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Dinâmica de Comunidades e Emissão de Metano por Cupins na Amazônia Central

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Manaus, Amazonas
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Dinâmica de Comunidades e Emissão de Metano por Cupins na Amazônia Central

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Tese apresentada ao Instituto Nacional de Pesquisas da Amazônia como parte dos requisitos para obtenção do título de Doutor em Ciências Biológicas, área de concentração em Entomologia.

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Sinopse:

Foi realizado um estudo de dinâmica de comunidades da fauna de cupins da reserva Ducke, correlacionando taxas de colonização e extinção das espécies com os padrões de beta diversidade espaciais. Realizamos estudos de emissão de metano por uma colônia e indivíduos de cupins. Adicionalmente, foi descrita uma espécie de cupim sem soldado.

Palavras Chave: Ecologia de Comunidades, Térmitas, Apicotermitinae, Isoptera.

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Dedico esta tese para minha mãe (Cleide),

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RESUMO

Os cupins, ou térmitas (Insecta: Blattodea:Isoptera) são insetos eussociais detritívoros, com espécies adaptadas para se alimentar ao longo de todo o gradiente de humificação. São extremamente abundantes nos trópicos e importantes engenheiros do ecossistema. Esta tese é composta em três capítulos. No primeiro, investigamos a contribuição relativa dos processos de colonização e extinção das espécies de cupins em criar e manter os padrões de beta diversidade espacial. Os cupins foram coletados em três (2008, 2009 e 2014) em 30 parcelas da Reserva Ducke. Depois, nós calculamos as taxas vitais (extinção e colonização) das 112 espécies encontradas correlacionamos com a posição média de ocorrência das espécies ao longo dos gradientes ambientais. Nós descobrimos que embora colônias de cupins morram e sejam substituídas ao longo do tempo, a colonização ocorre mais comumente em lugares semelhantes ao que as espécies já ocorrem($0.17 < r^2 < 0.40$). Por outro lado, as taxas de extinção não estão correlacionadas com o ambiente, exceto para fósforo em menor grau($r^2 = 0.28$), o que sugere que as diferentes pré-adaptações das espécies é crucial para que as espécies consigam se estabelecer em um ambiente, em especial nos estágios iniciais da colônia. Essa diferenciação mantém os padrões de beta-diversidade. No segundo capítulo, abordamos um dos aspectos que destaca os cupins dos demais insetos: a emissão de metano (CH4), proveniente da atividade metabólica de simbiontes que participam do processo da decomposição da celulose no sistema digestivo dos cupins. Em ambiente natural, a alta produção de CH4 desses simbiontes é contrabalanceada por bactérias metanotróficas que utilizam o CH4 como substrato para incorporação de biomassa, mais ativas em lugares de alta concentração de CH4, como cupinzeiros. Nós medimos uma emissão de of 9.97 CH4 nmol^{-1} s^{-1} de um ninho de *N. braziliensis*. Com base na biomassa da colônia, estimamos que a colônia produzia em torno de 36.58 CH4 nmol^{-1} s^{-1}, o que indica que três quartos do metano foi filtrado por ação de bactérias presentes no ninho e solo. Também reportamos a emissão por indivíduos de 14 espécies de cupins de um fragmento amazônico. Estes dados são importantes para melhor estimar a importância dos cupins no orçamento global de CH4. Por fim, descrevemos uma nova espécie de *Ruptitermes*, da subfamília Apicotermitinae, a menos conhecida taxonomicamente da região Neotropical.

ABSTRACT

Ttermites (Insecta: Blattodea:Isoptera) are eusocial detritivorous insects, with species adapted to feed along the entire humification gradient. They are extremely abundant in the tropics and important ecosystem engineers. This thesis consists of three chapters. In the first, we investigate the relative contribution of the colonization and extinction processes of termite species in creating and maintaining patterns of spatial beta diversity. Termites were collected in three periods (2008, 2009 and 2014) in 30 plots of Reserva Ducke. We then calculated the vital rates (extinction and colonization) of the 112 found species and correlated with the average position of occurrence of the species along the environmental gradients. We found that although termite colonies die and are replaced over time, colonization occurs more commonly in places similar to what the species already does ($0.17 < r2 < 0.40$). On the other hand, extinction rates are not correlated with the environment, except for phosphorus to a lesser degree ($r2 = 0.28$), which suggests that the different pre-adaptations of species are crucial for species to be able to establish themselves in an environment, especially in the early stages of the colony. This differentiation maintains the patterns of beta diversity. In the second chapter, we address one of the aspects that highlight termites from other insects: the emission of methane (CH4), from the metabolic activity of symbionts that participate in the cellulose decomposition process in the termites digestive system. In the natural environment, the high CH4 production of these symbionts is counterbalanced by methanotrophic bacteria that use CH4 as a substrate for biomass incorporation and are more active in places with a high concentration of CH4, such as termite mounds. We measured emission of 9.97 CH4 nmol^{-1} s^{-1} from a nest of *N. braziliensis*. Based on the colony's biomass, we estimated that the colony produced around 36.58 CH4 nmol^{-1} s^{-1}, which means that around three-quarters was filtered by the action of bacteria present in the nest and soil. We also report the emission by individuals of 14 species of termites from an Amazonian fragment. These data are important to better estimate the importance of termites in the overall CH4 budget. Finally, we describe a new species of *Ruptitermes*, from the subfamily Apicotermitinae, the least known taxonomically in the Neotropical region.

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INTRODUÇÃO GERAL

Os cupins, ou térmitas (Insecta: Blattodea:Isoptera) são insetos eussociais detritívoros, capazes de consumir substrato alimentar ao longo de todo o gradiente de humificação (Donovan et al., 2002) e extremamente abundantes em regiões tropicais, diminuindo em diversidade e biomassa em direção aos trópicos (Cereser et al., 2020). Em solos tropicais e subtropicais, os cupins são organismos-chave no funcionamento do ecossistema, pois ao modificarem o ambiente com a construção de bioestruturas como os ninhos e galerias, influenciam propriedades do solo, como fertilidade e dinâmica hídrica (Jouquet et al., 2016). Ao degradarem a matéria vegetal, são parte importante do ciclo de nutrientes desses ambientes (Holt e Lepage, 2000). Essa tese contém três capítulos, tendo em comum os cupins como objeto de estudo. No primeiro capítulo, estudamos a influência dos gradientes ambientais nas dinâmicas de colonizações e extinções locais de comunidades de cupins. Os cupins são um ótimo grupo para estudos ecológicos de monitoramento por serem abundantes, diversos e por suas colônias serem sésseis, além da influência que exercem no ambiente e a forma que respondem a ele (Brown, 1991). No segundo capítulo, abordamos um outro aspecto do grupo que os torna mais relevantes em um contexto mais amplo: o fato de serem um dos poucos grupos animais que emitem regularmente metano, embora indiretamente (por meio de simbiontes) com biomassa global suficientemente grande para ser relevante no contexto global de orçamento dos gases de efeito estufa (Kirschke et al., 2013). Nós medimos o quanto uma colônia de *Neocapritermes braziliensis* emite de metano em seu ambiente natural e estimamos o quanto é produzido e absorvido pelo ninho e solo, além de estimar a emissão por indivíduo para 14 outras espécies. No terceiro e último descrevemos uma nova espécie de *Ruptitermes* (Termitidae:Apicotermitinae), atualmente a subfamília menos conhecida taxonomicamente na região Neotropical (Bourguignon et al., 2016).

Primeiro capítulo

O primeiro capítulo dessa tese, intítulado “**Spatial turnover in termite species composition is maintained by differential colonization along environmental gradients in an Amazonian rainforest**”, lida com um tema corrente da ecologia: a mudança temporal de uma comunidade. Uma comunidade é definida como é um conjunto de organismos que compartilham o mesmo espaço-tempo (Vellend, 2010). No entanto, ela varia tanto no espaço quanto no tempo (Ontiveros et al., 2021). Embora os estudos de ecologia de comunidades tenham focado na importância do espaço e na dispersão de indivíduos e de espécies nas últimas décadas, o fator temporal ainda não está bem incorporado na teoria ecológica, apesar de um longo histórico de modelos teóricos sobre formação e estabilidade de comunidades (Gravel & Massol, 2020). De forma resumida, não entendemos ainda os mecanismos pelos quais as comunidades biológicas mudam e nem conseguimos quantificar estas mudanças (Mittelbach & McGill, 2019).

Para verificar o papel dos gradientes ambientais na forma como uma comunidade muda ao longo do tempo, nós estudamos a mudança temporal de uma comunidade de cupins de uma floresta amazônica central que foi coletada em três períodos: 2008, 2009 e 2014. Nós analisamos como a composição de espécies variou no tempo e calculamos as taxas vitais (extinção e colonização) das populações das espécies encontradas e as correlacionamos com os principais gradientes ambientais que estruturam a comunidade dos cupins. Esta não é uma abordagem clássica pois apenas recentemente há um acúmulo de dados de séries temporais de estudos de longa duração para que possamos fazer testagens de forma empírica (Ontiveros et al., 2018). Estudos de análise temporal são raros para invertebrados (Dornelas et al., 2014) e inédito para cupins, o que demonstra a relevância da investigação.

Nossas análises indicam que diferentes espécies de cupins estão adaptadas a intervalos diferentes dos gradientes ambientais demostrados, e que essa diferença tem efeito na fase de estabelecimento das colônias. Porém, uma vez que colonizam um local, a permanência das espécies não está associada aos gradientes ambientais. Estes resultados ajudam a entender a associação do turnover espacial e temporal, assim como a explicar como uma comunidade rica em espécies pode ser mantida em uma floresta tropical inalterada. As pré-adaptações de cada espécie a diferentes condições mantém o turnover espacial ao longo do tempo.

Segundo capítulo

Este capítulo intitulado “**Termites methane production and uptake: a case study for a Central Amazonia assemblage**”, é um estudo de métodos para o estudo da emissão de metano (CH4) por cupins. O CH4 é um dos principais gases do efeito estufa (GEE), que são gases que permanecem na atmosfera e absorvem e emitem a energia termal do espectro infravermelho, oriunda do reflexo da luz solar na terra. Estes gases causam um efeito análogo ao de uma estufa, pois a energia termal que normalmente se dissiparia fica retida no globo, aquecendo-o. O CH4 é particularmente importante por causa do seu poder radiativo, Muito maior que o do dióxido de carbono (CO^2) e pelo constante incremento na concentração de CH4 na atmosfera terrestre (Saunois et al, 2020). Por esse motivo, a concentração atmosférica de CH4 é monitorada globalmente e seus processos de geração e mitigação, estudados (Arias et al., 2021).

O CH4 pode ser emitido por fontes antrópicas ou naturais e é gerado através da ação de bactérias metanogênicas, que produzem grande quantidade de CH4 como resultado da conversão de alguns substratos em CH4 no metabolismo energético dessas bactérias (Whitman et al., 2006). Estas bactérias ocorrem em diversos ambientes hipóxicos e anóxicos, e são geralmente ligadas ao processo de decomposição da matéria orgânica (Kirschke et al., 2013). Alguns animais também emitem metano, mas não diretamente, uma vez que o metano é produzido por uma microfauna associada, presente no tubo digestivo desses animais (Hackstein, 2006). Grupos muito diversos coevoluíram para abrigar uma microbiota simbiótica que têm como um dos produtos do metabolismo a emissão do CH4. Porém dois grupos se destacam pelo alto grau de emissão de CH4 por esses animais, devido à grande biomassa global, que são os mamíferos ruminantes e os cupins (Saunois et al., 2020). Nos cupins, o sistema digestivo funciona como diminutos biorreatores extremamente eficientes (Breznak e Brune, 1994; Brune, 1998), organizado espacialmente em gradientes de metabólitos, oxigênio e hidrogênio, o que permite que eles abriguem comunidades de microorganismos com requerimentos ambientais diferentes. Nos cupins, a lignocelulose é decomposta por ação fermentativa de protozoários, nos cupins inferiores, e de bactérias, nos cupins superiores (Brune e Dietrich, 2015). Esse processo de fermentação gera compostos com uma única molécula de carbono e hidrogênio (H_2), que são utilizadas como substrato por Archaeae metanogênicas para incorporar biomassa, gerando ácidos graxos de cadeias curtas que são utilizados por seus hospedeiros e o CH4, que é emitido por difusão (Brune e Dietrich, 2015).

Essa grande produção de metano dos cupins é mitigada por bactérias metanotróficas (consomem CH4 para incorporar biomassa), que são naturalmente presentes no solo e cuja densidade e atividade é maior na estrutura do ninho e no solo adjacente, especialmente abaixo do ninho (Chiri et al., 2020). Para espécies australianas, cerca de metade do CH4 do metano produzido por uma colônia é filtrado pelo ninho e solo, sendo que para alguns casos, chega a 80% (Neuer et al., 2018). O desconhecimento desta relação entre o CH4 emitidos pelos cupins e assimilação do CH4 por bactérias metanotróficas é o principal impeditivo para uma estimativa mais precisa da contribuição dos cupins no orçamento global de metano (Neuer et al., 2018, Saunois et al., 2020). Outro problema é a distribuição geográfica dos estudos existentes sobre a emissão de metano por cupins: os estudos mais abrangentes foram realizados nos continentes africano, asiático e australiano. Os cupins são extremamente abundantes na região Neotropical, o que torna mais importante entender a importância deles no ciclo de CH4 desse ecossistema (Van Asperen et al., 2020). Atualmente, é a região onde a importância dos cupins no orçamento de CH4 global é menos compreendida (Kischke et al., 2013).

Nós reportamos a emissão individual de cupins de diferentes castas de 14 espécies de cupins, algo que era conhecido previamente apenas para uma espécie (Van Asperen et al., 2020). Também fazemos um estudo de caso para estimar a produção de CH4 de uma colônia de *Neocapritermes braziliensis*, uma espécie abundante na Amazônia Central (Pequeno et al., 2015; Dambros et al., 2017). Nós medimos a emissão do ninho in situ, em condições naturais, e depois a emissão dele extraído do solo. Depois, extraímos a biomassa de cupins e, com base nela, estimamos a produção total de metano da colônia. Nós medimos que o ninho em condições naturais emitiu 9.97 CH4 nmol^{-1} s $^{-1}$, enquanto que o ninho extraído do solo emitiu 17.71 CH4 nmol^{-1} s $^{-1}$. A biomassa seca da colônia foi de 70.53g, da qual estimamos que a colônia produzia 36.58 CH4 nmol $^{-1}$ s $^{-1}$. Desse resultado, estimamos que a estrutura do ninho filtrou metade do metano produzido, e o solo adjacente filtrou um quarto. Desse modo, apenas um quarto do metano produzido foi emitido na atmosfera.

