UNIVERSIDADE FEDERAL DE MINAS GERAIS
Instituto De Ciências Biológicas
Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre

TIAGO VINÍCIUS FERNANDES

THE LITTLE THINGS THAT RUN THE MOUNTAIN:
direct and indirect role of ants in ecosystem processes in forest and grassland

Belo Horizonte
2022

Tiago Vinícius Fernandes

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Tese apresentada ao Programa de Pós-Graduação em Ecologia, Conservação e Manejo de Vida Silvestre do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do título de Doutor em Ecologia, Conservação e Manejo de Vida Silvestre.

Orientador: Dr. Ricardo Ribeiro De Castro Solar

Belo Horizonte
2022

043 Fernandes, Tiago Vinícius.
The little things that run the mountain: direct and indirect role of ants in ecosystem processes in forest and grassland [manuscrito] / Tiago Vinícius Fernandes. – 2022.
84 f. : il. ; 29,5 cm.

Orientador: Dr. Ricardo Ribeiro de Castro Sola.
Tese (doutorado) – Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas. Programa de Pós-Graduação em Ecologia Conservação e Manejo da Vida Silvestre.

1. Ecologia. 2. Formigas. 3. Ecossistema. I. Sola, Ricardo Ribeiro de Castro. II. Universidade Federal de Minas Gerais. Instituto de Ciências Biológicas. III. Título.

CDU: 502.7

Ficha catalográfica elaborada por Fabiane Cristielle M. Reis - CRB: 6/2680

UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA, CONSERVAÇÃO E MANEJO DA VIDA SILVESTRE

ECMVS

Ata da Defesa de Tese

N° 218
Entrada: 2018/2

Tiago Vinícius Fernandes

No dia 16 de dezembro de 2022, às 08:30 horas, por vídeo conferência, teve lugar a defesa de tese de doutorado no Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, de autoria do(a) doutorando(a) Tiago Vinícius Fernandes, orientando(a) do Professor Ricardo Ribeiro de Castro Solar, intitulado: **"The little things that run the mountain: direct and indirect role of ants in ecosystem processes in forest and grassland"**. Abrindo a sessão, o(a) Presidente(a) da Comissão, Doutor(a) Ricardo Ribeiro de Castro Solar, após dar a conhecer aos presentes o teor das normas regulamentares do trabalho final, passou a palavra para o(a) candidato(a) para apresentação de seu trabalho. Estiveram presentes a Banca Examinadora composta pelos Doutores: Hannah M. Griffiths (University of Bristol), Filipe Machado França (University of Bristol), Fabricio Beggiato Baccaro (UFA), Fernanda Vieira da Costa (UFMG) e demais convidados. Seguiu-se a arguição pelos examinadores, com a respectiva defesa do(a) candidato(a). Após a arguição, apenas os senhores examinadores permaneceram no recinto para avaliação e deliberação acerca do resultado final, sendo a decisão da banca pela:

(X) Aprovação da tese, com eventuais correções mínimas e entrega de versão final pelo orientador diretamente à Secretaria do Programa, no prazo máximo de 30 dias;

( ) Reprovação da tese - (marcar se é a primeira ou segunda reprovação): ( )*primeira reprovação ( ) segunda reprovação

*Conforme o disposto no Artigo 80 da Resolução Complementar do CEPE/UFMG Nº 02/2017, de 04 de julho de 2017, caso seja a primeira reprovação, poderá ser concedido, a critério do Colegiado de Curso, um prazo para a realização de nova defesa de tese.

A banca indica esta tese aos Prêmios CAPES e UFMG de teses? ( ) SIM (X) NÃO

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Belo Horizonte, 16 de dezembro de 2022

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Dedico esta Tese aos meus pais Vitor e Simone
que nunca mediram esforços
para "estudar" seus filhos.

AGRADECIMENTOS

Esse documento marca o fim de mais uma fase na minha vida, mas essa tese não é uma conquista individual e tão pouco se resume apenas aos últimos quatro anos dessa jornada. Por isso, gostaria de agradecer a todos aqueles que cruzaram meu caminho, e que de alguma forma contribuíram para que eu chegasse até aqui. A começar pelos meus pais, Vitor Fernandes e Simone Conceição Fernandes, que são dois trabalhadores do campo sem diplomas, mesmo assim são meus maiores mestres. Agradeço a eles por reconhecerem a educação como o bem mais precioso para seus filhos, e nunca medirem esforços para que pudéssemos estudar. Finalmente, agradeço a eles pelo seu amor e orações que ultrapassam as barreiras geográficas, estando assim sempre presentes independente do local que eu estivesse.

Desde a primeira série do primário até o presente momento foram 24 anos dedicados à educação formal. Nessa jornada acadêmica eu tive que escalar muitos degraus, mas felizmente em cada degrau existia um Professor para me auxiliar. O meu muito obrigado aos educadores que me ajudaram a chegar até aqui. Em especial, agradeço ao Bob (Dr. Ricardo Solar) por ter aceitado me auxiliar em parte da minha jornada, e por todo o suporte para eu me desenvolver como pesquisador. Bob, obrigado pela orientação, não só acadêmica, mais de vida e pelos conhecimentos diversos que compartilhou comigo sobre ciência, estatística, academia, cafés, cervejas, hamburguers, filosofia... Me sinto honrado e muito feliz em dizer que fui orientado por você. Também gostaria de agradecer ao meu grande amigo e mentor Dr. Ricardo Campos por ter me apresentado ao maravilhoso mundo das formigas e da ciência, e por sempre me incentivar a cada vez ir mais longe. Também sou grato ao professor Dr. Frederico Neves e Dra. Tatiana Cornelissen, pelas ideias, conversas e conhecimentos compartilhados que engrandeceram esse trabalho. Finalmente, agradeço aos avaliadores dessa tese, Dr. Filipe França, Dra. Hannah Griffiths, Dra. Fernanda Costa, Dr. Fabricio Baccaro, Dra. Tathiana Guerra e Dra.Luciana Podgaiki, pelo seu tempo e valiosas contribuições.

Muito obrigado à Universidade Federal de Minas Gerais e ao Programa de Pós-graduação em Ecologia, Conservação e Manejo da Vida Silvestre pelo suporte, estrutura física e logística. Essa gratidão se estende a toda comunidade ECMVS, os funcionários, técnicos, terceirizados, professores, pós-doutorandos e colegas de pós-graduação do ECMVS pelos momentos de aprendizado e de descontração. Em especial gostaria de agradecer aos meus amigos “Marmiteiros” e do Centro de Síntese Ecológica e Conservação (CSEC) que me proporcionaram muita diversão e apoio durante esses mais de 4 anos de UFMG.

(Peço licença ao leitor em Língua Portuguesa para agradecer a algumas pessoas em uma língua em que elas entenderão).

I want to thank the extraordinary professor Dr. Kate Parr who kindly accepted me as a visitor in her lab at the University of Liverpool. I am very thankful for her contribution to this thesis, her help when I got COVID and for teaching me away more than science. I also would like to thank the ECOMAR family for the reception, particularly those that shared office 308. Thank you, Alice Lowry, Dina, Eve, Freddie, Stefano, Fin, Guillermo, Ros, Lauren, Joel,Alice Walker and Martjin, for the advice, pints, laughs, warm cups of tea with milk, and even cold days at the beach. Furthermore, I would like to thank Jack, who shared the yellow 38 door with me, and with much patience, helped me to improve my English and even understand some Scouse, "right mate?". Thank you all so much!

Minha gratidão a todas as pessoas que contribuíram de alguma forma para a produção deste trabalho científico. Agradeço a minha imensa equipe de campo e laboratório, composta por velhos e novos amigos que enfrentaram chuva, sol, cansaço, machucados, perigos diversos, horas de triagem e no computador e até um macarrão “alho e ódio”. Meu muito obrigado à: Gabriel Lopes, André Fernandes, Ícaro Rodrigues, Vanessa Monteiro, Samy Behle, Heitor Castro, Tulio Cota, Larissa Silva, Flavio Castro, Giselle Lourenço, Guilherme Lisbene, Tiago Vilas-Boas, Lorena Bueno, Isabella Araujo, André Pinto, Thais Matos, Gabriela, Bruna Campos, Gabriel Aguiar, Tomás, Sthefany Lorena, Milena Solto e Lucas Vieira. Fico grato também aos amigos Inácio Telles, Leticia Ramos e Leticia Boratto que se dispuseram a ler e dar sugestões para o desenvolvimento dessa tese. E finalmente, agradeço a minha mãe Simone e vó Fransisca (em memoria) que costuraram mais de 600 *litterbags* que foram utilizados nesse trabalho.

Eu também sou imensamente grato aos apoios financeiros que tornaram esse sonho possível. Primeiramente, agradeço ao Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (428298/2018-4) pela bolsa com a qual me mantive durante todo o período do doutorado, e pela taxa de bancada que auxiliou na minha formação e execução desse projeto. Em segundo lugar, agradeço ao programa de doutorado sanduiche CAPES-Print sendo financiado pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela oportunidade de expandir minha pesquisa para além das fronteiras do Brasil. Finalmente, sou grato pelos auxílios financeiros que possibilitaram a execução desse projeto concedidos pelo setor de Pesquisa & Desenvolvimento da Agência da Nacional de Energia Elétrica e Companhia Energética de Minas Gerais (P&D ANEEL/CEMIG, PROECOS GT-599) e pela Fundação de Amparo à Pesquisa do Estado de Minas Gerais - FAPEMIG (APQ-01424-15).

Gratidão a família da Casa 15, Paulinho, Luan e Tiago que não somente dividiram a casa comigo, mas fizeram um lar. Obrigado pela parceria, convívio, refeições, conversas e cervejas. Estendo meus agradecimentos às agregadas Lorena e Bruna que amenizaram o caos da pandemia de COVID com a suas presenças, e que ainda agraciam a casa com suas visitas. Também ao morador horário da casa e grande amigo que BH me deu, Carlos, que desde o primeiro dia sempre me estendeu a mão. Obrigado, tudo foi melhor com vocês.

Não menos importante, agradeço aos meus parceiros de longa data, que sempre estavam dispostos a afagar meus lamentos e que fizeram essa jornada mais leve e prazerosa. Obrigado a minha família sanguínea, minhas avós Francisca e Dona Lia e meus avôs Silvio e Zé Larindo, que sempre estarão em minha memória e coração. A meus tios, tias, primos, primas e meus irmãos, André e Aline, que são meu refúgio de segurança, paz e alegria. Sou muito grato também a família que Viçosa me deu: Vinicius, Talles, Eduardo (Dudu), Thiago, Inácio, Priscila, Aline, Tais, Miguel, Gil, Joel, Larissa, Bressane e Brenda. Finalmente, agradeço a Isabele Lima pelos bons momentos, por sempre apoiar e acreditar em mim, e também ao meu amigo formiguense Lucas Vieira pela parceria e fraternidade.

Sinto-me privilegiado por ter recebido tanto apoio e oportunidades que me foram dadas pelos inúmeros programas de incentivo à educação e desenvolvimento científico, tais como transporte escolar gratuito, bolsa família, alojamento universitário, iniciação cientifica, Ciências sem Fronteira, Bolsas de mestrado e doutorado, bolsa de doutorado sanduíche, dentre outras. Assim, agradeço aos órgãos municipais, estaduais e federais, que foram responsáveis pelas criações e manutenções desses programas. Eu espero que programas como esses façam parte da realidade de muitos outros como eu.

Resumo

As últimas décadas testemunharam uma aceleração desenfreada das atividades humanas, o que levou, entre outros estressores, a uma crescente perda de biodiversidade e interferência nas interações biológicas. No entanto, ainda entendemos pouco sobre as consequências da perda de grupos-chave no funcionamento do ecossistema. Nesse contexto, as formigas se destacam como o grupo de animais terrestres mais abundante do planeta. Além disso, elas estão envolvidas em diversos processos ecossistêmicos, tanto diretamente pelo consumo de detritos, bioturbação do solo, dispersão de sementes e polinização; quanto indiretamente em decomposição e herbivoria, predando insetos que executam essas funções (por exemplo, cupins e insetos herbívoros). Há muito tempo estamos cientes do envolvimento das formigas nesses processos, mas poucos estudos quantificaram especificamente a contribuição relativa das formigas para eles em larga escala. Isso deixa uma grande lacuna a ser preenchida; por exemplo, para esclarecer como as características do habitat podem modificar o papel das formigas nos processos do ecossistema. Por isso, neste estudo, avaliamos os efeitos diretos e indiretos das formigas nos processos ecossistêmicos em dois habitats contrastantes, campo e floresta. Mais especificamente, avaliamos o efeito direto de formigas, invertebrados não formigas e vertebrados na eliminação de recursos ricos em nitrogênio e carbono (Capítulo I); e medimos os efeitos indiretos - de cima para baixo - das formigas na herbivoria em comunidade de plantas e na decomposição de matéria orgânica por cupins (Capítulo 2). Para isso, realizamos um experimento de supressão de formigas em larga escala em dois habitats naturais contrastantes nas montanhas brasileiras, campo (campo rupestre) e floresta. Suprimimos 70% das formigas em ambos os habitats, o que diminuiu a remoção de detritos nas pastagens em 57% e em 31% nas florestas. Também mostramos que a supressão de formigas pode mais que dobrar a herbivoria no nível da comunidade de plantas em ambos os habitats e dobrar a decomposição por cupins em pastagens, mas não na floresta. Nós encontramos que a maior abundância e atividade em habitats abertos provavelmente aumenta a importância das formigas na remoção de detritos e decomposição, mas não na herbivoria. Por fim, destacamos o papel insubstituível das formigas, direta ou indiretamente, na manutenção do funcionamento do ecossistema em ambientes não perturbados. Consequentemente, sugerimos que distúrbios que afetam as comunidades de formigas, especialmente causados por humanos, podem impactar fortemente os ecossistemas.

Palavras-chave: Formicidae, serviços ecossistêmicos, interações biológicas, ciclagem de nutrientes, declínio de insetos

Abstract

The last decades have witnessed a rampant acceleration of human activities, which has led, among other stressors, to an increasing loss of biodiversity and interference in biological interactions. However, we still seldom an understanding of the consequences of loosing key groups participating in ecosystem functioning. In this context, ants stand out as the planet's most abundant group of terrestrial animals. Ants are involved in several ecosystem processes, both directly by promoting scavenging of nutrients, soil bioturbation, seed dispersal and pollination; and indirectly in decomposition and herbivory by preying upon insects that perform those functions (e.g. termite and herbivorous insects). Considering the ant's relative contribution to those processes on large scales, there are still few quantitative studies embracing it. This leaves a large gap to fill; for example, to clarify how habitat characteristics can modify ants' role in the ecosystem processes. In this study, we aim to evaluate ants' direct and indirect effects on ecosystem processes in two contrasting habitats, grassland and forest. More specifically, we assessed the direct effect of ants, non-ant invertebrates and vertebrates in scavenging Nitrogen and Carbon-rich resources (Chapter I); and measured the indirect – top-down – effects of ants on herbivory at the plant community level and decomposition by termites (Chapter 2). To do so, we run a large-scale ant suppression experiment in two contrasting natural habitats in the Brazilian mountains: grassland (*campo rupestre*) and forest (montane rainforest). We suppressed 70% of ants in both habitats, which impacted scavenging in the grassland by 57% and by 31% in the forest. We also showed that ant suppression can more than double herbivory at the plant community level in both habitats and double the decomposition by termites in grassland but not in the forest. We support that the higher abundance and activity in open habitats will likely increase their importance in scavenging and decomposition but not for herbivory. Finally, we highlight the irreplaceable, direct or indirect, role of ants in maintaining ecosystem functioning in undisturbed environments. Consequently, disturbances that impact ant communities, especially those caused by humans, could greatly impact ecosystem functioning.

Keywords: Formicidae, Ecosystem services, Biological interactions, Nutrient cycling, insect decline

CONTENTS

1. GENERAL INTRODUCTION

The escalation of human activities in recent decades has led to an increasing loss of biodiversity and profound interference in the functioning of ecosystems (Dirzo et al. 2014; Solar et al. 2015; Barlow et al. 2016; Gonzalez et al. 2020; Oakley and Bicknell 2022). Environmental degradation and its consequences, including climate change, have impaired essential processes, such as energy flow, biomass accumulation and nutrient cycling (Hooper et al. 2005; Newbold et al. 2016). Impacts on key ecosystem processes could be related to the reduction of key animal groups directly or indirectly linked to these processes (Keesing and Young 2014). For example, reducing large vertebrates' populations, a process called defaunation, mainly caused by hunting and habitat loss, directly affects seed dispersal and consequently affects carbon storage in tropical forests (Bello et al. 2015). Another example is the extinction of the megafauna in the Americas by the end of the Pleistocene, which may have led to an increase in fire intensity, due to the accumulation of fuel and the development of forests by the decrease in herbivory pressure (Malhi et al. 2016). However, our knowledge about the importance of some groups of organisms in ecosystems is still limited, making it difficult to predict and mitigate the impacts of human activities on natural environments.

Despite our growing knowledge about the role of biodiversity on ecosystem processes, this comprehension remains mostly obscure to the smaller organisms (e.g. invertebrates), even though they dominate our planet, both numerically and in biomass (Tuma et al. 2020; Schultheiss et al. 2022). Insects are present in virtually all terrestrial environments, with the highest number of described species among all living things (Mora et al. 2011). They are involved in several ecosystem processes, such as pollination, herbivory and decomposition, being considered “the little things that rule the world” (Wilson 1987; del Toro et al. 2012). Despite the lack of quantification of the effect of these organisms on ecosystems, the main hypothesis is that groups of insects with greater abundance make a greater contribution to the functioning of ecosystems (Gaston 1991; Tuma et al. 2020). Nevertheless, insects have been highly impacted by human activities and climate change, which compromise their ability to provide key processes for ecosystem function (Kehoe et al. 2020).

