

Extensive vs. semi-intensive grazing systems – are ants sensitive to this difference?

Pastagens extensivas e semi-intensivas – formigas são sensíveis a esta diferença?

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Resumo: Nosso objetivo foi avaliar as mudanças ambientais causadas pelo sistema de pastagem Voisin, usando formigas como bioindicadoras. Testamos a hipótese de que a riqueza e a composição de espécies são influenciadas pelo impacto. Amostramos as formigas em pasto extensivo, semi-intensivo Voisin e em um fragmento de mata, usando 16 armadilhas em cada área, na fazenda Barro Preto, Carrancas, Minas Gerais. A riqueza de espécies foi negativamente influenciada pelo impacto ambiental, sendo que os dois sistemas de pastagem tiveram a mesma riqueza de espécies, e o fragmento de mata obteve maior riqueza. A composição de espécies seguiu o padrão, a composição das pastagens foi similar, porém diferente do fragmento de mata. Sugerimos que não houve tempo suficiente para os dois sistemas de pastagens se diferenciarem em termos de impacto, embora a intensificação da agricultura afete negativamente a fauna de invertebrados terrestres.

Palavras-chave: Bioindicadores; perturbação; pastagem; impacto; voisin.

Abstract: Our aim was to evaluate environmental changes caused by Voisin grazing system, using ants as bioindicators. Our hypothesis was that species richness and composition are influenced by the impact. We sampled ants in an extensive grazing system, semi-intensive Voisin and a forest fragment using 16 pitfall traps in each area, on the Barro Preto Farm, Carrancas, Minas Gerais. Ant species richness was negatively influenced by the environmental impact, since both grazing systems had the same species richness, and we found a higher species richness in the forest fragment. Species composition follows the same pattern, as the composition between grazing systems was similar but different from the forest fragment. We suggest there was not enough time for the grazing systems to become different from each other in terms of impact, but the agriculture intensification negatively affect terrestrial invertebrate fauna.

Keywords: Bioindicators; disturbance; pastures; impact; voisin.

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Artigo recebido em: 25/08/2014 Aceito em 05/11/2014

Introduction

Bioindicators are used for measuring or the evaluation of conditions and changes in the environment (HEINK & KOWARIK, 2010). Their applications have shown efficiency in monitoring and analyzing environmental impacts (GARDNER, 2010). Detecting impacts in the environment by collecting abiotic variables may be troublesome, since many times it is difficult or expensive to measure them, thus, the use of individuals, or a group of taxa to measure these impacts becomes necessary (GOODSELL *et al.*, 2009).

Invertebrates have being widely used as bioindicators because of their high sensitivity to environmental changes (BOURN & THOMAS, 2002). Ants are used as bioindicators due to their wide distribution, ease and low-cost sampling, great species diversity, well known taxonomy and for performing key roles in the dynamics of an ecosystem (PHILPOTT *et al.*, 2010; RIBAS *et al.*, 2012). In biomonitoring studies of grazing areas and other soil uses, the use of ants has been efficient, responding negatively to the environmental impacts (MARINHO *et al.*, 2002; BRAGA *et al.*, 2010). The Voisin Rational Grazing System (VOISIN, 1974) is a semi-intensive system that follows the principles of Agroecology, which is the

science of sustainable development, where the exploitation of natural resources is undertaken with the knowledge of the ecological system (ALTIERI, 2007). This grazing system is expected to cause lower environmental impacts, maintain animal welfare and uphold the social and economic needs of the producer, through the use of a rotational grazing method. It is an ecological technique that favors the biodiversity while seeking to maintain the balance between soil, grass and cattle, with no detriment to any, increasing productivity without causing negative environmental impact and being profitable to the landowner (BRUCH *et al.*, 2007). An important observation made about the soil is compactation reduction (VOISIN, 1974). Therefore, aiming at sustainable cattle production, the Voisin is seen as the best alternative.

