UNIVERSIDADE FEDERAL DE LAVRAS

MARINA ACERO ANGOTTI

ANT ASSEMBLAGE AND ITS ECOLOGICAL FUNCTIONS IN COFFEE CROP SYSTEMS

LAVRAS - MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Entomologia, área de concentração em Entomologia, para obtenção de título de Doutora.

Profa. Dra. Carla Rodrigues Ribas
Orientadora

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Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Angotti, Marina Acero.
Ant assemblage and its ecological functions in coffee crop systems / Marina Acero Angotti. - 2018.
116 p.

Orientador(a): Carla Rodrigues Ribas. .
Tese (doutorado) - Universidade Federal de Lavras, 2018.

Bibliografia.
1. Ecological function. 2. Environmental variables. 3. Coffee crop. I. Ribas, Carla Rodrigues. . II. Título.

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ASSEMBLEIA DE FORMIGAS E SUAS FUNÇÕES ECOLÓGICAS EM SISTEMAS DE CULTIVO DE CAFÉ

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Entomologia, área de concentração em Entomologia, para obtenção de título de Doutora.

APROVADA em 23 de março de 2018.

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AGRADECIMENTOS

À minha família, minha mãe Edna, meu pai Luiz, minha irmã Luciana e meus queridos avôs Terezinha e Miguel. Agradeço por esta família linda, que sempre está ao meu lado me apoiando e me encorajando para passar por todos os obstáculos. Embora sempre quisessem que estivesse presente em momentos importantes, também entendiam e aceitavam minha ausência devido ao trabalho que “me esperava”. Agradeço meu cunhado Caio César pela pessoa linda que é e por me proporcionar momentos de risada e alegria. Amo vocês...

Ao meu namorado Július Cerqueira, “meu Amore”, meu companheiro e amigo que sempre esteve presente nessa minha jornada. Sem dúvida ter você por perto nesses quatro anos de Lavras foi fundamental para mim. Obrigada por ser essa pessoa linda e fazer parte da minha vida, Amo você! Agradeço também à Dona Neuza e Sr. Florindo Cerqueira que mesmo distante, sempre se preocuparam conosco.

À minha orientadora Carla R. Ribas, por ser um exemplo de pessoa, amiga, organizada, dedicada e sempre preocupada com seus orientandos. Você sem dúvida me deu um suporte enorme durante esses anos de convivência. Obrigada por sempre acreditar em mim e no meu trabalho.

Ao Laboratório de Ecologia de Formigas (LEF), o melhor que já conheci. Um povo diferente, meio assim “a”normal que fizeram meus dias mais felizes e divertidos. Levo cada um de vocês no meu coração! Ananza Rabello, Antônio Queiroz, Ariel Reis, Carolina Oliveira, Chaim Lasmar, Ernesto Canedo-Júnior, Felipe Ferreira, Gabriela Bandeira, Graziele Santiago, Guilherme Alves, Ícaro Gonzaga, Jonas Aguiar, Luana Zurlo, Mariana Comanducci, Mariana Rabelo, Mayara Imata, Rafael Cuissi, Rafael Casarino. Obrigada por todos os momentos compartilhados. Vocês são Maravilhosos!

As minhas três equipes de campo, só nós sabemos o que a chuva nós fez ir e vir... Carolina Oliveira, Mariana Comanducci, Ernesto Canedo-Júnior, Rafael Cuissi, Július Cerqueira. Agradeço imensamente o auxílio de vocês.

Aos meus amigos da Ecologia!!! Prefiro não citar nomes para não correr o risco de esquecer alguém, afinal nesses quatro anos foram muitas emoções! Muitas pessoas queridas saindo e outras chegando para compor esse ambiente de trabalho tão agradável e leve que essas pessoas proporcionaram a mim. Agradeço aos membros do Bond do RU que saia as 11 horas em ponto ou um pouquinho depois, quem sabe... onde sempre rolava altos papos e risadas. Aos membros dos Laboratórios de Ecologia: de Rola-Bosta, de Peixes, de Mamíferos, de Comunidades, Vegetal e das Plantas herbáceas que conviveram comigo nesses anos. Muitooo obrigada por todos os momentos divertidos, pelos altos churrascos e festinhas. Obrigada também aos agregados que sempre estiveram presentes.

À Universidade Federal de Lavras (UFLA) e ao departamento de Entomologia por terem me proporcionado essa experiência. Ao departamento de Ecologia que foi minha casa nesses anos e que me proporcionou diversas amizades. Obrigada aos dois programas e aos professores que contribuíram para essa formação. Não poderia deixar de agradecer a secretária Isabel (entomologia) e a Ellen (ecologia) que sempre deram um suporte e esclareciam todas as dúvidas que surgiam. À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela minha bolsa neste período de doutorado e à Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) pela bolsa da professora Carla Ribas que permitiu minhas coletas de campo.

À Cooperativa dos Agricultores Familiares de Poço Fundo (COOPFAM) pelo apoio e comunicação com os produtores da região de Machado e Poço Fundo, sul de Minas Gerais. Aos produtores que nos permitiram realizar minha pesquisa em suas propriedades e por sempre nos tratem tão bem. Sou imensamente grata a cada um de vocês: João, Paulo (In memorian), Messias, D. Manuela,Vânia, Maria Selma, Cássio e famílias. À Prof. Leda do Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas - Campus Machado por intermediar o contato para nossa estada no Instituto durante um período da coleta.

Aos membros da banca Luiz Fernando Silva Magnago, Ronald Zanetti, Renata Pacheco do Nascimento e Wallace Beiroz por aceitarem a dar sugestões e melhorar minha tese.

Às meninas da república, Angélica, Iara, Mariana Junqueira e Mariana Gonçalves por serem alegres e divertidas e sempre terem algo para contar e compartilhar. Levarei vocês comigo para sempre. Obrigada pelos anos de convivência.

Meu muito obrigada a todos que estiveram comigo nesse momento e puderam contribuir de alguma forma para minha tese e formação pessoal. Agradeço por ter tido vocês nessa minha caminhada!

RESUMO GERAL

A expansão de áreas agrícolas tornou-se uma das principais causas de perda da biodiversidade e a forma como o manejo das áreas é efetuado pode agravar ainda mais essa perda. Diante disso objetivou-se com essa tese avaliar a influência dos sistemas de café sobre a assembleia e funções ecológicas realizadas por formigas, bem como verificar a influência de variáveis de paisagem e locais sobre elas. Realizou-se o trabalho em áreas de monocultivo de café e fragmentos florestais adjacentes nos municípios de Machado e Poço Fundo, Minas Gerais, Brasil. No primeiro capítulo utilizou-se armadilhas do tipo *pitfall* para capturar a assembleia de formigas em áreas com manejo convencional e orgânico e avaliou-se a influência do manejo bem como de variáveis ambientais locais sobre a assembleia de formigas. Verificou-se que a utilização de agrotóxico no manejo convencional deve ter sido um dos fatores responsáveis pela mudança na composição das formigas e que a abertura de dossel explicou a variação nesta composição. No segundo capítulo avaliou-se a remoção de sementes artificias e a predação de insetos por formigas em áreas florestais e dois sistemas de café (orgânico e convencional). Destacou-se que o sistema agrícola independente do manejo é responsável pela mudança na composição de espécies predadoras e que a abertura de dossel novamente relacionou-se à mudança na composição das espécies predadoras. No terceiro capítulo comparou-se variáveis em diferentes escalas espaciais, a nível de paisagem e local e avaliou-se a influência dessas variáveis sobre a assembleia de formigas em fragmentos de floresta e em café. Verificou-se que as formigas respondem tanto a variáveis de paisagem como local e que a composição de formigas na floresta foi relacionada à porcentagem de cobertura de café, sendo esta a mais importante para explicar essa mudança, seguida da abertura de dossel. No monocultivo de café a composição foi relacionada apenas à abertura de dossel. Dessa maneira, conclui-se que o sistema agrícola é responsável pela mudança na assembleia de formigas e que se deve pensar em estratégias de manejo que levem em consideração práticas mais naturais e similares ao manejo orgânico, bem como a implantação de árvores nos cultivos a fim de fornecer um ambiente mais favorável a assembleia de formigas. Além disso, as formigas são bons organismos para serem utilizadas como modelos em avaliações de impactos ambientais relacionados tanto a assembleia como a função ecológicas, além de responderem tanto a variáveis em escala de paisagem como variáveis locais.

Palavras-chave: Agricultura, Café, Escalas espaciais, Formicidae, Função ecológica, Predação, Paisagem, Remoção de sementes, Variáveis ambientais.

GENERAL ABSTRACT

The agricultural land expansion has become one of the main causes for biodiversity loss, and this loss can be even greater depending on the type of crop management. Thus, the aim of this thesis was to assess the influence of different coffee crop managements on the assemblage and ecological functions of ants, as well as to evaluate the influence of landscape and local variables on these animals. The sampling was taken in coffee monocultures and surrounding forest fragments at the counties of Machado and Poço Fundo, Minas Gerais, Brazil. In the first chapter, I used pitfall traps to collect the ant assemblage at different coffee crops under conventional and organic managements. Then, I evaluated the influence of the management on the ant assemblage, as well as the local environmental variables. I found that the use of pesticide in the conventional crops might be one of the factors responsible for the changes on the ant assemblage, and that canopy openness explained the variation on the ant assemblage composition. In the second chapter, I evaluated the removal of artificial seeds and insect predation by ants in forest áreas and two coffee management (conventional and organic). I found that the coffee crop, independently of its management, was responsible for the composition of predator species, and that canopy openness was related with the changing of predator species composition. At last, in the third chapter I compared the influence of variables at landscape and local scales on the ant assemblage in forest fragments and coffee crops. I found that the ants responded to both landscape and local variables, and that the ant assemblage composition in the forest was related to the percentage of coffee crop. Percentage of coffee crop was the most important variable to explain ant composition in the forest, followed by canopy openness. In the coffee monocultures, the ant composition was related only to the canopy openness. Therefore, the agricultural system is responsible for the change on the ant assemblage and we should consider management more natural practices strategies, such as organic managements, and inclusion of trees in the crops to decrease the canopy openness, which proportionate a favorable environment to the ant assemblage. Moreover, ants are good organisms to be used as models for assessments of environmental impacts that are related to the assemblage and ecological functions. Besides, ants can respond to variables at both landscape and local scales.

Key words: Agriculture, Coffee, Spatial scales, Formicidae, Ecological function, Predation, Landscape, Seed removal, Environmental variables.

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1 INTRODUÇÃO GERAL

Diversos biomas mundiais tem sofrido uma drástica redução em suas áreas devido à atividade e exploração humana (ELLIS; RAMANKUTTY, 2008). No Brasil, os biomas da Mata Atlântica e do Cerrado estão entre os mais afetados pelas atividades antrópicas. Essas regiões possuem alta diversidade de espécies e alto endemismo, sendo considerados *hotspots* mundiais de biodiversidade (MYERS et al., 2000). Por serem biomas fortemente ameaçados pelas atividades antrópicas necessitam de esforços para conservação. Myers e colaboradores (2000) estimaram que restavam apenas 7,5% da vegetação primária da Mata Atlântica e 20% do Cerrado, porém esse nxúmero só diminui devido a continuidade da conversão de áreas e mudanças nos usos do solo (ESPÍRITO-SANTO et al., 2016). Essa drástica redução se deve, entre outras causas, a diversas atividades antrópicas como o desmatamento para fins de urbanização e expansão agrícola (SCHMITZ et al., 2015; MORAES et al., 2017).

A expansão agrícola ganhou forças por volta do século XX com a chamada Revolução Verde, quando houve uma maior possibilidade de ampliar áreas cultivadas, com intensa utilização de grandes maquinarias e de agrotóxicos (ALTIERI, 2004). Dessa maneira, com a facilidade de utilização de recursos e insumos para produção, houve uma crescente conversão de áreas naturais em extensas áreas de monocultivos. No Brasil existe uma vasta produção de café, sendo o país considerado o maior produtor mundial desta commodity e o estado de Minas Gerais responsável por cerca de 50% da produção nacional (CONAB, 2014). Dessa forma, é visível a importância que esta cultura tem para o mercado financeiro, contribuindo grandemente para o PIB brasileiro. No entanto, grande parte da produção de café advém de áreas de monocultivo, no qual áreas naturais são convertidas em ambientes homogêneos. Essa conversão leva à transformação do habitat e dos ecossistemas naturais (CROWDER; JABBOUR, 2014). Além disso, atrelado à simplificação do ambiente, alguns manejos de café, como o sistema convencional, utilizam insumos e maquinários agrícolas que podem contribuir ainda mais para a modificação do ambiente e perda de espécies. Nesse sentido, alguns produtores têm optado por produzir café utilizando manejo orgânico, no qual são utilizados produtos naturais para controlar possíveis pragas, além de capina manual que danifica menos o solo e utilização de adubo orgânico, como restos de outras culturas e de produção animal.

Com a conversão de áreas naturais para outras atividades, como o monocultivo, há também uma intensa fragmentação do habitat. Neste cenário, ambientes naturais sofrem recortes, sobrando paisagens contendo apenas algumas manchas com vegetação nativa (MYERS et al., 2000). Esses fragmentos normalmente têm tamanhos diferentes e podem ficar muito isolados um dos outros, dificultando ou até impossibilitando o fluxo entre os organismos que vivem nessas regiões (FAHRIG, 2007; MILLIGAN et al., 2016). Essa modificação pode resultar em um desequilíbrio ecológico, com perda da biodiversidade (PHILPOTT et al., 2008) e deriva genética. Além da perda de espécies, também pode haver perda de funções ecológicas desempenhadas pelos organismos como polinização, dispersão de sementes, controle biológico (HOPWOOD et al., 2015; OFFENBERG, 2015; RABELLO et al., 2015).

