Invertebrate mediated biodiversity-ecosystem functioning relationships: lessons from tropical forest dung beetles

MSc Hannah M. Griffiths

Lancaster University

Universidade Federal de Lavras

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Supervisors
Dr Jos Barlow (Lancaster University)
Professor Richard Bardgett (University of Manchester)
Professor Júlio Louzada (Universidade Federal de Lavras)

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DECLARATION

I hereby declare that this work has been originally produced by myself for this thesis and it has not been submitted for the award of a higher degree to any other institution. Inputs from co-authors are acknowledged throughout

Hannah Griffiths, Lancaster , March 2015

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ABSTRACT

Biological communities are changing across the globe as a result of anthropogenic pressures; abundances of individuals are declining within populations and species are becoming extinct. Biological diversity and trophic complexity in grasslands and soil food webs are positively associated with the cycling of nutrients in soil and water, primary productivity and decomposition. Since these ecosystem processes underpin a number of goods and services to society, human-driven changes in the structure of ecosystems could negatively impact upon human wellbeing. However, the majority of our knowledge of the role of biodiversity in ecosystem functioning comes from studies conducted in temperate grassland systems. Consequently, our understanding of how of higher-level organisms influence ecological processes in different ecosystems is limited. This thesis aims to address these knowledge gaps by investigating how dung beetle traits and functional diversity influence the secondary dispersal of seeds and the emergence and survival of seedlings in the northeastern Brazilian Amazon.

My first research aim was to understand the importance of intraspecific variability in dung beetle traits for the accuracy of functional diversity (FD) indices (Chapter 2). This chapter demonstrates that intraspecific differences in dung beetle traits are small compared to between species differences. However, failure to include intraspecific variability resulted in large errors in the calculation of FD indices when describing small and/or species poor communities. Second, I investigated how dung beetle diversity influences secondary seed dispersal, and the role of environmental context in modulating relationships. Here I reveal positive relationships between dung beetle functional diversity and both the probability of seed burial and the dispersion of seeds throughout the soil profile. However, these patterns were dependant on soil type and thus environmental context (Chapter 3). Finally, I explored the multitrophic significance of findings from Chapter 3 by testing how dung beetle communities affect the burial of different sizes of seeds and emergence and survival of seedlings (Chapter 4). Results from this chapter demonstrate how dung beetles could influence vegetation regeneration because beetle diversity negatively affected the likelihood that experimental seeds emerged from the soil surface, but positively impacted on the likelihood that emerged seeds survived until the end of the experiment period. Furthermore, I show that large seeds could be more vulnerable to anthropogenic driven changes in dung beetle communities than smaller seeds.

These research aims were realised through field-based experiments from which I sampled and identified approximately 2,650 dung beetles from 180 naturally formed communities, collected more than 17,000 morphological trait measurements and sieved approximately 11 tonnes of soil in search of 1800 seed mimics. Overall, this work demonstrates diversity in dung beetle communities is positively associated with the ecological processes they govern but that environmental context is instrumental in modulating biodiversity-ecosystem functioning relationships. I use the outcomes from this work to discuss the challenges in describing diversity-functioning relationships across trophic levels. Finally, I highlight that ecological processes are the product of complex species-specific interactions, dependent on the biotic and abiotic environment. Therefore, predicting the consequences of anthropogenic-driven species losses for the structure and functioning of natural systems is a major research challenge.

Key words: *Functional diversity indices; invertebrate traits; animal-mediated functioning; context dependency; soil; secondary seed dispersal; plant recruitment*

RESUMO

Comunidades biológicas estão sofrendo alterações em todo o globo como resultado das pressões antrópicas; as abundâncias de indivíduos em populações estão em declínio e espécies estão sendo extintas. A diversidade biológica e a complexidade trófica em vegetações campestres e as teias alimentares edáficas são positivamente relacionadas com a ciclagem de nutrientes no solo e na água, produtivi-

dade primária e decomposição. Uma vez que estes processos sustentam uma gama de benefícios e serviços para sociedade, mudanças condicionadas pelo homem na estrutura do ecossistema poderia impactar negativamente o bem-estar humano. Entretanto, a maioria da nossa compreensão do papel da biodiversidade no funcionamento do ecossistema é oriunda de estudos conduzidos em campos temperados. Consequentemente, nosso entendimento de como organismos de nível superior influenciam processos ecológicos nos diferentes ecossistemas é limitado. Essa tese tem por objetivo tratar destas lacunas, investigando como os atributos e a diversidade funcional de besouros rola-bosta influencia a dispersão secundária de sementes, bem como a emergência e sobrevivência de plântulas no nordeste da Amazônia brasileira.

Meu primeiro capítulo teve como objetivo entender a importância da variabilidade intraespecífica nos atributos de rola-bostas para a acurácia dos índices de diversidade funcional (DF) (Capítulo 2). Esse capítulo demonstra que as diferenças intraespecíficas nos atributos de rola-bostas são pequenas quando comparadas com as diferenças entre espécies. No entanto, a omissão da variabilidade intraespecífica pode resultar em grandes erros no cálculo dos índices de DF ao descrever comunidades pequenas e/ou pobres em espécies. Em segundo lugar, eu investiguei como a diversidade de rola-bostas influência a dispersão secundária de sementes, e o papel do contexto ambiental na regulação dessa relação. Neste capítulo eu mostro relações positivas entre a diversidade funcional de rola-bosta tanto com a probabilidade de enterrio de sementes, quanto com a dispersão de sementes através do perfil do solo, porém esses padrões são dependentes do tipo de solo. Finalmente, eu explorei a significância multitrófica dos achados do capítulo 2 através de testes para mostrar como as comunidades de rola-bostas afetam o enterrio de semente de diferentes tamanhos e a emergência e sobrevivência de plântulas (Capítulo 4). Os resultados deste capítulo demonstram como os besouros-rola-bostas podem influenciar a regeneração da vegetação, já que a diversidade de besouros afetou negativamente a probabilidade das sementes experimentais emergirem na superfície, e impactou positivamente a chance de sobrevivência das sementes que bro-

taram até o fim do período do experimento. Além disso, eu mostro que sementes grandes podem ser mais vulneráveis às alterações antropogênicas nas comunidades de besouros do que sementes pequenas.

Estes objetivos da pesquisa foram realizadas por meio de experimentos em campo, a partir do qual eu amostrei e identifiquei aproximadamente 2.650 besouros rola-bosta de 180 comunidades formadas naturalmente, coletei mais de 17.000 medidas de atributos morfológicos e peneirei cerca de 11 toneladas de solo, na busca por 1.800 sementes mímicas. No geral, este trabalho demonstra que a diversidade nas comunidades de rola-bosta está positivamente relacionada com os processos ecossistêmicos que eles regem, mas o contexto ambiental é fundamental na modulação da relação entre biodiversidade e funcionamento do ecossistema. Eu uso os resultados deste trabalho para discutir os desafios em se descrever as relações da diversidade com o funcionamento do ecossistema através dos níveis tróficos. Finalmente, eu destaco que os processos ecológicos são o produto de complexas interações espécie específicas, dependente do ambiente biótico e abiótico. Portanto, prever as consequências da perda de espécies conduzidas por ações antropogênicas para a estrutura e funcionamento dos sistemas naturais é um grande desafio para a pesquisa.

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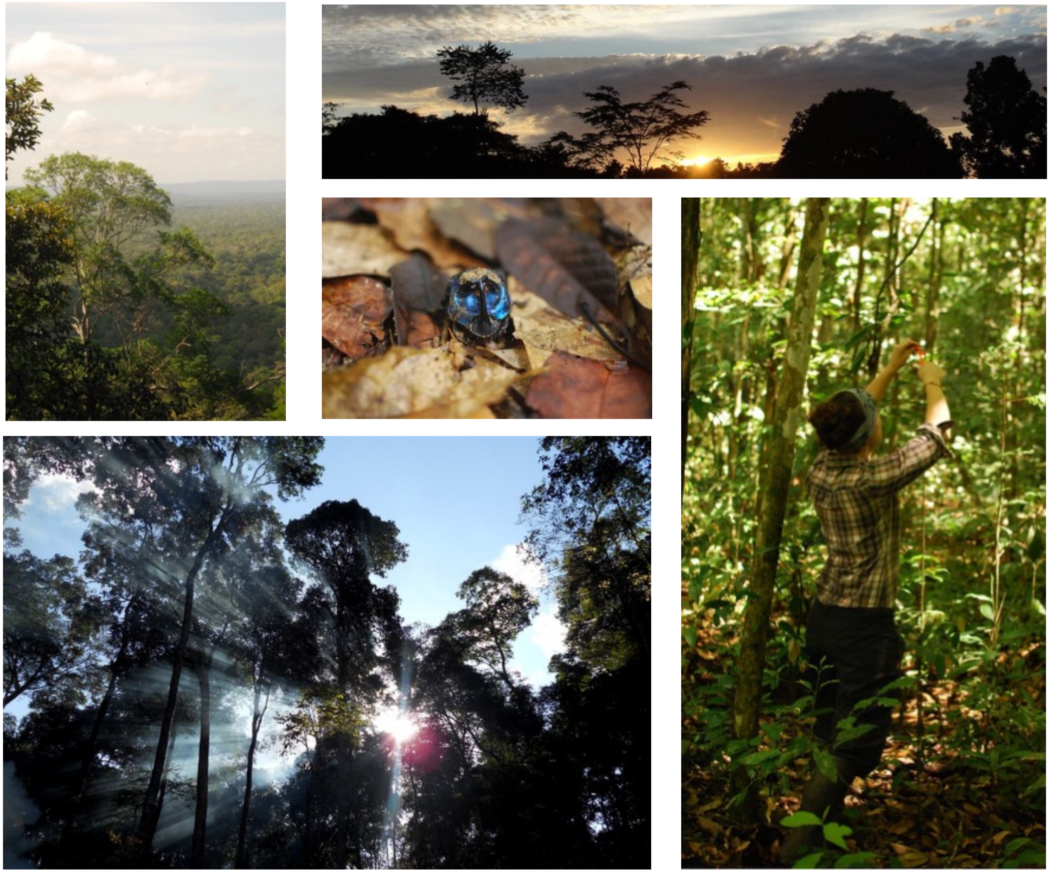
Chapter 1

GENERAL INTRODUCTION



1.1 BIODIVERSITY DECLINE AND IMPLICATIONS FOR ECOSYSTEM FUNCTIONING

Estimates suggest that the Earth is home to more than 450,000 species of plants and between 5 and 11 million species of animal, but that most organisms are currently unknown to science (Pimm *et al.* 2014). Whist we haven't described the majority of species, nor is there a consensus of how organisms there are on the planet, there is agreement that species are disappearing from ecosystems up to 1000 times faster than background extinction rates, largely because of human activities (Barnosky *et al.* 2011; De Vos *et al.* 2014). Of terrestrial animals, for example, currently 13% of birds, 41% of amphibians and 25% of mammals are threatened with extinction (IUCN 2014).

With every species extinction comes an extinction of interactions between that species and its biotic and abiotic environment (Valiente-Banuet *et al.* 2014). Humanity is reliant upon a number of goods and services provided by natural systems (MEA 2005) and these services are a product of ecological processes driven by species interactions. There is increasing evidence that biological diversity is positively associated with a number of ecosystem functions; in particular, species richness in producer systems has been demonstrated to positively influence primary productivity and the cycling of nutrients in the soil and water (Cardinale *et al.* 2011, 2012; Hooper *et al.* 2012), and in decomposer systems, the loss of trophic complexity in soil food webs have been shown to impair soil functions (Bardgett & van der Putten 2014). Since primary production, nutrient cycling and decomposition underpin a number of ecosystem functions and services (MEA 2005), declining species diversity and associated ecological interactions, has the potential to negatively impact human wellbeing (Cardinale *et al.* 2012).

The majority of our understanding about how biodiversity influences the functioning of ecosystems comes from the investigations into biomass assimilation in temperate grassland systems (Balvanera *et al.* 2006; de Bello *et al.* 2010; Cardinale *et al.* 2011). This means that our knowledge of the role of higher trophic level organisms in influencing a variety ecosystem processes in other habitats is limited. This bias towards biodiversity-ecosystem functioning (BEF) research in temperate grasslands should be addressed for a number of reasons. First, higher-level organisms are the agents of a number of important processes and services (e.g. Andresen & Levey 2004; Lavelle *et al.* 2006; Blouin *et al.* 2013), yet our understanding of BEF relationships governed by animal communities is based on a limited number of empirical investigations (Slade *et al.* 2007a; Dangles *et al.* 2011; Griffiths *et al.* 2015). Second, tropical forests are home to a large proportion of the Earth's biodiversity; the Amazon rainforest alone hosts more than 50,000 species of vascular plants (Hubbell *et al.* 2008) and c.15,000 species of tree (ter Steege *et al.* 2013). The role of diversity in governing ecosystem processes in these hyper-diverse systems could differ from less speciose environments. Therefore, inferring BEF relationships in tropical systems from work conducted in the temperate regions may not be meaningful. Third, while the erosion of species and populations is globally pervasive, tropical forests are experiencing higher current rates of losses of animal species than any other terrestrial ecosystem (Stork 2009; Dirzo *et al.* 2014). Humid tropical forests are globally important ecosystems; covering around 11 million km^2 of the Earth's surface (Mayaux *et al.* 2005), they sequester and store huge amounts of carbon (Saatchi *et al.* 2007; Berenguer *et al.* 2014) and influence regional and global weather systems (Gedney & Valdes 2000; Werth & Avissar 2002). However, we currently know very little about how declining biodiversity in these regions will impact the ecological processes that maintain tropical forests and thus their associated benefits to human societies.

Given the rate at which we are changing animal communities (Pimm *et al.* 2014; Dirzo *et al.* 2014) and the importance of tropical forests for global ecosystem services (e.g. Saatchi *et al.* 2007) it is critical that the gaps in our knowledge outlined above are addressed. This will not only further our theoretical understanding of ecological systems, but will also allow us to more accurately predict the consequences of human-driven declines in biodiversity. This thesis therefore uses a trait-based approach to investigate how functional diversity in dung beetle communities influences the secondary seed dispersal in a tropical rainforest.

1.2 FUNCTIONAL DIVERSITY

Functional traits are any behavioural, physiological, phenological or morphological characteristic measurable at the individual level (Violle *et al.* 2007). They form the fundamental building blocks of functional diversity (FD), which is described as the value, range and relative abundance of functional traits in a given ecosystem (Díaz & Cabido 2001). The collection of functional traits generally represents a greater investment in time and resources than simply counting the number of species in an assemblage. However, a functional approach can be justified because there is growing evidence that FD is a better predictor of ecosystem processes than taxonomic diversity (de Bello *et al.* 2010; Clark *et al.* 2012; Griffiths *et al.* 2015; Gagic *et al.* 2015). This is likely because not all species contribute equally to all functions i.e. the relationship between taxonomic and functional diversity is not predictable and linear. It will vary depending on the function or trait under consideration and is likely to be largely unique to the each system (Naeem & Wright 2003). One species may be functionally redundant when considering one process while singular (or distinct) when another is examined. These complexities are not captured with taxonomic indices. Consequently seeking a general relationship between taxonomic and functional diversity or using one as a proxy for the other may not yield useful results for BEF research (Díaz & Cabido 2001; Naeem & Wright 2003; Mayfield *et al.* 2010; Cadotte, Carscadden & Mirotchnick 2011). Therefore, a functional approach could increase the accuracy with which we link organisms to the ecological processes they govern and thus the accuracy with which we predict the consequences of species losses.

Since the beginnings of BEF research in the mid 1990s (e.g. Naeem *et al.* 1994; Tilman *et al.* 1997; Grime 1998) the mathematical sophistication and variety of indices available to calculate functional diversity has grown alongside interest in the concept. Reviews of the various indices, describing their evolution and providing critiques and recommendations for usage are provided by Petchey & Gaston (2006), Mouchet *et al.* (2010), Schleuter *et al.* (2010), and Casanoves *et al.* (2011). However, below I provide a brief summary of each multi-trait index used throughout this thesis. These have been categorised into richness, evenness, divergence, and dispersion as first suggested by Mason *et al.* (2005) and built upon by Villager, Mason, & Mouillot (2008) and Laliberté & Legendre (2010). Using an index from each category should complementarily describe the distribution of species and their abundances within functional space (Mouchet *et al.* 2010).

1.2.1 Functional richness - FRic

Functional richness describes the functional space occupied by a community (Fig. 1.1 (a)). This can be applied to a single trait approach by assessing the difference between the maximum and minimum trait values within a community (Mason *et al.* 2005). In order to do so for multiple traits (T), however, the minimum volume occupied by all species in the community in the T dimensional trait space is calculated (Villéger *et al.* 2008). This volume is known as the convex hull and was first proposed by Cornwell, Schwilk, & Ackerly (2006). It provides a multidimensional measure of the range in trait values in a community; is heavily influenced by the extreme values; it does not take into consideration species abundances; is not independent from species richness; and requires there to be more species (points) than traits (dimensions).

1.2.2 Functional evenness - FEve

Functional evenness is regularly of spacing between species and evenness in distribution of abundance in functional space (Fig. 1.1 (b)). The importance of incorporating a measure of species abundances in evenness measures of functional diversity was first highlighted, and the means to do so presented, by Mouillot, Mason, Dumay, & Wilson (2005). However, their Functional Regularity Index (FRO) is constrained in its capabilities as it allows for only the assessment of a single trait within a community. An alternative, multi-trait approach was suggested by Villager, Mason, & Mouillot (2008) in which the distribution of species in T dimensional trait space is converted onto a single axis using a minimum spanning tree (MST). To do this, the minimum distance between all the points (species) in the functional space are linked and then summed. Abundances are incorporated into the computation by dividing each branch by the sum of the abundances of the species that it connects (EW); the EW value for each branch is then divided by the sum of all other EW values in the MST, giving the PEW. A final calculation using the sum of the PEW values provides the functional evenness index (for full mathematical explanation see Villager, Mason, & Mouillot 2008). This measure decreases when distances between points are less regular or when abundance is distributed less evenly between species.

1.2.3. Functional divergence – FDiv

Functional divergence quantifies the distribution of abundances of functional traits within functional trait space (Fig. 1.1 (c)). As with the abovementioned indices there exists a single trait equivalent (FD_{var}), in this case, it was put forward by Mason *et al.* (2005). FD_{var} is a descriptor of how the abundance-weighted traits are spread within the range of traits occupied by the community. If the most abundant species are close the centre of the range, FD_{var} will be low, while if the dominant species display traits that are at extreme of the range it will be high. This concept is applied to a multi-trait model through assessing how the abundances of traits are distributed within a T dimensional functional volume (similar to the convex hull): Functional Divergence (FDiv) (Villéger *et al.* 2008). It is calculated based on the mean distance of all the species from centre of the functional trait space, and the sum of the abundance-weighted distances of all the points from the mean. Like FD_{var}, FDiv decreases if the most abundant species are close to the centre (relative to rare ones) and increases when dominant species are distant from the centre. All the indices developed by Villager, Mason, & Mouillot (2008) are independent from one another.

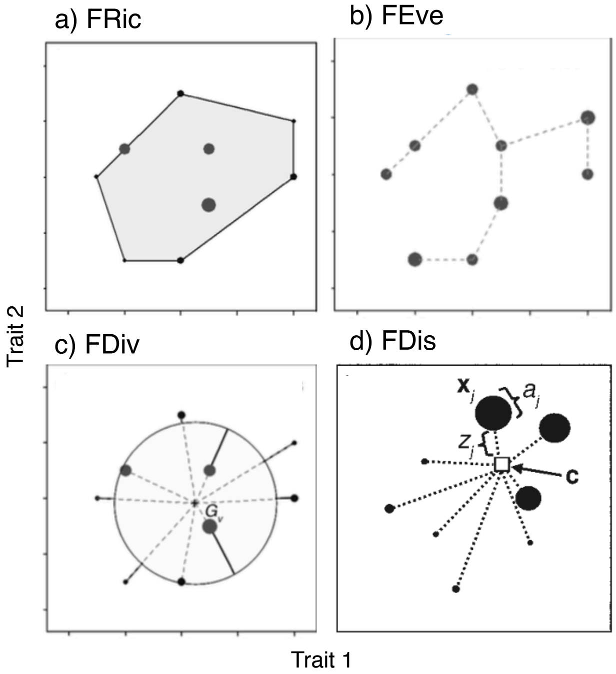


Figure 1.1 | Multi-trait functional diversity indices used in throughout this thesis: functional richness (a), functional evenness (b), functional divergence (c) and functional dispersion (d). Indices and diagrams (a) – (c) were developed by and taken from (Villéger *et al.* 2008) and (d) by (Laliberté & Legendre 2010).

1.2.4 Functional dispersion – FDis

Functional Dispersion calculates the mean distance of all species from the abundance-weighted centroid (Fig. 1.1 (d)). A criticism of FDiv presented by Laliberté & Legendre (2010) is that it takes into account the distribution of the species within the T dimensional trait space without considering the actual volume of the convex hull (this is inevitable because a goal of Villager, Mason, & Mouillot [2008] was to ensure independence between their indices). It is therefore not a measure of dispersion and as such cannot discriminate between the distributions of species in a small trait space compared to similarly distributed species in a larger trait space. As an alternative the authors present functional dispersion (FDis) that is conceptually similar to Rao's quadratic entropy (Rao's Q) (Rao 1982) another commonly used multidimensional measure of functional diversity (e.g. Ricotta 2005; Ricotta

& Moretti 2011). Rather than being calculated using the divergence of each point from the mean distance to the centroid, FDis is a product of the abundance-weighted mean distance of each point to the abundance-weighted centroid (i.e. the centre of the trait space is pulled towards the most abundant species and weight is given to the distances between this point based on abundances of species). Because both FDis and Rao's Q both calculate dispersion traits within a community weighted by their abundances, the two measures are strongly correlated. A correlation was also found by Laliberté & Legendre (2010) between FDis and FRic, FDis and FEve and FDis and FDiv.

1.3 FUNCTIONAL TRAITS

A common approach in functional diversity/ecosystem functioning studies is to categorise organisms into functional groups or guilds based upon shared behavioural, physiological, taxonomic, or morphological traits, and using the number of different groupings present (functional group richness) as an approximation of the functional diversity of the community (e.g. Díaz & Cabido 2001; Tilman *et al.* 2001; Vulinec, Lambert, & Mellow 2006; Slade *et al.* 2007; Barragán *et al.* 2011). However, this assumes that those traits most important in predicting ecosystem functioning are discrete rather than continuous (Naeem & Wright 2003), can result in loss of information (Villéger *et al.* 2008), and increases the level of subjectivity within the investigation (through the experimenter's classification of organisms into groups based on arbitrary thresholds) (Petchey, Hector & Gaston 2004). These shortfalls can be avoided through directly measuring continuous traits of the study organisms rather than using discrete functional groupings (e.g. Vandewalle *et al.* 2010; Hidasi-Neto, Barlow, & Cianciaruso 2012; Spasojevic & Suding 2012). The use of continuous traits for the calculation of functional diversity indices is growing in popularity, however, this approach remains comparatively uncommon in non-producer focused investigations (de Bello *et al.* 2010).

Trait selection is of critical importance for the outcome of any functional diversity study utilising any of the above mentioned FD indices because the metrics are a product of the numbers used to generate them; the traits. The greater the number of traits included, the greater the ability to differentiate between species (each species has an increased likelihood of being functionally singular), whereas the smaller the number included, the greater the incidence of functional redundancy (Petchey & Gaston 2006; Cadotte *et al.* 2011). Furthermore, inclusion of functionally uninformative traits or those that are positively correlated will increase the artificial congruence between taxonomic and functional diversity, while negative correlation between traits will lead to inflation of functional diversity values (Naeem & Wright 2003; Cadotte *et al.* 2011). It is important, therefore, that the investigator avoids, where possible, correlation between the traits (Cadotte *et al.* 2011) and considers only those that are linked to the function of interest. As a result, an understanding of how the community under scrutiny interacts with its environment and clear definition of the focal ecological process is necessary in order to select only appropriate traits (Petchey & Gaston 2006).

1.4 Study taxa – dung beetles

With just over 5,900 recognised species, belonging to 236 genera and inhabiting every continent except Antarctica, dung beetles (Coleoptera; Scarabaeidae) are a large, diverse and widely distributed group (ScarabNet 2008). The Scarabaeidae family is divided into two subfamilies, namely the Coprinae and Scarabaeinae, which are further divided into six tribes in each. Coprinae contains the tribes of: Coprini, Dichotomiini, Oniticellini, Onitini, Onthophagini and Phanaeini; Scarabaeinae contains: Canthonini, Eucraniini, Eurysternaini, Gymnopleurini, Scarabaeini and Sisyphini. True dung beetles appear in the fossil record around 40 million years BP, but the evolution of the group is thought to have coincided with the expansion of large mammals at the end of the Mesozoic period around 66 million BP (Hanski & Cambefort 1991).

The tropics are home to the highest generic and species rich assemblages of dung beetles where they are most abundant in savannah and forest environments (Davis & Scholtz, 2001; Hanski & Cambefort, 1991). Tropical forest dung beetle communities are known to be sensitive to land use change, which has been shown to lead to the differential loss of large bodied species (Larsen, Williams & Kremen 2005; Larsen, Lopera & Forsyth 2008) and result in reductions in abundances as well as taxonomic and functional diversity (Halffter & Arellano 2002; Larsen *et al.* 2005, 2008; Gardner *et al.* 2008b; Barragán *et al.* 2011). Furthermore, these changes in community structure have been demonstrated to impact upon beetle-mediated functioning as measured by dung burial rates and seed dispersal (Larsen *et al.* 2005; Santos-Heredia, Andresen & Stevenson 2011; Slade, Mann & Lewis 2011; Braga *et al.* 2013; Nichols *et al.* 2013b). Because dung beetles are the agents of a number of ecological functions (reviwed in Nichols *et al.* 2008), are sensitive to disturbance, are a cost effective indicator group of biodiversity change and can be manipulated in field investigations (Barlow *et al.* 2007a; Slade *et al.* 2007a; Gardner *et al.* 2008b), they are an ideal focal taxa for elucidating the impact of human pressures on biodiversity and ecosystem functioning.

1.5.1 Dung beetle meditated ecosystem processes

Dung beetles use mammalian dung resources for feeding and nesting purposes and most species fit into one of three broad nesting strategies: tunneler species (paracoprids) bury brood balls in vertical (or near vertical chambers) below to dung deposition site; roller species (telecoprids) create brood balls that are moved horizontally away from the dung before burial beneath the soil surface; dweller species (endocoprid) lay eggs within the dung mass, without relocating material under the soil (Halffter *et al.* 1982). Through the movement and burial of dung in these ways, dung beetles are the agents of a number of ecological processes, which are outlined below

Secondary seed dispersal

Dung beetles often relocate seeds from their deposition site within mammalian dung to beneath the soil surface (Andresen & Feer 2005) and in doing so can benefit relocated seeds in the following ways: (i) placement in a more suitable microclimate for emergence and establishment (Shepherd & Chapman 1998; Andresen

& Levey 2004); (ii) escape from predation and pathogen mediated mortality (Estrada & Coates-Estrada 1991; Shepherd & Chapman 1998; Feer 1999); and (iii) reduction in clumping and associated implications for seedling competition and density dependant mortality (Andresen & Feer 2005; Lawson, Mann & Lewis 2012). By influencing seed and seedling survival in these ways, dung beetle activity could have far reaching impacts upon forest regeneration and community composition.

However, despite the demonstrated advantages of secondary seed dispersal by beetles, complex species-specific and size (of both beetles and seeds) dependent processes determine if a beetle-seed interaction will result in net benefits to seed fitness. The size and composition of a seed's reserves reflects species-specific life-history strategies through differing mechanisms for germination, establishment and seedling growth (Vazquez-Yanes & Orozco-Segovia 1993; Poorter & Rose 2005; Gilbert *et al.* 2006; Soriano *et al.* 2011). Large seed reserves allow a longer period before seedling autotrophy is necessary (Kitajima 2002; Poorter & Rose 2005) whilst simultaneously increasing attractiveness to predators (Shepherd and Chapman 1998). Burial by dung beetles is one mechanism for predator escape (e.g. Estrada and Coates-Estrada 1991, Feer 1999), but can also lead to mortality through placement of individuals in unsuitable locations for germination and establishment. Estrada and Coates-Estrada (1991), for example, demonstrated that seeds avoided predation through burial by dung beetles, but Shepherd and Chapman (1998) showed that seeds buried too deep are unable to germinate and that only large seeds were able to survive when buried at depths greater than 10cm. This suggests that for each species there exists an optimal and unique microsite at which the probability of predation is significantly decreased whilst germination and establishment is maximised.

Large beetles bury large seeds whereas small beetles do not, and generally, they bury all seeds deeper (Feer 1999). These size mediated beetle-seed interactions suggest that in the absence large beetles, large seeds will not be buried/won't be buried sufficiently deeply to avoid predator detection, and if is the case, they could suffer comparatively higher rates of seed predation (Estrada & Coates-Estrada 1991; Feer 1999). Large seeds that are not buried may therefore have lower survival probabilities than smaller unburied seeds. As large bodied dung beetle species are disproportionately sensitive to anthropogenic disturbance (Larsen *et al.* 2005, 2008; Gardner *et al.* 2008c), this could lead to a differential reduction in survival of large seeded species, creating a filter effect (Grime 1998) on the seedling communities.

Soil properties

Given that nutrient availability is commonly a limiting factor for plant growth (Vitousek 2004), dung beetles, through the re-location of nutrient rich animal excreta, can instigate edaphic changes with important implications for vegetation. Dung beetle activity has been shown to: 1) increase the rate at which inorganic nitrogen, potassium and plant available phosphorous is lost from dung (Yamada *et al.* 2007); 2) increase plant available nitrogen in residual surface dung and the soil beneath dung (Yokoyama *et al.* 1991a; Yamada *et al.* 2007); 3) and reduce the loss of nitrogen as ammonia (NH_3) from dung to the atmosphere (volatilization) (Yokoyama, Kai & Tsuchiyama 1991b). These changes are postulated to be a consequence of the aeration of dung pats and brood balls creating more favourable conditions for the microorganisms responsible for nitrogen mineralization and nitrification, as well as the incorporation of dung into the soil (thus reducing the area exposed to the atmosphere). In addition to these nutrient cycling effects, Bang *et al.* (2005) provide the only empirical evidence that bioturbation as a result of tunnelling by tunnelling species alter soil physical properties and, in doing so significantly increased surface soil permeability on pasture-land.

Investigations linking these observed edaphic changes to a net positive response in plant growth are provided by Bang *et al.* (2005) and Yamada *et al.* (2007). These investigations demonstrate that dung beetle activity can result in both an increase in above ground crop yield and available digestible nutrients within plant tissues (if beetles were present before the sowing of seeds in a glasshouse experiment).

The only field-based study demonstrating the positive influence of naturally assembled beetle communities on non-crop plants reveals a similar pattern as the aforementioned works, with a significant plant growth enhancement effect in a heathland system (Borghesio, Luzzatto & Palestrini 1999) (although soil properties were not investigated so a mechanistic link cannot be made). It is not possible from these studies, however, to disentangle the mechanisms behind the observed changes in plant growth response, i.e. the relative contribution of increased aeration and permeability (physical changes) compared to altered nutrient availability.

There is, to date, no *in situ* manipulative experimental studies examining the influence of beetles on edaphic qualities in tropical forests. A descriptive investigation of howler monkey latrines (Dos Santos Neves *et al.* 2010) does, however reveal how dung (and inferred beetle activity) can impact upon soil properties beneath large quantities of mammalian dung; carbon and nitrogen was distributed evenly through the top 6cm of soil in the latrines, whereas nutrients decreased rapidly past the first 2cm in the controls.

While these studies do highlight the ways in which beetles may influence soil properties and thus vegetation, this is unlikely to be the most significant role carried out by dung beetles in tropical rainforests because: 1) investigations into the effect of dung and beetles on the nutrient content of tropical soils have exclusively been carried out in latrine areas (Pouvelle, Feer & Ponge 2008; Dos Santos Neves *et al.* 2010) where nutrients are likely to be of unusually high concentrations. This not typical of the majority of land area in a rainforest system where dung pats are usually small, patchily distributed resources (e.g. Peck 1984; Horgan 2005) and thus the impact of dung beetles is likely to be significantly less pronounced than reported in these areas; 2) in contrast, leaf litter is ubiquitous on the floor of tropical rainforests, commonly exceeding 12mg/ha (see Vitousek 1984). The decomposition of which is therefore likely to be the primary route for the transfer of nutrients above to below ground rather than the redistribution of faeces.

Parasite suppression

Another important ecosystem processed performed by dung beetles, that is not addressed in this thesis, is parasite surpression. Nichols & Gómez (2014) provide a recent review into the possible mechanisms by which dung beetles influence parasite transmission. These include competitive interctions over resouces (Hughes *et al.* 1975), damage to spores, larvae and eggs (Miller 1961; Bishop *et al.* 2005), and alteration of conditions within dung pats and brood balls (Ridsdill-Smith & Hayles 1987). Dung beetle activity has therefore been shown to reduce abundance of enteric parasites of livestock (e.g. Fincher 1973, 1975; Mathison & Ditrich 1999) and, in experimental manipulations, result in elevated dung-fly mortality (Bornemissza 1970; Bishop *et al.* 2005). Conversely, dung beetles as hosts of livestock parasites has also been suggested (Fincher, Stewart & Davis 1969; Fincher 1982), although this relationship is less well established (see Nichols *et al.* 2008). Evidence of these associations in tropical forest ecosystems are, to my knowledge, lacking in the literature.

