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## **RESEARCH ARTICLE - ANTS**

# Ant Community in Burned and Unburned Sites in Campos Rupestres Ecosystem

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#### Introduction

#### Abstract

Ground-dwelling ants have shown consistent resilience to fire in savanna environments. We carried out a study to investigate how ant community structure responds to fire in *campos rupestres* sites. We studied the ant communities' response to fire in two different elevations (800 m above sea level and 1300 m a.s.l.) in southeastern Brazil. Pitfall trap samples were set at three different periods: one, four, and ten months after a fire event. Overall, one hundred and fifteen ant species were collected. At a local scale, ant species richness and composition did not differ between burned and unburned plots in the lower elevation (800 m). At the higher altitude (1300 m) the burned sites presented higher richness and different ant composition in comparison to unburned sites. Moreover, ten months later no difference between the richness of ant species in burned and unburned plots was found at 1300 m, even though the plots showed distinct species composition. These data support the hypothesis that fire represents a structuring disturbance factor for ant assemblages and indicates that the recovering of the *campos rupestres* vegetation influences ant community structure at higher elevations.

Fire represents an important evolutionary factor in forest distributions and tree cover (Bond & Keeley, 2005; Hirota et al., 2011; Veldman et al., 2015). Ants play important interactions in ecosystem functioning and are good indicators of ecosystem response to fire disturbances (York, 2000; Hoffmann, 2003; Parr et al., 2004; Andersen et al., 2006). Ground dwelling ants have been reported to be resilient to burning in many tropical savanna ecosystems (e.g., Frizzo et al., 2011; Maravalhas & Vasconcelos, 2014; Anjos et al., 2015). Fire events in this ecosystem promote a high biodiversity of ants mediated by fire-induced changes in the vegetation structure (Maravalhas & Vasconcelos, 2014). In other savannas, the fire effect on ant assemblages also depends on vegetation structure (Farji-Brener et al., 2002; Parr et al., 2004). The increase in fire frequency regimes leads to ant community simplification and loss of biodiversity, while sensitive forest ant species are generally eliminated from the environment (Bond & Keeley, 2005; Hirota et al., 2011; Dantas et al., 2015). As a result, ant species more adapted to open habitats are selected (Andersen et al., 2006) and then the effect of fire on ant assemblages depends both on habitat traits and on the regional context of the ant fauna (Farji-Brener et al., 2002; Parr et al., 2004; Arnan et al., 2006).

In *campos rupestres* fire is a common phenomenon (Alves & Silva, 2011; Conceição et al., 2013; Fernandes et al., 2014; Veldman et al., 2015; Silveira et al., 2016). This ecosystem occurs mainly in Espinhaço mountain range and presents a rich and endemic flora and fauna (Lara & Fernandes, 1996; Giulietti et al., 1997). It is characterized by a variety of soil types, a mosaic of vegetation types, and many life forms, mostly adapted to fire (e.g., Kolbek & Alves, 2008; Neves et al., 2011; Alves et al., 2014; Silveira et al., 2016). The pyrophilic characteristic is specially due to the high abundance of a herbaceous stratum of Poaceae and Cyperaceae (Vitta, 2002; Ribeiro & Figueira, 2011; Neves et al., 2011). The herbaceous layer generally possess high postfire resilience, great capacity of rapid reestablishment and almost complete recovery after months since the fire event



(Neves & Conceição, 2010). Many graminoid species mainly found at higher altitudes sprout and blossom shortly after fire disturbances (Kolbek & Alves, 2008; Neves et al., 2011; Ribeiro & Figueira, 2011; Conceição et al., 2013; Alves et al., 2014). In contrast to the herbaceous stratum, shrubs and trees exhibit thick, corky, or exfoliating bark that protect the gems and guarantees their survival after fire. On the other hand, recovery time is greater when compared to the herbaceous stratum (Warming, 1892; Mistry, 1998; Miranda et al., 2002; Maurin et al., 2014).

The vegetation in characteristics exert a fundamental role in determining the ant community structure (Warming, 1892; Miranda et al., 2002; Ribas et al., 2003; Pacheco & Vascocelos, 2012). In campos rupestres, the biota quickly re-colonize burned sites, benefiting from vegetation products induced by fire (Conceição et al., 2013). This ecosystem presents a high diversity of ants (see Costa et al., 2015), which has been argued to be highly resistant and resilient to fire disturbance (Anjos et al., 2015). Nevertheless, human induced impact on the campos rupestres is increasing at fast rates (Fernandes et al., 2014). Fire has now been known to promote biological invasion in *campos rupestres* by exotic plant species as well as to have a deep effect on the depletion of the seed bank (Miranda et al., 2002; Medina & Fernandes, 2007). Hence, considering the complexity and dynamics of campos rupestres environment, the presence of frequent fire events may change this structural complexity affecting the availability of nesting sites and food resources for ants (see Fagundes et al., 2015).

