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# Does fipronil application on roots affect the structure of termite communities in eucalypt plantations?

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ABSTRACT

Fipronil is an insecticide used for termite control in forest plantations. Although pest species need to be restricted, the effects on non-target termites are unknown. The effects of fipronil application on the termite community composition, species richness and functional group diversity were assessed in four different treatment plots (new eucalyptus plantations respectively treated and untreated with fipronil; naturally regenerating secondary forest and the parent native Brazilian savanna) at three different times (90 days before and 90 and 180 days following) clonal seedling planting. A total of 53 termites species belongs to families Termitidae and Rhinotermitidae were collected. The mean richness of termite species differed significantly among the treatments and times. Grass feedings showed the highest overall richness in the plot under natural regeneration 90 days before planting. Soil feedings had higher richness in the control treatment (native savanna) and in the plot under regeneration at 180 days after planting. No significant differences in richness of wood feedings termites were detected in any comparison. Termite assemblage composition in the new eucalyptus plantations did not differ between the fipronil-treated and fipronil-untreated plots.



## 1. Introduction

Plantation forestry with *Eucalyptus* and *Pinus*, is an important economic activity in several regions of Brazil, reducing pressure on natural forests (Azevedo et al., 2011). However, such monoculture simplifies the natural environment, creating a homogeneous agroecosystem composed of one or at most a small number of species of economic value (Murray et al., 2009).

Evidence has accumulated throughout the humid tropics that termite species richness and assemblage structure can respond to even small changes in habitat conditions, including those imposed by human disturbance and conservation management. Such changes are reported in Brazilian savannas (Cunha and Silva Orlando, 2011; Dosso et al., 2012) and rain forests (DeSouza and Brown, 1994; Ackerman et al., 2009). Illustrating the sensitivity of termites, Vasconcellos et al. (2010) found a significant difference between areas of caatinga habitats with different levels of disturbance caused by the walking activities of cattle and goats.

Structurally complex habitats may provide more physical niches and a greater diversity of resources, thus supporting greater species richness (Stein et al., 2014), although this relationship is not always linear (Allouche et al., 2012). A decrease in habitat heterogeneity can lead to reduced availability of resources, changes in environmental conditions and eventually to loss of species and as a consequence the associated ecological functions (Kerr and Packer, 1997). Termites and ants are considered among the most important insects in tropical forest ecosystems (Luke et al., 2014), often at high density and biomass, and frequently described as ecosystem engineers (Bignell and Eggleton, 2000). The functional roles of termites include the distribution (and redistribution) of organic matter and soil particles, and the promotion of increased aeration and soil water infiltration by tunneling and gallery construction (Lavelle et al., 1997; Jouquet et al., 2011). However, despite these benefits to the ecosystem, a few species are nevertheless important pests of eucalyptus, causing large-scale damage (Sales et al., 2010; Rouland-Lefèvre, 2011).

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Fipronil is non-repelent, a broad-spectrum insecticide, which has proven effective in reducing populations of subterranean termites (Ibrahim et al., 2003). Fipronil works by contact and ingestion, acting in insects by blocking the gamma-aminobutyric acid receptor (GABA) in the chloride channels of neurons, interrupting the central nervous system activity and consequently causing the death of the insect (Cole et al., 1993). However, in Brazil, the Forest Stewardship Council (FSC), charged with meeting international certification requirements for forest products, prohibited the use of fipronil in all certified plantations. This decision was based on fipronil bioaccumulation and its high oral toxicity (FSC, 2007), reflecting studies in other continents, where soil and climatic conditions differ, and both plant and termite community structures are quite dissimilar (Hayasaka et al., 2012). Fipronil is formulated to create a chemical barrier around the plant above ground, inhibiting boring or consumption by insects (Wilcken et al., 2002). In forestry this is presumed to protect seedlings against the small number of termite species, which can attack young living trees but non-target species may be vulnerable if the insecticide preparation makes contact with the soil (Manzoor et al., 2012).

Thus, there is a need to evaluate the effects of fipronil on non-target species to assess the validity of management decision to prescribe it. Additionally, there is no work on the impact of the treatment on seedlings in the nursery setting or during early plantation growth. This study evaluates the effect of the application of fipronil in roots on the termite community in eucalyptus stands in the Native Brazilian savanna vegetational zone. We tested the hypotheses that: (i) the total richness of termites species and functional groups is negatively influenced by fipronil application and therefore differs between treated and non-treated plots (in the present study plantations); (ii) the composition of the termite assemblage changes with time after clear felling in treated sites and therefore differs between regenerating secondary forest and native Brazilian Savanna.

