Above- and belowground interactions drive habitat segregation between two cryptic species of tropical trees

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Abstract. In the lowlands of central Panama, the Neotropical pioneer tree *Trema micrantha* (sensu lato) exists as two cryptic species: "landslide" *Trema* is restricted to landslides and road embankments, while "gap" *Trema* occurs mostly in treefall gaps. In this study, we explored the relative contributions of biotic interactions and physical factors to habitat segregation in *T. micrantha*. Field surveys showed that soils from landslides were significantly richer in available phosphorus and harbored distinct arbuscular mycorrhizal fungal (AMF) communities compared to gap soils. Greenhouse experiments designed to determine the effect of these abiotic and biotic differences showed that: (1) both landslide and gap species performed better in sterilized soil from their own habitat, (2) the availability of phosphorus and nitrogen was limiting in gap and landslide soils, respectively, (3) a standardized AMF inoculum increased performance of both species, but primarily on gap soils, and (4) landslide and gap species performed better when sterilized soils were inoculated with the microbial inoculum from their own habitat. A field experiment confirmed that survival and growth of each species was highest in its corresponding habitat. This experiment also showed that browsing damage significantly decreased survival of gap *Trema* on landslides. We conclude that belowground interactions with soil microbes and aboveground interactions with herbivores contribute in fundamental ways to processes that may promote and reinforce adaptive speciation.

Key words: adaptive speciation; arbuscular mycorrhizal fungi; Barro Colorado Island, Panama; gaps; habitat differentiation; landslides; shadehouse experiment; soil microbial communities; Trema micrantha.

Introduction

Habitat segregation among sympatric sibling species that share similar morphology and life history can provide important insights into how diversity is generated and maintained (e.g., Arlettaz 1999, Moritz et al. 2000). Recent studies have shown that local adaptation leading to habitat segregation may result in incipient speciation (Smith et al. 1997, Fine et al. 2005). However, the relative roles and importance of abiotic and biotic factors in contributing to habitat segregation remain unclear, particularly in tropical habitats (Schemske et al. 2009). In temperate and tropical forest communities, much empirical attention has been placed on assessing how tree species might segregate along abiotic resources axes (Whittaker 1972). For example, spatial heterogeneity in light, combined with species-specific differences in light requirements, has traditionally been thought to play a key role in maintaining diversity (e.g., Kobe 1999, Montgomery and Chazdon 2002, Poorter and Arets 2003). Similarly, spatial heterogeneity in soil nutrients and moisture availability has been implicated as potentially important to tree species distributions at both local and landscape scales (Clark et al. 1999, Engelbrecht et al. 2007, John et al. 2007). However, in addition to the potential for performance trade-offs to drive resource partitioning directly, biotic interactions are also recognized as playing a role in maintaining plant diversity (Ashton 1969, Schemske et al. 2009) and may act in association with resource gradients (Fine et al. 2004).

Both above- and belowground biotic interactions can affect patterns of resource partitioning and habitat segregation. Above ground, foliar herbivores can contribute to species partitioning of light gradients (Louda and Rodman 1996, DeWalt et al. 2004) and have recently been shown to mediate the partitioning among congeneric species pairs of soil types with contrasting fertility (Fine et al. 2004, 2005). Below ground, soil organisms can influence survival and resource uptake rates in host plants. Importantly, it is increasingly apparent that the composition of tropical

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soil microbial communities are heterogeneous (Husband et al. 2002, Lovelock et al. 2003, Mangan et al. 2004) and that tree species differ in their response to different members of these microbial communities (e.g., Kiers et al. 2000, Herre et al. 2005, 2007, Augspurger and Wilkinson 2007, Mangan et al. 2010). Thus, given the observed spatial and functional heterogeneity, soil microorganisms potentially provide additional axes of habitat differentiation for plant species (Ettema and Wardle 2002, Reynolds et al. 2003). Nonetheless, evidence that the composition of soil microbial communities can influence plant community composition has come mostly from temperate herbaceous communities (Mills and Bever 1998, Klironomos 2002, Reynolds et al. 2003).

In this study, we investigated factors contributing to the observed habitat segregation of two cryptic species of the tropical pioneer tree *Trema micrantha* L. that regenerate either on landslides or in forest gaps on Barro Colorado Nature Monument (Silvera et al. 2003). Specifically, we investigated the relative degree to which differences in physical (i.e., nutrient availability) and biotic (i.e., composition of soil microbial communities) soil factors contribute to habitat segregation. To do this, we (1) compared soil nutrients and soil arbuscular mycorrhizal fungal (AMF) spore communities found in each habitat (landslides and gaps), (2) conducted greenhouse experiments to assess the relative effects of these abiotic and biotic soil properties on the growth and survival of seedlings of each *Trema* species, and (3) conducted a reciprocal transplant experiment to assess the response of seedlings of each *Trema* species to aboveground (herbivory) and belowground (soils) factors in the field.

METHODS

Study site and species

This study was conducted in seasonally moist lowland tropical forest on the Barro Colorado Nature Monument (BCNM) in Central Panama (9°10′ N, 70°51′ W), described in detail in Leigh et al. (1999). The total area of the BCNM is 5400 ha, comprised both of secondary and primary forest. The BCNM receives an average annual rainfall of 2600 mm, with <10% falling from December through mid-April (Leigh et al. 1999).

