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

Do the impacts of an ecosystem engineer vary along an environmental gradient? Tests with the leaf-cutter ant *Atta laevigata* in a Neotropical savanna.

Alan N. Costa[1*], Emilio M. Bruna[2,3†], Heraldo L. Vasconcelos[1]

[1]Instituto de Biologia, Universidade Federal de Uberlândia, Av. Pará 1720, 38405-320, Uberlândia, MG, Brazil

[2]Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, FL 32611, U.S.A.

[3]Center for Latin American Studies, University of Florida, Gainesville, FL 32611, U.S.A.

*Current Address: Instituto de Biotecnologia, Universidade Federal de Goiás - Regional de Catalão, Avenida Larmartine P. Avelar 1120, Setor Universitário, Catalão, GO, Brazil, 75704-020. Email: alan_nilo@yahoo.com

†Author for correspondence: Emilio Bruna, Department of Wildlife Ecology and Conservation, University of Florida, PO Box 110430, Gainesville, FL 32611, U.S.A. embruna@ufl.edu Phone: +1 (352) 514-3935 Fax: +1 (352) 392-6984

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1692v3 | CC-BY 4.0 Open Access | rec: 18 May 2016, publ: 18 May 2016

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 diversity 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 canopy cover locally or at the gradient scale. However, ants are modifying environmental conditions, with the magnitude and spatial extent of these changes consistent across the gradient. In contrast to prior studies, we found that both seedling abundance and diversity were independent of canopy-related environmental conditions. Instead, proximity to leaf-cutter nests had the strongest effect on both diversity and richness.

Discussion. *Atta laevigata* engineers environmental conditions in the Cerrado, with strong but spatially restricted consequences for seedling communities. We hypothesize that by clearing litter and reducing surface humidity ants indirectly reduce seedling establishment by increasing rates of seed dessication. The alteration of soil nutrients could also reduce seedling growth and survivorship, such that the indirect reduction of seedling abundance due to engineering could exacerbate the direct reduction due to herbivory. While the effects of *Atta* on environmental conditions and seedling communities appear restricted to the nest mound, they could be long-lasting because *Atta* mounds persist long after a colony has died or migrated.

These results underscores 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 populations, community composition, and ecosystem function (reviewed in Kleinhesselink et al. 2014; Wright & Jones 2006). Most research to date has focused on documenting the magnitude of engineer impacts on local biodiversity with more recent work evaluating how these impacts vary spatially (e.g., Badano & Marquet 2009; Baker et al. 2013; Dibner et al. 2015; Kleinhesselink et al. 2014; McAfee et al. 2016). Of particular 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 local biodiversity (e.g., John et al. 2007). Although disentangling the relative effects of engineers and gradients on biodiversity and how these vary spatially can be quite challenging, it is necessary to advance conceptual and mathematical models of ecosystem engineering (Hastings et al. 2007; Wright & 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, creating mounds with 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 & 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 & Wilson 2010;

Leal et al. 2014). However, they can be 2-3 times more abundant in Neotropical savannas than lowland forests (Costa & Vieira-Neto in press); in savannas they may also harvest up to 7-fold more plant biomass per hectare (Costa et al. 2008).

Brazil's *Cerrado* is a savanna woodland whose distribution of 2 million km^2 makes it South America's second largest biome. Like many savannas it 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 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. 2012a). For instance, areas with more cover can have cooler, shadier understories and more leaf-litter on the soil surface. This facilitates seedling establishment and enhances seedling survival by reducing soil water deficits and increasing nutrient availability (Salazar et al. 2012a). Closed-canopy sites also have lower cover of the native and invasive grasses that can inhibit seedling establishment (Hoffmann & Haridasan 2008).

In addition to directly reducing Cerrado seedling abundance via herbivory, leaf-cutter ants could indirectly reduce seedling abundance and diversity around their nests by creating canopy gaps, removing leaf-litter from the soil surface, decreasing soil fertility, or otherwise altering the environmental conditions that favor seedling establishment. The magnitude of *Atta*'s negative effects on seedlings should increase with canopy cover, however, since areas where trees are sparse are already hot, bright, and have limited leaf-litter on the soil surface. To test this prediction of context-dependent impacts of an ecosystem engineer we 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?

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; Figure 1A).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1692v3 | CC-BY 4.0 Open Access | rec: 18 May 2016, publ: 18 May 2016

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 & 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 A).

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 ≤ 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).

Because many of the biophysical variables we measured were highly correlated (Appendix B), we used Principal Components Analysis (PCA) to summarize the environmental conditions along the canopy cover gradient. We conducted two PCAs. The first, hereafter PCA-E, 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. The second, hereafter PCA-E&S, 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).

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 G and H) were ranked with Akaike Information Criteria (Burnham & 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).

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 I and J).

RESULTS

The canopy cover over plots is independent of plot proximity to leaf-cutter ant nests, indicating ants are not modifying either local canopy cover or the canopy cover gradient (Appendix C). Almost all environmental variables we measured varied with canopy cover, however, with the slope of the relationship influenced by proximity to *Atta laevigata* nests (Appendix D). For example, leaf-litter increased with canopy cover, but it was up to five-fold higher in plots far from nests and on nest edges than in plots on nest mounds. Similarly, grass biomass 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 varied with plot location, although some values were 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, with the conditions in plots adjacent to and far from nests overlapping in ordination space (Fig. 2a). In PCA-E the first axis explained 45.6% of the variance and was positively correlated with litter biomass and soil moisture (Fig. 2a). The second axis explained an additional 29.6% of the variance; it was negatively correlated with grass biomass and soil hardness (Appendix E). 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)

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. 2b). 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. 2b, Appendix F). 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 G). 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 H). 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. 3).