A fim de estudar essas modificações no ambiente, bem como na comunidade presente nesses locais, pesquisadores têm utilizado cada vez mais organismos bioindicadores (SIDDIG et al., 2016). Esses organismos são sensíveis a modificações ambientais e fornecem uma resposta confiável a diferentes impactos (PHILPOTT et al., 2010; RIBAS et al., 2012). Dentre os grupos utilizados, as formigas destacam-se por responderem a diversos tipos de impactos ambientais (PHILPOTT et al., 2010; RIBAS et al., 2012). Elas têm sido utilizadas não somente em estudos que avaliam a comunidade (ANJOS et al., 2017; SOLAR et al., 2016) mas também em avaliações de funções e serviços ecossistêmicos, visto que elas são consideradas boas removedoras e dispersoras de sementes (GALLEGOS et al., 2014; RABELLO et al., 2015) e predadoras de insetos, o que pode contribuir para o controle biológico de pragas (OFFENBERG, 2015).

A remoção de sementes pelas formigas ocorre principalmente devido a uma estrutura nutritiva e atrativa contida em algumas sementes conhecida como elaiossomo (NESS et al., 2010). As formigas removedoras de sementes se alimentam dessa estrutura sem causar danos à semente (HIGASHI et al., 1989; CANNER et al., 2012). Alguns estudos mostram que a remoção do elaiossomo pode facilitar a germinação e o processo de estabelecimento das plantas (PASSOS; OLIVEIRA, 2004; CHRISTIANINI et al., 2007; LEAL et al., 2007). Dessa maneira, as formigas removedoras de sementes podem beneficiar as plantas, uma vez que carregam suas sementes para locais distantes da planta mãe, minimizam o processo de competição entre plantas aparentadas, bem como a densidade de plantas locais (HIGASHI et al., 1989; GILADI, 2006). Ao remover as sementes do solo e levá-las para locais com menores concentrações de sementes, as formigas também acabam protegendo essa estrutura contra a ação de predadores (AULD; DENHAM, 1999; KWIT et al., 2012). Além disso, as formigas podem transportar sementes para dentro ou perto do seu ninho, em uma região que pode ter melhores características nutricionais para a semente no solo, uma vez que a "pilha de resíduos" deixada fora do ninho contém matéria orgânica e nutrientes que influenciam e facilitam o processo de germinação de sementes (PASSOS; OLIVEIRA, 2002).

As formigas podem ser presas de diversos grupos de animais, servindo como fonte alimentar para estes grupos. Além de presas muitas formigas atuam também como predadoras, sendo esta outra importante função ecológica exercida por este grupo. Uma vez que as formigas participam da cadeia alimentar ao predarem diferentes organismos elas podem consequentemente auxiliarem o controle populacional dos mesmos (OFFENBERG, 2015). Dessa maneira, as formigas podem se tornar importantes também para os produtores, uma vez que muitas delas predam organismos potencialmente pragas nos cultivos agrícolas (MORRIS et al., 2015; OFFENBERG, 2015).

Diante deste contexto, essa tese apresenta três capítulos em forma de manuscritos que tem como objetivo central testar como a assembleia de formigas, bem como a remoção de sementes e a predação de insetos realizadas pelas formigas são afetadas pelo monocultivo de café e fragmentos florestais sempre adjacentes a estes e como a assembleia de formigas responde a diferentes escalas espaciais.

No primeiro capítulo avaliamos como o manejo do café (convencional e orgânico) e variáveis ambientais locais (abertura de dossel, compactação do solo, peso seco e heterogeneidade de serapilheira) das plantações e das florestas podem influenciar a assembleia de formigas. Ainda neste capítulo, avaliamos qual o principal mecanismo responsável pela mudança na composição de espécies de formigas nos sistemas de café e florestais avaliados.

No segundo capítulo nós verificamos o efeito do manejo do café (convencional e orgânico) e áreas florestais bem como de variáveis locais (abertura de dossel, compactação do solo, peso seco e heterogeneidade de serapilheira) sobre duas importantes funções exercidas pelas formigas: remoção de sementes e predação de insetos.

No terceiro capítulo verificamos a influência de variáveis de paisagem (porcentagem de café e de floresta) e local (abertura de dossel) sobre a assembleia de formigas tanto em ambientes agrícolas (café) como em fragmentos de vegetação de transição Cerrado – Mata Atlântica.

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SEGUNDA PARTE

Artigo 1 – Conventional coffee growing negatively affecting the ant assemblage in relation to the organic system

Preparado de acordo com as normas da revista Agricultural Systems

Versão preliminar

Conventional coffee growing negatively affecting the ant assemblage in relation to the organic system

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Abstract

The implantation of monocultures and the management type adopted in these areas may be responsible for the great loss of biodiversity. Based on this, the study aimed to evaluate the effect of conventional and organic coffee management grown in full sun on the ant community and to verify whether the habitat structure can be responsible for influencing this community. Moreover, the objective was to evaluate the main mechanism responsible for the possible change in species composition among systems. It was expected that conventional coffee would negatively affect the ant community and that environmental variables would be related to community change. The study was performed in the municipalities of Machado and Poço Fundo, Minas Gerais, Brazil, in five areas of conventional coffee, four areas of organic coffee and nine areas of forest fragments (as control). The ant community was sampled with pitfall traps installed on those systems. Four environmental variables were measured to evaluate the habitat structure (canopy openness, dry weight of leaf litter, litter heterogeneity, and soil compaction). It was found that the ant richness was similar among all systems, but the composition was more dissimilar between the conventional coffee and the forest, and turnover was the mechanism responsible for this dissimilarity. The single variable related to the composition was the canopy openness; however, it explained only 12% of the variation. These results indicate that the use of pesticides in conventional management may have been one of the factors responsible for the change in the ant composition. This highlights the importance of using more natural and less aggressive techniques in the environment in order to minimize the impacts caused by agricultural systems.

Key words: Beta diversity, Coffee crop, Environmental variables, Formicidae.

1 Introduction

Coffee is one of the most economically important items for human consumption worldwide (Perfecto et al. 1996). Traditional coffee is grown in association with a diversity of trees that provide conditions and resources more similar to a forest environment (Perfecto & Snelling 1995; Philpott et al. 2008), and tends to have a lower abundance of pests and diseases (Pumariño et al. 2015). However, with the intensification of agriculture in the mid-20th century, in the so-called green revolution (Altieri, 2004), traditional coffee plantations and several native forest areas were converted into extensive monocultures with full sun cultivation (Philpott et al. 2008). This ecosystem simplification alters the environment structure by modifying the microclimate due to the loss of vegetation cover (Dovciak & Brown, 2014), and availability of litter, which can alter the whole trophic chain, favoring a higher incidence of pests, such as the coffee berry borer (*Hypothenemus hampei*) (Jonsson et al. 2015).

Large areas of monoculture use conventional management in which some agricultural inputs are used as agrochemicals in order to reduce damages caused by pests, besides increasing productivity and mechanization processes. In organic management, generally used in smallholdings, there are a number of restrictions on the use of synthetic agrochemicals, although machinery may be used (Gliessman, 2009; FAO, 1999). Both systems use the soil in different ways, but organic management may present better soil properties and higher macrofauna and microbial diversity in relation to conventional management (Tuck et al. 2014; Inclan et al. 2015; Velmourougane, 2016), besides favoring the abundance and richness of species. Moreover, organic management causes lower impact to the environment (Jerez-Valle et al. 2014; Masoni et al. 2017) and seems to be a healthier food alternative for consumers, and that has gained strength and visibility in agriculture (Assis, 2005; Altiere, 2004).

In order to evaluate the impact of environmental modifications, researchers have used bioindicator organisms, due to response reliability, low cost and sampling time, among other reasons (McGeoch, 1998; Jørgensen et al. 2013). Among the common bioindicators, ants have been widely used to evaluate different types of environmental impacts due to their sensitivity to several factors (Philpott et al. 2010; Ribas et al. 2012; Solar et al. 2016). Furthermore, it was already reported that the simplification of the coffee growing system (from shade to full sun) negatively affects the diversity of ants (Philpott et al. 2008; Urrutia-Escobar & Armbrecht, 2013). Some studies compare the impact of organic and conventional systems on the arthropod community (e.g. Caprio et al. 2015; Santos et al. 2017); however, studies that compare full sun coffee systems has not been found. These assessments might be important because, although both coffee systems are grown in full sun, probably, there is a greater environmental impact resuting from the management type .

Evaluating the impact of certain crops on the environment is important for decision-making, regarding conservation techniques and management type that should be adopted. Among the parameters that can be evaluated are the richness and composition of ants (Canedo-Júnior et al. 2016; Lasmar et al. 2017), ecological functions performed by these organisms (Offenberg et al., 2013; Gallegos et al. 2014), behavior (Ferreira et al. 2014), among others. Besides these parameters, the beta diversity partitioning (Baselga, 2010) can also be used to understand the ecological mechanisms and processes underpinning changes in species composition. In order to understand possible changes in the community, turnover and nestedness mechanisms were evaluated. On one hand, turnover is related to the substitution of species among the environments, which in turn can be related to the landscape structure, spatial configuration and habitat filtering, besides the environmental characteristics (Qian et al. 2005; Baselga, 2010, Bishop et al. 2014). On the other hand, nestedness is related to species loss due to environmental processes that may result in the extinction and colonization of new species (Gaston & Blackburn, 2000; Baselga, 2010).

Therefore, the aim of the present study was to evaluate the effect of different coffee management systems under full sun (conventional and organic) on the ant community (richness and composition) and to verify whether the habitat structure (environmental variables) influences such community. Moreover, the main mechanism responsible for the possible change in species composition among systems was evaluated. Thus, it is hypothesized that (1) ant richness will respond conversely to the impact degree (conventional coffee, organic coffee and forest); (2) ant species composition will also be different among systems and (3) these changes will be due to differences in habitat structure from each type of system. Regarding the mechanism responsible for the possible change in composition, it is hypothesized that, among the forest system and conventional coffee, the main mechanism will be turnover, with a greater substitution of species due to the more intensive management and use of agrochemicals. However, among the forest system and organic coffee, the main mechanism should be nestedness, due to the use of more natural methods in this system, which may lead to greater species similarity among them.

2 Methodology

2.1 Study area

The study was performed in the rainy season, from January to March 2015, in the municipalities of Machado (21°39'40” S, 45°55'30" W) and Poço Fundo (21°46'59” S 45°57'13” W), Minas Gerais, Brazil (Cidade Brasil, 2017). The annual rainfall is approximately 1430 mm, with maximum and minimum temperature of 28 ºC and 16 ºC (Inmet, 2017), respectively, with rainy season in summer (October to March) and dry season in winter (April to September). The state of Minas Gerais is one of the largest coffee producers in Brazil and the sampled regions have a large cultivated area (Conab, 2014). Forest fragment areas in the region are transitional vegetation between the Atlantic Forest and Cerrado biomes.

Nine areas of forest fragments were sampled, and four coffee areas with organic management and five coffee areas with conventional management. The natural areas correspond to fragments ranging from 0.5 to 6.5 ha approximately. In general, the areas under organic management showed coffee trees with greater spacing in relation to coffee with conventional management, and the producers used natural methods for pest control and fertilization. Agrochemicals are used in conventional coffee, even in a small amount and in a controlled manner.

2.2 Community of ants

In order to evaluate the ant community, 10 sampling points were established in each area, with a minimum distance of 20 m among them, totaling a transect of 200 m. It was established transects in the coffee area always as close as possible to the transect in the forest area, the minimum distance of 200 m among the coffee transects and among transects from the forest area.

An epigeic pitfall trap (at the soil surface) was installed at each sampling point (Bestelmeyer et al. 2000) containing 200 ml of water, salt (0.4%) and detergent (0.6%) (Canedo-Júnior et al. 2016). The digging-in effect was disregarded (Lasmar et al. 2017) and the traps were left open for 48 h, being then removed and the captured ants conserved in containers with 70% alcohol. The ants were sorted into genera using the keys Baccaro et al. (2015) identification and then morphospeciated. Subsequently, the lowest possible taxonomic level was verified by the taxonomist Dr. Rodrigo Feitosa from the Universidade Federal do Paraná (UFPR).

2.3 Environmental variables

Environmental variables were sampled in order to verify if there was a difference in habitat structure among the evaluated systems and whether these differences could influence the ant community. Four environmental variables (canopy openness, litter dry weight and heterogeneity, and soil compaction) were sampled at the same points in which the epigeic traps were installed. Canopy openness may be related to the amount of shading and microclimatic changes (Dovciak & Brown, 2014) which can affect the ant community (Chen & Robinson, 2014). Litter heterogeneity and its dry weight represent the quantity and diversity of ant nesting resources and sites (Queiroz et al. 2013). Soil compaction is responsible for influencing ant species composition (Schmidt et al. 2017).

Canopy openness was evaluated by hemispheric photographs taken at ground level by a digital camera coupled with fisheye lens. The percentage of canopy openness from each point was calculated in the Gap Light Analyzer® software. In order to sample the litter heterogeneity, all litter present within a 25 x 25 cm^2 quadrant was collected at each sampling point. The collected items were sorted according to their similarities, quantified and then calculated their heterogeneity (Queiroz et al. 2013) with the Simpson's index (Magurran, 1955). Afterwards, the items were taken to the oven at 60 °C for three days to measure the dry weight of the litter. At each point, were obtained the mean value from three measurements of soil compaction using a pocket penetrometer.

