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Biotic drivers of seedling establishment in Neotropical savannas: selective granivory and seedling herbivory by leaf-cutter ants as an ecological filter

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Summary

1. Herbivory has been shown to have prominent top-down effects on vegetation in Paleotropical savannas, where consumers of early stages of life history act as demographic bottlenecks. Such impact has been largely ignored in Neotropical savannas, however, despite insect consumption being linked to reduced recruitment of woody species.

2. We hypothesize that *Atta* leaf-cutter ants – the prevalent herbivores in the Neotropics – alter the establishment of woody plant seedlings in the Brazilian Cerrado by reducing seed availability and seedling survival. We also hypothesize that the demographic effects will be species-specific.

3. To test these hypotheses, we experimentally (i) compared seed removal in areas with and without *Atta* foraging and (ii) compared the survivorship of seedlings exposed to or protected from *Atta* foraging. Both experiments were performed with the same common Cerrado species which allowed us to evaluate the potential net effects of consumers at the population and community levels.

4. Overall seed removal rates in sites with *Atta* foraging were greater than those where ants were absent (59.2% and 39.2% of seeds removed, respectively). There were differences in removal for 10 of the 12 tested species, with per species removal 1.1- to 3.8-fold greater in areas with *Atta* foraging. On transplanted plants, 45% of seedlings exposed to *Atta* were attacked, resulting in a survival 7.6% lower than that of protected seedlings. Seedling survival was 11.8–31.5% lower in five species, with the largest differences in survival between treatments during the dry season.

5. Taken together, these results support the hypothesis that consumers can lead to demographic bottlenecks in Neotropical savanna plant species, with effects varying among life-history stages and between species.

6. *Synthesis.* Granivory by ants has been linked to reduced seedling recruitment of woody species in the Brazilian Cerrado. Our results show that leaf-cutter ants may largely limit early seedling establishment of woody species by reducing seed availability and seedling survival with differential species-specific effects. *Atta* ants may therefore be acting as an ecological filter, which coupled with potential selectivity in foraging on reproductive life-history stages, may ultimately influence the relative abundance of different species and hence the structure and composition of Cerrado vegetation.

Key-words: *Atta laevigata*, Cerrado, community ecology, granivory, insect herbivory, plant–herbivore interaction, seed predation, seedling establishment, top-down effect, vegetation dynamics

Introduction

Plants tend to be most susceptible to herbivore attacks at early stages of life history, greatly reducing their likelihood of survival (Marquis, Newell & Villegas 1997; Karban & Thaler 1999). This is particularly true for seeds, which can be consumed entirely and thus removed from the population, as well as seedlings for whom even partial defoliation can lead to mortality (Marquis 2004; Anderson, Ritchie & McNaughton 2007; Hanley & Sykes 2009). While these detrimental impacts of herbivores on recruits can vary between species, environments and spatio-temporally (Fenner & Thompson

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2005), they can nevertheless be impressive – it is not unusual for the proportion of seeds removed or consumed by granivorous to exceed 90% (e.g. Asquith, Wright & Clauss 1997; Crawley 2000; Wenny 2000; Figueroa *et al.* 2002). Likewise, seedling mortality direct or indirectly resulting from herbivores has been estimated at 30–40% (e.g. Asquith, Wright & Clauss 1997; Côté, Ferron & Gagnon 2003; Lindquist & Carroll 2004; Latorre, Larrinaga & Santamaría 2013). Mortality during early stages of regeneration is therefore thought to act as an important demographic bottleneck for many plant species (Côté, Ferron & Gagnon 2003; Lindquist & Carroll 2004; DeMattia, Rathcke & Curran 2006; Vaz-Ferreira, Bruna & Vasconcelos 2011).

Because consumers can be highly selective, in part due to species-specific seed (Plucinski & Hunter 2001; Vaz-Ferreira, Bruna & Vasconcelos 2011) and seedling traits (Burt-Smith, Grime & Tilman 2003; Elger *et al.* 2009), herbivory may affect plant community composition, population dynamics and vegetation structure (Huntly 1991; Lindquist & Carroll 2004; Marquis 2004; Kauffman & Maron 2006; Paine & Beck 2007). In Neotropical savannas, however, the paucity of large mammalian herbivores (Marinho-Filho, Rodrigues & Juarez 2002) has led to the generalization that vegetation is primarily structured by physical factors, such as fire, precipitation and soil chemistry (Henriques & Hay 2002; Hoffmann, Orthen & Franco 2004; Carvalho & Batalha 2013). However, this assumption largely ignores ‘cryptic consumers’ like insects (sensu Andersen & Lonsdale 1990; Keesing 2000), which are abundant and diverse in Neotropical savannas (Marquis, Morais & Diniz 2002; Costa *et al.* 2008; Mundim *et al.* 2012). Indeed, the few invertebrate exclusion experiments conducted in savanna habitats in the Neotropics and elsewhere have indicated high rates of seed removal and seedling mortality by insects, although the impact of consumers varies substantially among plant species (between 10–95% and 20–30%, respectively; Shaw, Keesing & Ostfeld 2002; Goheen *et al.* 2004; Moe *et al.* 2009; Vaz-Ferreira, Bruna & Vasconcelos 2011; Fischer, Kollmann & Wagner 2015).

