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The Re-colonization Ability of a Native Earthworm, *Estherella* spp., in Forests and Pastures in Puerto Rico

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ABSTRACT.—Populations of some native earthworm species are decreasing or disappearing due to human activities like habitat disturbance and introduction of exotic earthworms. Habitat disturbance can cause changes in soil physical structure and nutrient cycling, which may reduce native earthworm populations prior to the invasion of exotic earthworms. Our purpose was 1) to investigate habitat disturbance as a key process in the decline or extirpation of native earthworms, and 2) to measure the ability of native earthworms to re-colonize disturbed areas. We hypothesized that habitat disturbance will reduce the population of native earthworms and impede their re-colonization in those perturbed areas. We set up 48 soil mesocosms in three field sites representing different degrees of disturbance (abandoned pasture, young and mature forests) in the Cayey Mountains of Puerto Rico. Three individuals of the native earthworm, *Estherella* spp., were inoculated into each soil core to evaluate their re-colonization ability by measuring survivorship, growth rates and reproduction. We found that, in the absence of exotic earthworm species, the survivorship and growth rates of *Estherella* spp. in the pasture was not significantly different than that from young and mature forests during the first six months of re-colonization process. Our results suggest that habitat disturbance (changes in vegetation and soil properties) may not have significant influences on native earthworm (*Estherella* spp.) populations. We propose that biotic factors, such as competitive exclusion of native earthworms by exotic earthworms, may have considerable effects on retarding their re-colonization and/or causing the disappearance of native earthworm population in disturbed areas.

KEYWORDS.—exotic earthworm, invasion, Puerto Rico, soil aggregation, succession, survivorship, tropics

INTRODUCTION

Earthworms play a significant role in soil food webs. Earthworm activities (e.g., casting and burrowing) can change soil structure (porosity and aggregation) and stimulate microbial activities, and the release of nutrients from litter to be available for plant growth (Lee 1985; Lavelle et al. 1999; Edwards 2004). As estimated by Reynolds (1994), 3627 earthworm species have been described in the world, and this number is expected to increase at a predictable rate of 68 species per year given more field surveys, particularly in tropical regions (Reynolds 1994; Fragoso et al. 1997). However, the diversity of native earthworms is declining with the invasion of exotic earthworm species introduced widely due to human activities (González et al. 2006). Lavelle and Lapied (2003) found that many native earthworm species are in danger of extinction or have already disappeared in Amazonian areas that are now colonized by exotic species.

The disappearance and decline of native earthworm populations are caused mostly by human activities, especially habitat disturbance and the introduction of exotic earthworm species. Kalisz and Doston (1989) completed an earthworm survey in the Appalachian Mountains of Kentucky, USA, and found that populations of native earthworms were distributed far away from severely disturbed areas, whereas the occurrence of exotic earthworms was always related to land clearance, agricultural cultivation, and human habitations. Habitat disturbance, such as land-use changes

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(e.g., logging and cultivation), may cause sharp fluctuations in soil physical environmental conditions (e.g., soil temperature, moisture and structure) and in dynamics of soil nutrient cycling as vegetation types and litterfall input are altered. In disturbed habitats, these physical-chemical changes can cause the decline of native earthworm populations prior to the invasion of exotic earthworms, so that the exotics can occupy vacant habitats without interacting with native earthworm species (Kalisz 1993; Kalisz and Wood 1995). The interactions between native and exotic earthworm species, especially as related to resource competition, may become a determining factor in the decimation of native species in relatively undisturbed area (Kalisz 1993; Kalisz and Wood 1995). Yet, it is still unclear how significantly these two mechanisms affect the diversity of native earthworm species at a given site. Human-caused disturbance can affect the suitability of soil as a habitat for native earthworms, thereby degrading a basic requirement for sustaining native earthworm populations, while competitive intensity for finite resources between native and exotic earthworms may change the performance of native earthworm populations in their original habitats after invasion of exotic earthworms. Understanding the impacts of habitat disturbance on native earthworm species should be a priority for the conservation of biodiversity, and it should be a first step before considering the potential consequences of native and exotic interactions.

Due to soil degradation and/or changes in economic strategies, many tropical pastures have been gradually converted to grasslands or secondary forests following the abandonment of agricultural practices (e.g., Brazil, Buschbacher 1986). Puerto Rico, a Caribbean Island, is a typical example representing the history of land-use changes and forest regeneration in the past decades. Forests in Puerto Rico were converted to pastures and cultivated land in the early 1900's. By the late 1940's, the island started to shift from an agricultural to an industrial economy; abandonment of agricultural lands occurred across the whole island (Birdsey and Weaver 1987). Forest cover of the island has increased from 5% to over 30% (Birdsey and Weaver 1987; Helmer 2004).

