Costa AN, Bruna EM, Vasconcelos HL. 2018. Do an ecosystem engineer and environmental gradient act independently or in concert to shape juvenile plant communities? Tests with the leaf-cutter ant *Atta laevigata* in a Neotropical savanna. PeerJ 6:e5612
https://doi.org/10.7717/peerj.5612

Are the effects of an ecosystem engineer and environmental gradient on plant biodiversity independent, additive, or synergistic? Tests with the leaf-cutter ant *Atta laevigata* in a Neotropical savanna

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PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1692v4 | CC BY 4.0 Open Access | rec: 20 May 2017, publ: 20 May 2017

ABSTRACT

Background. Species that transform habitats or create new ones in ways that influence other species are known as Ecosystem Engineers. While the impacts of many engineer species have been well described, our understanding of how the impacts of engineers vary along or even alter environmental gradients remains limited. While disentangling the effects of gradients and ecosystem engineers on biodiversity is complicated – the gradients themselves can be altered by engineers – doing so is necessary to advance conceptual and mathematical models of ecosystem engineering. We used leaf-cutter ants (*Atta* spp.), a major engineer in the Neotropics, as a model system with which to investigate the relative influence of gradients and environmental engineers on the abundance and species richness of woody plant seedlings.

Methods. We conducted our research in the South American savanna woodlands known as the *Cerrado*. We used data from survey of seedlings along a canopy cover gradient and data on environmental conditions previously shown to influence seedling establishment collected along the gradient and at different distances from ant colonies to fit statistical models that addressed the following questions (1) Do the environmental conditions previously shown to influence Cerrado seedling establishment vary with canopy cover? (2) Does *A. laevigata* alter the canopy cover gradient and the related environmental conditions influencing seedling establishment? (3) If so, what is the spatial extent of *Atta* impact on the gradient and environmental conditions, and how does this vary along the canopy cover-cover gradient? (4) Do *A. laevigata* and canopy cover act independently or in synergy to influence seedling abundance and species richness?

Results. We found that the environmental conditions previously shown to influence seedling establishment in the *Cerrado* varied in concert with canopy cover, but that ants are not modifying the canopy cover gradient or canopy cover around nests. However, ants are modifying other local environmental conditions, and the magnitude and spatial extent of these changes is consistent across the gradient. In contrast to prior studies, we found that both seedling abundance and diversity were independent of canopy cover. Instead, ant-related factors (e.g., proximity to nests) had the strongest effect on both abundance and richness.

Discussion. *Atta laevigata* in the Cerrado modify local environmental conditions in ways that have strong but spatially restricted consequences for seedling communities. We hypothesize that by clearing litter and reducing soil moisture content, ants indirectly reduce seedling establishment by increasing rates of seed desiccation. The alteration of soil nutrients could also reduce seedling growth and survivorship; if so these indirect negative effects of engineering could exacerbate the direct effects of their herbivory. The effects of *Atta* on environmental conditions and seedling communities appear restricted to the nest mound, but they could be long-lasting because *Atta* mounds persist long after a colony has died or migrated.

These results underscore the hypothesis that leaf-cutter ants play a dominant role in Cerrado plant demography, and that the ecological and economic footprint of these engineers may increase dramatically in coming decades due to ongoing transformations of the Cerrado by human activities.

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 population dynamics, community composition, and ecosystem function (reviewed in Kleinhesselink et al. 2014; Wright & Jones 2006). Most research on engineers to date has focused on documenting the magnitude of their impacts on local biodiversity, with more recent work evaluating how these impacts vary spatially (e.g., Badano et al. 2006; Baker et al. 2013; Dibner et al. 2015; Kleinhesselink et al. 2014; McAfee et al. 2016). An emerging area of interest is identifying how the impacts of engineers vary along or even alter environmental gradients (Bertness & Callaway 1994; Crain & Bertness 2006), which are ubiquitous and can also exert strong effects on biodiversity (e.g., John et al. 2007). Experimental studies disentangling the effects of engineers and gradients are rare, however, in part because they are challenging to design and implement at the landscape scale. This makes surveys of biodiversity in landscapes where gradients and engineers overlap, coupled with measurements of ecologically relevant environmental parameters, an important tool for advancing conceptual and mathematical models of ecosystem engineering (Hastings et al. 2007; Wright & Jones 2006).

Brazil's *Cerrado* is a savanna woodland whose distribution of 2 million km^2 makes it South America's second largest biome. Like many other savanna biomes the Cerrado is a mosaic of plant physiognomies ranging from open grassland to forests (Oliveira-Filho & Ratter 2002). These vegetation types are often found in close proximity (Cardoso et al. 2009), resulting in broad and continuous gradients in canopy cover that can have important implications for local plant biodiversity. Canopy cover in a site is associated with both biotic and abiotic variables that exert strong effects on woody plant recruitment and survivorship (Salazar et al. 2012a); locations with more canopy cover have cooler understories and produce more leaf-litter, which facilitates seedling establishment and enhances seedling survival by reducing soil water deficits and increasing nutrient availability (Salazar et al. 2012a). In addition, closed-canopy sites also have less cover of the grasses that can inhibit seedling establishment (Hoffmann & Haridasan 2008).

Also found in the Cerrado is a prominent ecosystem engineer: leaf-cutter ants (*Atta* spp.). They transport tons of soil to the surface as they excavate their massive nests, create mounds whose surface area can reach 100 m^2 (Alvarado et al. 1981), harvest copious amounts of plant biomass, farm fungal colonies in chambers up to 10 m below the surface, and alter nutrient cycling and soil properties (reviewed in Farji-Brener & Illes 2000; Leal et al. 2014). *Atta* colonies have direct effects on plant populations and communities – they are major seed predators and harvest seedlings to use as the substrate for their fungal gardens (Farji-Brener & Illes 2000; Leal et al. 2014; Vasconcelos & Cherrett 1997). In addition to their direct impacts on plants, however, their alteration of the landscape may also indirectly influence plant growth, survivorship, or community composition (e.g., Sternberg et al. 2007). To date the potential for engineering by *Atta* to indirectly influence plant communities has primarily been studied in lowland tropical forests (Farji-Brener & Illes 2000; Leal et al. 2014). However, the abundance of *Atta* colonies can be 2-3 fold greater in the Cerrado (Costa & Vieira-Neto 2016), where they have the ability to completely defoliate trees (Mundim et al. 2012). This suggests a novel means by which this ecosystem engineer could indirectly shape plant diversity – by modifying canopy cover gradients, and therefore the local environmental conditions that influence seedling establishment. The magnitude of these indirect impacts should vary along the gradient, however, because areas where trees are sparse will already be hotter, brighter, and have limited litter on the soil surface.

