

# Association of Ant Predators and Edaphic Conditions with Termite Diversity in an Amazonian Rain Forest

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richness, and species composition of termites. We constructed a set of statistical models to tease apart the association of termites and ants from their simultaneous association with environmental variables. We also compared the association of termites and ants with a null expectation based on random predation. These analyses suggest that termite abundance and termite species richness are more strongly associated with the density of predatory ants than with measures of vegetation and soil chemistry. In addition, there was little evidence at the community level for non-random predation of termite species by ants.

## METHODS

**STUDY AREA.**—Sampling was conducted between December 2008 and May 2009 at Reserva Ducke (3°05'S, 60°00'W), a tropical forest reserve of 10,000 ha in central Amazonia, Brazil (Figure S1). Elevation within the reserve varies from 39 to 110 asl (PPBio 2009), with a moderate decrease in soil nutrient content along this gradient. The vegetation consists of relatively uniform dense evergreen tropical rain forest (terra firme forest; Chauvel et al. 1987) that is not subjected to periodic flooding (Hopkins 2005). The leaf litter depth varies among transects, but is typically ~20 cm, and the undergrowth is dominated by palms in the genera *Astrocaryum* and *Attalea* (Chauvel et al. 1987, Ribeiro et al. 1999). There is no record of logging or burning in this area, and a total of 1200 tree species have been recorded (see <http://ppbio.inpa.gov.br> for more information).

**SAMPLING DESIGN AND DATA COLLECTION.**—In 1998, a permanent array of 9 N–S and 9 E–W perpendicular trails was established in the reserve as part of the Program on Biodiversity Research (PPBio) of the Brazilian government (Magnusson et al. 2005). The PPBio survey strategy aims to make the sampling effective and efficient for a diversity of taxa from soil invertebrates to canopy trees (Magnusson et al. 2005). The minimum distance between the trails and the forest edge is 1 km. The trails allow access to a 5 km × 6 km grid of 30 transects, with 1-km spacing (Figure S1). Each transect is 250-m long and follows an elevation isocline to minimize variation in exposure and soil composition. Transects were established at least 10 m away from the nearest walking trail.

Termite sampling was performed using a modification of the standard sampling protocol developed by Jones and Eggleton (2000). To sample termites, we established 10 'sections' (5 m × 2 m) at 25-m intervals along each transect. Every section was actively searched for termites by three trained investigators for 20 min, yielding 1-man hour of search time per section, and 10 hours total for each of the 30 transects. We searched for termites in soil, leaf litter, rotting logs, and tree and shrub roots. However, nests higher than 2 m above ground level were not surveyed, and our results do not include termites living exclusively in the canopy. The upper layer of soil was completely dug down to a 50-cm depth or until the upper layer of humus was thoroughly searched. Most termite colonies were found in the soil, small branches, and inside dry leaves of *Astrocaryum* and *Attalea* palms. Termites were sampled in the wet season (December

2008) and in the dry season (May 2009), and the data were combined for analyses.

Termites were collected and preserved in 95 percent EtOH and were identified to genus using Constantino (1999). Individuals were then sorted to morphospecies and to species whenever possible by comparison with museum collections at the Federal University of Rio Grande do Norte and the National Institute of Amazonian Research (INPA), Brazil. For termites in the taxonomically problematic subfamily Apicotermittinae, we dissected worker guts for species identification based on diagnostic characters of the enteric valve (Noirot 2001), and compared our specimens with descriptions from Bourguignon et al. (2010). Voucher specimens from this survey were deposited in the Entomological Collection of the National Institute of Amazonian Research. Termite data are included in Table S1.

We analyzed termite community structure with predictor variables of ant density, tree density, and soil variables that were measured by other investigators for each transect. Ant data at the transect level were taken from Souza et al. (2012), who used pitfall traps, sardine baits, and litter samples extracted by the Winkler method. Sifted leaf litter samples of 1 m<sup>2</sup> surface area were collected from sampling stations located at 25-m intervals along the center line of each transect. Pitfall traps and sardine baits were placed at the same stations after litter collection, giving 10 sections for each method per transect (10 sections × 30 transects × 3 techniques resulted in 900 samples). Ants were extracted for 48 h from Winkler bags through a 1 cm<sup>2</sup> mesh sieve (Bestelmeyer et al. 2000).

The pitfall traps (95 mm diameter; 8 cm depth; 500 ml volume) were partially filled with water and detergent, buried with the rim at ground level, and left for 48 h. After removal of the pitfall traps, approximately 5 g of canned sardine was placed on a plastic card (10 cm × 7 cm) on the litter surface; after 45 min, all ants on the plastic card were collected and preserved in 90 percent EtOH. The baiting and litter-sampling were conducted between 0800 h

both ant predator and non-predator density as predictor variables. Ant density was quantified as the total number of ant nests detected per transect.

