UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE INVESTIGACIONES EN ECOSISTEMAS Y SUSTENTABILIDAD
ECOLOGÍA

EFECTOS DE LOS ESCARABAJOS COPRÓFAGOS SOBRE LA REGENERACIÓN DE PLANTAS EN EL BOSQUE TROPICAL

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

LINA ADONAY URREA GALEANO

TUTORA PRINCIPAL DE TESIS: DRA. ELLEN ANDRESEN
INSTITUTO DE INVESTIGACIONES EN
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INSTITUTO DE INVESTIGACIONES EN
ECOSISTEMAS Y SUSTENTABILIDAD, UNAM
DR. GABRIEL RAMOS FERNÁNDEZ
CENTRO DE CIENCIAS DE LA COMPLEJIDAD, UNAM

MORELIA, MICHOACÁN, SEPTIEMBRE, 2020

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POSGRADO CIENCIAS BIOLÓGICAS
UNAM POSGRADO

COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS

ENTIDAD IIES-M

OFICIO CPCB/471/2020

ASUNTO: Oficio de Jurado

M. en C. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
Presente

Me permito informar a usted que en la reunión ordinaria del Subcomité de Ecología y Manejo Integral de Ecosistemas del Posgrado en Ciencias Biológicas, celebrada el día **24 de febrero de 2020,** se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la estudiante **URREA GALEANO LINA ADONAY,** con número de cuenta **514350914,** con la tesis titulada, **"Efectos de los escarabajos coprófagos sobre la regeneración de plantas en el bosque tropical",** realizada bajo la dirección de la **DRA. ELLEN ANDRESEN,** quedando integrado de la siguiente manera:

Presidente:	DR. FEDERICO ESCOBAR SARRIA
Vocal:	DRA. JULIETA BENÍTEZ MALVIDO
Secretaria:	DRA. EK DEL VAL DE GORTARI
Suplente:	DR. LEONEL ARTURO LÓPEZ TOLEDO
Suplente:	DRA. LUCRECIA ARELLANO GÁMEZ

Sin otro particular, me es grato enviarle un cordial saludo.

ATENTAMENTE
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Cd. Universitaria, Cd. Mx., a 11 de agosto de 2020

COORDINADOR DEL PROGRAMA

DR. ADOLFO GERARDO NAVARRO SIGÜENZA

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A mis padres, hermano y esposo
A mi querida tía Ana

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RESUMEN

Los escarabajos coprófagos procesan y entierran las heces de otros animales, principalmente de mamíferos. Como consecuencia de este comportamiento desarrollan varias funciones ecológicas con implicaciones importantes para las plantas. En los bosques tropicales muchos estudios han justificado el uso de los escarabajos coprófagos como taxón focal o indicador debido, entre otras cosas, a su importancia funcional. Sin embargo, la información cuantitativa sobre sus funciones ecológicas viene principalmente de sistemas productivos, pastizales templados, y estudios bajo condiciones controladas, y son pocas las funciones ecológicas que se han cuantificado bajo condiciones naturales en los bosques tropicales. La única función evaluada con mayor detalle en bosques tropicales es la dispersión secundaria de semillas defecadas por mamíferos. Sin embargo, incluso para esta función el conocimiento es limitado, debido a que es principalmente sobre la dispersión vertical (enterramiento de semillas), y a que son pocos los estudios que han dado seguimiento al destino de las semillas hasta la etapa de plántula. Sobre otras funciones que desempeñan los escarabajos coprófagos y que pueden tener implicaciones para las plantas, existe poca información para bosques tropicales. El objetivo principal de esta investigación fue cuantificar los efectos de la actividad de los escarabajos coprófagos en diferentes fases de la regeneración temprana de las plantas en un bosque tropical. Para esto, se realizaron varios experimentos de campo en sitios del sotobosque (a > 50 m de bordes de bosque) de la Estación de Biología Tropical Los Tuxtlas, Veracruz, México. En el primer capítulo se evaluaron los efectos de la dispersión secundaria a través del movimiento horizontal de semillas por escarabajos coprófagos, sobre la agregación espacial de semillas y plántulas, y la probabilidad de establecimiento de plántulas de dos especies arbóreas (*Bursera simaruba* y *Poulsenia armata*). Para ambas especies estudiadas, se encontró que la actividad de los escarabajos coprófagos redujo la agregación espacial de semillas y plántulas, pero no tuvo un efecto positivo sobre el establecimiento. En el segundo capítulo se determinó el efecto de la actividad de los escarabajos coprófagos sobre la dinámica del banco de semillas y el establecimiento de plántulas provenientes del banco de semillas, comparando sitios con un patrón de defecación recurrente vs. sitios con un patrón de defecación única. Se encontró que los escarabajos coprófagos redistribuyeron en el eje vertical del suelo, hacia arriba y hacia abajo, a las semillas enterradas en el banco de semillas. Las semillas pequeñas tuvieron una mayor probabilidad de ser movidas hacia estratos más profundos, y lo contrario ocurrió para las semillas grandes. También, se encontró que la actividad de los escarabajos coprófagos tuvo un efecto positivo sobre el establecimiento de plántulas a partir de semillas presentes en el banco de semillas. Sin embargo, no se encontró efecto de la recurrencia de la defecación (y por lo tanto de la frecuencia de la actividad de los escarabajos coprófagos) sobre el establecimiento. Finalmente, en el tercer capítulo se evaluó si la actividad de los escarabajos coprófagos, bajo condiciones naturales, tiene algún efecto positivo sobre la asimilación de nutrientes foliares (N y P) y el desempeño (supervivencia, crecimiento) de plántulas de seis especies arbóreas (*Brosimum alicastrum*, *Calophyllum brasiliense*, *Cymbopetalum baillonii*, *Diospyros digyna*, *Omphalea oleifera* y *Poulsenia armata*). Se encontró que la actividad de los escarabajos coprófagos no tuvo efectos positivos sobre la asimilación de nutrientes ni sobre el desempeño de las seis especies de plántulas. Para dos especies, la actividad de los escarabajos coprófagos tuvo un efecto negativo sobre su probabilidad de crecer. En conjunto, los resultados de esta investigación demuestran que el efecto de la actividad de los escarabajos coprófagos sobre la regeneración temprana de las plantas en bosques tropicales depende del contexto y de la especie de planta. Hasta el momento, para los bosques tropicales, hay evidencia de que la dispersión secundaria de semillas por escarabajos coprófagos puede favorecer la supervivencia de semillas y el establecimiento de plántulas a partir de semillas defecadas. Asimismo, existe evidencia que puede promover el establecimiento de plántulas a partir del banco de semillas del suelo. Sin embargo, no hay evidencia de que la actividad de escarabajos coprófagos tenga efectos positivos sobre el desempeño de plántulas establecidas en el sotobosque. Finalmente, esta tesis destaca que los escarabajos coprófagos son un grupo focal e indicador muy útil y ecológicamente importante en muchos sistemas. No obstante, es necesario tener precaución con las extrapolaciones de los efectos positivos sobre las plantas observados en otros sistemas de estudio, hacia los bosques tropicales. Es importante estimar empíricamente las funciones de los escarabajos coprófagos en bosques tropicales bajo condiciones naturales, para evitar inferir importancias funcionales sin el adecuado soporte.

ABSTRACT

Dung beetles process and bury the feces of other animals, mainly that of mammals. As a consequence of this behavior, they perform some ecological functions with important implications for plants. In tropical forests many studies have justified the use of dung beetles as a focal or indicator taxon due to, among other reasons, their functional importance. However, the quantitative information about their ecological functions comes mostly from crops, temperate grasslands and studies done under controlled conditions, and there are few ecological functions quantified under natural conditions in tropical forests. The only function assessed in more detail in tropical forests, is the secondary dispersal of seeds found in mammal defecations. Nonetheless, even for this function knowledge is limited, because it is mostly for vertical dispersal (seed burial), and becausefew studies have followed the fate of these seeds until seedling establishment. Regarding other dung beetle functions that might have implications for plants, there is little information for tropical forests. The main objective of this research was to quantify the effects of dung beetle activity on different phases of plants' early regeneration in tropical a forest. To this end, field experiments were carried out in forest understory sites (> 50 m away from the forest border) at Los Tuxtlas Tropical Biology Station, Veracruz, Mexico. The first chapter, assessed the effects of secondary seed dispersal through the horizontal movement of seeds by dung beetles, on the spatial aggregation of seeds and seedlings, and on the probability of seedling establishment of two plant species (*Bursera simaruba* and *Poulsenia armata*). For both studied species, dung beetle activity was found to reduce the spatial clumping of seeds and seedlings; however, it did not have a positive effect on the probability of seedling establishment. The second chapter, determined the effect of dung beetle activity on the dynamics of the soil seed bank and the establishment of seedlings from the soil seed bank, comparing sites with a recurrent vs. single defecation pattern. Dung beetles were found to cause the re-distribution, both upwards and downwards, of soil seeds along the vertical axis. Small seeds had higher probability of being moved to deeper layers, and the contrary occurred for large seeds. Also, dung beetle activity was found to have a positive effect on the establishment of seedlings originating from the soil seed bank. However, there was no effect of the frequency of

defecation (and therefore of the frequency of dung beetle activity) on seedling establishment. Finally, the third chapter assessed whether the activity of dung beetles, under natural conditions, has any positive effect on nutrient assimilation in leaves (N and P) and on seedling performance (survival, growth), in six tree species (*Brosimum alicastrum*, *Calophyllum brasiliense*, *Cymbopetalum baillonii*, *Diospyros digyna*, *Omphalea oleifera* and *Poulsenia armata*). Dung beetle activity did not have positive effects on nutrient assimilation, or seedling performance in any of the six seedling species. For two species, dung beetle activity had a negative effect on the probability of growth. Overall, the results of this research show that the effects of dung beetle activity on the early regeneration of plants in tropical forests are context- and species-specific. Up to this moment, for tropical forests, there is evidence that secondary seed dispersal by dung beetles can favor seed survival and seedling establishment for defecated seeds. Also, there is evidence that it can promote seedling establishment from the soil seed bank. However, there is no evidence that the activity of dung beetles has positive effects on the performance of understory seedlings. Finally, this thesis highlights that dung beetles are a useful focal and indicador group that is ecologically important in many systems. Nevertheless, it is necessary to be cautious when extrapolating their positive effects on plants observed in other systems, into tropical forests. It is important to estimate the functions of dung beetles empirically in tropical forests under natural conditions, to avoid inferring functional importances without adequate evidence.

INTRODUCCIÓN GENERAL

La regeneración natural de los bosques es un proceso de reemplazamiento de árboles maduros, que eventualmente mueren, por nuevos árboles reclutas (Martínez-Ramos 1994). Los árboles reclutas se pueden establecer a partir de diferentes fuentes de propágulos como las semillas recién dispersadas, el banco de semillas del suelo, el banco de plántulas establecidas en el sotobosque, entre otros (Martínez-Ramos 1994). Sobre los propágulos ocurren varios procesos ecológicos (e.g., dispersión de semillas, germinación, establecimiento, supervivencia y crecimiento de plántulas) que determinan la transición entre las fases del ciclo de vida de las plantas, y por consiguiente, son importantes en la demografía de las poblaciones y la composición de las comunidades vegetales (Forget et al. 2005; Leck et al. 2008). Un buen entendimiento de los procesos ecológicos que actúan en las fases iniciales de la regeneración natural de los bosques tropicales es fundamental para comprender el funcionamiento de estos ecosistemas (Schupp 2007; Norden 2014; Arroyo-Rodríguez et al. 2015) y desarrollar estrategias eficientes de manejo (Shoo y Catterall 2013; Catterall 2018).

En los bosques tropicales muchos de los procesos de la regeneración natural son mediados por la fauna (Burslem et al. 2005; Neuschulz et al. 2016). Así, por ejemplo, en sistemas diplócoros donde los vectores de dispersión primaria (i.e., primer movimiento de una semilla fuera del área de influencia de la planta madre) y secundaria (i.e., cualquier movimiento de una semilla posterior a la dispersión primaria) son diferentes (Vander Wall y Longland 2004), existe una alta proporción de plantas que son dispersadas primariamente a través de las heces de aves y/o mamíferos, y secundariamente por insectos y/o roedores (Forget et al. 2005; Vander Wall et al. 2005; Fleming y Kress 2013). Un grupo de animales que puede afectar a la vegetación, directamente mediante la interacción con semillas, o indirectamente por los cambios que provoca en el suelo, son los escarabajos coprófagos (Scarabaeinae). Estos insectos procesan las heces de animales, preferentemente de mamíferos, y las entierran para su alimentación y reproducción (Halfter y Edmonds 1982). Las consecuencias funcionales de este comportamiento con implicaciones para la regeneración de las plantas incluyen: bioturbación del suelo, dispersión secundaria de semillas, reciclaje de nutrientes, entre otros (Nichols et al. 2008; Scholtz et al. 2009).

Este grupo de insectos ha sido usado como taxón indicador y focal en estudios ecológicos y de conservación a nivel mundial, especialmente en regiones tropicales (Spector 2006; Nichols y Gardner 2011; Fonseca Oliveira et al. 2017; Carvalho et al. 2020a). El uso como taxón indicador se debe a que los escarabajos coprófagos son sensibles a perturbaciones antropogénicas que provocan cambios en la vegetación (Halffter y Arellano 2002) y/o en las poblaciones de vertebrados que les proveen el recurso alimenticio (Andresen y Laurance 2007; Nichols et al. 2009; Culot et al. 2013). Como taxón focal se ha utilizado porque es un grupo taxonómico relativamente bien conocido, cuyo método de captura es sencillo y económico (Spector 2006). Entre otras características, su importancia funcional en los ecosistemas es una de las principales justificaciones para su uso como taxón focal o indicador de integridad ecológica (e.g., Carvalho et al. 2020b; Salomão et al. 2020). Sin embargo, mucha de la información cuantitativa sobre la mayoría de las funciones ecológicas que se les atribuye a estos insectos viene de sistemas productivos, pastizales templados, o estudios bajos condiciones controladas (e.g., Bang et al. 2005; Hanafy 2012; Johnson et al. 2016). Con excepción de la dispersión secundaria de semillas defecadas por mamíferos (Andresen y Feer 2005), son pocas las funciones que se han cuantificado bajo condiciones naturales en bosques tropicales (Nichols et al. 2008).

La dispersión secundaria de semillas por escarabajos coprófagos incluye tanto la dispersión vertical, como la horizontal. La primera comprende los movimientos de las semillas a lo largo del eje vertical del suelo (entierro), mientras que la segunda involucra los movimientos a lo largo del eje horizontal (Andresen y Feer 2005). La mayoría de los estudios que han evaluado la dispersión secundaria de semillas han cuantificado principalmente los porcentajes y distancias de dispersión, y el destino post-dispersión de las semillas a corto plazo (ver referencias en Andresen y Feer 2005). Dichos estudios, han permitido generalizar que la dispersión vertical de semillas por escarabajos aumenta la probabilidad de supervivencia de las semillas (Estrada y Coates-Estrada 1991; Shepherd y Chapman 1998; Andresen y Feer 2005; Santos-Heredia et al. 2010). Sin embargo, pocos estudios han dado seguimiento a las semillas hasta la etapa de plántula, encontrándose tanto efectos positivos, como negativos sobre la emergencia y/o establecimiento de las plántulas (Andresen y Levey 2004; Lawson et al. 2012; Culot et al. 2015, 2018; Griffiths et al. 2016). Es importante notar que, la mayoría de los estudios previos han evaluado principalmente los efectos de la dispersión vertical. En el caso de la dispersión horizontal, se ha sugerido que las cortas distancias (< 5 m) de movimiento horizontal que provocan los escarabajos coprófagos a las semillas defecadas, podrían ser suficientes para favorecer el establecimiento de plántulas tras reducir la agrupación espacial en que fueron depositadas por el dispersor primario (Estrada y Coates-Estrada 1991; Andresen 2002; Ponce-Santizo et al. 2006). Las altas agregaciones de semillas y plántulas se asocian con una alta mortalidad de individuos debido a un incremento en la competencia, ataque por patógenos, depredación y herbivoría (Forget et al. 2005; Leck et al. 2008; Gallagher 2014). Hasta la fecha sólo un estudio ha cuantificado las consecuencias de la dispersión horizontal de semillas presentes en heces y dispersadas secundariamente por escarabajos coprófagos, sobre el establecimiento de plántulas (Lawson et al. 2012). Dicho estudio encontró que, en parcelas con actividad de escarabajos coprófagos, las plántulas de dos especies (*Tabernaemontana donnell-smithii* Rose y *Guazuma ulmifolia* Lam) tuvieron una menor agregación espacial, la cual fue asociada con un efecto positivo sobre el establecimiento para una de las especies, pero con un efecto negativo para la otra especie (Lawson et al. 2012). En consecuencia, se requiere de más estudios que permitan establecer cuál es el efecto general de la dispersión horizontal de semillas por escarabajos en fases iniciales de la regeneración natural de las plantas en bosques tropicales.

La interacción entre escarabajos coprófagos y semillas no ocurre únicamente a través de la dispersión secundaria de semillas defecadas por mamíferos. Dos de los principales grupos funcionales de escarabajos coprófagos tropicales, los rodadores y cavadores, excavan grandes cantidades de suelo de estratos inferiores hacia la superficie (Braga et al. 2013, 2017; Carvalho et al. 2020a). Como consecuencia de la excavación, los escarabajos pueden traer hacia la superficie del suelo semillas enterradas en el banco de semillas (Santos-Heredia y Andresen 2014). Este proceso podría tener importantes efectos sobre el establecimiento de plántulas. Hasta la fecha, sólo dos estudios han abordado este tema, uno para bosque tropical húmedo (Santos-Heredia y Andresen 2014) y otro para bosque tropical seco (Ocampo-Castillo y Andresen 2018). Ambos estudios encontraron un mayor establecimiento de plántulas, a partir del banco natural de semillas, en sitios con heces y actividad de Scarabaeinae en comparación a sitios sin heces y por lo tanto sin actividad de escarabajos coprófagos. Sin embargo, los diseños experimentales usados en dichos estudios no permitieron determinar claramente el efecto de la actividad de los escarabajos coprófagos (i.e., por ausencia de un tratamiento control con heces pero sin escarabajos, Santos-Heredia y Andresen 2014; por un tamaño de muestra y poder estadístico limitado, Ocampo-Castillo y Andresen 2018). Por otro lado, podría esperarse que la actividad de los escarabajos coprófagos fuera mayor en áreas del bosque que reciben deposiciones recurrentes de heces, como ocurre en los sitios de descanso o letrina de algunos mamíferos (Irwin et al. 2004; Whitworth et al. 2019). Investigaciones previas han mostrado un efecto sobre la disponibilidad de nutrientes en el suelo, y sobre el uso de estos nutrientes por las plantas, en sitios en los que monos y murciélagos defecan de manera recurrente (Feeley 2005; Dos Santos Neves et al. 2010; Voigt et al. 2015; Bravo y Cueto 2020). Asimismo, se ha encontrado que los bancos de semillas (Pouvelle et al. 2009; Feer et al. 2013) y de plántulas (e.g., Anzures-Dadda et al. 2011; Bravo 2012; González-Zamora et al. 2015) son diferentes en áreas donde se acumula excremento. En estos estudios, los patrones de mayor cantidad de nutrientes y de mayor establecimiento de plántulas han sido atribuidos al mayor ingreso de materia fecal y de semillas defecadas, respectivamente. Sin embargo, estos efectos podrían verse acentuados por la actividad de los escarabajos coprófagos. Por ejemplo, dado el efecto de los escarabajos coprófagos en la dinámica del banco de semillas (Santos-Heredia y Andresen 2014; Ocampo-Castillo y Andresen 2018), estos insectos podrían estar siendo responsables, al menos en parte, de la mayor cantidad de plántulas establecidas en sitios de deposición recurrente de mamíferos.

Finalmente, los escarabajos coprófagos no sólo interactúan directamente con las plantas mediante el movimiento de semillas, sino que también pueden interactuar indirectamente. En sistemas agropecuarios y/o experimentos bajo condiciones controladas se ha comprobado que la actividad de los escarabajos coprófagos provoca cambios fisicoquímicos en el suelo

(Scholtz et al. 2009; Brown et al. 2010; Forgie et al. 2018), que a su vez pueden acelerar la entrada y mineralización de nutrientes provenientes de las heces al suelo (Yamada et al. 2007; Menéndez et al. 2016; Maldonado et al. 2019), y tener un efecto positivo en el desempeño de las plantas (e.g., crecimiento, contenido de nutrientes foliares; Behling Miranda et al. 2000; Bang et al. 2005; Hanafy 2012; Johnson et al. 2016; Badenhorst et al. 2018). Según mi conocimiento, solamente un estudio ha investigado estos efectos en un bosque tropical bajo condiciones naturales, aunque para una sola especie de plántula (*Brosimum lactescens* (S. Moore) C.C. Berg; Santos-Heredia et al. 2016). En dicho estudio, la actividad de los escarabajos coprófagos provocó un aumento en el contenido de nitrógeno y fósforo foliar, y se relacionó con un incremento en el crecimiento neto en el número de hojas, pero también con una mayor mortalidad de plántulas (Santos-Heredia et al. 2016). En la medida que este tipo de estudios se realicen en otros bosques tropicales y con un mayor número de especies de plantas, será posible tener un mejor entendimiento de la dirección y la magnitud del efecto de la actividad de los escarabajos coprófagos, mediante la bioturbación y el reciclaje de nutrientes, sobre el desempeño de las plántulas del sotobosque. Conocer las implicaciones ecológicas de esta función (i.e., favorecimiento del desempeño de las plantas) es importante, porque los estudios tropicales que la usan como justificación para utilizar estos insectos como taxón focal o indicador, lo hacen extrapolando información obtenida a partir de otros sistemas.

El objetivo general de esta investigación fue cuantificar los efectos de la actividad de los escarabajos coprófagos sobre diferentes fases de la regeneración temprana de las plantas en un bosque tropical (Figura 1). Primero, se determinó para *Bursera simaruba* y *Poulsenia armata* el efecto de la dispersión secundaria a través del movimiento horizontal de las semillas presentes en heces, sobre la agregación espacial de semillas y plántulas, y también sobre la probabilidad de establecimiento de plántulas (Capítulo I). Se seleccionó *B. simaruba* y *P. armata* porque las semillas de ambas especies germinan rápido (< 2 meses), son dispersadas en heces de mamíferos (Estrada y Coates-Estrada 1991) y su tamaño permite la dispersión secundaria por parte de los escarabajos coprófagos del sitio de estudio. Segundo, se evaluó el efecto de la actividad de estos insectos sobre la dinámica del banco de semillas y el establecimiento de plántulas provenientes del banco de semillas, comparando sitios con un patrón de defecación recurrente de mamíferos con sitios de defecación única (Capítulo II). Finalmente, se cuantificó el efecto de la actividad de los escarabajos coprófagos sobre la asimilación de nutrientes foliares (nitrógeno y fósforo), la asignación de recursos y el desempeño (supervivencia, crecimiento) de plántulas establecidas (individuos de ~ 3-8 meses de edad y 1-72 cm de altura) de *Brosimum alicastrum, Calophyllum brasiliense, Cymbopetalum baillonii*, *Diospyros digyna*, *Omphalea oleifera* y *Poulsenia armata* (Capítulo III). Se seleccionaron estas especies de plantas porque sus plántulas son tolerantes a la sombra del dosel del bosque (Coates-Estrada y Estrada 1988; González Soriano et al. 1997; CATIE 2000; Ricker et al. 2000; Zambrano et al. 2014), y por la facilidad de obtener el número de plántulas suficiente para los experimentos. En resumen, esta tesis contribuye de manera importante al conocimiento actual sobre las interacciones entre los escarabajos coprófagos y las plantas tropicales, llenando así algunos de los vacíos de información y avanzando en la comprensión acerca de la importancia funcional de estos insectos en los bosques tropicales (Figura 1).

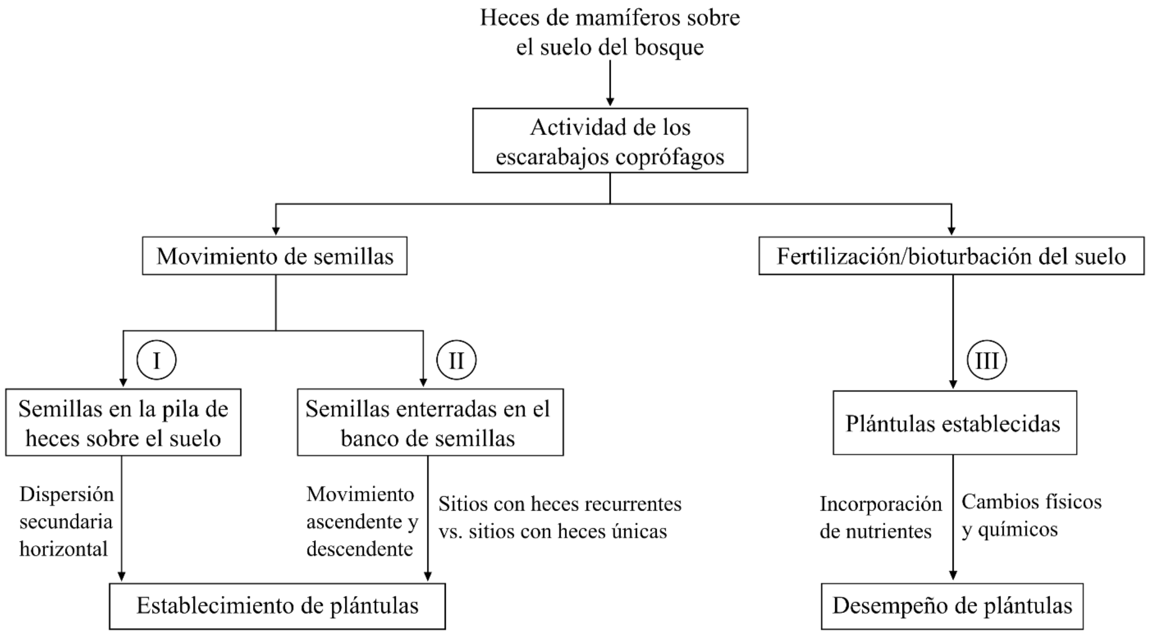

FIGURA 1. Diagrama que ilustra las interacciones escarabajo-planta evaluadas en esta tesis. El número dentro de cada círculo indica el capítulo de la tesis relacionado con él. Las heces de mamíferos sobre el suelo de los bosques tropicales atraen escarabajos coprófagos. Al procesar y enterrar las heces, estos insectos llevan a cabo importantes funciones ecológicas para las plantas, como el movimiento de semillas y la fertilización y bioturbación del suelo. En el caso del movimiento de semillas, la actividad de los escarabajos coprófagos puede, mediante la dispersión horizontal de semillas defecadas, afectar la agrupación espacial de semillas/plántulas y por ende la probabilidad de establecimiento de estas plántulas (Capítulo I). También, mediante la construcción de túneles y la excavación de suelo, puede afectar la dinámica del banco natural de semillas y el subsecuente establecimiento de plántulas; este efecto puede ser de diferente intensidad en sitios con defecaciones recurrentes en comparación con sitios con defecaciones únicas (Capítulo II). Con respecto a la fertilización y bioturbación del suelo, la actividad de los escarabajos coprófagos provoca cambios físicos y químicos en el suelo, que pueden afectar indirectamente la concentración de nutrientes foliares y por lo tanto el desempeño (e.g., supervivencia, crecimiento) de plántulas establecidas en el sotobosque (Capítulo III).

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CAPÍTULO I

Horizontal seed dispersal by dung beetles reduced seed and seedling clumping, but did not increase short-term seedling establishment

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RESEARCH ARTICLE

Horizontal seed dispersal by dung beetles reduced seed and seedling clumping, but did not increase short-term seedling establishment

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Abstract

Dung beetles are secondary seed dispersers, incidentally moving many of the seeds defecated by mammals vertically (seed burial) and/or horizontally as they process and relocate dung. Although several studies have quantified this ecological function of dung beetles, very few have followed seed fate until seedling establishment, and most of these have focused on the effects of seed burial. We know very little about the effects of horizontal seed movement by dung beetles, though it is generally assumed that it will affect plant recruitment positively through diminishing seed clumping. The objective of our study was to assess the effects of dung beetle activity on the spatial distribution of seeds and seedlings, and on the probability of seedling establishment. In a tropical rainforest in Mexico we carried out two complementary field experiments for each of two tree species (*Bursera simaruba* and *Poulsenia armata*), using seeds experimentally imbedded in pig dung and recording their fate and spatial location over time. For both species, dung beetle activity reduced the spatial clumping of seeds and seedlings; however, it did not increase the probability of seedling establishment. We discuss the context- and species-specificity of the combined effects of horizontal and vertical dispersal of seeds by dung beetles, and the need to quantify long-term seedling fates to more accurately determine the effects of seed movement by dung beetles on plant recruitment.

Introduction

Any movement of a seed after deposition by the primary seed dispersal vector constitutes secondary seed dispersal, a common process that affects seed fate and ultimately plant demography [1,2]. In diplochorous systems, where the primary and secondary dispersal vectors are

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different, a potential advantage of the second phase of dispersal can be the movement of the seeds to microsites that are predictably more adequate for seed survival and/or seedling establishment and survival (i.e., directed dispersal; [3,4]). In tropical forests, a very large proportion of plant species are dispersed primarily by frugivorous animals, mostly birds and mammals [5,6]. In the case of seeds dispersed through mammal defecation, a common diplochorous system occurs, in which dung beetles are often responsible for the secondary dispersal of those seeds [4,7,8].

Dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae) disperse seeds accidentally, as they process dung for feeding or ovipositing [9]. The way in which dung beetles process dung depends on the functional group they belong to. They are classified as dwellers, when all their activities are done in the dung pad or immediately underneath it; tunnelers, if they first construct tunnels in the soil under or near the dung pad and then push and pull portions of dung into the tunnels; and rollers, when they first shape dung portions into balls, which they then roll away from the dung pad to a location where they build a tunnel to bury the dung balls [9]. Competition for the ephimerous and patchily-distributed dung is intense, and in tropical rainforests all the fecal material is quickly relocated beneath the soil by tunneler and roller beetles [10]. Dung relocation is responsible for many of the ecological functions attributed to dung beetles [11], including the secondary dispersal of seeds embedded in the dung [7].

The interaction between dung beetles and defecated seeds can have strong effects on seed fate and seedling establishment, but the direction (positive vs. negative) and intensity of these effects are highly species- and context-specific [8,12,13]. For example, burial by beetles is known to greatly diminish the probability of seed predation [7,14–16]. However, while seed burial by beetles may increase seedling establishment for some plant species due to enhanced seed survival [8,12,17,18], others may be affected negatively due to reduced seedling emergence when seeds are buried too deeply [12,13,19]. Even though secondary seed dispersal by dung beetles has been relatively well studied in tropical forests, our ability to generalize on the ultimate net effect of the seed-beetle interaction on plant fitness is still limited, not only due to the context-dependence of the interaction's result, but also due to the fact that very few studies have followed seed fate until seedling establishment [8,12,13,18,20].

Secondary seed dispersal by dung beetles can include both seed burial (vertical dispersal) and/or horizontal seed dispersal [21], but most studies have focused on the effects of the former. However, it has often been suggested that horizontal movement of seeds by dung beetles, although generally restricted to small distances (< 5 m), may favor plant recruitment by decreasing the degree of spatial clumping in which defecated seeds are deposited by the primary disperser [14,21,22]. Spatial clumping of seeds and seedlings, generally associated with high densities, is known to negatively affect plants through various mechanisms, including decreased germination and increased competition, pathogen attack, predation and herbivory [2,23,24]. To our knowledge, only one study has so far quantified a decrease in the spatial clumping of seedlings of two species, following dung beetle activity, with an associated positive effect on the probability of seedling establishment for one of the species, but not for the other one [20]. However, this study used mixtures of both seed species in each dung pile, and seedling establishment could have been affected by interspecific competition [20]. Thus, considering that: (i) the outcome of the seed-beetle interaction is species- and context-specific, (ii) very few studies have followed seed fate until seedling establishment, and (iii) only one study has quantified the effects of horizontal seed dispersal by dung beetles on seedlings, we believe that additional information is necessary before we can reach a more general conclusion on when secondary dispersal by dung beetles has positive effects on plant regeneration, and when not.

Our main objective was to assess, for two tree species, the effects of secondary seed dispersal by dung beetles, with emphasis on the horizontal movement of seeds, on the spatial distribution of seeds and seedlings, and on the probability of seedling establishment. Our hypothesis was that dung beetle activity would favor seedling establishment because it reduces seed and seedling clustering through horizontal relocation of the seeds present in dung. To test this hypothesis we carried out field experiments to assess the following predictions: (i) dung beetle activity increases the horizontal distance between adjacent seeds deposited in dung, regardless of seed burial; (ii) dung beetle activity increases the distance between established seedlings; (iii) dung beetle activity increases the probability of seedling establishment.

Methods

Study site

The study was carried out in the Los Tuxtlas Biological Station (LTBS), in the Mexican state of Veracruz (18°35′5″ N, 95°4′34″ W; *ca*. 150 m a.s.l.). The LTBS, a protected area established in 1967, encompasses 640 ha of tropical rainforest that constitute, since 1998, one of the core areas of the 155,122-ha Los Tuxtlas Biosphere Reserve [25]. All field activities were conducted under full permission of the authorities of the LTBS, Institute of Biology, National Autonomous University of Mexico. No endangered or protected species were collected. Mean annual temperature is 24.1°C and mean annual rainfall is 4201 mm, with a short drier period ($<$ 100 mm per month) from March to April, and a long wetter period ($\geq$ 100 mm per month) from May through February, the latter representing *ca*. 95% of the total annual rainfall [26].