The successional processes of forest regeneration in Puerto Rico have been widely investigated for management and conservation purposes (Aide et al. 1995; Zimmerman et al. 1995; Pascarella et al. 2000). Interestingly, the composition of earthworm communities has also changed along with the vegetation successional processes. The recovery of native earthworm communities, which mostly disappeared in the pastures, occurred along with forest successional stages. An earthworm survey following secondary succession of abandoned tropical pastures in the Luquillo Mountains found that native *Estherella gatesi* existed only in successional stages older than 15 years (Zou and González 1997). Sánchez-de León et al. (2003) discovered that five native earthworm species were only found in the mature secondary forests of a chronosequence of old tropical pastures (pastures, young and mature forests) in the Cayey Mountains of Puerto Rico. Therefore, degrees of disturbance may determine the distributions of native earthworms among the successional stages and management practices in regenerated forests. Lower degrees of habitat disturbance may correlate with a greater opportunity for recolonization by native earthworm species. The characteristics of a habitat, including litter and/or root biomass, soil properties (water and nutrient content), and microbial biomass may contribute to a diverse earthworm community during forest regeneration (González et al. 1996; Zou and González 1997). Meanwhile, exotic earthworm species may affect the re-colonization by native species, since the exotic earthworm, *Pontoscolex corethrurus*, was distributed in all successional stages in these old tropical pastures in Puerto Rico. Sánchez-de León et al. (2003) proposed that competition between native and exotic earthworms may cause the disappearance of native earthworms in the disturbed areas. Reversibly, these combined effects may be the mechanisms retarding current re-colonization of native earthworms.

In this study, we investigated whether

habitat disturbance (changes in soil properties and vegetation) is a key mechanism for current re-colonization processes of native earthworms (*Estherella* spp.) in a chronosequence of regenerated forests in Puerto Rico. We also determined how native earthworms (*Estherella* spp.) change soil aggregation after colonization of the soil. Our hypothesis is that habitat disturbance will impede the re-colonization of native earthworms (*Estherella* spp.) by decreasing their survivorship, growth rate, and reproduction.

Materials and Methods

Study site

The study area is located in the Sierra de Cayey Mountains of southeastern Puerto Rico. All sites are 600-700 m above sea level. We chose three sites (pasture, young and mature secondary forests) on a chronosequence of naturally regenerated forest to include different degrees of habitat disturbance. The pasture site, abandoned 1-2 years before the study, had supported intense land use activities such as: cattle tramping and forest clearance, for the past few years. The young and mature secondary forests have steadily recovered from the impacts of human disturbances, and represent intermediate and slight perturbation, respectively. Grass species are dominant in the pasture site, while woody species become more prevalent in the young and mature secondary forests (Pascarella et al. 2000; Sánchez-de León et al. 2003).

A previous earthworm survey in this area had discovered exotic *Pontoscolex corethrurus,* which was widespread over all the sites (active pasture, young and mature secondary forests) (Sánchez-de León et al. 2003). They also only found a native earthworm community composed of *Borgesia sedecimestae*, *Estherella* sp., *Onychochaeta borincana*, *Neotrigaster rufa*, and *Trigaster longissimus* in the mature secondary forest (Sánchez-de León et al. 2003).

Sampling

During this study, we measured litterfall, litterfall carbon/nitrogen content, soil physical characteristics, and microbial biomass to examine the differences among the sites.

On August, 2004, four blocks were chosen (at least 3 m apart) within each of study sites, and four soil cores (soil samples contained within PVC tubes) were collected randomly (30 cm apart) from each of four blocks at each site. We collected intact soil cores (diameter = 11 cm, length = 30 cm) by inserting a PVC tube into the ground after the removal of the surface litter. This method allowed for the collection of physically undisturbed soil samples. All soil cores (n = 48) were put in a freezer (–30 °C) for 48 hours to eliminate earthworms. We covered the bottom and top of each soil core with 1-mm mesh screen to prevent the migration of earthworms (see below). Soil cores were replaced into exact sites from where they were collected.

Native earthworms, *Estherella* spp., were collected from the Bisley experimental watersheds, which is located at the northeast side of the Luquillo Mountains. The vegetation in the Bisley experimental watersheds includes primary and secondary tabonuco forests (*Dacryodes excelsa*) (Scatena 1989). Fresh weights of earthworms were recorded. A subsample of earthworms was used to void their gut for 24 hours to calibrate the weight of earthworms minus gut contents [Gut-voiding weight (g) = –0.0253 + 0.999 Fresh weight (g)]. Three individuals of *Estherella* spp. (mean ± S.D. = 3.17 ± 0.4 g) were inoculated into each of the soil cores in the field.