To elucidate how gradients and ecosystem engineers interact to influence plant biodiversity we used data on the distribution of over 1800 seedlings in a Cerrado landscape dominated by the leaf-cutter ant *Atta laevigata*. Our study addressed the following questions: (1) Does *Atta laevigata* modify the gradient in canopy cover found in our Cerrado site? (2) Do environmental conditions that influence Cerrado seedling establishment vary with canopy cover or proximity to *A. laevigata* nests? (3) Do *A. laevigata* and canopy cover act independently or in concert to influence seedling abundance and species richness?

MATERIALS AND METHODS

Study site and system

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 October-April (UFU Santa Mônica Climate Station). Most of the major Cerrado vegetation types can be found at Panga Station, including the two known as *cerrado ralo* and *cerrado denso* (Cardoso et al. 2009; Appendix A). *Cerrado ralo* has a dense layer of grasses and herbs and sparsely distributed shrubs and trees typically <3m tall;

the average canopy cover in *cerrado ralo* is ~30%. *Cerrado denso* has less grass cover and more abundant trees that can reach a height of ca. 6 m; average canopy cover in *cerrado denso* is ~60%. There is large variation in the canopy cover of both vegetation types, however, so there can be strong gradients in canopy cover in landscapes where they abut. At Panga Station, for instance, the canopy cover gradient in the *Cerrado denso* / *Cerrado ralo* mosaic ranges from 0-95% (Mean = 52% ± 33.1 SD; Figure 1A).

Our focal ecosystem engineer is *Atta laevigata*, whose large nest mounds are formed by workers depositing excavated soil around the main entrance to the nest. *Atta laevigata* is the most common *Atta* species in both *cerrado ralo* and *cerrado denso* (Costa & Vieira-Neto 2016); *A. sexdens* is also found at Panga Station but it is primarily found in closed-canopy forest. In 2010 we haphazardly selected 10 active *A. laevigata* nests in each vegetation type (range in nest surface area of the N=20 nests: 7-37 m^2, mean = 16.7 m^2 ± 6.7 SD); We then established three 1x2 m plots around each nest in which to measure environmental variables and survey seedlings: one on the center of the nest mound, one immediately adjacent to the mound, and one 10 m from the mound edge (Appendix A). Although there are also some abandoned nests in our site, we restrict our analyses to active colonies because the effects of time-since-abandonment on environmental variables is unknown.

Environmental data

Litter biomass in each plot was measured by collecting all litter from a randomly selected half of each plot once during the 2010-2011 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). In our analyses we used the average canopy cover in two photos taken during the same 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 content 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 used soil penetrability: 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, then measured the depth to which the rod penetrated the soil at each point. We 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 ≤ 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 = 5 nests in *Cerrado ralo* and N=5 nests in *Cerrado denso* nests for analyses of soil chemistry. For each nest we collected soils in the plots on nest mounds and the plots 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 Federal University of Uberlândia (UFU), where pH, P, K^+, Ca^{2+}, Mg^{2+}, Al^{3+}, and total organic matter were measured using their standard protocols (EMBRAPA 1997).

Statistical analyses: Does Atta laevigata *modify the gradient in canopy cover?*

To test for an effect of plot proximity to *A. laevigata* nests on logit-transformed canopy cover (Warton & Hui 2011) we used using Generalized Linear Mixed Models (GLMMs, Bolker et al. 2009). The significance of plot proximity was assessed by comparing the model including only the random effect of nest identity with models including this random effect, plot proximity to ant nests, nest mound area as a covariate, and plot location x covariate interactions. 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.

Statistical analyses: Do environmental conditions that influence Cerrado seedling establishment vary with canopy cover or proximity to A. laevigata nests?

We used Principal Components Analyses (PCA) to summarize environmental conditions in each plot because many of the biophysical variables we measured were highly correlated (Appendix B). The complete suite of environmental data was only collect in a subset of N = 10 nests, so we conducted two separate PCAs. The first was conducted using the environmental data collected in all N=60 plots (i.e., plots on, adjacent to, and far from all N=20 nests). These variables were: total grass biomass, total litter biomass, soil penetrability, surface soil moisture content, and percent canopy cover. The second was conducting using the subset of N=10 nests for which we also collected data on soil chemistry; it was therefore the most comprehensive with respect to the environmental variables included, but was more limited in nest number and plot location because it only included plots on and far from the N=10 nests. We hereafter refer to these PCAs as “PCA-1” and “PCA-2”, respectively. Both were conducted with correlation matrices because of the scales of each variable were different.

Each plot’s PCA scores are new variables that summarize local environmental conditions after controlling for correlation among the variables measured, and can therefore be used as dependent or independent variables in subsequent analyses (sensu Baiser et al. 2012). To determine if environmental conditions in a plot vary with canopy cover or proximity to *A. laevigata* nests we used a plot’s score on the 1st Principal Component Axis as the dependent variable in Generalized Linear Mixed Models with Gaussian errors. Plot location (i.e., on, adjacent to, or far from the nest) was a main effect with canopy cover included as a covariate. Although the effects of large colonies could potentially extend further from the nest boundary than those of smaller ones (Costa et al. 2008), we did not include nest area as a covariate because preliminary analysis indicated it did not improve the fit of models including just canopy cover. However, we did include nest identity as a random effect. The resulting models were ranked with Akaike Information Criteria (Burnham & Anderson 2002) to determine which model best fit the observed data.

Statistical analyses: Do A. laevigata *and canopy cover act independently or in concert to influence seedling abundance and species richness?*

We used two sets of Generalized Linear Mixed Models with Poisson error distributions to determine if the seedling abundance and seedling species richness in plots were best explained by proximity to leaf-cutter ant nests, canopy cover, or combinations of the two. The two analyses that were identical except for the PCA scores used to summarize local environmental conditions: the first group of models used the axis scores from 'PCA-1' (i.e., all nests and plots but fewer environmental variables), while the second used the axis scores from 'PCA-2' (all environmental variables but fewer nests and plot locations). Seedling abundance or richness were the dependent variables. Main effects included plot location and local environmental conditions (i.e., each plot's scores from the 1st Principal Component). Colony area was included as a covariate in these models, as was colony cover. Nest identity was again included as a random effect; because of significant overdispersion we also included a random per-observation term.

