ELENIR APARECIDA QUEIROZ

BOTTOM-UP AND TOP-DOWN EFFECTS SHAPING HERBIVORY IN A TROPICAL FOREST POST-FIRE: THE IMPORTANCE OF NITROGEN

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ecologia, para obtenção do título de *Magister Scientiae.*

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APROVADA: 28 de fevereiro de 2019.

Tatiana Cornelissen
Tatiana Garabini Cornelissen

Lucas Navarro Paolucci
(Coorientador)

José Henrique Schoereder
(Orientador)

Dedico novamente aos meus pais, Ana Cristina e George, e minha irmã, Eliane, por quem tenho eterna admiração, respeito e amor.

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SUMÁRIO

RESUMO

QUEIROZ, Elenir Aparecida, M.Sc., Universidade Federal de Viçosa, fevereiro de 2019. **Efeitos bottom-up e top-down moldando a herbivoria em floresta tropical pós-fogo: a importância do nitrogênio**. Orientador: José Henrique Schoereder. Coorientador: Lucas Navarro Paolucci.

Perdas de biodiversidade aumentaram em florestas tropicais devido ao uso do fogo para fornecer novas áreas para atividades agrícolas, urbanização e extração de madeira. Além disso, o aumento da ocorrência de incêndios florestais no futuro próximo é previsto por modelos de mudanças climáticas. Por meio de alterações nos efeitos bottom-up e top-down, incêndios florestais podem afetar diversas interações ecológicas como a herbivoria. Essa interação influencia uma variedade de fatores em um ecossistema, principalmente através de mudanças na sobrevivência, produtividade e crescimento das plantas. Assim, como a frequência do fogo aumentou nos trópicos e não compreendemos completamente seu impacto na herbivoria, é essencial conhecer mecanismos que afetam esse processo em florestas tropicais pós-fogo (efeitos bottom-up e top-down). Neste contexto, pretende-se determinar os mecanismos que levam ao aumento da herbivoria em uma floresta tropical alterada por fogo. Nossa hipótese é que a severidade do fogo, a abundância de insetos herbívoros mastigadores, a abundância de artrópodes predadores, o teor de nitrogênio e a dureza foliar, afetam diretamente a herbivoria em floresta tropical. Foi feito teste de predação (efeito top-down) para estimar a predação na floresta pós-fogo, uma vez que a predação influencia herbívoros que, por sua vez, influenciam a herbivoria. As amostragens e o teste de predação foram realizados em parcelas queimadas e não queimadas na floresta amazônica, Brasil. Análises da área foliar foram realizadas como medida de herbivoria, sendo feitas coletas de folhas das árvores para isso. Para determinar a abundância de insetos herbívoros mastigadores e artrópodes predadores, coletas foram feitas utilizando guarda-chuva entomológico ($50cm^2$). Para determinar a suscetibilidade das plantas, análises do teor de nitrogênio e medidas de dureza foliar foram realizadas. Por fim, para estimar a predação, lagartas artificiais feitas com massinha de modelar foram colocadas nas árvores e seus ataques por predadores foram avaliados. Para a análise de herbivoria e predação, um modelo linear generalizado (GLM) foi utilizado. Foram feitas ANOVA unidirecional para análise de herbivoria e análise de regressão para estimar a predação. 'Structure equation modeling' (SEM - análise de caminho) foi utilizada para verificar relações entre nossas variáveis e determinar seus impactos na herbivoria. Nossos resultados sugerem que não há diferença entre herbivoria em áreas não queimadas e em áreas queimadas pós-fogo e o fogo não afetou a predação. O teor de nitrogênio é a única variável medida que afeta de forma direta e significativa a herbivoria. Com base nisso, podemos concluir que o fogo pode afetar indiretamente a herbivoria através do teor de nitrogênio (um efeito bottom-up), já que o nitrogênio é mais encontrado em áreas queimadas do que em áreas não queimadas e os herbívoros preferem plantas com maior teor de nitrogênio. O nitrogênio é um importante fator modelador da herbivoria em uma floresta tropical pós-fogo. Assim, entender as interações entre fogo em floresta tropical e processos ecológicos tal como a herbivoria é importante, uma vez que contribuem significativamente para a produtividade, a biodiversidade e o funcionamento do ecossistema.

ABSTRACT

QUEIROZ, Elenir Aparecida, M.Sc., Universidade Federal de Viçosa, February, 2019. **Bottom-up and top-down effects shaping herbivory in a tropical forest post-fire: the importance of nitrogen**. Advisor: José Henrique Schoereder. Co-advisor: Lucas Navarro Paolucci.

Biodiversity losses have increased in tropical forests due to the use of fire to provide new areas for agricultural activities, urbanization and timber harvesting. Besides that, climate change models predict increases in forest fire occurrence in the near future. Through changes in bottom-up and top-down effects, wildfire occurrence may affect diverse ecological interactions as herbivory. The interaction influences a variety of factors in an ecosystem, mainly through changes in plant survival, productivity and growth. Thus, since frequency of fire has increased in the tropics and we do not completely understand its impact on herbivory, it is essential to know the mechanisms affecting this process in a tropical forest post-fire (bottom-up and top-down effects). In this context, we aim to determine the mechanisms that lead to increases in herbivory in a tropical forest altered by fire. Our hypothesis is that the fire severity, the abundance of chewing herbivorous insects, the abundance of predatory arthropods, the nitrogen content, and the leaf toughness, affect herbivory directly in a tropical forest. Predation test (top-down effect) was made to estimate predation in the post-fire forest, since predation influences herbivorous that in turn influence the herbivory. Samplings and the predation test were conducted in burned and unburned plots in the Amazon forest, Brazil. Leaf area analysis was performed as a measure of herbivory, and tree leaves were collected for this purpose. To determine abundance of chewing herbivorous insects and predatory arthropods, collections were made by using an entomological umbrella-beating sheet ($50cm^2$). To determine the susceptibility of plants, analysis of nitrogen content and leaf toughness measurements were performed. Finally, to estimate predation, artificial caterpillars made by modeling clay were put in trees and their attacks by predators were evaluated. To analyze herbivory and predation, a generalized linear model (GLM) was used. One-way ANOVA to analyze herbivory and regression analysis to estimate predation were performed. Structure equation modeling (SEM – path analysis) was used to see relationships among our variables and determine their impact on herbivory. Our results suggest that there is no difference between herbivory in burned and unburned plots post-fire and fire did not affect predation. Nitrogen content is the only variable measured that directly and significantly affects herbivory. Based on that, we can conclude that fire can affect herbivory indirectly through nitrogen content (a bottom-up effect) since nitrogen

is known to be higher in burned areas than in unburned ones and herbivorous prefer plants with higher nitrogen content. Nitrogen is an important factor shaping herbivory in a tropical forest post-fire. Thus, understanding interactions between fire in tropical forest and ecological processes such as herbivory is important since they contribute significantly to productivity, biodiversity and ecosystem functioning.

INTRODUÇÃO GERAL

As florestas tropicais são ecossistemas ricos em biodiversidade, porém vêm sofrendo grandes perdas de diversidade devido aos impactos antrópicos crescentes nesses ambientes (Strassburg et al. 2010). Diversas discussões reportam o aumento de importantes impactos antrópicos como o fogo em florestas, já que há uma crescente busca por novas áreas para cultivo agrícola e construções urbanas. Nesse contexto, esses impactos têm ganhado importância devido sua relação com a emissão de gases do efeito estufa afetando o aquecimento global (Fearnside, 1990) e principalmente pelos impactos ecológicos de incêndios (Cochrane 2003; Edwards and Krockenberger 2006).

Os principais efeitos do fogo em florestas tropicais são perdas de biomassa, mudanças no ciclo hidrológico e no ciclo de nutrientes (Salati and Vosep, 1984) e a diminuição das comunidades nativas de animais e de vegetais (Pinard et al., 1999). No entanto, além de afetarem a biodiversidade, os impactos antrópicos também podem afetar interações ecológicas como a herbivoria. Massad et al. (2015) demonstram que há um aumento da herbivoria em florestas tropicais pós-fogo. Tal interação é essencial ao ecossistema, uma vez que os efeitos de sua intensidade afetam a estrutura e a composição da vegetação.