Ants are the most abundant group of terrestrial animals on our planet and participate in several ecosystem functions (del Toro et al. 2012; Schultheiss et al. 2022). Estimates suggest that there are about 30,000 species (from over 16,000 already described), that would sum global biomass greater than all mammals and birds together (Schultheiss et al. 2022; Ant Web 2022). Due to this great diversity and abundance, ants contribute to ecosystem functioning in several ways such as through seed dispersal (Giladi 2006), scavenging (Griffiths et al. 2018), soil bioturbation (Frouz and Jilková 2008) and many other processes (del Toro et al. 2012; Fig. 1). Most ants are omnivorous scavengers and opportunistic predators, so they can control the population of other insects and consequently the role of those insects in other ecosystem processes (Parr et al. 2016; Tuma et al. 2020). For example, by controlling termites and herbivorous insect populations, ants have been shown to drive decreases in organic matter decomposition by termites within African savannas (Walker et al. 2022) and plant damage within many ecosystems (Rosumek et al. 2009; Ramos et al. 2022). Thus, ants also indirectly regulate other ecosystem functions through top-down control (Fig.1). Even though most studies are restricted to small scale investigations, at the plant or branch level focussing on a single or few species within a community, ant predation has been recorded as capable of controlling herbivory (Rosumek et al. 2009). At the same time, the role of ants controlling termites is well accepted, but their effect on decomposition is still unclear.

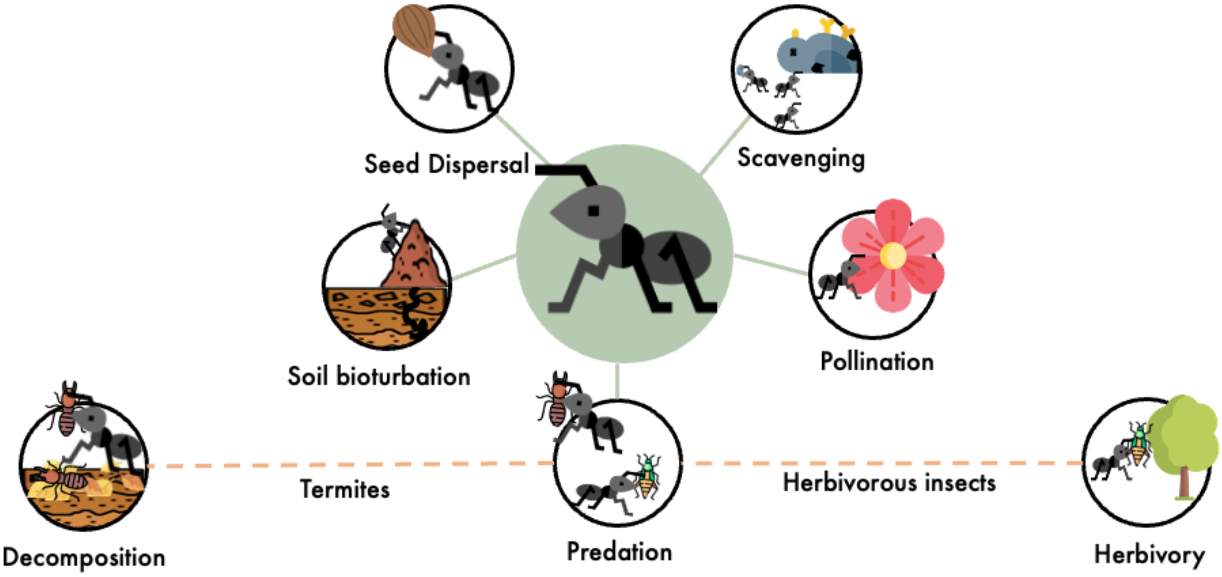

Figure 1: Ecosystem processes that ants are involved directly (solid lines) or indirectly (top-down control - dashed line)

The role of ants in ecosystem processes has been studied for over a century (Weiss 1908). By 2020, we had 1,626 papers explicitly studying the role of ants in at least one of the main processes they are involved: Scavenging (n = 37), Bioturbation (n = 77), Pollination (n = 139), Nutrient cycling (n = 173), Seed dispersal (n = 562), and Predation (n = 638) (Supplementary, Fig. 2). This demonstrates, the lack of support for ants acting as scavenging and a great amount of literature showing their role as predators (Fig. 2), which could affect many other processes through a cascading effect. Furthermore, few studies were able to quantify the effect of ants at large scales (but see, Evans et al. 2011; Parr et al. 2016; Griffiths et al. 2018; Walker et al. 2022). Yet, no large-scale studies were conducted in the neotropics, a region of high diversity and abundance of ants (Kass et al. 2022; Schultheiss et al. 2022).

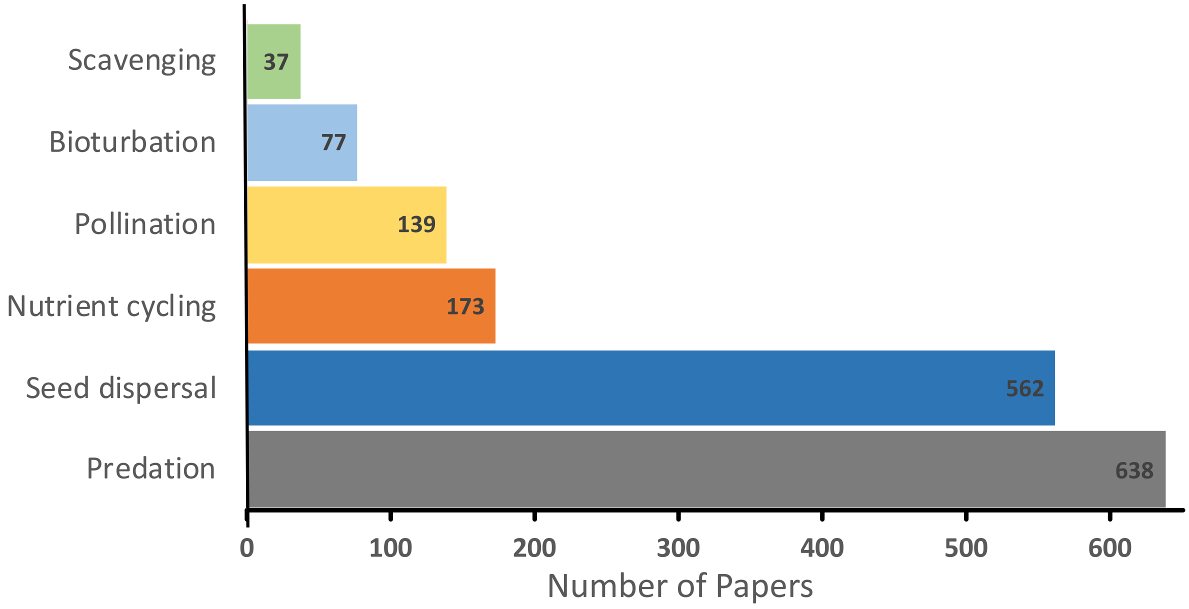

Figure 2: Number of studies evaluating the role of ants in each of the main ecosystem processes they are involved in until august 2020.

The few existing large-scale ant suppression experiments were able to measure ants' direct and indirect contribution to some ecosystem processes. The suppression of ants on a large scale (100m x 100m) was first validated by Parr *et al.* 2016. In this study, authors showed that ants, through a top-down control, affect two important ecosystem processes in African savannas, herbivory and decomposition. In the same study site, Walker *et al.* (2022) demonstrated that this ant suppression could almost double the wood decomposition. Using a similar approach, Griffiths *et al.* (2017) showed that ants are responsible for the removal of more than half of seeds and other resources in the Malaysian rainforest and that the ant role is not likely to be replaceable by any other animal taxa. Although these studies present a great advance in understanding the role of ants in ecosystems, there is still a large gap to be filled; for example, it still needs to be determined how the type of habitat can modify these effects.

Habitat openness and vegetation structures play an important role in ant communities, which can also affect their role in ecosystem processes (Andersen 2019; Castro et al. 2020; Fontenele and Schmidt 2021). When compared to forests (i.e. close ecosystems), grassland and savannas (i.e. open environments) usually present a dissimilar species composition (Dröse et al. 2019; Castro et al. 2020), higher epigeic ants biomass (Andersen 2019) and high ant activity (Bucy and Breed 2006; Parr and Bishop 2022). Thus, habitat openness might increase the contribution of ants to ecosystem functioning. However, the effect of habitat openness on processes performed by ants in undisturbed habitats remains unknown.

To address this gap, in this thesis, we aim to understand the direct and indirect role of ants as a dominant group of animals in the maintenance of terrestrial ecosystem functions. In addition, we seek to elucidate how habitat openness can affect the participation of ants in those processes. We approach those aims in two different manuscripts to be submitted for publication in scientific journals. In Chapter I, we assessed the direct effect of ants, non-ant invertebrates and vertebrates in scavenging Nitrogen and Carbon-rich resources in *campo rupestre* grasslands and in a montane forest. In chapter II, we measured the indirect - top-down - effects of ants in herbivory at the plant community level and decomposition by termites in those two habitats.

2. CHAPTER I

Ants rule the protein and carbohydrates scavenging on grassland but not in the forest.

Tiago Vinícius Fernandes[1,2]; Catherine L. Parr[3,4,5]; Ricardo Idelfonso Campos [6]; Frederico de Siqueira Neves[2], Ricardo Solar [2]

[1] Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre. Universidade Federal de Minas Gerais. Instituto de Ciências Biológicas, Belo Horizonte, Brazil. E-mail: fernandes.tiagov@gmail.com

[2]Departamento de Genética, Ecologia e Evolução. Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

[3] School of Environmental Sciences. The University of Liverpool, Liverpool, United Kingdom.

[4] School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Wits, South Africa

[5]Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa

[6]Departamento de Biologia Geral. Universidade Federal de Viçosa, Viçosa, Brazil

3. Abstract

Scavenging is a central process for the cycle of nutrients in ecosystems. When animals feed on dead organisms or part of them, they promote the transport and redistribution of nutrients in the soil and accelerate the decomposition process. Apart from the importance of specific groups of animals in scavenging, there have been few ecological studies that compare them. Here, we experimentally quantified the relative contribution of ants, non-ant invertebrates and vertebrates in scavenging insect carcasses and seeds in two contrasting habitats. We run a large-scale ant suppression experiment in two contrasting natural habitats that occur together in the Brazilian mountains, grasslands (*campo rupestre*) and forest (montane rainforest). Overall, baits were more likely to be removed on the forest floor than grasslands. Ants were the primary scavengers in grasslands, responsible for more than 57% of dead insects and seeds removals, while, in forests, non-ant invertebrates were the main ones, removing nearly 65% of all baits. Vertebrates had a minor role in scavenging dead insects and seeds in both habitats, with < 4% of removals. Finally, our study suggests that nitrogen is more limited in forests than carbon, and both nutrients seem to be equally limited in grassland. As such, we bring new insights into the role of three animal groups in maintaining key ecosystem processes and further our understanding of differences in ecosystems functioning among habitats.

Keywords: Bottom-up, Resource removal, nutrient cycling, protein, environmental stoichiometry

4. Introduction

Scavenging is a central process for the cycling of nutrients in ecosystems (Beasley *et al.* 2019, Eubanks *et al.* 2019). When animals feed on non-living animal or plant matter, they promote the transport and redistribution of nutrients and accelerate the decomposition process (DeVault *et al.* 2003a). The removal and deposition of nutrients from one place to another creates a flux of nutrients on the soil surface and modifies the original deposition site, which can benefit plants and other animals (Beasley *et al.* 2019, Potapov *et al.* 2022). Vertebrates are the most studied scavenging actors, removing large portions of carrion in all ecosystems. Some vertebrates, such as vultures and hyenas, have diets primarily based on carrion and usually consume the majority of large carrion sources (Beasley *et al.* 2019). Nevertheless, insects play an essential role in scavenging because they are prolific biomass consumers, primary colonisers, and sometimes the only or first ones to access those resources (DeVault *et al.* 2003a, Englmeier *et al.* 2022). For example, blowflies (Calliphoridae) can identify and dominate vertebrate carrion almost immediately, and their larvae can increase the decomposition rates up to nine times (Park *et al.* 2021). Despite the importance of vertebrates and invertebrates for scavenging and nutrient flux, few ecological studies have quantitatively compared them. However, this understanding is essential in the Anthropocene (Lewis & Martin 2015) when disruptions to ecological communities due to climate change, overhunting, and habitat modification can alter animal communities and, therefore, their relative role in ecosystem functions, such as scavenging.

Scavenging ecology has mainly focused on the consumption of large carrions, especially from dead vertebrates (Beasley *et al.* 2019, DeVault *et al.* 2003). Compared with dead vertebrates, dead invertebrates and plants - or their parts - present higher biomass, are small, widely distributed, and consistent. Therefore, they represent a valuable resource to scavengers (Seastedt *et al.* 1981, Tan & Corlett 2012). Invertebrate carcasses are nitrogen (N) rich, a limiting nutrient in almost all environments, that are usually rapidly removed by other animals (Elser *et al.* 2000, Kaspari & Yanoviak 2001). On the other hand, non-lignified plant parts, such as seeds, are a good source of carbon (C, carbohydrate, and fat) for scavengers (Janzen 1971, vander Wall *et al.* 2005). Although it is usually assumed that generalist scavengers consume whatever they find, the Resource-ratio theory (Tilman 1982) predicts that animals achieve optimal consumption of complementary nutrients by favouring the most limiting ones (Kay 2004). Therefore, resources rich in more limited nutrients, such as N-rich ones, are likely removed in great quantities (Craine *et al.* 2018). However, very few studies have compared the relative importance of different food sources to omnivorous scavengers.

Ants are responsible for a great proportion of forest scavenging (Griffiths *et al.* 2018). They are the most abundant group of terrestrial ground-foraging animals, and most species are omnivorous scavengers (Hölldobler & Wilson 1990). They can be found in almost all terrestrial habitats and represent more than 25% of the whole biomass of terrestrial animals (Schultz 2000, Tuma *et al.* 2020). Although the importance of ants to ecosystems is mentioned in several studies (review: Toro *et al.* 2012), few studies have quantified the effect of ants on ecosystem processes at a large scale (Holway & Cameron 2021, Evans *et al.* 2011, Parr *et al.* 2016, Griffiths *et al.* 2018, Walker *et al.* 2022.). To our knowledge, there is only one study, Griffiths et al. (2018), showing that ants are major scavengers on the forest floor and that no other animal group could compensate for their role in this habitat (e.g. no functional redundancy). Thus, there is still a lack of empirical data that quantifies their relative importance in ecosystem functioning in many other ecosystems, nor do we understand how it varies among habitats.

Ants are very sensitive to habitat openness and vegetation structure, which can also affect their role in the ecosystem (Andersen 2019, Castro *et al.* 2020, Fontenele & Schmidt 2021). Castro *et al.* 2020 showed that the species turnover between adjacent open (e.g. grasslands) and close environments (e.g. forests) could be as high as 98%. Furthermore, open environments usually sustain higher biomass of dominant epigeic ant species that can control and remove resources faster than subordinates (Andersen 2019). As ants are heterothermic organisms, the greater solar radiation in open areas increases ground temperature and, consequently, ant activity (Bucy & Breed 2006, Parr & Bishop 2022) – this could be especially important in colder environments such as mountains. Therefore, habitat openness might increase the ecological function provided by ants by increasing their relative role in ecosystem functioning.

We thus asked the following questions: (1) Do scavenging rates vary with habitat? (2) What is the contribution of vertebrates, non-ant invertebrates, and ants to scavenging in each of those habitats? (3) Is there evidence of functional redundancy among taxa? (4) Are the proportions of different resources removed at similar rates among habitats? To answer those questions, we established a large-scale ant suppression experiment in two contrasting natural habitats that occur together as a mosaic in the Brazilian mountain: grasslands (*campo rupestre*) and forest (montane rainforest). We predict that (1) baits are more likely to be removed from the forest floor than grassland due to its higher abundance of animal biomass. However, (2) ants would have higher relative importance in scavenging in open habitats (grasslands) than in closed (forests) because in open habitats, ants have higher activity (Bucy & Breed 2006), richness (Castro *et al.* 2020), and there are more dominant species (Andersen 2019). (3) The role of ants in scavenging is not compensated for by other taxa when ants are absent. Finally, (4) the removal rates of N-rich baits on the ground are higher than C-rich baits in both environments because N is more limiting than C in most habitats (Kaspari & Yanoviak 2001)

5. Methods

5.1.Study area

We undertook this study at the Serra do Cipó National Park, located in the southern portion of the Espinhaço Range Biosphere Reserve (19°14′19″S, 43°31′35″W), in the central region of Minas Gerais state, Brazil. It is a mountainous area with elevations ranging from 1300 m to 1350 m and two marked seasons, wet (October to March) and dry (April to September). The average annual temperature in this area ranges from 15.1 to 20.7°C, and rainfall from 1,250 and 1,550 mm (Fernandes & Madeira 1999). Two distinct and contrasting environments occur together in this area: the *campo rupestre* (grasslands) and semi-deciduous forest islands, allowing us two test our hypotheses within the same climatic conditions (Fig. 1). The *campo rupestre*, which dominated the landscape, is an ancient ecosystem, climatically buffered and, an infertile landscape (OCBIL; Hopper et al. 2021; Fig. 1A). It is characterised by the dominance of herbs and shrubs covering nutrient-poor quarzitic soil (Fernandes 2016). Within the *campo rupestre* matrix are natural islands of montane rainforest (locally called *Capões de Mata*), with floristic composition resembling the semi-deciduous Atlantic Forests (Coelho *et al.* 2018; Fig. 1B). Those habitats have a very diverse and well-study ant community (Castro *et al.* 2020, Nunes *et al.* 2020, Brant *et al.* 2021), with approximately 195 species/morphospecies (50 genera) sampled, a similar richness found in the Amazon rainforest areas (Majer & Delabie 1994, Vasconcelos & Vilhena 2006).

