Are the impacts of an ecosystem engineer context-dependent? Tests with the leaf-cutter ant Atta laevigata in a Neotropical savanna.

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ABSTRACT

Species that transform habitats or create new ones are known as Ecosystem Engineers. It has been posited that ecosystem engineers benefit other species by ameliorating conditions at the stressful end of environmental gradients, but that this beneficial impact diminishes as conditions along the gradient become more benign. We tested this hypothesis along a canopy-cover gradient in Brazil's *Cerrado* with a widespread engineer of Neotropical ecosystems – the leaf-cutter ant *Atta laevigata.* We tested for changes in environmental conditions influencing seedling establishment, seedling abundance, and seedling diversity at different distances from ant nests. Local environmental conditions are influenced by both canopy cover and proximity to nests. Furthermore, the negative effect of *Atta* on seedlings overwhelms that of the gradient, but these impacts restricted to the area covered by nest mounds. The results demonstrate that some engineers amplify rather than reduce stress along environmental gradients, but that these impacts can be context-dependent and spatially-complex.

INTRODUCTION

Species that transform habitats or create new ones are known as Ecosystem Engineers (Jones et al. 1994; Jones et al. 1997), and they can have major impacts on populations, community composition, and ecosystem function (reviewed in Hastings et al. 2007; Wright and Jones 2006). To date most attention has focused on documenting the type and spatial extent of engineer impacts; less well understood is the extent to which these impacts are context-dependent (Hastings et al. 2007; Wright and Jones 2006). The principal conceptual framework put forward to address this issue is the Stress-Gradient Hypothesis (Bertness and Callaway 1994; Crain and Bertness 2006). It posits that engineers enhance species diversity and abundance along environmental gradients by ameliorating stressful conditions, but that this beneficial impact diminishes as conditions become more benign. While there is some empirical support for this hypothesis (Badano and Marquet 2009; Kleinhesselink et al. 2014), not all engineers modify habitats in ways that reduce environmental stress for other species. Evaluating the impacts of engineers such as these, and how they vary spatio-temporally, is central to advancing conceptual models of ecosystem engineering (Crain and Bertness 2006; Wright and Jones 2006).

Leaf-cutter ants (genus *Atta*) are classic examples of ecosystem engineers. They transport tons of soil to the surface as they excavate their nests, which can create a mound covering a surface area of up to 100 m^2 (Alvarado et al. 1981). This, coupled with activities related to their farming of fungal food sources in chambers up to 10 m below the surface (Moreira et al. 2004), can dramatically alter plant population and community ecology and ecosystem processes such as decomposition and nutrient cycling (Farji-Brener and Illes 2000; Leal et al. 2014). In lowland tropical forests *Atta* are the dominant herbivore in terms of both abundance and total biomass consumption (Hölldobler and Wilson 2010; Leal et al. 2014). However, they can be 2-3 times more abundant in Neotropical savannas than lowland forests (Costa and Vieira-Neto in press); in savannas they may also harvest up to 7-fold more plant biomass per hectare (Costa et al. 2008).

Throughout much of South America the savanna biome is called the *Cerrado*, and with a distribution of 2 million km^2 it is second only to Amazonia in total area (Oliveira and Marquis 2002). Like most savannas the Cerrado is not homogenous, but rather a mosaic of plant physiognomies that range from open grassland to closed woodlands (Oliveira-Filho and Ratter 2002). These vegetation types are often found in close proximity (Cardoso et al. 2009), resulting in steep local gradients in canopy cover. Previous work has shown that the amount of local canopy cover is correlated with suite of biotic and abiotic variables that exert strong effects on seedling recruitment and community composition (Salazar et al. 2012). For instance, areas with more cover can have cooler, shadier understories and more leaf-litter on the soil surface. This facilitates seedling establishment of seedlings and enhances their survival by reducing soil water deficits and increasing nutrient availability (Salazar et al. 2012). Closed-canopy sites also have lower cover of the native and invasive grasses that can inhibit seedling establishment (Hoffmann and Haridasan 2008).

If leaf-cutter ants in the Cerrado create canopy gaps, remove leaf-litter from the soil surface, decreasing soil fertility, or otherwise alter the environmental conditions that favor seedling establishment, then this should reduce local seedling abundance and diversity around their nests. The consequences of this engineering will decrease in magnitude as canopy cover becomes sparser, however, because more open areas are already hotter, brighter, and have limited litter on the soil surface because there are fewer canopy trees. Put another way, we predict that *Atta laevigata* in Neotropical savannas exacerbates environmental stress for plants rather than ameliorate it, but that its amplification of stress decreases in magnitude with decreasing canopy cover. We test for this context-dependence impact of an ecosystem engineer by addressing the following questions: (1) Does *A. laevigata* alter the environmental conditions that influence seedling establishment, and if so, does the magnitude of these modifications vary along a canopy-cover gradient? (2) Does *A. laevigata*'s environmental engineering alter patterns of seedling abundance and species richness along a canopy cover gradient as predicted by our Stress Amplification Hypothesis?

MATERIALS AND METHODS

We conducted our study at Panga Ecological Station (19°10'45''S, 48°23'44''W), a 404 ha reserve (Bruna et al. 2010) administered by the Universidade Federal de Uberlândia (UFU). The climate at Panga is highly seasonal, with mean annual temperature of ~23º and most of the ~1600 mm of annual precipitation between Ocober-April (UFU Santa Mônica Climate Station). Most of the major Cerrado vegetation types can be found at Panga Station (Cardoso et al. 2009), including *cerrado ralo* and *cerrado denso*. *Cerrado ralo* has a dense layer of grasses and herbs and sparsely distributed shrubs and trees typically <3m tall; average canopy cover in *cerrado ralo* is ~30%. *Cerrado denso* has less grass cover and more abundant trees;

because these trees can reach a height of ca. 6 m the average canopy cover in *cerrado denso* is ~60%. Because there is large variation in canopy cover within these physiognomies, there can be strong gradients in canopy cover in landscapes where they abut. In our site canopy cover along this gradient ranges from 0-95% (Mean = 52% ± 33.1 SD; Appendix A). The canopy cover over plots along the gradient is independent of plot proximity to leaf-cutter ant nests (Appendix A).

Our focal ecosystem engineer is *Atta laevigata*, whose nests are formed by workers depositing excavated soil around the main entrance to create a large nest mound. Although *A. sexdens* is also found at Panga Station it is primarily in closed-canopy forest; *A. laevigata* is the most common *Atta* species in both *cerrado ralo* and *cerrado denso* (Costa and Vieira-Neto in press). In 2010 we haphazardly selected 10 active *A. laevigata* nests in each of these two vegetation types (range = 7-37 m^2, mean = 16.7 m^2 ± 6.7 SD, N = 20); although there are also some abandoned nests we restrict our analyses to active colonies because the effects of time-since-abandonment on environmental variables is unknown. We then established three 1x2 m plots around each nest: one on the center of the nest mound, one immediately adjacent to the mound, and one 10 m from the mound edge (Appendix B).

