

EARTHWORM INVASIONS ON THE GALAPAGOS: PATTERNS, PROCESSES
AND THREATS

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ABSTRACT

The Galapagos forest ecosystems are facing profound alterations as a result of plant and animal introductions. Historically the forests of Santa Cruz have been dominated by *Scalesia pedunculata* Hook. F. (Asteraceae), a fast-growing, shade-intolerant tree species endemic to the Galapagos. However, introduced vegetation has transformed these forests and Himalayan blackberry (*Rubus niveus*) is the most devastating introduced plant in the Galapagos. Non-native earthworms as invasive species have been overlooked on the Galapagos with potentially important consequences for native biota. We sampled in four areas of interest defined according to their management: 1) Agricultural: those private properties where agriculture and cattle ranching were allowed; 2) Park_(int): buffer zone of protected areas adjacent to agricultural areas where control activities for *R. niveus* and other invasive plant species are often implemented, 3) PA_(cont): protected area in Los Gemelos where mechanical and chemical control of *R. niveus* is implemented as a continuous activity and located within a 100m buffer area from the airport highway; and 4) PA_(int): protected area in Los Gemelos where mechanical and chemical control of *R. niveus* is implemented as an intermittent activity, based on the needs of certain scientific and conservation projects and located within 100m - 200m of the buffer area from the airport highway. We established seven 200 m long transects that crossed perpendicular from highly disturbed agricultural areas through the buffer zone into the protected areas of the Galapagos National Park (*i.e.*, from agricultural to Park_(int)). In addition, to evaluate effects of land management for conservation purposes (*i.e.*, mechanical and chemical control of *R. niveus*), we included five additional 200 m transects that crossed from a buffer zone into the remnant

Scalesia forests in Los Gemelos. Within each transect, we established 20 1 m² quadrats at different distances.

We investigated the effects of non-native earthworms in the Galapagos in several ways. First, we used a combination of parametric and spatial statistics to understand the invasion patterns of non-native earthworms and their relationships with invasive plants, specially *R. niveus*. Second, to understand the possible impacts of non-native earthworms on seed germination of invasive plants, we conducted a microcosm experiment. Finally, using a field experimental approach, we examined the growth of *S. pedunculata* seedlings in response to sites invaded by non-native earthworms and *R. niveus*.

Surprisingly, we found a highly significant positive correlation between abundance of non-native earthworms and *R. niveus* density, but only in the presence of invasive ants. We found that Park areas, where intermittent control of *R. niveus* is implemented, had a significantly higher non-native earthworm density compared with agricultural areas. Non-native earthworm density showed spatial autocorrelation, however this was highly dependent on land management. In areas where land management driven disturbances were occasional (i.e., Park_(int) and PA_(int)), we found that the probability of occurrence of *R. niveus* increased rapidly as a function of non-native earthworm biomass. Whereas, in areas continually managed for *R. niveus* invasion in Los Gemelos, we found a strong negative spatial correlation between non-native earthworm density and *R. niveus* at short ranges and a positive spatial correlation at long ranges. These results suggest that the spatial pattern of *R. niveus* invasion in the highlands of Santa Cruz might depend only to some degree on the spatial distribution of non-native earthworms, which in turn depend on land disturbances at the local scale (i.e., <100 m). A critical factor in this possible invasional

meltdown scenario is the presence of invasive ants that may alter non-native earthworm behavior to benefit *R. niveus*.

In the case of seed germination, we found a clear negative effect of non-native earthworm presence on *R. niveus* germination on soils collected in areas invaded by *R. niveus* outside of *Scalesia* forest and free from earthworm colonization. Our results suggested that there were strong effects of non-native earthworms on seed germination rates and species composition, however, the direction and magnitude of the effects depended on plant species, seed bank composition, and the invasion history of the soil.

Finally, we found that the main cause of mortality for the *Scalesia* seedlings was mechanical control of *R. niveus*. In addition, our results suggested that overall mortality of *Scalesia* seedlings decreased as non-native earthworm biomass increased. At the end of our study period, average height of *Scalesia* seedlings was highest in the Park_(int) areas compared with agricultural areas, PA_(int), and PA_(cont). However, we did not detect any significant difference in growth between the four areas of interest. We found that, at the end of our experimental period, mean height of *Scalesia* seedlings decreased as a function of earthworm biomass in Park_(int) and PA_(int) areas. These results suggest that earthworm invasion can play an important role on *Scalesia* growth, however these effects are mediated by land management activities (i.e. mechanical control of invasive plants).

Overall, our findings shed new light on the unintended effects of mechanical control of invasive plants and provide indicative patterns of the possible effects of non-native earthworms on invasive and native plant establishment on tropical forests and remote islands. However, the magnitude and direction of the effects of non-native earthworm on these ecosystems are mediated by (1) the local spatial distribution of non-native earthworm communities, (2) presence

of other invasive soil dwelling invertebrates (i.e. invasive ants), (3) seedbank composition, and (4) land management activities (i.e. control of invasive plants).

BIOGRAPHICAL SKETCH

Jaime Ortiz is a Galápagueño dedicated to preserving island ecosystems. Jaime research interests evolve around the effects of non-native earthworm invasions on the Galápagos, which may be contributing to the spread of invasive plant populations. Jaime's goal is to preserve the unique biodiversity of the Galapagos and aid in the construction of an adequate management strategy based on scientific information, prevention, and education to avoid the effects of invasive species on the islands.

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LIST OF ABBREVIATIONS

AIC: Akaike information criterion

ANOSIM: Analysis of similarities

ANOVA: Analysis of variance

CDF: Charles Darwin Foundation

DPNG: Galapagos National Park Directorate (in Spanish)

GLM: Generalized linear model

GLMM: Generalized linear mixed model

GNP: Galapagos National Park

LMC: Linear model of coregionalization

LSMEANS: Least-squares means

NMDS: Non-metric multidimensional scaling

PA: Protected area

PERMANOVA: Permutational multivariate analysis of variance

ZIP: Zero-inflated Poisson model

PREFACE

Around the world, one of the leading factors of environmental degradation is from non-native species (Sala et al. 2000). Their impact has been well documented (Mauchamp 1997a; Tye 2001, 2006; Campbell and Donlan 2005; Jäger et al. 2007; Bataille et al. 2009; Phillips et al. 2012; Gardener et al. 2013). The full extent of degradation is context dependent, determined by the interaction of non-native species with local climates, land-use history and native species (Atkinson 2001; Wardle et al. 2004; Vilà et al. 2011a; Atkinson et al. 2012; Walsh et al. 2016). Understanding all of these factors is a fundamental challenge for invasion ecology (Simberloff et al. 2013).

Non-native invasive plants are a major threat to biodiversity (Hobbs and Humphries 1995; Vitousek et al. 1997; Sax and Brown 2000), specially to island ecosystems (Pyšek and Richardson 2006; Sax and Gaines 2008; Pyšek et al. 2012). This threat is due to their superior performance-related traits such as rapid growth rate, size, and fitness among others (Van Kleunen et al. 2010). Such high performance allows non-native vegetation to transform local plant community composition and diversity by changing nutrient dynamics, community productivity, and disturbance regimes (Mack et al. 2000; Richardson 2001; Itow 2003; Jäger et al. 2007; Vilà et al. 2011a; Gardener et al. 2013). Thus, effective conservation of ecosystems requires identification of the underlying causes of non-native plant invasions and successful implementation of management strategies (Hobbs and Humphries 1995; Blossey 1999; Gardener et al. 1999; Rentería 2011; Rentería et al. 2012b; Buddenhagen and Tye 2015). While the ecological importance of interactions between below- and aboveground organisms and

ecosystem processes is recognized (De Deyn and Van Der Putten 2005; Kardol and Wardle 2010; Brussaard et al. 2012; Craven et al. 2017), the effects of non-native earthworms on aboveground communities are less understood than those of non-native aboveground organisms (Wardle et al. 2004; Craven et al. 2017).

Non-native earthworms are considered ecosystem engineers due to their ability to alter physical and chemical soil characteristics, as well as organic matter breakdown regimes (Lee 1985; Lavelle et al. 1997; Edwards 2004; Eisenhauer et al. 2009b). These changes in soil ecosystem processes, enhanced by non-native earthworms, can negatively impact local biota and other ecosystem processes. For instance, studies in temperate forests show that non-native earthworms change vegetation composition in favor of sedges and grasses over forbs (Holdsworth et al. 2007), and affect litter food webs, which in turn impacts invertebrates, salamanders (Maerz et al. 2009), bird communities (Loss et al. 2012) and interact with invasive plants to drive local biodiversity dynamics (Hendrix 2006; Dobson and Blossey 2015). In addition, It is increasingly recognized that non-native earthworms are important dispersers and predators of seeds (Eisenhauer et al. 2009b; Forey et al. 2011; Clause et al. 2011, 2015a), and they also have an indirect impact on germination (Scheu 2003; Eisenhauer et al. 2009b). Furthermore, non-native earthworms might have important direct and indirect effects on plant growth and they may structure plant-plant competition (Scheu 2003; Wurst et al. 2005; Eisenhauer and Scheu 2008; Laossi et al. 2009, 2011; Vos et al. 2014). While effects of non-native earthworms on temperate forests are becoming better understood, knowledge of their effects on tropical regions (Fragoso and Brown 2003; González 2006; González et al. 2006) and in remote islands is still incipient (Novo et al. 2015).

The Galapagos forest ecosystems are facing profound alterations as a result of plant and animal introductions (Hamann 1990; Mauchamp 1997b; Itow 2003; Toral-Granda et al. 2017). Historically the forests of Santa Cruz have been dominated by *Scalesia pedunculata* Hook. F. (Asteraceae), a fast-growing, shade-intolerant tree species endemic to the Galapagos (Itow 1971; Hamann 1979). However, invasive species have transformed these mono-dominant endemic forests into more heterogeneous ecosystems. Invaded forests inhibit *S. pedunculata* growth by preventing its natural regeneration following large diebacks after “El Niño” events (Itow 2003). *Scalesia* forests in the Galapagos humid region are threatened by introduced plant species invading from the agricultural zone (Tye 2006). To limit the distribution of invasive plants land managers usually have to rely on continuous mechanical and chemical control methods (Kettenring and Adams 2011). However, similar to other environments in the world, programs to control invasive plants on the Galapagos have had limited success (Buddenhagen 2006; Gardener et al. 2010, 2013; Coello and Saunders 2011; Buddenhagen and Tye 2015).

Here, we investigated the relationship between non-native earthworms and *R. niveus* density and distribution, and the influence of non-native earthworms on germination, and plant fitness of endemic and invasive species commonly found in *Scalesia* forest. Understanding the spatial variability and effects of non-native earthworms in the native and invasive vegetation of the Galapagos will provide greater insight into below- and above-ground processes interactions, which will help determine priorities for restoration management.

CHAPTER 1

INTERACTIONS AMONG LAND MANAGEMENT, INVASIVE ANTS, INVASIVE PLANTS, AND NON-NATIVE EARTHWORMS IN THE GALAPAGOS ISLANDS

Introduction

Invasive species are a well-documented, leading driver of global environmental degradation (Sala et al. 2000; Campbell and Donlan 2005; Bataille et al. 2009; Phillips et al. 2012). Environmental degradation, however, is context-dependent, and is influenced by interactions between non-native species and climate, land-use history, and native species (Atkinson 2001; Wardle et al. 2004; Vilà et al. 2011a; Atkinson et al. 2012; Walsh et al. 2016). In addition, human-driven disturbances such as climate change and land management driven transformations have the potential to enhance impacts of established non-native species (Hellmann et al. 2008) and to facilitate new invasions (Mack et al. 2000; MacDougall and Turkington 2005; Bellard et al. 2013). For instance, invasive vegetation can transform plant community composition and diversity by changing nutrient dynamics, community productivity, and disturbance regimes (Mack et al. 2000; Richardson 2001; Itow 2003; Jäger et al. 2007; Vilà et al. 2011a; Gardener et al. 2013). Thus, we must understand factors that contribute to establishment of invasive species, especially on island ecosystems where naturalization of invasive plants is rapidly increasing (Pyšek and Richardson 2006; Sax and Gaines 2008).

In the Galapagos National Park, regions bordering agricultural areas are especially vulnerable to invasion, because agricultural areas are often where invaders first become

established, and intensity and frequency of invasion to new habitats correlates with distance from the source of propagules (Nathan and Muller-Landau 2000). For example, *Rubus niveus* Thunb. has spread from agricultural lands on inhabited islands and has become one of the most widespread invasive species in the Galapagos Islands (Rentería et al. 2012a). It forms dense stands up to 4m high, which threatens endemic species such as *Scalesia pedunculata* Hook. f. (hereafter *Scalesia*) (Rentería et al. 2012b, a). However, similar to other efforts to control plant invaders in the world (Kettenring and Adams 2011), programs to control invasive plants on the Galapagos have had limited success (Buddenhagen 2006; Gardener et al. 2010, 2013; Coello and Saunders 2011; Buddenhagen and Tye 2015). Although chemical and mechanical approaches reduce density of invasive species temporarily, treatments must be repeated frequently and may not lead to habitat restoration and population increases of endemic biota (Buddenhagen 2004; Jäger et al. 2007; Rentería et al. 2012b). In addition, in the highlands of Santa Cruz, anthropogenic disturbances caused by activities to control invasive plants are common (Jäger and Kowarik 2010). Together with these anthropogenic disturbances and invasive plants such as *R. niveus*, invasive ants are major agents of change throughout *Scalesia* forests (Jourdan 1997; Causton et al. 2006). The dispersion of invasive ants into protected areas of Santa Cruz is also influenced by neighboring agricultural areas, where invasive ants first became established (Ulloa-Chacon and Cherix 1993). Two invasive ants, *Wasmannia auropunctata* Roger and *Solenopsis geminata* (F.) (Formicidae), can negatively impact native vegetation and invertebrates (Clark et al. 1982; Lubin 1984; Williams and Whelan 1991; Ulloa-Chacon and Cherix 1993; Roque-Albelo and Causton 1999; Causton et al. 2005).

In *Scalesia* forests, *R. niveus*, invasive ants, and non-native earthworms often co-occur (J. Ortiz, pers. obs.). How non-native earthworm invasions influence tropical forest dynamics

outside agroecosystems is not fully understood (Lavelle et al. 1999; Liu and Zou 2002; Dechaine et al. 2005; González 2006; Zou et al. 2006; Amador et al. 2010, 2013), but they have the potential to be strongly negative (Fragoso and Brown 2003; González 2006; González et al. 2006). In temperate forests, non-native earthworms are well documented ecosystem engineers that impact local biota negatively and other ecosystem processes through their influence on soils (Lee 1985; Lavelle et al. 1997; Edwards 2004; Eisenhauer et al. 2009b). For instance, non-native earthworms have shifted temperate forests from forb-dominated to sedge- and grass-dominated plant communities (Holdsworth et al. 2007), with cascading consequences on local food webs (Maerz et al. 2009; Loss et al. 2012). Although effects of non-native earthworms on invasive plants in temperate forests are becoming better understood (Nuzzo et al. 2009; Craven et al. 2017), knowledge of their effects on fragile ecosystems on volcanic islands is still incipient (Novo et al. 2015). Thus, non-native earthworms can exert potentially important consequences on native and non-native plant communities, which have been overlooked previously within the Galapagos and other islands worldwide.

In the Galapagos, there has been only one attempt to collect information about the distribution of earthworms (Nakamura 1997). The earthworm survey led by Nakamura (1997) confirmed presence of non-native *Amyntas corticis* (Kinberg, 1867), *Amyntas morrissi* (Beddard 1892), and *Pontoscolex corethrurus* (Muller 1856) in agricultural areas, but these species appeared to be absent in protected areas, such as Los Gemelos on Santa Cruz (Nakamura 1997).

Although individual impacts of anthropogenic disturbances, invasive plants, and invasive ants on *Scalesia* forests are well documented (Jourdan 1997; Causton et al. 2006; Jäger and Kowarik 2010; Rentería et al. 2012a), we lack information about their combined effect in the

presence of non-native earthworms. Strong relationships between underground and aboveground organisms and ecosystem processes (De Deyn and Van Der Putten 2005; Bohlen 2006; Kardol and Wardle 2010; Brussaard et al. 2012; Craven et al. 2017) indicate that co-occurrence of multiple stressors may produce complex non-additive interactive effects (Didham et al. 2007). These interactions, however, have seldom been studied, as in the case of land management driven disturbance, invasive ants, invasive plants, and invasive earthworms. Here, we investigated the relationship among anthropogenic disturbances (i.e., land management: agriculture and control activities for *R. niveus*), non-native earthworms, invasive ants, and *R. niveus* in the Galapagos within two areas: (1) historical land management interventions varying in disturbance intensity from agricultural areas to adjacent protected areas with intermittent control of invasive plants (Galapagos National Park area), and (2) more recent land management activities to control *R. niveus* through the protected area of Los Gemelos and varying in *R. niveus* control intensity (intermittent to continuous).

We used a combination of parametric and spatial statistics to test the following hypotheses: (1) non-native earthworms are more abundant in agricultural areas than in protected areas, (2) there is a positive relationship between non-native earthworm abundance and abundance and spatial distribution of *R. niveus* in protected areas, (3) there is a negative correlation between invasive ants and non-native earthworms, and (4) management activities to control *R. niveus* invasion have a positive effect on non-native earthworm abundance.

Methodology

Study area

We conducted our research in the humid zone of the highlands of Santa Cruz Island (90°20' W, 0°40' S; 986 km²; mean annual precipitation of 1845 mm) (Trueman and d'Ozouville 2010). The soils are well-weathered and sandy loam in texture (McBirney and Williams 1969; Lasso and Espinosa 2018) (Fig. 1), which support forests dominated by the endemic tree *S. pedunculata*. This habitat is critical for endangered endemic and native species (Itow 1971). Further, the *Scalesia* forest at Los Gemelos, a 200 ha fragment within the Galapagos National Park, is one of the last remnants of this moist vegetation type in Galapagos (Hamann 2001; Rentería et al. 2012b), because 99% of the original forest was cleared for agriculture (Mauchamp et al. 1998; Mauchamp and Atkinson 2009; Gardener et al. 2013).

Since creation of the Galapagos National Park Service in the 1950s, management of the border between agricultural areas and protected areas has been a priority. This area, known as the “Impact Reduction Zone”, includes sections of protected areas adjacent to agricultural or urban areas (DPNG 2006). Protected areas adjacent to agricultural areas have a high degree of disturbance caused primarily by invasive plant species (DPNG 2006, 2013). In the 1960s, introduced mammals (goats, feral pigs, dogs, and cats) were considered the main conservation challenges on the Galapagos (Bowman 1960; Acosta-Solís 1966; Perry 1970; Lavoie et al. 2006). More recently, introduced plant species such as *R. niveus*, *Cinchona pubescens* Vahl, *Cedrela odorata* L., *Lantana camara* L., *Pennisetum purpureum* Schumach, and *Tradescantia*

fluminensis Vell. have been a major focus for research and management (Tye 2006; Jäger et al. 2007; Guézou et al. 2010; Rentería et al. 2012a; Rivas-Torres and Rivas 2018).

Sampling Design

We sampled in four areas of interest defined according to their management: 1) Agricultural: those private properties where agriculture and cattle ranching were allowed; 2) Park_(int): buffer zone of protected areas adjacent to agricultural areas where control activities for *R. niveus* and other invasive plant species are often implemented, 3) PA_(cont): protected area in Los Gemelos where mechanical and chemical control of *R. niveus* is implemented as a continuous activity and located within a 100m buffer area from the airport highway; and 4) PA_(int): protected area in Los Gemelos where mechanical and chemical control of *R. niveus* is implemented as an intermittent activity, based on the needs of certain scientific and conservation projects and located within 100m - 200m of the buffer area from the airport highway (Fig. 1).

We established seven 200 m long transects that crossed perpendicular from highly disturbed agricultural areas through the buffer zone into the protected areas of the Galapagos National Park (*i.e.*, from agricultural to Park_(int)) (Fig. 1; Table 1). This approach allowed us to compare areas with and without plant invasions across different land management strategies to determine the spatial relationship of anthropogenic disturbances, non-native earthworms and invasive vegetation, especially *R. niveus*. In addition, to evaluate effects of land management for conservation purposes (*i.e.*, mechanical and chemical control of *R. niveus*), we included five additional 200 m transects that crossed from a buffer zone into the remnant *Scalesia* forests in Los Gemelos (*i.e.*, from continuous to intermittent control protected areas) (Fig. 1; Table 1).