Thus, the aim of this work was to evaluate the possible environmental changes caused by the Voisin Grazing System, when compared with the extensive grazing system, using ants as bioindicators. For this we tested the following hypothesis: (i) the number of species is negatively influenced by the impact caused by the cattle, which is higher in extensive grazing systems, (ii) the species composition is affected by

the impact, and (iii) there are bioindicator species in each evaluated impact. Furthermore, we also tested if the amount of organic matter in the soil is negatively affected by the impact of grazing and whether it positively influences ants species richness.

Materials and Methods

Study Area

We conducted the sampling in May 2009, in the Barro Preto Farm (21°28'24''S and 44°39'05''W) located in the rural area of Carrancas, Minas Gerais, Brazil. The average annual temperature in the area is 14.8°C and the average annual precipitation is 1483 mm (OLIVEIRA-FILHO *et al.*, 2004). The native vegetation is composed by Cerrado (Brazilian savanna), Semideciduous forests and Rupestrian fields (MATSUMOTO & MARTINS, 2005).

The Voisin System for dairy livestock farming was installed in this farm in December 2008 (five months before our sampling) in an area previously occupied by an extensive grazing system. The Voisin System consists in two modules of four paddocks each, based upon methodologies of Voisin (1974) and Melado (2003). These modules were implanted in an area of 4.7 hectares, each paddock with 5000 m². Of these

eight paddocks installed, two were made up of native grasses and the other six with *Brachiaria* spp. grasses.

Ant sampling

We sampled ant community in three areas: extensive grazing system, semi-intensive Voisin and forest fragment (control area). In each area we established 16 sampling points. At each sampling point we used non-baited pitfall traps in two microhabitats: one hypogaeic and one epigaeic pitfall per point. Pitfalls consisted of plastic bottles with 8 cm of diameter and 12 cm of height. The hypogaeic traps were installed underground, 20 cm deep and the epigaeic were left with the opening at the ground level. Traps were filled with 200 mL of a solution of glycerol (5%) and salt (0.9%) that worked as a killing and conservation agent, maintaining ants corporal constitution.

Pitfalls were left in the sites for 48 hours and then the ants were sorted, mounted and identified to the genus level with the use of the guide from Fernández (2003), with subsequent morpho-speciation. The morpho-speciation was later confirmed by the specialist Rodrigo Feitosa from the Universidade Federal do Paraná (UFPR).

We collected a topsoil sample from each plot (no more than 5 cm deep).

These soil samples were then submitted to the Soil Laboratory of the Universidade Federal de Lavras (UFLA) for organic matter quantification. This analysis aimed to estimate the availability of resources for ants.

Data Analyzes

To compare total species richness in each system we build species accumulation curves for each microhabitat using the observed number of species and the number of samples, performing 1000 randomizations with replacement of the samples, to generate confidence intervals, using EstimateS 8.2.0 (COLWELL, 1997).

To test the hypothesis that ant species richness is negatively influenced by grazing impact, being higher in the forest fragment, intermediate in Voisin and lower in the extensive grazing system, we performed variance analysis using Poisson distribution for each microhabitat. The response variable was the average number of species per pitfall and the explanatory variables were the different systems. The analysis was performed in R software (R DEVELOPMENT CORE TEAM, 2009), followed by residuals analysis to verify errors distribution and appropriateness of the model.

To verify if the composition varies

among the systems, we utilized NMDS (Non-metric multidimensional scaling), using the Raup-Crick index of similarity, which is the most suitable for presence/absence data, in each microhabitat.

To test the significance of possible differences in the composition indicated by NMDS, we compared the similarities among areas by ANOSIM (Analysis of Similarity), a test analogous to ANOVA. To assess the significance of differences among the areas we observed the R value. The significance of the R value was determined by comparison with the result of the permutation of samples 10,000 times. The composition analyzes were performed in PAST software.

We calculated the value of each species as an indicator of each system using the IndVal index (DUFRÊNE & LEGENDRE, 1997). This method combines degree measures specific of a species to a particular habitat and its fidelity within the habitat. Species with high specificity and fidelity in a habitat will have a high value indication. The significance of IndVal indexes for each species was tested by a Monte Carlo test, with 4999 permutations using the software PC-ORD 5.10 (MCCUNE & MEFFORD, 2006).