Terceiro capítulo

Neste capítulo, "***Ruptitermes melanocephalus*** **(Termitidae: Apicotermitinae), a new soldierless termite species from Amazonian Central Rainforest**" nós descrevemos uma espécie de cupim sem soldado (Apicotermitinae) de um gênero recém revisado (Acioli & Constantino, 2015). Esta é a subfamília menos conhecida taxonomicamente na região Neotropical, apesar de ser abundante e diversa, compondo em torno de um terço da diversidade amostrada em estudos ecológicos (Davies, 2002 ; Ackerman et al., 2009; Bourguignon et al., 2011; Palin et al., 2001; Dambros et. Al., 2017. Os estudos taxonômicos dos Isoptera tradicionalmente utilizaram a casta dos soldados para a descrição e diagnose dos táxons. Isto se deve ao fato dos soldados e alados possuírem maior distinção morfológica interespecífica, ao passo que operários de diferentes espécies se assemelham entre si de uma forma geral(Constantino, 2001).

Durante décadas, houve poucas publicações de novas espécies (Matthews, 1977; Fontes, 1982). Um esforço mais consistente para a resolução do grupo recomeçou com Bourguinon et al. (2010), com a redescrição da espécie-tipo, duas espécies comumente encontradas e descrição de uma nova espécie. Em 2016, Bourguinon et al. (2001) publicaram cinco novos gêneros com chave diagnóstica, avançando os estudos com o grupo. Novos trabalhos vêm sendo publicados regularmente (Acioli e Constantino, 2015; Scheffrhan et al., 2017; Castro et al., 2018; Constantini et al., 2018; Florian et al., 2019, Castro et al., 2020), com destaque para Constantini (2018), que em sua tese fez um esforço semelhante ao de Sands (1978) com os Apicotermitinae do continente africano, revisando a subfamília e redescrevendo espécies quando necessário e descrevendo 13 novos gêneros e 20 novas espécies para a Mata Atlântica. Atualmente são reconhecidos 14 gêneros e 54 espécies válidas (Constantino, 2019), além das descritas por Constantini (2018) que ainda aguardam publicação em periódicos.

Durante o desenvolvimento desta tese, exemplares de mais de 300 colônias de Apicotermitinae foram dissecados e tiveram suas válvulas entéricas fotografadas. Apenas para Maracá, Roraima, foram registrados 34 morfotipos. Porém, a situação atual do grupo não justifica um único trabalho com a descrição de um grande número de novos táxons, sem um alinhamento com outros especialistas que atualmente trabalham com o grupo. Dessa forma, os novos táxons serão publicados futuramente levando-se em conta esses fatores.

OBJETIVOS

- Verificar como as taxas vitais (colonização e extinção) de 112 espécies de 30 assembleias de cupins da Reserva Ducke ajudam a criar e a manter os padrões de beta-diversidade espacial;

- Medir a emissão de metano para a atmosfera produzido por uma ninho de *Neocapritermes braziliensis* e estimar o total produzido pela colônia e o quanto de metano é filtrado por bactérias metanotróficas;

- Reportar a taxa de emissão de metano de indivíduos de diferentes castas de uma assembleia de um fragmento amazônico;

- Descrever uma espécie nova de Apicotermitinae, do gênero *Ruptitermes*.

CAPÍTULO I

Alves-Oliveira, J.R., Azevedo, R.A., Morais, J.W., Franklin, E. & Dambros, C.S. **Differential colonization rates maintain beta diversity along environmental gradients**. *Manuscrito a ser enviado para Oikos Journal*.

Differential colonization rates maintain beta diversity along environmental gradients

Abstract

Species replace each other along gradients (beta diversity) and theoretical studies show that this spatial replacement result from the historical processes of colonization and extinction. However, few empirical studies have evaluated the relative contribution of these processes in creating and maintaining beta diversity. To measure the impact of differential species colonization and extinction on beta diversity, we investigate the temporal dynamics of colonization and extinction of termite assemblages along gradients of ant predator density, soil phosphorus, and altitude. Colonization and extinction rates were measured for each of the 112 species found using multi-season occupancy models. Although termite colonies die and are replaced over time within plots, species composition is relatively constant over time in the 30 studied assemblages. However, there are substantial differences in species composition across sites, which can be explained by the differences in species colonization along all measured gradients ($0.17 < r^2 < 0.40$). In contrast to colonization, extinction was relatively constant in space and only associated with soil phosphorous ($r^2 = 0.28$). Our results suggest that once a species has established in a given environment, it is likely to build a fortress, obtaining the necessary resources to persist even in the presence of predators. Species early colonization is likely the most decisive phase in the life cycle of a species. Species differentiate from one another in their ability to colonize a given biotic or abiotic environment, and this differentiation determines the structure of whole communities.

Keywords: Niche Theory, Species-Environment Relationship, Species composition, Metacommunity, Community assembly.

Introduction

Species spatial distribution has always been one of the focal points of community ecology. During the community ecology development, several well-known distribution patterns, such as the species-area relationship (Rosenzweigh 1995; Drakare et al. 2005) and the species turnover along environmental gradients (Whittaker 1967; Robroek et al. 2017) were extensively registered and studied. Those spatial configurations are the result of interactions between species and the environment through time. Despite a substantial theoretical body concerning community assembly, temporal community analysis received less attention, in part because comprehensive spatiotemporal community datasets only recently have become widely available (Dornelas et al. 2018). While analysis of snapshot community data (single season) allows measuring how species are distributed along gradients (pattern), temporal data reveal the process of assembly dynamics (Jabot et al. 2019); i.e. how colonization and extinction history created species beta diversity.

Each species carries a set of life story traits that makes them more or less pre-adapted to occupy any given place. The importance of those differences is well-known for temporal turnovers, such as plants with drought-resistant seeds having a higher chance of early colonization and plants with shade-resistant seeds having a higher chance of colonization in mature forests (Rees et al. 2001). However, patch dynamics are tough to evaluate in ecosystems near equilibrium, such as mature forests. In these ecosystems, the relative importance of colonization vs. extinction is much less precise than when comparing distinct habitats (Breugel et al. 2018), although the spatial turnover in plant species composition can be notable (Costa et al. 2005). Such habitats do not have clear dispersal barriers or habitat differences that could create colonization/establishment trade-offs.

Although it is clear that species differ in their adaptations to the environment, it is not clear if species distribution patterns emerge from differences in their colonization capacity or if they differ in their competitive capacity for space/resources once established. Besides preadaptations, factors such as the quantity of resources (Dallas et al. 2020), propagule pressure (Alzate et al. 2020), and the number of immigration events (Koontz et al. 2018) are important for a species to successful colonize an area against established competitors (Alzate et al. 2020). However, once established, species may resist extinction even if the conditions become unsuitable due to prolonged individuals' survival and population and metapopulation dynamics (Figueiredo et al. 2019). Species turnover along environmental gradients may be caused by colonization-extinction trade-offs or differential colonization, and extinction rates among species along environmental gradients create these spatial patterns. Analyze vital species rates (colonization and extinction) that vary according to the environment (Yakulic et al. 2015) could unveil which process is determinant in species distribution.

Termites are key soil organisms in tropical and subtropical soils, performing as engineers creating biostructures, influencing soil properties, soil fertility, and water dynamics (Jouquet et al. 2016). Their importance to decomposition process can be compared to those of the earthworms in the tropics (Bignell 2020), but differently of earthworms, termites can feed on a large range of the organic matter decomposition gradient, from sound wood to bare soil, making them essential to nutrients cycling in tropical areas (Donovan et al. 2001, Bourguignon et al., 2011?). Previous studies have shown strong environment-turnover associations and low spatial autocorrelation in termite species turnover in the Amazonian rainforest (Dambros et al., 2016; 2020), suggesting a low impact of dispersal limitation on the community's spatial structure. Predator density and gradients of altitude and soil chemistry are known to create spatial turnover in termite species composition (Dambros et al. 2016; 2020; Davies et al. 2003; Pequeno et al. 2015; Pequeno, 2017). Termites are the prey of opportunistic and specialized predators, and the colony's fitness depends on antipredator strategies (Traniello & Leuthold, 2000; Araújo et al. 2017). Predatory ants are the strongest predictor of local abundance and richness of termite species (Dambros et al. 2016). In the Amazon rainforest, the soil phosphorus's content is the strongest predictor of termite species composition, followed by clay content and tree diversity (Dambros et al. 2016).

This paper analyzes the dynamics of a termite assembly by calculating the components of species colonization and extinction rates and correlating their colonization/extinction probabilities with their median occupancy position related to predator density and gradients of altitude and soil P content. We hypothesize that (i) species of the termite assemblage have differential colonization and extinction rates along those environmental gradients associated with spatial turnover in species composition and (ii) those differential colonization/extinction rates conserve the relation between species distribution and the environment through time, ultimately maintaining the species spatial turnover. The understanding of the role of those species vital rates in a ubiquitous and ecologically relevant group will give insight into how species of a diverse assemblage can coexist in an undisturbed rainforest with no clear environmental barriers.

MATERIAL AND METHODS

Study area

The study was conducted in Ducke Reserve, a preserved and protected area located in Manaus, Brazil (Fig. 1a). The reserve covers an area of 10.000 ha of non-flooded rainforest (*terra-firme*) with a closed canopy (Ribeiro et al. 1999). The climate is classified as "Afi" under the Köppen system. The mean temperature is 26 ºC, with a mean annual rainfall of 2570 mm. The rainier period occurs from October/November to May, and the drier period from July to September/October, where precipitation is < 100mm, with meteorological series spamming from 1966 to 2018 (Costa et al., 2020). Elevation varies from 110 - 39 masl (PPBIO 2009), and it is highly correlated with soil clay content percentage (r = 0.94) (Luizão et al. 2004). At the higher areas (plateaus), the soil has a horizon of about 0.3 m of brown, clayey organic soil, followed by a 0.3 to 0.4 horizon of soil with very high clay content but permeable. The soil becomes progressively sandy towards the lower areas (Chauvel, 1987). There are approximately 1,200 species of trees (Costa et al. 2008). The undergrowth is characterized by abundant sessile palms, such as Astrocarium spp. and Attalea spp. (Chauvel et al. 1987). At the plateaus, the forest is higher, with canopy trees reaching 35 - 40 m, some even 60 m. Height of trees decreases towards the lower areas, where the soil is richer in sand. The vegetation of the reserve is more diversified accordingly to the soil composition and proximity of bodies of water, varying from a 25 - 30 m canopy height with high proportion of palm trees, to more sandy areas with 15-25 canopy height with few palm trees (Hopkins, 2005).

Sampling design

This study was conducted as part of the Research Program on Biodiversity (PPBio) in Brazil. PPBio is a Long-Term Ecological Research (LTER) project that uses a standardized sampling method applied to several taxonomic groups (RAPELD), such as ants and termites. A RAPELD (Magnusson et al. 2005) sample grid consists of 5 N-S and 5 E-W trails (http://ppbio.inpa.gov.br). The grid allows access to 30 uniformly distributed sample transects with a length of 250 m following the isoclines to minimize within-transect soil variation. This design reduces soil type variation, and depth to the water table since the topography is highly correlated with soil properties, tree species composition, and trait distributions in Amazonian forests (Fig 1.b-c) (Schietti et al., 2014).

Termite sampling

Termite sampling occurred in December of 2008, May of 2009, and December of 2014, at the 30 transects with equal sample effort. In each transect, five sections measuring 5 x 2 m were marked, one distant 45 m from another. The sections were thoroughly explored at all termites' habitats (soil, litter, rotten wood, palm trees, and trees) by three trained collectors for 20 min for each subplot, totalizing 5 h of sampling effort per plot. The soil was dug for about 50 cm to collect termites from underground nests and forage lines. The trees were inspected up to 2 meters, so the canopy termites were not collected (Fig 1.d-e). The termites were preserved in Ethanol 95% and later identified at species level, wherever possible, using the available taxonomic literature and by comparison with reference material from the collections of Federal University of Rio Grande do Norte and the National Institute of Amazonian Research (INPA), Brazil. The termites which identification was not possible were classified into morphospecies. For species with soldier caste, the external soldier morphology was used to assign the morphospecies to the lowest taxonomic rank possible. The soldierless termites (Apicotermitinae) were identified following recent taxonomic literature for this group, such as Bourguignon et al., 2016, Acioli et al., 2017 and Constantini (2018) and morphotyped when needed using the same taxonomic parameters used to describe new species in those cited references, such as mandibles, gut configuration, and enteric valve.

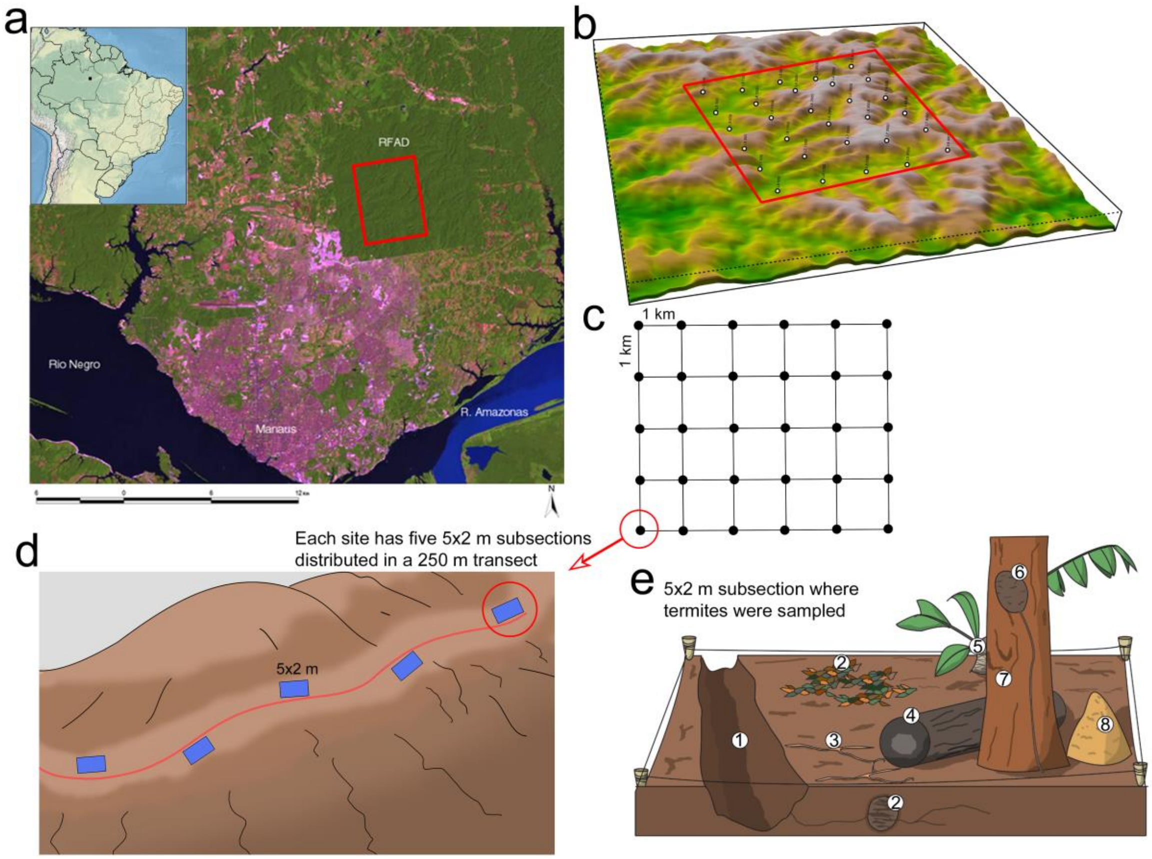

Figure 1: Study location and termite sampling scheme. a) The sampling grid is located at Ducke Reserve, a primary Rain Forest in Central Amazonia. b) Topography of Ducke Reserve, whereas edaphic condition is correlated with altitude and slope (39 m – 109 m). C) Sampling grid. Each sampling site is located 1 km distant of each other. d) Each sampling site consists in a 250 m transect , in which termites were collected in 5 subsections of 5 x 2 m, distant 45 m from each other. The transect is in a isocline, i.e., in the same altitude from beginning to end. e) subsection, where termites are actively sampled by examining all their microhabitats, such as 1 – soil, 2 – hypogeous nests, 3 – branches, 4 – fallen tree trunks, 5 – at palm trees, 6 – arboreal nests, 7 – at trees and at 8 – mounds.