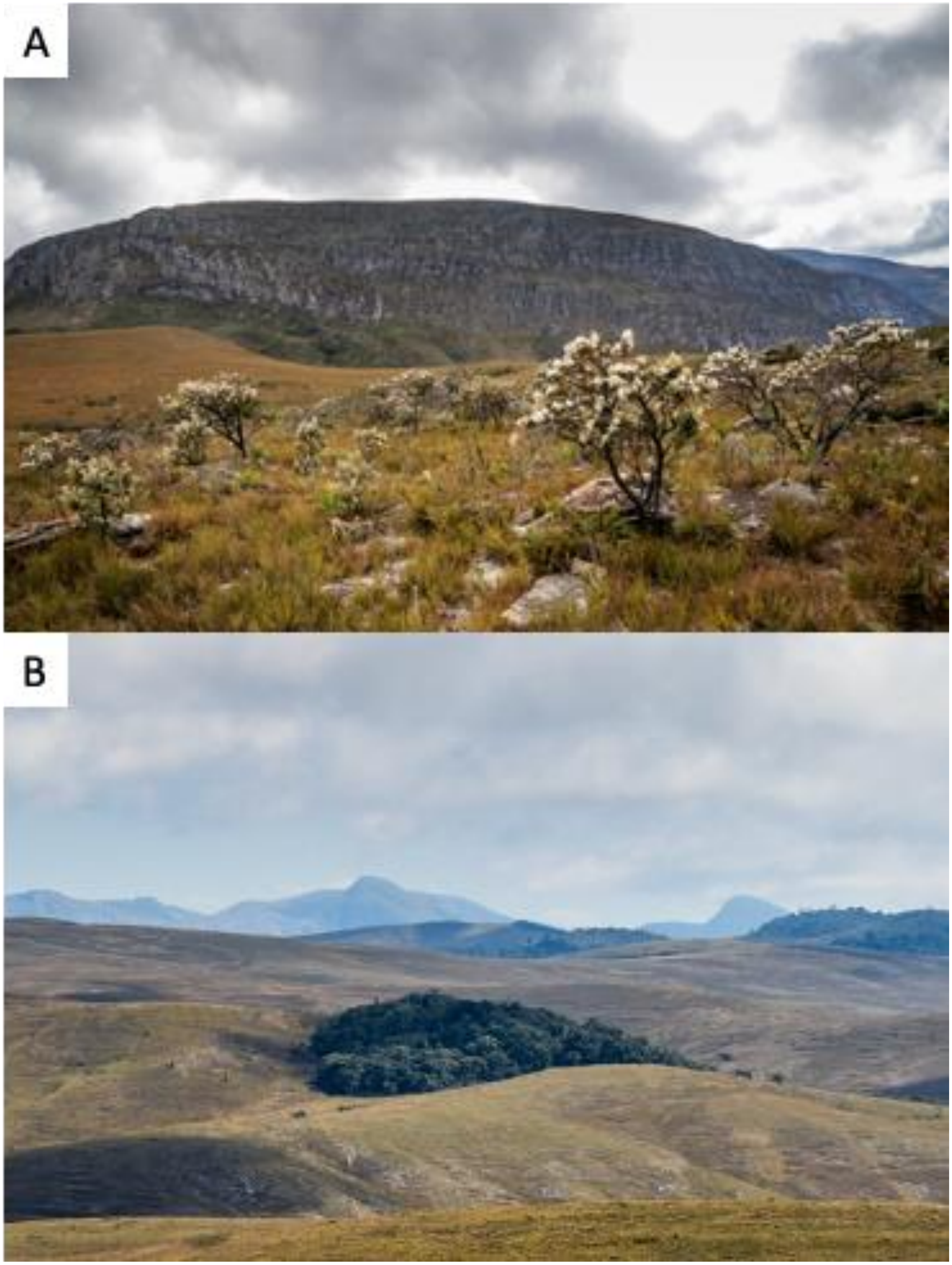

Figure 1. Study sites at Serra do Cipó National Park, Minas Gerais state, Brazil. A) *campo rupestre* (rocky grassland) and B) tropical montane forests islands surrounded by *campo rupestre* matrix.

5.2.Experimental design

We set a factorial experimental design with 14 plots divided into i) 4 forest control; ii) 4 forest ant-suppression; iii) 3 grassland control; iv) 3 grassland ant-suppression treatments. Each plot consisted of an experimental area of 50 x 50 meters and a buffer area of 15 meters on each side (80 x 80 m total). We aggregated the plots in pairs of control and treatment, where each control plot was located 100 m from its respective suppression plot. Each pair of plots was at least 1 km apart, and in the case of forest plots, each pair was placed on a different forest island. Therefore, we reduced variation between control and suppression plots, captured a more significant site variation within each habitat, and took independent samples.

To suppresses ants, we used two poison bait types: homemade imidacloprid ant bait (100 ppm w/v); and ATTA MEX-S® (Sulfonamide 300 ppm m/m). We prepared the homemade ant bait following Griffiths *et al.* (2018) and Parr *et al.* (2016) by soaking 1 kg of Whiskas® cat food (mostly grain-based carbohydrates, and protein) in a solution of 40 g of jelly (gelatin and sugar), 1 litre of distilled water, and 0.0285 g of Evidence 700 WG from Bayer AG (imidacloprid 70 % m/m). In December 2019 (wet season), we started the ant suppression by spreading 10 kg of homemade and 5 kg of commercial ant baits across each suppression plot, including the buffer area. The suppression remained active for 90 days. To minimise the use of chemical baits, we monitored ant abundance monthly, and when ant activity in suppression plots was 20% of the control plots, we reapplied both ant baits at the same amount as when we started, this only occurred once, 60 days after we started the ant suppression (Supplementary 1).

5.3.Caveats about ant suppression bait

We chose imidacloprid and sulfonamide as ant suppression agents due to their high specificity (Bai *et al.* 1991), the low concentration required (Rust *et al.* 2004, Parr *et al.* 2016), and their rapid degradation in the environment (Anhalt *et al.* 2007). Also, the method described here has already been tested in the studies done by Parr et al. (2016) in African savannas, and Griffiths et al. (2018) carried out in Bornean forests. Both studies showed that this method has no detrimental effect on other groups of invertebrates and was effective in suppressing 76% of ants in savannas and 90% in Borneo tropical forests. In addition, we emphasise that because we sought to minimise, or remove, any possible effect on other non-target organisms and the environment: I) Imidacloprid and sulfonamide are neocotinoid that specifically affect the receptors of insect motor neurons (Bai *et al.* 1991), and therefore have low toxicity to non-target organisms like vertebrates and plants. II) The doses used are very low (100 p.p.m and 300 p.p.m); for example, the Imidacloprid dose is four times lower than the lowest recommended dose for controlling pests that attack crops such as cauliflower, broccoli, and citrus (Wang *et al.* 2015, Bayer 2019). Therefore, they present a very low risk to other non-target organisms (Parr *et al.* 2016, Griffiths *et al.* 2018). III) The baits are designed to be highly attractive to ants that usually remove them quickly from the soil surface and carry them to their nests where consumption by other organisms is unlikely (Parr *et al.* 2016). IV) The application timetable was defined based on the increased activity of the ants, maximising the removal speed by these organisms (Hölldobler & Wilson 1990). Here, we also did not find a significant effect on the overall abundance of non-target epigeic invertebrates among treatments (Supplementary 2). V) The active ingredient Imidacloprid was chosen because it rapidly degrades microorganisms in the soil, reducing the persistence of this chemical in the environment (Anhalt *et al.* 2007). VI) We carried out periodic monitoring of ant abundance and only repeated the application of baits if necessary (Supplementary 1).

5.4.Monitoring ant abundance

We monitored ant activity monthly in each experimental area by setting three linear 50-m transects apart 25 m from each other. Each transect had three sampling stations, where we placed 0.3 g of the homemade bait (catfood without Imidacloprid) onto three white laminated cards (10 × 10 cm), each separated by 25 m. After one hour, we inspected and counted the number of ants on the card. This method allowed us to estimate ant activity in each plot rapidly. However, to quantify the effects of the ant suppression treatment more accurately on ant numerical abundance, in March 2020, we sampled ants using nine pitfall traps per plot. Pitfall traps consisted of 350 ml containers (8 cm diameter) buried flush with the ground level and 1/3 filled with a solution of water and detergent. They were also arranged in three linear equidistant transects and 25m apart. Pitfall traps remained in the field for 48 h, then we filtered their solution, placed the collected invertebrates in a 70% alcohol solution, and sorted and counted the ants in the lab.

5.5.Resource removal

To perform the resource removal trials, we set 40 stations per plot equally divided into: 20 stations with 2 g of dried *Tenebrio molitor* larvae (hereafter: protein stations) and 20 with 3 g of crushed sunflower seeds (*Helianthus annuus*; hereafter: seed stations). The baits were used to mimic natural resources used by scavengers. We also covered half of the stations with metal cages (cage dimension= 20 diameter × 20 height, mesh = 1 × 1 cm) to prevent vertebrates from accessing the resources. Each station consisted of an open petri dish (6 cm width; 0.5 cm depth) where we randomly placed one of the two food sources and covered it with metal mesh cages (caged stations) or not (open stations). With this design, we could separately estimate the resources removed: *Non-ant invertebrates* = bait mass removed in caged stations in ant-suppression plots; *Vertebrates*= the difference between bait mass removed by the whole community (open stations in control plots) and bait mass removed by invertebrates (caged stations in control plot); *Ants* = the difference between bait mass removed by invertebrates (caged stations in control plots) and bait mass removed by non-ant invertebrates;. The stations were distributed 5 m from each other in six equidistant transects within the 50 x 50 experimental plots. All stations were protected from the rain by a plastic cover and left in the field for 24 hours. We dried baits at 50°C for 48h and weighed all resources before placing them (initial dried weight before) and after collecting them in the field (final dried weight after). To calculate the mass of resource removed, we subtract the weight after from the initial weight of each sample. The scavenging experiment took place in March 2020, 90 days after we began the ant suppression.

5.6.Statistical analyses

We evaluated the success of ant suppression in each habitat (grassland/forest) using a Generalized Linear Mixed Models (GLMM) with a negative binomial error distribution. Ant suppression treatment (control/suppression) and habitat were set as explanatory variables, the number of ants collected in pitfall traps was selected as the response variable, and the plot identity as a random effect. We used the negative binomial error distribution to correct for the overdispersion presented by the Poisson error distribution.

To test whether the habitat type (grassland/forest), ant suppression treatments (control/suppression), cages (open/caged), bait type (protein/seeds) or the interaction between these factors affect resource removal, we used three Generalized Linear Mixed Models (GLMM) with Binomial distribution. We set ant suppression, habitat, cages, and bait type as explanatory variables, the proportion of dry mass removed as the response variable, and the plot identity as a random effect.

We conducted all analyses in R v 4.1.2 (Crawley 2013, R Core Team 2021). We graphically examined the residuals for model assumptions, including normality of errors and homogeneity of variances, to assess model suitability in all models using the package DHARMa. We used the lme4 package v 1.1-12 (R Core Team 2016) to build LME models. We computed the significance of the LME model using type II Wald F tests with Kenward–Roger degrees of freedom approximation, “mixlm” package v 1.2.3 (Liland 2018). We used a backward model selection approach based on likelihood ratio test to determine the simplest fitted models.

6. Results

We collected 776 ants from 49 morphospecies (22 genera), 33 morphospecies in the grassland and 31 in the forest with distinctive compositions (Supplementary 3, fig. 1). We suppressed a similar proportion of ants in both habitats. Ant suppression plots had 73% and 70% fewer ants than control plots in the forest and grassland, respectively (forest control= 14±12.46 mean±SD ants/pitfall, forest suppression = 3.9±2.9 ants/pitfall; Grassland control= 26.8±16.7 ants/pitfall, Grassland suppression = 8±5.25 ants/pitfall; Chi= 72.7; P<0.01). The average number of ants per pitfall in control and suppression plots combined was twice as high in the grassland than in the forest (grassland= 17.3±15.39 ants/pitfall; forest = 7.94± 7.02 ants/pitfall; Chi= 22.25, P<0.01; Figure 1).

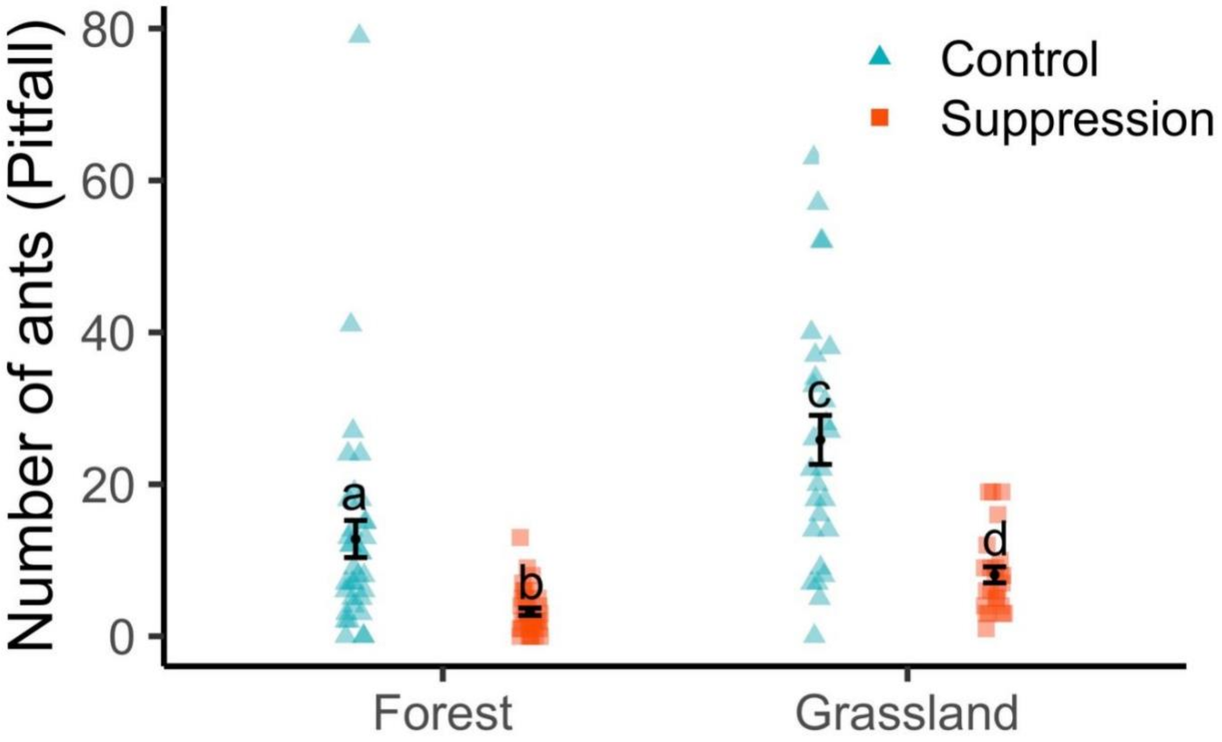

Figure 2. Number of ants per pitfall trap in ant suppression plots (suppression) and control plots set in two mountainous habitats in southeast Brazil, forest (Could Forest) and Grassland (*campo rupestre*). Pitfall traps were set 90 days after the beginning of ant suppression. Black points represent average and lines standard errors of groups. Statistical differences are represented by different letters.

Habitat type (forest/grassland; Chi = 27.64, P< 0.01), ant suppression treatment (ant suppression/control; Chi = 25.17, P< 0.01), and bait type (protein/seeds; Chi = 19.28, P< 0.01), all influenced the proportion of bait removed, but cage treatment did not (caged/open; Chi = 2.13, P=0.14). In 24 hours, 23.2% more baits mass was removed in forests than in grasslands; 24% more baits were removed in control plots than in ant suppression plots, and 18.9% more protein mass was removed than seed mass (Figure 3, figure 4A, Supplementary 4). There was an interaction between habitat and bait type (Chi= 33.27, P<0.01). This interaction shows that protein removal is higher in forests than in grassland, but protein removal in grasslands, seed removal in forests and grassland did not differ (figure 4A). Besides, the interaction between ant suppression treatment and bait type (Chi= 13.19, P<0.01), indicates that the effect of ant suppression is higher for protein than for seeds (figure 4A).

The difference between bait mass removed in closed stations in control plots (resources available to all invertebrates only) and open stations in control plots (full community access) suggests that vertebrates play a minor role in resource removal in both habitats, being responsible for ~4% and 3% of removals in forest and grassland respectively (Figure 4a). We also partitioned the scavenging by ants from non-ant invertebrates by subtracting caged stations in suppression plots (mainly non-ant invertebrates) from caged stations in control plots (resources available to all invertebrates only). We found that ants removed 31% of the forest and 57% of baits in grassland. Finally, non-ant invertebrates removed 65% and 40% of baits in forest and grassland, respectively (caged stations in suppression plots). However, it is essential to point out that our figures for the bait loss due to ants are likely to be underestimated while for non-ant invertebrates may be an overestimation as we could not wholly remove ants from suppression plots (suppression 70% of ants, Figure 2).

We checked the functional redundancy of vertebrates, ants, and non-ant invertebrates in scavenging, by comparing the relative (rather than absolute) contribution of each animal group in removing seeds and protein in forests and grassland (Figure 4b). To calculate the contribution of each group, we used the same estimation method presented above in relation to the total amount of protein and seed removed. The protein removal was twice as high in the forest than in the grassland, and the contribution of each animal group differed. In forests, protein removal was led by non-ant invertebrates (69.2%), followed by ants (27.1%) and vertebrates (2%). While in grassland, ants were the main protein removal (57.7%), followed by non-ant invertebrates (37.6%) and vertebrates

(4.7%, Figure 4b). Nearly half of the seeds were removed in both habitats, and the contribution of each animal group is similar to protein removal, with non-ant invertebrates (61.3%) leading seed removal in forest, followed by ants (35.7%) and vertebrates (5.6%). Whereas, in grassland, ants were the main seed removal (57.3%), followed by non-ant invertebrates (40.8%) and vertebrates (1.9%, Figure 4b). Although overall small, the contribution of vertebrates to seed removal was three times higher in the forest (5.6%) than in grassland (1.9%).