On October 31, 2004, we gathered initial soil data by sampling one soil core from every block at each site before inoculating with native earthworms. Every two months, one soil core from every block at each site was sampled (Core sample), and another soil sample (diameter = 5 cm, length = 30 cm) was also collected 1 m apart from the core sample within each block (Field sample).

At each sampling date, litterfall was collected in four baskets (57 × 43 cm in size) which were randomly set up at each site. Varied amounts of litter gathered from field litter baskets (see below) were added into the rest of soil cores in the field based

on the area of soil cores (95 cm^2) (e.g., pasture/young/mature = 0.12/0.90/0.88 g dry litter per core in March, 2005).

Litterfall and field soil data were collected at all the sites during the experimental period for determination of habitat characteristics (Table 1). Litterfall was recorded after air-drying for 48 hours. Field and Core samples were analyzed for soil water content and pH (0-5 and 5-10 cm deep). Ten grams of soil from each sample were oven-dried at 105°C for 48 h to determine soil water content. Soil pH was measured using a 1:2 ratio of dried soil and deionized water (Hendershot et al. 1993). Subsamples of litter and soil were finely ground for total carbon (C) and nitrogen (N) analysis. Total C and N in the soil samples (20 mg) and the litter samples (5 mg) were determined by dry combustion in a Carlo Erba model 1500 C/N analyzer.

Earthworms harvested from the experimental cores were used to calculate survivorship and vertical distribution of *Estherella* spp. We also calculated biomass growth rates (%) of earthworms by using the mean

TABLE 1. Habitat characteristics, including litterfall, soil properties, and microbial biomass C and N, at three study sites representing different degrees of disturbance in the Cayey Mountains of Puerto Rico. Data are mean ± standard error.

	Sites		
Variables	Pasture	Young forest	Mature forest
Litterfall			
Litterfall (g/m^2 per month)	7.56 ± 2.04[a]	59.33 ± 4.87[b]	57.45 ± 5.55[b]
Carbon (%)	43.44 ± 1.56[a]	44.48 ± 0.74[a]	43.40 ± 0.50[a]
Nitrogen (%)	1.06 ± 0.04[ab]	1.20 ± 0.04[b]	1.02 ± 0.05[a]
C/N	41.13 ± 1.15[ab]	37.78 ± 1.15[a]	44.34 ± 1.90[b]
Soil			
pH			
0-5 cm	4.76 ± 0.02[b]	4.64 ± 0.02[b]	4.28 ± 0.06[a]
5-10 cm	4.69 ± 0.07[b]	4.61 ± 0.04[b]	4.30 ± 0.04[a]
Water content (%)			
0-5 cm	94.7 ± 5.67[b]	37.6 ± 2.52[a]	47.5 ± 3.10[a]
5-10 cm	90.2 ± 5.04[b]	34.9 ± 2.18[a]	42.7 ± 2.26[a]
Carbon (%)			
0-5 cm	4.49 ± 0.02[a]	4.76 ± 0.29[a]	5.12 ± 0.29[a]
5-10 cm	3.58 ± 0.21[a]	3.84 ± 0.30[a]	3.26 ± 0.15[a]
Nitrogen (%)			
0-5 cm	0.38 ± 0.02[a]	0.37 ± 0.02[a]	0.34 ± 0.01[a]
5-10 cm	0.32 ± 0.02[b]	0.29 ± 0.01[b]	0.24 ± 0.01[a]
C/N			
0-5 cm	11.9 ± 0.25[a]	12.8 ± 0.26[a]	15.2 ± 0.35[b]
5-10 cm	11.3 ± 0.15[a]	12.9 ± 0.59[b]	13.7 ± 0.23[b]
Microbial biomass			
Carbon (μg/g dry soil)			
0-5 cm	1834.8 ± 110[a]	1626.3 ± 146[a]	1450.0 ± 135[a]
5-10 cm	1551.6 ± 134[a]	1298.8 ± 100[a]	1152.1 ± 152[a]
Nitrogen (μg/g dry soil)			
0-5 cm	201.4 ± 5.88[b]	162.3 ± 7.82[a]	158.9 ± 4.37[a]
5-10 cm	167.1 ± 12.10[b]	133.1 ± 6.78[a]	125.2 ± 5.56[a]
C/N			
0-5 cm	9.1 ± 0.43[a]	9.8 ± 0.61[a]	9.1 ± 0.77[a]
5-10 cm	9.6 ± 0.86[a]	10.0 ± 0.79[a]	9.0 ± 1.01[a]

Note: Data on soil properties and microbial biomass were collected in October (2004), January, March, and May, 2005, while litterfall data were from January, March, and May, 2005. Common letters indicate no significant difference between habitats [Tukey (HSD) multiple comparison method; $\alpha = 0.05$].

final weight of the worms (gut voided for 24 hours) divided by mean initial weight per core.