1.5 STUDY SITE

The Amazon rainforest spans nine South American Countries and covers more than 6.5 million km^2. It is the largest expanse of tropical forest on Earth, home to around 25% of terrestrial species (Dirzo & Raven 2003), stores 86 Pg of carbon (Saatchi *et al.* 2007) and influences weather patterns across the Americas (Werth & Avissar 2002; Malhi *et al.* 2008). The biome therefore plays a vital role in the provision of a number of regional and global services.

This project was carried within the 17,000km^2 landholding of Jari Florestal, located in the state of Pará in the north-eastern Brazilian Amazon (0°53S, 52°36W). The region contains roughly 530km^2 of *Eucalyptus urophylla* plantations and more than 500km^2 of regenerating native vegetation in areas that were cut and subsequently abandoned. These plantations and secondary forests are embedded within a matrix of undisturbed *terra firme* primary forest (Barlow *et al.* 2007b). Within this landscape, experiments were established in three primary forest sites.

1.6 RESEARCH OBJECTIVES

The overarching aim of this thesis is to better understand how diversity in higher trophic level organisms influences the ecological processes they govern. This was addressed in the following three topics:

1.7.1 Chapter 2 – Intraspecific variability in dung beetle traits and implications for functional diversity indices

The use of mean trait values in functional indices assumes that traits are robust, in that greater variability exists between than within species. While the assertion of robust traits has been explored in plants, there exists little information on the source and extent of variability in the functional traits of higher trophic level organisms. Consequently researchers adopting a trait-based approach to investigate animal mediated ecosystem functioning must make methodologically important decisions regarding the level of precision to employ without any empirical guidelines. Therefore, the first research objective was to assess the source and extent of variation in two functionally relevant dung beetle traits.

Chapter 2 research questions: (1) What is the relative contribution of between vs. within species differences in trait values? (2) How does sampling site influence intraspecific trait differences? (3) What sample size is needed to provide representative species mean trait values? (4) What impact does omission of intraspecific trait information have on the calculation of functional diversity indices from naturally assembled communities?

1.7.2 Chapter 3 – Functional diversity, dung beetle mediated secondary seed dispersal and environmental context

Biodiversity-ecosystem functioning research is dominated by studies carried out in temperate grassland systems under homogenous environmental conditions. We therefore have limited understanding of how diversity in higher trophic level organisms influence ecosystem processes in other habitats or the role of environmental conditions in shaping BEF relationships. The second research aim was therefore to explore dung beetle mediated BEF relationships in a tropical forest under real, heterogeneous field conditions.

Chapter 3 research questions: (1) How does dung beetle taxonomic and functional diversity influence the probability of seed burial and the dispersion of seeds throughout the soil profile? (2) What impact does environmental context have on the strength and/or direction of observed BEF relationships?

1.7.3 Chapter 4 – The influence of tropical forest dung beetle communities on the emergence and survival of seedlings

We have a very limited understanding about how species extinctions or populations declines in one trophic level could impact on the ecological functions governed by organisms in other trophic levels. Consequently our knowledge of the possible cascading effects of the modification of biological communities is limited. The final research aim was to address this by investigating how diversity in naturally formed dung beetle communities could impact upon the germination and survival of seedlings in a field experiment.

Chapter 4 research questions: (1) Are seeds of different size classes differentially vulnerable to the cascading effects of defaunation? (2) Does dung beetle diversity impact on vegetation regeneration through influencing seedling emergence and survival? (3) What is the species-specific optimal burial depth of experimental seeds to allow emergence from the soil?

1.7. THESIS STRUCTURE

Each of the data chapters of this thesis have been written for publication: Chapter 2 has been submitted to *PLOS ONE*, Chapter 3 is *in press* in *Ecology,* and I intend to submit Chapter 4 to *Journal of Animal Ecology* in due course. Therefore, this thesis is made up of stand-alone chapters linked by a common theme of dung beetle functional traits and diversity and their relevance for the secondary seed dispersal in tropical forests. Chapter 5 summarises the key findings resulting from each experimental chapter and highlights future research needs.

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Chapter 2

INTRASPECIFIC VARIABILITY IN DUNG BEETLE TRAITS AND IMPLICATIONS FOR FUNCTIONAL DIVERSITY INDICES

Intraspecific variability in dung beetle traits and implications for functional diversity indices

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Hannah M Griffiths*[1,2], Julio Louzada[1,2], Richard D Bardgett[3], Jos Barlow[1,4]

[1]Lancaster Environment Centre, Lancaster University, Bailrigg, Lancaster, LA1 4YQ, UK.
[2]Departamento de Biologia, Universidade Federal de Lavras, Lavras, Minas Gerais, 37200-000.
[3]Faculty of Life Sciences, Michael Smith Building, The University of Manchester, Oxford Road, Manchester, M13 9PT, UK.
[4] Museu Paraense Emilio Goeldi, Av. Magalhães Barata, 376, Belém-Pará-Brazil
** Corresponding author: han.m.griffiths@gmail.com*

2.1 ABSTRACT

Functional diversity indices are used to facilitate a mechanistic understanding of many theoretical and applied questions in current ecological research. The use of mean trait values in indices assumes that traits are robust, in that greater variability exists between than within species. While the assertion of robust traits has been explored in plants, there exists little information on the source and extent of variability in the functional traits of higher trophic level organisms. Here we investigated variability in two functionally relevant dung beetle traits, biomass and back leg length, to address the following questions: (i) what is the contribution of between vs. within species differences in trait values; (ii) how does sampling site influence intraspecific trait differences; (iii) what sample size is needed to provide representative species mean trait values; and (iv) what impact does omission of intraspecific trait information have on the calculation of functional diversity indices from naturally assembled communities? The error associated with calculating the FD of small and/or species poor communities, without inclusion of intraspecific variability was unpredictable, varying between 4 and 55%, and this error increased with a decrease in community size. However, at a larger scale, population level interspecific trait differences explained the majority of variability. Our findings suggest that the importance of inclusion of intraspecific variability for accuracy in FD indices increases as community size decreases. This is the case even when using traits that display very low within species differences at the population level. Consequently, complete sampling to capture variance information may be necessary when investigating the FD of small, naturally formed communities, such as in mesocosm or enclosure experiments. In these cases, the exclusion of intraspecific variability could ultimately lead to an underestimation of the role of biodiversity for ecosystem functioning.

Key words: *Functional diversity indices; invertebrate traits; mesocosm experiments; phenotypic plasticity; robust traits*

2.1 INTRODUCTION

Understanding how biological diversity influences ecosystem processes is crucial if we are to predict and thus mitigate the consequences of anthropogenic driven species losses (Barnosky *et al.* 2011). Functional diversity (FD) quantifies the value, range, and relative abundance of functional traits in a given ecosystem (Díaz & Cabido 2001) and has been used to link biodiversity with a suite of ecosystem functions and services (Hooper *et al.* 2005, 2012; Balvanera *et al.* 2006; Cardinale *et al.* 2011; Clark *et al.* 2012; Griffiths *et al.* 2015). Furthermore, FD has improved our understanding of species interactions and community assembly rules (McGill *et al.* 2006), as well as species responses to disturbance (Mouillot *et al.* 2013). Additionally, it has been proposed that FD and its links to ecosystem processes could be of value for defining a planetary threshold for biodiversity loss (Rockstrom *et al.* 2009; Mace *et al.* 2014). Functional diversity, therefore, has the capacity to facilitate a mechanistic understanding of the impact anthropogenic disturbances on biological communities and the processes they govern (McGill *et al.* 2006), and could ultimately inform conservation management and policymaking decisions.

Functional traits (physiological, morphological or phenological characteristics measurable at the individual level that impact upon fitness; (Violle *et al.* 2007) are the building blocks of FD and are generally incorporated into indices through the use of species mean trait values. This assumes that traits are 'robust', i.e. that greater variability exists between than within species (Garnier *et al.* 2001; McGill *et al.* 2006; Albert *et al.* 2011; Violle *et al.* 2012). There is, however, growing evidence that this is not necessarily the case (Lecerf & Chauvet 2008; Hulshof & Swenson 2010; Albert *et al.* 2010b; Messier, McGill & Lechowicz 2010), especially when considering the traits of individuals originating from spatially discrete locations (Lecerf & Chauvet 2008). Furthermore, intraspecific trait variability is increasingly recognised as an important component of diversity driving ecosystem functioning (Lecerf & Chauvet 2008) as well as functional responses to disturbances (Jung *et al.* 2014), and recent work has demonstrated that the failure to consider intraspecific trait variability in FD investigations has the potential to influence findings (Albert *et al.* 2010a, 2012; de Bello *et al.* 2011). There is, therefore, a clear need to better understand the magnitude and source of variability in the traits of functionally relevant organisms (Albert *et al.* 2010b, 2012; de Bello *et al.* 2011).

It is often not feasible, or necessary, to gather information on every trait, from every individual within a given community (Baraloto *et al.* 2010). Consequently, quantifying intraspecific trait variability (Albert *et al.* 2010b; de Bello *et al.* 2011) and understanding when and how it should be measured (Albert *et al.* 2011) has received reasonable attention in recent years. Concurrently, investigations have focussed on methods of incorporating within species variability into FD indices (Cianciaruso *et al.* 2009) and the impact of doing so for interpretation of results (Albert *et al.* 2012). However, to our knowledge, this work has been exclusively carried out on plant traits (Hulshof & Swenson 2010; Messier *et al.* 2010; Albert *et al.* 2011, 2012), likely because the use of functional traits as a tool to investigate diversity-functioning relationships in non-producer systems is comparatively uncommon (Slade *et al.* 2007b; Dangles, Carpio & Woodward 2012; Griffiths *et al.* 2015). Researchers adopting a trait-based approach using higher trophic level organisms must, therefore, make methodologically important decisions regarding the level of precision to employ without any empirical guidelines.

Here we investigated variability in invertebrate functional traits. Using data from the Chapter 3 biodiversity-function experiment (Griffiths *et al.* 2015), where morphological measurements were collected from dung beetle individuals (n = 1962), we quantified the source and extent of variation in two functionally relevant traits: biomass and back leg length. In doing so, we ask the following questions: (1) what is the relative contribution of between vs. within species differences in trait values; (2) how does sampling site influence intraspecific trait differences; (3) what sample size is needed to provide representative species mean trait values; and (4) what impact does omission of intraspecific trait information have on the calculation of functional diversity indices from naturally assembled communities?

2.3 MATERIALS AND METHODS

2.3.1 Field sites and sampling strategy

Sampling was carried out during July and August 2012 in the 17 000km^2 landholding of Jari Florestal, located in the State of Pará in the north-eastern Brazilian Amazon (0°53S, 52°36W). Dung beetles were sampled from three *terra firme* primary forests as part of a biodiversity-ecosystem functioning experiment (Griffiths *et al.* 2015); full permission was granted by the private land-owner, Jari Florestal, to carry out work at these sites, sampling did not involve any endangered species and permission to collect zoological material was granted to JL by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA). All sites were within 100km of one another, classified as dense lowland tropical rain forest, were subject to the same regional climatic conditions and contained distinct dung beetle communities (Fig. S2.1 for multidimensional scaling ordination plots of beetle communities).

Dung beetle communities were collected from within ninety 50 cm x 50 cm experimental plots baited with a 100g mixture of 50:50 human and pig dung, protected from the rain by a plastic cover. After baiting the plots were left open for colonisation by beetles for either 12 or 24 hours. Following colonisation, plots were closed to ensure beetles could not escape. Un-baited pitfall traps (13.5cm width, 9cm depth), buried flush with the ground surface and filled with salt and water were located inside each of the plots; these were opened when the plots were closed to capture the beetle communities following emergence from the soil. Internal pitfall traps were left in place for seven days in site 1 and site 3 but because logging operations in site 2 restricted access to the area, beetles were removed after two weeks at this site. More detailed sampling design and rationale are presented in Chapter 3.

2.3.2 Trait selection and measurement

Beetles were identified to species level using a reference collection held at the *Universidade Federal de Lavras* (UFLA) in Brazil and region-specific classification keys developed by F. Z. Vaz-de-Mello and T. A. Gardner (unpublished). Using traits to inform biodiversity-ecosystem functioning investigations involves defining the function of interest, identifying predictive traits for that function, and gathering representative values for those traits (Petchey, Gorman & Flynn 2006). The ecological functions provided by dung beetles result from the burial of mammalian dung (Nichols *et al.* 2008). We therefore measured morphological traits relevant to excavation and burial (Inward *et al.* 2011) from every individual (n = 1962); namely pronotum volume (pronotum area multiplied by pronotum height), front leg area, back: front leg lengths (Fig. 3.2 [Chapter 3]; measured using a Leica M250 microscope and Life Measurement software); and dry biomass (determined using a Shimatzu AY220 balance with precision to 0.0001g). Biomass, back: front leg lengths, biomass adjusted pronotum volume and biomass adjusted front leg area where used in Chapter 3 to successfully predict seed burial and dispersion throughout the soil profile. We therefore selected these traits for use in this study. However, as the non-biomass-adjusted traits are co-linear (Fig. S2.2) we present results from the two least correlated traits in the main text: biomass and back leg length (Pearson's $\rho = 0.89$). All other results are detailed in Appendix S2.1 (Fig. S2.3 – Fig. S2.6)

Sixty-one species and morphospecies were recovered during sampling, the abundance of each varied from 1 – 239 individuals. However, in order to assess the magnitude and source of variability of measured traits, we selected only the species from the complete dataset for which we collected 50 or more individuals (n = 13). To investigate the impact of sampling site on trait values we used only species from which we collected at least 15 individuals from each location (n = 2; Table S2.1 for species abundances at each site).

2.3.3 Statistical analyses

All analyses were carried out in R version 3.0.2 (R Core Team 2013). The first aim of this investigation was to quantify the extent and source of variability (intra vs. interspecific) in dung beetle functional traits. To do this, we performed variance component analyses following methods presented by Messier, McGill & Lechowicz (2010). Each trait was $\log_{10}$ transformed to normalise the data and general linear mixed models (lme) from the ‘nlme’ package (Pinheiro *et al.* 2013) were fitted to the variance within and between species. Individual was nested within species and included as random factors in the models. A variance component analysis (varcomp) from the ‘varComp’ package (Qu 2013) was performed on each model. To explore the impact of sampling location on intraspecific differences in trait values we performed analysis of variance models (using the aov function) for each trait and each species including site as a predictor. Where site significantly affected trait values, Tukey’s HSD tests were performed to assess the source of the differences.

Our third objective was to determine the number of individuals from which measurements should be taken in order to provide a representative value for each dung beetle functional trait. This was achieved through resampling (with replacement) the complete data set to create sub-sets containing 3 to 100 individuals for each species (n = 1000 per sub-set). This was possible up to a sub-set size of 50 individuals for every species, but where the target sub-set size was larger than the number of individuals collected for a particular species, re-sampling was stopped. From each resampled dataset the standard error (SE) of each trait was calculated and from these we created a mean SE for each sub-set size. These mean standard error values were compared to the overall mean trait value calculated using every individual in the dataset for each trait and each species. The number of individuals needed to create a mean standard error within 5% of the overall sample mean was considered the minimum necessary to provide a representative trait value. To assess if sample size can be reduced when considering a single population, this process was repeated but using only individuals collected from one of the experimental sites. Site was selected for each species based on where they occurred in the greatest abundance (Table S2.1 for species abundances at each site). The mean standard errors generated during resampling were compared to site-specific species mean trait values.

The final goal of this study was to better understand how omission of intraspecific trait variability influences functional diversity indices when assessing naturally formed communities. Our focal traits are used in Chapter 3 to calculate multidimensional functional diversity indices. However, in a plant-based investigation Albert *et al.* (2010a) demonstrated that functional traits are likely to display unequal variance. Combining multiple traits together to calculate multidimensional indices could, therefore, mask the differences in traits and species that we are seeking to better understand (de Bello *et al.* 2011). Consequently, for the purposes of this study we calculated functional diversity using two single trait indices: community weighted mean (CWM) and functional richness (FRic). Community weighted mean is the mean value of a trait within a community, weighted by the relative abundances of the species carrying that trait (Garnier *et al.* 2004; Violle *et al.* 2007). FRic describes the volume of functional trait space occupied by a community; when using single traits it is the range in values (Mason *et al.* 2005).

These two indices were calculated twice for each community, once using individual trait values from each beetle captured within experimental plots (inclusion of intraspecific trait variability) and subsequently using median species trait values (omission of intraspecific trait variability). We recognise that mean trait values are most commonly used in the calculation of FD indices (e.g. Albert *et al.* 2012) but because the median values of these traits were used Chapter 3, for consistency, here we use median values. Furthermore, the mean and median values are highly co-linear, we are therefore confident that use of median rather than mean traits does not impact upon conclusions drawn. Following methods presented in Lavorel *et al.* (2007), when calculating CWM traits with the inclusion of intraspecific variability, we calculated a mean for each community using values measured from each individual. Linear regressions were performed to assess the relationships and to estimate the possible bias in calculating FD indices without intraspecific trait information. R^2 values from these models provide the percentage of information excluded from the FD indices when intraspecific trait information is omitted (Albert *et al.* 2012).

2.4 RESULTS

2.4.1 Extent of trait variability

The complete measurement of biomass, pronotum volume, front leg area and back and front leg lengths from all 1962 dung beetle individuals amounted to around 480 hours of researcher time. We found large interspecific variability across both the biomass and back leg lengths of the thirteen focal species studied (Fig. 2.1). Species median values ranged from 0.006g to 0.847g for biomass (FRic = 0.841g; Fig. 2.1(a)) and from 2.34mm to 15.92mm for back leg length (FRic = 13.58mm; Fig. 2.1(b)). When individual, rather than median trait values were considered, variability increased by 73.2% for biomass, ranging from 0.003g to 1.460g (FRic = 1.457) and by 18.9% for back leg length, ranging from 1.68mm to 17.83mm (Fric = 16.15). This greater influence of intraspecific variability on the range in biomass values is reflected in differences in the coefficients of variation (CV) for both traits. The mean CV of all species for biomass was consistently larger than that of back leg length; 0.33 compared to 0.1, respectively (Fig. 2.1).

2.4.2 Source of trait variability

The partitioning of variance in the two traits revealed interspecific variance accounted for the vast majority of variability compared to intraspecific differences. Between species differences were responsible for 94% and 96% of variability for biomass and back leg length respectively, whereas intraspecific variation accounted for just 5% and 3% for biomass and back leg length.

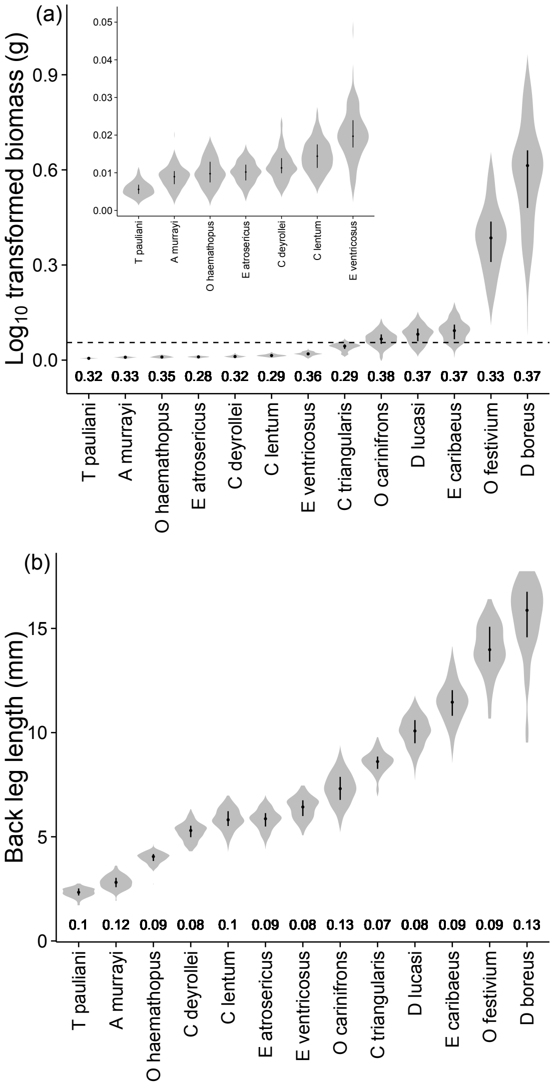

FIGURE 2.1 | Extent of intraspecific variability in dung beetle biomass (a) and back leg length (b). Violin plots display (i) the density of data estimated by kernel method (grey areas); (ii) the median value (black horizontal lines in the centre of violins); and (iii) the interquartile range (between the top and bottom of the vertical black lines). Results are presented by species, ordered by their median trait values and the coefficients of variation (calculated as the SD/mean) are given for each species below the violin. The horizontal dashed line on panel (a) shows the median biomass value (0.057) of all species collected during sampling (61 species). For clarity, the inset within the biomass panel (a) displays violins for species that had no individuals greater than the median biomass value.

2.4.3 Spatial differences in trait values

Biomass of *Eurysternus caribaeus* and *Dichotomius lucasi,* the two species collected in sufficient abundances (minimum of n = 15 per site), differed significantly across sites ($F_{2,137} = 17.47$, $P < 0.0001$ and $F_{2,226} = 10.76$, $P < 0.0001$ respectively, Fig. 2.2), whereas back leg length was unaffected. Post-hoc tests revealed that the biomass of *E. caribaeus* differed significantly between site 1 and site 3 ($P < 0.0001$) and between site 2 and site 3 ($P < 0.0001$); biomass was significantly higher at site 3 than at site 1 and site 2. The biomass of *D. lucasi* was significantly higher at site 3 than site 2 ($P < 0.0001$).

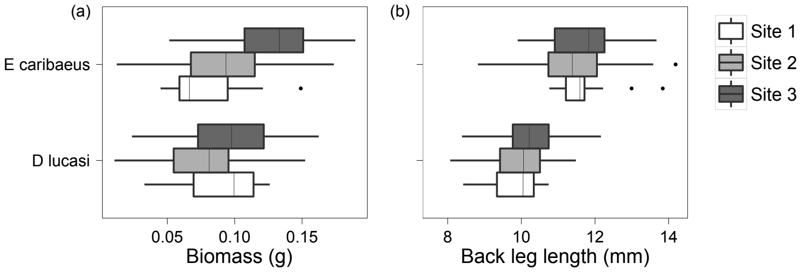

Figure 2.2 | Sampling site differences in dung beetle biomass (a) and back leg length (b). Medians (central vertical lines) interquartile ranges (boxes) and outliers (black points) are displayed for species collected from site 1 (white boxes); site 2 (light grey boxes) and site 3 (dark grey boxes).

2.4.4 Sample size selection

Between 35 and 60 individuals were needed to reduce the mean SE of biomass to within 5% of the total sample mean when individuals from all populations were included in resampling (Fig. 2.3). When analyses were repeated using individuals from just one population, 5 or 10 fewer individuals were required for 5 of the focal species (dashed lines Fig. 2.3). This resulted in between 30 and 60 individuals needed to attain an accurate estimate of the population mean. In both cases, 35 individuals was most frequently required sample size (Fig. 2.3).

The mean SE of back leg length fell to within 5% of the total sample mean when considering just 3 individuals for the majority of species (10 out of 13; Fig. 2.3). The 3 species with the highest CV for back leg length: *A. murrayi, O. carinifrons and D. boreus* (Fig. 2.2 (b)) required 5 (*A. murrayi*) or 10 (*O. carinifrons* and *D. boreus*) individuals to reduce mean SE to within our threshold value. For these same 3 species, a comparatively large number of individuals were also needed to attain mean SE within the 95% confidence intervals of biomass: 45, 60, and over 50 individuals (*A. murrayi, O. carinifrons and D. boreus* respectively; Fig. 2.3). Examining just one population did not reduce the number of individuals required to accurately estimate mean leg length in any species.

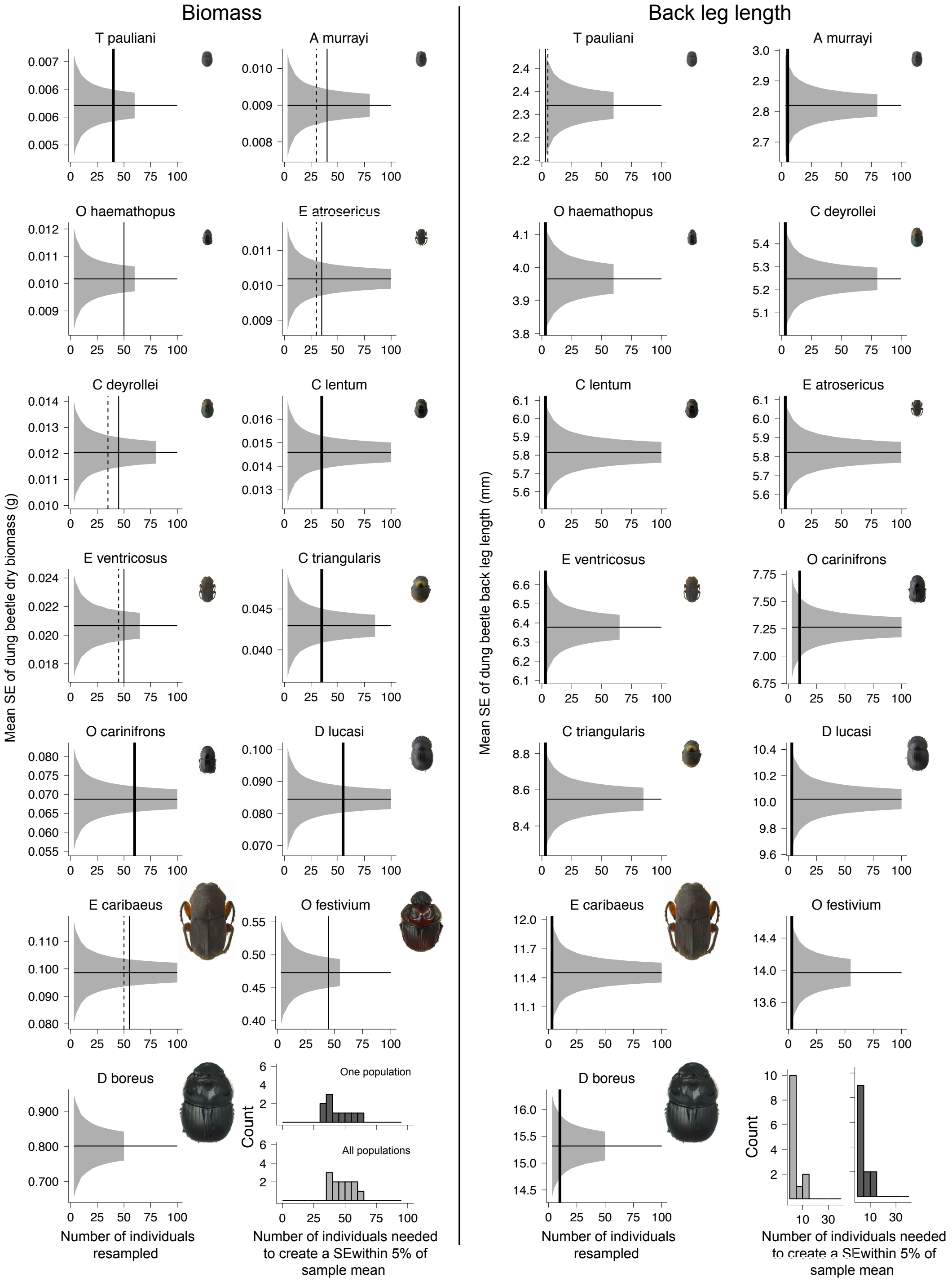

Figure 2.3 | Resampling of dung beetle biomass and back leg length. Total population mean (solid horizontal black lines; calculated using all individuals from each species, n = 51 - 229) and mean standard error (grey ribbons; calculated using resampled data from all populations) of dung beetle dry biomass (left panel) and back leg length (right panel) with species photographs. Photographs are scaled to each other; smallest species, *Trichillum pauliani,* length: 5.5mm; largest species, *Di-*

chotomius boreus, length: 24mm length. Data were resampled to create new datasets containing 3 to 100 (n = 1000 datasets per sample size) individuals and the mean standard error was calculated from the new datasets. Vertical lines indicate the number of individuals needed to create a mean standard error within 5% of the total population mean when considering individuals from every site (thin solid lines), one population only (dashed lines) and when there was no difference in the numbers needed between all and one population, thick solid lines are used. Histograms display the frequency with which each sample size created a mean standard error below the 5% threshold using all populations (light grey) and one population (dark grey). Results are presented by species, ordered by their median trait values.

2.4.5 The influence of intraspecific trait variability on functional diversity indices

Community weighted mean (CWM) was more sensitive to the omission of intraspecific trait variability than functional richness (FRic) for both biomass and back leg length (Fig. 2.4). The error associated with calculating CWM biomass without considering intraspecific trait information was 27% and 55% for biomass and back leg length respectively (Fig. 2.4(a) and (c)), whereas the error made in calculating FRic without individual trait information was 16% and 6% for biomass and back leg length (Fig. 2.4(b) and (d)). The strength of relationships between the indices calculated without and without intraspecific trait variability were both strongest ($R^2 = 0.96$) and weakest ($R^2 = 0.45$) when considering back leg length, even though this trait displays the lowest intraspecific variability (Fig. 2.1(b)).

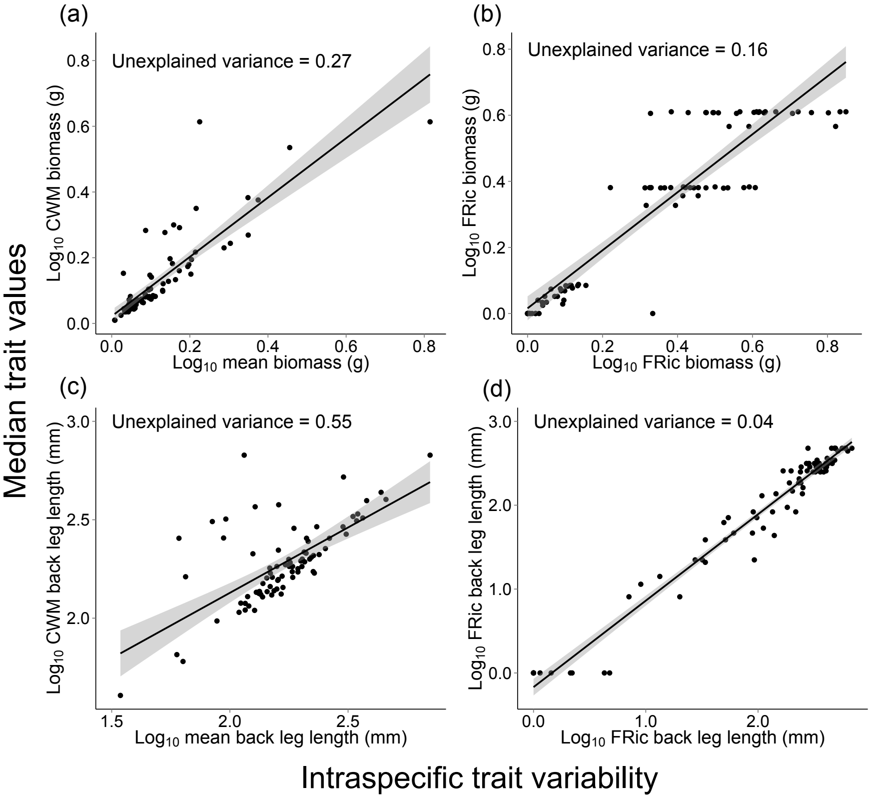

Figure 2.4 | Associations between functional diversity indices calculated with and without the inclusion of intraspecific trait variability. Community weighted mean (CWM) of biomass (a), functional richness (FRic) of biomass (b), CWM back of back leg length (c) and FRic of back leg length (d). Linear model outputs are displayed: regression lines (solid back lines), standard errors (grey ribbons, calculated using the predict function in R) and the inverse of R^2 values to describe the loss of information as a result of exclusion of intraspecific trait information.

2.5 DISCUSSION

Our study has taken the first steps in quantifying the importance of variability in invertebrate traits for the calculation of functional diversity (FD) indices. In doing so, we reveal that omission of within species differences in trait values can result in large reductions in the accuracy with which invertebrate communities are described in mesocosms, even though the dung beetle traits we examined displayed greater between than within species variability. Our results therefore support the use of mean trait values to summarise species trait information when considering trait diversity for whole populations, but clearly demonstrate the importance of considering within species trait differences when linking the functional diversity of small communities to the ecological processes they perform.

2.5.1 Are dung beetle functional traits robust?

We tested the assumption that dung beetle functional traits are robust, i.e. that they vary more between than within species (Garnier *et al.* 2001). Within species differences in trait values were responsible for between 2.6% and 5% of total variability for pronotum volume (Appendix S2.1; Fig. S2.3) and biomass, respectively. We are therefore confident that the assumption of robust traits in dung beetles is valid, at least at the spatial scale of this study. This finding is in contrast to a number of plant based studies that report greater (Lecerf & Chauvet 2008), equal (Messier *et al.* 2010) or less, but non-negligible contributions (Albert *et al.* 2010b; a) of intra, compared with interspecific variability.