Despite intense forest loss and fragmentation occurring in the Los Tuxtlas region, the LTBS and the other core areas of the Biosphere Reserve are an important refuge for animals, including dung beetles, with at least 34 species reported for LTBS [27], as well as many mammals [28,29]. Among mammals present at LTBS that are primary dispersers of seeds through defecation, figure most prominently the herbivorous-frugivorous howler monkeys (*Alouatta palliata*; [14]), but also other important omnivorous-frugivorous species such as coatis (*Nasua narica*), raccoons (*Procyon lotor*), tayras (*Eira barbara*), peccaries (*Pecari tajacu*) and kinkajous (*Potos flavus*) [30].

Effects of dung beetle activity on seed and seedling spatial distribution and on seedling establishment

To assess the effects of dung beetle activity on seeds and seedlings we carried out two complementary field experiments focusing on two tree species that are abundant in our study site (G. Ibarra-Manríquez, pers. comm.): *Bursera simaruba* (L.) Sarg. (Burseraceae) and *Poulsenia armata* (Miq.) Standl. (Moraceae), hereafter referred to by their genus names. Both experiments were carried out independently for each species.

Bursera is a dioecious canopy tree; it is a light-demanding species that grows mostly in treefall gaps, reaching heights of 30 m [31]. Fruiting occurs between October and May; fruits are dehiscent drupes that contain a single seed covered by a juicy aril [31,32]. *Bursera* seeds are 7–9 mm long, 5–7 mm thick and wide, and germinate in ~ 2 weeks [33]. *Poulsenia* is a monoecious late-successional tree species that can reach heights of 20–40 m [34,35]. Fruiting occurs between May and November; the fruit is soft and berry-like, containing 9–17 seeds [32]. *Poulsenia* seeds are 8–10 mm long, 5–7 mm thick and wide, and germinate in ~ 4 weeks [33].

We chose to focus on *Bursera* and *Poulsenia* because they can be dispersed through defecation by mammals (e.g., *Alouatta palliata*; [14]), because their seeds have no dormancy [36], and due to the ease of obtaining the necessary number of seeds for the experiments. Furthermore, the seeds of these two species are of a size large enough to allow marking (see below, Experiment 1), but small enough that beetles at our study site will secondarily disperse many of them while processing dung.

First, we carried out Experiment 2 and then Experiment 1; however, since the former corresponds to the seedling stage and the latter to the seed stage, we describe them in life-cycle order. All experimental seeds of *Bursera* and *Poulsenia* were obtained from freshly fallen fruits underneath parent trees in April and June 2017, respectively. For both species, half of the seeds were used within 2 days of collection to set up Experiment 2; the rest of the seeds were air-dried in the shade and stored at room temperature until the setup of Experiment 1 (six months of storage for *Bursera* seeds and four months for *Poulsenia* seeds). We did not test the viability of the seeds prior to our experiments, and seed storage might have negatively affected it. However, in Experiment 1 we did not require seeds to be viable as we only recorded seed condition and position after 48 h (see below).

In the experiments we used fresh domestic pig dung (collected from a nearby household on the same day it was used) to make experimental dung piles containing seeds, thus mimicking primary seed dispersal through mammal defecation. We used domestic pig dung because we needed large quantities of dung for our experiments, and because it is efficient in attracting rainforest dung beetles [37].

Experiment 1. Seeds: Secondary dispersal and spatial distribution. We established 30 experimental sites in the forest understory ($\geq$ 50 m apart and $\geq$ 10 m from fruiting adults of the focal plant species). In each site we had 3 circular plots 50 cm in diameter, with 2–3 m between adjacent plots. The border of each plot was delimited by burying a 30 cm wide metallic mosquito netting strip 10 cm into the soil (S1 Fig). From the inside of each plot we removed the few existing seedlings. However, we kept the leaf litter to avoid affecting the behavior of roller dung beetles, which often choose a spot hidden under litter to build their tunnel (E. Andresen, pers. obs.), and thus litter removal may cause them to roll the dung balls larger distances. Each plot was randomly assigned to one of the following treatment levels: (1) 50 g of dung with 20 seeds embedded in it and access to dung beetles (+Feces+Beetles), (2) 50 g of dung with 20 seeds but no access to dung beetles (+Feces–Beetles), (3) no dung, 20 seeds placed directly on the forest floor (–Feces–Beetles). Since the amount of seeds present in the defecations of rainforest mammals can vary tremendously, depending on the plant and animal species (e.g., [8,38,39]), we used seed numbers that can commonly be found in howler-monkey dung piles (e.g., [40,41]). Furthermore, to better mimic the generally smaller size of individual dung piles of frugivorous rainforest mammals that fall on the forest floor (5–25 g; [22,41,42]) we divided the 50 g of dung into 4 equal portions (S1A Fig), each containing 5 seeds. Portions were placed in the center of plots, with ~ 3 cm between portions; this layout was also used for seeds without dung (S1B Fig). In order to measure seed movement, we marked each seed by threading a 30 cm-long piece of fishing line through it (S1C and S1D Fig). After placing dung and seeds in the plots we covered the two control plots (+Feces–Beetles and –Feces–Beetles) with mosquito netting to exclude dung beetles (S1E Fig), but left the plots with dung beetle access uncovered (+Feces+Beetles). After 48 h, when all dung in the plots with dung beetle activity had disappeared from the soil surface (S1F Fig), we used a 2 cm grid to map the location of each seed in the three plots (S1G Fig); for seeds that were buried we mapped its location projected to the surface. To describe the short-term fate of the seeds we classified each seed into one of three categories: (1) seed intact, when seeds were unharmed; (2) seed predated, when seed remains were found; and (3) seed removed, when the seed could not be found. For seeds intact we also recorded if the seed was visible on the soil surface, under leaf-litter, or buried. In the case of buried and horizontally moved seeds we measured the vertical and horizontal distances to the nearest centimeter. Dung beetle movement was limited by the plot's fence, i.e., seeds could not be dispersed beyond the fence. While this allowed us to find most seeds, it probably caused some underestimation of horizontal distances (see Discussion). This experiment was carried out during the rainy season (October 2017), first with *Poulsenia* seeds, and after two weeks, the same sites and plots were used to repeat the experiment with *Bursera* seeds.

Experiment 2. Seedlings: Spatial distribution and probability of establishment. We used a similar experimental setup as above, but without thread-marking the experimental seeds, to allow for germination. We used the same 30 sites as in Experiment 1, but with different plots for each plant species. For *Bursera*, the experiment started in April 2017 (one of the driest months) and for *Poulsenia* in June 2017 (the beginning of the rainy season). Control plots were immediately covered with mosquito netting, while the plot with dung beetle activity was kept open. After 48 hours the latter plots were also covered, to have the same conditions affecting seed and seedling fates in all treatments. The netting remained throughout the experiment to avoid seed rain and to minimize seed removal by granivorous animals and seedling loss due to herbivory. Once seedling establishment occurred (20 and 32 days after the setup of the experiments for *Bursera* and *Poulsenia*, respectively; S1H and S1I Fig) we checked each plot once a week for 15 weeks. During each check we registered established seedlings of the focal species and we mapped the location of each seedling using the 2 cm grid. We assumed that all seedlings of the focal plant species that we recorded, originated from our experimental seeds because: (i) all plots were > 10 m away from any fruiting adult, and (ii) in a previous study in the same sites and with the same treatments, only two seedlings of *Bursera* and two of *Poulsenia* established, overall, from the soil seed bank during a time period of 8 months [33]. Finally, we acknowledge that using seeds extracted from fruits may yield different results compared to using seeds that have passed through the digestive system of a mammal. However, we expect that whatever difference there might be in terms of seed germination would equally have affected our three treatment levels.

Data analyses

To measure the spatial distribution of seeds (Experiment 1) and seedlings (Experiment 2), following Lawson *et al.* [20] we used the Clark-Evans nearest neighbor index R [43]: $R = \bar{r}_A/\bar{r}_E$, where $\bar{r}_A$ is the average observed distance from an individual to its nearest neighbor in the plot, and $\bar{r}_E$ is the expected mean distance between neighbors if the distribution were random. When $R < 1$ the spatial distribution of individuals is clumped, when $R = 1$ it is random, and when $R > 1$ it is overdispersed [43].

For Experiment 1 we lost one *Poulsenia* plot with the +Feces+Beetles treatment. The nearest neighbor index data were analyzed by fitting linear mixed models (LMMs), one for each species. Treatment (+Feces+Beetles, +Feces−Beetles, and −Feces−Beetles) was the fixed factor, while site was included as a random factor.

For Experiment 2, following Lawson *et al.* [20] we analyzed the values of the nearest neighbor index observed during the week of peak seedling abundance, which was determined separately for each species-treatment combination. We chose this approach because for *Bursera* the temporal pattern of seedling emergence differed strongly among treatment levels, and mortality of seedlings occurred rapidly after emergence (S2 Fig). The weeks of peak abundance were as follows: *Bursera*, +Feces+Beetles: 4 wk, +Feces−Beetles: 9 wk, −Feces−Beetles: 10 wk; *Poulsenia*, +Feces+Beetles: 11 wk, +Feces−Beetles and −Feces−Beetles: 6 wk. The nearest neighbor index data for seedlings was analyzed in the same way as for seeds (LMM) in the case of *Poulsenia*; for *Bursera*, due to singularity problems during model fitting, seemingly caused by almost zero variance estimation for the random effect, we excluded the random factor following Bolker [44] and fitted a simple linear model.

To analyze seedling establishment, we fitted Cox regression models with mixed effects (i.e., frailty models), following [45]. In these models we included treatment (+Feces+Beetles, +Feces

–Beetles, and –Feces–Beetles) as the fixed factor, and site and plot as random factors. For these survival analyses we used as response variable the number of days elapsed until seedling establishment occurred for each seed; when this event did not occur at the end of the experiment, we considered this observation as a right-censored datum (e.g., [46]).

Data analyses were carried out using the R statistical environment (v. 3.5.2; [47]). The nearest neighbor indices were calculated using the function *clarkevans.test* of package 'spatstat' [48]. Models for the nearest neighbor index were fitted using functions *lm* of package 'stats' [47], and *lmer* of package 'lme4' [49]. Models for seedling establishment were fitted with function *coxme* of package 'coxme' [50]. In all models, treatment effect was tested through a Wald Chi-square test using the *Anova* function in the 'car' package [51]. We obtained marginal mean and standard error values, and carried out *post hoc* tests using the function *emmeans* of the package 'emmeans' [52]. We adjusted *P*-values in all *post hoc* tests using the False Discovery Rate method [53], because this method controls for false positives while also minimizing false negatives (e.g., [54]). All processed and raw data used in this study are provided in Supporting Information.

Results

Experiment 1. Seeds: Secondary dispersal and spatial distribution

The percentages of experimental seeds lost due to disappearance and predation were very low (*Bursera*: 0.5% and 0.2%, respectively; *Poulsenia*: 2.1% and 0.2%, respectively). For seeds classified as 'intact', most were seeds buried by beetles (*Bursera*: 57.2% ± 26.7%; *Poulsenia*: 54.1% ± 26.4%; mean ± SD), followed by seeds visible on the soil surface (*Bursera*: 32.4% ± 25%; *Poulsenia*: 34.1% ± 25.3%), and by seeds hidden under the leaf litter (*Bursera*: 10.4% ± 11.5%; *Poulsenia*: 11.8% ± 12.4%). For buried seeds, mean depth for both species was 5 cm, but the distribution was asymmetrical with most seeds buried shallowly, although some were buried deeply (*Bursera*, N = 341, min = 1 cm, max = 30 cm, median = 3 cm; *Poulsenia*, N = 308, min = 1 cm, max = 25 cm, median = 2 cm, S3 Fig).

Of all seeds classified as 'intact', 97% of *Bursera* seeds and 98% of *Poulsenia* seeds were moved horizontally at least 2 cm by dung beetles. The mean dispersal distance was 6 cm for both species, again with an asymmetrical distribution (*Bursera*, N = 577, min = 2 cm, max = 24 cm, median = 5 cm; *Poulsenia*, N = 556, min = 2 cm, max = 25 cm, median = 5 cm, S3 Fig).

As we predicted, for both species the mean nearest distance between two seeds was higher in plots with dung beetle activity (*Bursera*: 1.23 cm ± 0.61 cm; *Poulsenia*: 1.15 cm ± 0.51 cm), compared to plots with dung added but beetles excluded (*Bursera*: 0.003 cm ± 0.02 cm; *Poulsenia*: 0.0 cm) and to plots with no dung or beetles (*Bursera*: 0.13 cm ± 0.57; *Poulsenia*: 0.0 cm). The treatment had a significant effect on the spatial distribution of both seed species (*Bursera*, $\chi 2 = 137.46$, df = 2, $P < 0.001$, Fig 1A; *Poulsenia*, $\chi 2 = 310.66$, df = 2, $P < 0.001$, Fig 1B). Although in all cases the nearest neighbor index values were <1, indicating spatial clustering of seeds, dung beetle activity significantly reduced the degree of seed aggregation, compared to both treatment levels with no dung beetles (*Bursera*, +Feces+Beetles vs. +Feces–Beetles: $t = 10.60$, df = 87, $P < 0.001$, +Feces+Beetles vs.–Feces–Beetles: $t = 9.64$, df = 87, $P < 0.001$; *Poulsenia*, +Feces+Beetles vs. +Feces–Beetles: $t = 15.30$, df = 86, $P < 0.001$, +Feces+Beetles vs.–Feces–Beetles: $t = 15.30$, df = 86, $P < 0.001$). The two control treatment levels were not significantly different from each other (*Bursera*, +Feces–Beetles vs.–Feces–Beetles: $t = -0.95$, df = 87, $P = 0.34$; *Poulsenia*, +Feces–Beetles vs.–Feces–Beetles: $t = 0$, df = 86, $P = 1$).

Experiment 2. Seedlings: Spatial distribution and probability of establishment

As observed for seeds, for both plant species we found that the nearest neighbor distance between seedlings was higher in plots with dung beetle activity (*Bursera*, 3.67 cm ± 1.43 cm;

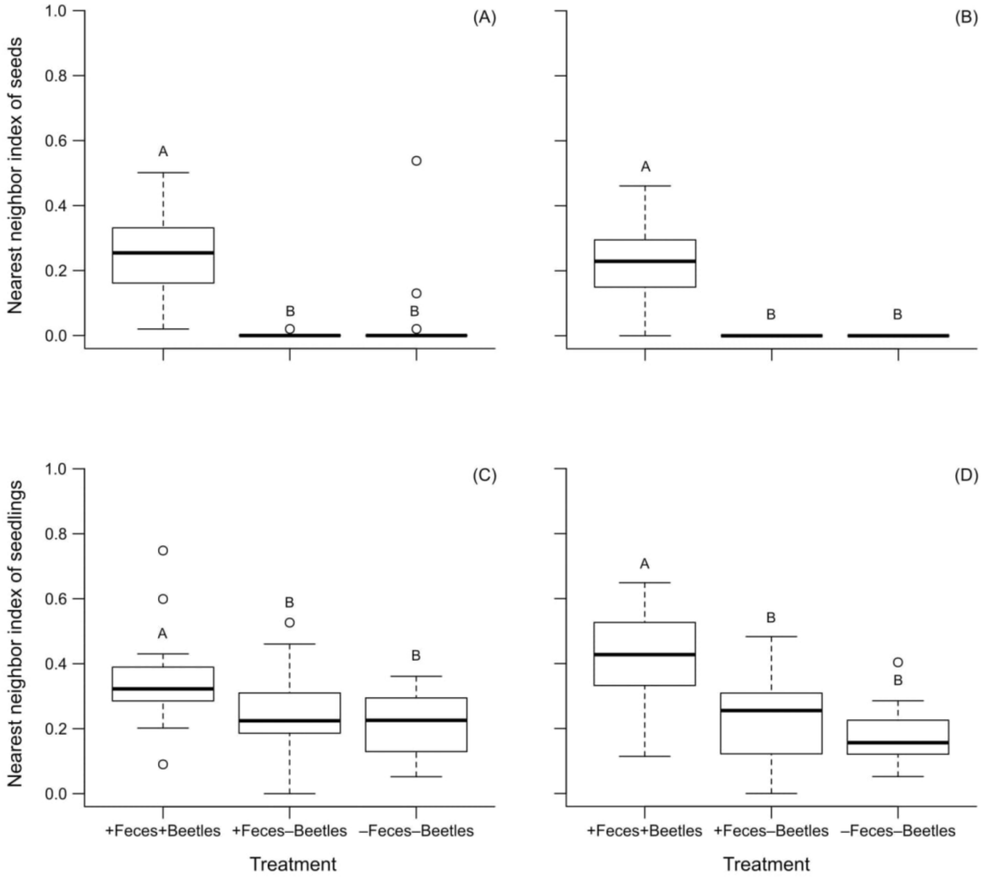

Fig 1. Box-plots of the nearest neighbor index for seeds and seedlings of two plant species. The index was measured for *Bursera* seeds (A) and seedlings (C) and *Poulsenia* seeds (B) and seedlings (D) in plots (50-cm-diameter) with three treatment levels: 50 g of feces and dung beetle access (+Feces+Beetles), 50 g of feces and dung beetle exclusion (+Feces–Beetles), and no feces or dung beetles (–Feces–Beetles). In the first two treatments 20 seeds were mixed in the dung, and in the last treatment seeds were placed on the soil surface. Independent experiments were carried out for seeds and seedlings. Seedling results are from data observed during the week of peak seedling abundance, which was determined separately for each species-treatment level combination (see text for details). Circles represent outliers; different letters above bars indicate statistical differences.

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Poulsenia, 4.39 cm ± 2.14 cm; S4 Fig), than in plots with dung added but beetles excluded (*Bursera*, 2.79 cm ± 1.84 cm; *Poulsenia*, 2.56 cm ± 1.84 cm) and plots with no dung or beetles (*Bursera*, 2.34 ± 1.54; *Poulsenia*, 1.86 cm ± 1.29 cm). The treatment had a significant effect on the spatial distribution of seedlings of both species (*Bursera*, $F = 5.57$, df = 2, $P = 0.006$, Fig 1C; *Poulsenia*, $\chi 2 = 31.88$, df = 2, $P < 0.001$, Fig 1D). As with seeds, dung beetle activity significantly reduced spatial aggregation of seedlings when compared to both control plots (*Bursera*, +Feces+Beetles vs. +Feces–Beetles: $t = 2.26$, df = 57, $P = 0.04$, +Feces+Beetles vs.–Feces–

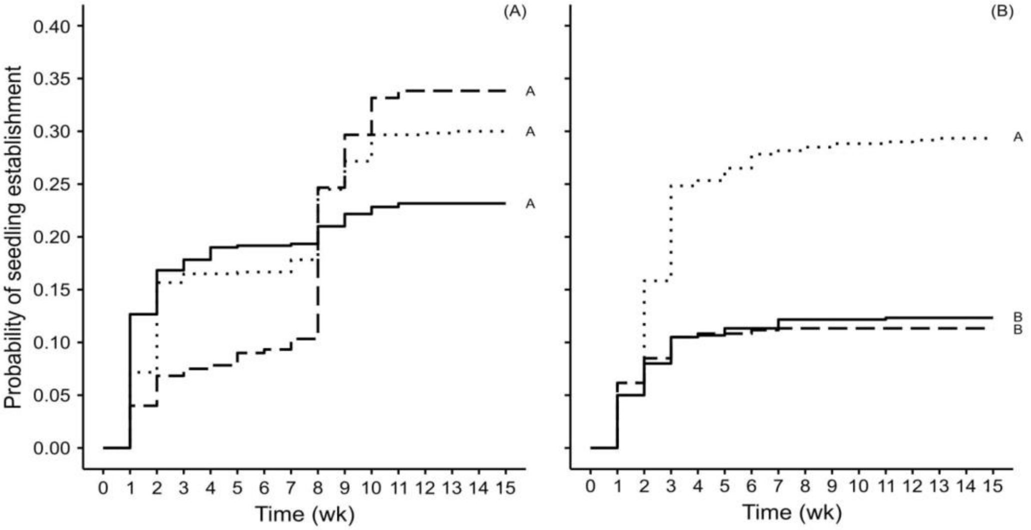

Fig 2. Kaplan–Meier curves for the probability of seedlings establishing from experimental seeds of two plant species. During 15 weeks, seedling establishment was monitored for *Bursera* (A) and *Poulsenia* (B) in plots containing: 50 g of feces and dung beetle access (solid black lines), 50 g of feces and dung beetle exclusion (dashed black lines), and without feces or dung beetles (dotted black lines). In the first two treatments 20 seeds were mixed in the dung, and in the last treatment seeds were placed on the soil surface. All curves have censored data. Different letters next to each curve indicate statistical differences.

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Beetles: $t = 3.30$, df = 57, $P = 0.005$; *Poulsenia*, +Feces+Beetles vs. +Feces–Beetles: $t = 3.89$, df = 53, $P < 0.001$, +Feces+Beetles vs.–Feces–Beetles: $t = 5.50$, df = 53, $P < 0.001$). The two control treatment levels were not significantly different from each other (*Bursera*, +Feces–Beetles vs.–Feces–Beetles: $t = 1.25$, df = 57, $P = 0.21$; *Poulsenia*, +Feces–Beetles vs.–Feces–Beetles: $t = 1.10$, df = 53, $P = 0.27$).

Of all experimental seeds, 29% of *Bursera* and 18% of *Poulsenia* seeds registered seedling emergence. During the 15 weeks in which we monitored seedling emergence, *Poulsenia* followed a steady pattern of increase until an asymptote was reached, while for *Bursera* we observed peaks of emergence followed by seedling death (S2 Fig). The treatment did not have a significant effect on the probability of seedling establishment in the case of *Bursera* ($\chi2 = 4.26$, df = 2, $P = 0.12$; Fig 2A). However, it had a significant effect for *Poulsenia* ($\chi2 = 69.90$, df = 2, $P < 0.001$); the probability of seedling establishment was higher in the plots with no feces, and it was equally low in the two treatment levels with feces, regardless of dung beetle activity (Fig 2B). In plots with no feces the probability of establishment increased by a factor of 2.70 compared to plots with dung beetle activity.

Discussion

Almost all experimental seeds (> 97%) were moved by dung beetles horizontally and more than half the seeds (> 55%) were moved vertically (i.e., buried). As predicted, dung beetle activity was associated with a decrease in the spatial clumping of both seeds and seedlings for both plant species. However, contrary to our expectation, dung beetle activity did not have a positive effect on the probability of seedling establishment. While the first result shows clearly the importance of dung beetle activity in diminishing the aggregation of seeds deposited in fecal clumps by fruit-eating mammals and of the seedlings establishing from those seeds, the second shows that this effect does not necessarily translate into increased seedling establishment. This stresses the need to be cautious when drawing conclusions regarding the effects of dung beetle activity on plant regeneration, based only on their effects on seeds.

Our result on reduced seed aggregation was expected, as several studies have determined and measured horizontal seed movement caused by dung beetles [12,16,21,22,55]. In terms of seedling spatial aggregation, our results are consistent with the only published study (that we are aware of) designed to quantify this consequence of dung beetle activity. In that study, Lawson et al. [20] also found that dung beetle activity decreased seedling spatial clumping in two plant species, *Tabernaemontana donnell-smithii* Rose and *Guazuma ulmifolia* Lam. For seedling establishment, they obtained mixed results: while one of the species had a higher percentage of establishment in plots with dung beetle activity, the other one had lower establishment in those plots [20]. In our study, one species had the highest probability of seedling establishment in plots with dung added but beetles excluded, while the other species had significantly more seedling establishment in plots without dung (Fig 2). Lawson's study used both seed species mixed together in their experiments and the authors argued that their results on seedling establishment might have been affected by competitive interactions between germinating seeds of the two species. Furthermore, they pointed out that given the short duration of their study (4 weeks after experimental setup), they could not disentangle potential mechanisms affecting seedling establishment mediated by dung beetle activity (e.g., effects of dung beetles on seed clumping vs. on seed germination; [20]). We carried out independent experiments for each seed species, and we monitored seedling establishment for a longer time period (15 weeks after onset of establishment), yet we also recognize that several factors could have influenced our results. Both our study and that of Lawson underscore the fact that the outcomes of seed-beetle interactions are species- and stage-specific, and can be affected by many factors [7,8,12,13,33].

So, why did we not find a positive effect of dung beetle activity on short-term seedling establishment? First, vertical seed dispersal by dung beetles might have hindered seedling emergence, counterbalancing a positive effect of decreased clumping due to horizontal dispersal. We found that over half of all experimental seeds in the plots with dung beetle activity were buried. Several studies in tropical forests have shown that, depending on seed species and burial depth, the effect of vertical seed dispersal by dung beetles can promote seedling establishment (through a dominant effect of avoiding seed predation) or it can hinder it (through a dominant effect of non-emergence of germinating seeds; [8,12,13,18,33]). It has been considered that burial depths ≤ 3 cm should favor seedling establishment of rainforest seeds dispersed by dung beetles [7]. In our study, the median burial depth was 2–3 cm, meaning that half of the seeds buried by dung beetles were located at greater depths (S3 Fig) and may have suffered from non-emergence or other types of seed mortality associated with burial [56]. In a parallel study in the same study region, in which we buried seeds of the focal plant species at 3, 5 and 10 cm, seedling establishment decreased with depth [33]. Furthermore, in that study, only 7% of *Bursera* and 14% of *Poulsenia* seeds buried at 3 cm emerged as seedlings, compared to 30% for seeds placed on the surface. This clearly indicates that even shallow seed burial depths have a strong negative effect on the recruitment of these two species. However, it could also be that seasonal differences in dung beetle assemblages caused a higher proportion of seeds to be buried and/or buried more deeply in Experiment 2, which was setup in April-June, than in Experiment 1, which was done in October. When we sampled dung beetles, we found higher abundances, particularly of large beetles (≥ 10 cm body length), in April than in October (S1 Table).

Second, it is possible that the decrease in seed and seedling spatial aggregation due to dung beetle activity, though statistically meaningful, might not have the necessary effect size to be of ecological significance. Though most seeds were moved horizontally by dung beetles, dispersal distances were short (~ 80% between 2 and 7 cm; S3 Fig). However, given our experimental setup, these distances could, to a certain degree, be underestimates, as the movement of beetles was limited by the edge of the plot, i.e., the plot radius of 25 cm was the maximum possible distance recorded. In our study site, roller dung beetles have been reported to move dung balls (often containing seeds) to a mean distance of 1.2 m, and up to 5 m [14]. On the other hand, tunneler dung beetles bury the dung relatively close to the dung source [10], but that does not mean that they do not cause any horizontal movement of seeds. Tunnelers sometimes push dung portions on the soil surface for a short distance (< 20 cm) before burying it; furthermore, underground tunnels are often dug with an angle < 90° such that seeds moved into the tunnels are displaced both vertically and horizontally (L.A. Urrea-Galeano and E. Andresen, pers. obs.). Given that 95% of all individuals captured in our pitfall traps were tunnelers (S1 Appendix and S1 Table), we believe that most seed movement we observed was carried out by this functional group and that most horizontal distances recorded were therefore accurate. Nonetheless, the few seeds that can be dispersed longer distances by roller dung beetles may be the ones establishing seedlings that have higher long-term survival probabilities. For example, a recent study found that, while dung beetle activity had a negative effect on seedling emergence of one plant species, it increased seedling survival [13]. Although the authors did not interpret the latter result as a possible consequence of reduced seedling clumping, this explanation remains plausible. Thus, studies following the fate of seeds moved by beetles in an unconstrained fashion and assessing seedling recruitment and survival, will be necessary to better understand the role of horizontal seed movement in decreasing the density-dependent processes causing seed/seedling mortality. Furthermore, since the life-stage at which plants suffer density-dependent mortality varies among species and contexts (e.g., [57]), future research will need to carefully determine the necessary duration of studies, in order to accurately assess the effects of reduced seed/seedling clumping caused by dung beetles, on plant fitness.

Related to the point above, it is important to mention that we protected experimental plots with mosquito netting, thus excluding most seed predators and seedling herbivores, while assuming that a positive effect of decreased clumping on seedling establishment might still be evident due to less intense seed/seedling competition and pathogen attack. Yet, density-dependent seed predation and seedling herbivory are known to be frequent processes affecting plant fitness [2,23,24]. Seed predation can often be very high, with 100% seed loss not being uncommon [58]; we used the netting precisely to avoid losing experimental seeds, and thus be able to have enough remaining seeds and seedlings for data analyses. Therefore, by excluding seed predators we also excluded the known positive effect of seed burial by dung beetles, i.e., seed predation avoidance [14–16,59]. To fully understand the effect of dung beetle activity on seed/seedling fates, we will need to design studies that allow us to simultaneously assess each of the positive and negative effects of horizontal and vertical seed dispersal by beetles, while disentangling the effects of both types of seed movement.

Finally, we want to stress once more how species-specific requirements for seed survival, germination, seedling establishment and survival, can strongly influence the results of the beetle-plant interactions. In our study, though seeds of both focal species were similar in size, a plant attribute that strongly affects the short-term fate of seeds after dung beetle activity (e.g., [33,60]), functional seedling attributes differed between species and were perhaps responsible for the different patterns of seedling establishment. For example, the timing of peak seedling establishment differed between treatment levels in *Bursera* but not in *Poulsenia* (S2 Fig). The temporal dynamics in *Bursera* seedlings was determined by their shade-intolerance [31], which caused the death of seedlings shortly after emergence. *Poulsenia* seedlings, on the other hand, are shade-tolerant [61], but the seeds suffered clear negative effects, both from remaining imbedded in dung (when beetles were excluded) and from being buried (when beetles were active; Fig 2). In the case of seeds that remained in dung, it is possible that merely dung presence could explain differences between the two species, as a few studies have shown that dung itself can have either positive or negative effects on germination or seed/seedling performance, depending on seed species [62,63]. In the case of seeds moved by dung beetles, many of which were buried, characteristics associated with the position, exposure and function of cotyledons can play an important role in determining the probability of seedlings emerging from buried seeds and surviving through the establishment period [64]. In this regard, *Poulsenia* seedlings are cryptocotylar hypogeal with reserve storage (CHR) while *Bursera* seedlings are phanerocotylar epigeal with foliaceous cotyledons (PEF; [65]). One study found that out of ten rainforest seed species tested, those that were CHR had the highest seedling establishment from buried seeds, while PEF species had the lowest [7]. In a previous study, CHR *Poulsenia* seeds buried at 3–5 cm did indeed have more seedling establishment, compared to PEF *Bursera* seeds [33]. It seems that in the present study, however, the lower seedling establishment observed for *Poulsenia* seeds in plots with dung beetle activity, compared to those of *Bursera*, is due, at least partly, to a cause different than seedling functional morphology. For example, differences between species could be due to seasonality in dung beetle activity, since the experiment for *Bursera* was started in April and the one with *Poulsenia* in June, a drier and a rainier month, respectively. Secondary seed dispersal by dung beetles has been shown to be affected by seasonality [7], not only because dung beetle assemblages vary seasonally [10], but also because in rainier months softer soils might favor deeper seed burial [21], which in turn might hinder seedling emergence.

In conclusion, our study confirms the important role dung beetles may play, through the horizontal secondary dispersal of seeds, in diminishing seed/seedling aggregation after seeds are deposited by mammals in fecal clumps. However, we did not find evidence indicating that this effect may have consequences for early seedling establishment. Longer-term studies will be necessary to ascertain if over time, the decreased seed/seedling clumping translates into increased seedling or sapling survival probabilities. Furthermore, since vertical and horizontal dispersal by dung beetles can simultaneously affect seeds and seedlings through different and sometimes opposing mechanisms, we must design studies that will allow us to assess each of them accurately, but in conjunction. Finally, given the species- and stage-specific outcome of the interactions between plants and dung beetles, more studies, including many seed species and their different stages of regeneration (seed bank, germination, emergence, establishment, survival), are necessary to fully understand the impact of dung beetle activity on plants.