Nesse contexto, o aumento da herbivoria pode estar relacionado aos processos que ocorrem dentro da chamada cadeia trófica, sendo esta a estrutura de relações alimentares que interliga os seres vivos. Cada organismo possui um papel importante nesse sistema, onde os que dão início e sustentam toda a cadeia são os organismos produtores (plantas e algas, por exemplo). Eles servem de alimento aos consumidores primários ou herbívoros, que nutrem os consumidores secundários ou carnívoros. Essa estruturação é importante para o funcionamento do ecossistema, uma vez que o funcionamento da cadeia trófica está ligado a fatores fundamentais para a viabilidade de qualquer ecossistema: a transferência de matéria e de energia (Ricklefs, 2003).

Numa cadeia trófica existem diferentes níveis tróficos. O efeito indireto que um nível trófico exerce nos demais níveis tróficos através do efeito direto em níveis tróficos intermediários é conhecido como cascata trófica (Carpenter, 1993). Esta definição contempla tanto o efeito bottom-up quanto o efeito top-down. O efeito bottom-up está relacionado à quando um nível trófico na base da cadeia trófica afeta de forma indireta um nível trófico superior. Enquanto que, o efeito top-down está relacionado a quando um predador afeta um nível trófico basal através do consumo direto de um nível trófico intermediário.

Um dos principais predadores generalistas de artrópodes importantes em ecossistemas terrestres são as formigas. Elas podem atuar como importantes predadores de herbívoros. Esses predadores são de grande porte e ocupam a maior posição trófica entre o grupo das formigas. Tais fatores levam esses insetos a serem considerados sensíveis às perturbações ambientais (Andrade et al., 2014; Filgueiras et al., 2011; Leal et al., 2014). Assim, é esperado que a degradação de florestas afete esses organismos quando isso está relacionado a mudança da estrutura do habitat, como abertura de dossel, mudança na estrutura da vegetação e na riqueza de plantas. Isso consequentemente irá afetar os herbívoros e a herbivoria.

Contudo, existem fatores responsáveis pelo aumento da herbivoria em ambientes queimados, sejam estes relacionados as plantas hospedeiras de herbívoros (efeitos bottom-up) ou aos predadores (efeitos top-down). Nesse sentido, como a frequência de incêndios tem aumentado nos trópicos, é importante a compreensão de suas interações com os efeitos bottom-up e top-down, os quais se relacionam à diversidade florestal (Laurance et al., 2011; Terborgh, 2012). Portanto, é importante compreender como os impactos antrópicos alteram as comunidades de consumidores, uma vez que esses consumidores contribuem significativamente para a produtividade do ecossistema, para a sua biodiversidade e o seu funcionamento (Hahn e Orrock, 2015). Vale ressaltar que, relacionado a herbivoria, destacam-se os insetos, os quais se alimentam de uma grande variedade de plantas, sendo o grupo de organismos que gera a maior perda de biomassa vegetal (Wilson, 1987) e, portanto, agentes do nosso estudo.

Neste estudo, procuramos entender porque a herbivoria é maior em florestas tropicais queimadas comparada as não queimadas, e os mecanismos (efeitos bottom-up e top-down) envolvidos nesse processo. Para determinar os mecanismos que controlam a herbivoria, medimos os seguintes fatores: danos causados por insetos herbívoros mastigadores, abundância de insetos herbívoros, abundância de artrópodes predadores, teor de nitrogênio e espessura foliar em plantas de floresta tropical queimada e não queimada na Bacia Amazônica. Esperamos que a herbivoria seja mais alta sob fogo, porque, primeiro, é esperado que a abundância de insetos herbívoros e a qualidade das plantas sejam altas e, segundo, a abundância de artrópodes predadores deve ser baixa, assim como as estimativas de predação. Além disso, predizemos que a abundância de insetos herbívoros mastigadores, a abundância de artrópodes predadores, o teor de nitrogênio, a espessura das folhas e a severidade do fogo afetam diretamente a herbivoria.

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BOTTOM-UP AND TOP-DOWN EFFECTS SHAPING HERBIVORY IN A TROPICAL FOREST POST-FIRE: THE IMPORTANCE OF NITROGEN

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Bottom-up and top-down effects shaping herbivory in a tropical forest post-fire: the importance of nitrogen

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Abstract

Biodiversity losses have increased in tropical forests due to the use of fire to provide new areas for agricultural activities, urbanization and timber harvesting. Also, climate change models predict increases in forest fire occurrence. Through changes in bottom-up and top-down effects, wildfire may affect diverse ecological interactions as herbivory, which influences plant survival, productivity and growth. Here, we aim to understand why herbivory is higher in burned than unburned tropical forests, and the mechanisms involved in the changes. Samplings of tree leaves, chewing herbivorous insects, predatory arthropods, nitrogen content and leaf toughness were conducted in burned and unburned plots in the Amazon forest, Brazil. Predation test was made to estimate predation. To analyze herbivory and predation, a generalized linear model (GLM) was used. One-way ANOVA to analyze herbivory and regression analysis to estimate predation were performed. Structure equation modeling (SEM – path analysis) was used to see relationships among our variables and determine their impact on herbivory. Our results indicate that there are no difference between herbivory in burned and unburned plots post-fire and fire did not affect predation. Furthermore, nitrogen content is the only variable measured that directly, significantly and positively affects the herbivory. Herbivory levels may return to previous levels due to forest regeneration or along the ontogeny of plants. Older plants can invest more in chemical defenses than younger ones, so they can be less susceptible to herbivorous. Predation of herbivorous may not show differences because of forest regeneration as well. Arthropod species composition, abundance and diversity measured in burned and unburned plots can show high resilience as well as the rapid recovery of the species composition that existed prior to fire. We show that fire can affect herbivory indirectly through nitrogen content (a bottom-up effect) since nitrogen is known to be higher in burned areas than in unburned ones and herbivorous prefer plants with higher nitrogen content. Then, nitrogen is an important factor shaping herbivory in a tropical forest post-fire in the Amazon Basin.

Key words: Burnings, leaf area consumed, insects, predation, nitrogen, leaf toughness, path analysis, structural equation modeling, SEM.

Introduction

Tropical forests are ecosystems rich in biodiversity, but they have suffered great losses of diversity due to anthropogenic disturbances (Strassburg et al. 2010). Among these disturbances we highlight the use of fire. It is known to be used by humans to open space in forests for agricultural cultivation, urban constructions and timber harvesting. Fire activity is predicted to increase in the near future because of global climate changes (Flannigan, et al., 2000). And, combined to forest canopy opening (caused by humans activity), climate changes leads to increases in burned tropical forests flammability (Alencar et al., 2015; Davidson and Martinelli, 2009).

Biomass loss, changes in the hydrological and in the nutrient cycle (Salati and Vosep, 1984) and decreases in natives communities of plants and animals (Pinard et al. 1999) are the main effects of fire in tropical forests. However, besides affecting biodiversity, fire can affect ecological interactions as herbivory, and Massad et al. (2015) described an increase of this interaction in burned tropical forests.

Herbivory is affected in burned tropical forests, probably because fire can affect bottom-up effects related to resource availability and top-down effects of predators, important mechanisms in determining community structure and functioning (Hairston et al. 1960). Bottom-up effects such as nitrogen content and leaf toughness may be changing and thus affect herbivorous directly, while top-down effects may also be affecting herbivorous directly by predator activity.

Bottom-up effects can decrease (Martin et al., 1999; Diniz et al., 2011) or increase the number of herbivorous insects (Fredericksen and Fredericksen, 2002; Carvalho et al., 2012; Bebi et al., 2003; Lopes and Vasconcelos, 2011) because these effects can directly shape plant communities. Opening in vegetation can benefit edge effects, which causes reductions in population sizes of plants and animals that leads to high likelihoods of extinction and ecosystems disturbance (Haddad et al., 2017). Bottom-up effects can act in host plant quality in burned forests through plant stress, which is an adverse reaction to environmental conditions that do not favor plant growth. Based on the hypothesis of plant stress, plants under fire are more nutritious as these plants invest more in nutritional quality than in chemical defenses (White, 1984). The hypothesis illustrates how abiotic factors can influence biotic interactions. In this way, stressed plants become attractive targets for herbivorous insects, as they prefer plants nutritionally better with less physical and chemical defenses (White, 1969; Mattson and Haack, 1987). Decreases in rates of photosynthesis and/or nitrogen content are important criteria in damage assessments caused by stress factors, being low nitrogen negatively related to preference and performance of insects (Stamp and Casey, 1993). Thus, plants that have low nitrogen content as well as high leaf toughness, for example, may lead to a decrease in their preference and performance of chewing herbivorous insects (Marquis, 2012) and hence less changing in vegetation structure.