Trema is a pantropical genus of fast-growing, light-demanding, short-lived pioneer trees in the Cannabaceae (Sytsma et al. 2002). Within central Panama, *T. micrantha* is largely restricted to landslides that occur primarily on lake shores, road embankments, and cuts where mineral soil is exposed (J. W. Dalling, *personal observations*) or to large light gaps in the forest interior (Silvera et al. 2003). Although once thought to be a single species, morphological, physiological, and genetic data indicate that these habitats support two distinct species (see Appendix A). "Gap *Trema*" has larger seeds, initial seed dormancy, and temperature-cued seed germination, whereas "landslide *Trema*" lacks seed dormancy and germinates in response to light quality (Appendix A: Table A1). A previous study explored whether differences in light requirements could provide an axis for segregation between *Trema* species, but no differences were found in seedling relative growth rates across a range of light conditions in a pot experiment using a common soil from the forest in BCNM (Silvera et al. 2003). Furthermore, historical colonization patterns and localized dispersal are unlikely to explain habitat segregation of these species because segregation between gaps and landslides is maintained across BCNM and even when gap and landslide recruitment sites are <100 m apart (Silvera et al. 2003).

Soil nutrient and mycorrhizal fungal communities in gaps and landslides

To address whether differences in soil nutrient status and AMF communities could account for differential habitat associations, we first compared nutrient availability (NO_3, NH_4, P, Al, Ca, Cu, Fe, Mg, Na, and Zn) and AMF spore communities between landslide and gap habitats. Landslide habitats had a coarse-grained sandy texture and completely lacked an organic-rich topsoil. Forest soils are variable across BCNM, but are generally loams or clay-loams (Baillie et al. 2007). Nutrients (see Appendix B: Table B1) were measured from 200-g composite soil samples collected from the top 15 cm of soil at four random locations within each of four landslide and four gap sites (same sites as the ones used in the field experiment). We used *t* tests to compare each soil nutrient concentration between landslides and gaps.

We examined AMF spore community composition in soils of five naturally occurring landslides and five gaps containing adult trees of landslide and gap *Trema*, respectively (sites differed from those used in the field experiment). Landslide sites were distributed along the lakeshore at Miller Cove and Peña Blanca peninsula, while gaps were located in the interior of the forest on Barro Colorado Island. Three soil cores (100 g each) were collected from the rooting zone of each of three adult *Trema* trees per gap or landslide. These three soil cores per tree were thoroughly mixed and AMF spores were extracted from a 10-g subsample using sucrose density gradient centrifugation (Daniels and Skipper 1982). The extracted spores were identified and counted using light microscopy. Spore abundances of each AMF species were averaged among the three composite samples per site. To meet assumptions of normality, AMF spore abundances were rank transformed across all sites prior to analysis. Bray-Curtis similarity of AMF spore communities was computed for all possible pairwise comparisons among the 10 sites. Unweighted pair-group cluster analysis was performed on Bray-Curtis indices and data were plotted using nonmetric multidimensional scaling. We then used a one-way analysis of similarity (ANOSIM; Clarke and Warwick 1994) to determine whether community composition of AMF spores found associated with *Trema* species on

landslides differed significantly from the composition found associated with *Trema* species in gaps. All analyses on spore composition were conducted using the software package PAST (Hammer et al. 2001).

Shadehouse experiments

We conducted three shadehouse experiments to investigate the relative importance of abiotic and biotic components of soil as potential determinants of habitat segregation between the *Trema* species. First, we assessed the importance of abiotic soil properties on seedling performance by growing seedlings in landslide and gap soil sterilized to eliminate soil organisms. Next, we tested for the effects of nitrogen (N) and phosphorus (P) addition, either with or without a common standardized AMF inoculum consisting of AMF species found at both sites on seedling growth. Finally, to investigate the potential importance of habitat-specific soil communities on seedling growth and survival, we grew seedlings in landslide and gap soils inoculated with the whole-soil community from each habitat. For all experiments, we examined roots for the presence or absence of AMF. Plants grown in sterile soil that were colonized by AMF were excluded from growth analyses. See Appendix C for details on general protocols of shadehouse experiments.

Pot experiment 1: response of Trema species to sterilized gap and landslide soils

In experiment 1, we eliminated the soil biota by sterilizing the soil to assess whether seedlings of the two *Trema* species respond differently to abiotic properties of landslide and gap soil. We grew 30 seedlings of each species in individual 2-L pots filled with an autoclaved sand–soil mixture, with soil originating from landslides or light gaps. Pots were fully randomized and seedlings were grown under 11% full sun for 154 d. Seedling survivorship curves for each species × treatment combination were compared using the Kaplan-Meier survival estimate, and a proportional hazards model was used to test for the effects of species, soil type, and the species × soil type interaction on survival (Fox 2001). Effects of species and soil type (and their interaction) on relative growth rate (RGR) were analyzed using a fixed-effect two-way ANOVA. In each experiment, RGR was calculated based on initial and total final biomass; RGR = [ln(final biomass) – ln(initial biomass)]/(no. days).