All analyses were conducted using the R statistical programming language (R Core Development Team 2014). For the GLMMs we used package lme4 (Bates et al. 2015), while PCAs were conducted with package ggbiplot (Vu 2015).

RESULTS

Does Atta laevigata *modify the canopy cover gradient?*

The model that best fit the data on the amount of canopy cover over a plot is the one including only the random effect of nest identity (Table 1). This indicates that *A. laevigata* colonies alter canopy cover around their nests, but not in a systematic way. In addition, there is no predictable change in canopy cover as a function of proximity to ant nests (Figure 1B).

Do environmental conditions that influence Cerrado seedling establishment vary with canopy cover or proximity to Atta laevigata *nests?*

Plots on nest edges and those far from nests overlapped in ordination space, indicating they had very similar environmental conditions (Fig. 2a). However, there was almost no overlap in ordination space between either of these locations and the plots located in the middle of *A. laevigata* nest mounds (Fig. 2A), even when the number of nests was reduced to include soil data (Fig. 2B). In 'PCA-1' the first axis explained 45.6% of the variance and was positively correlated with litter biomass and soil moisture content. The second axis explained an additional 29.6% of the variance; it was negatively correlated with grass biomass and soil penetrability (Table 2). In 'PCA-2' the first axis explained 42.9% of the variance and was positively correlated with litter and grass biomass, soil moisture content, and soil P, Al^{3+}, and organic material (Table 3). The second axis explained 21.4% of the variance and was positively correlated with all other environmental variables measured. In light of these results we used the scores from the first principal components as the dependent variable in subsequent analyses.

When using the results of 'PCA-1', canopy cover over a plot was positively correlated with a plot's PCA1 score ($\rho = 0.44$, $t = 3.77$, $df = 58$, $p < 0.001$), suggesting an association between canopy cover and local environmental conditions. This was supported by the GLM, in which the best fit to the data was by the model included canopy cover. However, the best model also included plot location, indicating leaf-cutter ants also influenced environmental conditions but that the magnitude of the effect varied with plot proximity to nests (Table 4, Fig. 3A). When data on soils were included in the PCA, however, there was no significant correlation between canopy cover over a plot and that plot's score on the 1st axis (ρ = 0.30, t = 1.35, df = 18, p = 0.20). The limited influence of canopy cover on environmental conditions was underscored by the results of the GLM, in which the model that best fit the data was the one including only the main effect of plot proximity to ant nests and the random effect of nest identity (Table 5, Fig. 3B). This indicates that once data on soil chemistry have been included in analyses, the impact of ants on local environmental conditions far outweighs that of canopy cover.

Do A. laevigata *and canopy cover act independently or in concert to influence seedling abundance and species richness?*

On average there were 27.5 ± 22.81 SD seedlings (range=0-86) in each 2 m^2 study plot. However, the mean number of seedlings $plot^{-1}$ decreased as one moved closer to the center of nests: plots far from nests had on average 45.1 ± 17.0 SD seedlings in them vs. 35.4 ± 18.5 SD seedlings $plot^{-1}$ on nest margins and 10.8 ± 12.7 SD seedlings $plot^{-1}$ in the center of nest mounds. The mean number of species per plot was also lowest in plots on the center of nests (4.25 ± 3.2 SD) with three-fold higher species richness in plots on nest margins (14.8± 4.9 SD) and 10 m from nests (16.6 ± 3.7 SD). 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).

Both seedling abundance and seedling species richness were best explained by how leaf-cutter ants have modified the environment than the gradient in canopy cover (Fig. 4). This was true when using the Axis 1 scores from ‘PCA-1’ (Table 6) or ‘PCA-2’ (Table 7) as a summary of environmental conditions in plots (although when using ‘PCA-2’ the dAIC scores for models including both ants and environmental conditions were <1; Table 7). The significant effect of nest identity also indicates that some nests exert larger or smaller effects on local seedling abundance and diversity than others of similar size.

DISCUSSION

Both ecosystem engineers and environmental gradients are known to exert strong effects on biodiversity, but it is unknown if their effects are generally independent, additive, or synergistic. This is because empirical studies simultaneously evaluating the relative influence of engineers and gradients remain rare (e.g., Badano & Marquet 2009; Kleinhesselink et al. 2014). We quantified seedling communities and environmental variables that influence seedling establishment along a canopy cover gradient and at different distances from nests of the ecosystem engineer *Atta laevigata*. Our results suggest that although the varying environmental conditions along a canopy cover gradient may indeed influence the recruitment and survival of Cerrado seedlings, the effects of canopy cover are relatively small compared to those of leaf-cutter ants. However, we also found that conclusions regarding the relative importance of these factors depend on whether data on soil chemistry are included in analyses. Although we only collected data on soils for a subset of nests and plots, the signal of ant effects on environmental conditions was striking (Fig. 2). We are therefore cautiously optimistic our conclusion would be supported by including analyses of soils from additional nests.

Leaf-cutter ants in our savanna site engineer the habitat in many of the same ways *Atta* species in lowland forests have been shown to – by transporting large amounts of soil to the surface, modifying soil chemistry (Meyer et al. 2013; Moutinho et al. 2003), clearing the soil surface of plant material (reviewed in Farji-Brener & Illes 2000; Leal et al. 2014), and stripping tree canopies of leaves (Leal et al. 2014). However, our spatially stratified sampling around nests also revealed that leaf-cutter ants do not modify canopy cover, even directly over nest mounds. This suggests that neither increased light penetration to the understory nor changes in abiotic conditions resulting from increased light are mechanisms by which *A. laevigata* indirectly modifies seedling communities in our site. This conclusion contrasts sharply with that of prior studies (Correa et al. 2010; Meyer et al. 2011), but most of these have been conducted in lowland forests where light limitation is often the principal factor limiting seedling recruitment and growth (Kitajima 1994). The relatively shorter stature of Cerrado tress results in far greater penetration of light to the understory, even in physiognomies like *Cerrado denso* where canopy cover can exceed 90%.