Supporting information

S1 Table. Dung beetles captured in the Los Tuxtlas Biological Station, Veracruz, Mexico. Beetles captured using 10 pitfall traps each baited with 50 g of fresh domestic pig dung and opened during 48 hours. Sampling was conducted in April, September and October 2016. Information about dung relocation behavior (tunneler 'T', roller 'R', dweller 'D'), diet (predominantly feces 'F' or carrion 'C'), and body measurements (dry weight, body length) are from Díaz & Favila [2009. Escarabajos coprófagos y necrófagos (Scarabaeidae, Silphidae y Trogidae) de la reserva de la biosfera Los Tuxtlas, México. Memorias VIII Reunión Latinoamericana de Scarabaeidología (Coleoptera: Scarabaeoidea). Pp. 34. Xalapa, Veracruz]. **Eurysternus* has a unique behavior in which dung is not relocated; unlike typical rollers, feeding takes place directly in the dung source, but unlike typical dwellers, dung balls are made for nesting and

are lightly covered by soil near the dung source [Halffter, G., & Edmonds, W. D. 1982. The nesting behavior of dung beetles (Scarabaeinae). An ecological and evolutive approach (Man and the Biosphere Program, Publication 10). Instituto de Ecología, Mexico City].
(DOCX)

S1 Fig. Methodological details of the two experiments carried out to assess the effects of dung beetle activity on the spatial distribution of seeds (Experiment 1) and seedlings (Experiment 2), and on the probability of seedling establishment (Experiment 2). (A) 50 g of fresh domestic pig dung used in plots with dung added; dung was divided into 4 equal portions, each containing 5 seeds of either *Bursera simaruba* or *Poulsenia armata*; (B) seeds inside the plots with no dung added; seeds were placed directly on the soil surface (as indicated by the red arrows); (C and D) experimental seeds of *Poulsenia* thread-marked with a 30 cm-long fishing line in Experiment 1 (for Experiment 2, seeds were not thread-marked); (E) mosquito netting excluded dung beetles from control plots during the first 48 h (both experiments), and also excluded seed rain and seed/seedling predators from all plots after the first 48 h (Experiment 2); (F) plot with dung beetle activity after 48h of having placed the dung piles containing seeds; no dung remains visible on the soil surface; (G) grid (2 cm) used to map the location of each seed (Experiment 1) and seedling (Experiment 2), to calculate the nearest neighbor index; (H and I) seedlings of *Bursera* and *Poulsenia*, respectively, establishing inside plots from experimental seeds.
(DOCX)

S2 Fig. Mean number of seedlings of two plant species registered weekly for 15 weeks. *Bursera simaruba* (A) and *Poulsenia armata* (B) seedlings established in 50-cm-diam plots (N = 30 for each species-treatment level) with three treatment levels: 50 g of dung and beetle access (black continuous line), 50 g of dung and beetle exclusion (black dashed line), and with no dung or beetles (black dotted line). In each plot of the first two treatment levels 20 seeds were mixed in the dung, in the last treatment level seeds were placed on the soil surface. Error bars represent ± 1 SE.
(TIF)

S3 Fig. Frequency distribution of dispersal distances for two seed species. Vertical (A and B) and horizontal (C and D) dispersal distances for experimental seeds that were secondarily dispersed by dung beetles (seeds that remained in their original position were not dispersed and thus were not included in these graphs). Seed species are *Bursera simaruba* (A and C) and *Poulsenia armata* (B and D). Dung beetle activity was restricted to circular plots 25 cm in radius; inside each plot 50 g of fresh pig dung containing 20 seeds of one species was placed.
(TIF)

S4 Fig. Mean nearest neighbor distance for seedlings of two plant species over time. Distance for seedlings of *Bursera simaruba* (A) and *Poulsenia armata* (B) over 15 weeks in plots (N = 30 for each species-treatment level) with three treatment levels: 50 g of dung and beetle access (black continuous line), 50 g of dung and beetle exclusion (black dashed line), and with no dung or beetles (black dotted line). Error bars represent ± 1 SE.
(TIF)

S1 Appendix. Methods and results of dung beetle sampling in the Los Tuxtlas Biological Station, Veracruz, Mexico.
(DOCX)

S1 Dataset. Data on the nearest neighbor index for seeds of *Bursera* in experiment 1. See Expe1RatioBursera in metadata file.
(XLSX)

S2 Dataset. Raw data on the coordinates for seeds of *Bursera* in experiment 1. See Expe1RawdataBursera_Spatialdistribution in metadata file.
(XLSX)

S3 Dataset. Raw data on the secondary seed dispersal for seeds of *Bursera* in experiment 1. See Expe1RawdataBursera_Secondaryseeddispersal in metadata file.
(XLSX)

S4 Dataset. Data on the nearest neighbor index for seeds of *Poulsenia* in experiment 1. See Expe1RatioPoulsenia in metadata file.
(XLSX)

S5 Dataset. Raw data on the coordinates for seeds of *Poulsenia* in experiment 1. See Expe1RawdataPoulsenia_Spatialdistribution in metadata file.
(XLSX)

S6 Dataset. Raw data on the secondary seed dispersal for seeds of *Poulsenia* in experiment 1. See Expe1RawdataPoulsenia_Secondaryseeddispersal in metadata file.
(XLSX)

S7 Dataset. Data on the nearest neighbor index for seedlings of *Bursera* during the week of peak seedling abundance in experiment 2. See Expe2RatioBursera in metadata file.
(XLSX)

S8 Dataset. Raw data on the coordinates for seedlings of *Bursera* during the week of peak seedling abundance in experiment 2. See Expe2RawDataBursera_Spatialdistribution in metadata file.
(XLSX)

S9 Dataset. Data on the germination of seeds of *Bursera* registered weekly for 15 weeks in experiment 2. See Expe2SurvivalBursera in metadata file.
(XLSX)

S10 Dataset. Data on the nearest neighbor index for seedlings of *Poulsenia* during the week of peak seedling abundance in experiment 2. See Expe2RatioPoulsenia in metadata file.
(XLSX)

S11 Dataset. Raw data on the coordinates for seedlings of *Poulsenia* during the week of peak seedling abundance in experiment 2. See Expe2RawdataPoulsenia_Spatialdistribution in metadata file.
(XLSX)

S12 Dataset. Data on the germination of seeds of *Poulsenia* registered weekly for 15 weeks in experiment 2. See Expe2SurvivalPoulsenia in metadata file.
(XLSX)

S13 Dataset. Data on the dung beetles captured in the Los Tuxtlas Biological Station, Veracruz, Mexico. See S1 Appendix_RawdataDungbeetle_Sampling in metadata file.
(XLSX)

S1 Metadata. Descriptive information about all datasets.
(TXT)

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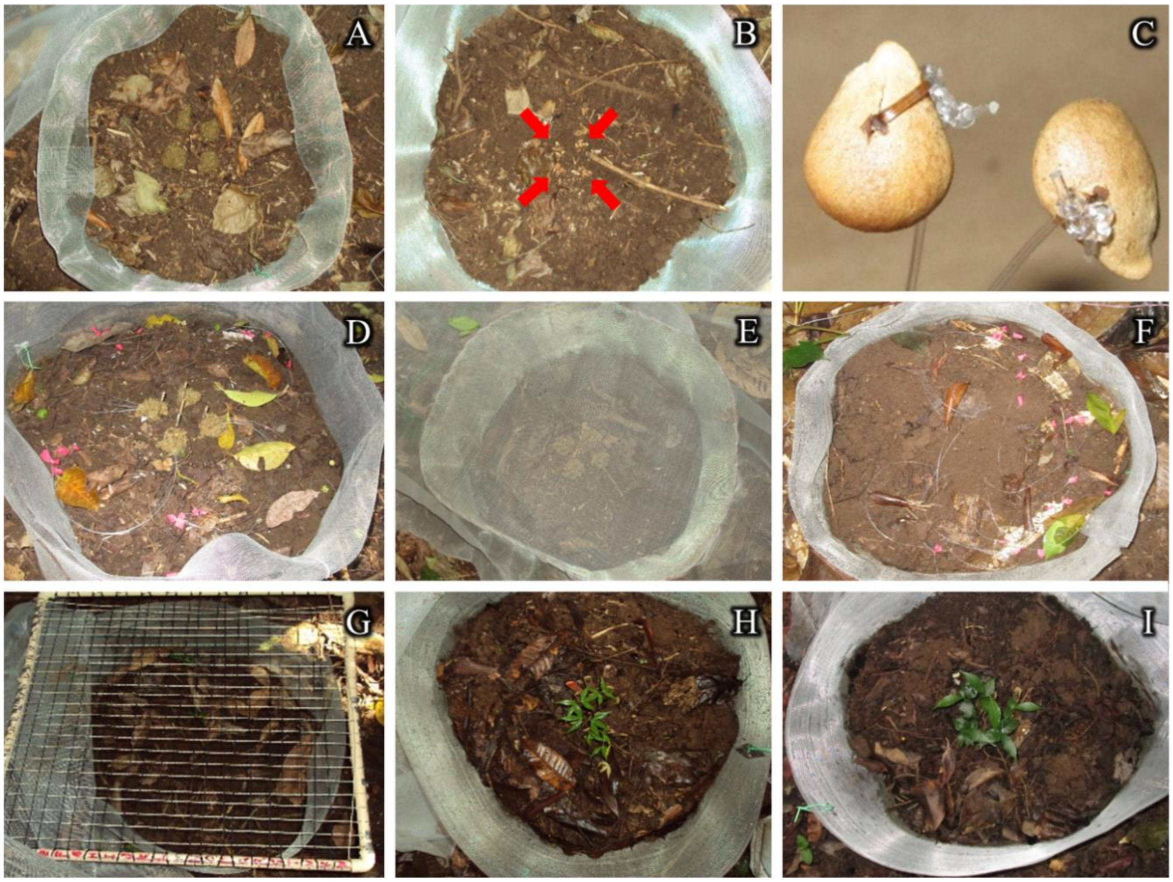
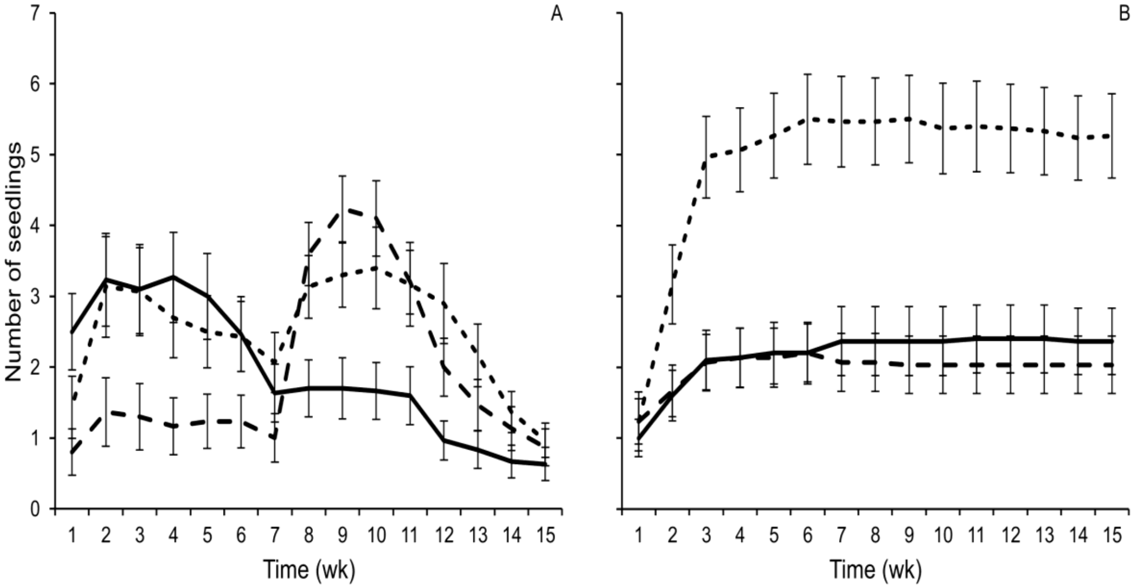
SUPPORTING INFORMATION

S1 Table. Dung beetles captured in the Los Tuxtlas Biological Station, Veracruz, Mexico.

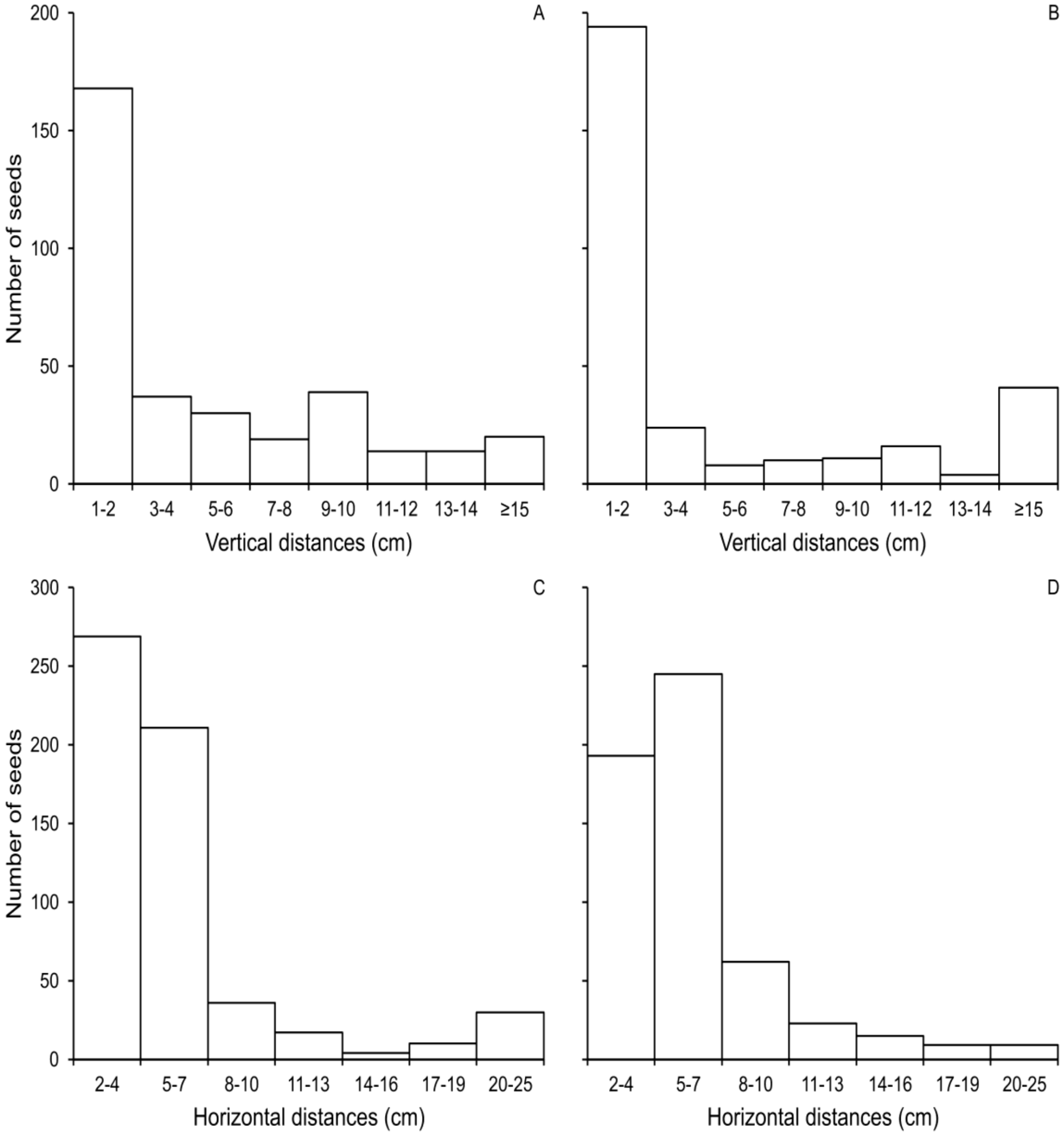
Species	**Abundance**				**Behavior**			**Diet**		**Body measurements**	
	Apr.	**Sept.**	**Oct.**	**Total**	**T**	**R**	**D**	**D**	**C**	**Weight (mg)**	**Length (mm)**
Ateuchus illaesum Harold, 1869	53	65	43	161	x			x		11	7
Bdelyropsis newtoni Howden, 1971	0	1	6	7	x			x		14	4
Canthidium centrale Boucomont, 1928	5	9	5	19	x			x		18	8
Canthon cyanellus cyanellus LeConte, 1859	5	1	0	6		x			x	23	8
Canthon femoralis (Chevrolat, 1834)	5	5	0	10		x		x		16	7
Canthon vazquezae Martínez, Halffter & Halffter, 1964	2	6	1	9		x		x		12	6
Copris laeviceps Harold, 1869	90	52	50	192	x			x		25	10
Deltochilum pseudoparile Paulian, 1938	3	2	3	8		x			x	38	10
Deltochilum sublaeve Bates, 1887	0	2	0	2		x			x	415	23
Dichotomius satanas (Harold, 1867)	42	101	7	150	x			x		173	20
Eurysternus angustulus Harold, 1869	4	0	0	4			x*	x		23	9
Eurysternus mexicanus Harold, 1869	7	2	0	9			x*	x		22	11
Onthophagus batesi Howden & Cartwright, 1963	44	1	4	49	x			x		9	7
Onthophagus incensus Say, 1835	97	6	137	240	x			x		8	7
Onthophagus rhinolophus Harold, 1869	24	16	30	70	x			x		9	7
Phanaeus endymion Harold, 1863	0	0	2	2	x			x		103	15
Uroxys boneti Pereira & Halffter, 1961	7	4	3	14	x			x		1	3
Uroxys platypyga Howden & Young, 1981	2	2	0	4	x			x		1	3
Total number of individuals	**390**	**275**	**291**	**956**	**908**	**35**	**13**	**940**	**16**		
Total number of species	**15**	**16**	**12**		**11**	**5**	**2**	**15**	**3**		



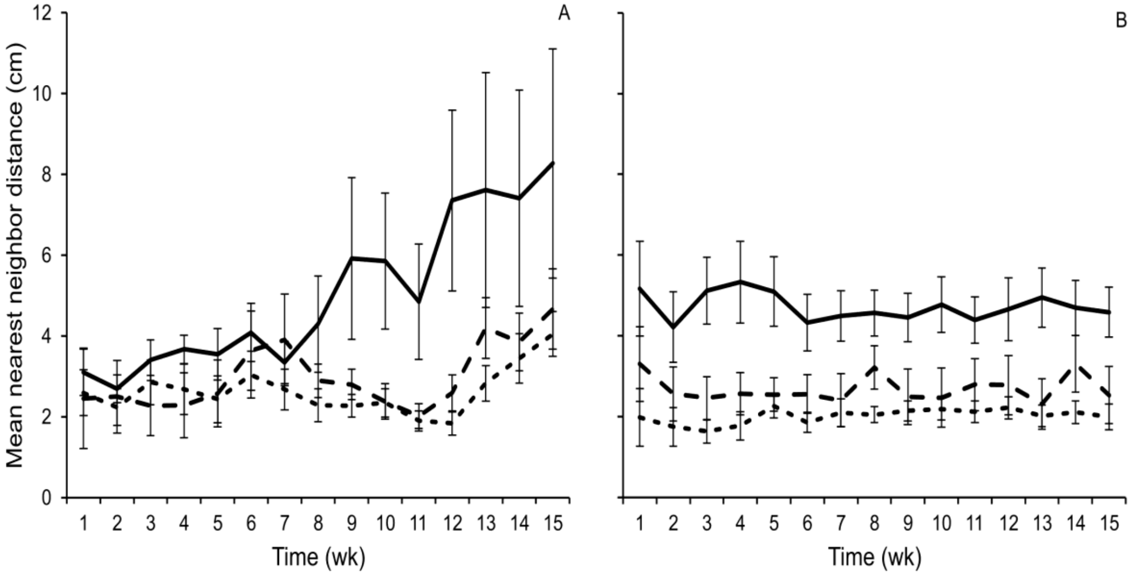
S1 Fig. Methodological details of the two experiments carried out to assess the effects of dung beetle activity on the spatial distribution of seeds (Experiment 1) and seedlings (Experiment 2), and on the probability of seedling establishment (Experiment 2).



S2 Fig. Mean number of seedlings of two plant species registered weekly for 15 weeks.



S3 Fig. Frequency distribution of dispersal distances for two seed species.



S4 Fig. Mean nearest neighbor distance for seedlings of two plant species over time.

S1 Appendix. Methods and results of dung beetle sampling in the Los Tuxtlas Biological Station, Veracruz, Mexico.

To determine the structure and composition of the dung beetle assemblage responsible for the results obtained in our two field experiments, we sampled dung beetles. We used 10 pitfall traps (separated from each other by ≥100 m), each baited with 50 g of fresh domestic pig dung, i.e., the same type of dung we used in our experiments. The 10 traps were deployed three times (without changing their location): in April, September and October 2016, and opened for 48 h every time. All individuals were counted and identified in the Laboratorio de Ecoetología at the Instituto de Ecología A.C., Xalapa, Veracruz, Mexico. Also, we used a dung beetle collection with specimens collected from LTBS and Los Tuxtlas region.

Overall, we captured a total of 956 dung beetles of 18 species (S1 Table); at the trap level, we captured an average of 32 (SD: ± 13) individuals and 7 (± 2) species in each 48-h sampling. According to their functional traits, 11 species were classified as tunnelers, 5 as rollers, and 2 as dwellers; 15 species were considered mainly coprophagous, while 3 species were considered primarily necrophagous. Beetle body length ranged from 3 to 23 mm, with 33% of all species and 38% of all individuals captured being relatively large (body length ≥10 mm, S1 Table).

CAPÍTULO II

Dung beetle activity affects rainforest seed bank dynamics and seedling establishment

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ORIGINAL ARTICLE

Dung beetle activity affects rain forest seed bank dynamics and seedling establishment

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Abstract

Dung beetles relocate vertebrate feces under the soil surface, and this behavior has many ecological consequences. In tropical forests, for example, seeds defecated by mammals that are subsequently buried by dung beetles are less likely to suffer predation. While the effects of dung beetles on the fate of defecated seeds have been relatively well studied, their effect on seeds already buried in the soil has not. To contribute to fill this gap, we designed a study with three objectives: (a) Describe the vertical re-distribution of soil seeds that occurs due to dung beetle activity; (b) Determine if beetle activity favors establishment of seedlings from the soil seed bank; and (c) Determine if the effect of dung beetles is stronger in sites of recurrent mammal defecation. We carried out three complementary field experiments, one with artificial seeds (plastic beads) of three sizes buried at known depths, one with two species of seeds buried at those same depths, and one with the natural soil seed bank in sites of single vs. recurrent defecation. Buried beads were moved by dung beetles along the vertical axis, both upwards (9.5%) and downwards (11.5%); smaller beads were more frequently moved downwards while the contrary occurred for larger beads. Dung beetle activity caused an increase in seedling establishment, both from experimentally buried seeds and from the natural seed bank. Defecation recurrence had no effect on seedling establishment. We conclude that dung beetle activity affects seed bank dynamics with important consequences for seedling establishment in tropical forests.
Abstract in Spanish is available with online material.

KEYWORDS
biotic interactions, Los Tuxtlas, Mexico, Scarabaeinae, seed burial, tropical rain forest

1 | INTRODUCTION

After dispersal, seeds that do not germinate or die, become part of the soil seed bank. The soil seed bank includes seeds in the litter, on top of the soil or buried (Simpson, Leck, & Parker, 1989), all waiting for microclimatic conditions that might trigger germination (Thompson, 2000). Seed banks play important roles in plant demography, as well as vegetation regeneration and restoration (Garwood, 1989; Skoglund, 1992). Seeds may be buried through the action of animals (e.g., invertebrates; Beattie & Culver, 1982) and physical factors (e.g., rain; Marthews, Mullins, Dalling, & Burslem, 2008). The time seeds remain buried prior to germination is highly variable, depending on species and context (Gallagher, 2014; Simpson et al., 1989). In tropical forests, seed germination and seedling establishment are strongly influenced by depth. For example, at depths > 3 cm seed germination and/or seedling emergence can be greatly reduced, leading to a decline in seedling establishment (Andresen & Levey, 2004; Dalling, Swaine, & Garwood, 1994; Pearson, Burslem, Mullins, & Dalling, 2002). Biotic or physical processes are again needed for moving deeply buried seeds to shallower depths, thus promoting germination and seedling emergence (Donath & Eckstein, 2012; Grant, 1983; Putz & Appanah, 1987).

Among animals that can influence seed bank dynamics are the dung beetles (Coleoptera: Scarabaeinae; Feer, Ponge, Jouard, & Gomez, 2013). Most species of tropical dung beetles remove the feces of vertebrates (mainly mammals) for food or oviposition (Hanski & Cambefort, 1991), relocating them under the soil surface. Dung burial is achieved through the construction of underground tunnels, and the consequent excavation of soil from deep layers to the surface (Pouvelle, Feer, & Ponge, 2008). Many important ecological functions are derived from this behavior (Nichols et al., 2008; Santos-Heredia & Andresen, 2014; Santos-Heredia et al., 2016), including the accidental movement of seeds, either when seeds in feces are buried (i.e., vertical secondary seed dispersal; Andresen & Feer, 2005), or when seeds already buried in the soil are moved along their tunnels (Pouvelle, Jouard, Feer, Tully, & Ponge, 2009). Secondary seed dispersal by tropical dung beetles has been the focus of numerous studies (e.g., Andresen & Feer, 2005; Culot, Huynen, & Heymann, 2018; Culot, Mann, Muñoz- Lazo, Huynen, & Heymann, 2011; Griffiths, Bardgett, Louzada, & Barlow, 2016; Lawson, Mann, & Lewis, 2012; Shepherd & Chapman, 1998). On the other hand, the vertical re-distribution (i.e., upward and downward movement along the vertical axis) of seeds already buried in the soil has been proposed (Pouvelle et al., 2009), and quantified in one study, but only for seed exhumation, that is, when buried seeds are brought back to the surface (Santos-Heredia & Andresen, 2014). The general re-distribution patterns of the buried soil seed bank due to dung beetle activity remain unassessed.

What determines whether a seed that is buried in the soil is moved by dung beetles? One of the main factors affecting secondary dispersal of defecated seeds by beetles is seed size; seeds are “dung contaminants” from the beetles’ perspective and larger seeds tend to be excluded (Andresen & Feer, 2005). It could be that larger seeds in the soil also represent obstacles to be avoided during soil excavation and tunnel building, and one study found that the probability of seed exhumation by beetles was lower for larger seeds (Santos-Heredia & Andresen, 2014). Also, the depth at which seeds are buried may affect their movement probability. While some dung beetle species commonly make deep tunnels (>20 cm), others use shallower depths (Hanski & Cambefort, 1991). Thus, it is conceivable that seeds buried closer to the soil surface will be subject to higher levels of dung beetle activity (as even deep-tunneling beetles will have to traverse through the shallow layers), and thus have a higher probability of being moved.

Is re-distribution of the seed bank through dung beetle activity likely to promote seedling establishment? An increase in seedling establishment from the seed bank in sites where dung beetles had been active has been observed in two studies (Ocampo-Castillo & Andresen, 2018; Santos-Heredia & Andresen, 2014). While the upward movement of seeds and a consequent increase in germination seems like a plausible mechanism explaining the observed pattern, it could also be that dung presence stimulates other processes that could promote germination and/or seedling establishment (e.g., increased activity of other soil organisms, increased soil fertility or water content; Warr, Thompson, & Kent, 1993; Freymann, Buitenwerf, Desouza, & Olff, 2008). Thus, whether dung beetle activity promotes seedling establishment from buried seeds needs to be tested more explicitly. Also, given that in tropical forests seed viability, germination, and seedling emergence decrease with increasing depth of seed burial (Dalling et al., 1994; Pearson et al., 2002; Pouvelle et al., 2009; Shepherd & Chapman, 1998), assessing the role of dung beetle activity at different seed bank depths becomes relevant.

Finally, it is well known that dung beetle communities, and consequently dung beetle activity show large spatial variability, even at local scales (Hanski & Cambefort, 1991). For example, forest sites where mammals defecate recurrently, sometimes referred to as latrines (Irwin, Samonds, Raharison, & Wright, 2004), have been found to have higher dung beetle activity (Feer et al., 2013). Studies on primate latrines have also found increased seed rain (González-Zamora et al., 2015; Muñoz-Lazo, Culot, Huynen, & Heymann, 2011) and seed banks in these sites (Dos Santos Neves, Feer, Salmon, Chateil, & Ponge, 2010; Feeley, 2005; Feer et al., 2013; Pouvelle et al., 2009). Further, some studies have found that as the frequency of latrine use increases, so does the density of seeds in the seed rain and seed bank (Feer et al., 2013; González-Zamora et al., 2015), and the activity of dung beetles (Feer et al., 2013). Seedling establishment has often been found to be higher in primate latrines, an effect attributed to the increase in the seed rain (e.g., Anzures-Dadda, Andresen, Martínez, & Manson, 2011; Bravo, 2012). However, it is possible that increased dung beetle activity in latrines also contributes to the increased seedling establishment in these sites (Santos-Heredia & Andresen, 2014).

Our main objective was to determine more accurately the effect of dung beetle activity on seed bank dynamics and how this effect, in turn, affects seedling establishment. We carried out three field experiments, each designed to test a group of hypotheses: (a) dung beetle activity causes vertical movement, both upward and downward, of seeds buried in the soil, and the probability of movement is inversely related to seed size and burial depth; (b) dung beetle activity enhances seedling establishment from buried seeds, and this effect will be proportionally larger as burial depth increases; and (c) seedling establishment from the natural soil seed bank will be enhanced by dung beetle activity, and this effect will be stronger in sites of recurrent defecation.

2 | METHODS

2.1 | Study site

The study was carried out between March 2016 and October 2017 in the Los Tuxtlas Biological Station (LTBS; 18°35′5″ N, 95°4′34″ W), in the Mexican state of Veracruz. The Station has an area of

640 ha covered by conserved tropical rain forest, and it is connected to a larger area of forest, together constituting the Los Tuxtlas Biosphere Reserve, which has a total area of ~155,000 ha (CONANP 2006). Mean annual temperature is 24.1°C, and rainfall is 4201 mm (Gutiérrez-García & Ricker, 2011).

Dung beetles are abundant and diverse in the study site, with at least 24 species recorded (Favila & Díaz, 1997), and an average of 32 individuals captured per 48-hr pitfall trap baited with 50 g of pig dung (L. Urrea-Galeano & E. Andresen, unpubl. data). The mammal fauna includes several herbivorous and omnivorous species whose feces are likely to attract dung beetles. Among these mammal species figure howler monkeys (*Alouatta palliata*), caviomorph rodents (*Cuniculus paca*, *Dasyprocta mexicana*), marsupials (*Didelphis marsupialis*, *Philander opossum*), tayras (*Eira barbara*), coatis and racoons (*Nasua narica*, *Procyon lotor*), and peccaries (*Pecari tajacu*) (Estrada, Halffter, Coates-Estrada, & Meritt, 1993; Flores, Coates, Sánchez-Cordero, & Mendieta, 2014). More information on the study site can be found elsewhere (Soriano, Dirzo, & Vogh, 1997).

2.2 | Experiment 1: Vertical re-distribution of different-sized artificial seeds buried at three depths

In all experiments, we used the fresh dung of domestic pigs as a “model dung” of an herbivorous/omnivorous mammal, due to the ease of obtaining large quantities. Rain forest dung beetles are considered generalists in terms of their food choices (Hanski & Cambefort, 1991), and previous studies have found that pig dung is effective in attracting dung beetles (e.g., Marsh, Louzada, Beiroz, & Ewers, 2013).

In this first experiment, we used plastic beads as artificial seeds buried at known depths in PVC cylinders 40 cm high and 15 cm diam (Supporting Information Figure S1). In the forest understory, we buried six cylinders (≥50 m between cylinders) level with the surface and filled the interior with forest soil up to 33 cm; the 7 cm rim prevented dung beetles from moving the dung out of the cylinder (modified from Estrada & Coates-Estrada, 1991). In each cylinder, we buried 150 plastic beads at one of three depths: 3, 5, or 10 cm. Tropical seed banks vary tremendously in terms of seed density (Garwood, 1989), but densities as the ones used in our experiments can be found in some forest areas (e.g., in latrines; Pouvelle et al., 2009). Spherical beads of three sizes were mixed and placed in each cylinder: 50 large (7 mm), 50 medium (4 mm), and 50 small (2 mm). The size of the largest bead used was determined after we observed in a pilot study that seeds 10 mm in diam placed in dung were not moved by dung beetles. Additionally, most seeds found naturally buried in the soil are usually not very large (≤4 mm; Pouvelle et al., 2009). On top of the soil in each cylinder, we placed 50 g of feces, allowing access to the naturally occurring dung beetles; after 24 to 48 hr (when no dung remained on the surface), we searched all the soil in each cylinder by removing layers of 1 cm and counted the number of beads in each layer. Between April and July 2017, the six cylinders were used repeatedly (although not always simultaneously) until we completed 15 replicates for each depth ($N = 15$). Depth treatments were assigned haphazardly to cylinders. A minimum of 48 hr passed between repeated use of a given cylinder. To describe the dung beetle community, we collected all the beetles we found inside 15 of the cylinders at the same time we registered bead location. We acknowledge that the use of a narrow cylinder could alter the normal tunneling behavior of dung beetles, making them tunnel more deeply since they are not able to make diagonal burrows. However, we do not think that this would affect the response variables we measured (proportion of beads moved upwards and proportion moved downwards).

2.3 | Experiment 2: Seedling establishment from seeds of two species buried at three depths

We used the seeds of two focal tree species which are abundant in our study site (G. Ibarra-Manríquez pers. obs.), *Bursera simaruba* (L.) Sarg. (Burseraceae), and *Poulsenia armata* (Miq.) Standl. (Moraceae), referred hereafter by their genus name. Both species are dispersed by frugivorous animals. Seed length × width and dry weight for *Bursera* are 7–9 × 5–7 mm and 71 ± 17 mg (mean ± *SD*), respectively, and for *Poulsenia* 8–10 × 5–7 mm and 103 ± 10 mg. *Bursera* seeds start germinating in ~2 week, and *Poulsenia* seeds in ~4 week (L. Urrea-Galeano & E. Andresen, pers. obs.). *Bursera* seeds were obtained from ripe fruits in April 2017; since these seeds are orthodox (desiccation-resistant; CATIE 2000) they were dried in the shade and stored for 2 months. *Poulsenia* seeds were obtained from fruits in June 2017 and used in the experiments within 2 days of collection. Seeds of both species were used simultaneously in the experiment, which started in June 2017.