Top down effects relate to predators play an important hole in reducing foraging activity of herbivorous insects leading to herbivory lower levels. For example, predators as spiders that produce silk avoid herbivorous insects contact with plants colonized by them (Tahir et al., 2017; Rypstra and Buddle, 2013). There is considerable impact of fire on litter-dwelling ant communities in the Amazon mainly decreasing abundance and richness of predatory ants, an important group of predators in tropical forests (Paolucci et al., 2016). Changes in forest structure affect predatory arthropods individuals because of their large size and the high trophic level occupied, being considered sensitive to disturbances (Andrade et al., 2014; Filgueiras et al., 2011; Leal et al., 2014). In this context, trophic cascade theory suggests that top predators action will be transmitted to lower trophic levels and, therefore, affect primary productivity.

Overall, since fire occurrence is increasing in tropical forests and it can lead to increases in herbivory, it is important to understand simultaneous mechanisms that directly influence this process, which include bottom-up and top-down effects. As we know, herbivory is responsible for creating lagged changes in vegetation growth and reproduction and hence, change its structure and composition. Thus, in this study, we aim to understand why herbivory is higher in burned than unburned tropical forests, and the mechanisms (bottom-up and top down effects) involved through this. In order to determine the mechanisms controlling herbivory, we measured the following factors: herbivorous damage in plants caused by chewing herbivorous insects, abundance of chewing herbivorous insects, abundance of predatory arthropods, nitrogen content, and leaf toughness in burned and unburned tropical forest in the Amazon Basin. We expect herbivory to be higher under fire, first because chewing herbivorous insects abundance and plant quality is expected to be high and, second, the abundance of predatory arthropods is expect to be low as well as the predation estimates. Moreover, we predict that abundance of chewing herbivorous insects, abundance of predatory arthropods, nitrogen content, leaf toughness and fire severity affect herbivory directly.

Material and Methods

Site description

The study site is established on a privately owned farm called "Fazenda Tanguro", which is located in the state of Mato Grosso – Brazil, in the southern part of the Amazon basin (13º 04' S; 52º 23' W; Fig. 1a). The climate is tropical humid, and the remaining transitional forest between the *Cerrado* and the southeast Amazon forest describe the forest in our experimental plots. Expanding agriculture threatens the experimental forest plots (Balch et al., 2015) and according to Soares-Filho et al. (2006) only about 25% of 400,000 km^2 of the transitional forest's original extent is supposed to remain until 2050.

Experimental fires were used in a previous study in three adjacent 50-ha (0.5 x 1.0 km) forest plots right in from of a soybean field (Fig. 1b). There is one plot (B1yr) burnt annually from 2004 to 2010, except 2008; a second one (B3yr) burnt triennially (2004, 2007 and 2010); and, finally, a third plot (Control), which was not burned. The burnings were conducted at the end of the dry season, which is between August and September, using kerosene drip torches to set the fire. Environmental responses show us that fire was more intense in the triennial burned plot because of higher litter accumulation (Balch et al., 2008). Fire severity leads to expressive changes in the biome and in its biological process, since vegetation is degraded. More details about the experimental burns can be found in Balch et al. (2008).

Wildfires that occur in the Amazon require a large-scale ecosystem approach. It transforms adequate experimental replication challenging (Oksanen, 2001). So, here we treat sampling within the 50-ha treatment plots as independent, since we did not replicate our treatment plots. That includes legal, logistical and financial constraints. Thus, differences between plots is our focus (Davies and Gray, 2015).

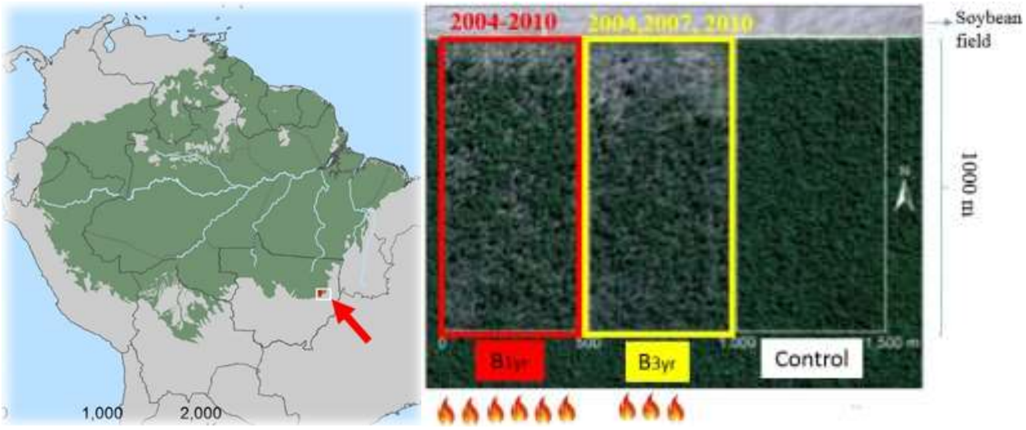


Figure 1. a. Study site location – MT/Brazil. b. Aerial view of the study site in 2010 (Adapted from Balch et al, 2015) with the following plots: B1*yr* = annually burned (2004-2010, except 2008); B3*yr* = triennially burned (2004, 2007 e 2010); Control. Each plot has 1000m in profundity and 500m in length.

Sampling design

The experimental plots are divided in transects and lines according to previous data report. Trees were identified in these transects according to specific diameter at breast height (DBH). For our sampling design, two linear transects of 500m and 750m of the experimental area were selected in order to avoid edge effects. To measure herbivory, abundance of chewing herbivorous insects, predatory arthropods abundance, nitrogen content and leaf toughness, a previous selection of trees were performed based on the following characteristics to avoid influencing the data: amount of nectary and chemical defenses, diameter at breast height (DBH <20cm), and plant family abundance. Five species of each five plant families were chosen: *Myrcia multiflora* (Lam.) DC. (Myrtaceae), *Micropholis egensis* (A. DC.) Pierre (Sapotaceae), *Sacoglottis guianensis* Benth. (Humiriaceae), *Sloanea eichleri* Schum. (Elaeocarpaceae) and *Tapirira guianensis* Aubl. (Anacardiaceae). Thus, totalized 25 trees being randomly chosen into both transect (Fig. 2) following the Herbivory Sampling Protocol provided by a Global Herbivory Network under Tatiana Cornelissen coordination (see supplementary material). Samplings were conducted between March and April of 2018.

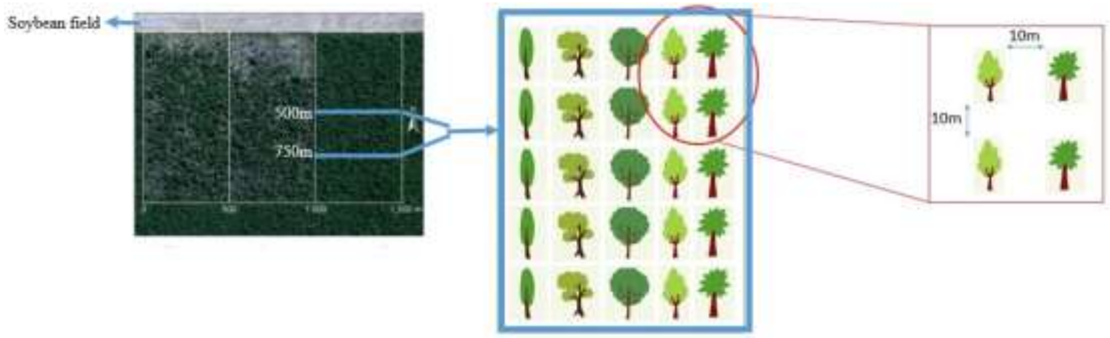


Figure 2. Experimental design to measure herbivory, abundance of herbivorous insects and predatory arthropods, nitrogen content and leaf toughness. Tree selection in both transects (500m and 750m) for each plot. Five species of trees and five individuals belonging to each species were previously selected (according to the Herbivory Sampling Protocol – see supplementary material). The plants are spaced between each other at least 10m.