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, best explained the patterns of seedling abundance and species richness (Fig. 4). This was true when using as a proxy for environmental conditions the scores from PCA-E or PCA-E&S (Appendices I and J), although when using PCA-E&S the dAIC scores for models including both ants and environmental conditions were <1 (Appendix J). 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 engineers and environmental gradients are known to exert strong effects on ecosystems, it has been suggested they could have synergistic effects on local species abundance and diversity. However, empirical studies evaluating the impacts of ecosystem engineers along gradients (e.g., Badano & Marquet 2009; Kleinhesselink et al. 2014) remain rare, especially in species-rich tropical ecosystems. 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 canopy cover locally or at the gradient scale. However, ants are modifying environmental conditions, with the magnitude and spatial extent of these changes consistent across the gradient. In sharp contrast to prior studies, our analysis of 1800 seedlings found that both seedling abundance and diversity were independent of canopy-related environmental conditions; proximity to leaf-cutter nests had the strongest effect on them. Taken together these results demonstrate that *Atta laevigata* engineers environmental conditions in the Cerrado, with strong but spatially restricted consequences for seedling communities.

Leaf-cutter ants engineer our savanna site in many of the same ways *Atta* species in lowland forests do – by 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 & Illes 2000; Leal et al. 2014). However, we did not find a significant effect of plot location on canopy cover, even at the highest end of the canopy cover gradient and despite *A. laevigata* removing 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 by clearing litter and reducing surface humidity (Appendix D Figures C & D) ants indirectly reduce seedling establishment by increasing rates of seed dessication (Salazar et al. 2012a). Furthermore, the growth and survivorship of seedlings established on mounds could be reduced as a result of modified soil nutrient levels or the burial of seedlings under excavated soil (Costa 2013). If so, it is possible the indirect reduction of seedling abundance resulting from environmental engineering could rival *A. laevigata*'s direct effects from granivory (Ferreira et al. 2011) and harvesting seedlings for fungal gardens.

It is notable that *Atta laevigata* impacts on seedling abundance and diversity appear restricted primarily to the nest mound. While this may limit the spatial extent of an individual colony's impact on seedlings communities, a salient feature of many engineers is that their localized impacts can persist long-term (Hastings et al. 2007). Because *Atta* mounds can persist long after a colony has died or migrated, both the short- and long-term footprint of *Atta laevigata* on a landscape will depend on changes in population size. Such demographically dependent engineer impacts may be particularly common in sites where engineer impacts have clearly delineated boundaries that scale with individual, colony, or population size (Hastings et al. 2007). If so, this suggests 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.

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 (e.g., Ferreira et al. 2011), as are those simultaneously assessing the effects of herbivores and edaphic conditions (e.g., Klink 1996). Leaf-cutter ants are the most prominent members of the *Cerrado*'s rich community of herbivorous insects and other 'cryptic consumers' (sensu Keesing 2000), and they have direct detrimental impacts on plant recruitment, growth, survivorship, and reproduction (Ferreira et al. 2011; Mundim et al. 2012). While we did not manipulate herbivores or environmental conditions directly, our study provides compelling evidence that by altering environmental conditions*, Atta* can also have indirect detrimental effects on plant recruitment. This further underscores our hypothesis that leaf-cutter ants play a dominant role in Cerrado plant demography (Costa et al. 2008; Ferreira et al. 2011; Mundim et al. 2012), and that ignoring these keystone herbivores will undermine attempts to develop general theory for vegetation dynamics in this biome (e.g., Gardner 2006) as well as conservation and restoration efforts.

Our results suggest three promising directions for future studies of ecosystem engineers in tropical ecosystems. First, we demonstrate that survey data coupled with rigorous statistical approaches can be used to test hypotheses about the impacts of engineers in high-biodiversity sites not amendable to landscape-scale experimental manipulation or in environments defined by correlated variables. Nevertheless, we suggest experiments manipulating both engineers and subsets of environmental conditions 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 (e.g., Crain & Bertness 2006) but conspicuously understudied, especially in the tropics (Hastings et al. 2007). Fire can affect many factors influencing seedling establishment in the Cerrado (e.g., soil nutrients, canopy and ground cover, plant reproduction), including the foraging of leaf-cutter ants (Lopes & Vasconcelos 2011). We suggest future studies explicitly consider time-since fire or compare burned and unburned sites, since the details of this disturbance disturbance could alter the relative importance for plant communities of environmental factors and *Atta* engineering. 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 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, and anonymous reviewers provided helpful feedback or comments on the manuscript.

LITERATURE CITED

Alvarado A, Berish CW, and Peralta F. 1981. Leaf-Cutter Ant *(Atta cephalotes*) influence on the morphology of Andepts in Costa Rica. *Soil Science Society of America Journal* 45:790-794.

Badano EI, and Marquet PA. 2009. Biogenic habitat creation affects biomass-diversity relationships in plant communities. *Perspectives in Plant Ecology Evolution and Systematics* 11:191-201.

Baker BW, Augustine DJ, Sedgwick JA, and Lubow BC. 2013. Ecosystem engineering varies spatially: a test of the vegetation modification paradigm for prairie dogs. *Ecography* 36:230-239.

Bates D, Mächler M, Bolker B, and Walker S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67:1-48.