Leaf-cutter ants are among the most prominent herbivores in the Neotropics, including in savannas (Wirth *et al.* 2003; Costa *et al.* 2008; Falcão *et al.* 2011). They can harvest up to 1 ton of plant material per hectare per year (Wirth *et al.* 2003; Herz, Beyschlag & Hölldobler 2007; Costa *et al.* 2008) to cultivate their symbiotic fungus gardens (De Fine Licht & Boomsma 2010; Hölldobler & Wilson 2011). Although fruits and seeds often represent a small portion of the plant material consumed by colonies (*c.* 2%; Costa *et al.* 2008), individual plants can lose a substantial proportion of their fruits/seeds when attacked by leaf-cutter ants (Wirth *et al.* 2003; Silva *et al.* 2007). Leaf-cutter ants can also act as important secondary seed dispersers because not all harvested seeds are consumed (Leal & Oliveira 1998; Passos & Oliveira 2003; Christianini & Oliveira 2009). While seed processing by leaf-cutter ants can increase rates of seed germination (Leal & Oliveira 1998; Christianini, Mayhé-Nunes & Oliveira 2007) and seedling recruitment (Dalling & Wirth 1998; Christianini & Oliveira 2010), defoliation of seedlings always reduces their growth and survival (Vasconcelos & Cherrett 1997; Meyer *et al.* 2011; Mundim *et al.* 2012). Nevertheless, the effects of leaf-cutter ants on vegetation are often context-dependent, with greater impacts on plants growing on poor soils and during periods of drought stress (Leal, Wirth & Tabarelli 2014). Furthermore, despite being considered generalist herbivores, leaf-cutter ants can be remarkably selective foragers (Vasconcelos & Fowler 1990). This selectivity, coupled with proportionally larger effects on early stages of life history (Leal, Wirth & Tabarelli 2014), could lead to species-specific changes in recruitment that ultimately influence local plant community composition (Vaz-Ferreira, Bruna & Vasconcelos 2011).

The Neotropical savannas known in Brazil as the Cerrado are the second largest biome in South America after the lowland forests of the Amazon (Pennington, Lewis & Ratter 2006) and a biodiversity hot spot with more than 10 000 plant species (Myers *et al.* 2000; Mendonça *et al.* 2008). Cerrado vegetation grows on well-drained and low-fertility acidic soils, with a rainfall highly seasonal and frequent wildfires (Oliveira-Filho & Ratter 2002). The Cerrado also harbours a particularly high density of *Atta* colonies (0.6–4.8 nest ha^{-1}; Costa & Vieira-Neto 2016). This has led to the suggestion that herbivory by leaf-cutter ants plays a role in dynamics of Cerrado vegetation comparable to that of physical factor or of large mammalian herbivores in other tropical savanna ecosystems (Costa *et al.* 2008; Vaz-Ferreira, Bruna & Vasconcelos 2011; Mundim *et al.* 2012). Here we test two important components of this paradigm: (i) that *Atta* foraging reduces seed availability and seedling survival for woody plant species and (ii) that *Atta* impacts on plants are species-specific, thereby setting the stage for consumer-driven effects on the structure and composition of Cerrado vegetation. To address these hypotheses, we conducted experiments to assess whether leaf-cutter ants reduce seed availability and seedling survival in 12 common Cerrado plant species.

Materials and methods

STUDY SITE AND SELECTED SPECIES

The study was conducted at the Reserva Ecológica do Panga (REP; 19°10′ S, 48°23′ W), a 404-ha reserve administered by the Universidade Federal de Uberlândia (UFU) located 30 km south of Uberlândia, Minas Gerais, Brazil. The region has a highly seasonal climate with a dry season from May to September; mean annual temperature and precipitation in the region are *c.* 23 °C and *c.* 1650 mm, respectively (UFU Santa Mônica Climate Station). The REP represents one of the best-preserved Cerrado sites in south-eastern Brazil, harbouring most Cerrado vegetation physiognomies and a highly diverse fauna (Cardoso *et al.* 2009). Our fieldwork was conducted in an area of *cerrado stricto sensu* (Oliveira-Filho & Ratter 2002), which represent the dominant vegetation type in the reserve (Cardoso *et al.* 2009). Within the study area, the canopy cover varied from 33% to 90% (mean = 67% ± 2 SD, $N = 40$), and the tallest trees were approx. 6 m in height.

Almost 25% of Cerrado’s flora is woody species (i.e. shrubs and trees; Mendonça *et al.* 2008), which tend to occur at low densities relative to herbaceous plants (1–2 ind m^{-2} vs. 120–286 ind m^{-2}, respectively; Batalha, Mantovani & Mesquita Júnior 2001). We selected for our experiments twelve of the most abundant woody species found at REP (Table S1 in Supporting Information); together, these species represent >50% of the individuals in demographic plots established in the area; E.M. Bruna and H.L. Vasconcelos unpublished data. All focal species are animal-dispersed with diaspores (i.e. fruits or seeds) whose mass varied from 28 to 7400 mg (based on measurements of n = 30 diaspores/species; Table S1). These species, hereafter referred by their generic names, are *Alibertia myrciifolia* (Rubiaceae), *Brosimum goudichaudii* (Moraceae), *Coussarea hydrangeaefolia* (Rubiaceae), *Eugenia calycina* (Myrtaceae), *Guapira graciliflora* (Nyctaginaceae), *Maprounea guianensis* (Euphorbiaceae), *Matayba guianensis* (Sapindaceae), *Miconia albicans* (Melastomataceae), *Myrcia rostrata* (Myrtaceae), *Siparuna guianensis* (Siparunaceae), *Tapirira guianensis* (Anacardiaceae) and *Virola sebifera* (Myristicaceae). For complete descriptions of the plants and diaspores, see Lorenzi (2002).

