecnica Nacional, Contrato Marco MAE-DNB-CM-2016-0068, and transported to Germany under beetle export authorization number 62-2019-EXP-CM-FAUDNBIMA. Beetles were collected using pitfall traps, baited with cow dung in Germany (seven pitfall traps, 41 beetles), and human dung in Ecuador (eight pitfall traps, 94 beetles). Beetles were also collected opportunistically by hand in Ecuador ($N = 41$).

Both cow and human dung are common attractants for dung beetle pitfall trapping (*Hanski & Cambefort, 1991*; *Whipple & Hoback, 2012*; *Marsh et al., 2013*; *Frank et al., 2018a*; *Frank et al., 2018b*). However, in preliminary trials, we realized that, in the Ecuadorian population, cow was less effective in attracting dung beetles than human dung. The latter attracted a higher number and diversity of dung beetles, so we decided to use human dung in Ecuador. We did not see a substantial difference between bait types in Germany and, therefore, decided to use cow dung as it is easier to handle. In all experiments, we euthanized beetle specimens quickly using either absolute ethanol or freezing to reduce the suffering of the specimens. Disposable nitrile gloves (VWR) were worn for all dung manipulations for personal protection, especially as human dung was used as a bait. After the baiting periods, human dung was disposed of in the same hole created for the pitfall trap following the Leave No Trace Principles for human waste disposal (www.lnt.org).

To minimize the contamination of beetle guts with cow or human dung, direct physical contact from dung beetles to baits was minimized. The pitfall traps consisted of a plastic cup leveled with the soil, a rain cover, and an overhanging tea bag with bait inside, mostly prohibiting unintended bait consumption (*Frank et al., 2018a*). Pitfall traps were installed 24 h before collection. When pitfall traps were collected, only dung beetles were collected. All other animals were released. After beetle collection in the field, specimens were either preserved in ethanol and then frozen (Ecuador) or simply frozen (Germany). Our field site in Ecuador is subject to occasional power outages and thus requires a second level of DNA preservation. Dung beetles were then identified morphologically by KMP using the latest species keys (*Edmonds, 2000*; *Solís & Kohlmann, 2002*; *Edmonds & Zídek, 2004*; *Edmonds & Zídek, 2012*; *Vaz-De-Mello et al., 2011*; *Chamorro et al., 2018*; *Nunes, Nunes & Vaz-de Mello, 2018*), and the reference collections of the Pontificia Universidad Católica (Ecuador) and the Escuela Superior Politécnica del Litoral (Ecuador).

Beetle dissection

After frequently amplifying human DNA in preliminary experiments before this study began, we developed a washing protocol in sterile conditions to minimize human DNA contamination, which markedly reduced the amplification of contaminants such as human DNA and prevented cross-contamination of samples. First, surfaces and tools were UV sterilized. Beetles were placed singly in distilled water and shaken for 30 s to remove dirt and external DNA from the outside. We transferred the beetles in a 2% NaClO (chlorine) solution under a fume hood where the air was constantly UV sterilized. The chlorine solution was washed off the beetles with 70% ethanol. Then, beetles were transferred to a dissection tray and dissected using various dissection tools. Tools were flamed, washed in 2% NaClO, rinsed in 70% EtOH, and then flamed again and cooled before each dissection. Cleaning solutions were changed every two beetles to prevent cross contamination and reduce waste liquid byproducts. After every beetle, the dissection tray was washed with 2% NaClO solution and 70% EtOH. We dissected 177 beetles, 135 from Ecuador and 42 from Germany.

For large beetles (body length > 1 cm), the digestive tract was removed and placed in the DNA extraction buffer of a Qiagen Blood and Tissue Kit (Qiagen, Hilden, Germany). For smaller beetles (body length < 1 cm), the entire abdomen was placed in the same DNA extraction buffer without further dissection. We used the entire abdomen for small specimens to prevent possible contamination. Their small size made it much harder to dissect, and as our first attempts at sequencing this way worked, we kept using the method. For a subset of 51 large beetles, we visually categorized gut fullness in the following way: (1) *full*—more than half the length of the intestines is full; (2) *half-full*—half or less than half the length of the intestine is full; (3) *empty*—there is no visible content in the intestine (Fig. S1).

DNA extraction and amplification

DNA was further purified using the Bio-RAD Micro Bio-Spin Columns P-30 Tris following the manufacturer's instructions. This results in 75 µl of purified DNA, which can be used for PCR. We then ran PCRs using the Qiagen multiplex kit (with 1.5 µl of molecular grade water), 5 µl of Qiagen multiplex PCR Master Mix, then 0.5 µl of 10 µM for the forward and reverse primers, and with the addition of 0.5 µl of bovine serum albumin (BSA) to counteract remaining PCR inhibitors and 2 µl of template DNA. The PCRs started with an initial activation period of 95 °C for 15 min, followed by 35 cycles with the following settings: denaturation at 94 °C for 30 s, the annealing temperature of 65 °C for 90 s; extension at 72 °C for one minute. A final elongation step was performed at 72 °C for 10 min. PCRs were repeated up to three times per sample. For all 177 beetles, we used vertebrate-specific PCR primers to amplify portions of the mitochondrially encoded 12S rDNA (*Ushio et al., 2017*). For the Ecuadorian beetles ($N = 135$ beetles), we additionally used the mammal-specific primers 16smama1 (forward) and 16smama2 (reverse) (*Taylor, 1996*). For a subset of samples ($N = 121$ DNA extractions), we measured the DNA concentration using a ThermoScientific Nanodrop Lite Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Of 177 beetles, 128 were amplified and sent to sequencing.

Proof of concept study

As a proof of concept, we offered a defined diet to *Anoplotrupes stercorosus* dung beetles consisting of a variety of mammals and amplified mammal DNA from the beetles' guts. We then amplified the mammal DNA from the beetles' guts. All 30 beetles were initially fed apples for five days to clear their guts. We then fed them with 10 grams of the following six dung types: tapir (*Tapirus terrestris*), fennec fox (*Vulpes zerda*), otter (*Aonyx cinerea*), porcupine (*Hystrix cristata*), macaque (*Macaca nigra*), and cow (*Bos taurus*). Beetles were fed for 24 h to ensure they had enough time to consume dung. Dung types were fed to five beetles each. Dung beetles were then euthanized in the freezer. The dung was contributed by a local zoo (Vivarium Darmstadt) and a local farm. We then applied our method as described above to identify dung beetle gut content. The amplified 12s rDNA fragment was then compared with a reference library using the Basic Local Alignment Search Tool (BLAST) to verify if it matched the consumed mammal dung (*Altschul et al., 1990*). Please note that we only used one species and a relatively low sample size for this proof-of-concept experiment, so caution should be taken in extrapolating the results to other dung beetle species.

Gut content fullness of beetles in pitfall traps after 8 h, 24 h, and 48 h

Pitfall trapping means that live beetles might empty their guts in the trap before being collected. At our German field site, we experimentally assessed how many beetles would still have full guts after pre-defined time periods. To approximate realistic gut fullness in pitfall traps, we set out 15 traps in the forest. The traps were baited with cow dung and randomly assigned to three different groups: five traps were emptied after 8 h, five traps were emptied after 24 h, and five traps were emptied after 48 h. Only *A. stercorosus* beetles were collected and immediately placed in 70% ethanol. In this proof-of-concept study, we solely used *A. stercorosus* to assess the level of gut fullness. Due to its larger size compared to *Aphodius sticticus*, the gut is easier to dissect reliably. In addition, they are also more readily available in Germany, and it is easier to identify the species in the field. The smaller *Aphodius* beetles are much harder to reliably identify. Beetle abdomens were then dissected to record gut fullness (at least 50% of the gut filled) or less than 50% filled. Data were analyzed with a Pearson's chi-squared test.

Gut content after 48 h of starvation

A. stercorosus beetles ($N = 20$) were collected from the forest, transferred alive into different enclosures, fed cow dung for five days, and then starved for two days to survey the proportion of empty guts. After 48 h, the beetles were frozen and then dissected. Contents of guts (empty, not empty, full) were recorded together (Fig. S1). As a control, 20 beetles with *ad libitum* access to cow dung were frozen simultaneously to survey how many beetles had an empty gut when provided a continuous supply of food. Data were analyzed with a Person's chi-squared test.

DNA sequence processing

For gut DNA analysis, we dissected 177 beetles from the two field sites belonging to 10 dung beetle species. Eight of the species were from Ecuador: *Deltochilum* sp.(8), *Oxysternon conspicillatum* (53), *Canthon angustatus* (58), *Onthophagus* sp. (8), *Dichiotomius* sp. (1), *Canthidium* sp.(3), *Sulcophanaeus notis* (1), and *Scybalocanthon trimaculatus* (2), as well as one unidentified species; two species were from Germany: *A. stercorosus* (19), and *A. sticticus* (23). PCR products of successful DNA amplifications, verified by gel electrophoresis and staining with ROTI® GelStain, were sent for Sanger sequencing to Macrogen Europe. Forward and reverse directions were sequenced for each amplicon. Post-processing was done using Codon Code Aligner 10.0.2 on macOS High Sierra. Low-quality base pairs (base pairs with a quality score lower than Phred 20) were clipped from the ends of the sequences. A Phred 20 quality score corresponds to 99% accuracy in a base call. At this stage, low-quality sequences were discarded (sequences with 50 or more base pairs with a score lower than Phred20) or sequences with a length of less than 50 base pairs. The resulting sequence length and quality scores were then recorded. Sequences were aligned, if possible, to create a consensus sequence. The resulting consensus sequences were then matched to reference sequences using the NCBI MegaBLAST search (*Morgulis et al., 2008*). We accepted the best match as our ID if the match was >90%, which is commonly used for 12s rDNA and 16s rDNA short sequences at the genus level for mammals (*Hoffmann et al., 2017*; *Kocher et al., 2017*; *Drinkwater et al., 2019*; *Saenz-Agudelo et al., 2022*). The best species match was recorded, along with the accession ID, percent identity, max score, and bit score. Mismatches between references and query sequences were often found in base pairs with a low-quality score. Lower matches might have partly arisen from DNA degradation as we analyzed DNA within digestive tracts. Due to this constraint and previously established protocols (*Hoffmann et al., 2017*; *Kocher et al., 2017*; *Drinkwater et al., 2019*; *Saenz-Agudelo et al., 2022*), we decided to identify the mammal species only to the level of the genus when there where multiple species within a genus otherwise we used the species name.

Data analysis

For the pitfall trap dataset, we ran a logistic regression with positive electrophoresis results as the dependent variable and gut fullness, collection method, PCR primers, DNA concentration, and beetle species as predictor variables. We then performed a dominance analysis (*Budescu, 1993*) to determine which of the predictor variables were the most important predictors of DNA amplification. A dominance analysis compares predictor variables in a pairwise fashion across all the subset models and generates a predictor hierarchy or the importance (dominance) of each predictor value. Higher values have a greater predictive power (*Azen & Budescu, 2006*; *Lee & Dahinten, 2021*).

We also constructed bipartite food networks (mammal dung–dung beetle) for each site to investigate the specificity of dung choice. For this, we counted the number of beetle individuals containing DNA of a given mammal genus. Our Ecuador data set includes 41 beetles whose gut content DNA successfully amplified and from which we successfully obtained sequence matches out of a total of 135 dissected beetles. The German network includes data from 21 beetles of the total 42 dissected beetles. Our 'interaction frequency' was the number of beetle individuals per species that contained a specific mammal DNA sequence. We calculated network specialization using the H_2' statistic for each plot (*Blüthgen, Menzel & Blüthgen, 2006*) as in *Frank et al. (2018b)*. We compared H_2' values to randomized networks using the Patefield null model (see *Blüthgen, Menzel & Blüthgen, 2006*). We excluded sequences matching the bait (human in Ecuador and cow in Germany). Beetle species with no measured trophic interactions with mammal dung were dropped from the network analysis. We included humans as dung beetle interaction partners in our German network for two reasons. First, we observed a fair amount of human excrement at the German study site. Second, we showed that the newly developed washing protocol drastically decreased the detection of human contamination in the samples. Although contamination with human DNA cannot be entirely excluded, we consider it likely that most of the detected interactions with human dung at the German study site are accurate. When displaying the networks, we use species names if only one species of the genus is present in the study site, or we use spp. to indicate that more than one species is possible; however, we caution that using species distribution information in this way could make investigators overlook species with expanding ranges, or closely related genera could be misidentified. Finally, to estimate the diversity of mammal dung consumed by beetle species within each habitat type, we calculated the Shannon index for each beetle species and their associated mammal diversity.

RESULTS

Proof of concept

We offered six dung types to a single dung beetle species. We verified the mammal genera for all six test dung types by matching DNA barcodes from beetle gut contents. The best DNA barcode match corresponded to the respective species for tapir, otter, porcupine, and cow (the top 10 best matches are the expected species), but macaque and fox have mixed species in the top 10 best matches. The 16s rDNA or 12s rDNA fragments for mammal identification are often used for genus level identification because this increases the accuracy of identification (*Hoffmann et al., 2017*; *Kocher et al., 2017*; *Drinkwater et al., 2019*; *Saenz-Agudelo et al., 2022*). Sequence quality was high, with a Phred score of 20 or higher from all base pairs. Data are available in the Supplemental Information.

Table 1 Results of dominance analysis. The dominance analysis suggests that digestive tract fullness and DNA concentrations are the best predictors of measurable DNA amplification. The r2m approximates the importance of different predictor variables for a positive result from the gel electrophoresis within the model and is the average contribution of each of the five variables.

Variable	Dominance statistic
Beetle species	0.051
Collection method	0.009
DNA concentration ng/ul	**0.291**
Gut fullness	**0.341**
PCR primers	0.009

Notes.
Bolded values are the best predictors of DNA amplification.