2.4 Analyses

In order to perform the analyses, the richness and composition data of epigeic ants and the environmental variables of each area (by transect) were considered, calculating the average of environmental variables and considering the total richness of the area in ten points of the transect. In order to evaluate whether the system (conventional and organic coffee) negatively influences ant richness, a generalized linear model (GLM) with Poisson distribution was done, considering the system as explanatory variable and richness as response variable, always comparing to the forest system. Contrast analysis was used to check which systems were different. Data overdispersion was evaluated and the models were fitted when necessary, using quasipoisson distribution. All the analyses were performed using software R 3.2.3 (R Core Team 2015).

The influence of systems on ant composition were evaluated using presence and absence data. The analysis of similarities (ANOSIM) was performed with Jaccard's similarity index with 999 permutations using the software Primer version 6.0 PERMANOVA +.

The difference of environmental variables among the systems were evaluated considering each of the environmental variables as response variables and the systems as explanatory variables. Normal (Gaussian) distribution for canopy openness and litter dry weight, binomial for litter heterogeneity and poisson for soil compaction were used. In order to evaluate the influence of each environmental variable on the ant richness, richness was considered as a response variable and the environmental variables as explanatory variable, using Poisson distribution. In both cases, generalized linear model (GLM) was performed and the data overdispersion was evaluated to fit the models when necessary. Contrast analysis was done to check which systems were different. All the analyses were performed using software R 3.2.3 (R Core Team 2015). A DistLM was performed to verify the influence of variables on the ant composition, considering presence and absence data in the software Primer version 6.0 PERMANOVA +.

In order to evaluate the main mechanism responsible for the change in the ant composition among the systems, the mechanisms of a coffee system (conventional or organic) were compared with its closest forest system (control). To this end, the beta diversity was partitioned through the method proposed by Baselga (2010), where the total beta diversity (β_{sor}) is additionally composed by β_{sim} corresponding to the turnover and the β_{sne} corresponding to the nestedness. In order to obtain the values, the betapart package in R software (R Core Team 2015) was used.

3 Results

A total of 116 species was captured belonging to 36 genera (Table 1). The most representative genera were *Pheidole* (28 species), *Camponotus* (14 species) and *Solenopsis* (10 species).

There was no difference in ant richness among any of the sampled systems ($F=3.41$, $p=0.06$). In relation to the ant composition, there was no difference between the organic coffee and the forest system ($R=0.06$, $p=0.31$) and between the conventional and the organic coffee system ($R=0.22$, $p=0.08$); however, ant composition was different when comparing the conventional coffee and the forest system ($R=0.31$, $p=0.01$).

Regarding environmental variables, no difference was observed among the systems in the litter dry weight ($F=2.81$, $p=0.09$) and soil compaction ($F=1.89$, $p=0.18$). However, the litter heterogeneity was different among systems ($F=4.48$, $p=0.03$), being higher in the forest system and similar among coffee systems (Figure 1). Canopy openness was different among all evaluated systems ($F=13.87$, $p<0.001$), being the forest system the one with the smaller openness, followed by conventional and organic coffee (Figure 1). However, there was no relationship between any environmental variable and the species richness: percentage of canopy openness ($F=1.06$, $p=0.31$), litter dry weight ($F=1.49$, $p=0.24$), litter heterogeneity ($F=1.83$, $p=0.19$) and soil compaction ($F=0.62$, $p=0.44$).

As for the influence of environmental variables on species composition, the canopy openness was related with the ant composition (pseudo-$F=2.17$, $p<0.01$), although the explanation percentage was only 12%. The other environmental variables did not influence the ant composition (litter dry weight: pseudoF=1.17, $p=0.23$, litter heterogeneity: pseudoF=1.27, $p=0.18$, soil compaction: pseudoF=1.04, $p=0.43$).

In relation to the mechanisms responsible for the change in composition, only the comparison between the conventional coffee system and the forest was analyzed, since variation in composition was observed only between these two systems. It was observed that turnover (β_{sim}) is the main mechanism responsible for the change in the species composition between the conventional and the forest coffee system with a variation from 75 to 92%.

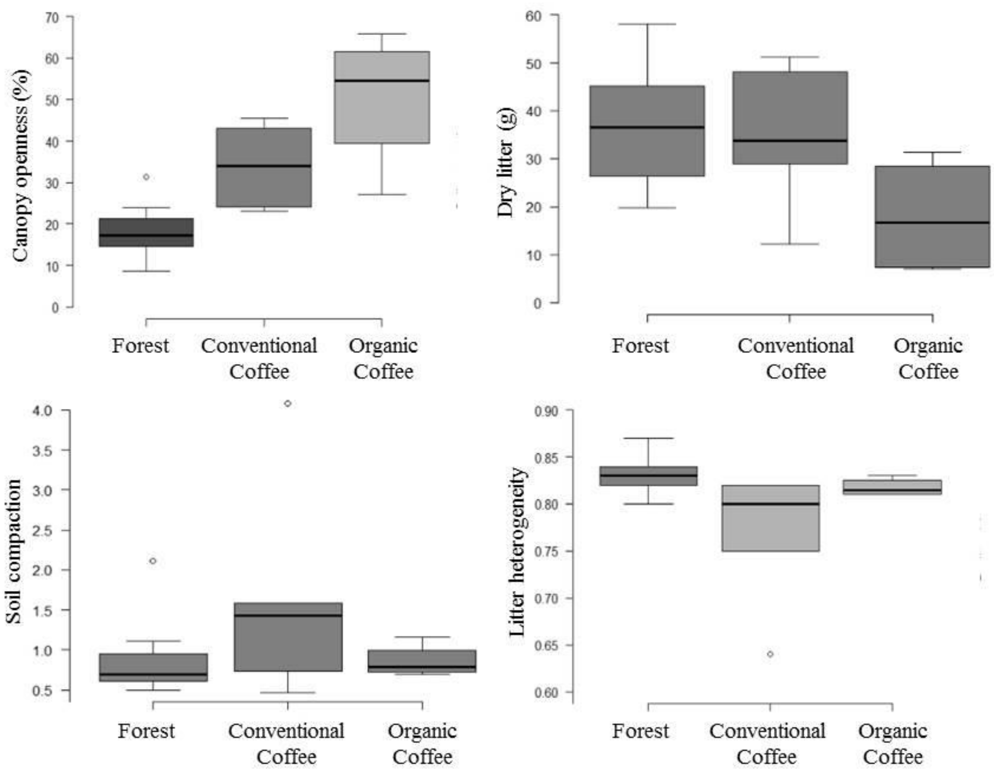

Figure 1. Difference in canopy openness, litter dry weight, soil compaction and litter heterogeneity among conventional, organic and forest coffee systems. Different color bars indicate difference among variables considering significance level 5% ($p < 0.05$).

4 Discussion and Conclusion

It was observed that coffee management adversely affects the ant composition and that organically managed systems is more similar to forest environments than conventional coffee systems. The richness was similar among the systems and the environmental variables did not affect this parameter sampled in our study.

The similarity of the richness between the sampled coffee systems and the forest area is possibly because coffee growing is a perennial crop with high amount of food resources, which can favor the colonization of several ant species. Other studies have also found no changes in the richness or abundance of ants using agrochemicals (Matlock Jr. & de la Cruz, 2003; Chong et al. 2007; De la Mora et al. 2013). In addition, evaluating species richness solely might not be sufficient to suggest some modifications related to environmental impacts and modifications (Ribas et al. 2012; Gomes et al. 2014), since this parameter seems to be more resistant to disturbances (Supp & Ernest 2014). Therefore, species composition seems to be a more adequate parameter for this type of response (e.g. Ribas et al. 2012; Gomes et al. 2014; Stork et al. 2017).

In the present study, the dissimilarity in the species composition between coffee with conventional management and the forest system may have been due to the use of pesticides. This hypothesis should be considered since the structure of systems were very similar, being probably this change caused by the management adopted in the conventional coffee that can suppress species from more conserved / natural environment (see Jerez-Valle et al. 2014; Masoni et al. 2017).

Although some measured environmental variables (canopy openness and litter heterogeneity) varied among the systems, they did not affect the species richness, and only the canopy openness influenced the composition. Another study also used environmental variables similar to the present study to verify the effect of fire on ant community at different recovery times of the savanna, also without relation of variables with species richness (Canedo-Júnior et al. 2016). The similarity in litter weight among systems may be related to the fact that the producers leave a good amount of litter on the soil regardless of the management (organic or conventional), as observed in the sampling period. The similarity of soil compaction may be related to the fact that most of the coffee systems sampled in this study use pruning and hand weeding, with sporadic use of machines, which cannot have affected soil compaction. Regarding litter heterogeneity, which was higher in the forest system and similar among coffee systems, the latter are cultivated in monoculture regardless of the management, with a low litter heterogeneity in relation to natural areas. Although some organic farmers have a greater plant diversity near the crops, this had no influence on the litter heterogeneity, since our collections occurred on the lines between the coffee trees.

The canopy openness was the only variable that varied among the systems and influenced the species composition. The forest system had a smaller canopy openness, precisely because it has a large number and diversity of trees with different heights and larger canopies. Trees in the organic coffee systems in our study areas are planted with a great spacing between the lines, possibly to facilitate management in an area that requires a more constant hand practice than in the conventional system, which is denser. Although the organic coffee system and the forest have the greatest differences in canopy openness, their composition was more similar. This fact may be due to the low explanation (only 12%) of canopy openness on the composition variation. Thus, although canopy openness has utmost importance in several studies by altering the ant community and ant-plant interactions (King et al. 1998; Dáttilo & Dyer, 2014), possibly the greatest impact caused by conventional management may have a greater influence on species composition.

It is worth noticing that in the studied conventional coffee systems, a low amount of agrochemicals and machinery is used. However, even considering this and the proximity between coffee systems and forest systems, the conventional management seems to suppress some more sensitive species. Non-selective agrochemicals can possibly affect ant species that have their nests on the soil surface, since they would be in direct contact with the product. This change in the species composition may have implications for the rural producer, who in the long-term would have higher costs in its production. This is due to alterations in the interactions and ecological functions in which the ants participate as seed removal, predation and biological control of insects, soil aeration, nutrient cycling, among others (Gallegos et al. 2014; Offenberg, 2015).

Finally, it was found that turnover was the main mechanism responsible for the change in composition between conventional coffee and the forest. Possibly, the most aggressive type of management excludes some forest species more demanding in terms of conditions and resources, besides more sensitive to agrochemicals, giving way for the colonization of opportunistic and generalist species. It is not possible to attribute this change in composition to the expected canopy openness. This is because although it was the only variable different among all systems, the conventional coffee system showed an intermediate canopy openness. This result supports the idea of the negative effect caused by conventional management, or even that this change may be related to other parameters not measured in our study.

In conclusion, it was observed that conventional coffee management is more harmful to the environment when compared to organic management. The organic coffee system showed an ant species composition more similar to forest areas, possibly due to the management adopted in these areas. Other authors have already demonstrated that natural environments converted into agricultural systems can alter and reduce the local species community (Philpott et al. 2008) and the intensification of management negatively affects the community (Velmourougane, 2016; Masoni et al. 2017). Apparently, one of the main factors that influenced the ant community was the practice of more intensive management and the use of agrochemicals. In this way, the importance of trying to minimize these damages and to use practices less aggressive to the environment and more similar to the forest environment are highlighted, such as organic management. The low variation in the richness and ant species composition can be explained by the fact that coffee monocultures occupy a large area in the study region. Thereby, there could be great pressure from the landscape of coffee plantations on forest fragments, which could gradually replace and even exclude forest species from the landscape. As a next step, further studies should verify how the effect of landscape can alter the ant community parameters in coffee monocultures.

Acknowledgements

We would like to thank all the producers from the municipalities of Machado and Poço Fundo (Cássio, João, Manuela, Maria Selma, Messias, Paulo -in memoriam- and Vânia) for providing access to their crops for our research. To Ernesto O. Cañedo-Júnior, Mariana C.S. Carvalho and to Julius S. Cerqueira for help in the field collections. To Júlio Louzada by the software license of Primer 6.0 Permanova+. We would like to thank the FAPEMIG (CRA PPM 00243/14) for funding part of our study and to the development agencies for the scholarships. The authors MAA, CJL grant a scholarship from CAPES, CSO from PIBID, and CRR from FAPEMIG.

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Appendices

Table S1. List of species collected in the municipalities of Machado and Poço Fundo, Minas Gerais, Brazil, in coffee systems with conventional management (CONV), organic management (ORG) and forest system (FOR).

Species	CONV	ORG	FOR
Acromyrmex coronatus		X	X
Apterostigma sp.1			X
Apterostigma sp.2	X		X
Apterostigma sp.3	X		X
Atta sexdens	X	X	X
Basiceros disciger			X
Brachymyrmex brasiliensis		X	X
Brachymyrmex sp.1	X	X	X
Brachymyrmex sp.2			X
Camponotus ager	X	X	X
Camponotus atriceps			X
Camponotus cingulatus			X
Camponotus crassus		X	X
Camponotus melanoticus	X	X	X
Camponotus renggeri		X	X
Camponotus sericeiventris			X
Camponotus vittatus			X
Camponotus sp.5		X	X
Camponotus sp.10			X
Camponotus sp.12			X
Camponotus sp.13		X	
Camponotus sp.16			X
Camponotus sp.17			X
Cephalotes pusillus		X	
Crematogaster aff. *acuta*			X
Crematogaster aff. *evallans*	X	X	
Crematogaster chodati		X	
Crematogaster sp.2		X	
Crematogaster sp.3			X
Cyphomyrmex minutus		X	
Cyphomyrmex rimosus	X	X	X
Dorymyrmex sp.1		X	
Ectatomma brunneum	X	X	X
Ectatomma edentatum	X	X	X
Gnamptogenys striatula	X	X	X

Table S1 (continuation). List of species collected in the municipalities of Machado and Poço Fundo, Minas Gerais, Brazil, in coffee systems with conventional management (CONV), organic management (ORG) and forest system (FOR).