The most common leaf-cutter ant species in the REP is *Atta laevigata* (F. Smith, 1858), which is found in an average density of 1.13 nest ha^{-1} (Costa & Vieira-Neto 2016). Like most leaf-cutter species, *A. laevigata* builds subterraneous nests and a complex system of chambers and galleries (Moreira *et al.* 2004), with the excavated soil deposited on the surface form conspicuous nest mounds (Moreira *et al.* 2004). For the experiments, we selected 16 nests with nest mound area ranging of 4–27 m^2. Each nest had above-ground foraging trails, some of which were >100 m long, extending from the exit/entrances holes of the galleries to the plants being harvested. The presence of these foraging trails was used as a reference for choosing the specific locations for the experiments. Foraging activities are mostly concentrated along the trails (Vasconcelos 2002; Kost *et al.* 2005).

EXPERIMENTAL DESIGN

To determine the effect of *A. laevigata* foraging on the availability of plant diaspores, we conducted an experiment in 2011 and 2012 with 15 nests in which we manipulated worker access to fruits and seeds collected in our study site. For each nest, we established two seed removal stations: one adjacent to an active foraging trail (treatment) and other at least 25 m away in a location with no evidences of recent *Atta* activity (control; Fig. S1). Each station was composed of 10 plastic plates (*c.* 10 cm in diameter) filled with local soil and litter. These plates were placed along two parallel lines in each aforementioned area, with each plate at least 1 m from the others (Fig. S1). Experiments were conducted with one plant species at a time, with the order based on their fruiting phenology. For most species, we placed three diaspores in each plate (n = 900 diaspores per species); the exceptions were *Brosimum* and *Eugenia*, for which only one fruit was placed in each plate due to limited fruit availability. After 24 h, we recorded the number of diaspores removed by the ants. Cleaned seeds and partially consumed fruits remaining on plates were recorded as ‘not removed’. Because other ant species have been observed removing diaspores (Vaz-Ferreira, Bruna & Vasconcelos 2011), we conducted observations at most stations throughout the experiment to document that leaf-cutter ants were removing the diaspores and determine diaspore fate. At the conclusion of each experiment, we inspected the area within and around the stations and all focal trails looking for abandoned diaspores, and inspected all leaf-cutter ant nests daily for 10 days to determine whether seeds were discarded on the nest mound surface or in near entrance/exit holes.

To determine the effects of herbivory by *A. laevigata* on the survival of seedlings in their first year following establishment, we conducted an experiment in which we manipulated leaf-cutter ant access to seedlings of our focal plant species. In March 2011, we selected 10 colonies and established four 1.2 m^2 plots along the foraging trails belonging to each colony. The plots were established in pairs, with one plot from each pair randomly selected as ‘leaf-cutter ant exclusion’ and the other as a control plot to which ants had complete access (two pairs of plots per colony, total N = 20 exclusion plots and N = 20 controls; Fig. S2a). Into each plot, we transplanted three seedlings of each focal species (N = 36 seedlings per plot; seedlings were grown in a greenhouse at the reserve from seeds collected in REP). The density of experimental seedlings (30 seedlings m^{-2}) is comparable to the density of naturally occurring seedlings in our study site (mean = 38.8 plants m^{-2} ± 17.3 SD, N = 80; Costa, Bruna & Vasconcelos 2016), and seedlings were randomly assigned to their location within plots; Fig. S2b). We censused seedlings every 15 days from April 2011 to March 2012, at which time we recorded if a seedling was attacked by leaf-cutter ants (identified with the characteristic semi-circular marks on leaves and clipped stems) as well as for damage by other foliar herbivores and pathogens. We also observed evidence of seedling desiccation.

STATISTICAL ANALYSES

To determine whether seed removal was influenced by proximity to *Atta* foraging trails, we used generalized linear mixed models with binomial errors (GLMMs; Bolker *et al.* 2009). The number of seeds removed and the number of seeds remaining from each station (all plates in a station pooled) were used as the dependent variables, with combinations of station location (i.e. adjacent to or far from trails), species identity and their interaction as fixed effects. Nest identity was included as a random effect in all models. The resulting models (Table 1) were ranked with Akaike information criteria (Burnham & Anderson 2002). We also used GLMMs with binomial errors to

Table 1. Generalized linear mixed model selection for the effect of proximity to leaf-cutter ant (*Atta laevigata*) foraging trails on the removal of seeds of 12 common Cerrado woody species. The significance of plot proximity and diaspore species identity was assessed by comparing the model including only the random effect of nest identity (model 1) with models including this random effect and these main effects and their interaction. All models used a binomial distribution with a logit function