Pitfall trap gut content fullness after defined hours

Among all beetles from traps emptied after eight hours, 75% had full guts ($N = 15$ total beetles, four beetles with empty guts and 11 beetles with full guts). After 24 h, 61% of the beetles had full guts ($N = 44$ total beetles, 17 beetles with empty guts and 27 beetles full guts), and 58% had full guts after 48 h ($N = 24$ total beetles, ten beetles with empty guts, and 14 beetles with full guts). Despite a decreasing proportion of full guts over sampling time, the three time points did not differ significantly in their proportion of full guts (Pearson's chi-squared test: $Chi^2 = 0.95$, $df = 2$, $p = 0.621$).

Gut fullness after 48 h of starvation

In a trial testing how many beetles could be expected to retain full guts after 48 h, we found that among the 20 beetles included in the starvation treatment, half the beetles ($N = 10$) had empty guts, nine guts were half full, and only one gut was full. Among the 20 beetles where food was available *ad libitum* for 48 h, five beetles had empty guts, five beetles had half full guts, and ten beetles had full guts. The proportion of full guts is thus lower in the starvation treatment than in the fed treatment. Accordingly, the two treatments differed (Pearson's chi-squared test: $Chi^2 = 10.17$, $df = 2$, $p = 0.006$).

Field collected dung beetles for gut content identification

Factors associated with successful DNA identification

Digestive tract fullness and DNA concentrations were the best predictors of measurable DNA amplification (Table 1). From the 177 beetles dissected across the two sites, we obtained 137 unique sequences of the rDNA gene fragments 12s and 16s. Success rates were higher for hand-collected dung beetles, 66% (27/41) than for dung beetles caught in pitfall traps, 49% (66/136) (Table S1).

The majority of dung beetles collected in pitfall traps had visibly full stomachs (31 out of 51), while fewer beetles had half-full or empty stomachs (20 out of 51). The success rate for amplifying mammal DNA dropped from 74% for beetles with full stomachs to 22% for empty stomachs (Table 2). Additionally, DNA concentration tended to be higher in cases of successful DNA amplification (median = 73 ng/μL mean = 86 ng/μL for positive result)

Table 2 Number of beetles with different gut fullness out of 51 specimens collected in total. Showing that visible gut fullness was a good indicator of successful DNA amplification within our study. Data derive from eight dung beetle species: Deltochilum sp. ($n = 5$), *O. conspicullatum* ($n = 21$), *C. anagustatus* ($n = 2$), *Onthophagus sp.* ($n = 1$), *Anoplotrupes stercorosus* ($n = 19$), *Dichiotomius sp.* ($n = 1$), *S. notis* ($n = 1$).

	Full	Half Full	Empty
Total Number of Beetles	31	11	9
Number of Amplified DNA sequences	21	6	2
Success rate	74%	55%	22%

versus those where DNA amplification failed (median = 34 ng/µL, mean = 46 ng/µL for negative results).

MegaBLAST search results

We amplified rDNA in 128 beetles out of 177 beetle guts. Of these 128 beetles, we only used 93 for the network analysis, either because the result from BLAST did not match a mammal (*e.g.*, match to bacteria *Klebsiella pneumoniae*), resulted in an NA (matched nothing in the database), or the best match was lower than 90% which we considered too low to make inferences about genera or species. With a mean percent sequence identity of 96% at the species level for the 12s rDNA primers and 95% at the species level for the 16s rDNA primers, the best MegaBLAST matches were almost identical for the two primer combinations. These sequence matches were considered too low to make mammal species-level identifications but high enough to infer genus-level identities.

The 12s rDNA fragment had a higher chance of matching a species in the reference database (94% matching a mammal species present in study sites), while 55% of sequences of the 16s rDNA fragments did not match a mammal species present at our study site. However, matches at the genus level were better for 16s rDNA. There are three monkey species *Ateles fusciceps*, *Alouatta palliata*, and *Cebus capucinus,* at the field site in Ecuador (*Tirira, 2017*). Therefore, it is reasonable to assume that a genus level match can be used to identify the species in monkeys. However, that will be harder for more species rich orders of mammals such as Chiroptera and Rodentia. In the German network, there are two genera from Rodentia with multiple species, *i.e., Apodemus* and *Myodes*. In Ecuador, one genus has multiple species, *i.e., Caloromys*. Due to the possible lack of reference sequences with 16s rDNA, we instead focused on the 12s rDNA locus for network analysis. With respect to genus, the two primers produced the same result, suggesting some consistency within the results (Table S2–Table S4). The 12s rDNA primers provided one additional mammal species when compared with the 16s rDNA primers. Additionally, the storage method may be important for successful DNA amplification and subsequent sequence matching. The beetles from Ecuador stored in EtOH and frozen, were less likely to amplify and pass all quality control steps (41/135 beetles; 30%) than our German or frozen only beetles (21/42 beetles; 50%). We caution that these data are highly confounded with beetle species, PCR primer, temperature, and transport time from the field to the lab and, thus, should not be used to justify one method over the other.

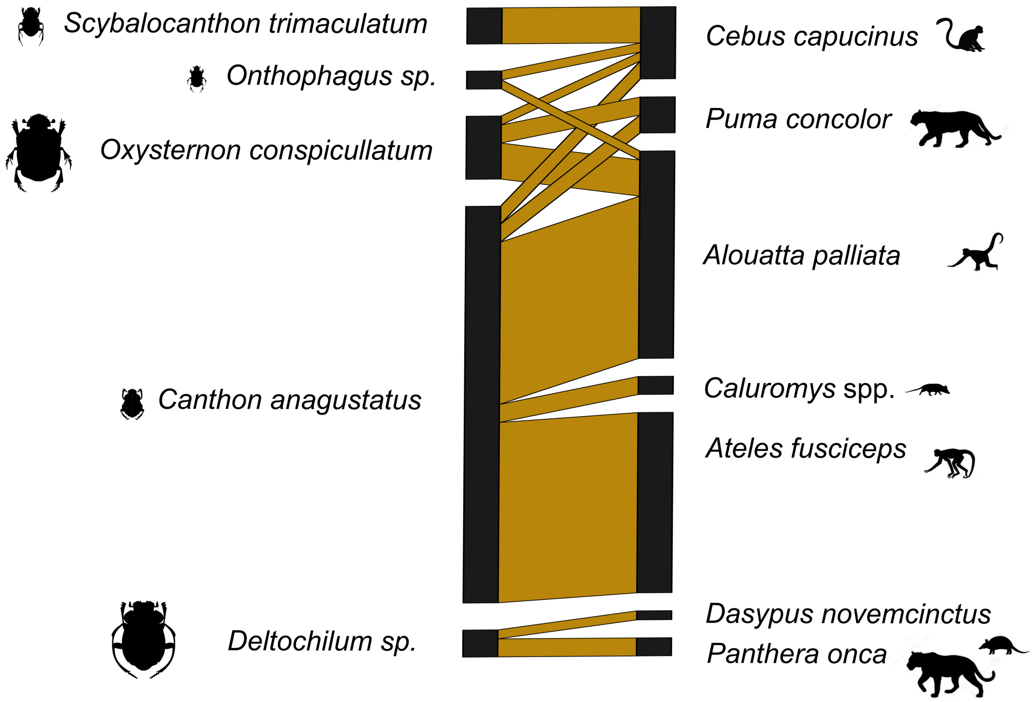

Figure 1 **Dung beetle mammal dung network Ecuador 12s and 16s Primers.** A bipartite network visualizing the links between dung beetle species and types of mammal DNA within their gut contents. The network consists of five dung beetle morphospecies and seven mammal genera. Sequences corresponding to the bait (human) were excluded. Line widths are proportional to the strength of the association, with thicker lines representing stronger observed links.

Full-size DOI: 10.7717/peerj.16627/fig-1

Networks

With the dung beetles from Ecuador, we used both 16s and 12s rDNA primers. The network for the 16s rDNA primers is much less complete than the network generated using the 12s rDNA primers (Figs. S2 and S3). The 16s rDNA Ecuador network has an $H_2{}' = 1$, and the 12s rDNA network has an $H_2{}' = 0.66$. Both are significantly different than the null model (both $p < 0.001$). However, after the data from both primers were combined, the level of network specificity dropped to $H_{2'} = 0.49$, also significantly different from the null model ($p < 0.001$) (Fig. 1). The mammal dung–dung beetle network from Germany showed a similar moderate degree of specificity ($H_{2'} = 0.41$, $p = 0.005$; Fig. 2). We detected dung of 14 mammal genera from 10 dung beetle species (Tables S2, S3 and S4). These included top predators (*e.g.*, *P. onca* (jaguar)), herbivores (*e.g.*, *Capreolus capreolus* (deer)), and omnivores (*e.g.*, *Caluromys sp.* (opossum)) (Tables S5 and Table S6). Per beetle species, we detected between one and six mammal genera (Tables 3 and 4).

DISCUSSION

We investigated resource specialization in dung beetles by constructing bipartite interaction networks based on the barcoding of mammal dung derived from the beetles' digestive tracts. Our method is broadly applicable, both geographically and phylogenetically. In the present

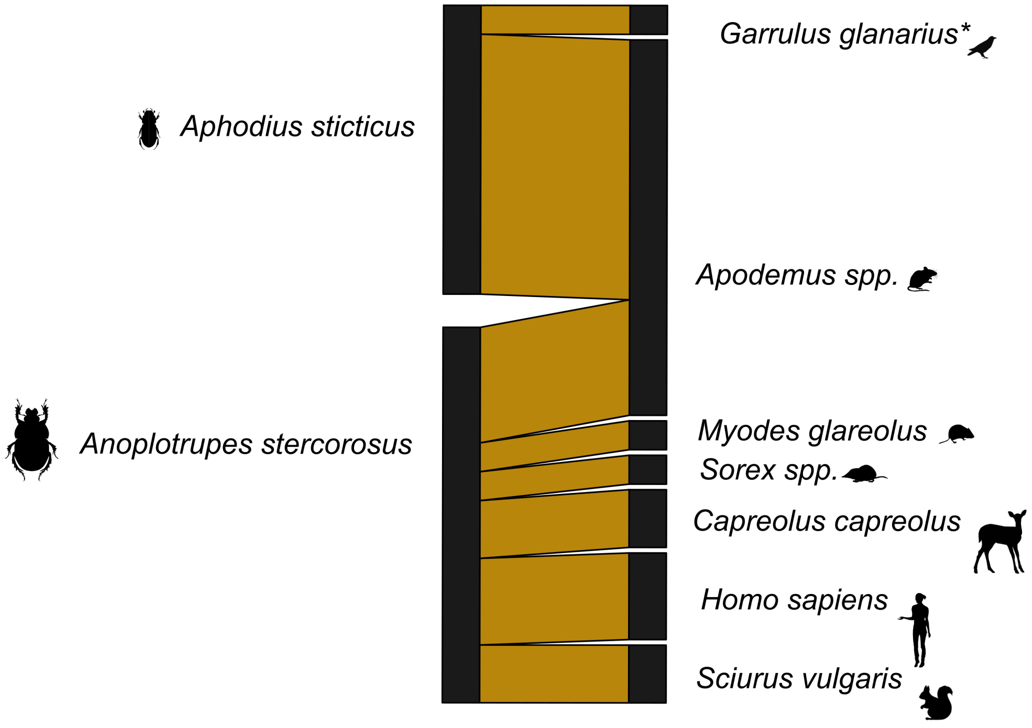

Figure 2 Dung beetle mammal dung network Germany 12s primers. A bipartite network visualizing the links between dung beetle species and types of mammal DNA within their gut contents. The network consists of two dung beetle species and seven vertebrate genera. Sequences corresponding to the bait (cow) were excluded. Line widths are proportional to the strength of the association, with thicker lines representing stronger observed links. *included one bird species.

Full-size DOI: 10.7717/peerj.16627/fig-2

Table 3 Ecuadorian forest network. Table summarizing the dung beetles species statistics including individual beetles per species, number of samples sequenced, match mammal species richness, and diversity.

Beetle species	*N* Beetles	DNA amplification with PCR	Mammal richness	$e^{H'}$
Canthon angustatus	29	23	5	3.11
Canthidium sp.	3	0	NA	NA
Deltochilum sp.	6	4	4	1.89
Dichiotomius sp.	1	0	NA	NA
Oxysternon conspicillatum	36	9	4	2.60
Onthophagus sp.	8	3	3	3.00
Sulcophaneus notis	1	0	NA	NA
Scybalocanthon trimaculatus	2	2	1	1.00

Table 4 **German forest network.** Table summarizing the dung beetles species statistics including individual beetles per species, number of samples sequenced, match mammal species richness, and diversity.

Beetle species	N beetles	Positive PCR	Mammal richness	$e^{H'}$
Anoplotrupes stercorosus	19	12	6	5.32
Aphodius sticticus	23	12	2[a]	1.38

Notes.

[a] One bird species included here, all other samples were mammals.

work, we analyzed a tropical and a temperate forest community of dung beetles, including species of all of the three prominent dung beetle taxa (Scarabaeinae, Aphodiinae, and Geotrupidae). The greatest degree of DNA amplification success was found in beetles with visibly full guts. Hence, future work should best consider extracting DNA preferentially from beetles with full guts, which has the potential to cut down on expenses and time investment.