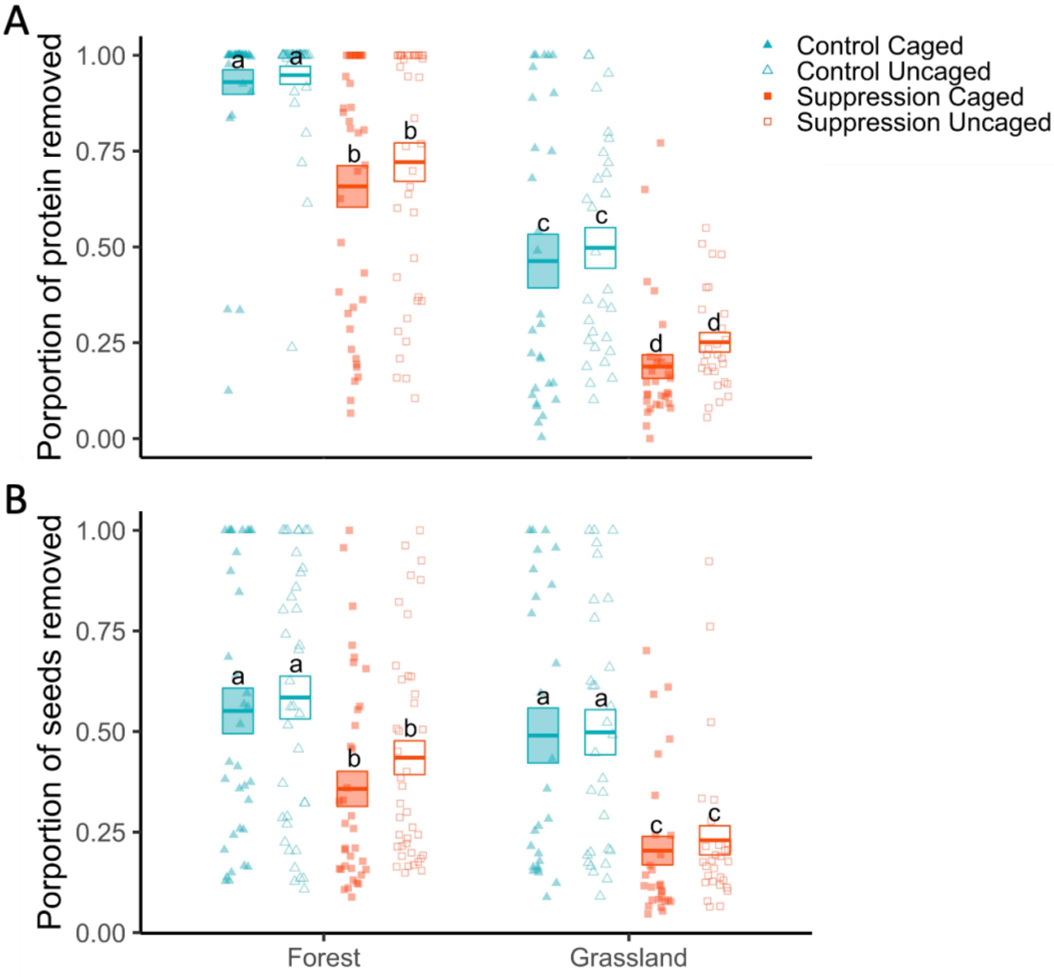

Figure 3. Proportion of **A)** protein (2 g of *Tenebrio molitor* larvae) and **B)** seeds (3g of sunflower seeds) removed per station according to ant suppression (suppression/control) and caged treatments (caged/open) in two mountainous habitats in southeast Brazil montane rainforest forest and Grassland (*campo rupestre*). Horizontal lines represent average, and boxes show standard errors. Statistical differences are represented by different letters.

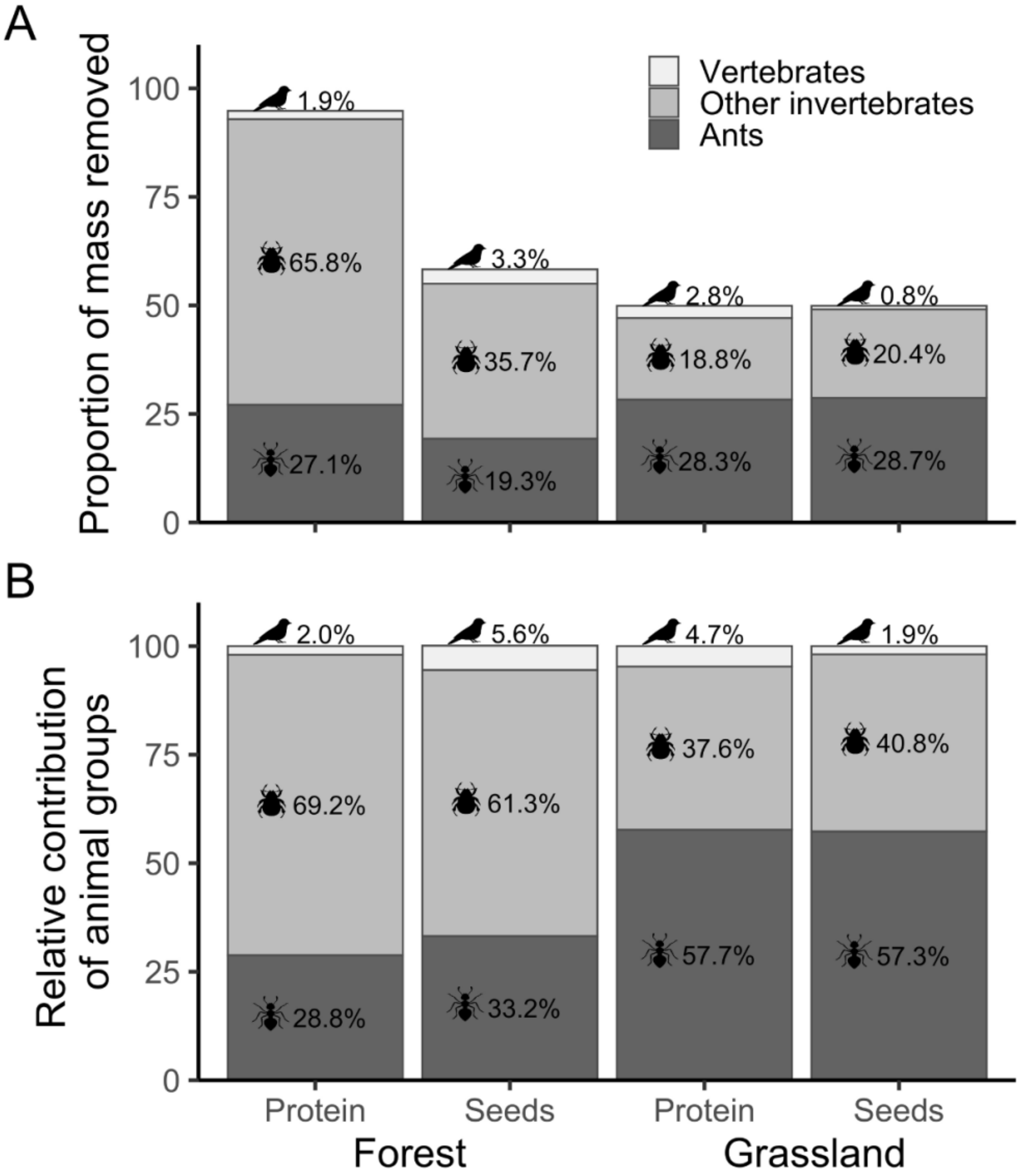

Figure 4. Estimations of *Tenebrio molitor* larvae (protein) and sunflower seeds (seeds) removal by ants, non-ant invertebrates and vertebrates in forest and grassland **A)** Proportion of mass removed from the total offered (protein 2g, seeds 3g) **B)** Relative contribution of each animal group to mass removal, based on the total removal for each bait type in each habitat. By: VERTEBRATES= the difference between bait mass removed in open stations in control plots and bait mass removed in caged stations in control plot; ANTS = the difference between the bait mass removed in caged stations in control plots and bait mass removed in closed stations in ant-suppression plots; NON-ANT INVERTEBRATES = bait mass removed in caged stations in ant-suppression plots.

7. Discussion

Here, we experimentally quantified simultaneously the relative contribution of ants, non-ant invertebrates and vertebrates in scavenging insect carcasses and seeds in two adjacent and contrasting natural habitats. We found less bait was taken on the grassland floor than on the forest. Ants are the primary scavengers of invertebrates carcasses and seeds in grassland, non-ant invertebrates are the main ones in forests, and vertebrates have a trivial role in both habitats. Although ant relative contribution to scavenging varies between habitats, their role in this process could not be replaced by any other group when they are absent. That might indirectly affect community dynamics and ecosystem structure (Parr *et al.* 2016, Griffiths *et al.* 2018). Finally, we showed that the removal of N-rich baits in forests was twice as higher as C-rich baits, while there was no difference in removal between bait types in grassland. As such, we bring new insights into the role of three animal groups in maintaining key ecosystem processes and further our understanding of differences in ecosystems functioning among habitats.

We found that 50% and 73% of the baits offered were removed in 24h in grassland and forest, respectively. Scavenging is an essential part of the decomposition process that links higher trophic level organisms, decomposers, and plants (Carter *et al.* 2007, Barceló *et al.* 2022). The consumption of dead organisms by scavengers releases nutrients into the trophic web, making them available to other levels of consumers (Wilson & Wolkovich 2011). Furthermore, what is not assimilated by those organisms is broken down into smaller parts, which facilitates the decomposition by microorganisms (Barceló *et al.* 2022). Therefore, scavenging small detritus (e.g. dead invertebrates and seeds) might play a significant role in ecosystem nutrient flux because, as we showed, at least half of those resources are taken by scavengers rather than being directly decomposed. Moreover, the impact of scavengers on nutrient flux seems stronger in forests than in grasslands, leading to a faster nutrient cycle in forests. While most scavenging studies focus on vertebrate carcasses (DeVault et al. 2003), we show that dead invertebrates and seeds are also essential resources for scavengers (Tan & Corlett 2012). Although, this needs to be taken with care because the amount and type of resources that naturally occur in each habitat may also differ (Saint-Germainet al., 2007; Lakka & Kouki, 2009).

Invertebrates were responsible for virtually all scavenging of small resources (e.g. invertebrates carcasses and seeds) in both forest and grassland, and vertebrates do not significantly contribute to this process. Among invertebrates, ants present higher relative importance in scavenging in grassland and non-ant invertebrates in the forest. Ants are social insects, mostly living in fixed colonies, usually with hundreds of individuals (Beckers *et al.* 1989). When foraging, ants typically take the food source to their colony before consuming it (Hölldobler & Wilson 1990). This characteristic behaviour contributes to the redistribution and concentration of nutrients in ecosystems, increasing the nutrients in the nest area (e.g., N, P, organic matter; Farji-Brener & Werenkraut 2017). Consequently, soil modifications by ants can increase environmental heterogeneity and impact soil biota and plant communities (Farji-Brener & Werenkraut 2017, Swanson *et al.* 2019, Lash *et al.* 2020). On the other hand, non-ant invertebrate scavengers, such as cockroaches and beetles, are solitary foragers that could either consume it on the site or remove small portions of the source (Durier & Rivault 2001, Ilardi *et al.* 2021). Therefore, ant and non-ant invertebrates might have different outcomes in the ecosystem's scavenging dynamic and nutrient cycle, with ants promoting greater heterogeneity of nutrients. Thus, we might expect soil nutrients to be patchier in grassland, where ants are the main scavengers, than in forests where non-ant vertebrates are the main ones. The nutrient patches created by ants could ultimately influence the plant community dynamic by increasing plant perforce and survival (Farji-Brener & Werenkraut 2017). This effect of nutrient patches could be even more prominent in *campo rupestre* because it has soils with low nutrient contents.

The insubstantial scavenging by vertebrates can decrease nutrient transport across great distances or even among ecosystems. By consuming resources, vertebrates usually transport and deposit nutrients and seeds over longer distances than invertebrates (Beasley *et al.* 2019, Potapov *et al.* 2022). Consequently, they are responsible for spreading nutrients at the landscape level and connecting ecosystems through the nutrient flux (DeVault et al. 2003, Almeida-neto et al. 2008). We found that vertebrates contribute to less than 4% of bait removals, limiting the nutrient flux within the studied ecosystem and other ecosystems. In contrast, Griffiths et al. (2017), using a similar experimental design, found that the role of vertebrates in scavenging in a Bornean rainforest is five times higher than we found here (25%). Such difference might be related to a low abundance of vertebrates in our experimental site, which could be explained by two hypotheses that need further investigation. First, this mountainous ecosystem might not support a high abundance of vertebrates because of adverse conditions, structure, and relatively low resource availability (Geise *et al.* 2004, Godoi & de Souza 2016). Second, human activities, such as cattle rise, increased fire frequency, and climate change that has profoundly impacted those habits could have led to a defaunation process (Dirzo *et al.* 2014, Fernandes, Arantes-Garcia, *et al.* 2020). However, those hypotheses still need empirical support. In either case, the lack of vertebrates can limit the Import and export of nutrients in that ecosystem and affect other ecosystem processes and services related to this group (e.g. seed dispersal and biological control).

Ant suppression decreases the scavenging of protein and seeds in both habitats studied. According to the redundancy hypothesis, species loss does not affect ecosystem processes if there are functionally similar taxa that can replace them (Grime, 1997, Yachi & Loreau, 1999). Based on this hypothesis, we support the findings of Griffiths et al. (2018) that indicate low functional redundancy of scavenging by ants. Because of their great diversity and abundance, ants are key actors in many ecosystem processes (del Toro *et al.* 2012). They contribute to processes either directly, for example, as seed dispersers (Fernandes, Paolucci, *et al.* 2020), in soil formation (Farji-Brener & Werenkraut 2017), and as resource removal agents (Griffiths *et al.* 2018) or indirectly controlling populations of other invertebrates (Parr *et al.* 2016) and protecting plants against herbivorous (Gomes *et al.* 2021). Therefore, the lack of functional redundancy of ants as scavengers could also extend to other ecosystem processes dominated by ants. It is also important to point out that we were unable to suppress all ants in our experimental area, so their role in scavenging is likely much greater than shown here.

We also support our hypothesis that ants are more critical for scavenging in grassland than in forest habitats. We have suppressed 70% of ants in both habitats, which impacts scavenging in the grassland by 57% and only 31% in forests. The habitat might openness seems to increases ants' role in other ecosystem processes. For example, Jones *et al.* (2017) showed that plant protection by ants is higher in patches with high light incidence. Therefore, we believe this pattern could be extended to other processes involving ants, such as the ones mentioned above, but we still need more studies. If true, human activities that affect the ant community (e.g. change in land use and climate change), especially their abundance, would greatly impact open ecosystems.

Our study suggests that N is more limited in forests than carbon, but both nutrients seem to be equally limited to grassland. Most terrestrial organisms are assumed to be limited by N availability (White 1978). According to the Resource-ratio theory (Tilman 1982), consumers achieve optimal ratios of complementary nutrients (Kay 2004). Here, we showed that there was no difference in the N-rich and C-rich baits removal proportion in grassland, indicating that both nutrients might equally limit those communities. The *campo rupestre* is a nutrient and water pour environment, so plant development is slow, and the production of reliable C sources to animals (fruit, nectar, seeds) is rare and/or sporadic (Silveira et al. 2015, Hopper et al. 2021). On the other hand, on the forest floor, N-rich baits were taken almost twice as much as C-rich ones, which is suggestive of N-limitation. That might be related to the increase in plant production of Carbon-rich food sources (fruits and nectar) in forests, which offer a consistent and reliable supply of carbohydrates compared to grasslands.

Most ants are omnivores, so they balance the N: C intake by favouring resources with the most limiting nutrient in a habitat. Kaspari and Yanoviak (2001) showed that canopy ants have a higher preference for N-rich resources when compared to ground ants, which is explained by the higher plant-based diet of canopy ants (C-rich). Similarly, we suggest that differences in habitat productivity could modulate the ant preference for nutrients. Ants inhabiting forests (high productive habitat) seem to be N-limited, while in grassland, N and C are equally limiting. This difference in habitat nutrient limitation can also guide other ecosystem processes (Davidson 2005). C-rich diets increase ant aggressiveness and predation, consequentially ant role in plant protection (Ness *et al.* 2009, Ribeiro *et al.* 2019). In accordance, the increase of carbohydrates in Ants-tended Hemiptera diet changes their interaction with their protective hemipteran. Then, ants could treating hemipterans as prey (N-rich source) instead of solely feeding on their exudate (C-rich source; Engel et al. 2001). Thus, ant predation on hemipterous and plant protection might be more intense in forests where N seems more limited than C.

We experimentally support Eduard Wilson’s statement (1987): “the little things that run the world” by showing that invertebrates rule the scavenging of small resource patches in two contrasting habitats. Invertebrates are an overlooked scavenging group, but here we showed that they remove more than half of all baits offered in forests and grasslands. That implies that most non-living insects or non-lignified plant matter might be consumed by invertebrate scavengers rather than decomposed. In such a way, nutrients are rapidly recycled and inserted back into the trophic chain. We also show a minor contribution of vertebrates in this process, which could limit the import and export of nutrients in these mountainous ecosystems. However, the cause and the effects of the lack of vertebrate contribution to scavenging need further investigation. Finally, we highlight that a single animal group, the ants, is responsible for most nutrient removal in mountainous grassland but not in forests. That shows the possible dominance of this group in governing processes in open habitats in mountainous environments. However, we need more studies in different forests and grasslands to asses if this statement is also applied to low-land environments. Thus, we further advance our knowledge on the relative importance of three major groups in scavenging and the differences in ecosystems functioning between two contrasting tropical habitats.

