



**ANANZA MARA RABELLO**

**REMOÇÃO DE SEMENTES POR FORMIGAS  
COMO BIOINDICAÇÃO DE IMPACTO PELA  
MINERAÇÃO E ESFORÇOS DE  
RECUPERAÇÃO**

**LAVRAS - MG**

**2013**

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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós - Graduação em Ecologia Aplicada, área de concentração Ecologia e Conservação de Recursos Naturais em Ecossistemas Fragmentados e Agrossistemas, para obtenção do título de Mestre.

Orientadora

Dr<sup>a</sup>. Carla Rodrigues Ribas

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Dr. Júlio Neil Cassa Louzada UFLA

Dr<sup>a</sup>. Claudia Bottcher UNICAMP

Dr<sup>a</sup>. Carla Rodrigues Ribas

Orientadora

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**2013**

*À minha família*

**DEDICO**

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*Seremos conhecidos  
Para sempre  
Pelas pegadas que deixamos*

## RESUMO GERAL

As formigas têm sido amplamente usadas como bioindicadoras de impacto pela mineração e sucesso de esforços de recuperação, mas as funções ecológicas desempenhadas por elas como a remoção de sementes ainda são pobremente utilizadas nesse contexto. Diante disso esse estudo avaliou a remoção de sementes por formigas em áreas sobre impacto pela mineração e em processo de recuperação após mineração com diferentes idades e técnicas, durante as estações chuvosa e seca. Foi analisado: riqueza e composição de formigas removedoras de sementes; taxa de remoção; influencia das variáveis ambientais na riqueza, composição e taxa de remoção; e espécies de formigas indicadoras das diferentes áreas em recuperação. As espécies de formigas foram amostradas em áreas impactadas pela mineração e em processo de recuperação com diferentes idades (2 a 10 anos), espécies de plantas exótica e nativa, com dois tipos de capim exótico e em cima de cava e pilha de estéril. Foi encontrado diferença na riqueza de espécies, composição e taxa de remoção entre áreas impactadas pela mineração e controles e também entre os diferentes tipos e idades de recuperação. Porém a sazonalidade teve influencia apenas na composição das formigas entre as áreas de recuperação e as áreas controles. As variáveis ambientais tiveram influencia sobre a riqueza, composição e taxa de remoção na análise das áreas impactadas pela mineração, enquanto que entre as áreas em recuperação essas variáveis influenciaram apenas a composição. Portanto a atividade de mineração ameaça a remoção de sementes pelas formigas, sendo necessária a recuperação eficaz dessas áreas. Mesmo após dez anos de recuperação a riqueza e a composição continuam diferentes das áreas controles. O uso de espécies de planta nativa e recuperação sobre cava são as melhores técnicas de recuperação. E por fim, as formigas e sua função ecológica de remoção de sementes podem ser usadas como bioindicadores de impacto pela mineração e esforços de recuperação após a mineração para analisar e monitorar processos de recuperação.

Palavras-chave: Mineração. Idade de recuperação. Técnicas de recuperação. Estrutura do habitat. Formigas bioindicadoras.

## ABSTRACT

Ants have been widely used as bioindicator of mining impact and success recovery efforts, but the ecological functions played by them like removal seeds still are poorly used in this context. Thereby, this study evaluated the seed removal by ants in mining sites and in recovery process after mining with different ages and techniques, during rainy and dry seasons. It was evaluated: seed-removing ant species richness and composition; rate of seed removal; influence of environmental variables on the richness, composition and rate of seed removal; and ant species indicator of different recovery areas. Ant species were sampled in impacted areas by mining and in recovery process with different ages (2 to 10 years), native and exotic plants species, two types of exotic grasses and upon mine and sterile material. It was found difference on the species richness and composition, and rate of seed removal between impacted areas and controls, and among different recovery techniques and age. But seasonality had influence only on the ant species composition between impacted areas by mining and control areas. Environmental variables had influence on the richness, composition and rate of seed removal on the analysis about impacted areas by mining, while among recovery areas these variables influenced only the composition. Therefore mining activity threatens the seed removal by ants, necessitating efficient recovery of these areas. Even after ten years of recovery, the richness and composition remain different of control areas. The use of native plant species and recovery upon mine are better recovery techniques. Finally, ants and their ecological function of seed removal can be used as bioindicator of mining impact and recovery effort after mining for analyzing and monitoring recovery process.

Palavras-chave: Mining site. Recovery age. Recovery techniques. Habitat structure. Ant bioindicator.

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**PRIMEIRA PARTE**

## 1 INTRODUÇÃO GERAL

Atividades antrópicas provocam impactos biológicos, químicos e físicos em ecossistemas naturais com consequências negativas na estrutura do habitat e na sua biota associada. A expansão dessas atividades causa uma desestruturação das comunidades ecológicas e reduz a integridade dos ecossistemas tornando esse um assunto de extrema importância, principalmente em relação aos efeitos sobre as funções ecológicas desempenhadas pela biodiversidade (MONTROYA et al., 2012). Dentre essas atividades a mineração é uma das mais impactantes, pois altera completamente a paisagem através da remoção total da vegetação e de camadas de solo resultando em uma drástica degradação do solo e da biota (HOLEC, 2005; RIBAS et al., 2012a).

A atividade de mineração no Brasil representa a dicotomia da sociedade brasileira entre as necessidades e desenvolvimento econômico e a conservação da biodiversidade. O Brasil possui um dos maiores patrimônios minerais do mundo, colocando-o como maior produtor e exportador de minérios. Dentre esses, o minério de ferro é o produto que gera maior renda nas exportações brasileiras, sendo que o estado de Minas Gerais é o maior produtor desse minério (48% da produção nacional). Por isso, esse estado apresenta a maior concentração de empresas mineradoras, como exemplo a Vale S.A. considerada a maior empresa de produção de minério de ferro no Brasil (81,7%) e no mundo. Em 2010, a Companhia Vale do Rio Doce produziu 372 milhões de toneladas de minério de ferro, equivalente a 15% da produção mundial (IBRAM, 2011).

Porém, a mineração pode causar poluição do ar, água e solo em ecossistemas próximos às áreas de mineração, fazendo com que essa perturbação seja mais difícil de remediar (DIAS et al., 2012). Esse tipo de impacto tem sido considerado maior que aqueles causados por outras atividades antrópicas como agricultura, corte seletivo e construção de hidroelétricas (PAROTTA &

KNOWLES, 2001). Diante desse cenário, estratégias de recuperação de áreas degradadas pela mineração são requeridas para a conservação da biodiversidade e das funções ecológicas por ela desempenhadas (DOMINGUEZ-HAYDAR; ARMBRECHT, 2011; MONTOYA et al., 2012).

Muitos esforços de recuperação se dedicaram ao estabelecimento de comunidades de plantas, levando em consideração que a fauna das áreas próximas colonizaria tais ambientes em recuperação (BISEVAC; MAJER, 1999). Esse fato não permitiu avanços no aprimoramento de técnicas de recuperação, e conseqüentemente na extrapolação dos resultados de um ecossistema para outro. Entretanto, a partir do ano 2000 ocorreu uma alta produção de estudos, sobre restauração de áreas degradadas pela mineração, que começaram a abordar a fauna em suas avaliações (MAJER, 2009). Um exemplo é o trabalho de FUNK et al. (2008) o qual demonstrou que áreas com espécies de plantas nativas são mais resistentes à invasão e ao fogo e proporcionam um ambiente mais heterogêneo para a biota local. O avanço nas publicações colaborou para a sustentação da ciência da Ecologia da Restauração, que se tornou uma importante ferramenta para a conservação da biodiversidade.

Porém a efetividade da recuperação das áreas degradadas tem sido pobremente estudada, principalmente no que diz respeito a estudos de monitoramento em longo prazo que são importantes para a avaliação do sucesso da recuperação (PAIS; VARANDA, 2010). Invertebrados é o grupo de animais mais comumente usados em estudos sobre recuperação de áreas degradadas, por serem capazes de indicar a qualidade e a complexidade do habitat (HOLEC; FROUZ, 2005). Dentre os invertebrados, as formigas são os organismos preferencialmente utilizados em trabalhos sobre monitoramento de impactos ambientais, incluindo impactos pela mineração (RIBAS et al., 2012b).

O sucesso das formigas como organismos bioindicadores é devido a sua ampla distribuição, alta abundância, facilidade de amostragem, sensibilidade e

resposta rápida às mudanças ambientais, e são taxonômica e ecologicamente bem conhecidas (PHILPOTT et al., 2010; RIBAS et al., 2012c). Em florestas tropicais, sazonalidade, heterogeneidade do habitat e disponibilidade de recursos são fatores que afetam a riqueza e composição das espécies de formigas (RIBAS et al., 2003; NEVES et al., 2010; SCHMIDT et al., 2013). Todos esses fatores são relevantes em estudos de ambientes com diferentes tempos de recuperação, os quais apresentam complexidade de habitat distinta gerando mudanças na assembleia de formigas (OTTONETTI et al., 2006).

Além de serem bons bioindicadores, as formigas desempenham funções ecológicas nos ecossistemas como dispersão de sementes (DOMINGUEZ-HAYDAR; ARMBRECHT, 2011) e ciclagem de nutrientes (SOUSA-SOUTO et al., 2007). Apesar do uso das formigas como bioindicadoras de impacto ambiental pela mineração ter crescido nos últimos anos, ainda poucos estudos incluem as funções ecológicas desempenhas por esse táxon nas suas avaliações, principalmente relacionadas ao impacto da mineração (DOMINGUEZ-HAYDAR; ARMBRECHT, 2011).

A dispersão de sementes por formigas (Mirmecocoria) é um mutualismo no qual as plantas têm suas sementes dispersadas e as formigas adquirem alimento através de um apêndice nutritivo da semente chamado de elaiossomo (BEAUMONT et al., 2011). Essa interação pode ser negativamente afetada por atividades antrópicas, como a mineração, capazes de provocar mudanças na riqueza e composição das espécies de formigas removedoras de sementes (GRIMBACHER; HUGHES, 2002). A remoção de sementes é particularmente importante dentro do contexto de áreas degradadas, já que as formigas podem remover uma grande quantidade de sementes favorecendo o processo de reflorestamento (SUAZO et al., 2013). Além disso, a umidade, matéria orgânica e aeração do solo proporcionados pelo ninho das formigas influenciam no sucesso da germinação das plantas (HENAO-GALLEGO et al., 2012).

Em condições ideais, a remoção de sementes por formigas pode acelerar o processo de recuperação de áreas degradadas e diminuir custos. Além disso, a mirmecocoria pode tornar os esforços de recuperação auto-sustentáveis por agir na distribuição das sementes pelo habitat e favorecer a germinação de novos indivíduos de plantas (LOMOV et al., 2009). Além de gerar grande benefício para as plantas por evitar que essas sementes sejam predadas por roedores e outros granívoros (ANDERSEN; MORRISON, 1998). Com isso, a mirmecocoria se torna uma poderosa ferramenta para indicar mudanças funcionais e estruturais na flora e na fauna dos ecossistemas, assim como para compreendermos a trajetória e o sucesso de processos de restauração, consolidando sua utilização como métrica em programas de monitoramento.

Como relatado acima, a mineração é uma atividade antrópica que causa mudanças drásticas no habitat e em sua biota associada. Tal atividade afeta a assembleia de formigas e pode prejudicar o desempenho da sua função ecológica de remoção de sementes (mirmecocoria). Dentro desse contexto, esta dissertação contém dois capítulos em forma de manuscritos. O primeiro capítulo trata a questão da utilização da mirmecocoria como métrica de bioindicação do impacto causado pela mineração, tendo como objetivos comparar a riqueza, composição e taxas de remoção pelas formigas removedoras de sementes, assim como a influência da complexidade da vegetação nesses parâmetros entre áreas impactadas (mineradas) e não-impactadas (florestas naturais). O segundo capítulo discute o processo de recuperação de áreas degradadas pela mineração com diferentes tempos e tipos de recuperação, avaliando a riqueza, composição e taxas de remoção de sementes pelas formigas. Além disso, analisa também a influência das variáveis ambientais sobre a composição de espécies de formigas removedoras de sementes, e aponta espécies dessas formigas que possam agir como indicadoras do tempo e tipos de recuperação.

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**SEGUNDA PARTE**

**ARTIGOS**

*For Ecological Indicators*

**ARTIGO 1**

**SEED REMOVAL BY ANTS: BIOINDICATION OF MINING IMPACT**

Rabello, A.M.<sup>1</sup>, Cuissi, R.G.<sup>2</sup>, Queiroz, A.C.M.<sup>1</sup>, Lasmar, C.<sup>2</sup>, Ribas, C.R.<sup>2</sup>

<sup>1</sup>Programa de Pós – Graduação em Ecologia Aplicada, Departamento de Biologia, Setor de Ecologia, Universidade Federal de Lavras, Caixa Postal 3037, CEP 37.200-000, Lavras – MG, Brasil.

<sup>2</sup>Laboratório de Ecologia de Formigas, Departamento de Biologia, Setor de Ecologia, Universidade Federal de Lavras, Lavras – MG, Brasil.