The distribution of soil aggregates was analyzed for both Core and Field samples by using a wet-sieving apparatus described in Beare et al. (1994). Three aggregate classes (>2000 μm, 250-2000 μm, and 53-250 μm) were measured in this study. Fifty grams of dried soil were evenly distributed on top of stacked 2000-μm and 250-μm sieves and wetted for 8 minutes with deionized water prior to wet-sieving. Then the stacked sieves were oscillated vertically within deionized water for 5 minutes (31 oscillation cycles per minute). Following the oscillations, the water column was drained through a 53-μm sieve. Soil aggregates with sieves were transferred to aluminum pans then air-dried. Soil aggregates distribution (%) was calculated on a dry weight basis for each size class.

Statistic analysis

All analyses were performed by using SAS software (version 9.1, SAS Institute Inc. USA). Data representing characteristics of the study sites (litterfall and soil data) were analyzed as one way factorial design by the GLM procedure to compare the difference among habitats. Soil pH and microbial biomass data were tested by the GLM procedure for the differences among habitats and treatments (Field and Core samples). If significant, Tukey (HSD) multiple comparison method was used. A logistic procedure was applied to analyze the vertical distribution and survivorship of earthworms because of the discrete responses. The distribution pattern of soil aggregates was tested by MANOVA of ANOVA procedure. The differences of earthworm growth rates among site and time factors were compared by repeated-measures GLM procedure. The significance level for all tests was set at $\alpha = 0.05$.

RESULTS

Site characteristics

Litterfall input in the pasture was significantly the lowest ($F_{site\ 2,21} = 14.9$, $p < 0.0001$; Table 1). Litter C was similar among all sites ($F_{site\ 2,45} = 0.74$, $p = 0.48$; Table 1), while the litter from the mature secondary forest had the lowest litter nitrogen (N) ($F_{site\ 2,45} = 4.3$, $p = 0.02$; Table 1) and highest C/N ratio ($F_{site\ 2,45} = 4.8$, $p = 0.01$; Table 1). Soil pH in the mature forest was significantly lower than those in the young forest and pasture in the top 10 cm of soil (0-5 cm: $F_{site\ 2,33} = 40.3$, $p < 0.0001$; 5-10 cm: $F_{site\ 2,33} = 15.8$, $p < 0.0001$; Table 1). Soil water content of the top 0-10 cm in the pasture was about 2-2.5 times higher than those in the young and mature forests (0-5 cm: $F_{2,45} = 58.2$, $p < 0.0001$; 5-10 cm: $F_{2,45} = 76.2$, $p < 0.0001$; Table 1). Soil C and N did not significantly differ among habitat types, except that soil N was lower at 5-10 cm soil in the mature secondary forest ($F_{site\ 2,47} = 8.9$, $p = 0.0005$; Table 1). Soil C/N was higher in mature forest than in the pasture, suggesting an increase with successional stage (0-5 cm: $F_{site\ 2,44} = 32.5$, $p < 0.0001$; 5-10 cm: $F_{site\ 2,47} = 11.0$, $p < 0.0001$; Table 1). Microbial biomass N was significantly higher in the pasture than in the young and mature forests (0-5 cm: $F_{site\ 2,45} = 14.6$, $p < 0.0001$; 5-10 cm: $F_{site\ 2,45} = 6.7$, $p = 0.003$; Table 1). However, microbial C and C/N were similar among all sites ($p > 0.05$; Table 1).

Soil water content of the top 10 cm soil in the Core samples of the young and mature forests were 2.5 and 2 times higher than those in the Field soil samples (t-test, $p < 0.0001$; data not shown), but not significantly different in the pasture ($p > 0.1$; data not shown). However, soil C, N and C/N did not significantly differ between the Core and Field samples at top 10 cm soil in all habitats (0-5 cm: $F_{1,17} = 1.27$, 2.03 and 0.07 for C, N and C/N data, respectively; all $p > 0.1$), as well as soil pH ($F_{1,17} = 1.5$, $p > 0.1$) at the end of the experiment.