Previous work on dung beetle diets mostly produced 'experimental' (artificial) mammal dung–dung beetle networks by using laid-out dung or providing direct observations on natural dung sources (*Young, 1981*; *Hanski & Cambefort, 1991*; *Frank et al., 2018a*; *Frank et al., 2018b*; *Raine & Slade, 2019*). We see the present work as a step forward as DNA gut barcoding allowed us to provide more representative and natural networks. For instance, networks using laid out dung were highly generalized (mean ± sd $H_2' = 0.23 \pm 0.17$ in 116 datasets, *Frank et al., 2018b*), while our two DNA-based networks showed a much higher level of specialization ($H_2' = 0.41$ and 0.49). Partly, this is because the studies included in the *Frank et al. (2018a)*, *Frank et al. (2018b)* meta-analysis and others were often limited by dung access and often used dung from available domestic animals or animals from local zoos rather than naturally occurring dung in a habitat (*Martín-Piera & Lobo, 1996*; *Errouissi et al., 2004*; *Korasaki et al., 2013*; *Frank et al., 2018a*; *Raine et al., 2018*; *Ong, Slade & Lim, 2020*). Experimentally laying out dung could change environmental variables that are important for both attractiveness (dung volume) and natural encounter rates on the landscape (activity windows). Dung attractiveness is driven by dung volume in pitfall traps. The volume is often standardized across species instead of using naturally occurring defecates that vary in size (*Errouissi et al., 2004*). Dung beetles are also active at different times of day and, in many ecosystems, extremely efficient dung removers. This means mammal dung from nocturnal mammals is more likely to be encountered and consumed by nocturnal dung beetles than by diurnal dung beetles. The reverse also stands. Using only direct observations of dung beetles and mammal dung is open to a lot of observer bias, including observations of the most obvious dung types (*e.g.*, elephant), or diurnal interactions over nocturnal interactions (*Hanski & Cambefort, 1991*; *Scholtz, Davis & Kryger, 2009*). The DNA-based method applied here minimizes these biases and helps to provide a more representative and detailed image of mammal dung–dung beetle trophic interactions. For example, our tropical interaction network suggests that nocturnal dung beetles are more likely to consume the dung of nocturnal mammals, *e.g. P. onca* and *Dasypus novemcinctus*. Furthermore, in the German temperate forest, we see less overlap in dung beetle diets than would be expected by random dung choice, under the expectation that dung beetles are generalist dung consumers (*Hanski & Cambefort, 1991*; *Nichols et al., 2009*; *Frank et al., 2018a*). Rodents were more dominant in the German mammal dung–dung beetle network than expected based on previous conclusions from the research of mammal dung–dung beetle networks (*Hanski & Cambefort, 1991*; *Nichols et al., 2008*; *Frank et al., 2018a*; *Raine & Slade, 2019*; *Chiew et al., 2022*). Previous research suggested that German networks are dominated by wild boar and deer, among other medium and large-bodied mammals, while rodents were mostly missing. However, a trend like that observed in the data might indicate excessive hunting or loss of large mammals like that suggested by *Nichols et al. (2009)*. Both the present study and the one of *Kerley et al. (2018)* suggest that rodents have been largely overlooked using traditional methods to study dung beetle diets.

Despite the many advantages of the DNA-based approach, there are also some limitations. First, these networks measure adult diets. While there is much overlap between adult and larval diets, there are some suggestions that adults might provide different dung to their young than they consume themself (*Byrne, Watkins & Bouwer, 2013*; *Shukla et al., 2016*; *Kerley et al., 2018*). However, there is more evidence to the contrary, suggesting instead that adults and larvae both consume the same dung. Larvae still have chewing mandibles and may be able to take advantage of the solid parts of dung (grass fibers and other undigested material), while the adults only consume the liquid parts (*Halffter & Edmonds, 1982*; *Hanski & Cambefort, 1991*; *Byrne, Watkins & Bouwer, 2013*; *Shukla et al., 2016*). Second, some of the detected gut DNA may come from carrion instead of dung. However, the amount of available carrion and the integrity of the DNA is probably much lower than that of dung, particularly for older carrion (*Itani et al., 2011*; *Yang et al., 2017*). In addition, our results match that of the South African study (*Kerley et al., 2018*), where carrion is not considered part of a dung beetle‘s diet. Third, our method, like all pitfall trapping sampling methods, is likely to miss attracting dung beetles with highly specialized diets such as sloth dung (*Young, 1981*), snail mucus (*Vaz-de Mello, 2007*), or millipedes (*Schmitt, Krell & Linsenmair, 2004*). Fourth, we focused on the amplification of mammal DNA, which does not provide a complete picture of the beetles' niche breadth. This may, in fact include plant material (*Halffter & Halffter, 2009*), other arthropods (*Schmitt, Krell & Linsenmair, 2004*; *Silva, Vaz-de Mello & Barclay, 2018*; *Giménez Gómez et al., 2021*), and snail mucus (*Vaz-de Mello, 2007*). By pointing out these deficiencies, we hope to inspire future research to tackle the method's limitations, for example, using a broader set of bait attractants and/or analyzing gut DNA of a broader phylogenetic spectrum.

Overall, we see great potential in the analysis of dung beetle gut contents for various research areas. One noteworthy aspect of gut content DNA analysis is the potential to use it as mammal monitoring (see also *Drinkwater et al., 2021*). Like carrion feeding flies (*Srivathsan et al., 2022*), dung beetles can serve as 'mammal samplers,' and they could potentially constitute a more cost-effective and complete method than the traditional and widely used camera trapping (*Drinkwater et al., 2021*). The method is both spatially and temporally informative regarding mammal presence because the DNA degrades quickly, and dung beetles do not usually travel great distances within one day (*Peck & Forsyth, 1982*; *Roslin et al., 2009*; *Silva & Hernández, 2015*). By covering a broad dung beetle phylogenetic diversity and distinct geographic areas, the result of the present work suggests that gut barcoding can be broadly applied as mammal detector across distinct ecosystems. Further, the method could be broadly applied to the study of mammal dung–dung beetle networks, for example, to study dung beetle resource specialization in various habitats. Local scale changes, such as disturbance in natural habitats due to human activities, could be examined in terms of biodiversity and interaction shifts.

CONCLUSIONS

This study presented a gut DNA-based method to uncover mammal dung–dung beetle trophic networks, which will hopefully provide many new insights into these ubiquitous interaction networks. To maximize the success of future studies, we recommend that beetles should not be kept alive for more than 24 h. The recommendation is a conservative one but based on the results of the 48-hour starvation trial of two days without food significantly reduced gut fullness. Fuller guts should have more target DNA. This was also found to be a good predictor of a successful PCR amplification. Thus ensuring that beetles have limited time to void their guts is an important factor to consider. We also recommend washing the beetles and performing the dissections in a sterile environment to reduce human contamination, which can swamp target DNA. Finally, checking the gut contents before DNA extraction should markedly improve the success rate of future studies. Using DNA analyses of dung beetle guts could greatly improve our understanding of dung beetle biology within ecosystems and potentially provide an additional tool for biodiversity monitoring.

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Author Contributions

- Karen M. Pedersen conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
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ORIGINAL ARTICLE

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Seed size and pubescence facilitate secondary dispersal by dung beetles

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Abstract

In tropical forests, primary dispersal by animals is the most important form of seed dispersal. Dung beetles are secondary seed dispersers attracted to mammal feces. When they bury dung of frugivorous mammals, they move seeds to new sites, possibly protecting them from seed predation or pathogens, or moving to better microclimates and away from conspecifics. As a result, secondary dispersal by dung beetles potentially increases rates of seed survival and germination. Previous studies examined how dung beetles filter seeds by size. However, other seed traits have not been examined. We discovered that pubescent seeds covered with hairs on their surface hold a thin layer of dung and possibly "trick" the dung beetles into burying them like a dung ball. In a lowland tropical forest (Chocó Ecuador), we collected dung balls from dung beetles (*Canthon angustatus*, *Oxysternon conspicillatum*, *Sulcophanaeus noctis*, and *Scybalocanthon trimaculatus*), and fecal samples from brown-headed spider monkeys (*Ateles fusciceps fusciceps*) and mantled howler monkey (*Alouatta palliata*). We characterized the traits of seed morphospecies found within samples and counted them. Our data show that larger size is coupled with a higher proportion of pubescence in seeds. The association between seed size and pubescence may extend beyond our study area supported by an analysis of the literature data for neotropical seeds at the genus level. Large pubescent seeds were more likely to be included in dung balls than smooth large seeds. Our results are consistent with the hypothesis that secondary dispersal by dung beetles exerts some selection pressure on the phenotype of endozoochorous seeds.

KEYWORDS
dung beetles, secondary dispersal, seed dispersal, seed pubescence, seed traits, *Spondias mombin*

1 | INTRODUCTION

Fruit morphology and seed (embryonic plant) dispersal mechanisms have been studied for many years (Hughes et al., 1994; Janson, 1983; Pijl, 1969; Ridley, 1930; Tamme et al., 2014; Thomson et al., 2010, among many others). Primary dispersal by frugivorous animals maximizes seed dispersion and distance from the parental tree. Accordingly, the Janzen–Connell hypothesis suggests that increasing space between conspecific seeds should increase their survival by decreasing seed predation and pathogen transmission (Connell, 1970; Janzen, 1970). The Janzen–Connell hypothesis is often used to explain seed dispersal, and the maintenance of diversity in

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tropical systems where up to 100% of trees rely on animal dispersers (Muller-Landau & Hardesty, 2005; Seidler & Plotkin, 2006). Most studies of seed dispersal and relevant seed traits have focused on *primary dispersal* by mammals and birds (Bodmer, 1991; Chapman, 1989; Estrada et al., 1993; Galetti et al., 2001; Matías et al., 2010; Milton, 1981). However, primary dispersal alone may have many downsides because seeds are often deposited in an aggregated fashion on the ground and only rarely in an appropriate micro-habitat. This may lead to a decrease in seedling establishment (Vander Wall & Longland, 2004). There are two types of animal seed dispersal, (a) endozoochorous, passing through the digestive system of an animal, and (b) epizoochorous, adhering to an animal usually in their fur. *Secondary dispersal* of endozoochorous seeds by dung beetles has also been studied but to a lesser degree. There is evidence that seedling survival is higher when both mammals and dung beetles act together as primary and secondary dispersers, respectively (Culot et al., 2018). Understanding secondary dispersal by dung beetles could be critical to our understanding of forest dynamics and seedling establishment because dung beetles reduce seed clumping (Lawson et al., 2012; Urrea-Galeano et al., 2019), which should decrease density-dependent seed mortality (e.g., pathogen and seed predation) (Andresen & Levey, 2004; Beaune et al., 2012; Bell et al., 2006; Vulinec, 2002). When dung beetles bury seeds, they drastically reduce seed discovery by seed predators. In at least one case, burial by dung beetles decreased seed predation rates by as much as 95%–98% (Vulinec, 2002). Primarily dispersed seeds suffer from seed predation accelerated by the fact that dung odors are attractive to seed predators such as rodents (Andresen & Levey, 2004; Beaune et al., 2012). For this reason, seed traits that promote acceptance by dung beetles might be advantageous for seeds.

Studies of seed traits related to the acceptability and burial of seeds by dung beetles have primarily focused only on seed size (Andresen, 2002; Andresen & Levey, 2004; Braga et al., 2017; Feer, 1999). One example of a trait other than size influencing seed acceptability to dung beetles is the odor trait used by *Ceratocaryum argenteum*, seeds mimic the odor of herbivore feces and are buried by dung beetles. These seeds are protected from seed predation by rodents because dung beetles bury them (Midgley et al., 2015). However, seeds do not provide either the adult or larval dung beetles with sustenance, and as a result, dung beetles have been observed cleaning and removing large seeds from their dung balls. For this reason, it is essential to understand what traits make seeds acceptable to dung beetles.

Small seeds are more likely to be included in dung balls and dispersed by dung beetles. Large beetles accept larger seeds in their dung balls than do smaller beetles (Andresen, 2001). Large seeds are also buried less deeply by dung beetles than small seeds (Shepherd & Chapman, 1998), which is important because seeds that are buried too deeply do not germinate (Andresen & Levey, 2004; Shepherd & Chapman, 1998). Methods for studying seed dispersal by dung beetles focus on size, often excluding other seed traits (e.g., surface or shape). Studies using beads as a proxy for seeds overlook potentially important seed traits, for example, surface or shape (Andresen, 2002; Andresen & Feer, 2005; Braga et al., 2017; Feer, 1999; Lawson et al., 2012).

Dung beetles disperse more small seeds than large seeds, resulting from a combination of rejecting large seeds and a higher abundance of small seeds (Andresen, 2002; Andresen & Feer, 2005; Andresen & Levey, 2004). Large dung beetles move a greater quantity of dung and are therefore expected to disperse more seeds and accept larger seeds than smaller dung beetles (Andresen, 2002; Nichols et al., 2008; Shepherd & Chapman, 1998).