## ABSTRACT

Myrmecochory (seed removal by ants) is major factor increasing plant recruitment, but yet there is a little understanding of how mining activities may influence this interaction. This study sought to evaluate if mining impact affect the seed-removing ant species. We analyzed: (1) richness and composition of seed removing ants, (2) rates of seed removal by ants and if (3) there is influence of the complexity of vegetation structure on the richness and composition of seed removing ants, and on rates of seed removal by ants. We selected four impacted areas by mining and four non-impacted areas (natural forest) at Nova Lima city, Minas Gerais, Brasil. Environmental variables measured were: canopy cover, understory, weight and diversity of litter, number, density, height and diameter of trees. We also measured soil temperature and humidity during the sampling. Using artificial fruits for attracting ants species, we collected 19 seed-removing ant species. We found differences on the seed-removing ant species richness, composition and rate of seed removal by ants between impacted and non-impacted areas. Only one ant species belonging to *Nylanderia* was observed removing seed in impacted areas. Seasonality, temperature and humidity did not have influence on the seed-removing ant species and its seed removal. The opposite occurred with environmental variables which had influence on the seed-removing ant species richness, composition and rates of seed removal. We concluded that mining impacts threatens ecosystems disrupting interactions like plant-ant. Moreover, the myrmecochory should be used as a tool for bioindication in order to monitor degraded habitats, associated to physical structure of environments.

Keywords: Mining. Myrmecochory. Habitat structure. Disruption interaction. Bioindication.

## INTRODUCTION

Mining activities are responsible for drastic changes in the landscape, particularly through complete deforestation and removal of topsoil. Other impacts from mining activity involve loss of biotic resources and pollution of the air, water and soil (Parrotta & Knowles 2001). These impacts have negative consequences in the environment around the mining site, threatening the integrity of nearby ecosystems such as rivers, lakes and natural forests, which can be more difficult to detect (Dias et al. 2012).

It is possible to analyze the degree of the impacts through the use of bioindicators, which provide information about environmental conditions and changes. In this way, these organisms are used for monitoring disturbed environments helping in the diagnosis of ecological problems (Mc Geoch 1998, Niemi & McDonald 2004). The use of bioindicators is also related to low cost, ease of sampling, sensibility to environmental conditions, ecological importance and rapid response to environmental changes (Gardner 2010).

Ants have been used as a powerful tool in studies about habitat impacts, because they attend the requirements of a good bioindicator (Philpott et al. 2010). These studies include disturbances like fire, simplification of landscape, deforestation and mining (Ribas et al. 2007, Philpott et al. 2010; Escobar-Ramírez et al. 2012). Moreover, they are important for play ecological functions as nutrient cycling (Sousa-Souto et al. 2007) and seed dispersal (Dominguez-Haydar & Armbrecht 2011) which are essential to a good performance of ecosystems.

Seed dispersal by ants (myrmecochory) is a primordial process in areas with poor soils, because it is the main way of plant propagation (Andersen & Morrison 1998). The seed removal by ants increases the probability of

germination and establishment of seedlings mainly in impacted areas, which do not have vertebrate dispersers (Christianini & Oliveira 2010). However few studies analyzed the relationship between the impacts caused by mining on ecological functions played by ants, as seed removal (Majer 1985, Andersen & Morrison 1998, Lomov et al. 2009, Dominguez-Haydar & Armbrecht 2011).

Some studies show that increasing the complexity of the vegetation structure may positively affect the ant assemblage (Majer et al. 1984, Majer & Nichols 1998, Ribas et al. 2003), and this reflects on the seed removing ants (Sorrells & Warren 2011). This increase of the complexity leads to an increase in the heterogeneity of the habitat and consequently in the conditions and availability of resources (Graham et al. 2009). Because of this, it is relevant consider that changes in ant assemblages can be related to changes in environmental variables, improving the use of the ants as bioindicator organisms (Schmidt et al. 2013).

The present study aimed to evaluate the seed removal by ants as a tool for bioindication of ecological functions in impacted areas by mining. We sought to compare: (1) richness and composition of seed removing ants, (2) rates of seed removal by ants and (3) influence of the complexity of vegetation structure on the richness and composition of seed removing ants, and on rates of seed removal by ants; between natural forests and impacted areas by mining.

## **METHODS**

### **Study site**

The study was carried out in the Nova Lima city at Minas Gerais state, southeast Brazil. Native vegetation is classified as a transition area of Atlantic Forest and Cerrado (savanna). The region has average altitude of 1400m, annual average temperature of 21.1°C, and local climate with dry winters (April to September) and rainy summers (October to March) (IBGE 2012). The company Vale S.A. was installed with goal to extract iron ore in this region. The field work was conducted on Tamanduá (S 20°5'968" e W 43°57'64") and Capão Xavier (S 20°2'770" e W 43°59'16") mines in February/March and July 2012, in four impacted areas by mining and in four natural forests (hereafter named non-impacted areas) (Fig.1). Non-impacted areas contain "Campo sujo" vegetation and the impacted areas contained the same type of vegetation before impact by mining. Inside each area, we established one transect of 200m containing five sampling points, 40m distant from each other, for sampling seed-removing ants and environmental variables.

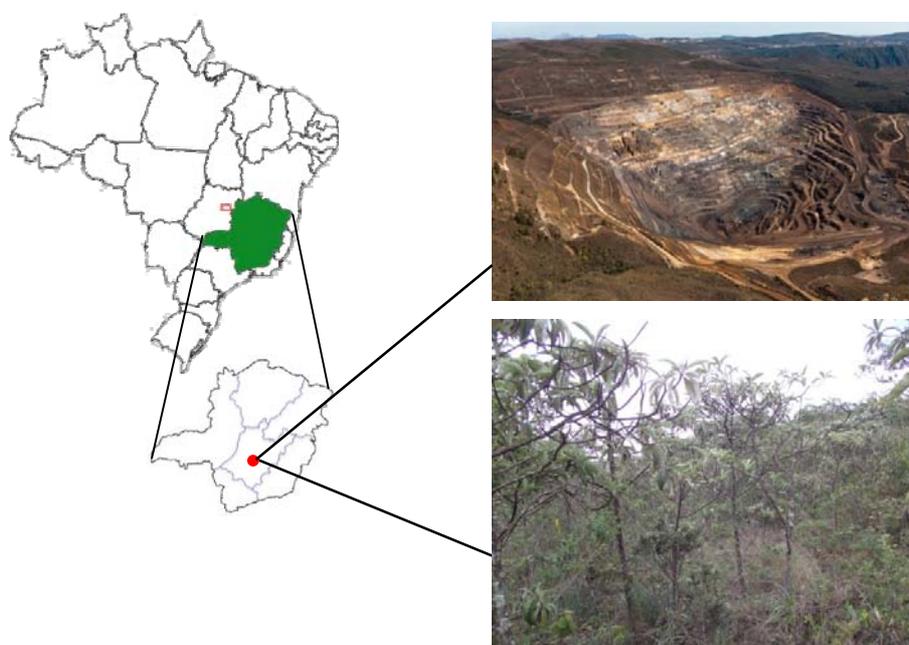


Figure 1 Location of the study site on mining sites of Vale S.A., at Nova Lima city, Minas Gerais state, southeast Brazil. (A) Aerial view of Tamanduá mine and (B) Aspect of a natural forest (non-impacted area).

### Sampling ants

In each sampling point we offered on the ground 50 artificial lipid-rich fruits (based in Raimundo et al. 2004) protected from vertebrate predation by a wire cage (1.5cm mesh). The artificial fruits contained one white fleshy part representing the elaiosome (part of natural fruit attractive for ants) and the “seed”. The fleshy part consisted of vegetal fat (75%), fructose (4.8%), sucrose (0.5%), glucose (4.7%), casein (7%), calcium carbonate (3%), and maltodextrin (5%), and was developed in the Laboratory of Chemical, Biochemical and Analyses of Food (Food Engineering Department, Federal University of Lavras,

Brazil). Representing the “seed” we used orange beads of 0.03g and 2mm diameter, which were attached to fleshy part. Thus our artificial fruits (hereafter fruits) reproduced natural fruits with a single seed covered by an elaiosome, with total weight of 0.2g and 5.5mm diameter. These characteristics include our fruits in small and medium categories proposed by Pizo and Oliveira (2001).

Fruits were set at 09:00h and checked for 15 minutes in each hour until 16:00h (maximum time allowed by company). In each observation, we collected and recorded the attracted ant species which removed the fruit over distances greater than 30cm of the original local in both season (dry and rainy) (Christianini & Oliveira 2010). During the same observations we recorded how many fruits had been removed. After 24 hours that the experiment was installed, we recorded the number of fruits removed for data about rates removal. These rates were ranged between 0 to 100%. Ants collected were identified in the Laboratory of Ant Ecology at Federal University of Lavras, Brazil. To identify genera we used the keys in Palacio & Fernández (2003) and Bolton (1994), and to separate in morphospecies we compared with specimens in the reference collection of the same Laboratory.

### **Environmental variables**

For vegetation structure we measured, in dry and rainy season, at each sampling point in all areas, canopy cover (CAC), understory (UND), weight (WL) and diversity (DL) of litter, number (NT), density (DT), height (HT) and diameter (DIT) of all trees with circumference at breast height (cbh) > 5cm. Soil temperature and humidity were measured every hour during observations of seed removing ants (09:00 to 16:00h) with a thermohygrometer.

Around each sampling point we delimited a quadrant of 6 x 6m and counted the number and height of different morphospecies of individuals of

trees. The litter samples were obtained at each sampling point in a delimited square of 50 x 50cm. Its weight was determined with a precision balance, and its diversity counting the number of different leaves, branches and sticks.

The canopy cover was estimated with digital images using fish-eye lens attached to a camera positioned at a height 1.5m, and analyzed on the software Gap Light Analyser 2.0 (GLA). For measuring the understory, we photographed four points, in different directions, around each sampling point using white cloth of 100 x 100cm. The white cloth was 3m away from the sampling point and 1m above ground level. We calculated the density of understory using the “Global Analysis” on the software Sidelook (Nobis 2005).

### **Data Analysis**

We used generalized linear models (GLM), with Poisson distribution, to determine whether the impact caused by mining and the season (explanatory variables) affect the species richness of seed-removing ants. The seed-removing ant species richness was calculated through the number of ants species by transect in each area. We also used GLM to evaluate the influence of the same explanatory variables on rates of seed removal, with binomial errors distribution. These analyses were performed in the R 2.14 statistical software (R development Core Team 2011).

To verify whether the composition of seed-removing ants changes between impacted and non-impacted areas and between seasons we carried out a non-metric multidimensional scaling (NMDS) based on Jaccard index, which uses species presence/absence data. The statistical significance of NMDS was verified by one-way ANOSIM performed with 999 permutations, which also uses Jaccard index to indicate the dissimilarity among groups. The program used was Primer v6 (Clark & Gorley 2006).

To determine the influence of environmental variables on species richness of seed-removing ants and on rates of seed removal, we carried out hierarchical partitioning which shows independent effects of eight environmental variables (CAC, UND, WL, DL, NT, DT, HT, DIT). Hierarchical partitioning consists in a multiple regression technique in which all possible linear models are used to verify the most likely causal factors, providing a measure of the effect of other variables (Chevan & Sutherland 1991; Mac Nally 2000). Analysis included Poisson errors, with the significance of independent effects obtained by using 500 randomizations, on the statistical program R (R development Core Team 2011). Data about seed-removing ant species between non impacted and impacted areas were correlated with humidity and temperature using Pearson correlation in Past program (Hammer et al. 2001).

The evaluation of the influence of environmental variables in the seed-removing ant composition between non-impacted and impacted areas was performed using distance-based linear modeling (DISTLM) with Jaccard index. Analyses were performed on the program Primer Permanova+ v6 (Anderson et al. 2008).

## RESULTS

We recorded 19 seed-removing ant species belonging to five subfamilies and nine genera (see Table 1). Myrmicinae was the subfamily with the highest number of species (12), followed by Dolichoderinae, Ectatomminae and Formicinae (two each), and Ponerinae (one). The richest genera were *Pheidole* with nine species (47.4% of the total). *Nylanderia* sp1 was the only and exclusive species found in mining impacted areas. All environmental variables differed between non-impacted and impacted areas. The same variables also differed between seasons (dry and rainy) only in non-impacted areas (Table 2).

We found greater seed removing ant species richness in non-impacted (NI) than in impacted (I) one ( $\chi^2 = 19.64$ ;  $df = 1$ ;  $p < 0.0001$ ) but not for the effect of season on species richness ( $\chi^2 = 17.02$ ;  $df = 1$ ;  $p = 0.1$ ) (Fig.2). The effect of interaction between impact and season on the seed-removing ant species richness was not significant ( $\chi^2 = 15.99$ ;  $df = 1$ ;  $p = 0.3$ ). The rate of seed removal in non-impacted was also greater than impacted areas, showing the effect of mining impact on this variable ( $F = 57.02$ ;  $p < 0.0001$ ). We did not detect any influence of the season on the rate of seed removal ( $F = 1.97$ ;  $p < 0.16$ ) (Fig. 3). However, evaluating the effect of impact jointly with the season we detected a significant difference in removal rates ( $F = 6.45$ ;  $p < 0.01$ ).

It was not possible to verify if there is difference on the composition of seed- removing ant species between impacted and non-impacted areas because in impacted areas we recorded only one species in both seasons. Thus we analyzed the difference on the composition of seed-removing ant between seasons in non-impacted areas, and this composition is similar ( $R = 0.26$ ;  $p = 0.7$ ) (Fig.4).

Impacted areas did not contain trees, litter and the canopy cover did not vary, always showing 100% opening. Therefore we were not able to evaluate the effect of environmental variables on seed-removing ant species richness and on rates of seed removal in these areas. But in the hierarchical partitioning analyzes carried out in non-impacted areas, any environmental variable affected significantly the seed-removing ant species richness in any season ( $p > 0.05$ ). On the other hand, understory (UND) positively affects ( $p < 0.05$ ,  $Z = 2.82$ ) the rate of seed removal during the rainy season (Fig.5a). During dry season, only diameter of trees (DIT) showed a positive effect ( $p < 0.05$ ,  $Z = 4.14$ ) on the rate of seed removal (Fig.5b).