Herbivory measurement

Following the Herbivory Sampling Protocol (see supplementary material), 50 leaves from each selected tree were collected in order to measure herbivory. We ended up with 1,250 leaves from each plot. Leaf area removed was analyzed by scanning each tree leaf. Each image was analyzed using the R package 'EBImage' (R Core Team 2018). Values of the area removed by herbivorous and values of total leaf area were the results from the analysis. From this, the percentage of leaf area consumed was calculated using the following formula: [(leaf area removed/total leaf area) x100]. Finally, the mean of leaf area consumed (herbivory in %) per tree was calculated.

Sampling of herbivorous insects and predatory arthropods

To measure abundance of herbivorous insects and predatory arthropods in each plot, insects and small invertebrate arthropods were collected from the trees using an umbrella-beating sheet ($50cm^2$), held bellow the canopy of each tree. Four trees around each selected tree received 10 soft beats given by a small piece of wood. All arthropods fallen on the umbrella were collected using manual entomological sucker, paintbrush and tweezers. Arthropods were sorted according to the sampled point (tree), being stored in alcohol 70% into tube Falcon type. Herbivorous insects and predatory arthropods samples were sorted to the level of family/gender and attributed morphospecies classification when family/gender identification was not possible. The herbivorous insects were separated into two guilds according to their eating habits: sucking and chewing insects. This was done based on the morphology of the mouthpiece of each insect. This separation is because chewing insects promote high loss of leaf area (90%) in comparison to sucking insects, miners and gallers (Morante-Filho et al., 2016). Therefore, only chewing insects were used for data analysis. All insects belonging to families with predominant herbivorous habit were considered herbivorous in this study (Moran e Southwood, 1982; Neves et al., 2013, 2014). Voucher specimens of the species are held at the Laboratório de Sistemática e Biologia de Coleoptera (LabCol) located in the Universidade Federal de Viçosa, State of Minas Gerais – Brazil.

Plant susceptibility

- Nitrogen content measurement

Nitrogen content is related to leaf photosynthetic rate, leaf extension, leaf aging and leaf chlorophyll concentration among others factors. To measure nitrogen content, one of the most important factors in plant growth physiology and insect nutrition, a device called SPAD meter or chlorophyll content meter (SPAD-502 - Spectrum Technologies, Inc., Plainfield, IL, USA) was used. Along with photosynthesis data, the SPAD equipment provides nitrogen content data since they are linearly correlated (Loh et al., 2002). One completely healthy leaf was sampled randomly for each selected tree (see the topic "*Sample design"*). The measurements were taken around the midpoint near the midrib of the leaves.

- Leaf toughness measurement

Leaf thickness was used as a proxy to measure leaf toughness. Groups of ten healthy leaves of each tree were sampled. To measure leaf thickness an equipment called External Digital Micrometer (0-25mm/0,001mm MDC-Lite 293-821-30) was used. The measurements were taken around the midpoint in both side of the midrib of the leaves. The mean of the leaf thickness measurements was calculated for each leaf. Finally, mean of the leaves thickness was calculated for each tree.

Predation

In order to estimate predation by insectivorous predators in the plots, a predation field experiment using artificial prey were performed to imitate natural prey of predators (Dátillo et al., 2016; Maas et al., 2015). Artificial caterpillars made with oil-based, odorless, non-toxic modeling clay, 3cm long and 5mm in diameter, were used. There was a wire passing through the center of the clay, with 5cm of leftover to attach the clay to the plant (Fig. 3). They were green in color, as predators usually do not attack preys of aposematic coloring.

The predation test had a specific sample design, being as follows. The experiment was made only in the transect of 500m from the edge bordering the soybean field. The plants selected were 1.5-2m in height being chosen arbitrarily two meters spaced from the transect. Two plants, one on each side of the transect, represented one point. Each plot had fifteen points spaced 10m apart (Fig. 4). One artificial caterpillar was fixed per plant. They were placed in distal branches of the plants and were exposed for four days. After this time, they were examined in the field. Preys were selected as predated if marks and not predated if no marks. This test was conducted in May, 2018.

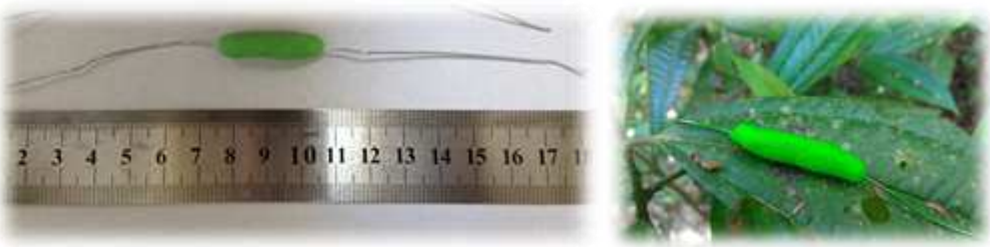


Figure 3. Artificial caterpillars made with oil-based, odorless, non-toxic modeling clay. a. It has 3cm long and 5mm in diameter and there is a wire passing through the center of the clay, with 5cm of leftover to attach the clay to the tree (credit of the first image: Tatiana G. Cornelissen). b. Illustrative picture.

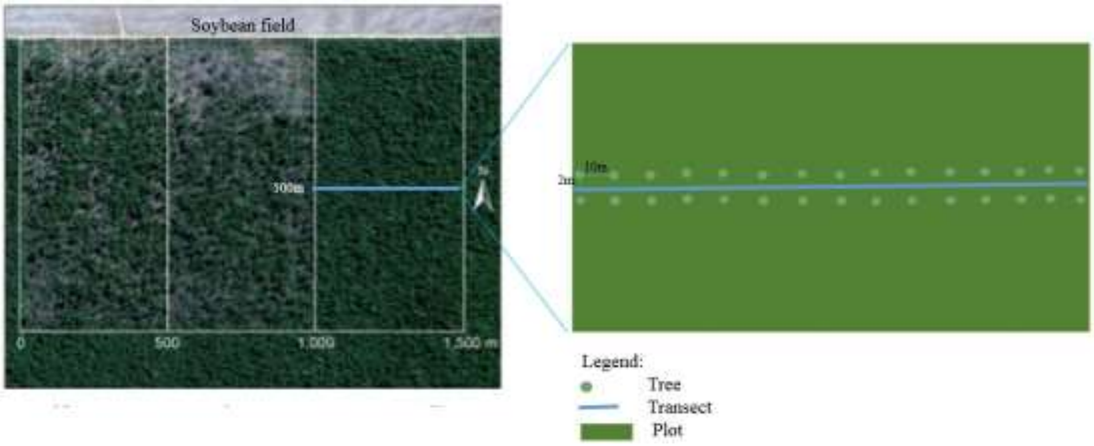


Figure 4. Experimental design for the predation test. The plants selected were 1.5-2m in height being arbitrarily chosen two meters spaced from the edge of the trail (represented in blue). Two plants, one on each side of the trail, represented one point (green circles). Each plot had fifteen points spaced 10m from each other.

Data analysis

- Effects of fire on herbivory

A generalized linear model (GLM) was performed to investigate herbivory levels in each plot. Assumptions of normality were assessed using Q-Q plots. One-way ANOVA was used to check if there is difference among means of each plots, being F-test chosen to test the equality of the means. This statistical analysis was carried out using the software R (R Core Team 2018).

- Path analysis

Structural Equation Modeling (SEM) was used to do a path analysis (R - package ‘lavaan’ (Rosseel 2012; R Core Team 2018) in order to estimate the relative importance of fire severity, herbivorous insect abundance, predatory arthropods abundance, nitrogen content, and leaf toughness (independent variables) on herbivory (dependent variable). Path analysis is a method to check for relationships among variables based on hypothetical pathways of interaction that are identified *a priori*. The paths between variables are relationships expressed in equation form, where the response variables are driven by one or more predictor variables. SEM tests if the variables in the path are interrelated by examining the variances and covariances/correlation of the variables.