Model	Resid. d.f.	Resid. dev	dAIC	Weight
1 Nest as a random effect	358	5250.043218	2353.1	0
2 Treatment + Random effect of nest	357	4793.979953	1899	0
3 Species + Random effect of nest	347	3578.279314	703.3	0
4 Treatment + Species + Random effect of nest	346	3027.501259	154.5	0
5 Treatment + Species + Treatment*Species interaction + Random effect of nest	**335**	**2850.955094**	**0**	**1**

The resulting models were ranked by delta Akaike information criteria (dAIC), while Akaike weight (weight) indicates the probability that model is the best among the candidate models. The model that best fit the data is in bold.

determine whether seedling survival was influenced by *Atta*. Seedling survival after 1 year was used as the dependent variable, with combinations of protection from *Atta* (i.e. exclusion, access), seedling species identity and their interaction included as fixed effects. Individual leaf-cutter nests (or experimental plot within nest) were considered random effects. The resulting models (Table 2) were again ranked with Akaike information criteria. All analyses were conducted using the R statistical programming language (R Core Development Team 2014) and packages LME4 (Bates *et al.* 2015).

Results

Overall, 52.9% of the diaspores exposed were removed during our seed predation experiments (all species combined, $n = 5080$ diaspores). We did not observe partial seed predation (i.e. seed only partial consumed) by leaf-cutter ants or any other predator. The model that best fit the data was the one including proximity to leaf-cutter ant foraging trails, diaspore species identity and their interaction (Table 1). Diaspore harvest rates in areas with leaf-cutter ants were on average 1.5-fold greater than in control areas (59.2 ± 31.2% vs. 39.3 ± 28.4% diaspores removed, respectively; mean ± SD), with ten of the twelve species having higher rates of seed removal in treatment plots. The greatest increment in removal – between 1.6- and 2.2-fold – occurred in *Coussarea*, *Guapira*, *Matayba*, *Myrcia* and *Tapirira* (Fig. 1c,e,g,i,k). These species have fruits of intermediate size (mass: 0.209–0.634 g) containing a single seed. In addition, *Atta* workers also intensely collected two other species whose fruits were of intermediate size, *Alibertia* and *Miconia* (mass: 0.617 and 0.295 g, respectively). However, their fruits are soft and have numerous small seeds, which even smaller ant species (mostly *Pheidole*) were able to fragment and carry. By this, there was a

Table 2. Generalized linear mixed model selection for the effect of protection from a leaf-cutter ant (*Atta laevigata*) and plant species identity on seedling survival of 12 common Cerrado woody species. The significance of protection and species identity was assessed by comparing the model including only the random effect of nest identity (model 1) with models including this random effect and these main effects and their interaction. All models used a binomial distribution with a logit function

Model	Resid. d.f	Resid. dev	dAIC	Weight
1 Nest as a random effect	1438	1878.881	142.1	0
2 Treatment + Random effect of nest	1437	1869.620	134.9	0
3 Treatment + Replicate nested in the random effect of nest	1436	1862.856	130.1	0
4 Treatment + Species + Random effect of nest	1426	1719.765	7	0.03
5 Treatment + Species + Replicate nested in the random effect of nest	**1425**	**1710.754**	**0**	**0.95**
6 Treatment + Species + Treatment*Species interaction + Random effect of nest	1415	1705.473	14.7	0
7 Treatment + Species + Treatment * Species interaction + replicate nested in the random effect of nest	1414	1696.300	7.5	0.02

The resulting models were ranked by delta Akaike information criteria (dAIC), while Akaike weight (weight) indicates the probability that model is the best among the candidate models. The model that best fit the data is in bold.

