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PEDRO ELIAS SANTOS NETO

EFEITO DAS FORMIGAS CORTADEIRAS *Atta* E *Acromyrmex* NOS ATRIBUTOS DO SOLO E NA REGENERAÇÃO DA CAATINGA

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Tese apresentada ao Programa de Pós-Graduação em Biologia Vegetal da Universidade Federal de Pernambuco, área de concentração de Ecologia e Conservação, como exigência para a defesa e obtenção do título de doutorado em Biologia Vegetal.

Orientadora: Inara Roberta Leal

Coorientador: Rainer Wirth

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RESUMO

As perturbações antrópicas e mudanças climáticas constituem a principal causa de depleção dos estoques de nutrientes nos solos e da interrupção na regeneração florestal. Apesar disso, alguns organismos se beneficiam das condições impostas por esses fatores e aumentam suas populações sob efeito dessas condições. Um desses organismos são as formigas cortadeiras, insetos herbívoros especializados no cultivo de um fungo simbionte em câmaras subterrâneas de seus ninhos. A manutenção dos ninhos e o acúmulo de material vegetal manejado pelas formigas disponibiliza nutrientes mineralizados no solo que são absorvidos pelas raízes das plantas nas proximidades. A Caatinga é um dos ecossistemas onde formigas cortadeiras têm aumentado suas populações associadas a regimes de perturbações humanas. Essa floresta seca do nordeste da América do Sul sofreu com perturbações agudas no passado e hoje é mais submetida ao tipo crônico de perturbação, o qual atrasa a regeneração florestal. Além disso, as previsões climáticas apontam para um aumento de aridez na região. Essa tese tem como objetivo geral investigar o efeito das perturbações antrópicas e da crescente aridez sobre as populações de formigas cortadeiras, sua influência nos solos, e no estabelecimento e desenvolvimento de plantas. Para isso, nós acompanhamos ninhos de formigas cortadeiras, acessando a densidade de ninhos em áreas com diferentes níveis de perturbação e aridez, e quantificando suas taxas de forrageamento. Escavamos ninhos a fim de quantificar os nutrientes nos solos desses ninhos em diferentes perfis do solo. Usando solos de ninhos, germinamos sementes de três espécies de plantas comuns da Caatinga a fim de investigar a influência dos solos dos ninhos no desenvolvimento vegetal. Nossos resultados indicam que os ninhos de áreas mais perturbadas e áridas tem maior concentração e estoque de nutrientes em relação a solos controle, devido a depleção de nutrientes ser maior nessas áreas. Encontramos também que plantas crescendo em solos de ninhos acumulam biomassa mais rapidamente, embora não tenham maior concentração de nutrientes em seus tecidos. Assim, o conjunto das alterações no solo promovidas pelos ninhos, aumentam a performance das plantas que acessam esses solos. Esses achados sugerem que em áreas perturbadas e secas, com solos mais pobres, as formigas podem promover uma concentração maior de matéria orgânica nos ninhos, retirando e tornando esse material inacessível em outras áreas. Esse processo pode aumentar o grau de depleção de matéria orgânica na matriz do solo, afetando a capacidade de retenção de água e nutrientes, e segregando nutrientes mineralizados para árvores adultas, inviabilizando o solo para indivíduos juvenis.

Palavras-chave: Florestas tropicais sazonalmente secas; Nutrientes do solo; Engenheiros de ecossitema; Mudanças climáticas; Perturbação antrópica crônica; Herbivoria.

ABSTRACT

Anthropogenic disturbances and climate change are the main causes of depletion of nutrient stocks in soils and the interruption of the forest regeneration. Nevertheless, some organisms can profit from the conditions imposed by these factors and increase their populations. One such organism is the leaf-cutting ant, herbivorous insects specialized in growing a symbiont fungus in underground chambers inside their nests. The maintenance of the nests and the accumulation of plant material managed by the ants make mineralized nutrients available in the soil, which are taken up by the roots of nearby plants. The Caatinga is one of the ecosystems where leaf-cutting ants have increased their populations associated with human disturbance regimes. This dry forest in northeastern South America has suffered from acute disturbances in the past and is now more subjected to the chronic type of disturbance, which slows down the forest recovery. In addition, climate predictions point to an increase in aridity in the region. The overall objective of this thesis is to investigate the effect of anthropogenic disturbances and increasing aridity on leaf-cutter ant populations, their influence on soils, and on plant establishment and development. To this end, we monitored leaf-cutter ant colonies, assessing nest density in areas with different levels of disturbance and aridity, and quantifying their foraging rates. We excavated nests in order to quantify nutrients in nest soils at different soil profiles. Using nest soils, we germinated seeds of three common Caatinga plant species in order to investigate the influence of nest soils on plant development. Our results indicate that nests in more disturbed and arid areas have a higher concentration and stock of nutrients compared to control soils, because nutrient depletion is higher in these areas. We also found that plants growing in nest soils accumulate biomass faster, although they do not have a higher concentration of nutrients in their tissues. Thus, all of the changes in the soil promoted by the nests increase the performance of the plants that access these soils. These findings suggest that in disturbed and dry areas with poorer soils, the ants may promote a higher concentration of organic matter in the nests, removing and making this material inaccessible in other areas. This process can increase the degree of organic matter depletion in the soil matrix, affecting the water retaining capacity and nutrients, and secreting mineralized nutrients to adult trees, making the soil unviable for juvenile individuals.

