

The relative importance of light and soil environment in seedling establishment should vary across habitats. In the forest understory light is likely to be most limiting for survival and growth, while in open sites, nutrients and water may be more likely to limit survival and growth (Burslem 1996). Root competition may be an important factor in both environments.

Soils can differ not only in their abiotic resources, but also biotic ones. Bacterial and earthworm diversity and functions are known to differ between forest and pasture (Nusslein and Tiedje 1999, Decaens et al. 2004). Fungi are also likely to be of importance to seedling establishment, yet their variability across these habitats is unknown. In black cherry, Packer and Clay (2000) found that soil fungi associated with adult trees reduced seedling survival.

During the last century, abandoned pastures have greatly increased in abundance in tropical forested landscapes. Despite their ubiquity, very few studies have used abandoned pastures to compare seedling establishment between forest and pasture (Gerhardt 1993). Yet, understanding factors which influence seedling establishment in pastures is essential to predicting population dynamics of plant species in abandoned pastures and the trajectory of secondary succession (Aide and Cavellier 1994, Marcano-Vega et al. 2002).

In this study, I used the tropical forest tree species *Terminalia amazonia* (J. F. Gmel.) Exell (Combretaceae) as a model forest tree to examine the role of soil environment in seedling establishment in habitats that differ dramatically in light availability. I conducted a reciprocal-transplant experiment in replicated field sites in southern Costa Rica, in which I planted seedlings of *T. amazonia* inoculated with either forest or pasture soil into forest and pasture, to test whether there were differences in seedling survival and growth between habitats and between soils. Based on previous work on seedling

establishment in light gaps and understory, it seemed likely that pasture would prove more favorable to seedling establishment than forest, due both to higher photosynthetic rates in pasture and to escape of seedlings from pathogens. I expected that forest soil would be more conducive to seedling establishment than pasture soil, due to the association of beneficial microorganisms found in forest soil with seedling root systems. I also expected to observe an interaction of habitat with soil inoculum, in which seedlings inoculated with pasture soil and grown in the forest understory would experience reduced mortality relative to seedlings inoculated with forest soil because fewer pathogens of tree seedlings would occur in pasture than forest soil.

## METHODS

### *Study species*

*T. amazonia* is a common canopy tree found in the humid tropics from Mexico to Brazil (Croat 1978). Seeds are small, wind-dispersed and appear to lack dormancy. Flowering and fruiting in southern Costa Rica occur from February-April; seeds are observed germinating on the forest floor at the start of the wet season (May-June). Mortality of germinating seeds may typically exceed 99% (*personal observation*). The genus has a pantropical distribution with many species of local importance for forestry (Record and Hess 1943).

### *Study sites*

The study was conducted on three adjacent farms in the community of Siete Colinas, 7 km NE of the town of San Vito, Coto Brus, Puntarenas Province, in southern Costa Rica at ~850 m of elevation (8°53' N, 82°57' W; Figure 1.1). Vegetation is a mosaic of coffee and corn fields, pastures, and small (0.5-7 ha) fragments of Tropical Premontane Wet Forest (Holdridge Life

Zone System; Tosi 1969). Soils are Andepts (Vásquez Morera 1983). The study area receives 3000-4000 mm of rainfall yearly, concentrated in a wet season lasting from May-December (Coen 1983).

At each site, seedlings were planted into paired pasture and forest habitats. Habitat pairs were <1 km apart (Figure 2.1). The three sites have similar land-use histories. Further detail on these sites is provided in Chapter One. Forest at sites currently in forest was mature at the time of Costa Rican settlement of the area ca. 1970 (Juan Cascante, *personal communication*; aerial photos). The largest canopy trees in the forest fragments are *T. amazonia*, with an understory dominated by species in the genera *Miconia* and *Psychotria*. Pastures were converted from forest in the mid-1970s and improved with introduced grass species during the 1980s and early 1990s (José Ramírez, *personal communication*). Common plant species in pastures include two introduced grasses in the genus *Urochloa* and bracken fern, *Pteridium aquilinum*. The three sites were fenced to exclude cattle for the duration of the experiment.