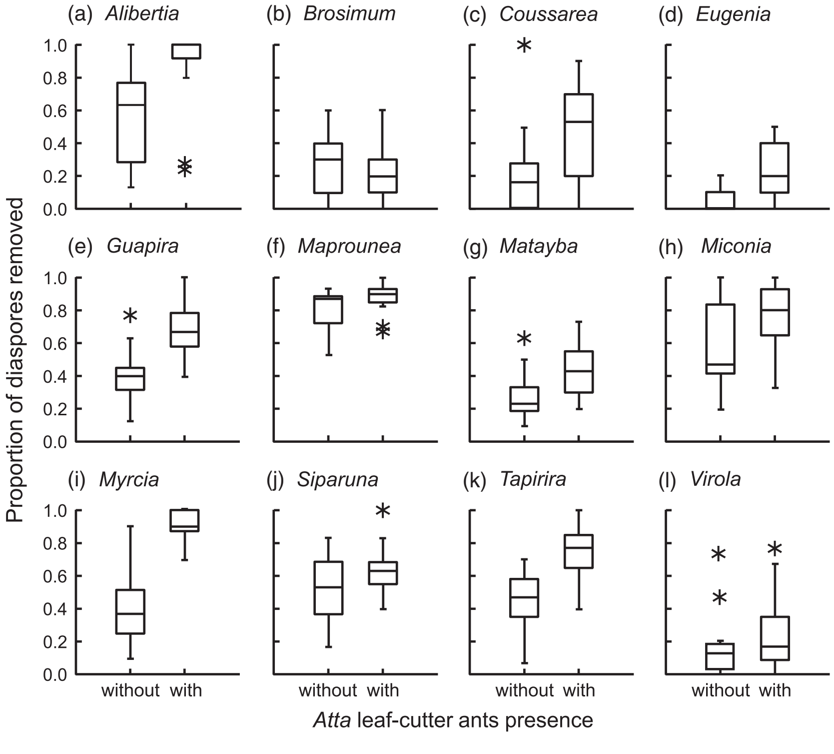

Fig. 1. Removal rates over a period of 24 h for seeds of 12 common Cerrado woody species (a–l) experimentally placed alongside 15 foraging trails of the leaf-cutter ant *Atta laevigata* (with) and 25 m away from these trails, where ants were not present (without). Same scales for all figures.

comparatively smaller difference in removal rates of *Alibertia* and *Miconia* seeds between treatment and control areas (1.3- and 1.5-fold, respectively; Fig. 1a,h). A similar pattern was observed for *Maprounea* and *Siparuna*, whose very small seeds (mass: 0.028 and 0.035 g, respectively) were readily collected by *Pheidole*, resulting in seed removal rates in areas with *Atta* foraging only 1.1- to 1.2-fold greater than in control areas (Fig. 1f,j). The fruits of *Eugenia* (mass: 2.186 g), although also large and with 1–5 seeds, were removed at a much higher rate in treatment areas (a 3.8-fold increase compared to control areas; Fig. 1d). However, most *Eugenia* fruits harvested by *Atta* ants were divided into smaller parts and loaded to foraging trails, where foragers removed the remaining pulp and left cleaned seeds intact. Two of the species with large seeds, *Brosimum* and *Virola* (mass: 7.399 and 0.743 g, respectively), presented no differences in harvest between treatment and control areas (Fig. 1b,l), but seed cleaning by *Atta* ants without removal was observed. Approximately 90% of removed *Brosimum* seeds were loaded few centimetres and then buried without pulp close to removal stations during the night by unknown agents. We observed that *Virola* seeds were more frequently removed by *Ectatomma* ants, but after cleaned, the seeds were dropped yet close to removal stations; the similar result was observed in interactions of *Pheidole* ants with seeds of *Maprounea* and *Siparuna*. We did not observe the cut of large seeds by leaf-cutter ants to enable or facilitate the harvesting. With exception for *Eugenia*, diaspores of all species harvested by *Atta* workers, and followed for us, were transported to the trail entrance/exit roles, and no clean seeds were found abandoned on trails or on nest mounds in subsequent days of the experiment.

In the seedling protection experiment, 38.0% (n = 547) of seedlings died after 12 months (all treatments pooled). Most of these seedlings passed by an evident drying process (2–6 weeks), and all plant that we recorded as dead failed to re-sprout; one exception was a seedling appeared to be completely consumed by an herbivore. *Virola* was the unique species that suffer evident attack by pathogens (likely a fungus), which caused death of 15% (n = 18) of seedlings. The model that best fit the data included both treatment and species identity, but not their interaction (Table 2). Leaf-cutter ants attacked 45.0% of the seedlings transplanted to unprotected plots (n = 720). Most of these ant attacks (69.1%) resulted in complete or nearly complete defoliation, with great part of plants failing to re-sprout. Overall survival was 7.6% lower in plots to which leaf-cutter ants had access than ant exclusion plots. Approximately 75% of the mortality recorded for all species occurred during the dry season, with the largest differences in survival between protected and unprotected seedlings occurring from the middle of the dry season until the beginning of the wet season (Fig. 2). Of the 12 studied species, five had significantly reduced survival (nonparametric log-rank survival analysis, $P \leq 0.003$) in areas with *Atta* foraging. Seedlings of *Coussarea* and *Myrcia* were the most frequently attacked and showed the greatest decline in survival (23.8% and 31.5%, respectively; Fig. 2c,i). The survival of *Guapira*, *Maprounea* and *Tapirira* was also reduced (11.8–22.8%; Fig. 2e,f,k), although only half of their seedlings were attacked. In contrast, *Eugenia* and *Brosimum* were frequently attacked but tolerated defoliation and showed no significant reduction in survival.

Discussion

It is widely recognized that herbivores have prominent top-down effects on savanna vegetation (Frost *et al.* 1986; Scholes & Archer 1997; Jeltsch, Weber & Grimm 2000;