We selected 30 independent sites in the forest understory (≥50 m apart, >10 m away from the crown border of an adult tree of the focal species, and avoiding treefall gaps), and used a similar setup as in Experiment 1, except that we used 3 L plastic bottles (30 cm high, 13 cm diam) with the bottom cut off and the cap removed, instead of the PVC cylinders (Supporting Information Figure S1). In each site, we buried three bottles (upside down, 2 m between bottles) with the rim of the open end level with the surface and filled with soil up to 25 cm. In each bottle, we buried 70 seeds, mixing 30 *Bursera* and 40 *Poulsenia* seeds at one of three depths: 3, 5, or 10 cm. Sites were assigned haphazardly to one of the three depths, with the same depth used for the three bottles in a site; we had 10 replicates (sites) per depth ($N = 10$). Then, in each site, each bottle was assigned to one of three treatment levels: (a) 50 g of feces on the soil surface within the bottle and allowing access to dung beetles; (b) 50 g of feces and exclusion of dung beetles; and (c) without feces, and consequently without beetles. Bottles with the two latter treatment levels were immediately covered with fine-mesh netting that allowed rain and light to enter, but not dung beetles or seed rain. The first treatment level was covered with netting after 48 hr, when all the dung had been buried by dung beetles. Once a week we registered established seedlings of the focal species. The duration of the experiment was 4 months because germination of both focal species under field conditions occurs within 14 weeks (L. Urrea-Galeano & E. Andresen, unpubl. data).

2.4 | Experiment 3: Seedling establishment from the natural soil seed bank in sites with recurrent vs. single defecation

For this experiment, we used 28 of the 30 sites used for Experiment 2; 14 months elapsed between the setup of the two experiments. In each site, we had five small circular 0.10-m^2 plots, each of which consisted of a plastic bucket 23 cm high and 36 cm diam (with bottom removed), buried 10 cm in the soil (Supporting Information Figure S1). Distance between adjacent plots was 2–3 m. We removed all seedlings and leaf litter from inside the plots and haphazardly assigned them to the following treatment levels: (a) 50 g of feces added once and beetle access; (b) 50 g of feces added once and beetle exclusion; (c) 50 g of feces added four times and beetle access; (d) 50 g of feces added four times and beetle exclusion; (e) without feces or beetles. In the two treatment levels simulating recurrent defecation, 50 g of fresh dung were placed four times within a period of 6 months to replicate defecation patterns reported for monkey latrines (spider monkeys, Chapman, 1989; howler monkeys, Julliot, 1996). Each replicate (site; *N* = 28) was started during a three-day period: on the first day we placed the feces in the two plots with dung beetle access; 48 hr later we placed dung in two of the remaining plots and immediately all five plots were covered with fine-mesh netting (to exclude dung beetles and seed rain). For the treatments of recurrent defecation, we placed feces three more times: 5 weeks after the initiation of the experiment, 10 weeks thereafter, and again 10 weeks thereafter. Biweekly during the duration of the experiment (32 weeks), we counted, marked and identified all the seedlings of woody species (trees, shrubs, lianas) and palms, established in each plot. Once seedlings were identified they were clipped at the base to avoid them interfering with the establishment of other seedlings.

2.5 | Data analyses

One outlier datum was removed from the 3 cm depth treatment in Experiment 1. Data from Experiments 1 (proportion of buried beads moved upwards and downwards) and 2 (proportion of seedlings established from experimentally buried seeds) were analyzed by fitting linear mixed models (LMMs) with normal error structure, using the logit transformation on the response variables (Warton & Hui, 2011). Two LMMs were fitted for Experiment 1, one for upward movement of beads, and one for downward movement. Fixed factors were burial depth (3, 5 and 10 cm) and bead size (small, medium, large), while the cylinder was included as a random factor. The interaction term between burial depth and bead size was included. LMM for Experiment 2 included burial depth, feces/beetle manipulation treatment, and plant species as fixed factors, while site and bottle were included as random factors. All possible interaction terms were included.

To analyze the data of Experiment 3, we grouped it into four periods: 5, 15, 25, and 32 weeks. We fitted generalized linear mixed models (GLMMs) with negative binomial error structure, one model for each response variable (number of seedlings and number of species). Response variables were cumulative values, that is, the number of seedlings and species accumulated in each time period, following Crawley (2012) for mixed-effects models with temporal pseudoreplication. The feces/beetle manipulation treatment with five levels was the fixed factor; site was added as a categorical random effect on the intercept, another random-effect term was added to indicate that the week of measurement (a continuous variable) represents pseudoreplication within each individual plot (Crawley, 2012). We carried out five post hoc contrasts: (a) three contrasts to compare dung beetle activity vs. the effect of only feces vs. no feces or beetles; for these tests we pooled levels with single dung placement with those that had recurrent dung placement; and, (b) two contrasts to determine the effect of recurrent defecation, that is, single vs. recurrent placement of feces and dung beetle activity, and single vs. recurrent placement of feces with no dung beetle activity.

In all models, fixed effects were tested using marginal Chi-squared tests. All analyses were carried out using the R statistical environment (v. 3.4.2; R Core Team 2017). Model fitting was done using functions *lmer* (Experiments 1 and 2) and *glmer.nb* (Experiment 3) of package "lme4" (Bates, Mächler, Bolker, & Walker, 2015). Test of fixed effects was done using the *Anova* function in the "car" package (Fox & Weisberg, 2011). For the models of all experiments, we obtained marginal mean and standard error values using the function *emmeans* of package "emmeans" (Russell, 2018). Post hoc tests for Experiment 2 were also carried out using *emmeans*. Post hoc tests for Experiment 3 required us to define a contrast matrix to test the planned comparisons; to do this we used function *glht* of package "multcomp" (Hothron, Bretz, & Westfall, 2008). *p*-Values in all post hoc tests were adjusted with the FDR method (False Discovery Rate; Benjamini & Hochberg, 1995). R codes are provided in the Supporting Information.

3 | RESULTS

3.1 | Experiment 1: Vertical re-distribution of different-sized artificial seeds buried at three depths

In 15 cylinders (out of the total of 45) that we examined for dung beetles we collected a total of 488 dung beetles of 17 species (Supporting Information Table S1); on average, the 50 g of dung were processed by 33 ± 11 individuals belonging to 8 ± 2 species. We acknowledge that these values may be underestimated since some dung beetles could have left the cylinders before we checked them. Dung beetles moved 21 ± 20 percent of all plastic beads buried in the PVC cylinders; 9.5 ± 12.2 percent were moved upwards, while 11.5 ± 16.3 percent were moved downwards. Most beads (79 ± 20%) remained at the depth of placement. In all cases, some beads were taken to the most superficial layers (0–1 cm) and some to the deepest (33 cm; Supporting Information

Figure S2). The mean proportions of beads moved upwards and downwards were not affected by the interaction between bead size and burial depth (upward: χ^2 = 1.33, *df* = 4, *p* = 0.85; downward: χ^2 = 8.60, *df* = 4, *p* = 0.07). Neither was the main effect of depth treatment significant (upward: χ^2 = 1.90, *df* = 2, *p* = 0.39; downward: χ^2 = 1.17, *df* = 2, *p* = 0.56). However, both types of bead movement were affected by bead size (upward: χ^2 = 13.10, *df* = 2, *p* = 0.001; downward: χ^2 = 78.34, *df* = 2, *p* < 0.001). The proportion of upward movement increased with increasing bead size, while the proportion of downward movement increased with decreasing bead size (Figure 1).

3.2 | Experiment 2: Seedling establishment from seeds of two species buried at three depths

The proportion of seedlings establishing from seeds buried between 3 and 10 cm was low overall (Figure 2). The feces and beetle treatment had no significant interaction with the other factors (feces and beetle treatment × depth: χ^2 = 8.62, *df* = 4, *p* = 0.07; feces and beetle treatment × species: χ^2 = 0.55, *df* = 2, *p* = 0.76; feces and beetle treatment × depth × species: χ^2 = 5.72, *df* = 4, *p* = 0.22). However, the main effect of the dung and beetle manipulation had a significant effect on seedling establishment (χ^2 = 14.24, *df* = 2, *p* < 0.001). More seedlings established when dung beetles were active compared to the other two treatment levels, while the treatment level with feces but exclusion of beetles had similar seedling establishment as the treatment level with no dung or beetles (+Feces+Beetles vs. +Feces−Beetles: *t* = 3.29, *p* = 0.003; +Feces+Beetles vs. −Feces−Beetles: *t* = 3.07, *p* = 0.003; +Feces−Beetles vs. −Feces−Beetles: *t* = −0.23, *p* = 0.81; Figure 2a). The interaction between burial depth and plant species had a significant effect (χ^2 = 11.18, *df* = 2, *p* = 0.003) because, although both species showed the same trend of decreasing establishment with increasing depth, the differences between depths were more pronounced for *Poulsenia* (Figure 2b).

3.3 | Experiment 3: Seedling establishment from the natural soil seed bank in sites with recurrent vs. single defecation

In all the 140 0.10-m^2 plots during 32 weeks we recorded 618 seedlings from 100 woody plant species establishing from seeds of different sizes present in the soil (Supporting Information Table S2). We found that the mean number of seedlings established per plot was affected by the treatment (χ^2 = 11.19, *df* = 4, *p* = 0.024; Figure 3). The first set of post hoc contrasts revealed that more seedlings established when dung beetles were active compared to only the presence of feces (+Feces+Beetles vs. +Feces−Beetles, *Z* = 2.69, *p* = 0.033) and compared to the control (+Feces+Beetles vs. −Feces−Beetles, *Z* = 2.47, *p* = 0.033), and also that the effect of the presence of dung by itself was not significant (+Feces−Beetles vs. −Feces−Beetles, *Z* = 0.30, *p* = 0.77; Figure 3). The second set of contrasts showed that the recurrence of dung had no effect, either when dung beetles were active (+Feces×1 + Beetles vs. +Feces × 4 + Beetles, *Z* = 0.60, *p* = 0.68), or excluded (+Feces × 1−Beetles vs. +Feces × 4−Beetles, *Z* = 1.05, *p* = 0.49; Figure 3). The mean number of species establishing in the five treatment levels showed the same general trends as observed for the number of seedlings, but the effect of treatment was not statistically significant (χ^2 = 8.67, *df* = 4, *p* = 0.069).

4 | DISCUSSION

4.1 | Movement of seeds buried in the soil

According to our expectation, we found that dung beetle activity caused not only seed exhumation as previously shown (Santos-Heredia & Andresen, 2014), but a more generalized upward and downward re-distribution of seeds buried in the soil. We had hypothesized that, because seeds represent "unwanted particles" from the beetles' perspective such that large seeds tend to be excluded during dung processing (Andresen & Feer, 2005), a similar behavior

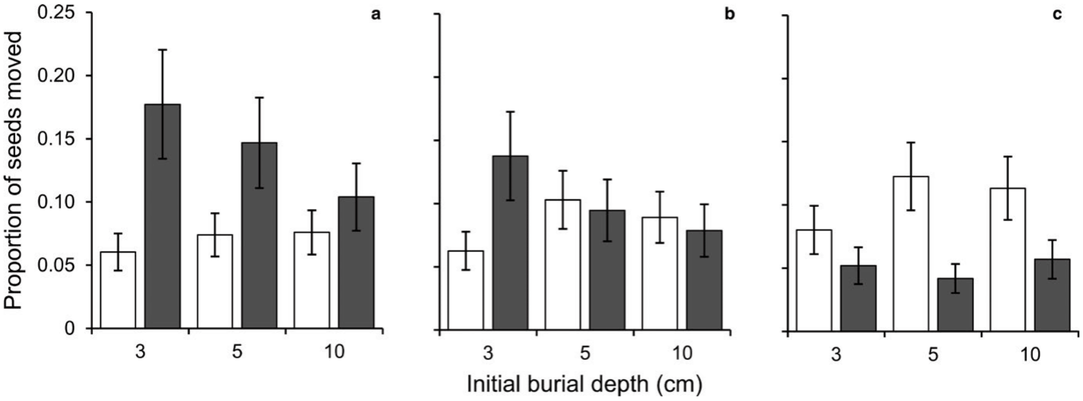

FIGURE 1 Proportion of beads of three sizes 2 mm (a), 4 mm (b), and 7 mm (c) buried at three known depths (3, 5, and 10 cm) and moved by dung beetles upwards (white bars) and downwards (gray bars). Mean values and error bars (±1 *SE*) are those predicted by the model

would occur for seeds present in the soil during tunnel building. The probability of downward movement of beads did indeed increase with decreasing bead size. However, the contrary was observed for upward movement. It is possible that larger seeds are perceived by beetles similarly to large soil aggregates that need to be removed by being pushed out of the tunnel, while smaller seeds are ignored and can fall to deeper layers through the tunnels created by beetles.

In a previous study in which seed exhumation was quantified, the probability of exhumation decreased with increasing seed size (Santos-Heredia & Andresen, 2014). However, the results of both studies are not directly comparable as the sizes of artificial seeds only coincided for the 4 mm beads, while the other beads used were larger (8 and 12 mm; Santos-Heredia & Andresen, 2014). One could speculate, that perhaps a lower and upper limit on seed size exists that maximizes the probability of upward movement. Another aspect that could influence the vertical movement of seeds is the body-size distribution of the different dung beetle communities, given that larger beetles (>1 cm) are more likely to bury large seeds present in dung (Andresen, 2002a; Braga, Korasaki, Andresen, & Louzada, 2013).

Finally, while the probability of downward movement of small and medium seeds diminished as the initial burial depth increased, this trend was not observed for upward movement. In other words, the initial burial depth of seeds did not affect the probability of upward seed movement. This means that even seeds buried deeply have a probability similar to that of shallowly buried seeds, of being moved upwards through beetle activity.

Overall, results of our first experiment suggest that the movement of buried seeds by dung beetles may have a positive effect on seedling establishment or a negative one. Further, it seems that while the former may be more common for larger seeds, the latter may predominate for small seeds. In tropical forests, the probability of seedling emergence is positively associated to seed size and negatively to burial depth (Andresen & Feer, 2005; Dalling, 2005; Dalling et al., 1994; Pearson et al., 2002). For large tropical seeds (>5 mm) burial depths of up to 3 cm allow seedling emergence (Andresen & Levey, 2004; Shepherd & Chapman, 1998), but this threshold is much lower for small (<3 mm) seeds, which at depths >2 cm may only produce ten percent of seedlings (Pearson et al., 2002). If the upward movement of buried seeds by beetles can have a positive effect on seedling establishment, while the downward movement can have negative consequences, what is the net effect?

4.2 | Seedling establishment from the soil seed bank

We found an overall net positive effect of dung beetle activity on seedling establishment from buried seeds, both for experimentally buried seeds, and the natural soil seed bank. For experimentally buried seeds the positive effect of dung beetle activity was strongest for seeds buried at 5 cm (almost a threefold increase in seedling establishment), compared to seeds at 3 and 10 cm (Supporting Information Figure S3). At 3 cm the negative effect of depth on seedling emergence is likely not as strong as at 5 cm; on the other hand, at depths of 10 cm, even if seeds are moved upwards by beetles, they most probably remain at depths that hinder emergence (Supporting Information Figure S2). It is also interesting to note that seedling establishment from seeds buried at 5 cm (both species pooled) was ~12% (Supporting Information Figure S3), which is the same percentage of large beads (7 mm, similar in size to *Bursera* and *Poulsenia* seeds) moved upwards by dung beetles in Experiment 1 (Figure 1c).

Although the effect of dung beetle activity was positive, it is important to note that, even at depths of 3 cm, seedling establishment was quite low (<15%), compared to a 30% of establishment for both *Bursera* and *Poulsenia* seeds placed on the forest surface and protected from vertebrate removal/predation (L. Urrea-Galeano & E. Andresen, unpubl. data). This underscores the fact that even

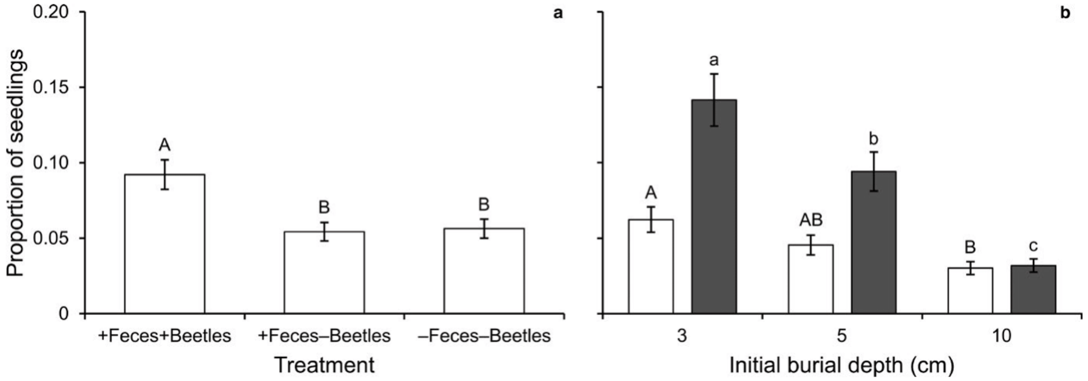

FIGURE 2 Proportion of seedlings establishing from two seed species buried at three depths and three treatment levels of feces and beetle manipulation: feces and dung beetle access (+Feces + Beetles), feces and beetle exclusion (+Feces–Beetles), and no feces or beetles (–Feces–Beetles). Feces and beetle manipulation (a), and the interaction between burial depth and plant species (b) had significant effects. Different letters indicate statistical difference; in (b) upper-case letters are used for *Bursera* (white bars), while lower-case letters are used for *Poulsenia* (gray bars). Mean values and error bars (±1 *SE*) are those predicted by the model

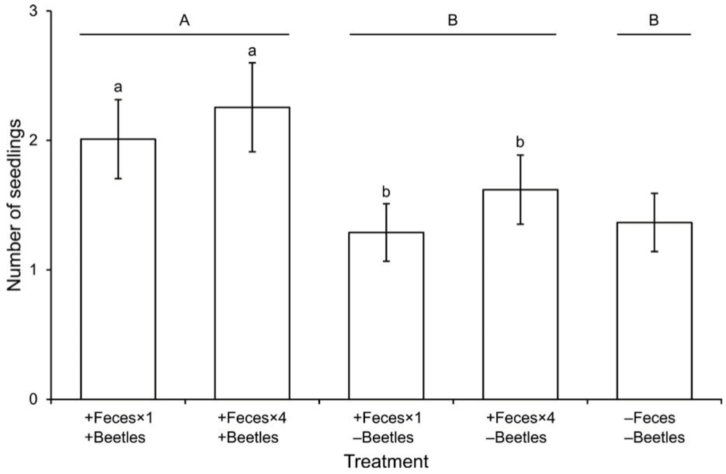

FIGURE 3 Seedling establishment from the natural seed bank in plots with: feces added once and beetle access (+Feces × 1 + Beetles), feces added four times within 6 months and beetle access (+Feces × 4 + Beetles), feces added once and beetle exclusion (+Feces × 1–Beetles), feces added four times and beetle exclusion (+Feces × 4–Beetles), and without feces or beetles (–Feces–Beetles). Different letters above bars indicate statistical difference; upper-case letters are for contrasts in which the ×1 and ×4 treatment levels were pooled, lower-case letters are for contrasts comparing the ×1 and ×4 treatment levels. Bars indicate predicted marginal means (with error bars as ±1 *SE*) from a model in which the response variable was the cumulative number of seedlings establishing in each 0.10-m^2 plot over time

relatively shallow burial depths can negatively affect seedling emergence of some seed species (e.g., Andresen & Levey, 2004). However, as tropical species display a tremendous variation in germination and seedling establishment requirements (Norden et al., 2009), more studies will be needed before we can determine which species are likely to benefit from dung beetle activity and which ones not. Also, we acknowledge that in the case of *Bursera* seeds, storage could have affected germination ability. In addition, as mentioned earlier, the use of narrow cylinders might have forced dung beetles to tunnel more deeply increasing the final burial depth for seeds moved downwards, with a negative effect on germination. Similarly, the high density of seeds used in the experiment could have had negative effects on seedling emergence of both species (Gallagher, 2014). However, none of this would have affected our general conclusions regarding the relative effects of the factors of interest (dung beetle activity and initial seed burial depth).

We also found a positive effect of dung beetle activity on seedling establishment from the natural soil seed bank. In our experiment with plastic beads, we found that larger beads were more likely to be moved upward than smaller beads, suggesting that a positive effect of dung beetle activity on seedling establishment may be relatively more frequent for larger seeds. Interestingly, in our experiment with seedling establishment from the natural seed bank, we observed that in plots with dung beetle activity 17% of seedlings were from species with seeds ≤3 mm, 29% from species with seeds 4–6 mm, and 54% from species with seeds >6 mm (Supporting Information Table S2). However, a similar trend was observed in plots with no dung beetles, and thus more studies will be necessary before a conclusion can be reached.

A positive effect of dung beetle activity on seedling establishment from the seed bank had been previously reported (Santos-Heredia & Andresen, 2014). However, that study did not include a treatment level in which dung was added but beetles were excluded and was thus not able to unequivocally relate the increase in seedling establishment to dung beetle activity. We found that merely adding feces had no effect on seedling establishment, both in the experiment with *Poulsenia* and *Bursera* seeds buried at known depths and the experiment with the natural soil seed bank.

Contrary to our expectation, we did not find an effect of the recurrence of dung beetle activity on seedling establishment. In a previous study, Santos-Heredia and Andresen (2014) found that recurrent dung increased seedling establishment fourfold. It is possible that differences in results are due to differences in the soil seed bank of both study sites. For example, if the soil seed bank in our study site was more dynamic (faster germination rates) and/or more depauperate (lower seed densities), it is possible that after the first placement of dung most of the viable seeds germinated in both treatments with dung beetle activity (single and recurrent). If this were the case, few viable seeds would have remained in the soil seed bank to interact with dung beetles in the event of a recurrent defecation.

It is also possible that methodological differences between the two studies could be responsible for the different results regarding the effect of recurrent defecation. For the recurrent treatment levels, we used 50 g of feces four times in a 6-month period, while in the other study 100 g of dung four times in a 1-month period were used. Dung beetle activity is positively related to both amounts of dung (Andresen, 2002b; Andresen & Levey, 2004; Culot et al., 2011) and defecation frequency (Feer et al., 2013). In turn, higher dung beetle activity could increase seedling establishment through other mechanisms, in addition to the more frequent upward movement of buried seeds, such as changes in physical and/or chemical characteristics of soil favoring germination and/or seedling performance (e.g., permeability, aeration, nutrient content, pH; Nichols et al., 2008).

Mammal species with latrine behavior vary greatly in the type and amount of dung they produce and in their defecation patterns (Irwin et al., 2004), which are factors known to affect dung beetle assemblages and their interactions with seeds (Andresen, 2002b; Culot et al., 2011; Ponce-Santizo, Andresen, Cano, & Cuarón, 2006; Santos-Heredia, Andresen, & Zárate, 2010). Even for the same mammal species in the same forest, frequency of use may vary greatly among latrines (e.g., between 1 and 7 uses per year for spider and howler monkey latrines; Chapman, 1989; Feer et al., 2013). Also, tropical seed banks vary greatly between and within forests (Dalling et al., 1994). Thus, more studies will be necessary before we can determine if, and to what extent, dung beetles contribute to the increased seedling establishment observed in latrines. Future studies should include experiments in both natural latrines, as well as simulated ones, to disentangle the effect of dung beetles.

5 | CONCLUDING REMARKS

Our study adds evidence strengthening previous assertions about the important role that dung beetles play in tropical seed bank dynamics, and that this role has consequences for early plant regeneration. The direct interaction between beetles and seeds, be it the secondary dispersal of defecated seeds or be it the movement of seeds in the seed bank, entails the relocation of seeds to microsites where conditions are different, which in turn changes the seeds' fate paths. Whether these new conditions affect plants positively or negatively, is species and context dependent, and may also vary according to the plant's developmental phase (Andresen & Levey, 2004; Culot et al., 2018; Griffiths et al., 2016). Further, dung beetle activity is likely to affect plants not only directly through seed relocation, but also indirectly by creating some of those new conditions encountered by either the relocated seed, or by an already established seedling (e.g., increased nutrient accessibility for plant uptake; Santos-Heredia et al., 2016). So, in all, given their ubiquitous presence in tropical forests, as well as the patchy distribution of their activity, dung beetles likely contribute to creating small-scale spatial heterogeneity in plant regeneration niches (Griffiths et al., 2016), perhaps even facilitating the co-existence of plant species. Still, we have much work ahead, as we have only started to understand some of the multiple effects of dung beetle activity on tropical plants.

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DATA AVAILABILITY

The data used in this study are archived at the Dryad Digital Repository https://doi.org/10.5061/dryad.fq5p33c.

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SUPPORTING INFORMATION

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

How to cite this article: Urrea-Galeano LA, Andresen E, Coates R, Mora Ardila F, Ibarra-Manríquez G. Dung beetle activity affects rain forest seed bank dynamics and seedling establishment. *Biotropica*. 2019;51:186–195. https://doi.org/10.1111/btp.12631

SUPPORTING INFORMATION

TABLE S1. Dung beetles found during April, June and July 2017 in 15 PVC cylinders (40 cm tall, 15 cm diam; see Fig. S1 A-C) filled with soil (up to 33 cm) and baited with 50 g of fresh pig feces at Los Tuxtlas Biological Station, in Veracruz, Mexico. Cylinders were used in Experiment 1 to quantify upward and downward movement of experimentally buried seed mimics; beetles were collected 48 h after placing the dung. The second column indicates the dung-relocating behavior (R = roller, T = tunneler, *unique behavior in which dung is not relocated, and dung balls, only made for nesting, are lightly covered by soil near the dung source). The last column has the mean dry weight per individual. Weight and dung-relocation behavior are according to Díaz & Favila (2009).

TABLE S2. Seedlings of woody plant species and palms established from the soil seed bank (seed rain was excluded) in 140 plots of 0.10 m2 during eight months (see Fig. S1 G-I). Plots were used in Experiment 3 to determine the effect of dung beetle activity and its recurrence on the establishment of seedlings from the seed bank. The last column contains information on seed size (largest dimension) obtained from the literature, internet and/or direct measurements.

FIGURE S1. Details of three experiments carried out to assess the effects of dung beetle activity on seed bank dynamics and seedling establishment. Experiment 1: (A) plastic beads of three sizes (2, 4 and 7 mm diam) used as artificial seeds were buried at known depths (3, 5 and 10 cm) in PVC cylinders 40 cm high and 15 cm in diam; (B) 50 g of fresh domestic pig feces were placed on top of the soil covering the beads; (C) after 48 h, all dung had been buried and some beads were visible on the surface. Experiment 2: (D) seeds of two focal plant species *Poulsenia armata* and *Bursera simaruba* were buried at three depths (3, 5 and 10 cm) in plastic bottles 30 cm high and 13 cm diam, and (E) the presence of feces and beetles was manipulated; (F) seedlings of the focal plant species establishing in the bottles were counted. Experiment 3: (G) circular 0.10-m^2 plots were used to quantify seedling establishment from the natural seed bank; presence and recurrence of feces and dung beetles were manipulated; (H) in plots with feces but exclusion of beetles, the dung remained on the soil surface until disintegrated by rain; (H and I) seedlings establishing inside plots were assumed to have originated from the seed bank as seed rain was excluded.

FIGURE S2. Vertical re-distribution of buried beads (used as artificial seeds) caused by dung beetle activity (i.e., soil excavation and tunnel building for dung burial). Beads were experimentally buried in PVC cylinders (see Fig S1 A-C) filled with soil at three known depths, 3 cm (A), 5 cm (B), and 10 cm (C), identified in figure panels by *. One depth was used per cylinder (N = 15 per depth), and beads of three sizes (50 of each) were buried together in each cylinder: 2 mm (dark gray bars), 4 mm (light gray bars), and 7 mm (white bars). 50 g of dung were placed on the soil surface of each cylinder and bead location was measured after 24 to 48 h. Error bars represent ±1 SE.

FIGURE S3. Percentage of seedlings of two focal plant species pooled (*Bursera simaruba* and *Poulsenia armata*) establishing from seeds buried experimentally at three depths in plastic bottles filled with soil, in which the presence of feces and beetles were manipulated. Three feces and beetle manipulation treatment levels were compared: 50 g of feces and beetle access (+Feces+Beetles; white bars), 50 g of feces and beetle exclusion (+Feces–Beetles; dark gray bars), and no feces or beetles (–Feces–Beetles; light gray bars). Error bars represent ±1 SE.

APPENDIX S1. R codes and model outputs.

TABLE S1. Dung beetles found during April, June and July 2017 in 15 PVC cylinders (40 cm tall, 15 cm diam; see Fig. S1 A-C) filled with soil (up to 33 cm) and baited with 50 g of fresh pig feces at Los Tuxtlas Biological Station, in Veracruz, Mexico.

Species	**Behavior**	**Number of individuals**	**Weight (mg)**
Ataenius sp.	*	5	1.38
Ateuchus illaesum Harold, 1869	T	38	10.85
Canthidium centrale Boucomont, 1928	T	8	17.88
Canthon cyanellus cyanellus LeConte, 1859	R	9	23.08
Canthon euryscelis Bates, 1887	R	55	8.51
Canthon femoralis (Chevrolat, 1834)	R	7	15.68
Canthon subhyalinus Harold, 1887	R	1	5.52
Canthon vazquezae Martínez, Halffter & Halffter, 1964	R	4	11.71
Copris laeviceps Harold, 1869	T	60	24.72
Coprophanaeus corythus (Harold, 1863)	T	1	589.46
Dichotomius satanas (Harold, 1867)	T	81	173.52
Eurysternus mexicanus Harold, 1869	*	3	22.14
Onthophagus rhinolophus Harold, 1869	T	38	9.11
Onthophagus batesi Howden & Cartwright, 1963	T	31	8.72
Onthophagus incensus Say, 1835	T	136	8.10
Uroxys boneti Pereira & Halffter, 1961	T	4	1.30
Uroxys platypyga Howden & Young, 1981	T	7	1.51
Total number of individuals		488	
Total number of species		17	

TABLE S2. Seedlings of woody plant species and palms established from the soil seed bank (seed rain was excluded) in 140 plots of 0.10 m^2 during eight months (see Fig. S1 G-I).