#### *Inoculation of experimental plants*

Seed was collected from five *T. amazonia* trees in the study area in April 1997, mixed and sown onto either forest or pasture soil in six germination beds under shade in a common garden (Plate 2.1) within 1 w of soil collection. Soil for germination beds was collected at a depth of 0-20 cm from 20 randomly chosen points in each habitat at each of the three sites, mixed and sieved through 1-cm diameter hardware cloth. Hardware cloth was sterilized by rinsing with 5% (v/v) sodium hypochlorite between sievings. Each germination bed was filled with soil from pasture or forest at a single site. Germination beds were kept moist with tapwater.

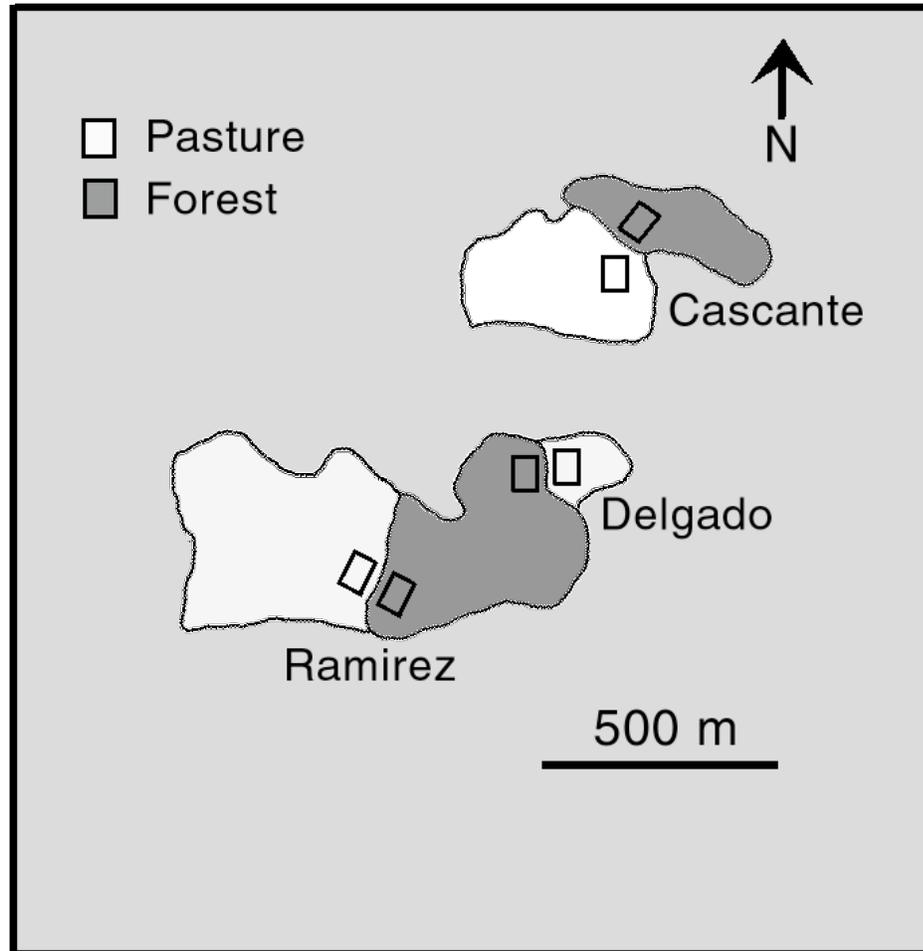


Figure 2.1 Locations of paired forest and pasture plots (rectangles, approximately to scale) in lower Siete Colinas, Coto Brus, Costa Rica. Surrounding vegetation (shown in light gray) is a mosaic of coffee and corn fields, pastures, and home gardens. Sites are named for the farms on which the pasture plots were established.



Plate 2.1 Germination beds with *Terminalia amazonia* seeds (left) and approximately one-month-old seedlings (right; photo by author).

Seedlings began to germinate after 1 mo. From late June to early July 1997, as seedlings produced first true leaves, they were transplanted from germination beds to individual 20-cm-deep plastic planting bags under shadecloth in the common garden. Bags contained pasture or forest soil collected <10 d previously in the same manner as outlined above; to minimize cross-contamination, they were grouped by habitat, with groups randomized by site. Seedlings were watered daily with tapwater. Two weeks prior to outplanting, shadecloth was removed from seedlings to be planted in pasture in order to facilitate acclimation.