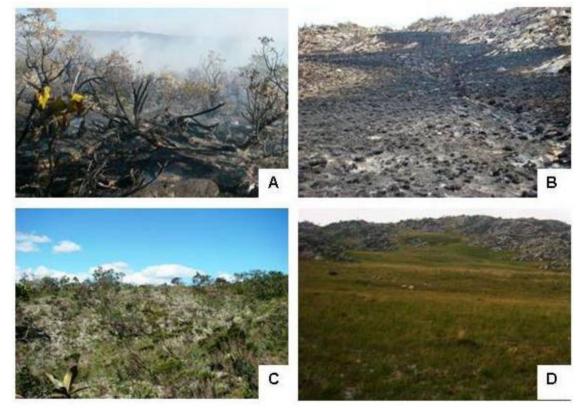
An extensive fire event occurred at Serra do Cipó in September 2011 offering an important opportunity to evaluate the effects of fire on ant community. Our goal is to determine the response of ant species richness and composition after a fire event on a local scale in *campos rupestres*. Moreover, we aimed to compare such effects at two different altitudes (800 and 1300 m a.s.l.) in this ecosystem. We postulated that ant species richness and composition would change after fire, and this variation would differ according to the altitude.

#### Materials and methods

#### Study site

This study was conducted in Serra do Cipó, Minas Gerais, Brazil. A study conducted by Madeira and Fernandes (1999) in the site reported mean annual precipitation of 1374 mm, with four distinguishable seasons: the rainy season from November to February; the 'post-rain' transition season from March to April; the dry season from May to September; and the 'post-dry' transition in October. The soil is acidic, poor in phosphorus, calcium, potassium and magnesium and rich in aluminum (Medina & Fernandes, 2007; Negreiros et al., 2009).

Our study sites comprised two distinct *campos rupestres* sites: the low elevation located at the Parque Nacional da Serra do Cipó (19°17'49.6" S 19°17'49.6"W) at 800 m (a.s.l.) characterized by several shrub species belonging to the *Vellozia* (Velloziaceae) and *Lychnophora* (Asteraceae) genera (De Carvalho et al., 2012) (Fig 1). At this altitude, the



**Fig 1**. Burned sites immediately after fire in September 2011 in *campos rupestres* at two altitudes at A) 800 and B) 1300 m. C and D: the same sites four months after fire, in the mid rainy season.

greater amount of available water, higher temperatures, deeper and fertile soils promote the presence of woody vegetation (Ribeiro & Figueira, 2011). The higher elevation site was at Reserva Natural Vellozia (19°16'45''S, 43°35'27.8''W), at 1300 m (a.s.l.). This was a more typical *campos rupestres* environment, presenting rocky grasslands, formations of contiguous graminoid cover, some rock outcrop islands, as well as spots of sandy grassland habitats (see De Carvalho et al., 2012) (Fig 1). The graminoid taxa have been argued to be responsible for the spread of fire in fire-prone ecosystems (Castro & Kauffman, 1998; Ribeiro & Figueira, 2011).

#### Ant sample design

A burned and an adjacent unburned site (control) were randomly chosen at 800 m and 1300 m elevations (Fig 1). Both elevations had the same experimental design: the burned and unburned sites were at least 500 m apart from each other and in each of them the sample design consisted of three parallel transects of 200 m long, spaced 200 m apart. This experimental design allowed the minimization of the effects of variation in soil structure, slope, topography, and other factors that could influence the diversity of soil organisms. Along each transect, five pitfall traps were installed with intervals of 50 m among them, totalizing 15 pitfalls in the burned site and 15 in the unburned site. Pitfall traps were 1 L volume plastic container, 15 cm of diameter, filled with 300 ml of soapy water, and left open for 48h in the field.

The first sample was taken in October 2011, just one month after the fire event; at the beginning of the wet season. The second sample was collected after the rainy period, in the middle of the wet season (January), four months after the fire event. Finally, the last sample was collected in the middle of the dry season (in July), ten months after the fire event. Ant specimens were identified to the species level whenever possible (Baccaro et al., 2015) and deposited in the collection at the Laboratório de Ecologia de Insetos, UFMG, Belo Horizonte, MG, Brazil.