Instead, it appears that *Atta laevigata* colonies create what Farji-Brenner and Illes (2000) refer to as ‘bottom-up’ gaps: patches of unique habitat resulting from *Atta*’s modifications of the understory and soil surface. We hypothesize that *A. laevigata* indirectly increases seed mortality due to desiccation (Salazar et al. 2012a) and granivory (Costa et al. 2017) by reducing soil moisture content and clearing away litter (Appendix D). We also hypothesize it reduces the growth or survival of seedlings that become established on nest mounds by altering soil chemistry through bioturbation, by altering nutrient availability (but see Sternberg et al. 2007), or burying them under excavated soil (Costa 2013). If so, *A. laevigata's* reduction of seedling abundance via environmental engineering of the Cerrado may rival its direct effects as a seed predator (Costa et al. 2017; Ferreira et al. 2011) and herbivore (Vasconcelos & Cherrett 1997).

It is notable that the impacts of *Atta laevigata* on seedling abundance and diversity appear restricted primarily to the nest mound itself, which may limit the spatial extent of an individual colony's impact. However, a salient feature of many engineers is that localized impacts such as these can often persist long-term (Hastings et al. 2007). *Atta* mounds remain long after a colony has died or migrated; both the short- and long-term footprint of *Atta laevigata* on a landscape may therefore be strongly influenced by on historical changes in population size. Such demographically dependent effects of engineers may be particularly common in sites where their activities have clearly delineated boundaries that scale with individual, colony, or population size (Hastings et al. 2007). If so, this suggests there is potentially an alternative framework for conceptualizing engineer impacts – one in which engineer life-history and population dynamics, rather than engineer impacts on underlying gradients – is central to understanding their landscape-level impacts.

Implications for Cerrado plant communities

In contrast to savannas in the Paleotropics, the density and diversity of large mammalian herbivores in the Cerrado is very low (Marinho-Filho et al. 2002). This has led many to conclude that plant population and communities in this biome are largely structured by edaphic factors (reviewed in Hoffmann & Moreira 2002; Mistry 1998; Ruggiero et al. 2002) and that the influence of herbivores is negligible (e.g., Gardner 2006). Although the key role of physical factors in Cerrado seedling recruitment is indisputable (Hoffmann 1996; Hoffmann 2000; Salazar et al. 2012a; Salazar et al. 2012b), studies evaluating the impacts of herbivores are rare (Ferreira et al. 2011; Mundim et al. 2012), especially those simultaneously assessing the effects of herbivores and edaphic conditions (e.g., Klink 1996). Our study supports the hypothesis that herbivores do indeed play a dominant role in Cerrado plant demography (Costa et al. 2008). Furthermore, it provides compelling evidence that leaf-cutter ants do so both directly as consumers and indirectly by altering environmental conditions influencing seedling recruitment, growth, and survival. As such, ignoring these keystone herbivores could undermine attempts to develop general theory for vegetation dynamics in this biome (e.g., Gardner 2006) as well as conservation and restoration efforts.

Future directions

Our results suggest three promising directions for future studies of ecosystem engineers, especially those carried out in high-diversity tropical systems. First, we demonstrate that models fit with diverse types of ecological data can be used to test hypotheses about the impacts of engineers at spatial scales not amendable to experimental manipulation, in high-biodiversity sites, or when the environment of interest is a best described by a combination of correlated variables. Nevertheless, we suggest experiments manipulating both engineers and subsets of environmental variables at smaller spatial scales will complement statistical approaches and provide important insights needed to test and guide theory (Hastings et al. 2007; Wright & Jones 2006). Second, how the spatio-temporal impacts of engineers are influenced by disturbance type, frequency, and intensity is conceptually critical (Crain & Bertness 2006) but conspicuously understudied (Hastings et al. 2007). The same is true for how the spatio-temporal impacts of disturbances might be influenced by engineers and their modification of the environment. Fire is an important form of disturbance that critically influences seedling recruitment in a diversity of temperate and tropical ecosystems, including savannas like the Cerrado. Fire has been shown to alter the foraging activity of leaf-cutter ants (Lopes & Vasconcelos 2011), but the density and abundance of ant nests can influence the spread of fire (Carvalho et al. 2012) and post-fire nutrient availability (Sousa-Souto et al. 2007). We suggest future studies explicitly consider potential for fire-*Atta* feedbacks and time-since-fire, both of which could influence the relative importance of environmental factors and *Atta* engineering for plant communities. Finally, human activities such as deforestation, habitat fragmentation, road creation, and nutrient deposition can alter environmental gradients (Broadbent et al. 2008; Tulloss & Cadenasso 2015) as well as the abundance of *Atta laevigata* and other ecosystem engineers (Cameron & Bayne 2009; Vasconcelos et al. 2006; Vieira-Neto et al. 2016). The ecological and economic footprint of these engineers may therefore increase dramatically in coming decades – especially in tropical regions – in ways that remain underappreciated and poorly understood.

ACKNOWLEDGEMENTS

We thank the Universidade Federal de Uberlândia for logistical support and M. D. Rodrigues for assistance in the field. G. M. Araújo and I. Schiavini provided invaluable help identifying plants, and B. Baiser, J. Ashander, Beatriz Sosa, and two anonymous reviewers provided helpful feedback or comments on the manuscript. Code used for the analyses and figures in this paper are available at Zenodo (doi -------).

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TABLE 1. 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 & Hui 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. The best model is noted in bold.

Model	Factors	Resid. Df	Resid. Dev	*d*AIC	wAIC
1	Nest Identity	57	97.525	0	0.999
2	Nest Identity, Plot Location, Nest Mound Area	54	94.098	14.567	6.8×10^{-4}
3	Nest Identity, Plot Location, Nest Mound Area, Plot Location*Nest Mound Area Interaction	52	90.144	29.186	4.6×10^{-7}

TABLE 2: 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%. The variables included in this PCA (referred to as PCA-1 in the text) were litter biomass, soil penetrability, grass biomass, and soil moisture content. Data for PCA-1 were collected in plots on the center of, adjacent to, and 10m from the edge of N = 20 all *Atta laevigata* nest mounds.