Pot experiment 2: response of species to AMF and nutrient addition across landslide and gap soils

In experiment 2, we examined the importance of both nitrogen and phosphorus on seedling growth in the presence or absence of a standardized AMF mix consisting of pure cultures of four AMF species (*Acaulospora scorbiculata*, *Glomus fasciculatum*, *G. geosporum*, and an undescribed *Glomus*; see Appendix C for inocula preparation). The purpose of the AMF treatment was to examine the overall effect of AMF on

seedling growth. Therefore, we used previously established cultures originally isolated from soils of BCI, but not from our gap or landslide locations. However, spores matching those of the cultures were found in both habitat types. We grew seedlings of each species in 2-L pots filled with sterile soil originating from either landslide or gap sites. Half of these pots received live AMF inoculum, while the remaining half received autoclaved inoculum. Pots with autoclaved inoculum also received 20 mL of a filtrate of live AMF inoculum washed with tap water through filter paper to control for any potential differences in nutrients and other microbes (e.g., bacteria) associated with the AMF inoculum (e.g., Reynolds et al. 2006). Finally, we added nitrogen or phosphorus (0.06 g of KNO_3 or 0.04 g of KH_2PO_4, respectively; application rate from Yavitt and Wright 2008) to a third of the pots of each AMF treatment, once at the beginning of the experiment and then again every month. Each soil–AMF–nutrient combination was replicated eight times, with two pots of each combination placed randomly on one of four benches (blocks) in the growing house. Plants were grown under 11% full sun for 60 d.

Two analyses of RGR were performed for this experiment. First, we tested whether RGR of the two species differed depending on soil type by constructing a four-way ANOVA in which soil type, species, AMF, and nutrient addition were included as fixed effects, while block was included as a random effect. Within the four-way interaction term, we constructed a priori contrasts to examine whether each *Trema* species performed differently depending on soil type (i.e., significant species × soil interaction), in the presence of AMF, and separately, in absence of AMF. Second, we constructed two separate three-way ANOVAs (per soil type) to explore seedling response to nutrient and AMF addition. Separate ANOVAs for each soil type were constructed to facilitate interpretation of treatment interactions. Both models included species, AMF, and nutrient addition as fixed effects, while block was included as a random factor. We then constructed a priori contrasts to explore the importance of N and P addition to seedling growth and its relation to the addition of AMF (see Appendix C for more details). Because these contrasts were not orthogonal, significance levels were adjusted for multiple comparisons using the Dunn-Sidak correction.

Pot experiment 3: response of species to live whole-soil inoculum from landslides and gaps

We used a whole-soil inoculum (i.e., fine roots, rhizosphere soil, and associated biota) originating from landslides or gaps to explore the effects of the entire soil microbial community (AMF and other microorganisms such as pathogens, parasites, and fauna) on the growth of the two species. This experiment was designed to (1) examine the potential contribution of habitat-specific differences in soil biota to differential seedling growth

between species and (2) compare the relative importance of abiotic vs. biotic soil components of landslides and gaps in influencing seedling performance of the two species.

Seedlings of each species were grown in 3.8-L pots that were filled 70% with autoclaved soil from landslides or light gaps. Half of the pots of each soil–species combination were inoculated with a whole-soil inoculum (100 g per pot) collected and pooled from beneath either sapling or adult *Trema* trees growing on five landslides or five forest gaps. Each of the eight soil–species–inoculum combinations was replicated eight times and plants were grown under 31% full sun for 90 d. Species and inoculum source effects on relative growth rate and root:shoot ratio was assessed using a three-way fixed effects ANOVA. We then used a priori contrasts to compare seedling growth between both species when each was grown in their own soil type and inoculum (see Appendix C for details).

Experiment 4: survival and growth of species transplanted to landslides and gaps

To examine whether growth and survival differences observed in pot experiments also occurred under field conditions, we transplanted seedlings of both *Trema* species previously grown in sterilized soil to forest gaps and landslides. We selected four 15×15 m canopy gaps in Buena Vista Peninsula of the BCNM (see Pearson et al. 2003 for site description), and in each we cleared $\sim$180 m^2 of regenerated vegetation prior to planting. Four recent landslides with exposed mineral soil were selected along the Lake Gatun shoreline on BCNM at Miller Cove, Peña Blanca Peninsula, and Bohio Peninsula. Landslide areas selected for seedling transplants were chosen to avoid gullies and areas of active soil movement and consisted of mineral soil without litter or vegetation. At each site, we transplanted 10 individuals of each species randomly within a grid with plants >2 m apart. Seedlings were $\sim$15-cm tall when transplanted and were placed in 30 cm high cylindrical poultry-wire cages covered with 50% shade cloth for the first two weeks to reduce transplant shock. Cages were intended to protect small seedlings from physical damage; cages were open-topped and therefore seedlings remained accessible to large browsers and insect herbivores. We replaced dead seedlings only during the first two weeks after transplanting; those that died after this period were not included in growth analyses (see Appendix C for details).