Species	Seedlings	Family	Habit	Seed size (mm)
Abuta panamensis (Standl.) Krukoff & Barneby	1	Menispermaceae	Liana	21-30
Acalypha diversifolia Jacq.	1	Euphorbiaceae	Shrub	2-3
Acalypha skutchii I. M. Johnton	93	Euphorbiaceae	Tree	2-3
Albizia purpusii Britton & Rose	2	Fabaceae	Tree	5-7
Alchornea latifolia Sw.	98	Euphorbiaceae	Tree	3-4
Ardisia compressa Kunth	1	Primulaceae	Tree	6
Ardisia tuerckheimii Donn. Sm.	4	Primulaceae	Shrub	5-6
Aristolochia ovalifolia Duch.	7	Aristolochiaceae	Liana	4-6
Astrocaryum mexicana Liebm. Ex Mart.	7	Arecaceae	Palm	45-70
Bunchosia lindeniana A. Juss.	4	Malpighiaceae	Tree	10
Bursera simaruba (L.) Sarg.	2	Burseraceae	Tree	7-8
Calatola uxpanapensis P. Vera & T. Wendt	1	Icacinaceae	Tree	35-52
Casearia sp.	1	Salicaceae	-	-
Casearia tacanensis Lundell	1	Salicaceae	Tree	9
Cecropia obtusifolia Bertol.	1	Urticaceae	Tree	1-2
Celtis iguanaea (Jacq.) Sarg	1	Cannabaceae	Liana	5-6
Chamissoa altissima (Jacq.) Kunth	1	Amaranthaceae	Liana	2
Cionosicys sp.	1	Cucurbitaceae	Liana	19-26
Cissus gossypiifolia Standl.	5	Vitaceae	Liana	8-10

TABLE S2 (continued)

Cissus microcarpa Vahl	4	Vitaceae	Liana	7-9
Cnidoscolus multilobus (Pax) I.M. Johnst.	1	Euphorbiaceae	Tree	7-8
Coccoloba hondurensis Lundell	2	Polygonaceae	Tree	6-7
Coccoloba montana Standl.	1	Polygonaceae	Tree	6-8
Croton schiedeanus Schltdl	36	Euphorbiaceae	Tree	4
Cymbopetallum baillonii R.E. Fr	3	Annonaceae	Tree	11-17
Cynometra retusa Britton & Rose	1	Fabaceae	Tree	25-31
Cyphomandra hartwegii (Miers) Walp.	1	Solanaceae	Tree	5-7
Dendropanax arboreus (L.) Decne. & Planch.	3	Araliaceae	Tree	4-5
Desmopsis trunciflora (Schltdl. & Cham.) G.E. Schatz	1	Annonaceae	Tree	11
Diospyros nigra (J.F. Gmel.) Perr.	3	Ebenaceae	Tree	19-22
Faramea occidentalis (L.) A. Rich.	2	Rubiaceae	Tree	6-10
Forsteronia viridescens S.F. Blake	29	Apocynaceae	Liana	12-20
Garcinia intermedia (Pittier) Hammel	1	Clusiaceae	Tree	38-45
Gouania lupuloides (L.) Urb.	5	Rhamnaceae	Liana	6-7
Guarea glabra Vahl	2	Meliaceae	Tree	12-20
Guarea grandifolia DC.	2	Meliaceae	Tree	13-25
Hampea nutricia Fryxell	3	Malvaceae	Tree	7-9
Heliocarpus appendiculatus Turcz.	11	Malvaceae	Tree	1-2
Heteropterys laurifolia (L.) A. Juss.	1	Malpighiaceae	Liana	6-8
Ipomoea phyllomega House	2	Convolvulaceae	Liana	5
Itzaea sericea (Standl.) Standl. & Steyerm.	1	Convolvulaceae	Liana	7-8
Koanophyllon pittieri (Klatt) R.M. King & H. Rob.	4	Asteraceae	Tree	2
Licaria velutina van der Werff	2	Lauraceae	Tree	20-30
Lonchocarpus cruentus Lundell	1	Fabaceae	Tree	10-20

TABLE S2 (continued)

Lunania mexicana Brandegee	1	Salicaceae	Tree	1
Lycianthes heteroclita (Sendtn.) Bitter	1	Solanaceae	Tree	1
Lycianthes purpusii (Brandegee) Bitter	5	Solanaceae	Liana	4
Marsdenia macrophylla (Humb. & Bonpl. ex Schult.) E. Fourn.	2	Apocynaceae	Liana	12
Marsdenia sp.	5	Apocynaceae	Liana	12-14
Mascagnia rivularis C.V. Morton & Standl.	1	Malpighiaceae	Liana	5-8
Mendoncia retusa Turrill	1	Acanthaceae	Liana	12-14
Mollinedia viridiflora Tul.	2	Monimiaceae	Tree	9-14
Nectandra ambigens (S.F. Blake) C.K. Allen	2	Lauraceae	Tree	15-23
Nectandra sp.	1	Lauraceae	Tree	-
Ochroma pyramidale (Cav. Ex Lam) Urb.	3	Malvaceae	Tree	3-4
Odontonema callistachyum (Schltdl. & Cham.) Kuntze	1	Acanthaceae	Shrub	4-5
Orthion oblanceolatum Lundell	3	Violaceae	Tree	5
Palicourea faxlucens (Donn. S.m.) C.M. Taylor & Lorence	5	Rubiaceae	Tree	10-14
Palicourea tetragona (Donn. Sm.) C.M. Taylor & Lorence	27	Rubiaceae	Tree	9-14
Paragonia pyramidata (Rich.) Bureau	8	Bignoniaceae	Liana	5-8
Parathesis conzattii (S.F. Blake) Lundell	1	Primulaceae	Tree	5
Passiflora Cookii Killip	2	Passifloraceae	Liana	5-6
Paullinia clavigera Schltdl.	4	Sapindaceae	Liana	10-16
Piper aequale Vahl	16	Piperaceae	Tree	1
Piper amalago L.	12	Piperaceae	Tree	1-2
Piper hispidum Sw.	1	Piperaceae	Shrub	1
Piper sanctum (Miq.) Schltdl. ex C. DC.	1	Piperaceae	Tree	1
Piper sp. 1	1	Piperaceae	-	1
Pisonia aculeata L.	1	Nyctaginaceae	Liana	10-15

TABLE S2 (continued)

Poulsenia armata (Miq.) Standl.	2	Moraceae	Tree	6-9
Prestonia mexicana A. DC.	11	Apocynaceae	Liana	15-20
Pseudolmedia glabrata (Liebm.) C.C. Berg	3	Moraceae	Tree	8-13
Psychotria chagrensis Standl.	13	Rubiaceae	Shrub	6
Psychotria flava Oerst. ex Standl	4	Rubiaceae	Tree	9-14
Psychotria limonensis K. Krause	8	Rubiaceae	Shrub	2-3
Psychotria papantlensis (Oerst.) Hemsl.	16	Rubiaceae	Tree	5-7
Psychotria sarapiquensis Standl.	2	Rubiaceae	Tree	4
Psychotria simiarum Standl.	26	Rubiaceae	Tree	3-4
Psychotria sp.	4	Rubiaceae	-	-
Psychotria veracruzensis Lorence & Dwyer	10	Rubiaceae	Shrub	4
Randia pterocarpa Lorence & Dwyer	9	Rubiaceae	Tree	8-11
Randia retroflexa Lorence & M. Nee	11	Rubiaceae	Liana	8
Rinorea guatemalensis (S. Watson) Bartlett	1	Violaceae	Tree	5-7
Robinsonella mirandae Gómez Pompa	2	Malvaceae	Tree	2-3
Sapium nitidum (Monach) Lundell	2	Euphorbiaceae	Tree	4-5
Senna multijuga (Rich.) H.S. Irwin & Barneby	1	Fabaceae	Tree	5-6
Sideroxylon portoricense Urb.	3	Sapotaceae	Tree	11-15
Siparuna thecaphora (Poepp. & Endl.) A. DC.	2	Siparunaceae	Shrub/Tree	3-4
Solanum schlechtendalianum Walp.	4	Solanaceae	Tree	2-3
Sparattanthelium amazonum Mart.	1	Hernandiaceae	Liana	14-20
Spondias radlkoferi Donn. Sm.	1	Anacardiaceae	Tree	21-30
Stigmaphyllon ellipticum (Kunth) A. Juss.	5	Malpighiaceae	Liana	7
Stigmaphyllon lindenianum A. Juss.	2	Malpighiaceae	Liana	4
Tabernaemontana donnell-smithii Rose	4	Apocynaceae	Tree	7-12

TABLE S2 (continued)

Trema micrantha (L.) Blume	2	Cannabaceae	Tree	2
Trichospermum mexicanum (DC.) Baill.	1	Malvaceae	Tree	3
Trichostigma octandrum (L.) H. Walter	4	Phytolaccaceae	Liana	4-5
Trophis mexicana (Liebm.) Bureau	1	Moraceae	Tree	3-6
Vachellia mayana (Lundell) Seigler & Ebinger	1	Fabaceae	Tree	8-11
Virola guatemalensis (Hemsl.) Warb.	1	Myristicaceae	Tree	18-22
Total number of individuals	618			
Total number of species	100			

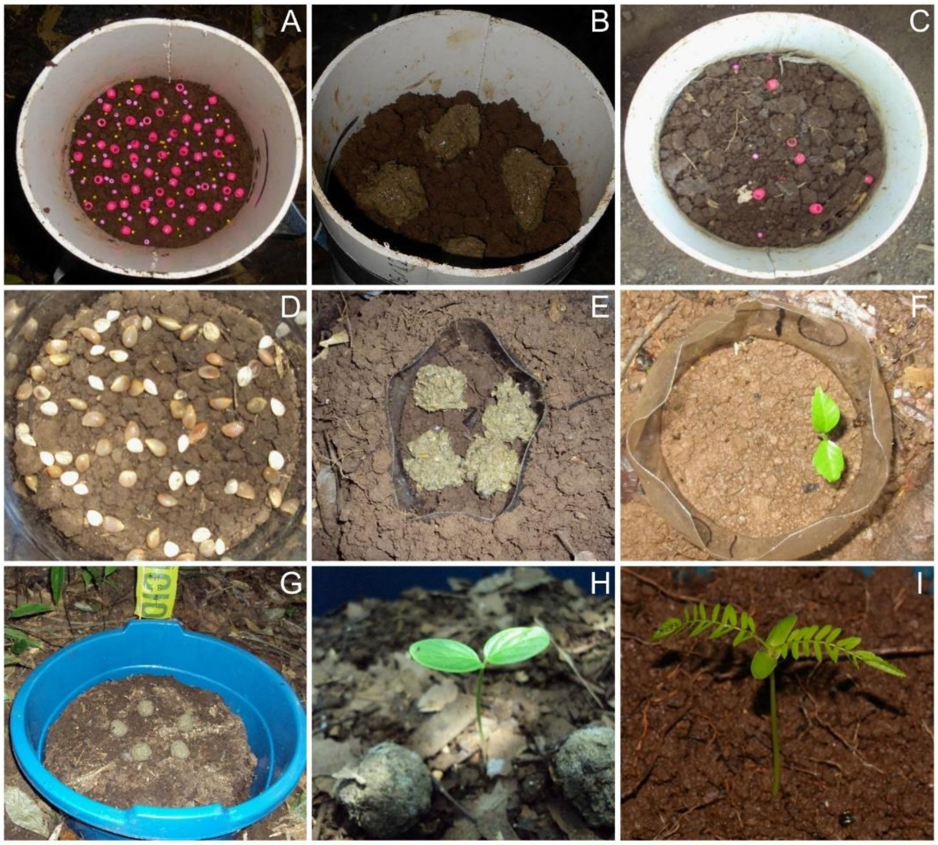

FIGURE S1. Details of three experiments carried out to assess the effects of dung beetle activity on seed bank dynamics and seedling establishment.

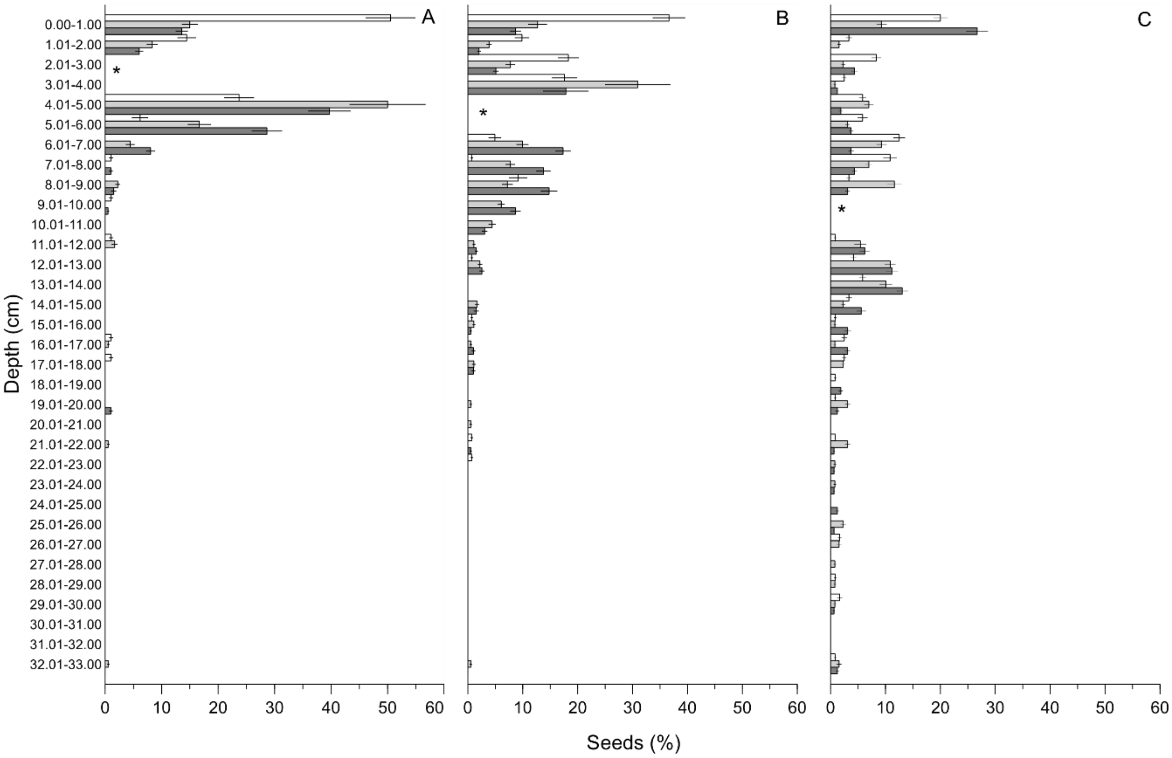

FIGURE S2. Vertical re-distribution of buried beads (used as artificial seeds) caused by dung beetle activity (*i.e.*, soil excavation and tunnel building for dung burial).

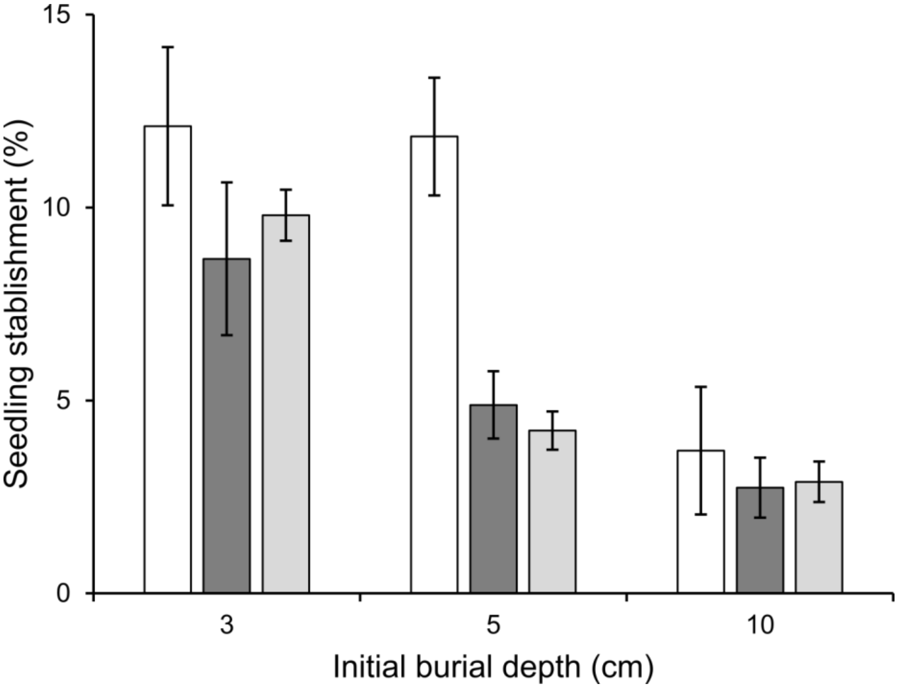

FIGURE S3. Percentage of seedlings of two focal plant species pooled (*Bursera simaruba* and *Poulsenia armata*) establishing from seeds buried experimentally at three depths in plastic bottles filled with soil, in which the presence of feces and beetles were manipulated.

APPENDIX S1. R codes and model outputs

```
#### 0. Load packages ####
library(lme4) #v. 1.1-13
library(car) #v. 2.1-4
library(emmeans) #v. 1.2.4
library(multcomp) #v. 1.4-6
library(tidyverse) #v. 1.2.1
```

```
#### 1. EXPERIMENT 1: Movement of buried seeds ####
# 1.0. Load data ####
Expe1 <- read.csv("Expe1.csv", header=TRUE, sep=",")
Expe1$burial_depth<-factor(Expe1$burial_depth)

# 1.1. Upward movement ####

# 1.1.1. Linear mixed model fit ####
mup <- lmer(logit(proportion_movedup) ~ burial_depth * bead_size +
        (1|cylinder), data=Expe1)
Anova(mup)
```

#Ouput mup:

```
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: logit(proportion_movedup)
                         Chisq Df Pr(>Chisq)
burial_depth            1.9001  2   0.386729
bead_size              13.1016  2   0.001429 **
burial_depth:bead_size  1.3359  4   0.855258
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
# 1.1.2. Calculation of marginal means ####
emmeans(mup, ~ burial_depth * bead_size, transform="response")
```

 # Output of marginal means mup:

```
burial_depth bead_size   response         SE    df   lower.CL   upper.CL
 3            Large     0.08026044 0.01903527 66.37 0.04225931 0.11826158
 5            Large     0.12254048 0.02678660 66.37 0.06906493 0.17601603
 10           Large     0.11315452 0.02499947 66.37 0.06324672 0.16306232
 3            Medium    0.06262937 0.01513847 66.37 0.03240763 0.09285112
```

```
 5           Medium    0.10289082 0.02299497 66.37 0.05698471 0.14879693
 10          Medium    0.08912517 0.02022414 66.37 0.04875062 0.12949971
 3           Small     0.06048380 0.01465331 66.37 0.03123059 0.08973701
 5           Small     0.07392487 0.01705484 66.37 0.03987736 0.10797238
 10          Small     0.07589073 0.01747121 66.37 0.04101200 0.11076945

Degrees-of-freedom method: kenward-roger 
Confidence level used: 0.95
```

```
# 1.2. Downward movement ####

# 1.2.1. Linear mixed model fit ####
mdown <- lmer(logit(proportion_moveddown) ~ burial_depth * bead_size + 
        (1|cylinder), data=Expe1)
Anova(mdown)
```

#Ouput mdown:

```
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: logit(proportion_moveddown)
                         Chisq Df Pr(>Chisq)    
burial_depth            1.1701  2    0.55708    
bead_size              78.3444  2    < 2e-16 ***
burial_depth:bead_size  8.6022  4    0.07185 .  
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
# 1.2.2. Calculation of marginal means ####
emmeans(mdown, ~ burial_depth * bead_size, transform="response")
```

 # Output of marginal means mdown:

```
burial_depth bead_size   response         SE    df   lower.CL   upper.CL
 3           Large     0.05197194 0.01455860 62.29 0.02287237 0.08107150
 5           Large     0.04191381 0.01146330 62.29 0.01900110 0.06482652
 10          Large     0.05697076 0.01533646 62.29 0.02631642 0.08762509
 3           Medium    0.13753099 0.03504884 62.29 0.06747578 0.20758621
 5           Medium    0.09464172 0.02445970 62.29 0.04575197 0.14353148
 10          Medium    0.07868847 0.02069501 62.29 0.03732353 0.12005341
 3           Small     0.17719857 0.04308091 62.29 0.09108896 0.26330819
 5           Small     0.14677463 0.03574891 62.29 0.07532014 0.21822912
 10          Small     0.10395813 0.02659100 62.29 0.05080834 0.15710791

Degrees-of-freedom method: kenward-roger 
Confidence level used: 0.95
```

```
#### 2. EXPERIMENT 2: Seedling establishment from buried seeds ####

#Beetle treatment levels are:
#masHmasE: plusFecesplusBeetles
#masHmenE: plusFecesminusBeetles
#menHmenE: minusFecesminusBeetles


# 2.0 Load data ####
Expe2 <- read.csv("Expe2.csv", header=TRUE, sep=",")
Expe2$burial_depth<-factor(Expe2$burial_depth)

# 2.1. Linear mixed model fit ####
m1 <- lmer(logit(proportion) ~ beetle_treatment * burial_depth * plant_species +
        (1|site/bottle), data=Expe2, na.action = na.fail)
Anova(m1)

# Output m1:
```

```
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: logit(proportion)
                                               Chisq Df Pr(>Chisq)    
beetle_treatment                             14.2441  2  0.0008071 ***
burial_depth                                 61.0046  2  5.663e-14 ***
plant_species                                29.3737  1  5.968e-08 ***
beetle_treatment:burial_depth                 8.6215  4  0.0712898 .  
beetle_treatment:plant_species                0.5499  2  0.7595923    
burial_depth:plant_species                   11.1831  2  0.0037292 ** 
beetle_treatment:burial_depth:plant_species  5.7211  4  0.2209699    
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
# 2.2. Calculation of marginal means and post-hoc comparisons ####

# 2.2.1. Between beetle_treatment levels ####
emmeans(m1, pairwise ~ beetle_treatment, transform="response", adjust="fdr")

# Output emmeans  beetle_treatment levels:
```

```
$emmeans
 beetle_treatment   response          SE     df   lower.CL   upper.CL
 masHmasE         0.09214572 0.009754023 158.15 0.07288076 0.11141067
 masHmenE         0.05431686 0.006078388 158.15 0.04231157 0.06632215
 menHmenE         0.05636411 0.006335459 158.15 0.04385108 0.06887713

Results are averaged over the levels of: burial_depth, plant_species 
Degrees-of-freedom method: kenward-roger 
```

```
Confidence level used: 0.95

$contrasts
 contrast                estimate          SE     df t.ratio p.value
 masHmasE - masHmenE  0.037828853 0.011492944 158.15   3.291  0.0037
 masHmasE - menHmenE  0.035781609 0.011630950 158.15   3.076  0.0037
 masHmenE - menHmenE -0.002047244 0.008779797 158.15  -0.233  0.8159

Results are averaged over the levels of: burial_depth, plant_species
P value adjustment: fdr method for 3 tests
```

```
# 2.2.2. Between burial_depth levels ####
emmeans(m1, pairwise ~ burial_depth, transform="response", adjust="fdr")
```

 # Output emmeans burial_depth levels:

```
$emmeans
 burial_depth   response          SE     df   lower.CL  upper.CL
 3            0.10192717 0.010135609 158.15 0.08190856 0.1219458
 5            0.06980233 0.007669151 158.15 0.05465516 0.0849495
 10           0.03109719 0.003267930 158.15 0.02464277 0.0375516

Results are averaged over the levels of: beetle_treatment, plant_species
Degrees-of-freedom method: kenward-roger
Confidence level used: 0.95

$contrasts
 contrast   estimate         SE     df t.ratio p.value
 3 - 5    0.03212484 0.01271009 158.15   2.528  0.0125
 3 - 10   0.07082998 0.01064941 158.15   6.651  <.0001
 5 - 10   0.03870514 0.00833638 158.15   4.643  <.0001

Results are averaged over the levels of: beetle_treatment, plant_species
P value adjustment: fdr method for 3 tests
```

```
# 2.2.3. Between plant_species levels ####
emmeans(m1, pairwise ~ plant_species, transform="response", adjust="fdr")
```

 # Output emmeans plant_species levels:

```
$emmeans
 plant_species   response          SE     df   lower.CL   upper.CL
 Bursera       0.04601414 0.003803098 158.15 0.03850273 0.05352556
 Poulsenia     0.08920365 0.007332071 158.15 0.07472224 0.10368505

Results are averaged over the levels of: beetle_treatment, burial_depth
Degrees-of-freedom method: kenward-roger
Confidence level used: 0.95

$contrasts
 contrast              estimate          SE     df t.ratio p.value
 Bursera - Poulsenia -0.0431895 0.007739563 158.15   -5.58  <.0001

Results are averaged over the levels of: beetle_treatment, burial_depth
```

2.2.4. Between burial_depth levels within plant_species levels (given its
significant interaction)

emmeans(m1, pairwise ~ burial_depth|plant_species, transform="response",
    adjust="fdr")

 # Output emmeans for interaction between burial_depth levels within plant_species:

```
$emmeans
plant_species = Bursera:
 burial_depth   response          SE     df   lower.CL   upper.CL
 3            0.06231227 0.008346527 158.15 0.04582723 0.07879730
 5            0.04548504 0.006519723 158.15 0.03260808 0.05836199
 10           0.03024513 0.004242718 158.15 0.02186543 0.03862483

plant_species = Poulsenia:
 burial_depth   response          SE     df   lower.CL   upper.CL
 3            0.14154207 0.017265648 158.15 0.10744108 0.17564307
 5            0.09411962 0.012910004 158.15 0.06862136 0.11961788
 10           0.03194924 0.004366074 158.15 0.02332591 0.04057258

Results are averaged over the levels of: beetle_treatment
Degrees-of-freedom method: kenward-roger
Confidence level used: 0.95

$contrasts
plant_species = Bursera:
 contrast   estimate          SE     df t.ratio p.value
 3 - 5    0.01682723 0.010591096 158.15   1.589  0.1141
 3 - 10   0.03206714 0.009362968 158.15   3.425  0.0024
 5 - 10   0.01523991 0.007778653 158.15   1.959  0.0778

plant_species = Poulsenia:
 contrast   estimate          SE     df t.ratio p.value
 3 - 5    0.04742245 0.021558544 158.15   2.200  0.0293
 3 - 10   0.10959283 0.017809132 158.15   6.154  <.0001
 5 - 10   0.06217038 0.013628310 158.15   4.562  <.0001

Results are averaged over the levels of: beetle_treatment
P value adjustment: fdr method for 3 tests
```

3. EXPERIMENT 3: Seedling establishment from seed bank

#Beetle treatment levels are:
#masHmasE1: plusFecesx1plusBeetles
#masHmasE4: plusFecesx4plusBeetles
#masHmenE1: plusFecesx1minusBeetles
#masHmenE4: plusFecesx4minusBeetles
#menHmenE: minusFecesminusBeetles

```
# 3.1. Number of seedlings ####

# 3.1.0. Load data ####
Expe3Abundance <- read.csv("Expe3Abundance.csv", header=TRUE, sep=",")

# 3.1.1. Generalized linear mixed model fit ####
modAbundance <- glmer.nb(number_seedlings ~ beetle_treatment +
                    (1|site) + (time_measurement|plot), data = Expe3Abundance)
Anova(modAbundance)

# Output modAbundance:
```

```
 Analysis of Deviance Table (Type II Wald chisquare tests)

 Response: number_seedlings
                   Chisq Df Pr(>Chisq)
 beetle_treatment 11.188  4    0.02453 *
 ---
 Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
# 3.1.2. Calculation of marginal means ####

emmeans(modAbundance, ~ beetle_treatment, transform="response")

# Output emmeans beetle_treatment levels:
```

```
beetle_treatment response        SE  df asymp.LCL asymp.UCL
 masHmasE1        2.009691 0.3048643 Inf 1.4121678  2.607214
 masHmasE4        2.255224 0.3440381 Inf 1.5809218  2.929526
 masHmenE1        1.288010 0.2217145 Inf 0.8534573  1.722562
 masHmenE4        1.618767 0.2661085 Inf 1.0972038  2.140330
 menHmenE         1.365813 0.2249695 Inf 0.9248812  1.806745

Confidence level used: 0.95
```

```
# 3.1.3. Contrasts between beetle_treatment levels

# Contrast matrix definition
mat.contrasmodAbunance <- rbind("just dung vs without feces or beetles " = c(0,0,0.5,0.5,-1),
                    " beetle access vs without feces or beetles" = c(0,0.5,0,0,-1),
                    " beetle access vs just dung" = c(0,1,-1,-1,0),
                    " beetle access single defecation vs. beetle access recurrent defecation "   = c(0,1,0,0,0),
                    " just dung single defecation vs just dung recurrent defecation " = c(0,0,-1,1,0)
)
```

```
# Contrasts calculations
comparisonAbundance <- glht(modAbundance, mat.contrasmodAbunance)
summary(comparisonAbundance, test = adjusted("fdr"))
```

 # Output contras test:

```
Simultaneous Tests for General Linear Hypotheses


Fit: glmer(formula = number_seedlings ~ beetle_treatment + (1 | site) + 
    (time_measurement | plot), data = Expe3Abundance, family = negative.binom
ial(theta = 250700.027422383))


Linear Hypotheses:

                                                                              Estimate Std. Error z value Pr(>|z|)

just dung vs without feces or beetles  == 0                                    0.05563     0.18786   0.296   0.7671

 beetle access vs without feces or beetles == 0                                0.44387     0.17981   2.469   0.0339 *

 beetle access vs just dung == 0                                               0.77647     0.28904   2.686   0.0339 *

 beetle access single defecation vs. beetle access recurrent defecation  == 0  0.11527     0.19217   0.600   0.6858

 just dung single defecation vs just dung recurrent defecation  == 0           0.22857     0.21717   1.052   0.4876

---

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Adjusted p values reported -- fdr method)
```

```
# 3.2. Number of species ####

# 3.2.0. Load data ####
Expe3Richness <- read.csv("Expe3Richness.csv", header=TRUE, sep=",")

# 3.2.1. Generalized linear mixed model fit ####
modRichness <- glmer.nb(number_species~ beetle_treatment +
                    (1|site) + (time_measurement|plot), data = Expe3Richness)
Anova(modRichness)
```