Variables

We used environmental data collected in previous studies and avaliable in PPBio's repository (ppbio.inpa.gov.br). Ant data was obtained from Souza et al. (2012), and altitude and soil properties are available for the study site from Costa et al., (2015). Ants were collected using pitfall traps and sardine baits and extracted from litter using the Winkler method. The samples occurred ten times for each method at intervals of 25 m at each transect. Thus, the total sampling effort was of 30 subsamples per transect, totaling 900 subsamples (3 sampling events x 10 subsamples x 30 transects = 900). A total of 1m² of litter was collected and placed within the Winkler collector, which consists of a bag, in which there is a coarse mesh sieve of 1cm² at the bottom. The bag was vigorously shaken and suspended for 48 h. As the litter dries up, the arthropods migrate vertically downwards, pass through the sieve, and fall in a collecting pot filled partially with alcohol (Bestelmeyer et al. 2000). At intervals of 25 m, a pitfall trap (95 mm diameter; 8 cm depth; 500 ml volume) was placed at the ground level filled partially with water and detergent for 48h, totalizing 10 per plot. After this time, the pitfall trap was removed, and 5 g of canned sardine was placed on a plastic card (10 cm x 7 cm) on the ground. After 45 minutes, all ants were collected from the plastic card and preserved in alcohol 90%. Ants were previously classified as "predators" and "non-predators" by Dambros et al. (2015), following Silva and Brandão 2010.

Data analysis

Colonization and extinction rates of termites

To estimate the changing of species colonization and extinction rates in response to environmental gradients, we used a Multi-season Occupancy Model (MacKenzie, 2003). This method estimates the probabilities of populations' vital rates, i.e. colonization and extinction change in response to the predictor variables (beta coefficients) while accounting for the fact that species for most cases are not detected with certainty. We considered each year we collected as a primary sampling period, and analyses are performed utilizing two sets of primary sampling. The termite community obtained in the dryer season of 2008 was first analyzed and paired with the community from the wetter season of 2009 to measure the population's vital rates (colonization and extinction) from one season to another. The community of the dryer season of 2008 was then compared with the community from the dryer season of 2014 to verify the populations' vital rates over six years without the seasonal influence. We ran the model for each species using three predictor variables as covariates (density of predatory ants, altitude, and P content).

To estimate how the changes in species colonization and extinction along the gradients are associated with species turnover along these gradients, we correlate the response coefficients of colonization and extinction obtained for each species (beta coefficients) with the mean position of the species along the gradients. We expected that species with differential occurrence along a gradient (e.g. distinct preferences for P content) would also have differential colonization and/or extinction along this gradient (e.g. higher colonization rates at higher P content for species with the higher occurrence at higher P content).

All analyses were performed in the R program (R Core Team 2019) using the “vegan” ver. 2.5-6 (Oksanen et al. 2019) and “unmarked” (Fiske and Chandler) packages.

Similarity of the termite communities

To find out how the termite community changed over time, we ran analyses of similarities between 2008, 2009, and 2014 communities. To place them in the same space, firstly, we reduced all three communities to 2 axes utilizing NMDS (Non-metric multidimensional Scaling) utilizing Bray-Curtis as dissimilarity index and 999 permutations to visualize data.

To check how assemblages change over time, first we created a single matrix with the assemblages of all years joined, resulting in a matrix with 90 rows (corresponding with each plot, i.ie, community). Then, we measured the Jaccard dissimilarity between each community, resulting in a dissimilarity matrix. Then, we performed a PERMANOVA (Permutational Multivariate Analysis of Variance utilizing the function "adonis" from the Vegan package, using the dissimilarity matrix as the dependent variable and plot and year as predictors. This function was implemented after Anderson (2001) and tests if centroids of each assemblage differ utilizing distance matrices. We used plot as predictor to test the intraplot variation between years, and the year as predictor to test if composition of all assemblages varied as a whole from one year to another.

Results

Termite community

A total of 928 colonies of termites species were collected, distributed in 349, 320, and 259 in 2008, 2009, and 2014, respectively. The highest number of species (64) was recorded in 2009, followed by 59 in 2008 and 59 in 2014. The wood-feeding termite *Cylindrotermes parvignathus* was the most frequent species (ranging from 45 to 68 occurrences in sections at each sample event). The occurrences of the ten most frequent species were similar among the years, and none of them was absent at any given year (Fig. S1).

Colonization and extinction rates along gradients

The termite assemblage response to colonization and extinction rates were consistent for both time periods (Table 1). Species colonization rates were correlated to all environmental gradients (predator density, altitude, and soil phosphorous), meaning that they colonized sites with similar conditions to the sites which they already occurred (Fig. 2). Only phosphorus was relevant to the extinction rate, albeit the weaker effect compared to the colonization rate (Fig. 3). Termites species colonization nor extinction rates are associated with the non-predatory ants gradient (Table S1).

Table 1: Slope coefficients and R^2 of a linear model between the dynamic occupancy models coefficients and the position of termites species along the environmental gradient.

Values in bold indicate a significant effect. * = $p < 0.05$, ** $p < 0.01$, *** = $p < 0.001$.

	Turnover-colonization relationship					
	Seasons 2008(dryer)-2009(wetter)			Years (2008-2014)		
Variables	Slope estimate (linear model)	R^2	Proportion of species responding positively	Slope estimate (linear model)	R^2	Proportion of species responding positively
Predatory ants	**0.501*** **	0.31	0.28	**0.410** **	0.17	0.19
Altitude	**0.535*** **	0.34	0.31	**0.430*** **	0.23	0.27
P	**0.503*** **	0.33	0.22	**0.527*** **	0.40	0.37
	Turnover-extinction relationship					
Predatory ants	-0.158	0.03	0.70	-0.183	0.05	0.81
Altitude	0.076	0.01	0.71	0.107	0.02	0.80
P	**-0.331** **	0.14	0.73	**0.445*** **	0.28	0.81

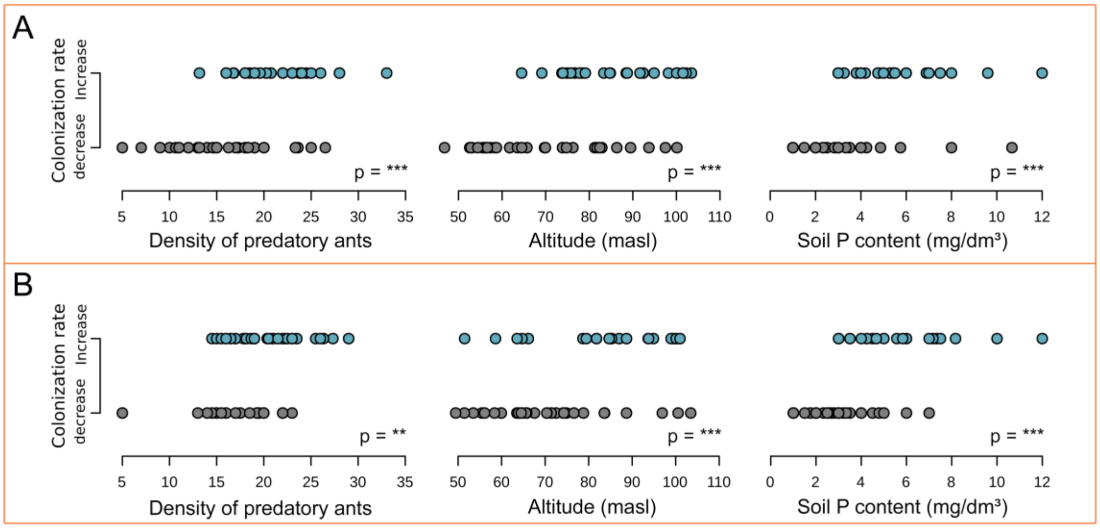

Figure 2: Species mean position along the environmental gradients divided according to the colonization rate sign (positive or negative) for each variable (predators ants, altitude and phosphorus) in the multispecies occupancy model. (A) Species colonization response to environmental variables comparing the dryer and wetter seasons of two subsequent years (2008 and 2009). (B) Species colonization response to environmental variables comparing the dryer season separated by six years (2008 and 2014).

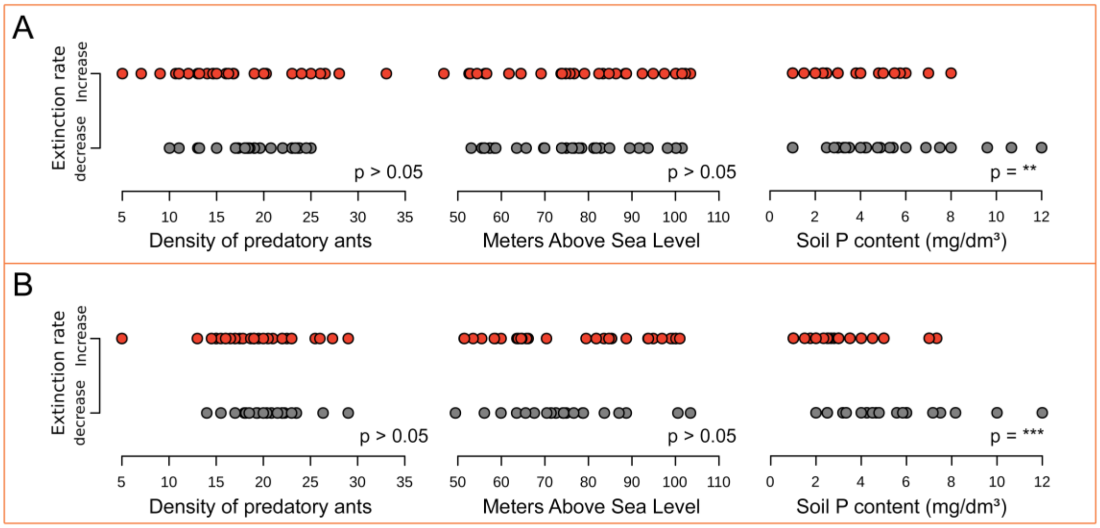

Figure 3: Species mean position along the environmental gradients divided according to the extinction rate sign (positive or negative) for each variable (predators ants, altitude and phosphorus) in the multispecies occupancy model. (A) for a comparison from 2008 dryer season to the 2009 wetter season, while (B) is a comparison between the 2008 dryer season termite community with the 2014 dryer season termite community.

The community was better structured along the phosphorus and altitude gradients (Fig. 4). The community structure is maintained by the colonization rate performance of species being dependent on their position along the environmental gradients. After colonizing a site, species extinction is generally not influenced by the environmental conditions, except for phosphorus soil content, whereas species position in gradient is correlated with their extinction rates (Fig. 4A).

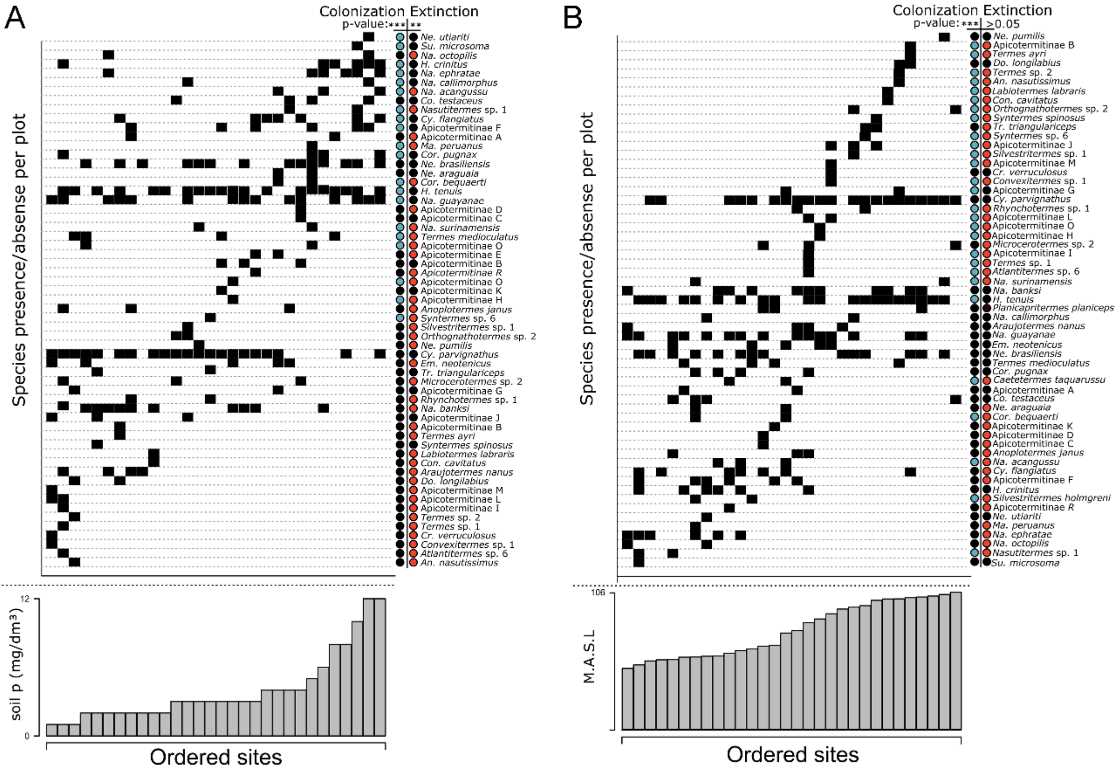

Figure 4: Species occurrence in 2014 organized according to the phosphorus soil content of each plot. Colored circles refer to the multi-season occupancy model's beta coefficients utilizing the dryer season communities of 2008 and 2014. Blue circles indicate species that colonization rates from the multispecies occupancy model are positive, while red circles indicate species where values were positive for the same model's extinction rates. Black circles from the left column indicate a negative colonization rate, and on the right column, a negative extinction rate.