Litter biomass in each plot was measured by collecting all litter from a randomly selected half of each plot once during the same rainy season, drying it at 50° C for 72 h, and weighing it with a microbalance. Similarly, we dried and weighed all grasses from a randomly selected half of each plot to estimate above-ground grass biomass. Canopy cover above each plot was estimated using photos analyzed with Adobe Photoshop (Adobe Systems Inc., San Jose, California, USA) and the method of Engelbrecht & Herz

(2001). We used in our analyses the average canopy cover in two photos taken during the 2010-2011 rainy season. Photos were taken with a Nikon Coolpix 950 from a height of 50 cm in either the early morning (6h) or early evening (18h).

At the end of the 2011 dry season we estimated surface soil moisture in plots by collecting a sample of the top 20 cm of soil from two points separated by 100 cm. These samples were bulked, weighed, dried at 50° C for 96h, then weighed again to estimate percent moisture content. As a proxy for soil compaction, we dropped a 1 m long x 5 mm diameter iron rod vertically from a height of 50 cm at three haphazardly selected points in each plot. We then measured the depth to which the rod penetrated the soil at each point and used the average of these values in our analyses; these data were recorded at the end of the 2010-2011 rainy season. Finally, at the end of the 2010-2011 rainy season we also counted all woody and herbaceous plants $\leq$ 120 cm tall in each plot and identified them with the help of local specialists and comparison with the collections of the UFU herbarium (HUFU). Of the 1827 stems recorded 25% could only be identified to genus and morphospecies.

During the 2011 rainy season we selected N = 10 nests for analyses of soil chemistry in plots on nest mounds and 10m from nests. We collected 5 soil samples of ~100 g samples each from each plot: one from the plot center and one from each corner. The 5 samples from each plot were bulked into a single sample and taken to the Soils Analysis Lab of the Brazilian Agricultural Research Corporation (EMBRAPA) in Uberlândia, where pH, P, K^+, Ca^{2+}, Mg^{2+}, Al^{3+}, and total organic matter were measured using their standard protocols (EMBRAPA 1997).

Does A. laevigata alter the environmental conditions that influence seedling establishment? Does the magnitude of these modifications vary along a canopy-cover gradient? Because many of the biophysical variables we measured were highly correlated (Appendix C), we used Principal Components Analysis (PCA) to summarize the environmental conditions along the canopy cover gradient. We conducted two PCAs. The first was conducted using environmental data collected in all plots (i.e., plots on, adjacent to, and far from all N=20 nests): total grass biomass, total litter biomass, soil hardness, soil surface moisture, and percent canopy cover (hereafter PCA-E). The second was conducting using the subset of plots for which we had these environmental measurements and data on soil chemistry (i.e., plots on and far from N=10 nests; hereafter PCA-E&S).

These PCAs result in composite values summarizing environmental conditions in each plot – the axis scores for principal components – that control for correlations among biophysical parameters. To determine if environmental conditions along the canopy gradient are altered by *A. laevigata* we used two sets of Generalized Linear Mixed Models with Gaussian errors (GLMMs; Bolker et al. 2009). The scores from Axis 1 from each of PCA were the dependent variables, plot location (i.e., on, adjacent to, or far from the nest) and canopy cover were fixed effects, and nest identity was a random effect. The resulting models (Appendices D and E) were ranked with Akaike Information Criteria (Burnham and Anderson 2002). All analyses were conducted using the R statistical programming language (R Core Development Team 2014, Zenodo Citation) and packages lme4 (Bates et al. 2015) and ggbiplot (Vu 2015).

Does A. laevigata's environmental engineering alter patterns of seedling abundance and species richness along a canopy cover gradient? We used Generalized Linear Mixed Models with Poisson error distributions to determine if seedling abundance and species richness in plots were best explained by combinations of factors related to leaf-cutter ants or the canopy cover gradient. Seedling abundance or richness were the dependent variables; fixed effects included plot location, canopy cover, and environmental conditions, i.e., a plots score on the first principal component of either PCA-E or PCA-E&S. The amount of plant biomass harvested by *A. laevigata* colonies is positively correlated with nest area (Costa et al. 2008), and hence large colonies could have an effect that extends further from the nest boundary than smaller ones. However, we did not include nest area as a covariate in our models because initial analyses showed it did not significantly improve model fit. However, nest identity was again included as a random effect, as was a per-observation term because of significant overdispersion (Appendices F and G).

RESULTS

Almost all environmental parameters varied with canopy cover, with the slope of the relationship influenced by proximity to *Atta laevigata* nests (Appendix H). For example, leaf-litter increased with canopy cover in all locations, but was up to five-fold higher in plots far from nests and on nest edges than in plots on nest mounds. Similarly, grass biomass always decreased with canopy cover, but plots on nest mounds had up to four times less grass biomass than those in other locations. Soil properties also

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varied with plot location, although some values higher far from nests (e.g., P, Al^{3+}, organic matter, soil humidity) while others were lower in these locations (e.g., Ca^{2+}, K^{+}).

Integrating these disparate data with PCA revealed that plots located on *Atta laevigata* nest mounds have environmental conditions that differ from those in plots adjacent to or far from nests, whose conditions overlap in ordination space (Fig. 1a). In PCA-E the first axis explained 45.6% of the variance and was positively correlated with litter biomass and soil moisture (Fig. 1a). The second axis explained an additional 29.6% of the variance; it was negatively correlated with litter biomass and soil hardness (Appendix I). Canopy cover over a plot was positively correlated with a plot's score on the first axis (ρ = 0.44, t = 3.77, df = 58, $p < 0.001$) and negatively correlated with its score on the second axis (ρ = -0.60, t = -5.7, df = 58, $p < 0.001$; Fig. 1a)

In PCA-E&S plots were again grouped in ordination space based on their proximity to nests, with those on nest mounds associated with positive scores on axis 1 (Fig. 1b). This axis explained 42.9% of the variance and was positively correlated with litter and grass biomass, soil moisture, and soil P, Al^{3+}, and organic material (Fig. 1b, Appendix J). Cover over a plot was not correlated with its score on the first axis (ρ = 0.30, t = 1.35, df = 18, p = 0.20) but was negatively correlated with its score on the second axis (ρ = -0.58, t = -3.0279, df = 18, p = 0.007).

When using a plot's scores from PCA-E as a proxy for local environmental conditions, the best fit to the data was by the model including plot location, canopy cover, and their interaction. (Appendix E). However, when using PCA-E&S scores as the response variable the model that best fit the data only included a main effect of plot proximity to ant nests (Appendix F). This indicates leaf-cutter ants and canopy cover both influence local environmental conditions until soil properties are taken into account, at which point it becomes evident the primary factor influencing environmental conditions in plots is their proximity to *Atta* nests. Whether or not data on soils are included, however, the effect of leaf-cutter ants is limited to the plots located on the nest itself (Fig. 2).