Within each transect, we established 20 1 m² quadrats at different distances (1, 10, 30 m) (Fig. 1) to increase the probability that we would capture the spatial correlation within and between each variable studied. Separations between sampling points were selected based on previous work on spatial distribution of invasive earthworms (Jiménez et al. 2001; Rossi and Nuutinen 2004; Snyder et al. 2011).

We collected vegetation data in July and August 2017 in all quadrats (N = 240). We estimated cover of *S. pedunculata*, five invasive plant species (*C. odorata*, *R. niveus*, *T. fluminensis*, *P. guajava*, and *P. purpureum*), and five plant functional groups (graminoids, herbaceous, ferns, vines, and shrubs) in 16 cover categories (0.01, 0.2, 0.5, 1, 3, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100%). We estimated mean leaf litter depth (cm) from measurements taken at four scattered locations within each quadrat. Leaf litter estimates included all dead vegetation present in the quadrat. To overcome confounding effects of ongoing management actions for *R. niveus* at our study sites (Filek et al. 2018) in our spatial analysis, we recorded *R. niveus* as present in quadrats where we observed small shoots (<10 cm), seedling regeneration, and/or signs of control activities such as soil disturbance by uprooting and dead plant material within the quadrat.

We sampled earthworms and recorded presence/absence of invasive ants (present if >10 individuals were observed) at the center of each vegetation sampling quadrat by hand-sorting small (25 × 25 cm) soil monoliths, which were excavated to a depth of 30 cm (Moreira et al. 2012). We washed earthworms with water and placed them in 20% ethanol for approximately 5 min to be killed. We dried and weighed earthworms on a balance (Smart Weigh SWS100; BetterBasics, Chestnut Ridge, NY) to determine total fresh biomass (g) per sampling quadrat (0.0625 m²), and we then preserved earthworms in 95% ethyl alcohol and identified sexually

mature individuals to species. However, due to the large number of juveniles in our samples, we do not present separate results per earthworm species but treat all non-native earthworms as a group. Additionally, quadrats separated by 1 m were grouped together, and the mean for each variable measured was used for the analyses (N = 100).

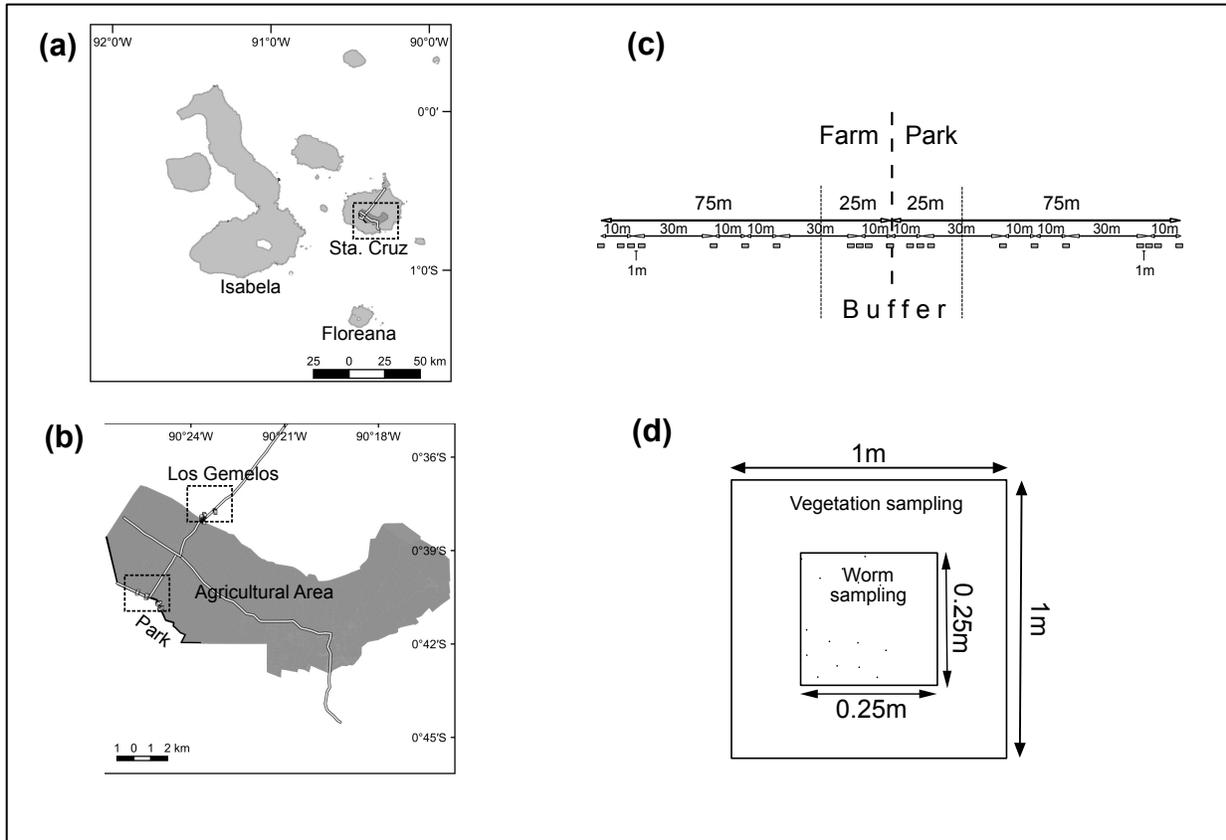


Fig. 1 Location of the different study sites in a) Santa Cruz Island, b) in the agricultural area and in Los Gemelos, and diagrams that show the c) transect and quadrat design, and d) vegetation and earthworm sampling design.

Table 1 Land management and vegetation characteristics of each transect in our study on Santa Cruz Island, Galapagos during August - September 2017

Id	Characteristics (dominant plant species)	
	Agricultural area	Park _(int)
1	Agroforestry + pastures (<i>C. odorata</i>)	Highly invaded forest + control of <i>R. niveus</i> (<i>C. odorata</i>)
2	Agroforestry + pastures (<i>C. odorata</i>)	Highly invaded forest + control of <i>R. niveus</i> (<i>C. odorata</i>)
3	Pastures + agriculture (non-native grasses and <i>P. guajava</i>)	Highly invaded forest + control of <i>R. niveus</i> (<i>C. odorata</i>)
4	Pastures + agriculture (non-native grasses and <i>P. guajava</i>)	Highly invaded forest + control of <i>R. niveus</i> (<i>C. odorata</i>)
5	Pastures (<i>P. purpureum</i> , non-native grasses)	Highly invaded forest + control of <i>R. niveus</i> (<i>C. odorata</i> , <i>T. fluminensis</i>)
6	Pastures (<i>P. purpureum</i> , non-native grasses)	Highly invaded forest + control of <i>R. niveus</i> (<i>C. odorata</i> , <i>T. fluminensis</i>)
7	Pastures + coffee production (<i>P. purpureum</i> , non-native grasses)	Highly invaded forest + control of <i>R. niveus</i> (<i>C. odorata</i>)
	PA _(cont)	PA _(int)
8	Well preserved <i>Scalesia</i> forest	Reforestation activities (<i>S. pedunculata</i> , <i>R. niveus</i> , non-native grasses, <i>T. fluminensis</i>)
9	Reforestation activities + Intense control of <i>R. niveus</i> (<i>S. pedunculata</i> , non-native grasses)	Invaded forest (<i>S. pedunculata</i> , <i>R. niveus</i> , non-native grasses)
10	Invaded forest (<i>S. pedunculata</i> , <i>R. niveus</i> , non-native grasses)	Invaded forest + endemic tree ferns: <i>Cyathea weatherbyana</i> (<i>S. pedunculata</i> , <i>R. niveus</i> , <i>P. guajava</i>)
11	Reforestation activities + Intense control of <i>R. niveus</i> (<i>S. pedunculata</i> , non-native grasses)	Some control of <i>R. niveus</i> (<i>S. pedunculata</i> , <i>T. fluminensis</i>)
12	Intense control of <i>R. niveus</i> (<i>S. pedunculata</i> , non-native grasses)	Intense control of <i>R. niveus</i> for research on <i>Philornis downsi</i> (<i>S. pedunculata</i> , <i>T. fluminensis</i>)

We investigated the invasion patterns of non-native earthworms in several ways. First, we used variograms to detect and quantify autocorrelated spatial processes related to earthworm presence (Rossi et al. 1992), non-native earthworm density, and presence of *R. niveus*. Non-native earthworm counts were \log_{10} transformed to improve data symmetry. Because some samples contained zero abundance data, data were transformed as $x' = \log_{10}(1+x)$. To model the spatial autocorrelation of non-native earthworms and *R. niveus* for each of the four areas of interest, we computed empirical variograms, which related the semivariance of the difference between points that were separated by a given distance h in space to that separation (Rossi et al. 1992). The empirical variogram is:

$$\hat{\gamma}(h) = \frac{1}{2m(h)} \sum_{i=1}^{m(h)} \{z(x_i) - z(x_i + h)\}^2$$

where $z(x_i)$ and $z(x_i + h)$ are the observed values of z at a point and at points separated by some distance range, and $m(h)$ is the number of paired comparisons at lag distance h (Cressie 1993). We standardized variograms by dividing the semivariance by the overall sample variance to allow comparisons at a common scale (Rossi et al. 1992). Due to the small number of points in our dataset, the number of variogram bins and the range of separation distances in each were determined empirically by narrowing the bins until at least 14 point-pairs were in a bin. According to Cressie (1993), to allow reliable estimation of the variogram model we need a minimum of 32 point-pairs per bin (Cressie 1993). Thus, our variogram estimations are necessarily suggestive but not conclusive.

We fitted a spatial model (i.e., exponential, spherical, or Gaussian, selected by eye according to the apparent form of the empirical variogram) to each variogram. We also computed empirical cross-variograms between non-native earthworm density and presence of *R. niveus* to detect any spatial interdependence between these variables:

$$2\hat{\gamma}(h) = \frac{1}{N(h) \vee \sum_{N(h)} (Z_A(s_j) - Z_A(s_i)) (Z_B(s_j) - Z_B(s_i))}$$

where Z_A and Z_B are the two random variables of interest. Unlike the semivariance, the cross-semivariance may be negative if there is a negative spatial correlation between variables (Rossi et al. 1992). Cross-variogram models were fitted with a linear model of coregionalization (LMC) and with a range set from the strongest direct variogram model. We computed and modelled empirical variograms and cross-variograms using the *gstat* package (Pebesma 2004) in R 3.4.3 (R Core Team 2017).

To determine the effect of land management categories on non-native earthworm abundance, we fit a generalized mixed linear model (GLMM) with negative binomial errors. Similarly, we fit a second set of models to evaluate the effect of land management on vegetation cover of each of six invasive plant species (*S. pedunculata*, *C. odorata*, *R. niveus*, *T. fluminensis*, *P. guajava*, and *P. purpureum*) and of plant functional groups (graminoids, herbaceous, ferns, vines, and shrubs) with a GLMM with Gaussian errors. We arcsine square-root transformed vegetation cover values (Sokal and Rohlf 1987). Finally, we fit a third set of models to evaluate the effects of land management on *R. niveus* presence/absence and on ant presence/absence with a GLMM with binomial errors. All GLMM models included transect number and land management within the transect as random nested terms to reflect the hierarchical structure of the

data to control for pseudo-replication, as the observed plots are considered a random sample from a larger population (Bolker et al. 2009). Differences in abundance of non-native earthworms, invasive ants, and *R. niveus* among areas were tested using Least-Square Means.

To detect relationships between non-native earthworm abundance and other suspected confounding predictive factors and the presence and density of *R. niveus*, we fitted a zero-inflated GLMM using a Poisson distribution to overcome the problem of a large abundance of zeros in our dataset. Zero-inflated GLMMs models combine a Poisson count model for positive counts and an expected number of zeroes with a logit model for modelling excess zeros, which allowed us to include random effects (Brooks et al. 2017). In this model, we regressed *R. niveus* density against land management, mean leaf litter depth, non-native earthworm abundance, and presence of invasive ants, and we included transect number and land management within the transect as random nested terms. We selected the best model by dropping non-significant variables from the full model until omitting variables did not result in further improvements in the Akaike Information Criterion (AIC) (Bolker et al. 2009). We examined assumptions (i.e., over-dispersion) of all models at each step of the model procedure. We fitted GLMM and zero-inflated GLMM models with the *glmmTMB* package (Magnusson et al. 2019). We fitted all models in R 3.4.3 (R Core Team 2017).

Results

Vegetation

We found the endemic tree *S. pedunculata* only at the site in Los Gemelos. The area coverage of *S. pedunculata* was, on average, higher in the PA_(cont) for *R. niveus* (20.39 ± 8.86 %) than in the PA_(int) (1.11 ± 1.11 % cover; Table 2). In addition, continuous control activities for *R. niveus* in Los Gemelos showed a significant positive effect on percent area cover of *S. pedunculata* ($P < 0.001$). Whereas, intermittent control activities for *R. niveus* in Los Gemelos showed a negative relationship ($P = 0.021$; Table 3).

R. niveus was present in 34, 4.3, 17, and 31% of the total quadrats in the Park_(int), Agricultural, PA_(cont), and PA_(int), respectively. Percent cover of *R. niveus* per m² was significantly higher in the Park_(int) area (8.59 ± 2.20 %; lsmeans, $P < 0.05$; Fig. 2) than in the Agricultural, PA_(cont), and PA_(int) (1.28 ± 0.76 %; 0.59 ± 0.55 %; 4.67 ± 2.85 %, respectively; Table 2). In addition, *R. niveus* presence was lower in agricultural areas (lsmeans, $P < 0.001$), compared to other land-use categories. The average number of *R. niveus* stems/m² was higher in Park_(int) compared with Agricultural area (1 ± 0.26 ; 0.05 ± 0.05 , respectively; $N = 120$). At Los Gemelos, *R. niveus* average stem count/m² was 0.47 ± 0.32 and 0.97 ± 0.46 for PA_(cont) and PA_(int), respectively.

Coverage of graminoids was, on average, higher in the PA_(cont) (46.17 ± 7.60 %) than in the Agricultural, Park_(int), and PA_(int) (25.99 ± 5.23 ; 1.03 ± 0.48 ; 22.36 ± 7.54 %, respectively; Table 2). In areas with continuous *R. niveus* control, graminoid, shrub, and herb cover was higher in comparison to other areas ($P = 0.035$, $P = 0.04$, $P = 0.005$, respectively; see Table 5 for

other plant functional groups). We found that herbaceous species showed a significantly higher area coverage in agricultural areas ($P < 0.05$).

Table 2 Percent coverage per m² (means \pm se) of plant functional groups and species found in agricultural, Park_(int), PA_(cont), and PA_(int) during August - September 2017 in the humid region of Santa Cruz (N = 120)

	Vegetation Cover (%)			
	Ag. area	Park _(int)	PA _(cont)	PA _(int)
Graminoids	25.99 \pm 5.23	1.03 \pm 0.48	46.17 \pm 7.60	22.36 \pm 7.54
Herbaceous	18.64 \pm 4.06	4.25 \pm 0.85	14.16 \pm 4.77	9.48 \pm 2.44
Ferns	2.80 \pm 0.97	5.21 \pm 1.27	4.00 \pm 2.44	3.19 \pm 1.76
Shrubs	5.76 \pm 1.79	4.95 \pm 1.43	5.83 \pm 3.01	8.68 \pm 3.81
Vines	1.42 \pm 0.91	0.24 \pm 0.07	0.24 \pm 0.09	0.06 \pm 0.06
<i>Scalesia pedunculata</i>	0	0	20.39 \pm 8.86	1.11 \pm 1.11
<i>Cedrela odorata</i>	4.08 \pm 2.19	5.09 \pm 2.12	0	0
<i>Rubus niveus</i>	1.28 \pm 0.76	8.59 \pm 2.20	0.59 \pm 0.55	4.67 \pm 2.85
<i>Tradescantia fluminensis</i>	9.69 \pm 3.19	15.38 \pm 4.55	21.56 \pm 8.44	34.28 \pm 8.87
<i>Pennisetum purpureum</i>	7.88 \pm 4.04	0	0	4.44 \pm 4.44
<i>Psidium guajava</i>	7.74 \pm 3.06	4.43 \pm 2.41	0	1.11 \pm 1.11

Table 3 Results of generalized linear model with Poisson errors on the effects of different land management ($PA_{(cont)}$, $PA_{(int)}$) on the coverage per m^2 of *S. pedunculata*. *Scalesia pedunculata* was present only in the $PA_{(int)}$ and $PA_{(cont)}$ sites (N = 36). Models included transect number and land management within the transect as random nested factors

<i>S. pedunculata</i> *				
	Estimates	SE	Z	P
$PA_{(cont)}$	0.34	0.09	3.51	<0.001
$PA_{(int)}$	-0.32	0.14	-2.29	<0.05

* arcsine square-root transformed coverage per m^2

Invasive Ants

Invasive ants were least common in the Park_(int) (we found ants in 20% of sampled quadrats), compared to Agricultural and $PA_{(int)}$ (52% each) and $PA_{(cont)}$ (57%) and these differences were significant (lsmeans, P = 0.017; Table 6)

Abundance of non-native earthworms

We collected 1753 non-native earthworms along our 12 transects. Average non-native earthworm fresh biomass (0.34 to 1.97 g per 0.0625 m²) and density (3.46 to 26 earthworms per 0.0625 m²) varied among transects. Our results indicated that management for *R. niveus* inside the Park_(int) area had a positive correlation with non-native earthworm density ($P < 0.001$; Table 6). Thus, contrary to our hypothesis, we found that Park_(int) areas had a significantly higher non-native earthworm density compared with agricultural areas ($P < 0.05$; Fig. 2). The mean fresh biomass of earthworms in these two areas reflected this same pattern. Non-native earthworm fresh biomass was higher, on average, in the Park_(int) (1.39 ± 0.12 g per 0.0625 m²) than in agricultural areas (0.74 ± 0.16 g per 0.0625 m²). On the other hand, there was no statistically significant difference in non-native earthworm fresh biomass between areas with continuous (0.68 ± 0.12 g per 0.0625 m²) and intermittent (0.70 ± 0.16 g per 0.0625 m²) *R. niveus* control. However, continuous management for *R. niveus* in Los Gemelos showed a positive correlation with non-native earthworm abundance ($P < 0.001$; Table 6). Non-native earthworms were more dense, on average, in the PA_(cont) (5.23 ± 0.97 earthworms per 0.0625 m²) compared with the PA_(int) (4.86 ± 1.01 earthworms per 0.0625 m²).