There was no correlation between species richness and humidity ( $p = 0.1$ ) and temperature ( $p = 0.2$ ) in both seasons. The humidity varied on non-impacted areas between 38.3 to 53.4% and 42.8 to 68.2% during dry and rainy season respectively. On impacted areas this variation was of 50 to 66% and 43.2 to 72% during dry and rainy season respectively. The temperature varied on non-impacted areas between 24.2 to 33.2°C and 23.7 to 35.6°C during dry and rainy season respectively, and non-impacted areas between 18.7 to 26°C and 23.4 to 35.3°C in dry and rainy season respectively.

The evaluation about the effect of environmental variables on the seed-removing ant species composition was performed only in non-impacted areas which showed vegetation unlike impacted areas. We detected only DL (diversity of litter) as an environmental variable that influences the seed-removing ant composition during rainy season ( $p = 0.04$ ). Diversity of litter was the one with the largest contribution (14%) to explain the composition variation (Table 3). The eight environmental variables together explained 34.3% of the total variation on the seed-removing ant species composition during rainy season. Environmental variables such as CAC ( $p = 0.02$ ), WL ( $p = 0.03$ ) and DL ( $p = 0.004$ ) affect the seed-removing ant composition during dry season (Table 3).

Diversity of litter showed the largest contribution (15%) followed by canopy cover (12%) and weight of litter (11%). All environmental variables together explain 29.1% of the total variation on the seed-removing ant composition during dry season.

Table 1 Seed-removing ant species and its corresponding subfamily collected in impacted (mining) and non-impacted areas (natural forests). Impacted areas had only one and exclusive seed-removing ant species (*Nylanderia* sp1).

<b>Subfamily</b>	<b>Species</b>
Dolichoderinae	<i>Linepithema</i> sp1
	<i>Linepithema</i> sp3
Ectatomminae	<i>Ectatomma edentatum</i>
	<i>Ectatomma</i> sp3
Formicinae	<i>Camponotus crassus</i>
	<i>Nylanderia</i> sp1
Myrmicinae	<i>Acromyrmex</i> sp1
	<i>Pheidole</i> sp1
	<i>Pheidole</i> sp2
	<i>Pheidole</i> sp6
	<i>Pheidole</i> sp7
	<i>Pheidole</i> sp14
	<i>Pheidole</i> sp16
	<i>Pheidole</i> sp19
	<i>Pheidole</i> sp20
	<i>Pheidole</i> sp27
Ponerinae	<i>Pogonomyrmex</i> sp1
	<i>Solenopsis invicta</i>
	<i>Pachycondyla striata</i>

Table 2 Mean and standard error (SE) of all environmental variables measured in impacted and non-impacted areas, on mining site of Vale S.A., Nova Lima-MG, Brasil. CAC = canopy cover; UND = understory; WL = weight litter; DL = diversity litter; NT = number trees; DT = density trees; HT = height trees; DIT = diameter trees.

Environmental Variables	Non-impacted		Impacted	
	Rainy (Mean $\pm$ SE)	Dry (Mean $\pm$ SE)	Rainy (Mean $\pm$ SE)	Dry (Mean $\pm$ SE)
CAC	85,5 $\pm$ 2,5	89,9 $\pm$ 1,4	100,0 $\pm$ 0,0	100,0 $\pm$ 0,0
UND	14,7 $\pm$ 1,9	18,1 $\pm$ 2,1	0,0 $\pm$ 0,0	0,0 $\pm$ 0,0
WL	32,8 $\pm$ 4,1	24,6 $\pm$ 3,5	0,0 $\pm$ 0,0	0,0 $\pm$ 0,0
DL	1,1 $\pm$ 0,05	1,1 $\pm$ 0,1	0,0 $\pm$ 0,0	0,0 $\pm$ 0,0
NT	1,8 $\pm$ 0,2	1,8 $\pm$ 0,2	0,0 $\pm$ 0,0	0,0 $\pm$ 0,0
DT	0,1 $\pm$ 0,03	0,1 $\pm$ 0,03	0,0 $\pm$ 0,0	0,0 $\pm$ 0,0
HT	1,7 $\pm$ 0,1	1,7 $\pm$ 0,1	0,0 $\pm$ 0,0	0,0 $\pm$ 0,0
DIT	13,3 $\pm$ 1,0	13,3 $\pm$ 1,0	0,0 $\pm$ 0,0	0,0 $\pm$ 0,0

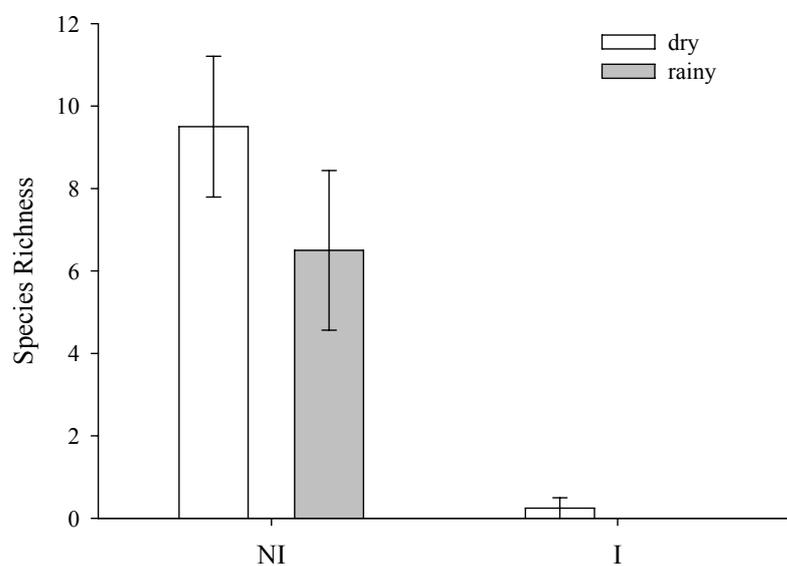


Figure 2 Seed-removing ant species richness in non-impacted areas (NI) and impacted areas (I) ( $\chi^2 = 19.64$ ;  $df = 1$ ;  $p < 0.0001$ ) in different seasons (dry and rainy) ( $\chi^2 = 17.02$ ;  $df = 1$ ;  $p = 0.1$ ). Bars are standard errors.

All ant species were collected on mining site of Vale S.A., at Nova Lima, MG, Brazil.

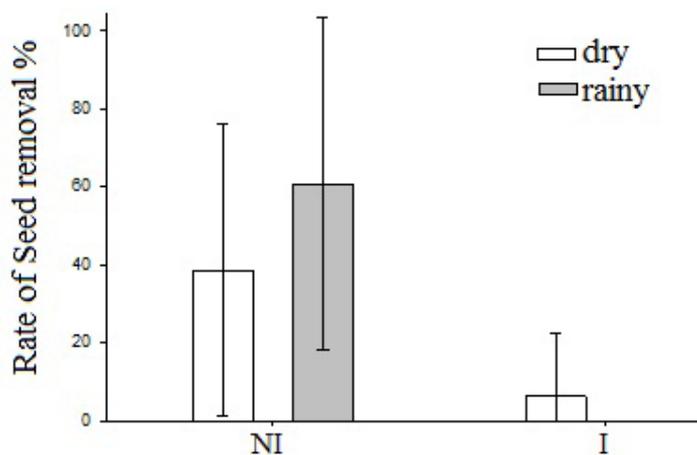


Figure 3 Rate of seed removal by ants in non-impacted (NI) and impacted (I) areas ( $F = 57.02$ ;  $p < 0.0001$ ), from mining site of Vale S.A., at Nova Lima, MG, Brazil. Season did not influence on the rate of seed removal by ants ( $F = 1.97$ ;  $p < 0.16$ ). Bars are standard errors.

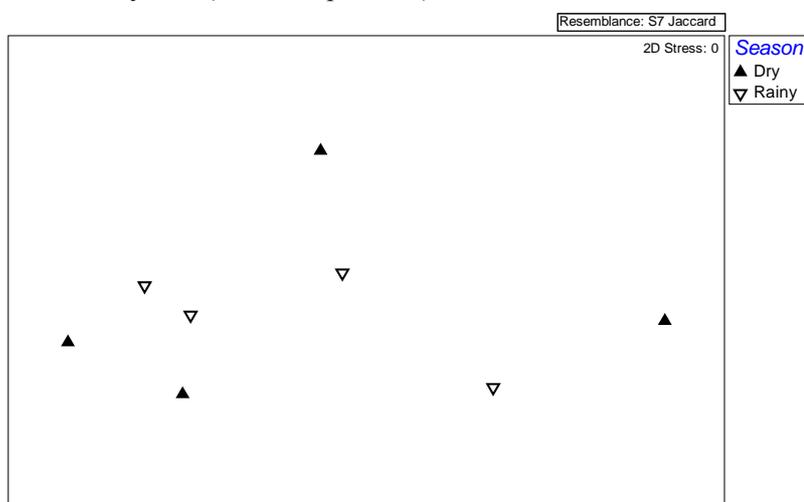


Figure 4 Non-metric multidimensional scaling (NMDS) performed on seed-removing ant species composition among seasons in non-impacted areas ( $R = 26$ ;  $p = 0.7$ ). All ant species were collected on mining site of Vale S.A., at Nova Lima, MG, Brazil.

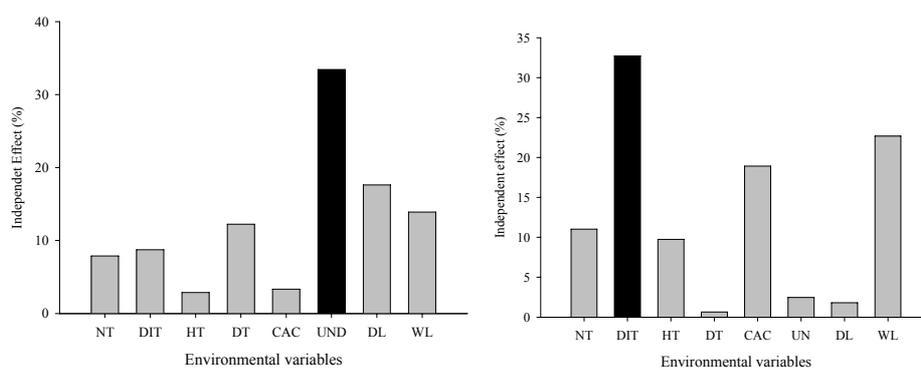


Figure 5 Independent effect of <sup>a</sup> environmental variables on the rate of seed removal by ants. Black bar indicates the significant and positive effect of: (a) understory in rainy season; (b) diameter of trees, in dry season. CAC = canopy cover; UND = understory; WL = weight of litter; DL = diversity of litter; NT = number of trees; DT = density of trees; HT = height of trees; DIT = diameter of trees. All environmental variables were measured on mining site of Vale S.A., at Nova Lima, MG, Brazil.

Table 3 Distlm of variation of seed-removing ant composition and environmental variables on the non-impacted areas. CAC = canopy cover; UND = understory; WL = weight of litter; DL = diversity of litter; NT = number of trees; DT = density of trees; HT = height of trees; DIT = diameter of trees. All environmental variables were measured on mining site of Vale S.A., at Nova Lima, MG, Brazil.

Environmental variables	Rainy		Dry	
	<i>p</i>	Proportion (%)	<i>p</i>	Proportion (%)
CAC	0.3	7.7	0.02	12
UND	0.7	4.6	0.7	4.7
WL	0.9	2.1	0.03	11
DL	0.03	14	0.004	15
NT	0.7	4.6	0.9	3.1
DT	0.8	4.4	0.5	6.2
HT	0.4	7.4	0.1	9.5

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DIT	0.7	4.8	0.4	6.4
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## DISCUSSION

Mining impact affect negatively the seed-removing ant species richness, composition and rate of seed removal by ants. However the seasonality did not influence the ant species richness and composition, neither on the rate of seed removal by them in impacted and non-impacted areas. Temperature and humidity also did not affect directly the seed-removing ant species richness in both types of areas and seasons. These last variables might have an indirect effect through changes on the vegetation. The environmental variables influenced the seed-removing ant species richness, composition and rates of seed removal by ants.

Ant assemblages are highly associated with the development of a diverse vegetation structure, mainly of some environmental variables like coverage of trees and shrubs, and increase in the leaf-litter layer (Ribas et al. 2003; Dekoninck et al. 2008). Mining areas do not have any vegetation and thus ants do not meet appropriate habitat neither diversification of conditions (e.g. light and humidity) and resources (e.g. food and nesting sites) for maintaining its colony established. This can explain the low seed-removing ant species richness and rates of seed removal by them in impacted areas.

*Nylanderia* was the only genus found removing seeds in mining areas, justifying the low seed removal rate in these areas compared with non-impacted areas. This genus has already been documented in anthropogenically-disturbed localities (Ivanov et al. 2011). It was not a surprise to find it in impacted areas, since it supports adverse conditions. *Pachycondyla striata* is environmental specialist ant species expected in non-impacted habitats and associated with a vegetation structure developed (Schmidt & Diehl 2008; Schmidt et al. 2013)

such as in our study. These last species were documented by Pizo & Oliveira (1998) as good seed removers in preserved habitats.

However the integrity of non-impacted areas in this study may be under threat by mining activity, due to presence of ant species like *Solenopsis invicta*. Though this ant species had been collected removing seeds, it is often associated to impacted habitats and trophic simplification (Epperson & Allen 2010). Its presence is considered as a threat due to its aggressive behavior that may cause negative impacts in seed remover species like *Ectatomma edentatum* and others seed-removing ants cited above, compromising the good performance of ecological function on ecosystems.