Each 2 m^2 study plot had in it an average of 27.5 ± 22.81 SD seedlings (range=0-86). Plots located on nest mounds had only 10.8 ± 12.7 SD seedlings in them, while those in other locations had abundances up to four-fold greater (35.4 ± 18.5 SD and 45.1 ± 17.0 SD in plots adjacent to and far from nests, respectively). The mean number of species per plot also increased with increasing distance from nests: plots on nests had in them an average of 4.25 ± 3.2 SD species vs. 14.8± 4.9 SD adjacent to nests and 16.6 ± 3.7 SD in plots 10 m from the nest boundary. The most common species recorded were *Miconia albicans* (Melastomataceae, N=239), *Eupatorium* sp. 3 (Asteraceae, N=139), *Tapirira guianensis* **(**Anacardiaceae**,** N=98), *Matayba guianensis* (Sapindaceae, N=66) and *Alibertia myrciifolia* (Rubiaceae, N = 65).

Leaf-cutter ants, and not the gradient in canopy cover, had best explained the patterns of seedling abundance and species richness (Fig. 3). This was true when using as a proxy for environmental conditions the scores from PCA-E or PCA-E&S (Appendices F and G), although when using PCA-E&S the dAIC scores for models including both ants and environmental conditions were <1 (Appendix G). The significant effect of nest identity also indicates some nests exert larger effects on local seedling communities than others in ways independent of nest area and how ants modify local environmental conditions.

DISCUSSION

Because both ecosystem engineers and environmental gradients are known to exert strong effects on ecosystems, it has been suggested they their synergistic effects on species abundance and diversity could be dramatic. To date, however, few empirical studies have evaluated the impacts of ecosystem engineers along gradients (e.g., Badano and Marquet 2009; Kleinhesselink et al. 2014), and none have been conducted in species-rich tropical ecosystems. We sampled over 1800 seedlings distributed along a canopy cover gradient in Brazil's *Cerrado* to elucidate if the ecosystem engineer *Atta laevigata* ameliorated or exacerbated environmental stress for plants. We found that environmental conditions known to exert strong effects on seedling communities are influenced by both the canopy cover gradient and ant activity, but that the negative effect of the ecosystem engineer on seedling abundance and species richness overwhelms that of the environmental gradient. Changes in both environmental conditions and seedling communities were strongly dependent on proximity to ant nests, however, with *Atta*'s impacts restricted to the area covered by nest mounds. Taken together, these results demonstrate that engineers can amplify stress along environmental gradients, but that these impacts can be context-dependent and spatially-complex in ways that are poorly understood.

Leaf-cutter ants engineer our savanna site in many of the same ways they do lowland forests – transporting large amounts of soil to the surface, modifying soil chemistry (Meyer et al. 2013), and reducing the amount of biomass on the soil surface (reviewed in Farji-Brener and Illes 2000; Leal et al. 2014). However, we did not find a significant effect of plot location on canopy cover (Appendix A), even at the highest end of the canopy cover gradient. Despite the fact that *A. laevigata* removes a large proportion of the leaves from trees in our site (Mundim et al. 2012), this leads us to conclude that in contrast to what has been observed with other *Atta* species (Correa et al. 2010; Meyer et al. 2011), modified light regimes and the associated abiotic changes are not the mechanism by which *A. laevigata* alters Cerrado seedling communities. Instead, it appears that *A. laevigata* create what Farji-Brenner and Illes (2000) refer to as 'bottom-up' gaps: patches of unique habitat resulting from *Atta*'s modifications of the soil surface and forest understory. We hypothesize that the differences in soil properties we observed on *A. laevigata* nests will reduce plant growth. Furthermore, by clearing litter they will also reduce surface humidity (Appendix H) and hence seed viability and seedling survivorship (Salazar et al. 2012). We also hypothesize that reducing seedling abundance directly, for instance by harvesting seeds (Ferreira et al. 2011) and seedlings for their fungal gardens or by burying seedlings under excavated soil (Costa 2013), *A. laevigata* will have effects on Cerrado plant communities whose magnitude is comparable to that of altering environmental conditions that alter seedling growth.

It is notable that *A. laevigata* impacts on seedling abundance and diversity appear restricted primarily to the nest mound, with plots on mound edges having values of abundance and species richness comparable to those in plots 10m from nests. However, a salient feature of many ecosystem engineers is that their localized impacts can persist long-term, even the engineer is no longer present (Hastings et al. 2007). Mounds can persist long after a colony has died so while localized, their impacts on species richness and abundance may persist long-term. This suggests that the magnitude of landscape engineering by *Atta* could stem not only from how it modulates local conditions along environmental gradients (Crain and Bertness 2006), but also as a function of its local density and population structure. We hypothesize this alternative type of context-dependent impact could be common where engineer effects have clearly delineated boundaries and scale with individual, colony, or population size (e.g., beaver dams, termite mounds). If so, it suggests an alternative framework for theoretical models in which engineer life-history and population dynamics, rather than local environmental conditions, is central to understanding their landscape-scale impacts (Hastings et al. 2007).

Unlike savannas in the Paleotropics, the density and diversity of large mammalian herbivores in the Cerrado is very low (Marinho-Filho et al. 2002). Consequently, it is thought that plant population and communities in this biome are largely structured by edaphic factors drivers (reviewed in Hoffmann and Moreira 2002; Mistry 1998; Ruggiero et al. 2002) with a negligible impact of herbivores (e.g., Gardner 2006). Although the key role played in Cerrado plant ecology by fire, drought, and competition for limited soil nutrients is indisputable (e.g., Gardner 2006; Henriques and Hay 2001; Hoffmann 1999), there is a rich diversity of insects and other ‘cryptic consumers’ (sensu Keesing 2000). Chief among these are leaf-cutter ants, which recent studies have shown to have large and direct detrimental impacts on plant recruitment, growth, survivorship, and reproduction (Ferreira et al. 2011; Mundim et al. 2012). Our study provides compelling evidence suggesting herbivores can also have indirect effects on plants by altering environmental conditions, and that the conventional wisdom regarding the primary drivers of Cerrado plant demography merits rigorous reevaluation.