Key-words: Seasonally dry tropical forests; Soil nutrients; Ecosystem engineers; Climate change; Chronic anthropogenic disturbance; Herbivory.

SUMÁRIO

1 INTRODUÇÃO

As perturbações humanas afetam negativamente a maioria dos ecossistemas da Terra, e são a principal causa da perda e fragmentação de habitat (LAURANCE et al., 2002), deterioração da biodiversidade (BARLOW et al., 2016), e mudanças negativas das propriedades físicas e químicas dos solos (BARBHUIYA et al., 2004; CARDELÚS et al., 2020; TIESSEN; SAMPAIO; SALCEDO, 2001). Os efeitos das perturbações humanas são suficientemente deletérios quando considerados isolados, mas podem ser potencialmente mais graves quando combinados com aqueles impostos pelas mudanças climáticas (PEREIRA et al., 2010; SALA et al., 2000). Por exemplo, os efeitos combinados das perturbações antrópicas e das mudanças climáticas podem modificar a composição da microbiota dos solos e alterar as suas concentrações de nutrientes (FERRENBERG et al., 2015). Todas essas alterações afetam o desenvolvimento das plantas, reduzindo as taxas de crescimento de árvores em locais sob adversidades climáticas e perturbação humana (TOLEDO et al., 2011).

As alterações ambientais promovidas pelas perturbações e mudanças climáticas são deletérias para muitas espécies de animas e plantas, mas podem reunir condições ideias para a proliferação de alguns organismos. Um dos organismos que proliferam nessas novas condições adversas são as formigas cortadeiras, insetos herbívoros dos gêneros *Atta* e *Acromyrmex*, dominantes das regiões neotropicais e capazes de incluir até 50% das espécies de plantas de uma área em sua dieta (WIRTH et al., 2003). As formigas cortadeiras aumentam suas populações em áreas perturbadas devido a perda de cobertura vegetal e consequente aumento de sítios para nidificação, assim como com o aumento na abundância de plantas com menos defesas anti-herbivoria, portanto mais palatáveis (relaxamento bottom-up), e diminuição de predadores (relaxamento top-down) (SIQUEIRA et al., 2017; URBAS et al., 2007; VASCONCELOS et al., 2006). Além de aumentarem suas populações, as formigas cortadeiras aumentam suas taxas de herbivoria em áreas perturbadas, exercendo maior pressão sobre a vegetação nessas áreas (SIQUEIRA et al., 2017; URBAS et al., 2007). As colônias de formigas cortadeiras cortam folhas e outros materiais vegetais, que são usados como substrato para o cultivo de seu fungo simbionte, a principal fonte de alimento da colônia (ABRIL; BUCHER, 2004). Parte do material vegetal que não é totalmente digerido pelo fungo é disposta em câmaras de lixeira dentro dos ninhos ou em pilhas de lixeira fora do ninho (WIRTH, R. et al., 2003).