According with our results, species richness of seed-removing ants, rates of seed removal and seed-removing ant species composition are not sensible to seasonality. This indicates that the impact is more important than seasonality to evaluate changes on the seed-removing ant assemblage. Campos et al. (2006) also observed that the arboreal ant assemblage responds more to loss of host trees than to seasonality. Others abiotic factors like humidity and temperature also did not have any relationship with seed-removing species richness. This reinforces the fact that the impact causes drastic changes on the ant assemblage more than others abiotic factors. Moreover, microsites differentiation in humidity and temperature may have little influence on the ant assemblage, which can contains some ant species with discrete abiotic tolerance (Warren et al. 2012).

Seed-removing ant species richness did not have relationship with any environmental variable. This can be explained due to the low number of ant species collected in both seasons. This low number may be attributed to the presence of opportunist species like *Linepithema* sp. which may prevent others species, more sensible to its presence, to colonize and survive in the same habitat (Hoffman & Andersen 2003). Another ant species that also can prevent

the presence of others ant species is the generalist predator *Solenopsis invicta*, which is able to alter food webs at soil surface (Wickings & Ruberson 2011). The low number of species also may be explained by the low degree of preservation of the non-impacted areas sampled. This can be the most likely explanation, due to the proximity with mining sites which cause pollution of biotic resources like air and water (Parrotta & Knowles 2005), and ease of access causing disturbance in these areas.

In the non-impacted areas, understory (UND) and diameter of trees (DIT) affect positively the rate of seed removal. However the first one influenced during the rainy season, when the density of understory is higher, which may increase resource availability. Ants may respond to resource availability increasing its population abundance (Ribas et al. 2003), and this favors the rate of seed removal. Diameter of trees influenced the rate of seed removal during dry season, and this may be explained by larger crown offering more resource and space for ant foraging. Larger crown has richer ant communities and allow the growth of larger colonies (Yanoviak & Kaspari 2000). During dry season, with loss of leaves, such ants may forage in epigeaic habitat contributing for the increase in the rate of seed removal.

Schmidt & Diehl (2008) argued that ant species composition is a parameter more adequate than species richness for evaluating changes on the structure of ant assemblages, mainly in studies about bioindication. Ribas et al. (2012) also showed that ant species composition is a better parameter for understanding the ant community response to habitat impact. Our results are similar with them, because we observed strong influence of vegetation structure on the seed-removing ant species composition, and do not for species richness in non-impacted areas. The main environmental variables responsible for this influence were diversity of litter (DL), canopy cover (CAC) and weight of litter (WL). These results are consistent with Grimbacher & Hughes (2002) who

showed the importance of physical characteristics of habitat, and emphasize the influence of litter and canopy cover on the seed-removing ant species composition.

Diversity and weight of litter are important variables to epigeic ants, like seed-removing ants, because litter is the main foraging local to these ants and represent a stock of nutrients and seeds (Dias et al. 2012). Diversity and weight of litter contribute to habitat complexity on the ground which may affect the presence or absence of some ant species (Otonetti et al. 2006). Canopy cover was an important variable on the dry season, possibly due to the loss of leaves by trees in this season allowing a higher intensity of sunlight on the ground. Thus the local temperature increases, mainly during dry season, decreasing ant foraging and causing changes in habitat conditions. Aranda-Rickert & Fracchia (2012) argued that most seed-removing ant species prefer warm temperatures for foraging and interacting with seeds, and these ants usually are dominant species. Personal observations allowed us to detect the same pattern, because any ant species removed seeds at midday, period with higher temperatures.

In conclusion, to understand the effects of human activity on biota is crucial to monitor degraded habitats. Ants are effective bioindicators and play an important role on ecosystems as seed removers. Moreover, the use of seed-removing ants is a fast and cost-effective metric for environmental monitoring programs. However few studies consider this function for evaluating mining impact. Here, we showed that seed-removing ant species richness and composition are negatively affected by this activity, since we observed with only one seed-removing ant species interacting with seeds in impacted areas. The rate of seed removal was also negatively affected by this human practice, indicating the need of recovery measures. Such results about the seed removal function role by ants in impacted areas are worrying, because the myrmecochory is an event

responsible by recruitment, spatial distribution and diversity of plants. Finally, linking physical structure of environments (through environmental variables, such as used in this study) and ecological functions, as seed removing by ants, are required for providing more reliable information about the ecosystem healthy.

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*For Restoration Ecology*

**ARTIGO 2**

**SEED REMOVAL AND RECOVERY AFTER MINING:  
IMPLICATIONS FOR THE SUCCESS OF RECOVERY EFFORTS**

Rabello, A.M.<sup>1</sup>, Cuissi, R.G.<sup>2</sup>, Queiroz, A.C.M.<sup>1</sup>, Lasmar, C.<sup>2</sup>, Ribas, C.R.<sup>2</sup>

<sup>1</sup>Programa de Pós – Graduação em Ecologia Aplicada, Departamento de Biologia, Setor de Ecologia, Universidade Federal de Lavras, Caixa Postal 3037, CEP 37.200-000, Lavras – MG, Brasil.

<sup>2</sup>Laboratório de Ecologia de Formigas, Departamento de Biologia, Setor de Ecologia, Universidade Federal de Lavras, Lavras – MG, Brasil.

## ABSTRACT

Ant community has been widely used as indicator of success recovery efforts, but the ecological functions played by them still are poorly used in this context. This study evaluated the seed removal by ants in recovery areas with different ages and techniques in dry and rainy season. We examined: (1) richness and composition, (2) rates of seed removal, (3) influence of environmental variables on seed removing ant species composition, and (4) indicator seed-removing ant species, in these different recovery areas. We sampled seed-removing ant in recovery areas with ages that ranged from two to ten years after mining. We also sampled in recovery areas with exotic and native plant species, with two types of exotic grasses, and upon sterile material and upon mining site. We did not find difference on the seed-removing ant species richness between seasons in all recovery areas. But seasonality affected the rate of seed removal and ant species composition in all recovery ages or techniques. All parameters used related to seed-removing ant species (species richness and composition and rate of seed removal) were affected by recovery ages and techniques. Environmental variables were important for explaining changes on the ant species composition in all recovery ages and techniques. Ant assemblage has still species richness and composition different of control areas, even after 10 years of recovery. The use of native plant and recovery upon mining site pointed the best results as recovery techniques. Our results showed that seed-removing ant species must be used as bioindicator of recovery areas for analyzing and monitoring recovery process.

Keywords: Recovery ages. Recovery techniques. Myrmecochory. Exotic plant species. Native plant species. Ant bioindicator.

## INTRODUCTION

Mining activity results in drastic changes in the landscape mainly through the excavation to obtain ore, leading to massive destruction of soil and its biota associated (Holec & Frouz 2005). Recovery techniques are required to avoid that environmental impacts caused by mining remain due to the high degradation level, and reach nearby ecosystems off-site the mining sites. Rivers, lakes, reservoirs and forests are ecosystems that may be affected by mining due to erosion, runoff and pollution of water and air (Dias et al. 2012). It is also important to monitor the environmental quality of recovery techniques used to evaluate the natural re-establishment of biodiversity (Ribas et al. 2012a).

However, the majority of studies about recovery techniques drive its efforts in quantitative measures related to the habitat structure, like techniques involving tree planting (Dias et al. 2012, Souza & Batista 2004, Parrota & Knowles 2001). Parrota & Knowles (2001) evaluated the restoration process with native and exotic plant species, but did not evaluate the influence of these techniques in the fauna. Few studies used qualitative measures, like ecological functions, to evaluate recovery efforts (Dominguez-Haydar & Armbrrecht 2011, Lomov et al. 2009, Andersen & Morrison 1998).

Most recovery programs are concerned only with the establishment of vegetation cover to avoid soil erosion. Thereby, these programs expect that animals will recolonize naturally the recovered areas, without to analyze the attributes needed for the return of fauna (Bisevac & Majer 1999). Rates of colonization of arthropods in recovery sites are associated with structure and diversity of plant community and after the first months of planting at these sites it is possible to observe high arthropods species richness (Pais & Varanda 2010). However, to assess if recovery techniques are sufficient for the re-establishment

the fauna, it is necessary a monitoring using bioindicator organisms (Ribas et al. 2012).

Studies involving the use of bioindicators are essential to provide information about environmental conditions and ecological integrity of habitats during recovery process (Majer & Moir 2007). Ants have been widely used in studies about impacts by mining throughout the world (Ribas et al. 2012b, So & Chu 2010, Ottonetti et al. 2006, Hamburg et al. 2004; Majer 1984). This is due to ants high diversity, wide geographic distribution, ease sampling, taxonomy and ecology relatively well known and their important roles in the ecosystem (So & Chu 2010).

Majer et al. (2009) showed that ants have much importance as bioindicator in restoration programs due to their role as seed predators and removers. Removal of seeds is an important ecological function performed by ants (myrmecochory) considered a critical factor for the maintenance of plant populations and its community structure (Berg-Binder & Suarez 2012). This interaction may accelerate the rehabilitation process, reduce costs and also contribute for self-sustaining ecosystems after disturbance (Dominguez-Haydar & Armbrrecht 2011). This plant-insect interaction provides information about structural and functional changes in plant and insect communities, and also detects if the ecological function is being restored (Lomov et al. 2009).

Ant-plant interactions may be influenced by environmental changes in space and time (Warren et al. 2012). This occurs, for instance, because changes on environmental heterogeneity affect the ant species richness, composition and abundance (Ribas et al. 2003; Vasconcelos et al. 2000). Difference on habitat structure, mainly vegetation cover, during successional process also influence on the ant species richness and composition, which positively responds to the increase in the successional age (Costa et al. 2010; Schmidt et al. 2013). Dominguez-Haydar & Armbrrecht (2011) also found this same positive response

of ant species richness to rehabilitation age. Thus, changes in ant community due to habitat structure and resources modifications by mining activities may cause imbalances on the ant-plant interaction due to the loss of species removing seeds (Sorrells & Warren 2011).

The present study evaluated the seed removal by ants in recovery areas after mining activity, in rainy and dry seasons. We compared areas with different recovery ages and techniques regarding their: (1) richness and composition, (2) rates of seed removal, (3) influence of environmental variables on seed removing ant species, and (4) indicator seed removing ant species.

## METHODS

### Study sites

Research was conducted in the Nova Lima city at Minas Gerais state, southeast Brazil. This region is located on the transition area of Atlantic forest and Cerrado (savanna), and shows average altitude of 1400m, annual average temperature of 21.1°C, and local climate with dry winters (April to September) and rainy summers (October to March) (IBGE 2012). The company Vale S.A. was installed, in this region, to extract iron ore. The study were carried out on *Mutuca* (20°1'43" S e 43°57'10" W), *Tamanduá* (20°5'17" S e 43°56'27" W) and *Capão Xavier* (20°2'47" S e 43°58'59" W) mines in February, March and July 2012.

For analyzing the influence of time on the recovery process, the areas were here classified according to the number of years since beginning of recovery: 2 years (R2), 4 years (R4), 6 years (R6), 8 years (R8) and 10 years (R10) (Fig.1). For evaluating the success of different recovery techniques, we selected two areas of each type of technique. Two different types of exotic grasses, the *Braquiaria* spp. (“braquiária”) (BEP) and *Melinis minutiflora* (“capim gordura”) (CEP), were used in one of the comparisons of different types of technique. All recovery techniques were compared with adequate control sites (natural vegetation in the area) for analyzing the success of each recovery technique (Table 1). Inside all areas we installed one transect of 200m containing five sampling points, 40m distant from each other, for sampling seed removing ants and environmental variables.

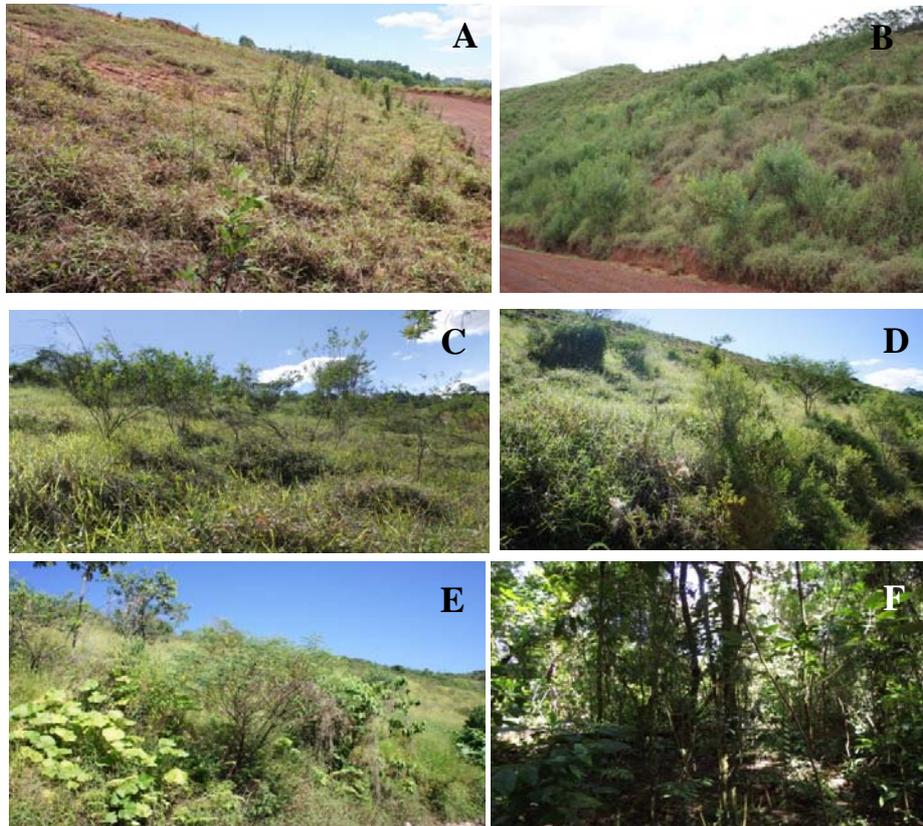


Figure 1 Recovery areas with different ages at mining sites of Vale S.A., at Nova Lima –MG, Brazil. (A) Two years of recovery (R2); (B) Four years (R4); (C) Six years (R6); (D) Eight years (R8); (E) Ten years (R10); (F) Riparian forest (RF). Such areas also represent: (A) and (B) CEP, SM; (C), (D) and (E) BEP.