Our study highlights four promising directions for future studies of ecosystem engineers. First, our study shows that coupling survey data with rigorous statistical approaches can yield unique insights into the impacts of ecosystem engineers along environmental gradients, especially for engineers not amendable to landscape-scale experimental manipulation or environments defined by correlated variables. Nevertheless, we suggest experiments manipulating both engineers and subsets of environmental conditions at smaller spatial scales can complement statistical approaches such as ours to provide important insights needed to test and guide theory (Hastings et al. 2007; Wright and Jones 2006). Second, although disturbances are ubiquitous across ecosystems they are rarely considered in theoretical models of ecosystem engineering or their empirical tests. An important caveat to our conclusions is that fire is a key element in the ecology and evolution of Cerrado biodiversity that can influence soil nutrients, canopy and ground cover, and plant reproduction (Miranda et al. 2002); it could conceivably influence leaf-cutter ant abundance or behavior as well. We suggest how engineer impacts are influenced by disturbance type, frequency, and intensity is an area of much needed empirical and theoretical attention; in future studies we will explicitly consider time-since fire and how it could potentially alter the relative importance of environmental factors vs. *Atta* engineering for plant communities. Third, previous work investigating the dynamics of Cerrado plants has focused almost entirely on physical factors and ignored plant-animal interactions. Our results underscore the hypothesis that leaf-cutter ants play a dominant role in Cerrado plant demography; ignoring these keystone herbivores will undermine attempts to develop general theory (Gardner 2006) as well as ongoing conservation and restoration efforts. Finally, human activities such as deforestation, habitat fragmentation, road creation, and nutrient deposition can alter environmental gradients (Broadbent et al. 2008; Tulloss and Cadenasso 2015) and the abundance of *Atta laevigata* and other ecosystem engineers across landscapes (Cameron and Bayne 2009; Vasconcelos et al. 2006). Their ecological and economic footprint may therefore increase dramatically in coming decades in ways that remain underappreciated and poorly understood.

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DATA AND CODE AVAILAIBILITY STATEMENT

Data used in this paper will be archived upon acceptance at the Dryad Digital Repository (datadryad.org; accession number -----). Code used for analyses is available at Github (githurb url); it will be archived as v1.0 at Zenodo.org upon the acceptance of the manuscript.

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FIGURE LEGENDS

Figure 2. (A) Principal component ordination (PCA-E) of plots located on (red), adjacent to (orange), or 10m from the edge of (blue) N=20 *Atta laevigata* nest mounds in Brazilian Cerrado. **(B)** Principal component ordination of the subset of plots located on each of N=10 nest mounds (red) and 10m from nests (blue) for which we also had data on soils chemistry (PCA-E&S). Symbol size in both PCAs indicates the percent canopy cover over the plot.

Figure 3. Regression of a plot's score on the first principal component summarizing environmental conditions vs. canopy cover over that plot. **(A)** Uses scores from PCA-E and **(B)** uses scores from PCA-E&S. Plots were located in the center of leaf-cutter ant (*Atta laevigata*) nest mounds (red), adjacent to nest (orange), or 10 m from nests (blue).

Figure 4. Regression of **(A)** the number of seedlings and **(B)** species richness in plots as a function of the plot's canopy cover. Plots were located in the center of leaf-cutter ant (*Atta laevigata*) nest mounds (red), adjacent to nest (orange), or 10 m from nests (blue) in Brazil's *Cerrado*.

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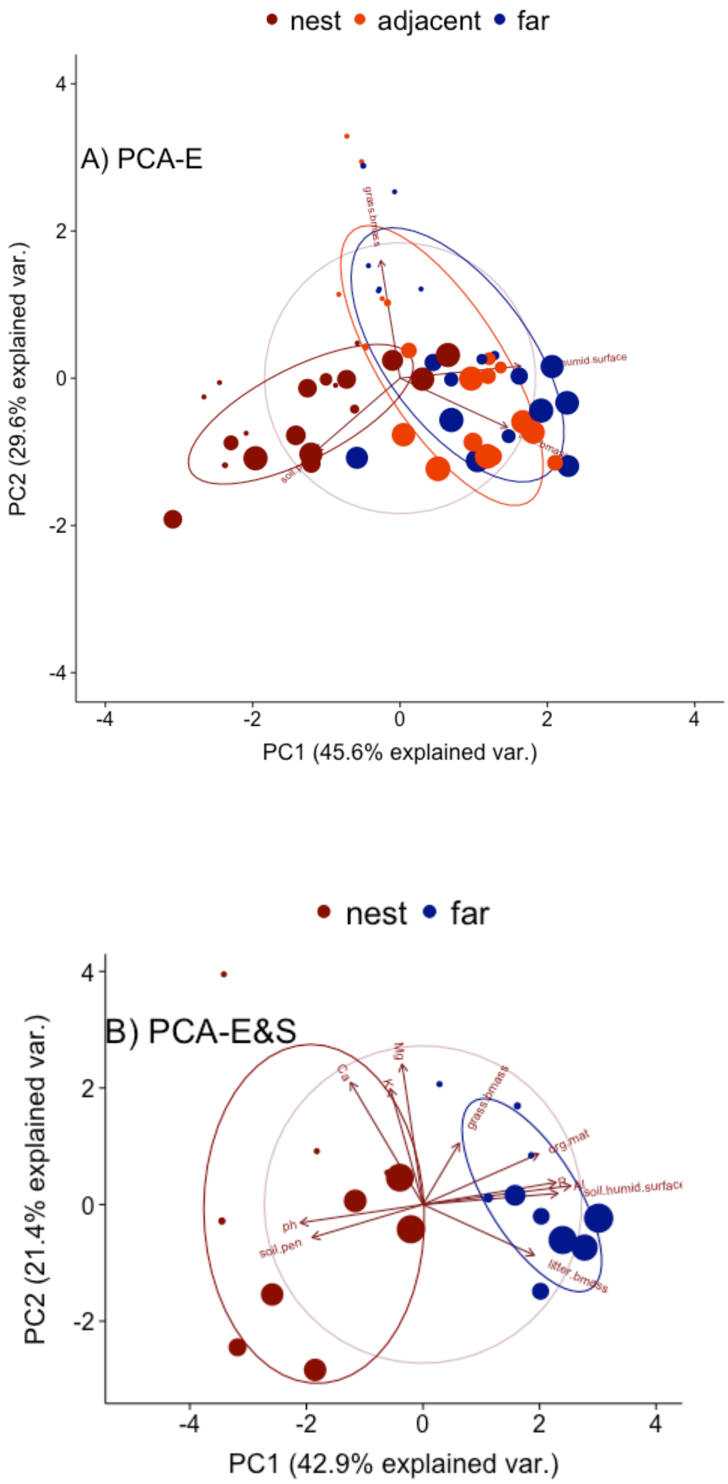
● nest ● adjacent ● far
A) PCA-E
grass.bmass
humid.surface
bmass
soil.p
PC2 (29.6% explained var.)
PC1 (45.6% explained var.)
4
2
0
-2
-4
-4
-2
0
2
4
● nest ● far
B) PCA-E&S
Mg
Ca
K
grass.bmass
org.mat
P
Al
soil.humid.surface
ph
soil.pen
litter.bmass
PC2 (21.4% explained var.)
PC1 (42.9% explained var.)
4
2
0
-2
-4
-2
0
2
4