As lixeiras, ricas em material orgânico, dispostas tanto dentro como fora dos ninhos, constituem uma fonte de nutrientes para os solos (FARJI-BRENER; WERENKRAUT, 2014). Esses nutrientes contidos nas lixeiras, podem ser mineralizados e absorvidos pelas plantas que crescem ao redor dos ninhos (STERNBERG et al., 2007). Devido ao processo de acúmulo de material orgânico via herbivoria e às atividades de escavação e manutenção dos ninhos, as formigas cortadeiras são consideradas organismos engenheiros de ecossistemas (HÖLLDOBLER; WILSON, 1990). No entanto, os murundus dos ninhos (i.e., os solos provenientes das escavações das câmaras subterrâneas (Figura 1)) podem sofrer depleção de nutrientes e diminuir o recrutamento de plântulas que crescem diretamente nesse solo nos ninhos em que as lixeiras são internas (MEYER et al., 2013). Assim, plantas que crescem em solos enriquecidos pelo material orgânico das lixeiras tem crescimento potencializado (FARJI-BRENER; WERENKRAUT, 2014), enquanto plântulas que crescem nos murundus dos ninhos com lixeiras internas não conseguem acessá-las e têm concentração de nutrientes e recrutamento reduzidos (MEYER et al., 2011b, 2013).

O conhecimento dos efeitos positivos das formigas cortadeiras enriquecendo os nutrientes dos solos e potencializando o crescimento de plantas nas redondezas dos ninhos é bem fundamentado (FARJI-BRENER & WERENKRAUT, 2014; MOUTINHO; NEPSTAD; DAVIDSON, 2003; STERNBERG et al., 2007). Pouco se sabe no entanto sobre o efeito das perturbações humanas e mudanças climáticas nessa influência que o ninho exerce nos solos e consequentemente nas plantas. Apesar das formigas cortadeiras aumentarem suas taxas de herbivoria, virtualmente aumentando as deposições de lixeiras (FARJI-BRENER, Alejandro G.; TADEY; LESCANO, 2017; SIQUEIRA et al., 2018), essas lixeiras podem ser de menor qualidade em áreas perturbadas, consistindo de material vegetal recalcitrante e outros compostos como fezes de animais (SIQUEIRA et al., 2018). Além disso, a maior parte do conhecimento sobre efeitos de perturbações humanas nas populações de formigas cortadeiras vem de floretas úmidas, com poucos estudos em floretas secas, onde suas populações são densas e podem infligir maior pressão à vegetação (SIQUEIRA et al., 2018).

A Caatinga é a maior e mais diversa floresta tropical seca do Globo (SILVA, Jose Maria Cardoso Da; LEAL; TABARELLI, 2017), sendo também a mais densa em população humana (26 habitantes/km², Medeiros et al. 2012), composta principalmente por famílias de baixa renda fortemente dependentes de recursos naturais para a subsistência

(AB’SABER, 2000). Devido processo de uso dos recursos naturais, a maior parte da Caatinga já sofreu perda de sua vegetação (perturbação aguda), restando hoje apenas 34% da vegetação original (SILVA, Jose Maria Cardoso Da; LEAL; TABARELLI, 2017). As regiões remanescentes passam por outro processo de exploração de recursos, denominado perturbação crônica (SINGH, 1998), caracterizado principalmente pelo uso da vegetação nativa para a criação de gado bovino e caprino, extração de lenha, e exploração de recursos florestais não madeireiros (ARNAN et al., 2018; RIBEIRO, Elaine M .S. et al., 2015; SOUZA et al., 2019). Além de causarem perda de biodiversidade (RIBEIRO, Elaine M.S. et al., 2016; RIBEIRO, Elaine M .S. et al., 2015; RITO et al., 2017), tanto o processo de perturbação aguda como crônica promovem diminuição de estoques de nutrientes nos solos (ALTHOFF et al., 2018; SANTANA et al., 2019; SCHULZ et al., 2016; TIESSEN; SAMPAIO; SALCEDO, 2001). Outro problema que atinge a Caatinga são os efeitos relacionados com as mudanças climáticas globais. A Caatinga vai enfrentar aumento de aridez com redução na precipitação de 22% e aumento de temperatura de 6°C até o final do século XXI (MAGRIN et al., 2014). Os efeitos das mudanças climáticas na Caatinga e em outras partes do mundo podem ser mais severos se associados com perturbações humanas, com recorrentes mudanças nos ciclos de nutrientes e perda de seus estoques nos solos (SARDANS; PEÑUELAS; ESTIARTE, 2006; ST.CLAIR; LYNCH, 2010).