8. References:

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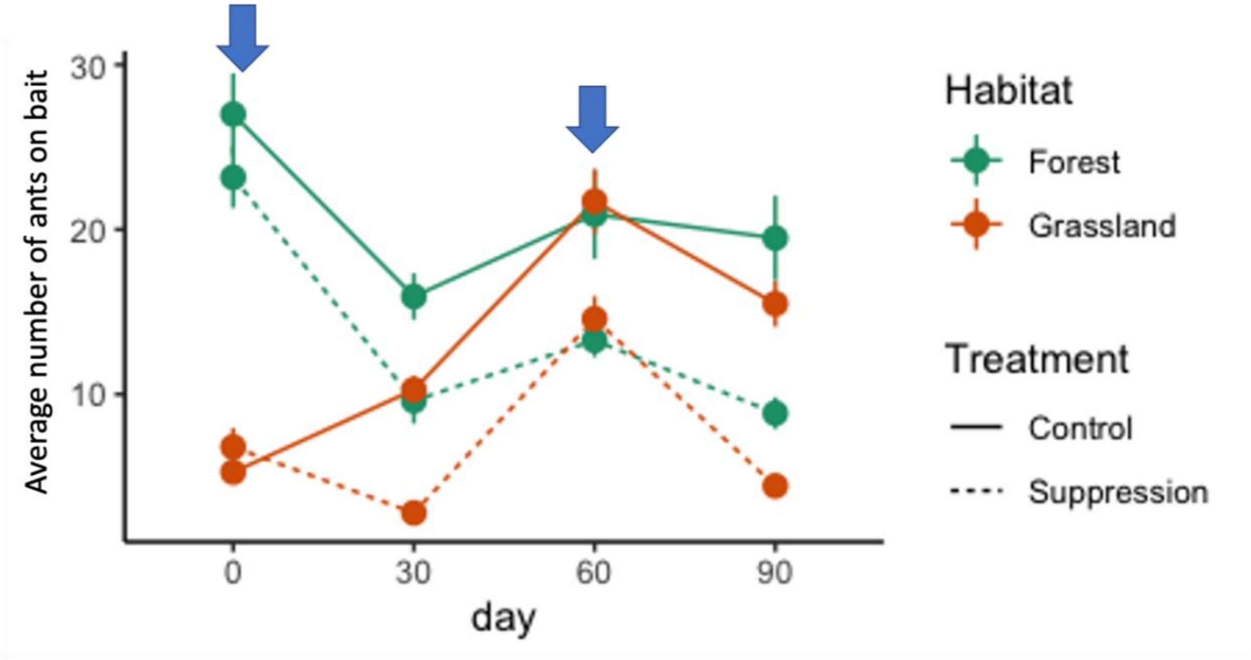
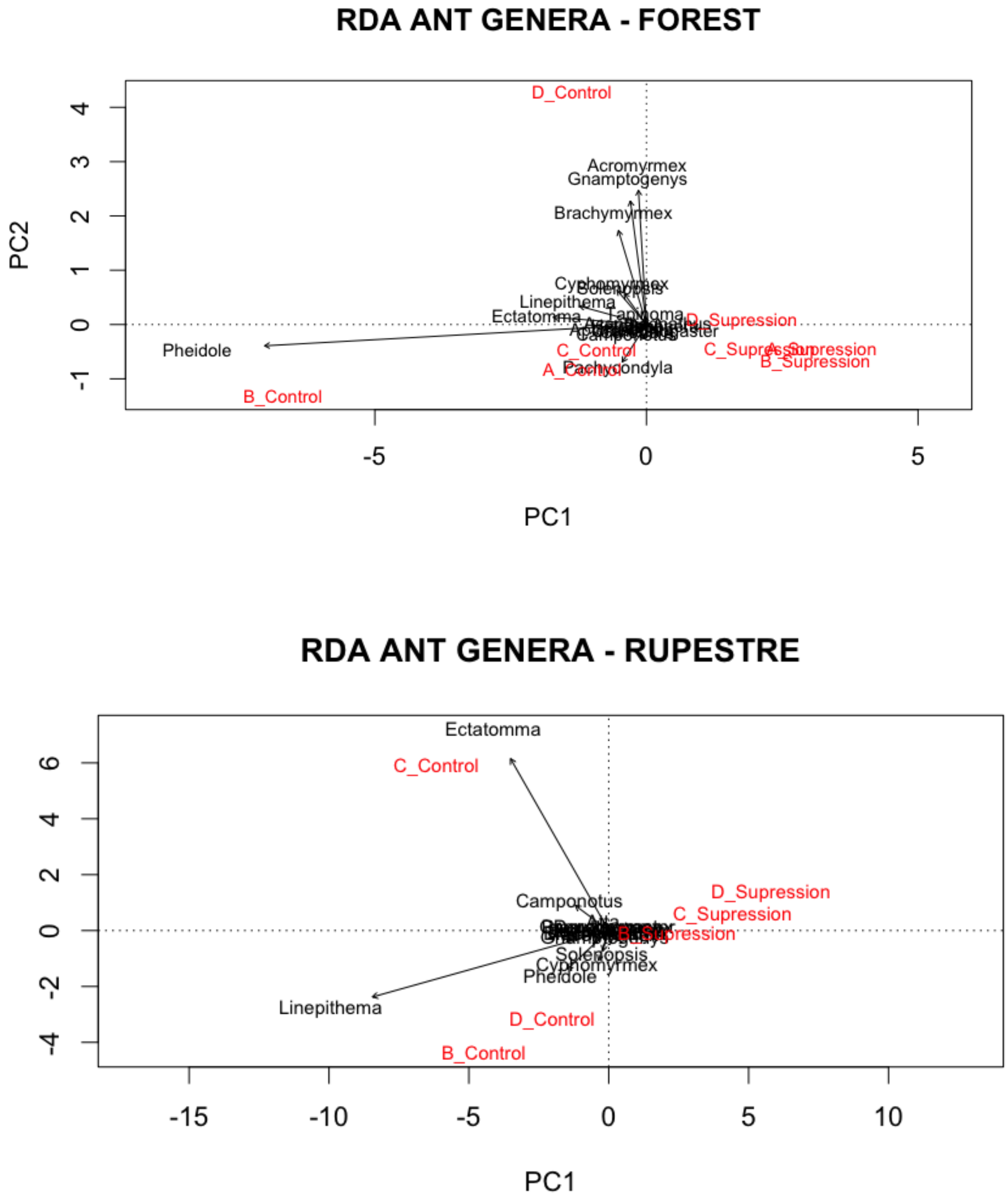
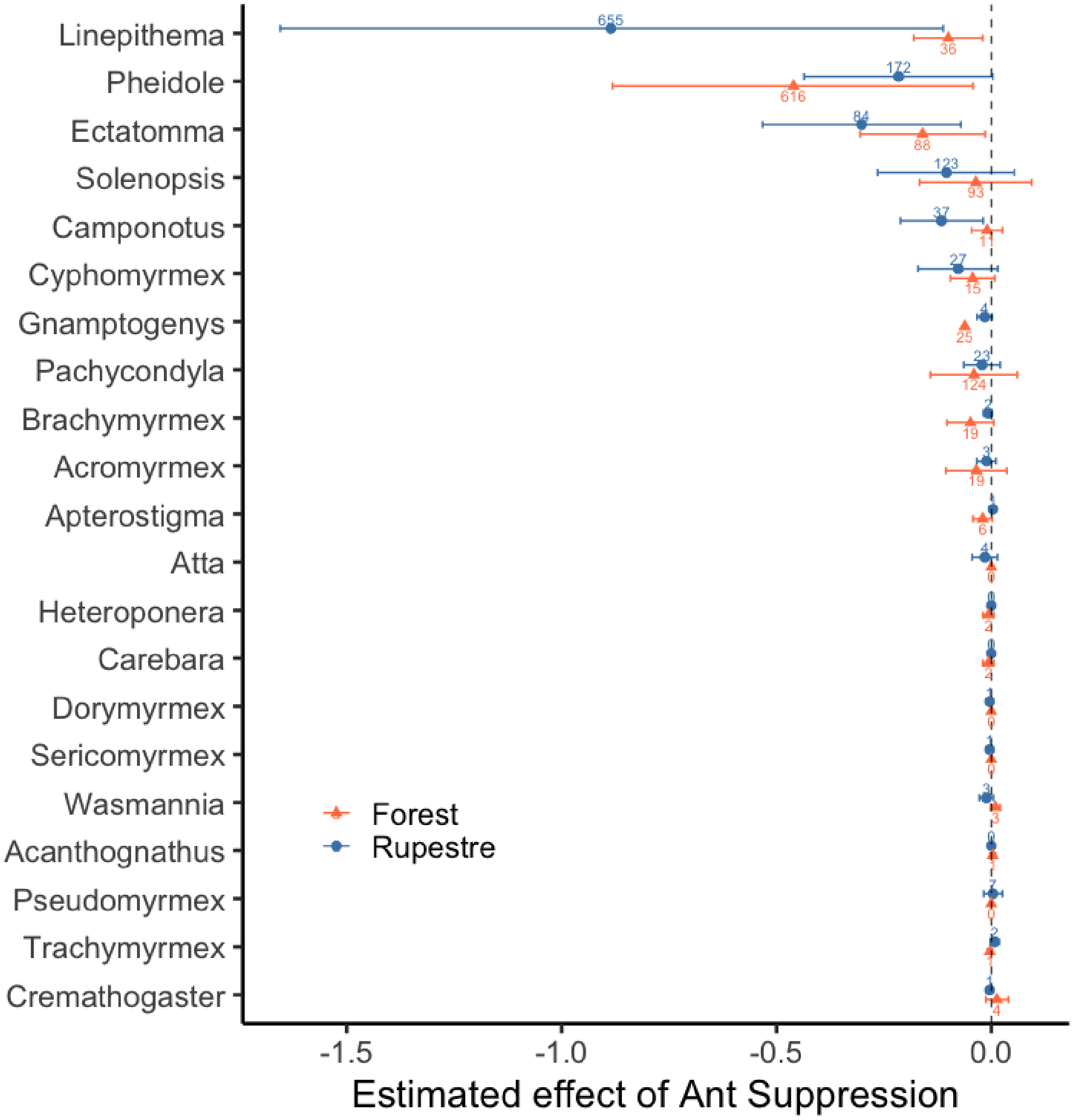
9. SUPPLEMENTARY 1



Supplementary 1 figure 1: Monthly ant activity in baits (cat food soaked in sugary water) from December 2019 to March 2020. Points are the average ants per bait in each plot, and lines represent the standard error. Blue arrows showed when we applied the ant suppression baits.



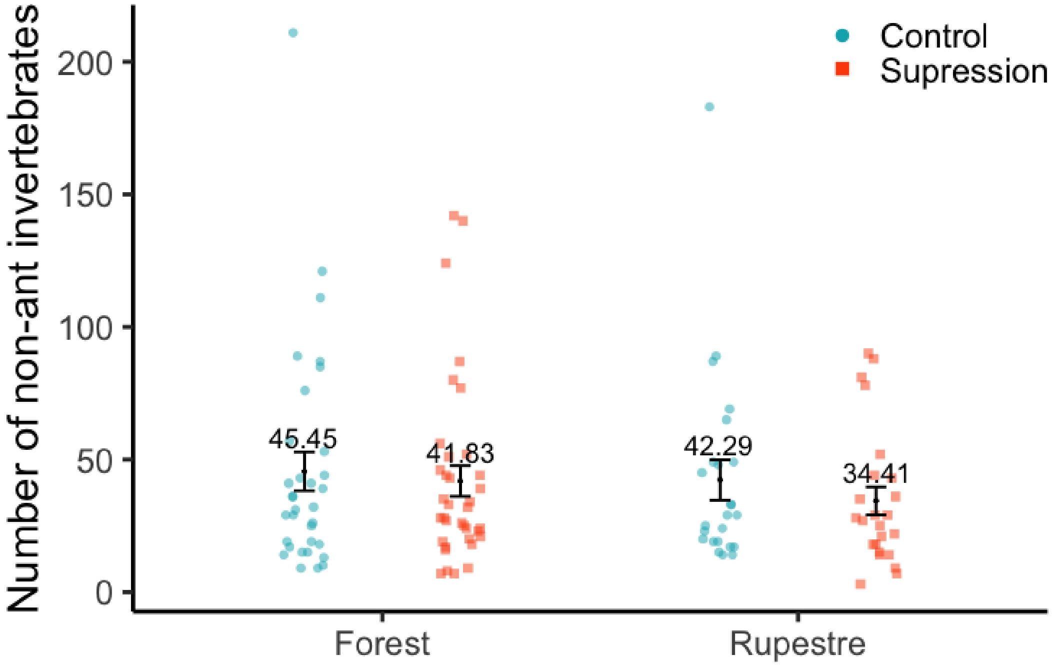
Supplementary 1 - Figure 2: Redundance Analysis (RDA) of the ant genera collected from pitfall traps post-treatment (March 2020) on ant suppression plots (suppression) and control plots set in two mountainous habitats in southeast Brazil: **A)** forest (montane rainforest forest) and **B)** Grassland (*campo rupestre*). Red words represent the plot's position, control plots on the left and suppression on the right.



Supplementary 1 - Figure 3: The estimated effect of ant suppression on forest (montane forest) and grassland (*campo rupestre*). Negative values are when the ant suppression negatively affects the genera and a positive value is when the ant suppression positively affects the group. If the interval bar overlaps the 0 the effect is not significant.

10. SUPPLEMENTARY 2

We access the effect of suppression and habitat on the abundance of non-target invertebrates in pitfall traps Using a Generalized Linear Mixed Models (GLMM) with a negative binomial error distribution. Ant suppression treatment (control/suppression) and habitat were set as explanatory variables, the number of non-ant invertebrates collected in pitfall traps was selected as the response variable, and the plot identity as a random effect. Ant suppression did not affect the overall non-ant invertebrates abundance (Chi=0.83, P=0.36). They suggested that the ant suppression treatment did not impact the overall non-target epigeic invertebrate community.



Supplementary 2 Figure 1: Number of non-ant invertebrates per pitfall trap in ant suppression plots (suppression) and control plots set in two mountainous habitats in southeast Brazil, forest (montane rainforest forest) and Grassland (*campo rupestre*). Pitfall traps were set 90 days after the beginning of ant suppression. Black points represent the mean and lines standard errors of groups.

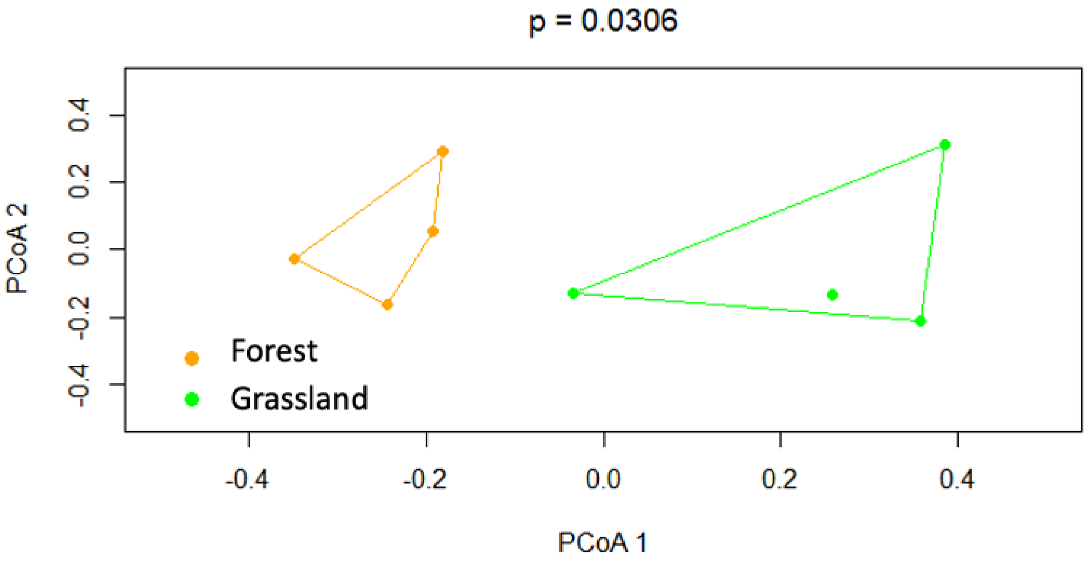
11. SUPPLEMENTARY 3

We collected 776 ants from 49 species (22 genera), 33 species in *campo rupestre* and 31 in Capões de Mata (Table 1). The richness between the two environments did not differ (Fig sup 2, p = 0.9282). However, habitats differed in their community composition (PERMANOVA, p = 0.0306, Fig 1).

Supplementary 3 - Table 1: Frequency of ant species in pitfall traps in all plots before ant suppression in tropical montane forests and *campo rupestre* (grassland). Samples were conducted in December using pitfall traps. Specimens were deposited in the EcoTrop Lab Collection at the Universidade Federal de Viçosa. The number in parentheses is the frequency of occurrence in pitfall traps.

Species	**Forest**	**Grassland**
Acromyrmex_cf.coronatus	5 (3)	0
Acromyrmex_sp.1	2 (1)	1 (1)
Acromyrmex_sp.2	2 (2)	3 (3)
Apterostigma_cf.pilosum	1 (1)	0
Brachymyrmex_cf.coactus	0	4 (2)
Camponotus_alboannulatus	1 (1)	0
Camponotus_lespesii	3 (1)	0
Camponotus_sp.1	0	8 (4)
Camponotus_sp.2	0	1 (1)
Camponotus_vittatus	0	1 (1)
Camponotus(Myrmobrachys)_cf.crassus	0	7 (3)
Camponotus(Myrmothrix)_rufipes	10 (4)	3 (3)
Crematogaster_sp.2	0	1 (1)
Crematogaster_sp.5	1 (1)	1 (1)
Crematogaster_sp.6	1 (1)	0
Crematogaster_sp.8	1 (1)	0
Crematogaster_sp.9	2 (1)	0
Cyphomyrmex_minutus	4 (2)	20 (7)

Ectatomma_permagnum	0	1 (1)
Ectatomma_sp.1	0	2 (1)
Ectatomma_tuberculatum	0	6 (1)
Gnamptogenys_striatula	27 (8)	3 (2)
Heteroponera_cf.mayri	2 (2)	0
Hylomyrma_sp.1	2 (2)	5 (3)
Hylomyrma_sp.2	3 (3)	0
Hylomyrma_sp.3	2 (2)	1 (1)
Labidus_praedator	1 (1)	0
Linepithema_cf.neotropicum	19 (8)	55 (8)
Linepithema_sp.1	0	2 (2)
Linepithema_UFV_sp.2	0	1 (1)
Neivamyrmex_sp.	0	1 (1)
Neoponera_latinoda	0	1 (1)
Nylanderia_sp.1	1 (1)	0
Nylanderia_UFV_sp.5	0	1 (1)
Pachycondyla_harpax	3 (2)	0
Pachycondyla_striata	33 (8)	12 (7)
Pheidole_cf.alexeter	3 (1)	0
Pheidole_cf.vafra	4 (3)	3 (3)
Pheidole_sp.2	23 (8)	8 (6)
Pheidole_UFV_sp.11	8 (4)	0
Pheidole_vafra	22 (8)	9 (4)
Pogonomyrmex_naegelli	0	12 (3)
Pseudomyrmex_cf.termitarius	0	1 (1)
Pseudomyrmex_sp.1	0	2 (2)
Solenopsis_geminata	1 (1)	2 (2)
Solenopsis_sp.1	11 (6)	6 (4)
Solenopsis_sp.2	1 (1)	0
Solenopsis_sp.3	2 (1)	0
Solenopsis_sp.5	0	15 (7)



Supplementary 3 – Figure 1: Principal coordinate analysis (PCoA) between ant communities collected in pitfall traps in control plots after ant suppression (March 2020) in tropical montane forests and *campo rupestre* (grassland).