FIGURE 1

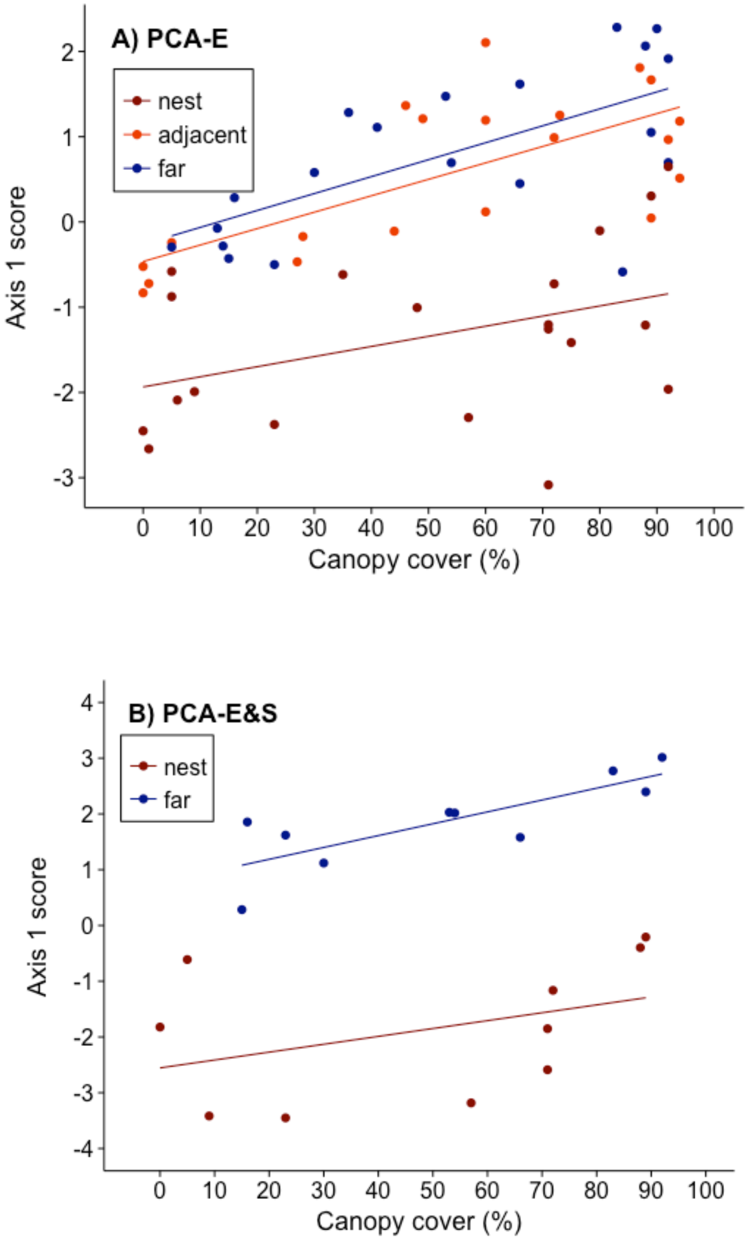

FIGURE 2

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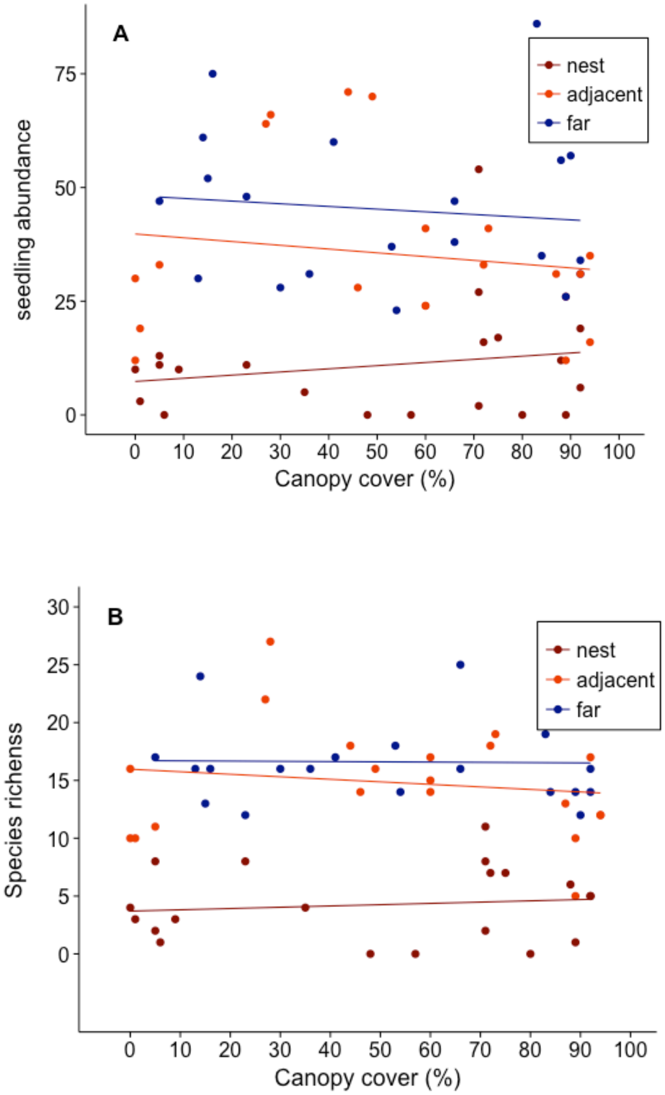
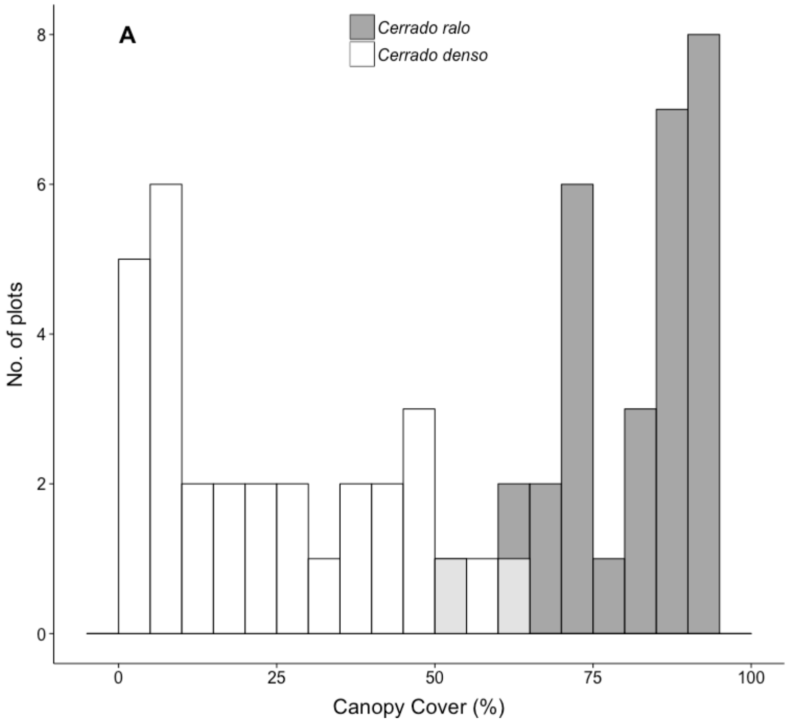