Bertness MD, and Callaway R. 1994. Positive interactions in communities. *Trends in Ecology & Evolution* 9:191-193.

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, and White JSS. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution* 24:127-135.

Broadbent EN, Asner GP, Keller M, Knapp DE, Oliveira PJC, and Silva JN. 2008. Forest fragmentation and edge effects from deforestation and selective logging in the Brazilian Amazon. *Biological Conservation* 141:1745-1757.

Bruna EM, Guimarães JF, Lopes CT, Pacheco R, Facure KG, Lemos FG, and Vasconcelos HL. 2010. Mammalia, Estação Ecologica do Panga, a Cerrado protected area in Minas Gerais state, Brazil. *CheckList: Journal of Species Lists and Distribution* 6:668-675.

Burnham KP, and Anderson DR. 2002. *Model selection and multimodel inference: a practical information-theoretic approach*. New York: Springer Science & Business Media.

Cameron EK, and Bayne EM. 2009. Road age and its importance in earthworm invasion of northern boreal forests. *Journal of Applied Ecology* 46:28-36.

Cardoso E, Cruzeiro Moreno MIB, E. M., and Vasconcelos HL. 2009. Mudanças fitofisionômicas no Cerrado: 18 anos de sucesão ecológica na Estação Ecológica do Panga, Uberlândia, MG. *Caminhos de Geografia* 10:254-268.

Correa MM, Silva PSD, Wirth R, Tabarelli M, and Leal IR. 2010. How leaf-cutting ants impact forests: drastic nest effects on light environment and plant assemblages. *Oecologia* 162:103-115.

Costa AN. 2013. Direct and indirect effects of leaf-cutter ants (*Atta*) on the dynamics of vegetation in a Neotropical Savanna Ph.D. Doctoral thesis. Universidade Federal de Uberlândia.

Costa AN, Vasconcelos HL, Vieira-Neto EHM, and Bruna EM. 2008. Do herbivores exert top-down effects in Neotropical savannas? Estimates of biomass consumption by leaf-cutter ants. *Journal of Vegetation Science* 19:849-854.

Costa AN, and Vieira-Neto EHM. in press. Species turnover regulates leaf-cutter ant densities in environmental gradients across the Brazilian Cerrado. *Journal of Applied Entomology*.

Crain CM, and Bertness MD. 2006. Ecosystem engineering across environmental gradients: Implications for conservation and management. *Bioscience* 56:211-218.

Dibner RR, Doak DF, and Lombardi EM. 2015. An ecological engineer maintains consistent spatial patterning, with implications for community-wide effects. *Ecosphere* 6.

EMBRAPA. 1997. *Manual de métodos de análise de solo, 2a edição*. Rio de Janeiro, Brazil: Produção de Informação-EMBRAPA.

Farji-Brener AG, and Illes AE. 2000. Do leaf-cutting ant nests make “bottom–up” gaps in neotropical rain forests? A critical review of the evidence. *Ecology Letters* 3:219-227.

Ferreira AV, Bruna EM, and Vasconcelos HL. 2011. Seed predators limit plant recruitment in Neotropical savannas. *Oikos* 120:1013-1022.

Gardner TA. 2006. Tree-grass coexistence in the Brazilian cerrado: demographic consequences of environmental instability. *Journal of Biogeography* 33:448-463.

Hastings A, Byers JE, Crooks JA, Cuddington K, Jones CG, Lambrinos JG, Talley TS, and Wilson WG. 2007. Ecosystem engineering in space and time. *Ecology Letters* 10:153-164.

Hoffmann WA. 1996. The effects of fire and cover on seedling establishment in a neotropical savanna. *Journal of Ecology* 84:383-393.

Hoffmann WA. 2000. Post-establishment seedling success in the Brazilian Cerrado: A comparison of savanna and forest species'. *Biotropica* 32:62-69.

Hoffmann WA, and Haridasan M. 2008. The invasive grass, *Melinis minutiflora*, inhibits tree regeneration in a Neotropical savanna. *Austral Ecology* 33:29-36.

Hoffmann WA, and Moreira AG. 2002. The role of fire in population dynamics of woody plants. In: Oliveira PS, and Marquis RJ, eds. *The cerrados of Brazil: ecology and natural history of a neotropical savanna*. New York: Columbia University Press, 159-177.

Hölldobler B, and Wilson EO. 2010. *The leafcutter ants: civilization by instinct*. New York: WW Norton & Company.

John R, Dalling JW, Harms KE, Yavitt JB, Stallard RF, Mirabello M, Hubbell SP, Valencia R, Navarrete H, Vallejo M, and Foster RB. 2007. Soil nutrients influence spatial distributions of tropical tree species. *Proceedings of the National Academy of Sciences of the United States of America* 104:864-869.

Jones CG, Lawton JH, and Shachak M. 1994. Organisms as Ecosystem Engineers. *Oikos* 69:373-386.

Jones CG, Lawton JH, and Shachak M. 1997. Positive and negative effects of organisms as physical ecosystem engineers. *Ecology* 78:1946-1957.

Keesing F. 2000. Cryptic consumers and the ecology of an African savanna. *Bioscience* 50:477-477.

Kleinhesselink AR, Magnoli SM, and Cushman JH. 2014. Shrubs as ecosystem engineers across an environmental gradient: effects on species richness and exotic plant invasion. *Oecologia* 175:1277-1290.

Klink CA. 1996. Germination and seedling establishment of two native and one invading African grass species in the Brazilian cerrado. *Journal of Tropical Ecology* 12:139-147.