Path analysis results in diagrams that consist of variables connected by wires and/or arrows. They represent, respectively, undirected and directed relationships between variables. These variables can be endogenous, a dependent variable that is present in at least one linear equation in the equation system under consideration, or exogenous, that is never a dependent variable. Endogenous variables have at least one arrow pointing to them, while there are no arrows pointing to exogenous variables. The variables must also be either manifest or latent. Observed variables can be measured directly, and latent variables cannot be measured directly, like factors in factor analysis, or residuals in regression (Rosseel, 2018).

Besides representing linear equations relationships with arrows, path diagrams contain variances and covariances of independent variables. Numbers on the lines indicate the standardized regression coefficients from regression used as path coefficients (effect sizes).

In path analysis, as all data is continuous, the default estimator in the lavaan package is maximum likelihood (estimator = "ML"). It is possible to identify the suitability of the model for the available data by using comparative fit index (CFI) and Bayes Information Criterion (BIC) estimated for the model.

Path analysis has previously been used to measure effect of fire severity on plant quality (Murphy et al., 2018) and to examine productivity and anthropogenic effects on a mesopredator (Elmhagen and Rushton, 2007). It is recommended for other cases where the knowledge of the natural history of the studied system is sufficient to construct a priori path diagrams (Palomares et al., 1998).

We expected that the following five general factors are important in determining herbivory: abundance of herbivorous insects, abundance of predatory arthropods, nitrogen content, leaf toughness, and fire severity. For the model, we included direct effects of each of those factors on herbivory as we predicted. Our data include measurements of herbivory, abundance of chewing herbivorous insects and predatory arthropods, nitrogen content, leaf toughness and plot effect (fire). To include fire in the analysis, a categorical variable, we had to transform it into an ordinal dummy variable. Variables in our analysis are treated as continuous. Assumptions of normality were assessed using Q-Q plots and a confirmatory factor analysis (CFA) was used to test the model.

Verifications if the model correct fits our data were done by using more than one model criteria because, unlike other statistical tests, structural equation modeling relies on several statistical tests to determine the adequacy of model fit to the data. The chi-square test indicates the amount of difference between expected and observed covariance matrices. A chi-square value close to zero indicates little difference between the expected and observed covariance matrices and so smaller values indicate better fit. The CFI ranges from 0 to 1 with a larger value indicating better model fit. Acceptable model fit is indicated by a CFI value of 0.90 or greater (Hu & Bentler, 1999). The Root Mean Square Error of Approximation (RMSEA) ranges from 0 to 1 with a smaller RMSEA value indicating better model fit. Acceptable model fit is indicated by an RMSEA value of 0.06 or less (Hu & Bentler, 1999). If model fit is acceptable, parameters estimates are examined. The ratio of each parameter estimate to its standard error is distributed as a z statistic and is significant at the 0.05 level if its value exceeds 1.96 and at the 0.01 level it its value exceeds 2.56 (Hoyle, 1995). Standardized estimates correspond to effect-size estimates.

- *Effects of fire on predation*

A generalized linear model was performed to estimate predation in each plot. Assumptions of normality were assessed using dispersion parameter for Gaussian family (deviance/df. residual). The data has a binomial distribution and chi-square was chosen to statistically test the equality of the means. This statistical analysis was carried out using the software R (R Core Team 2018).

Results

Effects of fire on herbivory

Herbivory was not influenced by fire according to its occurrence (Control plot: 2.35 ± 0.537) or its frequency (Burned 1 year: 2.92 ± 0.555) Burned 3 year: 3.62 ± 0.793) (Fig. 5).

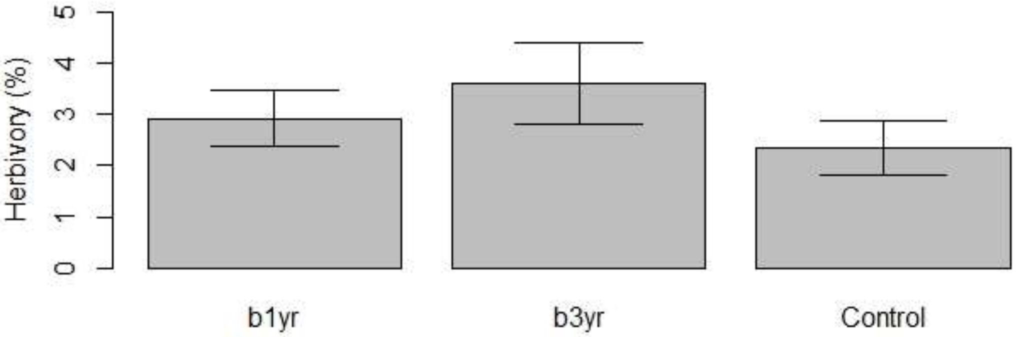


Figure 5. Percentage of herbivory on trees in b1yr (burned annually), b3yr (burned triennially), and Control (unburned) sites. Means ± Standard Error (SE) are given (ANOVA, $F_{(2, 72)} = 0.9918$, $P = 0.3759$).

Path analysis

Confirmatory factor analysis (CFA) suggests that abundance of herbivorous insects, predatory arthropods abundance, nitrogen content and leaf toughness provide a good fit for changes in herbivory levels. The data for the current study also includes fire severity effects, which is our major factor studied. Description for all observed variable is provided in Table 1 and 2 (see Appendices for Table 2).

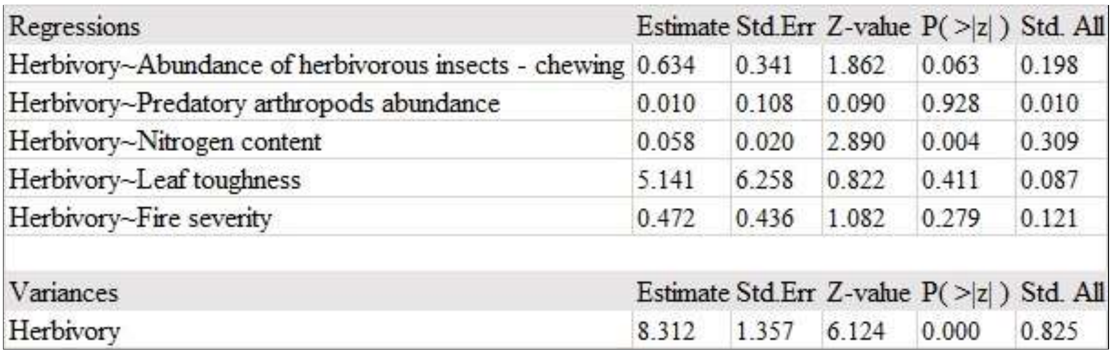
The model fit was perfect, with a CFI of 1.0; RMSEA ≤ 0.05; x^2 (5) =14.470, P = 0.013, df = 5; see Appendices, Table 2). Taken together, these results are consistent with the characterization of herbivory being affected by fire comprising the following distinct factors: herbivorous, predatory arthropods, plant quality and its physical defenses (bottom-up and top-down effects).

In Figure 6, we can see that abundance of herbivorous insects, abundance of predatory arthropods, nitrogen content, leaf toughness and fire severity affect herbivory directly. There are no indirect effects in our model. Direct effects of abundance of herbivorous insects, predatory arthropods abundance, nitrogen content, leaf toughness, and fire severity on herbivory are 0.20, 0.01, 0.31, 0.09, and 0.12 respectively (Table 1).

Nitrogen content is the only variable that represents a significant effect on herbivory (effect size=0.31; p=0.004; moderate positive relationship; see Table 1). Effect sizes are classified in weak (0.01 to 0.30), moderate (0.31 to 0.70), strong (0.71 to 0.99), perfect (1.00) and no effect (0.00) (Olabatuyi, 2006).