# Statistical analysis

Analyses were performed separately for each elevation. To test the influence of fire through time on ant species richness, we compared burned and unburned plots through a linear mixed effects model for each altitude. This analysis fits linear mixed-effects model with specified mixtures of fixed effects and random effects, which exclude temporal pseudo-replication of data (Crawley, 2013). Data were grouped by sample points (pitfalls) as random effect, whereas the burning and time effects were added to the model as a fixed effect. Sampling and burning dates were also incorporated as fixed units in order to test the effects of time and fire on ant species richness. We conducted all mixed effects models analyses with the package lmer in the R Language and Environment for Statistical Computing (R Development Core Team, 2014).

Moreover, for each elevation we performed separate analysis of similarity on a monthly basis (one way ANOSIM) to test changes in ant species composition in burned and unburned sites at each sample month. We used an absencepresence matrix, performing the similarity index of Jaccard and each R-value with a corresponding p-value. In addition, Non Metric Multidimensional Scale (NMDS) analysis was carried out to plot the significant differentiation in ant community composition. ANOSIM and NMDS analysis were performed with the program Past (Hammer et al., 2001).

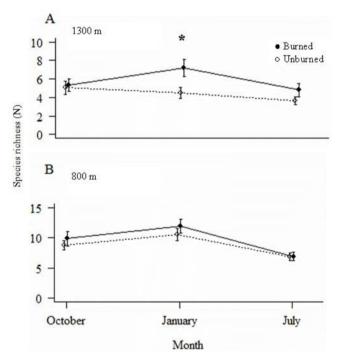
**Table 1**. Values of linear mixed effects model of burning and time since fire effects on ant species richness in *campos rupestres* at two altitudes (800 and 1300 m) and NMDS's values of dissimilarity between burned and unburned plots at each site (Jaccard index), separated by time since fire (in months).

Elevation	<b>Response variable</b>	Parameters	Chisq	d.f.	AIC	р
800 m	Ant richness	Time*Burn		5	143.44	
		Time + Burn	0.168	4	141.61	0.681
		Burn	1.134	3	140.75	0.287
		Time	8.532	3	148.15	0.003
1300 m	Ant richness	Time*Burn		7	125.03	
		Time + Burn	2.788	5	123.82	0.249
		Burn	4.304	4	126.12	0.038
		Time	8.918	3	128.74	0.011
Elevation	<b>Response variable</b>	Parameters	Time (moth)		R	р
800 m	Ant composition	Burn x Unburned	1		0.04	0.180
			4		0.01	0.391
			10		0.08	0.065
1300 m	Ant composition	Burn x Unburned	1		0.07	0.043
			4		0.60	<0.001
			10		0.59	<0.001

### Results

We recorded 115 morphospecies of ground-dwelling ants foraging in *campos rupestres* of Serra do Cipó (Appendix). These species were distributed into seven different subfamilies with Myrmicinae being the most diverse in terms of species number (Appendix). *Pheidole* was the most diverse genus with 21 species, followed by *Camponotus* with 20 ant species. As expected, the two elevations exhibited contrasting ant community composition and showed distinct patterns of ant community organization after the fire disturbance (Table 1).

Ant species richness per pitfall at 800 m (a.s.l.) changed throughout time (p <0.01, Table 1) but did not differ between burned and unburned plots in all sampling periods (p=0.681, Table 1, Fig 2). In January, incoming species that were absent in October such as *Trachymyrmex* sp.2, *Cyphomyrmex lectus* (F.), *Strumigenys schulzi* (E.), *Pheidole* sp.2, and *Tapinoma* sp.1 were equally found in both burned and unburned plots. Ant species composition at this elevation did not differ statistically between burned and unburned plots, regardless of time (Table 1, Fig 3).



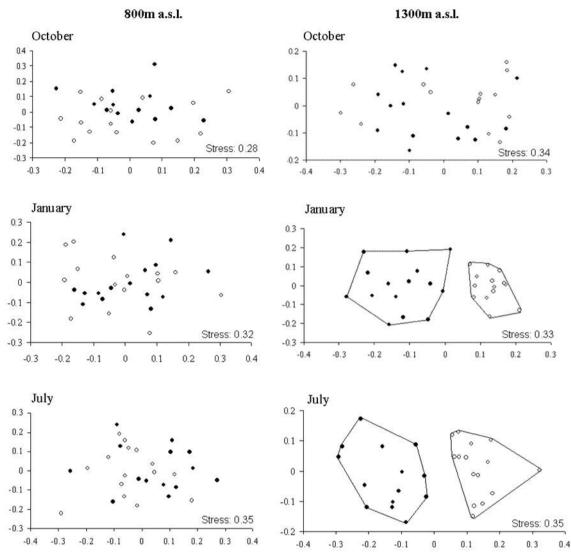
**Fig 2.** Mean number ( $\pm$  SE) of species per pitfall in burned and unburned plots A) site at 1300 and B) site at 800 m, one (October), four (January) and ten (July) months after fire. \* indicates significant difference (p<0.05) in mean number of species per pitfall between burned and unburned plots.