12. SUPPLEMENTARY 4

Resource	Habitat	Ant Suppression	Cage	Average Ofered (g)	Sd Ofered (g)	Averaged removed (g)	Sd Removed (g)	Proportion Removed %
Protein	Forest	Control	Caged	2.02	0.02	1.88	0.40	92.96
Protein	Forest	Control	Open	2.02	0.02	1.91	0.29	94.85
Protein	Forest	Suppression	Caged	2.01	0.01	1.32	0.68	65.82
Protein	Forest	Suppression	Open	2.02	0.01	1.45	0.64	72.10
Protein	Grassland	Control	Caged	2.04	0.16	0.95	0.79	47.12
Protein	Grassland	Control	Open	2.02	0.02	1.00	0.58	49.95
Protein	Grassland	Suppression	Caged	2.01	0.01	0.38	0.34	18.81
Protein	Grassland	Suppression	Open	2.04	0.17	0.51	0.27	25.15
Seed	Forest	Control	Caged	3.02	0.02	1.66	0.99	55.07
Seed	Forest	Control	Open	3.02	0.02	1.76	0.97	58.41
Seed	Forest	Suppression	Caged	3.02	0.03	1.07	0.78	35.75
Seed	Forest	Suppression	Open	3.02	0.02	1.31	0.81	43.51
Seed	Grassland	Control	Caged	3.01	0.01	1.48	1.05	49.08
Seed	Grassland	Control	Open	3.02	0.02	1.50	0.93	49.92
Seed	Grassland	Suppression	Caged	3.01	0.01	0.61	0.57	20.42
Seed	Grassland	Suppression	Open	3.02	0.02	0.69	0.59	23.00

13. CHAPTER II

Top-down effects of ants on decomposition and herbivory in two contrasting tropical montane habitats

Tiago Vinícius Fernandes[1,2]; Tatiana Cornelissen[2]; Catherine L. Parr[3,4,5]; Ricardo Ildefonso Campos [6]; Frederico de Siqueira Neves[2]; Tiago Vilas-Boas[7], Ricardo Solar [2]

[1] Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre. Universidade Federal de Minas Gerais. Instituto de Ciências Biológicas, Belo Horizonte, Brazil. E-mail: fernandes.tiagov@gmail.com

[2] Centro de Síntese Ecológica e Conservação. Departamento de Genética, Ecologia e Evolução. Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

[3] School of Environmental Sciences. The University of Liverpool, Liverpool, United Kingdom.

[4] School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Wits, South Africa

[5]Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa

[6] Departamento de Biologia Geral. Universidade Federal de Viçosa, Viçosa, Brazil

[7] Departamento de Botânica. Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

14. Abstract

Ants are among the most abundant terrestrial animals worldwide, especially in tropical open areas. Through predation, ants control herbivore and termite populations, which affects herbivory and decomposition ecosystem processes. Yet, the ant contribution for these processes is barely measured, so we still lack information on how their role varies across habitats. Here, we delve into this question to understand the role of ants in controlling decomposition and herbivory within two contrasting habitats, tropical montane forests and *campo rupestre* (a rocky grassland ecosystem). We predict that ants, through a top-down effect, decrease both herbivory at the plant community level and decomposition by termites in forests and grassland, with stronger effects in grasslands. To evaluate our prediction, we performed an ant suppression experiment with 16 plots (80 x 80 m) equally split into: forest control, forest ant suppression, grassland control, and grassland ant suppression (for plots each treatment). Using standardised global protocols, we assessed the effect of ant suppression on plant community-level herbivory and tea-bag decomposition. We found that in only three months, the suppression of ~70% of ground ants nearly doubled herbivory in grassland and four-fold in the forest. Moreover, ant suppression doubled termite decomposition in grassland but had no effect in forests. Our results reinforce the top-down role of ants in herbivory and decomposition and show for the first time that the top-down effects of ants in herbivory are consistent across habitats, while decomposition is guided by habitat openness. Thus, our findings contribute to the understanding of habitat characteristics shaping the top-down role of a key animal group on ecosystem functioning.

Keywords: ecosystem engineering, myrmecology, trophic interaction.

15. Introduction

Biodiversity loss is a major threat to ecosystem functioning (Oliver et al. 2015). Yet, understanding the feedback between biodiversity and ecosystem processes is still a notable challenge (Balvanera et al. 2006), mainly concerning the contribution of specific taxa. Studies linking species or species groups to ecological processes usually approach direct functions performed by them (Gonzalez et al. 2020; Elizalde et al. 2020). For example, the loss of bird species and abundance can directly impair seed dispersal (Fontúrbel et al. 2015), and changes in bee communities affect pollination success (Larsen et al. 2005). However, species loss could also affect other functions through cascading interactions (Pérez-Méndez et al. 2016). That is the case of the loss of big seed dispersers decreasing the recruitment of large trees and consequentially affecting the carbon storage of tropical forests (Bello et al. 2015). The stepwise nature of cascading effects makes them hard to be measured, which reduces the development of our knowledge about the indirect contribution of specific taxa to multiple ecological processes (Balvanera et al. 2006). Moreover, we still lack information on the cascading effects of key taxa for ecosystem function and how these are shaped by environmental factors (Loreau et al. 2001). It is especially important facing the continuous increase in habitat loss and climate change caused by human activities.

More than species loss by itself, ecosystem processes depend on abundance and species identities. Therefore, the decrease in species abundance also has a greater impact on ecosystem functioning (Spaak et al. 2017), and the loss of key species or groups of species can disproportionately affect some processes (Leitão et al. 2016). Ants are one of the most abundant groups of animals in the world. Recent estimation indicates that biomass of terrestrial ants alone (~12 megatons) exceeds the combined biomass of wild mammals and birds (Schultheiss et al. 2022). Ants are considered ecosystem engineers, directly acting in seed dispersal (Fernandes et al. 2020), soil bioturbation (Farji-Brener and Werenkraut 2017), nutrient flux (Griffiths et al. 2018), pollination (Del-Claro et al. 2019), among other key ecosystem processes and services (del Toro et al. 2012; Elizalde et al. 2020). Moreover, ants are one of the major generalist predators and can control other insect communities, which can ultimately have indirect consequences on ecosystem functioning (Hölldobler and Wilson 1990). For example, by preying upon herbivorous insects, ants decrease the plant herbivory levels (Rosumek et al. 2009; Ramos et al. 2022; Anjos et al. 2022), while termite predation by ants have been linked to decreases in decomposition rates (DeSouza et al. 2009; Parr et al. 2016; Walker et al. 2022). However, few studies have measured the role of ants in those processes on a large scale (Parr et al. 2016; Walker et al. 2022).

Herbivory is one of the key ecosystem processes driving plant establishment, development and reproduction, with impacts at the population, community and ecosystem levels (Kozlov and Zvereva 2015; Schowalter 2016; Agrawal and Maron 2022). Insects are the most abundant and widespread herbivorous, responsible for ~ 8% of total plant consumption globally (Rinker and Lowman 2004; Mendes et al. 2021). However, the effects of insects in herbivory can be modulated by top-down interactions – predation (Zvereva et al. 2020; Moura et al. 2021). Ants are a dominant group of predators, playing an important role in protecting plants against herbivorous insects (Rosumek et al. 2009). The protection of plants by ants against herbivory is well studied, especially in the context of mutualisms, where plants produce resources that attract ants (e.g. nesting site and food resources; Gomes et al. 2021). However, non-mutualistic ants are also suggested to protect plants with no apparent reward (Seifert et al. 2016). In addition, ants are found foraging on almost all plant species and in most cases, they are not observed feeding on plant sources (Costa et al. 2016; Ramos et al. 2022). Therefore, ants might chase or prey on herbivorous insects present on plants, which can decrease herbivory levels in the whole plant community; however, it lacks empirical support.

Organic-matter decomposition is fundamental to carbon and nutrient cycling, influencing local food webs and plant communities (Chambers et al. 2000; Moore et al. 2004; Ulyshen 2016). Termites play an important role in decomposition, being responsible for up to 64% of dead wood decomposition in tropical rainforests (Jouquet et al. 2011; Griffiths et al. 2019). Nevertheless, it is suggested that the decomposition performed by termites is limited by top-down forces (DeSouza et al. 2009), with ants recognised as the main termite predators (Sheppe 1970; Tuma et al. 2020). Still, we have limited information about the outcomes of this interaction on decomposition rates at larger scales – particularly in the tropics (Stroud *et al.* 2017; Clarke *et al.* 2017). To our knowledge, only two studies (Parr et al. 2016; Walker et al. 2022) experimentally demonstrated in the field that ant suppression increases termite decomposition, both conducted in African savannas. Therefore, we don’t know, for example, how habitat characteristics would change this outcome and if patterns in Neotropical forests and grasslands would mirror those from other ecosystems. Termite and ant communities are very sensitive to habitat and environmental conditions (Parr and Bishop 2022;

Zanne et al. 2022), so it is sound to expect that the indirect impact of ants on decomposition changes across habitats.

Open and closed habitats, such as forests and grasslands, can offer ideal contrasts to assess the context-dependency of the contribution of ants to ecosystem process. Forest and grassland ant communities present similar species richness but divergent species composition (Dröse et al. 2019; Castro et al. 2020). Because ants are exothermic organisms, the solar radiation in open areas increases ground temperature and, consequently, ant activity (Bucy & Breed 2006, Costa et al. 2018, Parr & Bishop 2022). Accordingly, a recent meta-analysis showed that ant activity is higher in open habitats (e.g. savannas, wetlands and shrubland) than in closed ecosystems (i.e. tropical forests; Schultheiss et al. 2022). Therefore, we can expect that habitat openness might increase the relative role of ants in ecosystem functioning.

Here, we perform a large-scale experiment to assess the role of ants in controlling leaf herbivory and organic matter decomposition in two contrasting habitats, tropical montane forests and *campo rupestre* (a rocky grassland ecosystem). We specifically tested the following hypothesis: H1) Ants decrease the herbivory at the plant community level through top-down effect. This hypothesis is based on Rosumek et al. (2009)'s meta-analysis, which points out that ant presence decreases herbivory at the plant species level through predation of herbivorous insects; and Costa et al. (2016) study showing that ants are present in almost all plant species within a community. H2) Ants decrease the organic matter decomposition through top-down effect. This hypothesis is based on previous research showingthe importance of termites in the processing of organic matter and ants in the control of termites (Walker et al. 2022); Tuma et al. 2020). H3) Ants would have a greater influence on herbivory and decomposition in open habitats (grasslands) than in closed (forests). We expect this because in open habitats ground ants present higher activity. Our results will help us to understand the indirect role of one of the most conspicuous groups of predator insects in ecosystem functioning and the possible functional consequences of their decline.

16. Methods

16.1. Study area

We set up this study at the Serra do Cipó National Park in the central region of Minas Gerais state, Brazil (19°14′19″S, 43°31′35″W). The area is within a mountainous environment in the southern portion of the Espinhaço Range Biosphere Reserve between 1300 m to 1350 m above sea level. It presents two marked seasons, wet (October to March) and dry (April to September), with a mean annual temperature ranging from 15.1 to 20.7° C, and rainfall from 1,250 and 1,550 mm (Fernandes and Madeira 1999). There we can find two distinct and contrasting environments: the *campo rupestre* (hereafter called grassland) and semi-deciduous montane forest islands (hereafter called forest). The natural occurrence of these two environments in the same area was crucial to test our hypotheses under similar conditions.

The *campo rupestre* – the main landscape component - is a fire-prone grassland dominated by herbs and shrubs covering extremely-impoverish nutrient quarzitic soil (Fernandes 2016). This is a megadiverse ecosystem with a high level of species endemism (Silveira et al. 2016). Although it occupies less than 1% of Brazil's territory (66,000 km^2), it has more than 5,000 native vascular plant species (14.7 % of all plants in Brazil) and approximately 195 species/morphospecies (50 genera) of ants (Costa et al. 2015; Fernandes 2016; Brant et al. 2021). This ecosystem is also classified as an OCBIL – old, climatically buffered, infertile landscape, with high priority for conservation and studies given its unique characteristics (Hopper et al. 2021). Within the *campo rupestre* matrix are natural islands of tropical montane forests (locally called *Capões de Mata*), with structurally, phylogenetically, and functionally distinct flora. Its floristic composition resembles the semi-deciduous Atlantic Forests, serving as a habit and refuge for the local fauna (Coelho et al. 2018; Nunes et al. 2020).

16.2. Experimental design

We measured the indirect effect of ants on decomposition and herbivory rates in grassland and forest ecosystems. To do so, we set up a factorial experimental design with 16 plots (80 x 80 m), divided into i) four forest control plots; ii) four forest ant suppression plots; iii) four grassland control plots; and iv) four grassland ant suppression plots. Each plot measured 80 x 80 m, divided into a central experimental area of 50 x 50 m and a surrounding buffer area. The buffer area was used to prevent the access of surrounding ant colonies to the experimental area. We set plots in pairs of control and ant suppression in each site, placing each control plot 100 m apart from its respective ant suppression plot. To capture habitat heterogeneity and take independent samples, we placed each pair of plots 1 km apart (grassland plots) or on a different forest island (forest plots).

16.3. Ant suppression

To suppress ants, we used two poison bait types: homemade ant bait (imidacloprid 100 ppm w/v) and ATTA MEX-S® (Sulfonamide 300 ppm m/m). The homemade bait is carbohydrate-protein-based, very effective to most ant species (especially the dominant ones), and has a low effect on other animal groups (for more information on the production of bait and caveats about ant suppression using this bait, please see Chapter I; Parr et al. 2016, Griffiths et al. 2018). ATTA MEX-S® is a bait specific for leaf-cutting ants. Although the occurrence of leaf-cutting ants in this area is very low (Chapter I, Castro et al. 2020), we used ATTA MEX-S® to control for the possible direct influence leaf-cutting ants would have in herbivory during the experiment. We spread 10 kg of homemade bait in each suppression plot and 5 kg of ATTA MEX-S® in the whole plot extension.

We conducted the ant suppression during the wet season, from December 2019 to March 2020 (90 days), the period of higher insect activity (Queiroz et al. 2022). We monitored ant abundance monthly and reapplied suppression baits only if the difference in ant abundance between control and suppression plots were less than 20% (only once after 60 days). Using this approach, we suppressed 73% of ants in forests and 70% in grassland (Chapter I).

16.4. Herbivory Measurement

To assess the effect of ant suppression on herbivory at plant community levels, we selected the three most abundant plant species in each plot. For each plant species, we randomly chose five individuals (a total of 15 plants per plot). To make the comparison between habitats possible, we only sampled plants with heights between 0.5 to 2.5 meters because that is the range of most plants found in the grassland. Because we were interested only in the post-ant-suppression accumulated herbivory, we used a Before-After-Control-Impact (BACI) approach , which is considered more robust for ecological research (França et al. 2016; Christie et al. 2020). Thus, we sampled the same plants before (December 2019) and 90 days after ant suppression (March 2020).

We measured herbivory following the Global Herbivory Protocol developed by Mendes *et al.* 2021. Therefore, we systematically collected 50 leaves from a randomly chosen branch of each plant. In case the branch did not have 50 leaves, we also collected leaves from the closest one (total of 750 leaves per plot). After collecting leaves in December 2019, we marked the branch and used a different one in the next sample 90 days after. Leaves were pressed and dried for 48 hours in an oven (50^{o}C). Thus, we placed all leaves on a white surface and photographed using a digital camera (12.1 Megapixels resolution) from 50 cm high. We then processed all images using Gimp 2.1 and determined the proportion of consumed area per plant using an R script function as described in Mendes (2022). Leaves damaged by the pressing processes or plants that died during the period of this study were excluded from this analysis. The herbivory levels (proportion of leaf area loss) were assessed by dividing the total leaf area loss by the total leaf area measured.

To measure the effect of ant suppression treatment on herbivory disregarding previous plant history, we used the Delta Herbivory (*i.e.* the proportion of leaf area lost in three months). We calculated Delta Herbivory ($\triangle_{herb}$) for each plant as follows:

$$\triangle_{herb} = Herb_{t2} - Herb_{t1}$$

Where $Herb_{t2}$ is the herbivory level after ant suppression (March 2020) and $Herb_{t1}$ is the herbivory level before ant suppression (December 2019). Therefore, $\triangle_{herb}$ is the proportion of leaf area lost per plant within the three experimental months.

This method was chosen for three reasons: I) It allows us to measure the role of ants controlling herbivory in the plant community scale rather than the population scale which is usually done (Parr, 2016); II) The *campo rupestre* and tropical montane forests are very heterogeneous and diverse habitats, which makes it impracticable finding plots with same plant species. III) The global herbivory protocol allows comparison of herbivory across different habitats (Mendes et al. 2021).

16.5. Decomposition Measurements

To assess the effect of ant suppression on organic matter decomposition, we measured termite contribution to decomposition using a similar approach to the tea bag decomposition protocols developed by Keuskamp et al. (2013) and validated by Teo et al. (2020). After 60 days we have started the ant suppression (February 2020), we placed 30 bags of green tea Lipton™ (1.8±0.04g) per plot (640 in total). To separate the contribution of termites and microorganisms in decomposition, we protected half of the tea bags with a Nylon net (mesh 0.5 mm). Therefore, we have two types of tea bags I) *unprotected*: accessed by termites and microorganisms; and II) *protected*: accessed only by microorganisms. Protected and unprotected tea bags were randomly and equidistantly distributed in 5 transects inside the experimental area of each plot. We placed tea bags in direct contact with the soil and did not bury them. We collected all tea bags and recorded if the tea bag had signs of termite damage (e.g. holes) or not (e.g. no holes) after 30 days of exposure in the field. Therefore, we could assess the probability of an unprotected tea bag being damaged by termites. Furthermore, we identified and excluded from our analysis protected tea bags occasionally damaged by termites able to circumvent the net protection.