Aside from sampling differences between this study and those of plant-based investigations, there are well-established biological reasons as to why the traits of animals should display less intraspecific variability than those of plants. Namely, most animals can move in response to environmental cues or pressures whereas plants cannot. Therefore, many plant species can quickly respond physiologically to changes in, for example, resource availability (Zhang *et al.* 2013; Bardgett & van der Putten 2014). Phenotypic plasticity (the capacity of a given genotype to adopt different phenotypes under varying environmental conditions; (Valladares, Gianoli & Gómez 2007) is therefore recognised as of a greater evolutionary advantage in sessile plants than in mobile animals. The differences in levels of phenotypic plasticity between plants and animals, in combination with results presented here, suggests that inclusion of intraspecific variability should be less important in animal based functional investigations compared with similar producer-focussed studies. However, to more thoroughly test our assertion that dung beetle functional traits are robust, further work is needed to quantify variability in traits derived from individuals originating from geographically distant sites, as well as sites distributed along longer gradients of environmental conditions, including anthropogenic disturbance (Mouillot *et al.* 2013).

Although not specifically tested, our findings caution against the categorisation of species based on continuous traits, unless the distribution of values within a community show clearly discrete clusters of species within which a threshold can be reasonably placed. This is because the median trait value of one species can frequently represent a small or large value of an individual from a species of a similar size, which is apparent from consideration of the violin plots of biomass. Furthermore, *D. lucasi, E. caribaeus, O. carinifrons* and *C. triangularis* all display biomass values that traverse the median value of all species collected. A number of previous dung beetle functional diversity investigations have categorised species as small or large based on thresholds such as body length (Escobar *et al.* 2008; Barragán *et al.* 2011; Braga *et al.* 2013) or ability to fit through a certain size mesh (Slade *et al.* 2007b; Dangles *et al.* 2012). Our threshold value artificially categorises three species as either large or small when in fact individuals have a high probability of displaying trait values that places them in a different category. Therefore, gathering species into groups artificially imposes a discrete structure on functional differences that are generally continuous, resulting in loss of information (Petchey & Gaston 2006). This could ultimately compromise efforts to determine patterns between organisms and the ecosystem processes they govern if the miss-categorisation of individuals involved leads to an underestimation of relationships.

2.5.2 How many individuals are enough?

We have demonstrated that intraspecific trait variability in dung beetle traits is negligible, suggesting that average values should accurately represent species functional characteristics. But how many individuals per species should be assessed to provide a realistic estimation of the actual sample mean, whilst minimising sampling effort? Our results suggest that this depends on the trait and species of interest. Biomass was the most variable, and consequently the trait from which most individuals must be measured in order to provide a reliable mean. Considering individuals from just one population reduced the sample size needed to estimate biomass, but had no impact on the numbers needed for back leg length. This implies that caution is needed when designing a sampling regime based on the assumption that individuals will display less intraspecific trait variability if originating from the same population. While this may be true (for some traits), it appears that this does not necessary translate into a large reduction in the numbers of individuals needed to gain a representative estimate of mean trait values.

An explanation for the different levels of variability in traits, and thus the number of individuals required in sampling, could lie in differing levels of plasticity displayed by each trait. Biomass can change in response to short term environmental cues, and as such it displays phenotypic plasticity (Valladares *et al.* 2007). Morphological characteristics such as leg length or leg area are, however, determined during larval development (Hunt & Simmons 1997) and are fixed during adult life. Thus, fluctuations in resources over very small spatiotemporal scales (e.g. weeks or kilometres) would have little impact on the variability of these fixed traits compared with biomass. This is supported when considering pronotum volume and front leg area (Fig. S2.3 – Fig. S2.5), both of which, like back leg length, are non-plastic traits in adult beetles. The number of individuals needed for these traits to reduce variability to within 5% of the total sample mean was also fewer than was needed for biomass. These findings are further supported by the significant influence of site on the biomass of both species tested, compared to the generally non-significant effect of sampling location on the other traits. Trait databases (e.g. Kattge *et al.* 2011) are increasingly important tools in facilitating large-scale functional investigations in plant-focussed studies (e.g. Pietsch *et al.* 2014) but equivalent trait collections are lacking for higher trophic level organisms. Further work is therefore needed to understand the ability of plastic versus fixed traits to predict animal-mediated ecosystem functioning. This would facilitate the targeted development of much-needed trait databases for non-producer organisms.

2.5.3 The influence of intraspecific variability on functional diversity indices

Our final objective was to assess how the omission of intraspecific information in dung beetle traits influenced the accuracy with which functional diversity indices described naturally formed communities. Interestingly, despite the very low contribution of intraspecific compared to interspecific trait variability at the population level, ignoring within species differences in trait values at the small-scale community level led to non-negligible loss of information. In development of their 'spatial variance partitioning' hypothesis Albert *et al.* (2011) suggest that at broader spatial scales, interspecific variability will be relatively more important than intraspecific variability. This is because increasing the area over which a study is conducted will generally increase the genetic and environmental variability that is included. In this investigation, individuals were collected from three locations separated by between 48 and 90km. When we examine the structure of variability in traits over this relatively large spatial scale, intraspecific variation appears insignificant, but it has important impacts on the outcome of a finer scale investigation. Moreover, our results are in agreement with those of Baraloto *et al.* (2010) who proposed that investment in complete sampling is necessary in order to quantify vital variance at a local scale. It is, after all, the individuals that carry out the functions of interest. Our results suggest that increasing accuracy in trait information will increase the precision with which organisms can be linked to ecological processes when sample sizes are small (e.g. in mesocosms or other enclosure experiments). However, in larger scale population studies, the significant investment involved in complete sampling may not be necessary.

For all traits CWM was more sensitive to the omission of intraspecific trait variability (ITV) than FRic. This is incongruent with the findings of Albert *et al.* (2012) who report CWM to be less sensitive than FRic to the exclusion of ITV. Albert *et al.* (2012) calculated FD with and without varying levels intraspecific trait variability for communities consisting of between 22 and 51 species, covering an area of 1% - 87% of the sampling plots. In contrast, this investigation considered communities containing between 1 and 11 species with abundances of between 1 and 95 individuals. It is feasible, therefore, that the differences we see in the sensitivity of the indices to the exclusion of intraspecific trait information between this study and that of Albert *et al.* (2012) could arise from a reduction in precision of metrics as community size decreases; a possibility that has not been previously explored. Species richness was closely related to species abundance (Fig. S2.7), both of which were negatively correlated to the residuals from models assessing relationships between indices with and without intraspecific information (Fig. S2.8). Therefore, we cannot disentangle the mechanism behind the decrease in the influence of intraspecific variability on functional diversity indices with increasing species richness and numbers of individuals. Nevertheless, these findings concur with previous studies, suggesting that the inclusion of intraspecific information increases in importance with a decrease in the scale of the investigation (Albert *et al.* 2011). Further work on which index performs best under contrasting community size and diversity will provide useful guidelines for the investigator faced with the choice of multiple functional diversity indices (e.g. Mouchet *et al.* 2010).

A surprising outcome was the high sensitivity of CWM of back leg length to omission of intraspecific trait information. This is counterintuitive given that back leg length is the trait for which every species displayed the lowest coefficient of variation (CV). The relatively poor association between CWM back leg length with and without the inclusion of intraspecific trait information is a result of an over estimation of the CWM back leg lengths of a small number of communities. This result further demonstrates the increased importance of quantifying and including intraspecific variability in functional investigations dealing with small communities. We demonstrate that the stochastic occurrence of individuals displaying extreme species trait values appear to influence the accuracy of indices in unpredictable ways, resulting in large losses of information.

2.5.4 Conclusion

Our exploration into the sources of trait variability in a functionally important invertebrate group has demonstrated that using dung beetle mean trait values when dealing individuals from the same geographic region is likely the most ecologically meaningful approach (Albert *et al.* 2011). To accurately estimate mean trait values, however, we urge thoughtful consideration of the variability of the focal traits and the sampling location(s) from which individuals are collected. We reveal that considering small communities with low species richness and/or abundances without incorporating intraspecific trait variability risks the loss of potentially large amounts of information. This is the case even when using traits that display very low intraspecific variability at the population level, and is particularly important in the growing number of mesocosm or mesoclosure studies that involve small naturally assembled or experimental communities (Lahteenmaki *et al.*). If the aim of an investigation is to describe links between organisms and the ecological processes they govern this could have large implications for the accuracy of results. The importance of functional diversity is increasingly recognised as a tool for predicting the consequences of human impacts on ecosystems (Dirzo *et al.* 2014; Mace *et al.* 2014), and functional traits are the fundamental building blocks of this fast developing field. Research efforts should therefore be directed at increasing the accuracy with which we describe species characteristics in order to develop functional ecology into a more quantitative and predictive science (McGill *et al.* 2006).

2.6 ACKNOWLEDGEMENTS

We thank our field assistants Edivar Dias Correa, Jucelino Alves dos Santos, Filipe França and Daniel Tregidgo, as well as Tom Walker for essential statistical advice. We are also grateful to Jari Forestal for permission to work on their landholding and logistical support throughout the duration of the project. This work was funded by a studentship awarded to HG by the National Environment Research Council (U.K.) and CNPq-PELD site 23. JB thanks CNPq 400640/2012-0.

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2.8 SUPPLEMENTARY INFORMATION

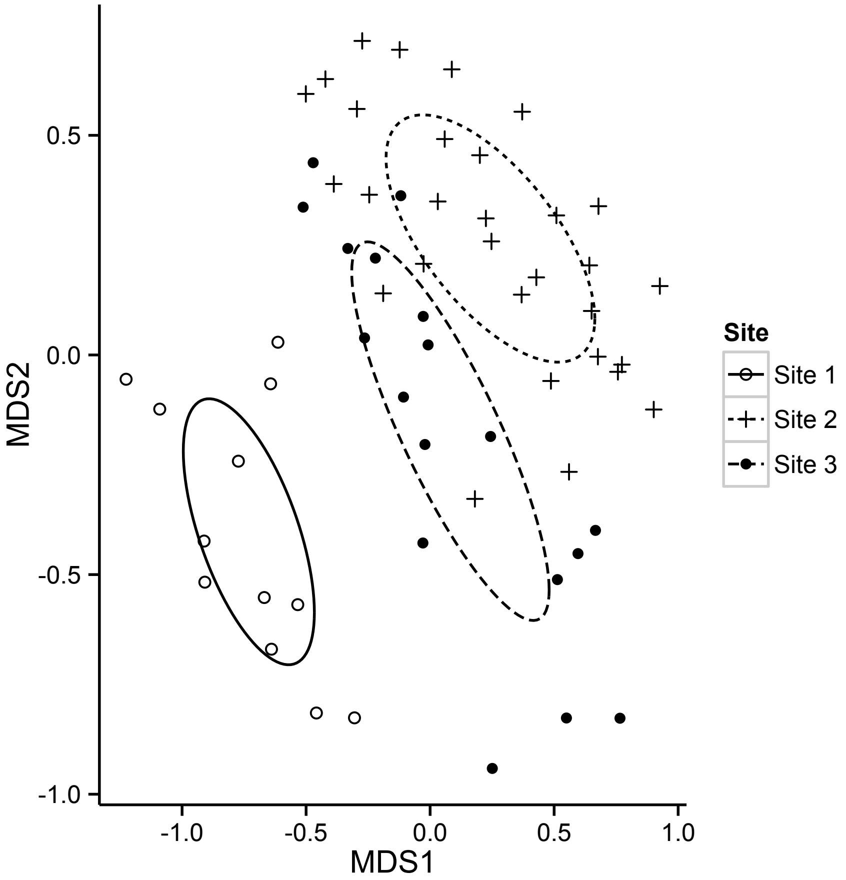


Figure S2.1 | Non-metric multidimensional scaling (MDS) ordinations of dung beetle communities in site 1 (open circles); site 2 (closed circles); and site 3 (crosses). Stress value = 0.23.

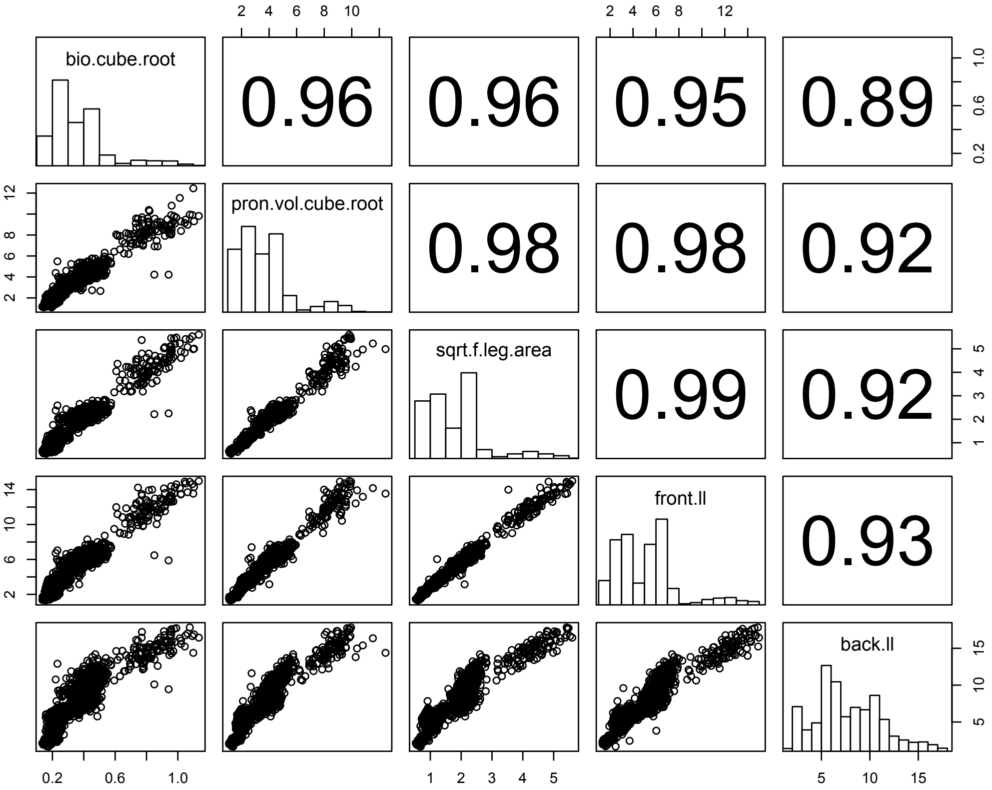


Figure S2.2 | Associations between dung beetle functional trait values: bivariate plots (lower panels), distributions (diagonal), and Pearson's ρ (upper panels). Biomass and pronotum volume were cube-rooted, and front leg area square-rooted to convert values to the same scale. Trait abbreviation definitions: bio.cube.root: cube-root dry biomass; pron.vol.cube.root: cube-root pronotum volume; sqrt.f.leg.area: square-root front leg area; front ll: front leg length; back ll: back leg length.

Appendix S2.1 – Analyses on front leg area and pronotum volume

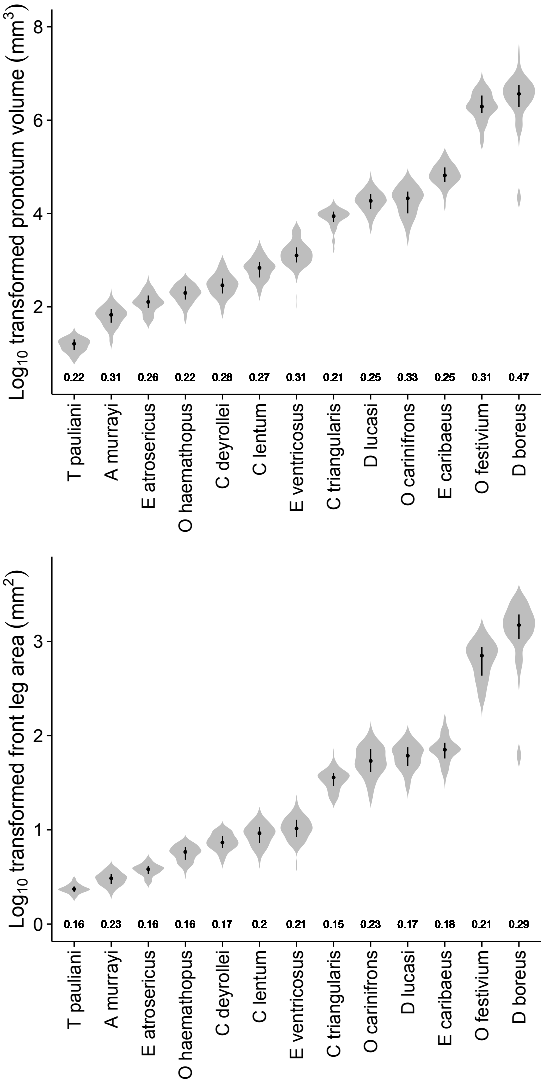


Figure S2.3 | Extent of intraspecific variability in dung beetle pronotum volume and front leg area for thirteen dung beetle species collected from the north-eastern Brazilian Amazon, state of Pará. Violin plots display (i) the density of data estimated by kernel method (grey areas); (ii) the median value (black horizontal lines in the centre of violins); and (iii) the interquartile range (between the top and bottom of the vertical black lines). Results are presented by species, ordered by their median trait values and the coefficients of variation (calculated as the SD/mean) are given for each species below the violin.

Variance partitioning

Interspecific differences were responsible for 96.6% and 96.7% of variability for pronotum volume and front leg area respectively, whereas intraspecific variation accounted for just 2.6% in both traits.

Spatial differences in trait values

Pronotum volume of *Eurysternus caribaeus* did not differ significantly between sites, but that of *Dichotomius lucasi* did ($F_{2,137} = 6.63$, $P = 0.002$; Fig. S2.4); conversely the front leg area of *D. lucasi* was unaffected by site, but the leg area of *E. caribaeus* differed significantly ($F_{2,226} = 3.507$, $P = 0.033$; Fig. S2.4). Post-hoc tests revealed that the pronotum volume of *D. lucasi* differed significantly between site 1 and site 2 ($P = 0.001$) and between site 1 and site 3 ($P = 0.003$). The front leg area of *E. caribaeus* differed significantly between site 2 and site 3 ($P = 0.03$).

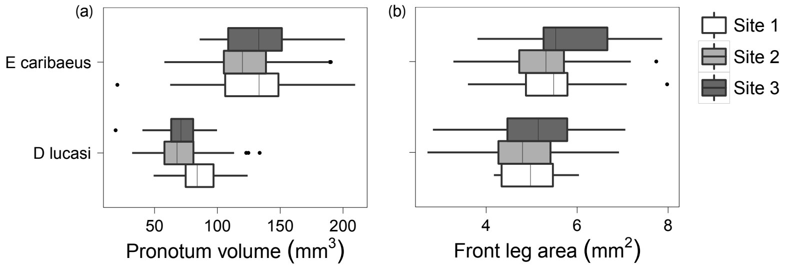


Figure S2.4 | Median pronotum volume (a) and front leg area (b) and interquartile range of two dung beetle species for which more than 15 individuals were collected from each sampling location in Brazil, State of Pará; site 1 (white boxes); site 2 (light grey boxes) and site 3 (dark grey boxes).

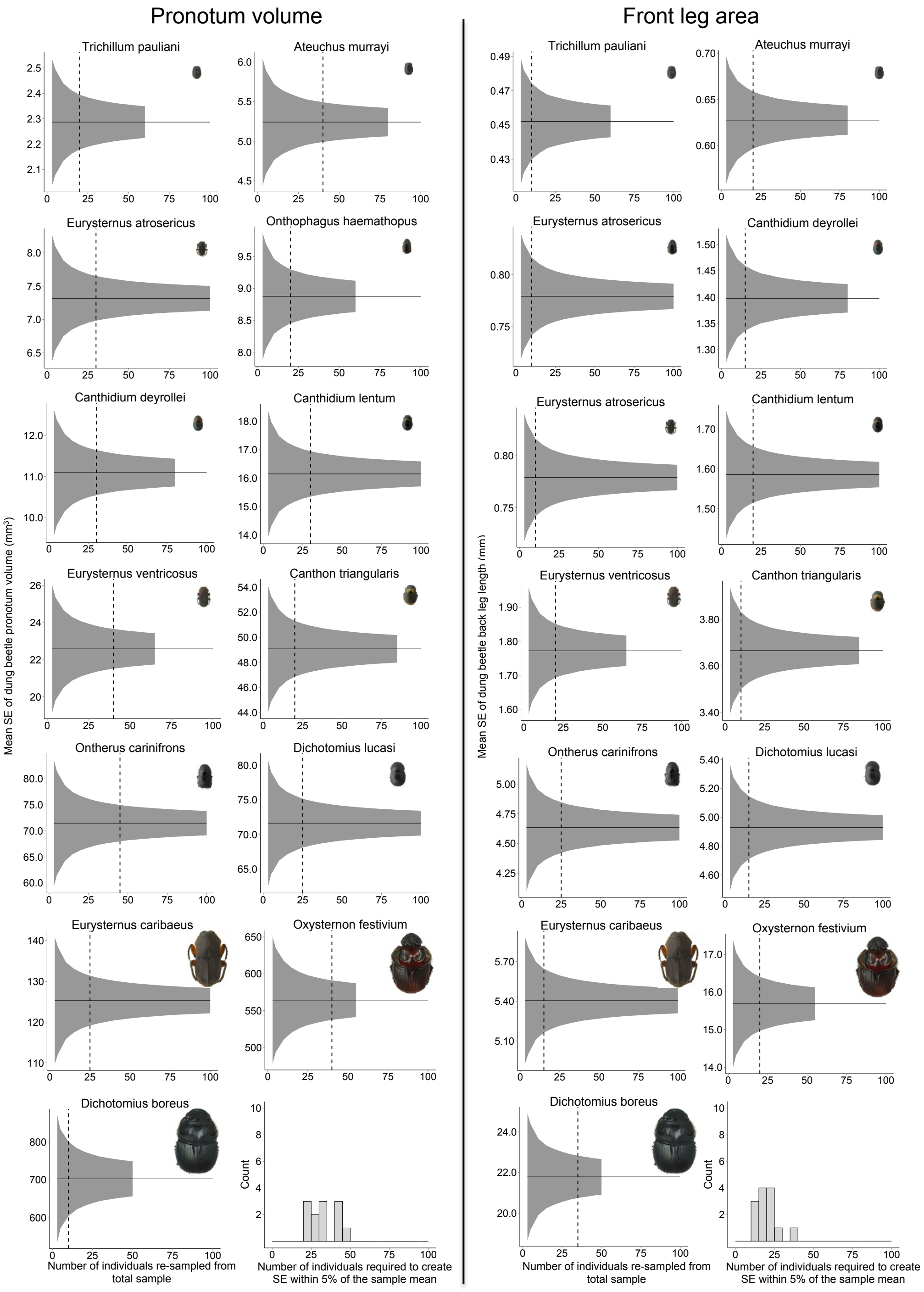


Figure S2.5 | Total population mean (solid horizontal black lines; calculated using all individuals from each species (n = 51 - 229) and mean standard error (grey ribbons; calculated using resampled data) of dung beetle pronotum volume and front leg area with species photographs. Photographs are scaled to each other; smallest species, *Trichillum pauliani,* length: 5.5mm; largest species, *Di-*

chotomius boreus, length: 24mm length. Data were resampled (with replacement) to create new datasets containing 3 to 100 individuals (n = 1000 datasets per sample size) and the mean standard error was calculated from the new datasets. Vertical dashed back lines on each panel show the number of individuals needed to create a mean standard error within 5% of the total population mean. Histograms in the bottom right corners display the frequency with which each sample size created a mean standard error below the 5% threshold. Results are presented by species, ordered by their median trait values.

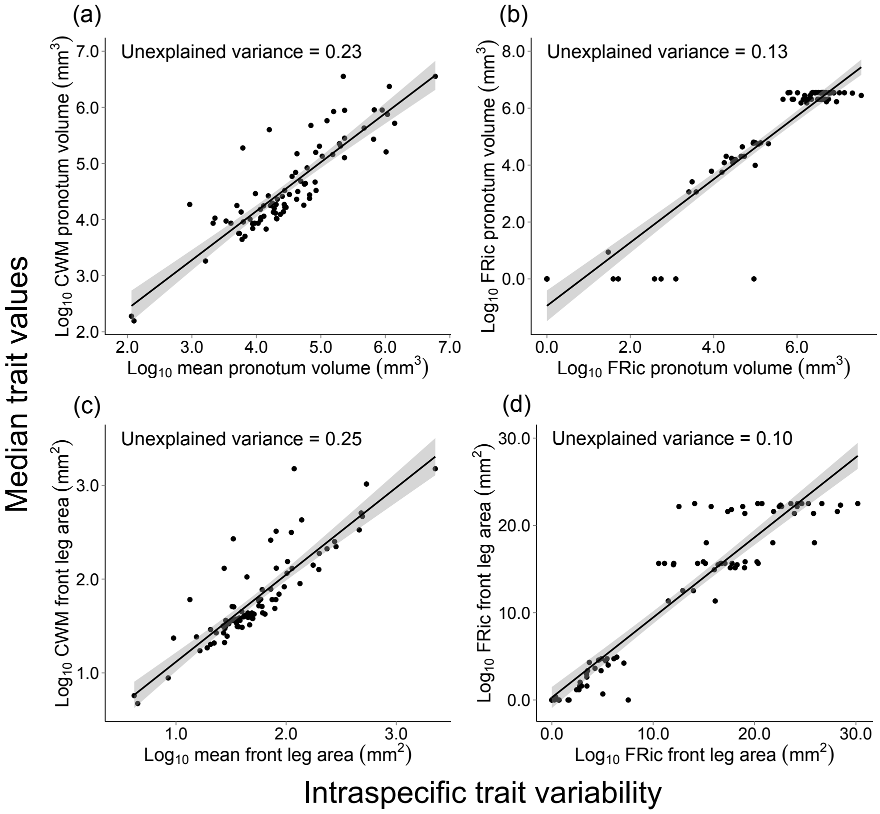


Figure S2.6 | Associations between single trait functional diversity indices calculated without the inclusion of intraspecific trait variability (ITV): community weighted mean (CWM) pronotum volume (a), functional richness (FRic) of pronotum volume (b), CWM front leg area (c) and FRic front leg area (d). Linear model outputs are displayed: regression lines (solid back lines), standard errors (grey ribbons, calculated using the predict function in R) and the inverse of R^2 values to describe the loss of information as a result of exclusion of ITV information.

Table S2.1 | Abundances of species at each site, highlighted sites are those where species were sampled at the greatest abundances and were thus used in the single population analyses. Species highlighted in bold are those from which at least 15 individuals were sampled from each site and were therefore used to assess the influence of sampling site on median trait values.

Species	Total abundance	Site 1 abundance	Site 2 abundance	Site 3 abundance
Ateuchus murrayi	82	2	77	3
Canthidium deyrollei	82	0	76	6
Canthidium lentum	138	0	135	3
Canthon triangularis	89	87	1	1
Dichotomius boreus	51	0	16	35
Dichotomius lucasi	229	18	135	76
Eurysternus atrosericus	114	2	108	4
Eurysternus caribaeus	140	16	98	26
Eurysternus ventricosus	66	6	51	9
Ontherus carinifrons	151	0	147	4
Onthophagus haemathopus	62	6	22	34
Oxysternon festivum	59	2	22	35
Trichillum pauliani	62	0	0	62

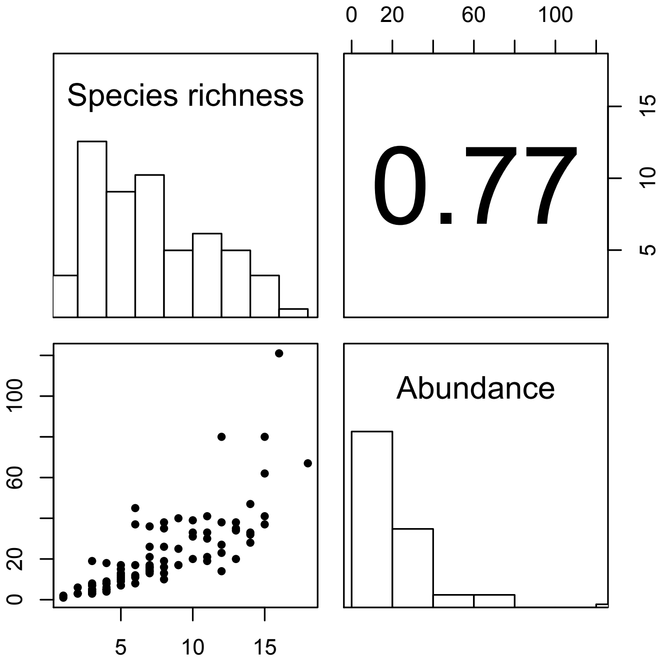


Figure S2.7 | Associations between dung beetle species richness and abundance: bivariate plot (lower panel), distributions (diagonal), and Pearson's ρ (upper panels).

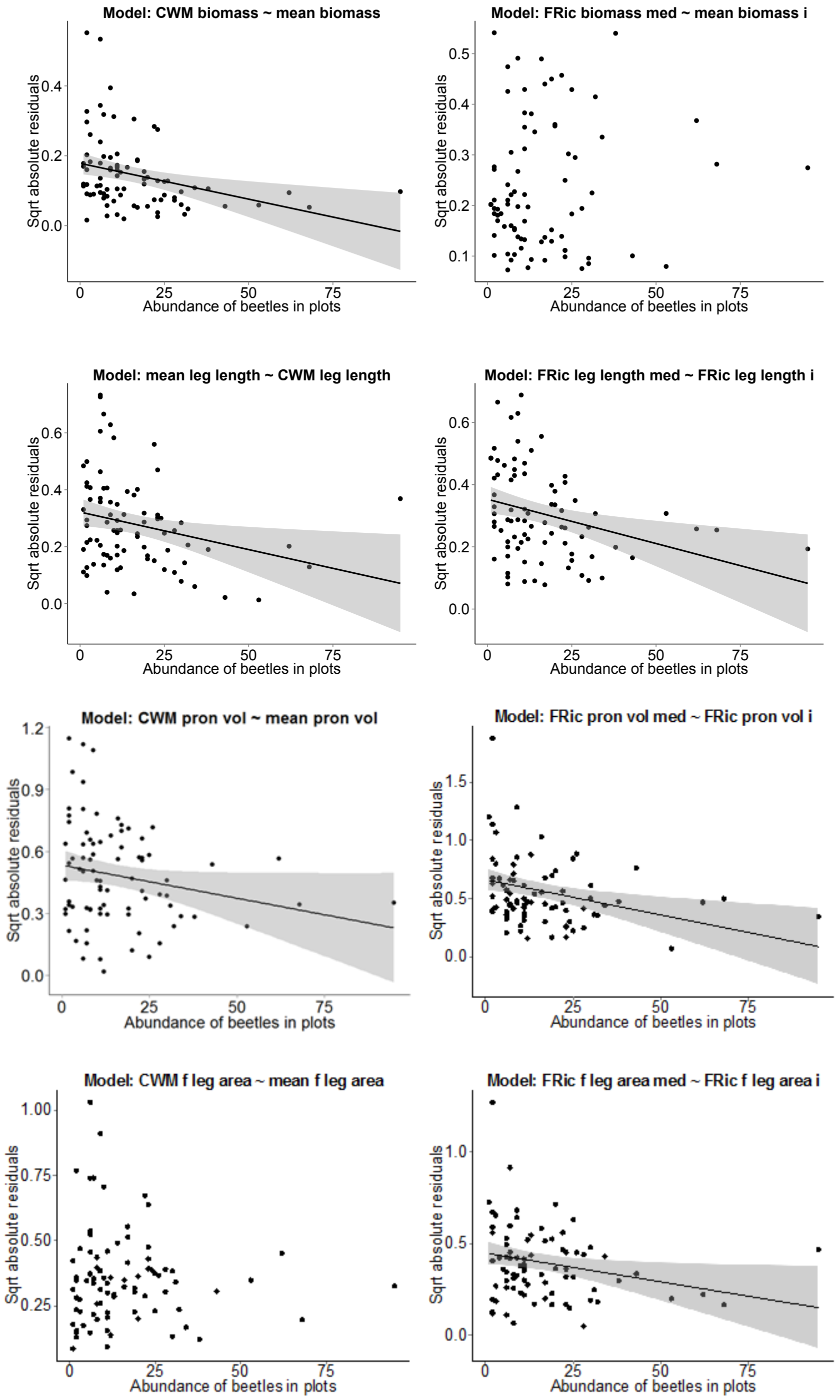
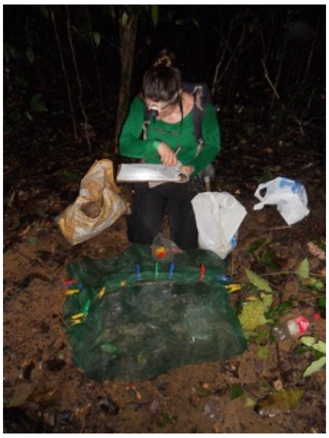


Figure S2.8 | Associations between dung beetle abundance and the absolute residuals from models assessing the relationship between functional diversity indices calculated with and without inclusion of intraspecific trait information. Linear model outputs are displayed: regression lines (solid back lines), standard errors (grey ribbons, calculated using the predict function in R).