Prior to transplanting, we grew seedlings in a shade house for four weeks in a 1:1 mixture of autoclaved soil and live inocula (i.e., whole soil) collected from habitats to which each seedling was subsequently transplanted. At transplant, a subsample of 10 seedlings of each species was dried at 60°C to determine initial biomass. We monitored total leaf area, percentage of leaf loss to herbivores, and seedling survival every two weeks. Surviving seedlings (including roots) were harvested after five months in the field. We determined percentage of AMF colonization (Giovannetti and Mosse 1980) for all surviving seedlings, while foliar nutrient concentrations were measured from a single seedling of each species surviving at each site. Total N was determined using a CHN analyzer (Costech Analytical Technologies, Valencia, California, USA); total P, K, Ca, and Mg were determined by ICP (Perkin Elmer Instruments, Shelton, Connecticut, USA) following 1:200 (mass/volume) digestion in concentrated nitric acid.

We compared seedling survival curves for each species × habitat combination using the Kaplan-Meier method (Fox 2001). We analyzed RGR, percentage of leaf herbivory, and AMF colonization separately using mixed-model ANOVA for split-plot design. Each mixed model included species and habitat (and their interaction) as fixed effects, while site(habitat) and species × site(habitat) were included as random effects. Because models were unbalanced due to differential seedling mortality across sites, models were fitted using the restricted maximum likelihood (REML) method (Littell et al. 1996). We analyzed foliar nutrient concentrations using a two-way MANOVA.

RESULTS

Soil nutrient and mycorrhizal fungal communities on gaps and landslides

Field soil analyses showed that landslides had almost threefold higher contents of P compared to gaps and similar contents of all other measured nutrients (NO_3, NH_4, Al, Ca, Fe, K, and Mg) (Appendix B: Table B1). We isolated an average of 1196 ± 809.5 (mean $\pm$ SE) AMF total spores in each gap and 1005 ± 641.6 spores in each landslide. We identified 29 AMF species based on spore morphology across all landslide and gap sites, including 20 species from the genus *Glomus*, eight species from the genus *Acaulospora*, and a single species of *Scutellospora*. Of these, five species were restricted to gap sites, while two species were restricted to landslides. When considering both presence and absence of species and differences in spore abundances, the community composition of AMF spores differed significantly between landslide and gap sites (ANOSIM, $R = 0.376$, $P = 0.0261$; Appendix B: Fig. B1).

Experiment 1: response of species to sterilized gap and landslide soils

We found that seedlings grown in sterilized soil from landslides had higher total survival than seedlings grown in sterilized soil from gaps ($\chi^2 = 45.1$, $P < 0.001$; Fig. 1A). Relative survival differed depending on soil source and species identity. In sterilized landslide soil, 59 of 60 seedlings survived and there was no difference in survival between species (Wilcoxon $\chi^2 = 1.0$, $P = 0.317$; Fig. 1A). However, in sterilized gap soil, survival of gap *Trema* (20 of 30 seedlings) was significantly higher than that of landslide *Trema* (4 of 30 seedlings) (Wilcoxon $\chi^2 = 15.7$, $P < 0.001$; Fig. 1A). Seedlings also
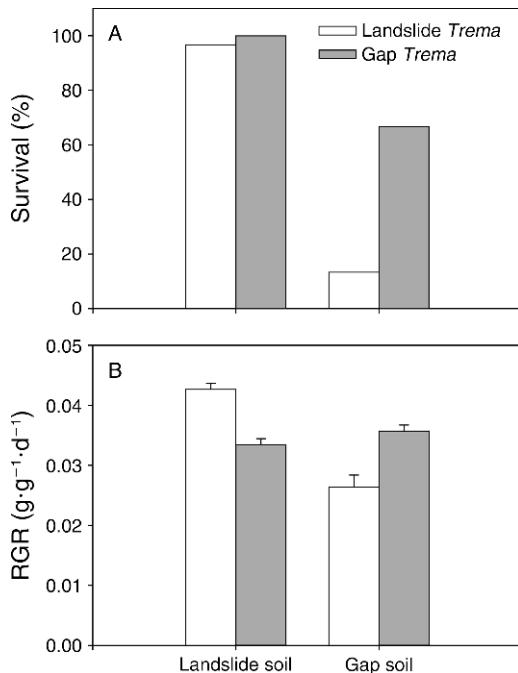
FIG. 1. (A) Survival and (B) relative growth rate (RGR) of surviving seedlings of two cryptic species of *Trema micrantha* (Neotropical pioneer trees) when grown on sterilized landslide and gap soils in the shadehouse (experiment 1; $N = 6–30$ seedlings) at Barro Colorado Nature Monument in Central Panama. The RGR data are given as least-square means + SE.

grew faster in sterilized soil from landslides than in sterilized soil from gaps (soil type, $F_{1,78} = 37.17$, $P < 0.001$; Fig. 1B). Furthermore, we found a significant species × soil interaction ($F_{1,78} = 65.34$, $P < 0.001$) indicating that each species grew fastest in sterilized soil from its corresponding habitat (Fig. 1B).

Experiment 2: response of species to AMF and nutrient addition across landslide and gap soils

Consistent with experiment 1, we found that seedlings of each species grew fastest in soil from their own habitat (species × soil contrast interaction, $F_{1,165} = 5.15$, $P = 0.025$; Fig. 2), but only when inoculated with AMF. In contrast to experiment 1, there was no evidence for an interaction between soil type and species in treatments in the absence of AMF (species × soil contrast interaction, $F_{1,165} = 1.10$, $P = 0.297$; Fig. 2).