Essa tese tem como objetivo investigar como as perturbações humanas e o aumento da aridez afetam as populações de formigas cortadeiras, sua influência nos estoques de nutrientes dos solos e consequente efeitos nas plantas com implicações na dinâmica de regeneração da floresta na Caatinga. Dessa forma, a tese foi dividida em dois capítulos. No capítulo I, focamos nos efeitos de perturbação humana crônica e aridez (1) nas populações de cortadeiras, (2) na biomassa vegetal que as formigas levam para dentro dos ninhos, e (3) nos solos das colônias em diferentes profundidades, avaliando as propriedades físicas (densidade aparente) e químicas (concentração e estoques de nutrientes) dos solos. No capitulo II, nosso foco foi no efeito de dois tipos de perturbação, aguda e crônica, juntamente com os efeitos da aridez nos solos dos ninhos e consequentemente nas plantas que crescem sobre eles, investigando (1) o crescimento das plantas em termos de biomassa e comprimento acima do solo, e (2) a concentração de nutrientes na biomassa dessas plantas. Nossos resultados nos permitem tirar conclusões sobre como as perturbações humanas, incluindo os efeitos das mudanças climáticas, afetarão os solos sob influência de um organismo dominante nas paisagens perturbadas como as formigas cortadeiras, e como isso se reflete nas comunidades de plantas pioneiras também nessas áreas perturbadas.

2 REFERENCIAL TEÓRICO

2.1. Perturbações humanas nos solos e na regeneração florestal dos ambientes tropicais

Nas últimas décadas, uma grande porção da paisagem das regiões tropicais foi convertida em lavouras ou pasto para suprir as demandas de uma população humana crescente (HANSEN, 2013). No entanto, grande parte dessas áreas tem sido abandonada devido ao declínio da fertilidade do solo e consequentemente da produtividade vegetal, entrando em processo de sucessão secundária (BUSCHBACHER; UHL; SERRAO, 1988; NEPSTAD; UHL; SERRAO, 1991; NEPSTAD et al., 1996; ARROYO-RODRÍGUEZ et al., 2017). Apesar de ser amplamente aceito que as áreas em sucessão secundária se regeneram naturalmente, o tempo para que a floresta adquira novamente as propriedades de uma floresta primária pode superar 200 anos (COLE; BHAGWAT; WILLIS, 2014). Um importante fator que afeta o tempo de regeneração de uma floresta é a intensidade da perturbação que a área sofreu durante o tempo que foi usada (NEPSTAD; UHL; SERRAO, 1991; ARROYO-RODRÍGUEZ et al., 2017). A intensidade de perturbação pode diminuir a quantidade de nutrientes do solo e influenciar o caminho da regeneração, alterando o papel do tempo de abandono na sucessão ecológica (BUSCHBACHER; UHL; SERRAO, 1988).

Os solos desempenham papel fundamental no ciclo global dos nutrientes, além de ser (1) a terceira maior reserva de carbono (depois dos oceanos e da litosfera abaixo do solo) com aproximadamente 2400 Pg de C nos primeiros 2 m de profundidade (KIRSCHBAUM, 2000); e (2) abrigar a maior reserva de Nitrogênio dos ecossistemas, com uma grande porção em formas moleculares (N_2) mas que podem ser mineralizadas e então absorvidas pelas raízes das plantas (BALDOCK, 2007). A influência da perturbação no processo de regeneração é intimamente ligada aos estoques e à ciclagem de nutrientes nos solos dos ecossistemas perturbados, porque a maior parte da matéria orgânica que compõe os estoques e entra no ciclo de nutrientes vêm da biomassa acima do solo (BALDOCK, 2007; VITOUSEK; SANFORD, 1986). O problema é que as perturbações humanas ligadas ao uso da terra provocam exportação e perda de biomassa acima do solo, subtraindo nutrientes dos estoques dos solos dessas áreas (BUSCHBACHER; UHL; SERRAO, 1988; KAUFFMAN et al., 2013; MENEZES et al., 2020; SCHULZ et al., 2016; TCHIOFO LONTSI et al., 2019).

Os estoques de C na biomassa acima do solo em áreas perturbadas por extração de madeira, e por extração de madeira seguida de queima, podem sofrer redução de 35% e 57% respectivamente em relação à áreas naturais preservadas (BERENGUER et al., 2014). De forma semelhante, o uso da terra, como agricultura e pasto, são responsáveis pela diminuição dos estoque de C no solo, sendo as monoculturas ainda mais deletérias (GUO; GIFFORD, et al., 2022). Os efeitos deletérios da perturbação sobre os estoques de nutrientes do solo podem ser ainda maiores quando há sucessivos eventos de perturbação, ainda que esparsos no tempo. Por exemplo, os estoques de C são ainda mais reduzidos quando áreas de sucessão secundária são novamente convertidas em algum tipo de uso da terra como plantação para extrativismo (BLÉCOURT, DE *et al.*, 2013). Outros macronutrientes também têm seus estoques afetados, como P e S, principalmente com diminuição, mesmo que alguns tipos de perturbação em nível moderado não provoquem mudança significativa (TCHIOFO LONTSI *et al.*, 2019).