To verify if the amount of organic matter in the soil is higher in the forest

fragment, intermediate in Voisin and lower in the extensive pasture grazing system, we also performed a variance analysis. The response variable was the amount of organic matter in the soil and the explanatory variables were the different systems. To verify the relationship between the amount of organic matter in the soil and the number of ant species, we performed a linear regression with Poisson errors distribution in each microhabitat. The response variable was the number of species and the explanatory variable was the amount of organic matter. These analyzes were performed in R software (R DEVELOPMENT CORE TEAM, 2009), followed by residual analysis to verify the distribution of errors and appropriateness of the model.

Results

33 species were sample, 29 epigaeic and 12 hypogaeic (electronic supplement). 21 species were found in the forest fragment, 12 species in the Voisin system, and eight species in the extensive grazing system.

The total number of epigaeic species was higher in the forest fragment than in the two grazing systems (Figure 1a) and the total number of hypogaeic species was higher in the two grazing systems than in the forest fragment (Figure 1b).

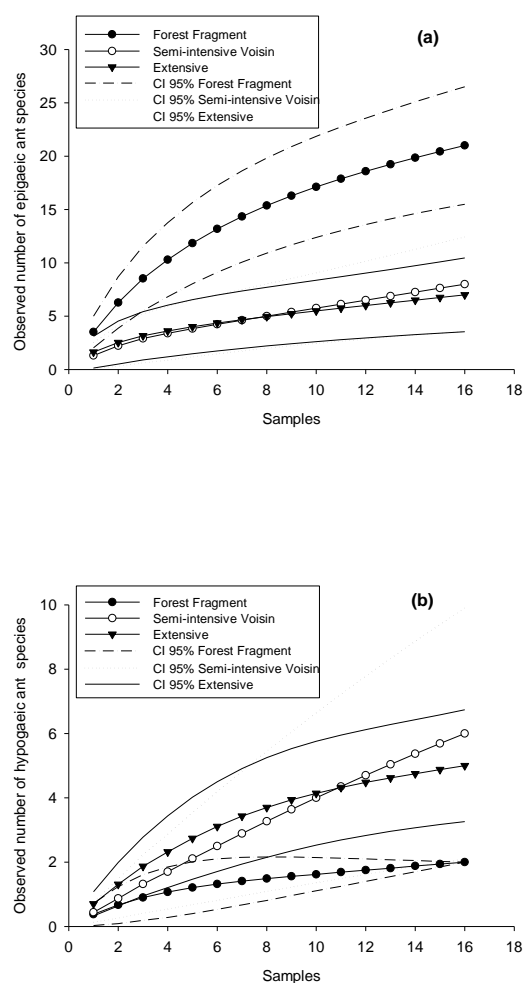


Figure 1 - Observed number of (a) epigaeic ant species and (b) hypogaeic ant species. CI – confidence intervals.

The average number of epigaeic species per pitfall was also higher in the forest fragment than in the two grazing systems ($\chi^2=20.14$, $p<0.0001$), but it was similar between the grazing systems ($\chi^2=0.53$, $p=0.47$; Figure 2). The number of hypogaeic species did not vary among the systems ($\chi^2=1.68$, $p=0.43$).

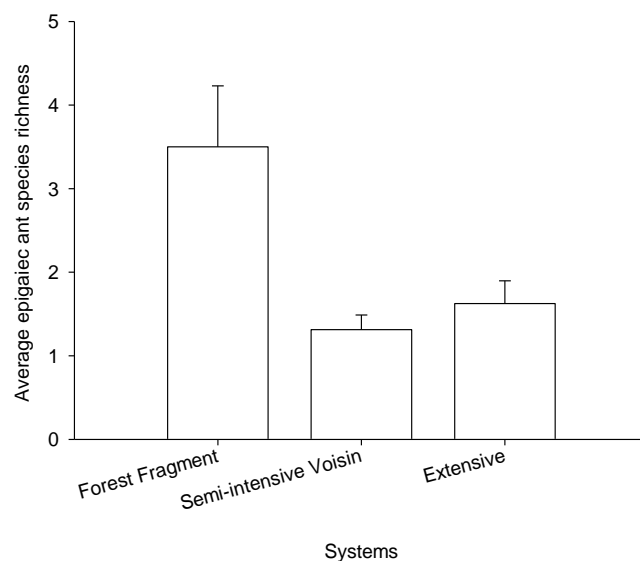


Figure 2 - Average epigaic ant species richness collected in Carrancas, Minas Gerais state, Brazil. Bars are standard errors. Difference between forest fragments and grazing systems - $p < 0.0001$. The difference between the grazing systems is nonsignificant - $p = 0.47$.