There was a temporal variation in species richness per pitfall in burned and unburned plots at 1300 m (a.s.l.), and this variation differed among plots in January, but did not differ in October and July (Table 1, Fig 2). One month after the fire (October) burned and unburned plots at 1300 m presented similar number of ant species and similar species composition. On the other hand, in January, burned plot exhibited significantly more species per pitfall compared to the unburned plot, as well as a dissimilarity of 60% in the community composition between them (Table 1, Fig 2 and Fig 3). While the mean number of species per pitfall was 4.62 +0.55 (n=15) in unburned plots in this period, in burned sites it was equal to  $7.13 \pm 0.93$  (n=15). Ten months after the fire disturbance (July), the number of species per pitfall decreased and both plots exhibited again a non-significant difference in ant richness, although was presented distinct species composition. The ant species found more frequently in burned sites four and ten months after fire at 1300 m were Dorvmvrmex goeldii (F.). Camponotus rufipes (F.) and Ectatomma edentatum (R.), while Brachymyrmex. sp 4. Solenopsis sp. 6 was found exclusively in burned sites and completely absent from samples in unburned plots. We also observed the presence of the harvester ant Pogonomyrmex naegelii (F.) in the burned plots, and its absence at the unburned site in these months. Linepithema sp.1 and the Argentine ant L. humile were by far the most frequent species in both unburned and burned plots at higher elevation of campos rupestres, in all studied months. In January, at this altitude, L. humile was more frequent in burned site than in the adjacent unburned site.

#### Discussion

We found a resilient ground-dwelling ant community on a local scale at the altitude of 800 m (a.s.l.) considering that there was not a significant alteration in ant community structure after fire in this altitude. Similar results were obtained in burned sites of the Cerrado *stricto sensu* (see Frizzo et al., 2011; Vasconcelos et al., 2009; Maravalhas & Vasconcelos, 2014). Therefore, ground-dwelling ants that nest and forage underground seem not to suffer from the negative effects of fire (Frizzo et al., 2011; Vasconcelos et al., 2009; Maravalhas & Vasconcelos, 2014; Anjos et al., 2015). On the other hand, arboreal ant species may hide and protect themselves inside thick trunks of fire-adapted plant species in the Cerrado (Fagundes et al., 2015).

The variation in ant richness at 800 m correlates with the climatic and phenological traits of vegetation (e.g., Belo et al., 2013). We observed an increase in ant species richness in January, the warmer and wetter period, followed by a significant decrease in July, the colder and dryer period. Many plant species bloom and disperse in the rainy season in Serra do Cipó (Madeira & Fernandes, 1999; Ranieri et al., 2012; Belo et al., 2013). The availability of food resources, mediated by plant phenology, influences positively the ant-plant interactions (see Rico-Gray et al., 2011; Lange et al., 2013), which could reflect in greater ant species richness. In the same way, during the winter the number of ant species decreased, probably due to a combination of lower availability of plant-resources produced and lower ant activity due to cooler temperatures (Rico-Gray et al., 2011; Lange et al., 2013). Therefore, cold temperatures during winter may act as another environmental factor filtering ant species colonization and success.



**Fig 3**. Ant species spatial distribution in Non Metric Dimensional Scale (NMDS) in *campos rupestres* at two altitudes (800 and 1300 m); filled symbols correspond to burned plots. The polygons represent significant differences between the plots (P < 0.05).

At higher altitude (1300 m a.s.l.), ant community showed a distinct organization pattern from the one detected at lower altitude. Fire apparently caused a positive effect on ant species richness and triggered a distinct organization on the community in burned plots from higher elevation. Burned plots exhibited an increase in ant richness in January and different species composition from unburned plots. The new post fire ant community organization in January could have been related to the sharp increase in the graminoid resprouting biomass and to the blooming of some species plant induced by fire (see Maurin et al., 2014; Maravalhas & Vasconcelos, 2014). The high plant regeneration capacity after fire (Coutinho, 1990; Maurin et al., 2014) provides quick recovery of ants in campos rupestres (see Anjos et al., 2015). In this study, the reestablishment of grasslands of the burned sites was almost complete four months after the fire event (Lana personal observation). Moreover, the typical superficial layer of the campos rupestres, which is composed by rocky and sandy soils, offers protection against fire heat (Coutinho, 1990; Kolbek & Alves, 2008; Alves et al., 2014).