Spatial and temporal stability in termite species composition

We found that species composition varied temporally ($p < 0.001$, $R^2=0.41$, Adonis test). Plotted compositions, however, show that centers of distributions are similar (Fig 4. A). The variation in species composition for the same plots increased with the time span of the comparison

– species composition of the assemblage of 2008 was more similar to the assemblage of 2009 than with the assemblage of 2014, although the assemblages of 2008 and 214 were collected at the same season (wetter) (Fig. 4 B-C).

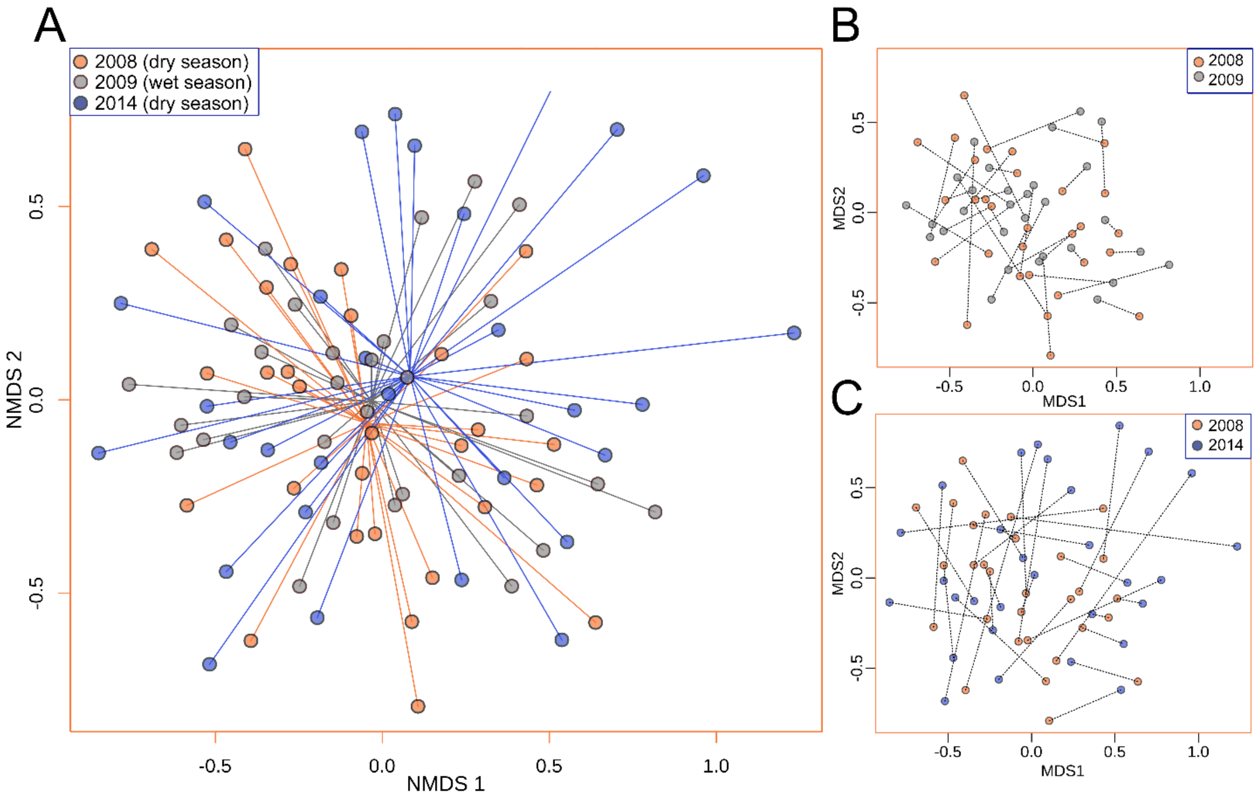

Figure 5: Similarity between termites communities. (A) centroids of reduced assemblages of 2008, 2009 and 2014 are near each other, showing overall composition stability through time. (B) Positions of each plot in 2008 and 2009. (C) Position of each plot in 2008 and 2014.

5. DISCUSSION

Many ecological studies describe a turnover in species identity (beta diversity) over time and space (Whitaker, 1972; Anderson et al. 2011; Magurran et al. 2019), but do not integrate temporal and spatial dynamics to understand how species colonization and extinction over time create spatial turnover. We found that termites species within an assemblage have differential colonization capacities. Moreover, their colonization depends on the local environment and is related to species occurrence, i.e. species tend to occur mostly in those environments where they easily colonize. The turnover in colonization rates across species along environmental gradients creates spatial turnover in species composition. After established, an assembly of species tends to persist, regardless of the environment (Table 1; Fig. 2-3). Ultimately, differential colonization rate, not extinction rate, is the driver that creates and maintains species spatial turnover.

Differential colonization and extinction rates along environmental gradients

We found that predator density and gradients of altitude and soil phosphorus were all influential to termite colonization both over seasons and years. Species have high colonization in those environments where they are most frequent (Fig. 2). The reduction in termite colonization in areas far from the species center of distribution could result from a limit on species dispersal or a limit on colony establishment in conditions where it is not well adapted. The termite reproductive strategy maximizes the number of propagules (alates) to be able to disperse (Bourguignon et al. 2009; Messenger and Mullins, 2005, Dambros et al. 2017; Fogeyrollas et al. 2018). However, termites are unlikely to be dispersal limited at the scale of the study. Moreover, both the environment and termite species composition are not strongly spatially structured in the study area (Dambros et al. 2016). Therefore, the association of colonization rate and the conditions where the species occur likely depends on species early colonization capacity in a given environment or condition. Although extinction was also associated with soil phosphorous, this effect was much weaker than the colonization effect (r = 0.4 vs. r = 0.28; Fig. 2).

Our results reveal that multiple factors are associated with species colonization success, as during these early stages, species better adapted to explore the available resources and resist predator attacks are more likely to establish (Fig. 2). Termites colonies at the early stages of development are very vulnerable (Lepage, 1991; Soki et al. 1996). Colonies have a slow initial phase and are highly dependent on the energy accumulated by the founders, until the sterile castes increase numerically to perform work and forage (Lepage and Darlington, 2000). Only established colonies, with positive energetic budget, are able to produce propagules, and this process can take up to months (Lepage and Darlington, 2000). However, once established, these species seem to be able to maintain a fortress colony resistant to the temporal fluctuation of resources to predatory attacks (Fig. 3).

The observed distinct performance of species according to the environment likely reflects different species requirements for resources. Termites are well known for their diversified feeding and nesting habits in the tropics, ranging from wood-consumers who live inside their food to soil-feeders that forage far from their nests (Eggleton & Tayasu, 2001). Species that build more resistant (clayey) nests occur more frequently in clayey sites, while species that build fewer resistant nests occur more in sandy sites, probably due to a trade-off between colony defense and foraging (Pequeno et al. 2015; Cornelius and Osbrink 2010). Soil composition is largely related to topography at the study site (Castilho et al. 2006), so differential colonization capability between species according to altitude may be influenced by this trade-off between building and feeding requirements in a heterogeneous soil, especially as altitude, alongside phosphorus soil content, best structured the assemblages (Fig. 4).

Although relevant both for colonization and extinction, the assemblages are not well structured along with the predatory ant density. Most of the defense repertoire of termites seems to be aimed to provide defense against ants (Prestwich 1984). Although predatory ant density is negatively correlated with termite abundance and species richness (Dambros 2016), the effect of predators on termites seems to be diffuse (Dambros 2016; Tuma et al. 2020). Many of the 75 predator ant species included in our study are opportunistic with no preference for particular termite species. Therefore, it seems that predatory ants as a whole exercise a generalist predation pressure on termites assemblages. Previous studies have shown little correlation of predator density with termite species composition (Dambros et al. 2016; Basset et al. 2020) and our results indicate that this is related to the similar effect that predators enforce on the colonization of multiple termite species. Although termites have distinct defense mechanisms, they possibly have evolved defenses against ants that are equally efficient. This contrasts with the differentiation in traits related to resource use and acquisition, because species replace each other and differentiate on colonization capacity along environmental gradients.

Spatial and temporal stability in species composition

Spatial variation in species composition was higher than the temporal variation (high dispersion of points in Fig. 5A, but small difference in centroid between years). The temporal variation observed in some plots (Fig. 5B-C) was mostly associated with changes in species with intermediate to low occurrence, while a core group of species of high occurrence had a low fluctuation in prevalence over time (Fig. S1). These differences among species indicate that some species maintain larger and well-established populations over time. These species with high occurrence might also produce a higher number of propagules, which increases colonization probability and, as a consequence, species persistence, whereas low occurrence species are more vulnerable to local stochastic extinction events (Sgarbi and Melo 2017).

Our results indicate that some species were more successful in surviving and/or colonizing new sites within certain portions of the environmental gradients than others. Termites compete for nesting and foraging space and the scarce nutrients in the wood (Shellman-Reeve 1994). At rainforest, mature arboreal colonies of soil-eater *Anoplotermes banksi* show high mortality at a young age, clumpered distribution of young nests and overdispersed mature nests, indicatives of intraspecific competition (Bourguignon et al. 2011). Workers of soil termites are highly aggressive and evasive when encountering other termites, which creates a well-defined spatial separation between neighboring soil nests (Jost et al. 2012). For colonies occupying ephemeral substrates as pieces of wood, encounters are common, with varying degrees of aggression and even fusion of unrelated colonies (Korb and Roux 2012). This scenario of fierce competition and stable environmental conditions is favorable to the development of adaptations and niche-partitioning.

Although it is well known that competition and adaptation to resources drive colony establishment, we demonstrate that species differentiate in colonization along a gradient of resource availability and conditions. These results may indicate that spatial turnover in species composition depends mostly on differential species colonization along environmental gradients and to a less extent on species response to predators or persistence in a given area. Species that are abundant in one extreme of a gradient are those that easily colonize these sites when absent. However, once species are established, their persistence is less dependent on the environment or the presence of predators. Several studies have demonstrated how colonization and extinction rates create temporal turnover in species composition (Nuovoloni et al. 2016; Jones and Gilbert, 2017; Tatsumi et al. 2020). In addition, studies on niche differentiation and species coexistence demonstrate that the ability of a species to invade a community when in low abundance (colonization rate in our study) can be determinant for species occurrence (Chesson 2000). Nevertheless, species are heterogeneously distributed in space and previous studies have not properly associated the temporal dynamics of communities with the spatial heterogeneity in species composition. Our study demonstrates that this spatial heterogeneity is created by how species colonize and establish along gradients. Our results might indicate that colonization and early establishment are the most limiting phases of an organism's life cycle, affecting the fate of individual species and the structure of entire communities.

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SUPPLEMENTARY MATERIAL

APÊNDICE A:

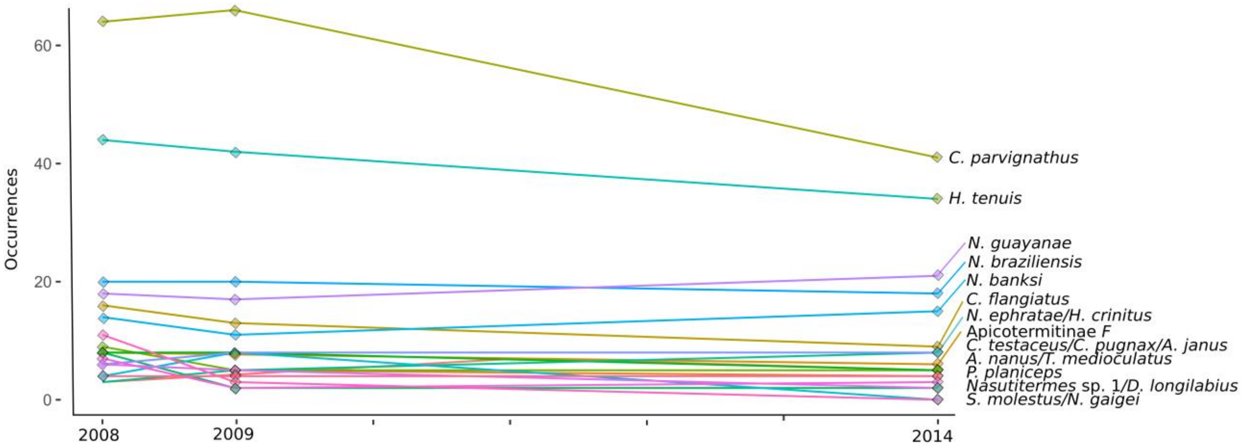

Figure S1: 20 most occurring termite species during the three sampled periods (2008, 2009 and 2014).

APÊNDICE B:

Table S1: Slope coefficients and R^2 of a linear model between the dynamic occupancy models coefficients and the position of termites species along the environmental gradient, using the non-predatory ants instead of the predatory ants as an independent variable. Values in bold indicate a significant effect. * = $p < 0.05$, ** $p < 0.01$, *** = $p < 0.001$.

	Seasons				**Years**			
	Species differential response to:				**Species differential response to:**			
	Colonization		Extinction		Colonization		Extinction	
	Estimate	R^2	Estimate	R^2	Estimate	R^2	Estimate	R^2
Altitude	**0.4081*****	0.18	**-0.24614***	0.06	**0.4095****	0.17	-0.21276	0.05
P	**0.39876*****	0.20	0.022470	0.00	**0.4403*****	0.24	-0.06647	0.01
NonPred. Ants	0.01758	0.00	-0.07353	0.00	-0.17316	0.04	0.17397	0.03

APÊNDICE C:

Table S2: List of termite species occurences per year.