 # Output modRichnes:

```
Analysis of Deviance Table (Type II Wald chisquare tests)
Response: number_species
                  Chisq Df Pr(>Chisq)
beetle_treatment 8.6764  4    0.06972 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

CAPÍTULO III

Dung beetle activity had no positive effect on seedling performance or nutrient concentration in six rainforest tree species

[Enviado a Biotropica]

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Dung beetle activity had no positive effect on seedling performance or nutrient concentration in six rainforest tree species

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Keywords:	ecological functions, foliar nitrogen, foliar phosphorus, Los Tuxtlas, natural fertilization, Scarabaeinae, seedling growth, seedling survival

Dung beetle activity had no positive effect on seedling performance or nutrient concentration in six rainforest tree species

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ABSTRACT

Dung beetles cause physical and chemical changes in the soil when they bury dung, which may in turn have positive effects on plants. These effects have been studied mainly in temperate agriculture and cattle-pasture systems through controlled experiments. But, information of these effects under natural conditions in tropical forests is practically nonexistent. In this study, we assessed whether dung beetle activity has any positive effect on foliar nutrient assimilation and performance of seedlings (survival, growth) in a tropical rainforest (Los Tuxtlas, Mexico). We carried out field experiments with seedlings (individuals ~ 3-8 months old and 1-72 cm height) of six tree species (*Brosimum alicastrum*, *Calophyllum brasiliense*, *Cymbopetalum baillonii*, *Diospyros digyna*, *Omphalea oleifera* and *Poulsenia armata*), manipulating the presence of feces and dung beetles. For each species, we measured foliar nitrogen and phosphorus, seedling survival, growth and resource allocation (root/shoot biomass ratio). Dung beetle activity had no positive effect on either nutrient concentration or seedling performance, for any of the species. Furthermore, in two cases dung beetle activity had negative effects on growth. In one case, dung beetle activity modified resource allocation. We propose that in rainforests with relatively fertile soils, such as our study site, dung beetle activity does not confer a nutrient boost to seedlings, and thus has no effect on seedling performance, whereas these effects might occur under nutrient-poor conditions. Until this hypothesis is tested, we lack convincing evidence to assert that dung beetle activity, through its effects on soil physical and/or chemical attributes, has positive effects on tropical seedlings established in the understory.

RESUMEN

Los escarabajos coprófagos provocan cambios en las propiedades físicas y químicas del suelo cuando entierran heces, los cuales, a su vez, pueden tener efectos positivos para las plantas. Estos efectos se han estudiado principalmente en sistemas agropecuarios de latitudes templadas, mediante experimentos controlados. Sin embargo, nuestro conocimiento sobre estos efectos para plántulas tropicales bajo condiciones naturales es prácticamente inexistente. En este estudio evaluamos si la actividad de los escarabajos coprófagos tiene algún efecto positivo sobre la asimilación de nutrientes foliares y el desempeño de las plántulas (supervivencia, crecimiento) en un bosque húmedo tropical (Los Tuxtlas, México). Realizamos experimentos de campo con plántulas (individuos de ~ 3-8 meses de edad y 1-72 cm de altura) de seis especies arboreas (*Brosimum alicastrum*, *Calophyllum brasiliense*, *Cymbopetalum baillonii*, *Diospyros digyna*, *Omphalea oleifera* y *Poulsenia armata*), manipulando la presencia de heces y de escarabajos coprófagos. Para cada especie medimos nitrógeno y fósforo foliar, supervivencia, crecimiento y asignación de recursos (relación biomasa radicular/biomasa aérea) en las plántulas. La actividad de los escarabajos coprófagos no tuvo efectos positivos sobre la asimilación de nutrientes ni sobre el desempeño de las seis especies de plántulas. Además, para dos especies la actividad de los escarabajos provocó efectos negativos en el crecimiento. Para una especie, la actividad de los escarabajos modificó la asignación de recursos. Proponemos que, en bosques tropicales con suelos relativamente fértiles, como nuestro sitio de estudio, la actividad de los escarabajos coprófagos no otorga beneficios para las plántulas, en comparación con sitios de suelos pobres en nutrientes. Hasta que se pruebe esta hipótesis, no existe evidencia contundente para afirmar que los escarabajos coprófagos tienen efectos positivos sobre las plántulas tropicales del sotobosque, mediante los cambios físicos y químicos que provoca su actividad en el suelo.

KEYWORDS

ecological functions, foliar nitrogen, foliar phosphorus, Los Tuxtlas, natural fertilization, Scarabaeinae, seedling growth, seedling survival

1. INTRODUCTION

Seedlings are key components of plant demography and natural forest regeneration (Leck, Parker, & Simpson, 2008). The seedling stage in a plant’s life cycle is characterized by high mortality due to numerous biotic and abiotic causes (Moles & Westoby, 2004). Thus, any factor or ecological process that increases seedling performance (e.g., survival, growth) can have a strong effect on plant demography and consequently on plant communities. Among factors known to influence seedling performance, soil nutrients figure prominently (Alvarez-Clare, Mack, & Brooks, 2013; Santiago et al., 2012; Wright, 2019). Soil nutrients are not distributed evenly, and their availability to plants is highly variable among habitat types (Vitousek & Sanford, 1986; Wright, 2019). Nutrient availability may even vary at small spatial and temporal scales within habitats due to processes that cause discrete pulses in their concentration or their uptake by plants. Consequently, such processes could have positive effects on plant performance.

One process that can create discrete patchy pulses of nutrient availability in ecosystems that sustain a high biomass of mammals, is the natural fertilization of soils through the nutrient-rich feces produced by these animals (e.g., Kalbitzer, McInnis, & Chapman, 2019; Kalbitzer, McInnis, Omeja, Bortolamiol, & Chapman, 2019; Stanbrook, 2018). This process can be particularly relevant in tropical forests, where mammals are abundant and the soil is often nutrient-poor (Dalling, Heineman, Lopez, Wright, & Turner, 2016; Vitousek & Sanford, 1986; Wright, 2019). Furthermore, studies have shown that experimental increases of soil macronutrient concentrations (e.g., P, N, K) enhances seedling survival and performance (Alvarez-Clare et al., 2013; Santiago et al., 2012), and modifies resource allocation (allocation to shoot growth increases relative to the allocation to root growth; Hermans, Hammond, White, &

Verbruggen, 2006). Natural fertilization occurs through the deposition of mammal feces on the soil surface (Kalbitzer, McInnis, & Chapman, 2019; Kalbitzer, McInnis, Omeja, et al., 2019), but it may be further enhanced by the activity of animals, such as dung beetles, that incorporate the feces into deeper soil layers (Santos-Heredia et al., 2016).

Dung beetles (Coleoptera: Scarabaeinae) remove mammal feces from the soil surface and use it for feeding and nesting. Dung removal by these insects is associated to numerous ecosystem functions (Nichols et al., 2008), several of which can affect plants. For example, tunneller and roller dung beetles excavate soil to build underground galleries where they bury the dung; this bioturbation changes the soil's physical and chemical properties (e.g., increased soil aeration, permeability and pH; Bertone, Green, Washburn, Poore, & Watson, 2006; Brown, Scholtz, Janeau, Grellier, & Podwojewski, 2010). Also, by burying the fecal material, dung beetles accelerate and deploy nutrient input into the soil (Maldonado, Aranibar, Serrano, Chacoff, & Vázquez, 2019), as well as nutrient mineralization (Yokoyama, Kai, Koga, & Aibe, 1991).

Bioturbation and nutrient cycling caused by dung beetles has been shown to improve plant resource assimilation and performance, but evidence comes mostly from studies under controlled conditions (e.g., greenhouse experiments) and temperate regions, generally related to agriculture and cattle pastures (Badenhorst, Dabrowski, Scholtz, & Truter, 2018; Bang et al., 2005; Hanafy, 2012). We do not know if results from these studies can be extrapolated to natural conditions in tropical forests. To our knowledge, only one study has assessed these effects on tropical plants under natural conditions, and only for one species (*Brosimum lactescens*; Santos-Heredia et al., 2016). That study found that dung beetle activity increased foliar concentrations of phosphorous and nitrogen. It also reported an increase in growth (number of leaves), but a decrease in seedling survival, both associated to dung beetle activity. The latter results, however, were not conclusive due to possible confounding factors in the experimental design (see Supplementary Information in Santos-Heredia et al., 2016). Thus, the following question remains basically unanswered: Do dung beetles have any positive effect on tropical forest plant performance (e.g., assimilation of nutrients, survival, growth) due to bioturbation and nutrient cycling caused by their activity? Finding the answer to this question is important because dung beetles are very commonly used as a focal or indicator group in studies assessing the effects of disturbances in tropical forests (Nichols & Gardner, 2011). Those studies very frequently justify using Scarabaeinae by stressing their ecological functions in ecosystems (including the enhancement of plant performance and nutrient assimilation), but they do this by extrapolating results from other systems to tropical forests.

The objective of this study was to determine whether dung beetle activity has any positive effect on tropical seedling performance under natural conditions. We carried out field experiments with seedlings of six tree species to test the hypotheses that dung beetle activity would influence seedling performance because it increases nutrient input and changes soil properties through relocation of dung. We assessed the prediction that dung beetle activity (i) has a positive effect on the concentration of foliar nutrients (N and P), seedling growth (height and number of leaves) and seedling survival, and (ii) modifies resource allocation by increasing the relative allocation of resources to shoot growth. Specifically, (1) seedlings in sites with feces and dung beetle activity (+Feces+Beetles) will have higher foliar nutrient concentrations compared to sites with feces but dung beetle exclusion (+Feces–Beetles), and the lowest concentration will occur in sites with no feces and consequently no beetle activity (–Feces–Beetles); (2) seedling growth and survival will have the highest values in +Feces+Beetles sites, followed by +Feces–

Beetles and –Feces–Beetles sites; (3) the root/shoot biomass ratio will have the lowest values in +Feces+Beetles sites, followed by +Feces–Beetles and –Feces–Beetles sites. An alternative hypothesis regarding survival is that, if seedlings in sites with dung beetle activity have indeed higher nutrient concentrations, they could be more attractive to herbivores and/or pathogens, and thus would suffer higher mortality, as suggested previously (Santos-Heredia et al., 2016). If this hypothesis were true, we would expect higher mortality in +Feces+Beetles sites, followed by +Feces–Beetles, and the lowest mortality in –Feces–Beetles sites.

2. METHODS

2.1 Study site

We conducted this study in the Los Tuxtlas Tropical Biology Station (18°35′5.46″ N, 95°4′34.896″ O), in the Mexican state of Veracruz, between September 2016 and February 2019. The station encompasses 640-ha of tropical rainforest with altitudes that range from 150 to 650 m a.s.l. (González Soriano, Dirzo, & Vogt, 1997), and it is part of a larger protected area, the Los Tuxtlas Biosphere Reserve (155,122.46-ha; CONANP 2006). Mean annual temperature and precipitation are 24ºC and 4,200 mm, respectively, and there is a relatively drier period (< 100 mm per month) between March and April (Gutiérrez-García & Ricker, 2011).

Although anthropogenic disturbances in the region have caused the local extinction of some large/medium mammal species (Dirzo & Miranda, 1990), the study site is still inhabited by a diverse fauna of herbivorous/omnivorous mammals (rodents, marsupials, howler monkeys, coaties, racoons, tayras, kinkajous, deer, peccaries; Flores, Coates, Sánchez-Cordero, & Mendieta, 2014). The feces of these animals constitute an important food resource for the local dung beetle community (e.g., Estrada, Halffter, Coates-Estrada, & Meritt Jr, 1993), which consists of at least 34 species (Favila & Díaz, 1997). These beetles are abundant and readily attracted to fresh mammal defecations; on average, 32 individuals of seven species are captured in a pitfall trap baited with 50 g of dung during 48 hr (Urrea-Galeano et al., 2019). In terms of functional groups related to dung-relocation and nesting behaviors, the tunnellers dominate the community (63% of species, 95% of individuals), followed by rollers (26% of species, 3.6% of individuals) and dwellers (11% of species, 1.4% of individuals; Urrea-Galeano et al., 2019).

2.2 Seedling species

We worked with seedlings of six midstory or canopy tree species: *Brosimum alicastrum* Sw. (Moraceae), *Calophyllum brasiliense* Cambess. (Calophyllaceae), *Cymbopetalum baillonii* R.E. Fr. (Annonaceae), *Diospyros digyna Jacq.* (Ebenaceae), *Omphalea oleifera* Hemsl. (Euphorbiaceae) and *Poulsenia armata* (Miq.) Standl. (Moraceae), referred to by their genus name from here on. *Poulsenia* and *Omphalea* are the two most abundant species in the study site, followed by *Brosimum* and *Cymbopetalum*, while *Diospyros* and *Calophyllum* are less abundant (Ek Rodríguez, 2019). We chose these species because their seedlings are reported to have at least some shade tolerance (CATIE 2000; Coates-Estrada & Estrada, 1988; Ricker et al., 2000; Soriano et al., 1997; Zambrano, Coates, & Howe, 2014), and because it was feasible to obtain a large numbers of seedlings for the experiments.

2.3 Experimental design

We carried out independent experiments for each plant species. Due to differences among species in seedling availability (timing and number) and size, several aspects of the experimental design also differed among species, such as time of seed collection and sowing, seedling number, size and age, size of plots, number of sites (Table 1).

We obtained all seedlings, except in the case of *Diospyros*, by sowing seeds collected from fruiting trees. Seeds of *Brosimum*, *Calophyllum*, *Cymbopetalum* and *Poulsenia* were sowed in plastic germination trays consisting of 40 cells filled with forest soil (one seed per cell; Figure S1a), which were placed in a shade house. *Omphalea* seeds were sowed individually in plastic germination bags filled with forest soil that were placed in the understory (Figure S1b). When necessary, germination trays and bags were watered. *Diospyros* seedlings were obtained by directly collecting individuals from four natural seedling banks; we selected young seedlings with cotyledons attached (i.e., they were of the same cohort).

Seedlings were transplanted to three plots in experimental sites. Experimental sites were separated by ≥ 50 m and located > 10 m from the border of the canopy projection of any adult tree of the focal species. Plots within sites were separated by 2-3 m. For *Omphalea*, *Brosimum* and *Calophyllum* plots were 40 cm x 40 cm cages (area of 0.36 m^2) built using plastic mosquito screen buried 10 cm and reaching a height of 90 cm (Figure S1c). For the other three species, plots were bottom-less buckets with their lower rim buried 10 cm into the soil and covering an area of ~ 0.04 m^2 (Figure S1d,e). For *Diospyros* and *Poulsenia*, each plot consisted of two buckets placed together (Figure S1d). After ~ 4 weeks of the transplant, each surviving seedling was marked by attaching a label at the base of its stem; additionally, each leaf was marked by attaching a small wire at the base of the petiole (Figure S1f,g). For each seedling we measured initial height and number of leaves.

In each site we randomly assigned plots to three treatment levels: (a) 20 g of feces placed at the base of each seedling and access to dung beetles (+Feces+Beetles; Figure S1h), (b) 20 g of feces per seedling and dung beetle exclusion (+Feces−Beetles), and (c) without feces and consequently with no dung beetle activity either (−Feces−Beetles). In all experiments we used fresh pig dung collected from a nearby household (pigs were fed with food waste). We acknowledge that pig dung is not part of the local biota of our site, but we chose it due to the ease of obtaining large quantities, and because it has been shown to be effective in attracting tropical forest dung beetles (e.g., Marsh, Louzada, Beiroz, & Ewers, 2013) and fertilizing plants (e.g., Nweke, Ijearu, & Igili, 2013).

Immediately upon dung placement, we covered the top of the +Feces−Beetles and −Feces−Beetles plots with plastic mosquito screen (Figure S1c,d). +Feces+Beetles plots remained uncovered during 48 hr, to allow for complete dung burial by beetles. The design of the plots and the behavior of beetles ensured that dung beetles could enter and leave the plots while they were uncovered, bury the dung inside the plot area, but they could not move dung outside the plot. After 48 hr the tops of these plots were also covered with screen. In plots where beetles were excluded, the dung dried out and then started to disintegrate with rain; activity of other soil insects such as termites or ants was not observed, at least aboveground. We kept the top screen in all plots until the moment in which we harvested some of the seedlings for nutrient measurements; afterwards the top screen was removed from all plots to allow for natural processes affecting seedling survival and growth to occur.

2.4 Seedling response variables

2.4.1 Nutrient concentration and resource allocation

Several weeks after dung placement (4–8 weeks, depending on species; Table 1) we harvested some of the seedlings in each plot. The number of seedlings harvested and the number of replicates for chemical analyses also varied among species, depending on the size of seedlings and the number of surviving seedlings available (Table 1). For *Cymbopetalum* we had few surviving seedlings and thus we harvested them all.

Harvested seedlings were washed to remove soil particles and then dried at 35°C for 72 hr. We weighed aerial parts (stem and leaves) and roots, separately, and calculated the root/shoot biomass ratio for each seedling. Leaves (*Brosimum*, *Calophyllum*, *Omphalea*) or leaves and stems (*Cymbopetalum*, *Diospyros*, *Poulsenia*) were pulverized; pulverized plant matter obtained from all the seedlings in a plot was mixed to obtain the minimum sample necessary for analyses (0.25 g). We obtained N and P concentrations using a Kjeldahl wet digestion (Technicon Industrial System, method No. 696-82 W) and colorimetric analysis (Bran+Luebbe AutoAnalyzer 3).

2.4.2 Survival and growth

We registered seedling survival weekly during six weeks for *Cymbopetalum* (at which time all seedlings of this species were harvested, see above), and during 26 weeks for the other five species. For the latter species we also measured seedling height, number of leaves lost and number of new leaves, every four or five weeks.

2.5 Data analyses

For the analyses of nutrient data we removed four outlier values, which could have been the result of some problem during the digestion process. To analyze N and P concentrations we fitted linear mixed effects models (LMMs), one model for each nutrient and species combination. The feces and beetle manipulation treatment was the fixed factor and site was the random factor over the intercept. We encountered singularity problems for N models in the case of *Brosimum* and *Cymbopetalum*. This problem may arise when the random variable has small effects on the variance; thus, following Bolker (2015), we simplified these two models by removing the random factor and fitting linear models (LMs).

For the root/shoot biomass ratios we log-transformed the response variable and fitted LMMs with treatment as the fixed factor. For *Brosimum*, *Calophyllum*, *Cymbopetalum* and *Omphalea* two random factors were added over the intercept: site and plot, with the latter nested within the first. For *Diospyros* and *Poulsenia* each plot consisted of a pair of buckets, thus we added bucket as a third random factor (nested within plot). As in the case of nutrients we had singularity problems in some of the models, which were simplified as described above. Thus, final models varied among species regarding the random factors included (Table S1).

For seedling survival, we fitted Cox regression models with mixed effects, following Austin (2017). Treatment was the fixed factor; random factors were the same as for the previous variable (Table S1). The response variable was the number of days elapsed until death; seedlings that had not died by the end of the experiment were included as right-censored data (e.g., Onofri, Gresta, & Tei, 2010).

To analyze net growth, we only used data from seedlings that had survived by the end of the experiment (26 weeks). For each of these seedlings we calculated net growth in height (final height minus initial height) and net growth in the number of leaves (final number of leaves minus initial number of leaves). Many seedlings did not grow in height during the study period and the resulting large number of zeros did not allow us to adequately model the continuous response variable. Thus, we analyzed this variable as binary, considering if a seedling did or did not grow in height. For both growth response variables (probability of growing in height, net growth in number of leaves) we included in the models the initial height and the initial number of leaves, respectively, as a second fixed predictor (covariable), because it has been shown that seedling growth rates depend on seedling size (e.g., Rees et al., 2010). We analyzed the probability of growing in height using generalized linear mixed-effects models (GLMMs) with a binomial error distribution. Models for *Calophyllum* and *Omphalea* had singularity problems and we fitted simplified generalized linear models (GLMs), without the random factors (Table S1). Net growth in the number of leaves was analyzed by fitting LMMs, with treatment as a fixed factor. Random factors were defined as in the case of the root/shoot biomass ratio (Table S1).

Models for nutrient concentration, root/shoot biomass ratio, and growth in leaves were fitted using functions *lmer* and *lm*. Models for the probability of growth in height were fitted with functions *glmer* and *glm*. Survival models were fitted with function *coxme*. In all models the effect of the feces and beetle manipulation treatment was determined with the Wald-Chi test, using function *Anova*. Marginal means and standard errors, as well as the significance of *post hoc* tests, were obtained with the function *emmeans*. All analyses were carried out in R (v. 3.6.1; R Core Team 2019) using packages ‘lme4’ (Bates, Mächler, Bolker, & Walker, 2015), ‘stats’ (R Core Team 2019), ‘coxme’ (Therneau, 2019), ‘car’ (J. Fox & Weisberg, 2011) and ‘emmeans’ (Lenth, 2019).

3. RESULTS

3.1 Nutrient concentration and resource allocation

Depending on the species, foliar N concentration varied between 7.39 and 41.90 mg/g, but in no case was it affected by the feces and beetle manipulation treatment (Table 2; Figures 1 and 2). Concentration of P varied between 0.54 and 5.83 mg/g, and no treatment effect was detected for four of the species. For the other two species, *Calophyllum* and *Cymbopetalum*, we found a significant treatment effect (Table 2; Figures 1 and 3), but not the one we had expected. In *Calophyllum* the concentration of P was higher in plots with feces and exclusion of dung beetles, but equally low in the other two treatments (Table 3; Figure 3). In *Cymbopetalum* we found a different pattern: seedlings in plots with dung beetle activity did have higher P concentration than seedlings with feces but beetles excluded; however, none of these P concentrations differed significantly from those observed in plots with no feces or beetles, which had intermediate values (Table 3; Figure 3).

In terms of resource allocation, the values of the root/shoot biomass ratio varied between 0.15 and 0.44, depending on species. No effect of treatment was found in five of the species. A treatment effect was observed for seedlings of *Poulsenia* (Table 2; Figures 1 and 4). Concordant with our prediction, this species had lower values of root/shoot biomass ratio in plots where dung beetles had been active, compared to the other treatment levels. Values in the latter two levels did not differ (Table 3; Figure 4).

3.2 Survival and growth

Seedling survival was high for *Calophyllum* (97%), *Cymbopetalum* (91%) and *Diospyros* (83%), intermediate for *Brosimum* (63%) and *Poulsenia* (43%), and low for *Omphalea* (28%). The treatment had no effect on the survival of any of the species (Table 2; Figures 1 and 5).

As mentioned previously, many seedlings did not show any height growth during the 26-week time period; those that grew had only very small increases in height in the whole period (0.9-1.3 cm). The treatment did not affect the probability of seedlings growing in height in the cases of *Calophyllum* and *Poulsenia*; the treatment had an effect in the cases of *Brosimum*, *Diospyros* and *Omphalea*, but these effects were not in accordance with predictions (Table 2; Figures 1 and 6). Seedlings of *Brosimum* and *Diospyros* had a lower probability of growing in height when they were in plots with dung beetle activity, compared to the other plot types (Table 3; Figure 6). For *Brosimum* there was no difference between plots with feces added but beetles excluded, and plots with no feces added. In the case of *Diospyros*, growth probability was higher in plots with no feces (Table 3; Figure 6). Finally, for *Omphalea* seedlings the probability of growing was similar in both treatment levels that had feces, regardless of beetle activity, but was higher in plots with no feces Table 3; Figure 6).

Regarding net growth in the number of leaves, during the 26-week period all species had negative growth, losing between 0.42 and 3.48 leaves during that time (Figures 1 and 7). The treatment had an effect on *Brosimum* seedlings but none of the other species (Table 2; Figures 1 and 7). The effect on *Brosimum* was, again, not in accordance with our prediction; seedlings in plots without feces lost the least amount of leaves compared to the other two plot types. There was no difference between the two latter types (Table 3; Figure 7).

The initial seedling height had a significant negative effect when modelling the probability of growth in the cases of *Calophyllum* and *Diospyros,* i.e., seedlings that were larger at the beginning of the experiment had lower probability of growing in height. In the case of the initial number of leaves, this covariable had a significant negative effect on the net growth in number of leaves, for all the species analyzed (Table S2).

4. DISCUSSION

Contrary to our predictions we found that dung beetle activity had no positive effects on seedling growth, survival or nutrient assimilation, for any of the studied species. Furthermore, dung beetle activity had negative effects on the growth of two of the species. Dung beetle activity modified resource allocation as predicted (decreased allocation to roots relative to shoots), but only in one of the six species. Thus, our results warn against assuming that dung beetle activity has positive effects on seedlings established in the tropical forest understory. Next, we discuss possible causes of our findings.

Nutrient concentrations in the six seedling species were not increased in plots with dung beetle activity when compared to seedlings with no feces added. These results are contrary to those of the single study that has to date quantified this effect for one tropical tree species (*Brosimum lactescens*) under natural conditions (Santos-Heredia et al., 2016). Nutrient dynamics can vary greatly among tropical forests, as it strongly depends on the conditions (soil, climate, vegetation, etc.) of each site (Wright, 2019). Consequently, it is possible that the difference between our results and those of the previous study are due to differences in the availability of nutrients to plants between both study sites. In our study site, the soil is andosol derived from volcanic ash, with relatively high concentrations N and P (N: 5.00 ± 1.62 mg/g y P: 1.34 ± 0.11