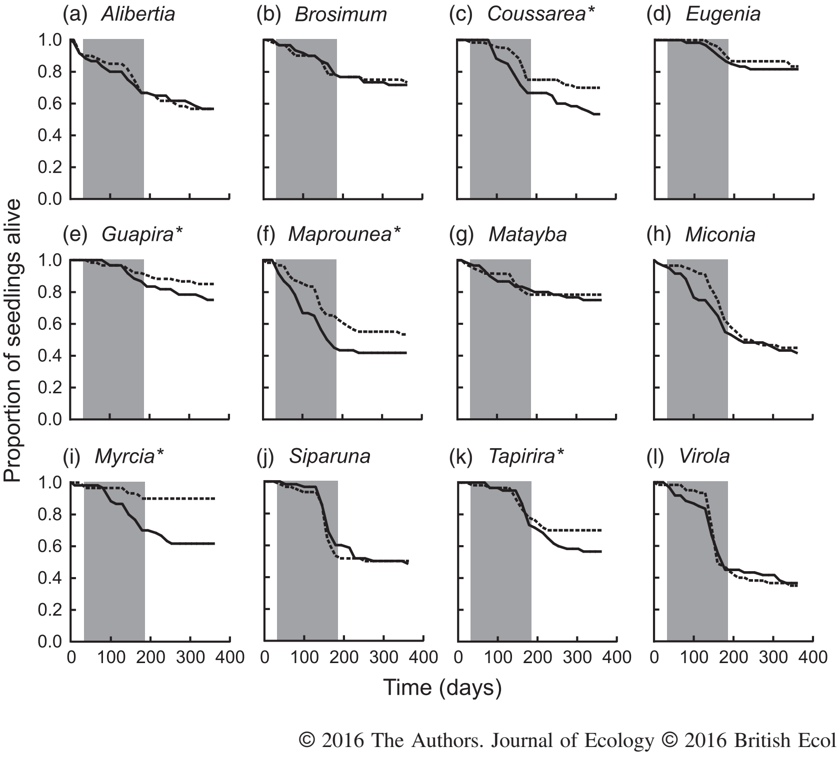

Fig. 2. Seedling survival curves of 12 common Cerrado woody species (a–l) in experimental plots that the leaf-cutter ant *Atta laevigata* were excluded (dashed line) or not (solid line). Grey areas show the May–September dry season. *Indicates species with significantly different survival curve between treatments ($P \leq 0.003$). Same scales for all figures.

Langevelde *et al.* 2003; Knegt *et al.* 2008). Part of this impact may come from plant consumption by insects (Keesing 2000), which even in African savannas can be comparable to that of large grazers and browsers (Crawley 1983). Where the mammalian herbivore fauna is depleted, such as the savannas of Australia, insects figure as the main grazers, folivores and seed predators, but their impacts have been largely ignored (Andersen & Lonsdale 1990). Insect herbivory in the Brazilian Cerrado, which is predominantly by ants, was recently linked to reduced seedling recruitment of woody species, with potential implications for local patterns of plant community composition (Vaz-Ferreira, Bruna & Vasconcelos 2011). Our results indicate that leaf-cutter ants do indeed limit seedling establishment through a combination of high seed predation and seedling defoliation. Most importantly, our data demonstrate that selective foraging by *Atta* means that some plant species are more affected than others. Ultimately, we identified three response groups after analysing the potential net effect of leaf-cutter ants on seed availability and seedling survival (Table 3). The first group comprises five species that suffer moderate to intense impact on recruitment in areas with *Atta* foraging activity (i.e. reduction in seed abundance and lowered seedling survival). The second group comprises five species that suffer low seed predation. Finally, the third group contains two species not affected by leaf-cutter ants.

Many experimental studies have shown that seed predation can negatively affect seedling abundance from grassland to temperate forest (Edwards & Crawley 1999; DeMattia, Rathcke & Curran 2006; Orrock *et al.* 2006; Paine & Beck 2007; Vaz-Ferreira, Bruna & Vasconcelos 2011), especially for species whose recruitment is seed-site rather than safe-site limited (revised by Crawley 2000). The overall removal/predation rate of diaspores we recorded (52.9%) is similar to those previously reported for the Cerrado (42–66%; Christianini, Mayhé-Nunes & Oliveira 2007; Vaz-Ferreira, Bruna & Vasconcelos 2011; Salazar *et al.* 2012a; Lima, Oliveira & Silveira 2013). Most of this consumption has been attributed to ant species with large workers, such as *Atta* and some species of *Acromyrmex, Ectatomma*, *Pachycondyla* and *Pheidole* (Christianini, Mayhé-Nunes & Oliveira 2007; Vaz-Ferreira, Bruna & Vasconcelos 2011; Lima, Oliveira & Silveira 2013). Given the presence of other granivorous ant genera in our study site, it was perhaps surprising to find that seed removal by *Atta* was of overwhelming importance – on average, removal rates were approx. 1.5-fold higher in areas with *Atta* foraging activity. Plants in the Cerrado – as a fine-prone ecosystem – have the capacity to reproduce vegetatively (Henriques & Hay 2002; Hoffmann & Moreira 2002); however, such reproduction strategy is most common in more open and frequently burned areas and in the years immediately after fire. Since the recruitment of woody plant species in the Cerrado is often severely seed-limited (Salazar *et al.* 2012a), the consume reported to *Atta* ants may be critical for the plant early establishment.