			Number of ocurrences		
Family	**Subfamily**	**Species**	**2008**	**2009**	**2014**
Rhinotermitidae	**Coptotermitinae**	*Coptotermes testaceus*	8	8	5
Rhinotermitidae	**Heterotermitinae**	*Heterotermes crinitus*	3	5	8
Rhinotermitidae		*Heterotermes tenuis*	44	42	34
Rhinotermitidae	**Rhinotermitinae**	*Dolichorhinotermes longilabius*	8	2	2
Rhinotermitidae		*Rhinotermes hispidus*	1	2	0
Rhinotermitidae		*Rhinotermes marginalis*	1	0	0
Termitidae	**Apicotermitinae**	*Ruptitermes arboreus*	0	1	0
Termitidae		*Anoplotermes Janus*	3	7	5
Termitidae		Apicotermitinae A	1	2	2
Termitidae		Apicotermitinae B	0	1	1
Termitidae		Apicotermitinae C	3	1	1
Termitidae		Apicotermitinae D	0	3	1
Termitidae		Apicotermitinae E	0	2	0
Termitidae		Apicotermitinae F	8	7	6
Termitidae		Apicotermitinae G	2	4	2
Termitidae		Apicotermitinae H	0	1	1
Termitidae		Apicotermitinae I	0	0	1
Termitidae		Apicotermitinae J	0	2	3
Termitidae		Apicotermitinae K	0	0	1
Termitidae		Apicotermitinae L	0	1	2
Termitidae		Apicotermitinae M	0	0	1
Termitidae		Apicotermitinae N	0	1	0
Termitidae		Apicotermitinae O	0	1	1
Termitidae		Apicotermitinae P	1	0	0
Termitidae		Apicotermitinae Q	0	0	0
Termitidae		Apicotermitinae R	0	0	1

			Number of ocurrences		
Family	**Subfamily**	**Species**	**2008**	**2009**	**2014**
Termitidae	**Nasutitermitinae**	*Angularitermes nasutissimus*	1	0	1
Termitidae		*Araujotermes nanus*	6	5	4
Termitidae		*Atlantitermes* sp. 6	0	0	1
Termitidae		*Atlantitermes* sp. 1	4	1	0
Termitidae		*Atlantitermes* sp. 2	1	0	0
Termitidae		*Caetetermes taquarussu*	3	1	2
Termitidae		*Coatitermes* cf. *clevelandi*	1	1	0
Termitidae		*Coatitermes* sp. 1	1	1	0
Termitidae		*Constrictotermes cavifrons*	0	1	1
Termitidae		*Convexitermes* sp. 1	0	0	1
Termitidae		*Nasutitermes acangussu*	0	0	3
Termitidae		*Nasutitermes banksi*	14	11	15
Termitidae		*Nasutitermes callimorphus*	4	5	2
Termitidae		*Nasutitermes corniger*	1	0	0
Termitidae		*Nasutitermes ephratae*	6	8	8
Termitidae		*Nasutitermes gaigei*	4	8	0
Termitidae		*Nasutitermes guayanae*	18	17	21
Termitidae		*Nasutitermes macrocephallus*	1	2	0
Termitidae		*Nasutitermes octopilis*	2	4	3
Termitidae		*Nasutitermes* sp. 1	6	5	2
Termitidae		*Nasutitermes* sp. 3	1	1	0
Termitidae		*Nasutitermes* sp. 4	1	1	0
Termitidae		*Nasutitermes* sp. 6	1	0	0
Termitidae		*Nasutitermes surinamensis*	0	1	2
Termitidae		*Nasutitermes wheeleri*	1	2	0
		Paraconvexitermes junceus	0	1	0
Termitidae		*Subulitermes microsoma*	0	1	1
Termitidae		*Triangularitermes triangulariceps*	4	3	4
Termitidae		*Velocitermes* sp. 1	2	0	0

			Number of ocurrences		
Family	**Subfamily**	**Species**	**2008**	**2009**	**2014**
Termitidae	**Syntermitinae**	*Cornitermes bequaerti*	0	0	2
Termitidae		*Cornitermes ovatus*	5	6	0
Termitidae		*Cornitermes pugnax*	9	5	5
Termitidae		*Cyrilliotermes angulariceps*	1	0	0
Termitidae		*Embiratermes neotenicus*	2	4	5
Termitidae		*Embiratermes spissus*	1	2	0
Termitidae		*Labiotermes labralis*	2	0	1
Termitidae		*Mapinguaritermes peruanus*	3	0	1
Termitidae		*Rhynchotermes* sp. 1	0	0	2
Termitidae		*Rhynchotermes* sp. 2	5	2	0
Termitidae		*Rotunditermes bragantinus*	0	1	0
Termitidae		*Silvestritermes holmgreni*	0	2	2
Termitidae		*Silvestritermes* sp. 1	0	0	1
Termitidae		*Syntermes longiceps*	5	0	0
Termitidae		*Syntermes molestus*	11	3	0
Termitidae		*Syntermes* sp. 6	0	0	1
Termitidae		*Syntermes spinosus*	3	2	1
		Uncitermes teevani	0	1	0
Termitidae	**Termitinae**	*Amitermes exellens*	2	1	0
Termitidae		*Cavitermes tuberosus*	0	1	0
Termitidae		*Crepititermes verruculosus*	6	2	1
Termitidae		*Cylindrotermes flangiatus*	16	13	9
Termitidae		*Cylindrotermes parvignathus*	64	66	41
Termitidae		*Dihoplotermes* sp. 2	1	0	0
Termitidae		*Dihoplotermes* sp. 2	1	0	0
Termitidae		Gen.n. sp.n	0	1	0
Termitidae		*Microcerotermes* sp. 2	0	0	3
Termitidae		*Microcerotermes strunkii*	1	3	0
Termitidae		*Neocapritermes angusticeps*	0	1	0

Family	Subfamily	Species	Number of ocurrences 2008	 2009	 2014
Termitidae		*Neocapritermes araguaia*	3	0	2
Termitidae		*Neocapritermes braziliensis*	20	20	18
Termitidae		*Neocapritermes opacus*	3	1	0
Termitidae		*Neocapritermes pumilis*	2	1	1
Termitidae		*Neocapritermes* sp.1	0	1	0
Termitidae		*Neocapritermes taracua*	4	0	0
Termitidae		*Neocapritermes utiariti*	0	0	1
Termitidae		*Orthognathotermes* sp. 2	0	0	2
Termitidae		*Orthognathotermes* sp.n.	0	1	0
Termitidae		*Planicapritermes planiceps*	7	2	3
Termitidae		*Spinitermes nigrostomus*	1	0	0
Termitidae		*Termes ayri*	0	1	1
Termitidae		*Termes medioculatus*	4	4	4
Termitidae		*Termes* sp. 1	0	0	1
Termitidae		*Termes* sp. 2	0	0	2
		Total	349	320	259

CAPÍTULO II

Alves-Oliveira, J.R., van Asperen, H., Azevedo, R.A., Heleodoro, R.A., Morais, J.W., Franklin, E. & Dambros, C.S. **Termites methane production and uptake: a case study for a Central Amazonia assemblage**. *Manuscrito a ser enviado para Acta Amazonica.*

Termites methane production and uptake: a case study for a Central Amazonia assemblage

Abstract

Termites are one of the major natural sources of the potent greenhouse gas methane (CH4) in Neotropics, but the least known of them. CH4 released by termites is a byproduct of lignocellulose degradation by symbiont protists or methanogenic bacteria in the gut. However, recent estimates of termites contribution to the global CH4 budget is much lower than the previous, in order of 1-3% of the global CH4 budget, due to recent findings on the role of bacteria that oxidizes CH4 to incorporate biomass (methanotrophs) that are highly active inside termites nests and surround soil, filtering about half of a termite colony emission. Here, we do a study case to estimate the original production of CH4 and in-situ CH4 emission of a mound of *Neocapritermes braziliensis*, an abundant mound-builder species in Central Amazonia. We have found an in-situ mound emission of 9.97 CH4 nmol^{-1} s $^{-1}$, while the nest alone emitted 17.71 CH4 nmol^{-1} s $^{-1}$. Colony dry biomass was 70.53g, from which we estimate that colony originally produced 36.58 CH4 nmol $^{-1}$ s $^{-1}$. From this result, we estimated that nest alone filtered (by CH4 uptake of methanotrophic bacteria) about half of the colony CH4 production. Adjacent solo filtered about a quarter. In this way, the *N. braziliensi*s colony only emitted about a quarter of its production. Additionally, we discuss two methods of using closed chamber flux to measure termites' CH4 emission rates of termite individuals. The first method measures the emission of termites inside nest pieces and the second, inside a Petri dish. We report the emission rates of individuals of different castes of 14 species from a Central Amazonia assemblage.

Introduction

Methane is a potent global warming gas released by both anthropogenic and natural sources. Most CH4 from natural sources are mainly a byproduct of the decomposition of organic matter by methanogenic archaea in anoxic or hypoxic sites such as wetlands and the guts of animals, such as ruminants and termites (Hanson & Hanson, 1996). Considering natural methane sources (CH4), termites derived CH4 emission is the least known (Saunois et al., 2020; Kirsche et al., 2013). There is a high degree of uncertainty of those values, as there are few empirical studies to provide both data about biomass and emission values (Kirsche et al., 2013). Although not the only arthropod capable of emitting CH4, termites are the only relevant to the global CH4 budget due to their high biomass (Brune, 2019; Bignell et al., 2010). This uncertainty is especially true in the tropical region, where termites are ubiquitous decomposers, adapted to feed on sound wood to bare soil and are among the most abundant animals (Bignell, 2016). Recent estimates estates that termites contribute about 1-3% of the global CH4 budget, whereas the main contributors are tropical America and Africa, with a contribution of 23% and 28%, respectively, to this value (Saunois et al., 2020).

Termites CH4 is a product of lignocellulose degradation through fermentation in the gut, performed mainly by symbiotic flagellates in lower termites and methanogenic archaea in upper termites (Brune, 2014). Termites act as bioreactors, harboring ideal conditions to microbiota symbiont performance. Termites guts present steep gradients of metabolites and a counter gradient of H2 and O2, enabling spatial separation of microbial communities with different requirements (Brune and Ohkuma, 2011). Although termites undoubtedly produce a large quantity of CH4, not all produced CH4 is released into the atmosphere. Instead, a portion of it is oxidized in CO^2 by methanotrophs, bacteria that use CH4 as the substrate to incorporate biomass and gain energy (Hanson and Hanson, 1996). They occur naturally in upland soils, and recently has been shown that termite nests have a great capacity to oxidize CH4 produced by the colony, in the range of 20-80% (Nauter et al., 2018). Termites build their mounds with soil, and physicochemical changes in the soil during nest construction and high intern CH4 concentration in the nest filters the soil microbial community, increasing the proportion, and abundance of methanotrophs (Chiri et al., 2020; Chen et al., 2021).

Despite the importance of the Neotropics to the CH4 global termite estimate budget, there are few empirical studies conducted in the region, which have not been able to precisely quantify the association of termite nest structure and species identity with CH4 emission (Zimmerman, 1982; Martius et al., 1993, Van Asperen et al., 2021). The CH4 turnover in termites colonies depends on soil microbial composition, nest structure, and termite species (Ho et al., 2013; Neuer et al., 2018; Chiri et al., 2021). In addition, changes in nest structure and species identity occur due to human activities, such as deforestation (Ackerman et al., 2009), road construction (Dambros et al., 2014). T hus, the understanding of these associations might help predict changes in CH4 emissions.

Here, we do a study case in an urban Amazonian rainforest fragment. First, we measured in-situ CH4 emission of a nest of *Neocapritermes braziliensis*, a common species in Central Amazonia. We then measured nest emission with nest removed from the soil. Later, we estimated total colony emission based on its biomass, extracted by flotation, to estimate how much of the CH4 uptake occurs both in the nest and adjacent soil (in situ conditions) and how much of the uptake occurs in nest alone, removed from the soil. For this specific case, we have found that termites colonies only emit about one-quarter of what was produced, whereas half uptake occurred in the nest and a quarter in the adjacent soil. We also provide individual emission estimates for 14 species from Central Amazonia.

Material and methods

Study Site: The study was performed in an urban forest fragment located within the National Institute for Amazonia Research (INPA) campus. This area covers about 13 ha of primary Ombrophilous Forest with patches of secondary forest in Central Amazonia. CH4 measurements and nest collection occurred in November of 2020, and species sampling for measuring individual termite species occurred from April to June of 2021.

Nest selection and measurements: we selected a nest of *N. braziliensis*, an abundant mound-builder species at Central Amazonia (Dambros, 2016; supp. mat) and most commonly encountered in sandy soils (Pequeno et al., 2015), which occurs in lowlands (Chauvel et al., 1987). This species feeds from rotten wood (Constantino, 1992), being one of the diverse group of Termitinae species that feeds on the wood-soil interface (Eggleton & Tayasu, 2001). The used emission rate for the species estimates was 0.2927 μmol termite^{-1} s^{-1} (Van Asperen et al., 2021). The species that feed on rotten wood to soil are usually termites with more significant emissions (Bignell, 2010).

CH4 emission measurements: We measured the colony CH4 emission in-situ and the CH4 emission of the nest removed from the soil, using a flux chamber. To measure in situ colony emission, we selected a suitable *N. braziliensis* nest, located in a place without near obstacles which could prevent the flux chamber installation. Then, we placed a steel stainless collar around the nest, 5 cm deep, to fixate the flux chamber. The flux chamber consists of an adapted 57.5 diameter 220 L polyethylene bucket, with two one-touch fittings on the sides. Internally, there is a four-inlet vertical sampling tube at different heights (about 10, 25, 35, and 50cm height) to assure an even sampling of internal gases. Around the internal bucket aperture, a strip of closed-pore foam (1 cm × 1 cm × 57.5 cm) was attached to seal any space between the bucket and the collar. The chamber was connected at a Los Gatos Ultraportable Greenhouse Gas Analyser in a closed loop. The air circulated from the chamber to the equipment by two 2 m PTFE tubing of 1/4in, connected at the bucket one-touch fitting. The air circulated with a flow of ~0.35 L min−1 using the internal pump of the Los Gatos instrument. We measured the nest emission during 20 minutes, in which the instrument registered the concentration value at intervals of 10 s. After the in-situ measurements, the nest was excavated using a pickaxe and removed undamaged. The nest was placed inside the bucket, and its emission was measured for 20 minutes.

To calculate CH4 flux, we used the following equation:

$$F = \frac{\mathrm{d}C}{\mathrm{d}t} \cdot \mathrm{CV}$$

where F is the mound emission (mol s^{-1}), d*C*/d*t* is the concentration change (mol m^{-3} s^{-1}), and CV is the corrected chamber volume (m^3). Concentration change was derived using linear regression. Details of the measurement method can be seen in Van Asperen et al. (2021).