1.1 | Hypothesis and predictions

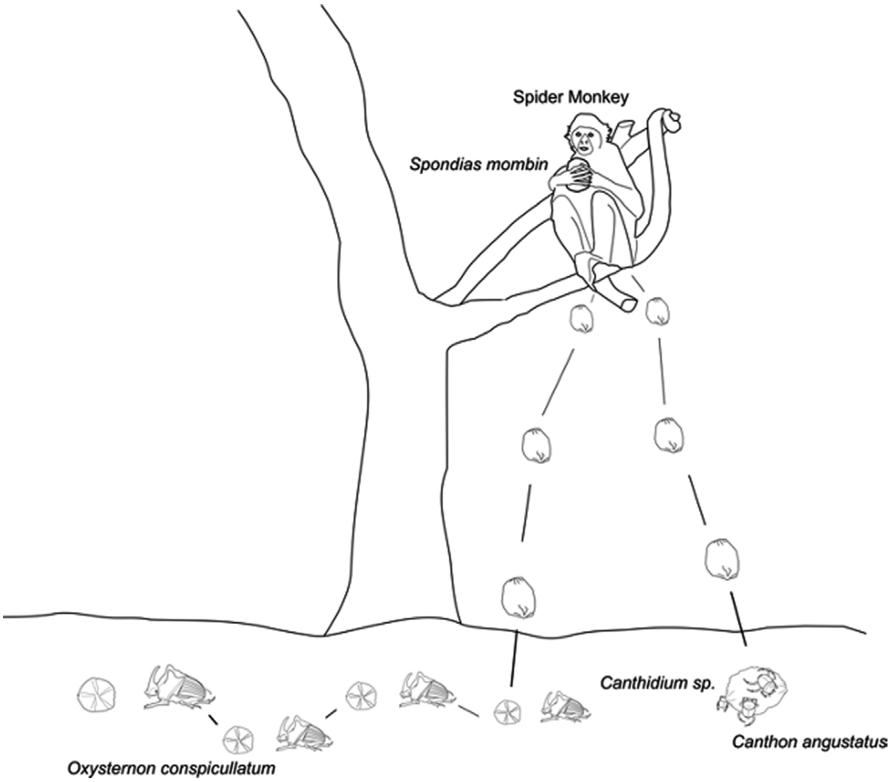
Our field observations of large beetles suggest that they accept larger seeds than smaller beetles. We also made a novel observation that made us consider traits apart from size that might be important in determining a seed's acceptability to a dung beetle. *Oxysternon conspicillatum*, *Canthon angustatus*, and *Canthidium* sp. treated a very large seed *Spondias mombin* as though it was dung. *S. mombin* is a large seed covered in hairs (pubescent), and dung sticks to the hairs, possibly misleading the beetles as to its makeup (Figure 1). We formulated a general hypothesis from our field observations: secondary seed dispersal by dung beetles, where advantageous, might be facilitated by some aspects of seed morphology. We focus our analysis on three seed traits: (a) round shape, (b) pubescent surface, and (c) seed size. We tested this hypothesis with the following predictions. (1) Larger dung beetles accept larger seeds in their dung balls, (2) large endozoochorous seeds are rounder, (3) large endozoochorous seed species are more likely to be pubescent than smaller ones, and (4) large pubescent seeds are over-represented in dung balls.

2 | METHODS

2.1 | Seed traits from in situ collections of dung balls and fecal samples

Our study site is in the Ecuadorian Chocó, a tropical lowland forest on the western side of the Andes within the Reserva Canadé and Tesoro Escondido (00°31′2″N, 79°12′13″W). During our observations of dung beetles in the field from January 2019 to June 2019, we opportunistically collected 82 dung balls from dung beetles = 2, *Scybalocanthon trimaculatus* $N = 1$) from under troops of brown-headed spider monkeys (*Ateles fusciceps fusciceps*) and mantled howler monkeys (*Alouatta palliata*). We calculated the range of seed sizes for the two common beetle species. Tunneling species *O. conspicillatum* and *S. noctis* had more individual balls ($N = 33$), but they only came from 11 individual beetles. We also collected 29 fecal samples from brown-headed spider monkeys and mantled howler monkeys. In the laboratory, we dissected and removed the seeds from these dung ball and fecal samples ($N_{\text{dungball}} = 3659$ seeds and $N_{\text{fecal}} = 2793$ seeds). The seeds from both the dung balls and fecal samples were then grouped by morphospecies, and the seeds belonging to each morphospecies within each sample were counted. We determined morphospecies visually, and seeds that look similar throughout both

FIGURE 1 (a) Illustration of *Spondias's* movement from the spider monkey to the dung beetles *Oxysternon conspicillatum*, *Canthon angustatus*, and *Canthidium* sp. (b) Photograph of an (*O. conspicillatum*) beetle with the *Spondias mombin* seed (*Ateles fusciceps fusciceps*) feces that is pushed into a crevasse, from a brown-headed spider monkey's defecate. (c) Screen grab from a video of *C. angustatus* and *Canthidium* sp. trying to use a second *S. mombin* seed as they normally would use dung



(b) *Spondias mombin* seed path

(b) *Oxysternon conspicillatum angustatus* and *Canthidium*

(c) *Canthon angustatus and Canthidium sp.*

the data set of seeds in dung balls and fecal samples were grouped in the same morphospecies. For each morphospecies, we measured seed length (longest side to the nearest millimeter) and seed width (widest point 90 degrees from length) for up to 20 seeds per morphospecies (as is common in studies of this nature, there are many singlets). We also characterized seed surface as pubescent, striate, or smooth. Pubescent seeds are defined by hairs, or hair-like projections coming from their surface, striate seeds by their grooves or indentations in their surface, smooth seeds by their relatively uninterrupted surface. The shape was characterized with a roundness index $R(\text{roundness}) = L/W$ where L *is the* length of seed along the longest axis and W is the widest part of the seed at 90° from L. The closer to 1, the more rounded the seed is, and a larger number correspond to a more elongated shape. Additionally, seed source (fecal sample or dung ball), monkey species, and beetle species were also recorded for each sample. From the set of these seed traits and sources, we examined how dung beetles filter seeds from feces by comparing the traits of seeds in dung balls to the traits of seeds in fecal samples. We also related the range of seed sizes in dung balls of both *O. conspicillatum* and the smaller *C. angustatus*. We used a Mann–Whitney *U* test to compare morphospecies average values in all cases. We then used the seed length as a variable to predict the roundness, using Spearman's rank correlation coefficient. Finally, we calculated the proportion of large pubescent seeds in a dung ball or a fecal sample using the set of seeds in dung balls, compared to seeds in fecal samples as a reference. For both proportions, we calculated 95% confidence intervals. To define large seeds, we used the upper quartile of morphospecies length.

2.2 | Seed traits from literature

To test whether the seed traits for our subset of seeds are similar to the wider Neotropics, we compiled a data base of Neotropical seeds from 123 families and 427 genera, which are not wind dispersed from the reference work "Seeds of Amazonian Plants" (Cornejo & Janovec, 2010). We then added dispersal type data from the Royal Botanic Gardens Kew Seed Information Database (SID, Royal Botanic Gardens Kew, 2020) and our field observations where available. This date set covers a much larger geographic area than our study and could indicate if the results extend beyond our study site. Our final seed trait data base consisted of seed shape, size class, seed surface, and type of seed dispersal. Only mammal dispersed genera or families were included in our statistical analysis (70 families and 176 genera). We eliminated genera without mammal dispersal, or dispersal data, because if they are not in mammal feces, they are most likely not secondarily dispersed by dung beetles. It should be noted here that this might be too conservative because we eliminated over a half of the genera and many trees in tropical forests are mammal dispersed (Muller-Landau & Hardesty, 2005). Seed trait values are categorical and were predetermined by the categories from the "Seeds of Amazonian plants" or the KEW data base. Seed shape was broken into four categories: (1) elongate (1.5 times as long as wide), (2) round (less than 1.5 times as long as wide), (3) flat (width is 1.4 times thickness), and finally (4) irregular (a shape that cannot be easily measured or categorized). Size classes were broken down as follows: 0–0.49, 0.5–0.99, 1–1.99 cm, and finally greater than or equal to 2.00 cm. Seed surface categories for statistical analysis were pubescent vs non-pubescent, similar to the previously described categories but for this analysis we combined the categories smooth and striate.

We tested the following predictions on this data set. (1) Large endozoochorous seeds have a disproportionate number of pubescent genera (or families) because they are unlikely to be incidentally included in dung balls, unlike small seeds (prediction 3 introduction). (2) Rounded seeds should have a disproportionate number of pubescent genera (or families), because of their increased "rollability." (3) The combination of large size and round shape should be an even better predictor of seed pubescence. To test trait associations, we created contingency tables with the number of genera (or families) of pubescent/non-pubescent seeds in each size or shape class. We then used the Patefield's algorithm to test whether associations were significant, for example, to evaluate whether pubescence occurred more often than predicted by chance in large or rounded seeds (Patefield, 1981). In conjunction with the null model, the $H2^0$ value was used as an indicator of the strength of trait partitioning (Blüthgen et al., 2006) (range from 0 to 1, with 0 showing traits are evenly distributed and 1 is the maximal degree of trait partitioning given the totals). Our first and second contingency tables test (1) whether rounded seeds are disproportionately pubescent and (2) whether larger seeds are disproportionately pubescent, respectively. Finally, we created a third contingency table to test whether the combination of roundness and large size better predict pubescence. The latter table was created using genera with the following trait combinations: (1) Seeds larger than 0.99 cm, elongate or round in shape and pubescent, (2) all other pubescent seeds, (3) large seeds, round or elongate but non-pubescent, (4) all other seeds (Table 1).

TABLE 1 Contingency table showing the number of plant genera with particular combinations of seed traits (i.e., large size greater than 0.99 cm, round or elongate) with the presence or absence of pubescence

	Large round or elongate seeds	All other seeds
Pubescent seeds	17[a]	7
Non-pubescent seeds	112	132

[a] *Spondias* is in this cell of the contingency table.

3 | RESULTS

3.1 | Field observations

3.1.1 | Natural history observation

On the 20th of March 2019, around 1100 h, during the opportunistic collection of dung beetle brood balls from the dung of brown-headed spider monkeys (*A. fusciceps fusciceps*) and mantled howler monkeys (*A. palliata*), we observed three different dung beetle species interacting with large (≈2.1 cm long) seeds from *S. mombin* as though they were dung. The behavior of three different dung beetles is described here. Each dung beetle handled dung and the seeds differently. First, we observed *O. conspicillatum* (2.5 cm body length) pushing *S. mombin* (Figure 1b). *Oxysternon conspicillatum* is a common tunneling dung beetle in the Reserva Canandé. Second, we observed *C. angustatus* (0.7 cm body length) trying to pull a section of dung off the *S. mombin* seed to form a ball. We observed them removing seeds of about 0.5–1 cm length from their balls and then rolling their ball on multiple occasions. Finally, we observed *Canthidium* sp. (0.6 cm body length) tunnel under the *S. mombin* (Figure 1a). Occasionally, *O. conspicillatum* and *C. angustatus* abandoned the seed after they could not remove dung from the seed. The pubescent (hairy) surface of *S. mombin* holds dung more effectively than smooth seeds of equivalent size and shape in the same site. During our field observations, dung beetles never accepted non-pubescent, endozoochorous seeds of the same size as *S. mombin*.

3.2 | Morphospecies seed traits

3.2.1 | Beetle size and seed length

Our prediction (1) that larger dung beetles accept larger seeds in their dung balls was confirmed. We first tested whether seed morphospecies in dung balls were smaller than seed morphospecies in the reference fecal set. The mean length of morphospecies in fecal samples ($N = 31$, $M = 6.6$ mm ± SD = 6.7 mm) was 1.6 times longer than morphospecies in dung balls ($N = 35$, $M = 4.2 \pm 4.8$ mm), but this difference was not significant using a Mann–Whitney *U* test ($W = 637.5$, $df = 1$, $p = .22$). To test whether the larger beetle *O. conspicillatum* accepts larger morphospecies than the smaller *C. angustatus*, we compared the mean length of morphospecies in both beetle's dung balls. The length of morphospecies acceptable to *O. conspicillatum* ($N = 14$, $M = 6.8 \pm 6.3$ mm) was a statistically significant 2.7 times longer than morphospecies acceptable to *C. angustatus* ($N = 29$, $M = 2.5 \pm 2.1$ mm, $W = 108$, $p = .014$). However, most seeds in the dung balls of both species were small, and when means were weighted by the number of seeds in each morphospecies, the mean seed lengths from both species were smaller than the unweighted means ($M = 4$ mm and $M = 1.7$ mm) for *O. conspicillatum* and *C. angustatus*. To test whether the set of morphospecies in each beetle's dung balls differed from morphospecies in the set of fecal samples, we compared the lengths of morphospecies within dung balls of the smaller *C. angustatus* and the larger *O. conspicillatum* to the lengths of morphospecies in fecal samples. The mean length of morphospecies in *C. angustatus* dung balls was 2.6 times shorter than seeds in fecal samples and the difference is statistically significant ($W = 303$, $p = .03$). As expected, the range of seed sizes in *C. angustatus* dung balls (min = 0.7 mm, max = 11 mm) was much smaller than the size range of fecal samples (min = 0.6 mm, max = 23 mm). *Oxysternon conspicillatum*, in contrast, accepted longer morphospecies, with a mean morphospecies length similar to that of fecal samples ($W = 235.5$, $p = .66$). The range of morphospecies lengths was also similar to the range seen in fecal samples (min = 1 mm, max = 20.5 mm) (Figure 2).

3.2.2 | Morphospecies and shape

We tested the prediction (2) that large endozoochorous seeds were rounder and did find a weak correlation between larger seed size and a less elongated shape ($\rho = -0.19$, $p = .15$; Figure 4a) though it was not significant. We also tested whether the shape of pubescent seeds was different from non-pubescent seeds. Pubescent morphospecies ($N = 17$, $M = 1.6 \pm 0.48$) were not significantly different in roundness to non-pubescent morphospecies ($N = 38$, $M = 1.8$, SD = 0.60) ($W = 280$, $p = .44$; Figure 3b). We also compared the shape of morphospecies in dung balls with the reference to morphospecies in fecal samples. Morphospecies in dung balls ($N = 35$, $M = 1.7 \pm 0.59$) were not significantly different in shape from morphospecies in fecal samples ($N = 31$, $M = 1.7 \pm 0.45$) ($W = 638$, $p = .22$).