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

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TABLE 3: 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%. The variables included in this PCA (referred to as PCA-2 in the text) were litter biomass, soil penetrability, grass biomass, soil moisture content, soil pH, several soil macronutrients, and soil organic material, and the data were collected in plots in the center of and 10m from N = 10 *Atta laevigata* nest mounds.

Variable	PC1	PC2	PC3	PC4
Litter biomass	0.3227	-0.2081	0.3570	-0.1962
Soil penetrability	-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 content	0.3914	0.0460	0.1600	0.2902

TABLE 4: 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-1 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), plot location 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 (bold) was the one that included Plot Location, Canopy Cover, and the random effect of Nest Identity.

Model	Factors	Resid. Df	Resid. Dev	*d*AIC	wAIC
4	**Plot Location, Canopy Cover, Nest Identity**	**54**	**134.13**	**0**	**0.796**
2	Plot Location, Nest Identity	55	148.95	2.72	0.205
5	Plot Location, Canopy Cover, Plot Location*Canopy Cover Interaction, Nest Identity	52	132.09	19.88	3.8×10^{-5}
3	Canopy Cover, Nest Identity	56	192.10	49.03	1.8×10^{-11}
1	Nest Identity	57	205.28	51.12	6.2×10^{-12}

TABLE 5: 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-2 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 (bold) was the one that included the fixed effect of plot location and the random effect of nest identity.

Model	Factors	Resid. df	Resid. Dev	*d*AIC	wAIC
2	**plot location, nest identity**	**16**	**56.15**	**0**	**0.906**
4	canopy cover, nest identity	15	49.82	4.55	0.093
5	plot location, canopy cover, plot location*canopy cover interaction, nest identity	14	49.47	13.75	9.4×10^{-4}
1	nest identity	17	86.77	26.67	1.5×10^{-6}
3	canopy cover, nest identity	16	84.86	33.41	5.0×10^{-8}

TABLE 6: 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 location, local environmental conditions, i.e., axis 1 scores from PCA-1). 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 (bold) included factors and covariates related ants and their activity.

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

Model	Factors	Resid. df	Resid. Dev	dAIC	wAIC
3	**nest mound area, plot location, local environmental conditions, random effect of nest identity, per-observation random effects**	**54**	**29.89**	**0**	**0.828**
4	nest mound area, plot location, local canopy cover, environmental conditions, random effect of nest identity, per-observation random effects	52	29.92	3.13	0.173
1	random effect of nest identity, per-observation random effects	57	23.34	43.97	2.3×10^{-10}
2	canopy cover, random effect of nest identity, per-observation random effects	56	23.39	45.92	8.8×10^{-11}

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

Model	Factors	Resid. df	Resid. Dev	dAIC	wAIC
3	**nest mound area, plot location, environmental conditions, random effect of nest identity, per-observation random effects**	**54**	**77.72**	**0**	**0.869**
4	nest mound area, plot location, canopy cover, environmental conditions, random effect of nest identity, per-observation random effects	52	78.76	3.787	0.131
1	random effect of nest identity, per-observation random effects	57	32.72	76.527	2.1×10^{-17}
2	canopy cover, random effect of nest identity, per-observation random effects	56	32.70	78.448	8.0×10^{-18}

TABLE 7: 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 location, 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-2), 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 (in bold) included factors and covariates related ants and their activity.

Seedling Abundance (*Environment = PCA-2 axis 1*)

Model	Factors	Resid. df	Resid. Dev	dAIC	wAIC
3	**nest mound area, plot location, local environmental conditions, random effect of nest identity, per-observation random effects**	**15**	**11.65**	**0**	**0.773**
4	nest mound area, plot location, local canopy cover, environmental conditions, random effect of nest identity, per-observation random effects	13	10.55	2.47	0.224
1	random effect of nest identity, per-observation random effects	17	7.31	12.04	0.002
2	Canopy Cover, random effect of nest identity, per-observation random effects	16	7.09	13.75	0.001

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Species Richness *(Environment = PCA-2 axis 1)*

Model	Factors	Resid. df	Resid. Dev	dAIC	wAIC
3	**nest mound area, plot location, local environmental conditions, random effect of nest identity, per-observation random effects**	**15**	**29.40**	**0**	**0.777**
4	nest mound area, plot location, canopy cover, environmental conditions, random effect of nest identity, per-observation random effects	13	31.90	2.49	0.223
1	random effect of nest identity, per-observation random effects	17	8.50	23.16	7.3×10^{-4}
2	canopy cover, random effect of nest identity, per-observation random effects	16	8.78	24.86	3.1×10^{-6}

Figure 1 (A). Number of plots in our *Cerrado* study site with different amounts of canopy cover. Dark gray bars represent plots in the *cerrado denso* vegetation type, while white bars light refer to plots in *cerrado ralo.* Light gray bars indicate overlap in habitat types. Three plots were arranged around each of N = 20 leaf-cutter ant (*Atta laevigata*) nests: one in the center of the nest mound, one on the edge of the nest, and one10m from the edge of the nest (N=60 plots total).