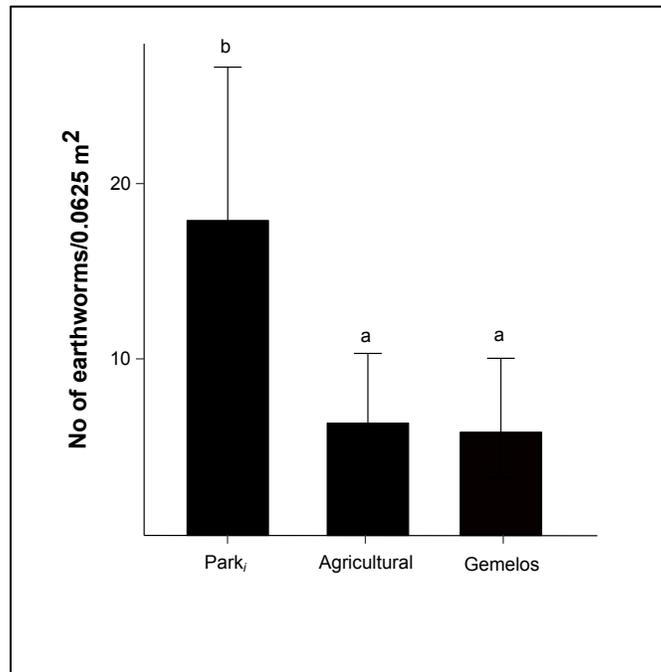


Fig. 2 Mean non-native earthworm abundance (in 0.0625 m²) per study site. Bars show least-square means, and error bars indicate 95% confidence intervals based on estimated parameters presented in Table 2. Different letters indicate a statistically significant difference ($p < 0.05$) between sites

Table 4 Results of generalized linear model with Poisson errors on the effects of different land management activities (Ag. area, Park_(int), PA_(int)) on the coverage per m² of key invasive plant species *R. niveus*, *T. fluminensis*, and *P. guajava*. Models included transect number and land management within the transect as random nested factors (N = 120)

	<i>R. niveus</i> *				<i>T. fluminensis</i> *				<i>P. guajava</i> *			
	Estimates	SE	Z	P	Estimates	SE	Z	P	Estimates	SE	Z	P
<i>PA</i> _(cont)	0.02	0.05	0.53	0.59	0.33	0.16	2.08	< 0.05	-1.03e-7	0.06	0.00	1.00
<i>Ag. area</i>	0.02	0.06	0.31	0.76	-0.17	0.19	-0.89	0.37	0.14	0.07	2.02	< 0.05
<i>Park</i> _(int)	0.17	0.06	3.08	< 0.01	-0.07	0.19	-0.37	0.71	0.08	0.07	1.14	0.25
<i>PA</i> _(int)	0.08	0.07	1.19	0.23	0.20	0.23	0.86	0.39	0.03	0.09	0.30	0.76

* arcsine square-root transformed coverage per m²

Table 5 Results of generalized linear model with Gaussian errors on the effects of different land management activities (Ag. area, Park_(int) area, PA_(int)) on the coverage per m² of vegetation functional groups. Models included transect number and land management within the transect as random nested factors. Plant species in tables 2 and 3 were excluded from the analysis (N = 120)

	Graminoids*				Herbaceous*				Ferns*				Vines*				Shrubs*			
	Est.	SE	Z	P	Est.	SE	Z	P	Est.	SE	Z	P	Est.	SE	Z	P	Est.	SE	Z	P
<i>PA(cont)</i>	0.17	0.08	2.11	<0.05	0.26	0.09	2.78	<0.01	0.08	0.05	1.86	0.06	0.03	0.02	1.11	0.27	0.12	0.06	2.04	<0.05
<i>Ag. area</i>	-0.02	0.09	-0.25	0.80	0.08	0.11	0.74	0.46	-0.01	0.06	-0.09	0.93	0.01	0.03	0.32	0.75	0.02	0.07	0.22	0.83
<i>Park(int)</i>	-0.17	0.09	-1.76	0.07	-0.09	0.11	-0.77	0.44	0.07	0.06	1.22	0.22	-0.001	0.03	-0.05	0.96	0.01	0.07	0.20	0.84
<i>PA(int)</i>	-0.11	0.11	-0.99	0.32	0.004	0.13	0.03	0.97	0.01	0.05	0.15	0.87	-0.02	0.04	-0.63	0.53	0.05	0.07	0.68	0.49

* arcsine square-root transformed coverage per m²

Table 6 Results of generalized linear model on the effects of different land management activities (Ag. area, Park_(int) area, PA_(int)) on the presence of *R. niveus* and ants, and in the density of non-native earthworms. Models for *R. niveus* and ants with binomial errors, and models for non-native earthworms with negative binomial errors. All models included transect number and land management within the transect as random nested factors (N = 120)

	<i>R. niveus</i>				Ants				Non-native earthworms			
	Estimates	SE	Z	P	Estimates	SE	Z	P	Estimates	SE	Z	P
<i>PA_(cont)</i>	1.08	0.50	2.14	<0.05	0.27	0.37	0.73	0.47	1.75	0.28	6.16	<0.001
<i>Ag. area</i>	-4.95	1.19	-4.16	0	-0.17	0.48	-0.36	0.72	0.11	0.36	0.31	0.75
<i>Park_(int)</i>	-0.71	0.64	-1.11	0.27	-1.69	0.54	0.54	<0.01	1.13	0.34	3.32	<0.001
<i>PA_(int)</i>	-1.01	0.63	-1.62	0.11	-0.20	0.52	0.52	0.70	-0.06	0.35	-0.16	0.87

Relationship between non-native earthworms and R. niveus

Non-native earthworm density was spatially correlated up to a distance of 90 m in Park_(int) areas and up to a distance of 86 m in the PA_(cont) (Fig. 3; Table 7), but there was no sign of spatial autocorrelation for non-native earthworm density in the PA_(int). Spatial autocorrelation at zero separation explained 65% and 68% of the variance in the Park_(int) and PA_(cont), respectively (Table 7).

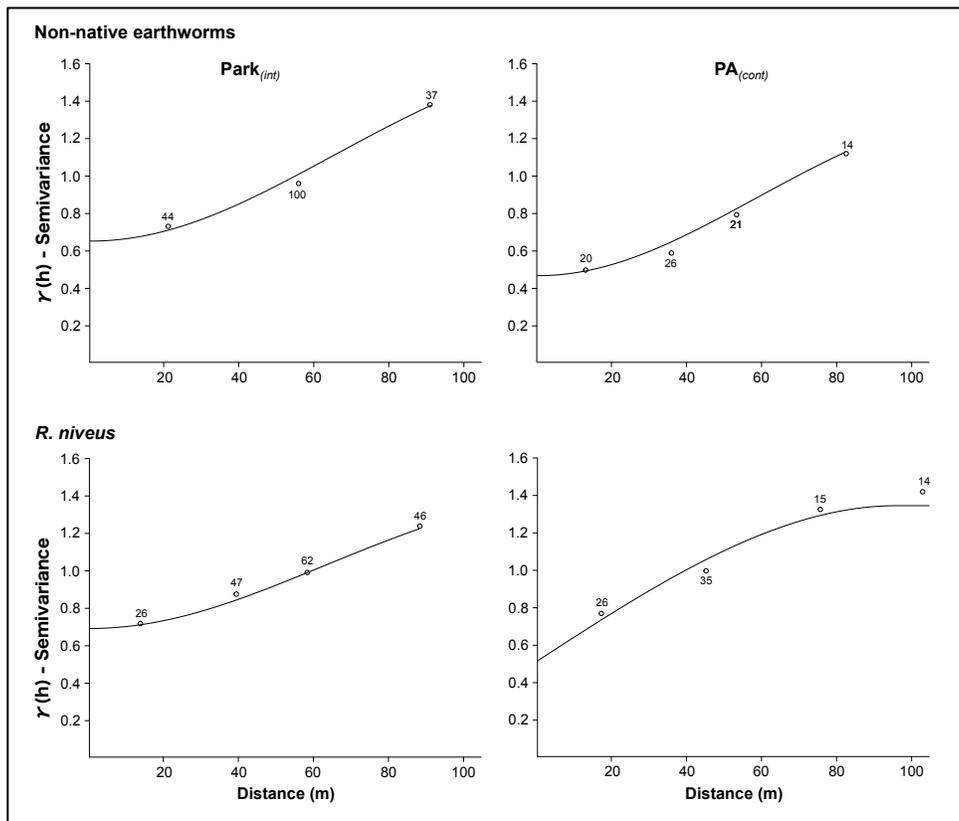


Fig. 3 Empirical variograms (open circles) of non-native earthworm density per 0.0625m² with a Gaussian model (solid line) within Park_(int) and within the PA_(cont). Presence of *Rubus niveus* is shown with a Gaussian model (solid line) in the Park_(int) area and with a spherical model (solid line) in the PA_(cont)

We found that presence of *R. niveus* was spatially auto-correlated up to 88 m and explained 55% of the variance at zero separation in the Park_(int) area (Fig. 3). We did not find a spatial auto-correlation for the presence of *R. niveus* in agricultural areas. In the PA_(cont), *R. niveus* was spatially auto-correlated up to a distance of 105 m, and 62% of the variance at zero separation was explained as spatial dependence (Fig. 3; Table 7).

Table 7 Variogram and cross-variogram model parameters for the different variables under study, log₁₀ transformed data for earthworm (EW) density

Variable	Location	Model	C_0	C	a	a'	$C_0 + C$	$C/(C_0 + C)$
EW Density (indiv 0.0625m ⁻²)	Park _(int)	Spherical	0.05	0.18	88	-	0.23	0.78
	PA _(cont)	Gaussian	0.35	0.72	-	90	1.07	0.67
<i>R. niveus</i> (Presence/absence)	Park _(int)	Spherical	0.001	0.031	120		0.032	0.97
	PA _(cont)	Gaussian	0.001	0.027	-	90	0.028	0.96
EW Density vs <i>R.</i> <i>niveus</i>	Park _(int)	Gaussian	0.007	-0.078	-	158	-	-
	PA _(cont)	Gaussian	-0.09	0.34	-	149	-	-

C_0 : nugget variance, C : structural variance, a : range; the distance (m) over which autocorrelation is expressed, $C_0 + C$ = sill variance or the variogram plateau, $C/(C_0 + C)$ = relative structural variance, - no convergence of model, a' : effective range.

In areas where land management driven disturbance was greater (i.e., Agricultural and PA_(cont)), we found that the increase in the probability of occurrence of *R. niveus* was slower but converged at a lower biomass of non-native earthworms at around 2 g (Fig. 4a). However, in areas where land management driven disturbances were occasional (i.e., Park_(int) and PA_(int)), we found that the probability of occurrence of *R. niveus* increased rapidly as a function of non-native earthworm biomass (Fig. 4a). In Los Gemelos, PA_(int) had a positive correlation with *R. niveus* density ($P = 0.03$; Table 8). We found a highly significant positive correlation between abundance of non-native earthworms and *R. niveus* density, but only in the presence of invasive ants ($P = 0.007$; Table 8). Density of *R. niveus* increased exponentially in scenarios where non-native earthworm biomass surpassed 1 g and invasive ants were present; whereas in the absence of invasive ants an increase on non-native earthworm biomass had no effect on *R. niveus* density (Fig. 4b).

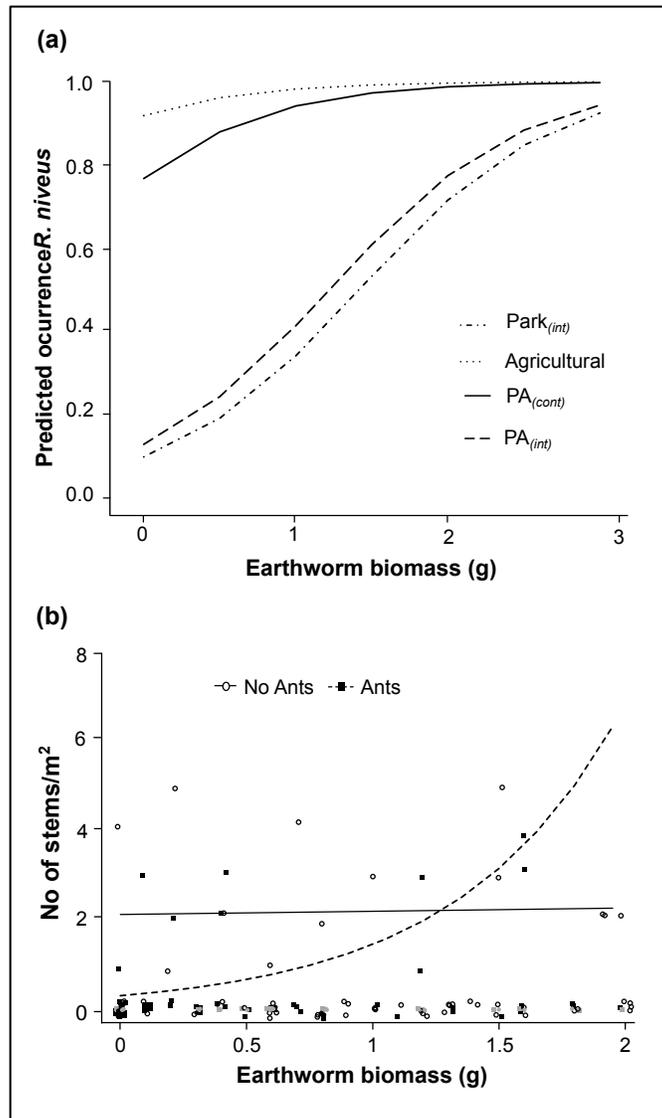


Fig. 4 Effects of non-native earthworm biomass (g per 0.0625m²) on a) presence of *Rubus niveus* under different land management, and b) density of *R. niveus* (number of stems per 1m²) in the presence of invasive ants. We sampled our sites during August - September 2017 (N=141). Data are means based on estimated parameters presented in Table 7. Grey symbols represent quadrats where *R. niveus* was cut during our study period and were omitted from the analyses

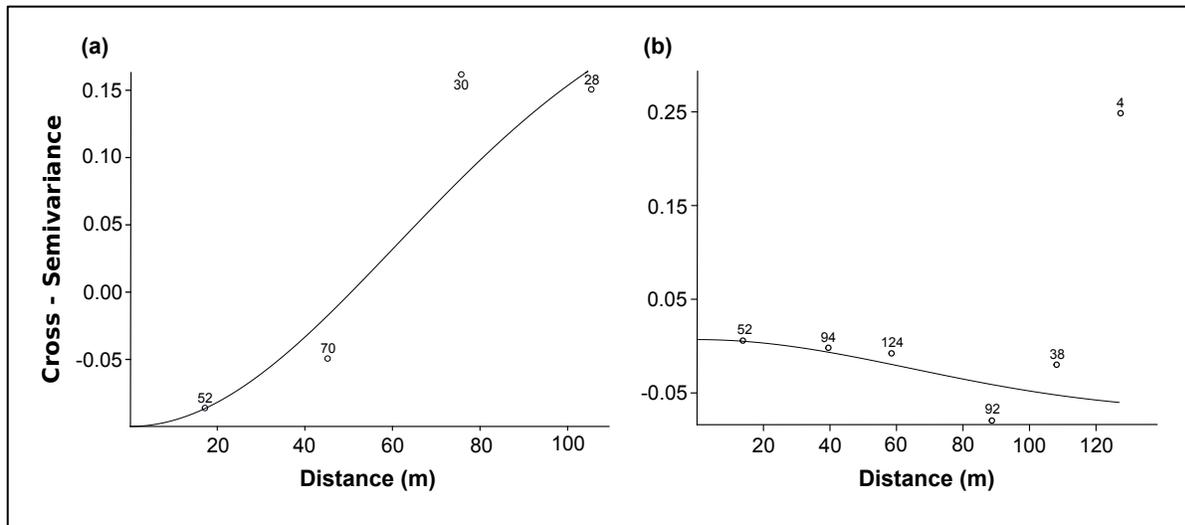


Fig. 5 Cross-variograms of non-native earthworm density per 0.0625m^2 and presence of *Rubus niveus* in a) the $\text{PA}_{(cont)}$ with a Gaussian model (solid line), and b) the $\text{Park}_{(int)}$ area with Gaussian model (solid line)

We found a strong spatial correlation between non-native earthworm density and *R. niveus* occurrence only in the $\text{PA}_{(cont)}$ of Los Gemelos. The cross-variogram analysis showed a strong negative spatial correlation at short ranges (<50 m; semivariance < 0) and a positive spatial correlation at long ranges (80-100 m; semivariance > 0). The spatial range was estimated at 256 m with a strong local continuity (Gaussian model; effective range = 149 m; Fig. 5a). On the other hand, we found almost no cross-correlation between non-native earthworm density and *R. niveus* occurrence in the $\text{Park}_{(int)}$ area (Gaussian model; effective range = 157 m; Fig. 5b).

Table 8 Results of zero-inflated Poisson regression on the effects of different land management (Ag. area, Park_(int) area, PA_(int)), presence of ants, non-native earthworm biomass per 0.0625 m², mean litter depth, and non-native earthworm – ant interaction on the presence and abundance of *R. niveus*. Model included transect number and land management within the transect as random nested factors (N = 120)

	Conditional Model				Zero-inflation Model			
	Estimates	SE	Z	P	Estimates	SE	Z	P
(Intercept)	1.70	0.73	2.33	<0.05	3.02	2.04	1.48	0.14
Ants 1	-1.74	0.63	-2.74	<0.01	-4.29	2.68	-1.60	0.11
Biomass (g)	-0.09	0.28	-0.33	0.74	0.36	0.55	0.65	0.51
Land Management:								
<i>Ag. area</i>	-0.01	1.05	-0.01	0.99	2.99	1.65	1.81	0.07
<i>Park_(int)</i>	-0.94	0.68	-1.40	0.16	-3.60	2.02	-1.78	0.08
<i>PA_(int)</i>	-1.39	0.66	-2.12	<0.05	-3.60	2.05	-1.75	0.08
Mean litter depth (cm)	0.12	0.06	1.94	0.05	0.17	0.20	0.86	0.39
Ants x Biomass	1.39	0.53	2.65	<0.01	2.53	1.85	1.37	0.17

Discussion

Understanding causes for and impacts of non-native earthworm abundance and distribution will provide greater insight into complex below- and above-ground interactions of multiple stressors. This, in turn may will determine priorities for invasive plant management. Non-native earthworms, invasive ants, and invasive plants co-occurred at all study sites. We do not have any historical evidence to believe that we will find native earthworms in the Galapagos, based on taxonomic identification of adult specimens. However, the non-native earthworm community in the study area was composed mainly of *A. corticis*, *A. morrisis* (Megascolecids), and *P. corethrurus* (Glossoscolecid). Contrary to our expectations, we found that non-native earthworms were more abundant in the Park_(int) area than in the agricultural area (Fig. 2). This finding may appear counterintuitive, because, at least in other tropical areas, non-native earthworms are often larger and more abundant in old agricultural fields than in nearby natural forests (Lee 1983; Fragoso et al. 1997; Lavelle et al. 1999; Zou et al. 2006). In addition, our results provide evidence that the highly invasive shrub *R. niveus* was positively associated with non-native earthworms, at the local scale, in the protected areas within the humid zone of the Galapagos Islands. These results agree with studies showing a positive association between non-native earthworms and invasive plant species in temperate forests (Kourtev et al. 1999; Ehrenfeld et al. 2001; Nuzzo et al. 2009) and Pacific Islands (Aplet 1990). However, we found that the effects of non-native earthworm biomass on *R. niveus* density was mediated by invasive ants, especially inside areas where land management driven disturbances were mainly driven by mechanical control of invasive plants (Fig. 4).

In our study, the surprising significant relationship between two non-native, soil dwelling invertebrate species and invasive plants, which was also associated to land disturbances created by invasive species management, suggested that (1) *R. niveus* density was positively associated to ant-earthworm interactions, (2) non-native earthworm abundance was highly correlated to *R. niveus* control activities, and (3) the spatial distribution of non-native earthworm, and the co-occurrence of non-native earthworms and *R. niveus* were mediated by the intensity of activities to control *R. niveus*.

First, both non-native earthworms and invasive ants are considered ecosystem engineers (Bohlen 2006; Brussaard et al. 2012), important agents of change, and of great concern for conservation of native biodiversity (Lubin 1984; Ness and Bronstein 2004; González et al. 2006; Hendrix 2006; Nuzzo et al. 2009). There are various ways in which non-native earthworms and invasive ants may directly or indirectly facilitate invasion by non-native plants and affect native plant biodiversity. Changes in soil nutrients driven by these non-native soil dwelling species can alter the competitive relationships between native and non-native plant species (Scheu 2003; Seaman and Marino 2003; Lafleur et al. 2005; Frouz and Jilková 2008; Craven et al. 2017). Studies that consider the relationship between earthworms and other invertebrates and their effects on plants are almost non-existent. According to Zhao et al. (2013), in the absence of predators (*Agonum impressum*) of earthworms, earthworms (*Aporrectodea nocturna* and *Pheretima aspergillum*) improved soil properties, but they did not affect plant biomass significantly in the Himalayas. The presence of predators on earthworms, however, altered the system resulting in a positive effect of earthworms on plant biomass (Zhao et al. 2013).

This positive direct effect of non-native earthworms on plant growth might be driven by (1) the inability of predators to suppress overall earthworm density and (2) the predator-induced,

habitat shift by earthworms from the upper to the lower soil layer that increased their soil-modifying and growth-enhancing effects on plants (Zhao et al. 2013). The surprising positive relationship of the non-native earthworm - ant (i.e., *W. auropunctata*) interaction and density of *R. niveus* in the protected areas of Santa Cruz (Fig. 4b) might suggest the presence of a similar predator-earthworm-plant process. However, ant-worm-plant interactions and their final outcome may be highly context dependent on particular species, their abundance and site characteristics (Fig. 3). For instance, in the Galapagos, most of the vegetation native to the transition and humid zones has adapted to phosphorus-poor soils (Kitayama and Itow 1999; Jäger et al. 2013). Thus, by escaping ant predation, non-native earthworms may increase phosphorus availability (i.e., casts) in the lower soil layers, altering phosphorus cycling processes that could facilitate the displacement of native plant species (sensu Jäger et al. 2013).