Chapter 3

Functional diversity, dung beetle mediated secondary seed dispersal and environmental context



Functional diversity, dung beetle mediated secondary seed dispersal and environmental context

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Hannah M. Griffiths[1,2], Julio Louzada[1,2], Richard D. Bardgett [1,3], Wallace Beiroz[1,2],
Filipe França[1,2], Daniel Tregidgo[1,2] and Jos Barlow[1,4]

[1]Lancaster Environment Centre, Lancaster University, Bailrigg, Lancaster, LA1 4YQ, UK.
[2]Departamento de Biologia, Universidade Federal de Lavras, Lavras, Minas Gerais, 37200-000.
[3]Faculty of Life Sciences, Michael Smith Building, The University of Manchester, Oxford Road, Manchester, M13 9PT, UK.
[4] Museu Paraense Emilio Goeldi, Av. Magalhães Barata, 376, Belém-Pará-Brazil

3.1 ABSTRACT

Biodiversity-ecosystem functioning (BEF) literature is dominated by investigations conducted in temperate grassland ecosystems under homogenous environmental conditions. Consequently, studies concerned with the functional importance of higher trophic levels, or with the role of environmental conditions in shaping BEF relationships, are comparatively uncommon. To address this, we assessed dung beetle diversity-functioning relationships *in situ*, in a field experiment in the Brazilian Amazon. Dung beetles perform a number of ecological functions in habitats across the globe; in tropical forests they play a key role in the secondary dispersal of seeds. We therefore experimentally tested how the functional diversity of dung beetle communities affects seed dispersal and how BEF relationships varied with environmental context, by replicating the experiments under contrasting soil conditions. Relationships between dung beetle diversity and function were examined using diversity indices calculated using continuous morphological traits of the individuals involved in experiments, and functioning was measured as the dispersion of artificial seeds throughout the soil profile and the probability of burial. Ninety experimental plots were established across three distinct primary forest sites. We collected, identified and measured almost 2000 beetles, and sieved around eleven tonnes of soil to quantify the dispersion of 1800 seed mimics. There was a significant effect of dung beetle functional diversity on both seed dispersion and seed burial, although this depended on environmental context, with the strength or direction of responses differing across the contrasting soils. Regardless of soil type, functional richness, but not species richness, predicted seed dispersion. We therefore advocate the use of functional diversity indices over taxonomic approaches in dung beetle focused BEF investigations. Furthermore, we highlight the difficulties in generalising BEF relationships, even considering a single function within the same ecosystem.

Keywords: *Functional diversity; animal-mediated functioning; context dependency; BEF; secondary seed dispersal; functional traits; dung beetles: soil; functional diversity indices*

3.2 INTRODUCTION

Species are disappearing from the planet at a faster rate than would be expected from the fossil record (MA 2005, Barnosky et al. 2011), and this trend is likely to continue throughout the 21st century (Pereira *et al.* 2010). These high extinction rates are largely a result of human activities (Steadman 1995; Dirzo & Raven 2003), but aside from moral considerations, a key issue in ecology is, does it matter? This question sparked the initial biodiversity-ecosystem functioning (BEF) studies (e.g. Naeem *et al.* 1994; Tilman & Downing 1994), and there is now a wealth of research demonstrating that biodiversity in producer systems matters for the maintenance of primary productivity, as well as the cycling of nutrients and water in soil (Cardinale *et al.* 2011, 2012; Hooper *et al.* 2012). This has important implications because primary production and nutrient cycling underpin the provision of a number of goods and services to society (Cardinale *et al.* 2012). Disruption of them, therefore, has the potential to negatively impact upon human well-being (MA 2005).

Despite progress made in our understanding of the role of diversity for ecosystem functioning (Hooper *et al.* 2005; Cardinale *et al.* 2012), three key areas remain under-represented in the literature. First, there is a clear bias towards the role of diversity on resource capture (particularly biomass assimilation) in terrestrial plant communities (Balvanera *et al.* 2006; de Bello *et al.* 2010; Cardinale *et al.* 2011). Our knowledge of diversity effects in higher trophic levels is therefore patchy, being based on a limited number of investigations (e.g. Slade et al. 2007, Dangles et al. 2011, 2012, Braga et al. 2013, Nichols et al. 2013b). As higher trophic level organisms are the agents of many ecosystem processes and services (e.g. Andresen and Feer 2005, Blouin et al. 2013), the functional importance of non-producers merits further research. Second, the majority of investigations to date have been conducted in temperate regions (e.g. de Bello et al. 2010, Cardinale et al. 2011; but see also Slade et al. 2007, Dangles et al. 2011, Gray et al. 2014), yet tropical forests are experiencing globally high rates of species losses (Stork 2009). Given that diversity effects vary across habitats (Schmid *et al.* 2009), our understanding of the impact of biodiversity loss in tropical forests may not be accurately inferred from BEF research conducted primarily in temperate systems. Third, individual BEF studies have typically focussed on how species influence ecosystem processes in single sites with homogenous environmental conditions (Roscher *et al.* 2004; Clark *et al.* 2012), but there is growing evidence that the size and direction of diversity effects depend on environmental context (Lensing & Wise 2006; Dangles *et al.* 2012; Steudel *et al.* 2012).

Dung beetles (Coleoptera: Scarabaeinae) are a practical group of organisms for use in ecological investigations (Slade *et al.* 2007b; Gardner *et al.* 2008a). Through their movement and burial of mammalian dung for feeding and nesting purposes (Hanski & Cambefort 1991), they perform a number of ecosystem functions (Nichols *et al.* 2008). In tropical forests, one of their key roles is the secondary dispersal of seeds by relocating them from their deposition site in mammalian dung to beneath the soil surface (Andresen & Feer 2005). This can result in the following benefits to the relocated seeds: (i) placement in a more suitable microclimate for emergence and establishment (Shepherd & Chapman 1998; Andresen & Levey 2004); (ii) escape from predation and pathogen mediated mortality (Estrada & Coates-Estrada 1991; Shepherd & Chapman 1998; Feer 1999); and (iii) reduction in clumping and associated implications for seedling competition and density dependant mortality (Andresen & Feer 2005; Lawson *et al.* 2012). By influencing seedling survival in these ways, dung beetle activity could have far reaching impacts upon forest regeneration and community composition.

Although dung beetles have previously been used as a model system for animal mediated BEF research (Slade *et al.* 2007b, 2011; Dangles *et al.* 2012; Braga *et al.* 2013; Nichols *et al.* 2013b), we are not aware of any studies that have established direct links between an ecological process of interest and the community that was directly responsible. For example, previous field-based dung beetle investigations (e.g. Slade et al. 2007, Dangles et al. 2012) sampled communities associated with the location of the experiment, but not the actual individuals that were involved in the manipulations and that therefore performed the function. Moreover, the functional diversity indices calculated to express the BEF relationships have been exclusively derived from categorical trait information based on nesting behaviour, diet, diurnal activity, and size (e.g. Slade et al. 2007, Barragán et al. 2011, Dangles et al. 2012). This is despite the fact that categorising species in this way could overlook important morphological traits, and risks loss of information (Petchey & Gaston 2006; Villéger *et al.* 2008).

The overarching aim of this study was to explore dung beetle mediated BEF relationships in a tropical forest under real, heterogeneous field conditions. Specifically we tested two hypotheses. First, in accordance with positive relationships reported between diversity and functioning in previous investigations (e.g. Slade et al. 2007, Cardinale et al. 2011, Dangles et al. 2011), we hypothesised that dung beetle diversity positively impacts secondary seed dispersal. We used seed mimics to measure both the likelihood that seeds were buried and, uniquely, the variety of depths and distances that seeds were moved. We advance realism by calculating our beetle functional diversity metrics using detailed morphological traits that were measured from the individuals responsible for seed burial, i.e. the communities that assembled within our experimental treatments. Second, we hypothesised that the strength and/or direction of BEF relationships depend on environmental context, in this case contrasting soil conditions in different primary forest sites. Previous dung beetle BEF studies have investigated the relationship between diversity and functioning either through exclusion of different functional guilds (Slade *et al.* 2007b; Dangles *et al.* 2012) or through correlative associations between proxy communities (sampled either temporally or spatially close to experimental plots) and an ecological process of interest (Slade *et al.* 2011; Braga *et al.* 2013; Nichols *et al.* 2013b). However, to our knowledge, this is the first study to assess dung beetle mediated diversity-functioning relationships using the realised, naturally assembled communities that were directly responsible for functioning.

3.3 MATERIALS AND METHODS

3.3.1 Study site

The study was carried out in the 17 000km^2 landholding of Jari Florestal, located in the state of Pará in the north-eastern Brazilian Amazon (0°53S, 52°36W). This area consists of a matrix of *Eucalyptus* plantations, regenerating secondary forests, and large expanses of largely undisturbed primary rainforest. Within this landscape, experiments were established in three *terra firme* primary forests sites with differing soil conditions: Clay soil site: clay textured Oxisol (mean clay content ± SE: 67.3 ± 1.5%, silt: 14.4 ± 1%, sand: 14.1 ± 1.1%), with aluminium sesquioxides; Sand soil site 1: sand textured Oxisol (mean clay content ± SE: 5.8 ± 0.2%, silt: 1.5 ± 0.2% sand: 86.3 ± 1%) with aluminium sesquioxides; Sand soil site 2: medium textured Oxisol (mean clay content ± SE: 8.2 ± 0.7%, silt: 2.8 ± 0.2%, sand: 77.8 ± 1.9%) with aluminium sesquioxides. The sites are all within 100km of one another (distance between Clay and Sand 1: 90km; Clay and Sand 2: 38km; Sand 1 and Sand 2: 75km), classified as dense lowland tropical rain forest and subject to the same regional climatic conditions.

3.3.2 Experimental design

During July and August 2012 we established a grid of thirty experimental plots separated by 100m at each experimental site (n = 90 in total). Within this grid, plots were created by burying nylon netting 10cm into the forest floor in a 50cm x 50cm square (Fig. 3.1, Fig. S3.1). Each plot contained a non-baited pitfall trap (13.5cm width, 9cm depth), buried flush with the ground surface and filled with salt and water. Experimental plots were baited with a 100g mixture of 50:50 human and pig dung containing 20 plastic seed mimics (hereafter referred to as seeds) of 4 different sizes: 2 large (20mm diameter, 4.12g), 6 medium (10mm diameter, 0.50g), 6 small (5mm diameter, 0.09g), and 6 very small (2mm diameter, 0.06g). The dung and seeds were placed on the floor in the centre of the plots. We used artificial, rather than real seeds, because they were easier to find in the soil during destructive sampling. Threads we not attached to the seeds to facilitate their location (e.g.

Andresen and Levey 2004, Santos-Heredia et al. 2010) because it appeared to alter beetle behaviour in pre-experimental trails. Previous investigations indicate that there are no differences in removal rate or burial depths between seed mimics and real seeds (Koike *et al.* 2012). The quantity of dung and number, size, and "species richness" of seeds were based on mean values from howler monkey (*Alouatta seniculus*) defecations reported by Feer (1999) and Andresen (2002).

To test our hypothesis that dung beetle diversity positively influences seed dispersal we attempted to manipulate dung beetle diversity across the experimental plots whilst retaining naturally assembled mixtures of species. To achieve this, we randomly assigned plots to three different dung baiting procedures: at each site, plots were either baited and left open for beetle colonisation for 12 hours during the day (n = 10), 12 hours during the night (n = 10), or 24 hours (baited either during the day, n = 5; or night, n = 5). Differences in plot opening times are henceforth referred to as community manipulation, and the effect of these treatments on the dung beetle community attributes can been seen in Fig. S3.2 and Fig. S3.3. We took this approach because of the known difficulties in artificially manipulating biological communities to create realistic and meaningful assemblages whilst disentangling species effects from functional diversity effects (Huston 1997; Naeem & Wright 2003). Baiting involved placing dung pats containing seeds on the ground in the centre of each plot, and protected from rain by a plastic cover. After 12 or 24 hours, the experimental plots were closed, using pegs to hold the netting together in a way that ensured the beetles could not leave and prevented further colonisation by beetles that had not buried the dung (Fig. S3.1). At the same time, the internal, non-baited pitfall trap was opened to capture the beetle community that had carried out the function following emergence from the soil. Plots were opened and closed between 06:30 and 08:30 or 18:00 and 20:30.

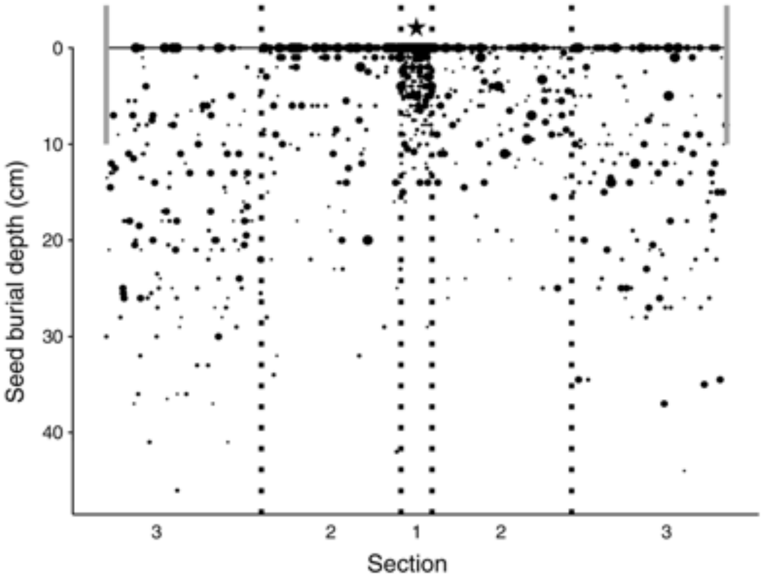

FIGURE 3.1 | Experimental plots were created by the burial of nylon netting 10cm into the soil in a 50 x 50cm square (grey vertical lines) and baited with dung and seeds (star above the centre of plot). For plotting purposes seed depth and horizontal movement data were pooled (n = 1800) for all plots (n = 90) and each seed was assigned a random horizontal movement value within the section from which it was recovered. Seed size is represented with different circle sizes. Seeds that were not found during destructive sampling were given a size and site specific burial depth, which were the median values of seeds found below 10cm in section three of experimental plots (Clay site, very small seeds: 19cm, small seeds: 13cm, medium seeds: 15.25cm; Sand site 1, very small seeds: 21cm, small seeds: 19cm, medium seeds: 17.5cm; Sand site 2, very small seeds: 21cm, small seeds: 22.75cm, medium seeds: 18cm).

After closure, plots in the Clay and Sand 2 sites were left for seven days before they were destructively sampled. Because logging operations in Sand 1 restricted access to the area, destructive sampling took place two weeks after the establishment of the experiments at this site. We divided the forest floor within the plots into three sections: section one formed a central 5cm x 5cm square; section two was the surrounding 25cm x 25cm square (minus section one), and section three the 50cm x 50cm surrounding section one and section two (Fig. 3.1). Dung that remained on the soil surface was washed to locate seeds within. Following this, seeds visible on the leaf litter surface were removed. Finally, leaf litter, fine roots and soil were removed in approximately 1cm layers and sieved using a sieve with 1mm^2 metal mesh to a soil depth 50cm. In total, around eleven tonnes of soil was screened in search of 1800 seeds. When a seed was located, the section, and depth at which it was found (if it was in the soil) was recorded. Any beetles recovered from the soil or the internal pitfall traps were assumed to have been involved in the dung and seed burying process. This was justified by the length of time that experimental plots were open for colonisation (either 12 or 24 hours), during which time casual visitors that were only feeding on the dung (and therefore not contributing to the function of interest, seed dispersal) were expected to feed and leave. This was supported by observational studies of beetle behaviour during experimental design and by reports of reduced visitation time at dung pats by beetles when they are feeding rather than nesting (Halffter & Edmonds 1982). All individuals were then oven dried and stored for laboratory processing.

3.3.3 Beetle traits

Beetles were identified to species level using a reference collection held at the *Universidade Federal de Lavras* (UFLA) in Brazil and region-specific classification keys developed by F. Z. Vaz-de-Mello and T. A. Gardner (unpublished). We measured pronotum area, front tibia and femur area, and front and back leg length (Fig. 3.2) using a Leica M250 microscope and Life Measurement software; with digital callipers (0.01mm resolution) we measured pronotum height; and dry biomass was determined using a Shimatzu AY220 balance with precision to 0.0001g. All traits were measured from every individual captured in the study (n = 1962). Front tibia and femur area were summed to provide a total front leg area, and pronotum height and area were multiplied to estimate pronotum volume. As these traits were colinear with biomass and correlated traits should not be used in the calculation of functional diversity indices (Naeem & Wright 2003; Cadotte *et al.* 2011), we divided front leg area and pronotum volume by the dry biomass of the same individual (biomass adjusted traits), and back leg length was divided by front leg length (back: front leg length). Biomass, biomass adjusted front leg area, biomass adjusted pronotum volume, and back: front leg length were then used in the calculation of the multi-trait diversity metrics described below. Median (rather than mean) traits (calculated using values from every individual in a species, n = 1 – 239) were used because they did not always display a normal distribution (see Fig. S3.4 for species biomass distributions). Because these morphological traits may not fully capture all functionally relevant behavioural information (Inward *et al.* 2011) we also included three categorical traits: nesting strategy (roller, tunneller, or dweller, for description see Hanski and Cambefort 1991), activity period (nocturnal, diurnal, crepuscular, or generalist), and diet (coprophagous or generalist). Categorical trait information was gathered from Nichols *et al.* (2013a) and Beiroz (2013). Where information on a species was unavailable, we assumed behavioural traits to be common between the species belonging to the same genus. This was necessary for 18 species for diet and 19 for activity period (Table S3.2 for species list, abundances and median trait values).

3.3.4 Calculation of diversity metrics

We used seven traits (biomass, biomass adjusted front leg area, biomass adjusted pronotum volume, back:front leg length, nesting strategy, diet and diurnal activity) to calculate four diversity metrics using the “FD” package (Laliberté, Shipley & Laliberté 2012) for R (R Core Team 2013). Each metric describes a different functional aspect of biological communities: (1) functional richness (FRic) is the range of traits in a community quantified by the volume of functional trait space occupied; (2) functional evenness (FEve) is the regularity in spacing and abundances of species in trait space; (3) functional divergence (FDiv) is the distribution of abundances in trait space relative to an abundance weighted centroid (Villéger et al. 2008); and (4) functional dispersion (FDis) is the distribution of abundances in trait space relative to an abundance weighted centroid, and the volume of space occupied (Laliberté and Legendre 2010). For calculation of each of the indices, traits were given equal weighting and species were weighted by their relative abundance. Species richness and total biomass were also calculated for each community.

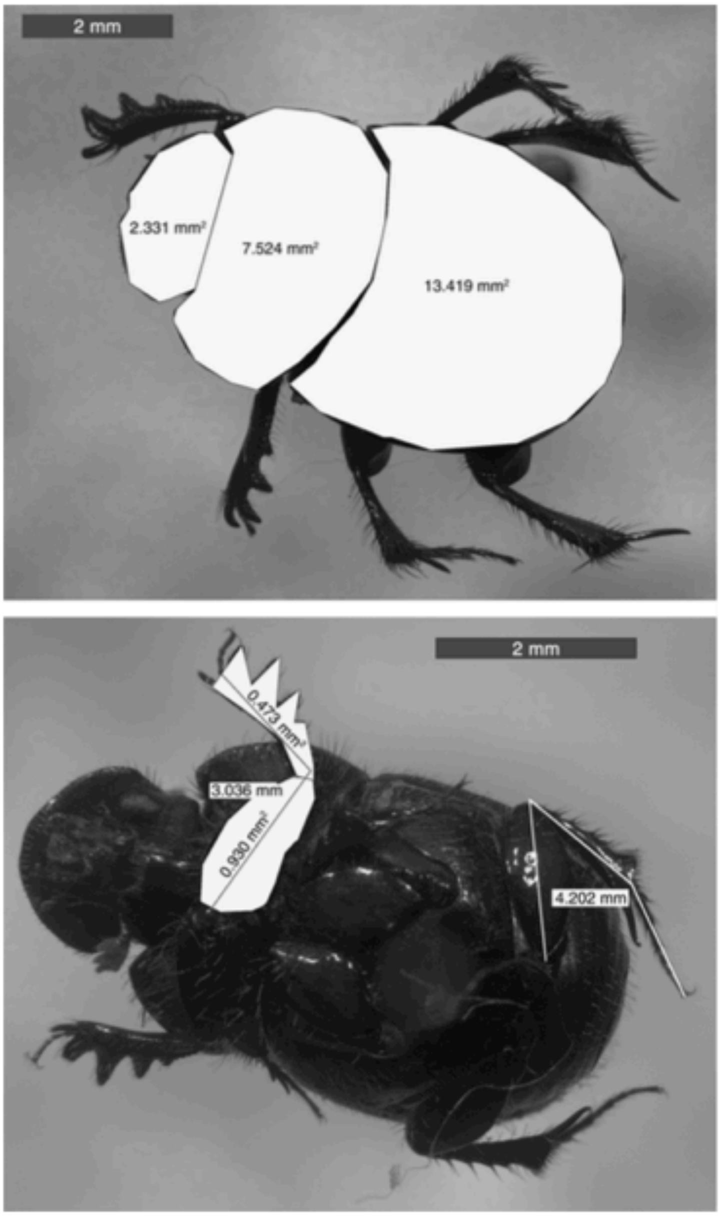

Figure 3.2 | Dung beetle trait measurements collected using Leica M250 microscope and Life Measurement software. Dorsal measurements (top panel): pronotum area and elytra area; ventral measurements (bottom panel): front and back leg length, femur area, tibia area. The scale bar represents 2mm, the species shown is *Cathidium deyrollei.*

3.3.5 Seed dispersal

We quantified seed dispersal in two ways. First, we used a binomial metric of buried or not to assess the probability of burial. Second, we created a novel metric of seed dispersion that provides an indication of both the mean horizontal and vertical distance that seeds were moved from a central point and quantifies the volume of soil occupied by all seeds. This was carried out using the FD package in R to calculate the “Functional Dispersion” (Laliberté & Legendre 2010) of seeds in the soil. We included seed burial depth and horizontal movement (section 1, 2, 3, or 4) as “traits”, and treated each plot as a separate community. Each seed represented a separate “species” of equal abundance.

Of the 1800 seeds used in this study, 284 (15.78%) were not recovered from the soil (2.2% were lost from the clay site; 5.7% from Sand 1; and 7.9% from Sand 2; Table S3.3). We are confident that lost seeds were unrecovered because dung beetles moved them beyond the 50cm x 50cm sampling area below the 10cm deep plot walls (Fig. 3.1) and not because they were buried deeper than 50cm (just six seeds, 0.38% of the total, were found below a depth of 40cm), removed by seed predators (Appendix 3.1 for details of additional experiments to test this possibility), or remained undetected during soil screening (1mm^2 sieve mesh size was smaller than the diameter of the smallest seed 2mm). To incorporate these missing data points into analyses, each unrecovered seed was given a horizontal movement value of 4 (the section outside the outer edges of the plots) and a size and site specific burial depth, which was the median depth of seeds found below 10cm in section 3, calculated separately for each seed size class within each site. Seed dispersion with and without imputed values were highly correlated (Pearson’s $\rho = 0.84$) and inclusion of plausible missing values was considered better than the removal of them, which could reduce the seed dispersion values of the plots with the most variability. See Appendix 3.1 for further justification for this approach, Fig. S3.5 for associations between seed dispersion with and without imputed seeds and Table S3.4 and Fig. S3.6 for the influence of imputation on model results.

3.3.6 Statistical analyses

Statistical analyses were carried using R version 2.14.1 (R Core Team 2013). We used generalised least squares models (gls) in the “nlme” package (Pinheiro *et al.* 2013) to quantify the effect of community manipulation (opening period and opening time), site (Clay, Sand 1, Sand 2) and the two-way interaction between the two factors on the dung beetle community attributes (species richness, functional diversity indices and biomass). We also used gls models to examine how the dung beetle community attributes, site and the two-way interaction between each of the community attributes and site affected seed dispersion. For gls models, appropriate variance structures were applied for site and community attribute (using the varIdent function as described by Zuur et al., 2009) to account for heteroscedastic variance in model residuals. Species richness and total beetle dry biomass were $\log_{10}$-transformed to ensure models satisfied assumptions of normality.

To explore how dung beetle communities influenced probability of seed burial, we used generalised linear mixed effects models (glmm) in the “lme4” package (Bates, Maechler & Bolker 2012). Seeds were assigned a 0 if they were buried and 1 if they remained on the soil surface; as such a binomial error distribution was specified with a logit link function. Bead size was nested within plot as random factors. Plots from which three or fewer beetles were recovered were removed ($n = 4$) from models with functional diversity as a predictor because no functional diversity index could be calculated. From models exploring seed dispersion and probability of burial we also removed plots in which no seeds were moved either horizontally or vertically ($n = 3$).

We used a top-down approach to arrive at the best descriptive model for each of the response variables (Zuur *et al.* 2009). Models were created including all fixed effects and interactions, which were then sequentially removed, until a reduced minimum model was attained, containing only significant terms with $P < 0.05$. Chi-squared likelihood ratio tests (LRT) were used for the gls and glmm models to assess the loss of explanatory power following the removal of an interaction or a single term predictor.

3.4 RESULTS

3.4.1 Beetle communities

A total of 1962 individuals of 61 dung beetle species and morphospecies were recovered from 90 experimental plots within the three sites (Table S3.2 for species list, medium trait values, and abundances). Eighty-nine per cent of beetles were collected from internal pitfalls within the plots, the rest were found in the soil during destructive sampling. The beetle community attributes (total biomass, species richness, FRic, FEve, FDiv, FDis) across the experimental plots were uncorrelated, except for species richness and FRic (Pearson's $\rho = 0.79$, n= 90, Fig. S3.7); however, because species richness is the most commonly used diversity index, we retained it in the analyses. Species richness was highest in Sand 1, FEve was lowest in Sand 1 and biomass was lowest in the Clay site (Table S3.1, Fig. S3.2 and Fig. S3.3).

The beetle community manipulation helped enhance the variation in community attributes across plots. Opening period (whether plots were opened during the day or night) significantly affected species richness, FDiv and FDis: plots that were opened during the day displayed higher values in these diversity indices than those open during the night. There was a significant interaction between opening period and site on FRic: it was higher in plots opened during the day in Clay 1 and Sand 2, but lower in plots opened during the day in Sand 1 (Table S3.1, Fig. S3.2). Opening time (whether the plots were open for 12 or 24 hours) significantly affected species richness, FRic and biomass: plots that were open for 24 hours had higher values than those that were open for 12 hours. FDis was involved in a weakly significant interaction with site: in Clay and Sand 2 FDis is higher in plots opened for 24 hours whereas in Sand 1 opening time had no effect on FDis (Table S3.1, Fig. S3.3).

3.4.2 Dung beetle diversity and seed dispersal

FDiv and total beetle biomass had a consistent positive effect on the likelihood of seed burial across all sites (Table 3.1; Fig. 3.3, (d) and (f)), whereas species richness and FRic had a significant positive effect on burial in the clay soil site only. FEve and FDis had no effect on burial probability. The only dung beetle community attribute that impacted upon seed dispersion was FRic, which, in accordance with its effect on burial (Fig. 3.3, (b)), had a positive effect, but only in the clay soil site (Table 3.1; Fig. 3.4).

Table 3.1 | Model outputs to assess the influence of dung beetle community, site and the interaction between beetle community and site on the probability of seed burial (determined by generalised linear mixed effects models; top section) and seed dispersion (determined by generalised least squares models; bottom section). Significant predictors are highlighted in bold.

	Effect terms								
Model tested	Beetle community			Site			Beetle community × site		
	LRT	df	*P*	LRT	df	*P*	LRT	df	*P*
glmm (seed burial ~ effect terms)									
Species richness	*0.37*	*1*	*0.5450*	*39.40*	*2*	*<0.0001*	**6.67**	**2**	**0.0360**
FRic	*0.00*	*1*	*0.9600*	*39.40*	*2*	*<0.0001*	**11.17**	**2**	**0.0040**
FEve	0.76	1	0.3831	**39.40**	**2**	**<0.0001**	4.12	2	0.1280
FDiv	**3.98**	**1**	**0.0460**	**39.40**	**2**	**<0.0001**	1.52	2	0.4670
FDis	0.11	1	0.7444	**39.40**	**2**	**<0.0001**	2.94	2	0.2301
Total biomass	**4.60**	**1**	**0.0320**	**39.40**	**2**	**<0.0001**	1.04	2	0.5934
gls (seed dispersion ~ effect terms)									
Species richness	0.64	1	0.4228	**21.68**	**2**	**<0.0001**	1.28	2	0.5264
FRic	*0.11*	*1*	*0.7389*	*21.68*	*2*	*<0.0001*	**9.38**	**2**	**0.0086**
FEve	0.00	1	0.9686	**21.68**	**2**	**<0.0001**	4.42	2	0.1100
FDiv	3.83	1	0.0505	**21.68**	**2**	**<0.0001**	3.15	2	0.2066
DFis	0.01	1	0.9348	**21.68**	**2**	**<0.0001**	1.66	2	0.4361
Total biomass	2.65	1	0.1038	**21.68**	**2**	**<0.0001**	1.03	2	0.5963

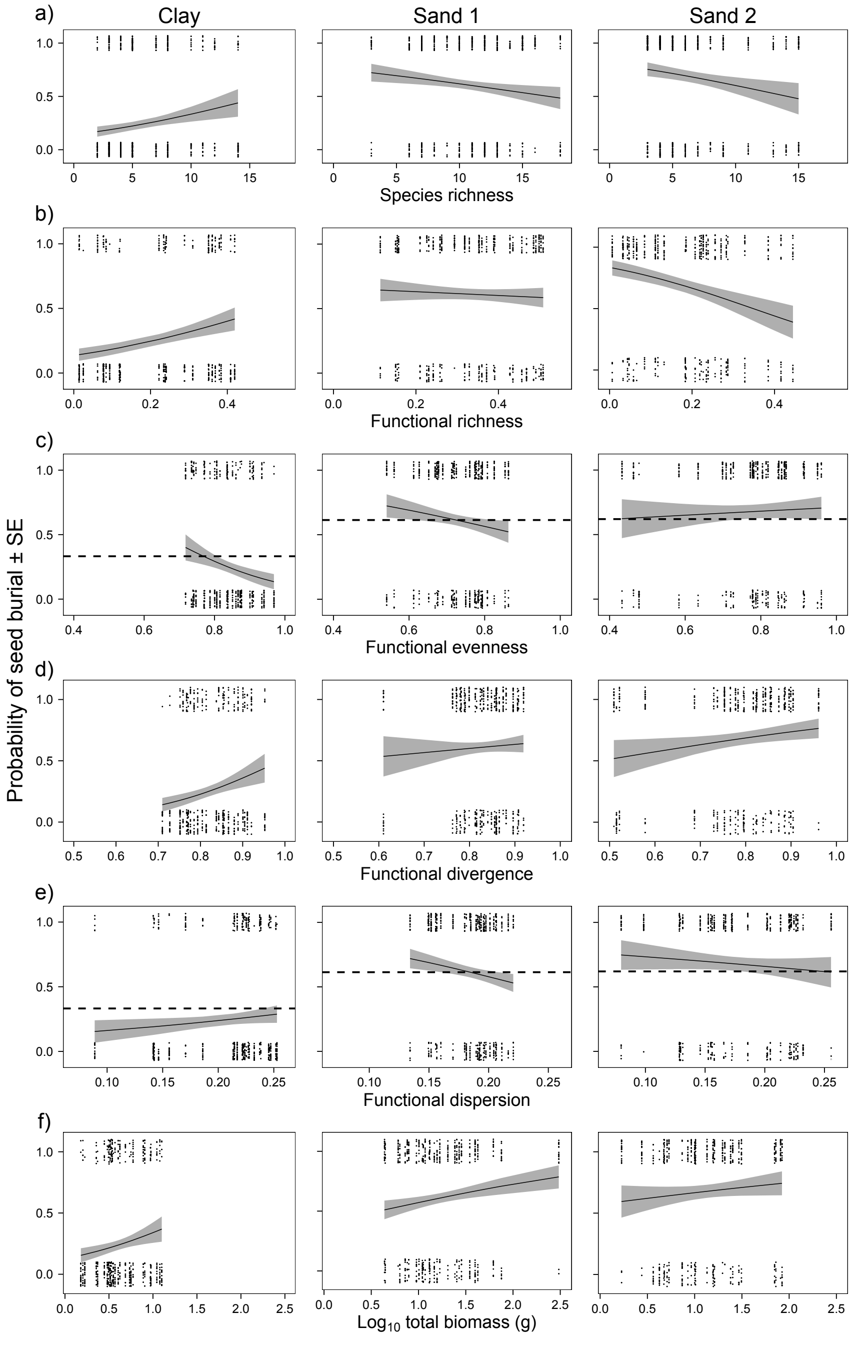

Figure 3.3 | The effect of six different dung beetle community attributes on the probability of seed burial in Clay (left panel), Sand 1 (middle panel) and Sand 2 (right panel) forest sites. Models were generalised linear mixed effect models with binomial error distributions. Predicted values (solid black lines) ± SE (ribbons, calculated using the predict function in R) are displyed for all models. In cases where dung beetle community attribute did not impact upon seed burial (functional evenness and functional disperion) but site did the percentage of seeds bured in each

site is marked on plots (horizontal dashed line) in addition to the direction of response. Individual seeds (small black points) are displayed on plots as either buried (1) or not buried (0).