Table 3. Net effect of ecological filters carried by a leaf-cutter ant (*Atta laevigata*) on early recruitment (seed and seedling stages) of 12 common Cerrado woody species

Species	Filter effect (reduction)		Impact on plant recruitment (net effect)
	Seed abundance	Seedling survival	
Alibertia myrciifolia	+	0	Low
Brosimum gaudichaudii	0	0	No
Coussarea hydrangeaefolia	++	++	Moderate-intense
Eugenia calycina	+	0	Low
Guapira graciliflora	++	+	Moderate-intense
Maprounea guianensis	+	+	Moderate-intense
Matayba guianensis	++	0	Low
Miconia albicans	+	0	Low
Myrcia rostrata	++	++	Moderate-intense
Siparuna guianensis	+	0	Low
Tapirira guianensis	++	+	Moderate-intense
Virola sebifera	0	0	No

0, absent effect; +, low effect; ++, high effect.

To determine the real impact of seed harvesting by leaf-cutter ants on plant recruitment, however, it is necessary to identify the destination of the collected seeds (Leal, Wirth & Tabarelli 2014). Seeds can be (i) abandoned along the foraging trails, (ii) carried into the nest and later discarded outside the nest in refuse piles or next to exit/entrance holes, or (iii) carried into the nest and discarded in internal refuse chambers. In the first two cases, the effects of leaf-cutter ants on seedling communities tend to be positive since seed processing can increase seed germination (Leal & Oliveira 1998; Christianini, Mayhé-Nunes & Oliveira 2007) and seedlings that become established on the nutrient-rich soil on nest mounds often grow at faster rates than seedlings on non-nest soils (Farji-Brener & Silva 1996; Leal & Oliveira 1998; Christianini & Oliveira 2009; but see Silva *et al.* 2007). In contrast, seeds that are discarded inside internal refuse chambers are effectively 'depredated' since these chambers are located several metres below the soil surface where seeds are unable to germinate or grow (Moreira *et al.* 2004). Our field observations indicate that most seeds collected by *A. laevigata* were carried into the nest and not transported back to the surface. We therefore conclude that, irrespective of whether seeds were being consumed, our focal ant species acts as a seed predator in our study system. The exception is *Eugenia* seeds commonly abandoned along trails, for which *Atta* acts as secondary seed dispersers.

Our experiments with seedlings further showed that 45% of the seedlings in plots accessible by *A. laevigata* ants were attacked, resulting in a 7.6% reduction in seedling abundance after 1 year. The increase in mortality was only observed in five of the 12 plant species tested. This can be in part explained by ant selective foraging, with difference in seedling survival among treatments occurring in most attacked species. However, even some of the frequently attacked species, notably *Brosimum* and *Eugenia,* suffered low mortality. This indicates some species are tolerant of herbivory by leaf-cutter ants. This relatively high level of tolerance to leaf-cutter ant herbivory also may explain why rates of seedling mortality reported here are lower than those reported for seedlings from tropical lowland forests (Vasconcelos & Cherrett 1997; Corrêa *et al.* 2010; Meyer *et al.* 2011; Silva *et al.* 2012). Although the overall seedling abundance was only slightly reduced by leaf-cutter ant attacks, when coupled with *Atta*-related declines in seed abundance there is the potential for large species-specific effects on the seedling community composition.

In addition to the direct effects of consumers, there also appears to be an interaction between herbivory by leaf-cutter ants and water deficits during the dry season. The woody species recruitment can be dramatically reduced in the Cerrado during the dry season (Hoffmann 1996; Salazar *et al.* 2012b). Our results show that most mortality (approx. 75%) occurred in this period. However, seedlings emerging from large seeds can better tolerate herbivore attacks, particularly during the dry season, as they tend to invest largely in roots or have high reserves compared to seedlings emerging from small seeds (Moreira & Klink 2000; Hoffmann, Orthen & Franco 2004; Saboya & Borghetti 2012). This hypothesis was supported by the observed faster and higher rates of mortality of species with smaller seeds, such as *Alibertia*, *Maprounea*, *Miconia* and *Siparuna*. Indeed, higher mortality during drought may mask or overwhelm any mortality due to defoliation. If there is a trade-off between seed size and seedling tolerance to herbivory in Cerrado woody plants, we would predict increased effects of leaf-cutter ant defoliation on seedling survival in species with intermediate size seeds and in exceptional years with atypically long dry periods. We suggest future experiments to examine this potential relationship between seed mass, root growth and tolerance to drought and herbivory.