Species	CONV	ORG	FOR
Gnamptogenys lavra	X	X	X
Gnamptogenys sp.2		X	
Gnamptogenys sulcata		X	
Hylomyrma reitteri			X
Hypoponera sp.1	X	X	X
Hypoponera sp.2			X
Hypoponera sp.4			X
Hypoponera sp.5		X	
Labidus coecus			X
Labidus praedator	X		X
Leptogenys sp.1			X
Linepithema cerradense	X	X	
Linepithema gallardoi	X	X	X
Linepithema iniquum			X
Linepithema leucomelas		X	X
Linepithema micans	X	X	X
Linepithema neotropicum	X		X
Mycetarotes carinatus	X	X	X
Mycetophylax strigatus			X
Mycocepurus goeldii	X	X	
Mycocepurus smithii	X		
Myrmelachista gallicola			X
Neoponera verenae		X	X
Nylanderia sp.1		X	X
Octostruma stenognatha			X
Odontomachus chelifer	X		X
Odontomachus meinerti		X	X
Oxyepoecus reticulatus		X	X
Pachycondyla harpax			X
Pachycondyla striata	X	X	X
Pheidole aff. *radoszkowskii*	X	X	X
Pheidole aff. *subarmata*	X	X	
Pheidole alpinensis			X
Pheidole gertrudae	X	X	X
Pheidole sp.2	X	X	X
Pheidole sp.3	X	X	X
Pheidole sp.4	X	X	X
Pheidole sp.8		X	X

Table S1 (continuation). List of species collected in the municipalities of Machado and Poço Fundo, Minas Gerais, Brazil, in coffee systems with conventional management (CONV), organic management (ORG) and forest system (FOR).

Species	CONV	ORG	FOR
Pheidole sp.9		X	
Pheidole sp.11	X		
Pheidole sp.12			X
Pheidole sp.14			X
Pheidole sp.15	X	X	X
Pheidole sp.16	X	X	X
Pheidole sp.17	X		
Pheidole sp.18			X
Pheidole sp.19	X	X	X
Pheidole sp.20	X	X	X
Pheidole sp.21	X	X	X
Pheidole sp.23			X
Pheidole sp.24			X
Pheidole sp.25			X
Pheidole sp.26		X	
Pheidole sp.27			X
Pheidole sp.28	X		
Pheidole sp.29	X		X
Pheidole sp.30			X
Pheidole sp.31			X
Pogonomyrmex naegellii	X	X	
Pseudomyrmex gr *pallidus* sp.			X
Pseudomyrmex phyllophilus			X
Pseudomyrmex schuppi			X
Pseudomyrmex termitarius		X	
Solenopsis gr. *geminata* sp.			X
Solenopsis invicta	X	X	X
Solenopsis sp.2	X	X	X
Solenopsis sp.3			X
Solenopsis sp.4	X	X	X
Solenopsis sp.6	X	X	
Solenopsis sp.7			X
Solenopsis sp.10			X
Solenopsis sp.11			X
Solenopsis sp.12		X	
Strumigenys aff *lousianae*	X		X
Strumigenys hindenburgi			X
Strumigenys oglobini		X	

Table S1 (continuation). List of species collected in the municipalities of Machado and Poço Fundo, Minas Gerais, Brazil, in coffee systems with conventional management (CONV), organic management (ORG) and forest system (FOR).

Species	CONV	ORG	FOR
Trachymyrmex oetkeri			X
Typhlomyrmex pusillus			X
Wasmannia affinis			X
Wasmannia auropunctata		X	X
Wasmannia lutzi			X
Total of species	44	58	92

Artigo 2 - The role of ants in tropical coffee agroecosystems: seed removal and predation

Preparado de acordo com as normas da revista Entomologia Experimentalis et applicata

Versão preliminar

The role of ants in tropical coffee agroecosystems: seed removal and predation

The role of ants in coffee agroecosystems

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Abstract

The conversion of natural areas into agricultural systems is responsible for a great loss of biodiversity. Based on this, our aim was to evaluate the influence of two types of coffee management and environmental variables (canopy openness, soil compaction, dry weight, and litter heterogeneity) on the ecological functions of seed removal and insect predation by ants as well as their richness and composition. We evaluated differences in environmental variables among the sampled systems. In order to estimate the seed removal, we provided artificial seeds in the soil in the morning period hence counting the number of seeds removed and collecting ants, after 4 h of field exposure. To estimate the occurrence of predation in the systems, we provided a beetle larva (*Tenebrio* sp.) at each point and collected ants that were preying on the larva. The different types of coffee management did not influence any evaluated parameter. However, we observed a difference in the composition of predatory ants between the natural system and the coffee systems regardless of the management. We verified that there was difference in litter heterogeneity and canopy openness with the conversion from the natural system to coffee monoculture and the canopy openness was related with the composition of predatory ants. The conversion of natural habitat into coffee monoculture areas can alter the ant community due to changes in canopy openness. Additionally, this study emphasizes the importance of relating the composition of species with environmental parameters and of evaluating more of an ecological function in order to verify the environmental changes. We emphasize the importance of management in the agricultural systems related to the canopy openness in order to promote an environment more similar to native areas.

Keywords: Coffee monoculture, sun coffee, Formicidae, artificial seed, seed-removing ants, predatory ants, Tenebrionidae, ecosystem function.

Introduction

Agricultural expansion and consequent habitat fragmentation are responsible for a great loss of biodiversity (Perfecto et al., 1996; Philpott et al., 2008). Due to the establishment of agricultural areas and intensification of management in these systems, natural areas are often converted into extensive areas of monoculture, which leads to a simplification of the habitat and consequent landscape homogenization (Tscharntke et al., 2005; Armbrecht et al., 2006; Moorhead et al., 2010; Solar et al., 2016). The increase in the extension of monoculture areas causes an increase in the use of agrochemicals and fertilizers (Moguel & Toledo, 1999) to combat pests and improving productivity. However, habitat modification leads not only to species reduction (Perfecto et al., 1996; Philpott et al., 2008) but also to the loss of ecological functions and ecosystem services (De Beenhouwer et al., 2013), such as seed dispersion, predation, and biological control (Gallegos et al., 2004; Offenberg, 2015; Pacheco et al., 2017).

Understanding the effects that different agricultural managements have on the biotic communities is important for the conservation of the ecosystem. Therefore, we can make more decisions aiming to reduce environmental negative impacts caused by these activities. One of the tools and approach to assess these impacts is the use of bioindicator organisms that are sensitive to disturbances (McGeoch, 1998). Several researchers have used ants because this group responds rapidly to environmental changes as well as has low costs to be sampled (Underwood & Fisher, 2006; Philpott et al., 2010; Ribas et al., 2012). The study seeks to evaluate how the impacts change the diversity (e.g. Rivera et al., 2013; Solar et al., 2016) and the ecological functions exerted by ants in the environments (e.g. Offenberg, 2015; Rabello et al., 2015).

Among the ecological functions performed by ants, one of the most studied is the secondary removal of seeds (e.g. Lima & Antonialli Jr., 2013; Gallegos et al., 2014), which occurs due to the ant attraction to the elaiosome, a nutritive structure present in some seeds (Ness et al., 2010). Seed-removing ants usually carry seeds to the nest, where they will consume the elaiosome and leave the embryonic structure intact (Canner et al., 2012). Thus, seed removal by ants may result in seedling dispersal and thus contributing to a better distribution, establishment, and growth of seedlings (Gallegos et al., 2014) as well as preventing seed predation by other animals (Kwit et al., 2012).

Another important role played by ants is the predation of other arthropods, which may result in the biological control of pests in agricultural systems (Philpott & Armbrecht, 2006; Offenberg, 2015), such as in cocoa, citrus, coffee, mango, palm oil, cashew, among others (Philpott & Armbrecht, 2006; Offenberg, 2015). Thus, the maintenance of ants in the agricultural systems is also important for the producer since their presence can minimize the production costs due to a reduction in the use of agrochemicals to control agricultural pests (Peng et al., 2004; Offenberg et al., 2013).

In this context, our aim was to evaluate the effect of coffee cultivation management on two ecological functions carried out by ants: seed removal and insect predation. Therefore, we verified whether the different coffee managements (conventional or organic) change in terms of: (1) the number of seeds removed and the predation of insects by ants, and (2) the richness and composition of seed-removing and insect predators ants. Furthermore, we verified if (3) the different ecological functions are correlated and (4) if there is a structural difference among systems. We hypothesized that (1) both the number of removed seeds and the predation would be higher in natural forest systems, followed by the organic and the conventional coffee systems. This is because the forest suffers less anthropic interference and the coffee with organic management has a management system less aggressive to the environment when compared to the coffee with conventional management, with possible structural differences among them; (2) the richness of removing and predatory ants will be higher in forest systems, followed by organic and conventional coffee and the composition of removing and predatory ants will be different among all systems. We expected this due to the use of agrochemicals in conventional management that can suppress more sensitive species; (3) there is no correlation between functions possibly because the nutritional need of predatory ants is different from the seed-removing ants and (4) there are differences between systems.

Materials and Methods

- Study area

We took our collections in the municipalities of Poço Fundo (21°46'59"S 45°57'13"W) and Machado (21°39'40"S 45°55'30"W), in southern Minas Gerais, Brazil. The climate of the region is dry in winter and humid in summer with annual rainfall of approximately 1430 mm, maximum temperature of 28 ºC and minimum of 16 ºC (Inmet, 2017). Coffee is among the main products traded in the world, with Brazil being one of the largest producers and exporters of this commodity (Conab, 2014). The State of Minas Gerais is Brazil's largest coffee producer and represents about 50% of the country's total production. The southern region of Minas Gerais is considered one of the main producers in the country (Conab, 2014).

Due to this agricultural importance in the region, we evaluated seed removal and insect predation by ants in nine coffee monoculture areas, five with conventional management and four with organic management. We considered both managements as full sun coffee, because although some areas had some trees, they were rare and isolated, which does not characterize shade-grown coffee (Philpott et al., 2008). We also sampled nine areas of vegetation fragments adjacent to the sampled coffee areas (as close as possible) with an area of approximately 0.5 to 6.5 ha. These fragments are in a transition area composed predominantly by native plants from Cerrado and Atlantic Forest biomes and are considered in this study as natural systems. In all cases, we compared the types of coffee management with natural systems. The natural systems are secondary forest, with high degree of anthropization.

- Seed removal by ants

In each area, we set a transect with 10 sample points, spaced 20 m apart. We placed 10 artificial seeds at each point consisting of a plastic bead with 1.8 mm size and 0.03 g weight. We chose these beads due to their small size (Pizo & Oliveira, 2001), which allows both large and small ants to carry out the beads. We used artificial seeds, and in order to simulate elaiosome (part of the ant-attractive seed), these beads were covered with an artificial fleshy part containing 75% hydrogenated vegetable fat, 4.8% fructose, 0.5% sucrose, 4.7% glucose, 7% casein, 3% calcium carbonate, and 5% maltodextrin (Raimundo et al., 2004; Rabello et al., 2015). Moreover, artificial fruits have been used in studies due to the ease of acquisition, besides being attractive for ants (Bieber et al., 2014; Rabello et al., 2015; Angotti et al., in preparation). All seeds were protected with a 1.5 cm mesh metal cage in order to avoid predation by small vertebrates (Henao-Gallego et al., 2012; Rabello et al., 2015). We provided the artificial fruits in the morning from 07:30 a.m. to 11:30 a.m, and in this period we collected all the ants we observed carrying seeds to evaluate seed-removing ant assemblage composition. At the end of this period, we removed the remaining seeds and counted the number of removed seeds. We also provided artificial seeds in the afternoon to assess whether there was a difference in the number of artificial seeds removed by ants between the morning and afternoon periods (supplementary material).

Thus, to evaluate the composition and richness of the seed-removing ants, we observed each sampling point three times for 8 min each, with a minimum interval of 1 h between each observation for each point. We captured the specimens observed carrying the seeds, placed them in a tube containing 70% alcohol and took them to the Laboratory of Ant Ecology of the Federal University of Lavras, Minas Gerais, Brazil. We identified the ants to the lowest possible taxonomic level based on the identification key of Baccaro et al. (2015) and compared the morphospecies with the ants from the reference collection of the Laboratory of Ant Ecology (UFLA).

- Predation of insects by ants

We performed the insect predation experiment at the same points selected for the removal of seeds by ants, however, on different days. To evaluate the presence of predation, we placed an alive beetle larva (*Tenebrio* sp.) about 3 cm long (modified from Pacheco et al., 2017) at each sampling point. We tied a string between the third and fourth abdominal segments of each larva and attached it to a wooden stick fixed to the ground to prevent the larva from escaping or burying, but allowing it to move on the soil surface. To avoid larval predation by small vertebrates, we also used a metal cage, as described for seed removal.

The larvae were placed on the ground in the morning from 07:30 a.m. to 11:30 a.m. and observed for 5 min each, with a minimum interval of 1 h among surveys. We collected the specimens of ants that were seen attacking the larvae, placed them in tubes containing 70% alcohol and took them for identification to the same laboratory mentioned above in order to determine the richness and composition of the potentially predatory ants. We identified the ants up to the lowest possible taxonomic level based on the identification key of Baccaro et al. (2015) and on the reference collection of the Laboratory of Ant Ecology (UFLA).

- Sampling of environmental variables

To evaluate if the systems had structural differences among them and if these supposed differences could affect the measured ecological functions (seed removal and predation) and ant community parameters. We measured four environmental variables in each system: dry weight and heterogeneity of the leaf litter, soil compaction, and canopy openness percentage. These variables may reflect the structural difference of the systems and may be important to the ant community.