Table1 Recovery areas with different ages and techniques at mining sites of Vale S.A., at Nova Lima –MG, Brazil. R2 = two years of recovery; R4 = four years; R6 = six years; R8 = eight years; R10 = ten years; CEP = “capim gordura” exotic plant; BEP = “braquiária” exotic plant; EP = exotic plant; NP = native plant; RF = “Riparian Forest” control; CS = “Campo Sujo” control; CR = “Campo Rupestre” control.

Mine	Recovery time	Recovery technique	Control area
<i>Mutuca</i>	R2	CEP, SM	RF
	R4	CEP, SM	RF
	R6	BEP	RF
	R8	BEP	RF
	R10	BEP	RF
<i>Tamanduá</i>		EP, MS	CS
<i>Capão Xavier</i>		EP, NP	CR

### Ant sampling

Each sampling point contained 50 artificial lipid-rich fruits (based in Raimundo et al. 2004) on the ground protected from vertebrate removal by a wire cage (1.5cm mesh). Artificial fruits were produced to represent a natural situation, thus they contained the “seed” and one white fleshy part representing the “elaiosome” (part of natural fruit attractive for ants) with all compounds usually met in fruits *in situ*. The fleshy part consisted of vegetal fat (75%), fructose (4.8%), sucrose (0.5%), glucose (4.7%), casein (7%), calcium carbonate (3%), and maltodextrin (5%), and was developed in the Laboratory of Chemical, Biochemical and Analyses of Food (Food Engineering Department, Federal University of Lavras, Brazil). This fleshy part was attached to orange beads (“seeds”) of 0.03g and 2mm diameter representing a true natural fruit. In total our artificial fruits had 0.2g weight and 5.5mm diameter, and such

characteristics allocated them in small and medium categories proposed by Pizo & Oliveira (2001).

Observations of ant-fruit interactions started at 09:00h and were checked for 15 minutes in each hour until 16:00h, which was the maximum time that the company allowed. During these observations we recorded and collected all ant species removing the fruits over distances greater than 30cm of the original local (Christianini & Oliveira 2010). At the same moment, we also recorded how many fruits had been removed to calculate the rates of removal, which were counted visually and ranged between 0 to 100%. Ants collected were identified in the Laboratory of Ant Ecology at Federal University of Lavras, Brazil. We used the keys in Palacio & Fernández (2003) and Bolton (1994) for identification to genera level, and grouped in morphospecies according to specimens in the reference collection of the same Laboratory.

### **Vegetation structure**

Vegetation structure was measured, at each sampling point in all areas, through environmental variables like: understory (UND), canopy cover (CAC), weight (WL) and diversity (DL) of litter, density (DT), diameter (DIT) and height (HT) of all trees with circumference at breast height (cbh) > 5 cm.

In each sampling point we also obtained the litter samples in a delimited square of 50 x 50 cm. Its weight was determined with a precision balance, and its diversity counting the number of different leaves, branches and sticks. The others environmental variables were measured using a quadrant of 6x6m around at each sampling point, where we counted the number and height of different morphospecies of individuals of trees.

Inside this same quadrant, we also measured the canopy cover with digital images using fish-eye lens attached to a camera positioned at a height

1.5m, and calculated on the software Gap Light Analyser 2.0 (GLA). Understory was measured through photographs in four points, from different directions, around each sampling point using white cloth of 100 x 100cm. The white cloth was 3m away from the sampling point and 1m above ground level. We analyzed the density of understory using the “Global Analysis” on the software Sidelook (Nobis 2005).

### **Statistical Analysis**

We used species accumulation curves to compare the species richness among areas with different recovery ages and techniques and between seasons. The EstimateS version 8.20 (Colwell 2010) was used for obtaining the curves. Moreover, we compare graphically the rates of seed removal by ants among all areas.

To check if areas with different recovery techniques were similar in relation to their ant species composition among each other and among control areas, we plotted a two dimensional ordination using non-metric multidimensional scaling (NMDS) based on Jaccard index. NMDS is a powerful method for ordinating similarity matrices which uses species presence/absence data. Then we evaluated the significance of NMDS through one-way ANOSIM performed with 999 permutations. We also verified if the same differences occurred in all areas between seasons (rainy and dry). These analyses were carried out in Primer v6 (Clark & Gorley 2006).

Changes on seed-removing ant composition among areas with different recovery ages and controls were carried out with principal coordinate analyze (PCO) based on the Jaccard’s similarity matrix. The program used for the above analyses was Primer v6 (Clark & Gorley 2006).

To determine the influence of environmental variables (predictor variables) on species composition of seed-removing ants among areas with different ages and recovery techniques, we used distance-based linear modeling (DISTLM) with Jaccard index. DISTLM shows the variation in the species composition matrix according to regression models based on quantitative predictor variables (CAC, UND, WL, DL, DT, HT, DIT). The program used was Primer Permanova+ v6 (Anderson et al. 2008).

Indicator species analyses (IndVal) were used to obtain the value of each species as an indicator for all areas (Dufrière & Legendre 1997). This analyze evaluate the association of each species with the areas which contains different environmental conditions. The specificity and fidelity of species are measured within that area. The indication value and the statistical significance of analyzes are obtained through the Monte Carlo test with 4999 permutation. The software used was PC-ORD 5.10 (McCune & Mefford 2006).

## RESULTS

We collected 42 seed-removing ant species from six subfamilies and 14 genera (Table 2). The most richness subfamily was Myrmicinae (26), in second was Formicinae (five) followed by Ponerinae (four), Ectatomminae and Dolichoderinae (three each), and Ecitoninae with only one species. *Pheidole* was the richest genera with 21 species (50% of the total). The most broadly distributed genera were *Pheidole* and *Camponotus*, which were collected in all areas. The “Riparian forest” (RF) control harbored the highest number of species with 18 seed-removing ant species.

We did not detected difference on the seed-removing ant species richness between seasons in any of different recovery ages or techniques, and then comparisons among recovery sites were made using ant richness of two seasons. In relation to species accumulation curves among areas with different recovery ages, RF showed a larger number of ant species followed by R4 e R8 (four and eight years of recovery, respectively). The areas R2, R6 e R10 had a lower number of ant species (Fig.2).

Comparing species richness between recovery with native and exotic plant species, we can note that recovery with exotic species showed a higher species richness than recovery with native species and “Campo Sujo” control. However, the “Campo Rupestre” control, adequate for recovery with native species since the species implemented in the recovery were “campo rupestre” species, had no seed-removing ant species, so we can observe that this recovery area had a better performance than its control (Fig.3). Comparing the two different types of exotic grasses there is no difference in the success of these techniques in relation to species richness, but they presented a smaller ant species richness than control areas (Fig.4). Finally, recovery upon mining site

(MS) showed better performance than upon sterile material (SM) and its “Campo Sujo” control area (CS). SM had the lowest number of species richness, even in relation to its RF control area (Fig.5).

Table 2 Seed-removing ants and its subfamily, associated with type of habitat in which it was captured in mining sites of Vale S.A., Nova Lima-MG, Brazil. Acronyms related to recovery age and type: R2 = two years of recovery; R4 = four years; R6 = six years; R8 = eight years; R10 = ten years; NP = native plant species; EP = exotic plant species; BEP = “braquiária” exotic grass; CEP = “capim gordura” exotic grass; MS = upon mining site; SM = upon sterile material; RF = “Riparian Forest” control; CS = “Campo Sujo” control.

Subfamily	Species	Type of habitat
Dolichoderinae	<i>Linepithema</i> sp.4	R8, BEP
	<i>Dorymyrmex</i> sp.1	R4, CEP, SM
	<i>Dorymyrmex brunneus</i>	EP, MS
Ectitoninae	<i>Labidus</i> sp.1	EP, MS
Ectatomminae	<i>Ectatomma edentatum</i>	RF, CS
	<i>Ectatomma</i> sp.3	CS
	<i>Gnamptogenys</i> sp.1	RF
Formicinae	<i>Camponotus</i> sp.1	R10, BEP, EP, MS
	<i>Camponotus rufipes</i>	RF, R4, CEP, EP, MS, SM
	<i>Camponotus crassus</i>	CS, R2, CEP, NP, EP, SM, MS
	<i>Camponotus</i> sp.5	RF
	<i>Nylanderia</i> sp.1	NP
Myrmicinae	<i>Acromyrmex</i> sp.1	R8, BEP
	<i>Acromyrmex</i> sp.2	CS, R2, CEP, SM
	<i>Pheidole</i> sp.1	RF, CS, EP, MS
	<i>Pheidole</i> sp.2	RF, CS, MS, EP, NP
	<i>Pheidole</i> sp.3	RF, R8, BEP
	<i>Pheidole</i> sp.5	RF
	<i>Pheidole</i> sp.6	CS, R8, R10, BEP
	<i>Pheidole</i> sp.7	RF, CS, R4, CEP, NP, EP, MS, SM
	<i>Pheidole</i> sp.8	R6, R8, R10, BEP
	<i>Pheidole</i> sp.11	R6, BEP
	<i>Pheidole</i> sp.12	R8, BEP
	<i>Pheidole</i> sp.13	RF
	<i>Pheidole</i> sp.14	RF, CS, R2, R4, CEP, SM, EP, MS, NP

“Table 2, conclusion”

	<i>Pheidole</i> sp.15	RF
	<i>Pheidole</i> sp.16	CS, EP, MS
	<i>Pheidole</i> sp.20	CS, R8, BEP, EP, MS
	<i>Pheidole</i> sp.21	RF, R8, BEP
	<i>Pheidole</i> sp.22	R4, CEP, SM
	<i>Pheidole</i> sp.23	R4, CEP, SM, NP
	<i>Pheidole</i> sp.25	R10, BEP
	<i>Pheidole</i> sp.26	RF
	<i>Pheidole</i> sp.29	RF
	<i>Pheidole</i> sp.30	R6, R8, R10, BEP, EP, MS
	<i>Pogonomyrmex</i> sp.1	CS
	<i>Solenopsis invicta</i>	R2, R4, R6, CEP, SM
	<i>Wasmannia</i> sp.1	R10, BEP
Ponerinae	<i>Odontomachus chelifer</i>	RF
	<i>Pachycondyla striata</i>	RF
	<i>Pachycondyla verena</i>	EP, MS
	<i>Pachycondyla</i> sp.3	RF

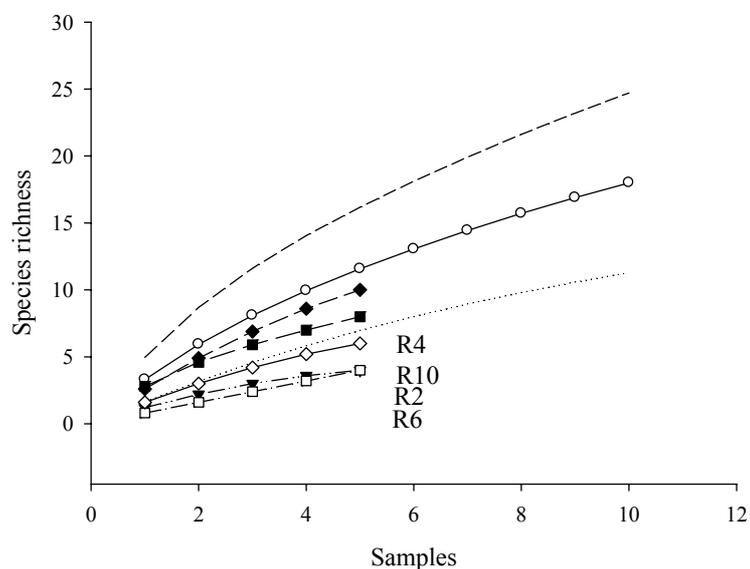


Figure 2 Species accumulation curves in areas with different recovery ages and controls areas from mining sites of Vale S.A., at Nova Lima, MG,

Brazil. R2 = two years of recovery; R4 = four years; R6 = six years; R8 = eight years; R10 = ten years; RF = “Riparian Forest” control area.

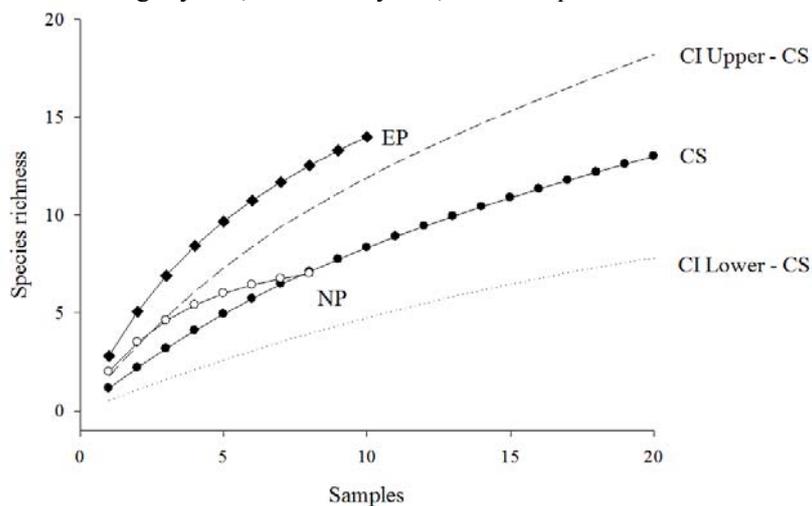


Figure 3 Species accumulation curves in recovered areas with native and exotic plant species and in control areas from mining sites of Vale S.A., at Nova Lima, MG, Brazil. NP = native plant species; EP = exotic plant species; CS = “Campo Sujo” control area.