Second, our results provide enough evidence that there is an important unintended effect of activities to control invasive plant species that may facilitate non-native earthworm invasion, which in turn might benefit the spread of *R. niveus* in the protected areas adjacent to farmlands in the Galapagos. We predicted that agricultural land management activities in the highlands of Santa Cruz would clearly show a higher abundance of non-native earthworms through direct and indirect effects of common land management practices linked to high earthworm abundance in the tropics, such as conventional tillage agroecosystems (Brown et al. 2003), agroforestry (Huerta et al. 2009), forest-coffee agroecosystems (Amador et al. 2010), conversion to grasslands (Zou et al. 2006), and enhanced nutrient availability from cattle manure (Leroy et al. 2007). For example, the non-native *P. corethrurus* prevailed in areas where forest had been converted to pastures (i.e., due to an increase in soil pH) (García and Fragoso 2002). Inside protected areas, however, establishment of non-native invasive biota facilitated by disturbances created by the

control of invasive plants is commonly reported (Mason and French 2007; Buckley and Han 2014). For instance, Jäger and Kowarik (2010) found that land disturbances derived from mechanical control of invasive plants in the protected areas of Santa Cruz facilitated *R. niveus* invasion rather than recolonization by native vegetation such as *S. pedunculata*. Similarly, our cross-variogram analysis (Fig. 5a) showed that *R. niveus* and non-native earthworm are negatively spatially correlated at short ranges <50m and positively spatially correlated at longer ranges between 80 and 100m. This suggests that within a radius of about 40m, increasing earthworm density at a point implies less occurrence of *R. niveus*. Conversely, the opposite is true within a radius 80-100m. The mechanism that might explain these results could be related to the spatial scale and periodicity of control actions within Los Gemelos rather than mechanism resulting from ecological processes (i.e. earthworm seed predation, growth facilitation). Therefore, we hypothesized that shifts in below- and above-ground invasive species communities, which included shifts in abundance and spatial patterns of non-native earthworms and *R. niveus*, were highly associated with management activities to prevent the spread of invasive vegetation in the protected areas of Galapagos. Nevertheless, to understand the ecological implications of these conservation side effects for the community composition of *Scalesia* forests will require further research.

Third, In tropical regions, non-native earthworm abundance and local spatial distribution was affected by many factors, which included leaf litter quality, chemical content of soils, and land disturbance (González et al. 2006; Brown and Fragoso 2007). However, their influence on non-native earthworm communities was highly dependent on the spatial and temporal scale of the study (Fragoso and Lavelle 1992; Fragoso et al. 1997; Barros et al. 2002; Sánchez-de León and Johnson-Maynard 2009, but see Jiménez et al. 2001). In our study, the spatial auto-

correlation range for non-native earthworms was similar among all sites (~90m), which suggested that, at the local scale, conditions (i.e., land disturbance) that facilitated earthworm establishment and growth were not significantly different between agricultural and protected areas. Although non-native earthworms in the tropics benefit from high levels of disturbance in agricultural fields (Fragoso and Lavelle 1992; Fragoso et al. 1997; Barros et al. 2002; Sánchez-de León and Johnson-Maynard 2009, but see Jiménez et al. 2001), the co-occurrence of disturbances created by the control of invasive plants and differences in invasive plant community composition and abundance within the buffer zone in the humid region of Santa Cruz might provide a better micro-habitat for non-native earthworm populations. Although the variograms fitted might not be robust because of the low number of data points, our results suggested that associations occurred at the local scale and at one point in time (Fig. 3); thus, the lack of temporal and regional variation might have confounded agricultural-driven effects. In fact, because of the harsh terrain and logistical difficulties, we worked mainly on the southwest portion of the buffer zone of the protected area where non-native earthworms and invasive plants were common and were adjacent to agricultural areas. In addition, it is important to mention that our data may have underestimated the full impacts of agriculture-related activities on non-native earthworm abundance because we did not sample in “pristine” areas free from earthworms and invasive vegetation, our approach of combining spatial statistics with traditional statistical techniques provided enough evidence to suggest that non-native earthworms were an important factor that contributed to the spread and distribution of *R. niveus* in the protected areas of the Galapagos.

Finally, field workers who perform the control and monitoring activities of invasive plants could also have involuntarily contributed to the dispersal and spatial distribution of non-

native earthworms by attachment of cocoons to footwear and tools (Tiunov et al. 2006; Callaham et al. 2006; Hale 2008). Thus, the spatial pattern of *R. niveus* invasion in the highlands of Santa Cruz might depend only to some degree on the spatial distribution of non-native earthworms, which in turn depends on land disturbances at the local spatial scale (i.e., <100 m) and the presence of ants that alter non-native earthworm behavior that would facilitate the transfer of nutrients into deeper soil layers to benefit *R. niveus*. Thus, this process could close a loop in a process that results in an invasional meltdown scenario (Simberloff and Holle 1999; Green et al. 2011). Studies that investigate effects of predators on non-native earthworms are almost non-existent, but such studies are needed to further our ecological understanding of the mechanisms of the effects of non-native earthworms on plant communities. More detailed work that includes community composition of earthworms and ants, physical and chemical characteristics of soils, and their responses to invasive species control activities will be required to further understand these interactions.

CHAPTER 2

NON-NATIVE EARTHWORM EFFECTS ON SEED GERMINATION OF NATIVE AND INVASIVE PLANTS OF THE GALAPAGOS ISLANDS

Introduction

The introduction, spread, and establishment of non-native species has become a global problem, but it is of particular concern on oceanic islands (Vitousek et al. 1997; Sax and Gaines 2008; Kueffer et al. 2010). On oceanic islands, invasive plant species have the potential to affect native biodiversity and ecosystem functioning (Allison and Vitousek 2004; Denslow et al. 2009). The impacts produced by plant invasions include changes in biogeochemical processes, species composition, nutrient availability, and seedbank composition (Levine et al. 2003; Ehrenfeld 2003, 2010; Vilà et al. 2011b).

Traits such as rapid growth of seedlings, high production of seeds, persistent seed banks, and adaptations for long- and short-distance seed dispersal are common in successful invasive plants (Lloret et al. 2004; Richardson 2004; Van Kleunen et al. 2010; Richardson et al. 2014). According to Traveset and Richardson (2006), seed dispersal and patterns of plant establishment are strongly related to the co-occurrence of non-specialized seed dispersers, which often benefit invasive plants over native vegetation. Seed dispersal facilitates plant invasion by allowing invasive plants to colonize new areas, find suitable conditions for germination, and expand their distribution (Gosper et al. 2005). In addition, persistent seed banks allow invasive plants to avoid intermittent unsuitable habitat conditions for germination and establishment (Pakeman and Small

2005). Thus, seed predation at both stages, after dispersal and while in seed banks, can be critically important drivers of forest structure and composition (Fenner and Thompson 2005; Pakeman and Small 2005). However, detailed information on the entire species assemblages of invasive plants, non-native seed predators/dispersers, and their interactions are rarely available (Traveset and Richardson 2006; Buckley et al. 2006). For example, despite their potential importance, the impacts of invertebrates such as non-native earthworms on seed dispersal and seed bank composition outside northern temperate forests remain poorly understood (Decaëns et al. 2003).

In North American temperate forests, invasive earthworms facilitate plant invasion and drive the decline of native plant species through the alteration of physical and chemical properties of soils and the community composition of microbiota (Hale et al. 2006; Frelich et al. 2006; Nuzzo et al. 2009; Eisenhauer et al. 2009c). Furthermore, non-native earthworms interact with seeds and, thus, are likely to impact plant community structure by selective seed ingestion and digestion, acceleration or deceleration of germination (i.e., break seed dormancy) (Ayanlaja et al. 2001), and seed transport (Asshoff et al., 2010; Eisenhauer et al., 2009a). Studies in temperate forests showed that non-native earthworm seed predation altered seed bank composition (Nuzzo et al. 2014), which led to changes in plant community composition (Pakeman and Small 2005; Cassin and Kotanen 2016). However, in the Galapagos, birds and reptiles are considered the main seed predators and dispersers of invasive plants (Buddenhagen and Jewell 2006; Heleno et al. 2011, 2012; Blake et al. 2012). Thus, understanding the impacts of non-native earthworms in the Galapagos is important given their success as invasive species globally (Hendrix et al. 2008). For instance, seed dispersal and predation by non-native earthworms can provide crucial information for understanding the dynamics of plant invasions

(Gosper et al. 2005; Forey et al. 2011). Ingestion of seeds by non-native earthworms is often species-specific, with different earthworm species selecting specific seeds according to traits such as size or oil content (Clause et al. 2011) or by selecting particular plant functional groups (Zaller and Saxler 2007). For instance, Clause et al. (2015b) found that seed-plant-earthworm interactions within grasslands in California had a positive effect on the abundance of non-native plants (Clause et al. 2015b). In addition, seedlings that emerge from casts are likely to benefit from the higher nutrient content of the medium and physical protection (Decaëns et al. 2003). However, seedling growth and survival are largely dependent on the combination of plant and earthworm species (Milcu et al. 2006; Eisenhauer et al. 2009a).

There is increasing evidence that earthworm-seed interactions are likely to impact plant community composition fundamentally in favor of invasive plants (Forey et al. 2011; Nuzzo et al. 2014). On remote oceanic islands, such as the Galapagos, native biodiversity is threatened by invasive plants (Gardener et al. 2013). For example, in the Galapagos more than 700 non-native plant species have been recorded, of which >40 have an impact on native vegetation (Mauchamp 1997a; Guézou et al. 2010). Historically the forests of Santa Cruz have been dominated by *Scalesia pedunculata* Hook. F. (Asteraceae), a fast-growing, shade-intolerant tree species endemic to the Galapagos (Itow 1971; Hamann 1979). However, introduced non-native biota have transformed these mono-dominant endemic forests into more heterogeneous ecosystems, which inhibit *S. pedunculata* growth and natural regeneration cycles after large diebacks following “El Niño” events (Itow 2003). For example, *Rubus niveus* Thunb. is one of the most widespread invasive species in the Galapagos Islands (Rentería et al. 2012a). It forms dense stands up to 4m high, shading out other plants, especially young *Scalesia spp.* (Rentería et al. 2012b, a).

More recently, aggressive invasive plant species such as *Tradescantia fluminensis* Vell. have been a major focus for research and management (Tye 2006; Guézou et al. 2010; Trueman 2014). This newly introduced invasive plant forms a thick mat on the forest floor which inhibits recruitment of native herbaceous species (Trueman 2014). In addition, both *R. niveus* and *T. fluminensis* can spread vegetatively and its seeds are dispersed long distances in the guts of giant tortoises (Blake et al. 2012) and birds (Heleno et al. 2012). However, little is known about additional factors that facilitate their establishment or may limit its distribution (Atkinson et al. 2012; Rentería et al. 2012b; Gardener et al. 2013; Trueman 2014). Although different aspects of the seed dispersal/predation of native and invasive plants by frugivores in the Galapagos have been documented (Buddenhagen and Jewell 2006; Heleno et al. 2011, 2012; Blake et al. 2012, 2015), the consequences of non-native earthworms as seed dispersers/predators are unknown. Therefore, our study aimed to understand the effects of non-native earthworms on seed germination of different native and introduced plant species in the Galapagos.

To understand the possible impacts of non-native earthworms on seed germination, we conducted a microcosm experiment to test the following hypotheses: non-native earthworms 1) suppress germination of native plants, 2) increase germination of introduced plant species.

Methods

We conducted a microcosm experiment from July 2017 to October 2018 to assess the effect of earthworms on germination rates of three native and three highly invasive plants. We selected the most common native species of the *Scalesia* forests: the tree *S. pedunculata* (Asteraceae) and the woody shrubs *Chiococca alba*, *Psychotria rufipes* (Rubiaceae), and

Tournefortia pubescens Hook. f (Itow 1971, Atkinson et al. 2009) plus the invasive *R. niveus*, *Sambucus nigra* L., and *Lantana camara* L. (Guézou et al. 2010).

Experimental Design

We collected three soil types that differed in land-use and invasion history from the highlands of Santa Cruz. Soil-1 was collected inside *Scalesia* forests with an existing earthworm invasion. Soil-2 was collected in areas invaded by *R. niveus* outside of *Scalesia* forest and there was no earthworm colonization. Soil-3 was collected from an area inside *Scalesia* forests free from earthworms and *R. niveus* invasion (Fig. 6). In each area identified for soil collection we selected five 0.5 m² quadrats in areas and removed leaf litter and humus before collecting the top 30 cm of soil. We transported soils in sealed plastic bags to the Galapagos National Park shade house where we sieved each soil sample (5 mm) to homogenize soils and to remove rocks, woody debris, macroinvertebrates, and earthworms. We then combined the five samples from each area, which yielded three unique soil types (Fig. 7). We used PVC tubes to create 120 (10.5 cm diameter, 30 cm length) earthworm microcosms (Fig. 8). We fixed fine mesh 1 mm window screening using PVC caps to the bottom of each tube to permit drainage. We filled each tube with 20 cm of the appropriate soil according to treatment. To provide earthworms with a food source, we collected grass, air-dried the leaves in a greenhouse, and then added 5g of a standardized leaf litter food source as the top layer to our microcosms each week. To standardize moisture, we placed each microcosm in a shallow pool of water for 1 min and sprayed leaf litter with 10 ml of tap water each week to facilitate conditioning and decomposition. We placed a fine mesh on top of each experimental unit to prevent earthworm

escape and secured the mesh with rubber bands. We allowed leaf litter to condition for 1 week before adding seeds and earthworms.

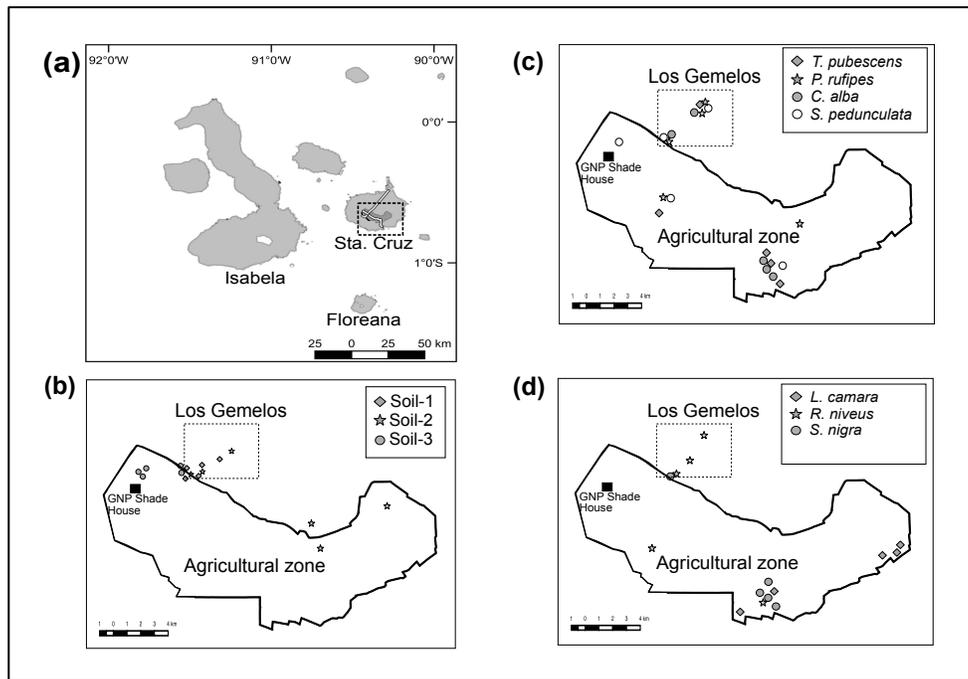


Fig. 6 Soil type (b) and seed collection locations on Santa Cruz Island in the Galapagos archipelago.

Further identified are the location of the experiment (GNP shade house, the agricultural zone and the protected area Los Gemelos). Seed collection locations for native (c) and introduced (d) plant species. For GPS coordinates and full species names please see Annex Tables 2 and 3

We collected approximately 1500 seeds from each focal plant species during 21-30 June 2017 at 15 different field sites in the highlands of Santa Cruz (Fig. 6c, d). We created 100 batches that contained 10 seeds each of the seven species (70 seeds per batch). We added one batch of 70 seeds to each experimental unit. We established 10 additional control replicate containers for each of the three soils and the two earthworm treatments, without adding seeds to account for the seed bank already present in each soil treatment.



Fig. 7 Soil collection with, top-left: Soil type 1 (Soil-1), collected inside *Scalesia* forests with an existing earthworm invasion; top-center: Soil type 2 (Soil-2), collected in areas invaded by *R. niveus* outside of *Scalesia* forest and there was no earthworm colonization; and top-right: Soil type 3 (Soil-3), collected from an area inside *Scalesia* forests free from earthworms and *R. niveus* invasion



Fig. 8 Experiment PVC units (10.5 cm diameter, 30 cm length) used in germination trials (N = 120)

We subsequently collected 150 earthworm individuals (*Amyntas* spp.) using the hand sorting method at five different sites in the vicinity of Bellavista (Rural town of Santa Cruz) during 6-7 July 2017. We chose only sexually mature individuals with a fully developed clitellum. Before inoculation (a single worm per microcosm in half of the total experimental units), we immersed each individual worm in water to remove soil particles, gently placed it on a paper towel to remove excess moisture and determined its mass (Smart Weigh SWS100; BetterBasics, Chestnut Ridge, NY). This procedure also allowed us to check the viability of each individual; we used only specimens that moved vigorously.

We positioned 120 microcosms randomly under controlled shade house conditions to achieve our overall design (3 soil types x 2 invasion levels (with and without earthworms) x 2 seed manipulations (seeds added or not) x 10 replicates/treatment). We identified and recorded the number of emerging seedlings every 3 d for a total of 4 mon. We identified and removed emerging seedlings to avoid double counting or suppression of late germinating seedlings by shading. We monitored the presence of earthworms in our microcosms, and every 3 d we examined microcosms for disappearance of grass clippings and other signs of earthworm presence. When there was no longer any sign of earthworms in a microcosm, seedlings were recorded and removed, and we added a new earthworm to maintain the appropriate treatment.

Data analysis

To investigate the effect of our treatments (i.e., soil type: Soil-1, Soil-2, Soil-3), non-native earthworm invasion (presence/absence), and seed inoculation (yes/no), and their interactions) on seed germination, we used a generalized linear model (GLM) with a negative

binomial error structure for each focal plant species. We used this model structure to overcome any issues that may have arisen from the large number of zeroes in our dataset and to adjust for data over-dispersion. Subsequently, to determine if treatments differed significantly ($P < 0.05$), we conducted pairwise comparisons of the fixed effects (all combinations of soil history, earthworms, seeds, and their interactions) using least-squares means (LSMEANS) with the *lsmeans* function in the package *lsmeans* (Lenth 2016). We fitted all models in R 3.4.3 (R Core Team 2017).

In addition, we applied non-parametric methods to minimize the statistical assumptions about our dataset and facilitate the graphical representation of multivariate abundance data (Clarke 1993). To examine the relationship of the resulting composition of invasive plant species and treatments after 4 mon, we used non-metric multidimensional scaling (NMDS) using Bray-Curtis distance based on the number of seedlings germinated across all replicates for each treatment. We conducted two NMDS procedures, one for the effects on the earthworm treatment and another for the interaction between all treatments. NMDS is a robust ordination procedure that produces a plot in which different replicates are placed far apart in the ordination space, and similar replicates are placed close together based on values in a distance matrix. This distance matrix is a summary of the difference in species composition (species identity) among experimental units (Gotelli 2013). Because the graphical representation is based on rank difference rather than numerical distance, NMDS is less susceptible than other ordination methods to outlying responses to underlying gradients (Clarke 1993). To analyze the differences between earthworm treatments, we used Analysis of Similarities (ANOSIM) with 999 permutations. ANOSIM produces an overall test statistic (R) that indicates if differences exist between treatments. As R approaches 1, there is less similarity between replicates. A test statistic

>0.75 indicates that replicates are highly different, whereas $R > 0.5$ and $R > 0.25$ indicate that replicates are different or different with some overlap, respectively (Clarke 1993).