Leal IR, Wirth R, and Tabarelli M. 2014. The multiple impacts of leaf-cutting ants and their novel ecological role in human-modified Neotropical forests. *Biotropica* 46:516-528.

Lopes CT, and Vasconcelos HL. 2011. Fire increases insect herbivory in a Neotropical savanna. *Biotropica* 43:612-618.

Marinho-Filho J, Rodrigues FHG, and Juarez KM. 2002. The cerrado mammals: diversity, ecology, and natural history. In: Oliveira PS, and Marquis RJ, eds. *The cerrados of Brazil; ecology and natural history of a neotropical savanna*. New York: Columbia University Press, 266-284.

McAfee D, Cole VJ, and Bishop MJ. 2016. Latitudinal gradients in ecosystem engineering by oysters vary across habitats. *Ecology* 97:929-939.

Meyer ST, Leal IR, Tabarelli M, and Wirth R. 2011. Ecosystem engineering by leaf-cutting ants: nests of Atta cephalotes drastically alter forest structure and microclimate. *Ecological Entomology* 36:14-24.

Meyer ST, Neubauer M, Sayer EJ, Leal IR, Tabarelli M, and Wirth R. 2013. Leaf-cutting ants as ecosystem engineers: topsoil and litter perturbations around *Atta cephalotes* nests reduce nutrient availability. *Ecological Entomology* 38:497-504.

Mistry J. 1998. Fire in the cerrado (savannas) of Brazil: an ecological review. *Progress in Physical Geography* 22:425-448.

Moreira A, Forti LC, Andrade AP, Boaretto MA, and Lopes J. 2004. Nest Architecture of *Atta laevigata* (F. Smith, 1858) (Hymenoptera: Formicidae). *Studies on Neotropical Fauna and Environment* 39:109-116.

Mundim FM, Bruna EM, Vieira-Neto EHM, and Vasconcelos HL. 2012. Attack frequency and the tolerance to herbivory of Neotropical savanna trees. *Oecologia* 168:405-414.

Oliveira-Filho AT, and Ratter JA. 2002. Vegetation physiognomies and woody flora of the cerrado biome. *The Cerrados of Brazil: Ecology and natural history of a neotropical savanna*:91-120.

R Core Development Team. 2014. R: A language and environment for statistical computing. In: R Foundation for Statistical Computing, editor. 3.1.0 ed. Vienna, Austria.

Ruggiero PGC, Batalha MA, Pivello VR, and Meirelles ST. 2002. Soil-vegetation relationships in cerrado (Brazilian savanna) and semideciduous forest, Southeastern Brazil. *Plant Ecology* 160:1-16.

Salazar A, Goldstein G, Franco AC, and Miralles-Wilhelm F. 2012a. Differential seedling establishment of woody plants along a tree density gradient in Neotropical savannas. *Journal of Ecology* 100:1411-1421.

Salazar A, Goldstein G, Franco AC, and Miralles-Wilhelm F. 2012b. Seed limitation of woody plants in Neotropical savannas. *Plant Ecology* 213:273-287.

Tulloss EM, and Cadenasso ML. 2015. Nitrogen deposition across scales: hotspots and gradients in a California savanna landscape. *Ecosphere* 6:12.

Vasconcelos HL, Vieira-Neto EHM, Mundim FM, and Bruna EM. 2006. Roads alter the colonization dynamics of a keystone herbivore in neotropical savannas. *Biotropica* 38:661-665.

Vieira-Neto EHM, Vasconcelos HL, and Bruna EM. 2016. Roads increase population growth rates of a native leaf-cutter ant in Neotropical savannahs. *Journal of Applied Ecology*:n/a-n/a.

Vu V. 2015. ggbiplot. Available at https://github.com/vqv/ggbiplot.

Warton DI, and Hui FKC. 2010. The arcsine is asinine: the analysis of proportions in ecology. *Ecology* 92:3-10.

Wright JP, and Jones CG. 2006. The concept of organisms as ecosystem engineers ten years on: Progress, limitations, and challenges. *Bioscience* 56:203-209.

Zuidema PA, Jongejans E, Chien PD, During HJ, and Schieving F. 2010. Integral Projection Models for trees: a new parameterization method and a validation of model output. *Journal of Ecology* 98:345-355.