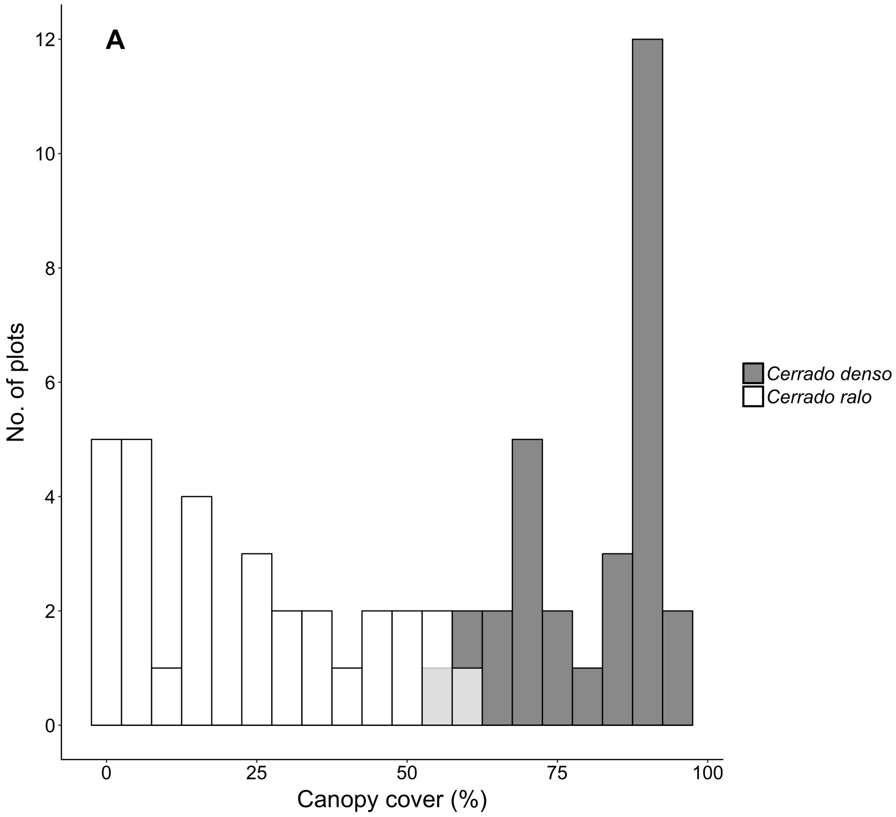


Figure 1 (B). Canopy cover over plots on *Atta laevigata* nests (blue circles), adjacent to nests (blue triangles), and far from nests (gray squares). Canopy cover is independent of plot proximity to the N=20 nests (Table 1), indicating ants are not responsible for or modifying the canopy cover gradient in our study site.

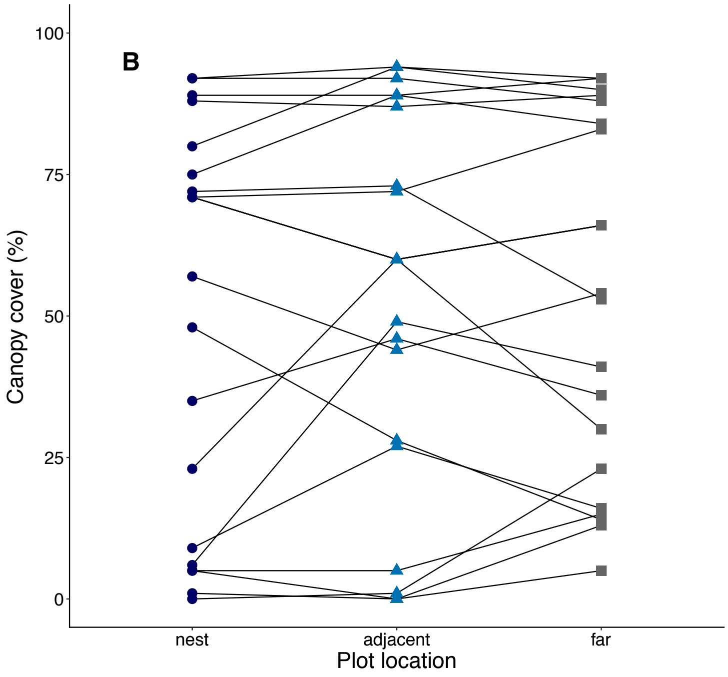


Figure 2 (A). Principal component ordination of the environmental conditions in plots located on (blue circles), adjacent to (blue triangles), or 10m from the edge (gray squares) of N = 20 *Atta laevigata* nests. The variables included in this PCA (referred to as PCA-1 in the text) were litter biomass, soil penetrability, grass biomass, and soil moisture content. Symbol size indicates the percent canopy cover over that plot.

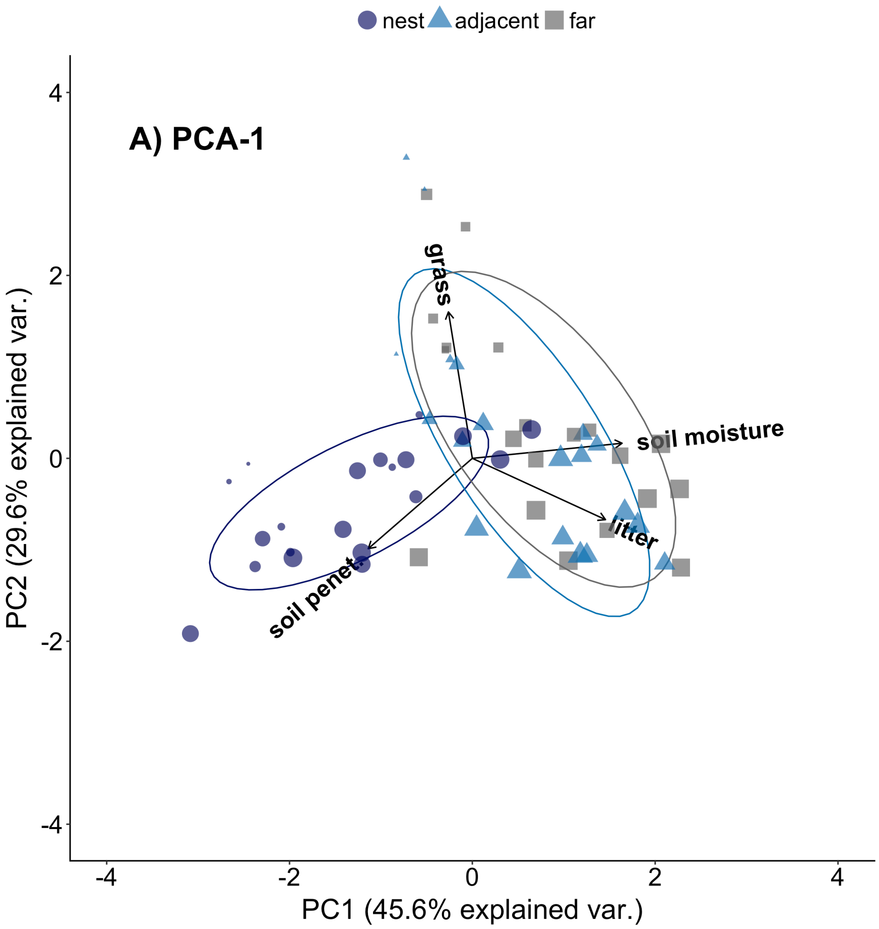


Figure 2 (B). Principal component ordination of the environmental conditions in plots located on (blue circles) or 10 m from (gray squares) each of N=10 *Atta laevigata* nests. The variables included in this PCA (referred to as PCA-2 in the text) were litter biomass, soil penetrability, grass biomass, soil moisture content, soil pH, several soil macronutrients, and soil organic material. Symbol size indicates the percent canopy cover over that plot.