3.2.3 | Morphospecies length and surface

Our prediction (3) that large endozoochorous seeds species have more pubescent seed species than smaller seed species was supported. We compared the mean length of pubescent morphospecies with non-pubescent morphospecies. Pubescent morphospecies ($M = 8.7 \pm 6.8$ mm) were a statistically significant 2.23 times longer than non-pubescent morphospecies ($M = 3.8$ mm, SD = 4.5 mm, $W = 513$, $p < .001$; Figure 4b). To clarify whether the smaller mean of non-pubescent species was associated with smooth or striate surfaces, we also compared the mean length of pubescent versus smooth and striate morphospecies. Smooth morphospecies ($N = 18$, $M = 3.9 \pm 4.1$ mm) were 1.5 times shorter than pubescent ones, which

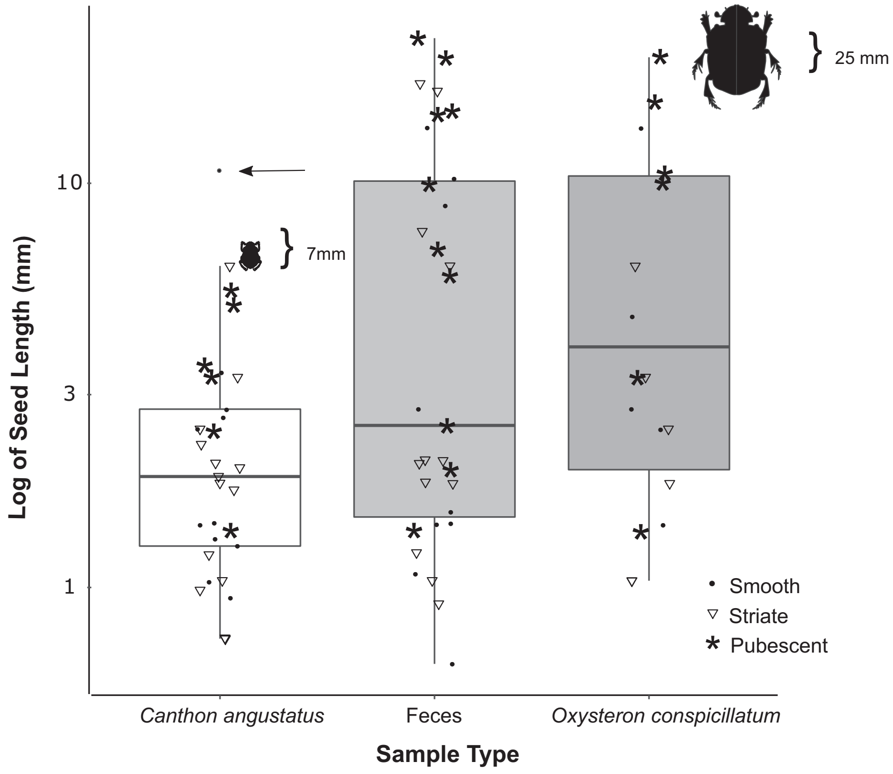
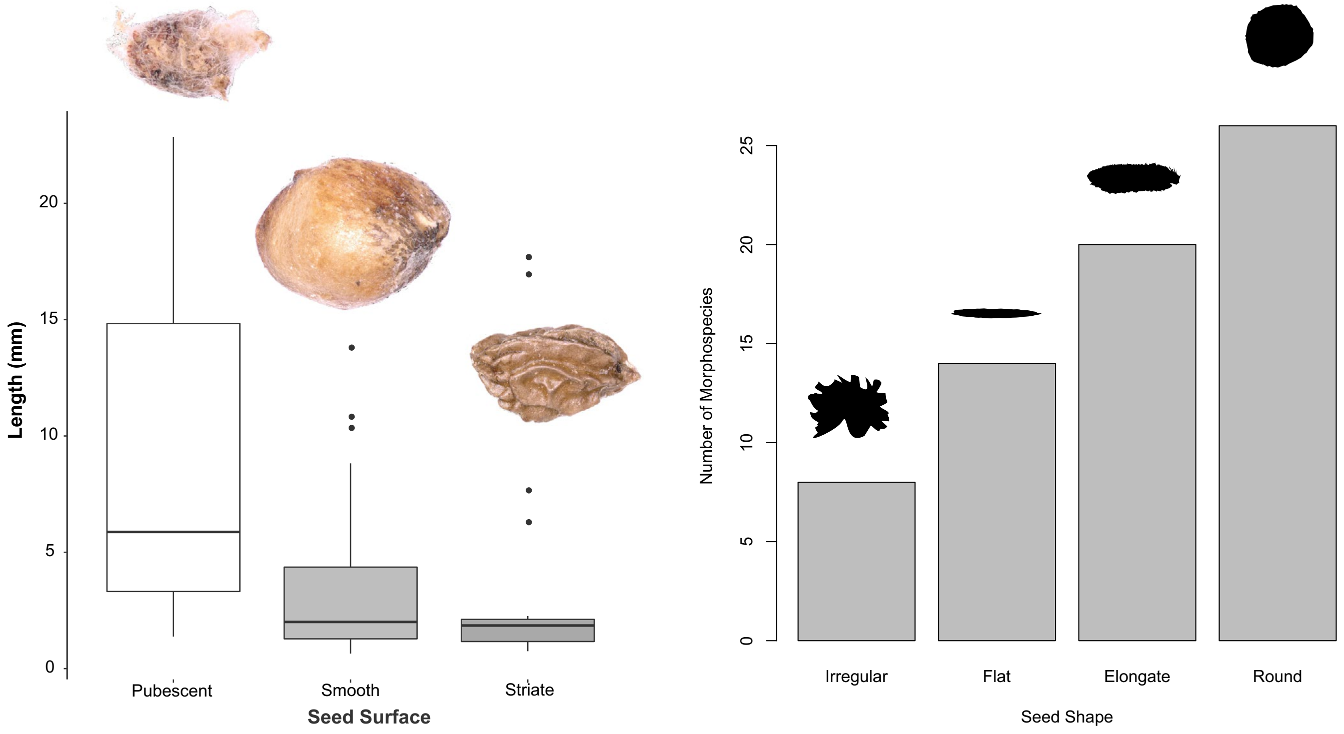


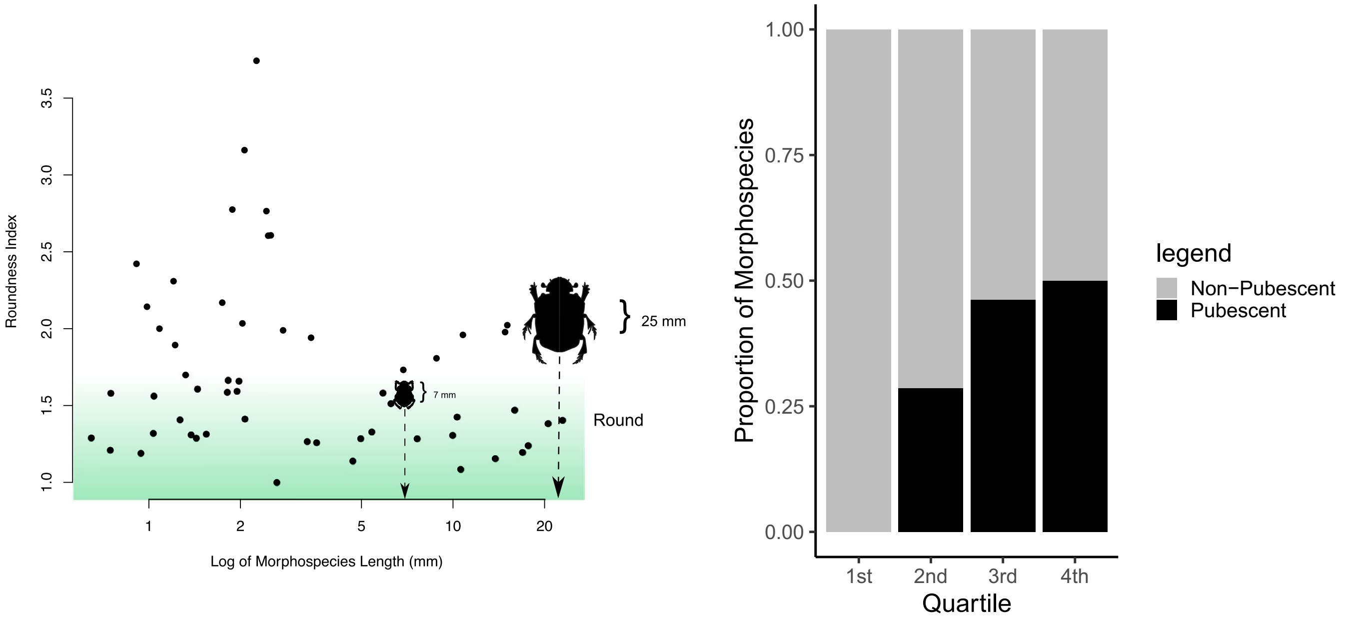
FIGURE 2 Distribution of seed size across samples. Each plant morphospecies is represented by a data point. Pubescent morphospecies are denoted with an asterisk, smooth morphospecies are denoted by a filled circle, and striate morphospecies are indicated with an inverted triangle. Beetles are placed along the *x*-axis at a height that indicates their body length. Seed length is plotted on a log scale. The arrow points to an outlier seed in *Canthon angustatus* dung balls which was flat, and possibly pubescent, though it is unclear as the surface was damaged (Figure S1). Image of seed is provided in Supporting Information.



(a) Seed Size and Surface

(b) Morphospecies Shape

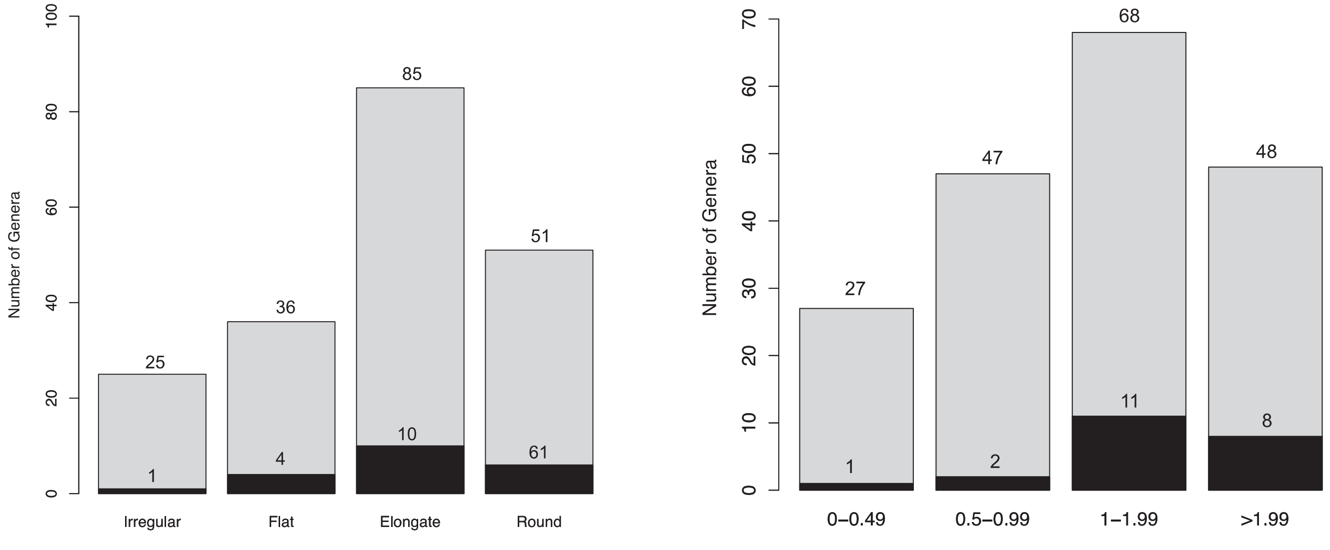
FIGURE 3 (a) Range of size and surface traits of seeds from fecal samples and dung balls. Pubescent seeds were larger than either smooth or striate seeds. A photograph of a typical seed for each surface type is provided. (b) When we categorized seeds in the same way as in the literature, rounded and elongated seeds were represented by a large number of morphospecies



(a) Seed size and Shape

(b) Proportion of morphospecies in different size classes

FIGURE 4 (a) Size and roundness were weakly correlated. The length of *Canthon angustatus* and *Oxysternon conspicillatum* is indicated on the *x*-axis. The shaded green area (below 1.5) marks "round" seeds based on the literature definition. (b) Size distributions of non-pubescent morphospecies where smaller seeds make up a larger proportion, whereas (c) pubescent morphospecies showed a clear trend toward more morphospecies with increasing size categories within the data set



(a) Seed Shape

(b) Seed Size

FIGURE 5 Neotropical seeds with zoochorous mammal dispersal, and the distribution of genera belonging to each shape and size class. Pubescent seeds in each size or shape class are in black, and all other seeds are in gray. (a) The number of genera in each shape category. Shape was not strongly associated with seed pubescence $H2^0 = 0.011$, $p = .26$. (b) The number of genera in each size class. Seed size was also not coupled to seed pubescence, although trait syndromes showed a higher degree of association than for shape $H2^0 = 0.056$, $p = .12$

is significant ($W = 236$, $p < .01$). The mean length of striate morphospecies ($M = 22\ 3.6 \pm 4.7$ mm) and the mean length of smooth morphospecies ($W = 213$, $p = .68$) are not significant. Both are shorter than pubescent morphospecies ($W = 304$, $p < .001$; Figure 3a).

Our final prediction (4) that large pubescent seeds (upper quartile) would be over-represented in dung balls in relation to fecal samples was supported. There were 52 large seeds in 21 fecal samples of which 20 were pubescent; hence, the proportion of pubescent seeds in fecal samples was 0.38 95% CI [0.17, 0.59]. The proportion of large pubescent seeds in dung balls was much higher, with 0.94 95% CI [0.80, 1.0], comprising 23 dung balls were made by 11 beetles with 84 large seeds, of which 79 were pubescent and statistically significantly different.