Juntamente com perturbação, outros fatores como clima e composição de espécies também atuam influenciando os padrões de estoque e ciclagem de nutrientes, já que estes interferem nos processos de decomposição de matéria orgânica e liberação de nutrientes no sistema (LU; MORAN; MAUSEL, 2002; MURPHY; LUGO, 1986). Por exemplo, solos de ecossistemas que recebem altos níveis de precipitação anual podem apresentar menores estoques de nutrientes comparado a áreas mais secas devido a maiores perdas por lixiviação (AUSTIN; VITOUSEK, 1998). Esses processos têm sido bem descritos e entendidos em florestas tropicais úmidas, mas relativamente menos estudados em florestas tropicais secas (FTSs). Nas florestas tropicais secas é a disponibilidade de água que desempenha um papel dominante na estrutura e dinâmica do ecossistema (MURPHY; LUGO1986; JAMARILLO; SANFORD 1995). Nessas regiões, a estação seca provoca drástica redução na produtividade primária e consequentemente diminuição da matéria orgânica no solo, diminuindo as taxas de ciclagem de elementos (BURINGH, 1984; AUSTIN et al., 2004; SCHULZ et al., 2016). No entanto, é evidenciado que em florestas secas a perturbação também é a principal responsável pela perda de biomassa e nutrientes (KAUFFMAN et al., 2013), e que perturbação e aridez podem interagir e reduzir a funcionalidade dos solos (GAITÁN et al., 2018).

2.2. Os efeitos da perturbação antrópica crônica nos ambientes tropicais secos

Além de causarem a perda de nutrientes dos solos, as perturbações humanas são fortemente deletérias para as comunidades vegetais e animais (SOUSA, 1984). Perturbações humanas, como a conversão de florestas em lavouras e pastagens, são a principal causa da perda de biodiversidade nos ecossistemas e aumento potencial dos riscos de extinção em fragmentos florestais (ROQUE et al., 2018). No entanto, nas regiões tropicais semiáridas do Globo ocorre principalmente um tipo de perturbação que não produz fragmentação florestal (i.e. perturbação aguda), mas ao invés disso, consiste na retirada contínua de produtos florestais produzindo uma perturbação crônica (SINGH, 1998). A perturbação crônica se caracteriza pela remoção contínua de produtos florestais, incluindo a caça contínua, a remoção de madeira para produção de lenha, coleta de produtos florestais não madeireiros e a criação extensiva de animais domésticos (e.g. gado caprino, ovino e bovino) (ARNAN et al., 2018). O problema com a forma crônica de perturbação é que os organismos, e o ecossistema como um todo, não têm tempo de se recuperar da contínua retirada de produtos, causando mudanças profundas nas áreas perturbadas, mesmo se as taxas de remoção de biomassa estiverem dentro da capacidade suporte do ambiente (SINGH, 1998). De fato, a perturbação do tipo crônica é responsável pelo empobrecimento das comunidades vegetal e animal nas FTSs (OLIVEIRA et al., 2017; RIBEIRO-NETO et al., 2016; RIBEIRO et al., 2019; RIBEIRO et al., 2015), pela ruptura de interações ecológicas (CÂMARA, Talita et al., 2018, 2019), e pela redução dos estoques de nutrientes nos solos (SCHULZ et al., 2016).

Os efeitos das perturbações nos estoques de nutrientes dos solos podem ser mais duradouros que o esperado nas FTSs. Por exemplo, os estoques de carbono nos solos podem levar até 60 anos para serem restabelecidos às condições pré-perturbação nessas regiões (ARAÚJO-FILHO et al., 2018). Isso pode se dever a potenciais interações das perturbações com efeitos climáticos, como a aridez (GAITÁN et al., 2018), já que os efeitos da redução de precipitação (aridização) são promotores da depleção de nutrientes nos ecossistemas secos de todo o mundo (MORENO-JIMÉNEZ et al., 2019). Além disso, a baixa produtividade juntamente com baixo intemperismo são fatores determinantes que limitam a disponibilidade de nutrientes (AUSTIN; VITOUSEK, 1998). Por exemplo, sabe-se que a diminuição da precipitação, ou aumento da aridez, tende a diminuir a quantidade de carbono orgânico e as concentrações de nitrogênio no solo, diminuir a razão C:N, além de desequilibrar as razões de C:P e N:P (AUSTIN; VITOUSEK, 1998; DELGADO-BAQUERIZO et al., 2013). Todos esses fatores podem suprimir a regeneração de áreas de florestas secas, fazendo com que mesmo áreas maduras e que tiveram seus episódios de perturbação há muitas décadas apresentem flora semelhante àquela de áreas recentemente perturbadas (BARROS et al., 2021).