In order to sample the dry weight and heterogeneity of the leaf litter, we placed a 25 x 25 cm quadrat at each point and collected all the litter present in this area. Afterwards, to determine the heterogeneity, we separated the items according to their similarities and counted the amount of equal and different items contained in the litter (Queiroz et al., 2013). Subsequently, we took the litter samples to the oven at 60 °C for three days and weighed them to obtain the dry weight.

To measure soil compaction, we used a pocket penetrometer, in which we made three measurements at each point and obtained the average per point. We calculated the canopy openness through hemispheric photos recorded at ground level. We used a digital camera equipped with fisheye lens and later we estimated the opening percentage in the Gap Light Analyzer® software.

Analyses

We grouped the ant species a seed-removing or predators, according to our observations. The same species could be assigned to both groups. To have the data for each area, we sum the seeds removed and the predated larvae in each transect. In order to evaluate the influence of the system (natural, organic coffee, conventional coffee) on the number of seeds removed by ants and on the richness of seed-removing ants, we constructed generalized linear models (GLMs) with Poisson distribution. We considered the systems as the explanatory variables and the number of seeds and the richness of ants as the response variables, and we evaluated the influence of systems on insect predation and on the richness of predatory ants, both with Poisson distribution. In this case, we considered the systems as the explanatory variable, the amount of larvae predated in the system and the richness of predatory ants as the response variable. To verify if the functions (of removal and predation) varied in the same way, we correlated the number of artificial seeds removed in the areas with the occurrence of predation.

In all cases, we did residue analysis to verify the distribution of errors and adjust the models to the most appropriate distribution when necessary (Quasipoisson). We performed all analyses using R 3.2.3 software (R Core Team 2015).

In order to evaluate the influence of the systems on the composition of seed-removing ants and predatory ants, we used the composition data (presence and absence of removing or predators). Following, we ran the analysis of similarities (ANOSIM) with the similarity index of Jaccard with 999 permutations, in the software Primer version 6.0 PERMANOVA +.

To verify if there were structural differences among the systems, we compared four environmental variables according to the system. We used GLMs, in which the system was our explanatory variable and each of the environmental variables (canopy openness, litter dry weight, litter heterogeneity, and soil compaction) were the response variables. We used the normal distribution (Gaussian) for the first two variables, Binomial distribution for litter heterogeneity and Poisson for soil compaction. To adjust the models, we made residue analyses and performed these analyses using R 3.2.3 software (R Core Team 2015).

To evaluate if structural differences among the systems affect the parameters of the sampled ants (ecological functions, richness and ant composition), we evaluated only the variables and the parameters of ants that were different among the systems in the previous analyses. We tested the relationship between ant richness (predators or removing) or ecological functions (removal or predation), which are response variables, with environmental variables (explanatory variables), through a GLM. We performed a linear model based on distance (DistLM) with Jaccard index when the composition was different. For this analysis, we used the software Primer version 6.0 PERMANOVA +.

Results

- Seed removal

There was no difference in the number of seeds removed between the morning and afternoon periods (supplementary material). Thus, we opted to perform analyses considering only the data collected in the morning in order to standardize, since the predation experiment was performed only during the morning.

We captured 18 species of seed-removing ants in the morning, 11 species in natural systems, eight in organic coffee, and nine in conventional coffee (supplementary material, Table S1). There was no difference among systems in the number of seeds removed (F=1.83, df=15, p=0.19) nor in the richness of seed-removing ants (F=0.28, df=15, p=0.76) and composition of seed-removing ants (global R=0.15, p=0.07).

- Predation of insects by ants

We captured 21 species of predatory ants in the morning, 13 species in natural systems, 10 in organic coffee, and 15 in conventional coffee (supplementary material, Table S1). The occurrence of predation was equal in all systems (F=0.89, df=15, p=0.43) as well as the species richness of predatory ants (F=2.02, df=15, p=0.17). However, the composition of predatory ants (global R=0.26, p=0.01) was the same among the coffee systems (conventional x organic R=0.11, p=0.21), but differed between the natural system and conventional coffee (R=0.31, p=0.02) and between the natural system and organic coffee (R=0.27, p=0.04) (Figure 1).

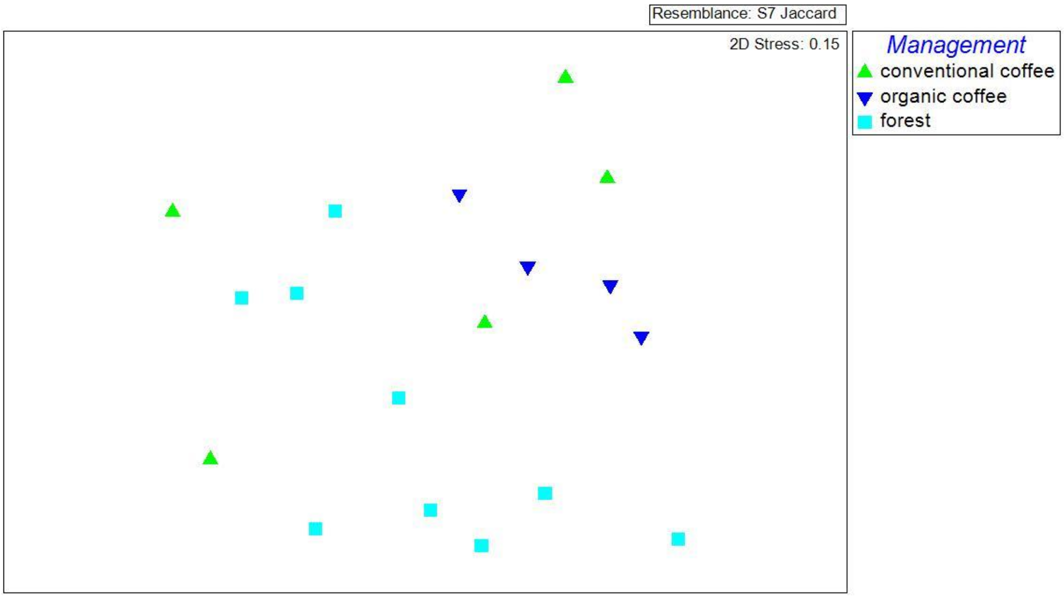

Figure 1. Non-metric multidimensional scaling (NMDS) of ant composition at conventional coffee (green), organic coffee (dark blue) and forest (light blue). Ant assemblage composition of conventional and organic coffee are similar and both are different from forest.

- Correlation between removal and predation

We found no correlation between the functions of seed removal and insect predation (Spearman 0.31).

- Difference of variables among systems

The management (conventional or organic) of coffee systems did not influence any parameters (seed-removing ants richess and composition and their function) of the sampled ants. In this way, in order to verify the possible differences among the systems, we only evaluated if the environments were natural (forest) or agricultural (coffee, regardless of management).

Among the sampled environmental variables (canopy openness, dry weight, litter heterogeneity, and soil compaction), only the canopy openness ($F=17.13$, $p<0.01$) and litter heterogeneity ($F=4.61$, $p<0.05$) were different among systems. We observed that the coffee system has a larger opening compared to the natural system and that there is greater litter heterogeneity in the natural system (Figure 2, Table S2, supplementary material).

Once only the composition of predatory ants was different among systems, we evaluated if the composition of predatory ants was influenced by the canopy openness and by the litter heterogeneity. We found that the composition of predatory ants related with the canopy openness ($PseudoF=3.196$, $p<0.01$) accounted for 16% of the explanation of this change, whereas the species composition did not related with litter heterogeneity ($PseudoF=1.22$, $p=0.24$).

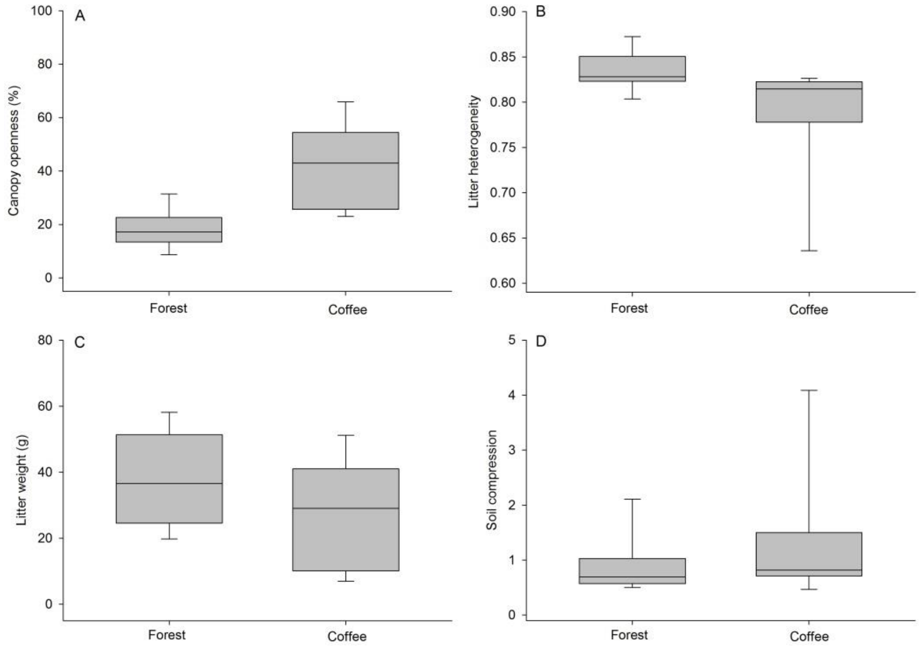

Figure 2. Comparison of environmental variables: A) canopy openness, B) litter heterogeneity, C) dry weight, D) soil compaction in the different evaluated systems (forest and coffee = organic and conventional), with significance level 5% ($p < 0.05$).

Discussion

We verified that the type of coffee management (organic or conventional) did not relate with any of the sampled parameters (richness, composition, and ecological functions of seed removal and predation). However, the agricultural systems (in this case, no matter the management just if it is a coffee monoculture) influenced the composition of predatory ants when compared to the natural system (forest). Functions of seed removal and insect predation are independent, since we did not observe any correlation among them.

- Seed removal by ants

The number of seeds removed by ants and the richness and composition of ants did not differ among the studied systems (natural, organic, and conventional coffee). In general, intensification of coffee management leads to a loss of arthropod diversity possibly due to loss of resources and less shading soil (Perfecto et al., 1996; Philpott et al., 2008). Nevertheless, we believe that coffee, because it is a perennial crop and can act as a source of individuals for a long period, besides having fruits and a relatively large canopy that can provide a greater shade in the soil.

The similarity found in our study for seed-removing ants can also be related to fragmentation of forest areas and to the little variation in the environmental variables. Probably in primary forest, more preserved, we would have a different result. Only the canopy openness and the litter heterogeneity were different among systems, but did not influence the number of removed seeds. Other studies found greater seed removal in areas with lower degree of disturbance (with forest vegetation) with higher canopy cover (Grimbacher & Hughes, 2002; Dominguez-Haydar & Armbrecht, 2011). However, the fact that the other measured variables (litter dry weight, soil compaction) were similar among systems may also be related to the similarity of seed-removing ants found in our study. These variables are also of great importance for the maintenance of the microclimatic characteristics of the soil and may be important for the seeds removal. Variables related to litter, such as the roughness may influence the foraging of ant species (Farji-Brener et al., 2004) and soil compaction, which can have negative effect in the ant community (Rivas-Arancibia et al., 2004; Schmidt et al., 2017) and affect the nesting and settlement process of the colonies, which, in this case, could indirectly influence the removal of seeds and the presence of seed-removing ants.

Most of the coffee systems sampled in our study used weeding to control weeds (personal communication). The farmers mentioned that the use of herbicides is sporadic, occurring only during periods of flowering and fruiting or when some pest appears, but the use is quite limited. In addition, some properties that grow conventional coffee have export certification seals, which minimizes the use of agrochemicals. Regarding the use of agrochemicals, De la Mora et al. (2013) showed that the use of agrochemicals in coffee also did not influence the richness or abundance of ants colony. Other studies have also found no direct effect of pesticides on the ant community (Kwon et al., 2005; Chong et al., 2007; De la Mora et al., 2013). This may be due to the use of selective agrochemicals that appear to have little effect on non-target invertebrates compared to broad-spectrum products, however, some more sensitive groups may be reduced (Jenkins et al., 2013). This low intensity and frequency in the use of these products can affect their action in insect communities, especially in the ants, because they have high resilience and resistance to disturbances (Folgarait, 1998; Andersen et al., 2014). The fact that the agrochemical did not affect the functions exerted by ants may also be related to the means of survival. Many ants that removed seeds or predated the larva of *Tenebrio* sp. (e.g. *Pachycondyla striata, Ectatomma lugens)* can nest underground (Antonialli Jr. & Giannotti, 2001; Silva-Melo & Giannotti, 2010) and this may hinder the contact of the agrochemical with ants. Furthermore, neither all individuals leave the nest to forage, and this fact can preserve the colony and allow the nest's resilience and maintenance in the environment, as suggested by De la Mora et al. (2013).

Thereby, the coffee systems can maintain a community of seed-removing ants similar to the natural ones, being able to perform the function of seed removal, which does not mean that this would imply in a greater dispersion of seeds and establishment of plants in monocultures. This function is important to the grower due aerating soil in ants nests and soil richer in nutrients by the deposit of organic matter made by the ants. Some studies showed that there is a drastic reduction in the richness of ants species, the greater the distance between the coffee system and the natural system (e.g. Perfecto & Vandermeer, 2002; Armbrecht & Perfecto, 2003), possibly due to resource limitations and dispersion. However, we sampled in coffee systems that were always close to natural systems and this may be another factor that contributed to this low variation in the sampled parameters. Thus, natural systems could serve as a source of species for the coffee plantations or, due to the proximity of coffee and natural systems, there could be a spillover whereby movement among these environments may occur, in which the ant would move to the agricultural system in search of resource and return to the natural environment.