After, we brought the tea bags to the lad, dried them out in an oven (50°C) for 48 hours and accessed their content mass using a precision scale (BEL engineering M214A, precision = 0.001). We calculated the decomposition rate by subtracting the tea mass lost from the initial mass of tea content (1.8 g).

Because we were interested in the impact of ant suppression on decomposition by termites, we used a estimate. We addressed all decomposition rates of protected tea bags to microbes and to assess the relative contribution of termites to decomposition, we calculated the difference in decomposition between protected (decomposition by microbes only) and unprotected tea bags (microbes + termites), using the formula:

Decomposition by termites = decomposition of unprotected tea bags - decomposition of protected tea bags.

16.6. Statistical analysis

To test whether the habitat type (grassland/forest), ant suppression treatments (control/suppression) or the interaction between these factors affect the △ Herbivory in the plant community, we used Linear Mixed Models (LMM). We set ant suppression and habitat as explanatory variables, △ Herbivory as the response variable and plot identity as a random effect.

We assessed whether the habitat type (grassland/forest), ant suppression treatments (control/suppression) or the interaction between these factors affected the probability of an unprotected tea bag being damaged by termites. We used Generalized Linear Mixed Models (GLMM) with Binomial distribution. We set habitat and ant suppression as explanatory variables, and probability of termite damage (whether or not the tea bag was damaged by termites) as the response variable, and the plot identity as a random effect.

We evaluated whether the habitat type (grassland/forest), ant suppression treatments (control/suppression), tea bag protection against termites (Protected/Unprotected) or the interaction between these factors affect the decomposition rates. We also used a GLMM with Binomial distribution, having habitat, ant suppression, and protection as explanatory variables, the proportion of tea mass lost as the response variable, and the plot identity as a random effect.

We use R version 4.1.2 to perform all analyses (Crawley 2013; R Core Team 2021) and the *lme4* package version 1.1-12 (R Core Team 2016) to build LMM and GLMMs. We graphically examined the residuals for model assumptions, including normality of errors and homogeneity of variances, to assess model suitability using the package *DHARMa*. Finally, we computed the significance of the LME model using type II Wald F tests with Kenward–Roger degrees of freedom approximation and GLMM using a type-II Wald Chi test, using the *mixlm* package v 1.2.3 (Liland 2018).

17. Results

We evaluated the herbivory of 21,289 leaves (before and after ant suppression) from 223 stems belonging to 27 plant species (14 in grassland and 13 in the forest; supplementary 1). The delta herbivory (i.e. the difference in proportion of leaf area lost in three months) was higher in ant suppression than in control plots (Chi = 4.98, p = 0.02, Fig 1). Furthermore, there was no difference in delta herbivory between forest and grassland (Chi= 1.17, p = 0.27, Fig 1). There was no statistical interaction between habitat and ant suppression treatments on delta herbivory (Chi = 1.82, p = 0.17, Fig 1). Finally, the suppression of ants almost four-folded herbivory in forests compared to control plots, while it doubled in grassland (Fig 1).

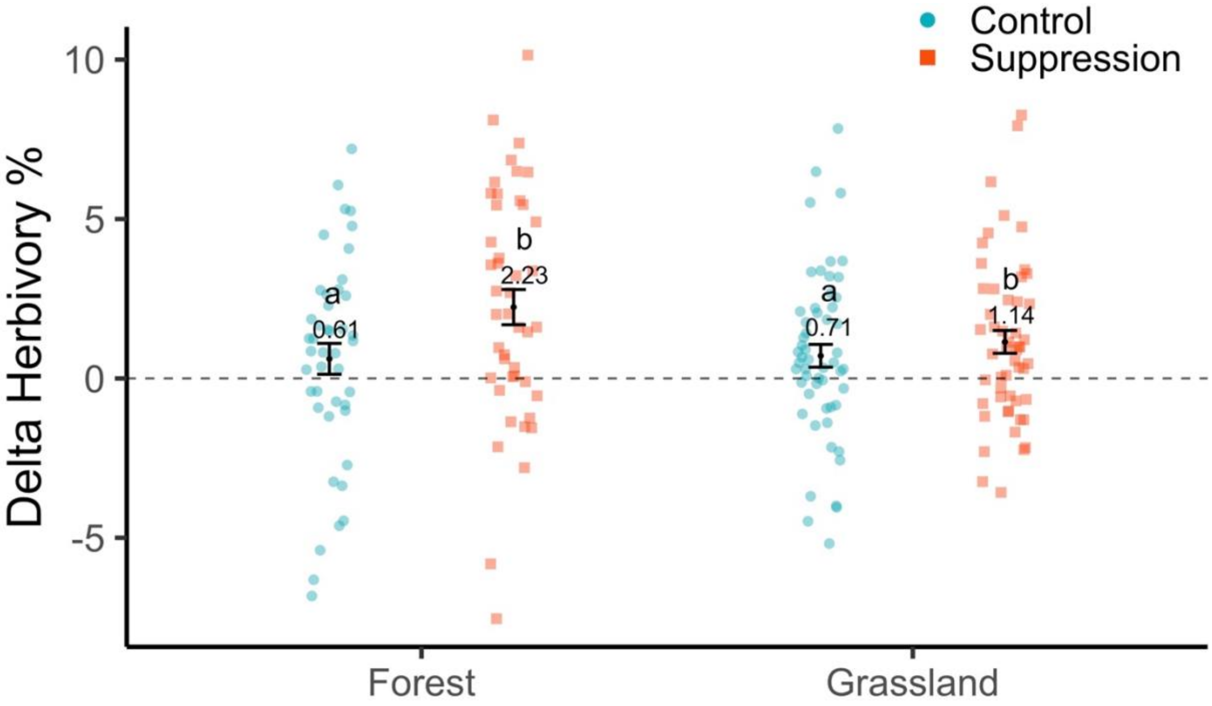


Figure 1. The proportion of leaf area loss on plant community within three months (Delta herbivory), in ant suppression plots (suppression) and control plots set in the forest (montane forest) and grassland (*campo rupestre*). The dashed line (zero) indicates the absence of herbivory; and negative values represent the growth of new leaves or differences in herbivory between branches sampled. Black points and numbers over the bars represent the average and line standard errors of groups. Statistical differences are represented by different letters.

Ant suppression showed divergent effects on the probability of termite damage to unprotected tea bags according to habitat type (Chi = 3.54, p = 0.05). The probability of termite damage to unprotected tea bags did not vary with ant suppression treatment in forests (Chi= 1.03, p=0.31), while in grassland, ant suppression decreased it by 54% (Chi= 14.79, p<0.001; Fig 2A). Similarly to the effect of ant suppression on the probability of termite damage, the bait mass loss through decomposition also depended on habitat type (Chi= 5.60, p=0.01). We also found no effect of ant suppression in bait mass loss in forests (Chi= 1.62, p=0.20). However, we observed an opposite effect in grassland, where ant suppression increases the bait mass loss decomposition by 8% (Chi= 18.32, p<0.001). Furthermore, baits in the forest lost more weight than in grassland (Chi = 85.94, p< 0.01; Fig 2. B), and unprotected tea bags were more decomposed than protected ones (Chi = 25.37, p< 0.01; Fig 2. B). Finally, there was no interaction between ant suppression and tea bag protection (Chi= 1.62, p=0.20) and habitat and tea bag protection (Chi= 3.2, p=0.07; Fig 2. B).

We discriminate the impact of ant suppression on decomposition performed by termites and microbes. To do so, we calculated the difference between protected (decomposition by microbes only) and unprotected tea bags (microbes + termites). In general, the contribution of termites to decomposition in the two ecosystems was low, less than 5% in control plots. Ant suppression plays an insignificant role in termite-driven decomposition in forests, with a 0.5% difference between suppression and control plots. On the other hand, decomposition performed by termites in the grassland was twice as higher in ant suppression plots (10%) than in control plots (5%). Furthermore, comparing control plots between grassland and forest, termites seem to present relatively higher importance in grassland decomposition (5,1%) than in forests (3.2%; Fig 3. A).

We also compare the relative contribution of termites and microbes to decomposition. We found that microbes were the main decomposer in both habitats and treatments (control and ant suppression plots). The relative contribution of termites almost doubled in grassland control plots (8.5%) compared to forest control plots (4.5%), while microbes contribution had small decrease between habitats (95.5% in forest *vs*. 91.5% in grassland). For forest, ant suppression was almost unaffected, and the contribution of each taxon was similar to control plots (termites = 5% and microbes = 95% to microbes). Contrarily, in grassland, ant suppression almost doubled the relative contribution of termites to decomposition compared to control plots (15.2%) and decreased microbes' relative contribution (84.8%; Fig 3. B).

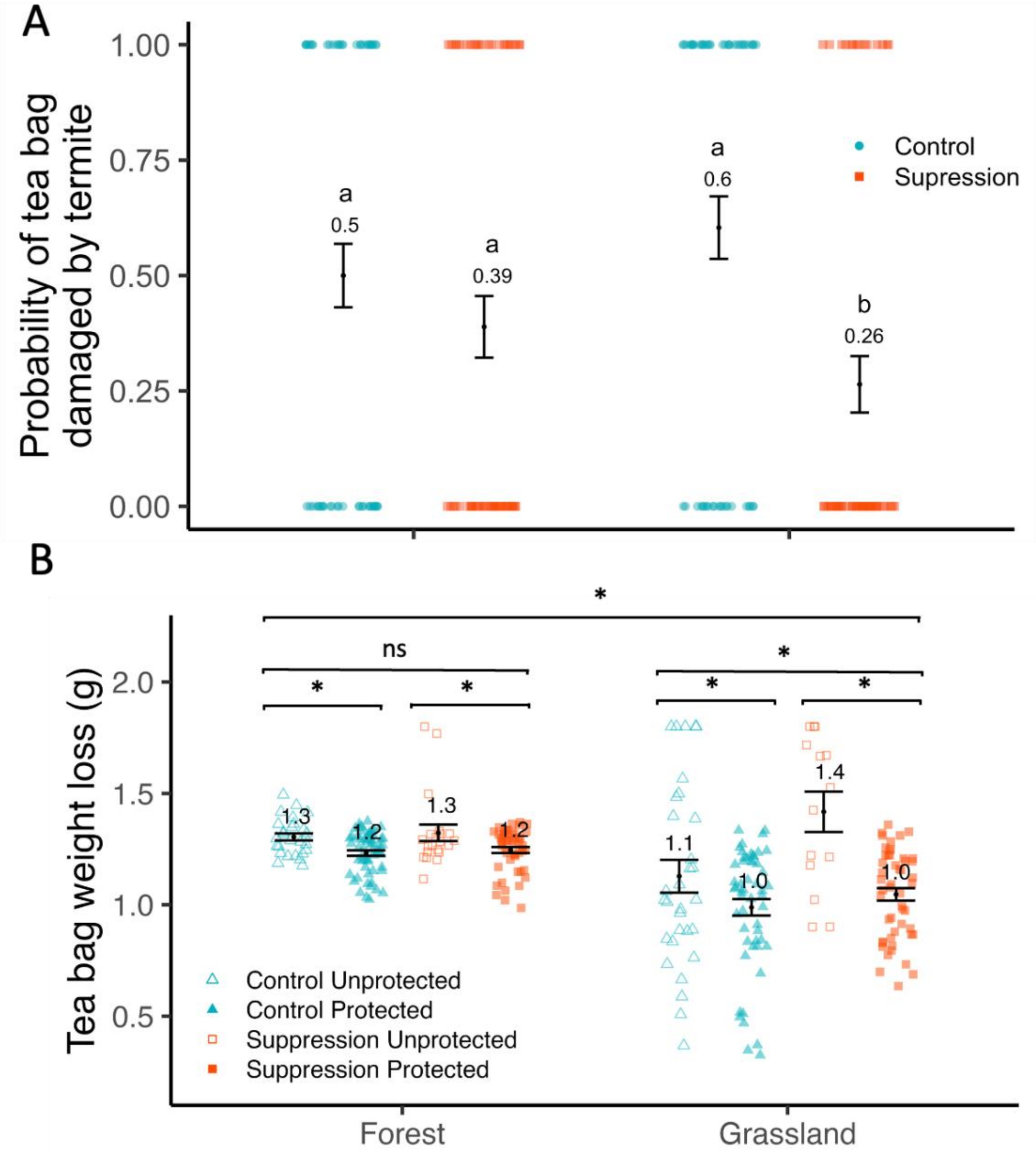


Figure 2. Tea bag decomposition left 30 days in the field according to ant suppression (suppression/control) in two mountainous habitats, montane rainforests and grassland (*campo rupestre*). **A)** Probability of unprotected tea bag to be damaged by termite **B)** Green tea mass loss (g) according to ant suppression (suppression/control) and net protection against termite (protected/unprotected). Black points and numbers over the bars represent the average and lines standard errors of each group. Statistical differences are represented by different letters in A and by * in B.

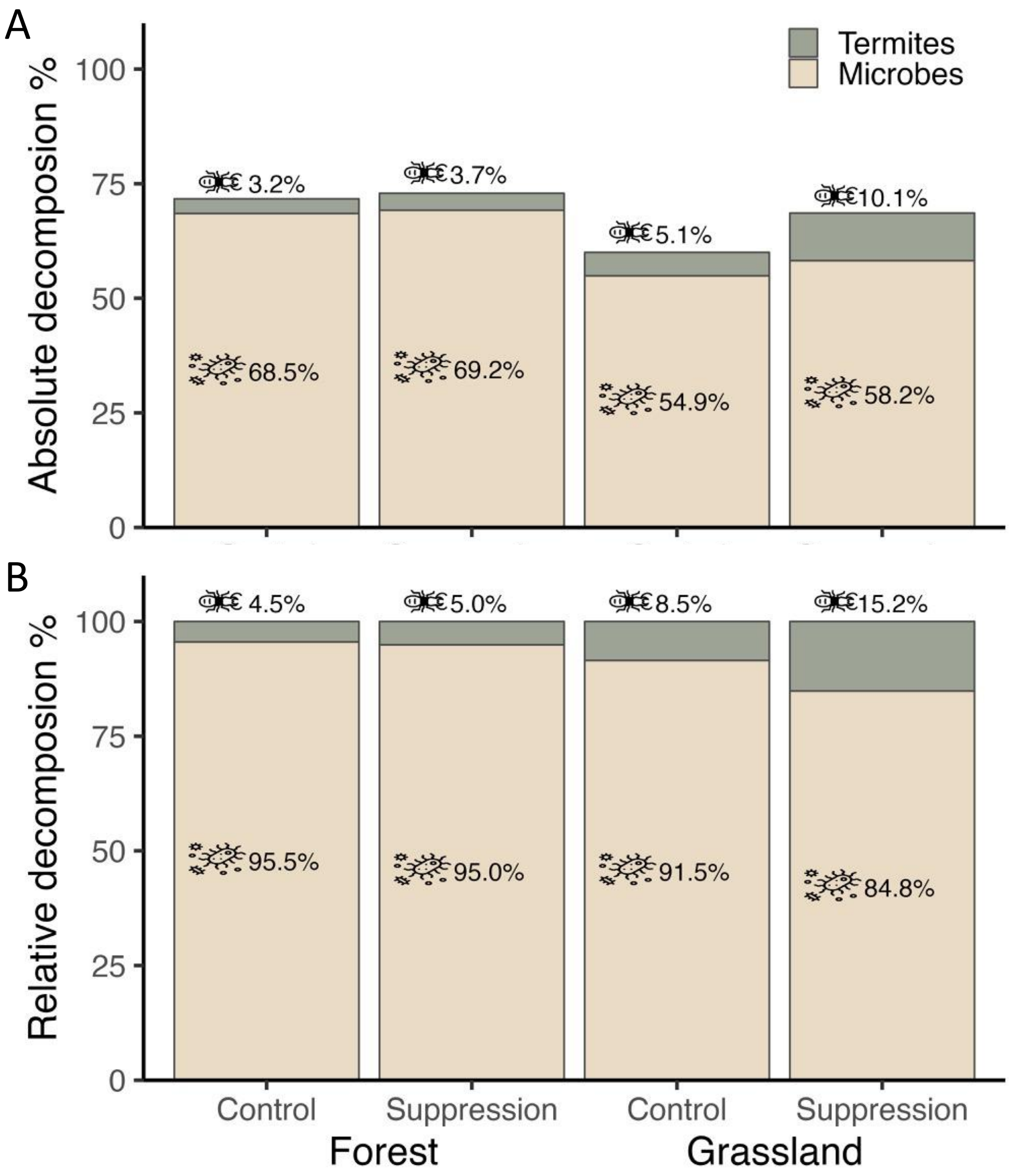


Figure 3. The estimated termites and microbes contribution to the tea bags decomposition according to ant suppression (suppression/control) in two mountainous habitats, montane rainforests and grassland (*campo rupestre*). The decomposition by termites was considered as the difference in decomposition between protected (decomposition by microbes only) and unprotected tea bags (microbes + termites) **A)** Proportion of tea bag mass loss from the initial weight (1.8g). **B)** Relative contribution of each taxon to decomposition, based on the decomposition in each habitat and treatment**.**

18. Discussion

Using a large ant suppression field experiment, we compared the top-down effects of ants in two key ecosystem processes, herbivory and decomposition, between forest and grassland. We suppressed nearly 70% of ants in both habitats, which increased herbivory at the plant community level, following our first hypothesis. Moreover, ant suppression had an insignificant impact on the decomposition performed by termites in tropical montane forests, while it doubled in grassland. This finding partially supports our hypothesis that ants control termite decomposition, as this effect was found only in one habitat, the grassland. Finally, we reject our hypothesis that ants would have higher effects on herbivory in the grassland than in forests, as the effects of ant suppression on leaf consumption were not different between habitats. Those results reinforce the cascading effects of a key predator group in controlling ecosystem processes. We also bring insight into the intrinsic factors of each habitat that could modulate the impact of ants in herbivory and decomposition (e.g., ant-plant traits and termite activity). Thus, we improved our understanding of ant top-down effects on ecosystem processes and how habitats might shape them.