Esses efeitos negativos das perturbações sobre os nutrientes do solo são particularmente preocupantes nos ambientes secos porque essas áreas abrigam grandes populações humanas que usam os recursos naturais para diversas atividades econômicas (ARNAN et al., 2018; MURPHY; LUGO, 1986; SILVA; LEAL; TABARELLI, 2017; SINGH, 1998a). Nesses ambientes, atividades econômicas e de subsistência, como a criação de rebanhos caprino e ovino, são reportados como promotores de empobrecimento tanto da diversidade vegetal como dos nutrientes do solo (SCHULZ *et al.*, 2016, 2019). No caso da agricultura, o problema é que uma vez que têm seus solos exauridos, essas áreas são abandonadas para o plantio, mas permanecem sendo exploradas para pastagem (solta de rebanho) e retirada de outros produtos florestais (SILVA; LEAL; TABARELLI, 2017). Dessa forma, a floresta sofre uma perturbação aguda e em seguida abandono, entrando em processo de sucessão secundária, mas permanece sob pressão da perturbação crônica (ARROYO-RODRÍGUEZ et al., 2017; BARROS et al., 2021).

2.3. Organismos que se beneficiam com perturbação: As formigas cortadeiras como engenheiras de ecossistema

Apesar de as perturbações antrópicas produzirem efeitos negativos nas comunidades de plantas e animais e no funcionamento do ecossistema, alguns organismos são capazes de contrapor esses efeitos negativos e aumentarem suas populações em áreas perturbadas (SIQUEIRA et al., 2017; VASCONCELOS et al., 2006). Dentre esses organismos, se destacam as formigas cortadeiras dos gêneros *Atta* (Fabricius, 1805), *Acromyrmex* (Mayr, 1865), e *Amoimyrmex* (Cristiano, Cardoso e Sandoval, 2020), insetos sociais cultivadores de fungos (SWANSON et al., 2019; WIRTH, et al., 2003; WIRTH, et al., 2007; CRISTIANO et al., 2020). Essas formigas são os principais herbívoros da região neotropical, coletando entre 12 e 17% da produção anual de folhas (CHERRET, 1986; URBAS et al. 2007) em uma floresta, e incluindo até 50% da flora local (WIRTH et al., 2003) ao longo de suas áreas de forrageamento. Todo esse material vegetal é usado para cultivar o fungo simbionte dentro dos seus ninhos, a principal fonte de alimento das colônias (HÖLLDOBLER; WILSON 1990).

As formigas cortadeiras estão entre os animais que mais provocam modificações no ambiente (FARJI-BRENER; GHERMANDI, 2000). A construção e manutenção dos seus ninhos provocam uma série de modificações no regime de luz e nas condições do solo, por isso são consideradas importantes engenheiros do ecossistema (MEYER et al., 2011a, 2013). Por exemplo, o ambiente mais aberto e dessecado que elas produzem promove o estabelecimento de plantas pioneiras em detrimento de plantas tolerantes à sombra, causando mudança na composição taxonômica e funcional da comunidade de árvores (MEYER et al. 2011b). As alterações edáficas provocadas por formigas cortadeiras também configuraram entre as mais importantes nos ambientes naturais. A construção e a manutenção dos ninhos podem representar a realocação de mais de 40 toneladas de solo mineral para a superfície (formando o murundu do ninho, **figura 1**) (ALVARADO et al., 1981). Este novo horizonte pode, também, ter sua gênese alterada pela remoção contínua de serapilheira da superfície do murundu e no entorno dos mesmos (MEYER et al. 2011a). Por outro lado, camadas mais profundas do solo são enriquecidas em nutrientes devido ao (1) acúmulo de material orgânico proveniente da cultura do fungo simbionte e de outras atividades das colônias, (2) remoção de material mineral para a superfície e (3) difusão/percolação de nutrientes (ALVARADO et al., 1981; MOUTINHO et al., 2003; WIRTH et al., 2003).