A
seedling abundance
0
25
50
75
0 10 20 30 40 50 60 70 80 90 100
Canopy cover (%)
nest
adjacent
far
B
Species richenss
0
5
10
15
20
25
30
0 10 20 30 40 50 60 70 80 90 100
Canopy cover (%)
nest
adjacent
far

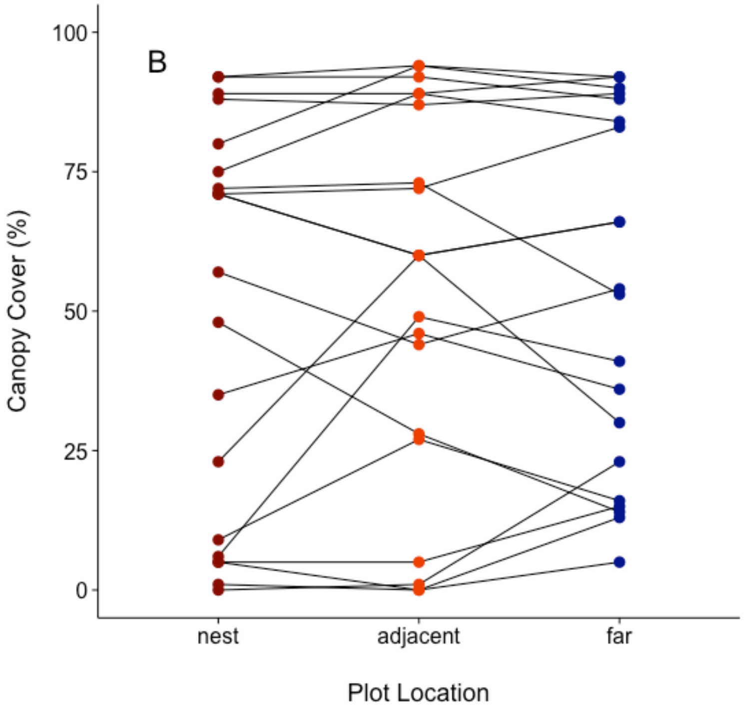
FIGURE 3

APPENDIX A: Canopy Cover at Panga Ecological Station

Appendix A Figure 1A. Histogram of canopy cover over N=60 plots in our Brazilian *Cerrado* study site. We established three plots established at each of the N = 20 leaf-cutter ant (*Atta laevigata*) nests we studied: one in the center of the nest mound, one on the edge of the nest, and the third 10m from the edge of the nest. Dark gray bars represent plots located in the *cerrado denso* vegetation type, while light gray bars refer to plots located in *cerrado ralo*.



Appendix A Figure 1B. Canopy cover over plots on, adjacent to, and far from each nest of N=20 nests. Canopy cover over plots is independent of plot proximity to ant nests, indicating ants are not responsible for the gradient in canopy cover (Appendix A).

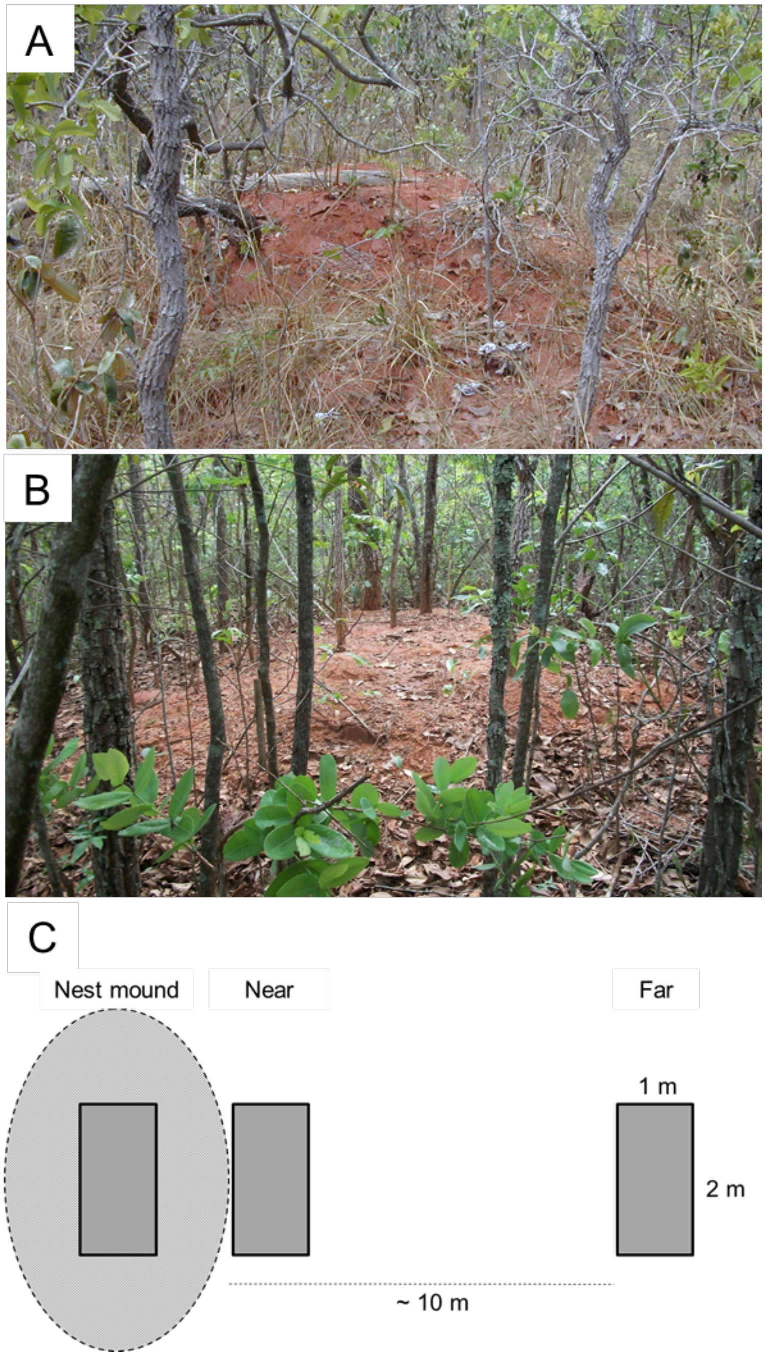


Generalized Linear Mixed Model selection for the effect of plot proximity to leaf-cutter ant (*Atta laevigata*) nests on canopy cover in plots (logit-transformed, following (Warton and Hui 2010; Zuidema et al. 2010). The significance of plot proximity was assessed by comparing the model including only the random effect of nest identity (model 1) with models including this random effect, plot proximity to ant nests, and nest mound area as a covariate (model 2: no plot location x covariate interaction; model 3: main effects of plot location, the covariate, and a plot location x covariate interaction). All models used a Gaussian distribution with an identity function; nest mound area was not included as a covariate because preliminary analyses indicated it did not improve the fit of models. Considering the location of plots or nest mound area does not improve the fit to the data, indicating canopy cover is independent of proximity to ant nests and nest mound size.