## 2. Materials and methods

### 2.1. Study area and experimental design

Our study was conducted in eucalyptus plantations established in areas of originally Native Brazilian savanna, in the municipality of João Pinheiro (17°56′49.1″S, 46°05′50.5″W), Minas Gerais State, in southeastern Brazil. The average annual temperature is 24.3 °C and annual rainfall is 1485 mm. The climate of the study area is humid tropical savanna (Aw Köppen classification), with a dry winter from April to September and rainy summer from October to March, 2007. The soil type study area was predominantly sandy.

Four different treatment plots were used, Native regeneration forest (REF), Native Brazilian savanna (NBS), eucalyptus seedlings with fipronil in roots (EWF) and eucalyptus seedlings without fipronil (ENF) (Table 1). In booth eucalyptus plot clonal seedlings of the hydrid *Eucalyptus urophylla* ST Blake (Myrtales: Myrtaceae) were used. In EWF treatment before plating, clonal seedlings were immersed for 30 s in fipronil insecticide solution (800 WG) at a concentration of 0.4%. Routine management practices included chemical fertilization with nitrogen, phosphorus and potassium macronutrients; herbicide (glyphosate), gypsum (phosphorgypsum) applications and 3 × 2 m eucalyptus spacing were used. Sampling took place 90 days before the eucalyptus planting with or without fipronil and at 90 and 180 days after this planting, and, on the same dates the REF and NBS areas were also collected.

### 2.2. Termite sampling

Termites were sampled in two plots per treatment, using three transects of 20 × 2 m per plot, totaling six transects per treatment. Transects were at least 50 m from each other and the same distance from the edge of the plot. Each was divided into four 5 × 2 m sections (10 $m^2$), to give a total sampling area of 960 $m^2$/transect (Jones and Eggleton, 2000).

Each transect section was sampled by two people for 30 min, making a total sampling effort of four person-hours per transect (Jones and Eggleton, 2000). Termites were collected by manual searching of litter, twigs and fallen logs, under the bark of trees, in galleries on the trunks of live or dead trees, on the surface of the ground, and by hand excavations to approximately 30 cm depth. Specimens were preserved in 80% alcohol and separated into morphospecies following Constantino (1999), at the Forest Entomology Laboratory of the Department of Entomology at the Federal University of Lavras (UFLA). Formal identifications of species were confirmed by Dr. Reginaldo Constantino, in the University of Brasília (UNB). Voucher specimens were deposited in Section Isoptera of the Department of Zoology at UNB and the Laboratory of the Forest Pest Department of Entomology at UFLA. The termites were allocated to four functional groups following Constantino (2005), based on presumed feeding habits: (1) wood feeding, termites that consume living, freshly dead or moderately decomposed wood; (2) Soil feeding, feeding on humus, generally found only in the soil column; (3) grass feeding, termites feeding on living plants or leaf litter; (4) intermediate, species that are not clearly defined into any of the other groups (Calderon and Constantino, 2007).

### 2.3. Data analysis

We considered the transect replications as n = 6. The average number of species for each transect was fitted to general linear models (GLM) using the Poisson distribution. The treatments were compared by analysis of aggregation levels and comparison of variances. This assessed the differences in total species richness and functional groups of termites in the four treatments at each sampling time. Analyses were performed using the software R 3.2.1 (R Development Core Team, 2015).

To test whether the termites species composition varied within the treatment at each sampling time (90 days before seedling planting, 90 days after and 180 days after), we used a non-metric multidimensional scaling (NMDS) ordination, which visualizes patterns within the data. This was done using root square transformed and standardized relative abundance, employing a Bray-Curtis

**Table 1**
Description of the four treatments where termites were sampled in the municipality of João Pinheiro, Brazil.

| Treatment | Parent vegetation | Plantation regime | Fipronil application | Area (ha) |
|---|---|---|---|---|
| (ENF) | Eucalyptus plantation | Seedlings from nursery | No | 25.47 |
| (EWF) | Eucalyptus plantation | Seedlings from nursery | Yes | 25.47 |
| (REF) | Regenerating forest | Naturally regenerating secondary | No | 30.00 |
| (NBS) | Native Brazilian savanna | None | No | 30.00 |