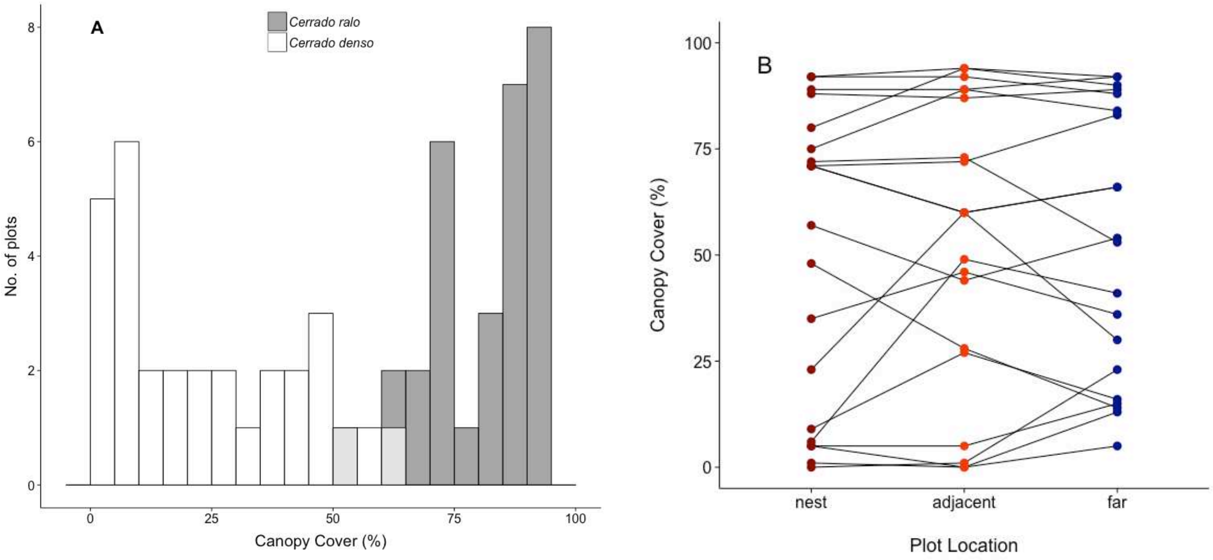


Figure 1 (A). Histogram of canopy cover over N=60 plots in our Brazilian *Cerrado* study site. We established three plots for each of N = 20 leaf-cutter ant (*Atta laevigata*) nests: one in the center of the nest mound, one on the edge of the nest, and the third 10m from the edge of the nest. Dark gray bars represent plots in the *cerrado denso* vegetation type, while light gray bars refer to plots in *cerrado ralo*. **(B).** Canopy cover over plots on, adjacent to, and far from each nest of N=20 nests. Canopy cover is independent of plot proximity to ant nests (Appendix C), indicating ants are not responsible for or altering canopy cover gradient.

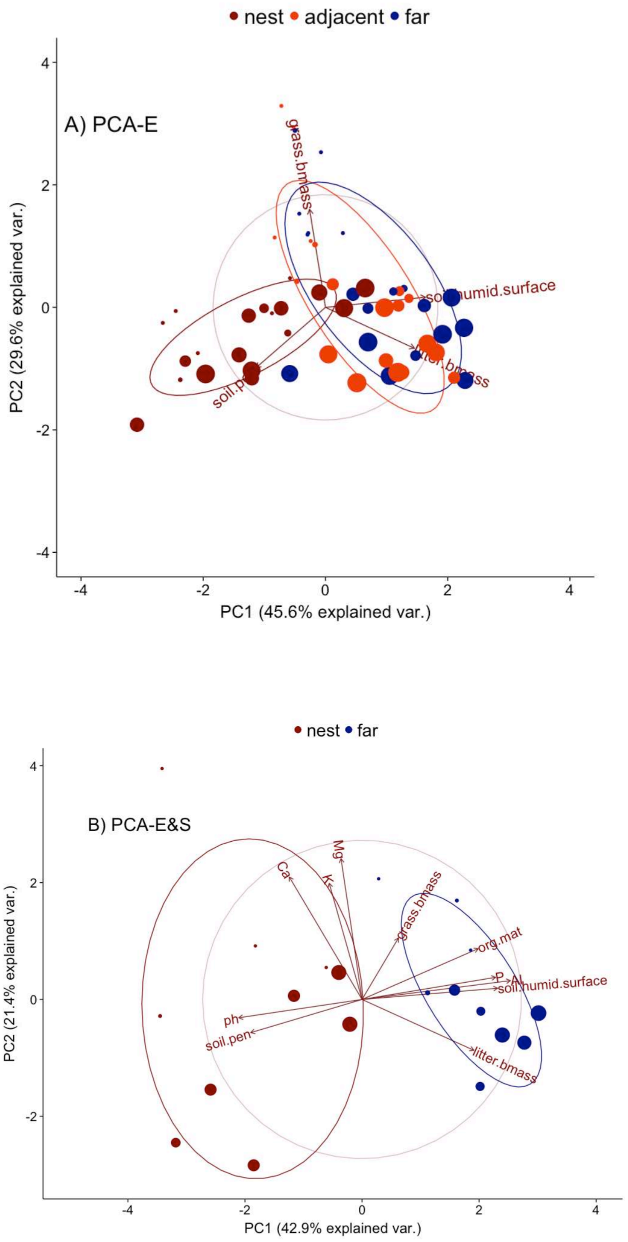


Figure 2. (A) Principal component ordination (PCA-E) of plots located on (red), adjacent to (orange), or 10m from the edge of (blue) N=20 *Atta laevigata* nest mounds in Brazilian Cerrado. **(B)** Principal component ordination of the subset of plots located on each of N=10 nest mounds (red) and 10m 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.