Later, the nest was brought to the laboratory, measured and weighted. Nest volume was estimated based in Ribeiro 1999 (Ribeiro, 1999; Pequeno et al., 2015)**,** which uses the following equation:

$$V = \frac{\pi HWT}{6}$$

where V is the mound volume (cm^3), H is the height (cm), W is the width (cm), and T is the thickness (cm) of the mound. Later, the termites were extracted from the nest by flotation, which was performed by breaking and washing nest pieces under flowing water into a bucket. The floating termites were collected with a sieve and reserved in alcohol 70%. A subset of 1200 workers was dried out and weighted. *Neocapritermes* species have a low ratio of soldiers-to-workers, of about 1:100, which we observed for this case, so we did not consider soldiers' biomass. Later, we cleaned the samples from nest debris and dried all the collected termites, and weighed them. Based on the weighted subset, we estimated the colony density. We estimated colony CH4 production multiplying the estimated colony density by *N. braziliensis* single termite emission (Van Asperen, 2021). We used the same Los Gatos instrument, with a small chamber of 1 L adapted with two one-touch fittings to measure the emission of single termites of different species,. We tested two methods of measuring termites CH4 emission: The first one measuried the emission of termites in Petri dishes. We broke pieces of nests in a white tray and separated four groups of 50 individuals of each caste. Next, we covered the bottom of the petri dish with humid sterilized soil and measured 50 individuals, adding 50 more after 5 minutes, measuring the emission of 50, 100, 150, and 200 individuals. We then estimated the emission of a single termite by using linear regression with a forced intercept at y = 0.

The second method we tested is to measure the emission of inhabited small nests pieces and later count the number of termites inside the piece of the nest. Later, we estimated the emission of a single termite using the same equation as before. We tried to measure the emission of five pieces of nest whenever possible; however, it was impossible for all nests. Therefore, the minimum we used was three pieces. Although measuring termites in a Petri dish seems ideal due to the better control of the conditions, it is not appliable to all species as some species are too sensitive to the dry and windy conditions inside the chamber. On the contrary, inside the nest pieces, termites remain in good conditions for a long time. Besides, it is faster and easier to measure the nest pieces than to do measurements in a Petri dish. For this reason, it is important to verify if both methods can be used. For the species measured in the urban forest fragment, we also weighted 100 live individuals of measured castes to provide the mean weight of workers and soldiers of that given colony and provide the CH4 emission rate as µmol g^{-1} h^{-1}, as it is standardized in literature. In addition, we also provided emission values of species measured along 2020, that we did testing the methods. However, those species were not weighted, and thus, their body weight and emission factor (µmol g^{-1} h^{-1}) are not provided.

Results:

Workers and soldiers CH4 emission of different species: We measured CH4 emission of workers of 12 species in Bosque da Ciencia, from April to May, utilizing the nest piece method, petri dish method, or both, when possible. Some species (as *Termes* sp. 1 and *Cavitermes cavifrons*) were only found in mounds cohabitated by two or more species, so could only be separated measured in a petri dish, as nest pieces contained more than one species. We also report here early measurements, from October 2020 at ZF-2, of some species we measured while the methods were being developed. Those species do not have their body weight value and emission factor in the Table 1.

Table 1: Emission values of a single termite of different species from a Central Amazonia assemblage, measured in nest pieces, petri dish, or both. Values are in μmol $^{-1}$ s^{-1} per termite. Measurements of single casts were made only with Petri dish method, while the nest piece method has a mix of castes. Only adult individuals were counted.

Species	**Nest Piece Method**	**Petri Dish Method**	**Body weight (mg)**	**Emission factor (μmol g^{-1} h^{-1})**	**Trophic Group**
Apicotermitinae sp. 1	0.339		3.1	0.28	Soil
Cavitermes tuberosus	--	0.497	3.6	0.5	Soil
Cyriliotermes sp. 1		0.531	4.3	0.45	Soil
Embiratermes neotenicus	0.381				Soil
Curvitermes sp. 1	0.149				Soil
Curvitermes sp. 1 (Workers)		0.292	3.5		Soil
*Labiotemes longilabius (*Workers)		0.831			Soil
L. longilabius	0.788				Soil
Nasutitermes corniger	0.151				Wood
Nasutitermes corniger (larger worker)	--	0.198	3.8	0.19	Wood
Nasutitermes corniger (small worker)	--	0.137	2.5	0.2	Wood
Nasutitermes corniger (soldier)	--	0.029	1.7	0.06	Wood
Nasutitermes gaigei	0.1791				Wood-soil
*Nasutitermes gaigei (s*oldier)		0.039			Wood-soil
*Nasutitermes gaigei (w*orker)		0.156	2.4	0.24	Wood-soil
Neocapritermes braziliensis	0.298		3.0	0.35	Wood-soil
Neocapritermes taracua	--	0.618	8.5	0.27	Soil
Rotunditermes bracantinus	0.116				Wood
Spinitermes sp. 1 (workers)	--	0.176	-		Soil
Termes sp. 1	--	0.215	1.8	0.44	-
Termes sp. 2	0.162	0,085	1.1	0.29	-
Silvestritermes holmgreni soldiers		0.161			Soil
Silvestritermes holmgreni workers		0.192			Soil
Silvestritermes holmgreni	0.416		-		Soil

Nest and colony biomass: The nest weighed 23 kg and measured 46 cm in height. The estimated volume was of 27 L. We measured a total colony dry weight of 70.53g. Each termite worker weighted an estimate 3mg. Therefore, the total number of workers estimated per colony was about 125.000.

Colony CH4 production and emission:

We estimate, based on the *N. braziliensis* emission rate reported in Van Asperen et al., 2021, and the measured nest biomass, that the colony produced 36.58 CH4 nmols $^{-1}$ s $^{-1}$. However, the above-ground in-situ nest emitted at normal conditions only 9.9698 CH4 nmols^{-1} s $^{-1}$. Even when the entire nest removed from the soil is considered, the emission is still lower – 17.71 CH4 nmols^{-1} s $^{-1}$.

Discussion

Workers and soldiers CH4 emission of different species:

Here, we presented data on the emission rate of 15 species from a Central Amazonian assemblage. Previously, those rates were only studied in Amazon for *Nasutitermes*, although not at the species level (Martius et al., 1993) and *N. braziliensis* (Van Asperen et al., 2021). All sampled species belongs to the Termitinae subfamily, with food habit ranging from wood to soil. The found range of emission factor of workers, from 0.2 (*Nasutitermes corniger*) to 0.45 (*Cyrilliotermes* sp. 1), is within the emission range of termites from other biogeographic regions, which spams from 0.1 to 1.60 (Bignell, 2010). Most species were collected at mounds or intermediate nests (nested in a piece of wood), except for *N. corniger* and *N. gaigei*, of which arboreal nests are abundant in modified landscapes (Dambros et al., 2013). We have sampled only a small proportion of Amazonian species, so the actual range for Amazonian species is certainly wider. Neotropical termite's composition differs most from the other regions (Cereser et al., 2020), as the group adaptatively radiated after isolation from other biogeographical regions (Bourguignon, 2014). So, it makes it more important to measure those species, as those rates can be used as data in other studies and climates surveys. Although we did not sample hypogeal nesters, which compose most of the soil feeders (Bourguignon, 2011), their emissions are expected to be mostly if not all oxidized by soil bacteria (Sugimoto and Inoue, 1998; Macdonald et al., 1999). For this reason, emission rates of arboreal and mound builder species are more relevant in the context of GHG emissions in the atmosphere.

We have found that both methods tested (measuring termites in nest pieces or in a petri dish) are viable, both having advantages and disadvantages. We tried to test both methods for all species when possible, but some species can only be measured by one of them. The nest piece method cannot be used for species that do not build nests. In some cases, we could not test the nest piece measurement method due to a high number of sampled mounds containing more than one species, Frequently *Cavitermes cavifrons* being one of them. The high proportion of mixed nests is possibly due to the edge effect, as the study area is relatively small (Dambros et al., 2013). On the other hand, other species could be measured only using the nest piece method, as termites killed themselves when placed in the petri dish or could not survive to the air exposition for a time long enough to the measurements occur. This may be the case for the lower emission of *Silvestritermes holmgreni* band *Termes* sp. 2 in the Petri dish, presumably by the exposure stress in chamber conditions.

Overall, measuring termite emission from nest pieces is easier for some reasons. First, individuals of most species survive well for long periods if maintained inside a nest piece. Second, it makes work in the field faster, and nest pieces can be saved for later measurements or counting of termites. The major caveats are that nest pieces emit CO^2(if the intention is to collect CO^2 rates), and not all species are nest builders. An inconvenience is that at one nest piece, generally, there is more than one caste present. However, in most cases, the presence of soldiers can be ignored, as they emit much less than the workers and are frequently outnumbered. Finally, the biology of each species must be taken into account when choosing the measuring method. In some cases, as for *Cyrilliotermes* sp., soldiers emitted more than half of workers' emissions, although we did not represent it on results due to the low sampling number. If the objective is to measure CH4 emission per colony biomass instead of measuring CH4 emission of specific castes, then measuring the emission of nest pieces can be even better, as the nest pieces samples may have a similar ratio of castes of the nest. Also, depending on its size, the nest piece must be accounted for because it diminishes the chamber's volume, which is used to calculate the emission. We recommend small pieces with 200 termites or less.

For the measurements in a Petri dish, we found out that this method seems to work well with species resistant to open-air exposure, as the Nasutitermitinae species sampled and more sclerosed soil termites as *Cyrilliotermes.* Some others, such workers of *Labiotermes* sp., will kill each other under those conditions. The major problem is that some individuals of sensible species may diminish activities and die during measurements, difficulting estimates. However, it is the only form to measure some species which do not build nests based on clay. Therefore, we recommend measuring termite's emissions using nest pieces instead of workers in Petri dish whenever possible, as some species tend to emit lower at Petri dish. Nevertheless, the preferred method depends on measured species.

This study has limitations. Unfortunately, due do the COVID-19 restrictions, we were limited to a small sampling area. The termite colonies were usually small, which prevented us to sample a large number of specimens for our tests. For this reason, we could not test both methods for all species, nor did we managed to sample a larger number of species. We intend to improve the methods and publish the emission values of a higher number of species in the future. For improvements on the nest piece method, we intend to calculate the volume of the nest piece by submerging the piece after measurements in a graduated glass measuring recipient filled with water and estimate nest voume by the change in the water column. For the petri dish method, we need to improve survivability of termites of some species to the measuring chamber inner conditions. We intend to design a rearing container in which the termites remains alive and well during the experiments.

Colony CH4 production and emission:

We compared termite CH4 production at the individual and nest levels to determine the CH4 produced and oxidized during the decomposition process. The colony CH4 production does not equal the colony nest emission at normal conditions. We have found that three-quarters of the CH4 produced by the colony was not emitted in *in-situ* conditions. When the nest was removed from the soil and measured alone, the nest emitted the equivalent of half of its production. Based on those measurements, is likely that CH4 is oxidized before emission both in the nest structure and in the adjacent soil. In this case, the nest alone likely oxidizes about 50% of colony CH4 production, and the adjacent soil oxidizes the other 25%. Therefore the total nest emission to the air is only 25% of the total produced by the colony.

The difference in production and emission we observed can be explained by the action of methanotroph bacteria which consume part of the CH4 both in the soil and inside the nest (Nauer et al., 2018). The results observed are similar to a recent evaluation of an Australian assemblage, in which nests of the three species filtered between 20-80% of nest emissions, with an average of 50% (Nauer et al., 2018). However, a later assessment of the methanotroph communities in those nests and surrounding soil revealed that termite mounds contained soil-derived methanotrophs kinetically adapted to elevated CH4 concentrations. Further, the bacteria composition of the nest and surrounding soil is different, with most CH4 oxidation occurring in the core of nests and soil below the nest (Chiri et al., 2020).

The estimated amount of CH4 reabsorbed by the nest and surrounding soil is in the high end of the observed in previous studies (Nauer et al., 2018). This amount could be explained by the differences in the environments investigated. Forests, as investigated here, tend to have a higher activity of bacterial methanotroph community, which might be reduced in modified environments and differ in grasslands and other ecosystems (Wu et al., 2020; Shuckla et al., 2013). For this reason, in Amazonia, primary and secondary forests are frequently CH4 sinks, whereas cattle pastures are CH4 sources (Meyers, 2020). In Cameroon, soil alteration by anthropogenic impact turn CH4 sinks into CH4 sources due to impaired soil CH4 uptake capacity (Macdonald et al., 1998). We know that the soil from which termites build their nests is the major factor of nest bacterial composition (Chen et al., 2021). Although there is no study investigating differences in the composition of methanotroph bacteria for most termites species, differences in the associated microbial community likely explain differences in CH4 emission between regions and environments (Chiri et al.,2020 Ho et al., 2013). If the hypothesis that nests composed and surrounded by impoverished soil emit more CH4 due to lesser CH4 uptake, it would reinforce the early hypothesis that termites from degraded areas can have greater emission of CH4 (Zimmerman, 1982). Our results support that those termite colonies in forests efficiently decompose organic matter while emitting only a small fraction of the CH4 produced. In addition, the number and density of termite workers could also potentially be estimated from CH4 emissions (Nauer et al., 2018; Van Asperen et al., 2021). Termites' colony size estimates are rarely performed as there is no easy, non-destructive method to measure termites population size within the nest or temporal changes in their population, which difficult populational studies (Jones et al., 2005). The CH4 turnover process in this system composed of the termites, the nest, and the soil is unknown. It is possible that collecting more data about termite emission, biomass, and others possibly relevant parameters such as internal CH4/CO2 concentration, a species-specific mechanist model can be created to estimate biomass.

In conclusion, our results indicate that both mound and adjacent soil filtered three-quarters of the colony CH4 emission for this case study. Also, we provided emission values of individuals of different species of the Amazonian fauna. This result is especially relevant as considering all sources of CH4, termites estimates are the most uncertain (Saunois et al., 2020), especially because of the lack of information on the CH4 turnover processes (Nauer, 2018) and lack of emission and biomass data (Kirsche, 2013), which causes divergences in termites global net emission estimates (Bignell, 2020). Local idiosyncrasies must be taken into account to better estimate termites' role on the GHG budget, mainly considering that Amazon termites may compose a third of the natural CH4 emission sources of this region (Kirschke et al., 2013) and termites' role in the Amazon Forest ecosystem is poorly understood and probably, underestimated (Van Asperen et al., 2021).