Soil microbial biomass C in the field mesocosms [Core samples; 0-5 cm (mean ± S.E): 1459.7 ± 140, 571.4 ± 101, 605.2 ± 124 ug/g dry soil ; 5-10 cm (mean ± S.E.): 1088.1 ± 127, 638.63 ± 80, 257.2 ± 44 ug/g dry soil in the pasture, young and mature forests, respectively] was significantly lower than in the Field samples for the top 10 cm soil at all sites (in average 75%, 42%, 32% of the field data in the pasture, young and mature

forests, respectively; F test and Tukey comparison, $p < 0.0001$). Soil microbial biomass N in the Core samples [0-5 cm (mean ± S.E.): 153.9 ± 16.35, 101.2 ± 10.51, 83.0 ± 11.51 ug/g dry soil ; 5-10 cm (mean ± S.E.): 119.4 ± 10.78, 79.0 ± 10.10, 46.9 ± 5.77 ug/g dry soil in the pasture, young and mature forests, respectively] was also lower compared to the Field samples (in average 74%, 61%, 45% of the field data in the pasture, young and mature forests; F test and Tukey comparison, $p < 0.0001$). However, soil microbial biomass C and N in the Core samples showed the similar patterns among all sites as the Field samples. Lower soil microbial C/N of young and mature secondary forests were found in the Core samples [microbial C/N (mean ± S.E.): 9.85 ± 0.61, 5.70 ± 0.74, and 7.95 ± 1.16 in the pasture, young and mature forests, respectively] than those in the Field samples at 0-5 cm soils ($F_{1,82} = 6.5$; $p = 0.01$), but no significant difference at 5-10 cm of the soils [Core samples: microbial C/N (mean ± S.E.): 9.09 ± 0.72, 8.43 ± 0.66, and 7.52 ± 2.19 in the pasture, young and mature forests, respectively; $F_{1,82} = 1.6$; $p = 0.20$].

Earthworm population

The survivorship of *Estherella* spp. in the pasture was not significantly different than those from the young and mature forests ($n = 36$, $\chi_2 = 1.12$, $p = 0.57$; Fig. 1A). *Estherella* spp. had higher mortality at later samplings (March and May) in all sites ($\chi_2 = 6.65$, $p = 0.04$; Fig. 1A). No significant difference in the growth rate of earthworms was found among the different habitats ($n = 25$, $F_{site\ 2,7} = 0.19$, $p = 0.84$; $F_{time\ 2,7} = 0.69$, $p = 0.53$; Fig. 1B). Highly individual variations in growth rates (wide error bars in Fig. 1B) was observed. The total abundance of *Estherella* spp. harvested during the experiment in the pasture, young and mature forests were 23, 29, and 22 worms, which represented 64, 81, and 61% of total worms that were inoculated in each habitat, respectively. Most *Estherella* spp. were found in the top 10 cm of the soils, especially 5-10 cm ($n = 108$, $\chi_3 = 18.8$, $p = 0.0003$; Fig. 2). There was no significant difference among site and time factors ($n = 108$, $\chi_{site,2} = 0.09$, $p = 0.96$; $\chi_{time,2} = 0.66$, $p = 0.72$; Fig. 2). No cocoon was found in all the Core samples during the experimental period.