When each soil type was analyzed separately, we found that seedlings of landslide *Trema* had significantly higher RGR than seedlings of gap *Trema* when grown in landslide soil (species, $F_{1,81} = 71.96$, $P < 0.001$; Fig. 2, Appendix B: Table B2). However, RGR did not differ between the two species when grown in gap soil (species, $F_{1,81} = 0.17$, $P = 0.69$). Seedlings had much higher RGR when AMF was added to gap soil (AMF, $F_{1,81} = 608.79$, $P < 0.001$; Fig. 2). In contrast, AMF inoculation did not significantly improve seedling RGR in landslide soil (AMF, $F_{1,81} = 3.64$, $P = 0.060$; Fig. 2).

Nutrient addition significantly affected seedling performance, but the relative importance of N and P addition on growth depended on soil type. In landslide soil, only N addition significantly increased seedling RGR ($F_{1,81} = 7.44$, $P = 0.016$; Fig. 2), with the increase consistent across AMF treatments (no AMF × N addition interaction). In gap soil, only P addition increased RGR (P addition, $F_{1,81} = 38.98$, $P < 0.001$), especially in the absence of AMF (AMF × P addition, $F_{1,81} = 12.22$, $P = 0.002$; Appendix B: Table B2).

Consistent across *Trema* species grown with AMF, both nutrient addition and soil type significantly influenced AMF colonization (species, $F_{1,74} = 0.67$, $P = 0.416$; nutrient addition, $F_{1,74} = 16.34$, $P < 0.001$; soil type, $F_{2,74} = 8.81$, $P = 0.004$; all interactions were nonsignificant). Phosphorus addition, but not N addition, significantly reduced AMF colonization relative to seedlings not receiving additional nutrients. Consistent across species and nutrient treatments, seedlings grown in landslide soil had lower AMF colonization (Appendix B: Fig. B2). In addition, we detected low levels of a superficial "brown" septated fungus (~6% colonization)
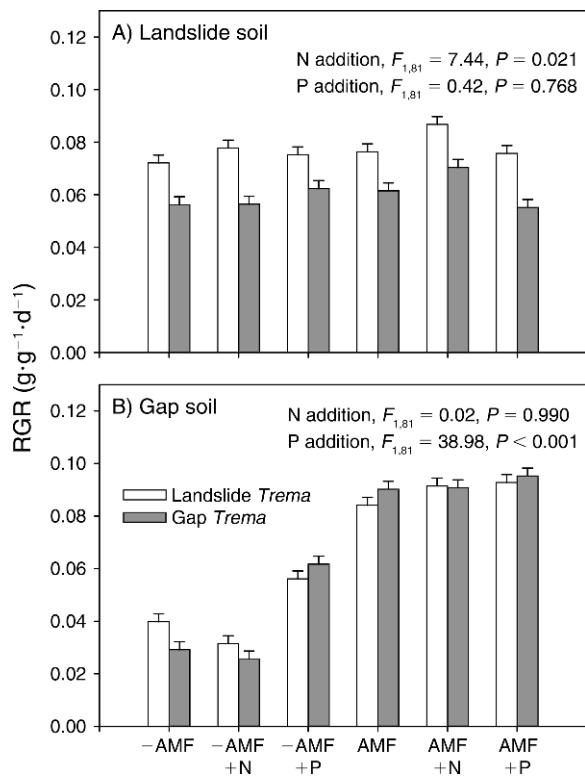

FIG. 2. Relative growth rate of two *Trema micrantha* species grown on sterilized soil collected from either (A) landslides or (B) gaps and with or without the addition of arbuscular mycorrhizal fungal (–AMF and AMF, respectively), phosphorus (P), and nitrogen (N) (experiment 2; least-square means + SE; $N = 8$ seedlings). Seedlings in the AMF treatment received an identical mixture of inoculum of four AMF species. Seedlings in the –AMF treatment received sterilized AMF inoculum plus 20 mL of microbial filtrate.
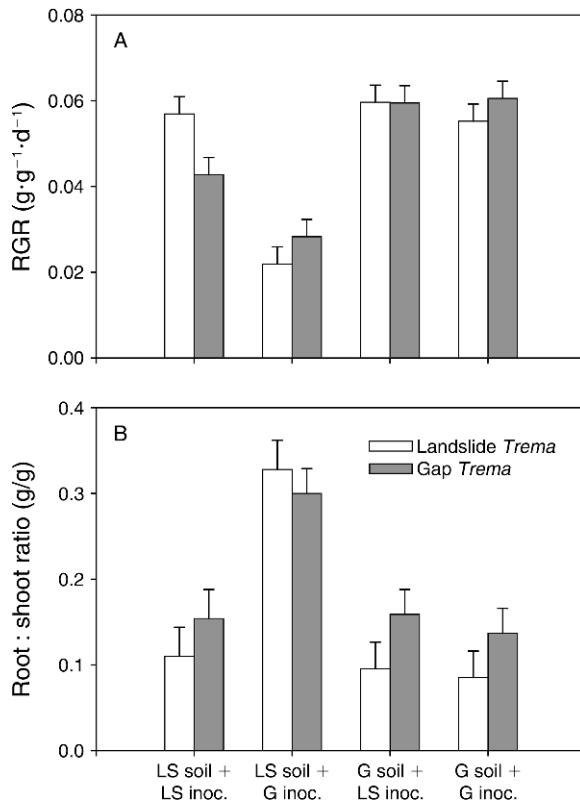
Fig. 3. (A) Relative growth rate (RGR) and (B) root : shoot ratios of the two *Trema micrantha* species grown in either sterilized soil from gaps (G) or landslides (LS) and with live whole-soil inocula (inoc.) obtained from *Trema micrantha* adults established in either gaps or landslides (experiment 3; least-square means + SE; $N = 8$ seedlings).