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SUPPLEMENTARY MATERIAL

APÊNDICE A:

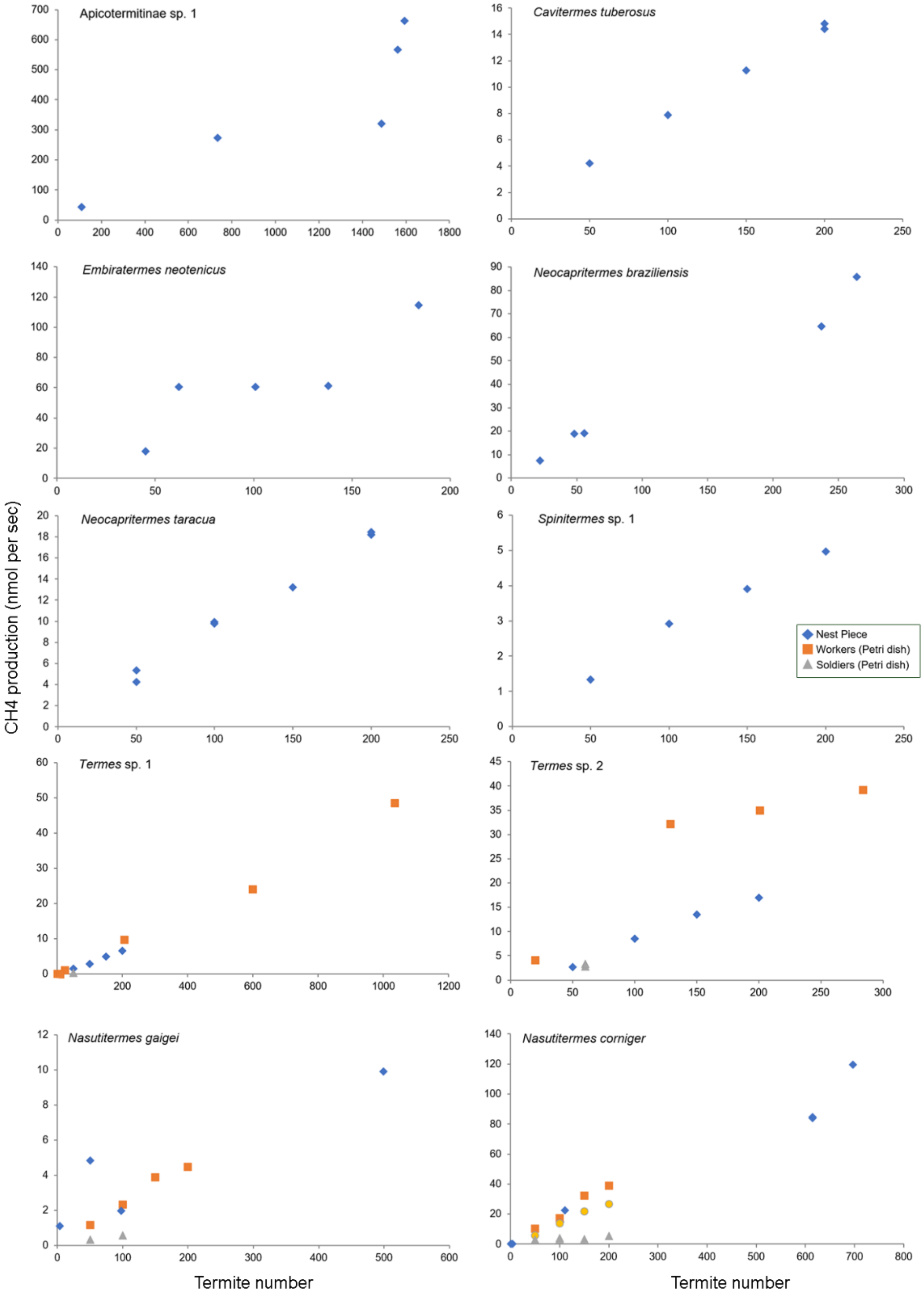

Fig S1: Graphs of the linear regression of CH4 production in function of the number of termites for different species, emitted by termites inside nest pieces (blue diamonds) or placed inside petri dishes (orange squares for workers and gray triangles for soldiers). Results are presented in Table 1.

CAPÍTULO III

João R. Alves-Oliveira, Renato Azevedo, José W. Morais, Elizabeth Franklin, Cristian S. Dambros. ***Ruptitermes melanocephalus*** **(Termitidae: Apicotermitinae), a new soldierless termite species from Amazonian Central Rainforest**. *Manuscrito a ser enviado para Acta Amazonica.*

REJEIÇÃO PARA FINS DE NOMENCLATURA

O ato nomenclatural proposto serve apenas para o exercício de avaliação da banca e não deve ser considerado como um ato nomenclatural taxonômico válido. Essa recomendação é mencionada no Código Internacional de Nomenclatura Zoológica (edição 1999), capítulo três, artigo 8.2 e 8.3.

A new soldierless termite species (Termitidae: Apicotermitinae) from Amazonian Central Rainforest

Abstract

A new termite (Termitidae: Apicotermitinae) species, *Ruptitermes melanocephalus* **sp. nov.** Alves-Oliveira & Azevedo, is described from the Brazilian Central Amazonian Forest. The new species is morphologically related to *Ruptitermes arboreus*, but differs mainly from it by having a darker, blackish coloration, enteric valve morphology, and the nest structure, made with clay instead of being cartonated, and by not burst their abdomen to release the content of the dehiscent organs as a defense mechanism when threatened. Illustration of workers, nest, behavioral notes, and one report of a termitophile millipede Pyrgodesmidae are provided.

Keywords: Isoptera, *Anoplotermes*, Anoplotermes-group, termitophily

Ruptitermes melanocephalus (Termitidae: Apicotermitinae) uma nova espécie de cupim sem soldado da Floresta Amazônia Central

Resumo

Uma nova espécie de cupim (Termitidae: Apicotermitinae), *Ruptitermes melanocephalus* **sp. nov.** é descrita para a Floresta da Amazônia Central Brasileira. A nova espécie é morfologicamente parecida com *Ruptitermes arboreus*, mas difere desta principalmente pela coloração mais escura e enegrecida, pela morfologia da válvula entérica e pela matéria-prima do ninho ser a argila, ao invés do ninho ser cartonado, além de não romperem o abdome para liberar o conteúdo de seus órgãos deiscentes como defesa. Ilustrações dos operários, do ninho, notas comportamentais e um registro de um diplópode Pyrgodesmidae termitófilo são fornecidos.

Palavras-Chave: Isoptera, *Anoplotermes*, grupo-Anoplotermes, termitofilia

Introduction

Apicotermitinae are termites known by being soil-feeders and having a less-sclerotized abdomen, turning its walls translucid, making the digestive tube visible, in which the first proctodeal forms a loop between the rectum and the paunch in ventral view (Constantino 1999). *Ruptitermes* Matthews 1977 distinguishes from other Apicotermitinae genera by being reported to feed on litter and not on soil, as other Apicotermitinae (Constantino 1999; Cancello et al., 2014). Furthermore, some remarkable external morphological characters aid the differentiation, such as having the abdomen not as translucid (as it is more sclerotized), the longest tibiae/body size ratio among Apicotermitinae (making the tibiae visually longer when compared with other genera). In addition, they are generally more active than other Apicotermitinae (*Ruptitermes* species (wanders more around litter while co-familiar species into the soil) (Acioli and Constantino, 2015). The most striking defensive feature is a *Ruptitermes* exclusive dehiscent organ that gives them the capability of burst their own body releasing a sticky secretion (Poiani et al., 2016), which contains toxins, defensins, and proteolytic enzymes that may immobilize or even kill opponents such as ants (Costa-Leonardo et al., 2020).

Ruptitermes has 13 described species, subdivided into two species groups: the group *arboreus*, composed only by *R. arboreus* (Emerson, 1925), which is restricted to forests, has a darker body coloration, vestigial compound eyes, and has long-curved setae on the head; and the group *Xanthochiton*, which includes the remaining 12 species and is known by having the procoxae with at least four spine-like setae and with short and straight setae on the head (Acioli and Constantino, 2015).

When collecting in the Reserva Ducke (Manaus, Amazonas, Brazil), we found a *Ruptitermes* nest that caught our attention due to its visually not of cartonate texture, as expected of a *Ruptitermes* arboreal nest. We brought the nest to the laboratory, and by analyzing its specimens, we confirmed through morphology that they belonged to the group *arboreus*. However, probing in more specific morphologic characters, we confirmed these specimens as a new species. Thus, we are describing this new species, discussing its relation to both groups of species, reporting a myriapod Pyrgodesmidae cohabiting the nest, and making laboratory-based behavioral notes.

Material and Methods

We found the nest on a recently fallen trunk tree during a collection at Ducke Reserve, Manaus, Brazil. We photographed it and sampled a piece of the nest. At the laboratory, nest pieces were put on a white plastic tray, and live termites were collected with a featherweight tweezer and preserved and killed in alcohol 90%. Several individuals were kept alive on a large Petri dish and were manipulated using tweezers and brushes to observe their behavior when stressed to check if their abdomen would explode, as other *Ruptitermes* species (Costa-Leonardo, 2004). Later they were also killed and preserved in alcohol 90%.

Specimens were photographed using a Leica DFC295 attached to a stereoscopic microscope M205. To access the gut, the specimen was positioned in a Petri dish containing alcohol, and the tergites and sternites were removed. Then, the enteric valve was dissected by removing the first proctodeal segment from the paunch and cleaning the internal content by slightly pressing the enteric valve against the Petri. Later, the enteric valve has its lumen to spread the structure. Subsequently, the enteric valve was transferred to a slide containing a PVA Mounting Medium (BioQuip Products Inc., #6371A), manipulated to detach the musculature, adjusted, and then permanently mounted on it. The illustrations were made or edited using Inkscape and Adobe Photoshop CS6.

RESULTS

Ruptitermes Melanocephalus **sp. nov.** Alves-Oliveira & Azevedo

Etymology. The name derives from the Greek words *melanos* (black) and *kephale* (head), referring to the dark head of the species.

Material examined

Holotype. Worker, in alcohol 90%, separated in microvial with the remaining sample. Original label: “Brazil, Amazonas, Manaus, Ducke, 02°57'50.63"S, 59°57'18.21"W. Coleta Manual 15-ii-

2020. J. A. Oliveira and R. A. Azevedo coll". The holotype was deposited in the Invertebrate Collection of the Instituto Nacional de Pesquisas da Amazônia.

Paratypes. 200 workers with the same data as the holotype.

Description

Imago. Unknown.

Worker (Figures 1 – 3). Head capsule black, densely covered with bristles of different sizes (Figure 1 a-b). Fontanelle barely visible, with the same color as the head. Postclipeus strongly inflated, with many bristles; anteclypeus length half the length of the postclypeus. Labrum density of bristle similar to the postclypeus (Figure 1 a). Left mandible with a short apical tooth, half the size of tooth M1 + 2; M3 the same size as the Apical tooth. Molar prominence long. Right mandible with apical and M1 tooth very similar to the left mandible; M2 shorter than M1 and larger than A; molar plat large and concave. Microscopic hairs on the pronotal anterior portion, long bristles on the anterior margin of pro-, meso- and metanotum. Body wall opaque, dehiscent organs visible, ranging from small to very large, occupying a large part of the abdomen (Figure 2). Digestive tube (Figure 3). Gut forming a dense mass, slightly longer than wide. Crop with a circular shape and practically occupying a third of the size of the gut (Figure 3 a). Left side margin of the crop with a constriction forming a primitive gizzard (Figure 3 b). In ventral view, mesenteron slightly enlarged, connected with the ileum near the left lateral margin, (Figure 3 c). Ileum tubular, slender, long, and forming an arc connecting with the paunch on the left margin in ventral view (Figure 3 d). Enteric valve with six similar and well-defined pads formed by scale-like lobes; each pad has one to three spines in its middle, at least one well-developed.

Scales form a reticulated pattern. Borders of scales are serrated and sometimes form a small spine at the top of scale (Figure 3 e-f).

Coloration (based on specimens preserved in alcohol)**.** Head black. Thoracic nota dark brown. Dehiscent organ forms a yellowish to white mass that can occupy up to half the abdomen of specimens in ventral view, visible through the body wall. Pleural and abdominal nota lighter than thoracic nota, due to being less sclerosed. Proximal portion of legs (coxae, trochanter, and femora) dark brown, tibiae becoming increasingly lighter towards tarsus, distal portion of tibiae and tarsus light yellow (Figure 2).

Behavioral notes. Like other *Ruptitermes*, the individuals of the new species are very active in relation to other Apicotermitinae species. The workers could climb plastic surfaces and even climb a few inches on glass. They are very aggressive, biting and holding the tweezers and brushes used to manipulate them. Even though some specimens of the new species have quite large dehiscent organs, we could not induce the bursting of the abdomen on workers even when considerable pressure was applied with featherweight tweezers on the head or abdomen.

Nest. The nest was located on a recently fallen trunk crossing a trail. The nest is small, just over 30 cm in height. It was active and had foraging lines leading to the ground. The top of the nest seemed to be recently built, consisting of yellowish clay. As it was, we do know if they made their nest on the already fallen tree or if it was an arboreal nest that survived the fallen and was reconstructed (Figure 4).

Termitophiles. When we were collecting the termites from the piece of nest put on the white trail, we found a specimen of a myriapod Pyrgodesmidae (Figure 5) living inside the galleries of the nest. It was not isolated from the termites, as some termitophiles do.

Distribution and habitat. The colony was sampled at Ducke Reserve, Manaus, Brazil. This reserve covers 10,000 ha, has a rugged topography with an altitudinal gradient of 30-140 m, and its vegetation is "terra-firme" non-flooded rain forest with a closed canopy (Ribeiro et al., 1999). The mean temperature is 25.5 ºC, with a mean annual rainfall of 2,400 mm, mainly between November to May (Marques-Filho et al., 1981).

Comparison

The main difference between the two arboreus-group species besides the nest is the coloration of the specimens. While *R. arboreus* body has a brown hue and dark brown head, *Ruptitermes melanocephalus* **sp. nov**. body has a dark brown shade and blackhead. *R. arboreus* possess a small, barely visible circular structure near the fontanelle (see Acioli and Constantino, 2015; Figure 22-B) that is not present or visible on *Ruptitermes melanocephalus* ***sp. nov..*** Probing into internal characters, *Ruptitermes melanocephalus* **sp. nov** has 1-2 spines on the middle of the ridges, at least one well-developed, while *R. arboreus* presents only a single spine, always well-developed.

DISCUSSION

Although termites nests generally show some degree of plasticity, mostly related to local climate and edaphic conditions, for most species they retain a basic structure, and only a few species are known for building very different nest types (Noirot, 2000). Nest structure is used as a reliable taxonomic character for some taxa, such as morphologically similar *Nasutitermes* species (Thorne, 1980) and African Apicotermitinae (Bourguignon, 2016). Moreover, subtle external morphological differences in workers were already used to differentiate Apicotermitinae species (Constantini, 2018) and even coloration alone for *Nasutitermes* (Emerson, 1935).