### *Outplanting*

From 14-18 August 1997, 50 seedlings inoculated with forest soil and 50 seedlings inoculated with pasture soil were planted into each habitat at each site, for a total of 598 seedlings (due to low germination of seedlings in Cascante pasture soil, only 98 pasture soil-inoculated seedlings were available for this site). Seedlings were planted into the same site from which soil used to inoculate them originated (e.g. seedlings inoculated with forest soil from the Cascante site were planted into forest and pasture only at the Cascante site). Each seedling was planted into a 20 × 20 × 20 cm hole filled with either forest or pasture soil collected 1-2 d previously, as described above (Plate 2.2). Planting holes were randomly assigned to either soil inoculation treatment. Seedlings were planted 1.5-2 m apart. In the pastures, aboveground vegetation within ~30 cm of each seedling was cleared at the time of planting and periodically clipped throughout the experiment to reduce competition of seedlings with pasture grasses.



Plate 2.2 A three-month-old seedling of *Terminalia amazonia* being transplanted into pasture at the Cascante site. Author is kneeling on *Urochloa ruziziensis*, a non-native forage grass referred to by local farmers as guinea rastrea (photo by Steve Travers).

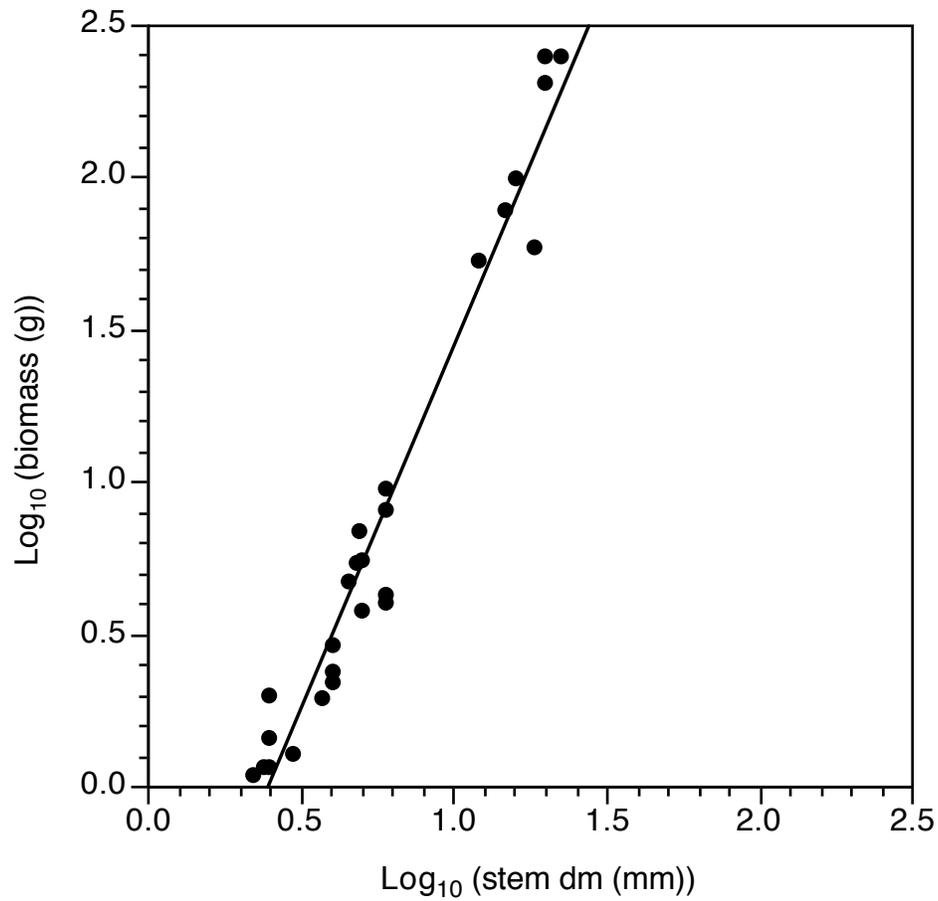


Figure 2.2 Relationship between stem diameter and biomass in *Terminalia amazonia* seedlings at three study sites in Siete Colinas, Coto Brus, Costa Rica ( $n = 29$ ;  $r^2 = 0.952$ ;  $P < 0.0001$ ).