Finally, we performed multivariate analysis using Permutational Multivariate Analysis of Variance (PERMANOVA, 999 permutations) to test for significant differences in patterns of invasive plants (total number of germinates from invasive species) germinated between treatments (three-way model) (Anderson 2001, 2017). PERMANOVA was conducted on the Bray-Curtis dissimilarity matrix. GLM models were constructed in R using the package *lme4* (Bates et al. 2015). NMDS ordinations and PERMANOVA were constructed in R using package *vegan* (Oksanen et al. 2019). We conducted all tests in R 3.4.3 (R Core Team 2017).

Results

Among the germinants, we identified a total of 20 plant species and there were significant differences in germination rates among treatments (Table 9, Fig. 9). However, we do not present results for plant species that showed an extremely low germination rate (between 1 – 3 germinates) during our experiment (see Annex Table 1).

Soil-1 had a significant positive effect on seed germination of *R. niveus* ($Z = 4.75$, $P < 0.001$, Table 10, Fig. 9). In addition, in Soil-1 without seed addition that was free of earthworms, *R. niveus* showed a significantly higher seedling abundance, compared with the other three species, ($P < 0.05$). Seed addition had a significant negative effect on seed germination of *S. americanum* ($Z = -0.25$, $P < 0.05$; Table 10, Fig. 9). We found that *R. niveus* germination decreased in Soil-2 invaded by earthworms and seed addition ($Z = -1.11$, $P < 0.01$; Table 10, Fig. 9). In soil-2, seedling abundance of *S. americanum* was significantly higher than the other three

Table 9 Number of seedlings of five different plant species that emerged over from September to November 2017 from three different soil type treatments (Soil type: Soil-1 was collected inside *Scalesia* forests with an existing earthworm invasion; Soil-2 was collected in areas invaded by *R. niveus* outside of *Scalesia* forest and there was no earthworm colonization; and Soil-3 was collected from an area inside *Scalesia* forests free from earthworms and *R. niveus* invasion). Additional treatments included adding 10 seeds of each plant species (+ seed) or earthworms (+EW). Data are means \pm se with N = 10 replicates per treatment

Treatment	Germinated Seeds				
	<i>Scalesia pedunculata</i>	<i>Rubus niveus</i>	<i>Tradescantia fluminensis</i>	<i>Solanum americanum</i>	<i>Oxalis corniculata</i>
Soil-1	0	1.88 \pm 0.20	0.45 \pm 0.05	0.50 \pm 0.05	0.24 \pm 0.04
Soil-2	0	0.95 \pm 0.11	0.13 \pm 0.03	0.15 \pm 0.04	0.01 \pm 0.01
Soil-3	0	0.43 \pm 0.06	0.30 \pm 0.05	0.43 \pm 0.07	0.24 \pm 0.04
Soil-1 + EW	0	0.64 \pm 0.05	0	0.08 \pm 0.03	0.23 \pm 0.04
Soil-2 + EW	0	0.80 \pm 0.04	0	0.48 \pm 0.05	0.07 \pm 0.02
Soil-3 + EW	0	0	0.05 \pm 0.02	0.32 \pm 0.04	0.57 \pm 0.05
Soil-1 + Seed	0	2.36 \pm 0.22	0.38 \pm 0.04	0.32 \pm 0.06	0.24 \pm 0.04
Soil-2 + Seed	0	0.30 \pm 0.06	0.14 \pm 0.03	0.04 \pm 0.02	0
Soil-3 + Seed	0	0.65 \pm 0.08	0.08 \pm 0.03	0.08 \pm 0.03	0.16 \pm 0.04
Soil-1 + Seed + EW	0	0.72 \pm 0.05	0	0.08 \pm 0.03	0.24 \pm 0.04
Soil-2 + Seed + EW	0	0.56 \pm 0.04	0	0.16 \pm 0.04	0.08 \pm 0.02
Soil-3 + Seed + EW	0.07 \pm 0.02	0	0.10 \pm 0.03	0.24 \pm 0.04	0.63 \pm 0.05

species with the absence of earthworms and no seed addition ($P < 0.05$), whereas seedling abundance of *R. niveus* was only significantly higher compared with *O. corniculata* and *T. fluminensis* ($P < 0.05$). Finally, in Soil-3, seed germination of *S. americanum* was significantly greater than the other three species in the absence of earthworms and no seed addition ($P < 0.05$). However, germination for *O. corniculata* was significantly higher than any other species in the same soil conditions (Soil-3), when earthworms were present irrespective of seed addition.

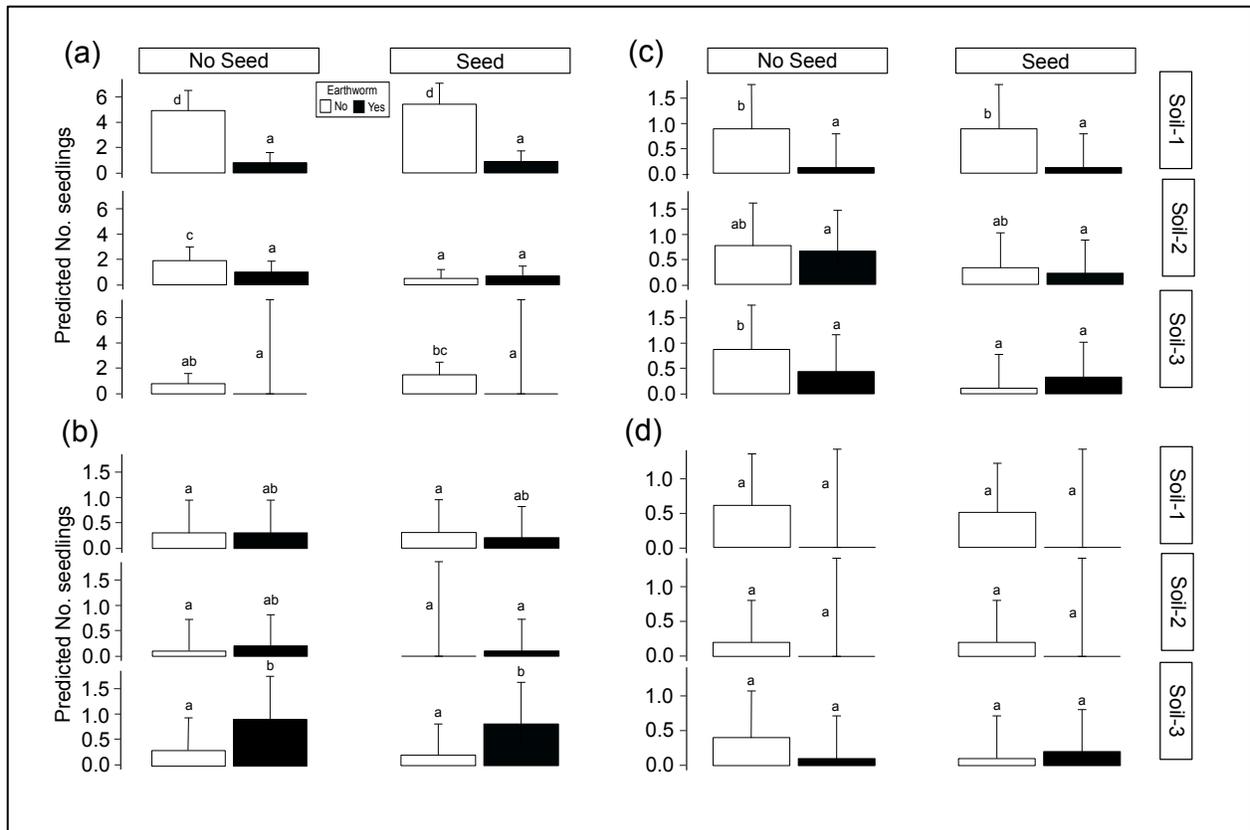


Fig. 9 Number of seedlings that emerged for (a) *R. niveus*, (b) *O. corniculata*, (c) *S. americanum*, and (d) *T. fluminensis* from different soil types in the presence/absence of non-native earthworms or with and without seed addition. Soil type: Soil-1 was collected inside *Scaevola* forests with an existing earthworm invasion; Soil-2 was collected in areas invaded by *R. niveus* outside of *Scaevola* forest and there was no earthworm colonization; and Soil-3 was collected from an area inside *Scaevola* forests free from earthworms and *R. niveus* invasion. Data are LSMEANS and error bars indicate 95% confidence intervals based on estimated parameters from GLM model presented in Table 10. Different letters indicate statistically significant pairwise comparisons ($P < 0.05$) between treatments. Note different scale on Y axis

Table 10 Results of generalized linear model with negative binomial errors on the effects from three different soil types (Soil type: Soil-1 was collected inside *Scalesia* forests with an existing earthworm invasion; Soil-2 was collected in areas invaded by *R. niveus* outside of *Scalesia* forest and there was no earthworm colonization; and Soil-3 was collected from an area inside *Scalesia* forests free from earthworms and *R. niveus* invasion). Additional treatments included adding 10 seeds of each plant species (+ seed) or earthworms (+EW) to assess germination for the invasive plant species *R. niveus*, *S. americanum*, *O. corniculata*, and *T. fluminensis* (N = 10 replicates per treatment)

	<i>R. niveus</i>			<i>S. americanum</i>			<i>O. corniculata</i>			<i>T. fluminensis</i>		
	Estimates	Z	P	Estimates	Z	P	Estimates	Z	P	Estimates	Z	P
(Intercept)	-0.22	-0.63	0.52	-0.22	-0.63	0.52	-1.20	-2.09	<0.05	-0.92	-1.83	0.07
<i>Soil-1</i>	1.81	4.75	<0.001	0.00	0	1.00	0.00	0.00	1.00	0.41	0.63	0.53
<i>Soil-2</i>	0.86	2.05	<0.05	-0.13	-0.25	0.79	-1.10	-0.95	0.34	-0.69	-0.80	0.42
<i>Seed</i>	0.62	1.43	0.15	-2.08	-1.96	<0.05	-0.41	-0.44	0.66	-1.39	-1.24	0.22
<i>EW</i>	-19.07	-0.01	0.99	-0.69	-1.13	0.25	1.10	1.65	0.10	-1.39	-1.24	0.22
<i>Soil-1*Seed</i>	-0.53	-1.11	0.27	2.08	1.77	0.07	0.41	0.33	0.74	1.20	0.95	0.34
<i>Soil-2*Seed</i>	-1.96	-2.95	<0.01	1.23	0.97	0.33	-16.59	-0.01	1.00	1.39	0.92	0.36
<i>Soil-1*Seed*EW</i>	0.65	0	0.99	-1.79	-0.90	0.36	-0.69	-0.43	0.67	-1.90	0.00	1.00
<i>Soil-2*Seed*EW</i>	1.61	0	0.99	-2.04	-1.21	0.22	16.02	0.01	1.00	-2.08	0.00	1.00

Seedling abundance of *R. niveus* and *S. americanum* was significantly higher in Soil-1 free from earthworms, regardless of seed addition ($P < 0.05$). Whereas, pairwise comparisons of seedling abundance of *T. fluminensis* in different treatments did not show any significant effect for any combination of treatments ($P > 0.05$ for all combinations, Fig. 9d).

NMDS sample ordinations revealed clearly that replicates inoculated with earthworms can be separated, with some overlap, from control replicates based on plant species composition (Fig. 10). Our results indicated that germination of *R. niveus* was affected by the presence of non-native earthworms. However, germination of *P. purpureum* and *T. fluminensis* was more related to the absence of earthworms. Finally, *O. corniculata* and *S. americanum* exhibited a positive correlation with non-native earthworm presence (ANOSIM, $R = 0.29$, $P < 0.001$, permutations = 999) (Fig. 10). A three-way PERMANOVA test showed that there was a positive correlation between invasive plant germination and the interaction between soil and earthworm treatments ($P = 0.0049$; permutations = 999, Table 11; Fig. 11). There were no significant associations between other combinations of treatments.

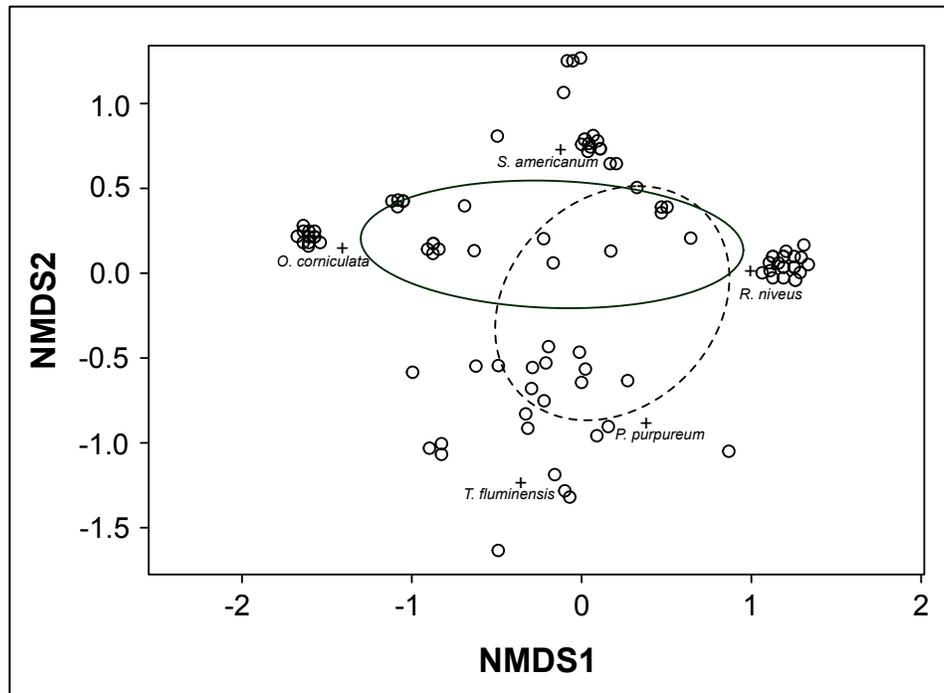


Fig. 10 Non-metric multidimensional scaling (nMDS) of seed germination at the end of the 4-month experiment, using the Bray–Curtis similarities coefficients. Each circle represents a replicate (N=120) and crosses represent the centroids for the species. Dotted-line ellipse indicates the maximum extent of earthworm absence, and solid line ellipse indicates the maximum extent of earthworm presence in “plant community space” (stress = 0.11, $R^2 = 0.95$). The distances between points approximate the dissimilarities among the communities at those experimental units in terms of species composition and relative abundance (Analysis of similarity, ANOSIM, $P = 0.001$)

Discussion

Our results suggested that there were strong effects of non-native earthworms on seed germination rates and species composition, however, the direction and magnitude of the effects depended on plant species, seed bank composition, and the invasion history of the soil (Figs. 9, 11). For instance, non-native earthworms reduced *R. niveus* germination rates in all three soil treatments, whereas effects of non-native earthworms were visible only in two soil treatment

categories for *S. americanum*, and only in one soil treatment category for *O. corniculata* (Fig. 9). Earthworms are known to change conditions for seed germination (Forey et al. 2011). Non-native earthworms might affect seed germination directly by selective ingestion of seeds (i.e., damage seeds) or by facilitating germination in their casts (Clause et al. 2015a). These mechanisms can affect the composition of plant communities from the germination stage to plant maturity (Eisenhauer et al. 2009b; Clause et al. 2015a).

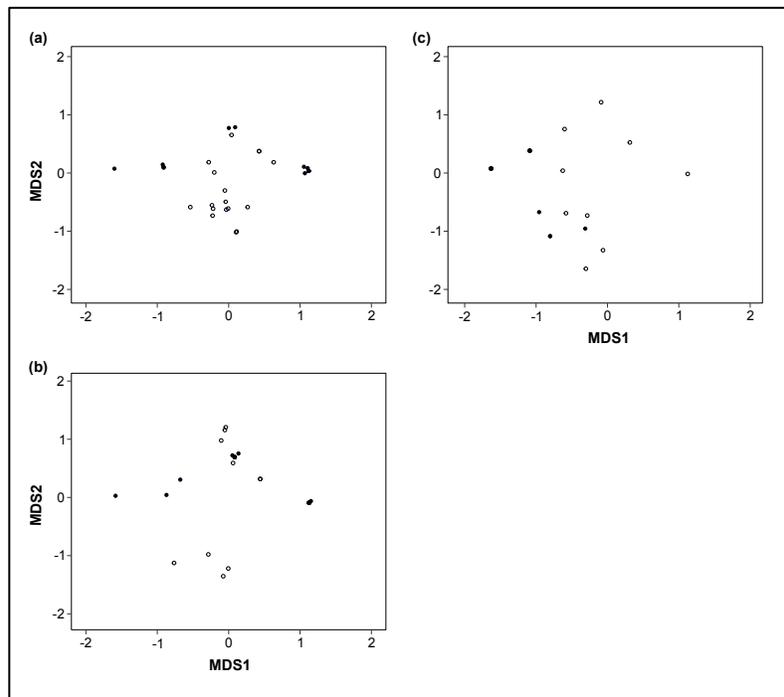


Fig. 11 Non-metric multidimensional scaling (NMDS) of seed germination at the end of the 4-month experiment, using the Bray–Curtis similarities coefficients as a function of earthworm treatment (presence/absence) and Soil type: (a) Soil-1 was collected inside *Scalesia* forests with an existing earthworm invasion; (b) Soil-2 was collected in areas invaded by *R. niveus* outside of *Scalesia* forest and there was no earthworm colonization; and (c) Soil-3 was collected from an area inside *Scalesia* forests free from earthworms and *R. niveus* invasion. (same species as Fig. 9) (stress = 0.11, $R^2 = 0.95$). There was a significant correlation of earthworm presence and soil history interaction on seed germination (PERMANOVA, $P = 0.004$, permutations = 999). Open circles indicate earthworm absence and filled circles indicate earthworm presence (N = 120)

Table 11 Results of three-way PERMANOVA analysis (Permutations = 999) on the effects of three different soil conditions (Soil type: Soil-1 was collected inside *Scalesia* forests with an existing earthworm invasion; Soil-2 was collected in areas invaded by *R. niveus* outside of *Scalesia* forest and there was no earthworm colonization; and Soil-3 was collected from an area inside *Scalesia* forests free from earthworms and *R. niveus* invasion), earthworms (+EW), and seed (+ seed) treatments and their interactions on the composition of invasive plant species seed germination

Source	df	MS	F	P
<i>Soil</i>	2	2.38	18.24	<0.001
<i>EW</i>	1	4.11	31.53	<0.001
<i>Seed</i>	1	0.11	0.83	0.47
<i>Soil*EW</i>	2	1.95	14.92	<0.001
<i>Soil*Seed</i>	2	0.03	0.21	0.93
<i>EW*Seed</i>	1	-0.01	-0.05	0.95
<i>Soil*Seed*EW</i>	2	0.13	1.00	0.42
<i>Residuals</i>	92	0.13		
<i>Total</i>	103			

It is increasingly recognized that earthworms are important dispersers and predators of seeds (Forey et al. 2011; Craven et al. 2017). We found that *R. niveus* germination rates were significantly higher in soils invaded by earthworms (Soil-1) in comparison to soils that were free from earthworm invasion (Soil-2 and Soil-3; Fig. 9a). However, once earthworms were present

germination rates of *R. niveus* decreased significantly (Fig. 9a). Thus, suggesting that earthworm presence decreases germination rates of *R. niveus*. A possible explanation for these results might be the ability of earthworms to predate on seeds found in seedbanks. For example, Nuzzo et al. (2014) found that seeds in seed banks were highly vulnerable to predation by non-native earthworms in North American eastern temperate forests. In addition, results from a field enclosure experiment also suggested that non-native earthworms may have influenced the species composition of forest plant communities by acting as an ecological filter due to seed predation (Cassin and Kotanen 2016). In some cases, passage through the digestive tract of earthworms broke seed dormancy and, thus, increased the chances of germination (Eisenhauer et al. 2009b). Hence, the ability of non-native earthworms to break seed dormancy might explain some of our results, for example for *O. corniculata* which benefitted from the presence of non-native earthworms (Fig. 9b).

It is, however, worth stating that the ingestion of seeds by non-native earthworms may also lead to the loss of viability when they are damaged while passing through the gut (Milcu et al. 2006; Forey et al. 2011). Eisenhauer and Scheu (2008) remarked that the invasion by earthworms might lead to a disproportionate reduction in viable seeds. In our study, we observed that the resulting seedling community was composed mainly of invasive plant species, however because of the extremely low germination rate of native plants we cannot suggest a direct negative effect of non-native earthworms on native plant regeneration through seed damage or ingestion (Table 9, Fig. 9). Thus, our results can provide only suggestive evidence that earthworm invasion can favor invasive plants within the ecosystem through interaction with seeds as reported in temperate forests (Nuzzo et al. 2014; Clause et al. 2015b).