- Predation of insects by ants

We also found no influence of coffee management on any of the parameters sampled for predatory ants (richness and composition of predatory ants and predation of larvae by ants). It seems that coffee systems are capable of supporting a great diversity of species possibly due to their complexity, as found for the seed-removing ants. However, these systems, regardless of management, were not able to support a predatory ant composition similar to the natural system, although richness and predation were similar.

Since the composition of predatory ant species was similar between organic and conventional coffee systems, we cannot attribute this difference to the use of agrochemicals. Possibly one of the factors that may have influenced this difference between agricultural systems and natural systems was the structure of the environment. The canopy openness of the agricultural systems was higher than in the natural systems and this variable was related with the change in the composition of predatory ants between coffee and natural systems. The litter heterogeneity was higher in the natural system, which was expected since the coffee systems are cultivated in the form of monoculture, therefore has a more homogeneous vegetation, with lower plant diversity, less availability and diversity of litter in the soil.

More abundant and diverse litter becomes an important source of resources for the ants (Queiroz et al., 2013) and can maintain microclimate conditions in the soil and as a shelter for several species. However, we did not find any influence of litter heterogeneity on changing the composition of predatory species. Some studies show that greater complexity in coffee systems (such as tree shading) may also support a greater diversity of organisms (e.g. Moorhead et al., 2010), resembling a natural system. However, in our study we evaluated only sun-grown coffee, and the difference in the composition of predatory ant species may be due to the lower shading in the coffee systems, since the canopy openness was higher in coffee systems (from 23 to 66 % of opening) when compared to natural systems (from 09 to 31% of opening). The effect of solar radiation on the soil can change the microclimate and change the temperature and humidity, which may modify the behavior and favor different ant communities (Chong & Lee, 2009; Fitzpatrick et al., 2014). Perfecto & Vandermeer (1996) pointed out differences in the ant community due to variation in canopy openness among systems and changes in shading.

It is believed that predatory ants may be more sensitive to environmental changes (Velasco et al., 2010; Andersen et al., 2003; Hoffmann & Andersen, 2003) and that the reduction in canopy cover may negatively affect the richness of predatory ants (Armbrecht & Perfecto, 2003). However, we found no difference in species richness or ecological functions, possibly because the other environmental variables of the systems were similar. Additionally, we considered the ants that attacked the larvae as predators, however, they may be experts, generalists or omnivores. In this way, the richness may also have been similar, since we considered all ants that were collected attacking the larvae as predators. In spite of this, Way & Khoo (1992) mentioned that expert predatory species do not seem to have an impact on the biological control, different from the generalist species, which are considered important for this function. This may explain why we found no difference in predation among systems, since less complex and more open environments tend to support more generalist and opportunistic species (King et al., 1998; Lassau & Hochulli, 2004).

The main approach of the studies in function diversity in coffee plantations has focused on the performance of ants as predators of insect pests in this crop (Philpott & Armbrecht, 2006; Gonthier et al., 2013; De la Mora et al., 2015; Morris et al., 2015; Milligan et al., 2016). However, we believe that it is increasingly important to assess the different ecological functions performed by ants in order to verify the impact of environmental degradation under community structure and ecosystem functioning. In this case, we observed that although parameters and functions of the seed-removing ants were not influenced by the systems, the composition of predators was sensitive to the variation in the structure of the environment. When we related these two functions, we found that they are independent and thus, when evaluated a particular function, it cannot be inferred that others would behave in the same way. Thus, when analyzing the two functions concurrently, we can have a greater reliability regarding the impact caused by the anthropogenic activities. Although some species may perform both the function of seed removal and insect predation, we observed that some perform only one function or another. Thus, *Pheidole gertrudae*, *Pheidole* aff. *triconstriata*, *Pheidole* sp. 3, *Pheidole* sp. 5 and *Heteroponera* sp. 1 were found only removing seeds and *Crematogaster* sp. 1, *Camponotus rufipes*, *Solenopsis* sp. 3, *Pheidole* sp. 4, *Pheidole* sp. 10, *Pheidole* sp. 11 and *Pheidole* sp. 13 only predating insect larvae. This strengthens the importance of assessing more than one function within systems for a better understanding of the caused impact.

Although some studies show that seed removal and insect predation by ants are altered depending on the use of the soil and impact on the environment (e.g. Parr et al., 2007; Pacheco et al., 2017), they do not compare these two functions concurrently. In this way, we are one of the first to relate the two ecological functions of seed removal and predation, which may be considered as the functions of seed dispersal and biological control, depending on the context. In this study, we showed that although coffee management did not alter the sampled parameters (richness, composition and functions), the difference in the structure of the environment may lead to a change in the composition of predatory ants (Pacheco et al., 2017). Possibly this change is related to an influence of the canopy openness on the predatory ant community in these systems. In this sense, it is important to manage and maintain trees in these agricultural systems by providing greater shading and increasing the availability of resources. This management can guarantee a occurrence of species similar to natural systems (Frizzo & Vasconcelos, 2013) and the maintenance of ecological functions.

In conclusion, we observed that the difference in the composition of predatory species between the agricultural and natural systems shows that the modification in the environmental structure is capable of altering the species that perform a certain function within the environment. Even if the ecological function (seed removal and insect predation) continued to be maintained in the systems, this modification in the community can alter an entire ecological chain with possible dominance and extinction of some species in the long term. Additionally, in the face of found results, we highlight the importance of relating the composition of species with environmental parameters and of evaluating more than one ecological function in order to study different environmental impacts. The fact that coffee management (organic or conventional) did not relate directly with the functions performed by the ant community studied here may be associated with the proximity of agricultural systems to natural systems, however, this will be a next hypothesis to be considered.

Acknowledgements

This project was partially financed by FAPEMIG (CRA PPM 00243/14). We are very thankful to the coffee producers in the regions of Machado (Maria Selma, Cássio) and Poço Fundo (João, Paulo, Messias, D. Manuela and Vânia) - MG, for all their affection and for providing the areas for collection. To Edimar of COOPFAM for putting us in contact with the producers. To the Dr. Luis Fernando Silva Magnago and Dr. Wallace Beiroz for their help with statistical analysis, to Chaim Lasmar for assisting in the morphospeciation of ants. To the Laboratory of Chemical, Biochemical and Food Analysis (Department of Food Engineering of the Federal University of Lavras) for providing the space and products to produce the artificial seeds. To Júlio Louzada by the software license of Primer 6.0 Permanova+. MAA, RGC and ACMQ receive financial assistance from CAPES, CSO and CRR from FAPEMIG, and EOCJ from CNPq.

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Supplementary material

Appendix S1. Supplementary methodology - Comparison Seed removal Morning X Afternoon

We verified and compared the number of seeds removed by ants in the morning and afternoon for organic, conventional, and natural coffee systems. The points and methodology used were the same as described in the article for the morning period. The afternoon sampling period was from 3:30 p.m. to 7:30 p.m. We evaluated in each system the influence of the period (morning or afternoon) on the number of seeds removed by ants using mixed generalized linear models (GLMM) in the *lme4* package, using the R software (R Core Team 2015). For each system, we considered the periods as the explanatory variables, the number of seeds removed as the response variable and the area as a random variable, using Poisson distribution. The results showed that the number of seeds removed by ants did not differ between the morning and afternoon periods ($F = 0.12$, $p = 0.77$).

Appendix S2. Collected species

Table S1. List of seed removing-ants (R) and insect predator (P) species collected in the morning, in the conventional and organic coffee and forest systems, in the municipalities of Machado and Poço Fundo, Minas Gerais, Brazil.

Species / Management	Conventional	Organic	Forest
Camponotus rufipes		P	P
Crematogaster acuta	P		
Ectatomma permagnum	P	R/P	P
Ectatomma edentatum	R/P		R/P
Gnamptogenys striatula	R/P	R	R/P
Heteroponera sp. 1			R
Linepithema aff. *aztecoides*	P	P	
Odontomachus chelifer	R/P		P
Pachycondyla striata	P	R/P	R/P
Pheidole radoszkowskii	R/P	R/P	P
Pheidole oxyops	R/P	P	
Pheidole gertrudae			R
Pheidole aff. *triconstriata*		R	
Pheidole sp. 1	P		R/P
Pheidole sp. 2		R/P	R/P
Pheidole sp. 3	R		
Pheidole sp. 4	P		
Pheidole sp. 5	R	R	R
Pheidole sp. 7	R	R	P

Table S1 (continuation). List of seed removing-ants (R) and insect predator (P) species collected in the morning, in the conventional and organic coffee and forest systems, in the municipalities of Machado and Poço Fundo, Minas Gerais, Brazil.

Species / Management	Conventional	Organic	Forest
Pheidole sp. 8		P	R
Pheidole sp. 9			R/P
Pheidole sp. 10	P	P	
Pheidole sp. 11	P		
Pheidole sp. 13			P
Solenopsis gr. *geminata* sp. 1	R/P	P	R/P
Solenopsis sp. 3	P		
Total of seed removing-ants	9	8	11
Total of predator ants	15	10	13

Appendix S3. Difference among environmental variables

Table S2. Comparison of environmental variables (canopy openness, dry weight and litter heterogeneity, soil compaction) between natural system (forest) and coffee plantations. Values with an asterisk indicate difference of environmental variables among systems with significance level of 5% ($p < 0.05$). Environmental variables: CO (canopy openness), LH (litter heterogeneity), LW (litter weight), SC (soil compression).

Environmental variables	df	F value	*p*-value
CO	**16**	**17.13**	**0.0008***
LH	16	4.61	0.0470
LW	16	2.16	0.1600
SC	16	1.18	0.2900

Artigo 3 - Canopy openness and percentage of coffee growing land are important to ant assemblages in agricultural landscapes

Preparado de acordo com as normas da revista Environmental Entomology
Versão preliminar

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Canopy openness and percentage of coffee growing land are important to ant assemblages in agricultural landscapes

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Abstract

Agriculture is one of the main human activities responsible for the loss and fragmentation of habitats. These alterations at the landscape and local scales may change the assemblage of organisms at shifted habitats. Therefore, the aim of this study was to compare and evaluate variables at different spatial scales: landscape (percentage of forest and coffee crops) and local (canopy openness) to verify which of them has the greater effect on ant assemblages (species richness and composition). We aimed at evaluating if these variables have the same effects in ants assemblage on forest fragments and coffee monocultures. We took our samples in the counties of Machado and Poço Fundo, Minas Gerais state, Brazil. We sampled six forest fragments and six monocultures of coffee. The forest fragments were transitions of the biomes Cerrado and Atlantic Forest. At each area, we set up 10 pitfall traps, 20 meters apart from each other, and let the traps for 48h in the field. We found that any variable influenced ant species richness. The ant species composition in forest fragments was related to the percentage of coffee in the landscape. Percentage of coffee was the most important variable to explain the variation of species composition, followed by canopy openness. As for the coffee mocultures, the ant compostion was related only to the canopy openness. We found that the influence of these variables on the ant assemblage may vary according to the studied environment (forest fragment or coffee monoculture). This work demonstrates the importance of evaluating different environments at the same time, as well as different spatial scales.

Keywords: Formicidae, Coffee crop, landscape variables, local variables.

Introduction

The landscape shifts might be responsible for changes of many ecological interactions and patterns, as well as for alterations in many assemblages of organisms. As an example, the smaller the natural fragment included in an agricultural landscape, the smaller the insect predation by birds (Jordani, et al. 2015). The distance of the forest fragment, the amount of habitats, among other factors, can alter the living assemblages at these environments (Soga et al. 2015; Egerer et al 2017). The number of studies evaluating the influence of the landscape structure on biodiversity and organism dynamics has increased in the last years (e.g. De la Mora et al. 2013; Evans et al. 2016). This is because the way the landscape is structured can facilitate, or halt, the permanency and movement of individuals between the patches of a given area (Frizzo & Vasconcelos 2013; Mortelliti, et al. 2014).

However, variables of a more restrict spatial scale, such as the local ones, also have great influence on the organisms (Audino, et al. 2017; Marín, et al. 2016; Queiroz, et al. 2013). The reason is that many organisms are sensitive to slight variations of conditions, such as the canopy openness that can influence the sun radiation on the ground and alterations of air temperature and humidity (Perfecto & Vandermeer 1996; Fitzpatrick et al., 2014), which may directly affect the activity of many organisms. The agriculture expansion has been highlighted as one the main causes of biodiversity loss and forest fragmentation (Philpott et al. 2008). When a native forest area is replaced by an agriculture, which are more homogenous, there is a simplification of the environment and of the organisms living in it (Moorhead et al., 2010; Fahrig et al. 2011; Solar et al., 2016), reducing the amount and quality of resources available. Moreover, this environmental simplification may be responsible for many microclimatic alterations, changing essential conditions for the survival of some organisms (Perfecto & Vandermeer 1996; Fitzpatrick et al., 2014). The vegetation mosaics that are present in landscape, as well as the quality of these habitats, may interfere on the flux of organisms (Fahrig, 2007), and even cause local extinction. Thus, it is important to consider variables that might influence organisms either in a local scale, or in a broader scale, considering the surrounding matrix.