Epigaic species composition is different among systems (stress=0.2, ANOSIM - $p < 0.0001$, $R = 0.4$), but only between the forest fragment and the two grazing systems ($p < 0.0001$ for both systems). The hypogaic species composition is different among systems

(stress=0.3, ANOSIM - $p = 0.0047$, $R = 0.3$), but also only between the forest fragment and the two grazing systems (Voisin - $p = 0.01$; Extensive - $p = 0.02$).

Seven epigaic indicator species and one hypogaic indicator species (Table I) were found.

Table I - Ant species indicators of a forest fragment (FF) and an extensive grazing system (EGS) in Carrancas, Minas Gerais, Brazil. Microhabitat indicates where the ants were sampled. IndVal - indicator value. p - probability, resulting from the permutation test.

Species	Microhabitat	Areas	IndVal	P
<i>Linepithema iniquum</i> (MAYR, 1870)	hypogaic	FF	83.3	0.001
<i>Camponotus cingulatus</i> (MAYR, 1862)	epigaic	FF	60.0	0.0002
<i>Linepithema iniquum</i> (MAYR, 1870)	epigaic	FF	40.0	0.002
<i>Atta sexdens</i> (LINNAEUS, 1758)	epigaic	FF	33.3	0.007
<i>Camponotus lespesii</i> (FOREL, 1886)	epigaic	FF	26.7	0.02
<i>Nylanderia</i> sp. (EMERY, 1906)	epigaic	FF	26.7	0.03
<i>Pachycondyla striata</i> (FR.SMITH, 1858)	epigaic	FF	26.7	0.03
<i>Dorymyrmex brunneus</i> (FOREL, 1908)	epigaic	EGS	57.0	0.0006

The amount of organic matter was higher in the forest fragment than in the two types of grazing systems ($F=14.1$, $p=0.0005$; Figure 3), however it was similar between the two grazing systems

($F=1.40$, $p=0.24$). The epigaeic species richness ($F=1.40$, $p=0.24$) and hypogaeic species richness ($\chi^2=0.12$, $p=0.73$) were not influenced by the amount of organic matter in the soil.

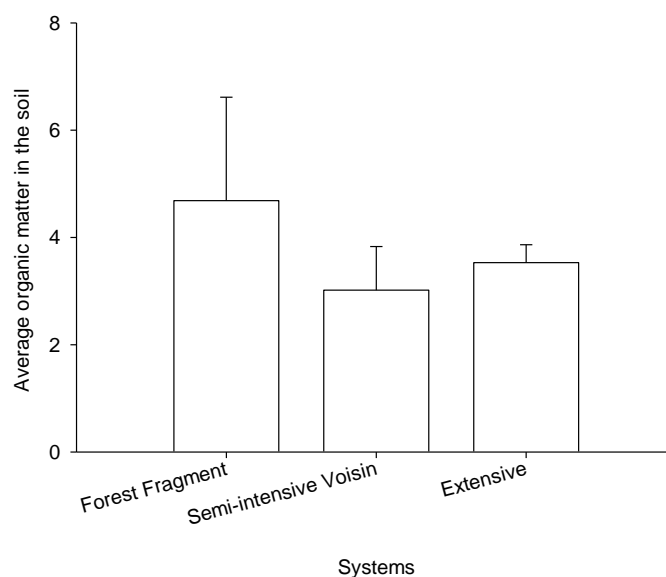


Figure 3 - Average organic matter in the soil in three areas in Carrancas, Minas Gerais state, Brazil. Bars are standard deviations. Difference between forest fragments and grazing systems - $p=0.0005$. The difference between the grazing systems is nonsignificant - $p=0.24$.