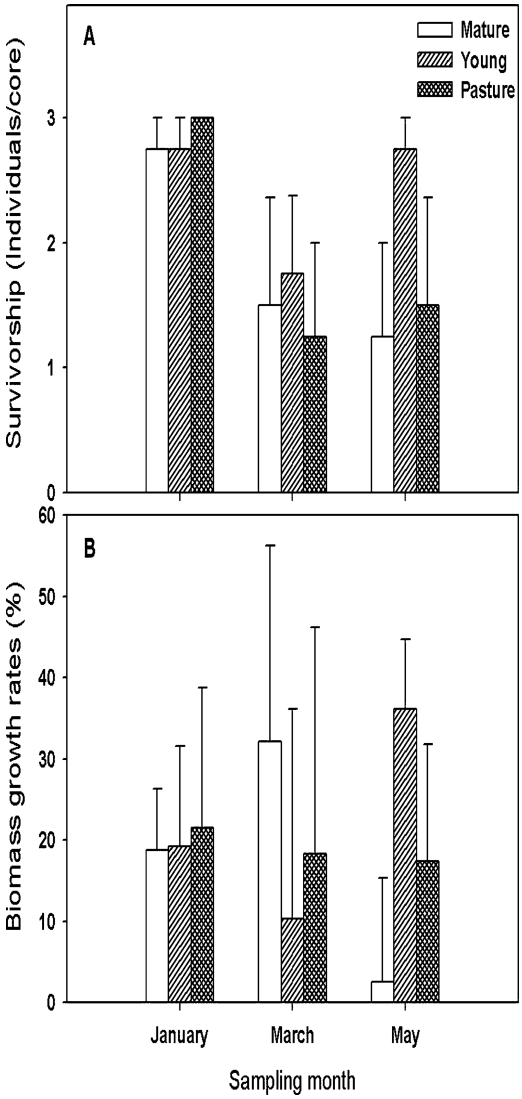

FIG. 1. Population dynamics of native earthworms, *Estherella* spp., during the experimental period (January, March, and May, 2005). A) Survivorship (mean ± S.E.) and B) percentage of biomass growth rate (mean ± S.E.) of native earthworms, *Estherella* spp., in three study sites representing different degrees of habitat disturbance in the Cayey Mountains of Puerto Rico.

Earthworm effects on soils

Estherella spp. significantly changed distribution patterns of macro- and microaggre-

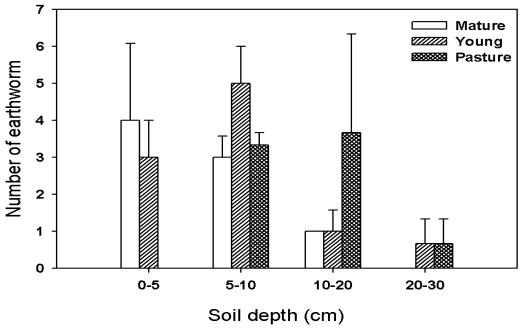

FIG. 2. Vertical distribution (mean ± S.E.) of native earthworms *Estherella* spp., in the top 30 cm of the soil at three study sites representing different degrees of habitat disturbance in the Cayey Mountains of Puerto Rico.

gates from the beginning (October, 2004) to the end (May, 2005) of the experiment ($F_{time\ 3,16}$ = 4.1, p = 0.02; initial and final data in Fig. 3A and 3B). In the presence of *Estherella* spp., the percentages of larger macro-aggregates (>2000 μm) in the Core samples showed a decline over the experimental time, from 80.1 to 63.2% and from 71.4 to 66.4% in the young and mature forests, respectively (Fig. 3A), which is in contrast to a rising trend observed in Field samples (increased from 68.8 to 78.9% and from 56.1 to 74.3% in the young and mature forests, respectively; Fig. 3B).

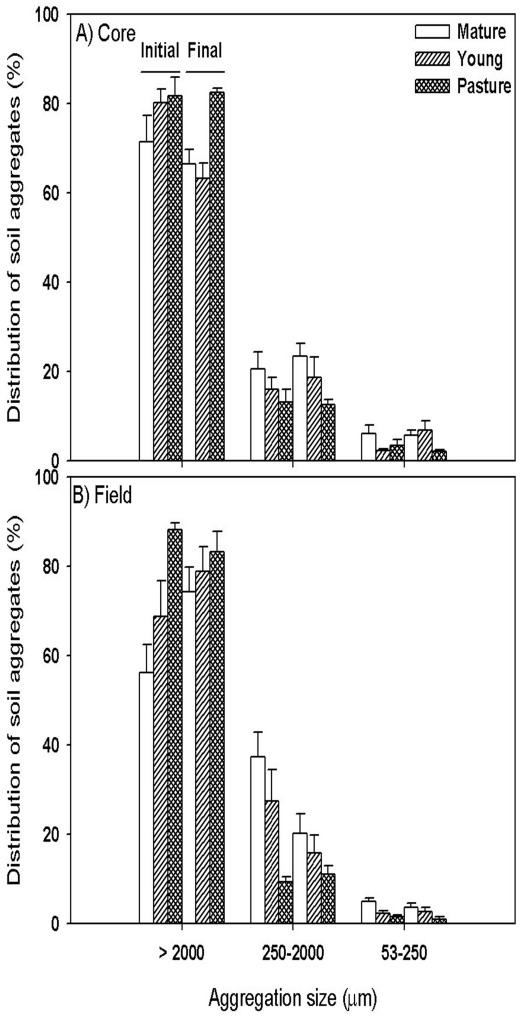

FIG. 3. Effects of the native earthworm, *Estherella* spp., on soil aggregate distribution (mean ± S.E.) in A) Core with *Estherella* spp. and B) field soil samples at three study sites representing different degrees of habitat disturbance in the Cayey Mountains of Puerto Rico. Initial and final data were collected on October, 2004 and May, 2005, respectively.