that could have contributed to plant performance. However, we found no visual evidence that this fungus caused root damage.

Experiment 3: response of species to live whole-soil inoculum from landslides and gaps

We found a significant interaction between inoculum and soil source (inoculum × soil, $F_{1,48} = 17.71$, $P < 0.001$), indicating that the RGR of seedlings (averaged across species) differed depending on the combination of soil and inoculum provided (Fig. 3A, Appendix B: Table B3). Seedlings obtained the highest RGR when grown in sterilized soil from gaps inoculated with live-soil inoculum from landslides (Fig. 3A). In contrast, RGR was greatly reduced by the combination of sterilized landslide soil and live-soil inoculum from gaps (Fig. 3A), indicating an apparent incompatibility between the abiotic soil component of landslides and the soil microbial community of gaps. Similarly, shoot : root ratios differed depending on the soil type and origin of the microbial community (inoculum × soil, $F_{1,48} = 20.33$; $P < 0.001$; Fig. 3B, Appendix B: Table B3); seedlings allocated a larger fraction of their biomass to roots when

grown with landslide soil and gap soil microbial community.

Consistent with experiments 1 and 2, the a priori contrast comparing RGR for the soil and inoculum combinations that occur naturally indicated that each species grew fastest with both the soil and inoculum from their native habitat ($F_{1,48} = 6.16$, $P = 0.017$). This response was largely due to the biotic component of the soil. We found that each *Trema* species responded differently depending on the source of live inoculum, with each species growing faster with soil biota from their corresponding habitat (species × inoculums, $F_{1,48} = 5.76$, $P = 0.021$; Fig. 3A). In contrast, the lack of a species × soil type interaction (Appendix B: Table B3) indicated that in the presence of microbes, each species responded similarly to the abiotic component of gap and landslides soils.

Experiment 4: survival and growth of species transplanted to landslides and gaps

We found that survival of seedlings of each species was highest in its corresponding habitat type; more seedlings of the landslide species survived on landslides (Wilcoxon $\chi^2 = 6.86$, $P = 0.009$) and more seedlings of the gap species survived in gaps (Wilcoxon $\chi^2 = 4.10$, $P = 0.043$; Fig. 4A). Stem damage, apparently due to herbivory either by browsing mammals or possibly by large insects, was the primary cause of seedling mortality in both species when grown on landslides. Browsing eliminated a larger number of gap *Trema* individuals (19
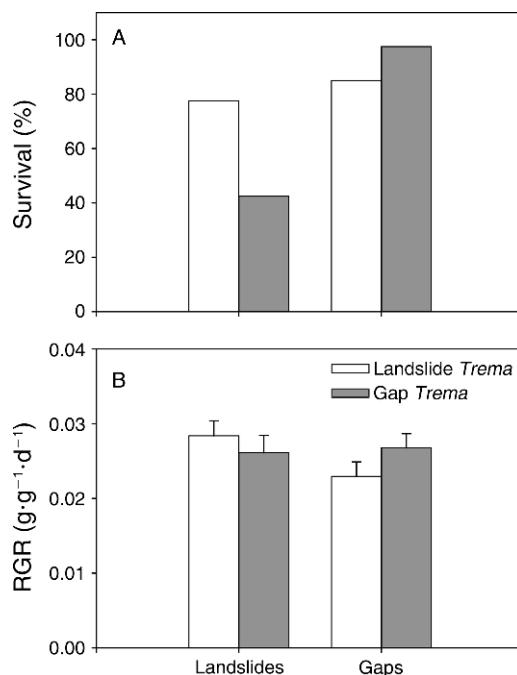

Fig. 4. (A) Survival and (B) relative growth rate (RGR) of the two *Trema micrantha* species transplanted into either landslides or light gaps in the field (experiment 4; least-square means + SE).

of the 23 seedlings that died), while such damage caused all mortality of landslide *Trema* (nine seedlings). No mortality due to stem damage occurred in seedlings of either species grown in gaps. There was no significant effect of habitat or species on the proportion of leaf area consumed by herbivores (habitat, $F_{1,6} = 0.54$, $P = 0.492$; species, $F_{1,5} = 2.88$, $P = 0.146$; habitat $\times$ species, $F_{1,5} = 1.29$, $P = 0.304$). Among the surviving seedlings, neither the main effect of habitat or species influenced RGR (habitat, $F_{1,6} = 0.96$; $P = 0.369$, species, $F_{1,7} = 1.21$; $P = 0.308$). However, as indicated by the significant habitat $\times$ species interaction ($F_{1,7} = 5.38$; $P = 0.046$), each *Trema* species had higher RGR in habitat in which it occurs naturally (Fig. 4B).