Tree data at the transect level were taken from Castilho et al. (2006), who measured the number of trees and palm trees per transect at breast height (dbh) using transects of 0.5-ha (20 m × 250 m) and 0.1-ha (4 m × 250 m) to sample trees with dbh of 10–30 cm and 1–10 cm, respectively.

From a previous survey, we obtained measures for each transect of soil phosphorus (mg/dm<sup>3</sup> of soil) and soil clay (%). Other variables were correlated in some degree with soil phosphorus and clay content, and their relation with termite community structure is shown in Table S3; Figure S3. These data are available at <http://ppbio.inpa.gov.br>. For the measurements of soil nutrients and texture, five soil samples were collected at a depth of 5 cm at 50-m intervals along each transect. The five samples from each transect were pooled for texture and chemical analyses. Before analysis, samples were cleaned of roots, air-dried, and sieved through a 2-mm sieve. Soil texture analyses were conducted at the Soil Laboratory of the Agronomy Department at INPA and chemical analyses at the Soil Laboratory of the Brazilian Enterprise of Research of Livestock and Agriculture, Manaus (Embrapa 1997).

**DATA ANALYSIS.**—In each transect, we counted the number of sections (0–10) in which a termite species occurred and treated these data as a measure of termite abundance. We quantified species diversity by using Hurlbert's (1971) Probability of an Interspecific Encounter (PIE; also known as Simpson's Diversity Index). The PIE index measures the probability that two randomly chosen individuals represent two different species. The PIE index is unbiased by sample size (Gotelli & Ellison 2012), and is an estimate of the slope of the individual-based rarefaction curve at its base (Olszewski 2004). We calculated the PIE index using the total abundance of each termite species recorded in a transect.

The dissimilarity in species composition among transects was measured by the Bray–Curtis dissimilarity index between all possible pairs of transects. We used the scores of the first two axes of a Non-Metric Multidimensional Scaling (NMDS; Faith et al. 1987) to summarize the changes in overall species composition among transects.

Using multiple regression models, we tested for the relationship between termite abundance, termite species richness, termite PIE, and termite species composition (response variables) versus ant predator density, tree density, and soil phosphorus and clay content (predictor variables). Because termite density and species richness represent count data and cannot take negative values, we used Generalized Linear Models (GLMs) with log-link functions, and a Poisson distribution of errors in the residuals for termite abundance and species richness. For the remaining analyses, we used multiple ordinary least squares regression models (OLS), which assume normally distributed errors in the residuals.

Ants and termites could both be affected by the same spatial and environmental variables, which could result in a spurious correlation between ants and termites that does not reflect a cause-and-effect relationship. Structural Equation Models (SEMs) can

be used to test for associations between variables, while controlling for potential confounding effects (Rosseel 2012). To disentangle the direct association of ant predator density with termite abundance and species richness from the simultaneous association of termite and ants with measured environmental variables, we created a set of Structural Equation Models (SEM). The models were created including direct and indirect links among soil phosphorus, clay content, tree density, ant predator density, and termite abundance and species richness. Finally, we also tested for the association of termite abundance, species richness, and species composition with the density of non-predatory ants. The results from the analyses using non-predatory ants are described in the Supplementary Material (Tables S4 and S5; Figures S4 and S5).

**PREDICTIONS OF EFFECTS OF PREDATION BY ANTS.**—To disentangle the potential effects of random versus selective predation of ant species on termite diversity, we examined the relationship between ant predator density and termite PIE.

If ant predators specialize on some termite species, ant predator density should be strongly associated with termite PIE (Figure S2, left and right panels). In contrast, if predators are generalists, ant predator density should not be strongly associated with termite PIE (Figure S2, middle panel). Although ant predators may reduce termite abundance, PIE will remain nearly constant when samples are randomly rarefied (Chao et al. 2014). The constancy arises because PIE is determined primarily by the relative abundance of the most common species in the assemblage, and these relative abundances are almost invariant to sample size effects.

As a further check, we rarefied Generaxtuassocipreda-

mite PIE or termite species richness are significantly smaller than expected by chance, whereas values  $> 1.96$  indicate that the observed values of PIE or richness are significantly larger than expected. If ants are selective predators on the rarest termite species, ants should lead to a reduction in termite PIE and species richness that is greater than predicted by the rarefaction curve (negative SES values). In contrast, if predators target the commonest termite species, ants should reduce termite species richness less than predicted by the rarefaction curve (positive SES values).