Figura 1: Murundu de uma colônia da espécie *Acromyrmex balzani* (Emery, 1890) no Parque Nacional do Catimbau, PE, Brasil.

solos ao redor dos ninhos. Esse acúmulo de material, resultante da atividade de corte e transporte de material vegetal para a cultura do fungo, se materializa na existência de uma estrutura importante dos ninhos: as lixeiras (**Figura 2**). As lixeiras são regiões dos ninhos onde as formigas dispõem a porção do material vegetal incorporado nos jardins de fungo que não foi totalmente metabolizado pelo fungo simbionte, juntamente com formigas mortas e restos do próprio fungo morto ou contaminado (ABRIL; BUCHER, 2004). Esse material de refugo é disposto em câmaras internas no ninho, separadas das câmaras de fungo, ou em pilhas externas na superfície próxima ao ninho (ABRIL; BUCHER, 2004; WIRTH, R. et al., 2003). A disposição desse material em partes isoladas das câmaras de fungo é uma medida sanitária tomada pelas colônias, já que é um material contaminado por bactérias e fungos diversos que podem infectar a cultura do fungo simbionte ou causar doenças nas operárias do ninho (ABRIL; BUCHER, 2004). No entanto, as lixeiras contêm muitos compostos orgânicos ricos em nutrientes que contribuem para efeitos positivos na estrutura e fertilidade do solo (FARJI-BRENER; ILLES, 2000). Isso porque os nutrientes contidos nas lixeiras podem ser mineralizados e ficarem disponíveis para serem absorvidos pelas plantas adultas crescendo nas proximidades dos ninhos, mesmo que as lixeiras estejam em câmaras profundas no solo (STERNBERG et al., 2006). Apesar de plantas lenhosas adultas experimentarem maior disponibilidade de macro e micronutrientes próximo aos ninhos de formigas cortadeiras (SOUSA-SOUTO et al., 2007), plântulas crescendo diretamente sobre os ninhos experimentam solos superficiais menos férteis e têm seu recrutamento reduzido em comparação com solos controle (MEYER et al., 2011B; CORRÊA et al., 2010).

Figura 2: Lixeira de uma colônia da espécie *Acromyrmex rugosus* (Smith, 1858) no Parque Nacional do Catimbau, PE, Brasil.

2.4. Influência da perturbação nas populações de formigas cortadeiras e nos seus efeitos nos solos e na vegetação em florestas úmidas e secas

Formigas cortadeiras, juntamente com outros herbívoros generalistas, proliferam em áreas modificadas pelas atividades humanas tanto em áreas úmidas (WIRTH et al., 2008; LEAL et al., 2014), como em regiões semi-áridas (SIQUEIRA et al., 2017). Esses organismos têm aumentando suas populações em áreas perturbadas devido ao (1) aumento na disponibilidade de alimento, pela colonização por plantas pioneiras, com menos defesas e mais palatáveis (URBAS et al., 2007); à (2) redução das populações de predadores e parasitóides, como tatus, tamanduás e dípteros forídeos (ALMEIDA; WIRTH; LEAL, 2008); e (3) ao aumento na disponibilidade de sítios para nidificação pela perda de cobertura vegetal em áreas perturbadas (VASCONCELOS et al., 2006; VIEIRA-NETO; VASCONCELOS; BRUNA, 2016). Os efeitos de perturbação não somente beneficiam as populações de formigas cortadeiras como aumentam os regimes de forrageamento das colônias. Tanto os efeitos de borda da perturbação aguda em floresta úmida (URBAS et al., 2007), como os efeitos da perturbação crônica em florestas secas (SIQUEIRA et al., 2018) são responsáveis pelo aumento das taxas de herbivoria das colônias. Esses efeitos podem estar ligados tanto ao aumento da densidade de plantas mais palatáveis e menos defendidas em áreas perturbadas (URBAS et al., 2007), como ao aumento de temperatura e potencialização dos picos de atividade das colônias em áreas mais abertas usadas como pasto (BUSTAMANTE; AMARILLO-SUÁREZ; WIRTH, 2020).