Discussion

Ant species richness was negatively influenced by environmental impact, which is partially in accordance with our hypothesis since both grazing systems (extensive and semi-intensive Voisin) had the same species richness, but we found a higher species richness in the forest fragment. The species composition follows the same pattern, as the compositions of both grazing systems were similar and different from the composition of the forest fragment. The

amount of organic matter follows the same pattern when comparing the three sites, but it didn't influence ant species richness.

The higher total and mean epigaeic species richness in the forest can be explained by its higher productivity and heterogeneity in comparison with the grazing systems. A higher productivity usually means abundance and diversity of resources and conditions, leading to a heterogeneous habitat which, consequently, can support a higher

number of species (CALCATERRA *et al.*, 2010). The same pattern were found by other authors (e.g. BRIDLE *et al.*, 2009; BRAGA *et al.*, 2010; OTTONETTI *et al.*, 2010), elucidating that simplifying of habitat may lead to decrease the species richness. The impact caused by the cattle negatively affects the epigaeic ants because of the removal of all the natural vegetation in the area. The removal of the plant biomass caused a change in the habitat, modifying the resources and conditions of it, furthermore the trampling by cattle influences on the species richness. This kind of impact can destroy the nests and individual ants when the ants were already presents locally, or it can affect the colonization of new areas because it compacts the soil hindering the establishment of new nests. Few epigaeic species can adapt to this new environment, and this fact is reflected in a lower number of epigaeic species in both grazing systems in comparison with the forest.

For the total number of hypogaieic ant species per system, we found an opposite pattern to that found in epigaeic ants: there is a higher number of hypogaieic species in both grazing systems than in the forest fragment. Higher species richness on both grazing systems can indicate that the species found in those

environments are habitat generalists that occupy the area soon after a disturbance, since these ants can disperse easily by establishing nests and colonies in places where other species are not able to live, such as a created grazing area. On the other hand, the presence of some generalist species such as *Linepithema inicuum*, and other epigaeic generalists in the hypogeic forest microhabitat (Table 1), could indicate that this area is disturbed, which in turn helps to explain its smaller number of hypogaieic species.

Contrary to the total number of hypogaieic ant species, the mean species richness per pitfall was similar in the three areas studied. Two hypotheses can explain this result: (i) the hypogeic layer is scarcely affected by the outside environment, i.e. the cattle trampling is not enough to disturb this microhabitat, and (ii) the impact caused by the cattle is an intermediate disturbance, allowing a higher number of species in the grazing systems and preventing competition to exclude hypogaieic species. Schimdt *et al.* (2013) already noticed that hypogaieic ant disturbance is only expressed when soil conditions are drastically modified, which we believe is not occurring in the study area.

In spite of the total hypogaieic species richness being higher in the grazing systems, the mean hypogaieic species

richness per pitfall was the same in all the three environments. These contrasting patterns indicate that what contributes to the total higher diversity of ant species in the grazing systems is the difference between the ants sampled in each pitfall trap (beta diversity). Higher beta diversity in grazing systems means a high species turnover among pitfalls, which in turn could be explained by each local habitat being colonized by a different set of species, soon after a disturbance, as already pointed out.

According to Ribas *et al.* (2012), species richness as a parameter to analyze environmental impacts on ant communities should be used with caution, as the response is not always consistent. This could occur because ant foraging is very diverse, implicating in a constant change in the species. Using other parameters, like species composition, is more reliable in environmental indicator studies (RIBAS *et al.*, 2012).

Hypogaeic and epigaeic species composition is the same in both grazing systems and different in the forest fragment. Nakamura *et al.* (2007) also found the same pattern, i.e. pastures with different grazing intensity did not affect species composition. Our results show that species composition on pastures is made up of a different group of ants,

which is distinct from the forests, the same result found by Braga *et al.* (2010) and by Schimdt *et al.* (2013). The divergence between the group of species found in grazing systems and the forest fragment may be caused by the difference in vegetation complexity of these habitats, as already mentioned.

Since species richness and composition did not differ between the grazing systems, we set up the following hypothesis to explain these results: (i) there was not enough time for the grazing systems to become different from each other in terms of impact; (ii) ants are not responsive to this impact (iii) the grazing systems are not ecologically different for ants.