### *Seedling survival and growth*

The number of seedlings surviving in each treatment at each site was assessed once every 1-3 mo. Stem length was measured on all seedlings at outplanting, and stem length and diameter at base were measured at months 5, 7, 12, 20, 22 and 24 after outplanting (stem diameter could not be determined at outplanting, as seedlings were too small and delicate to measure accurately). To determine the relationship of stem characteristics to biomass, over the course of the experiment twenty seedlings across a range of sizes (and ages) were harvested and dried to constant weight. Stem length proved to be a very poor predictor of biomass (*data not shown*). However, biomass was closely related to stem diameter (Figure 2.2). Using the formula derived from the relationship of stem diameter to biomass in these individuals ( $\log_{10}y = 2.453(\log_{10}x) - 1.003$ ), stem diameter measurements were converted to estimated biomass for ease of interpretation in the figures.

Differences in survival between habitats (forest vs. pasture), soil inocula (forest vs. pasture) and sites were detected by proportional hazards analysis. Using proportional hazards analysis, mortality risk ratios were calculated for the two levels (forest vs. pasture) of the two factors of principal interest (habitat and soil inoculum). Differences between soil inocula and sites in stem diameter (indicator of biomass) of seedlings six and 24 mo after outplanting were detected by fixed-effects two-factor ANOVA, followed by linear contrasts. Due to large differences between habitats in sample size as a result of differential mortality, stem diameter data were analyzed separately for forest and pasture. No analysis was conducted for seedlings in forest at 24 mo, since only nine individuals remained. To satisfy model assumptions, stem diameter data were  $\log_{10}$ -transformed prior to analysis. Statistical analyses

were conducted in JMP version 3 (SAS Institute 1994). Differences in biomass within treatments over the course of the experiment were also detected separately for pasture and forest seedlings using repeated measures ANOVA. For pasture seedlings, soil inoculum and site were treated as between-subject effects, and time, soil inoculum and site as within-subject effects. For forest seedlings, site was not included (since seedlings only survived to the end of the experiment at a single site). These two analyses were conducted using the generalized linear models procedure in SAS version 8 (SAS Institute 2002).

#### *Light and relative humidity*

Seedling light environment was evaluated by measuring photosynthetically active radiation (PAR) with a Decagon Sunfleck Ceptometer (Decagon, Pullman, Washington, USA). PAR was measured once directly above each (living or dead) seedling in forest habitats in the late dry season (April 1998). Light measurements were taken between 930-1230 h under cloudless skies. A PAR measurement was taken immediately above vegetation in the center of the pasture every 30 min, so that the percentage of full sunlight reaching each forest understory seedling could be determined. All pasture-grown seedlings were assumed to be in full sunlight, since no seedlings were shaded by other individuals at the time of sampling. The effects of light environment and soil inoculum on mortality of forest seedlings at the time of sampling were tested by logistic regression in JMP (SAS Institute 1994).

Relative humidity (RH) in pasture and forest was evaluated using two HOBO® data loggers (Onset Computer Corporation, Pocasset, Massachusetts, USA). Each data logger was placed 1 m above the ground in

the center of the area where seedlings were planted in each habitat, shielded from rain and direct sunlight, and alternated between sites and habitats every 1-3 d. Because dry season climate is more variable than wet season climate in southern Costa Rica, RH was measured hourly in pasture and forest at each site for 14-17 d during the dry season (April) and 4-6 d during the wet season (August). Differences in mean daily RH, minimum daily RH, and maximum daily RH between habitats, seasons and sites were detected by three-factor fixed-effects ANOVA. As site had no effect on RH, a reduced model with habitat and season was used for statistical analyses presented here. To satisfy model assumptions, mean daily RH and maximum daily RH were arcsine-transformed prior to analysis.