Model	Resid. Df	Resid. Dev	*d*AIC	wAIC
1	57	97.525	0	0.999
2	54	94.098	14.567	6.8×10^{-4}
3	52	90.144	29.186	4.6×10^{-7}

APPENDIX B: ***Atta laevigata*** nest mounds and the arrangement of sampling plots.

Leaf-cutter ant (*Atta laevigata*) nest mounds in (A) *cerrado ralo* and (B) *cerrado denso* vegetation types. (C) the location of sampling plots relative to nest mounds.



APPENDIX C: Correlations of biophysical properties measured in a Brazilian Cerrado site with each other and canopy cover.

	Litter biomass	Soil Hardness	Grass biomass	pH	P	K	Ca	Mg	Al	Org. Material	Soil moisture
Canopy cover (%)	**0.5584**	0.0070	**-0.6734**	-0.3087	0.3396	-0.4265	**-0.5505**	-0.3775	0.1611	-0.1829	**0.3095**
Litter biomass	-	-0.0995	-0.0524	**-0.5070**	**0.6308**	-0.1796	**-0.5274**	**-0.2458**	**0.6477**	**0.2411**	**0.6165**
Soil hardness		-	0.102	0.4222	**-0.5584**	0.1673	0.0828	-0.0034	-0.6115	-0.5999	**-0.4438**
Grass biomass			-	-0.1094	0.1032	0.1639	0.0234	0.0234	0.2511	**0.5737**	-0.0137
pH				-	**-0.6329**	**-0.1229**	0.3483	0.0000	**-0.7300**	**-0.4653**	**-0.6108**
P					-	-0.0492	-0.2137	0.0705	**0.7420**	0.4995	**0.7301**
K						-	**0.4850**	**0.6382**	-0.1128	-0.0435	-0.2032
Ca							-	**0.7882**	-0.3531	-0.1257	-0.2392
Mg								-	0.0331	0.0494	0.0417
Al									-	**0.7113**	**0.7521**
Org. material										-	**0.5788**
Soil moisture											-

APPENDIX D: Generalized Linear Mixed Model selection for the effect of plot proximity to leaf-cutter ant (*Atta laevigata*) nests vs. canopy cover on environmental conditions in plots (based on PCA-E scores for the 1st axis). The significance of these factors was assessed by comparing the models including only the random effect of nest identity (model 1) with models including this random effect and plot location (model 2), canopy cover (model 3), nest identity and canopy cover (model 4), or nest identity, and plot location, canopy cover, and a plot location x canopy cover interaction (model 5). All models used a Gaussian distribution with an identity function; nest mound area was not included as a covariate because preliminary analyses indicated it did not improve the fit of models. The best fitting model was the one that included plot location, canopy cover, and their interaction.

Model	Resid. Df	Resid. Dev	*d*AIC	wAIC
5	52	103.89	0	0.999
4	54	138.43	9.55	0.0083
2	55	154.53	13.44	0.0012
3	56	152.78	16.21	3.0×10^{-4}
1	57	168.23	19.27	6.4×10^{-5}

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APPENDIX E: Generalized Linear Mixed Model selection for the effect of plot proximity to leaf-cutter ant (*Atta laevigata*) nests vs. canopy cover on environmental conditions in plots (based on PCA-E&S scores for the 1st axis). The significance of these factors was assessed by comparing the models including only the random effect of nest identity (model 1) with models including this random effect and plot location (model 2), canopy cover (model 3), nest identity and canopy cover (model 4), or nest identity, and plot location, canopy cover, and a plot location x canopy cover interaction (model 5). All models used a Gaussian distribution with an identity function. The best fitting model was the one that included only the fixed effect of plot location.

Model	Resid. Df	Resid. Dev	*d*AIC	wAIC
2	16	56.15	0	0.906
4	15	49.82	4.55	0.0932
5	14	49.47	13.75	9.4×10^{-4}
1	17	86.77	26.67	1.5×10^{-6}
3	16	84.86	33.41	5.0×10^{-8}

APPENDIX F: Model selection for the effects on seedling abundance and species richness in plots of canopy cover vs. leaf-cutter ant (*Atta laevigata*) activity (i.e., nest mound area, plot proximity to nests, local environmental conditions, i.e., axis 1 scores from PCA-E). The significance of these factors was assessed by comparing the models including only the random effect of nest identity and per-observation random effects (model 1) with models including these random effects and canopy cover (model 2), random effects and those related to ants (model 3), or random effects and both canopy-cover and ant-related variables (model 4). All models used a Poisson distribution with a logit link function. The best fitting model included factors and covariates related ants and their activity.

Seedling Abundance (*Environment = PCA-E axis 1*)

Model	Resid. Df	Resid. Dev	dAIC	wAIC
3	54	500.6514	0.0000	0.8272
4	52	499.7834	3.1320	0.1728
1	57	550.6256	43.9742	2.3×10^{-10}
2	56	550.5752	45.9237	8.8×10^{-11}

Species Richness (*Environment = PCA-E axis 1)*

Model	Resid. Df	Resid. Dev	dAIC	wAIC
3	54	333.9756	0.0000	0.8691
4	52	333.7626	3.7869	0.1309
1	57	416.5026	76.5269	2.1×10^{-17}
2	56	416.4239	78.4482	8.0×10^{-18}

APPENDIX G: Model selection for the effects on seedling abundance and species richness in plots of canopy cover vs. leaf-cutter ant (*Atta laevigata*) activity (i.e., nest mound area, plot proximity to nests, local environmental conditions. The significance of these factors was assessed by comparing the models including only the random effect of nest identity and per-observation random effects (model 1) with models including these random effects and canopy cover (model 2), random effects and those related to ants (model 3), or random effects and canopy-cover and ant-related variables, and local environmental conditions (axis 1 scores from PCA-E&S), which analyses indicated were influenced by both canopy cover and proximity to ant nests (model 4). All models used a Poisson distribution with a logit link function. The best fitting model included factors and covariates related ants and their activity.