18.1. Ants control herbivory at the plant community level.

Our experiment is the first to demonstrate that the role of ants in protecting plants is noticeable at a plant community level regardless of plant species. Ground ants usually forage on soil, but they also climb plants seeking resources and eventually prey on herbivorous insects present on those plants (Sam et al. 2015; Leal and Peixoto 2017). While it is a consensus that ants can protect plants by predating or chasing herbivorous insects, this interaction or partnership is usually based on studies with one or a few species on small scales (Rosumek et al. 2009; Anjos et al. 2022). Also, the role of ants in protecting plants that do not produce rewards (e.g. non-myrmecophytes and non-myrmecophilic plants) is rarely measured (Rosumek et al. 2009), although ants also forage in great numbers in those plants (Costa et al. 2016). Parr *et al.* (2016) was the first study to show that the suppression of ants on large scales can increase the herbivory of a dominant non-mymecophilic plant species in African savannas. The effect of ants in controlling herbivory at the community plant level is suggested by studies with caterpillar models and dead insects that highlight the importance of ants as predators of insects – however, studies often do not measure the effect on herbivory (Sam et al. 2015, Seifert et al. 2016). Thus, we support those studies and demonstrate that ants can decrease herbivory in plant communities in forests and grassland.

The effects of ant suppression on herbivory were consistent across habitats. By using a BACI approach, we demonstrate that within only three months, plants in ant suppression plots in forests and grassland experience more herbivory than control plots in both habitats. Contrary to our hypothesis, the role of ants controlling herbivory is similar between grassland and forest. It is also important to remember that although not statistically different, the effect of ant suppression in increasing herbivory was twice as high in forests than in grassland (ant suppression resulted in 1.6% and 0.4% more herbivory than control plots in forest and grassland, respectively). A possible explanation relies on the redundancy of defences against herbivory presented by plants (Coley and Barone 1996; Gomes et al. 2021). Plants can have biotic (e.g. ants), chemical (e.g. tannins, alkaloydes, phenolics) and intrinsic physical (e.g. lignin, trichomes, spikes) defences against herbivory (Schowalter 2016). Those defences are compensatory and plants with less intrinsic chemical and physical defences are beneficiated from biotic protection (Gomes et al. 2021; Ramos et al. 2022). The *campo rupestre* (grassland) is a harsh environment with poor nutrient soil, water availability and high solar incidence (Carvalho et al. 2012). Plants in this habitat present adaptation to prevent desiccation, so it is common to find high levels of sclerophyll and trichomes (Silveira et al. 2016). At the same time, those traits also protect plants against herbivores (Coley and Barone 1996; Hanley et al. 2007). Therefore, biotic protection by ants might be less important in *campo rupestre* habitat. On the other hand, plants in forests usually have palatable leaves with low physical defences, so they might receive greater benefits from ant protection (Zvereva et al. 2020). Thus, this indicates that the effects of ants on herbivory might be guided by differences in plant traits across habits rather than solely differences in ant community characteristics.

Measuring herbivory effect on long-term and large scales is complex (Agrawal and Maron 2022). It involves many intrinsic factors related to plant identities, such as palatability, plant life history, and chemical and physical traits (Coley and Barone 1996; Barton and Koricheva 2010; Schowalter 2016). Furthermore, species richness, composition, and abundance of each species could influence herbivory levels at community levels. Still, we showed that ants influence the total area consumed in the plant community regardless of plant species, showing the strength of ants at controlling herbivory. Furthermore, it is important to point out that our study was restricted to understory vegetation. Canopy ants are more protein-limited and therefore more aggressive than ground ones (Ness et al. 2009, Law & Parr 2020), so the impact of ants in controlling herbivory in forest canopies could be even stronger than shown here. Finally, future studies should control plant composition and plant traits to help elucidating the mechanisms involved in possible differences between habitats.

18.2. Role of ants in decomposition by termites

Our experiment reveals that ants suppression increases the termite consumption of baits cellulose in grassland but has no effect in the forest. Ants are one of the major termite predators in nature and are known to control termites' populations and activity (DeSouza et al. 2009; Lima Pequeno and Pantoja 2014; Tuma et al. 2020; Potapov et al. 2022). As a result, the top-down control of termites by ants decreases the cellulose decomposition in tropical savannas (Parr et al. 2016; Walker et al. 2022). We support those findings by showing that partial ant suppression (~70%) can double the cellulose decomposition by termites in grassland. Moreover, our results indicate that the ant effect in decomposition is habitat-dependant because we did not find any effect of ant suppression in forest habitats. Therefore, disturbance in ant communities in grassland would present a greater impact on the decomposition performed by termites than in the forest. This reinforces the importance of ants in controlling decomposition in open environments, such as savannas and tropical grasslands.

Compared to microbes, termites have a small contribution to decomposition in both habitats studied here. Nevertheless, we notice a relatively higher importance of termites to decomposition in grassland (8.5% in control plots) than in forests (5% in control plots). Contrary to our findings, preview studies in tropical environments have attributed a much larger relative contribution of termites to decomposition than found here. For example, Walker *et al*. 2022 showed that in African savannas, the relative contribution of termites to wood, grass and elephant dung decomposition were, respectively 65%, 36.4% and 41.9%. Similarly, in the tropical rainforest, termites contribute to 58–64% of woodblock decomposition, while microbial decomposition is between 36% and 42% (Griffiths *et al.* 2019). A recent global study suggests that wood decomposition by termites is temperature-dependent, with decreasing wood decay by ~6.8 times each 10°C reductions (Zanne et al. 2022). The habitats studied here are mountainous and present lower annual temperatures (15.1 to 20.7° C) than lower land tropical ecosystems (Fernandes and Madeira 1999). Therefore, we propose that the lower temperatures in those habitats may impair termite activity and, therefore, their role in decomposition. Furthermore, the difference in temperature between grassland and forest could help explain the lack of ant suppression effects in decomposition within forests. Grassland presents higher solar incidence and lower vegetation statures compared to forests, so soil temperature in these habitats tends to be higher, which could increase termite activity (Villegas et al. 2010). If so, the top-down impact of ants in decomposition might also depend on termite activity, with ant suppression effects being hard to detect or absent in habitats with low termite activity (montane forest). However, because we lack data about the termite community in those habitats, we still need further investigation to elucidate these mechanisms.

Termites changed their foraging in ant suppression plots, decreasing the detection of cellulose baits but increasing the consumption of baits found in grassland. The optimal foraging theory states that predators tend to minimise the time locating prey and maximise food intake (Stephens and Krebs 1986). The predation risk also affects the foraging decision of animals that usually avoid or abandon resources in the imminence of predation (Verdolin 2006). Accordingly, Korb & Linsenmair (2002) showed that predation pressure on *Macrotermes bellicosus* (termite) increases the abandonment of resource patches, which impairs their consumption rate. Our data support their findings showing that control plots present less consumption of baits and a higher number of damaged baits. Suggesting that termites had to abandon baits before emptying them because of ant predation on the control plots. On the other hand, ant suppression plots present more termite bait consumption and less number of damaged baits. Therefore, further than control termite abundance as suggested by many studies (Sheppe 1970; Tuma et al. 2020), ants also modulate termite foraging, which ultimately can affect termite role in decomposition.

18.3. Conclusion

Ants are one of the most abundant animal groups in terrestrial habitats worldwide and are involved in many ecosystem processes (del Toro et al. 2012; Schultheiss et al. 2022). For this reason, they are considered part of “the little things that run the world” (Wilson 1987). Here, using a large-scale ant suppression experiment, we brought new contributions to support E. O. Wilson's statement, showing that the ground ants, through a top-down effect on other invertebrates, control herbivory by chewing insects and organic matter decomposition by termites. Also, we explored how different habitats could shape those interactions, which is an important gap in ant ecology literature (Tuma et al. 2020). For the first time, we showed that ants decreased herbivory at a community level disregarding plant species and this effect is consistent in grassland and forest. Additionally, we suggest that further than a top-down control through predation on termites, ants also play a role in termite foraging in grassland, decreasing the decomposition of organic matter. Therefore, our findings contribute to the understanding of the key importance of biological interactions on ecosystem functioning. The changes in land use, pollution and global change have significantly impacted insect communities, their interactions and the ecosystem process they are directly or indirectly involved (Sánchez-Bayo and Wyckhuys 2019; Kehoe et al. 2021). That is also true for ants, especially in the context of climate change, ants are more likely to decrease their abundance in warmer tropical environments and increase in temperate regions (Parr bishop 2022). Our study suggests that these changes in ant communities could indirectly affect two key ecosystem processes, and these consequences tend to be even more significant in open habitats. Finally, we highlight that the loss of key taxa in ecosystems can knock-on effects that multiply the initial impact of disturbances, thereby largely adding to the degradation of ecosystems caused by anthropogenic actions.

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20. SUPPLEMENTARY 1

Plant Species used to measure herbivory

	Speceis	**Family**	**Plot Occurrence**	
			cont	**sup**
Grassland	*Symphyopappus reticulatus Baker*	Asteraceae	0	2
	Roupala montana Aubl.	Proteaceae	1	1
	Baccharis platypoda DC.	Asteraceae	3	0
	Coccoloba acrostichoides Cham.	Polygonaceae	3	3
	Eremanthus glomerulatus Less.	Asteraceae	2	1
	Eremanthus incanus (Less.) Less.	Asteraceae	0	1
	Lychnocephalus mellobarretoi (G.M. Barroso) Loeuille, Semir & Pirani	Asteraceae	0	1
	Psychotria stachyoides Benth.	Rubiaceae	1	0
	Trembleya parviflora (D.Don) Cogn.	Melastomataceae	0	1
	Asteraceae_NO_ID	Asteraceae	1	0
	Accara elegans (DC.) Landrum	Myrtaceae	1	0
	Byrsonima variabilis A.Juss.	Malpighiaceae	0	2
	Leandra sp.	Melastomataceae	1	0
Forest	*Leandra glabrata (Bunbury) Cogn.*	Melastomataceae	1	0
	Myrtaceae_NO_ID	Myrtaceae	1	0
	Celastraceae_NO_ID	Celastraceae	1	1
	Guatteria sp.	Annonaceae	1	0
	Trembleya parviflora (D.Don) Cogn.	Melastomataceae	1	0
	Leandra aurea (Cham.) Cogn.	Melastomataceae	1	0
	Baccharis rufidula (Spreng.) Joch.Müll.	Asteraceae	1	0

	Casearia obliqua Spreng.	Salicaceae	1	1
	Miconia sclerophylla Triana	Melastomataceae	1	3
	Annona sp.	Annonaceae	1	1
	Miconia flammea Casar.	Melastomataceae	1	2
	Psychotria stachyoides Benth.	Rubiaceae	1	1
	Melastomataceae_NO_ID	Melastomataceae	0	3

21. SUPPLEMENTARY 2

Herbivory Levels

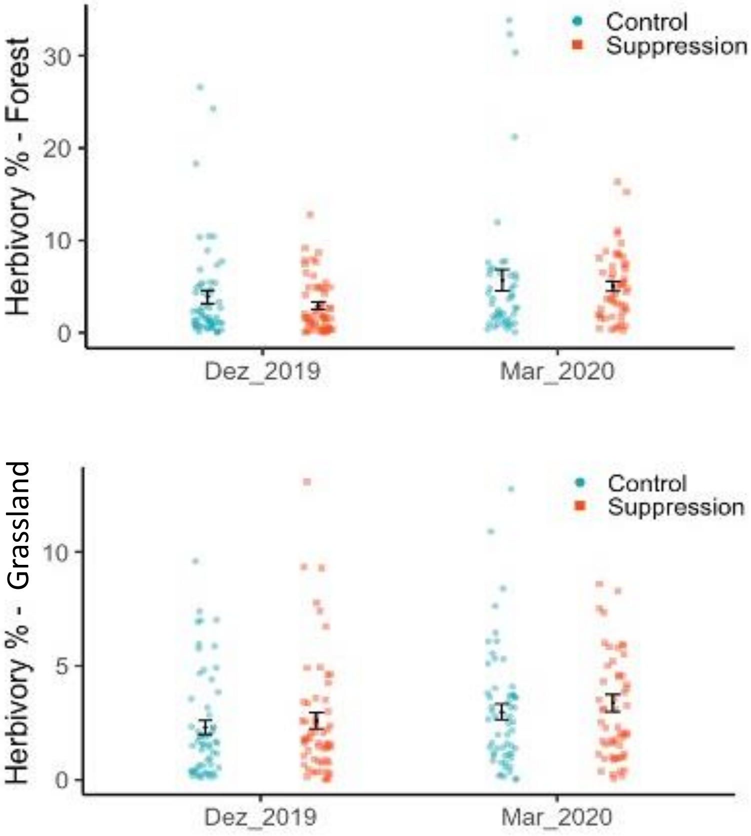

Figure Supplementary I - Proportion of area lost in Dec 2029 and March 2020 in ant suppression plots (suppression) and control plots set in forests and Grassland (*campo rupestre*). Herbivory levels are generally higher in forests than grassland (Control Grassland = 2.64%; Control Forest = 4.14%; Wald χ2 (1, N = 195) = 5.01 p = 0.02; supplementary 2).

22. GENERAL CONCLUSION

Here, we compare ants' direct and indirect contribution to ecosystem processes between open (grassland) and closed habitats (forest). We used a large-scale ant suppression to quantify the direct role of ants in the scavenging N-rich and C-rich baits. Furthermore, we also measure the cascading effects of ant suppression on herbivory at the plant community level and decomposition by termites. Although ants are a notorious animal group found in great numbers in both habitats and influenced all processes studied here, their role in ecosystems is habitat-depended. We showed that compared to the forest, in grasslands, ants have a higher importance in scavenging and decomposition but not herbivory. Thus, we highlight the unreplaceable role of this group in maintaining ecosystem functioning in a mountainous environment, especially in open habitats.

In addition, we demonstrate the invertebrates' superiority in scavenging compared to vertebrates. In our study system, vertebrates have only a trivial contribution to scavenging (<5%), implying that invertebrates have a major role in this process, although they are still overlooked in the literature. The high contribution of insects to scavenging could accelerate nutrient recycling, inserting nutrients back into the trophic chain while leaving fewer resources for decomposition. Ants present an outstanding contribution to this process in grasslands, where they are responsible for 57% of baits removed, and although smaller in forests (31%), they also have a significant contribution. Moreover, habitat seems to modulate ant preference for nutrients. We showed that ants inhabiting grasslands seem equally limited by Nitrogen and Carbon, while forest ants are N-limited. Thus, here we further advance our knowledge of ants, vertebrades e non-ants invertebrates and their role in scavenging across two contrasting tropical habitats.

Regarding the indirect influence of ants on ecosystem processes, the suppression of ground ants increased herbivory four-fold at the plant community level in forest and almost doubled it in grassland. We also showed that the suppression of ants doubled the decomposition of cellulose baits by termites in grassland but had no effect in the forest. That emphasises the unreplaceable role of ants in protecting plants against herbivory in both habitats and controlling decomposition by termites in grassland. Furthermore, our results imply that the effect of ants in decomposition is not limited to a decrease in termite population through predation but also a change in termite foraging behaviour. As observed in grassland, ant suppression, fewer cellulose baits were damaged by termites but were more consumed. Finally, our data indicate that other habitat factors other than ant community characteristics – activity and abundance - might also modulate the indirect contribution of ants to herbivory and decomposition, but we could not provide evidence for that. Thus, we suggest that future studies focus on how biotic protection (e.g. ant protection) interacts with plant community constitutive defence (e.g. trichomes and sclerophylly) between habitats and how termite community characteristics modulate the top-down role of ants in decomposition.

Thus, we reaffirm that ants are an important group of “little things that run the world” and add that disturbance that affects the ant communities could significantly impact ecosystem functioning. Therefore, disturbances in ant abundance due to climate change (Parr & Bishop, 2022) can lead to direct and indirect consequences for the ecosystem, especially in open habitats. In addition, these consequences can also extend to many other processes in that ants are directly or indirectly involved, such as soil bioturbation, pollination, and seed dispersal, carbon flux with unknown consequences. Finally, we highlight that the loss of key taxa in ecosystems can knock-on effects that multiply the initial impact of disturbances, thereby largely adding to the degradation of ecosystems caused by anthropogenic actions.

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24. Supplementary - General Introduction

Bibliometric assessment on the Role of ants in Ecosystems

Tiago V. Fernandes, Luane Fontenele, Tatiana Cornelissen, Ricardo I. Campos, Fernando Schmidt, Carla Ribas, Ricardo R. Solar.

We systematically accessed the number of publications that evaluated the ecosystem processes performed by ants. We searched for papers written in English in Web of Science, Scopus and Scielo databases, using all available years up to July 2020. We set two groups of keywords in our search: I) Ant, Ants and Formicidae II) Ecological role*, Service*, Function*, Productivity, Ecosystem engineer*, Pollination, Biological control, Decomposition, Bioturbation, Nutrient, Myrmecochory, Seed, Diaspore, Ecosystem process*, Scaveng*, Predator, Predation. We used the combination of all words from the first keywords group with the second keywords. We also filter the research to natural sciences subjects. We just selected studies that directly investigated the role of ants in at least one ecosystem process. Our initial search identified 16,519 studies (7,427 in Web of Science, 8,944 in Scopus and 148 in Scielo) that were potentially appropriate for our review. Of these, 3,061 were eliminated because they were duplicates and 9,912 because they were not about the subject of interest (not about ants and ecosystem processes). Then, we excluded 1,747 papers that have not measured the direct impact of ants (e.g. richness, composition, abundance, presence) in at least one ecosystem process Fig.1). Finally, we separated the selected 1799 papers on one of the main processes ants are involved: Scavenging (n=37), Bioturbation (n=77), Pollination (n=139), Nutrient cycling (n=173), Seed dispersal (n=562), and Predation (n=638; Fig 2 in General introduction).

Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart, summarising the sequence of information gathering and selection.