In addition, a massive flowering in burned site at 1300 m took place in January. This phenomenon followed by fruit dehiscence and germination of seeds has already been reported for campos rupestres (see Mistry, 1998; Kolbek and Alves, 2008; Neves et al., 2011; Conceição et al., 2013). This boom of resources are attractive for many insects (Seyffarth et al., 1996; Vieira et al., 1996), representing higher quality resources offered by the herbaceous stratum for herbivores and pollinators (Vieira et al., 1996; Conceição et al., 2013). Some ant species are seeds' harvesters and such resource represents a high-nutritional food for those ants (Passos & Oliveira, 2004). Ectatomma edentatum, Acromyrmex subterraneus (F.) and C. rufipes were found abundantly in burned site at 1300 m. Species of those genera were already reported to remove and transport Miconia seeds in campos rupestres of Serra do Cipó (Lima et al., 2012), and also to collect seeds from feces in Brazilian forest formations (Pizo et al., 2005). Moreover, ants were already reported to benefit from the seeds fallen after the fire event in other fire prone savannas worldwide (Andersen, 1988; Arnan et al., 2006). On the other hand, we are unaware

of any detailed experimental or even field study focusing on the resilience of the *campos rupestres* productivity and phenological shifts influencing the fauna.

The presence of the harvester ant *P. naegelii* in burned site at 1300 m in January supported our hypothesis of greater availability of food. In cerrado, *P. naegelii* has a generalist and season-dependent diet comprised of many seed species and arthropod preys, as well as pieces of plant and animal matter (Belchior et al., 2012). In California, abundant seeds of forbs appeared immediately after fire (Underwood & Christian, 2009). Also, an increase of seed harvester ants was reported one year after burning and related to greater food availability represented by plant re-sprouting (Underwood & Christian, 2009; Maravalhas & Vasconcelos, 2014). Also, harvester ants remove carcasses of insects killed by fire (Zimmer & Parmenter, 1998), and are associated with seed dispersion and establishment of "islands of fertility" in soil (MacMahon et al., 2000).

During the sampling we observed *E. edentatum* and *C. rufipes* widely interacting with trophobiont insects and extrafloral nectaries (EFNs) in the burned site. In the Cerrado, higher sugar concentration in EFNs was found in fire induced re-sprouting plants when compared to unburned ones, and this fact affected positively abundance of ants on those plants (Alves-Silva & Del-Claro, 2013). Previous studies also revealed that frequency of visiting EFNs by ants was higher in sites with more frequent fire events compared to rarely burned ones (Knoechelmann & Morais, 2008; Vasconcelos et al., 2009).

In summary, the increase of ant species only at higher elevations may have been influenced by fire-induced sprouting, blooming, and seed dispersion which occurred rapidly in the wet season resulting in a flush of resources highly palatable to ants (Vieira et al., 1996; Alves-Silva & Del-Claro, 2013; Conceição et al., 2013; Maravalhas & Vasconcelos, 2014; Anjos et al., 2015). Thus, we argue that fire adaptive traits of herbaceous stratum and rocky island plants at higher altitudes in *campos rupestres* may offer ants attractive and ephemeral resources after burning events (Frizzo et al., 2011; Alves-Silva & Del-Claro, 2013; Conceição et al., 2013), reflecting in more ant species foraging.

Regardless the shift from the original and indistinct situation of ant fauna richness between burned and unburned sites, of both altitudes, ten months after fire, burned sites at higher elevation still presented a distinct ant species composition in comparison to unburned plots. Similar results were found in other study with *campos rupestres* (Anjos et al., 2015). In this way, frequent fire events may provide a new community of ants in this system, and may also change the relationship with plants (Del-Claro & Marquis, 2015) extending their effects on other trophic levels. This study reinforces that ants respond quickly to fire disturbance and the changes persists for at least some months after burning, which could promote a dynamic and diverse ecosystem. At the same time, rapid colonization of burned sites by ants may be positive for plants since they are potential seed dispersers in these environments (Lima et al., 2012, Anjos et al., 2015). However, long-term studies are needed to verify these hypotheses. Even though, ant nests are known to be an important source of nutrients that contributes to accelerate the vegetation recovery after burning (Sousa-Souto et al., 2007). In *campos rupestres* the interaction among ants, plants and fire represent new avenues for future studies on the role of ants in this threatened fire-prone ecosystem.

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