Two-headed dotted arrows between two independent variables indicate correlation, but not causal relationship between them. Positive and negative correlations between independent variables are indicated by green and red two headed dotted arrows, respectively. Therefore, they refer to the degree of numerical independence between two random variables. Then, independent variables have zero correlation. Arrows entering and leaving the same variable indicate the variance of the variable under consideration. As our model is standardized, it fixes mean equal zero (0) and standard deviation equal to one (1) for independent variables.

Table 1. Regression herbivory (dependent variable) on abundance of chewing herbivorous insects, predatory arthropods abundance, nitrogen content, leaf toughness and fire severity (independent variables). The pathway between nitrogen content and leaf area consumed was the only one significant in this model (p=0.004) with a direct moderate effect. (Std.Err = Standardized error, Std.All = Effect size).



Regressions	Estimate	Std.Err	Z-value	P(>|z|)	Std. All
Herbivory~Abundance of herbivorous insects - chewing	0.634	0.341	1.862	0.063	0.198
Herbivory~Predatory arthropods abundance	0.010	0.108	0.090	0.928	0.010
Herbivory~Nitrogen content	0.058	0.020	2.890	0.004	0.309
Herbivory~Leaf toughness	5.141	6.258	0.822	0.411	0.087
Herbivory~Fire severity	0.472	0.436	1.082	0.279	0.121
Variances	**Estimate**	**Std.Err**	**Z-value**	**P(>|z|)**	**Std. All**
Herbivory	8.312	1.357	6.124	0.000	0.825

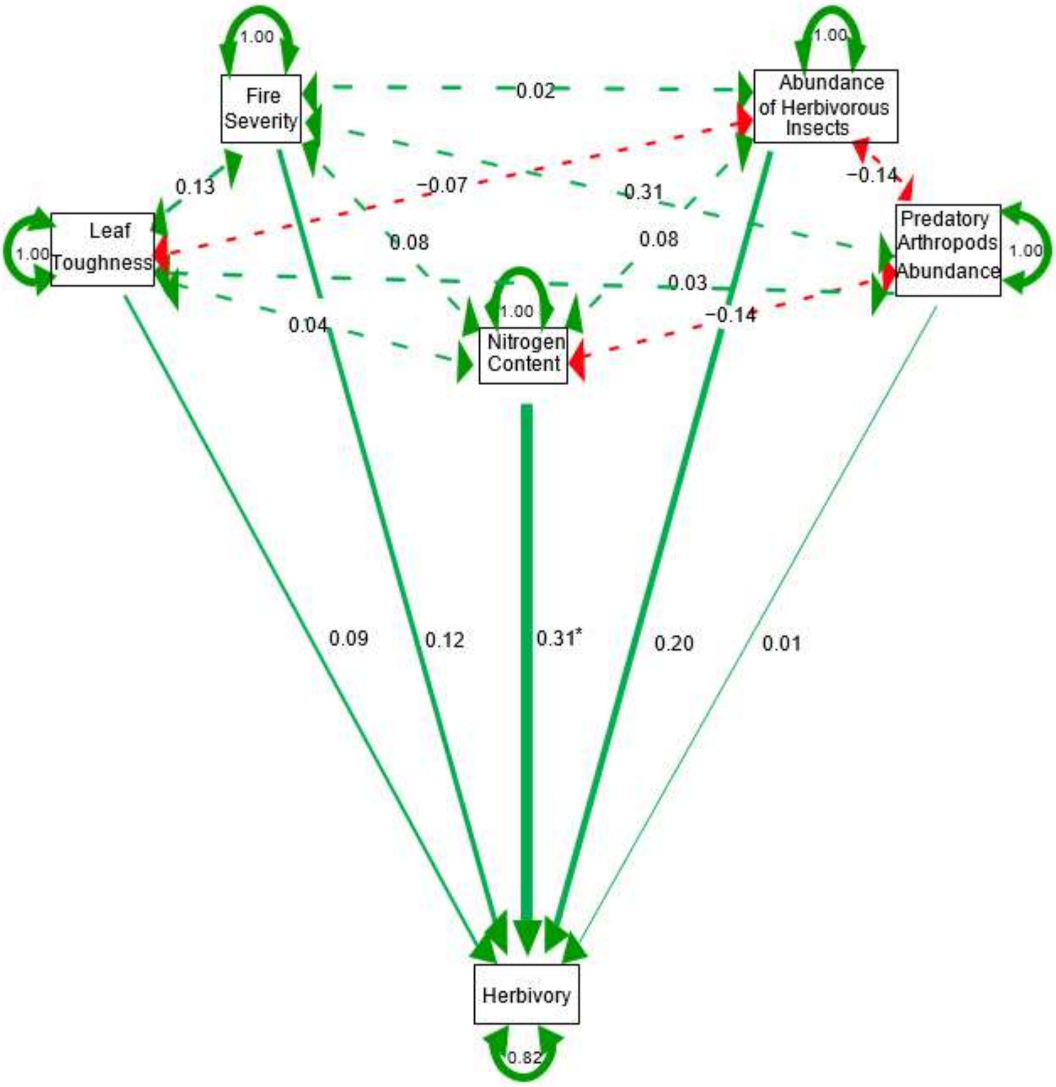


Figure 6. Diagram from path analysis. Single-pointed dark green full arrows indicate causal paths and path coefficients (effect sizes). Green and red dotted double-arrows indicate respectively positive and negative correlation between independent variables. Dark green double-pointed arrows pointed to the variable itself indicate its variance. Effect sizes: low=0.1, medium=0.3, and large=0.5. The strength of the correlation depends on the magnitude of the effect size, which also can be explained as follow: a weak relationship: 0.10 to 0.30; moderate relationship: 0.31 to 0.70; a strong relationship: 0.71 to 0.99; a perfect relationship: 1.00; and no relationship: 0.00 (Elifson, et al., 1998). The thickness of the single pointed headed arrows indicates the strength of the effect size of the independent variables on the dependent variable. The model result assumes that nitrogen content influences directly leaf area consumed being the major driver of change in herbivory levels among the tested variables (Independent variables: Leaf Toughness, Fire Severity, Nitrogen Content, Abundance of Herbivorous Insects, Predatory Arthropods Abundance; dependent variable: Herbivory). The asterisk (*) in the diagram indicates statistical significance of the path coefficient (effect size) at the 0.05 level.

Effects of fire on predation

The proportion of predation was not different among the plots (Control, B1yr and B3yr) (x^2= 3.0074, d.f. = 2, P = 0.2223).

Discussion

Our results do not support the hypothesis that herbivory is higher in burned tropical forests. Thus, the fact that herbivory is not different in the plots can be explained by the process of forest recovery. The year of 2010 marked the end of the prescribed fires and the experimental forest plots entered a recovery period that is characterized by post-fire mortality of large trees that continued higher comparing to our Control plot and by a vigorous regrowth of the understory vegetation (Brando, unpublished data). Despite differences in structure and ecosystem functioning between burned and unburned areas, Brando et al. (unpublished data) shows us that their H_2O fluxes and CO_2 uptake were similar considering few years after the end of fire, likely reflecting optimized resource use by post-fire incoming and fire-surviving vegetation. It can be possible for environment interactions as herbivory, since trees are growing and habitat structure is recovering.

Moreover, we measured herbivory in full-grown trees, which can be different from herbivory measured in seedlings, as reported in a previous study that shows higher herbivory in burned tropical forest (Massad et al., 2015). Annual herbivory measurements are higher on young leaves than on mature ones, because there are considerable differences in herbivory rates among leaf age-classes and among life history groups (Coley, 1983). Young leaves of pioneer and persistent plants are not so tough or fibrous comparing to mature leaves, for example. Indeed, they have higher nitrogen content and water (Coley, 1983). Depending on plant chemical defenses and physical structures (bottom-up effect), adult trees can be less susceptible to herbivorous attack than seedlings, which can justify why we did not find higher herbivory in our burned plots compared to the unburned one.

Concentrations of nitrogen in plants can be higher in burned plots in the first season and decrease later, existing no differences in nitrogen concentrations between plant from burned and unburned plots (Delwiche, 2010; Palviainen, 2017). Based on that, we can conclude that in a long-term, burned areas show vigorous regrowth, and nitrogen levels in these areas can be similar to the unburned ones. In this context, herbivory levels in burned and unburned plots become similar after years since fire.