Aiming to evaluate environmental changes, ants has been largely used as a study model (Anjos, et al. 2017, Solar et al., 2016). This is due to the cosmopolitan characteristic of this group, with large geographical distribution, easy sampling methods, and reliability with their response to several environmental impacts (Philpott, et al. 2010; Ribas, et al. 2012, Rabello, et al. 2015). Additionally, ants respond to both local variables, such as canopy cover of primary forests, heterogeneity and dry weight of leaf litter, biomass above ground (Queiroz, et al. 2013; Solar, et al. 2016), and to landscape variables, for example distance of the matrix and the fragment, and the components of the landscape (De la Mora et al. 2013; García-Martínez et al. 2017).

Therefore, the aim of this work was to compare the variables landscape and vegetation (local) scales, to verify which of them has the biggest effect on the ant assemblage (species richness and composition). Moreover, we aimed to evaluate whether the effects of these variables would be the same for forest areas and coffee monocultures. We hypothesized that at forest fragments, the local variables are more important to the ant assemblage, due to the greater structural complexity of these habitats (greater quantity and quality of resources). In contrast, we hypothesized that at coffee monocultures the landscape variables would be more important to ant assemblage, since the amount of forest habitat in the landscape may work as a source of species to the coffee crop.

Methods

- Study Area

The work was carried out at the counties of Machado and Poço Fundo, Minas Gerais state, Brazil. This region has a dry season during winter, and rainy season during summer. The maximum temperature is 28°C and the minimum is 16°C, with annual precipitation of approximately 1430 mm (Inmet, 2017). The region is characterized by old settlements and conversion of land to coffee crops. The southern region of Minas Gerais state is one of the biggest coffee producers in Brazil (Conab, 2014). The samples were taken in six forest fragments and six coffee monocultures that were grown in full sun, and these plots occupied about 14 to 32% and 12 to 68% of the evaluated area, respectively (Table S2). The forest fragments were always adjacent to the coffee crops and are classified as a transition between Cerrado and Mata Atlântica biomes.

- Ant sampling

We collected the ants at the twelve areas (six forest fragments and six coffee monocultures) using pitfall traps. At each area we established a transect to set up 10 pitfall traps at each 20m. Each trap stayed in the field for 48 h, containing a solution of 200 ml of water, salt (0.4%), and detergent (0.6%) (Canedo-Júnior, et al. 2016). After this period, the traps were collected and conserved in alcohol 70%. The material was sorted and the ants pinned at the Laboratório de Ecologia de Formigas, in Universidade Federal de Lavras. The ants were then classified into genera based on the key of Baccaro et al. (2015), and we used morphospecies to get the lowest level of identification. Posteriorly, the identification was confirmed by the specialist Rodrigo Feitosa, from Universidade Federal do Paraná (UFPR).

- Environmental variables

We selected canopy openness as local variable for this work. This choice was based on previous works carried out at the same area, where the canopy openness was the only variable affecting the ant assemblage (Angotti et al. In preparation – chapter 1 of this thesis). Thus, canopy openness is a good explanatory variable for the sampled areas and would work to the comparisons between local and landscape variables. We estimated the percentage of canopy openness using hemispherical images, taken with a camera attached to fisheye lens, on the ground level at each sampling point. These images were analyzed on the software Gap Light Analyzer (GLA). Then, we used the mean canopy openness by transect, and used this value as our local variable.

The selected landscapes were defined stablishing a buffer around the central pitfall trap in the transect, at both forest and coffee habitats. The buffers had 300 m radius (Figure S3) based on the foraging area of ants (Traniello 1989). We made the mapping of forest fragments and coffee crops with manual vectorization on a scale of 1:4. We did the visual interpretation of high resolution images available on Google Earth, using the software QGis 2.18. Then, we used the amount of forest cover and the amount of coffee growing area as our landscape variables.

- Statistical analyzes

First, we did the correlation test among landscape variables (percentage of coffee growing area and forest cover) and local (canopy openness), using as central point the forest or the crop to avoid collinearity among them. We used the Spearman correlation for comparisons between variables with non-normal distribution, and Pearson correlation between those with normal distribution. We considered the variables correlated when the correlation index was higher than 0.70. When the forest fragment was used as central point, no variables were correlated: percentage of coffee and forest (Pearson 0.20), percentage of coffee and canopy openness (Pearson -0.31), percentage of forest and canopy openness (Pearson -0.24). In the same way, when we considered the coffee monoculture as the central point, no variables were correlated: percentage of coffee and forest (Spearman 0.08), percentage of coffee and canopy openness (Spearman -0.54), percentage of forest and canopy openness (Pearson -0.32).

Second, we did a hierarchical partitioning to verify the influence of environmental variables (landscape and local) on ant species richness in forest areas and coffee crops. This analysis was made on R software 3.2.3 version (R Core Team 2015).

Finally, to verify which variables (landscape or local) would be more related to the change of species composition in forest and coffee areas, we did a linear model based on distance (DistLM) using the Jaccard index. This analysis was made on Primer software 6.0 version PERMANOVA+.

Results

We collected a total of 97 ant species. 79 of these species occurred in forest fragments, and 51 in coffee crops (Table S1, support material).

Considering the forest fragment as the central point, we obtained the following variation for landscape occupancy by each variable: percentage of coffee crop of 13 to 68% and forest cover of 14 to 32% (Table S2, support material). On the other hand, when we considered the coffee crop as the central points, the percentage of coffee area varied between 13 to 52%, and the percentage of forest cover 16 to 30% (Table S2, support material). As for the canopy openness, the variation was between 9 to 24% in forest fragments, and 23 to 66% in coffee crops.

For both forest and coffee areas, there was no influence of any landscape variables in ant diversity (Figure 1). Similarly, there was no influence of local variables on ant species richness (Figure 1).

Regarding the change on ant species composition, we found that, for forest fragments, both the percentage of coffee and canopy openness were related to this change. The percentage of coffee was the most important variable for ant species composition, responsible for 27% of this explanation (Table 1). Considering the coffee crop as the central point of the study, only the canopy openness was significant, responsible for 29% of the change on ant species composition (Table 1).

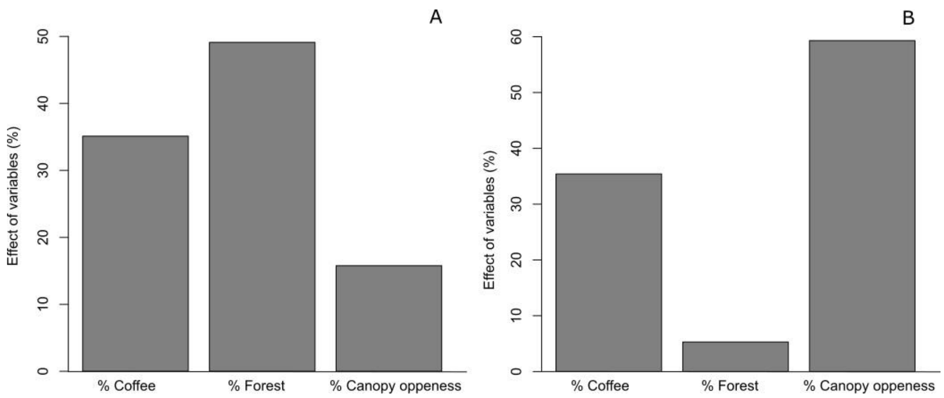

Figure 1. Hierarchical partitioning to evaluate the influence of landscape variables (percentage of coffee crop and percentage of forest cover) and local variable (canopy openness) on ant species richness, considering: (A) forest fragment or (B) coffee monoculture as central point. Figure 1A: Percentage of coffee Z=0.47, p>0.05; Percentage of forest Z=1.24, p>0.05; Canopy openness Z=-0.36, p>0.05. Figure 1B: Percentage of coffee Z=0.29, p>0.05; Percentage of forest Z=-0.82, p>0.05; Canopy openness Z=1.15, p>0.05.

Table 1. Influence of landscape and local variables on ant species composition considering the forest fragment or the coffee crop as central point for evaluation. Significance value ($p<0.05$). Bold font indicates variables that influence species composition. Percentage of coffee = % Coffee; Percentage of forest = % Forest.

Central Point	Environmental Variable	Pseudo-F	p	Percentage of explanation
Forest	**% Coffee**	**1.47**	**0.02**	**0.27**
	% Forest	0.84	0.84	0.17
	Canopy Openness	**0.86**	**0.04**	**0.17**
Coffee	% Coffee	1.27	0.29	0.24
	% Forest	0.86	0.59	0.18
	Canopy Openness	**1.64**	**0.05**	**0.29**

Discussion

We evaluated the influence of environmental variables at landscape and local scales on the ant assemblage. Moreover, we tested how these variables would influence the ant assemblage in two different landscapes, forest fragments and coffee monocultures. Our results indicate that no variable was related to ant species richness, independently of the sampled environment. However, the percentage of coffee crops in the landscape, and the canopy openness were responsible for the changing on ant species composition.

Ant species richness was not influenced by any variable tested in this work, at landscape or local scale, and independently of the habitat (forest fragment or coffee crop). Other works have already reported that species richness by itself has a low power of explanation on environmental changes and impacts (Angotti et al. in preparation – chapter 1 of this thesis; Ribas et al. 2012; Gomes et al. 2014). This has been attributed to the fact that many ant species are generalists, and when a habitat lose some sensitive species, they are swapped by other generalist and opportunistic species (Ribas et al. 2012). Therefore, our study corroborates the choice of many researchers of working with different parameters of assemblages, such as species composition that are more sensitive to environmental changes.

Different from what we expected, when we evaluated the forest fragments, we observed that the percentage of coffee in the landscape was the most important variable for the changing on ant species composition, followed by the canopy openness. Possibly, the amount of coffee in the landscape contributed the most for this change because the monoculture occupies an extensive area in the region, and in the sampled landscapes. The variation on the amount of coffee neighboring the forest fragments may increase the occurrence of generalist and opportunistic species in the forest. It was already reported that coffee monocultures can harbour distinct assemblages, besides of loss of species, trees, and birds due to the negative effects that the culture brings to the environment, such as the homogenization of the landscape (Philpott et al., 2008). De la Mora et al. (2013) show that ants may respond to both landscape and local scales, but mostly the groups responded strongly to local variables. The local variable canopy openness was the second most important variable for the changing on ant species composition in the forest habitats. As we expected, canopy openness can be related to the amount and quality of resources in the environment. This is due to the variation on the canopy and the height of trees in the forest, which may influence the solar radiation that reaches the ground, and consequently affect the ant assemblage that inhibits and forages in these locals.

Again, different from what we expected, any landscape variable was related to ant species composition when we considered the coffee crops. It is likely that the absence of relation is due to the fact that these habitats are more homogeneous and simplified (Philpott et al. 2008), and may present the occurrence of more generalist species. Although we predicted that forest areas could work as a source of species, those sensitive and specialist species may not be able to occur in the monocultures. We can infer this looking at the relation between the canopy openness and the change on ant assemblage composition in the forest areas, which shows that local variations are more important to ant species that colonize coffee crops, and that the variation of shadowing could allow a different colonization pattern of ants. Another study at the same sampled area found a similar result, showing that canopy openness influences the composition of epigeic ants (Angotti et al. in prep – chapter 1 of this thesis). Thus, this variation of the assemblage composition may occur due to differences on solar radiation, which is caused by different canopy openness. Consequently, microclimatic conditions above the ground may also differ. There are studies reporting how alterations of temperature and humidity can favor distinct ant assemblages (Perfecto & Vandermeer 1996; Fitzpatrick et al. 2014), changing the species composition according to the local conditions (Frizzo & Vasconcelos 2013). This reinforces that ants are extremely sensitive to slight local variations (Frizzo & Vasconcelos 2013).

We found that, although the habitats (forest fragments and coffee crops) are close to each other, there is a difference on the influence of environmental variables at landscape and local scale on ant assemblages. This shows us that organisms that live in the forest or in coffee crops, respond differently, due to their different requirements. Moreover, the management of the agricultural area may lead to landscape modifications, and be responsible for the change on ant assemblages and other organisms (Inclan et al. 2015; Velmourougane, 2016, Masoni, et al. 2017). The conservation and preservation of forest areas are important because they can guarantee the survival of different group of species that are responsible for the functioning of the environment. It has been related that even few spaced trees can guarantee the occurrence of some ant species that are not common in more simplified environments (Frizzo & Vasconcelos, 2013), as well as to provide a different microclimatic habitat that may attract several other organisms (Siqueira, et al. 2017).

Therefore, we observed the importance of carrying out the experiments in two environments of distinct characteristics, and verifying the influence of variables at different spatial scales on the ant assemblage. The response of ant assemblages may vary depending on the studied environment, forest fragment or coffee monoculture, and it seems plausible to evaluate more than one type of habitat whenever this is possible. Choosing only local variables would have made us to conclude that canopy openness was the responsible for the change on ant assemblage composition, both in forest fragments and coffee crops. However, when we added variables of another spatial scale, we found that these new variables had an influence even greater than the local ones on ant assemblages in forest habitats. This exemplifies the sensitivity of ants to respond to local variables as much as to landscape ones. Thus, we conclude that we must think of diversified conservation strategies for each type of environment, and that the variables to be chosen for evaluations of environments should consider different spatial scales.

Acknowledgments

We would like to thank Ernesto O. Canedo-Junior, Carolina S. Oliveira, Július S. Cerqueira, Mariana C, S. Carvalho, and Rafael G. Cuissi for helping with field work. To the producers from the counties of Machado and Poço Fundo for providing access to their crops, and to COOPFAM for support and intermediate the work with the producers. To André L. Tavares for discussions and help with landscape analyses. To FAPEMIG (CRA PPM 00243/14) for funding part of this work, and to the funding agencies for the scholarships. To the authors MAA, CJL, CARFB, and RGS were granted scholarships from CAPES.