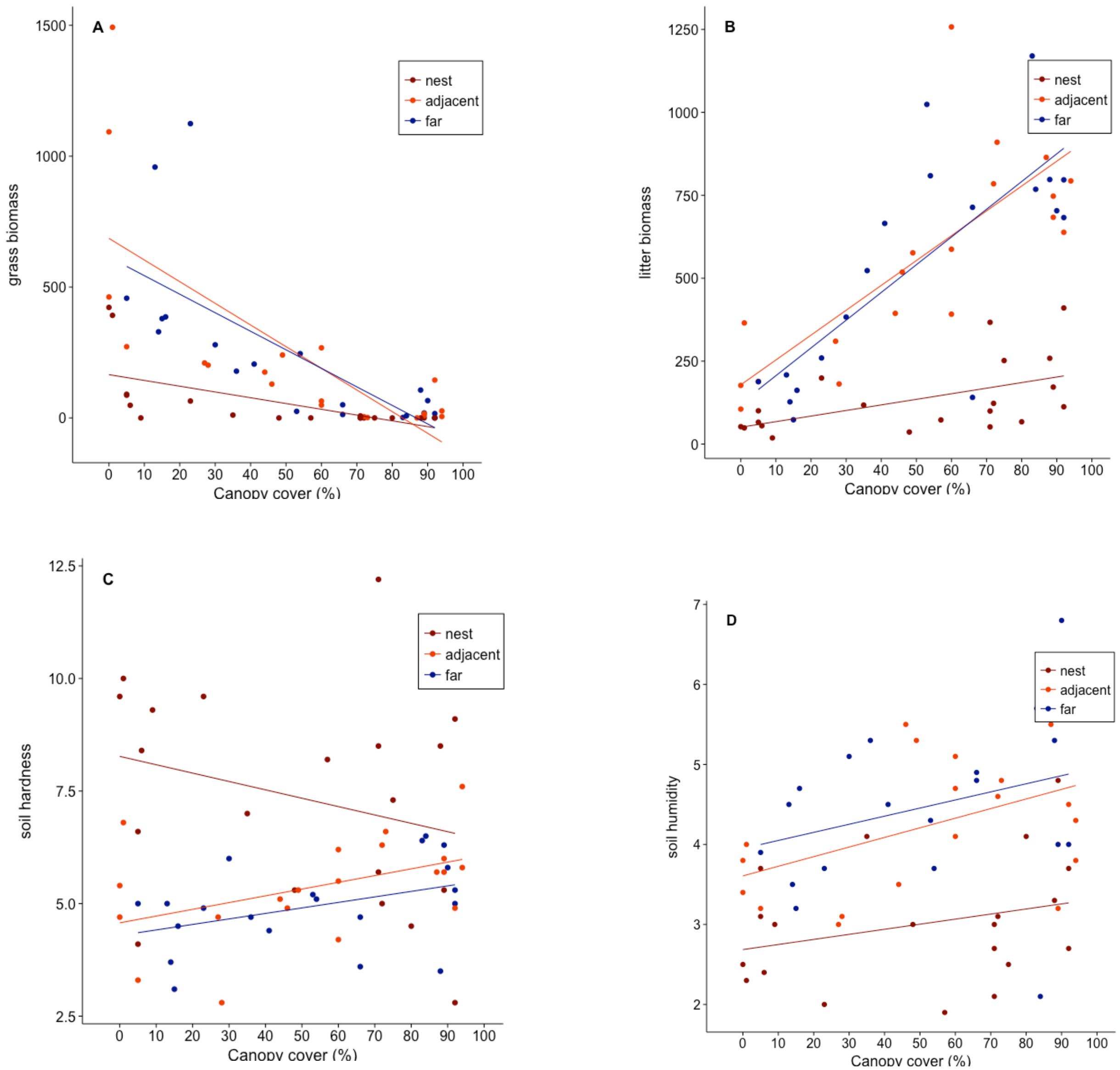
Seedling Abundance (*Environment = PCA-E&S axis 1*)

Model	Resid. Df	Resid. Dev	dAIC	wAIC
3	14	164.6	0	0.598
4	13	163.422	0.8219	0.397
1	17	180.983	10.383	0.003
2	16	180.696	12.097	0.001

Species Richness *(Environment = PCA-E&S axis 1)*

Model	Resid. Df	Resid. Dev	dAIC	wAIC
3	14	108.4128	0.0000	0.6263
4	13	107.4459	1.0331	0.3737
1	17	136.1146	21.7018	1.2×10^{-4}
2	16	135.8064	23.3936	5.2×10^{-6}

APPENDIX H: Scatterplot of **(A)** grass biomass, **(B)** litter biomass, **(C)** soil hardness (i.e., penetration distance), and **(D)** soil surface humidity along a canopy cover gradient in Brazilian *Cerrado*. Regression lines represent plots located at different distances from *Atta laevigata* nest mounds (i.e., plots located in the center of the nest mound, adjacent of the nest, and 10m from the nest).



APPENDIX I: Factor loadings for the four principal components axes summarizing environmental variables measured in study plots located in Brazilian *Cerrado*; the cumulative proportion of the variance explained by these axes = 100%. This PCA includes all *Atta laevigata* nests (i.e., plots on the center of the nest mound, adjacent to the nest mound, and 10m from the edge of the nests) but no data on soil chemistry (referred to as PCA-E throughout the manuscript).

Variable	PC1	PC2	PC3	PC4
Litter biomass	0.5864	-0.3345	0.4237	-0.6039
Soil hardness	-4.4584	-0.4926	0.6753	0.3016
Grass biomass	-0.1053	0.7990	0.5749	-0.1415
Soil moisture	0.6594	0.0855	0.1845	0.7241

APPENDIX J: Factor loadings for the first four principal component axes summarizing environmental variables measured in study plots in Brazilian *Cerrado*; the summed proportion of the variance explained by these axes is 84.9%. This PCA is for plots in the center of and 10m from N = 10 leaf-cutter ant (*Atta laevigata*) nest mounds for which data on soil properties were collected (referred to as PCA-E&S throughout the manuscript).

Variable	PC1	PC2	PC3	PC4
Litter biomass	0.3227	-0.2081	0.3570	-0.1962
Soil hardness	-0.3214	-0.1359	-0.2194	-0.4393
Grass biomass	0.1052	0.2517	-0.6646	-0.3390
pH	-0.3559	-0.0750	-0.1816	0.3599
P	0.3858	0.0906	0.2009	0.0639
K^+	-0.0948	0.4755	0.2186	-0.5393
Ca^{2+}	-0.2101	0.5013	0.0961	0.3533
Mg^{2+}	-0.0607	0.5764	0.2763	0.1293
Al^{3+}	0.4283	0.0770	0.0190	-0.0571
Organic material	0.3354	0.2080	-0.3874	0.0090
Soil moisture	0.3914	0.0460	0.1600	0.2902

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