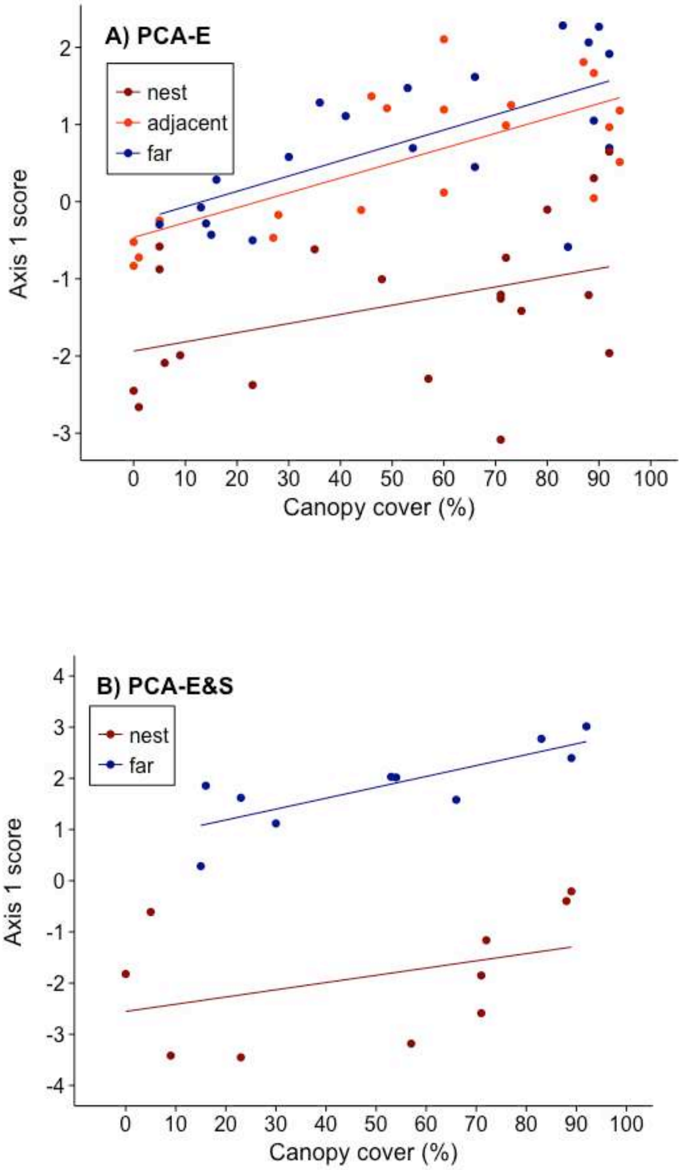


Figure 3. Regression of a plot's score on the first principal component summarizing environmental conditions vs. canopy cover over that plot. **(A)** Uses scores from PCA-E and **(B)** uses scores from PCA-E&S. Plots were located in the center of leaf-cutter ant (*Atta laevigata*) nest mounds (red),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

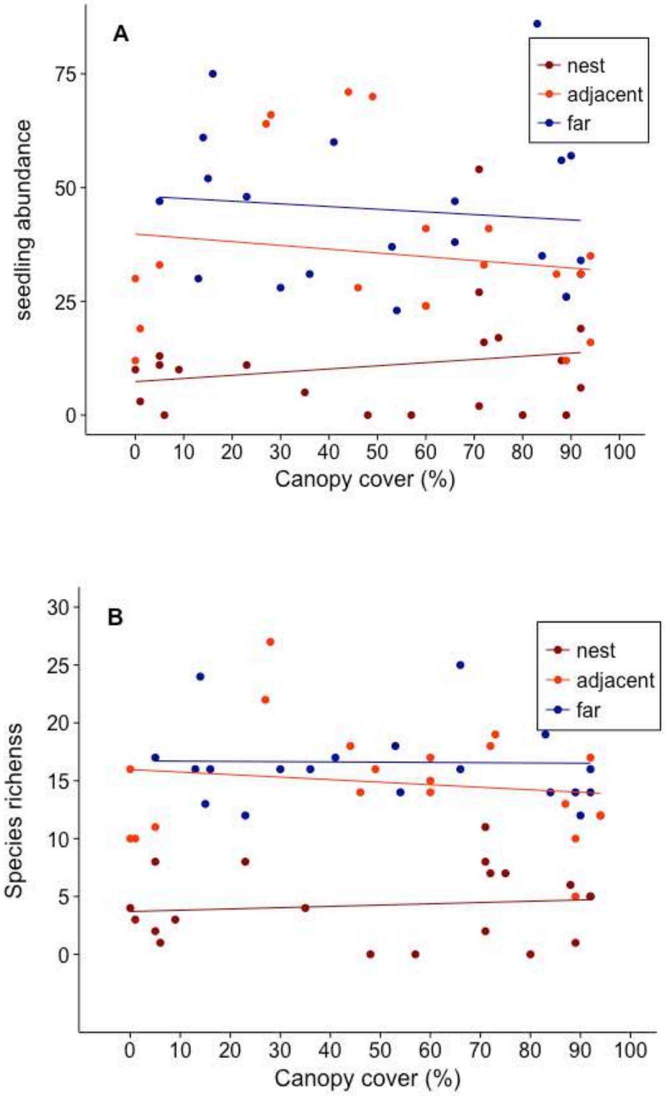
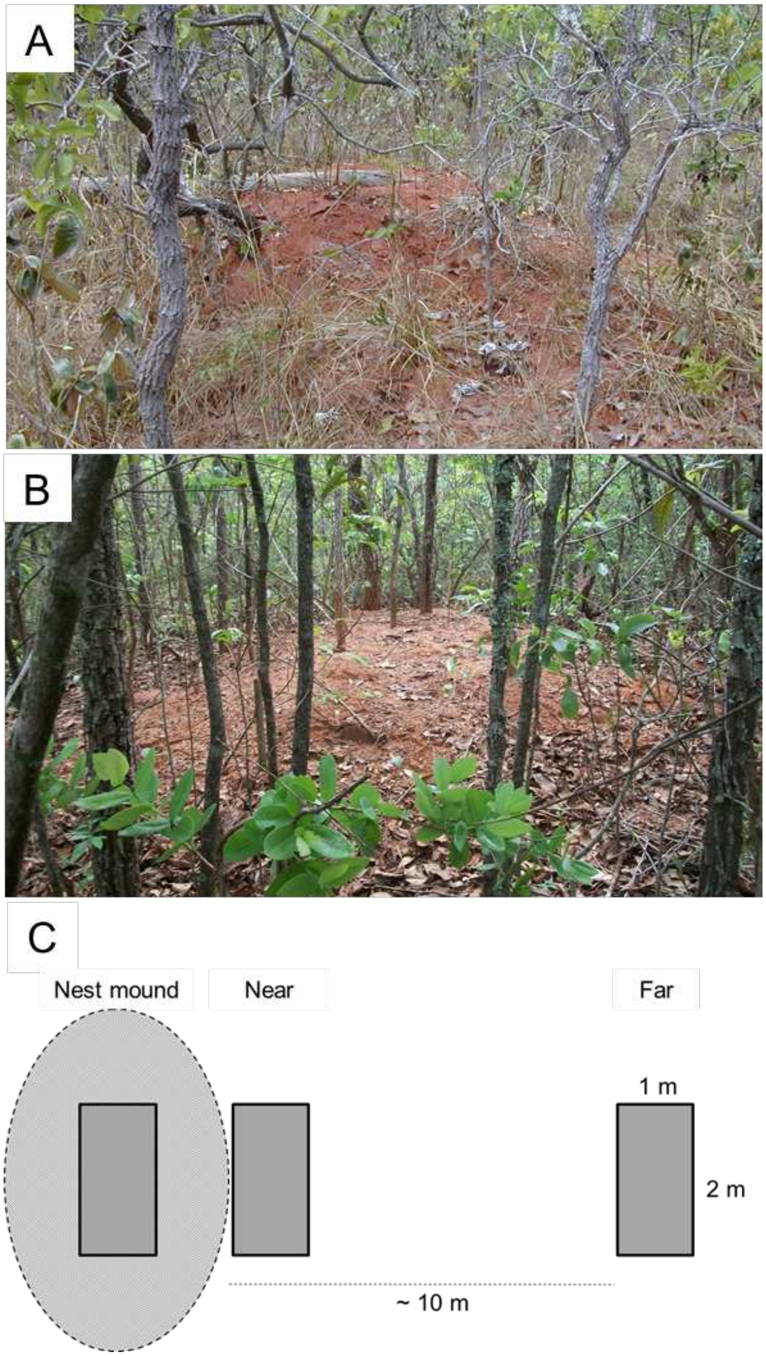