3.3 | Seed shape and pubescence from the literature

Using the trait data from "Seeds of Amazonian Plants" and the dispersal data from KEW SID (Cornejo & Janovec, 2010) to examine patterns at the family and genus level, we found that seeds with a combination of large size and rounded shape at the genus level are slightly more likely to be pubescent than other seeds ($H2^0 = 0.147$, $p = .048$). However, neither shape ($H2^0 = 0.011$, $p = .26$) nor size ($H2^0 = 0.056$, $p = .13$) alone were sufficient to predict pubescence. The pattern was similar at the family level (shape: $H2^0 = 0.014$, $p = .35$, size: $H2^0 = 0.049$, $p = .13$; Figure 5). However, if we do not exclude all genera without dispersal data from our data set, both size and the combination of size and shape are significant predictors of seed pubescence at the genus level (Tables S1–S3).

4 | DISCUSSION

Endozoochory is the dominate form of seed dispersal in the Neotropics, with 65%–100% of seeds being dispersed by animals (Muller-Landau & Hardesty, 2005; Seidler & Plotkin, 2006). Primates are important seed dispersers, and seeds passing through their digestive tract like seeds in our study have an equal or greater chance of germinating (Fuzessy et al., 2016; Stevenson et al., 2002), suggesting that this is a desirable trait for of seed dispersal. This may be augmented by secondary dispersal by dung beetles because of the aggregated deposition of seeds by some primates (e.g., Russo & Augspurger, 2004). At least one study of tamarins and associated dung beetles did find some increase in rates of germination with secondary dispersal by dung beetles in secondary forests (Culot et al., 2018) and in forests like our study site where spider monkeys are one of the most important frugivores secondary dispersal by dung beetles might be even more important because of the high density of seeds in their dung.

We examined seed traits in relation to dung beetle acceptance of seeds in their dung balls. Our results confirmed the importance of seed size which has been the main trait considered previously (Andresen, 2002; Andresen & Levey, 2004; Braga et al., 2017). In addition, we also highlighted two traits (seed surface and seed shape) that have not previously been studied concerning secondary dispersal. We considered both in situ observations of seed inclusion in dung balls and overall trends in seed trait associations from the literature. Our results support our hypothesis that seed traits size and pubescence facilitate secondary seed dispersal by dung beetles, but not shape. Three out of four predictions from our hypothesis were supported.

Our data are also consistent with the prediction (1) that larger beetles accept larger seeds (Andresen, 2001). *Oxysternon conspicillatum* is ≈3.6 times longer than *C. angustatus*, and the average length of morphospecies in *O. conspicillatum* dung balls was almost three times the mean length of seeds in *C. angustatus* dung balls. The maximum acceptable seed length for both beetles was close to their body length. Together, both beetles covered the entire range of seed sizes found in the feces of both howler and spider monkeys (Figure 2). This has ecological consequences for those seeds.

One of the benefits of secondary dispersal is escape from seed predation, and burial even at shallow depths of 1 cm has been shown to drastically reduce seed removal by rodents (Feer, 1999). The greater the burial depth, the lower the chance that a seed is removed by a seed predator, but the chance that a seed will germinate may be reduced. Small seeds are both less likely to germinate and more likely to be buried at greater depths which forms scenario where secondary dispersal may be detrimental. Small seeds are much less likely to germinate at depths greater than 3 cm, and large seeds are unlikely to germinate at depths greater than 5 cm (Shepherd & Chapman, 1998). Previously recorded seed burial depths by a *Canthon* species equal 4 cm for seeds smaller than 5 mm. They did not bury larger seeds. For *O. conspicillatum*, mean burial depths for seeds <5 mm were 5.65 cm and for larger seeds 2.57 cm (Vulinec, 2002). However, the reduced density of seeds on the surface or at lower depths might still create a net benefit for hundreds of small seeds (<2 mm) found in some of our spider monkey fecal samples.

Our prediction (2) that large mammal dispersed seeds are rounded was not supported and most seeds, regardless of size, tended to be somewhat rounded (Figure 4a). Additionally, seeds in dung balls did not differ in shape compared to seeds in fecal samples, suggesting that this may not be the most crucial seed trait for inclusion in dung balls by dung beetles. While shape was not found to be important here, it is possible that the number of morphospecies we have in our data set is too small because there does appear to be a trend toward less elongate seeds at larger sizes. In addition, it was an important variable in the literature data though only in combination with shape and size.

Our prediction (3) that large seeds are more pubescent than smaller seeds was more strongly supported by the field data (Figure 4b). At the genus level (literature data), the combination of large size and pubescence occurred more often than expected by chance suggesting that the trend might extend beyond our study system. However, further work is required. We also excluded many genera from the literature data set for the lack of dispersal data. This may be a mistake given that the majority of Neotropical trees are animal dispersed. A more complete data set with a continuous size variable and more complete dispersal data is needed. Finally, our prediction (4) that large pubescent seeds are more acceptable to dung beetles than large non-pubescent seeds was supported by our direct field observations of *O. conspicillatum*, *C. angustatus*, and *Canthidium* sp. and their interaction with *S. mombin*. In addition, large seeds in dung balls were disproportionately pubescent, especially when compared to the reference set of seeds in fecal samples. The disproportionate acceptance of large pubescent seeds by dung beetles indicates that they filter seeds with respect to both size and surface. Together, these findings suggest that this combination of seed traits is advantageous for secondary dispersal.

Of the 11 species of dung beetles which we observed on primate dung over 6 months, three were observed to treat the dung covered *S. mombin* as though it were feces. This unproductive foraging likely led to a delay in a feeding or reproductive opportunity, while the surrounding dung was buried by competitors (Figure 1). The cost may not be severe, because monkeys are a reliable source of dung, and we often saw dung beetles perch on leaves below monkey troops, apparently waiting for them to defecate. Despite *S. mombin* being 1.6 times longer than the next largest acceptable seed included in *O. conspicillatum* dung balls, and 2.3 times than even the longest outlier in *C. angustatus* balls, both beetles were attracted to and attempted to move *S. mombin* seeds. The combination of seed traits, large size, roundness, and pubescence, appears to make *S. mombin* an ideal candidate for secondary dispersal by large dung beetles.

Large seeds representing a larger parental investment (K-selection rather than r-selection) are more likely to respond strongly to conditions that increase survival. Secondary dispersal and burial by dung beetles increase their distance from conspecifics and their protection against seed predators and pathogens. These benefits could favor pubescence and thus dung attachment to seeds. Most mammals consume multiple fruits from the same tree. Personal observations in the field suggest there is a lot of synchronous defecation of howler and spider monkeys. Consequently, seeds from the same tree are often concentrated together within the mammal dung which should increase the relative benefits of secondary dispersal (Howe & Smallwood, 1982; Seidler & Plotkin, 2006). In the case of *S. mombin*, we observed many seeds falling close to each other, suggesting that dispersal away from conspecifics should be beneficial. However, in the case of small primates (e.g., tamarins), seeds may be deposited singly or in small numbers (Culot et al., 2018), which would weaken selective pressure for secondary dispersal by dung beetles because there is no dung to attract the dung beetles. In cases where there is dung it acts like a glue sticking seeds together. In these cases, dung beetles should be the most beneficial. For this reason, further study is required to fully understand in which habitats, and with which monkey species dung beetles are most beneficial as secondary dispersers.

Our evidence from the literature data that larger seeds are more likely to be pubescent suggests that this trend extends to other sites in the Neotropics, which will require further local studies. At least for primate-dispersed seeds, however, we would expect a weaker trend in the old world because of a larger number of seed spitting primates (Dominy & Duncan, 2005). When seeds are spat, they do not pass through the digestive system of a primate and are thus not in the dung for dung beetles to interact with. Mammals are more

TABLE 2 Genera of large, rounded, pubescent seeds that might exploit dung beetles as secondary dispersers in a similar manner as *Spondias mombin*

Family	Genus	Size class	Shape	Endozoochory
Anacardiaceae	*Spondias*	2+	Round	Yes
Arecaceae	*Astrocaryum*	2+	Round	Yes
Arecaceae	*Attalea*	2+	Elongate	Yes
Arecaceae	*Bactris*	0.5–2+	Elongate	Yes
Arecaceae	*Chamaedorea*	1.00–1.99	Elongate	Yes
Arecaceae	*Desmoncus*	1.00–1.99	Elongate	Yes
Arecaceae	*Euterpe*	1.00–1.99	Round	Yes
Arecaceae	*Oenocarpus*	1.00–2+	Round	Yes
Bombacaceae	*Matisia*	2+	Elongate	Yes
Bombacaceae	*Quararibea*	1.00–1.99	Elongate	Yes
Caryocaraceae	*Caryocar*	2+	Round	Yes
Chrysobalanaceae	*Couepia*	2+	Elongate	Yes
Dipteryx	*Dipteryx*	2+	Elongate	Yes
Quiinaceae	*Quiina*	1.00–1.99	Elongate	Yes
Verbenaceae	*Vitex*	1.00–1.99	Elongate	Yes
Violaceae	*Rinorea*	0.5–0.99	Round	Yes

likely to spit larger seeds; hence, seed spitting may blur the relative advantage of pubescence.

There are examples of dung beetle dispersal syndrome. One example is of *C. argenteum* seeds rolled by the *Epirinus flagellatus* dung beetles in De Hoop Nature Reserve, South Africa. Seeds with morphologies similar to *C. argenteum* are exposed on the surface and dispersed or eaten by small rodents. Instead, *C. argenteum* seeds smell strongly of herbivore feces and are dispersed and buried by dung beetles (Midgley et al., 2015). For *S. mombin* and other pubescent seeds, we suggest that these seeds use the actual dung to increase their attractiveness to dung beetles. Another example comes from an observation in the Ivory Coast where 26 dung beetles from five species mistook single seeds as dung, though in the observation seeds were only characterized by size (Kunz & Krell, 2011). Our results together with the aforementioned studies suggest that this phenomenon deserves further study.

5 | CONCLUSION

Dung beetles treat the *S. mombin* seeds similarly to dung, attempt to move the whole seed, or remove a part of it to form a ball, even though the seed is well outside the typical, acceptable seed size range for the beetle species. We suggest some cost to the beetle because of the lost opportunity and the need to spend more time searching for new dung. The finding that large pubescent seeds were accepted while large smooth seeds were excluded suggests that surfaces are essential. It is still unknown how widespread this phenomenon is. The combination of large size and a rounder, easier to roll shape corresponds to an increased proportion of pubescence across plant genera. Also, pubescence and shape may be variable traits in some seeds, capable of responding to environmental conditions. A clumped distribution and predation risk may be particularly costly to large seeds where secondary dispersal by dung beetles is particularly beneficial. We suggest that additional observations with a particular focus on large, round, pubescent seeds in the genera listed in Table 2 would be useful to elucidate how important this phenomenon is in the evolution of seed traits.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.cjsxksn6p (Pedersen & Blüthgen, 2021).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Appendix 5

The preliminary dung beetle guide was created using an automated pipeline (Figure A). It is still a work in progress, so I present here just the preliminary draft. This will be improved with better quality photos and a dichotomous key. The current product included here has photos and the published morphological characteristics for identification.

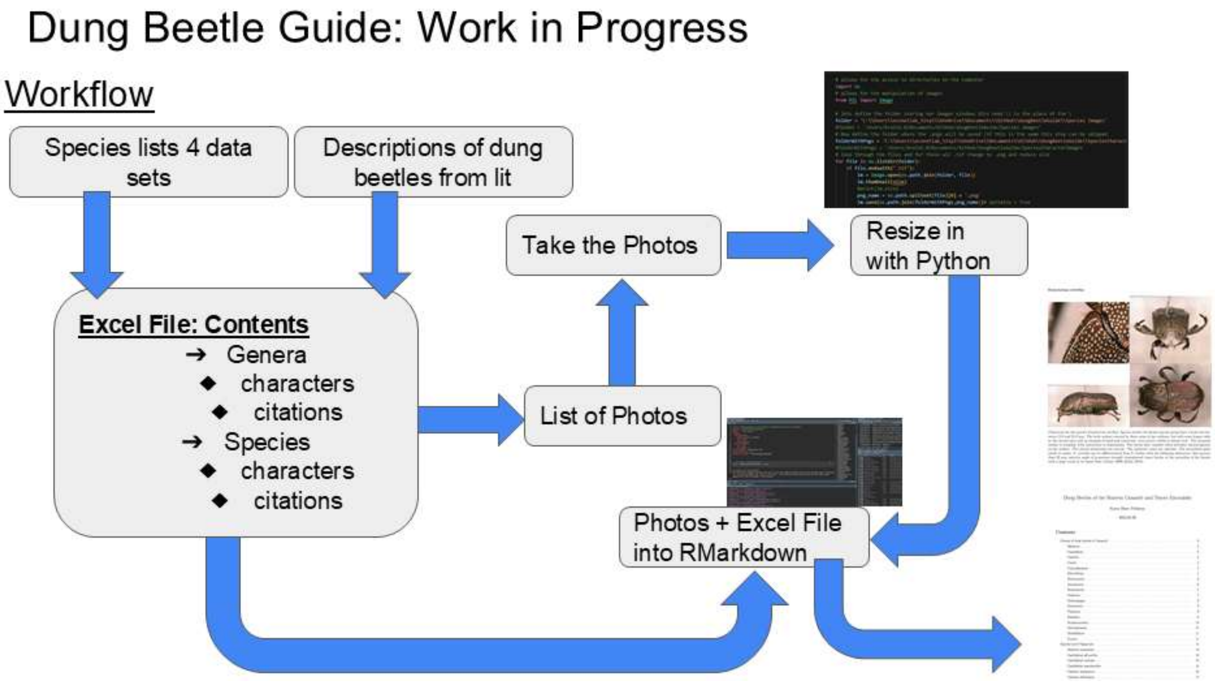

Figure A: Diagram of the workflow used to generate the key. This workflow should make it easier to update as the information becomes out of date, species are described etc.