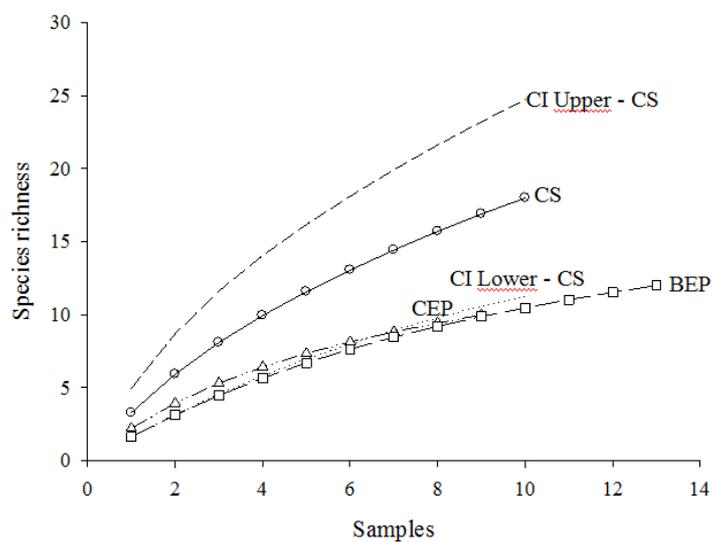


Figure 4 Species accumulation curves in recovered areas with different types of exotic grasses, CEP (“capim gordura”) and BEP (“braquiária”), and in

“Campo Sujo” control area (CS), within mining sites of Vale S.A., at Nova Lima-MG, Brazil.

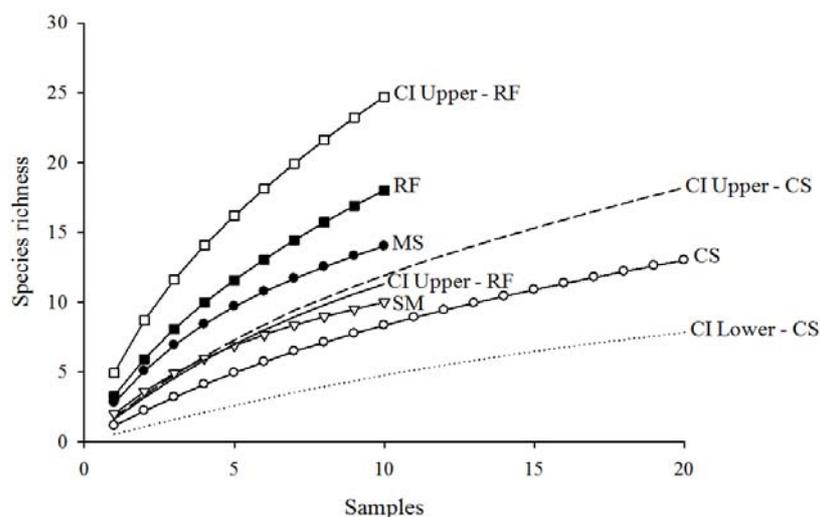


Figure 5 Species accumulation curves in recovered areas upon sterile material and upon mining site and control areas, from mining sites of Vale S.A., at Nova Lima-MG, Brazil. SM = upon sterile material; MS = upon mining site; RF = “Riparian Forest” control area; CS = “Campo Sujo” control area.

Rates of seed removal were different between seasons in all areas, including all recovery ages and techniques and, in the most cases, highest during rainy season (Fig. 6). The percentage of removal did not increase according to recovery age, being highest in the early recovery during rainy season and smallest in intermediate areas during dry season. Control areas showed a removal rate higher than all recovery areas (Fig.6a). On the recovered areas with exotic species the removal rate was higher than in recovered areas with native species and in control areas, in both seasons (Fig.6b). Comparing the two types of exotic grasses in the two seasons we met a higher removal rate in “capim gordura”, but again the removal rate of control areas was higher than in the recovery ones (Fig.6c). Finally, when evaluating the removal rates between

recovery areas upon sterile material and upon mining site, the first showed a higher removal rate than mining site in rainy and dry seasons. But both recovery areas presented rates of seed removal lower than its respective control areas in both seasons (Fig.6d).

In relation to seed-removing ant species composition, we detected a significant difference among different recovery ages and controls between seasons ( $p = 0.01$ ;  $R = 0.18$ ) (Fig.7). But in rainy season we only met differences on species composition between R2 and R8, and among control and all recovery areas ( $p = 0.001$ ;  $R = 0.27$ ). During dry season we detected differences among recovery ages and control areas ( $p = 0.002$ ;  $R = 0.10$ ), and R4 was different of all others areas.

Recovery techniques with native and exotic species caused significant differences on the species composition, which also varied between seasons ( $p = 0.008$ ;  $R = 0.061$ ) (Fig.8a). The same was detected on species composition using different exotic grasses comparing with control areas ( $p = 0.001$ ;  $R = 0.11$ ) (Fig.8b). This pattern also remained when we evaluated differences on the species composition between recovery upon sterile material and upon mining site ( $p = 0.001$ ;  $R = 0.09$ ) (Fig.8c).

Changes on the seed-removing ant composition among different recovery ages may be explained through five environmental variables (DIT-8.8%, HT-9.2%, DT-8.3%, CAC-10.5%, and DL-8.5%) which together explained 19.9% of total change during dry season. Variation on the species composition during rainy season was explained by almost all variables (DIT-7.4%, HT-10.2%, DT-9.7%, CAC-12%, UND-7.8%, DL-10.8%), exception to the weight of litter ( $p = 0.2$ ) (Table 3). Together these variables represented 20.5% of total variation on the species composition. Considering the species composition variation between recovered areas with exotic and native species during dry season, CAC (9%) and WL (10%) were important to explain the

species composition variation, and all variables explained 21.6% of total variation. Already in the rainy season, DIT (7.8%), HT (6.4%) and UND (5.7%) were variables that showed influence on the species composition, explaining 17.1% (Table 4).

Moreover, variables like CAC (9.4%), HT (9.2%), DIT (9%), DT (8.3%) and DL (8.5%), explained the variation on the composition between recovered areas with different exotic grasses, and such variables represented 18.8% of total variation during dry season. In relation to rainy season, environmental variables represented 22.3% of total changes on the species composition and they were CAC (12%), DL (12%), HT (11%), DT (11%), DIT (6.8%), UND (6.8%) (Table 5). Only WL ( $p = 0.2$ ) had not contribution on the variation of species composition. Evaluating the species composition variation between recovery upon sterile material and upon mining site during dry season, environmental variables explained 13.8%, being the variables CAC (7.1%), HT (6.7%), DIT (6.3%), WL (6.3%) and DT (5.7%) more important for this. During rainy season the only environmental variable not significant for explaining the species composition variation was WL ( $p = 0.5$ ). The percentage of explanation of others variables were: HT (7.3%), CAC (7.3%), DL (6.9%), DIT (6.2%), DT (5.3%) and UND (4%) (Table 6).

We detected two seed-removing ant species indicators of R4 (four years of recovery) and four of "Riparian Forest" control area. Recovery with native species showed three indicator ant species and recovery with exotic species had two indicator species. In the two types of exotic grasses BEP did not have indicator species, while CEP had two indicator species. Finally, Recovery upon sterile material showed two indicator ant species, the same occurring for recovery upon mining site. All these results may be seen in the table 7.

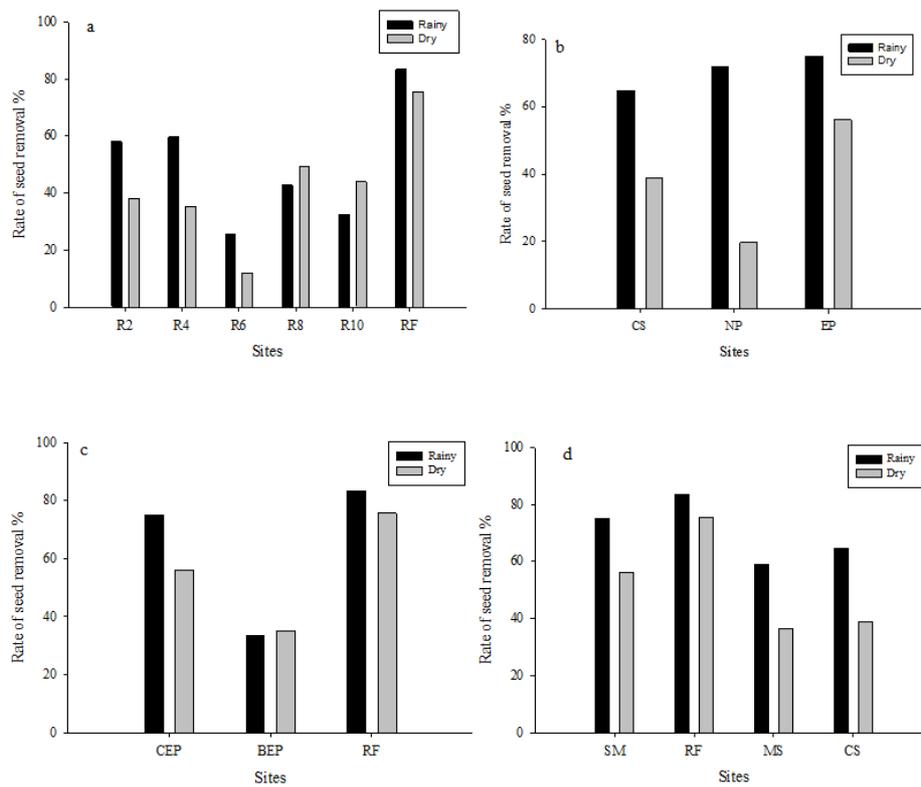


Figure 6 Rates of seed removal in areas with different recovery ages and techniques and its respective areas, in dry (D) and rainy (R) season. (a) R2 = two years of recovery; R4 = four years; R6 = six years; R8 = eight years; R10 = ten years; RF = “Riparian Forest” control; (b) NP = native plant species, EP = exotic plant species, CS = “Campo Sujo” control area; (c) CEP = “capim gordura” grass, BEP = “braquiaria” grass; (d) SM = upon sterile material, MS = upon mining site. All areas were located within mining sites of Vale S.A., at Nova Lima-MG, Brazil.

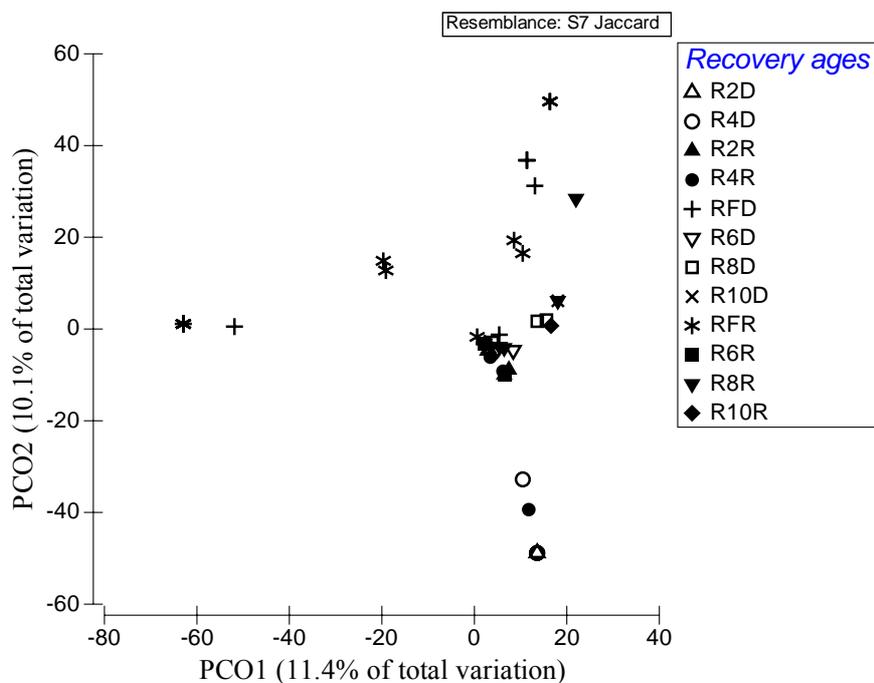
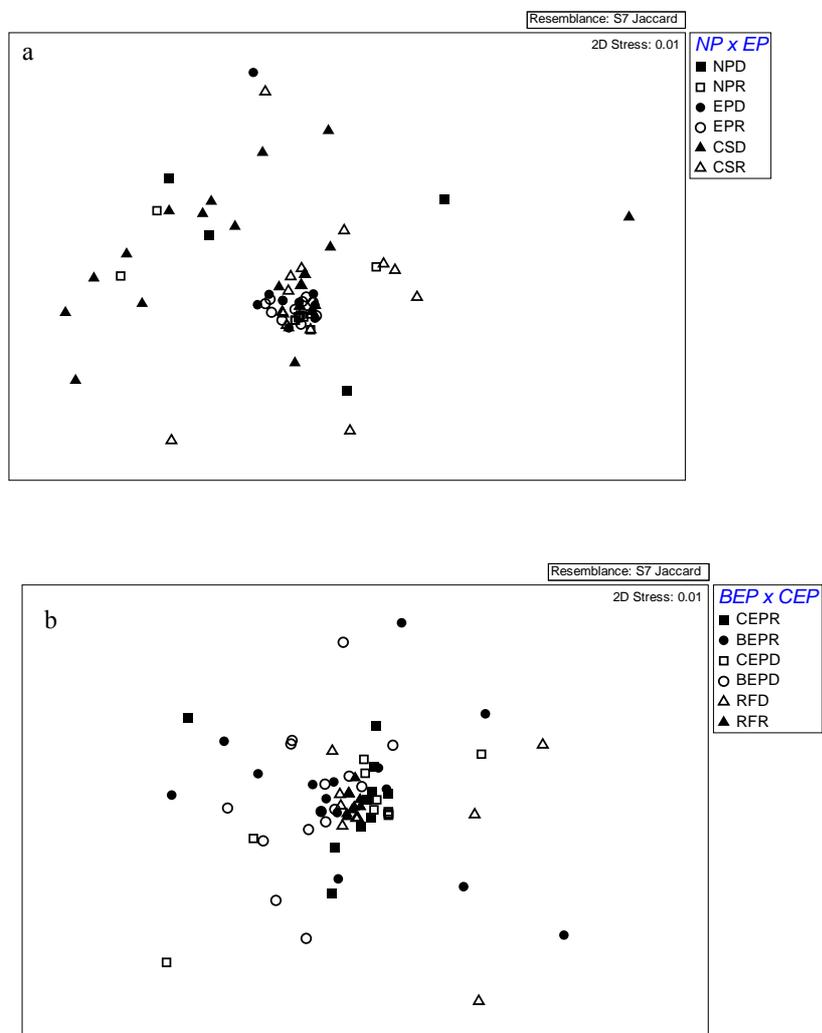