mg/g; Hughes, Kauffman, & Jaramillo, 2000; Ricker et al., 2000). The other study site, the Lacandona rainforest, is characterized by fluvial cambisols with relatively low concentrations of both macronutrients (N: 0.02-0.03mg/g y P: 0.009 mg/g; Ibarra-Manríquez & Martínez-Ramos, 2002; Navarrete-Segueda et al., 2017). We believe that this difference in soil fertility could cause the observed discrepancy in results, and therefore suggest that dung beetle activity could have a positive effect on seedling nutrient concentrations in low-fertility forests, but none in forests with fertile soil. Future studies could assess this hypothesis.

The fact that we did not observe any positive effect of dung beetle activity on seedling survival or growth, could simply be because dung beetles did not increase nutrient concentrations. Dung beetle activity could improve plant performance through mechanisms other than nutrient assimilation, such as increased aeration and permeability caused by bioturbation, as has been shown in pastures and controlled greenhouse experiments (Bertone et al., 2006; Brown et al., 2010; Forgie, Paynter, Zhao, Flowers, & Fowler, 2018). Since we did not find any evidence of improved seedling performance, we suggest that either dung beetle activity does not have these effects on tropical forest soils, or it does, but these effects are inconsequential for seedling performance under natural conditions. Again, future studies might evaluate these possible scenarios.

Regarding seedling survival, it is worth mentioning that Santos-Heredia et al. (2016) found a negative effect of dung beetle activity, seemingly due to higher pathogen-caused mortality, which in turn could have been a response to higher foliar nutrient concentrations in *Brosimum lactescens* seedlings (see Supplementary Information in Santos-Heredia et al., 2016). It is interesting to note that we found the same trend (though not significant) in the survival of seedlings of a different species in the same genus, *Brosimum alicastrum* (Figure 5), but without an associated trend in nutrient concentrations. Some studies have found a positive association between nutrient concentrations and herbivory in seedlings (Andersen, Corre, Turner, & Dalling, 2010). Nevertheless, for seedlings of *Brassica oleracea* the nutrient uptake caused by dung beetle activity did not increase plant suitability for herbivores (Johnson et al., 2016). Thus, more studies are needed to elucidate through which mechanisms dung beetle activity may, if at all, decrease seedling survival.

In terms of growth, Santos-Heredia et al. (2016) found some evidence suggesting that dung beetle activity was associated with increased growth in the number of leaves of *Brosimum lactescens*. Similarly, Johnson et al. (2016) found that dung beetle activity enhanced seedling growth in *Brassica oleracea*. These effects are likely due to the increase in nutrient assimilation associated with dung beetle activity, which was also observed in these two studies. In tropical forests it has indeed been found that nutrient additions boost aerial biomass production in seedlings (Santiago et al., 2012; Wright, 2019). Thus, since we did not find an increase in nutrient assimilation in our study site (possibly due to high soil fertility), it is, again, not surprising that we did not find an effect of dung beetles on seedling growth. Unexpectedly, however, dung beetle activity had a negative effect on the probability of growth in height for two of the species (*Brosimum* and *Diospyros*; Figures 1 and 6). Furthermore, *Poulsenia* had a lower root/shoot biomass ratio in plots with dung beetles, which can be assumed to be the result of reduced root growth rather than increased shoot growth, since the number of leaves was not affected by dung beetle activity. We also observed some results that point towards a negative effect of dung by itself, i.e., with or without dung beetle activity. In particular, the probability of growth in height for *Diospyros* and *Omphalea* seedlings, and of growth in number of leaves for *Brosimum* seedlings was negatively affected by dung presence (Figure 1). Future studies will need to assess if negative effects of dung beetle activity and/or dung by itself, such as those observed here, occur in other plant species, and try to determine the causal mechanisms.

In conclusion, we found no evidence to support the idea that dung beetle activity has positive effects on the performance and nutrient content of seedlings established in the tropical forest understory. Instead, we found some evidence suggesting that dung beetle activity and/or dung by itself, may have negative effects on seedling growth for some plant species. Our study demonstrates that results on the effects of dung beetle activity obtained in other systems (e.g., temperate grasslands and agricultural systems) cannot be extrapolated to tropical forests. Even within tropical forests, the effects of dung beetles on seedlings may vary depending on soil fertility, such that they may favor plants in sites where nutrients are limiting resources, but not in sites with fertile soils. Future studies are needed to test this hypothesis. Also, given that regeneration strategies vary greatly among plant species (e.g., shade tolerant vs. light demander), it would be relevant that future studies assess the relationship between the effect of dung beetles and seedling functional traits related to their regeneration strategy. Finally, future studies will need to determine to what degree the negative effects of dung beetles and/or dung observed here can be generalized, and if so, what mechanisms are responsible for those effects.

Dung beetles are very abundant in tropical forests, and so is the dung (and other decomposing matter) that they incorporate into the forest soil. Thus, it strikes us as surprising that we know so little about the ecological consequences of dung beetle activity for plants and other ecosystem components of tropical forests. We hope that our study will encourage others to start filling these gaps.

TABLE 1 Experimental details for each plant species, including the number of sites and seedlings used for each response variable. In each site we used three plots, each randomly assigned to one treatment level: 20 g of feces at the base of each seedling and access to dung beetles (+Feces+Beetles), 20 g of feces per seedling and dung beetle exclusion (+Feces–Beetles), and no feces added and consequently no dung beetle activity (–Feces–Beetles). For *Cymbopetalum* we did not have enough seedlings to measure growth. In the case of *Poulsenia* we carried out two complementary experiments, one to evaluate survival and growth (*Poulsenia*1) and the other to measure nutrients and root/shoot biomass allocation (*Poulsenia*2).

	Brosimum	***Calophyllum***	***Cymbopetalum***	***Diospyros***	***Omphalea***	***Poulsenia*1**	***Poulsenia*2**
Dates							
Seed collection & sowing	Apr. 2017	Dec. 2017	Apr. 2016		Apr. 2016	Jun.16	Apr. 2018
Transplant	Aug. 2017	Jul. 18	Sept. 2016	Apr. 2017	Aug. 2016	Oct. 2016	Jul. 18
Start of experiment	Sept. 2017	Aug. 2018	Oct. 2016	Jul. 17	Sept. 2016	Apr. 2017	Aug. 2018
End of experiment	Mar. 2018	Feb. 2019	Dec. 2016	Jan. 2018	Mar. 2017	Oct. 2017	Oct. 2018
Plot size							
Area (m^2)	0.36	0.36	0.04	0.08[a]	0.36	0.08[a]	0.08[a]
Seedlings							
Age (months)	4	7	4	~ 3	3	8	3

TABLE 1 (continued)

Initial height (cm)	10-38	5-40		9-16	27-72	1-11	
No. seedlings per plot	6	6	3-4	6	6	4	6
No. total of seedlings	540	540	87	540	540	360	270
Time of harvest (weeks)	8	8	6	8	4		8
No. sites (n), No. seedlings (s)							
Foliar nutrients	n=18, s=162	n=30, s=180	n=8, s=79	n=27, s=243	n=26, s=147		n=15, s=213
Root/shoot	n=19, s=171	n=30, s=180	n=8, s=69	n=27, s=243	n=26, s=147		n=15, s=216
Survival	n=30, s=369	n=30, s=360	n=8, s=87	n=30, s=297	n=30, s=375	n=30, s=360	
Growth	n=30, s=233	n=30, s=348		n=30, s=245	n=24, s=97	n=29, s=154	

[a] Each plot consisted of two buckets (see text).

TABLE 2 Results of the models assessing the effect of the feces/beetle manipulation treatment on seedling performance of six tropical tree species. We fitted a model for each response variable and plant species. χ^2 is for all mixed-effects models or simplified generalized linear models, and F is for linear models.

Response variables	**Species**					
	Brosimum	***Calophyllum***	***Cymbopetalum***	***Diospyros***	***Omphalea***	***Poulsenia***
Concentration of foliar nitrogen	$F_{2,51} = 0.49$ $P = 0.615$	$\chi^2 = 4.20$ $P = 0.122$ $df = 2$	$F_{2,21} = 0.27$ $P = 0.762$	$\chi^2 = 0.12$ $P = 0.941$ $df = 2$	$\chi^2 = 1.27$ $P = 0.527$ $df = 2$	$\chi^2 = 2.72$ $P = 0.256$ $df = 2$
Concentration of foliar phosphorus	$\chi^2 = 3.78$ $P = 0.150$ $df = 2$	$\chi^2 = 8.36$ $P = 0.015$ $df = 2$	$\chi^2 = 6.81$ $P = 0.033$ $df = 2$	$\chi^2 = 1.59$ $P = 0.449$ $df = 2$	$\chi^2 = 0.02$ $P = 0.99$ $df = 2$	$\chi^2 = 0.35$ $P = 0.837$ $df = 2$
Root/shoot biomass ratio	$\chi^2 = 3.75$ $P = 0.153$ $df = 2$	$\chi^2 = 0.05$ $P = 0.972$ $df = 2$	$\chi^2 = 1.99$ $P = 0.368$ $df = 2$	$\chi^2 =1.23$ $P = 0.539$ $df = 2$	$\chi^2 = 2.21$ $P = 0.330$ $df = 2$	$\chi^2 = 18.17$ $P < 0.001$ $df = 2$
Probability of survival	$\chi^2 = 3.11$ $P = 0.210$ $df = 2$	$\chi^2 = 0.0001$ $P = 0.999$ $df = 2$	$\chi^2 = 3.42$ $P = 0.180$ $df = 2$	$\chi^2 = 5.06$ $P = 0.079$ $df = 2$	$\chi^2 = 1.24$ $P = 0.537$ $df = 2$	$\chi^2 = 1.91$ $P = 0.384$ $df = 2$
Probability of growth in height	$\chi^2 = 10.94$ $P = 0.004$ $df = 2$	$\chi^2 = 1.66$ $P = 0.436$ $df = 2$		$\chi^2 = 32.67$ $P = < 0.001$ $df = 2$	$\chi^2 = 9.82$ $P = 0.007$ $df = 2$	$\chi^2 = 0.56$ $P = 0.752$ $df = 2$
Net growth in number of leaves	$\chi^2 = 6.36$ $P = 0.041$ $df = 2$	$\chi^2 = 0.57$ $P = 0.748$ $df = 2$		$\chi^2 = 3.46$ $P = 0.176$ $df = 2$	$\chi^2 = 0.50$ $P = 0.775$ $df = 2$	$\chi^2 = 4.81$ $P = 0.090$ $df = 2$

TABLE 3 Results of the *post hoc* tests performed to compare between pairs of treatment levels of the feces/beetle manipulation treatment. The three treatment levels were: feces added and access to dung beetles (+Feces+Beetles), feces added and dung beetles excluded (+Feces–Beetles), no feces added and consequently no dung beetle activity either (–Feces–Beetles). *Post hoc* tests were performed only in those cases in which the complete models showed a statistically significant effect of the feces and beetle manipulation treatment ($P < 0.05$; see Table 2).

Response variables	**Species**					
	Brosimum	***Calophyllum***	***Cymbopetalum***	***Diospyros***	***Omphalea***	***Poulsenia***
Concentration of foliar phosphorus:						
+Feces+Beetles vs. +Feces–Beetles		$t = -2.15$ $P = 0.035$	$t = 2.60$ $P = 0.020$			
+Feces+Beetles vs. –Feces–Beetles		$t = 0.63$ $P = 0.530$	$t = 1.13$ $P = 0.276$			
+Feces–Beetles vs. –Feces–Beetles		$t = 2.75$ $P = 0.007$	$t = -1.47$ $P = 0.163$			
Root/shoot biomass ratio:						
+Feces+Beetles vs. +Feces–Beetles						$t = -3.99$ $P < 0.001$
+Feces+Beetles vs. –Feces–Beetles						$t = -3.03$ $P = 0.003$
+Feces–Beetles vs. –Feces–Beetles						$t = 1.21$ $P = 0.232$

TABLE 3 (continued)

Probability of growth in height:			
+Feces+Beetles vs. +Feces–Beetles	z = -2.26	z = -4.26	z = 0.07
	P = 0.023	P = < 0.001	P = 0.937
+Feces+Beetles vs. –Feces–Beetles	z = -3.51	z = -7.39	z = -3.19
	P < 0.001	P = < 0.001	P = 0.001
+Feces–Beetles vs. –Feces–Beetles	z = -1.21	z = -2.82	z = -3.10
	P = 0.223	P = 0.004	P = 0.001
Net growth in number of leaves:			
+Feces+Beetles vs. +Feces–Beetles	t = -0.01		
	P = 0.989		
+Feces+Beetles vs. –Feces–Beetles	t = -2.14		
	P = 0.036		
+Feces–Beetles vs. –Feces–Beetles	t = -2.17		
	P = 0.034		

FIGURE LEGENDS

FIGURE 1 Synoptic schematic summary of all results obtained for seedlings of six tropical tree species and six response variables measured. Size of bars do not represent actual values of the response variables (these can be found in Figures 2-6), but merely indicate statistical differences among treatment levels. Bars of the same size indicate no statistical difference ($P \geq 0.05$); short vs. tall bars indicate statistical differences ($P < 0.05$); statistical interpretation in the two panels that also contain intermediate-sized bars are indicated by letters above the bars. Three treatment levels were compared: feces added and access to dung beetles (+Feces+Beetles; black bars), feces added and dung beetles excluded (+Feces–Beetles; grey bars), no feces added and consequently no dung beetle activity either (–Feces–Beetles; white bars). Response variables are the concentration of foliar nitrogen (Foliar [N]), the concentration of foliar phosphorus (Foliar [P]), the ratio of root and shoot biomass (Root/shoot), seedling survival probability (P(survival)), the probability of seedlings growing in height (P(growth)), and net growth in the number of leaves (No. leaves). Tree species are *Brosimum alicastrum*, *Calophyllum brasiliense*, *Cymbopetalum baillonii*, *Diospyros digyna*, *Omphalea oleifera* and *Poulsenia armata*.

FIGURE 2 Mean foliar nitrogen in seedlings of six plant species. Experimental seedlings were subject to three treatment levels: feces and access to beetles (+Feces+Beetles), feces and beetle exclusion (+Feces–Beetles), and no feces added and consequently no dung beetle activity either (–Feces–Beetles). Different letters above bars indicate statistical differences ($P < 0.05$) based on *post hoc* tests.

FIGURE 3 Mean foliar phosphorus in seedlings of six plant species. Experimental seedlings were subject to three treatment levels: feces and access to beetles (+Feces+Beetles), feces and beetle exclusion (+Feces–Beetles), and no feces added and consequently no dung beetle activity either (–Feces–Beetles). Different letters above bars indicate statistical differences ($P < 0.05$) based on *post hoc* tests.

FIGURE 4 Mean ratios of root biomass/shoot biomass for seedlings of six plant species. Experimental seedlings were subject to three treatment levels: feces and access to beetles (+Feces+Beetles), feces and beetle exclusion (+Feces–Beetles), and no feces added and consequently no dung beetle activity either (–Feces–Beetles). Different letters above bars indicate statistical differences ($P < 0.05$) based on *post hoc* tests.

FIGURE 5 Survival curves for seedlings of six plant species. Experimental seedlings were subject to three treatment levels: feces and access to beetles (+Feces+Beetles; solid lines), feces and beetle exclusion (+Feces–Beetles; dashed lines), and no feces added and consequently no dung beetle activity either (–Feces–Beetles; dotted lines). Censored data are denoted by crosses. Same letters to the right of each line indicate no significant differences ($P \geq 0.05$) based on *post hoc* tests.

FIGURE 6 Mean height growth probabilities for seedlings of six plant species. Experimental seedlings were subject to three treatment levels: feces and access to beetles (+Feces+Beetles), feces and beetle exclusion (+Feces–Beetles), and no feces added and consequently no dung beetle activity either (–Feces–Beetles). Different letters above bars indicate statistical differences ($P < 0.05$) based on *post hoc* tests.

FIGURE 7 Box-plots showing the net growth in number of leaves for seedlings of six plant species, after 26 weeks. Experimental seedlings were subject to three treatment levels: feces and access to beetles (+Feces+Beetles), feces and beetle exclusion (+Feces–Beetles), and no feces added and consequently no dung beetle activity either (–Feces–Beetles). Different letters above bars indicate statistical differences ($P < 0.05$) based on *post hoc* tests.

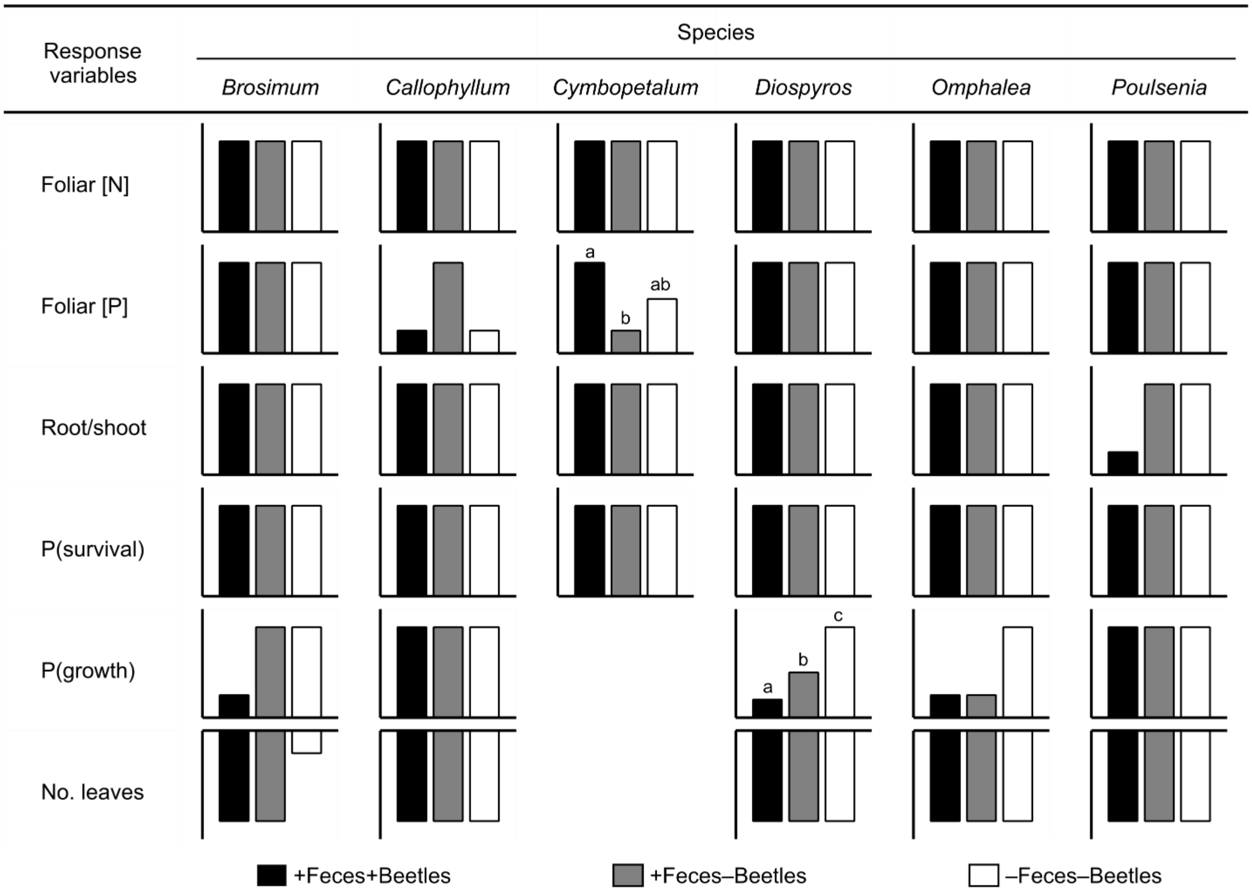

Response variables
Species
Brosimum
Callophyllum
Cymbopetalum
Diospyros
Omphalea
Poulsenia
Foliar [N]
Foliar [P]
a
b
ab
Root/shoot
P(survival)
P(growth)
a
b
c
No. leaves
+Feces+Beetles
+Feces–Beetles
–Feces–Beetles

FIGURE 1

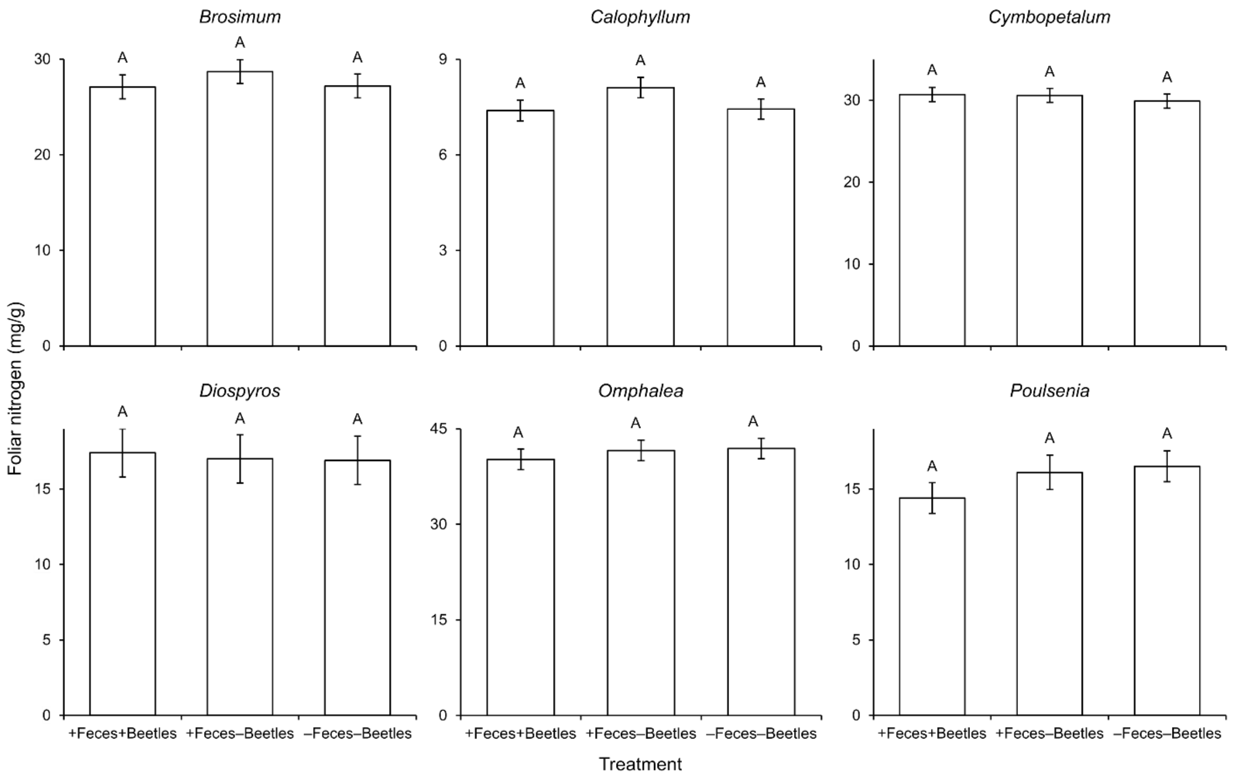

Brosimum
Calophyllum
Cymbopetalum
Diospyros
Omphalea
Poulsenia
Foliar nitrogen (mg/g)
A
30
20
10
0
9
6
3
15
5
45
+Feces+Beetles
+Feces–Beetles
–Feces–Beetles
Treatment

FIGURE 2

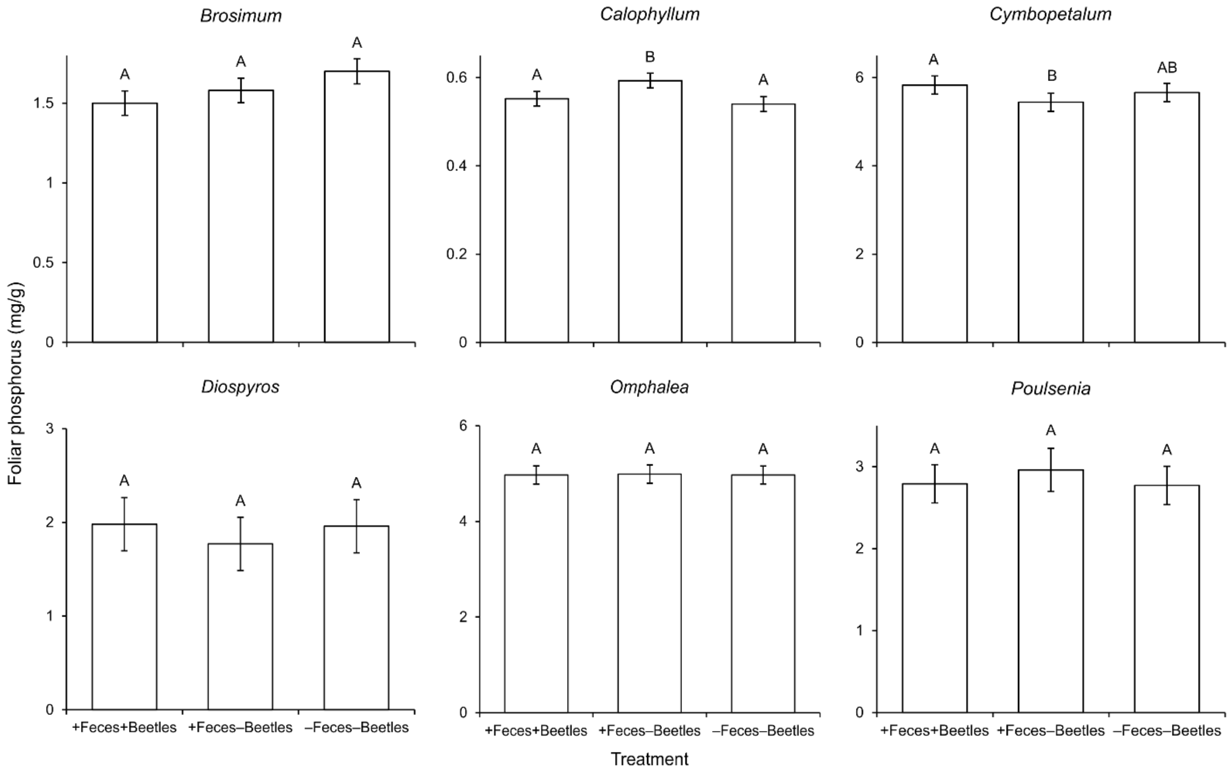

Brosimum
Calophyllum
Cymbopetalum
Diospyros
Omphalea
Poulsenia
Foliar phosphorus (mg/g)
Treatment
+Feces+Beetles
+Feces–Beetles
–Feces–Beetles
A
B
AB
0
0.5
1
1.5
0.2
0.4
0.6
2
3
4
6

FIGURE 3

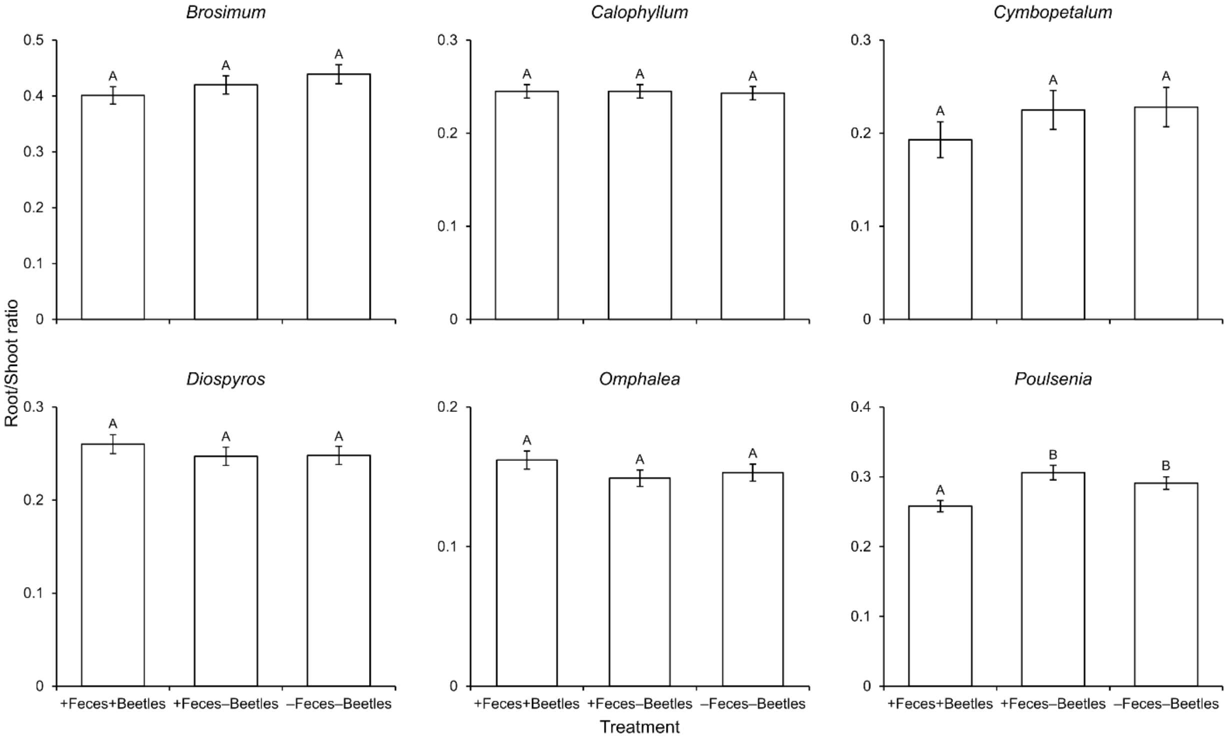

Brosimum
Calophyllum
Cymbopetalum
Diospyros
Omphalea
Poulsenia
Root/Shoot ratio
0.5
0.4
0.3
0.2
0.1
0
A
B
+Feces+Beetles
+Feces–Beetles
–Feces–Beetles
Treatment

FIGURE 4

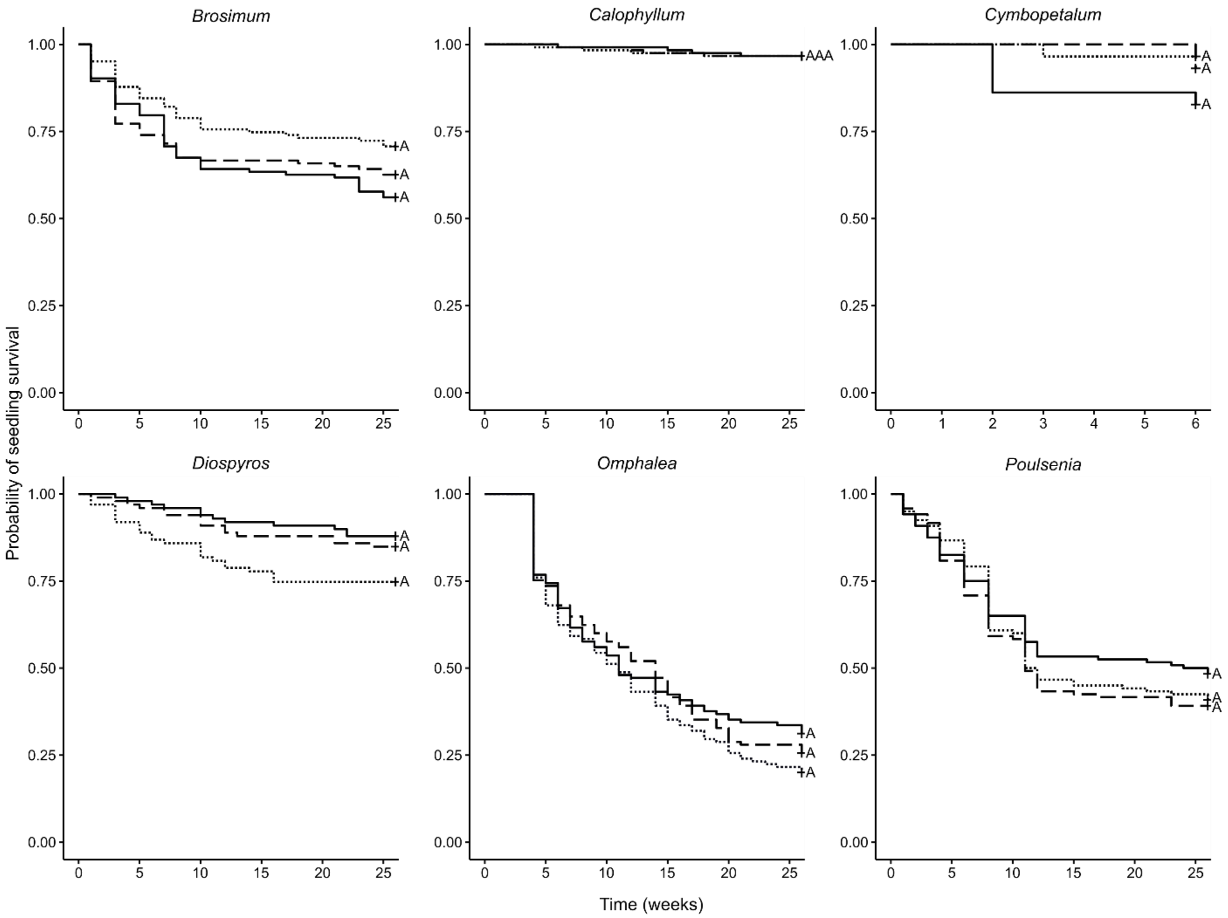

Brosimum
Calophyllum
Cymbopetalum
Diospyros
Omphalea
Poulsenia
Probability of seedling survival
Time (weeks)
1.00
0.75
0.50
0.25
0.00
0
5
10
15
20
25
0
1
2
3
4
5
6
A
AAA

FIGURE 5

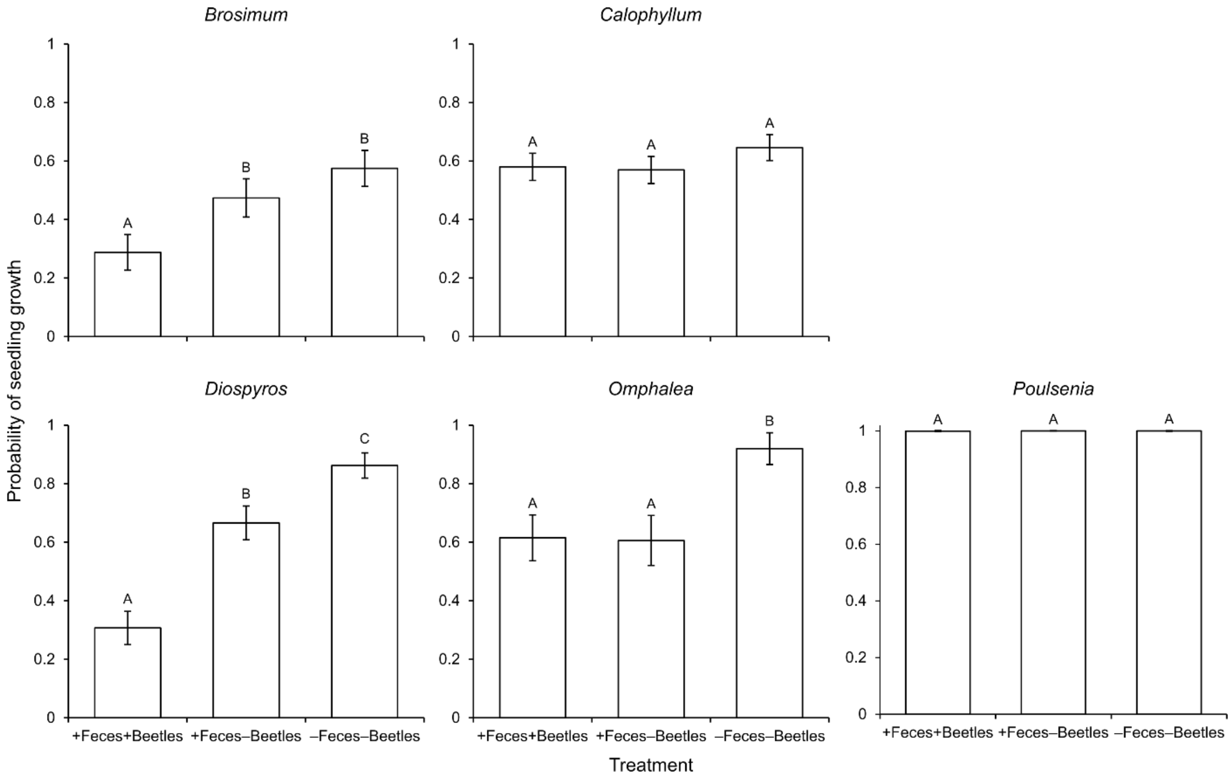
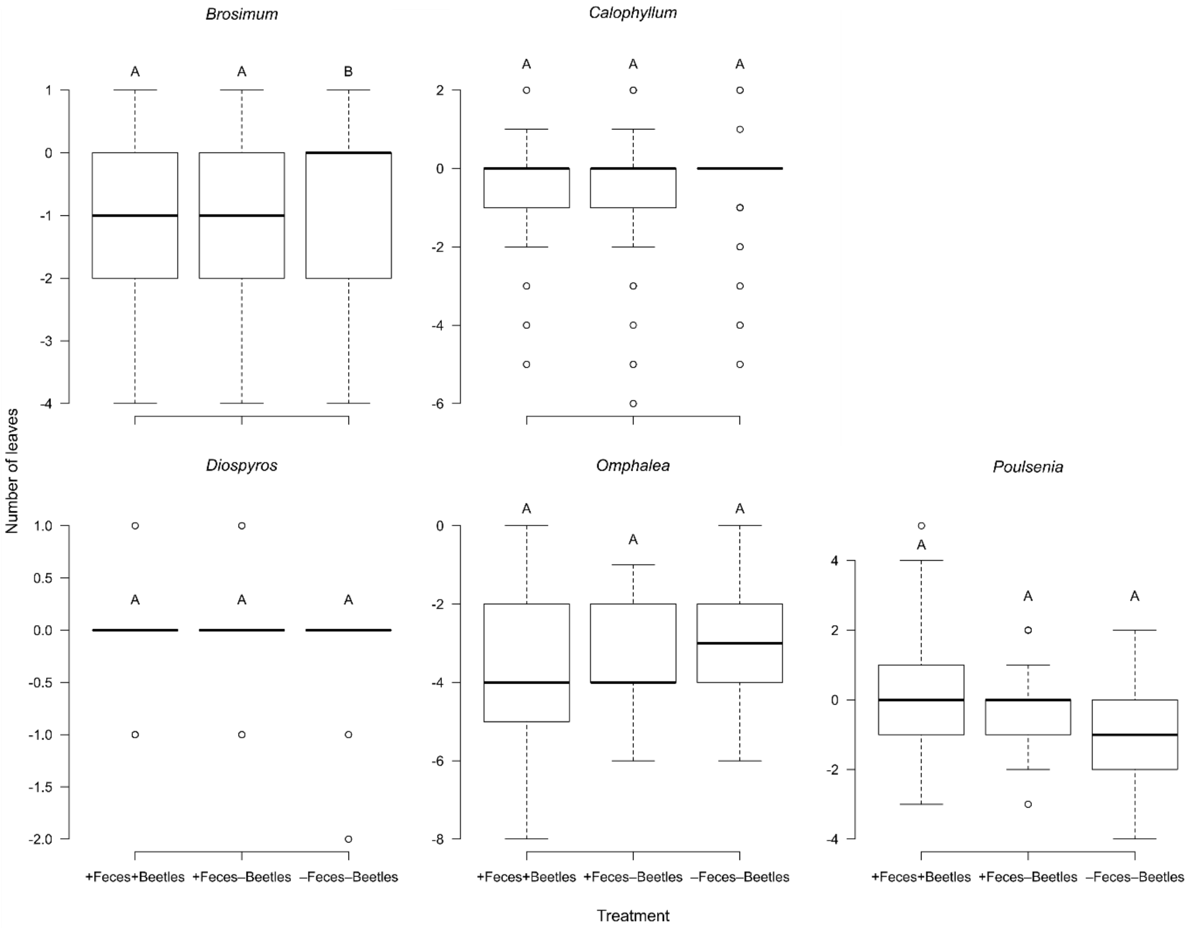

Brosimum
Calophyllum
Diospyros
Omphalea
Poulsenia
Probability of seedling growth
0
0.2
0.4
0.6
0.8
1
A
B
B
C
+Feces+Beetles
+Feces–Beetles
–Feces–Beetles
Treatment

FIGURE 6



Brosimum
Calophyllum
Diospyros
Omphalea
Poulsenia
Number of leaves
Treatment
+Feces+Beetles
+Feces–Beetles
–Feces–Beetles
A
B

FIGURE 7

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DATA AVAILABILITY

The data that support the findings of this study are openly available in Dryad Digital Repository.

AUTHOR CONTRIBUTION

LAUG, EA, Ed-V originally formulated the idea; LAUG, EA and RC developed the methodology; LAUG conducted fieldwork with support from RC; LAUG and MNM carried out nutrient analyses; LAUG and FMA analysed the data; LAUG and EA wrote the first version of the manuscript; all authors contributed to the final version of the manuscript.

CONFLICT OF INTEREST

The corresponding author confirms on behalf of all authors that no conflict of interest exists.

ETHICAL GUIDELINES

All applicable institutional and/or national guidelines for the care and use of animals were followed.

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SUPPLEMENTARY INFORMATION

Additional supporting information may be found online in the Supplementary Information.

Figure S1: Images of experimental details. Table S1: Random factors included in mixed models. Table S2: Effects of covariables in the growth models.

SUPPLEMENTARY INFORMATION

FIGURE S1 Images of experimental details: (a) *Poulsenia* seedlings obtained from seeds sown in germination trays and placed in a shade-house; (b) *Omphalea* seedlings obtained from seeds sown in germination bags and placed in the understory; (c) *Omphalea* seedlings in experimental plots built with mosquito screen; (d) for *Poulsenia* and *Diospyros* one plot consisted of two bottom-less buckets; plots were covered with mosquito screen until the time of seedling harvest; (e) *Cymbopetalum* seedlings in experimental plot consisting of a bottom-less bucket; (f) *Brosimum* seedlings with a wire and tag at their base; (g) *Calophyllum* seedlings with wire marks on each leaf; (h) 20 g of fresh pig feces placed at the base of each *Diospyros* seedling.

TABLE S1 Random factors included in mixed models. Due to singularity problems during model fitting some random factors had to be excluded. Random factors included in final models are shown in bold.

Response variable	Species	Random factors
Root/shoot biomass ratio	*Brosimum*	**Site**/**Plot**
	Calophyllum	Site/**Plot**
	Cymbopetalum	**Site**/**Plot**
	Diospyros	**Site**/Plot/**Bucket**
	Omphalea	Site/**Plot**
	Poulsenia	**Site**/Plot/**Bucket**
Probability of seedling survival	*Brosimum*	**Site**/**Plot**
	Calophyllum	**Site**/**Plot**
	Cymbopetalum	**Site**/**Plot**
	Diospyros	**Site**/**Plot**/**Bucket**
	Omphalea	**Site**/**Plot**
	Poulsenia	**Site**/**Plot**/**Bucket**
Probability of growth in height	*Brosimum*	**Site**/Plot
	Calophyllum	Site/Plot
	Diospyros	Site/**Plot**/Bucket
	Omphalea	Site/Plot
	Poulsenia	**Site**/**Plot**/Bucket
Net growth in number of leaves	*Brosimum*	**Site**/**Plot**
	Calophyllum	**Site**/**Plot**
	Diospyros	**Site**/**Plot**/**Bucket**
	Omphalea	**Site**/**Plot**
	Poulsenia	**Site**/**Plot**/**Bucket**

TABLE S2 Effects of covariables in the growth models. Models for the probability of growth in height included seedling initial height (IH) as a covariable; models for the net growth in number of leaves included seedling initial number of leaves (INL) as a covariable (see text for further explanation). Estimated coefficients that were statistically significant are shown in bold.

	Species				
Response variables	***Brosimum***	***Calophyllum***	***Diospyros***	***Omphalea***	***Poulsenia***
Probability of growth in height	IH = -0.002797	IH = **-0.03605**	IH = **-0.15305**	IH = 0.0004368	IH = -0.3518
	χ^2 = 0.0075	χ^2 = 3.5659	χ^2 = 5.084	χ^2 = 0.0002	χ^2 = 0.5521
	P = 0.931111	P = 0.05898	P = 0.02415	P = 0.98896	P = 0.4575
	df = 1	df = 1	df = 1	df = 1	df = 1
Net growth in number of leaves	INL = **-0.405208**	INL = **-0.10504**	INL = **-0.20848**	INL = **-0.6929**	INL = **-0.49918**
	χ^2 = 26.5154	χ^2 = 10.5656	χ^2 = 6.4635	χ^2 = 34.0176	χ^2 = 34.6794
	P = 0.0000002614	P = 0.001152	P = 0.01101	P = 0.000000005462	P = 0.000000003887
	df = 1	df = 1	df = 1	df = 1	df = 1

DISCUSIÓN GENERAL Y CONCLUSIONES

La presente tesis exploró algunos efectos que la actividad de los escarabajos coprófagos puede tener sobre fases tempranas de regeneración de las plantas del bosque tropical. Se encontró que la actividad de los escarabajos coprófagos provocó la dispersión vertical y horizontal de una gran proporción (56 y 97%, respectivamente) de semillas de tamaño medio (7.5─9.5 mm; Capítulo I). Además, que la dispersión horizontal disminuyó la agregación espacial de semillas y plántulas; sin embargo, bajo las condiciones de los experimentos realizados, no favoreció el establecimiento de plántulas (Capítulo I). También se encontró que la actividad de los escarabajos coprófagos provocó la redistribución vertical, tanto ascendente como descendente, de semillas que son parte del banco de semillas del suelo, promoviendo un mayor establecimiento de plántulas en el sotobosque (Capítulo II). Sin embargo, en sitios con un patrón de defecación recurrente, donde la actividad de los escarabajos coprófagos es más frecuente, el establecimiento de plántulas provenientes del banco de semillas, fue igual que en sitios con defecación única (Capítulo II). Finalmente, se encontró que la actividad de los escarabajos coprófagos, mediante el enterramiento de heces, no parece tener efectos positivos sobre el desempeño de plántulas del sotobosque (Capítulo III). ¿Qué tanto avanzan estos resultados, nuestro entendimiento general sobre los efectos que los escarabajos coprófagos pueden tener sobre la regeneración de las plantas? Para ayudar a contextualizar las contribuciones de esta tesis, se realizó una revisión sistemática de todos los trabajos publicados que han evaluado las interacciones escarabajo-planta a nivel global. En la primera parte de esta sección se presentan los resultados de esta revisión, y en la segunda parte se discuten los principales hallazgos de esta tesis, enfatizando su contribución en relación al conocimiento global y resaltando los vacíos de información que aún existen.

Efecto de los escarabajos coprófagos sobre la regeneración de plantas en condiciones naturales: Una revisión global

Se realizó una revisión sistemática de todos los artículos publicados desde 1991 hasta la fecha. Para tal fin, se hizo una búsqueda en las bases de datos de Web of Science (enero 30, 2020), de artículos que contenían en el tema de referencia las siguientes combinaciones de palabras: (dung beetle* O scarabaeinae) Y (plant* O seed* O seedling*). Los estudios adicionales que fueron citados en los artículos también fueron consultados. Solo se consideraron los estudios que utilizaron semillas naturales (i.e., se excluyeron aquellos que únicamente usaron cuentas a manera de semillas artificiales) y fueron hechos bajo condiciones naturales (i.e., se excluyeron estudios realizados en condiciones altamente controladas como en laboratorio, invernadero, entre otros).

Para cada artículo se identificaron las interacciones (escarabajo-semilla y/o escarabajo-plántula), las variables de respuesta y las especies de plantas evaluadas. Se hizo un registro independiente para cada especie de planta y variable de respuesta (Tabla 1). Por ejemplo, si un estudio evaluó dos variables de respuesta en tres especies de plantas, se hicieron seis registros. Las variables de respuesta incluyeron: semillas en heces dispersadas vertical y/u horizontalmente; distancias de dispersión de las semillas; supervivencia y/o germinación de semillas dispersadas verticalmente (enterradas); emergencia, establecimiento y/o supervivencia de plántulas a partir de semillas en heces y/o enterradas; establecimiento de plántulas a partir del banco natural de semillas; concentración de nutrientes foliares en plántulas; relación de biomasa radicular/biomasa aérea en plántulas, crecimiento y/o supervivencia de plántulas. Posteriormente, se resumieron los resultados registrando si estos eran descriptivos o si determinaban el efecto de algún tratamiento. Para los resultados del segundo tipo se registró la significancia del efecto de la actividad de los escarabajos coprófagos sobre la variable de respuesta, así como la dirección de los efectos significativos (positivo, negativo). Para cada registro se incluyó información del tamaño de semilla, el tipo de ecosistema, el estado de la vegetación, el país y la referencia del artículo.

La revisión literaria mostró que un total de 35 artículos evaluaron la interacción escarabajo-planta bajo condiciones naturales (Tabla 1). Los estudios fueron distribuidos en 19 países, pero la mayoría (74%) fueron realizados en regiones Neotropicales. Este resultado indica que hay un importante vacío de investigación sobre este tipo de interacciones, bajo condiciones naturales, en bosques Paleotropicales y en otros ecosistemas y regiones. Con respecto a los ecosistemas tropicales, más de la mitad de los estudios se realizaron exclusivamente en bosque primario (BP; 24 estudios) y muy pocos estudios en el bosque secundario (BS; 2). Un bajo número de estudios se realizó en sistemas con dos tipos de vegetación (i.e., BP y BS, 4; sabana y bosque de galería, 1). Todos los estudios (4) fuera de los trópicos se realizaron únicamente en pastizales templados (Tabla 1). Sobre el estado de la vegetación, la mayoría de los estudios tropicales se realizaron únicamente en sistemas conservados (vegetación continua de > 200 ha; 23 estudios), seguido de sistemas fragmentados (< 200 ha; 6). Muy pocos estudios (2) fueron hechos en sistemas con ambos estados de vegetación. En las regiones templadas, 3 de los 4 estudios se realizaron en sistemas tanto conservados como fragmentados (Tabla 1). Lo anterior, sugiere que el conocimiento sobre la interacción escarabajo-planta bajo condiciones naturales se ha generado, en gran medida, a partir de estudios hechos en bosques tropicales primarios conservados, principalmente en el Neotrópico. Más estudios en otros biomas con diferentes estados de conservación de la vegetación son necesarios para determinar en qué casos y hasta qué punto, la interacción escarabajo-planta es mayor o menor en frecuencia y/o intensidad, en comparación con los bosques tropicales.

En relación a las especies, en dichos estudios se evaluó un total de 65 especies de plantas. El número de especies evaluadas por estudio varió entre 1 y 12. Un mayor número de estudios evaluó el efecto de la actividad de los escarabajos coprófagos en una sola especie de planta (28.5% de los estudios), o entre 2 y 4 especies (28%); solo 15% evaluó entre 5 y 12 especies. El resto de los estudios (28.5%) evaluó el efecto de la actividad de los escarabajos coprófagos a nivel comunitario (Tabla 1). En particular, en 27 de las 65 especies de plantas (42%) solamente se cuantificó un efecto, en 21 especies (32%) dos efectos y en 7 especies tres efectos (10%). Para el resto de especies (16%) se cuantificaron entre 4 y 11 efectos. A nivel comunitario se evaluó un total de 10 efectos (Tabla 1).