The most striking defensive feature of *Ruptitermes* is the capability of bursting their abdomen when attacked. This defensive feature is possible due to the presence of dehiscent organs (previously denominated dehiscent glands), which are whitish rounded laterally paired structures located between the metathorax and abdomen, which accumulate a viscous adhesive substance (Poiani, 2016). When threatened, they burst the abdominal wall, releasing the organ content onto the aggressor. The sticky substance thickens when in contact with air and immobilizes the enemy, then kills it due to the presence of toxins (Costa-Leonardo, 2020). Although the bursting mechanism is easily triggered for *R. arboreus*, we could not induce this behavior even squeezing the largest workers vigorously with tweezers. Therefore, we hypothesize that *R. melanocephalus* **sp. nov**. does not have a bursting mechanism. Instead, it seems that the workers have quite a fragile abdomen, prone to be ripped by acute damage such as caused by the mandibles of a predator, such as ants. This may be the most striking difference between *R. melanocephalus* **sp. nov**. and *R. arboreus*, and unfortunately, is can not be used to differentiate specimens conserved in alcohol. One polydesmid was found living with the termites. In fact, there are several descriptions of polydesmids that were collected inhabiting termites nests from diverse genera (ex. *Macrotermes*, *Nasutitermes*, *Microcerotermes*), and the same polydesmid species can cohabit with different termite genera, sometimes distant phylogenetically (Kistner, 1982). Those reports are important since we know very little about the biology of those species.

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Figure legends

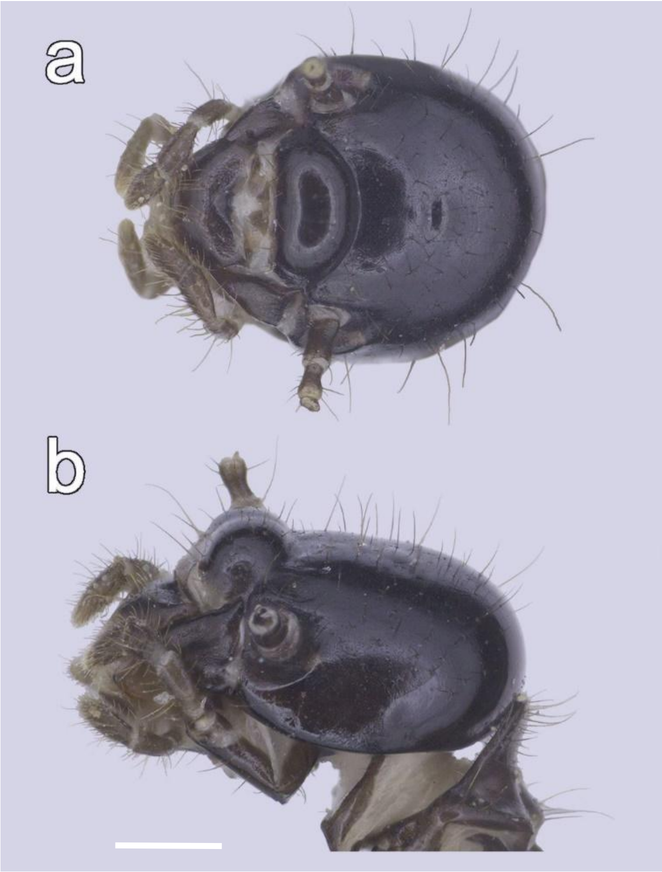

Figure 1: (a) Dorsal view of head and (b) head profile of *Ruptitermes melanocephalus* **sp. nov.** Scale = 0.5mm.

Figure 2: Variation of body size and dehiscent organs development in workers of the same colony of *Ruptitermes melanocephalus* **sp. nov**. Scale = 1 mm.

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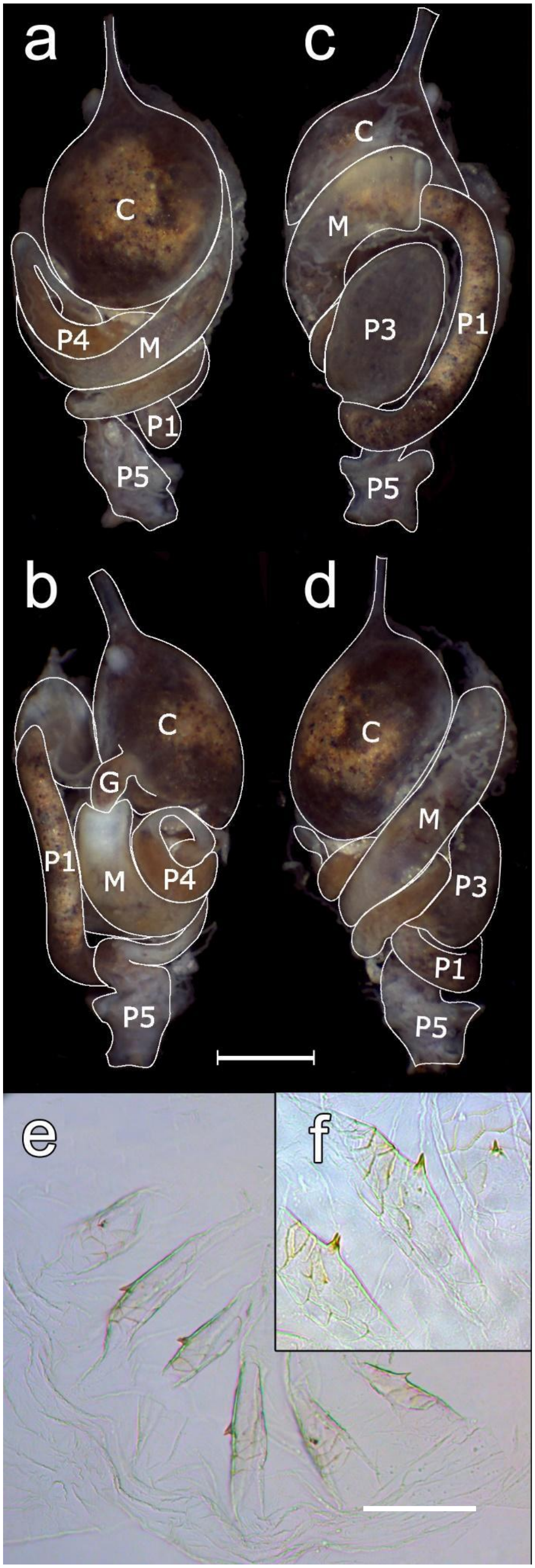

Figure 3: Digestive tube of *Ruptitermes melanocephalus* **sp. nov.** (a) Dorsal view, (b) left view, (c) Ventral view, (d) Right view, (e) enteric valve spread on slide, and (f) section of enteric valve showing a pad with two well- developed spines and one pad with two spines of different size. Scale for a-d = 1 mm. Scale for e = 100µm

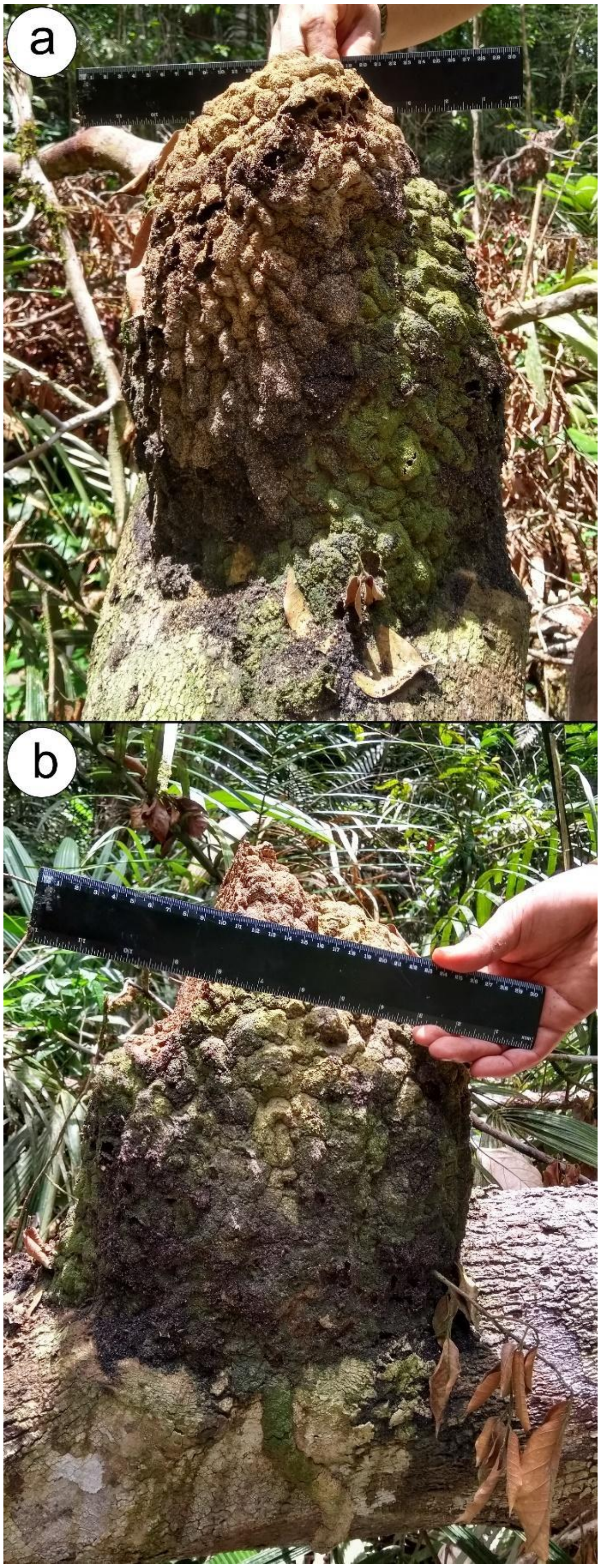
a
b

Figure 4: Nest of *Ruptitermes melanocephalus* **sp. nov.,** found on a recently fallen tree and with galleries leading to the ground.

Figure 5: Pyrgodesmidae sp. in (a) dorsal view and (b) ventral view, found in the nest galleries of the *Ruptitermes melanocephalus* **sp. nov.**

Table 1. Range of the measurements of 10 workers of the same colony of _Ruptitermes melanocephalus_ **sp. nov.**

	Worker (mm)
Head length to end of clypeus	1.04-1.10
Head width	1.02-1.08
Pronotum length	0.32-0.44
Pronotum width	0.69-0.75
Hind tibia length	1.61-170
Fore tibia width/length	0.1-0.118

SÍNTESE

No primeiro capítulo, investigamos como a dinâmica temporal de colonização e extinção de espécies cria o padrão espacial de distribuição de espécies, especialmente em relação à substituição de espécies de um local para outro (beta diversidade espacial). Nós demonstramos que as espécies diferem principalmente na capacidade de colonização ao longo dos gradientes ambientais e essa diferença cria e mantém o turnover espacial de espécies ao longo dos gradientes. Esses resultados estão alinhados com a teoria de nicho ecológico, que prediz que, em equilíbrio, espécies fora de seu nicho ótimo não seriam capazes de competir com outras espécies e invadir uma comunidade. Também demonstra a importância do efeito de prioridade, que indica que espécies tendem a permanecer em locais em que já estão estabelecidas. Porém, diferentemente de outros estudos, nós demonstramos que a capacidade de colonização e estabelecimento das espécies mudam continuamente ao longo dos gradientes ambientais. Essa diferenciação de nicho gera o turnover espacial ao longo dos gradientes e possibilita que as diferentes espécies possam coexistir, levando-se em conta escalas geográficas mais amplas. Nós esperamos resultados similares para outros grupos, especialmente aqueles com taxa reprodutiva alta, como outros invertebrados e a maioria das plantas.

Em relação ao segundo capítulo, testamos métodos para estimar a taxa de emissão de metano média de cupins de diferentes espécies. Também medimos o quanto uma colônia emite em condições naturais e estimamos que cerca de três quartos foi filtrado pela estrutura do cupinzeiro e pelo solo adjacente. Das fontes naturais de metano com relevância global, a emissão por cupins é a menos compreendida (Saunois et al., 2020). Os cupins podem contribuir com cerca de um terço do metano produzido por fontes naturais na Região Neotropical (Kirschke et al., 2013). No entanto, essas estimativas são baseadas em poucos dados empíricos e provavelmente subestimadas (Van Asperen et al., 2021). Décadas atrás, quando se descobriu que os cupins emitiam metano, houve um grande interesse e aumento no número de pesquisas com o grupo, porém este interesse diminuiu quando ficou claro que bactérias metanotróficas amenizava a maior parte das emissões dos cupins, diminuindo sua importância relativa em relação a outras fontes (Bignell, 2020). No entanto, essa rica interação entre cupins, simbiontes metanogênicos e bactérias metanotróficas no ambiente é muito pouco conhecida.

Apenas recentemente foi quantificado que a estrutura do ninho e o solo consegue filtrar de 20-80% do metano antes de ser emitido para atmosfera, para espécies de uma assembleia australiana (Nauer et al., 2018). Os fatores que governam essa diferença, no entanto, permanecem majoritariamente incompreendidos, embora se saiba que a espécie de cupim, a estrutura do ninho, parâmetros químicos do solo e composição original de bactérias metanotróficas no ambiente são alguns dos fatores que influenciam nessa diferença. Com os métodos testados nessa tese, pretendemos continuar a investigar e fornecer uma lista mais completa da emissão média das espécies de cupins e estudar a relação entre produção e emissão de metano a nível de colônia, para melhorar as estimativas futuras da importância dos cupins na Amazônia no balanço desse importante gás do efeito estufa. Nós sabemos que solos alterados na Amazônia apresentam uma diminuição na composição e abundância de bactérias metanotróficas. Uma questão futura interessante e relevante no contexto da preservação ambiental é se os ninhos e solos de áreas alteradas tem capacidade menor de filtrar a produção de metano de seus habitantes. Acreditamos que o método atual pode ser adaptado para responder esta questão, com o desenho amostral e esforço de amostragem corretos.

No terceiro capítulo, publicamos uma espécie de *Ruptitermes,* um dos gêneros mais característicos dentro da subfamília. O plano inicial desta tese pretendia dar um foco muito maior à taxonomia dos Apicotermitinae, que naquele momento retomava com os trabalhos de Bourguignon e colaboradores, que deram base para que os trabalhos com o grupo pudessem ser retomados. No entanto, certamente esta lacuna no conhecimento foi percebida e começou a ser trabalhada por outros pesquisadores, que publicaram novos táxons da subfamília nos últimos anos (Acioli e Constantino, 2015; Scheffrhan et al., 2017; Castro et al., 2018; Constantini et al.,2018; Florian et al., 2019, Castro et al., 2020). No total, foram dissecadas mais de 300 colônias da Amazônia Central brasileira e classificadas em dezenas de morfótipos. Essas amostras abrangem uma área pouco representada nos trabalhos mais recentes com o grupo: a Amazônia brasileira. e serão importantes para a elucidação desse grupo que ainda desafiará os termitólogos por vários anos.

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