Before beginning analyses, we tested for potential collinearity of independent variables across the sampling grid by calculating pairwise correlations among all possible independent variables. Ant predator density, soil phosphorus, clay content, and tree density were not correlated with each other and were therefore used as independent predictors in the regression analysis. Among the remaining variables included in the supplementary analysis, only 20 percent of the pairwise comparisons were statistically significant ( $P < 0.05$ ), mostly for associations of nutrient concentrations and elevation (see Figure S3). These variables were combined with a principal components analysis (PCA), and the PCA scores were used as predictor variables of termite community structure. The results of regression and SEM analyses using these PCA axes as predictor variables are presented in Table S3 and Figure S3, respectively.

For both independent and dependent variables, there could be spatial trends or spatial autocorrelation in the grid of sampled transects. To detect spatial trends, we regressed each variable against the x- and y-coordinates of the sample grid. To detect spatial autocorrelation, we binned the data into 1.5 km distance classes and calculated Moran's I for each variable. None of these analyses were significant ( $P > 0.05$ ), so we used each transect within a grid as an independent sample in regression models.

All statistical analyses were performed in R (R Development Core Team 2013), using the *vegan* (Oksanen et al. 2008) and *lavaan* (Rosseel 2012) packages. The randomization functions and all the tests performed in this paper are available as an annotated

R script (Appendix S1). Termite data are publicly available at <http://gshare.com/download/le/1320575/1> under CC-BY licence.

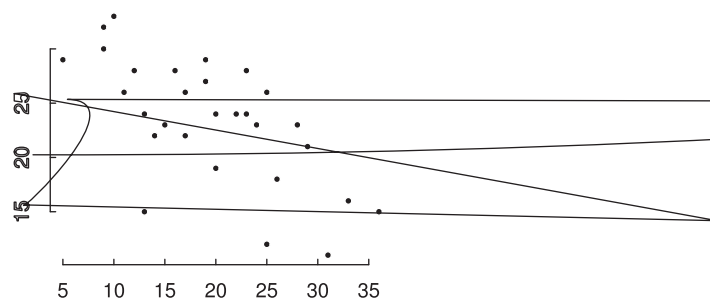
## RESULTS

Among the 30 censused transects, we recorded 702 termite occurrences and a total of 79 termite species. Ant predator density was negatively correlated with termite abundance ( $r = -0.66$ ;  $z = -3.92$ ;  $P < 0.001$ ; Fig. 1A) and termite species richness ( $r = -0.44$ ;  $z = -2.93$ ;  $P = 0.003$ ; Fig. 1B; Table 1) but was not significantly related to PIE ( $r = -0.15$ ;  $t = -0.80$ ;  $P = 0.42$ ; Table 1) or termite species composition (first and second ordination axes, respectively; Table 1; Table S3). The multiple regression models for termite abundance and species richness explained 49 and 32 percent of the variation in the data, respectively.

For PIE (Fig. 2A) and termite species richness (Fig. 2B), the declines in diversity with abundance matched those that would be expected with random predation by ants, based on rarefactions of the pooled termite transect data. Termite species composition (first ordination axis of NMDS) was correlated with soil phosphorus ( $r = 0.79$ ,  $t = 3.81$ ;  $P < 0.001$ ), and soil clay content ( $r = -0.75$ ,  $t = -2.82$ ;  $P = 0.009$ ; Table 1; Fig. 3). The density of trees per transect was weakly correlated with the PIE index of termite species diversity ( $r = -0.44$ ,  $t = -2.00$ ;  $P = 0.056$ ). The multiple regression model for PIE explained 26 percent of the variance. The explained variance for termite species composition was 76 and 13 percent for the first and second ordination axes, respectively.

Deviations from the rarefaction curve in termite PIE and species richness were not associated with any measured environmental variable ( $P > 0.07$ ; Table 1).

For both termite abundance and termite species richness, the Structural Equation Models (SEMs) indicated a much stronger effect of edaphic variables on ants (slope coefficient  $b = -0.40$ ;  $P < 0.001$  for soil clay content) than on termites



( $b = 0.25$ ;  $P = 0.30$  for soil phosphorus; Fig. 4A), and a strong direct effect of ant predators on termites ( $b = -0.43$ ;  $P = 0.001$  for abundance and species richness; Fig. 4). The SEM was able

The density of non-predatory ants was not associated with termite abundance and species richness (Table S4). The density of non-predatory ants alone explained 7 percent of the variance



random versus non-random predation by ants.

FIGURE S3. Structural equation models with regressions between the environmental variables, the density of ant predators and the termite abundance and richness.

FIGURE S4. Structural equation models with regressions between environmental variables, the density of non-predatory ants, and the termite abundance and termite species richness.

FIGURE S5. Structural equation models with regressions between the environmental variables, the density of non-predatory ants, and the termite abundance and richness.

APPENDIX S1. Annotated script on the termite analysis.

## LITERATURE CITED

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