En el caso de los efectos, a partir de los 35 estudios se identificaron 17 efectos de la actividad de los escarabajos coprófagos sobre la regeneración temprana de plantas (Tabla 1). La mayoría de los estudios evaluaron principalmente uno o dos efectos (42% y 30% de los estudios, respectivamente), mientras que el resto de los estudios evaluaron entre tres y seis efectos (28% en total; Tabla 1). En general, para los 17 efectos se identificó un total de 233 registros (Tabla 1). Se encontró un número de registros similar tanto con información únicamente descriptiva (116 registros), como con información en que se hizo al menos una comparación entre tratamientos que permitieron evaluar el efecto de la actividad de los escarabajos coprófagos (117; Tabla 1). Para este último grupo, el número de registros con efectos significativos positivos fue mayor que aquellos con efectos significativamente negativos (25 y 17, respectivamente). Sin embargo, el número de registros con efectos no significativos fue más alto que los dos anteriores (75).

Los resultados previos reflejan que en el estudio de la interacción escarabajo-planta, los diseños metodológicos (e.g., cantidad de semillas y/o plántulas, disponibilidad de heces, tiempos de seguimiento del experimento), entre otros factores, limitan la cuantificación de más de tres efectos de la actividad de los escarabajos coprófagos, así como la evaluación de un alto número de especies de plantas por estudio. Además, que en la medida que aumente el número de estudios que cuantifiquen, bajo condiciones naturales y estadísticamente, los distintos efectos de la actividad de los escarabajos coprófagos sobre la regeneración de las plantas, mayor será nuestro entendimiento de las implicaciones ecológicas de estos insectos en el funcionamiento de los ecosistemas.

Resultados encontrados, su contribución y los vacíos de información

Se ha asumido que la actividad de los escarabajos coprófagos tiene un efecto generalmente positivo sobre la regeneración de las plantas tropicales (Andresen y Feer 2005; Nichols et al. 2008). No obstante, es importante notar que la evidencia a favor de este supuesto es escasa. Hasta la fecha, la única función ecológica con implicaciones para las plantas que se ha estudiado con mayor detalle es la dispersión secundaria de semillas defecadas por mamíferos, y únicamente la dispersión vertical (enterramiento de semillas) cuenta con un mayor número de estudios (en comparación con la dispersión horizontal; Efectos 1-2; Tabla 1). A partir de estos estudios, se ha encontrado que la dispersión vertical de semillas por escarabajos coprófagos tiene frecuentemente un efecto positivo para las plantas, pero en algunos casos también se han reportado efectos negativos (e.g., para los Efectos 3-7; Tabla 1). Por lo tanto, aún no es posible generalizar sobre el efecto del enterramiento de semillas por escarabajos coprófagos para las plantas. Por un lado, son pocos los estudios que han dado seguimiento a las semillas hasta la etapa de establecimiento de plántulas y han evaluado estadísticamente este efecto (Andresen 2001, 2003; Andresen y Levey 2004; Santos-Heredia et al. 2010, 2011; Lawson et al. 2012; Griffiths et al. 2016; Culot et al. 2018; Urrea-Galeano et al. 2019a) y, por otro lado, el resultado de la interacción escarabajo-semilla es dependiente del contexto (e.g., especies de escarabajos, profundidad de entierro) y de la especie de planta (e.g., tamaño de semilla; Efectos 3-17; Tabla 1). Además, es importante notar que, el conocimiento actual sobre el efecto del enterramiento de semillas por escarabajos coprófagos para las plantas se restringe, principalmente, a las especies de plantas con semillas ≥ 6 mm de largo (97% de los registros para los Efectos 3-7, excluyendo aquellos a nivel comunitario; Tabla 1). Así que, aún se desconoce cuáles podrían ser las consecuencias de la actividad de este grupo de insectos para las plantas con semillas < 6 mm en fases posteriores a la dispersión secundaria.

En el caso de la dispersión secundaria horizontal, generalmente se ha asumido un efecto positivo debido a una supuesta disminución en la agregación espacial de las semillas defecadas. Sin embargo, solamente dos estudios, la presente tesis (Capítulo I) y un estudio previo (Lawson et al. 2012) han medido cómo la actividad de los escarabajos modifica la agregación espacial de semillas y plántulas, y los efectos sobre la probabilidad de establecimiento de plántulas. Los hallazgos de ambos estudios coinciden en que la actividad de los escarabajos coprófagos disminuye la agregación espacial de plántulas, aunque este hecho no necesariamente afecta positivamente la probabilidad de establecimiento. Es interesante destacar que en ambos estudios el establecimiento de las plántulas se evaluó dentro de un periodo de tiempo relativamente corto (2-4 semanas, Lawson et al. 2012; 4-11 semanas, Capítulo I). Por lo tanto, es necesario que estudios futuros evalúen este efecto a más largo plazo, ya que los factores de mortalidad denso-dependientes podrían actuar en estadíos posteriores del ciclo de vida de las plantas (e.g., Forget et al. 2005; Leck et al. 2008; Gallagher 2014). Esta es una hipótesis que requiere ser evaluada. Por otro lado, la dispersión vertical y horizontal de semillas ocurren al mismo tiempo y además se ha visto que la dispersión vertical puede tener efectos sobre semillas y plántulas tanto positivos como negativos (Tabla 1). Por lo tanto, es necesario diseñar estudios que consideren el efecto general de ambos tipos de dispersión al mismo tiempo, pero que también sean capaces de discernir el efecto relativo de cada uno.

Los escarabajos coprófagos no sólo interactúan con semillas que se encuentran en las heces, pero también con las semillas que se encuentran en el suelo formando parte del banco de semillas. Al igual que el efecto de la actividad de los escarabajos coprófagos sobre la dispersión horizontal de semillas, el efecto sobre la dinámica del banco de semillas apenas se ha comenzado a explorar (Santos-Heredia y Andresen 2014; Ocampo-Castillo y Andresen 2018; Capítulo II). Los tres estudios existentes han mostrado un efecto positivo (Efecto 10; Tabla 1) de los escarabajos sobre el establecimiento de plántulas a partir de semillas enterradas en el banco natural de semillas (Santos-Heredia y Andresen 2014; Ocampo-Castillo y Andresen 2018; Capítulo II). En estos tres estudios, el establecimiento se evaluó en parcelas donde se excluyó la lluvia de semillas, pero también la presencia de depredadores. Entre más cerca se encuentre una semilla de la superficie del suelo, mayor es su probabilidad de mortalidad por depredación (e.g., Estrada y Coates-Estrada 1991; Shepherd y Chapman 1998; Andresen 1999; Andresen y Levey 2004). Por lo tanto, es deseable que estudios futuros evalúen si la ventaja de una semilla ser movida hacia estratos superficiales del suelo o quedar sobre la superficie de éste, tras la actividad de los escarabajos coprófagos, no se contrarresta con la desventaja de ser fácilmente depredada.

Relacionado con lo anterior, también es deseable que estudios futuros relacionen las profundidades de relocalización ascendente y descendente que provocan los escarabajos coprófagos, con las características morfo-fisiológicas de las semillas. Esta información sería útil para predecir bajo qué condiciones una semilla tendría una mayor probabilidad de exhumación, de supervivencia y de establecimiento como plántula. Asimismo, es importante considerar que la probabilidad de movimiento hacia capas profundas del suelo parece incrementar al disminuir el tamaño de la semilla (Capítulo II), y que los escarabajos coprófagos grandes (≥ 10 cm largo corporal) entierran las heces a profundidades mayores que los escarabajos pequeños (Andresen 2002a). Por lo tanto, es muy probable que el efecto de los escarabajos sobre la dinámica del banco natural de semillas y el subsecuente establecimiento de plántulas, varíe con la estructura de la comunidad de escarabajos coprófagos, en conjunto con el tamaño de las semillas. Por ejemplo, es posible que, en sitios con actividad de escarabajos coprófagos de tamaño grande, el establecimiento de plántulas provenientes de semillas pequeñas sea menor, en comparación con sitios con escarabajos más pequeños. Los tres estudios que han evaluado este tipo de interacción se han realizado únicamente en México (Efectos 10-11; Tabla 1), cuyas comunidades de escarabajos coprófagos suelen tener un número relativamente más bajo de especies, de individuos y de escarabajos grandes (e.g., Halffter et al. 1992; Estrada y Coates-Estrada 1991; Andresen 2005), en comparación con bosques más ecuatoriales. Sería interesante evaluar la hipótesis previa en otros bosques tropicales con comunidades contrastantes, como las reportadas para la Amazonia brasilera (e.g., Andresen 2002a).

Por otra parte, solo en dos de los tres estudios que han estudiado el efecto de la actividad de los escarabajos coprófagos sobre el establecimiento de plántulas a partir del banco de semillas, se ha evaluado el efecto de la frecuencia de la actividad de los escarabajos coprófagos (Efecto 11; Tabla 1). En uno de los estudios se encontró un efecto positivo de la recurrencia de la defecación, y consecuentemente mayor frecuencia de actividad de escarabajos coprófagos

(Santos-Heredia y Andresen 2014), pero en el otro estudio no se observó efecto alguno (Capítulo II). Por lo tanto, son necesarias más investigaciones que evalúen este efecto, para determinar su generalidad. Además, en los estudios mencionados, la frecuencia de la actividad de los escarabajos coprófagos se evaluó en sitios con un patrón de defecación simulado. Por lo tanto, aún se requiere hacer esfuerzos metodológicos para evaluar la ocurrencia y dirección de este efecto en sitios con defecaciones naturales de mamíferos.

Finalmente, el resultado de las interacciones entre escarabajos coprófagos y plántulas establecidas en el sotobosque tropical es aún menos conocido, ya que sólo dos trabajos han estudiado esta interacción bajo condiciones naturales (Santos-Heredia et al. 2016; Capítulo III). Además, ni en el estudio de Santos-Heredia et al. (2016) ni en la presente tesis (Capítulo III), se midieron los cambios físicos y químicos que la bioturbación, causada por la actividad de los escarabajos coprófagos, provoca en el suelo. La bioturbación se ha asociado, en ecosistemas templados y estudios controlados, con efectos positivos para las plantas (Behling Miranda et al. 2000; Bang et al. 2005; Hanafy 2012; Badenhorst et al. 2018). Por consiguiente, para conocer con mayor detalle cómo los escarabajos coprófagos podrían afectar a las plántulas tropicales, sería deseable que estudios futuros cuantifiquen directamente los cambios físicos y químicos que su actividad genera en el suelo (e.g., pH, capacidad de intercambio catiónico, compactación del suelo, tasa de infiltración de agua, humedad, niveles de los nutrientes) y los relacionen con el desempeño de las plántulas.

En el estudio de Santos-Heredia et al. (2016), los autores encontraron que los escarabajos coprófagos provocaron un incremento en las concentraciones de N y P foliar de una especie de plántula, y mostraron resultados preliminares de un posible efecto positivo sobre el crecimiento, pero negativo sobre la supervivencia de las plántulas (Santos-Heredia et al. 2016). Contrario a estos resultados, en la presente tesis la actividad de los escarabajos coprófagos no aumentó la asimilación de N o P, y tampoco tuvo efectos sobre la supervivencia o crecimiento de las seis especies de plántulas evaluadas (Capítulo III). Es más, en dos especies, la actividad de los escarabajos coprófagos provocó una menor probabilidad de crecimiento en altura. La diferencia entre ambos estudios, con respecto al efecto de la actividad de los escarabajos sobre el contenido de nutrientes foliares podría ser explicada por diferencias en la fertilidad del suelo de ambos bosques. Una posible hipótesis sería que la actividad de los escarabajos coprófagos en suelos poco fértiles tiene un efecto positivo sobre el contenido de nutrientes foliares de las plántulas, mientras que no tiene ningún efecto en suelos con mayor disponibilidad de nutrientes. Sería interesante que estudios futuros, para poner a prueba esta hipótesis, evaluaran el desempeño de una misma especie de planta en áreas de bosque tropical que tengan niveles contrastantes de fertilidad del suelo.

Una explicación alternativa para la diferencia observada entre ambos estudios podría estar relacionada con la especie de planta. Es posible que la especie de plántula (*Brosimum lactescens*) del estudio de Santos-Heredia et al. (2016) responda positivamente al incremento de nutrientes del suelo, pero que esta respuesta no sea generalizada para otras especies del sotobosque, cuyo desempeño esté limitado por factores abióticos distintos al suelo (e.g., luz; Montgomery y Chazdon 2002). Con la información existente hasta el momento (Efectos 12-17; Tabla 1), no es posible dilucidar bajo qué circunstancias podría ocurrir un efecto positivo (o negativo) de la actividad de los escarabajos coprófagos sobre el desempeño de plántulas tropicales.

Asimismo, las 7 especies de plantas evaluadas hasta la fecha (Efectos 12-17; Tabla 1) son tolerantes a la sombra del dosel en estadio de plántula, y para ninguna se consideró la influencia del tipo funcional de plántula (según la posición, exposición y función de los cotiledones) sobre el desempeño. Por lo tanto, sería deseable que estudios futuros evaluaran el efecto de la actividad de los escarabajos coprófagos en relación al tipo funcional de plántula, considerando diferentes estrategias de regeneración (e.g., demandantes de luz vs. tolerantes a la sombra). Así, por ejemplo, se podría esperar que la actividad de los escarabajos coprófagos favoreciera a las especies demandantes de luz, las cuales, al tener una tasa de crecimiento rápido, podrían aprovechar pulsos efímeros de nutrientes, en comparación con las especies tolerantes a la sombra, cuya tasa de crecimiento es más lenta. Un patrón de este tipo se observó en un estudio en África, donde en sitios con heces de primates, las plántulas demandantes de luz crecieron más que las tolerantes a la sombra (Kalbitzer et al. 2019).

En conclusión, en estudios tropicales se ha justificado el uso extenso de los escarabajos coprófagos como taxón focal e indicador, entre otros motivos, por su importancia en el funcionamiento de estos ecosistemas (Spector 2006; Nichols y Gardner 2011; Fonseca Oliveira et al. 2017; Carvalho et al. 2020a). Sin embargo, dicha justificación funcional frecuentemente carece de una evidencia contundente que la soporte. La función que más frecuentemente se cuantifica, es la remoción de heces (Raine y Slade 2019), y a partir de ésta se hacen deducciones acerca de las demás funciones. Aunque se ha encontrado una relación directa entre la remoción de heces y otras funciones, ésta no siempre ocurre (Slade et al. 2007; Braga et al. 2013; Hosaka et al. 2014; Santos-Heredia et al. 2018; Milotić et al. 2019; Carvalho et al. 2020b). En estudios más recientes, otras funciones que también se han cuantificado en los trópicos, son la excavación de suelo y la dispersión secundaria de semillas (Braga et al. 2017; Raine y Slade 2019), pero raramente se han determinado sus consecuencias en el ecosistema. Sobre otras funciones que se atribuyen a estos insectos (e.g., reciclaje de nutrientes, incremento en la productividad primaria, reducción de la emisión de gases de efecto invernadero), la información se ha obtenido generalmente a partir de estudios hechos fuera de los trópicos con experimentos altamente controlados, donde además la actividad de los escarabajos coprófagos se ha medido, prácticamente, a partir de una o pocas especies de escarabajos en particular (Nichols et al. 2008; Slade et al. 2016). En general, nuestro conocimiento acerca de las funciones ecológicas y los servicios ecosistémicos que realizan los escarabajos coprófagos en bosques tropicales, es aún muy escaso.

Nuestra limitada comprensión de las funciones ecológicas de los escarabajos coprófagos no es un caso excepcional. Por el contrario, existe una escasez generalizada para la mayoría de los grupos faunísticos, sobre los papeles funcionales que juegan en los ecosistemas de la Tierra (Kunz et al. 2011; Wenny et al. 2011; Davidson et al. 2012; Noriega et al. 2018). Es necesario comprobar y medir empíricamente las funciones específicas que los animales realizan en cada ecosistema, tanto en estudios que los usan como grupos focales e indicadores, como en estudios que relacionan la biodiversidad con el funcionamiento de los ecosistemas. No hay duda de que los animales son de crucial importancia para el funcionamiento de los ecosistemas y que ante la crisis de defaunación que ocurre en el planeta, un colapso funcional es inminente (Dirzo et al. 2014). Para lograr mitigar e incluso revertir los efectos negativos de la defaunación, es fundamental que incrementemos nuestra comprensión de cómo los animales interactúan con los demás componentes bióticos y abióticos de los ecosistemas, formando parte de los engranajes que son la base de su funcionamiento.

TABLA 1 Listado de todos los estudios que han determinado, bajo condiciones naturales, los efectos de la actividad de la comunidad de escarabajos coprófagos en distintas fases de la regeneración temprana de las plantas. Las primeras dos columnas contienen el nombre de la especie, la familia y el tamaño de sus semillas (diámetro mayor en mm); las últimas tres columnas contienen información sobre el ecosistema (Eco; BTP = bosque tropical primario; BTS = bosque tropical secundario; ST = sabana tropical; BTG = bosque tropical de galería; PT = pastizal templado) y estado de la vegetación (EV; C = continuo > 200 ha; F = fragmento < 200 ha), el país (MX = México; UG = Uganda; PE = Perú; CO = Colombia; GF = Guyana Francesa; BR = Brasil; PA = Panamá; BE = Bélgica; EU = Europa; BZ = Belice; CM = Camerún; JP = Japón; CI = Costa de Marfil; CD = RD Congo), y la cita (Ref). Las demás columnas contienen información sobre los efectos medidos en los estudios (*E1–E17*) presentando un signo y una letra; el signo indica la significancia y dirección del efecto (**+** : efecto positivo significativo de los escarabajos sobre la variable de respuesta, **–** : efecto negativo significativo, **=** : sin efecto significativo) mientras que la letra indica los tratamientos comparados (**a**: semillas con heces y escarabajos que fueron enterradas vs. semillas con heces y escarabajos que no fueron enterradas; **b**: semillas con heces y escarabajos que fueron enterradas vs. semillas sin heces; **c**: semillas/plántulas con heces y escarabajos vs. semillas/plántulas sin heces; **d**: semillas/plántulas con heces y escarabajos vs. semillas/plántulas con heces pero sin escarabajos; **e**: sitios con defecación única vs. sitios con defecación recurrente). Cuando para algún efecto no se realizó una comparación entre tratamientos o si la comparación no tuvo prueba estadística asociada, se considera como resultado descriptivo (**D**). Los efectos estudiados son: ***E1***: efecto de la actividad de los escarabajos coprófagos sobre la dispersión secundaria vertical de semillas; ***E2***: efecto de la actividad de los escarabajos coprófagos sobre la dispersión secundaria horizontal de semillas; ***E3***: efecto del entierro de semillas en heces sobre la supervivencia de semillas tras el escape de la depredación por roedores; ***E4***: efecto del entierro de semillas en heces sobre la germinación; ***E5***: efecto del entierro de semillas en heces sobre la emergencia de plántulas; ***E6***: efecto del entierro de semillas en heces sobre el establecimiento de plántulas; ***E7***: efecto del entierro de semillas en heces sobre la supervivencia de semillas/plántulas; ***E8***: efecto de la actividad de los escarabajos coprófagos sobre el establecimiento de plántulas a partir de semillas en heces; ***E9***: efecto de la actividad de los escarabajos coprófagos sobre la supervivencia de plántulas establecidas a partir de semillas en heces; ***E10***: efecto de la actividad de los escarabajos coprófagos sobre el establecimiento de plántulas a partir del banco de semillas, en sitios con defecación única en sitios aleatorios; ***E11***: efecto de la actividad de los escarabajos coprófagos sobre el establecimiento de plántulas establecidas a partir del banco de semillas en sitios con defecación recurrente; ***E12***: efecto de la actividad de los escarabajos coprófagos sobre la concentración de N foliar de plántulas; ***E13***: efecto de la actividad de los escarabajos coprófagos sobre la concentración de P foliar de plántulas; ***E14***: efecto de la actividad de los escarabajos coprófagos sobre la asignación de recursos (relación biomasa radicular:biomasa aérea) en plántulas; ***E15***: efecto de la actividad de los escarabajos coprófagos sobre el crecimiento (altura) de plántulas; ***E16***: efecto de la actividad de los escarabajos coprófagos sobre el crecimiento (número de hojas) de plántulas; y ***E17***: efecto de la actividad de los escarabajos coprófagos sobre la supervivencia de plántulas. Celdas vacías representan no información.

Especie y familia	Tamaño semilla	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15	E16	E17	Eco; EV	País	Ref [d]
Brosimum alicastrum Sw., Moraceae	18	D		D a			D a												BTP; C	MX	1
													= c = d	= c = d	= c = d	– c – d	– c = d	= c = d			2
Cordia stellifera I.M. Johnst. Boraginaceae	6	D		D a			D a														1
Dialium guianense Steud. Fabaceae	10	D		D a																	
Dipholis minutiflora Pittier Sapotaceae	15	D		D a			D a														
Poulsenia armata (Miq.) Standl. Moraceae	8	D		D a			D a														
		D	D						– c = d												3
													= c = d	= c = d	– c – d	= c = d	= c = d	= c = d			2
Pseudolmedia glabrata (Liebm.) C.C. Berg, Moraceae	11	D		D a			D a														1

TABLA 1 (continuación)

Especie y familia	Tamaño semilla	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15	E16	E17	Eco; EV	País	Ref ^{d}
Rollinia jimenezii Saff., Annonaceae	13	D		D a			D a												BTP; C	MX	1
Spondias mombin L., Anacardiaceae	15	D		D a																	
Mimusops bagshawei S.Moore Sapotaceae	16	D	D																	UG	4
Monodora myristica (Gaertn.) Dunal Annonaceae	19	D	D																		
	19	D																			5
Uvaria sp., Annonaceae	10	D	D																		4
Batocarpus amazonicus (Ducke) Fosberg, Moraceae	17	D																		PE	6
Borismene japurensis (Mart.) Barneby, Menispermaceae	17	D																			
Brosimum lactescens (S. Moore) C.C. Berg, Moraceae	9	D	D																		
													+ c = d	+ c + d		= c = d	+ c + d	– c = d		MX	7
		D																			8
Chrysophyllum venezuelanense (Pierre) T.D. Penn., Sapotaceae	28	D																		PE	6
Clarisia racemosa Ruiz & Pav. Moraceae	20	D																			
		D	D	+ a^{b}		D													BTS; F	CO	9
Pseudolmedia laevis (Ruiz & Pav.) J.F. Macbr., Moraceae	8	D	D	+ a^{b}		D															
Coussapoa ovalifolia Trécul Urticaceae	3	D																	BTP; C	PE	6
Diospyros pavonii (A.Dc.) J.F.Macbr., Ebenaceae	15	D																			
Ficus spp., Moraceae	1	D																			
Guatteria acutissima R.E. Fr. Annonaceae	10	D																			
Micropholis egensis (A. DC.) Pierre Sapotaceae	16	D																			
Trichostigma octandrum (L.) H. Walter, Petiveriaceae	5	D																			
Virola calophylla (Spruce) Warb. Myristicaceae	16	D																			
Chrysophyllum lucentifolium Cronquist, Sapotaceae	31	D																		GF	10
		D		D a																	11
Doliocarpus sp., Dilleniaceae	8	D																			
Duguetia sp., Annonaceae	8	D																			
Micropholis guyanensis (A. DC.) Pierre, Sapotaceae	14	D		D a																	
	18	D																		BR	12
		D					+ a												BTP; F-C		13
Parahancornia fasciculata (Poir.) Benoist, Apocynaceae	12	D																	BTP; C	GF	11
Pouteria laevigata (Mart.) Radlk. Sapotaceae	46	D																			

TABLA 1 (continuación)

Especie y familia	Tamaño semilla	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15	E16	E17	Eco; EV	País	Ref [d]
Protium sp., Burseraceae	16	D																	BTP; C	GF	11
Schefflera decaphylla (Seem.) Harms, 1894, Araliaceae	9	D																			
Solanum sp., Solanaceae	4	D																			
Tetragastris spp., Burseraceae	18	D		D a																	
Micropholis guyanensis spp. *guyanensis* (A.DC.), Sapotaceae	18	D	D	+ b		– b	+ a		= c											BR	14
Byrsonima crispa A. Juss. Malpighiaceae	8	D																			15
		D																			12
Buchenavia grandis Ducke Combretaceae	21	D																			
Minquartia guianensis Aubl. Olacaceae	22	D																			
Ocotea percurrens A. Vicentini Lauraceae	12	D																			
Pourouma guianensis Aubl. Moraceae	11	D																			
		D					= a												BTP; F-C		13
Tetragastris altissima (Aubl.) Swart Burseraceae	15	D					D a													GF	16
Helicostylis scabra (J.F.Macbr.) C.C.Berg, Moraceae	5	D																		BR	13
Pouteria durlandii (Standl.) Baehni Sapotaceae	27	D					+ a														
Cecropia obtusifolia Bertol. Urticaceae	8	D	D																BTP; C	PA	17
Cecropia insignis Liebm., Urticaceae	1	D	D																		
Miconia argentea (Sw.) DC. Melastomataceae	1	D	D																		
Agrostis capillaris L., Poaceae	2								– d[b]										PT; C	BE	18
Poa pratensis L., Poaceae	3								– d[b]												
	4	D																	PT; F-C		19
		D																	PT	EU[c]	20
Rollinia edulis Triana & Planch. Annonaceae	7	D	D	+ a															BTP; F	CO	21
Guazuma ulmifolia Lam., Malvaceae	3								= c + d										BTP; C	BZ	22
Stemmadenia donnell-smithii (Rose) Woodson, Apocynaceae	12								– c – d												
Terminalia viridiflora (Ducke) Gere & Boatwr., Combretaceae	13	D						D a											BTP; F BTS; F	PE	23
Byrsonima poeppigiana A. Juss. Malpighiaceae	7	D						D a													
Inga loretana J.F.Macbr., Fabaceae		D						D a													
Parkia panurensis Benth. ex H.C.Hopkins, Fabaceae	17	D						D a													

TABLA 1 (continuación)

Especie y familia	Tamaño semilla	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15	E16	E17	Eco; EV	País	Ref [d]
Paullinia sp., Sapindaceae	10	D						D a											BTP; F BTS; F	PE	23
Uapaca spp., Phyllanthaceae	15	D	D																BTS; C	CM	24
Myrciaria dubia (Kunth) McVaugh Myrtaceae	14								– d	+ d									BTP; C	BR	25
Alopecurus myosuroides Huds. Poaceae	8	D																	PT; F-C	BE	19
		D																	PT	EU[c]	20
Galium aparine L., Rubiaceae	7	D																	PT; F-C	BE	19
		D																	PT	EU[c]	20
Bursera simaruba (L.) Sarg. Burseraceae	9	D	D																BTP; C	MX	3
									= c = d												35
Calophyllum brasiliense Cambess. Calophyllaceae	20												= c = d	= c – d	= c = d	= c = d	= c = d	= c = d			2
Cymbopetalum baillonii R.E. Fr. Annonaceae	17												= c = d	= c + d	= c = d			= c = d			
Diospyros digyna Jacq., Ebenaceae	22												= c = d	= c = d	= c = d	– c – d	= c = d	= c = d			
Omphalea oleifera Hemsl. Euphorbiaceae	28												= c = d	= c = d	= c = d	– c = d	= c = d	= c = d			
Nivel comunitario[a]	11 - 27	D	D	+ a = b		D	+ a		= c											BR	26
	> 4	D																	BTP; F BTS; F	PE	27
	> 4	D																			28
	≥ 4	D			= a		= a	= a + a													29
									+ d										PT; F	JP	30
			D																ST; C BTG; C	CI	31
									D										BTP; C	CD	32
	< 5 - 17										+ c	+ e + c								MX	33
											+ c = d										34
	1 - 70										+ c + d	= e									35

[a] Incluye varias especies de plantas, pero los datos fueron analizados a nivel comunitario

[b] Efecto evaluado para el total de plántulas establecidas de ambas especies en el mismo estudio

[c] Solo Alemania, Bélgica, Estonia, Francia, Reino Unido y Rumanía

[d] Siendo 1. Estrada y Coates-Estrada (1991); 2. Este estudio (Cap. III); 3. Urrea-Galeano et al. (2019a); 4. Shepherd y Chapman (1998); 5. Balcomb y Chapman (2003); 6. Andresen (1999); 7. Santos-Heredia et al. (2016); 8. Zárate et al. (2019); 9. Santos-Heredia et al. (2011); 10. Feer et al. (2001); 11. Feer (1999); 12. Andresen (2002a); 13. Andresen (2003); 14. Andresen (2001); 15. Andresen (2002b); 16. Hingrat y Feer (2002); 17. Wehncke y Dalling (2005); 18. D'hondt et al. (2008); 19. Milotić et al. (2017); 20. Milotić et al. (2019); 21. Santos-Heredia et al. (2010); 22. Lawson et al. (2012); 23. Culot et al. (2015); 24. Petre et al. (2015); 25. Griffiths et al. (2016); 26. Andresen y Levey (2004); 27. Culot et al. (2009); 28. Culot et al. (2011); 29. Culot et al. (2018); 30. Ishikawa (2011); 31. Kunz y Krell (2011); 32. Beaune et al. (2012); 33. Santos-Heredia y Andresen (2014); 34. Ocampo-Castillo y Andresen (2018); 35. Urrea-Galeano et al. (2019b). La información completa de cada trabajo se encuentra en la sección de referencias de la discusión general.

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