In the Galapagos, fast germination and growth may enable the invasive *R. niveus* to outgrow native shrub and tree species with which it is associated, which enables *R. niveus* to access resources more rapidly than native species such as *S. pedunculata* (Gardener et al. 1999; McDowell and Turner 2002; Itow 2003; Rentería et al. 2012a). We found that *R. niveus*, on average, had a higher germination rate than native species regardless of the presence of non-native earthworms that was mediated by soil invasion history suggesting the possibility of a legacy effect on soil nutrient cycling (Table 9). Furthermore, we found that in experimental units that contained soil from *Scalesia* forest invaded by non-native earthworms, the germination rate of *R. niveus* was greater than in the other two soil treatments in the absence of earthworms (EW-) and irrespective of seed addition, which suggested that earthworm presence facilitated *R. niveus* germination (Fig. 9a). Thus, our results provide further evidence to support the hypothesis that invasive plant species might have an early (i.e. germination rate) advantage over native species (Rejmánek and Richardson 1996; Pyšek and Richardson 2008), which can be enhanced by earthworm invasion (Nuzzo et al. 2009; Clause et al. 2015b; Craven et al. 2017).

Surprisingly, contrary to our expectations, we found that the highly invasive *T. fluminensis* did not have a significant response to non-native earthworms in any treatment combination (Table 9; Figs. 9d, 10). This highly aggressive plant invader is now widespread in the transition and humid zones of Santa Cruz, and it formed a thick mat on the forest floor that inhibited recruitment of native herbaceous species (Trueman 2014). Standish *et al.* (2004) found that *T. fluminensis* greatly increased leaf litter decomposition rates and soil nutrient availability (i.e., Nitrogen) in New Zealand forests. Leaf litter decomposition rates and nutrient availability in these forests changed due to alterations in microclimate conditions (i.e., temperature and humidity), leaf litter chemistry, and the composition of the decomposer community in sites

invaded by *T. fluminensis* (Maule et al. 1995; Heal et al. 1997; Standish et al. 2001). This invasive species can spread vegetatively and through seeds (Trueman 2014). In the Galapagos, *T. fluminensis* seeds are dispersed long distances in the guts of giant tortoises (*Chelonoidis nigra*) (Blake et al. 2012) and birds (Heleno et al. 2012).

It is important to consider that one of the main challenges that we faced during our experiment was the extremely low germination of native plants from our seed addition treatment and from the seedbank within our soil samples. It is not possible to suggest a unique cause for these results. According to Philipp *et al.* (2004), self-compatibility and seed set after selfing are the most common reproductive strategy of endemic flora in the Galápagos Islands. However, this strategy results in a low production of viable seeds in *Scalesia* species and other endemic species (Philipp and Nielsen 2010). For instance, it was reported that *Scalesia cordata* Stewart produces only 0.6 viable seeds per floret on average (florets per capitulum = 17.7; number of capitula = 5) (Philipp and Nielsen 2010). Similarly, Lindhardt (2006) reported that seed set after selfing resulted in low production of viable seeds for *S. pedunculata* in samples collected in Santa Cruz island. This pattern of low seed viability of endemic plants was observed during our experiment, especially of the key species *S. pedunculata*. In addition, our results also showed an extremely low germination from seeds within the seedbank. These results are in accordance to seedbank germination experiments in the Galapagos. For instance, during a seedbank germination experiment from soil collected in the same areas as our soil samples in Santa Cruz, Rentería (2011) reported that the germination of native plants (including *S. pedunculata*) was very low resulting in a poor representation of native species in the final germinant composition. The author suggested that this result might be the effect of continuous chemical control of *R. niveus*, which could negatively impact native seedbank (Rentería 2011). Finally, laboratory

essays showed that extremely low germination due to non-viable seeds of *S. pedunculata* can be expected due to their susceptibility to fungal contamination (McMullen 1986). To overcome this issue of low germination rate from seeds, park rangers search and collect small seedlings from natural forests and rear them in the GNP greenhouse for 2 months prior to reforestation activities (Ortiz, J pers. observations). Thus, it is crucial to consider this tendency for low seed viability when designing future germination experiments.

The extrapolation of the findings of this study to a broader geographic scale needs to be done with caution. The current study focused on analyzing and understanding the effect of non-native earthworms on the germination of particular invasive and native vegetation found in the Galapagos ecosystem. Furthermore, the current study did not explore other confounding factors such as the prevailing climatic conditions on the rate of germination. Even though our study did not find consistent information on the effects of non-native earthworms on native vegetation, our results provide strong evidence to suggest that non-native earthworms are a key factor that could shape plant community composition in the humid forests of the Galapagos similar to what has been observed in temperate forests of North America. For instance, we found that non-native earthworms suppressed the germination of *R. niveus* in infested areas (Figs. 9, 10).

The present study further provides experimental evidence to show the direct and indirect impact of invasive non-native earthworms on non-native plant species with respect to the rate of germination. Also, the data gathered in this study supports the emerging idea that non-native earthworms may be one of the primary drivers for plant community composition in a given habitat (Fig. 10). Non-native earthworms can be regarded as ecosystem engineers that are capable of accelerating nutrient cycling, altering plant community diversity, changing microbial and fungi community abundance, and affecting ecosystem composition (Lavelle et al. 1999,

2016; Jouquet et al. 2006; Eisenhauer et al. 2009c). The results of our study provide evidence to show the importance of continuing with future research to fully understand the impacts of earthworm invasion on non-native plant diversity. Also, it highlights the need to take into account such factors as soil invasion history and the nature of the plant species when addressing issues related to a reduction native biodiversity. Hence, further research is needed to fully understand the effects of non-native earthworm species currently present in the Galapagos on native vegetation to have a full picture of earthworm effects in plant community composition on tropical humid forests.

CHAPTER 3

EFFECTS OF NON-NATIVE EARTHWORMS ON THE PERFORMANCE OF AN ENDEMIC TREE FROM THE GALAPAGOS ISLANDS

Introduction

Invasive species are a serious ecological problem for ecosystems around the world (Simberloff 2014). In recent years, the unparalleled growth of globalization rapidly increased the spread of invasive species in all corners of the world challenging our efforts to control these species (Mack et al. 2000). Effective control of invasive species is particularly important for the preservation of native biodiversity in oceanic islands (Reaser et al. 2007), where biodiversity loss has been attributed to invasive species (Donlan et al. 2003; Sax and Gaines 2008). It is important to recognize that it is the impact and not the presence of the invader that threatens native biodiversity (Levine et al. 2003). There is, however, a general misconception that quantifying the removal of invasive plants can be used as evidence for conservation success (Downey 2011; Blossey 2016). This notion of success, especially for invasive plant management, fails to consider that alterations in the ecosystem might persist long after the invader is removed (Corbin and D'Antonio 2004). These persistent changes in biological or soil physical-chemical conditions and measurable even after the invader has been removed from the ecosystem are known as legacy effects or *legacies* (Corbin and D'Antonio 2012). One of the most important legacy effects of plant invasions are negative plant-soil feedbacks (Ehrenfeld et al. 2005). These

negative feedbacks are the result of invader-mediated changes in soil microbial and fungal communities, which might drive plant-soil interactions, and plant abundance and community composition (Klironomos 2002; Reinhart and Callaway 2006; Inderjit and van der Putten 2010).

In addition to these legacy effects from invasive plants, non-native earthworms are also capable of driving changes in soil systems that can be long-lived (Madritch and Lindroth 2008; Simmons et al. 2015). Earthworms are considered ecosystem engineers due to their ability to alter soil physical and chemical characteristics and for breaking down organic matter (Lee 1985; Lavelle et al. 1997; Edwards 2004; Eisenhauer et al. 2009b). These changes in soil processes, when modified or accelerated by non-native earthworms, have the potential to drive detrimental impacts, such as the decline in plant diversity (Hale et al. 2008; Szlavecz et al. 2011; Dobson et al. 2017; Bal et al. 2018). For instance, studies in temperate forests in New York showed that non-native earthworms decreased seedling survival of native understory plants (Dobson and Blossey 2015). Nevertheless, impacts of non-native earthworms on plant growth in tropical forests remain poorly studied or unknown and have the potential to be strongly negative (Fragoso and Brown 2003; Scheu 2003; González 2006; González et al. 2006).

The endemic vegetation of the Galapagos Islands is well preserved in comparison to other tropical islands. Among the endemic plants of the Galapagos, the genus *Scalesia* is the most iconic and comprises 15 species. These endemic species possess many traits associated with colonizing vegetation (i.e., fast growth), and they have adapted to the harsh environmental conditions of the Galapagos. The *Scalesia* species are all erect, single-stemmed, non-deciduous, and fast-growing shrubs or small trees that range 0.3-15m tall. *Scalesia* species have evolved to occupy particular ecological niches on the islands. For instance, the smaller species tend to occupy the arid zone near sea level, whereas the larger trees occur in the higher altitude humid

zone (500-700 m.a.s.l.) (Itow 1971, 1992; Eliasson 1974; Adersen 1989, 1990; Hamann 1991). As a result of evolutionary processes, the endemic *Scalesia* species are resilient to natural disturbances, such as volcanic eruptions and El Niño phenomena (Hamann 2001).

This unique tropical forest ecosystem, however, is facing profound alterations as a result of more recent anthropogenic disturbances, such as agriculture and non-native plant and animal introductions (Schofield 1989; Hamann 1990; Mauchamp 1997b; Itow 2003; Toral-Granda et al. 2017). Historically, the humid forests of Santa Cruz have been dominated by *Scalesia pedunculata* Hook. F. (Asteraceae), which is the largest of the *Scalesia* species (up to 15m) (Itow 1971; Hamann 1979). However, introduced non-native biota, such as the highly invasive plant *Rubus niveus* Thunb., has reduced *S. pedunculata* populations dramatically by altering patterns of plant succession and ecosystem processes (Itow and Mueller-Dombois 1988; Hamann 2001; Itow 2003). However, little is known about factors that facilitate its spread or may limit its distribution of *R. niveus* (Atkinson et al. 2012; Rentería et al. 2012c; Gardener et al. 2013).

Large control programs for invasive plants such as *R. niveus* are a key component in the conservation efforts to preserve the *Scalesia* forest in the Galapagos (DPNG 2013). Evaluations of these programs are largely based on indicators created to measure the decrease in cover or abundance of invasive plants (i.e. *R. niveus*). However, this approach does not properly evaluate the ecological success of invasive species management. For instance, there is no scientific evidence that areas cleared of invasive plants increases the resilience of native forests (Gardener et al. 2009). For instance, Jäger and Kowarik (2010) reported that native vegetation failed to re-occupy areas where mechanical control of invasive plants were implemented in the protected areas of Santa Cruz. Thus, invasive species management must evaluate the performance of species affected by invasive plant species and their legacy effects (Corbin and D'Antonio 2012;

Blossey 2016). Currently, in the Galapagos there is no data to assess the effects of invasive plant legacies, following post-control reforestation. Thus, the assessment of more adequate indicators, such as *S. pedunculata* seedling growth, are important for conservation management of these unique forest ecosystems.

The presence of introduced earthworms in agricultural areas of the Santa Cruz Island in the Galapagos and their overlap with the distribution of invasive plant species spreading from these agricultural areas; coupled with the important disturbances caused by the control of invasive plants in protected areas, raises questions about the role of invasive species legacies and plant-soil feedbacks facilitating non-native plant spread similar to mechanisms observed in temperate forest ecosystem. Exploring links between above- and below-ground communities illuminates the broader ecological implications of species invasions (Bohlen 2006; Inderjit and van der Putten 2010; Corbin and D'Antonio 2012; Paudel et al. 2016). Using a field experimental approach, we examined the growth of *S. pedunculata* seedlings in response to sites invaded by non-native earthworms and *R. niveaus*. We tested whether (1) *S. pedunculata* seedling mortality was greater in sites with greater non-native earthworm and *R. niveus* abundance, and (2) *S. pedunculata* seedling growth was lower in sites cleared from *R. niveus* and invaded by non-native earthworms.

Methods

Experimental Design

Technicians from the Galapagos National Park Service (GNPS) harvested nearly 500 *S. pedunculata* (hereafter *Scalesia*) seedlings in the humid region of Santa Cruz during May -June 2017. Technicians transferred seedlings to individual plastic bags (1500 cm³) filled with earthworm-invaded soil from local farmlands (Ortiz, J. unpublished data) and grew them inside a shade house in elevated cages to restrict access by birds and rodents. Once *Scalesia* seedlings were 6 w old, we randomly selected approximately 300 similar-sized specimens for our experiment.

We transplanted seedlings along our previously established transects (see Chapter 1 for details): Agricultural area (N = 70), Park_(int) (N = 70), protected areas of continuous control of *R. niveus* (PA_(cont)) (Los Gemelos, N = 50), and protected areas of intermittent control of *R. niveus* (PA_(int)) (Los Gemelos, N = 50) (Fig. 12). We planted one individual 1 m to the right of each quadrat position within 12 transects that were established previously for earthworm sampling (see Methods section on Chapter 1 for detailed information on transect and quadrat location), and we labelled each transplant individually. To avoid shade, we clipped competing vegetation up to 1 m away from each *Scalesia* seedling. Approximately 2 weeks after the planting, we surveyed seedlings and replaced seedlings that had died from apparent transplant shock. We measured *S. pedunculata* height (cm) five times over the duration of the study: September 2017 (t₀), October 2017 (t₁), November 2017 (t₂), February 2018 (t₃), and March 2018 (t₄). Seedling growth (cm) was assessed as the difference between initial height (t₀) and height at each time period (t₁-t₄).

We also assessed seedling survival and damage from herbivory, animal trampling, and chemical and mechanical control of *R. niveus* or other invasive plants. A seedling was considered present if any green part was visible above the leaf litter. Data on fresh biomass of non-native earthworms corresponded to previous measurements from our spatial analysis study (see Chapter 1 for full details on non-native earthworm data collection).

Statistical analysis

We evaluated the effect of non-native earthworm biomass (g per 0.0625 m⁻²), land management, time since transplant (t₁-t₄), and their interactions on the survival, and height (cm) of *Scaevola* seedlings using generalized linear mixed models (GLMMs). A first set of models was constructed with survival of *Scaevola* seedlings as the predictor variable with a logit link function and binomial distribution. One model included fresh biomass of earthworms as the predictor variable, and the second model included the interaction between earthworm biomass (g per 0.0625 m⁻²) and land management as predictors. Finally, a third GLMM included seedling height (cm) as the response variable and the interaction between earthworm biomass (g per 0.0625 m⁻²), time period (t₁-t₄), and land management as the response variables with Gaussian errors. All models included transect number and land management within the transect as random nested terms to reflect the hierarchical structure of the data (Bolker et al. 2009). Post-hoc differences were determined by comparing contrasts of least-squares means using the *lsmeans* function in the package *lsmeans* (Lenth 2016). Plots of fitted and observed values and residuals were examined to ensure deviations from homoscedasticity and normality did not occur (Pinheiro

2000). All models were constructed in R using the package *lme4* (Bates et al. 2015) in R version 3.4.3 (R Core Team 2017).

Results

For our analyses we used the earthworm biomass data previously collected. In addition, we tried to assess earthworm influence statistically. However, due to the large number of juveniles in our samples, we do not present separate results per earthworm species but treat all non-native earthworms as a group. We may thus have failed to detect specific relationships among species that might differ in magnitude and direction of effects on plant growth.

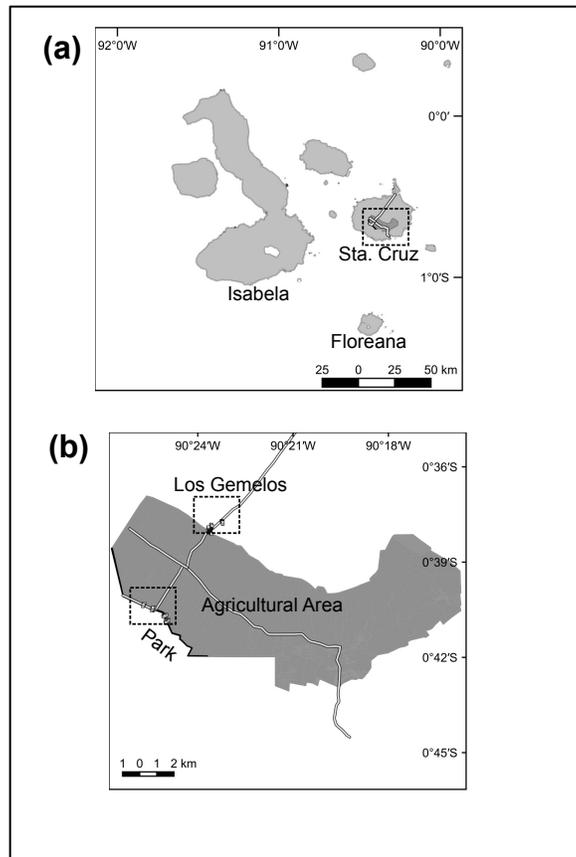


Fig. 12 Location of the different study sites in a) Santa Cruz Island, b) in the agricultural and adjacent protected areas and in Los Gemelos

At the end of our study, only 96 *Scalesia* seedlings survived (40%). Total survival was heterogeneous among the four areas of interest; survival was highest in the Park_(int) area with 41.7% compared with 18.8%, 24%, and 15.6% in the agricultural, continuous control of *R. niveus*, and intermittent control of *R. niveus* areas, respectively. Results from visual inspection of seedlings suggested that the main cause of mortality for the *Scalesia* seedlings (N = 144) was mechanical control of *R. niveus*, which resulted in 43.8% of deaths, followed by animal damage (19.4%), chemical control of invasive plants (6.9%), *R. niveus* invasion (6.9%), *Pennisetum purpureum* L. invasion (3.5%), and unknown causes (19.4%) (Table 12). In the protected region

of Los Gemelos, mortality caused by mechanical control of *R. niveus* accounted for 88.9% and 77.1% of total seedling deaths for the PA_(cont) (N = 27) and PA_(int) (N = 35) areas, respectively (Table 12). In the Park_(int) area (N = 30), however, the main cause of mortality was animal damage with 70%, and only 6.7% of deaths were due to mechanical control of invasive plants (Table 12). For the agricultural area (N = 52), the main cause of mortality was attributed to unknown causes (32.7%) followed by mechanical control of invasive plants (19.2%).

Table 12 Percentage of *S. pedunculata* seedling mortality in different areas of interest and according to causes identified in field experiment during September 2017 – March 2018 (N = 144)

Land management	Cause of Seedling Mortality					
	<i>R. niveus</i> invasion	Animal trampling	Chemical control	Mechanical control	<i>P. purpureum</i> invasion	Unknown
Agriculture	11.5%	13.5%	13.5%	19.2%	9.6%	32.7%
Park _(int)	13.3%	70%	10%	6.7%	-	-
PA _(cont)	-	-	-	88.9%	-	11.1%
PA _(int)	-	-	-	77.1%	-	22.9%

Overall, mortality of *Scalesia* seedlings decreased as non-native earthworm biomass increased (g per 0.0625 m⁻²), which was predicted by our first model (Fig. 13). Non-native

earthworm biomass (g per 0.0625 m⁻²) had a significant negative effect on plant mortality ($Z = -2.49$, $P = 0.013$; Table 13). In addition, our model showed that Park_(int) areas had a significantly negative association with *Scalesia* seedling mortality ($Z = -2.13$, $P = 0.033$; Table 14, Fig. 14). However, we did not detect any significant difference in predicted probabilities of plant mortality as a function of non-native earthworm biomass (g per 0.0625 m⁻²) among our four areas of interest by comparing contrasts of least-squares means ($P > 0.05$).