Dung Beetles of the Reserva Canandé and Tesoro Escondido

Karen Marie Pedersen

2025-03-14

Table of Contents

Description of contents

Species of dung beetles collected in the *Reserva Canandé* and *Tesoro Escondido*. Photos by KMP and characters are collected from published dichotomous keys. This serves as a list of the relevant publications (works cited), list of species (table of contents), and reference for future identification (photos). The species are ordered alphabetically, and the characters all come with a citation of the original description if avalible. The description was translated if the key was not published in English. Most species have three photos (apical, lateral and dorsal views). Exceptions include: *Deltochilum loperae, Eurysternus aff. lanuginosus* and *Eurysternus caribaeus* which have, no photos. Additionally, *Eurysternus plebejus* lacks an apical photo. Some species have additional photos of key identification characters.

Species of Dung Beetles

Below are the dung beetle species and the supporting literature for each species.

Bdelyrus seminudus

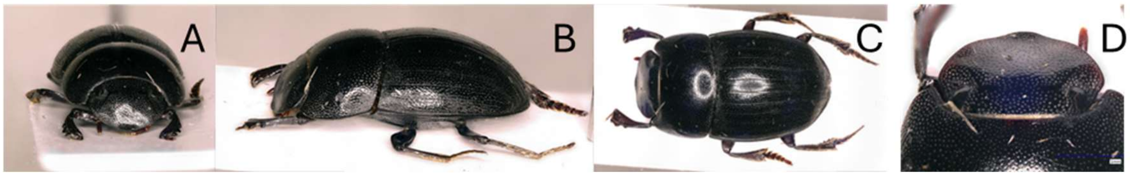

Figure 2 Photos of *Bdelyrus seminudus* from A) apical, B) lateral, C) dorsal views, and D) clypeus.

Characters for the species *Bdelyrus seminudus*: Total length is usually more than 8 mm; apex of clypeus weakly emarginate (fig 2D) (Cook 2000).

Canthidium aff. *aurifex*

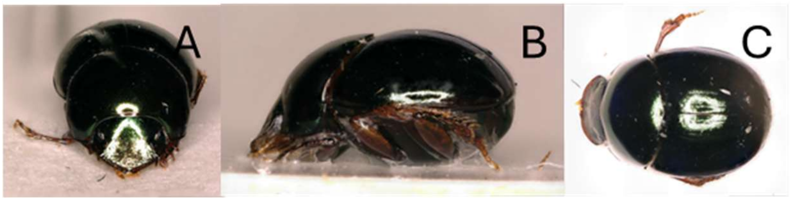

Figure 3 Photos of *Canthidium* aff. *aurifex* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Canthidium aurifex*: Interstriae with inconspicuous punctuation (fig 4A); pygidium of both sexes with crude punctuation (fig 4B); the protibia of the female with the apical tooth at an open angle in relation to the second tooth; distributed from 0-500 masl in the low tropical humid forests of Costa Rica (Solís and Kohlmann 2004).

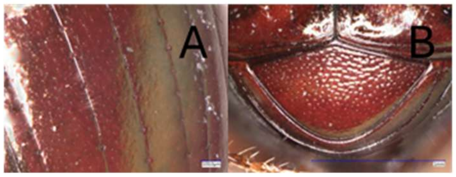

Figure 4 Photos of diagnostic characters of *Canthidium aurifex* A) the interstriae and B) the pygidium.

Canthidium centrale

Figure 5 Photos of *Canthidium centrale* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Canthidium centrale*: Head with slightly widened as if swollen, clypeus with coarse punctation and widely spaced eyes(fig 6A); granulated pronotum with crude punctuation (fig 6b); elytra with a poorly developed and non-shiny granulation, narrow striae; pygidium granulated only at the base and covered by fine punctuation; lateral edges of the metasternum, mesopimeron and metaepisternum with slightly arched points, without reaching a crescent shape (Solís and Kohlmann 2004).

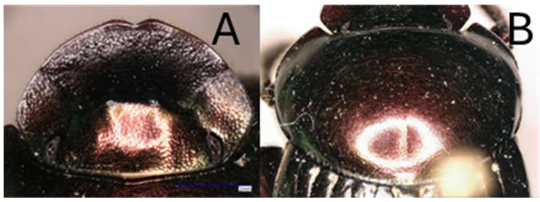

Figure 6 Photos of *Canthidium centrale* A) dorsal view of the head, and B) dorsal view of the pronotum.

Canthidium pseudaurifex

Figure 7 Photos of *Canthidium pseudaurifex from* A) apical, B) lateral, C) dorsal views.

Characters for the species *Canthidium pseudaurifex*: Not found in the literature but smaller and with shinier elytra than *Canthidium centrale*.

Canthon angustatus

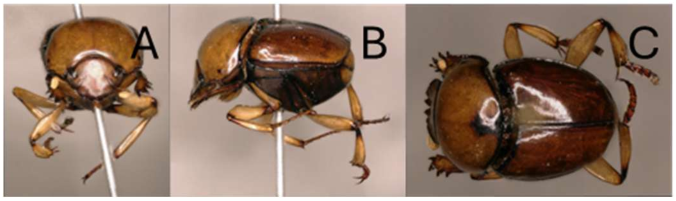

Figure 8 Photos of *Canthon angustatus from* A) apical, B) lateral, C) dorsal views.

Characters for the species *Canthon angustatus*: Anterior tibia with continuous internal border(fig 9) (Solís and Kohlmann 2002). Pronotum typically with a dark medial spot.

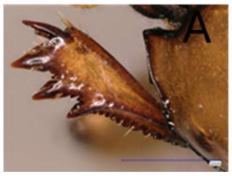

Figure 9 Photo of the left anterior tibia.

Canthon delicatalus

Figure 10 Photos of *Canthon delicatalus* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Canthon delicatalus*: Not found in the literature. Species in the genus Canthon are often determined with the dorsal patterning, which is darker with the elytra ringed by a lighter coloration highly variable among individuals.

Copris davidi

Figure 11Photos of *Copris davidi* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Copris davidi*: Cephalic horn indistinct or truncate and conical. Absence of pronotal armament. Punctation of pronotal disk medially interrupted by an impunctate band extending anteroposteriorly across the pronotal disc (fig 12A). Prosternum lacks longitudinal carina. Tuft of setae present along margin of mesotrochanter (fig 12B) and metatrochanter . Posteroventral surface of mesofemur and metafemur coarsely punctate. Male protibial spur distally expanded with a rounded apex. Parameres separated by a wide, angular ventrobasal gap, and lacking acute teeth at apex. Frontolateral peripheral sclerite elongate, right lateral frontolateral peripheral sclerite process acute, spiniform and gently curved (Darling and Génier 2018).

Figure 12 Photos of diagnostic of characters A) the pronotal disk, and B) right mesotrochanter.

Coprophanaeus morenoi

Figure 13 Photos of *Coprophanaeus morenoi* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Coprophanaeus morenoi*: Pronotal prominence of male, viewed dorsally, broad, width exceeding interocular distance; bimorphic, either cleat-shaped (upper view) or quadrilobate (lower view) with lobes linked by thick carina, middle lobes more salient than lateral lobes; prominence flanked by broad concavities descending to anterior pronotal margin (Edmonds and Zidek 2010).

Deltochilum loperae

Characters for the species *Deltochilum loperae*: Small calluses, scattered in the central 1/3 of the interstria, dimples of the stria are subcircular interstrial calluses larger than interstrial punctures. Pronotal medial lateral angle rounded. Striae broad, width of third stria, in species with the narrowest striae, 1/15th of the distance between striae II and III. Metaventrite with a small, weak posterior excavation occupying basal fourth (González-A., Molano-R., and Medina-U. 2009; González-Alvarado and Vaz-de-Mello 2014).

Deltochilum panamensis

Figure 14 Photos of *Deltochilum panamensis* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Deltochilum panamensis*: Posterior margin (between the dorsal and ventral faces) of the posterior femur excavated (fig 15 B). Interstrial calluses similar in size to interstrial punctures (fig 15 a). (González-Alvarado and Vaz-de-Mello 2014).

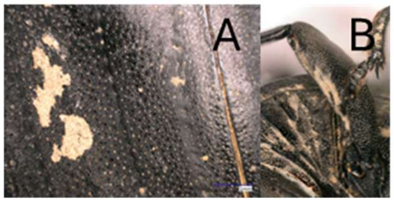

Figure 15 Diagnostic characteristics of *Deltochilum panamensis* A) interstical calluses and B) posrerior margin of the femur.

Deltochilum sp.1

Figure 16 Photos of *Deltochilum sp 1* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Deltochilum sp1*: Not found in literature (closest approximation described in González-Alvarado and Vaz-de-Mello 2021).This is the smallest *Deltochilum* species in Canandé.

Dichotomius divergens

Figure 17 Photos of *Dichotomius divergens* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Dichotomius divergens*: Cephalic protuberance located in the anterior area of the clypeus in the form of a transversely flattened horn or conical horn located between the base of the eyes and the beginning of the genes (fig 18A). For males, the pronotum has a strong flat slope, reaching around ¾ of the length of the pronotum.

There are also three protuberances. The central protuberance is usually the largest and is shaped like a horn with a slight forward projection. The two smaller protuberances are rounded and displaced anteriorly (Sarmiento-Garcés and Amat-García 2014).Strongly marked striae.

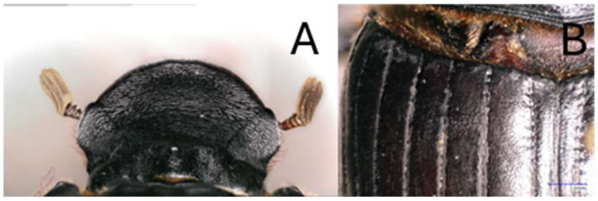

Figure 18 Diagnostic characteristics of *Dichotomius divergens* A) dorsal view of the head with the cephalic protuberance and B) striae.

Dichotomius fortepunctatus

Figure 19 Photos of *Dichotomius fortepunctatus* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Dichotomius fortepunctatus*: Obvious punctuation on the pronotum (fig 20), also smaller than the other *Dichotomius* species in Canandé (Chamorro, Lopera-Toro, and Rossini 2021).

Figure 20 Photo of the pronotum of *Dichotomius fortepunctatus*.

Dichotomius quinquedens

Figure 21 Photos of *Dichotomius quinquedens* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Dichotomius quinquedens*: Cephalic protuberance located in the anterior area of the clypeus in the form of a transversely flattened horn or conical horn located between the base of the eyes and the beginning of the genes. Males with pronotum with a strong slope, forming a smooth horizontal concavity, consisting of three protuberances located along the posterior margin of the slope, being the largest central horn, which constricts when reaching the tip. Females with opaque elytra, and faint striae. The pronotal lobe's anterior angles project laterally (Sarmiento-Garcés and Amat-García 2014).

Figure 22 Photo of *Dichotomius quinquedens* striae.

Dichotomius reclinatus

Figure 23 Photos of *Dichotomius reclinatus* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Dichotomius reclinatus*: Anterior edge of head rounded-elongated; horn flattened transversely and spina forma, and projects from the inner edge of the gena to the base of the eyes where it rises. Sometimes accompanied of two spine-shaped protuberances at the base of the main horn. Interstriae with heavily shagreened microsculpture giving an opaque appearance (Sarmiento-Garcés and Amat-García 2014).

Eurysternus aff. *lanuginosus*

Characters for the species *Eurysternus aff lanuginosus*: Lateral borders of the pronotum parallel; anterior angle of the subangular pronotum; outer edge gives tibia posterior sinuate; male com a smell of light silks on the internal face of the posterior tibia; angle anterior of the female pronotum dentiform, glossy, and dorsally curved; Ventrolateral keel of posterior tibia smooth and shiny; lateral keel of protibia of male normally curved; inner surface of the metatibia of the male with abundant easily visible thin seta (Génier 2009; Rubio 2010).

Eurysternus caribaeus

Characters for the species *Eurysternus caribaeus*: Posterior femur with a single tooth or tubercle on the posterior surface, rarely with an additional keel or tooth near the base, but the latter obtuse and usually poorly developed; posterior angle of male pronotum simple (straight line angle); two teeth basal parts of the male anterior tibia directed obliquely downwards. Genas with clear ocellated punctuation on its entire surface; posterior surface of metafemur mostly light in color; posterior angle of pronotum of male smooth; pronotum with a central smooth callus (Génier 2009; Rubio 2010).

Eurysternus foedus

Figure 24 Photos of *Eurysternus foedus* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Eurysternus foedus*: Species within the foedus species group have a body size between 12.0 and 25.0 mm. The body surface is covered by short uniform setae, but with some longer tufts at the elytral apex and on margins of head and pronotum. eyes poorly visible in dorsal view. The pronotal surface is irregular with concavities or depressions. The elytra have variable often alveolate microsculpture on the surface. The elytral interestriae are narrow. The posterior coxae (metacoxae) are unicolor (fig 25 B). The metatibial spine sessile in males. *E. foedus* can be differentiated from *E. streblus* with the following characters. Body size is between 15 and 20 mm. The anterior angle of the pronotum is narrow (fig 25A). The inner edge of the metatibia of the female is smooth (Génier 2009; Rubio 2010).