3.4.3 Environmental context: soil conditions and biodiversity-ecosystem functioning relationships

In cases where all other terms and interactions were removed from models during stepwise deletion, site always remained as the only significant fixed term influencing seed burial and had a consistent significant effect in all other models (Table 3.1). The probability of seed burial was almost twice as high in the sandy soil sites (61.4% and 62% in Sand 1 and Sand 2 respectively) than in the clay soil site (33%) (Fig. 3.3, (c) and (d)). Moreover, the highest probability of burial in the clay soil (≈ 40%, at FRic = 0.42) was lower, or roughly equal to, the lowest probability of burial in both of the sandy soils sites (Fig. 3.3, (b)). Site also significantly affected the magnitude and variability in seed dispersion values; the median was 13% and 17% lower in the clay soil site than in Sand 1 and Sand 2 respectively (Table 3.1, Fig. S3.8), while the interquartile range in the clay soil site was 63% and 70.4% higher than in Sand 1 and Sand 2 (Fig. S3.8).

The direction of the influence of species richness and FRic on probability of seed burial differed between sites as demonstrated by a weakly significant species richness × site and a stronger FRic × site interaction (Table 3.1; Fig. 3.3, (a) and (b)). We found a similar effect of FRic on seed dispersion in that it was involved in a significant interaction with site (Table 3.1); in the clay soil FRic increased the dispersion of seeds, whereas in sandy soils there was no effect (Fig. 3.4). In contrast, FDiv had a consistent positive effect on the likelihood of burial across all soils (Table 3.1), but the strength of these relationships differed. The strongest effects were seen in the clay soil site where seeds were roughly 25% more likely to be buried at the highest compared the lowest FDiv values, whereas the probability of burial increased by only around 10% and a little under 20% at the highest compared to the lowest FDiv values in Sand 1 and Sand 2 respectively (Fig 3, (d)).

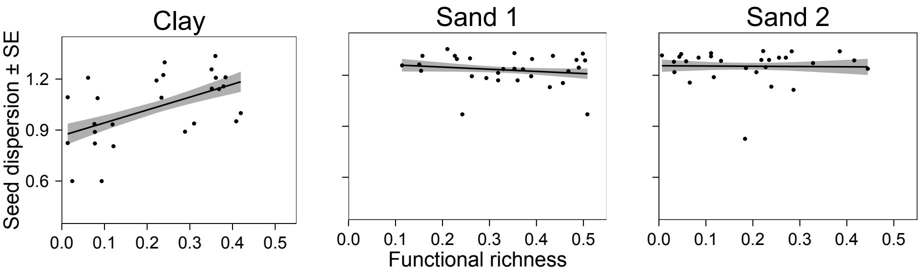

Figure 3.4 | Model predicted values (solid black lines) and SE (ribbons, calculated using the predict function in R) for the significant interaction between dung beetle functional richness and site; Clay (left panel), Sand 1 (middle panel) and Sand 2 (right panel) on the dispersion of seeds in the soil after dung beetle burial.

3.5 DISCUSSION

By using a functional trait-based approach, our study provides new insights into dung beetle mediated BEF relationships. In particular, we reveal a significant positive effect of dung beetle functional diversity on the secondary dispersal of seeds in soil, although we also demonstrate how the strength and direction of relationships can depend on soil type and hence environmental context. Our results show that, under certain soil conditions, a reduction in diversity in this key group of organisms can result in both a decreased likelihood of seed burial in soil and a reduction in the variety of depths and distances that seeds are moved from a central point.

3.5.1 Context dependency in biodiversity-ecosystem functioning relationships

Context dependency in BEF relationships is not a new concept (Lensing & Wise 2006; Dangles *et al.* 2012; Steudel *et al.* 2012), but has been generally overlooked in both producer and animal focussed investigations. Our results demonstrate that the functional consequences of increasing dung beetle species richness and functional richness (FRic) on the probability of seed burial varied in contrasting soil types. The effects were negligible or negative in sandy soils, but positive in clay soil, and, although functional divergence (FDiv) and total biomass positively influenced burial across all sites, the effects were stronger in clay soil. This implies an enhancing effect of diversity on functioning in the clay soil site, but suggests functional redundancy (Harrington *et al.* 2010) in sandy soils, raising the possibility that dung beetle diversity is of most importance for ecosystem functioning where processes are harder to deliver.

Properties of sandy soils, such as increased pore space and reduced cohesion (Marshall, Holmes & Rose 1996), are likely responsible for the generally higher proportion of seeds buried in the sand sites compared with the clay site. These qualities could create favourable, less energetically demanding digging conditions compared with clay soils, and this could lead to the generally higher proportion of seeds buried in the sandy soil sites; a finding in keeping with previous investigations that report reduced dung removal by beetles in clay versus sandy soils (Davis, Doube & McLennan 1988; Davis 1996a). Furthermore, given that clay soils have been found to increase in hardness with depth (Davis 1996b), it is possible that digging extensively in these soils could be restricted to certain species. The reduced importance of FRic for seed dispersion in sandy soils could, therefore, be because a larger number of species were able to utilise a greater variety of nesting depths within this soil type, whereas fewer species could access a full range of depths in the heavier clay soils. Thus a community with low FRic could fully exploit the soil profile in the sandy soil sites, but only a diverse community could achieve the same levels of functioning in clay soils. These results, considering processes mediated by the same focal taxa in the same ecosystem, demonstrate the difficulties in generalising BEF relationships across systems and taxon.

3.5.1 Potential implications for forest regeneration

The positive association between FRic and the variety of seed placements in the soil could affect plant regeneration in a number of ways. Previous investigations have demonstrated that burial by dung beetles is a mechanism for seed predator escape (e.g. Estrada and Coates-Estrada 1991, Feer 1999), but can also lead to mortality through placement of individual seeds within the soil in unsuitable locations for germination and establishment (Estrada & Coates-Estrada 1991; Shepherd & Chapman 1998; Andresen 1999; Andresen & Levey 2004; Koike *et al.* 2012). This suggests that for each species there exists an optimal and unique microsite at which the probability of predation is significantly decreased, whilst germination and establishment is maximised. Decreasing beetle diversity could, therefore, decrease the probability that any particular seed is relocated to its optimal species-specific site for predator avoidance, germination, establishment, and survival. Given that dung beetle taxonomic and functional diversity is known to be sensitive to land use change in the tropics (Barlow *et al.* 2007a; Nichols *et al.* 2007; Gardner *et al.* 2008c; Barragán *et al.* 2011; Beiroz 2013; Edwards *et al.* 2013; Gray *et al.* 2014), our results suggest that such changes in community composition could translate into changes in forest regeneration. However, we acknowledge the limitations of artificial seeds, and additional work is needed that follows real seeds from dispersal to germination and establishment. Although this would undoubtedly be challenging, it would increase our understanding of the multi-trophic links between secondary dispersers and plant communities in tropical forests.

3.5.2 Which community attributes link to ecological function?

Of the indices considered in this investigation, FRic alone significantly impacted on the dispersion of seeds in the soil. This unique association between FRic and the variety of depths and distances that seeds were moved from a central point is likely because FRic represents a multivariate range, describing the volume of multidimensional trait space filled by species. In contrast to the other functional diversity metrics explored here, it is unaffected by species abundance (for a full description of indices seeVilléger et al. 2008 and Laliberté and Legendre 2010). For a dung beetle community to bury seeds within the soil, resulting in a large range in depths and distances in final seed placements, it seems reasonable to hypothesise that individual beetles within that community should display a large range of morphological characteristics. As long as this community displays a wide variety of different traits, the abundance of each species could be comparatively less influential in this process. This could explain why the other indices such as FEve and FDiv did not associate with seed dispersion; they place emphasis on the distribution of abundances within a species assemblage, at the expense of describing the range in values. For the process of distributing seeds widely throughout the soil profile, our results indicate that a large range in functional traits is more important than the distribution of the abundances of traits within the community.

The incorporation of abundance information in dung beetle community attributes may also explain some of the relationships with seed burial. The relative numbers of functionally similar species exploiting the same resource within a biological community could describe levels of competition experienced by individuals within the assemblage, which in turn is known to alter seed burial behaviour. When competition is high, seeds are more frequently buried by dung beetles in brood balls, despite the fact that they represent contaminants by occupying space that would otherwise be filled with food for the developing larvae (Nichols *et al.* 2008). However, in less competitive environments, beetles have been observed to 'clean' the dung of seeds prior to burial (Andresen & Feer 2005). This could shed light on the tendency for FEve to negatively associate (although not significantly) with seed burial and dispersion. A community displaying low FEve is one in which species traits are unevenly distributed, which could increase competitive interactions, resulting in high levels of seed burial. The converse would be true when FEve is high. The discrepancy between the ability of species richness and FRic to predict seed dispersion is also noteworthy given that the two measures of diversity are closely correlated (Pearson's $\rho = 0.79$; Fig. S3.7) and inherently linked (Cadotte *et al.* 2011). Making inferences about ecosystem functioning using a taxonomic approach is, however, problematic because although a speciose community is more likely to display a wider variety of trait values than a species poor community, not every species in that group is necessarily functionally singular. This can result in a non-linear relationship between functional and taxonomic measures meaning that a reduction in taxonomic diversity will not always equate to equal reductions in functional diversity (Naeem & Wright 2003). Our results therefore present important validation for functional approaches (c.f. Clark et al. 2012), which is important because the collection of detailed traits is, in most cases, more costly and time consuming than calculating species richness.

3.5.3 Conclusion

Our field-based investigation into the effects of biodiversity on animal-mediated ecosystem functioning has taken a unique approach to mechanistically link dung beetle communities to secondary seed dispersal, which is an important ecological process in tropical forests. In doing so, we have demonstrated that reductions in dung beetle functional diversity could reduce both the likelihood that seeds are buried and the variety of depths and distances that they are moved in the soil. We also show the importance of environmental context in modulating observed patterns, given that effects of dung beetle functional richness and species richness differed between clay and sandy soils, highlighting the need for further research to provide a more precise description of the mechanisms driving seed burial in different environmental contexts.

An emerging area of interest in BEF research is focussed on understanding how diversity at one trophic level impacts upon diversity or functioning at another (Lavorel et al. 2013, Moretti et al. 2013). Our study provides some interesting insights into how invertebrate functional diversity could impact upon vegetation regeneration via secondary seed dispersal in a species rich tropical system. In particular, the use of detailed continuous trait values measured directly from the naturally assembling community responsible for the process of interest provides a novel platform for further experimental work to examine linkages between invertebrate communities and the functions they perform.

The idiosyncratic patterns reported here between dung beetle mediated seed dispersal, soil type and diversity metric demonstrate the need for continued research into the effects of biodiversity on ecosystem processes in varying environmental conditions, performed by a greater diversity of organisms. These divergent associations are of particular interest because, at present, a precise description of the mechanisms driving these patterns eludes us. Our results therefore highlight important avenues for future investigation, because the concurrent examination of relationships between contrasting diversity metrics, environmental context and ecological processes allows precise hypothesis testing centred around which aspects of community composition matter for functioning, under different abiotic conditions.

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3.8 SUPPLEMENTARY INFORMATION

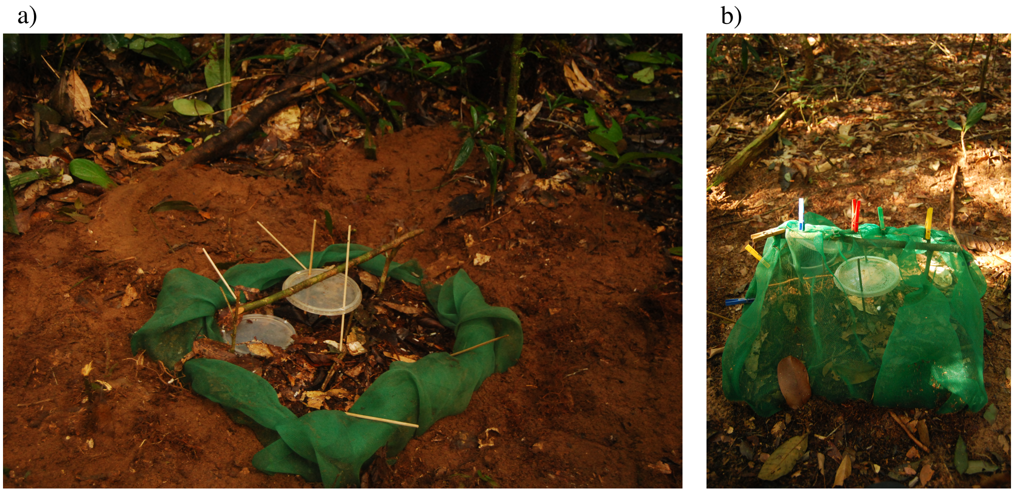


Figure S3.1 | Examples of experimental plots created by burying nylon letting 10cm into the soil in a 50cm x 50cm square on the forest floor. Photo a) shows a plot open for colonisation by dung beetles, b) is an example of a plot closed using pegs following dung beetle colonisation; no beetle can enter or leave. Photographs taken by H.M. Griffiths.

Table S3.1 | Outputs from generalised least squares models (gls) to assess the effect of community manipulation (opening time: 12 or 24 hours, and opening period: day or night, that plots were accessible for beetle colonisation) and site (Clay, Sand 1, Sand 2) on dung beetle community attributes. Significance was assessed by Chi-squared likelihood ratio tests (LRT) using single term deletions.

	Opening period			Opening time			Site			Site x Opening period			Site x opening time		
Index	LRT	df	*P*	LRT	df	*P*	LRT	df	*P*	LRT	df	*P*	LRT	df	*P*
Species richness	15.88	1	<0.0001	18.51	1	<0.0001	31.14	2	<0.0001	2.34	2	0.3110	3.65	2	0.1615
Functional richness	11.65	1	<0.0001	13.36	1	<0.0001	17.55	2	<0.0001	12.52	2	0.0019	0.99	2	0.6101
Functional evenness	0.26	1	0.6136	0.95	1	0.3305	23.82	2	<0.0001	2.91	2	0.2329	4.16	2	0.1248
Functional divergence	5.66	1	0.0173	0.34	1	0.5587	1.35	2	0.5086	0.91	2	0.6355	5.11	2	0.0776
Functional dispersion	14.22	1	<0.0001	3.27	1	0.0707	8.02	2	0.0181	3.58	2	0.1670	6.10	2	0.0474
Biomass	0.21	1	0.6457	4.66	1	0.0309	42.18	2	<0.0001	0.37	2	0.8304	0.79	2	0.6721

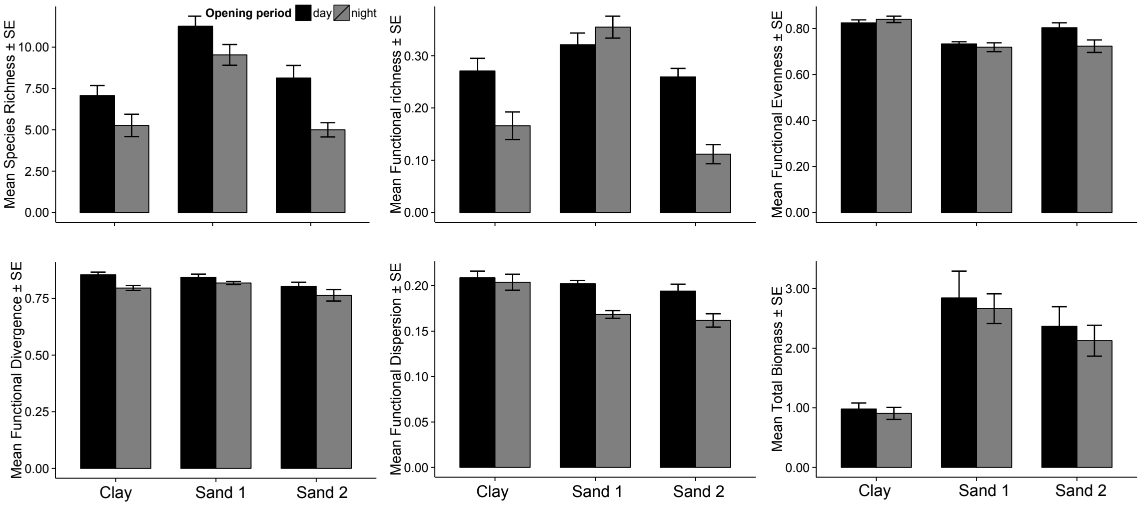


Figure S3.2 | The effect of opening period (day: black bars, or night: grey bars) and site (Clay, Sand 1 and Sand 2) on dung beetle community attributes ± SE (SD/√n)

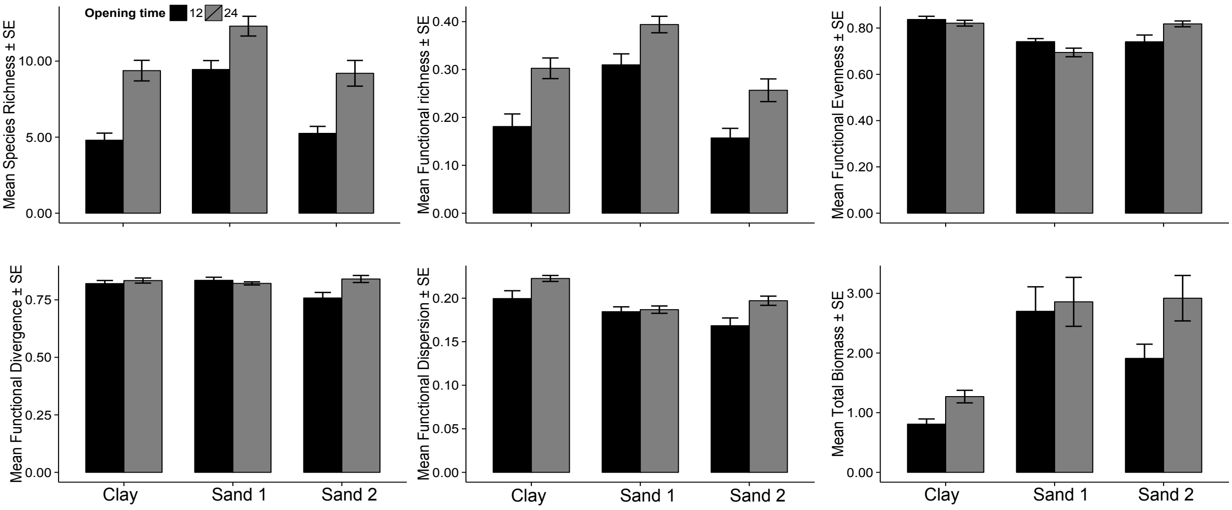


Figure S3.3 | The effect of opening time (12 hours: black bars, or 24 hours: grey bars) and site (Clay, Sand 1 and Sand 2) on dung beetle community attributes ± SE (SD/√n)

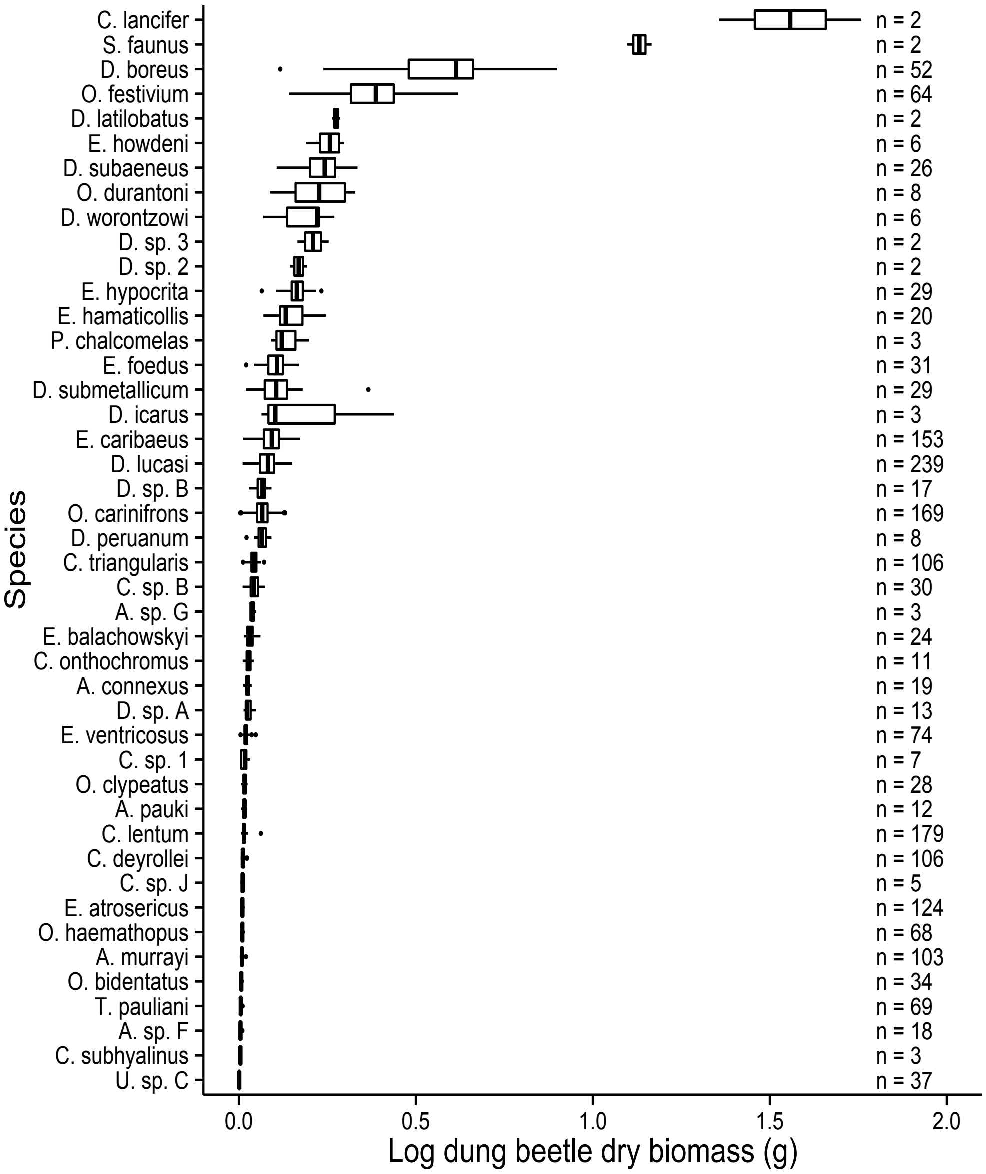


Figure S3.4 | Non-normal distributions in dung beetle dry biomass. Log_{10} median dry biomass and interquartile range of 44 dung beetle species collected from the Brazilian Amazon, Pará. Only species for which more than one individual was collected are displayed.

Table S3.2 | Species abundances, median trait values and categorical traits

Species	Taxonomic authority	Abundance	Biomass (g)	Biomass adjusted front leg area	Biomass adjusted pronotum volume	Back leg to front leg ratio	Nesting strategy	Activity period	Diet
Ateuchus connexus	Harold (1986)	19	0.026	76.84	930.81	1.24	Tunneller	Nocturnal	Coprophogus
Ateuchus murrayi	Harold (1986)	103	0.009	72.64	603.64	1.35	Tunneller	Nocturnal	Coprophogus
Ateuchus pauki	Balthasar (1939)	12	0.016	75.93	717.58	1.33	Tunneller	Nocturnal	Generalist
Ateuchus sp	NA	2	0.002	250.00	1479.27	1.33	Tunneller	Nocturnal	Coprophogus
Ateuchus sp D	NA	1	0.003	124.00	609.00	1.34	Tunneller	Nocturnal	Coprophogus
Ateuchus sp F	NA	18	0.005	110.00	721.28	1.26	Tunneller	Nocturnal	Coprophogus
Ateuchus sp G	NA	3	0.037	56.53	799.94	1.44	Tunneller	Nocturnal	Coprophogus
Canthidium deyrollei	Harold (1967)	106	0.011	115.88	920.99	1.55	Tunneller	Nocturnal	Coprophogus
Canthidium lentum	Erichson (1847)	179	0.015	108.00	1114.08	1.55	Tunneller	Diurnal	Coprophogus
Canthidium sp 1	NA	7	0.018	147.51	1107.26	1.50	Tunneller	Diurnal	Coprophogus
Canthidium sp 3	NA	1	0.008	165.00	1595.30	1.53	Tunneller	Diurnal	Coprophogus
Canthidium sp B	NA	30	0.044	73.11	948.41	1.53	Tunneller	Nocturnal	Coprophogus

Canthidium sp. J	NA	5	0.011	100.94	890.24	1.59	Tunneller	Diurnal	Coprophogus
Canthon subhyalinus	Harold (1867)	3	0.005	172.14	957.96	1.63	Roller	Nocturnal	Generalist
Canthon triangularis	Drury (1770)	106	0.045	79.80	1175.81	1.58	Roller	Nocturnal	Generalist
Canthonella sp	NA	1	0.004	123.81	585.29	1.29	unknown	Unknown	Unknown
Coprophaneus lancifer	Linnaeus (1767)	2	3.844	14.20	524.53	1.23	Tunneller	Generalist	
Deltochilum icarus	Olivier (1789)	3	0.109	126.01	2310.19	1.69	Roller	Generalist	
Deltochilum peruanum	Paulian (1938)	8	0.068	39.98	789.63	1.62	Roller	Nocturnal	Generalist
Deltochilum septemstriatum	Paulian (1938)	2	0.013	130.53	1624.05	1.77	Roller	Generalist	
Deltochilum sp	NA	1	NA	NA	NA	NA	Roller	Diurnal	Generalist
Deltochilum sp A	NA	13	0.023	71.24	1101.08	1.61	Roller	Diurnal	Generalist
Deltochilum sp B	NA	17	0.069	54.86	811.13	1.66	Roller	Diurnal	Generalist
Deltochilum submetallicum	Castelnau (1840)	29	0.112	44.76	838.06	1.67	Roller	Nocturnal	Generalist
Dendropaemon sp 1	NA	1	NA	NA	NA	1.24	unknown	Unknown	Unknown
Dendropaemon sp 2	NA	1	0.040	79.75	985.08	1.21	unknown	Unknown	Unknown

Dichotomius boreus	Olivier (1789)	52	0.847	27.31	801.39	1.22	Tunneller	Nocturnal	Coprophogus
Dichotomius imitator	Felshe (1901)	1	0.228	33.09	663.36	1.83	Tunneller	Diurnal	Coprophogus
Dichotomius latilobatus	Luederwaldt (1931)	2	0.317	44.30	1144.36	1.36	Tunneller	Diurnal	Coprophogus
Dichotomius lucasi	Harold (1869)	239	0.086	57.15	842.66	1.58	Tunneller	Nocturnal	Generalist
Dichotomius sp 1	NA	1	0.141	67.82	1246.37	1.43	Tunneller	Nocturnal	Coprophogus
Dichotomius sp 2	NA	2	0.185	45.31	863.92	1.33	Tunneller	Nocturnal	Coprophogus
Dichotomius sp 3	NA	2	0.235	37.22	597.99	1.03	Tunneller	Nocturnal	Coprophogus
Dichotomius sp 4	NA	1	0.205	36.78	668.25	1.24	Tunneller	Nocturnal	Coprophogus
Dichotomius sp 5	NA	1	0.295	24.37	780.34	1.46	Tunneller	Nocturnal	Coprophogus
Dichotomius sp 7	NA	1	0.057	126.84	1559.39	NA	Tunneller	Nocturnal	Coprophogus
Dichotomius sp 8	NA	1	0.262	43.90	928.00	NA	Tunneller	Nocturnal	Coprophogus
Dichotomius sp 9	NA	1	0.100	67.30	906.34	1.24	Tunneller	Nocturnal	Coprophogus
Dichotomius subaeneus	Castelnau (1840)	26	0.275	35.47	926.50	1.34	Tunneller	Nocturnal	Coprophogus
Dichotomius worontzowi	Pereira (1942)	6	0.247	63.28	1216.25	1.25	Tunneller	Nocturnal	Coprophogus

Eurysternus atrosericus	Génier (2009)	124	0.010	76.30	706.78	2.26	Dweller	Nocturnal	Coprophogus
Eurysternus balachowskyi	Halftter & Halftter (1976)	24	0.031	74.20	1028.41	1.83	Dweller	Diurnal	Coprophogus
Eurysternus caribaeus	Herbst (1789)	153	0.098	54.03	1213.30	1.75	Dweller	Diurnal	Coprophogus
Eurysternus foedus	Guérin-Ménéville (1830)	31	0.114	44.91	988.31	1.82	Dweller	Nocturnal	Coprophogus
Eurysternus hamaticollis	Balthasar 1939)	20	0.142	41.36	1285.62	1.76	Dweller	Nocturnal	Generalist
Eurysternus howdeni	Génier (2009)	6	0.293	41.55	1500.20	1.78	Dweller	Generalist	Coprophogus
Eurysternus hypocrita	Balthasar (1939)	29	0.178	38.93	1002.75	1.66	Dweller	Generalist	Coprophogus
Eurysternus ventricosus	NA	75	0.020	83.00	1115.03	1.76	Dweller	Diurnal	Coprophogus
Ontherus carinifrons	Luederwaldt (1930)	165	0.069	68.67	997.24	1.26	Tunneller	Nocturnal	Coprophogus
Onthophagus bidentatus	Drapiez (1819)	34	0.007	146.55	1401.71	1.33	Tunneller	Diurnal	Coprophogus
Onthophagus clypeatus	Blanchard (1843)	28	0.017	86.57	1069.22	1.33	Tunneller	Diurnal	Generalist
Onthophagus haemathopus	Harold (1875)	68	0.010	109.22	922.92	1.36	Tunneller	Generalist	Coprophogus
Onthophagus onthochromus	NA	11	0.028	122.68	2090.35	1.32	Tunneller	Diurnal	Coprophogus

Onthophagus sp 3	NA	1	0.006	195.00	1640.60	1.47	Tunneller	Diurnal	Coprophogus
Oxysternon durantoni	Arnaud (1984)	8	0.256	42.45	1666.65	1.31	Tunneller	Diurnal	Generalist
Oxysternon festivium	Linnaeus (1767)	64	0.473	31.20	1159.15	1.24	Tunneller	Diurnal	Generalist
Oxysternon silenus	Castelnau (1840)	1	0.196	58.00	1121.30	1.20	Tunneller	Diurnal	Generalist
Phanaeus chalcomelas	Perty (1830)	3	0.129	58.77	1457.00	1.29	Tunneller	Diurnal	Generalist
Sulcophaneus faunus	Fabricius (1775)	2	2.103	22.96	929.45	1.23	Tunneller	Diurnal	Coprophogus
Trichillum pauliani	Balthasar (1939)	69	0.006	82.91	425.01	1.40	Dweller	Nocturnal	Coprophogus
Uroxys sp C	NA	37	0.001	218.75	770.45	1.30	Tunneller	Nocturnal	Coprophogus

Table S3.3 | Number of seeds unrecovered from in each site and size class with values imputed to calculate seed dispersion

Site	Bead	Number below 10cm	Number lost	Median depth of seeds below 10cm	Max depth in section 3	Imputed value
Clay	Med	1	7	NA	20.5	**15.25**
Clay	Small	7	7	13	28	**13**
Clay	Very small	2	25	NA	28	**19**
Sand 1	Med	25	17	17.5	37	**17.5**
Sand 1	Small	27	32	19	35.5	**19**
Sand 1	Very small	11	53	21	44	**21**
Sand 2	Med	34	5	18	30	**18**
Sand 2	Small	44	57	22.75	46	**22.75**
Sand 2	Very small	32	81	21	41	**21**

Appendix S3.1. Unrecovered seed information and calculation of seed dispersion

The assumption that seeds were buried outside of the 50cm x 50xcm sampling area was based on observed burial depths of seeds within the plots. As only 6 seeds (0.38%) were found deeper than 40cm it is unlikely that a significant proportion of the unrecovered seeds were within the sampling area but below 50cm depth. Furthermore, seeds were generally buried progressively deeper, the further from the central dung pat they travelled (main text, Fig. 3.1). Seeds in the section three, therefore, were those most likely to be buried deeper than the 10cm limits of the plot edges.

Appendix S3.2 Trials to assess seed removal by seed predators

Following completion of destructive sampling at site Sand 2, a trial was established to assess the possibility that unrecovered seeds were not found because of removal by seed predators (birds, other vertebrates, or invertebrates). Ten plastic pots containing 100g of dung and twenty beads from the four size classes were placed on the forest floor and left for 24 hours (the maximum time that experimental plots remained open during the study). Dung beetles could not the bury dung and beads because the base of the pots prevented excavation. After 24 hours dung was washed to locate the seeds. All 200 seeds from the ten pots were recovered, indicating that seed predators did not remove beads in experimental plots.

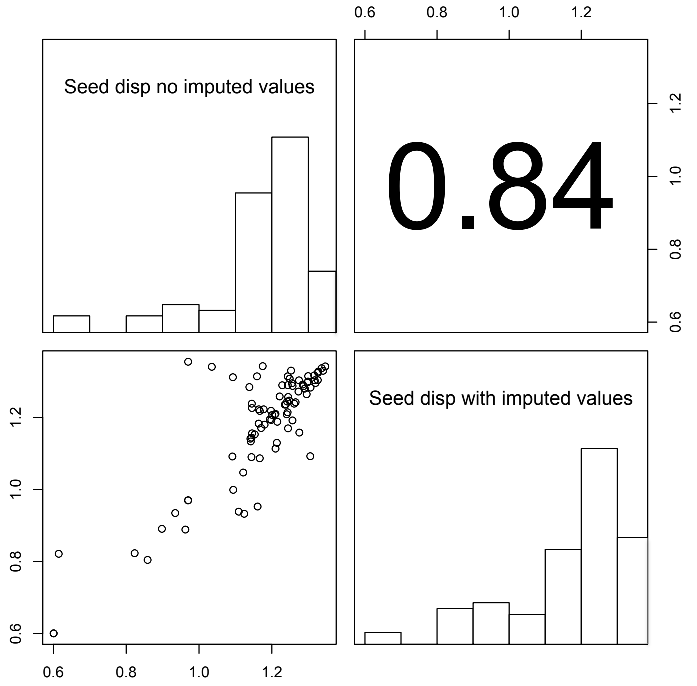


Figure S3.5. | Associations between seed dispersion values calculated with and without imputed values for missing seeds. Bivariate plots (bottom left panel), distributions (top left and bottom right panels), and Pearson's ρ (top right panel).