By the time we set up this study, the semi-intensive Voisin system had been implanted for five months, meaning it was possible that there was not enough time for the grazing systems to become different in terms of impact, since the area converted to semi-intensive Voisin was previously an extensive grazing system. In their study about anthropic impacts in the Amazon, Vasconcelos (1999) found that the recovery of the soil was directly related to its uses in the past, meaning that areas with the extensive grazing system in the past would take longer to recover than areas that did not suffer this impact. We do not

believe that ants are not responsive to the impact, since several studies have already demonstrated their sensibility to soil use (e.g. NAKAMURA *et al.*, 2007; SCHMIDT & DIEHL, 2008; BRAGA *et al.*, 2010; OTTONETTI *et al.*, 2010). Another hypothesis is that the semi-intensive grazing system could not cause a significant ecological change in the conditions and resources for ants. In the particular case of our study, we think that it is likely that there was not enough time for the soil to recover.

We found out that there is a higher amount of organic matter in the forest fragments, which can be explained because in these areas we also found a higher amount of litter. However, the organic matter was not related to ant species richness, maybe because it does not have a direct impact on ants since it is not a resource consumed directly by this group of animals, only influencing the litter and the other components of its fauna.

We found eight indicator species: one of them is an indicator of the extensive grazing system and the others of the forest fragment. *Dorymyrmex brunneus* was considered as an extensive grazing indicator. This ant has strong associations with anthropized areas (FARNEDA *et al.*, 2007) and disturbed landscapes (LUTINSKI *et al.*, 2008).

Few species are indicators of forest fragments: *Camponotus cingulatus* is commonly found in ombrophilous forests and savannas (RIBAS *et al.*, 2012). *Pachycondyla striata* is usually found on savannas, in secondary forests and in primary succession areas (RIBAS *et al.*, 2012). The *Linepithema* genus are generally found in disturbed areas such as *Eucalyptus* forests, burned areas and forest fragments (RIBAS *et al.*, 2012). *Nylanderia* genus is represented by invasive species and can occur in many habitats from deserts to rain forests, meaning that they are generalist ants (LAPOLLA *et al.*, 2011). The occurrence of *Linepithema* and *Nylanderia* genus in the forest fragment in our study can be an indicative of human disturbance in this area, such as harvesting by the residents, which could lead to a coexistence of disturbed area and non-disturbed area species. *Atta*, which was also found as a forest indicator, is a genus which is easily found in disturbed landscapes (SANTOS *et al.*, 2008) such as forests fragments or in habitats where we can find a great amount of sunlight and plants. The lack of an indicator species on Voisin system may infer some kind of environment change. Sampling was done at just five months after the implementation of the Voisin system, this small period of time

could be marked by initial changes in the habitat and there could be no specific species for this particular environment, not even *Dorymyrmex brunneus*, which presented a higher indicator value (83.3) on extensive system.

Based on the results found in this study, we were able to confirm that agriculture intensification affects terrestrial invertebrate fauna, because even in the forest fragment with some level of disturbance, epigaeic ant diversity still is higher than in the grazing systems. This is the first study that used ants as bioindicators to evaluate potential changes in the Voisin grazing system. At this point, there is no doubt that ants have a high potential for bioindication, however there is no standard response for monitoring grazing systems by this group of insects. The response, by itself, is highly dependent on the context (HOFFMANN, 2010). However, to verify if the Voisin system is really ecologically different from an extensive grazing system to ants, it is necessary to perform a longer term study on the area, keeping in mind that the changes in these kinds of habitats can require more time to be noticed.

Acknowledgments

The authors are indebted to the Barro Preto owners for allowing the sampling

on the farm, and to Henrique P. Samsonas, Carolina G.S. Moraes, Mateus S. Bello, Clarisse L. Valentim and Danilo M. Soares for helping with the fieldwork. We are also extremely grateful to Dr. Rodrigo Feitosa, who helped with the ants identifications. This study resulted from the research project: FAPEMIG - CRA - APQ-01084-12 - “Impactos ambientais e sua recuperação – Resposta das comunidades de formigas e suas funções ecológicas”.

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