Figure 7 Principal coordinate analyze (PCO) based on the Jaccard's similarity matrix of recovered areas with different ages and control areas according to seed removing ant species composition in dry (D) and rainy (R). R2 = two years of recovery; R4 = four years; R6 = six years; R8 = eight years; R10 = ten years; RF = "Riparian Forest" control areas. Areas were localized in mining sites of Vale S.A., at Nova Lima-MG, Brazil.



"Fig. 8, continues"

“Fig. 8, conclusion”

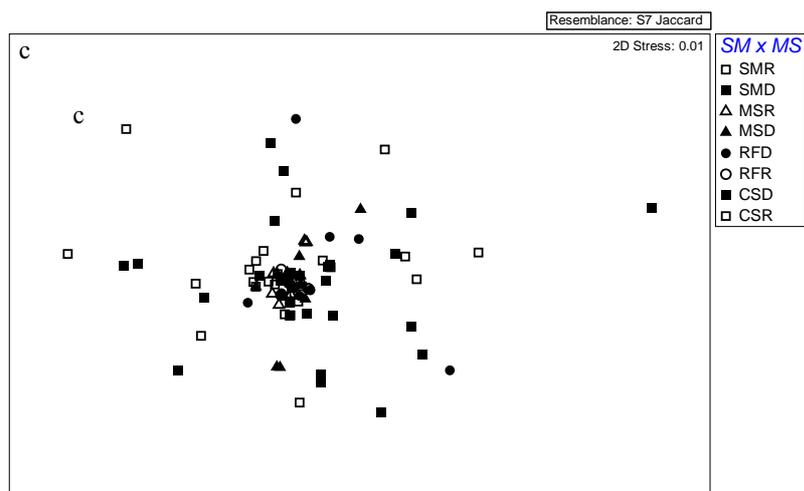


Figure 8 Non-metric multidimensional scaling (NMDS) performed on seed-removing ant species composition, in dry (D) and rainy season (R). (a) native and exotic plant species; (b) types of exotic grasses; (c) upon sterile material and upon mining site. NP = native plant species; EP = exotic plant species; BEP = “braquiaria” grass; CEP = “capim gordura” grass; SM = sterile material; MS = mining site; RF = “Riparian Forest” control; CS = “Campo Sujo” control. Areas were localized in mining sites of Vale S.A., at Nova Lima-MG, Brazil.

Table 3 Results of Distlm among different recovery ages, during dry and rainy seasons. CAC = canopy cover; UND = understory; WL = weight of litter; DL = diversity of litter; DT = density of trees; HT = height of trees; DIT = diameter of trees. All environmental variables were measured on mining site of Vale S.A., at Nova Lima, MG, Brazil.

Environmental variables	Dry		Rainy	
	<i>p</i>	Proportion (%)	<i>p</i>	Proportion (%)
DIT	0.01	8.8	0.02	7.4
HT	0.01	9.2	0.002	10.2
DT	0.02	8.3	0.001	9.7
CAC	0.001	10.5	0.001	12
UND	0.5	4.9	0.009	7.8
DL	0.01	8.5	0.001	10.8
WL	0.1	6.8	0.2	5.1

Table 4 Distlm between recovery with native and exotic plant according to ant species composition during dry and rainy season. CAC = canopy cover; UND = understory; WL = weight of litter; DL = diversity of litter; DT = density of trees; HT = height of trees; DIT = diameter of trees. All environmental variables were measured on mining site of Vale S.A., at Nova Lima, MG, Brazil.

Environmental variables	Dry		Rainy	
	<i>p</i>	Proportion (%)	<i>p</i>	Proportion (%)
DIT	0.3	6	0.005	7.8
HT	0.2	6.6	0.03	6.4
DT	0.6	4.9	0.4	3.6
CAC	0.05	9	0.1	5
UND	0.7	4.2	0.05	5.7
DL	0.5	5.1	0.1	4.9
WL	0.02	10	0.7	2.9

Table 5 Result of Distlm in relation to variation of seed-removing ant composition and environmental variables in recovery sites with different types of exotic grasses during dry and rainy season. CAC = canopy cover; UND = understory; WL = weight of litter; DL = diversity of litter; DT = density of trees; HT = height of trees; DIT = diameter of trees. All environmental variables were measured on mining site of Vale S.A., at Nova Lima, MG, Brazil.

Environmental variables	Dry		Rainy	
	<i>p</i>	Proportion (%)	<i>p</i>	Proportion (%)
DIT	0.004	9	0.04	6.8
HT	0.005	9.2	0.001	11
DT	0.02	8.3	0.001	11
CAC	0.005	9.4	0.001	12
UND	0.6	4.7	0.05	6.8
DL	0.02	8.5	0.001	12
WL	0.08	7	0.2	5.2

Table 6 Distlm between recovery upon sterile material and upon mining, from mining site during dry and rainy seasons. CAC = canopy cover; UND = understory; WL = weight of litter; DL = diversity of litter; DT = density of trees; HT = height of trees; DIT = diameter of trees. All environmental variables were measured on mining site of Vale S.A., at Nova Lima, MG, Brazil.

Environmental variables	Dry		Rainy	
	<i>p</i>	Proportion (%)	<i>p</i>	Proportion (%)
DIT	0.01	6.3	0.001	6.2
HT	0.009	6.7	0.001	7.3
DT	0.03	5.7	0.002	5.3
CAC	0.003	7.1	0.001	7.3
UND	0.8	2.5	0.05	4
DL	0.06	5.1	0.001	6.9
WL	0.006	6.3	0.5	2.6

Table 7 Seed-removing ant species indicators of all recovery areas with different ages and techniques, and controls areas from mining sites of Vale S.A. at Nova Lima-MG, Brazil. R2 = two years of recovery; R4 = four years; R6 = six years; R8 = eight years; R10 = ten years. SM = sterile material; MS = mining site; NP = native plant species; EP = exotic plant species; CEP = “capim gordura” exotic grass; BEP = “braquiária” exotic grass; RF = “Riparian Forest” control; CS = “Campo Sujo” control.

Species	Site	Value (IV)	<i>p</i>
<i>Pachycondyla striata</i>	RF	50.0	0.002
<i>Ectatomma edentatum</i>	RF	50.0	0.004
<i>Pheidole</i> sp.3	RF	85.7	0.04
<i>Pheidole</i> sp.5	RF	100.0	0.04
<i>Pheidole</i> sp.14	R4, SM	64.0	0.003
<i>Pheidole</i> sp.7	R4, NP, SM	45.0	0.01
<i>Pheidole</i> sp.6	BEP	22.5	0.02
<i>Pheidole</i> sp.8	BEP	100.0	0.0006
<i>Camponotus</i> sp.1	CEP, MS, EP	37.9	0.002
<i>Pachycondyla verena</i>	CEP, MS, EP	40.0	0.001
<i>Nylanderia</i> sp.1	NP	25.0	0.01
<i>Solenopsis invicta</i>	NP	32.4	0.002

## DISCUSSION

There is no difference on the seed-removing ant species richness between rainy and dry season, but seasonality affects ant foraging, decreasing ant activity during dry season, which was observed through the variation on the rate of seed removal. Seasonality is able to affect ant species composition, independent of recovery ages or techniques. All parameters used of seed-removing ant species (species richness, species composition and rate of seed removal) were affected by recovery ages and techniques. Environmental variables, except weight of litter in most cases, can explain changes on the seed-removing ant composition. These factors are associated with changes on the resources and conditions important to ant distribution.

The most commonly ants observed removing seeds belong to *Pheidole* genus, like in our study, making it important in the context of seed removal (Dominguez-Haydar & Armbrecht 2011; Bieber et al. no prelo). *Odontomachus chelifer* and *Pachycondyla striata* are specialist of preserved and natural habitats (Schmidt et al. 2013), and considered as good seed removers (Christianini & Oliveira 2010). Moreover, these last species carry the seed individually to long distances contributing to the establishment of seeds and maintenance of plant populations after disturbances (Lomov et al. 2009; Pizo & Oliveira 1998). This evidences that 10 years of recovery (our older recovery area) and the different techniques used for recovery evaluated in this study were not re-colonized by important specialist species. Here such species were collected only in control areas, showing that recovery efforts were still not enough to harbor specialist species. On the other hand, the high rates of seed removal and the high number of seed-removing ant species in recovery areas, indicate that the ecological function of seed removal is been recovered. However this ecological function is

being performed by generalist species like *Solenopsis* spp. which are not considered good removers (Christianini & Oliveira 2010), compromising the full recovery success.

Seasonality is a strong regulator of ecological communities, like plant and insect communities in tropical forests (Castro et al. 2012). We did not meet differences on the seed-removing ant species richness between dry and rainy season in all areas, although there was differences on the species composition between seasons in all areas. Therefore, species richness is not a good parameter for evaluating seasonal effects in sites after mining disturbance. Within this context the species composition appears as an ant community factor more sensible and appropriate to analyze seasonality influence in disturbed areas. Abiotic factors may change the ant distribution due to the intolerance of some ant species to changes in conditions and resources (Coelho & Ribeiro 2006).

Air and soil temperature and humidity are abiotic factors, which change among seasons, important for ant foraging activity (Warren et al. 2012). In almost all areas, rates of seed removal during rainy season were higher than in dry season, indicating that seasonality affects ant behavior, but not at the level of ant species richness. This difference can be explained by the increased abundance of ant workers on the rainy season, leading to increase of removal rates. Suazo et al. (2012) also observed this pattern of increase of seed-removing ants in restoration sites after burnt.

### **Recovery ages**

Contrary to our expectations, the seed removing ant species richness did not increase according to increase of recovery age, being higher in R4 and R8. After a great disturbance, the first species pool that colonizes is adapted to open habitats with generalist species consuming a wide variety of resources. With the increase of recovery time, occurs the appearance of new plant species and

replacement of others, and consequently new ant species are able to colonize (Schmidt et al. 2013). This new species added to pioneer ant species, still present, leads to an increase in the number of ant species, explaining the greatest ant species richness in the intermediate area with four years (R4). The youngest (R2) and oldest (R10) areas showed the same seed-removing ant species richness, indicating that these ants are not affected by recovery age. This it was also detected by others authors in studies about gradient succession in sites after human impacts (Schmidt et al. 2013; Costa et al. 2010; Ottonetti et al. 2006). Such outcome is related with the presence of only species tolerant to open habitats in the beginning of recovery, and in the oldest area to the presence of only species specialized in shadow habitats (Andersen et al. 2012). This pattern in ant species richness reinforces the intermediate disturbance hypothesis (Connel 1978).

Recovery with six years (R6) did not fit the intermediate disturbance hypothesis, possibly due to the change in the use of plant species from “capim gordura” to “braquiária” grass, which provide others conditions and resources for ants. The high species richness in the recovery area with eight years (R8) may be explained by its proximity with a control area. It is possible that this fact led to ant re-colonization in R8 at the same level, in relation to the number of species, of controls areas which may had been a source of ant species for this area. But it is important to point out that control areas showed a higher number of seed-removing ant species than all recovery areas, corroborating with Ribas et al. (2012c) who already recorded this pattern in Cerrado control sites.

The opposite was observed for removal rates, during rainy season, where youngest areas showed higher rates than others recovery areas, but not higher than control ones which contains more species for removing seeds. This can be explained by the presence, in R2, R4 and R8 areas, of *Solenopsis invicta* with its dominance and aggressive behavior, having large recruitment and foraging, and

increasing its abundance in disturbed habitats (Wickings & Ruberson 2011). So, it is possible that they were able to remove a large number of seeds in these areas.

Otherwise, during dry season, the oldest recovery areas showed higher rates than youngest ones, which may be explained by the presence of more developed vegetation. In dry season, arboreal ants present in this vegetation can forage in epigeic microhabitat, increasing the removal rates, since there is a small amount of resources in trees in this season. Coupled to it, in this season we also collected in R8 and R10 a larger number of species belonging to *Pheidole* genus than in youngest areas, which is documented to be a seed collector and to recruit a large number of ant workers for this activity (Bieber et al. no prelo), increasing the removal rates in these areas.