The identification of the relationships among variables that can affect herbivory and their effect on this process in tropical forests post-fire is important. Based on that, the path diagram allowed us to see direct and indirect effects on herbivory and the intensity of each effect. Results showed by this analysis corroborate our hypothesis that abundance of herbivorous insects, abundance of predatory arthropods, nitrogen content, leaf toughness and fire severity affect herbivory directly. However, they demonstrate that, in a long-term, only nitrogen content in trees affects herbivory significantly, an important bottom-up effect shaping this ecological interaction.

According to the plant stress hypothesis, plants stressed by fire should be more nutritious than non-stressed plants (White, 1984). Thus, plants in burned forests seem to be more nutritious and consequently will be preferred by herbivorous than plants in unburned forests, since herbivorous insects prefer nutritionally better plants. As nitrogen content has a medium impact on herbivory as showed by our results, it negatively impacts plant productivity in burned places compared to unburned ones.

Burned places cannot be as favorable as unburned for plant growth due to its poor soil quality, but post-fire time leads to a better growth because of the nitrogen content recovery in the soil (Harden et al., 2002). Together with the hypothesis of plant stress, it implies in host-plants attractiveness to herbivorous insects, what can increase herbivory. It consequently leads to changes in forest structure and composition over time, if top down effects did not act on herbivorous. Based on that, we conclude that fire can influence herbivory indirectly by nitrogen increases over time. Nitrogen in trees from burned areas are known to increase because of increases in plants nitrogen fixation (Palviainen, 2017).

Plant quality involves variability in plant nutrients. When there is heterogeneity related to plant nutrients, it can negatively influence performance and survivorship of herbivorous insects (Wetzel et al., 2014). In this context, the analysis of nutrients composition of the trees would be interesting to be correlated to herbivorous insects' performance.

Contrary to our expectation, predation was not different in the plots, which can be also explained by the recovery of the forest and its functioning. Disturbances such as fires can strongly affect organisms that are in the higher trophic levels in ecosystem, although this effect may change in a long-term (Paolucci et al., 2016). Spiders were the major groups of predatory arthropods sampled (see Appendices for Table 3). These groups of organisms have short life cycle and they can reproduce fast, increasing rapidly their populations in good conditions such as when fire occurrence stops (r-strategist species) (Wilson and MacArthur, 1967). Hence, predation in unburned tropical forests can become similar to predation post-fire. In addition, arthropod species composition, abundance and diversity measured in burned and unburned plots, where the time after single or repeated fires varied, show high resilience as well as the rapid recovery of the species composition that existed prior to fire (Moretti et al., 2006).

Fire can cause changes in ecological processes as herbivory and so it is important to understand the mechanisms behind them. In long-term, herbivory post-fire in tropical forests is positively influenced by nitrogen content in the trees (bottom-up effects). Nitrogen affects how herbivorous interact with their host plants influencing their feeding and consequently impacting their population sizes and community structure by changes in herbivory and forest productivity. Although the fire does not affect the herbivory over time due to recovery of the forest, it is important to note that if the fire is frequent the environment may not be able to recover. In addition, despite direct effects on vegetation structure, our results suggest for the first time that long-term fire has indirect effects on plant attractiveness for herbivorous insects through nitrogen content in the plants because nitrogen shows moderate positive impact on herbivory and plant quality increases in the presence of fire (plant stress hypothesis). Therefore, besides direct effects that are more commonly studied, there are indirect effects behind the interplay between fire and ecological interactions that need to uncover.

Nowadays there are concerns on how global climate changes and El Niño phenomenon influence fire occurrence in tropical forests (Flannigan, et al., 2000). Also, fire is mostly used for site preparation and for weed control that, combined, these disturbances alter fire regimes with projected large changes in fire intensity and severity, And how these changes affect species interactions is unknown (Koltz et al., 2018). However, our results help to fill out this knowledge gap emphasizing that fire deserve our attention since it affects nitrogen content that refer to plant nutritional quality, a key feed driver of herbivorous insects. Due to its predominance in causing herbivory, it is responsible for creating lagged changes in vegetation growth and reproduction and hence, changes in its structure and composition.

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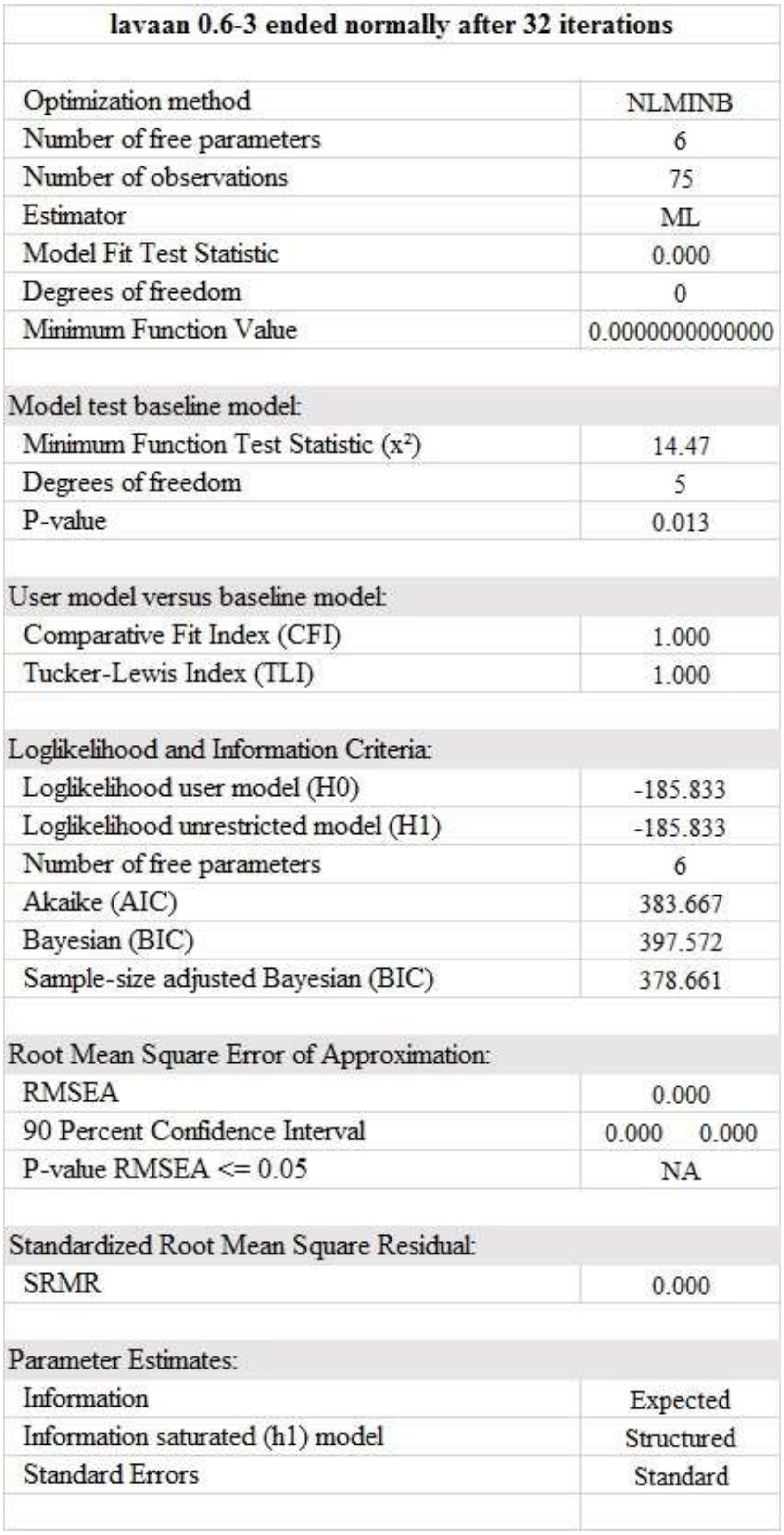
Appendix