#### *Nutrients and mycorrhizal colonization*

Soil samples for chemical analyses were obtained once during the dry and wet seasons in each habitat at each site from August 1996-April 1999. In each habitat, ~ 600 mL soil samples were taken at a depth of 0-20 cm at ten randomly selected points and then mixed to create a single pooled sample. Pooled samples were air-dried in paper bags at room temperature, then a 300-mL subsample of each was transported in a sealed plastic bag to the Nutrient Analysis Laboratories at Cornell University, Ithaca, New York, USA for analysis. Amounts of major and minor plant nutrients were determined by extraction with Morgan's solution, followed by atomic absorption (K, Mg, Ca, Mn, Fe, Al, Zn, Cu) or colorimetry (P, NO<sub>3</sub>-N, NH<sub>4</sub>-N). Available N was estimated as NO<sub>3</sub>-N + NH<sub>4</sub>-N. Soil pH was determined using a 1:2 (v/v) suspension of sample in water. Differences in pH and extractable nutrients between habitats, seasons and sites were detected by three-factor fixed-effects ANOVA. To meet model assumptions, N, K, Zn, and Al were log<sub>10</sub>-

transformed prior to analysis. Differences between seasons across habitats were detected by linear contrasts. Other interactions were not significant.

To compare early mycorrhizal colonization between forest and pasture soil inocula, 10-11 seedlings chosen at random were destructively sampled from each germination bed in the common garden on 5-6 August 1998, 10 d prior to outplanting; no seedlings inoculated with pasture soil from the Cascante site could be sampled, due to low germination). Roots were rinsed in water to remove debris and stored in 1% KOH at 4 °C for 1 wk. The root system of each seedling was then cleared in 10% KOH (*w/v*) at 90 °C for 30 min, rinsed in water, acidified in 2% HCl (*v/v*) at room temperature for 30 min, stained with 0.05% (*w/v*) trypan blue in lactoglycerol at 90 °C for 30 min, rinsed in water for 2 h and stored in water at 4 °C (modified from Phillips and Hayman 1970, Grace and Stribley 1991). To compare effects of soil inocula and habitat on mycorrhizal colonization after outplanting, fine roots were collected on 26-28 January 1998 from three randomly-chosen points along the root system of each of 5-6 seedlings selected at random from each soil inoculum treatment in pasture and from the forest inoculum treatment in the Delgado forest. All surviving seedlings were sampled in the pasture inoculum treatment in the Delgado forest and in both soil inocula in forest at the other two sites, since fewer than five seedlings per treatment remained. Roots were prepared and stained as above.

The percentage of root length of *T. amazonia* seedlings colonized by AM fungi was estimated by the grid intersect method at 80× magnification (Giovannetti and Mosse 1980). AM fungal hyphae were distinguished from non-AM hyphae by the presence of arbuscules, vesicles, and hyphal coils, and the absence of septa. Differences between soil inocula in the percentage of

root length colonized (%RLC) one week prior to outplanting were detected by one-way ANOVA. Differences in %RLC between habitats, soil inocula and sites six months after outplanting were detected by three-factor ANOVA. As site had no effect on %RLC, a reduced model with habitat and soil inocula was used for statistical analyses presented here. To satisfy model assumptions, %RLC data were arcsine-transformed prior to analysis.

## RESULTS

### *Effects of habitat and soil inocula on seedling survival and growth*

Seedling survival differed markedly between habitats. Survival was much lower in forest (3%) than in pasture (43%) (Table 2.1; Figure 2.3). Nine months after outplanting, all 200 seedlings planted into forest at the Cascante and Ramírez sites had perished, and only 12 of the 100 seedlings planted into forest at the Delgado site were still alive. Fitting a proportional hazards model to the data yielded a risk ratio of 1.77: for every ten seedlings that die in the pasture, nearly twice that number (17.7) would be expected to die in forest (Table 2.1). The effect of habitat on survival differed slightly between sites: seedling mortality in pasture was higher, and seedling mortality in the forest was lower, at the Delgado site than at the other two sites (Table 2.1; Figure 2.3). Sources of seedling mortality differed between habitats. In forest, seedlings usually disappeared from one census to the next. When seedlings were observed dying, symptoms of damping-off were apparent (sunken lesions at base of stem, followed by rapid wilting and disappearance of leaves). In pasture, seedlings were primarily killed by herbivore damage (e.g. repeated cutting of the apical meristem by a weevil) or competition with grasses (smaller seedlings were engulfed by grasses). I also observed leaf