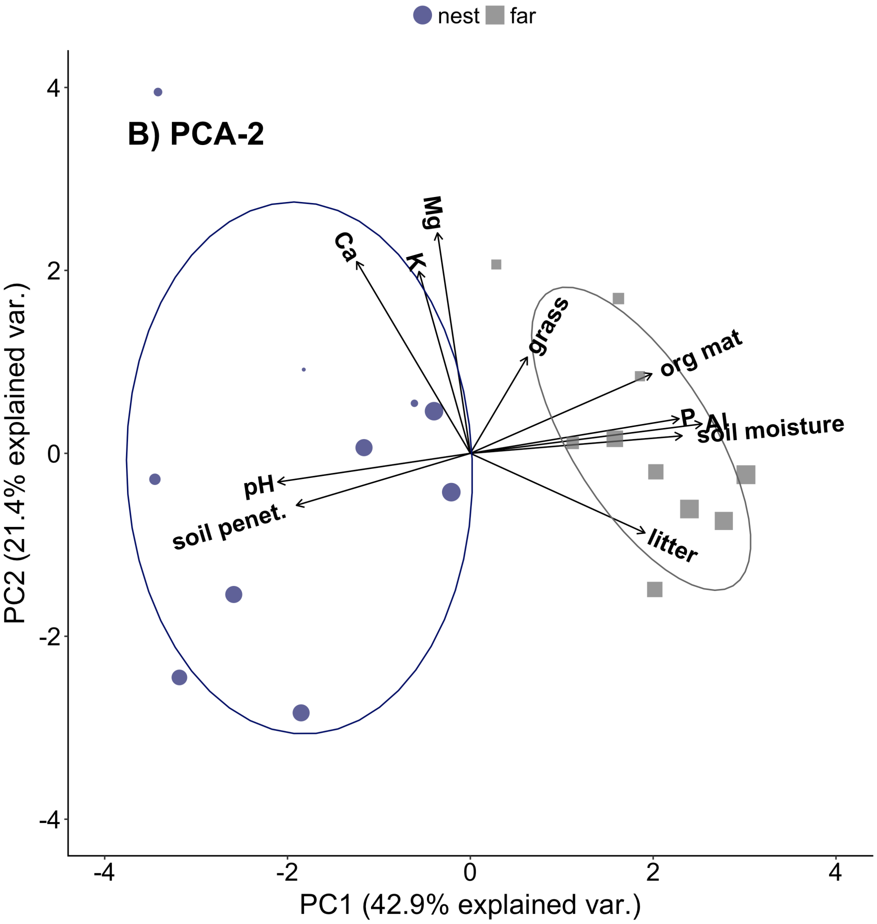


Figure 3. Relationship between canopy cover over a plot and environmental conditions in that plot. “Environmental conditions” were each plot’s score on the 1st principal component of either **(A)** PCA-1 (N= 5 environmental variables measured for N=20 nests) or **(B)** PCA-2 (N= 12 environmental variables measured for N=10 nests). Plots were on the middle of nest mounds (blue circles), adjacent to nests (blue triangles), or 10 m from the edge of nests (gray squares). The linear regression lines for each group of plots are shown in the corresponding colors.