DISCUSSION

Habitat disturbance is defined as any major event that alters resource availability and/or causes changes in the physical environment (Chapin III et al. 2002). In this study, we chose three habitat types representing a chronosequence of successional stages as sites differing in the degree of disturbance due to cattle ranching. In the pasture, we found the general characteristics of grass-domination, low litterfall input, and higher soil pH and microbial biomass (Aide et al. 1995; Guariguata and Ostertag 2001; Chapin III et al. 2002; Pregitzer and Euskirchen 2004; Bautista-Cruz and del Castillo 2005). Not only habitat characteristics change along succession, but also the composition and abundance of native and exotic earthworm communities varied within this chronosequence. Most native earthworms in Puerto Rico, such as *Estherella* spp., are distributed in mostly undisturbed areas (dwarf forests and tabonuco forests), and less disturbed habitats (later successional stages: naturally regenerated young and mature secondary forests) (González et al. 1996; Zou and González 1997; Hendrix et al. 1999; Sánchez-de León et al. 2003; Sánchez-de León and Zou 2004). In contrast

to the more limited distribution of native earthworms, the pantropical earthworm, *Pontoscolex corethrurus*, has expanded to the pastures, successional forests, tree plantations, and undisturbed forests in Puerto Rico (González et al. 1996; Zou and González 1997; Hendrix et al. 1999; Sánchez-de León et al. 2003). The mechanisms to explain this unequal distribution of native and exotic earthworm communities might be related to the changes of habitat characteristics due to habitat disturbance. González et al. (1996) proposed that soil water content, phosphate availability, root and microbial biomass contributed to the difference in earthworm abundance and composition between plantation and natural secondary forests. Zou and González (1997) suggested that changes in chemistry of litter biomass, rather than soil water content and soil pH, altered earthworm density and diversity along the successional gradient. Our field mesocosm experiment did not support these contentions, as the survivorship and growth rates (re-colonization ability) of the native earthworm, *Estherella* spp., were not significantly different among the sites that represented different habitat characteristics. Based on these data, it is fair to say that the re-colonization ability of *Estherella* spp. in the pasture was as good as in the young and mature forests, at least in the short-term. The soil environment in the pasture (lower litterfall input, higher soil water content and higher pH) did not prevent the survival of *Estherella* spp., even though the field mesocosm method (PVC tubes) caused artificial effects on soil properties such as increased soil water content and reduced microbial biomass C and N in the Core samples of all habitats. Interestingly, the reduction of food resource (litter input) in the pasture did not retard the survivorship and growth of the native *Estherella* spp. Previous research has suggested that plant fine roots and soil microbial biomass might provide additional food resources for earthworms (Lee 1985; González et al. 1996). Wright (1972)'s laboratory study found that *Lumbricus terrestris* used bacteria as food resource, and he suggested that bacteria could be important in its diet. Fraser et al. (2003) speculated that the reduction of fine root mass observed in one treatment of a laboratory experiment with the presence of the earthworm (*Aporrectodea caliginosa*) partly resulted from the direct feeding by the earthworm. At our pasture site, higher soil microbial biomass C and N, and more plant fine roots (Sánchez-de León et al. 2003) indirectly supports this possibility. The growth and survivorship of *Estherella* spp. in the pasture may be due to the utilization of soil microbial and root biomass as alternative food resource instead of surface litter. More field and controlled experiments are needed to specify the allocation and importance of different food resources (litter, microorganisms, and fine root) in earthworm's diets.

The mechanisms controlling the recovery of native earthworm populations also include biotic factors, particularly interactions with exotic earthworms (González et al. 1996; Sánchez-de León et al. 2003; Sánchez-de León and Zou 2004). The successful survival of *Estherella* spp. in our field mesocosm experiment may be due to the lack of competition pressure from exotic earthworm species, *P. corethrurus*. Hendrix et al. (1999) suggested a potential interspecific competitive relationship between *Estherella* sp. and *P. corethrurus* at a tabonuco forest by observing the completely overlapping ^{15}N enrichment of these two species. Winsome et al. (2006) conducted field and laboratory experiments in a California grassland, and found that native earthworms (*Argilophilus marmoratus*) performed well both in the nutrient-amended and unamended habitats, but performed poorly when co-existing with an exotic earthworm (*Aporrectodea trapezoides*). They further concluded that the exclusion of native *A. marmoratus* from the pastures might be due to the inter-specific competition with exotic *A. trapezoides*, rather than changes in soil properties (nutrient amendment) or physical disturbance. In Puerto Rico, Zou and González (1997) found that the densities of *P. corethrurus* were as high as 831 and 403 individuals/m^2 (at 25 cm depth of soil) in a pasture and grass-vine-fern site, respectively, whereas less than 141 individuals/m^2 were collected in the