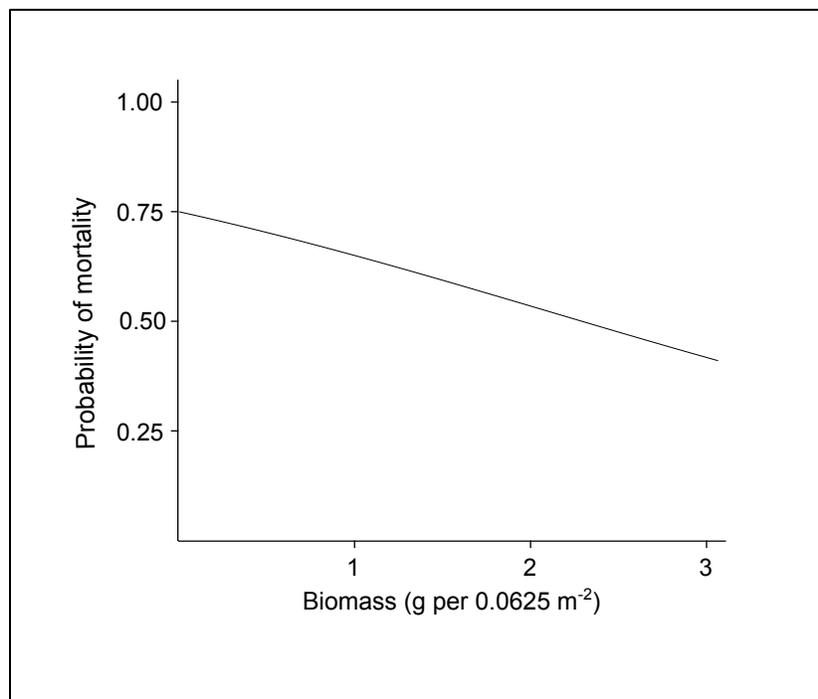


Fig. 13 Effects of non-native earthworm biomass (g 0.0625 m⁻²) on mortality of *Scalesia pedunculata* seedlings over time September 2017 (t₀), October 2017 (t₁), November 2017 (t₂), February 2018 (t₃), and March 2018 (t₄) (N=231). Data are probabilities based on estimated parameters presented in Table 13

Table 13 Results of GLMM with binomial errors on the effects of non-native earthworm biomass (g per 0.0625 m⁻²) on mortality of *S. pedunculata* seedlings. Models included transect number and area within the transect as random nested factors (N = 231)

	Seedling mortality			
	Estimates	SE	Z	P
(Intercept)	1.27	0.53	2.40	<0.05
Biomass	-0.65	0.26	-2.49	<0.05

Table 14 Results of GLMM with binomial errors on the effects non-native earthworm biomass (g per 0.0625 m⁻²) and area category on mortality of *S. pedunculata* seedlings. Models included transect number and area within the transect as random nested factors (N = 231)

	Seedling mortality			
	Estimates	SE	Z	P
(Intercept)	2.29	0.92	2.49	<0.05
Biomass	-1.04	0.54	-1.95	0.05
PA _(cont)	-1.57	1.34	-1.17	0.24
PA _(int)	-0.36	1.38	-0.26	0.79
Park _(int)	-2.59	1.22	-2.13	<0.05
Biomass * PA _(cont)	0.34	0.73	0.47	0.64
Biomass * PA _(int)	0.39	0.75	0.53	0.59
Biomass * Park _(int)	1.00	0.71	1.42	0.16

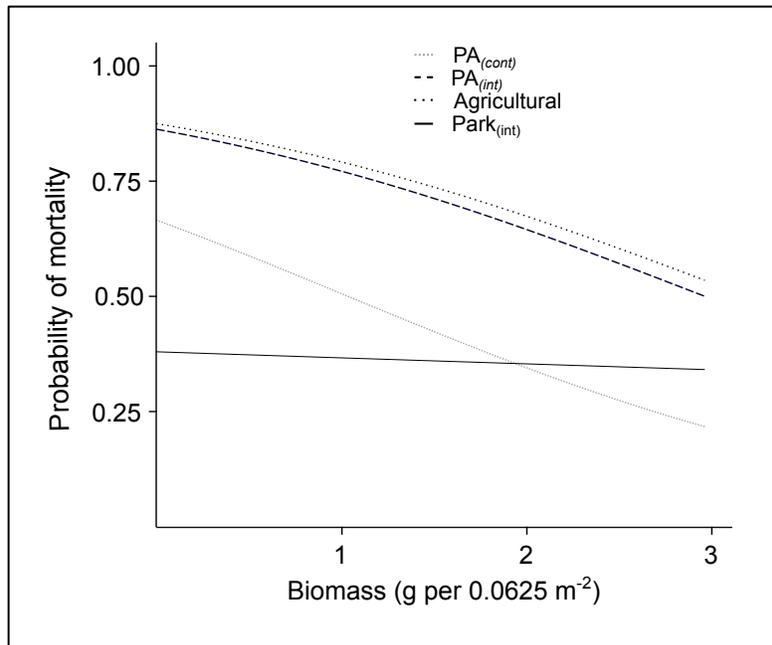


Fig. 14 Effects of non-native earthworm biomass (g per 0.0625m²) on mortality of *S. pedunculata* seedlings under different land management activities. We sampled our sites during August - September 2017 (N=231). Data are probabilities based on estimated parameters presented in Table 14

Final average height of *Scalesia* seedlings (70.5 - 157.21 cm) varied among transects. At the end of our study period, average height of *Scalesia* seedlings was highest in the Park_(int) areas (132.95 ± 7.93 cm; mean ± se) compared with agricultural areas (115.06 ± 9.78 cm), PA_(int) (92.27 ± 5.31 cm), and PA_(cont) (78.09 ± 6.42 cm). However, we did not detect any significant difference in growth between the four areas of interest ($P > 0.05$, not shown). Height of *Scalesia* seedlings was affected by greater non-native earthworm biomass over time. Our GLMM model showed a significant negative effect of time ($t = -4.37$, $P < 0.001$; Table 15). In addition, we found a significant interactive effect of Park_(int) areas and time t_4 on *Scalesia* height ($t = 4.48$, $P < 0.001$; Table 15; Fig. 15). We found that, at the end of our experiment period, mean height of *Scalesia* seedlings decreased as a function of earthworm biomass in Park_(int) and PA_(int) areas

(Fig. 15). In contrast, at the end of the study period, the mean height of *Scalesia* seedlings increased as a function of earthworm biomass in the PA_(cont) areas, mean *Scalesia* height was significantly higher when non-native earthworm biomass increased to >3g per 0.0625 m² (lsmeans, $P < 0.05$; Fig. 15).

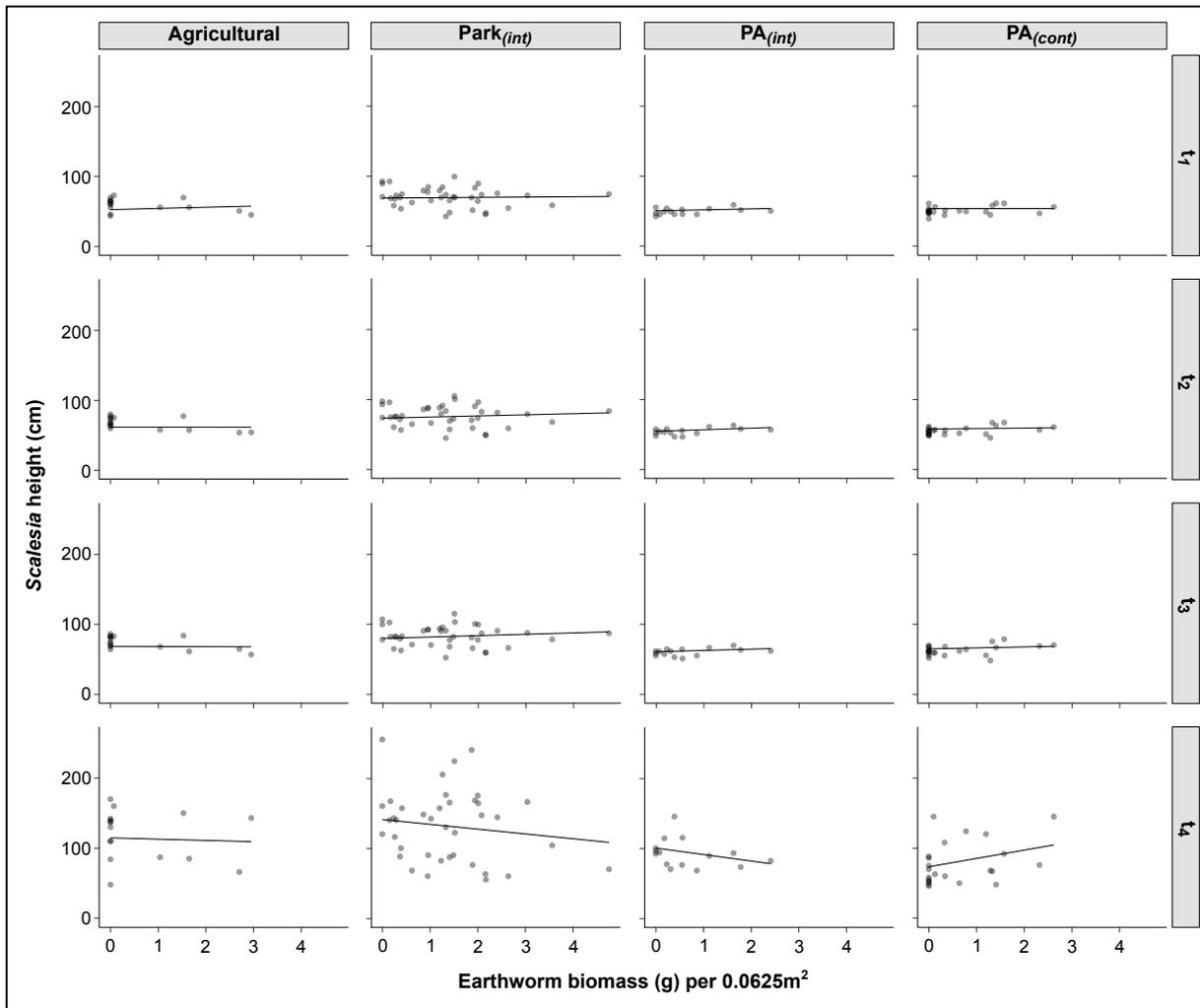


Fig. 15 Effects of non-native earthworm abundance (g) on *S. pedunculata* seedling height (cm) per study site. Grey points show observed data. Black line indicates best fit line based on estimated parameters presented in Table 15 and Annex Table 6

Table 15 Results of GLMM with Gaussian errors on the effects of non-native earthworm biomass (g per 0.0625 m²), time (t_1 - t_5) and land management, and their interactions on *S. pedunculata* height (cm). Model included transect number and area within the transect as random nested factors (N = 360). Only significant terms are shown ($P < 0.05$) (see Annex Table 6 for full details)

	Seedling height			
	Estimates	SE	<i>t</i>	P
(Intercept)	53.44	6.84	7.82	<0.001
Time t_4	-26.53	6.07	-4.37	<0.001
Time t_4 * Park _(int)	49.04	10.95	4.48	<0.001

Discussion

In contrast to our prediction that *Scaevola* seedling mortality will increase as a function of non-native earthworm abundance, our results suggested that non-native earthworm invasion reduced *Scaevola* seedling mortality overall (Fig. 13). It is well established that earthworms affect plant growth by increasing soil nutrient availability through their burrowing and casting activities (Lavelle et al. 1999; Scheu 2003; Brown et al. 2004). However, it is important to recognize that the degree and type of impact on plant performance depends on earthworm size, feeding guild, and location in the soil profile (Ransom 2012). Furthermore, our results suggested that increasing non-native earthworm biomass enhanced *Scaevola* growth in areas where invasive plants were eradicated recently (Fig. 15). However, it is difficult to ascribe changes in plant growth to a

single mechanism because invasive plants, invasive earthworms, and their legacy effects alter various below-ground processes that may affect plant growth simultaneously (Scheu 2003; Ehrenfeld 2003; Corbin and D'Antonio 2012; Simmons et al. 2015; Dobson et al. 2017).

Galapagos endemic plants are particularly vulnerable to anthropogenic changes (i.e. soil disturbance, invasive species) because of their small ranges and genetically homogeneous populations (Porter 1976; Lawesson et al. 1987; Tye and Francisco-Ortega 2011). Although feral goats, feral pigs, donkeys and free-ranging domestic cattle were relatively well controlled in the protected areas of Santa Cruz (Campbell and Donlan 2005; Carrión et al. 2006), our results showed that the greatest factor contributing to seedling mortality in the Park_(int) areas during our experiment was physical damage from large mammals (see Table 12). These results are in agreement with other research on the Galapagos reporting that physical damage from large mammals (i.e. trampling) is an important factor threatening the endangered endemic vegetation of the Galapagos (Simbaña and Tye 2009). For instance, decrease in growth and high mortality of *S. pedunculata* seedlings was commonly reported after local ecosystems were invaded by large mammals (Hamann 1975, 2001; Schofield 1989). In the other hand, the main factor contributing to the high mortality of *S. pedunculata* seedlings in our experiment in the PA_(cont) and PA_(int) areas was the mechanical control of *R. niveus* (see Table 12). Mechanical control is one of the most common techniques implemented to deal with invasive plants (Kettenring and Adams 2011). However, although continuous mechanical control campaigns are implemented in the Galapagos, their success is very limited (Buddenhagen 2006; Gardener et al. 2010, 2013; Coello and Saunders 2011; Buddenhagen and Tye 2015); failing to produce the desired conservation outcomes of habitat restoration and population increase of endemic vegetation (Buddenhagen 2004; Jäger et al. 2007; Rentería et al. 2012b). For instance, Jäger and Kowarik

(Jäger and Kowarik 2010) found that high disturbance created by the mechanical control of invasive plants in the protected areas of Santa Cruz may actually facilitate *R. niveus* re-invasion. In addition, our results showed that *S. pedunculata* regeneration in Los Gemelos can be limited by increased seedling mortality resulting from aggressive mechanical control of *R. niveus* (see Table 12).

Failed regeneration of native vegetation has also been reported in cases where soil ecosystems were invaded by non-native earthworms (Eisenhauer et al. 2009c). For instance, in North American temperate forests, research suggested that invasive earthworms might facilitate plant invasion and drive the decline of native plant species through the alteration of soil physical and chemical properties, and of microbiota community composition (Hale et al. 2006; Frelich et al. 2006; Nuzzo et al. 2009; Eisenhauer et al. 2009c). We predicted a negative effect of non-native earthworms on *Scalesia* performance because this endemic tree from the Galapagos has evolved without the influence of earthworms, which might result in a reduction of plant performance in sites where earthworm invasion occurred (Frelich et al. 2006; Forey et al. 2011). Among these mechanisms, earthworm interactions with arbuscular mycorrhizal fungi (AMF) is perhaps the main driver of plant community structure and diversity by changing patterns of plant recruitment, growth, and relative abundance (Paudel et al. 2016). In many cases, this benefits non-native vegetation (Nuzzo et al. 2009; Clause et al. 2015b) over native plant communities (Hale et al. 2006; Holdsworth et al. 2007). This is an important indirect effect of earthworm invasions in plant growth increase when we consider that nearly 90% of all terrestrial plant species in temperate and tropical ecosystems form a symbiotic association with AMF (Smith 2008). However, it is important to consider that this effect of earthworm invasions on AMF might

negatively impact the endemic *S. pedunculata* because this endemic tree does not depend on mycorrhizal fungi (Schmidt and Scow 1986).

In addition, our results, in general, do not agree with experimental studies that suggested the absence of impacts of invasive earthworms on native or resident plant species (Whitfield et al. 2014). Instead, we suggest that the direction and magnitude of non-native earthworm-*Scalesia* interactions might be highly context-dependent (i.e., legacy effects, earthworm species, and time since transplant). Non-native earthworms currently present in Galapagos, such as *P. corethrurus*, can inflict long-lasting legacy effects due to their ability to change soil pH and nutrient (Ca, Mg, P, K, NO₃) availability (Marichal et al. 2010; Taheri et al. 2018), soil physical properties (Chauvel et al. 1999; Barros et al. 2002), and microbial populations and activity (Araujo et al. 2004). For instance, we found that after several weeks we can expect strong negative effect of non-native earthworm abundance on *Scalesia* growth in sites where control for *R. niveus* was intermittent. We found that the non-native earthworm community in the study area was composed mainly of *A. corticis*, *A. morrisis* (Megascolecids), and *P. corethrurus* (Glossoscolecid) (Ortiz, J., pers. obs.). However, due to the difficulties to identify juvenile individuals we did not carry out further analysis by earthworm species. Thus, more intensive sampling and experiments will be needed to understand earthworm species and soil influence on native and invasive plant performance in these tropical forests.

Therefore, in the context of our study, we suggest that non-native earthworms are one of the driving forces behind changes in *Scalesia* growth in these areas. An alternative explanation for our results might be the long-lasting legacy effects of *R. niveus* invasion. Although, it is well documented that negative impacts of invasive plants might persist even after their removal (Corbin and D'Antonio 2012), we are not aware of any study on the impacts of current and

legacy effects of *R. niveus* invasion on soil processes in the *Scalesia* forest of the Galapagos. Legacy effects of non-native invasive plants include changes in soil chemical and physical properties, and microbial community composition (Ehrenfeld 2003; Corbin and D'Antonio 2004; Inderjit and van der Putten 2010). Hence our results might provide a preliminary glimpse of the importance of invasive species legacy effects and urge for future research in this area of critical importance for successful endemic plant restoration programs (Corbin and D'Antonio 2004).

Finally, even though we did not consider other important factors that affect plant performance, such as climatic conditions and soil chemical and physical characteristics, our results clearly indicated that earthworm invasion can play an important role on *Scalesia* growth. In addition, we found that animal damage and control activities for invasive plants were an important factor mediating *Scalesia* seedling survival, establishment and shoot growth.

CONCLUSION

EARTHWORM INVASIONS ON THE GALAPAGOS: A CONSERVATION VIEWPOINT

In the Galapagos National Park (GNP), regions bordering agricultural area are especially vulnerable to invasion, as agricultural areas are often where invaders first become established, and the intensity and frequency of invasion to new habitats correlates to the distance from the propagule source (Nathan and Muller-Landau 2000). This problem is not unique to the Galapagos. Areas adjacent to natural reserves often experience anthropogenic disturbance, which facilitates colonization of invasive plants (Pauchard and Alaback 2004) and, in turn, invasion into adjacent protected areas (Sakai et al. 2001). However, invasions by soil invertebrates, such as earthworms, have gone largely unnoticed (Ehrenfeld and Scott 2001). Interactions between invasive species may be paramount in understanding biological invasions (Belote and Weltzin 2006). These interactions, however, have seldom been studied, as in the case of invasive earthworms and invasive plants. Multiple stressors may interact to generate ecological impacts in a way more complex than simple additive effects (Didham et al. 2007; Darling and Côté 2008). Earthworm invasions are often strongly correlated with anthropogenic disturbances (Hobbs and Huenneke 1992; Lavelle et al. 1999; Zou et al. 2006; Winsome et al. 2006; Hendrix 2006; Loss et al. 2017). However, previous studies suggest that the impact of land disturbances on species' distribution is dependent on the spatial pattern and distribution of the disturbance itself (Moloney and Levin 1996; Cameron et al. 2007; Paudel et al. 2016; Loss et al. 2017).

In this study we evaluated the role of non-native earthworms in the Galapagos native *Scalesia* forests to understand their effects on plant invasion. Our main objective was to provide

the first scientific evidence for the negative effects of non-native invasive earthworms in the Galapagos and their role on facilitating invasive plant establishment at different life stages.

To this end, first we implemented a field survey to understand the relationships between non-native earthworm abundance, and the presence and abundance of the invasive shrub *R. niveus*. Surprisingly, our results are the first to suggest an important interaction between non-native earthworms and invasive ants, which might benefit a third invasive species (i.e. *R. niveus*) establishment and density. Thus, we urge the scientific community to consider the interactions between non-native earthworms and other soil invertebrates to fully understand the negative effects of non-native earthworms on forest ecosystems. Second, we established a microcosm experiment to understand the effects of non-native earthworms and their legacy effects on seed germination. During our experiment we found that non-native earthworm presence might suppress *R. niveus* germination, however this effect is mediated by legacy effects in soils and might be negligible due to the large amount of seeds within seedbanks. Thus, our results provides further evidence to suggest that copious production of seeds and deposited on seedbanks are an important strategy for invasive shrubs such as *R. niveus*. Finally, we implemented a field experiment to evaluate the effects of non-native earthworm abundance and *R. niveus* legacy effects on *S. pedunculata* seedling growth. Our results showed that the main factor contributing to *S. pedunculata* mortality inside protected areas were mechanical control of *R. niveus* and animal-related damage (i.e. trampling). Furthermore, we found that effects of non-native earthworm are an important determinant for *Scalesia* growth, which can act in concert with *R. niveus* invasion and/or its legacy effects. However, the direction and magnitude of the combined effect of invasive plant legacy effects and non-native earthworm invasion was mediated by land management actions.