1. Path analysis:

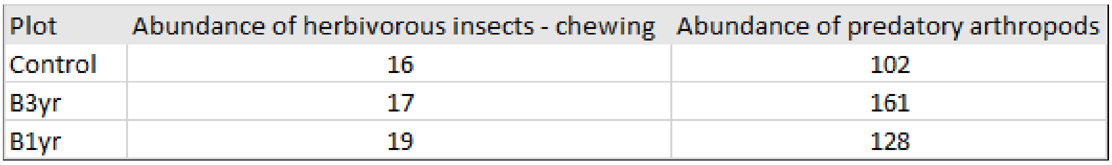
Table 2: Descriptive statistics for the observed variables.



lavaan 0.6-3 ended normally after 32 iterations	
Optimization method	NLMINB
Number of free parameters	6
Number of observations	75
Estimator	ML
Model Fit Test Statistic	0.000
Degrees of freedom	0
Minimum Function Value	0.0000000000000
Model test baseline model:	
Minimum Function Test Statistic (x^2)	14.47
Degrees of freedom	5
P-value	0.013
User model versus baseline model:	
Comparative Fit Index (CFI)	1.000
Tucker-Lewis Index (TLI)	1.000
Loglikelihood and Information Criteria:	
Loglikelihood user model (H0)	-185.833
Loglikelihood unrestricted model (H1)	-185.833
Number of free parameters	6
Akaike (AIC)	383.667
Bayesian (BIC)	397.572
Sample-size adjusted Bayesian (BIC)	378.661
Root Mean Square Error of Approximation:	
RMSEA	0.000
90 Percent Confidence Interval	0.000 0.000
P-value RMSEA <= 0.05	NA
Standardized Root Mean Square Residual:	
SRMR	0.000
Parameter Estimates:	
Information	Expected
Information saturated (h1) model	Structured
Standard Errors	Standard

2. *Herbivorous and predatory arthropods sampled:*

Table 3. Arthropods collected by using the entomological umbrella-beating sheet.



Plot	Abundance of herbivorous insects - chewing	Abundance of predatory arthropods
Control	16	102
B3yr	17	161
B1yr	19	128

Table 4. Abundance of arthropods from different taxonomic group collected by using the entomological umbrella-beating sheet in each plot. Only predatory ants are included in the analysis.

	Plot		
Taxonomic group	Control	B3yr	B1yr
Araneae	98	149	113
Coleoptera	1	3	4
Dermaptera	1	2	0
Hemiptera	0	0	2
Hymenoptera	2	6	6
Mantodea	0	0	2
Neuroptera	0	0	1
Orthoptera	0	1	0

Supplementary material

Herbivory Sampling Protocol:

Global Herbivory Network

Coordinator: Tatiana Cornelissen (Tatiana@ufsj.edu.br)

Herbivory Sampling Protocol – adapted from Kozlov et al. (2015) on GEB

a) Samples should be conducted in natural areas. The area covered by natural vegetation should be at least 1 ha and outside of settlements and agricultural areas, and no big industrial enterprise within 2 km of the selected locality.

b) Get GPS data (exact latitude and longitude), as well as altitude, for the selected locality.

c) Select at least 5 leaf-bearing woody plant species that are the most common species at the locality. They can be trees with accessible branches or shrubs. Record Latin name, growth type, data of collection and any other information you judge might be important.

d) After deciding on the 5 plant species to be recorded for each locality, the choice of individual plants to be sampled should be random.

e) For each species, choose 5 individual plants as replicates, at least 10m apart, and from a distance of about 5 m from you, choose an intermediate (not too tall, not too low) branch to be sampled. It is important that you choose the branch from a distance and do not change your choice later on.

f) From each branch, collect the first 50 leaves, starting from the base of the branch to the apex. If the selected branch has less than 50 leaves, take extra leaves from the nearest branch of same height.

g) Number the 50 leaves from each plant replicate with a four-digit code that indicate the species, the individual plant and the leaf (example: *Bauhinia brevipes* should be Bb 1.1 to 1.50, BB 2.1 to 2.50…Bb 5.1 to 5.50). A suggestion is to use the pen Pilot Super Color Silver Permanent (http://www.pilotpen.eu/en/SUPERCOLORPIM___0_EFT.html), which shows clear colors when the leaves are dry.

h) If you cannot write the code with a permanent pen on the leaf blade, attach a piece of tape to the petiole and write the code in the tape with a permanent pen.

i) Press all coded leaves in newspaper in such a way that leaves are not folded. Note that the 5 individual replicates per species should be stored separately. You will end up with 25 samples per locality (5 species X 5 replicates x 50 leaves).

j) Place samples at an oven at 45oC for 2-3 days or leave them in a dry place.

k)After drying, classify each leaf sampled (n=250 per locality) following the widely used methodology proposed by Dirzo & Dominguez (1989, 1995), according to damage classes of leaf area lost to herbivores. Damage classes are scaled according to Kozlov & Zvereva (2017):

0 – intact leaf
1- 0.01 to 1%
2- between 1 and 5%
3 - between 5 and 25 %
4- between 25 and 50%
5- between 50 and 75%
6- between 75 and 100% of leaf area lost

l) Data should be recorded for each plant species, each individual replicate and each of the 50 leaves per plant in an excel spreadsheet (attached)

m) Record also the time (in minutes) you need to classify each sample of 50 leaves. This information will be later required by the project coordinator.

n) Data is being recorded for defoliators (=chewers) but if leaves also exhibit gall-formers or leaf-miners, please record gall abundance and mine abundance for each leaf in the same datasheet, in a separate column. Please save mined and galled leaves for future studies regarding leaf area attacked by these guilds.

o) Take digital pictures of *each* leaf and use the software ImageJ to measure leaf area lost. Photograph leaves with a ruler on a side for scale.

p) Save at least 10 dried and intact leaves from each species of each of your sites. The leaves should be secured between sheets of cardboard and mailed into an envelope to the project coordinator for analysis of SLA. Include in the packet species name, site and date of collection.

CONCLUSÃO GERAL

O fogo pode causar mudanças em importantes interações ecológicas tal como a herbivoria e, portanto, a compreensão dos mecanismos por trás dessas relações é importante. Embora o fogo não afete a herbivoria e nem a predação (efeito top-down) ao longo do tempo, é importante ressaltar que, se o fogo for frequente, o ambiente pode não ser capaz de se recuperar. A longo prazo, a herbivoria pós-fogo nas florestas tropicais é positivamente influenciada pelo conteúdo de nitrogênio das plantas (efeito bottom-up). Isso implica em mudanças na forma como os herbívoros interagem com suas plantas hospedeiras, já que o nitrogênio é um nutriente essencial ao crescimento e reprodução desses organismos. Assim, esse nutriente influencia a alimentação de insetos herbívoros e, consequentemente, afeta o tamanho das populações e a estrutura da comunidade de plantas.

Apesar dos efeitos diretos do fogo na estrutura da vegetação, nossos resultados sugerem pela primeira vez que, a longo prazo, o fogo tem efeitos indiretos sobre a atratividade de insetos herbívoros através do nitrogênio nas plantas. Isso porque o nitrogênio apresenta um impacto positivo moderado na herbivoria, já que ele proporciona aumento da qualidade da planta na presença de fogo (hipótese de estresse vegetal). Portanto, além dos efeitos diretos mais comumente estudados, há efeitos indiretos por trás da interação entre o fogo e processos ecológicos que precisam ser estudados.

Atualmente, preocupações importantes como as mudanças climáticas e o fenômeno El Niño influenciam a ocorrência de incêndios, beneficiando-o em uma floresta tropical. Além disso, o fogo é muito utilizado para preparação de local para cultivo agrícola e para o controle de ervas daninhas. Esses fatores combinados alteram os regimes do fogo com grandes mudanças em sua intensidade e severidade. A forma como essas mudanças afetam as interações entre espécies é desconhecida. No entanto, nossos resultados ajudam a preencher essa lacuna do conhecimento, enfatizando que o fogo merece nossa atenção, uma vez que afeta o conteúdo de nitrogênio que se refere à qualidade nutricional das plantas, um fator chave na alimentação de insetos herbívoros. Devido a sua predominância em causar herbivoria, esses insetos são responsáveis por criar mudanças retardadas no crescimento e reprodução da vegetação e, consequentemente, causar mudanças em sua estrutura e composição.