shrub-small tree and forest sites of Luquillo Mountains. Sánchez-de León et al. (2003) documented a similar gradient of *P. corethrurus* densities along with successional stages in Cayey Mountains, decreasing in the order 244.4, 151.1, and 52.4 individuals/m^2 (at 25 cm depth of soil) in the pasture, young and mature forests, respectively. This density trend implies potentially stronger competitive pressure from *P. corethrurus* to native earthworm species in the pastures. Competitive exclusion by exotic *P. corethrurus* may result in relatively low abundance of the native earthworm species (0.9-14.2 individuals/m^2 in Sánchez-de León et al. 2003) in the mature forests and/or the complete disappearance of the native earthworm communities in the pasture sites.

Although *Estherella* spp. populations performed (survivorship and growth rates) equally well in all three habitats in this study, we found that their survivorship decreased during the first six months of the experiment. It is unlikely that the dry season of the year (from January to April) resulted in the loss of individuals (Brown et al. 1983), because soil water content in the Core samples remained higher than in the field. This decline in the population may be partly due to the cost of adapting to a new and/or restrained environment in the soil mesocosms.

Earthworms can have significant effects on both macro- and micro-aggregate formation and stability through their casting and burrowing activities (Marinissen and Dexter 1990; Ketterings et al. 1997; Jongmans et al. 2001; Fraser et al. 2003; Bossuyt et al. 2004). The aging and drying-rewetting cycles of earthworm casts increase the stability and formation of micro-aggregates in the casts and protect soil organic matter from rapid decomposition (Shipitalo and Protz 1988). Fraser et al. (2003) discovered that an endogeic species, *Aporrectodea caliginosa*, increased soil aggregate size (mean weight diameter-millimeter), but had little effect on aggregate stability. Bossuyt et al. (2004) found that the same species (*A. caliginosa*) enhanced the formation of stable micro-aggregates (53-250 μm) through their casting activities. Higher percentages of carbon (^{13}C) storage within large macro-aggregates after only 12 days of incubation suggested that earthworms rapidly incorporated fresh residues into micro-aggregates as the soils passed through their guts. In our study, the observation of fresh casts and burrows within the Core samples indicated high activities of *Estherella* spp. during the experimental period. The casting and burrowing activities of *Estherella* spp. seemed to inhibit the formation of large macro-aggregates (>2000 μm) at the end of the experiment, compared to soil aggregate data in the field. Besides, the increased formation of larger macro-aggregates (>2000 μm) in the field soil may have resulted from high densities of *P. corethrurus* distributed in all sites [see above, 52.4-244.4 individuals/m^2 at same study area in Sánchez-de León et al. (2003)]. The differential effects on soil aggregation by earthworms imply that the impact of earthworms on soil aggregate distribution is species dependent. For example, endogeic earthworm species, e.g., *A. caliginosa* and *P. corethrurus*, live in the soil and consume organic residues and soil, while epigeic and anecic species feed on surface litter and inhabit litter or upper soil layer (Lee 1985; Edwards 2004). Different ecological groups of earthworms may have differential effects on soil aggregates because of their utilization of different food and space resources. This relative inhibitory effect on soil macro-aggregates by *Estherella* spp., which is in contrast to the facilitation effect by the endogeic *P. corethrurus*, may be due to its identity as an epigeic and anecic species by the dark pigmentation and its inhabitation of top soils (Hendrix et al. 1999; Sánchez-de León et al. 2003; Sánchez-de León and Zou 2004). Our preliminary observation suggests that the influences on soil aggregate distribution and formation by earthworms depend on their feeding behavior and/or the soil environment they inhabit.

Conclusion

In this study, successional status and/or conversion of forest to pasture did not impede the survival of native earthworms (*Es-*

therella spp.) in disturbed areas (pasture site) in the absence of exotic earthworm species. The lack of re-colonization of native earthworms (*Estherella* spp.) into pastures may be explained by low propagule pressure/introduction opportunities of native earthworms and/or as a consequence of competition by the exotic worm, *P. corethrurus*. We suggest that changes in vegetation and soil properties resulting from habitat disturbance reduce native earthworm populations (*Estherella* spp.), but can not prevent their survival or cause their extinction. Biotic factors, particularly competitive interactions with exotic earthworms, may have significant effects on the populations of native earthworms (*Estherella* spp.). Further research is needed to determine if the competitive relationship with exotic earthworm has additive impacts with habitat disturbance on the native earthworm communities.

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