Table S3.4 | The influence of dung beetle community on seed dispersion when unrecovered seed depths were not imputed for the calculation of seed dispersion. Significant effects are highlighted in bold. Model results remain the same with and without the imputation of missing seed values with the exception of FEve, which changes from having no effect to significantly impacting on seeds dispersion.

	Effect terms								
Model tested	Beetle community			Site			Beetle community × site		
	LRT	df	*P*	LRT	df	*p*	LRT	df	*P*
gls(seed dispersion ~ effect terms									
Species richness	0.00	1	0.9936	**11.74**	**2**	**0.003**	3.48	2	0.1757
FRic	*0.61*	*1*	*0.4332*	*11.74*	*2*	*0.003*	**9.56**	**2**	**0.0084**
FEve	**8.86**	**1**	**0.0029**	**11.74**	**2**	**0.003**	1.04	2	0.5944
FDiv	2.86	1	0.0909	**11.74**	**2**	**0.003**	5.12	2	0.0773
FDis	1.50	1	0.2203	**11.74**	**2**	**0.003**	0.33	2	0.8493
Total biomass	3.58	1	0.0585	**11.74**	**2**	**0.003**	1.55	2	0.4617

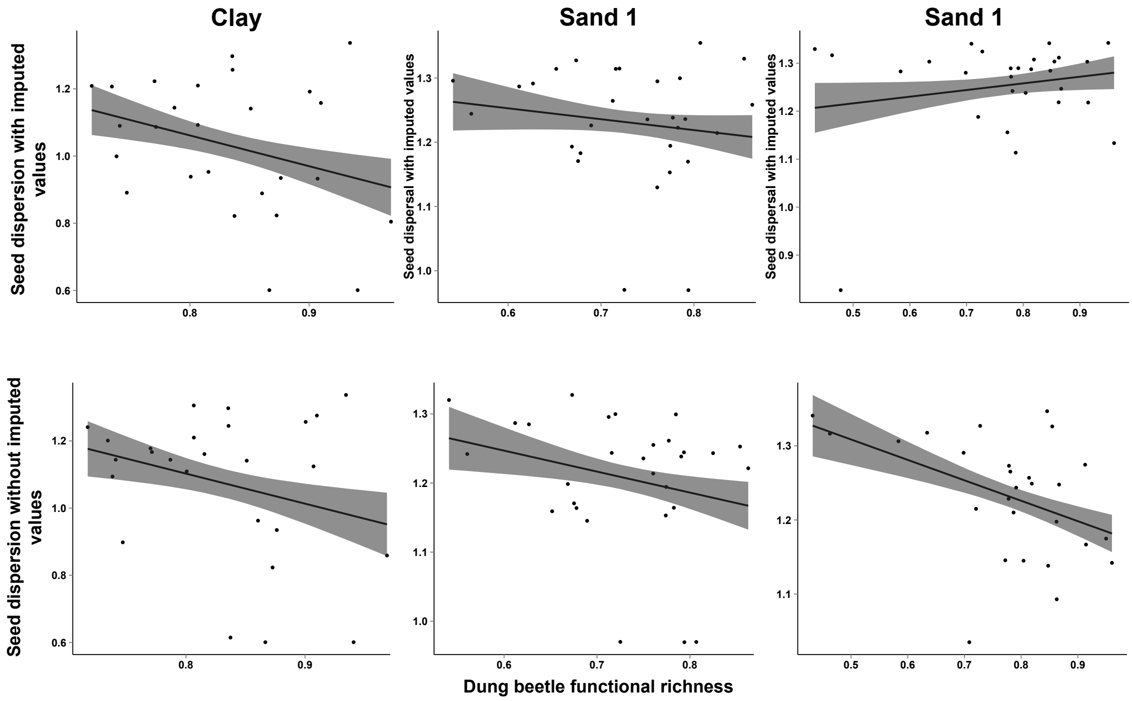


Figure S3.6 | The effect of FEve on seed dispersion in the three experimental sites when seed dispersion is calculated with (top panels) and without (bottom panels) imputation of missing seed value with standard errors (ribbons, calculated using the predict function in R)

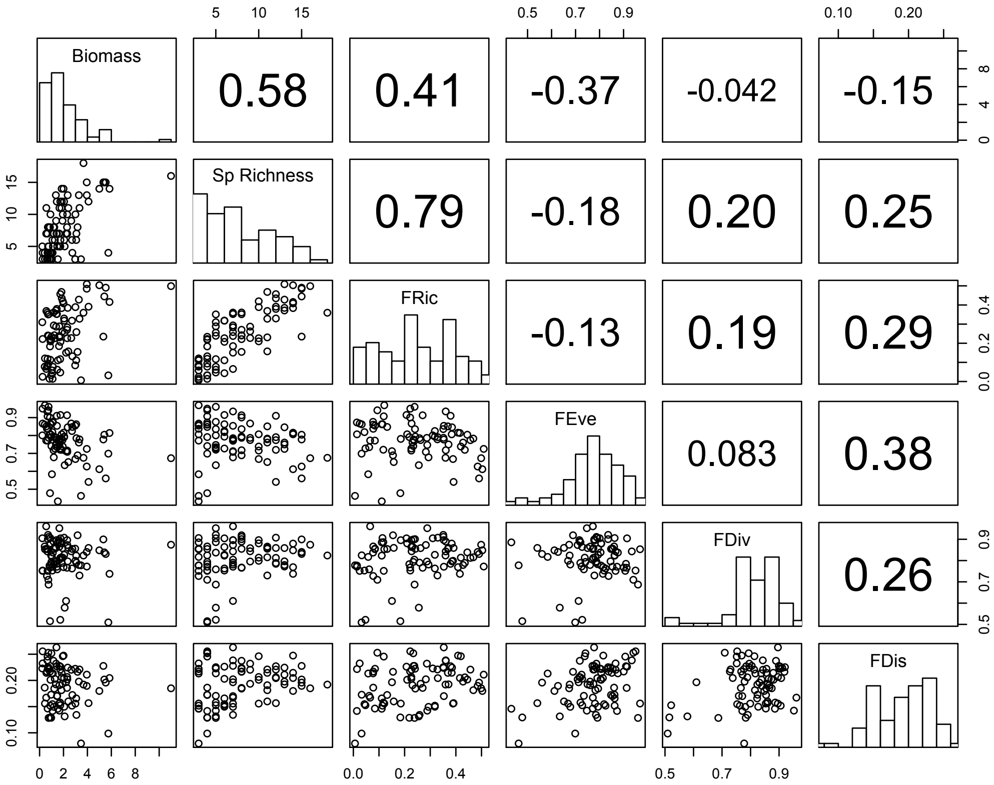


Figure S3.7 | Associations between dung beetle community attributes: bivariate plots (lower panels), distributions (diagonal), and Pearson's ρ (upper panels).

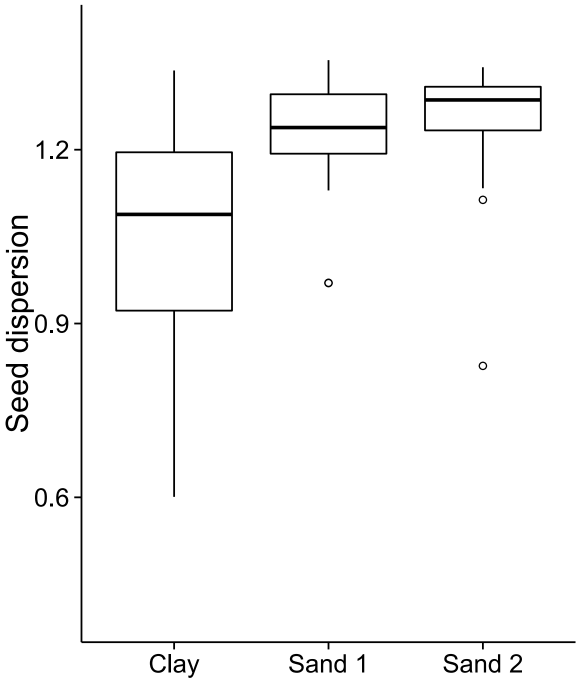
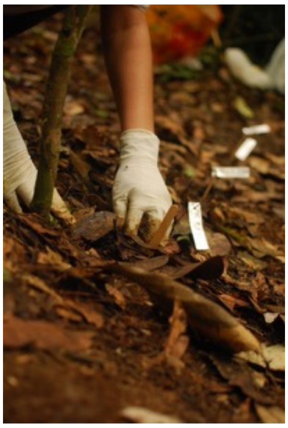
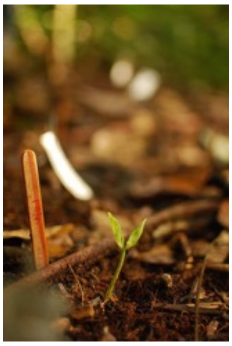


Figure S3.8 | Seed dispersion and interquartile range of seeds buried by dung beetles in the three sites in Brazil, Pará; Clay, Sand 1, and Sand 2. Outliers are displayed (open circles)

Chapter 4

THE INFLUENCE OF TROPICAL FOREST DUNG BEETLE COMMUNITIES ON THE EMERGENCE AND SURVIVAL OF SEEDLINGS





The influence of tropical forest dung beetle communities on the emergence and survival of seedlings

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Hannah M Griffiths*[1,2], Julio N Louzada[1,2], Richard D Bardgett[3], Jos Barlow[1,4]

[1]Lancaster Environment Centre, Lancaster University, Bailrigg, Lancaster, LA1 4YQ, UK.
[2]Departamento de Biologia, Universidade Federal de Lavras, Lavras, Minas Gerais, 37200-000.
[3]Faculty of Life Sciences, Michael Smith Building, The University of Manchester, Oxford Road, Manchester, M13 9PT, UK.
[4] Museu Paraense Emilio Goeldi, Av. Magalhães Barata, 376, Belém-Pará-Brazil
** Corresponding author: han.m.griffiths@gmail.com*

4.1 ABSTRACT

There is a growing consensus that anthropogenic activities are causing widespread species extinctions across the globe, raising concerns about the ecological consequences of changes in animal community composition. Yet we have a very limited understanding about how species loss in one trophic level could impact on the ecological functions governed by organisms in other trophic levels. Here we explore the complexity of multitrophic species interactions by analysing data from field experiments in the Brazilian Amazon that examined the effect of dung beetle communities on seed burial and the emergence and survival of *Myrciaria dubia* seeds. We found that biomass positively influenced the probability of seed burial of all size classes, but had a stronger effect on the burial of larger seeds. This suggests that large seeds may be differentially vulnerable to the loss of their secondary dispersers through anthropogenic driven reductions in large bodied dung beetles. However, dung beetle diversity and biomass negatively influenced the emergence success of *M. dubia,* suggesting that secondary seed dispersal has the potential to inhibit rather than promote the emergence of some species. These findings contrast with the results from seedling survival experiments where dung beetle community attributes positively affected the survival of emerged seedlings. This study therefore advances our understanding of the idiosyncratic nature of plant responses to changes in the community composition of their secondary dispersers in tropical forests. Furthermore, our findings demonstrate for the first time that small-scale soil modification by dung beetles could influence seedling recruitment. We thus present a novel way in which dung beetle communities could influence tropical forest regeneration and ultimately influence future vegetation composition.

Key words: *defaunation; plant recruitment; biodiversity-ecosystem functioning; soil environment; animal-mediated ecosystem processes*

4.2 INTRODUCTION

Human activities over the past 500 years have caused the known loss of 322 vertebrate species and many more animals are threatened with extinction (Dirzo *et al.* 2014). The complete loss of species or populations of wildlife is of concern for the maintenance of ecosystem processes that provide vital goods and services to humanity (Cardinale *et al.* 2012). So too is the on-going decline in the abundances of animals that remain. Defaunation describes both the extinction of species or populations as well as local declines in abundances (Dirzo *et al.* 2014). The well-established primary drivers of defaunation include overexploitation, land-use change, and the associated impacts of species losses and gains due to invasive species (Hoffmann & Al. 2010; Wardle *et al.* 2011). These anthropogenic drivers of change in biological communities are likely to continue throughout the 21st Century (Pereira *et al.* 2010), but will be compounded by emerging global threats to biodiversity such as climate change (Sala *et al.* 2000), and disease resulting from the introduction of novel pathogens (Smith, Sax & Lafferty 2006). The ecological consequences of this biological erosion are not fully understood, but it is increasingly recognised that they will lead to the extinction of species interactions and a loss of associated ecosystem processes (Cardinale *et al.* 2012; Hooper *et al.* 2012; Valiente-Banuet *et al.* 2014).

The geographic pattern of defaunation is non-random (Pimm *et al.* 2014), with tropical forests displaying the highest documented rates of population declines in bird and mammal species (Dirzo *et al.* 2014). Unsustainable hunting, in conjunction with habitat loss and modification, has been identified as one of the primary causes of vertebrate species loss in tropical forests (Corlett 2007; Peres & Palacios 2007; Fa & Brown 2009). Hunting often affects vertebrate species differentially as a function of their body size, with large-bodied mammals such as ungulates and large primates being most at risk because they are preferred game species (Peres & Palacios 2007; Kurten 2013). They also display a suite of life history traits such as a low reproductive rate and large home range that increase their vulnerability to exploitation and habitat loss (Cardillo *et al.* 2005; Öckinger *et al.* 2010).

The disproportionate removal of large-bodied species from tropical forest ecosystems can have indirect and cascading effects on ecosystem ecology. First, density compensation can occur, where the abundances of medium and small bodied vertebrates increase as they are released from competitive interactions for food and habitat (Peres 2000; Wright 2003). This structural modification of the tropical forest vertebrate community may then impact upon plants, through changes in the abundance of frugivores, granivores and/or folivores. Consequently, defaunation alters animal-plant interactions, such as seed dispersal, predation, herbivory and trampling, altering plant demography and community composition (Harrison *et al.* 2013; Kurten 2013) via changes in seedling recruitment (Wright *et al.* 2000; Galetti *et al.* 2006), survival (Asquith *et al.* 1999; Guariguata, Adame & Finegan 2000) and distribution (Wright & Duber 2001). There is growing evidence that this ultimately alters seedling density (Terborgh *et al.* 2001; Nunez-Iturri & Howe 2007; Harrison *et al.* 2013), abundance (Royo & Carson 2005; Beck, Snodgrass & Thebpanya 2013) and diversity (Wright, Hernandéz & Condit 2007; Stevenson & Aldana 2008; Harrison *et al.* 2013).

The secondary dispersal of seeds by dung beetles represents an extra layer of complexity in the linkages between vertebrates and plants (e.g. Culot, Huynen & Heymann 2014). Seeds contained within mammalian dung are frequently relocated to beneath the soil surface because dung beetles move and bury faeces for feeding and nesting purposes (Hanski & Cambefort 1991). This can benefit buried seeds by placing them in a more suitable microsite for germination and growth (Shepherd & Chapman 1998; Andresen & Levey 2004), or through the avoidance of density dependent competition and mortality (Andresen & Feer 2005; Lawson *et al.* 2012) as well as escape from seed predation (Estrada & Coates-Estrada 1991; Shepherd & Chapman 1998; Feer 1999). Because dung beetles are dependent on mammalian faeces, they are sensitive to changes in dung resource availability following defaunation (Andresen & Laurance 2007; Nichols *et al.* 2009, 2013b; Culot *et al.* 2013). Changes in dung beetle community composition have been reported in defaunated forests where the abundance of small-bodied species increase as large-bodied species decline (Culot *et al.* 2013). Both seed burial and the dispersion of seeds throughout the soil profile are positively linked to dung beetle taxonomic and functional diversity (Slade *et al.* 2007b; Braga *et al.* 2013; Griffiths *et al.* 2015), and large-bodied beetles have a disproportionally important role in seed and dung burial (Slade *et al.* 2007b; Gregory *et al.* 2014). Therefore, it seems likely that the cascading effect of mammal defaunation on dung beetle diversity and community structure could impact upon the germination and establishment of secondarily dispersed seeds. However, to our knowledge this has not yet been empirically tested.

The aim of this investigation was to explore how dung beetle taxonomic and functional diversity influences the burial, germination and survival of seeds in a tropical forest under realistic field conditions. To do this, we carried out mesocosm experiments in which naturally assembled communities of beetles buried either seed mimics or live seeds to test the following hypotheses. First, because large bodied dung beetles are instrumental in the dispersal of large seeds (Feer 1999), we predicted that large seeded species are more sensitive to reductions in dung beetle biomass and diversity than smaller seeds. Second, we build upon previous work from Chapter 3 where dung beetle diversity positively influenced the likelihood of seed mimic burial and dispersion throughout the soil profile (Griffiths *et al.* 2015). Because our ability to make inferences about the consequences of these patterns was limited by the use of artificial seeds, we carried out experiments using real seeds. We tested the hypothesis that dung beetle diversity positively influences seedling emergence and survival because: 1) burial decreases seed predation (Estrada & Coates-Estrada 1991; Shepherd & Chapman 1998; Feer 1999) and 2) the greater the variety of depths and distances a seed community is dispersed from a central point, the higher the likelihood that each individual seed will be placed in its optimal species-specific microsite for germination and growth. These experiments were complemented by germination trials where experimental seeds were placed at different depths within the soil profile, either with or without dung. This allowed us to examine the optimal burial depth of seeds and investigate how the presence of dung influences emergence and survival. Here, we hypothesised that highest germination would occur in microsites near the surface (from 1cm to 4cm), that are deep enough to reduce predation, yet shallow enough to avoid soil depth preventing emergence following germination (c.f. Shepherd & Chapman; 1998 and Andresen & Levey; 2004).

4.3 METHODS

4.3.1 Study site

The study was conducted from July to August 2012 and February to June 2014 in the 17 000-km^2 Jari Florestal landholding, located in the State of Pará, north-eastern Brazilian Amazon (0°53S, 52°36W). This area consists of a matrix of *Eucalyptus* plantations, regenerating secondary forests, and large areas of largely undisturbed primary *terra firme* rainforest. Within this landscape, experiments were established in three primary forests sites, classified as dense lowland tropical rain forest and subject to the same regional climatic conditions (see Chapter 3 for detailed site description).

4.3.2 Seed mimic burial in mesocosms

During July and August 2012 we established a grid of thirty mesocosms, separated by 100m, at each experimental site (n = 90 in total). Mesocosms were created by burying nylon netting 10cm into the forest floor in a 50cm x 50cm square and were baited with 100g mixture of 50:50 human and pig dung containing 20 plastic seed mimics (beads) of 4 different sizes: 2 large (20mm diameter, 4.12g), 6 medium (10mm diameter, 0.50g), 6 small (5mm diameter, 0.09g), and 6 very small (2mm diameter, 0.06g). The dung and seed mimics were placed on the floor in the centre of the plots and protected from the rain by a plastic cover. After baiting, mesocosms were closed using pegs to hold the netting together in a way that ensured the beetles could not leave and prevented further colonisation by beetles that had not buried the dung. Each mesocosm also contained an internal, non-baited pit-fall trap (13.5cm width, 9cm depth), buried flush with the ground surface and filled with a salt-water solution. Internal traps were opened when mesocosms were closed to capture the beetle community that had carried out the function following emergence from the soil. After closure, mesocosms were left for between 7 and 14 days before the soil beneath the dung was destructively sampled to a depth of 50cm in search of the seed mimics buried by the beetles. Internal pitfall traps were removed and the beetles were oven dried and stored for laboratory processing. See Chapter 3 for detailed experimental design and rationale.

4.3.3 Seed emergence and survival in mesocosms

Following the procedure described above, in February 2014, we created a further 90 mesocosms in one of the experimental sites characterised by clay textured Oxisols (mean clay content ± SE: 67.3 ± 1.5%, silt: 14.4 ± 1%, sand: 14.1 ± 1.1%) with aluminium sesquioxides (0°38` 46.418"S, 52°34` 11.125"W). This site was selected based on findings from Chapter 3 that demonstrated a stronger influence of dung beetle diversity on secondary seed dispersal in this site compared with other sites in the region (Griffiths *et al.* 2015). Each mesocosm was baited with 100g of human and pig dung containing two seeds each of five animal-dispersed, commercially important Amazonian fruit species: *Genipa americana, Malpighia emarginata, Myrciaria dubia*, *Psidium guajava* and *Rubus chamaemorus.* These species were chosen based on local availability and to approximately represent the range in sizes of seed mimics used in the previous investigation (Table S4.1 for species mean weights and dimensions). These species were chosen based on local availability and to approximately represent the range in sizes of seed mimics used in the previous investigation (Table S4.1 for species mean weights and dimensions). The quantity of dung and number, size, and species richness of seeds were based on mean values from howler monkey (*Alouatta seniculus*) defecations reported by Feer (1999) and Andresen (2002). Furthermore, 20 seeds (and not a greater sample size) were selected as an appropriate number for placement in baits because dung beetles are known to reject a dung food source if too many seeds are present, presumably because this represents too small a dung: seed (contaminant) ratio (*pers comm.* J. N. Louzada).

Dung and seeds were placed on the forest floor at the centre of the mesocosms between 07:00 and 09:00 and were protected from the rain by a plastic cover. To enhance variation in the diversity of dung beetle communities, we randomly assigned mesocosms to one of three experimental treatments (n = 30 in each): control: baited and closed immediately, using pegs to hold the nylon netting together, thus preventing any beetles from accessing dung and seeds; exclusion treatment: a 50cm x 50cm wire cage was placed over the dung and seeds (mesh size 15mm x 8mm) within the mesocosms, preventing the largest beetles from entering plots (see Slade *et al.* 2007; Dangles, Carpio & Woodward 2012); open treatment: baited and left open for colonisation by all beetles. During the establishment of mesocosms, nine were baited each day for 10 days (n = 3 per treatment, per day). The exclusion and open treatments were left for 24 hours following baiting before closure.

Internal pitfall traps were opened when mesocosms were closed to capture the beetle community that had buried dung and seeds following their emergence from the soil. Mesocosms were left closed in this way for two weeks, during which time internal pitfall traps were emptied of beetles and refilled with saltwater once. After two weeks, we removed the pitfall traps and nylon netting covering mesocosms to prevent light level or microclimate interference within and to allow seed predator and herbivore access to seeds/seedlings. All beetles recovered from within the mesocosms were dried and stored for laboratory processing. After initial baiting, mesocosms were monitored weekly for 18 weeks to assess emergence and survival of seedlings. The binary metric of dead/alive (survival) was based on an emerged seedling remaining present and alive until the end of the 18-week monitoring period.

4.3.4 Germination trials

Concurrently with the seed emergence and survival experiments in 2014, we created nine plots in the field to assess how burial depth and the presence of dung influenced emergence and survival of experimental seeds and seedlings. In each 120cm x 200cm plot we planted seeds at 10 different depths (n = 40 per species; n = 200 seeds per plot). The burial mesocosms (carried out in 2012) demonstrated that seed mimics were more frequently buried near the soil surface compared with deeper placements in the soil profile (Griffiths *et al.* 2015). We therefore chose a higher resolution between planting depths near the soil surface to reflect this, namely: above the leaf litter, below the leaf litter, 1cm, 2cm, 3cm, 5cm, 7cm, 10cm, 15cm and 20cm. At each depth, seeds were either planted alone or in the centre of a 1g ball of 50:50 human and pig dung (n = 2 per treatment, per depth). Plots were divided into 10cm^2 sections, seeds were assigned a depth x treatment (dung or alone) and placed randomly within the plots. This resulted in the planting of 1800 seeds in total (200 seeds x 9 plots). Following planting, germination plots were monitored weekly for emergence and survival of seedlings.

4.3.5 Dung beetle traits and diversity metrics

We identified beetles to species using a reference collection held at the Universidade Federal de Lavras (UFLA), Brazil, and region specific identification keys developed by T. A. Gardner and F. Z. Vaz-de-Mello. To calculate functional diversity, we used four continuous morphological traits: biomass, biomass adjusted pronotum volume, biomass adjusted front leg area, back: front leg length; as well as three categorical behavioural traits: nesting strategy (tunneller, roller, dweller), diurnal activity (diurnal, nocturnal, crepuscular, or generalist) and diet (coprophagus or generalist). These seven traits were selected because they were linked to dung beetle mediated seed dispersal in Chapter 3 (Chapter 3; Griffiths *et al.* 2015). Furthermore, dung beetle body size, diurnal activity and nesting strategy have been previously linked to beetle mediated processes, with large nocturnal tunnelling beetles being more instrumental in dung and seed removal than other guilds (Slade *et al.* 2007). We selected pronotum volume because this is the part of beetle anatomy where the muscles responsible for moving the front legs and therefore, the digging apparatus are located; front leg area was selected as the front legs are the dung beetle digging tools (along with head area, but as this was collinear with leg area, we selected only one trait to describe digging capacity); and back : front leg length was selected as an measure of the dung beetle's capacity to roll dung balls (rolling and tunnelling beetles have a larger back : front leg ratio compared to tunnelling beetles; Inward *et al.* [2011]). All traits were measured from every individual sampled from the 2012 bead burial experiments (n = 1962) and where possible, we used these data for the species median trait values and categorical information for beetles collected in the 2014 emergence and survival trials. However, biomass displays the greatest intraspecific variability in dung beetle traits, therefore information is required from a greater number of individuals compared with other traits in order to accurately summarise species trait values (Chapter 2). As such we measured the biomass of every individual collected in 2014 (n = 1-88 per species) and combined these data with the biomass values of individuals collected in 2012 and created new species median values. Additionally, where species median pronotum volume, front leg area and leg lengths were calculated from fewer than 15 individuals in 2012, if additional individuals were collected during 2014, we measured these traits from sufficient individuals to create a sample size of n = 15 for all species where possible (Table S4.2 for species abundances and trait information). See chapter 3 for details of trait measurements.

Species richness and total biomass were calculated for all mesocosms that contained beetles. Median biomass, biomass adjusted pronotum volume, biomass adjusted front leg area, back: front leg length, nesting strategy, diurnal activity and diet were used to calculated functional richness (FRic), which is a multidimensional measure of the range of traits in a biological community (Villéger *et al.* 2008). We selected FRic because out of four functional diversity metrics previously assessed for their association with beetle mediated seed dispersal, FRic was consistently the strongest predictor of seed burial and dispersion of seeds through the soil profile (Chapter 3; Griffiths *et al.* 2015). Additionally, we calculated community weighted means (CWM) of the individual traits used to calculate FRic: biomass, adjusted pronotum volume, adjusted front leg area and back: front leg length for each beetle community. These describe the mean value of each trait in the communities, weighted by the relative abundances of the species carrying that trait (Garnier *et al.* 2004; Violle *et al.* 2007). Functional richness and CWM traits were calculated using the "FD" package in R 3.0.2 (Laliberté *et al.* 2012; R Core Team 2013).

4.3.6 Focal seed species

Fifty-seven per cent of *M. dubia* seeds emerged from within mesocosms and 18% from within germination plots, compared to an emergence success of less than 10% and 5% from within mesocosms and germination plots respectively for the other four species (Fig. S4.1). Therefore, although we used five seed species in experiments, we focus results on only *M. dubia* (similar in dimensions to the medium bead used in burial trials; Table S4.1) because emergence of the other species was too low to allow analyses. *M. dubia* (HBK) McVaugh, is a small, dicotyledonous tree, belonging to the *Myrtaceae* family. It is widely distributed across the north-eastern Brazilian Amazon, Peru and Venezuela where it forms high density conspecific stands (Peters *et al.* 1989). It grows to a height of between 3-8m, and produces spherical fruits 2-5cm in diameter. Each fruit contains 2 seeds (Cavalcante 1996, see Table S4.1 for average weight and dimensions of seeds). The flowers of *M. dubia* are largely pollinated by *Apidae, Anthophorida* and *Halictidae* bees, and the fleshy fruits are exploited by primates and other forest vertebrates (Gressler, Pizo & Morellato 2006). *M. dubia* fruits are also an economically important non-timber forest product, used both locally in the preparation of fruit juices and alcohol (Peters *et al.* 1989) and exported on international markets because of high levels of nutritionally important minerals (Akter *et al.* 2011).

4.3.7 Statistical analyses

Analyses were carried out in R version 3.0.2 (R Core Team 2013). Our first objective was to test the hypothesis that large seeds are more vulnerable than smaller seeds to reductions in beetle biomass and diversity. To do this we used generalised linear mixed effects models (glmm) from the "lme4" package (Bates *et al.* 2012) to investigate if bead size, dung beetle community metric (species richness, functional richness, total biomass and CWM traits) and the interaction between the two factors affected the probability that beads were buried (2012 experiment). Each community metric was included in a separate model and mesocosm was nested within site as random factors.

Our second aim was to examine if dung beetle diversity positively influences seedling emergence and survival until the end of the 18-week monitoring period. First, we used linear models (lm) to assess if treatment (with or without exclusion cage) had succeeded in enhancing the variety in beetle community metrics across mesocosms (2014 experiment). Three of the mesocosms (one open, and two exclusion cage treatments) were not colonised by any beetles; these were removed from analyses because they created a distribution in values that could not be fitted to models (Fig. S4.2 for distributions of community metrics). We used glmms to assess how the presence (open and exclusion cage treatments pooled, n = 60) or absence (closed treatments, n = 30) of beetles, and beetle community metrics within mesocosms influenced the probability of seed emergence and survival until the end of the 18-week experimental period. Mesocosm was included as a random factor.

Our final goal was to assess the optimal burial depth of *M. dubia* seeds and to investigate if the presence of dung influences seedling emergence or survival. First, we tested if burial depth influenced the week that seeds emerged from the soil using linear mixed effects models (lmer) from the "lme4" package (Bates *et al.* 2012). Second, we used glmms to ascertain if burial depth, the presence of dung and the interaction between the two factors influenced probability that seeds emergence from the soil and subsequently survived until the end of the 18-week monitoring period. Third, glmms were also used to investigate if the week that seedlings emerged influenced the likelihood that they survived until the end of the experimental period. Germination plot was a random factor in lmers and glmms.

Within glmm models assessing the likelihood of bead burial, beads were assigned a 1 if they were buried and a 0 if they remained on the soil surface; in seed emergence models, seeds were assigned a 1 if they emerged from the soil surface and a 0 if they did not; in models assessing the likelihood of survival, seedlings that emerged where assigned a 1 if they survived until the end of the monitoring period and a 0 if they did not. As such a binary error distribution with a logit link function was specified for all glmms. All community metrics were $\log_{10}$-transformed to ensure models satisfied assumptions of normality. Models were created using all fixed terms and interactions, we then used a top-down approach to arrive at the best descriptive model (Zuur *et al.* 2009) in which only significant terms ($P < 0.05$) remained. Chi-squared likelihood ratio tests (LRT) were used for glmm models and anovas for lm models to assess the loss of explanatory power following removal of an interaction or a single term predictor.

4.4 RESULTS

4.4.1 Seed mimic burial in mesocosms

Bead size had a highly significant impact on the likelihood that dung beetles buried beads (LRT = 398.99, Df = 6, $P < 0.0001$) and significantly affected the depth at which they were placed within the soil (LRT = 325.91, df = 7, $P < 0.0001$). Both the proportion of beads buried and burial depth decreased with increasing bead size (Fig. 4.1). Biomass and CWM back: front leg length were the only dung beetle community metrics that significantly affected the probability of bead burial. Biomass had a consistent positive effect on the likelihood that beads of all size classes were buried (LRT = 4.53, df = 7, $P = 0.033$). However, the effect was stronger for the burial of medium sized seeds: the probability of burial increased from around 20% at the lowest biomass values to around 70% at the highest values for medium seeds compared to an increase from 70% to 90% for very small seeds and a 60% to 80%

increase for small seeds (Fig. 4.2 (a)). There was a significant interaction between CWM back: front leg length and bead size (LRT = 9.23, df = 10, $P = 0.0264$). An increase in CWM back: front leg length had a negative effect on the likelihood that small and very small beads were buried (a reduction of 80% to 55% and 90% to 65%, respectively, but did not affect the probability that medium seeds were buried (Fig. 4.2 (b)).