We found that overall concentrations of foliar nutrients differed significantly between habitats, but not between species (habitat, Wilks' $\lambda = 0.14$, $F_{5,7} = 8.32$, $P = 0.007$; species, Wilks' $\lambda = 0.47$, $F_{5,7} = 1.59$, $P = 0.279$; habitat $\times$ species, Wilks' $\lambda = 0.49$, $F_{5,7} = 1.44$, $P = 0.319$). In particular, foliar N concentrations were higher in seedlings grown in gaps, whereas foliar P concentrations were higher in seedlings grown on landslides (Appendix B: Table B4). We found that landslide *Trema* had higher percentage of root colonization by AMF than did gap *Trema* in both habitats (species, $F_{1,6} = 7.50$; $P = 0.034$; habitat, $F_{1,6} = 3.18$, $P = 0.125$; species $\times$ habitat, $F_{1,6} = 0.29$, $P = 0.61$).

DISCUSSION

Our study demonstrates the contribution of both abiotic (soil nutrients) and biotic (soil microbes and herbivores) factors influencing habitat segregation between cryptic plant species in a tropical forest. We found that different properties of the soil interacted and thereby augmented one another's contribution to the segregation observed between forest gap and landslide habitats of two species of *Trema* in central Panama. Specifically, we found that: (1) nutrient properties of these habitat types differed and seedlings of each species benefited from the sterilized soil from their own habitat (experiment 1); (2) growth response to a standardized mixture of AMF in both *Trema* species was site dependent, with higher response found in P-poor gap soils (experiment 2); (3) soil microbial communities from each habitat favored the corresponding *Trema* species (experiment 3); and (4) the effects of soil biotic and abiotic properties were further augmented on landslides by the effect of herbivores, where >50% of gap *Trema* were killed by browsing (experiment 4).

Landslides and forest gaps differ in the availability of phosphorus and nitrogen

We found that landslides and large forest gaps occupied by *Trema* differed in the availability of two key soil resources. Extractable P concentrations in gaps were less than half that of landslides, whereas N concentrations were marginally lower in landslides (Appendix B: Table B1). We found strong evidence for P limitation in gap soils and N limitation in landslide soils. In experiment 2, the addition of P significantly increased growth rates of both *Trema* species grown in gap soils, whereas such addition provided no benefit for seedlings grown in landslide soil. Further, foliar P concentrations were lower in seedlings of both species transplanted into gaps than those transplanted into landslides. In landslide soil, we detected a significant effect of N addition on seedling growth. Further, field-grown seedlings of both species on landslides had one-fifth of the foliar N concentrations that seedlings had when grown in gaps. Greater availability of phosphorus in landslides was most likely due to increased exposure to weathering of bedrock (Dalling and Tanner 1995), whereas greater availability of nitrogen in gap soil was most likely due to higher inputs of organic matter that can be mineralized into plant-available forms of N (Guariguata 1990, Dalling 1994, Dalling and Tanner 1995). Alternatively, it is possible that autoclaving promoted higher nutrient availability in gap soils because of its higher organic content. However, this is inconsistent with growth patterns found in experiments 1 and 2 in which overall seedling growth was higher in landslide soils compared to gap soils.

Habitat differences in nutrient limitation observed in our study could lead to habitat segregation in *Trema* if species differed in their N and P requirements (Tilman 1977, Baltzer et al. 2005). Specialization to some aspect of abiotic soil properties was evident in the first experiment as each *Trema* species exhibited increased growth and survival when grown in sterilized soil of their corresponding habitat. However, specialization to N and P was not supported by our subsequent experiments in which seedlings were exposed to only AMF or complete soil communities. In the fertilization experiment (experiment 2), both species responded similarly to P and N addition, and seedlings of both species planted in the field (experiment 4) did not differ in P and N foliar concentrations. Growth response detected in the first experiment could have been due to unmeasured soil properties such as texture or other soil nutrients. However, differences in background availabilities of P and N, alone, appear to be insufficient to explain habitat segregation in more realistic conditions in which soil biota are present.

General effects of AMF and their interaction with soil nutrients in influencing Trema performance

Consistent with previous studies (Smith et al. 2004, Reynolds et al. 2006), we found that the benefit that AMF provided to both *Trema* species was habitat-specific and was correlated with P availability (experiment 2). Further, the significant reduction in AMF colonization both in response to phosphorus addition and when seedlings were grown in P-rich landslide soils further supports the importance of P in influencing *Trema*–AMF interactions. The addition of AMF greatly improved seedling growth of both species in P-limited

gap soil. However, AMF provided no advantage to seedlings grown in P-rich landslide soil. The dependence of *Trema* on AMF for early growth in P-limited gap soil is typical of other small-seeded pioneer tree species for which seed reserves are quickly exhausted and roots alone are insufficient to uptake scarce availabilities of P (Kiers et al. 2000, Zangaro et al. 2003).