Nevertheless, it is important to recognize that due to logistic difficulties we were not able to sample in sites free from earthworm invasion. We may thus have failed to detect specific relationships among species that might differ in magnitude and direction of effects on invasive and native plant growth. In addition, this study draws conclusions from work carried out on seeds under controlled conditions for a limited period, but due to very low germination rates we did not consider responses of endemic plant species. As such, it cannot address the total complexity of non-native earthworm invasion within the *Scalesia* forest, although the findings conform to established invasive earthworm-plant interaction theories. These limitations notwithstanding, the current research provides relevant evidence to suggest that non-native earthworms have an important effect on plant community composition in *Scalesia* forests. However, the magnitude and direction of the effects of non-native earthworm on these forests are mediated by (1) the local spatial distribution of non-native earthworm communities, (2) presence of invasive ants, (3) seedbank composition, and (4) land management activities (i.e. control of invasive plants). For instance, our study provides enough evidence to reinforce the idea for the implementation of a holistic approach to ecological restoration in which above- and below-ground relationships are considered to improve strategies for the successful restoration of terrestrial ecosystems (Eviner and Hawkes 2008; Kardol and Wardle 2010; Boyer et al. 2016).

Overall, our findings shed new light on the unintended effects of mechanical control of invasive plants and provide indicative patterns of the possible effects of non-native earthworms on invasive and native plant establishment on tropical forests and remote islands. We suggest that the invasion of soil dwelling invertebrates and their interaction with land disturbances caused by invasive species management, may act in complex interactions (Didham et al. 2007; Darling and Côté 2008) to ultimately facilitate expansion of invasive plants such as *R. niveus*.

However, because our results are correlative, future research on plant-earthworm-ant interactions at the species level is needed to fully understand the complicated and interactive processes among these multiple stressors. This is important because effects on the environment by invasive plants, earthworms, ants, and their legacy effects are highly context-dependent and species-dependent.

Finally, we recommend that (1) plant-earthworm interactions are further studied and understood at the local scale to better inform new reforestation programs for *S. pedunculata* because soil biological activity and land management activities (i.e., agricultural areas) can mediate the success of seedling establishment and growth; (2) that *S. pedunculata* seedlings from the Galapagos National Park nurseries are checked carefully for the presence of earthworms before transplanting them into non-invaded areas; (3) that cars visiting farmlands have their tires cleaned before going to Los Gemelos; (4) mechanical control of *R. niveus* should be minimized and seek other methods for control such as bio-control to increase the survival of *S. pedunculata* seedlings; and (5) to include non-native earthworm species in the black list of prohibited species in the Galapagos. In addition, management decisions will have to prioritize actions that decompose the interactions found in this study. For instance, a management effort towards the control of invasive ants could help to break the three-way interaction among invasive species benefiting *R. niveus*. Biological control instead of mechanical control for *R. niveus* might generate better conservation results by reducing disturbances that facilitate other invasions.

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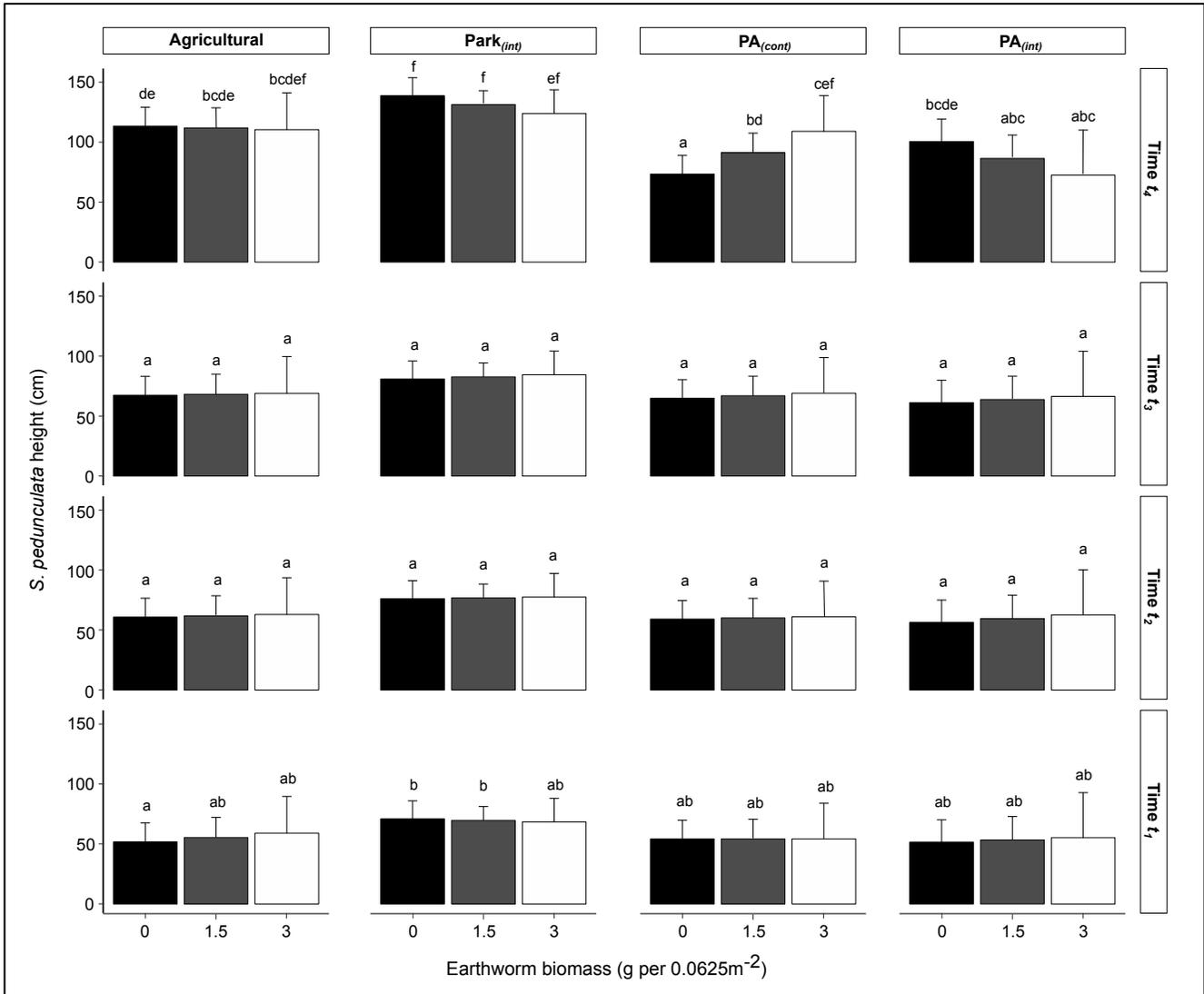
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ANNEX



Annex Fig. 1. Effects of non-native earthworm abundance (g per 0.0625m²) on *Scaevola pedunculata* seedling height (cm) per study site. Bars show least-square means, and error bars indicate 95% confidence intervals based on estimated parameters presented in Annex Table 6. Different letters indicate a statistically significant difference (p<0.05) between and within sites. Duration of the study: September 2017 (t₀), October 2017 (t₁), November 2017 (t₂), February 2018 (t₃), and March 2018 (t₄).

ANNEX Table 1. Number of seedlings of different plant species that emerged from September to November 2017 from 3 different soil types (soil 1 = Dominated by *R. niveus* (>90% cover) and earthworm-free; soil 2 = Earthworm-invaded *Scalesia* forest; soil 3 = Earthworm-free and *R. niveus*-free *Scalesia* forest). Additional treatments included adding 100 seeds of each plant species (+ seed) or earthworms (+EW). Data are means \pm se with N = 10 replicates per treatment.

Treatment	Germinated Seeds				
	<i>Scalesia pedunculata</i> *	<i>Rubus niveus</i>	<i>Tradescantia fluminensis</i>	<i>Solanum americanum</i>	<i>Oxalis corniculata</i>
Soil-1	0	1.88 \pm 0.20	0.45 \pm 0.05	0.50 \pm 0.05	0.24 \pm 0.04
Soil-2	0	0.95 \pm 0.11	0.13 \pm 0.03	0.15 \pm 0.04	0.01 \pm 0.01
Soil-3	0	0.43 \pm 0.06	0.30 \pm 0.05	0.43 \pm 0.07	0.24 \pm 0.04
Soil-1 + Seed	0	2.36 \pm 0.22	0.38 \pm 0.04	0.32 \pm 0.06	0.24 \pm 0.04
Soil-2 + Seed	0	0.30 \pm 0.06	0.14 \pm 0.03	0.04 \pm 0.02	0
Soil-3 + Seed	0	0.65 \pm 0.08	0.08 \pm 0.03	0.08 \pm 0.03	0.16 \pm 0.04
EW + Soil-1	0	0.64 \pm 0.05	0	0.08 \pm 0.03	0.23 \pm 0.04
EW + Soil-2	0	0.80 \pm 0.04	0	0.48 \pm 0.05	0.07 \pm 0.02
EW + Soil-3	0	0	0.05 \pm 0.02	0.32 \pm 0.04	0.57 \pm 0.05
EW + Soil-1 + Seed	0	0.72 \pm 0.05	0	0.08 \pm 0.03	0.24 \pm 0.04

EW + Soil-2 + Seed	0	0.56 ± 0.04	0	0.16 ± 0.04	0.08 ± 0.02
EW + Soil-3 + Seed	0.07 ± 0.02	0	0.10 ± 0.03	0.24 ± 0.04	0.63 ± 0.05

* Native species

Treatment	Germinated Seeds				
	<i>Borreira ericaefolia</i> *	<i>Zanthoxylum fagara</i> *	<i>Brassicaceae Sp1</i>	<i>Pennisetum purpureum</i>	<i>Physalis Sp1</i>
Soil-1	0	0	0.32 ± 0.04	0.01 ± 0.01	0.05 ± 0.02
Soil-2	0	0	0.32 ± 0.04	0.02 ± 0.01	0
Soil-3	0	0	0.08 ± 0.03	0	0
Soil-1 + Seed	0	0	0.08 ± 0.03	0.03 ± 0.02	0
Soil-2 + Seed	0	0	0	0	0
Soil-3 + Seed	0	0.09 ± 0.03	0.16 ± 0.04	0	0
EW + Soil-1	0.24 ± 0.04	0	0.59 ± 0.05	0	0
EW + Soil-2	0	0	0.55 ± 0.05	0	0

EW + Soil-3	0	0	0.24 ± 0.04	0	0
EW + Soil-1 + Seed	0.27 ± 0.04	0	0.42 ± 0.05	0	0
EW + Soil-2 + Seed	0	0	0.37 ± 0.05	0	0
EW + Soil-3 + Seed	0.06 ± 0.02	0.07 ± 0.03	0.38 ± 0.05	0	0.07 ± 0.03

* Native species

Treatment	Germinated Seeds				
	<i>Poaceae Sp1</i>	<i>Poaceae Sp2</i>	<i>Sp1</i>	<i>Sp2</i>	<i>Sp3</i>
Soil-1	0.19 ± 0.04	0	0.08 ± 0.03	0.41 ± 0.05	0.08 ± 0.03
Soil-2	0	0.04 ± 0.02	0	0.51 ± 0.05	0
Soil-3	0	0	0	0.22 ± 0.04	0
Soil-1 + Seed	0.21 ± 0.04	0	0	0.31 ± 0.05	0
Soil-2 + Seed	0	0	0	0.43 ± 0.06	0
Soil-3 + Seed	0	0	0	0.36 ± 0.05	0
EW + Soil-1	0	0.05 ± 0.02	0.08 ± 0.03	0.08 ± 0.03	0
EW + Soil-2	0	0.33 ± 0.05	0	0.08 ± 0.03	0
EW + Soil-3	0.22 ± 0.04	0	0	0	0
EW + Soil-1 + Seed	0	0	0	0	0
EW + Soil-2 + Seed	0	0.34 ± 0.05	0	0.13 ± 0.03	0
EW + Soil-3 + Seed	0.30 ± 0.05	0.26 ± 0.04	0	0	0

* Native species

Treatment	Germinated Seeds				
	<i>Sp4</i>	<i>Sp5</i>	<i>Sp6</i>	<i>Urtica dioica</i> L.	<i>Urticaceae Sp1</i>
Soil-1	0	0	0	0	0
Soil-2	0	0	0	0	0
Soil-3	0	0.07 ± 0.03	0	0	0
Soil-1 + Seed	0	0.05 ± 0.02	0	0	0
Soil-2 + Seed	0	0	0	0	0
Soil-3 + Seed	0	0.13 ± 0.03	0	0	0
EW + Soil-1	0.07 ± 0.03	0	0	0.16 ± 0.04	0
EW + Soil-2	0	0	0	0	0
EW + Soil-3	0.23 ± 0.04	0	0.12 ± 0.03	0.08 ± 0.03	0
EW + Soil-1 + Seed	0.08 ± 0.03	0	0	0	0
EW + Soil-2 + Seed	0	0	0.06 ± 0.02	0	0
EW + Soil-3 + Seed	0.24 ± 0.04	0	0.18 ± 0.04	0	0.08 ± 0.03

* Native species

ANNEX Table 2. List of GPS coordinates of each soil collection plot (soil 1 = Dominated by *R. niveus* (>90% cover) and earthworm-free; soil 2 = Earthworm-invaded *Scalesia* forest; soil 3 = Earthworm-free and *R. niveus*-free *Scalesia* forest) in our study of seed germination on Santa Cruz Island, Ecuador in 2017.

GPS Soil-1		GPS Soil-2		GPS Soil-3	
S	W	S	W	S	W
0° 37.2872'	90° 22.8165'	0° 37.3005'	90° 22.8254'	0° 38.0304'	90° 23.6588'
0° 38.0584'	90° 23.6148'	0° 39.7496'	90° 19.6148'	0° 38.0215'	90° 23.6558'
0° 37.3711'	90° 22.9302'	0° 40.0184'	90° 19.4532'	0° 38.0162'	90° 25.5803'
0° 37.5061'	90° 23.1264'	0° 39.2575'	90° 17.3595'	0° 38.0012'	90° 25.6414'
0° 38.0217'	90° 23.6173'	0° 37.9942'	90° 23.6314'	0° 37.9796'	90° 25.7009'

ANNEX Table 3. List of GPS coordinates of each native plant seed collection point in our study of seed germination on Santa Cruz Island, Ecuador (21 - 26 June 2017).

GPS <i>S. pedunculata</i>		GPS <i>C. alba</i>		GPS <i>P. rufipes</i>		GPS <i>T. pubescens</i>	
S	W	S	W	S	W	S	W
0° 38.0256'	90° 23.6584'	0° 37.2985'	90° 22.828'	0° 37.9372'	90° 23.546'	0° 37.289'	90° 22.8127'
0° 39.0992'	90° 24.4202'	0° 37.9329'	90° 23.5428'	0° 39.1343'	90° 24.4114'	0° 37.3084'	90° 22.8284'
0° 41.647'	90° 19.4962'	0° 42.2813'	90° 19.3297'	0° 38.0656'	90° 23.6143'	0° 39.7988'	90° 24.878'
0° 42.5452'	90° 18.9944'	0° 42.5367'	90° 19.0717'	0° 40.4895'	90° 19.3022'	0° 42.0688'	90° 19.3267'
0° 37.3976'	90° 22.9785'	0° 42.1464'	90° 19.333'	0° 37.3526'	90° 22.8832'	0° 41.946'	90° 19.3907'

ANNEX Table 4. List of GPS coordinates of each invasive plant seed collection point in our study of seed germination on Santa Cruz Island, Ecuador (26 - 30 June 2017).

GPS <i>R. niveus</i>		GPS <i>S. nigra</i>		GPS <i>L. camara</i>	
S	W	S	W	S	W
0° 37.7317'	90° 23.339'	0° 37.9795'	90° 25.7012'	0° 42.7658'	90° 20.596'
0° 39.9404'	90° 24.9658'	0° 38.1116'	90° 23.5684'	0° 42.4405'	90° 19.3178'
0° 40.4598'	90° 19.2956'	0° 42.0155'	90° 19.3358'	0° 42.4405'	90° 19.3178'
0° 40.4935'	90° 19.3072'	0° 42.4928'	90° 19.3543'	0° 40.4895'	90° 19.3022'
0° 37.3527'	90° 22.8765'	0° 42.3269'	90° 19.6927'	0° 40.2025'	90° 15.1465'

Annex Table 5. Height of *S. pedunculata* seedlings (cm) according to area of interest (N = total number of surviving seedlings) at different time periods: September 2017 (t_0), October 2017 (t_1), November 2017 (t_2), February 2018 (t_3), and March 2018 (t_4). Data are means \pm se

Time period	Area of interest			
	Agricultural (N = 18)	Park _(int) (N = 40)	PA _(cont) (N = 23)	PA _(int) (N = 15)
t_0	43.92 \pm 1.17	44.17 \pm 0.87	45.59 \pm 0.90	44.69 \pm 1.00
t_1	60.17 \pm 2.34	70.48 \pm 2.17	51.65 \pm 1.18	50.41 \pm 1.16
t_2	68.79 \pm 2.26	77.30 \pm 2.36	56.92 \pm 1.20	55.79 \pm 1.25
t_3	75.09 \pm 2.31	83.00 \pm 2.30	63.13 \pm 1.53	60.43 \pm 1.33
t_4	115.06 \pm 9.78	132.95 \pm 7.93	78.09 \pm 6.42	92.27 \pm 5.31

Annex Table 6. Results of GLMM with Gaussian errors on the effects non-native earthworm biomass (g per 0.0625 m²), time land management on *S. pedunculata* seedling height (cm). Model included transect number and area within the transect as random nested factors (N = 231)

	Seedling height (cm)			
	Estimates	SE	<i>t</i>	P
(Intercept)	53.44	6.84	7.82	<0.001
Biomass	4.04	4.03	1.00	0.32
Time <i>t</i> ₂	6.81	6.07	1.12	0.26
Time <i>t</i> ₃	5.51	6.07	0.91	0.36
Time <i>t</i> ₄	-26.53	6.07	-4.37	<0.001
PA _(cont)	-3.57	10.76	-0.33	0.74
PA _(int)	-4.67	10.62	0.94	0.35
Park _(int)	9.54	10.09	0.94	0.35
Biomass * Time <i>t</i> ₂	-0.56	5.11	-0.11	0.91
Biomass * Time <i>t</i> ₃	-1.92	5.11	-0.38	0.71
Biomass * Time <i>t</i> ₄	-2.75	5.11	-0.54	0.59
Biomass * PA _(cont)	-0.87	7.04	-0.12	0.90
Biomass * PA _(int)	-1.81	6.37	-0.28	0.78
Biomass * Park _(int)	-3.09	5.62	-0.55	0.58
Time <i>t</i> ₂ * PA _(cont)	-1.59	9.91	-0.16	0.87

Time t_3 * PA _(cont)	2.35	9.91	0.24	0.81
Time t_4 * PA _(cont)	8.23	9.91	0.83	0.41
Time t_2 * PA _(int)	-0.74	9.64	-0.08	0.94
Time t_3 * PA _(int)	1.36	9.64	0.14	0.89
Time t_4 * PA _(int)	6.05	9.64	0.63	0.53
Time t_2 * Park _(int)	-1.95	10.95	-0.18	0.86
Time t_3 * Park _(int)	1.44	10.95	0.13	0.89
Time t_4 * Park _(int)	49.04	10.95	4.48	<0.001
Biomass * Time t_2 * PA _(cont)	0.95	9.48	0.10	0.92
Biomass * Time t_3 * PA _(cont)	3.16	9.48	0.33	0.74
Biomass * Time t_4 * PA _(cont)	6.28	9.48	0.66	0.51
Biomass * Time t_2 * PA _(int)	0.37	8.57	0.04	0.97
Biomass * Time t_3 * PA _(int)	3.82	8.57	0.45	0.66
Biomass * Time t_4 * PA _(int)	-0.39	8.57	-0.05	0.96
Biomass * Time t_2 * Park _(int)	1.39	7.45	0.19	0.85
Biomass * Time t_3 * Park _(int)	4.30	7.45	0.58	0.56
Biomass * Time t_4 * Park _(int)	-5.08	7.45	-0.68	0.49