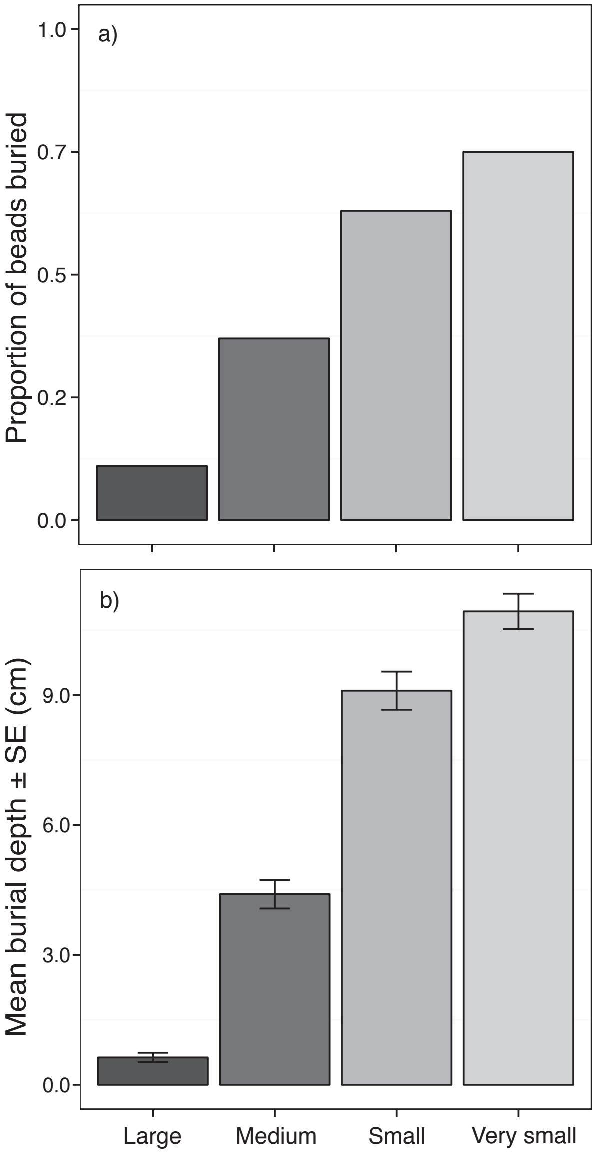

Figure 4.1 | The proportion of seed mimics of different size classes buried by dung beetles (a) and mean burial depths ± SE (SD/√n) (b) the 2012 mesocosm experiments

4.4.2 Beetle communities in emergence and survival in mesocosms

A total of 690 dung beetles of 45 species were collected from pitfalls within the 60 mesocosms that were open for beetle colonisation (open and exclusion cage treatments). No beetle activity was recorded within the 30 closed treatment mesocosms. Associations between beetle community metrics varied from Pearson's $\rho = 0.07$ (CWM biomass and species richness) to Pearson's $\rho = 0.85$ (FRic and species richness; Fig. S4.2). Mesocosm treatment (presence or absence of an exclusion cage) was successful in enhancing the variation in community attributes across the mesocosms: treatment significantly influenced the FRic, species richness, total biomass and CWM leg area of the beetle communities within (Table S4.3). Although there was a continuous distribution in community metric values between the treatments, mesocosms with exclusion cages had significantly lower FRic, species richness and total biomass, but significantly higher CWM front leg area values (Fig. 4.3).

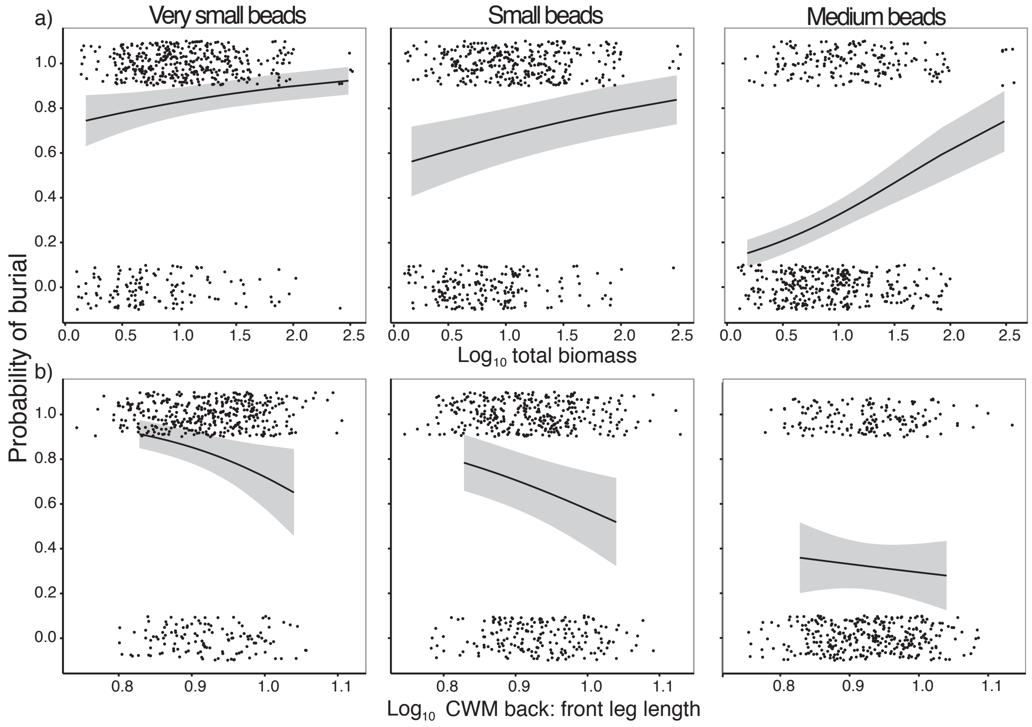

Figure. 4.2 | Significant effects of dung beetle total biomass (a) and CWM back: front leg length (b) on the probability of burial from 2012 burial experiments of very small beads (left panels), small beads (middle panels) and medium beads (right panels). Significance determined by generalised linear mixed effects models. Predicted values (solid black lines) ± SE (ribbons, calculated using the predict function in R) are displayed along with individual seeds (black points), which were either buried (1) or remained on the soil surface (0).

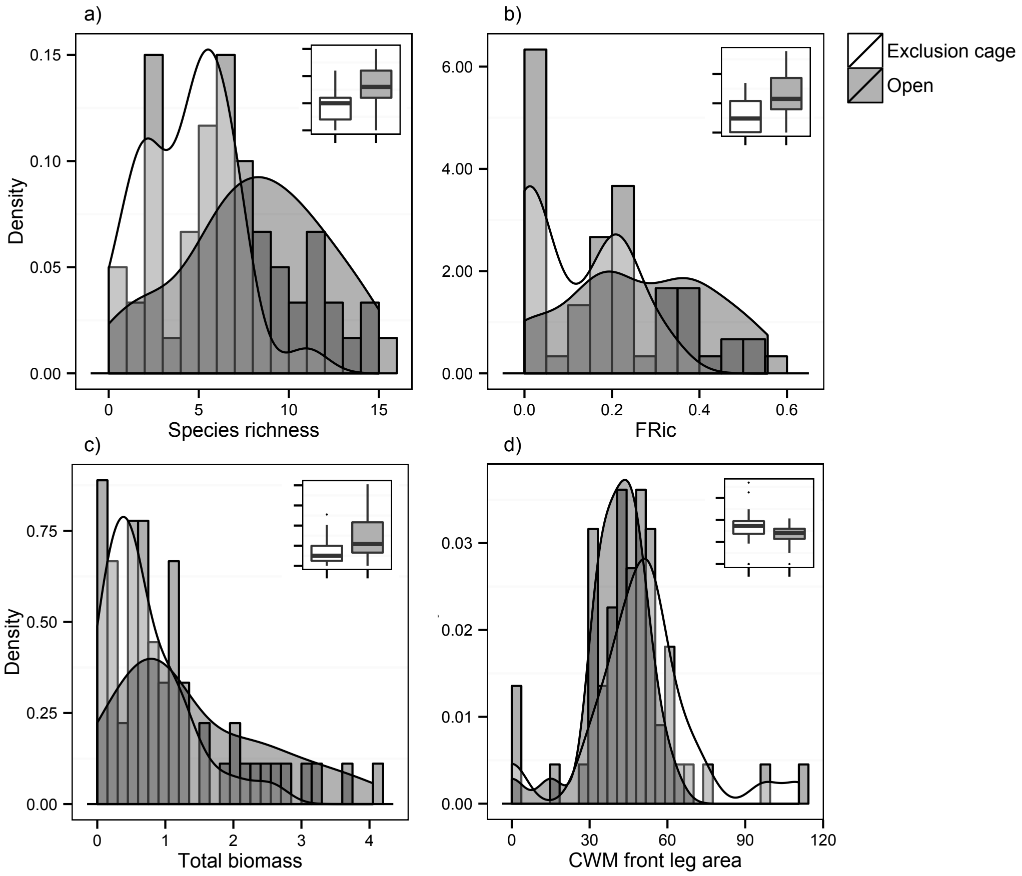

Figure 4.3 | Frequency distributions of dung beetle community metrics collected from mesocosms with exclusion cages (clear ribbons) and without exclusion cages (Open: grey ribbons). Species richness (a); functional richness (b); total biomass (c) and community weighted mean front leg area (d). Boxplots in top right corners display significant effects of treatment on community metrics.

4.4.3 Seedling emergence and survival in mesocosms

The presence or absence of beetles had no effect on the emergence or survival rates of *M. dubia* within mesocosms. The closed mesocosm treatments (no beetles) were therefore removed from further analyses. Functional richness, species richness and total biomass had a significant negative effect on the likelihood of *M. dubia* emergence. Eighty per cent of seeds emerged from mesocosms displaying the lowest FRic, species richness and total biomass values, compared to around 20% emergence from mesocosm displaying the highest values for FRic, species richness and total biomass. Community weighted mean biomass, pronotum volume, front leg area and back: front leg length had no significant effect on emergence success (Table 4.1; Fig. 4.4 (a) – (c)). In contrast, CWM back: front leg length, total biomass and species richness had a significant positive affect on the likelihood that emerged seedlings survived until the end of the 18-week monitoring period (Fig.4.4 (d) – (f)). The strongest predictor of survival was CWM back: front leg length (Table 4.1): 0% of seedlings buried by beetle communities displaying the lowest CWM back: front leg length values survived until the end of the monitoring period whereas 100% of seedlings within mesocosms with the highest values were alive at the end of the experiment. Functional richness, CWM biomass, CWM front leg area and CWM pronotum volume had no effect on seedling survival (Table 4.1), nor did the week that seedlings emerged from the soil surface (LRT = 1.19, d.f. = 1, $p = 0.275$).

Table 4.1 | Generalised linear mixed effects model outputs to assess the influence of dung beetle community attributes on the probability of seed emergence (left section) and seedling survival until the end of the 18-week experimental period (right section). Significant predictors ($P < 0.005$) are highlighted in bold

glmm(seed emergence ~ beetle community)	LRT	df	P	glmm(seedling survival ~ beetle community)	LRT	df	P
FRic	**6.3**	**3**	**0.0124**	**CWM back: front leg length**	**8.4**	**3**	**0.0038**
Total biomass	**5.7**	**3**	**0.017**	**Total biomass**	**6.5**	**3**	**0.0107**
Species richness	**4.6**	**3**	**0.0326**	**Species richness**	**3.9**	**3**	**0.0495**
CWM biomass	0.3	3	0.6119	CWM front leg area	1.8	3	0.18
CWM pronotum volume	0.1	3	0.7924	CWM biomass	1.3	3	0.2598
CWM front leg area	0.1	3	0.7416	CWM pronotum volume	0.9	3	0.3373
CWM back: leg length	0	3	0.9733	FRic	0.7	3	0.3994

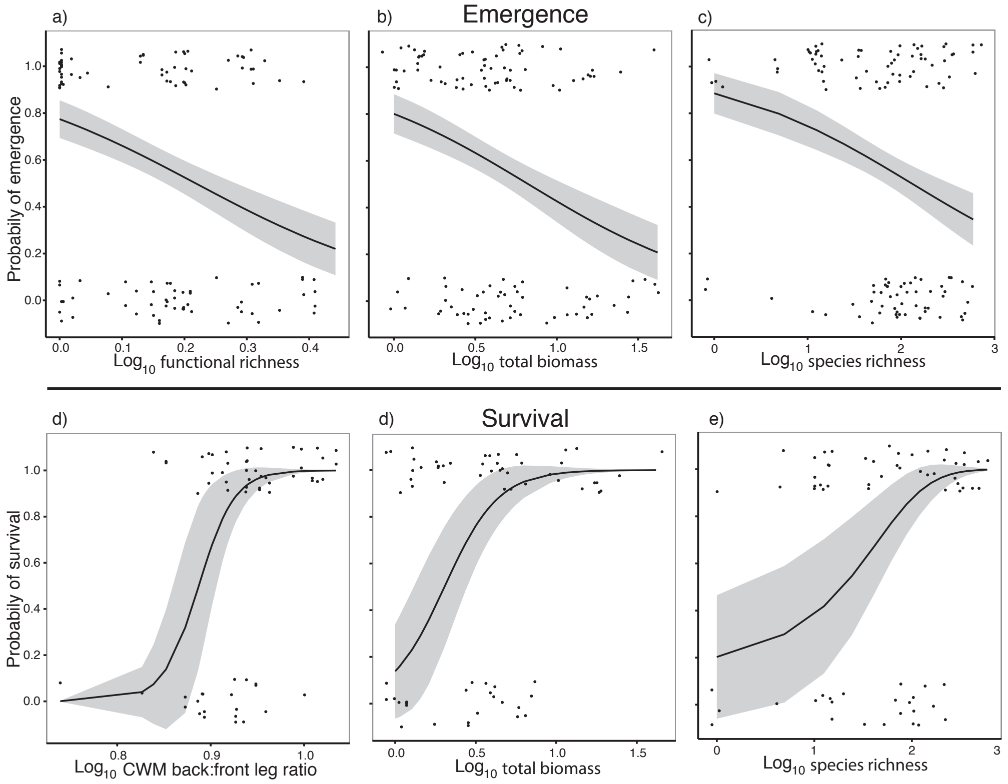

Figure 4.4 | Significant negative effect of dung beetle functional richness (a), total biomass (b) and species richness (c) on the probability of seed emergence (top panels) and the significant positive effect of community weighted mean (CWM) back: front leg length (d), total biomass (e), and species richness (e) on the likelihood that emerged seeding survived until the end of the 18-week experimental period (bottom panels). Significance determined by generalised linear mixed effects models. Predicted values (solid black lines) ± SE (ribbons, calculated using the predict function in R) are displayed along with individual seeds (black points), which either emerged (1) or did not emerge (0); and survived (1) or died after emergence (0).

4.4.4 Seedling emergence and survival in germination plots

There was a significant positive effect of burial depth on the week that *M. dubia* seeds planted within the germination plots emerged (LRT = 59.2, df = 11, $P <$ 0.0001; Fig. S4.3). However, emergence week had no effect on the probability that seedlings survived to the end the monitoring period (LRT = 2.8, df = 1, $P = 0.0921$). Burial depth was the only factor that significantly influenced the likelihood of emergence (LRT = 69.4, D.f. = 11, $P < 0.0001$) but the presence of dung had no significant effect. Seeds that were buried below the soil surface were less likely to emerge as seedlings than those placed above or below the leaf litter: there was a 44.4% and 52.8% emergence rate for seeds above and below the litter respectively, compared to between 19.4% and 5.6% for seeds buried at 1cm and 20cm respectively (Fig. 4.5) No factor or interaction had a significant effect on the probability of seedling survival. No seeds emerged from mesocosms after week 16 or from germination plots later than week 14 (Fig. S4.4.), and as such we are confident that all emergence events were captured during the monitoring period.

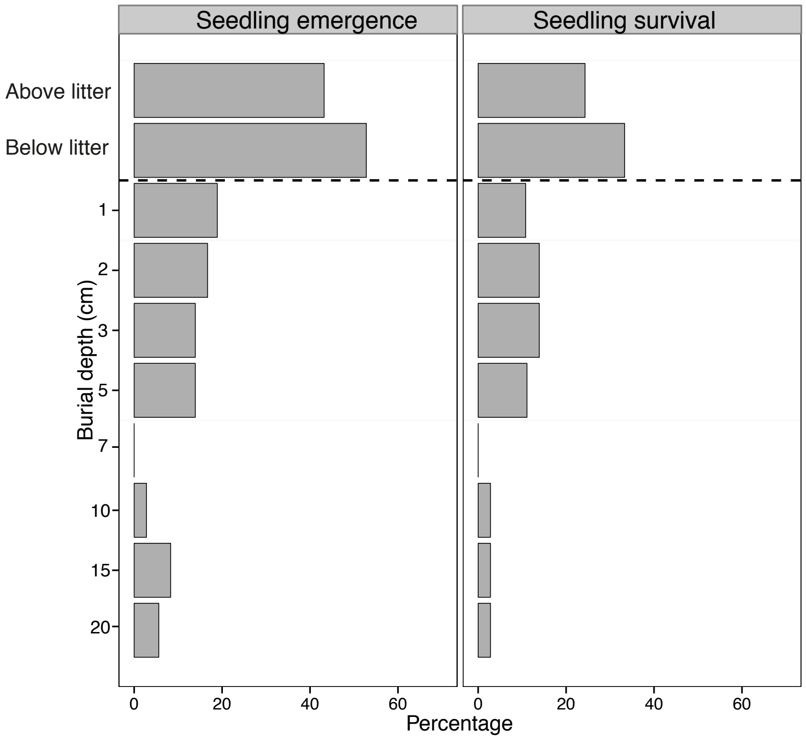

Figure 4.5 | Percentage *M. dubia* that emerged from the soil surface after being experimentally planted to ten different depths, n = 36 at each depth; left panel) and percentage of emerged *M. dubia* seedlings at each burial depth that survived until the end of the 18-week experimental period (right panel). The soil surface is shown with a horizontal dashed line.

4.5 DISCUSSION

The aim of this investigation was to explore the multitrophic consequences of changes in dung beetle communities for secondary seed dispersal and the emergence and survival of tropical seedlings. We found a stronger positive effect of beetle biomass on the likelihood of burial for medium sized beads compared to smaller beads. This suggests that large-seeded species may be more vulnerable to the loss of their secondary dispersers than smaller seeds. Furthermore, dung beetle diversity and biomass negatively affected the likelihood that experimental seeds emerged from the soil surface. Therefore, secondary seed dispersal has the potential to inhibit rather than promote the emergence of some species. Conversely, seedling survival was positively influenced by beetle diversity, biomass and the CWM of back: front leg length. This demonstrates that changes in the composition of dung beetle communities may influence tropical soil environments at a small scale, which could impact upon seedling recruitment and ultimately affect future vegetation composition. To our knowledge this is the first study to link naturally assembled dung beetle communities to the emergence and survival of seedlings. We therefore provide novel insights into how defaunation could impact tropical forest plant regeneration through cascading changes in multitrophic species interactions.

Although we found a positive association between dung beetle biomass and the probability that beads of all size classes were buried, effects were strongest for the burial of medium beads. Previous work has demonstrated that large beetles are functionally more efficient in the removal of dung and seeds compared to smaller species (Larsen *et al.* 2005; Slade *et al.* 2007) and that they are instrumental in the movement of large seeds (Feer 1999). It is likely, therefore, that the stonger relationship we observed between biomass and medium bead burial, compared to small bead burial, is driven by the presence of large beetles in high biomass communities driving the burial of large seeds. Large-bodied dung beetle species are known to be more prone to extinction and decline in response to human modifcation and defaunation (Larsen *et al.* 2005; Gardner *et al.* 2008c; Culot *et al.* 2013). These results, therefore, support our first hypothesis, that changes in dung beetle community structure are likely to differentially affect the secondary dispersal of seeds depending on their size. This adds weight to suggestions that large seeded species are most at risk from the extinction of complex animal-plant interactions as a result of human pressures (c.f. Harrison *et al.* 2013).

Secondary dispersal by dung beetles has been demonstrated on a number of occasions to be beneficial to buried seeds (Shepherd & Chapman 1998; Feer 1999; Andresen & Levey 2004; Nichols *et al.* 2008; Santos-Heredia *et al.* 2010). However, for the first time, and contrary to our predictions, we show that the functional richness (FRic), species richness and total biomass of dung beetle communities negatively influence emergence success, suggesting that dung beetle activity may be detrimental for some species. Previous beetle-mediated seed dispersal experiments in tropical forests demonstrate that burial depths of between 1cm and 4cm result in increased germination success compared to seeds that remained on the soil surface or were buried to deeper depths (Shepherd & Chapman 1998; Feer 1999; Andresen & Levey 2004). We show that *M. dubia* emergence rates within the germination plots were highest when seeds were placed either above or below the leaf litter, but immediately reduced by over 50% when seeds were buried within the soil profile. Therefore, it seems likely that the negative relationship between beetle community attributes and emergence of *M. dubia* seeds is a consequence of higher biomass and diversity resulting in higher rates of seed burial (c.f. Braga *et al.* 2013; Nichols *et al.* 2013b; Griffiths *et al.* 2015) and net disadvantages to the fitness of this species. Furthermore, results from the 2012 bead burial experiments demonstrate that small seeded species are buried deeper (mean ± SE: 10.93 cm ± 0.41) than larger seeds. Shepherd & Chapman (1998) demonstrated that only the largest seeds were able to germinate from burial depths of 10cm or more, we therefore also expect negative consequences of beetle activity for other, smaller seeded species. Yet caution must be taken when generalising results based on just one species. This is perhaps particularly relevant when one considers the ecology of *M. dubia*, a largely riparian species. *M. dubia* is known to be dispersed by a wide range of forest vertebrates, including primates (Gressler *et al.* 2006), which means that the seeds of this species are likely to be secondarily dispersed by dung beetles from time to time. However, seeds that are primarily water dispersed, such as those of *M. dubia* often display greater germination success on the soil surface (Lopez 2001); environments where they would naturally come to rest having been transported by water. This then could offer some explanation as to why dung beetles had natively impacted upon the germination success of *M. dubia.* A different outcome, more inline with previous dung beetle, seed dispersal investigations (e.g. Shepherd *et al.* 1998) may have been realised using a different species with a different ecology. Had greater number of the other experimental seeds used in this investigation germinated, further testing of the generality of results for other seed species would have been possible.

It is likely that the need for the secondary seed dispersal function will vary along gradients of anthropogenic impact. In this investigation, we established emergence and survival experiments in a primary forest with no recent logging, relatively low hunting pressure and therefore a full complement (but probably a reduced density) of large mammals (Parry, Barlow & Peres 2007). More heavily disturbed forests differ in that they can harbour elevated populations of seed predators (Adler 1996; Bodmer, Eisenberg & Redford 1997; Terborgh *et al.* 2001) and higher seed predation pressure (Asquith, Wright & Clauss 1997). Seed predator escape is a key mechanism underpinning the increased germination success observed in seeds secondarily dispersed by dung beetles in tropical forests (e.g. Shepherd & Chapman 1998; Feer 1999; Andresen & Levey 2004). If seed predation pressure was sufficiently high, burial by beetles could impart net benefits rather than disadvantages to *M. dubia* and other large-seeded species.

Anthropogenic disturbance may also affect the ability of dung beetles to deliver the secondary seed dispersal function. Dung beetle diversity often declines in response to defaunation or forest degradation (Andresen & Laurance 2007; Gardner *et al.* 2008; Culot *et al.* 2013). This could have major implications for the survival of seeds because beetle diversity is positively associated with secondary seed dispersal (Braga *et al.* 2013; Nichols *et al.* 2013b; Griffiths *et al.* 2015). The combination of increased seed predation and reduced beetle diversity means that beetle mediated seed dispersal could be least likely in disturbed sites where it has the potential to be more important for predator escape compared intact forests. However, further work is needed to investigate relationships between dung beetles communities and seedling emergence in forests with differing levels of defaunation and disturbance. This is important because it will facilitate an understanding how biological communities and anthropogenic disturbance indirectly influence dung beetle's ecological role as efficient seed dispersers or agents of seed mortality.

We found a positive affect of dung beetle total biomass, species richness and CWM back: front leg length on the probability that emerged seedlings remained present and alive until the end of our 18-week monitoring period. There are myriad processes acting both above and below ground that influence whether a seedling lives or dies following germination (e.g. Khurana & Singh 2001). However, the most plausible way in which beetles could influence seedling survival is through their impact on soil nutrients and physical properties (reviewed Nichols *et al.* 2008). Due to their small root system, seedlings are reliant on the nutrient and water availability in their immediate surroundings (Poorter & Hayashida-Oliver 2000) and nitrogen is a mineral element that can become insufficient in seed reserves (Fenner 1986). Dung beetle activity and abundance has been shown to positively influence N mineralisation, raising concentration of inorganic N in the soil as well as the concentrations of other limiting nutrients such as P and K (Yokoyama *et al.* 1991a; Yamada *et al.* 2007). It is possible, therefore, that through altering soil nutrients at a stage when seed reserves are becoming exhausted, dung beetles could create favourable conditions for seedling growth and thus positively influence survival.

The impact of dung beetles on soil physical structure may offer additional insights into the positive relationships between seedling survival and beetle community attributes. Bang *et al.* (2005) demonstrated that the activity of large bodied dung beetles has a positive effect on soil permeability in surface layers, which is positively associated to air and water movement and greater soil pore space (Marshall *et al.* 1996). These soil characteristics could facilitate root and shoot growth and result in benefits to seedling survival. However, studies investigating the effect of dung beetle activity on soil nutrients and structure have been exclusively carried out in grassland or heathland systems. Different minerals are limited in the soils tropical forests compared to grasslands and heaths, and nutrients are added to the soil via different pathways within each of the systems (Townsend *et al.* 2007; Vitousek *et al.* 2010). Therefore, making inferences about the role of dung beetles in modifying tropical soils based on evidence from temperate systems is problematic. Investigations are needed to elucidate the small scale impact of dung beetles on tropical soils where highly heterogeneous distributions in soil nutrients are important factors structuring plant communities (John *et al.* 2007).

Intriguingly, CWM of back: front leg length was positively associated with seedling survival and involved in a significant interaction with bead size on the probability of seed mimic burial. The abundance of dwellers within these dung beetle communities was positively related to CWM back: front leg length (Fig. S4.5), and as such an increase in the ratio between back to front leg length indicates an increase in the number of dweller species present. Dwelling species of dung beetle do not bury dung or seeds, but feed and nest within it. The burial of medium beads (of similar dimensions and weight to *M. dubia;* Table S4.1) was low compared to smaller beads and was always unaffected by leg length. Therefore it is unlikely that the relationship we found between seedling survival and CWM back: front leg length is a consequence of dwellers decreasing the likelihood that seeds are buried. Instead it is possible that mixing and breakdown of dung on the soil surface increases with an increase in the abundance of dwelling species. This could give raise to similar processes described above where altered biochemical cycling and structural changes in seed substrates provide benefits to seedling growth and thus survival. No study to date has investigated how the morphological traits of dung beetles influence nutrient cycling, soil structure and plant growth. Therefore, while mechanisms suggested here are speculative, our results illuminate exciting avenues for further research into the role dung beetle diversity and functional traits on ecosystem processes spanning multiple trophic levels.

4.5.1 Conclusion

This investigation aimed to better understand the ecological consequences of defaunation for dung beetle-mediated secondary seed dispersal in tropical forests. Conceptual frameworks predict that large seeded species are most at risk from the negative impacts of defaunation due to the extirpation of their large-bodied primary dispersers (Wright 2003; Stoner *et al.* 2007). We show that large seeds may also be differentially vulnerable to the loss of their secondary dispersers through anthropogenic driven reductions in large bodied dung beetles (Larsen *et al.* 2005; Gardner *et al.* 2008c; Culot *et al.* 2013). However, we demonstrate that decreases in dung beetle biomass and diversity could result in net advantages to some seed species, if seed burial negatively impacts upon their emergence success. Furthermore, we present intriguing novel evidence suggesting that dung beetle activity could modify conditions within the soil and/or dung in a way that promotes seedling survival. However, a mechanistic explanation of this eludes us because no studies to date have investigated the contribution of diversity versus key traits or species in driving dung beetle's impact on the soil environment in temperate or tropical systems.

The benefits imparted on seeds via two-phase dispersal are context dependent, driven by of a variety of interrelated biotic and abiotic factors (Culot *et al.* 2014) and the outcomes for plant fitness are governed by species-specific requirements. Therefore, to understand the idiosyncratic responses of tropical plant communities to defaunation (reviewed in Kurten 2013) further work is needed using a greater variety of seed species in sites subject to varying and different types of disturbance. However, here we demonstrate that investigation of plant responses under realistic field conditions is challenging because the functional changes underpinning patterns observed are often a product of complex, multitrophic species interactions. Experimental work aimed at gaining a mechanistic understanding of the processes driving the outcomes of these ecological interactions is vital if we are to appreciate the consequences of human modification of biological communities.

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4.8 Supplementary information

Table S4.1 | Weights and dimensions of experimental seed species (top panel) and seed mimics (bottom panel) used in mesocosms and germination trials. Means and standard errors were calculated using 15 individuals of each species, and measurements were taken at the widest and longest part of the seeds. The focal experimental species is highlighted (*M. dubia*) along with the seed mimic of similar dimensions (medium) to *M. dubia*.

Species	Mean weight (g)	SE	Mean width (mm)	SE	Mean length (mm)	SE
R chamaemorus	0.0034	0.0001	1.9713	0.0463	2.8580	0.0627
M emarginata	0.0394	0.0027	8.1973	0.2299	9.0647	0.2757
G americana	0.0413	0.0019	5.2827	0.1922	7.4020	0.3525
P guajava	0.0735	0.0640	2.4660	0.0917	3.4160	0.1082
M dubia	0.4462	0.0323	10.6767	0.2594	13.7600	0.2585
Seed mimics						
Very small	0.0600	NA	2.0000	NA	2.00000	NA
Small	0.0900	NA	5.0000	NA	5.00000	NA
Medium	0.5000	NA	10.0000	NA	10.0000	NA
Large	4.1200	NA	20.0000	NA	20.0000	NA

Table S4.2 | Species abundances (in sampling periods 2012 and 2014), median trait values and categorical information used to calculate functional richness

Species	Taxonomic authority	Abundance 2012	Abundance 2014	Biomass (g)	Biomass adjusted protum volume	Biomass adjusted front leg area	Back: front leg length	Nesting strategy	Activity period	Diet
Ateuchus connexus	Harold (1986)	5	25	0.023	937.64	36.25	1.24	Tunneller	Nocturnal	Coprophogus
Ateuchus murrayi	Harold (1986)	8	89	0.010	858.94	18.80	1.30	Tunneller	Nocturnal	Coprophogus
Ateuchus pauki	Balthasar (1939)	0	1	0.015	1299.95	41.16	1.33	Tunneller	Nocturnal	Generalist
Ateuchus sp A	NA	0	1	0.006	1934.13	47.86	1.36	Tunneller	Nocturnal	Coprophogus
Ateuchus sp C	NA	0	1	0.026	1057.80	13.37	1.50	Tunneller	Nocturnal	Coprophogus
Ateuchus sp D	NA	0	5	0.009	1057.04	18.65	1.33	Tunneller	Nocturnal	Coprophogus
Ateuchus sp E	NA	0	1	0.005	922.74	25.62	1.24	Tunneller	Nocturnal	Coprophogus
Ateuchus sp G	NA	3	9	0.046	830.18	23.14	1.43	Tunneller	Nocturnal	Coprophogus
Ateuchus sp. B	NA	0	1	0.002	1754.76	26.80	1.35	Tunneller	Nocturnal	Coprophogus
Canthidium sp A	NA	0	1	0.022	606.09	21.62	1.49	Tunneller	Diurnal	Coprophogus
Canthidium sp F	NA	0	1	0.024	1047.93	25.39	1.45	Tunneller	Diurnal	Coprophogus
Canthidium sp H	NA	0	2	0.010	1607.10	27.57	1.33	Tunneller	Diurnal	Coprophogus
Canthidium sp K	NA	0	2	0.008	1888.63	44.00	1.22	Tunneller	Diurnal	Coprophogus

Canthidium sp L	NA	0	1	0.007	1561.00	21.75	1.53	Tunneller	Diurnal	Coprophogus
Canthon triangularis	Drury (1770)	104	20	0.046	1186.59	73.70	1.58	Roller	Nocturnal	Generalist
Coprphanaeus lancifer	Linnaeus (1767)	0	3	1.621	2128.26	345.24	1.26	Tunneller	Crepuscular	Generalist
Deltochilum carinatum	Westwood (1848)	0	2	0.284	1000.52	76.15	1.72	Roller	Nocturnal	Generalist
Deltochilum orbiculare	Lansberge (1874)	0	1	0.383	1810.84	699.39	1.78	Roller	Crepuscular	Coprophogus
Deltochilum peruanum	Paulian (1938)	7	4	0.069	941.48	29.67	1.58	Roller	Nocturnal	Generalist
Deltochilum septemstriatum	Paulian (1938)	0	5	0.058	1046.74	28.55	1.68	Roller	Crepuscular	Generalist
Deltochilum sp A	NA	0	1	0.058	1237.03	28.00	1.53	Roller	Diurnal	Generalist
Dichotomius apicalis	Luederwaldt (1931)	0	51	0.224	1183.98	74.38	1.56	Tunneller	Nocturnal	Generalist
Dichotomius latilobatus	Luederwaldt (1931)	2	2	0.317	1323.22	150.64	1.35	Tunneller	Nocturnal	Coprophogus
Dichotomius lucasi	Harold (1869)	18	18	0.107	965.35	64.80	1.47	Tunneller	Nocturnal	Generalist
Dichotomius mamillatus	Felsche (1901)	0	1	0.232	1077.76	73.52	1.36	Tunneller	Nocturnal	Generalist
Dichotomius robustus	Luederwaldt (1935)	0	1	0.311	920.85	53.83	1.39	Tunneller	Nocturnal	Coprophogus
Dichotomius subaeneus	Castelnau (1840)	22	23	0.281	867.99	67.75	1.33	Tunneller	Nocturnal	Coprophogus
Eurysternus atrosericus	Génier (2009)	5	39	0.010	814.86	9.03	2.15	Dweller	Nocturnal	Coprophogus
Eurysternus caribaeus	Herbst (1789)	19	55	0.107	1601.99	85.58	1.81	Dweller	Diurnal	Coprophogus
Eurysternus cayennensis	Castelnau (1840)	0	8	0.028	1008.03	26.97	1.74	Dweller	Generalist	Coprophogus

Eurysternus foedus	Giérin-Méneville (1830)	6	53	0.134	1073.70	42.57	1.91	Dweller	Nocturnal	Coprophogus
Eurysternus hamaticollis	Balthasar (1939)	3	1	0.211	1183.47	46.70	1.71	Dweller	Nocturnal	Generalist
Eurysternus hypocrita	Balthasar (1939)	21	15	0.177	1075.52	58.58	1.66	Dweller	Generalist	Coprophogus
Eurysternus vastiorum	Martínez (1988)	0	18	0.023	1066.77	24.46	1.68	Dweller	Generalist	Coprophogus
Eurysternus ventricosus	NA	9	48	0.027	1308.46	27.69	1.88	Dweller	Diurnal	Coprophogus
Onthophagus bidentatus	Drapiez (1819)	31	87	0.008	1432.42	35.34	1.33	Tunneller	Diurnal	Coprophogus
Onthophagus clypeatus	Blanchard (1843)	22	15	0.015	1097.82	30.87	1.35	Tunneller	Diurnal	Generalist
Onthophagus haemathopus	Harold (1875)	9	45	0.007	1274.24	27.18	1.45	Tunneller	Generalist	Coprophogus
Oxysternon durantoni	Arnaud (1984)	8	8	0.335	1581.26	103.44	1.30	Tunneller	Diurnal	Generalist
Oxysternon festivum	Linnaeus (1767)	4	7	0.550	1626.81	103.30	1.18	Tunneller	Diurnal	Generalist
Phanaeus chalcomelas	Perty (1830)	3	5	0.189	1368.63	69.25	1.35	Tunneller	Diurnal	Generalist
Sulcophanaeus faunus	Fabricius (1775)	0	2	1.717	2155.10	294.16	1.19	Tunneller	Diurnal	Coprophogus
Uroxys sp A	NA	0	7	0.009	738.04	15.04	1.09	Tunneller	Nocturnal	Coprophogus
Uroxys sp B	NA	0	2	0.006	542.26	9.07	1.33	Tunneller	Nocturnal	Coprophogus

Table S4.3 | Model outputs to assess the influence of mesocosm treatment (presence or absence of an exclusion cage, mesh size 15mm x 8mm) on dung beetle community metrics (determined by linear models). The metrics that were significantly (*P* < 0.05) affected by treatment are highlighted in bold.