Species-specific differences in the general response to AMF can reinforce segregation across habitats differing in phosphorus. For example, Schultz et al. (2001) showed that one ecotype of the grass *Andropogon gerardii* occurred in soils with low P availability and showed a high dependency on AMF. In contrast, a second ecotype occurred in soils with high P availability and showed low dependency on AMF, but instead was characterized by a finer root system. Similarly, in experiment 2, we found some evidence for greater dependence of gap *Trema* on AMF. Whereas landslide *Trema* exhibited a growth advantage in gap soil lacking AMF, this advantage was lost with the addition of AMF. Instead, greater responsiveness to AMF by gap *Trema* resulted in a growth advantage that favored this species in its own soil.

Effect of soil microbial communities on Trema *growth performance*

Although additional molecular work is required to characterize root-associated AMF communities, we showed that AMF spore communities sampled near naturally occurring *Trema* trees differed significantly between habitat types. Such differences in AMF composition (and other components of the soil microbial community) are known to correlate with variation in both soil chemical properties (Johnson and Wedin 1997) and in host species composition (Bever 2002, Husband et al. 2002, Mangan et al. 2010), which we observed between these two habitats.

In experiment 3, we found that each species grew fastest with its own soil type and soil community and that this pattern was linked to soil microbial composition rather than soil abiotic properties. Specifically, the significant species × inoculum interaction indicated that the relative performance between the two *Trema* species shifted depending on the source of soil biota. In contrast, we found no evidence that such shift in performance between the two *Trema* species was due to soil type (i.e., lack of a species × soil interaction; Appendix B: Table B3). Differences in plant growth in response to differing community composition of soil biota is well documented in both temperate (Mills and Bever 1998, Klironomos 2002, Reynolds et al. 2003) and tropical (Kiers et al. 2000, Mangan et al. 2010) plant communities.

Interestingly, we detected a strong depression in growth and a large corresponding increase in root : shoot ratios when both *Trema* species were grown in landslide soil with gap inoculum (Fig. 3). Growth rates of seedlings with this treatment combination were compa-rable to seedlings grown in sterile soil, and biomass allocation to roots was twofold higher compared to other treatments. Increased allocation of total plant biomass to roots in both *Trema* species suggests that the AMF species naturally occurring in forest gaps are less efficient in uptake of available forms of nutrients found in landslide soils. Similar AMF-dependent effects on plant allocation have been previously reported for grassland plant species (Bever et al. 2009). Alternatively, the physical environment of landslide soil may favor organisms found in gap inoculum that directly exert negative effects on plant roots (see Ross 1980, Tommerup 1985) or are detrimental to the establishment of beneficial rhizosphere mircoorganisms (Wilson et al. 1988). Further experiments are needed to choose among these potential alternatives.

Trema *growth and survivorship in the field*

In our reciprocal transplant experiment, we found that differences in susceptibility to herbivory occurred even between cryptic species. Differences in survival between the two *Trema* species on landslides were caused by apical browsing damage, which resulted in twice the mortality of gap *Trema* seedlings over landslide *Trema* seedlings over five months. Differences in susceptibility to herbivory between *Trema* species on landslides were consistent with differences in their physical defenses, as landslide *Trema* had significantly tougher leaves and stems than gap *Trema* (Appendix A). This finding suggests that the two *Trema* species are adapted to differential herbivory pressure on landslides compared to gaps, such that landslide *Trema* allocates more resources to defense (i.e., tougher stems and leaves). Alternatively, susceptibility to herbivory could be linked to soil fertility, such that plants adapted to nitrogen-poor landslide soils are better defended against herbivores compared to plants adapted to nitrogen-rich gaps soils (Fine et al. 2004, 2005).

In contrast, browsing damage was not observed on either *Trema* species grown in treefall gaps. Thus, although differential herbivory can account for the absence of gap *Trema* in landslides, it cannot account for the absence of landslide *Trema* in gaps. Additional factors such as the experimentally demonstrated home-site advantage effects due to different soil abiotic and biotic properties need to be invoked. Indeed, we detected an identical pattern of home-site advantage in growth (and survival) when *Trema* seedlings were planted in the field, thereby suggesting an important role of soil in determining gap *Trema*'s success in treefall gaps.

Implications for speciation

Increased opportunities for the evolution of specialized interspecific interactions in species-rich tropical forests suggest that these ecosystems are likely to harbor many currently undescribed cryptic species that are nonetheless ecologically and functionally distinct (Bickford et al. 2007). Although largely ignored in the tropics,

our study demonstrates the importance of specialized interactions with abiotic and biotic soil properties as potential forces leading to incipient speciation. Coupled with aboveground herbivory, we can account for habitat segregation and ecological differentiation between these closely related tree species. If studies on gene flow between these two habitat types corroborate our findings, then we have uncovered an ideal model system for studying the importance of above- and belowground processes to plant speciation in the tropics.

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APPENDIX A

Characteristics, distribution, and phylogenetics of the two *Trema* cryptic species (*Ecological Archives* E092-005-A1).

APPENDIX B

Soil characteristics of gaps and landslides, statistical analyses of experimental results, composition of arbuscular mycorrhizal fungal (AMF) communities in the field, and AMF colonization in experiment 2 (*Ecological Archives* E092-005-A2).

APPENDIX C

Methods used for experiments 2 and 3 and the field experiment (*Ecological Archives* E092-005-A3).