After 10 years of recovery, seed-removing ant species composition still pointed differences in relation to control areas in both seasons. Such result corroborate with Lomov et al. (2009) that showed that 10 years of recovery presents a seed removing ant composition different from forest remnants . Domínguez-Haydar & Armbrrecht (2011) met an ant assemblage similar to forest habitats with 14 years of recovery after mining, indicating that more time is necessary for evaluating ant assemblage composition. This shows that the impact caused by mining creates a great disturbance and that ant species composition did not recover for a long time. Ant species like *Odontomachus chelifer*, *Pachycondyla striata* and *Gnamptogenys* sp.1 were restricted only to control areas and could be responsible for this difference. Some seed-removing ant species are restricted and specific to undisturbed habitats due to physical differences in vegetation structure (Grimbacher & Hughes 2002).

During dry season we found differences on the species composition between R2 and R8 recovery areas, indicating the effect of the proximity between R8 and control areas. Thereby, we can note that control areas functions

as a source of species to R8 since in this recovered area we observed the presence of ant species documented to prefer Cerrado preserved, like *Acromyrmex* spp. (Ramos et al. 2003). This shows that the proximity with natural habitat may be more important than the age for evaluating the recovery process, whereas we did not find differences on the seed-removing ant composition between R2 and R10.

However, during rainy season, the only different recovery area with respect to ant composition was R4, what may be explained by the presence of pioneer and intermediate ant species in this stage of recovery (as previously mentioned), causing its distinction with areas that harbor ant species tolerant to open or shadow habitats. It is also important highlight that R4 and R6 were different in relation to ant species composition in both seasons. The change of species grass used in the recovery (above cited), must has caused this difference indicating the influence of different techniques for the recovery process.

#### **Recovery techniques: native and exotic plant species**

The ant species richness with exotic species was higher than native species and “Campo Sujo” control (CS). Exotic species are many times judged a threat for native ecosystems, being able to alter interspecific interactions. But otherwise these plants provide habitat, increase structure complexity supplying resources and conditions for increasing species abundance and richness, important points in restoration programs (Schlaepfer et al. 2011). The use of native species plant also showed a good performance because its “Campo Rupestre” control area had not any seed-removing ant species. Indeed the greater species richness with exotic species explains the rates of seed removal being higher in these areas than in areas recovered with native species and control areas in both seasons.

We did not find difference on the species composition between recovery areas with exotic and “campo sujo control”, neither recovery with native species and its control area during dry season. The similarity during dry season can occur because some ant workers may migrate for areas with more developed vegetation, like that present in natural habitats, which offers better conditions and more resources, even during dry season. The proximity of recovery with native species area with one “campo sujo” control area possibly contributed for this migration and consequently similarity on the ant species composition. This indicates again the importance of the proximity with natural habitats for ant species composition in recovery process, such as observed in recovery with different ages.

During rainy season, ant species composition of exotic plant species area remained similar to control area, pointing to an efficient use of exotic plant species for recovering areas in the “campo sujo” domain. But the recovery with native plant species had an ant species composition different from exotic species area and “campo sujo” control. The ants of the native recovery may not migrate to another areas looking for resources and conditions, finding such attributes in its own territory.

#### **Recovery techniques: “capim gordura” and “braquiária” exotic grasses**

Recovery with different exotic grasses does not affect the number of ant species present on the area. This fact draws attention to what, for human perspective, seems to affect the re-colonization by ants, but in reality has no difference for ants. This re-colonization shows the resilience of ants to mining impact, even using different plant species. Maybe in this case, the most important is if these areas will develop any other vegetation for providing resources and conditions to ants establish and grow their colonies. Ribas et al.

(2003) and Campos et al. (2006), for instance, recorded the positive influence of habitat complexity on ant community.

Higher removal rates in recovery with “capim gordura” than with “braquiária”, in both seasons, indicates that the type of recovery may influence the ant species abundance. Increased abundance favors the increase of seed removal due to a large number of ant workers (Dominguez-Haydar & Armbrrecht 2011).

We detected difference on the seed-removing ant composition among recovery with two exotic grasses and control areas in both seasons. These difference were expected, because the type of recovery (here with different exotic grasses) can lead to more or less heterogeneous environmental, causing difference on the ant species composition (Ribas et al. 2012c). The difference of these two types of recovery with the control was also expected, because exotic species cause changes on the soil conditions, quantity and quality of litter and become a habitat more susceptible to invasion and fire (Funk et al. 2008). Almeida et al. (2011) also observed, in Cerrado, that an exotic grass modifies environmental conditions and affect the local species composition.-

#### **Recovery techniques: upon sterile material and upon mining site**

Seed-removing ant species richness responded different to recovery upon sterile material and upon mining site, being higher upon mining site. Recovery upon mining site also showed ant species richness higher than “campo sujo control”, indicating to be the best option as recovery technique than upon sterile material. The type of uppermost layer topsoil used for recovering areas will define the soil microbial community. This community is responsible by decomposition processes and quality of organic matter in soil, that are important compounds for ants which have its species richness correlated with soil microbial biomass in recovery areas after mining (Andersen & Sparling 2008).

However, as mentioned in the last topic, the type of technique also can affect the ant species abundance explaining the higher rate of seed removal in recovery upon sterile material than upon mining site, during dry and rainy seasons.

In relation to ant species composition, in both seasons, recovery upon sterile material and upon mining site was different of “riparian forest” and “campo sujo” control areas respectively. Independent of season, topsoil handling has been considered an essential prerequisite for a good growth of plant species and subsequent natural regeneration (Parrota & Knowles 2001). Moreover, natural habitat is more complex structurally, offering a larger amount of resources and conditions adequate for the establishment of an ant assemblage more diverse than in recovery areas (Costa et al. 2010, Neves et al. 2010).

### **Environmental Variables**

Environmental variables can be used for explaining changes on the seed-removing ant species composition (Dominguez-Haydar & Armbrrecht 2011; Grimbacher & Hughes 2002). Independent of season, canopy cover was the most important environmental variable, influencing the ant species composition in all recovery areas, because it is associated to soil exposition and, consequently, to microclimate conditions. This variable already has been cited as influencing soil surface humidity and temperature, affecting survival and reproduction of insects (Gries et al. 2011). Such relationship becomes more delicate during dry season in which the lack of leaves causes habitat structural simplification, decrease humidity and shadow levels and increase temperature (Neves et al. 2010). The opposite occur during rainy season providing better conditions and quantity of resource for ants, confirming this variable as a major factor for ant diversity (Schmidt et al. 2013).

Except in recovery with native and exotic species, in all others recovery areas, diameter and height of trees were important variables influencing the

species composition in both seasons, because they are associated with plant growth and crown development. Larger plants provide crown architecturally more complex (with all leaves) increasing available resources and better microclimatic conditions for ants (Campos et al. 2006a). This can have contributed for the influence of these variables on the species composition in recovery with native and exotic plants only in rainy season.

Again, except in recovery with exotic and native species, the density of trees is important to species composition independent of season in all recovery areas. The increase in tree density will increase availability of resources and modify conditions for the successful establishment of ants. Environments with higher tree density offer more potential niches and microclimatic conditions more appropriate for ant communities (Vasconcelos et al. 2000; Ribas et al. 2007). Moreover, higher tree density means more root penetration and water retention (Herath et al. 2009), very important for the establishment of ant nests.

In recovery with different ages and with the two types of exotic grasses, diversity of litter was also a relevant environmental factor, independent of season. Litter is the main local of foraging of seed-removing ants. High diversity of litter provides a greater number of suitable microhabitats and new nesting sites for ants, and this can be responsible by distribution of ant species (So & Chu 2010). Moreover, disturbance able to alter the litter causes changes in soil chemical characteristics, place where ants construct their nest (Sousa-Souto et al. 2012).

During dry season, weight of litter had importance to species composition in recovery with native and exotic species and upon sterile material and upon mining site areas. Litter layer may improve the environmental conditions making the soil less exposed, and able to store seeds during this season (Dias et al. 2012). During this season the fall of leaves and twigs increase the weight of litter, and provide more available niches for ants. Grimbacher and

Hughes (2002) showed that litter characteristics, in general, affect the seed removing ant composition.

Finally, the understory was important to explain species composition in all recovery areas during rainy season, because it is related with more structurally complex habitats, which affects positively the rates of ant colonization (Bisevac & Majer 1999). Habitat more heterogeneous harbor larger diversity of ant species (Campos et al. 2006). Moreover, in this season, the leaves are present, increasing physical contact among plants, facilitating the displacement of ants through plants, and thus contributing for its distribution.

### **Indicator species**

Some seed-removing ant species, as *Pachycondyla striata* and *Ectatomma edentatum*, were indicator of control areas and these ant species was already documented as indicators of forest and less altered habitats (Schmidt et al. 2013; Ribas et al. 2012c), and in events of myrmecochory (Pizo & Oliveira 1998). *Pheidole* spp. was an indicator genus of many sites in our study, due to its wide distribution and frequency on the sampling. This genus was also registered removing seeds in rehabilitation sites and forest remnants (Dominguez-Haydar & Armbrecht 2011). *Solenopsis invicta* is an aggressive and territorial species that inhibits the presence of other ant species (Epperson & Allen 2010), and may indicate incomplete rehabilitation (Ribas et al. 2012c). This species was found in recovery with native species indicating that the process of recovery in this area must be accompanied by more time.

*Nylanderia* sp.1 was already documented in anthropogenically-disturbed habitats and localities with non-native plants (Ivanov et al. 2011). It is worrying because this ant species was also found in this study as indicator of a recovery area with native plant species, showing that: or the recovery process is not complete yet or it is being done improperly. Some ant species belonging to

*Camponotus* and *Pachycondyla* genera were documented as indicator of open sites and in an recovery gradient (Vasconcelos et al. 2000; Schmidt et al. 2013), like in our study.

### **Implications for practice**

Seed removal by ants can be considered when evaluating the recovery process, linking information about ant assemblage and habitat structure. Information about seed removal by ants effectiveness must include quantitative (rates of seed removal) and qualitative (ant species richness and composition) factors. Indeed for achieving the short-time goals in monitoring recovery programs one should focus in seed-removing ant species, which are a fast, efficient and cost effective way to obtain information about quality and effectiveness of recovery actions. Moreover, seed removal by ants is one main process driving plant population ecology which promotes plant distribution and establishment. For this, evaluation of seed removal is an accessible metric to assess recovery process, and may be used in monitoring recovery programs.

During the recovery process, the ages of sites must be considered for successful evaluating the recovery. Even 10 years of recovery seems to be insufficient to correct drastic changes in ant assemblage, which showed composition and rates of seed removal different of natural forests (control areas). The use of native plant species in the recovery sites is the better option, but the plantation must be done after a detailed study about natural forests in the region and about the interaction between native plant species and the local fauna. Use of plant species attractive for ants is a good method, because this interaction has the ability of become recovery efforts more sustainable in a long-term. Determination about the type of layer topsoil is also a critical factor for ants and

effectiveness of recovery process. Our results showed that recovery upon mining site is better than upon sterile material.

For choosing about which technique to use, it is very important to think about those that will provide a greater vegetation cover, diversity of litter and size of trees. These attributes are related with seed removal ant species composition and removal rates by them at a high percentage, factors which may benefit further recovery efforts. However it is extremely important to think about the use of exotic species in recovery programs, because it can disorganize ant assemblage and inhibit the presence of native ants.

Finally, we highlight the importance of ecological function of seed removal by ants as a powerful tool of bioindication in monitoring recovery programs. Analyzes about the quality of recovery efforts using habitat structure and ecological function offer a more accurate and efficient knowledge about environments in recovery process.

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## CONCLUSÃO GERAL

A mineração, através da remoção completa da vegetação e da camada de solo, tem consequências negativas no habitat e na assembleia de formigas. Assim, a função ecológica de remoção de sementes desempenhadas pelas formigas também se torna comprometida por essa atividade antrópica. Tal fato é extremamente preocupante, pois a mirmecocoria (remoção de sementes por formigas) é um evento crucial para o recrutamento, distribuição espacial e diversidade de plantas no ambiente. Isso mostra a necessidade de recuperação das áreas que estão ou sofreram impacto pela mineração.

Na avaliação envolvendo o processo de recuperação, informações sobre a estrutura física do habitat e funções ecológicas, como a remoção de sementes por formigas, fornecem informações mais precisas sobre a qualidade do habitat e a efetividade desse processo. Fatores como a idade e as técnicas de recuperação são essenciais para o monitoramento do sucesso de esforços de recuperação. Esse trabalho detectou que 10 anos não são suficientes para reparar os danos na assembleia de formigas, sendo necessário um maior tempo de implantação da recuperação para uma análise mais eficaz. Dentre as técnicas de recuperação, aquelas com espécies de plantas nativas e a recuperação sobre área de mina (cava) mostraram melhor desempenho como técnicas eficientes no sucesso da recuperação. É necessário cautela para o uso de espécies de plantas exóticas, pois essas podem desestruturar a assembleia de formigas e impedir a colonização por espécies de plantas nativas, comprometendo o sucesso da recuperação.

Por fim, a especificidade e a fidelidade das espécies de formigas removedoras de sementes a um determinado tipo de habitat classificam-nas como indicadoras ambientais no processo de recuperação. Além disso, a remoção de sementes por formigas em combinação com a estrutura física do

habitat é uma ferramenta eficaz de bioindicação em programas de monitoramento em áreas após impacto pela mineração.