Herbivory by leaf-cutter ants can act as a bottleneck in at least three ways. First, herbivory on mature plants has a negative effect on fruit production (Mundim *et al.* 2012). Secondly, the harvesting of fruits/seeds that fall into the ground can have a negative effect on soil seed banks (Christianini, Mayhé-Nunes & Oliveira 2007; Vaz-Ferreira, Bruna & Vasconcelos 2011). Finally, defoliation can increase seedling mortality (Vasconcelos & Cherrett 1997; Meyer *et al.* 2011; Silva *et al.* 2012). The magnitude of *Atta* ant effects on the population of an individual plant species – that is their capacity to act as an ecological filter – depends on how many seeds are removed, how often seedlings and mature plants are attacked, and how tolerant they are. Almost half of the studied species were moderately to strongly affected by *Atta* granivory and seedling herbivory, and all of these are often attacked as adults as well (Mundim *et al.* 2012; A.N. Costa, H.L. Vasconcelos, E.H.M. Vieira-Neto and E.M. Bruna unpublished data). However, some species were less intensively selected suggesting *Atta* does indeed have the capacity to act as an ecological filter in the Cerrado potentially resulting in remarkable alterations in plant assemblages in explored sites. Changes in vegetation diversity as consequence combination of *Atta* herbivory and ecosystem engineering have been reported from habitats ranging from grasslands to forests (Farji-Brener & Silva 1995, 1996; Terborgh *et al.* 2001; Sosa & Brazeiro 2010, 2012; Silva *et al.* 2012). Some of these sites have similar *Atta* densities to the Cerrado (Costa & Vieira-Neto 2016); for example, small forested islands with leaf-cutter ant densities of 1.1–6.7 nest ha^{-1} show strong changes in seedling abundance and species richness when compared with large island with lower herbivores densities (Terborgh *et al.* 2001). These *Atta*-driven seedling declines were considered the primary factor underlying reduced sapling and adult abundance of preferred tree species (Rao, Terborgh & Nuñez 2001). Long-term studies are clearly needed to establish the demographic impacts of *Atta*-induced declines in seedling abundance on the structure and composition of Cerrado vegetation. While large-scale and long-term experimental studies excluding focal herbivores insect are likely challenging, a combination of large-scale demographic and small-scale experiments may provide insights into the demographic impacts of individual consumer species.

The inclusion of nest identity as a random effect improved the fit of models suggesting that *Atta*'s population- and community-level impacts can also be spatially heterogeneous. *Atta* colonies are non-randomly distributed – they are more common in successional habitats such as gaps, ecotones and disturbed sites like roads (Vasconcelos & Cherrett 1995; Wirth *et al.* 2007; Meyer, Leal & Wirth 2009; Silva *et al.* 2009; Dohm *et al.* 2011; Vieira-Neto, Vasconcelos & Bruna 2016) – and in the Cerrado, they are aggregated at densities ranging from 0 to 6.7 nests ha^{-1} (Costa & Vieira-Neto 2016). Moreover, the foraging of *Atta* workers also varies spatially, with foraging concentrated close to foraging trails, while other areas surrounding the nest remain unexplored (Vasconcelos 2002; Wirth *et al.* 2003; Kost *et al.* 2005; Silva *et al.* 2007). Finally, there is also temporal heterogeneity in their foraging, with high harvesting rates in the dry season (Vasconcelos 1997, 2002; Wirth *et al.* 1997; A.N. Costa, H.L. Vasconcelos, E.H.M. Vieira-Neto and E.M. Bruna, unpubl. data). The spatio-temporal heterogeneity in the distribution of herbivory has been shown to modify the vegetation of Paleotropical savannas at finer scales (Knegt *et al.* 2008). Given that, we suggest that changes of microclimate and soil traits (i.e. litter-cover, hardness and chemistry) in *Atta* leaf-cutter ant nest (Costa, Bruna & Vasconcelos 2016) and colony foraging will create a species-specific mosaic of enhanced or depressed seedling recruitment, which coupled with the effects of ants on the growth, reproduction and mortality of adult trees (Mundim *et al.* 2012) could have impacts on Cerrado vegetation comparable to those of soil chemistry, fire and other abiotic factors.

Finally, our results suggest a new and previously unexplored means by which the severe and continuing fragmentation of the Cerrado (Klink & Machado 2005) can alter plant diversity. Leaf-cutter ants are favoured by road creation and other forms of disturbance (Vasconcelos & Cherrett 1995; Rao 2000; Meyer, Leal & Wirth 2009; Vieira-Neto, Vasconcelos & Bruna 2016), so we would expect higher consumption rates (Urbas *et al.* 2007; Wirth *et al.* 2007) and an elevated pressure on plant recruitment in Cerrado remnants. This may lead to convergence on low-density and low-diversity seedling assemblages in fragments and degraded areas

(Silva *et al.* 2012). The *Atta* density increase will alter the relative importance of bottom-up and top-down forces acting on plant communities (Moreau *et al.* 2006) and hence must be considered when planning the recovery of degraded areas (Hobbs & Norton 2004); and the active control of leaf-cutter ant colonies may be necessary in the early stages of plant restoration efforts (e.g. Nave *et al.* 2009). Finally, our results provide support for the hypothesis that consumers – and especially ants – are fundamental to the dynamics of Cerrado plant species by acting as ecological filters. Elucidating how consumer impacts vary spatio-temporally and the way in which herbivory interacts with physical factors to influence plant performance help also to understand how plant diversity in Neotropical savannas will respond to climate change and other human-induced changes in the landscape.

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Data accessibility

Seed removal and seedling survival data: Dryad Digital Repository http://dx.doi.org/10.5061/dryad.1hj56 (Costa, Vasconcelos & Bruna 2016).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of woody plant species and characteristics of their diaspores used to perform seed and seedling predation experiments target to test the impacts of leaf-cutter ants foraging activity on early plant recruitment in the Brazilian Cerrado.

Figure S1. Design to seed removal stations (n = 15 pairs) established to perform a seed predation experiment with 12 common woody plant species in an area of the Brazilian Cerrado.

Figure S2. Design to ant exclusion experiment plots (n = 20 pairs) to determine the effects of leaf-cutter ant herbivory on the seedling survival of 12 common woody plant species in an area of the Brazilian Cerrado.