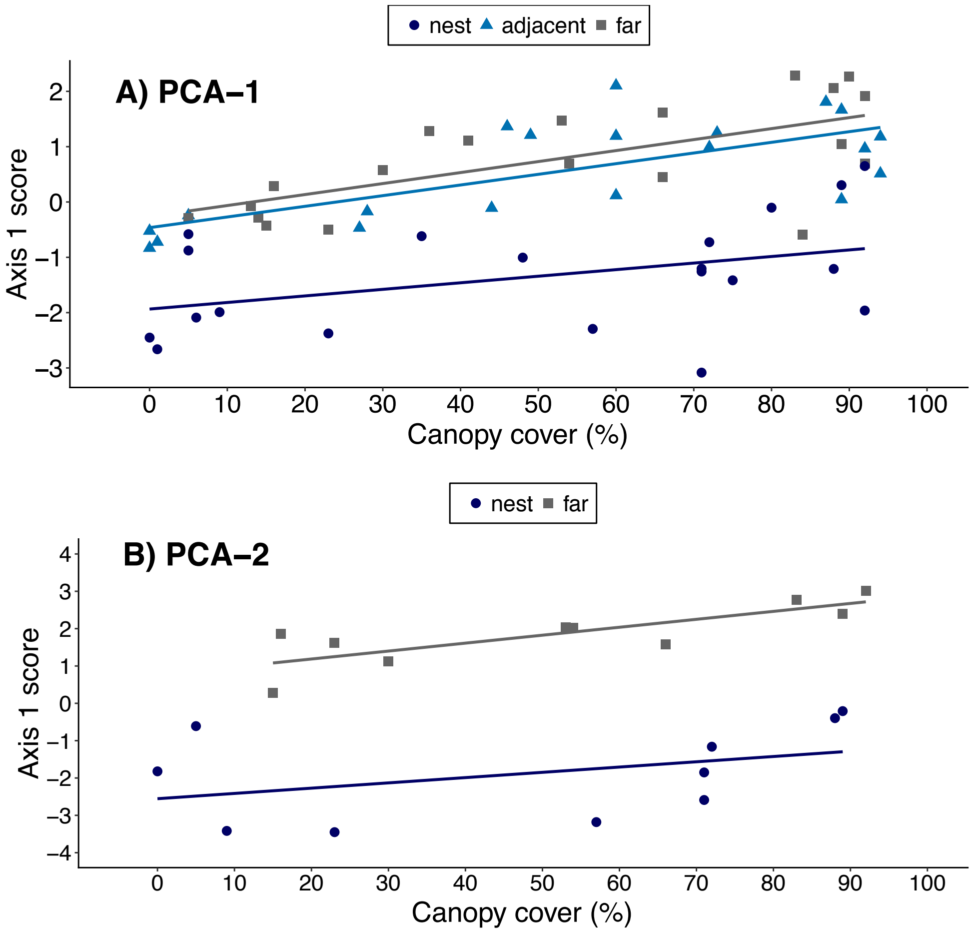
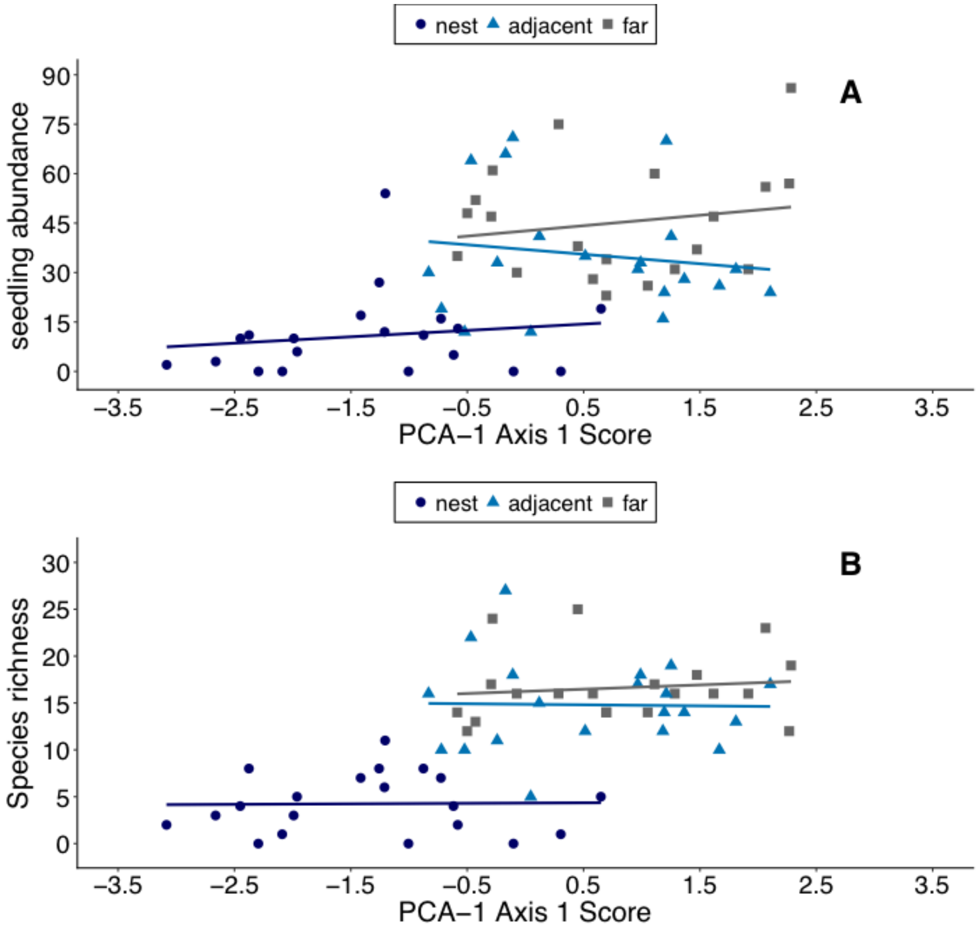
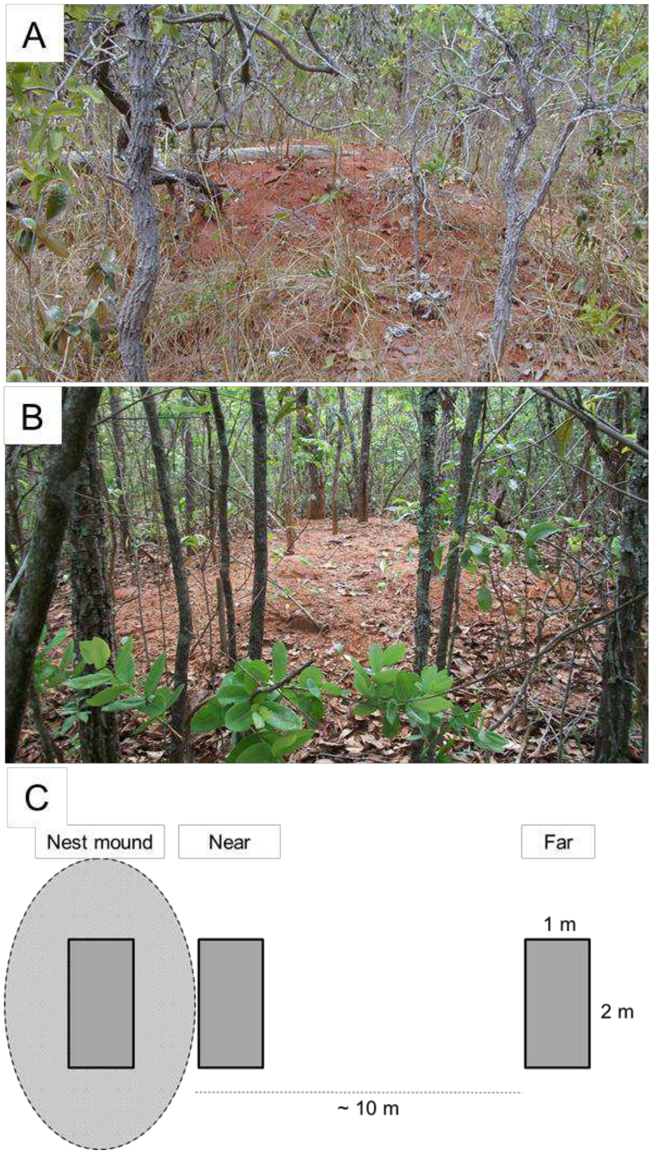


Figure 4. The number of **(A)** seedlings and **(B)** species in plots with different environmental conditions (defined as a plot's score on the first principal component axis of PCA-1). Plots were in the center of *Atta laevigata* nest mounds (blue circles), adjacent to nests (blue triangles), or 10 m from the edge of nests (gray squares). The linear regression lines for each group of plots are shown in the corresponding colors.



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

	Litter biomass	Soil penetrability	Grass biomass	pH	P	K	Ca	Mg	Al	Org. Material	Soil moisture content
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 penetrability		-	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 content											-

APPENDIX C: Scatterplot of **(A)** grass biomass, **(B)** litter biomass, **(C)** soil penetrability (i.e., penetration distance), and **(D)** surface soil moisture content 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).