Community metric	df	f	*P*
Species richness	**1**	**10.8**	**0.0018**
FRic	**1**	**15.3**	**0.0002**
Total biomass	**1**	**8.7**	**0.0045**
CWM biomass	1	1.5	0.2221
CWM pronotum volume	1	3.2	0.0784
CWM leg area	**1**	**9.0**	**0.0040**
CWM leg ratios	1	0.0	0.9776

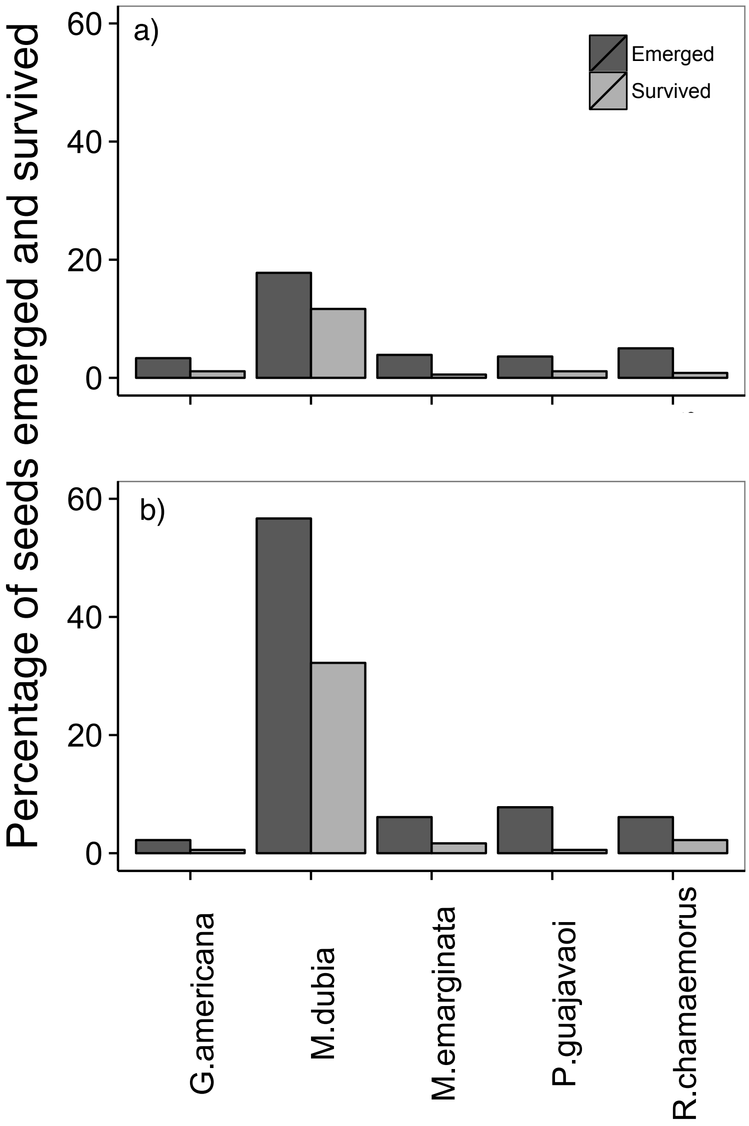

Figure S4.1 | Emergence (dark grey bars) and survival (light grey bars) of seed species within germination plots (a) (n = 360 per species) and mesocosms (b) (n = 180 per species).

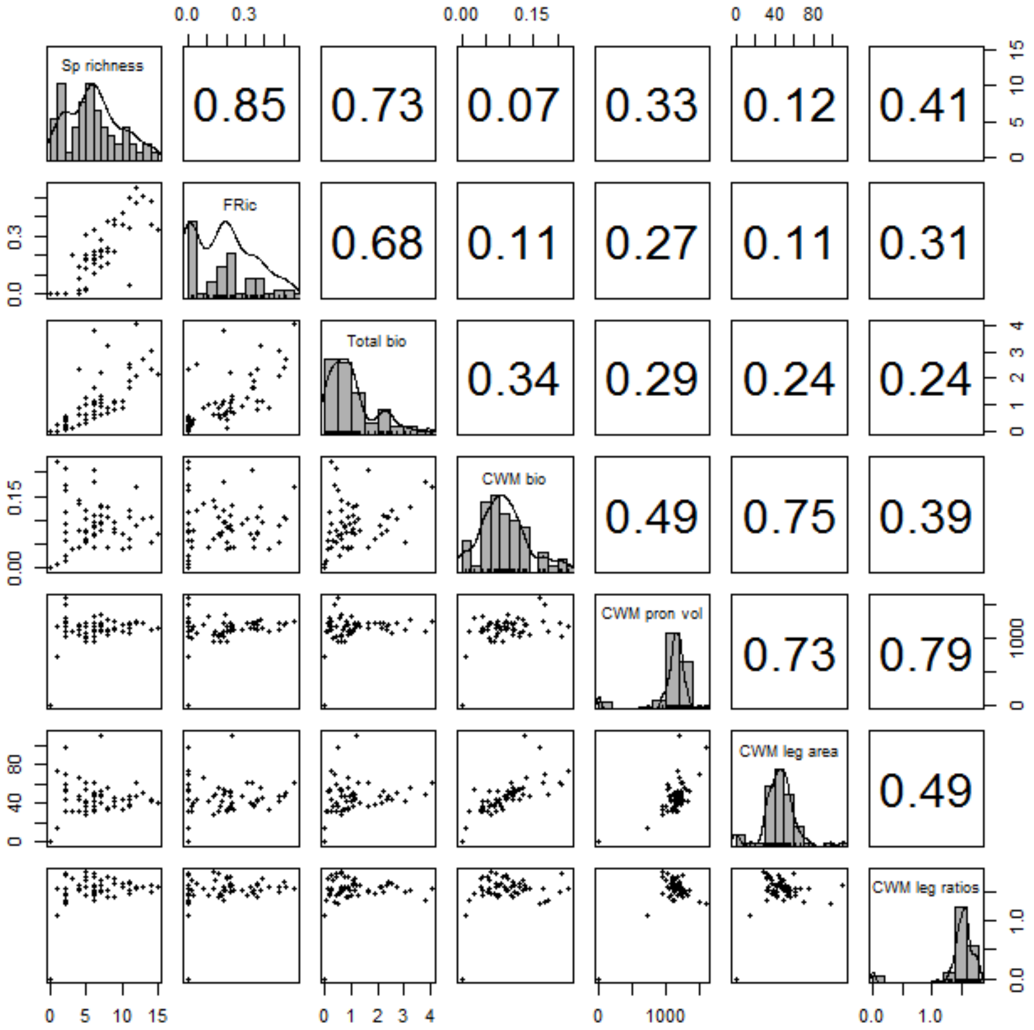

Figure S4.2 | Associations between dung beetle community metrics within seedling emergence and survival mesoscosm (2014 experiment): bivariate plots (lower panels), distributions (diagonal) and Pearson's ρ (upper panels).

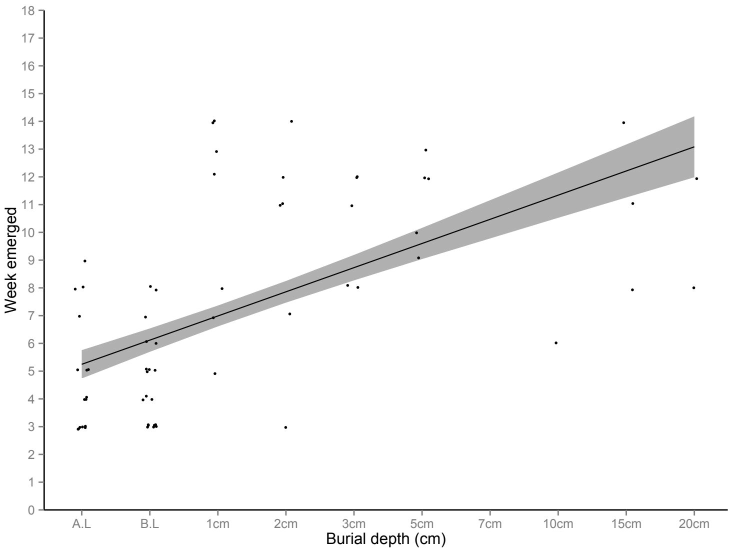

Figure S4.3 | Model predicted values (solid black lines) and SE (ribbons) for the significant effect of burial depth on the emergence week of *Myrciaria dubia* seeds within germination trials

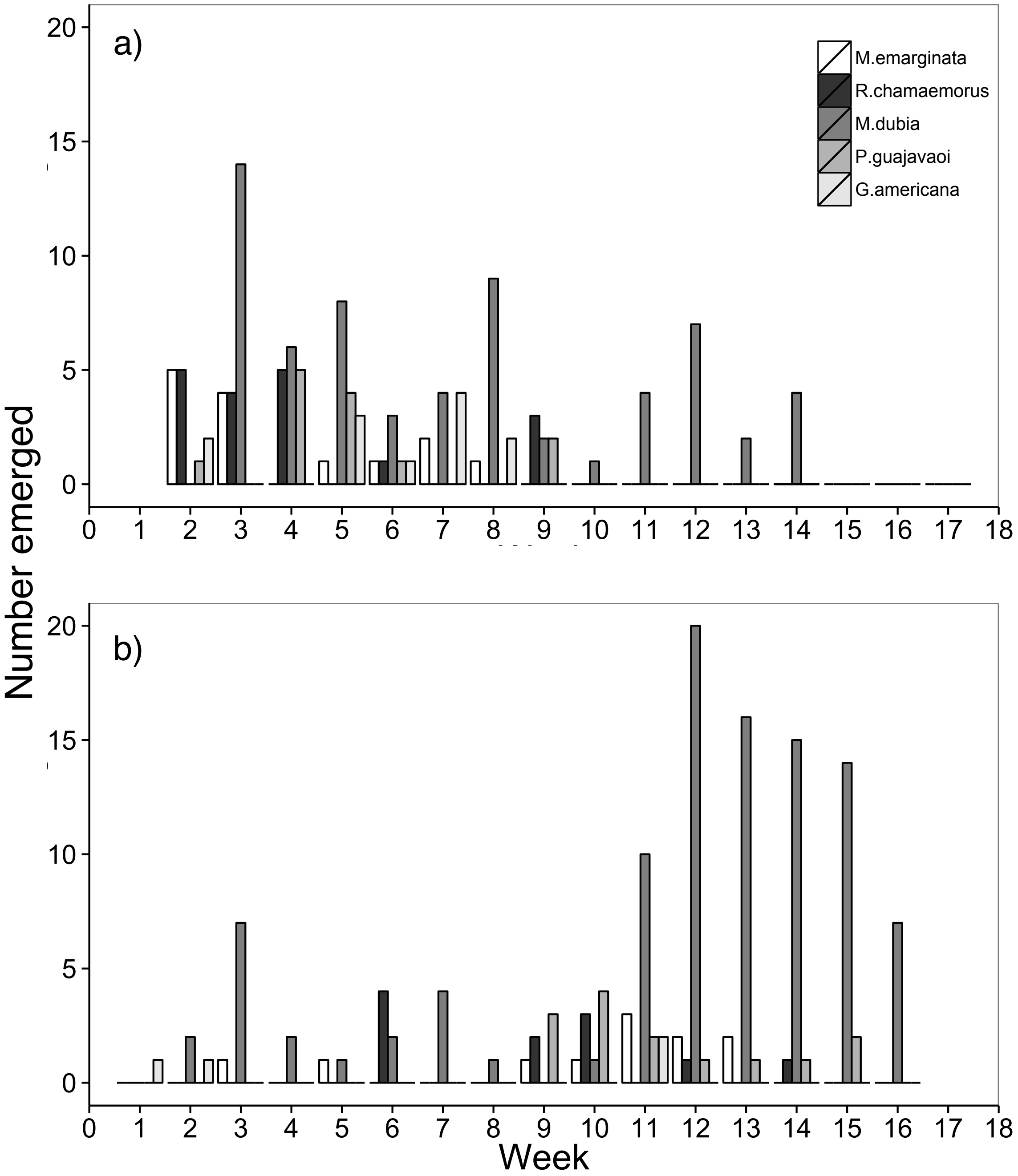

Figure S4.4 | Numbers of seeds that emerged per week within germination plots (a) and mesocosms (b), grouped by species

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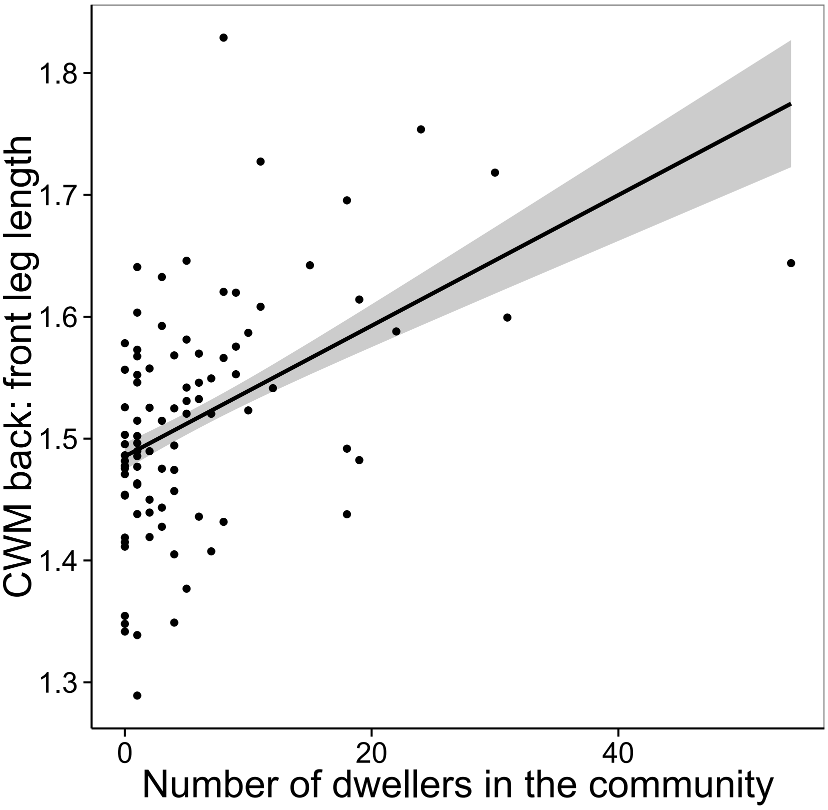
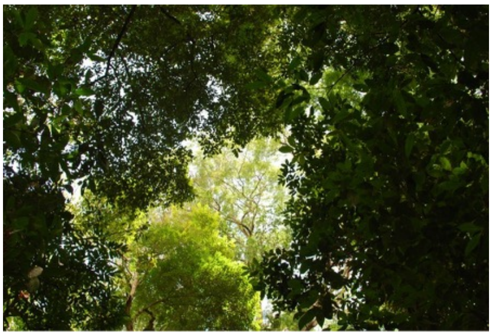


Figure S4.5 | The significant positive relationship between community weighted mean back: front leg length and the number of dwellers in dung beetle communities ($F_{1, 88} = 25.2$, $P < 0.0001$) as determined by a linear model ± SE (ribbon, calculated using the predict function in R). Although longer back compared to front legs are generally indicative of rolling dung beetle species (see Hanski & Cambefort 1991 for dung beetle resource use strategies), dwelling species are morphologically indistinguishable from rolling species in the Neotropics (Inward *et al.* 2011)

Chapter 5

General Discussion



5.1 KEY FINDINGS

The aim of this thesis was to better understand how anthropogenic modification of biological communities influences the structure and functioning of ecosystems. In particular, given that the majority of biodiversity-ecosystem functioning (BEF) research has been conducted in temperate grassland systems, I sought to investigate these relationships in a non-producer tropical system. To do this, I developed new methodologies to carry out in depth investigations into the role of dung beetle traits and functional diversity for the secondary dispersal of seeds in tropical rainforests. Overall, each chapter combines to demonstrate that continuous, morphological traits in dung beetle communities can be used to successfully predict ecosystem processes. Using this framework, emergent cross-chapter findings reveal that relationships are complex and dependent on biotic and abiotic environment, and that the consequences of dung beetle mediated BEF relationships for vegetation regeneration is unpredictable and species-specific. This work therefore provides several novel advances of how diversity in animal communities can influence the ecological processes they govern. Below I outline the key findings from each experimental chapter that contribute to these overall outcomes:

5.1.1 Variability in dung beetle traits and implications for functional diversity indices (Chapter 2)

Chapter 2 research questions: (1) What is the relative contribution of between vs. within species differences in trait values? (2) How does sampling site influence intraspecific trait differences? (3) What sample size is needed to provide representative species mean trait values? (4) What impact does omission of intraspecific trait information have on the calculation of functional diversity indices from naturally assembled communities?

A key outcome of Chapter 2 was that omission of intraspecific trait information could result in large errors in the calculation of functional diversity (FD) indices, even when using traits with very low variability. These errors were negatively associated with community size and/or species richness, suggesting that the smaller the community, the greater the importance of incorporation of within species trait information. Therefore, if the aim of an investigation is to describe links between organisms and the ecological processes they govern at the community scale, complete sampling of individual traits may be necessary. Failure to do so could have impact on the accuracy of results and ultimately conclusions drawn. This has methodological implications for the growing number of mesocosm or mesoclosure studies that involve small naturally assembled communities (such those sampled employed by Gregory *et al.* [2014] and in Chapters 3 and 4) and for methods highlighted by Lahteenmaki *et al.* (*in press*). In this chapter, I also demonstrate that intraspecific variability in dung beetle functional traits is insignificant compared to between species differences. Therefore, at the scale of this investigation, dung beetle functional traits appear to be robust, suggesting that at the population level complete trait sampling of all individuals is probably unnecessary. Finally, this chapter provides practical information on number of individuals from which measurements should be taken in order to accurately summarise dung beetle trait information. These findings provide useful guidance to future researchers adopting a trait-based approach when working with this key group of organisms.

5.1.2 Functional diversity, dung beetle mediated seed dispersal and environmental context (Chapter 3)

Chapter 3 research questions: (1) How does dung beetle taxonomic and functional diversity influence the probability of seed burial and the dispersion of seeds throughout the soil profile? (2) What impact does environmental context have on the strength and/or direction of observed BEF relationships?

In Chapter 3 I link, for the first time, the functional diversity of naturally formed dung beetle communities to the secondary dispersal of seeds across contrasting soil conditions. The most striking outcome of this study was the significant role that environmental context played in modulating the BEF relationships observed; the effect of diversity on seed burial and dispersion throughout the soil profile was stronger in sandy soils compared to clay soils. Patterns were context dependent despite the fact that I investigated the same processes, carried out by a single focal taxa, in just one ecosystem. Aside from highlighting the clear need to consider BEF relationships under varying environmental conditions; these results demonstrate the difficulties in generalising BEF relationships across biomes and taxa, thus illustrating some of the challenges in scaling up relationships to describe relationships over large spatial scales. Additionally, I show that decreases in dung beetle diversity as a result of anthropogenic pressures is likely to reduce both the probability that seeds are buried and the range in the depths and distances that they are moved from a central point. This has the potential to influence the seed community that successfully becomes established following dung processing by beetles. Therefore, this chapter demonstrates the possible impact that changes in beetle composition could have on future forest regeneration. Finally, these positive BEF relationships are also important from a theoretical perspective because studies empirically linking functional diversity in animal communities to the ecological processes they govern are conspicuously uncommon in the literature (but see Slade *et al.* 2007). I therefore provide evidence that the patterns emerging from two decades of BEF research in producer systems can apply to higher trophic level organisms.

5.1.3 The influence of tropical forest dung beetle communities on the emergence and survival of seedlings (Chapter 4)

Chapter 4 research questions: (1) Are seeds of different size classes differentially vulnerable to the cascading effects of defaunation? (2) Does dung beetle diversity impact on vegetation regeneration through influencing seedling emergence and survival? (3) What is the species-specific optimal burial depth of experimental seeds to allow seedling emergence from the soil?

In this final experimental chapter, I sought to understand how the diversity-functioning patterns observed in Chapter 3 translate across trophic levels to influence seedling emergence and survival. In accordance with previous dung beetle-seed dispersal experiments I demonstrate that large seeds are more vulnerable to the loss of large bodied dung beetles than smaller seeds (Feer 1999). This adds weight to the suggestion that large seeded species are most at risk from the extinction of complex plant-animal interactions as a result of human pressures (Harrison *et al.* 2013). However, an unexpected outcome was that dung beetle diversity resulted in net disadvantages for the fitness of *Myrciaria dubia* by negatively impacting on the probability of seedling emergence from the soil. This was because the optimal burial depth for germination of this species was on the soil surface, either above or within the leaf litter. I therefore demonstrate that for some species of animal-dispersed seeds, anthropogenic reductions in dung beetle communities could be beneficial. This is noteworthy because, in general, it is thought that endozoochorously dispersed seeds will suffer from the loss of their primary and secondary dispersers due to defaunation and habitat modification (Harrison *et al.* 2013; Kurten 2013). Therefore, I provide a further example of the idiosyncratic and species-specific nature of plant responses to defaunation. The final key finding from this chapter was the positive relationship between dung beetle community attributes and the survival of seedlings over the timescale (18 weeks) of this experiment. This is the first time that beetle activity has been linked to the survival of seedlings and suggests that small-scale soil modification by dung beetles could influence seedling recruitment. I therefore present a novel way in which dung beetle communities could influence tropical forest regeneration and ultimately influence future vegetation composition.

5.2 FUTURE RESEARCH NEEDS

In addition to increasing our knowledge of BEF relationships, this thesis also illuminates several areas in which more work would further our understanding of diversity-functioning relationships and thus help predict and mitigate the consequences of anthropogenic changes to natural systems. In this section I discuss six key research priorities. Development of the first three will facilitate the implementation and/or accuracy of future BEF investigations, while exploration of the final three will advance our understanding of complex BEF relationships.

5.2.1 Development of a large-scale dung beetle trait database

Trait databases (e.g. Kattge *et al.* 2011) are increasingly important tools in facilitating large-scale functional investigations in plant-focussed studies (e.g. Pietsch *et al.* 2014), but equivalent collections are lacking for most higher trophic level organisms. I have demonstrated that the use of dung beetle morphological traits can be successfully linked to the ecological processes they govern and that a functional approach is often a better predictor of processes than a taxonomic approach (Chapter 3; Griffiths *et al.* 2015). Dung beetles are known to be a cost effective indicator taxa in tropical forests (Gardner *et al.* 2008a) and they are the agents of a number of key ecosystem processes (Nichols *et al.* 2008). Therefore, the development of a large-scale dung beetle trait database would facilitate the rapid assessment of the influence of human activities on biological communities and allow us to predict the consequences of change in beetle community structure for ecosystem processes. Efforts should therefore be directed at collating trait information into global databases to ensure that the time and financial resources spent on projects such this doctoral research are not wasted. Findings from Chapter 2 highlight the need for work focussed on understanding the ability of plastic versus fixed traits to predict animal-mediated ecosystem functioning. This would facilitate the targeted development a dung beetle trait database. Furthermore, investigation into intraspecific trait variability between individuals originating from geographically distant sites, with contrasting environmental conditions is needed to test the assertion that dung beetle traits are robust. Finally, a measure of intraspecific variability associated with each species and trait would increase the utility of trait information within trait databases (see section 5.2.2 below) as well as allow assessment of the accuracy with which average values summaries species information.

5.2.2 Intraspecific variability in functional diversity indices

In Chapter 2 I show that the omission of intraspecific trait information can result in large errors in functional diversity indices yet, to my knowledge, there is currently no straightforward way to incorporate within species trait variability into multi-trait diversity metrics. Plants traits, in general, display greater variability than animal traits (Albert *et al.* 2010b; Messier *et al.* 2010). Therefore failure to include intraspecific trait information in indices is likely to have even greater consequences than reported here, for the accuracy of results in producer-based investigations. The complete trait sampling of large numbers organisms from an ecological investigation is time and resource intensive (for example, I spent more 480 hours collecting almost 17,000 measurements for these investigations). Furthermore, if the use of large-scale trait databases is to be employed, trait information from individuals sampled within a study may not be available. Therefore, rather than including trait information from every individual in a community, the calculation of indices should allow an extra component, which describes the level of variability in each trait (c.f. Cianciaruso *et al.* 2009). This would greatly improve the accuracy with which we link organisms to the ecological processes they govern and has been addressed previously by Cianciaruso *et al.* (2009). The authors present a possible method to do so, however, the process requires a level of programming not shared by all ecologists and a solution is only offered for a single FD index. An extension to the FD package (Laliberté *et al.* 2012), for example, in R (or development of a new package) to allow the incorporation of the level intraspecific trait variability into a functional diversity indices seems to be a key next step. This would encourage a greater number of investigators to consider within species trait differences and would help to develop functional ecology into a more precise and predictive science.

5.2.3 Which functional diversity index?

A key challenge facing any investigator using functional diversity indices is the selection of an ecologically meaningful index for their study system given the large number available (e.g. Schleuter *et al.* 2010). Although some are related (e.g. Rao's quadratic entropy; Rao [1982] and functional dispersion; Laliberté & Legendre [2010]), others are not, and therefore they cannot all be associated with a particular ecological process. Indeed, in Chapter 3 I show that of the four indices I used, the strength, direction and predictive power of their link to seed dispersal depended on the index. At present, there is a lack of academic discourse regarding the complexities associated with choice of index. Moreover, I am not aware of any investigation into the mechanisms driving these idiosyncratic responses. For example, I posit that competition within a community is negatively associated with functional evenness (FEve), which underpinned the negative association (although not significant) between FEve and seed burial in Chapter 3. However, I am not aware of any information about how known changes in community structure actually translate into changes in FD indices. This information, in combination with a taxa-specific understanding of the mechanisms driving diversity-functioning relationships, is important for the selection of the appropriate metric and would allow more meaningful interpretation of results and the construction of robust conclusions. Additionally, in Chapter 2 I demonstrate that community size and/or species richness impacts upon the accuracy with which an index describes a community, and that different indices perform better than others with small communities. Biodiversity-ecosystem functioning investigations are conducted at a range of spatial scales, from square meters (Lahteenmaki *et al. in press*) to landscapes (Pietsch *et al.* 2014). Therefore work into which index performs best under contrasting community size and diversity would provide useful guidelines for the investigator based on the specifics of their study system.

5.2.4 Environmental context and diversity-functioning relationships

Global ecosystem models (Harfoot *et al.* 2014) and the concept of planetary boundaries (Rockstrom *et al.* 2009) seek to describe and predict biome- or global-scale ecological processes and the responses of biological communities to anthropogenic habitat modification. However, the usefulness of these global concepts has been questioned because of the large spatial heterogeneity in the drivers and responses of biodiversity loss (e.g. Brook *et al.* 2013). Results from Chapter 3 further illustrate the difficulties in scaling up BEF relationships from small-scale experiments because I report contrasting relationships when studying just one ecological process in different soils within a single ecosystem. Establishing a well-replicated, robust experimental design in which diversity-functioning relationships can be empirically tested, whilst also investigating the role of environmental heterogeneity in driving patterns observed is undoubtedly challenging. Therefore, a large proportion of our understanding of BEF relationships originates from experiments carried out at single homogenous experimental sites (e.g. from experiments such as the Cedar Creek grassland experiment in Minnesota, US, and the Jena Diversity Experiment in Germany). Despite challenges, however, in order to truly appreciate how biological diversity influences the functioning and maintenance of ecosystems, future BEF investigations should specifically investigate relationships under realistic, heterogeneous environmental conditions. This would increase realism in the development of global models and allow for greater accuracy in predicting the ecological consequences anthropogenic species extinctions.

5.2.5 Diversity-functioning relationships between trophic levels

Ecological systems are the product of interactions between multiple trophic levels. However, most BEF investigations focus on how diversity influences biophysical processes directly linked to a focal group and do not consider how species assemblages at one trophic level influence diversity and functioning at another (but see Moretti *et al.* 2013; Dinnage *et al.* 2012). The unexpected results from Chapter 4 demonstrate the complex, species-specific nature of multitrophic BEF relationships. Further experimental work is needed to better understand of the ecosystem-level response to current and future changes in biological communities.

5.2.6 Dung beetle effects on tropical soils

The most commonly investigated ecological function carried out by dung beetles in tropical forests is their role as secondary seed dispersers, but what are the other subterranean consequences of beetle activity? Currently, there exists no information on how beetles modify the biophysical environment of tropical forest soils, probably because dung is patchily distributed in forest environments (Peck & Howden 1984) compared to leaf litter, which can exceed 12mg/ha (Vitousek 1984). Therefore, the decomposition of leaves is likely the primary source of nutrient cycling in these systems. However, in Chapter 4, I reveal novel data that suggests that dung beetles could influence soil properties in a way that influences local-scale seedling establishment. This indicates that in addition to seed burial, beetles could impart additional benefits to secondarily dispersed seeds in the tropics. However, at the moment this is speculative as my study does not provide firm insights into this process, but the results do highlight an exciting avenue for future work. Finally, all experimental work to date has investigated the effect of the presence/absence or abundance of beetles on soil properties without considering the role of diversity (e.g. Yokoyama *et al.* 1991; Bang *et al.* 2005; Yamada *et al.* 2007). Consequently, a mechanistic explanation of what aspects of beetle communities are driving the observed effect of beetles on soils are lacking. These knowledge gaps could be addressed concurrently through the design of field and complementary laboratory experiments that link dung beetle mediated edaphic changes in tropical soils to the functional characteristics of the community responsible.

5.3 CONCLUDING REMARKS

This thesis provides just one example of the overwhelming complexity integral to natural systems. It also empirically demonstrates how diversity in invertebrate communities can drive ecosystem processes and influence organisms belonging to different trophic levels. Ecosystems are a product of their abiotic environment and the biophysical processes governed by multitrophic species interactions. Since human activities are almost certainly erasing species from the planet before they are known to science (Giam *et al.* 2012; Lees & Pimm 2015), we must also be erasing species interactions (and therefore processes) before they have been observed. It is becoming increasingly apparent that the loss of ecosystem processes has the potential to negatively impact upon human wellbeing through disruption of the ecosystem services upon which we rely (Cardinale *et al.* 2012; Hooper *et al.* 2012). It is probable that we are unaware of countless species interactions underpinning these services. Therefore, since we are unlikely to describe the full array of diversity-functioning relationships prior to their expiration, the continued loss of species will likely have unforeseen, negative consequences for the functioning and maintenance of ecosystems in their current states.

The study of functional ecology allows us to document associations between organisms and facilitates a mechanistic understanding of how biological communities shape their environment. However, understanding the role of diversity in maintaining ecosystem processes will not prevent the loss of functioning associated with anthropogenic species extinctions. In order to mitigate the consequences of human-driven alternations of ecosystems and prevent or even reverse cascading ecological changes, the field must continue to develop to more precisely describe biological communities and the processes they govern. This will undoubtedly be challenging given the complexities illustrated in this thesis. However, the continued development of functional ecology will allow the formation of evidence-based conservation and policy decisions. This is essential for the persistence of diverse, functioning and self-sustaining ecosystems in an increasingly human-modified world. Finally, the erosion of biological diversity and ecological interactions represents a great loss to all those who are captivated by the startling intricacy of natural systems. The examination of species functional role in ecosystems increases our ability to describe these complexities, and in doing so increases in our appreciation of what we are unravelling.

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