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Support material

S1. Ants collected with pitfall traps in coffee monocultures and forest fragments (transition between the Cerrado and Mata Atlântica biomes) at the counties of Machado and Poço Fundo, southern Minas Gerais state, Brazil. Letter “X” indicates the presence of a species at each habitat.

Species richness	Coffee plantations	Forest fragments
Acromyrmex coronatus		X
Apteroestigma sp.2	X	X
Apteroestigma sp.3	X	X
Atta sexdens	X	X
Basiceros disciger		X
Brachymyrmex brasiliensis		X
Brachymyrmex sp.1	X	X
Brachymyrmex sp.2		X
Camponotus ager	X	X
Camponotus atriceps		X
Camponotus cingulatus		X
Camponotus crassus	X	X
Camponotus melanoticus	X	X
Camponotus renggeri	X	X
Camponotus sericeiventris		X
Camponotus sp.12		X
Camponotus sp.17		X

S1 (continuation). Ants collected with pitfall traps in coffee monocultures and forest fragments (transition between the Cerrado and Mata Atlântica biomes) at the counties of Machado and Poço Fundo, southern Minas Gerais state, Brazil. Letter "X" indicates the presence of a species at each habitat.

Species richness	Coffee plantations	Forest fragments
Camponotus sp.5	X	X
Camponotus vittatus		X
Crematogaster aff. *acuta*		X
Crematogaster aff. *evallans*	X	
Crematogaster sp.3		X
Cyphomyrmex rimosus	X	X
Dorymyrmex sp.1	X	
Ectatomma brunneum	X	
Ectatomma edentatum	X	X
Gnamptogenys lavra	X	X
Gnamptogenys sp.2	X	
Gnamptogenys striatula	X	X
Gnamptogenys sulcata	X	
Hylomyrma reitteri		X
Hypoponera sp.1	X	X
Hypoponera sp.2		X
Hypoponera sp.4		X
Labidus coecus		X

S1 (continuation). Ants collected with pitfall traps in coffee monocultures and forest fragments (transition between the Cerrado and Mata Atlântica biomes) at the counties of Machado and Poço Fundo, southern Minas Gerais state, Brazil. Letter "X" indicates the presence of a species at each habitat.

Species richness	Coffee plantations	Forest fragments
Leptogenys sp.1		X
Linepithema cerradense	X	
Linepithema gallardoi	X	X
Linepithema iniquum		X
Linepithema leucomelas		X
Linepithema micans	X	X
Linepithema neotropicum	X	X
Mycetarotes carinatus	X	
Mycetophylax strigatus		X
Mycocepurus goeldii	X	
Myrmelachista gallicola		X
Neoponera verenae	X	X
Nylanderia sp.1		X
Octostruma stenognatha		X
Odontomachus chelifer	X	X
Odontomachus meinerti		X
Oxyepoecus reticulatus		X
Pachycondyla harpax		X

S1 (continuation). Ants collected with pitfall traps in coffee monocultures and forest fragments (transition between the Cerrado and Mata Atlântica biomes) at the counties of Machado and Poço Fundo, southern Minas Gerais state, Brazil. Letter “X” indicates the presence of a species at each habitat.

Species richness	Coffee plantations	Forest fragments
Pachycondyla striata	X	X
Pheidole aff. *radoszkowskii*	X	
Pheidole aff. *subarmata*	X	
Pheidole alpinensis		X
Pheidole gertrudae	X	X
Pheidole sp.11	X	
Pheidole sp.12		X
Pheidole sp.14		X
Pheidole sp.15	X	X
Pheidole sp.16	X	X
Pheidole sp.17	X	
Pheidole sp.18		X
Pheidole sp.19	X	X
Pheidole sp.2	X	X
Pheidole sp.20	X	X
Pheidole sp.21	X	X
Pheidole sp.24		X
Pheidole sp.25		X

S1 (continuation). Ants collected with pitfall traps in coffee monocultures and forest fragments (transition between the Cerrado and Mata Atlântica biomes) at the counties of Machado and Poço Fundo, southern Minas Gerais state, Brazil. Letter “X” indicates the presence of a species at each habitat.

Species richness	Coffee plantations	Forest fragments
Pheidole sp.26	X	
Pheidole sp.29		X
Pheidole sp.3	X	X
Pheidole sp.30		X
Pheidole sp.4	X	X
Pheidole sp.8		X
Pheidole sp.9	X	
Pogonomyrmex naegellii	X	
Pseudomyrmex schuppi		X
Pseudomyrmex termitarius	X	
Solenopsis gr. *geminata* sp		X
Solenopsis invicta	X	X
Solenopsis sp.10		X
Solenopsis sp.11		X
Solenopsis sp.12	X	
Solenopsis sp.2	X	X
Solenopsis sp.3		X
Solenopsis sp.4	X	X

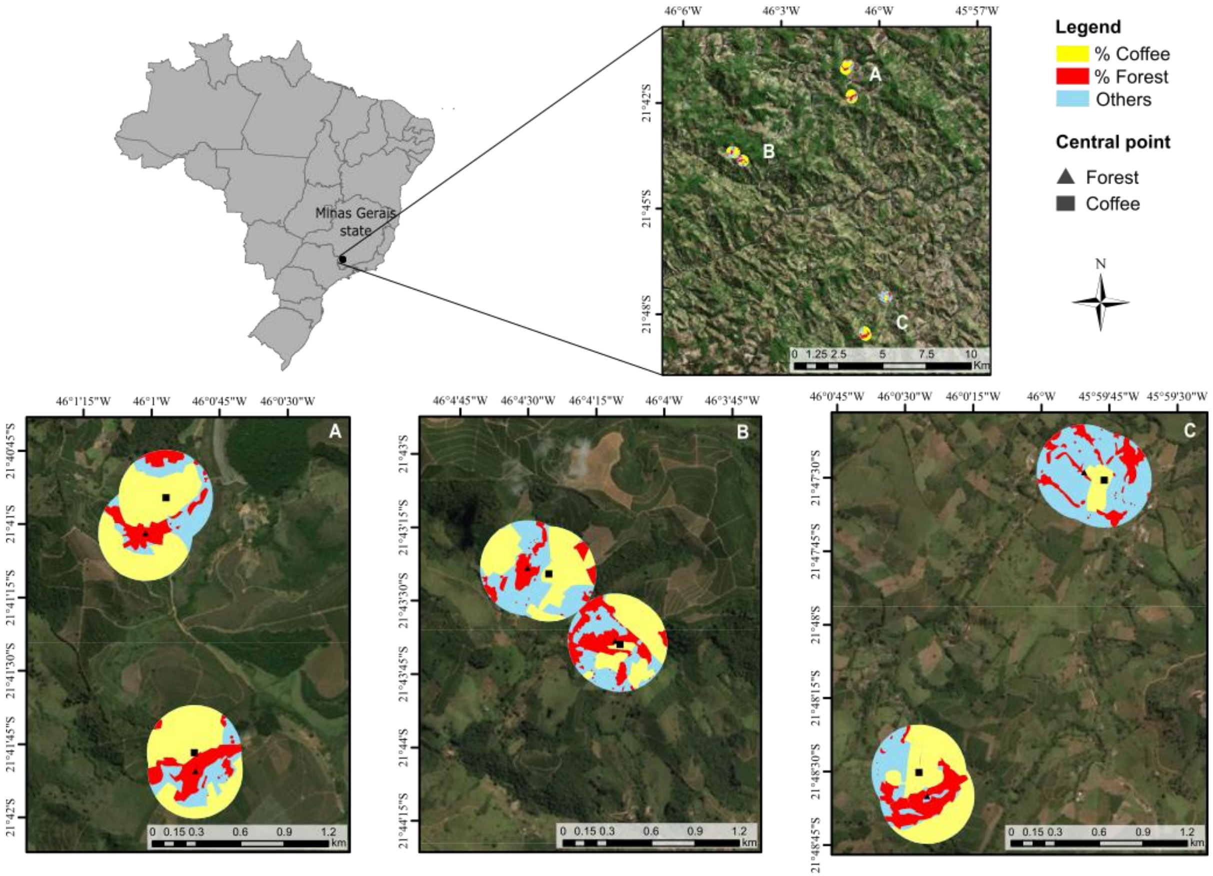
S1 (continuation). Ants collected with pitfall traps in coffee monocultures and forest fragments (transition between the Cerrado and Mata Atlântica biomes) at the counties of Machado and Poço Fundo, southern Minas Gerais state, Brazil. Letter “X” indicates the presence of a species at each habitat.

Species richness	Coffee plantations	Forest fragments
Solenopsis sp.6	X	
Solenopsis sp.7		X
Strumigenys aff. *lousianae*	X	X
Trachymyrmex oetkeri		X
Typhlomyrmex pusillus		X
Wasmannia affinis		X
Wasmannia auropunctata		X
Wasmannia lutzi		X
Species Richness (total)	**51**	**79**

S2. Percentage of occupied area by coffee monocultures (% coffee), and by forest fragments (% forest), from a buffer of 300 m radius with its center at each habitat (forest fragment and coffee monoculture). The percentage of canopy openness (mean value per transect) was also measured at each habitat.

	% coffee	% forest	% canopy openness
Coffee	12.58	17.87	45.44
	41.11	26.98	51.78
	41.77	30.47	42.96
	43.28	16.12	65.87
	50.07	28.20	23.00
	51.92	19.04	24.16
Forest	12.58	16.50	23.96
	39.16	29.89	21.21
	44.25	14.31	19.20
	49.64	27.77	12.20
	53.81	31.62	14.65
	68.13	17.17	8.68

S3. Buffers of 300 m with their central points established in forest areas (triangles), and coffe monocultures (quadrats), at the counties of Machado and Poço Fundo, Minas Gerais State, Brazil.



CONSIDERAÇÕES GERAIS

O objetivo geral da tese foi verificar a influência do monocultivo de café com diferentes tipos de manejo (café orgânico ou convencional) sobre a assembleia e funções ecológicas exercidas pelas formigas e a influência de variáveis da paisagem e locais sobre a assembleia de formigas. Diante disso, primeiramente nós avaliamos a influência do manejo do café e das variáveis ambientais locais sobre a assembleia de formigas e verificamos qual o principal mecanismo responsável pela mudança na composição de espécies. Apesar da riqueza de espécies não sido afetada pelo manejo e variáveis ambientais nós observamos que a composição das espécies foi mais dissimilar entre o café convencional e a floresta, e o mecanismo responsável foi o *turnover*. A abertura de dossel foi relacionada à mudança da composição e explicou 12% da variação, porém nosso resultado indica que a utilização de agrotóxico no manejo convencional pode ter sido um dos fatores responsáveis pelo efeito negativo encontrado na composição das formigas. Nesta primeira parte nós ressaltamos a importância da utilização de técnicas mais naturais e menos agressivas ao ambiente, a fim de minimizar os impactos causados pelos sistemas agrícolas nas assembleias de formigas.

Posteriormente, nós verificamos a influência do manejo e de variáveis ambientais locais sobre as funções ecológicas de remoção de sementes e predação de insetos por formigas, assim como na riqueza e composição das mesmas. Nós verificamos que embora a heterogeneidade de serapilheira e a abertura de dossel tenham sido diferentes entre os sistemas, apenas a abertura de dossel foi relacionada com a composição de formigas predadoras. Assim, a conversão de ambientes naturais para ambientes agrícolas altera a assembleia das formigas que exercem determinadas funções no habitat. Recomendamos que seja avaliada mais de uma função ecológica, sempre que possível, para que possam ser inferidos melhores conclusões a respeito dos impactos ambientais. Além disso, destacamos a importância do manejo das áreas de monocultivo agrícola em relação à abertura de dossel, com o objetivo de promover um ambiente mais complexo e com características mais similares às áreas nativas que suportem assembleias de formigas que realizem funções também mais similares.

Por fim, avaliamos a influência de variáveis de paisagem e local sobre a assembleia de formigas presentes nos monocultivos de café e nas áreas florestais. Mostramos que a resposta da assembleia de formigas depende do ambiente focal de estudo e que as formigas respondem tanto as variáveis de paisagem como locais. Nas área florestais a porcentagem de café na paisagem foi a principal responsável pela mudança na composição de espécies de formigas, seguida pela variável local abertura de dossel. Já nos monocultivos de café somente a variável local abertura de dossel foi importante para a mudança na composição de formigas. Ressaltamos a importância de se estudar em mais de uma escala espacial, devido a diferentes respostas que elas podem nos fornecer e que isso deve ser levado em consideração em planos e estratégias para conservação e manejo de ambientes agrícolas.

Em resumo, mostramos que as formigas respondem aos impactados da conversão de ambientes naturais para monocultivos agrícolas e que esse sistema agrícola (café) pode afetar a composição das assembleias de formigas, e a longo prazo pode afetar negativamente as funções ecológicas exercidas pelas mesmas. O manejo convencional pode suprimir algumas espécies de formigas, alterando mais a assembleia local em relação a fragmentos naturais quando comparado ao manejo orgânico. Além disso, as variáveis na paisagem e locais que compõem o ambiente são importantes para o bom funcionamento do mesmo. Verificar quais são estas variáveis em diferentes ambientes impactados e naturais nos leva a definir estratégias que visem reduzir os impactos causados pelo monocultivo, como estratégias mais similares ao manejo orgânico, bem como a implantação de algumas árvores dentro do cultivo. Essas medidas nos permitiriam criar um micro-habitat mais favorável à assembleia de formigas e provavelmente de outros organismos que poderiam se utilizar deste meio, propiciando um funcionamento ecológico mais similar a ambientes naturais. Demonstramos também que as formigas são bons modelo ecológicos a serem estudados como indicadores de impactos ambientais, tanto relacionado a assembleia como as funções por elas desempenhadas, e elas respondem as variações tanto em escalas de paisagem como local.