Figure 4. Regression of **(A)** the number of seedlings and **(B)** species richness in plots as a function of the plot's canopy cover. Plots were located in the center of leaf-cutter ant (*Atta laevigata*) nest mounds (red), adjacent to nest (orange), or 10 m from nests (blue) in Brazil's *Cerrado*.

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.



PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1692v3 | CC-BY 4.0 Open Access | rec: 18 May 2016, publ: 18 May 2016

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

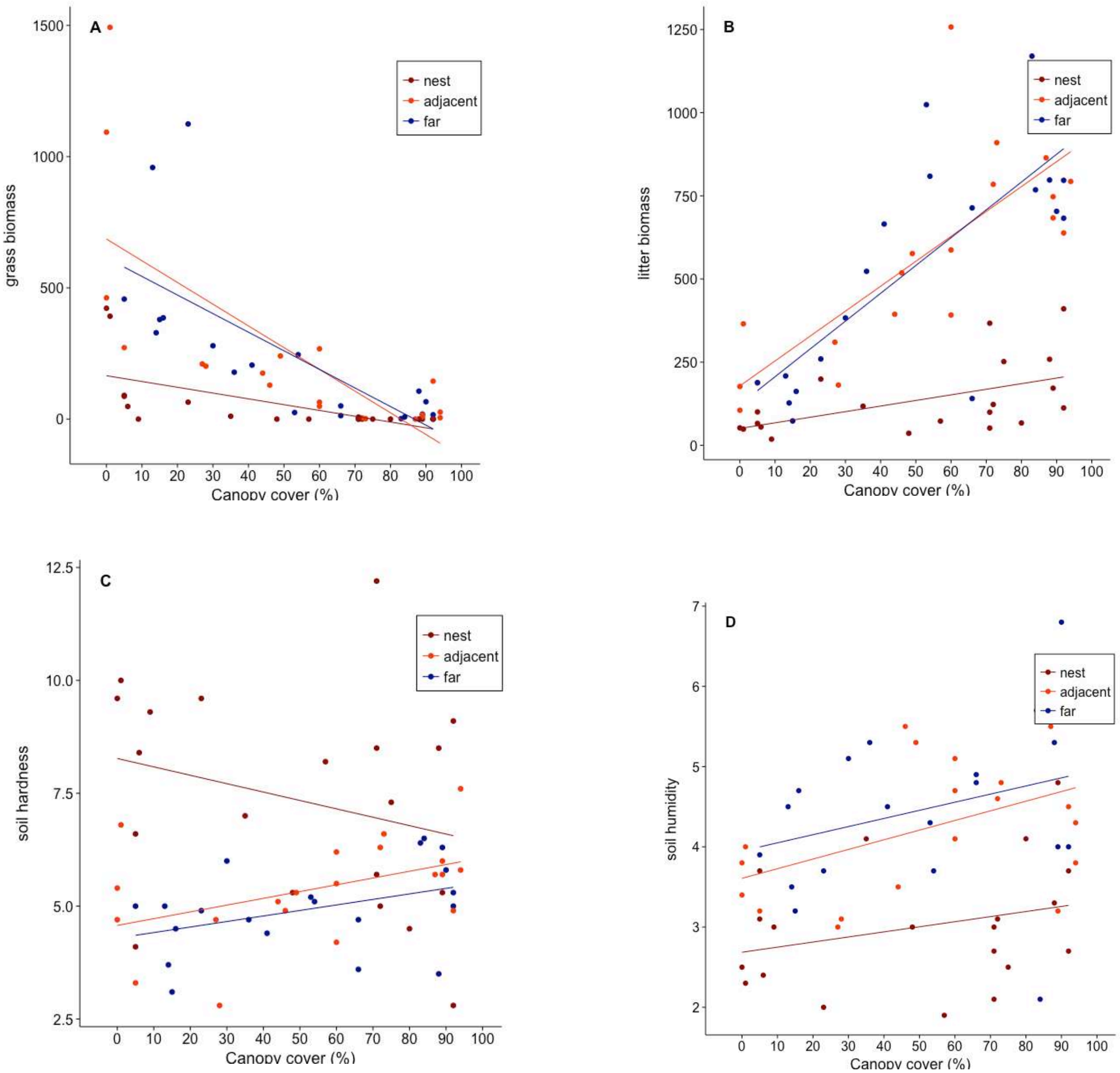
	Litter biomass	Soil Hardness	Grass biomass	pH	P	K	Ca	Mg	Al	Org. Material	Soil moisture
Canopy cover (%)	**0.5584**	0.0070	**-0.6734**	-0.3087	0.3396	-0.4265	**-0.5505**	-0.3775	0.1611	-0.1829	**0.3095**
Litter biomass	-	-0.0995	-0.0524	**-0.5070**	**0.6308**	-0.1796	**-0.5274**	**-0.2458**	**0.6477**	**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											-