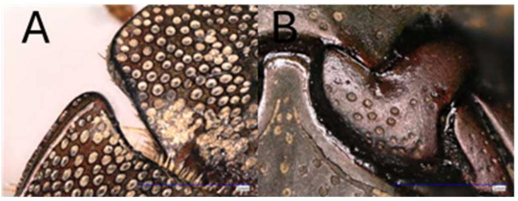

Figure 25 Photos of A) anterior angle of the pronotum, and B) the posterior coxae.

Eurysternus plebejus

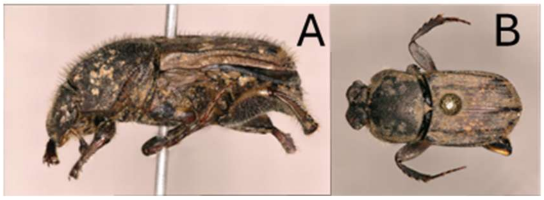

Figure 26 Photos of *Eurysternus plebejus* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Eurysternus plebejus*: Pronotum matte and without concavities or roughness on its surface; anterior edge of pronotum of female without lateral folds. Anterior carina of the proepisternum arched behind the anterior angle; third curved elytral interestria fat the apex (fig 27), semi small tubercle acute, setae similar to the rest of the surface; board anterior clypeus semi dente on each side of the middle (Génier 2009; Rubio 2010).

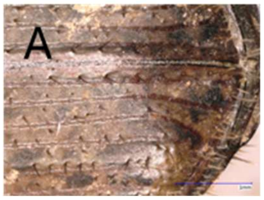

Figure 27 Photo of the apex of the third elytral interstria.

Eurysternus streblus

Figure 28 Photos of *Eurysternus streblus* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Eurysternus streblus*: Species within the foedus species group have a body size between 12.0 and 25.0 mm. The body surface covered by short setae of size uniform, but with some longer tufts at the elytral apex and on margins of head and pronotum. eyes poorly visible in dorsal view. The pronotal surface is irregular with concavities or depressions. The elytra have variable often alveolate microsculpture on the surface. The elytral interestriae are narrow. The posterior coxae are unicolor. The metatibial spine sessile in males. *E. streblus* can be differentiated from *E. foedus* with the following characters. Size greater than 20 mm; anterior angle of pronotum strongly emarginated (fig 29); inner border of the metatibia of the female with a large tooth in its basal third (Génier 2009; Rubio 2010).

Figure 29 Photo of the anterior angle of the pronotum.

Neoathyreus sp.1

Figure 30 Photos of *Neoathyreus sp1* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Neoathyreus sp1*: No description in literature (mentioned in Howden and Gill 1984). Not often found in pitfall traps.

Ontherus trituberculatus

Figure 31 Photos of *Ontherus trituberculatus* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Ontherus trituberculatus*: Three protuberances on the pronotum reduced in females(fig 32A). Median lobe of metastemum with at least few setae similar to those on mesosternum, especially anteriorly, along mesocoxa (fig 32B). Median lobe of metasternum completely marginate anteriorly, margin wide .(Génier 1996).

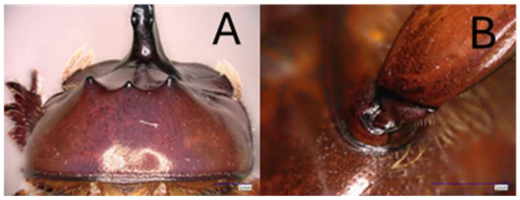

Figure 32 Photos of A) pronotal protuberances, and B) mesocoxal setae.

Onthophagus acuminatus

Figure 33 Photos of *Onthophagus acuminatus* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Onthophagus acuminatus*: Size between 4 and 8 mm; metasternum in its anterior part forming a gibbosity; developed males with the clypeus forming a small tooth-like projection. Coloration with lots of variation from black to a metallic copper or green. The dorsal and anterior area of the head has a transverse carina clearly visible in underdeveloped males, or poorly visible or with a small elevation in well-developed males and with a pair of straight horns between the eyes on the back of the head. (or instead of horns with a pair of tubercles or with a less elevated transverse carina in the middle of its course in poorly developed males). Rest of the description in the cited document (Kohlmann and Solís 2001).

Onthophagus aff. *clypeatus*

Figure 34 Photos of *Onthophagus* aff. *clypeatus* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Onthophagus clypeatus*: Major males with a clypeal projection, and horns, both sexes show some sculpturing of the pronotum, and have metallic pronota (Moctezuma, Sánchez-Huerta, and Halffter 2020).

Oxysternon conspicillatum

Figure 36 Photos of *Oxysternon conspicillatum* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Oxysternon conspicillatum*: Color almost always metallic emerald green. In other geographic locations sometimes with blue highlights or dark blue green; some individuals with deep royal blue coloration. Ventral surfaces of femora with metallic coloring matching that of dorsum (occasionally from front femora is not metallic, or the area is reduced to small areas of femoral surface). In large males, the pronotal surface between the lateral horns with a prominent posterior concavity behind a rounded convex area (Edmonds and Zídek 2004).

Figure 37 Photo of the ventral surface of the metafemora.

Phanaeus pyrois

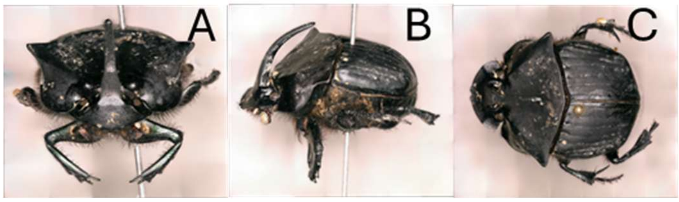

Figure 38 Photos of *Phanaeus pyrois* from A) apical, B) lateral, C) dorsal views.

Characters for the species *Phanaeus pyrois*: Elytral interstriae distinctly flattened and uniformly dull (however in Central American populations, more convex and shiny); striae not strongly impressed basally (fig 39 C), anterior ends in most specimens bearing deep punctures rather than large fossae (fig 39 B). Male: Pronotal disk dull, velvety smooth medially, finely textured, brighter laterally (fig 39A). Female: Pronotum evenly convex, lacking anteromedial concavity even in largest specimens, bearing three round, smooth tubercles in transverse line near anterior margin. Head and pronotum largely highly shiny metallic red to nearly completely dull black with metallic red restricted to ridges and isolated areas on anterior part of pronotum; elytra dull to weakly shiny black; pygidium usually metallic red medially, green peripherally, in some completely red or green (Edmonds and Zídek 2012).

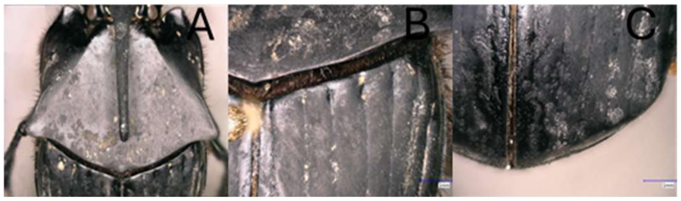

Figure 39 Photos of A) male pronotum, B)right anterior elytra, C) right basal elytra.

Scatimus sp.1

Figure 40 Photos of *Scatimus* sp.1from A) apical, B) lateral, C) dorsal views.

Characters for the species *Scatimus* sp.1: There are no published characters (see for other species descriptions Génier and Kohlmann 2003).

Scybalocanthon trimaculatum

Figure 41 Photos of *Scybalocanthon trimaculatum* 1from A) apical, B) lateral, C) dorsal views.

Characters for the species *Scybalocanthon trimaculatum*: Pronotum with three black spots (two rounded spots on each side of midline anteriorly and one quadrate spot on midline posteriorly). In some individuals, anterior spots are missing, in others spots are fused and two smaller additional spots are present laterally, endophallus with microbristles right beside the FLP sclerite (Silva and Valois 2019).

Sulcophanaeus noctis

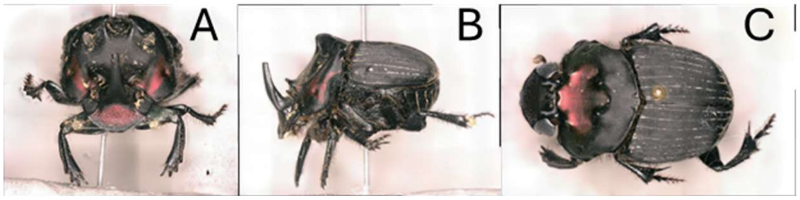

Figure 42 Photos of *Sulcophanaeus noctis* 1from A) apical, B) lateral, C) dorsal views.

Characters for the species *Sulcophanaeus noctis*: Pronotum with very fine texture (can be largely effaced in worn specimens and highly colored areas) in addition to puncturing, asperities or rugosities. Pronotal sculpturing strongest laterally, densely punctate to punctatorugose ; in females and small males distinctly weakest posteromedially, where usually only weakly punctate. Pronotum almost entirely dull black, sometimes partially dull metallic red or yellowish red; if color present at all, only seldom present posteromedially. Posteromedian protuberances of pronotum in large males more or less pyramidal, with flattened or concave anterior surface, apices directed forward (fig 43) (Edmonds 2000).

Figure 43 Photo of male pronotum.

Trichillidium pilosum

Figure 44 Photos of *Trichillidium pilosum* 1from A) apical, B) lateral, C) dorsal views.

Characters for the species *Trichillidium pilosum*: No description in lit (mentioned in Vaz-De-Mello 2008).

Uroxys gorgon

Figure 45 Photos of *Uroxys gorgon* 1from A) apical, B) lateral, C) dorsal views.

Characters for the species *Uroxys gorgon*: Meso-metasternal suture broadly arched anteriorly. The dorsal interocular distance, approximately seven times eye width (fig 46). The posterior margin of metatrochanter continuous with posterior margin of femur; 9 to 11 mm long (Solís and Kohlmann 2013).

Figure 46 Photo of the dorsal view of the head.

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Appendix 6

Karen Marie Pedersen
Doctoral Curriculum Vitae (2019-2025)
Biology Faculty Technische Universität Darmstadt

Peer-Reviewed Publications 2019-2025

Publications in bold are included in the thesis

- Escobar S, Newell FL, Endara MJ, Guevara-Andino JE, Landim AR, Neuschultz EL, Hausmann R, Müller J, Pederen KM, Schleuning M, Tremlett CJ, Villa-Galaviz E, Schaefer HM, Donoso DA, Blüthgen N. (2025) Reassembly of a tropical rainforest ecosystem: A new chronosequence in the Ecuadorian Chocó tested with the recovery of tree attributes. Ecosphere 16:e70157. DOI: 10.1002/ecs2.70157
- Gould, E., Fraser, H.S., Parker, T.H. ... Pedersen KM, ... Zitomer RA (2025) Same data, different analysts: variation in effect sizes due to analytical decisions in ecology and evolutionary biology. BMC Biology 23, 35 DOI: 10.1186/s12915-024-02101-x
- **Grella N, Pedersen K, Blüthgen N, Busse A, Donoso DA, Falconí-López A, Fiderer C, Heurich M, Kriegel P, Newell FL, Püls M, Rabl D, Schäfer HM, Seibold S, Tremlett CJ, Feldhaar H, & Müller J (2025) Vertebrate diversity and biomass along a recovery gradient in a lowland tropical forest. Biotropica, 57(1), e13417. DOI: 10.1111/btp.13417**
- **Pedersen KM, von Beeren C, Oggioni A, Blüthgen N (2024) Mammal dung–dung beetle trophic networks: an improved method based on gut-content DNA. PeerJ 12:e16627 DOI: 10.7717/peerj.16627**
- Marín-Armijos D, Chamba-Carrillo A, Pedersen KM (2023) Morphometric changes on dung beetle Dichotomius problematicus (Coleoptera: Scarabaeidae: Scarabaeinae) related to conversion of forest into grassland: A case of study in the Ecuadorian Amazonia. Ecology and Evolution 13 e9831. DOI 10.1002/ece3.9831
- **Pedersen KM, Blüthgen N (2022) Seed size and pubescence facilitate secondary dispersal by dung beetles. Biotropica DOI: 10.1111/btp.13052**
- Pedersen KM, Shanee S, Olivera Tarifeño CM (2019) Evidence of opossum (*Didelphi*s sp.) Predation by white-fronted capuchins (*Cebus yuracus*) in Copallín Private Conservation Area Amazonas, Perú. Neotropical Primates 25(1)

Conference Presentations

European Conference for Tropical Ecology	Amsterdam *Feb 2025* (Poster)
German Ecological Society	Freising, Germany *Sep 2024* (Talk)
European Conference for Tropical Ecology	Lisbon, Portugal *Mar 2024* (Talk)
Association for Tropical Biology and Conservation	Cartagena, Columbia *Jul 2022* (Talk)
Ecological Society of America	Online, *Jun 2021* (Talk)
Association for Tropical Biology and Conservation	Online, *Feb 2021* (Talk)

Course Work

Meta-analysis Workshop by Julia Koricheva	Biodiversity Exploratories Online Workshop *2024*
Introduction to Data Science with Python	TechAcademy, Goethe Universität Frankfurt Online Course *2024*
Principles of Research Software Engineering	HeFDI Code School Online Course *2024*
Data Science: Probability	Harvardx, edx Online Course *2024*
Python Data Structures	Michiaganx, edx Online Course *2023*
Programming forEverybody (getting started with Python)	Michiganx, edx Online Course *2023*
Data Science: Visualization	Harvardx, edx Online Course *2023*
Data Science: R Basics	Harvardx, edx Online Course 2023
Hyperspectral Data for Land and Coastal Systems	NASA, Online Course, *2021*
Species Distribution Models with GIS and Machine Learning in R	UDEMY, Online Course, *2021*
Fundamentals of Remote Sensing and Geospatial Analysis	UDEMY, Online Course, *2021*