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1692v3 | CC-BY 4.0 Open Access | rec: 18 May 2016, publ: 18 May 2016

APPENDIX C. 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; 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	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}

APPENDIX D: 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 E: 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 F: 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

APPENDIX G: 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	Factors	Resid. Df	Resid. Dev	*d*AIC	wAIC
5	Nest Identity, Plot Location, Canopy Cover, Plot Location*Canopy Cover Interaction	52	103.89	0	0.999
4	Nest Identity, Canopy Cover	54	138.43	9.55	0.0083
2	Nest Identity, Plot Location	55	154.53	13.44	0.0012
3	Canopy Cover	56	152.78	16.21	3.0×10^{-4}
1	Nest Identity	57	168.23	19.27	6.4×10^{-5}

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1692v3 | CC-BY 4.0 Open Access | rec: 18 May 2016, publ: 18 May 2016

APPENDIX H: 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	Factors	Resid. df	Resid. Dev	*d*AIC	wAIC
2	nest identity, plot location	16	56.15	0	0.906
4	nest identity and canopy cover	15	49.82	4.55	0.0932
5	nest identity, plot location, canopy cover, plot location*canopy cover interaction	14	49.47	13.75	9.4×10^{-4}
1	nest identity	17	86.77	26.67	1.5×10^{-6}
3	canopy cover	16	84.86	33.41	5.0×10^{-8}

APPENDIX I: 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	Factors	Resid. df	Resid. Dev	dAIC	wAIC
3	nest mound area, plot proximity to nests, local environmental conditions, random effect of nest identity, per-observation random effects	54	500.6514	0.0000	0.8272
4	nest mound area, plot proximity to nests, local canopy cover, environmental conditions, random effect of nest identity, per-observation random effects	52	499.7834	3.1320	0.1728
1	random effect of nest identity, per-observation random effects	57	550.6256	43.9742	2.3×10^{-10}
2	canopy cover, random effect of nest identity, per-observation random effects	56	550.5752	45.9237	8.8×10^{-11}

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

Model	Factors	Resid. df	Resid. Dev	dAIC	wAIC
3	nest mound area, plot proximity to nests, environmental conditions, random effect of nest identity, per-observation random effects	54	333.9756	0.0000	0.8691
4	nest mound area, plot proximity to nests, canopy cover, environmental conditions, random effect of nest identity, per-observation random effects	52	333.7626	3.7869	0.1309
1	random effect of nest identity, per-observation random effects	57	416.5026	76.5269	2.1×10^{-17}
2	canopy cover, random effect of nest identity, per-observation random effects	56	416.4239	78.4482	8.0×10^{-18}

APPENDIX J: 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	Factors	Resid. df	Resid. Dev	dAIC	wAIC
3	nest mound area, plot proximity to nests, local environmental conditions, random effect of nest identity, per-observation random effects	14	164.6	0	0.598
4	nest mound area, plot proximity to nests, local canopy cover, environmental conditions, random effect of nest identity, per-observation random effects	13	163.422	0.8219	0.397
1	random effect of nest identity, per-observation random effects	17	180.983	10.383	0.003
2	Canopy Cover, random effect of nest identity, per-observation random effects	16	180.696	12.097	0.001

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

Model	Factors	Resid. df	Resid. Dev	dAIC	wAIC
3	nest mound area, plot proximity to nests, local environmental conditions, random effect of nest identity, per-observation random effects	14	108.4128	0.0000	0.6263
4	nest mound area, plot proximity to nests, canopy cover, environmental conditions, random effect of nest identity, per-observation random effects	13	107.4459	1.0331	0.3737
1	random effect of nest identity, per-observation random effects	17	136.1146	21.7018	1.2×10^{-4}
2	canopy cover, random effect of nest identity, per-observation random effects	16	135.8064	23.3936	5.2×10^{-6}

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1692v3 | CC-BY 4.0 Open Access | rec: 18 May 2016, publ: 18 May 2016