Apesar da perturbação antrópica produzir efeito de aumento tanto das populações de formigas cortadeiras como das suas taxas de forrageamento, a maioria das informações sobre a influência dessas formigas no estoque e ciclagem de nutrientes dos solos é proveniente de estudos em florestas úmidas (MOUTINHO; NEPSTAD; DAVIDSON, 2003; SOSA; BRAZEIRO, 2010; VERCHOT; MOUTINHO; DAVIDSON, 2003), pouco sendo conhecido sobre este tema para florestas tropicais secas (mas veja SOUSA-SOUTO et al., 2007). Uma importante diferença no comportamento de colônias de formigas cortadeiras em ambientes secos e úmidos é que algumas espécies podem preferir fundar colônias em áreas mais abertas quando em ambientes úmidos, mas podem mudar o comportamento em ambientes secos, nidificando preferencialmente em áreas com maior cobertura vegetal (SIQUEIRA et al., 2017). Outra importante característica é a localização das lixeiras (ver seção 2.3), que na maioria das espécies é interna em florestas úmidas e externa em florestas secas e outros ambientes semiáridos (FARJI-BRENER; TADEY, 2016). De fato, as espécies que ocorrem na floresta seca da Caatinga do nordeste do Brasil têm sido reportadas colocando suas lixeiras na superfície, para fora do ninho (Felipe F. S.

Siqueira dados não publicados). Além do efeito do solo profundo do ninho (STERNBERG et al., 2007), o conteúdo das lixeiras externas tem efeitos positivos na estrutura e fertilidade do solo, porque também mineralizam nutrientes que podem ser absorvidos pelas plantas (FARJI-BRENER; TADEY, 2017; FARJI-BRENER; WERENKRAUT, 2014).

Em florestas úmidas, a interação de herbivoria promovida por formigas cortadeiras produz efeitos negativos nas plantas atacadas, com a perda de tecido vegetativo e partes reprodutivas dos indivíduos (CÂMARA, T. et al., 2019; WIRTH, Rainer et al., 2003). Por exemplo, as plantas que têm suas flores e folhas cortadas pelas formigas passam a produzir até duas vezes menos flores (CÂMARA, T. et al., 2019). Mais recentemente, um estudo na floresta tropical seca da Caatinga encontrou que colônias de formigas cortadeiras podem ser responsáveis pelo retardo na regeneração da floresta em áreas que sofreram perturbação aguda para a criação de pasto (KNOECHELMANN et al., 2020). No entanto, em contraponto aos efeitos negativos experimentados pelas plantas-alvo das colônias, a absorção de nutrientes mineralizados nos solos enriquecidos pelas lixeiras dos ninhos promove efeitos positivos nos indivíduos de plantas que conseguem acessar esses solos. Plantas crescendo sobre solos enriquecidos pelas lixeiras apresentaram maior crescimento do que aquelas crescendo sobre solos dos ninhos sem influência das lixeiras (FARJI-BRENER; WERENKRAUT, 2014). Além de aumentar as taxas de crescimento diretamente, as lixeiras de formigas cortadeiras também podem aumentar o fitness das plantas de forma indireta pelo aumento do tamanho das flores e prolongamento do período de floração, potencializando os eventos de visitação por polinizadores (FERNANDEZ; TADEY; FARJI-BRENER, 2019).

A heterogeneidade espacial causada pelas formigas cortadeiras e seus efeitos no solo precisam ser entendidos levando em conta a interferência da perturbação humana, já que com o aumento de suas populações também aumenta a potencial interação das colônias com o meio biótico e abiótico, e consequentemente a modificação de ambientes em escala local (LEAL; WIRTH; TABARELLI, 2014). Além disso, mais de um terço das florestas tropicais do Globo está em processo de sucessão secundária e/ou sofre algum grau de perturbação (ARROYO-RODRÍGUEZ et al., 2017; HANSEN, 2013; POORTER et al., 2021). A lacuna se dá porque há muitas evidências dos efeitos positivos que as lixeiras produzem nos solos e no crescimento das plantas (FARJI-BRENER, Alejandro G.; WERENKRAUT, 2014), mas a interferência que as perturbações humanas podem infligir nesse papel que as colônias desempenham como enriquecedoras do solo ainda foi pouco estudada. Por exemplo, na Patagônia, em áreas que sofreram perda total da cobertura vegetal, como para abertura de estradas, as colônias produzem lixeiras ainda mais ricas em nutrientes que contribuem positivamente para o fitness das plantas que crescem nos arredores dos ninhos (veja a **Figura 3**) (FARJI-BRENER; GHERMANDI, 2008; FARJI-BRENER; TADEY; LESCANO, 2017). De forma contrária, fontes de perturbação como a pressão de pastagem são responsáveis pela diminuição na qualidade do conteúdo de nutrientes nas lixeiras dos ninhos (TADEY; FARJI-BRENER, 2007b).

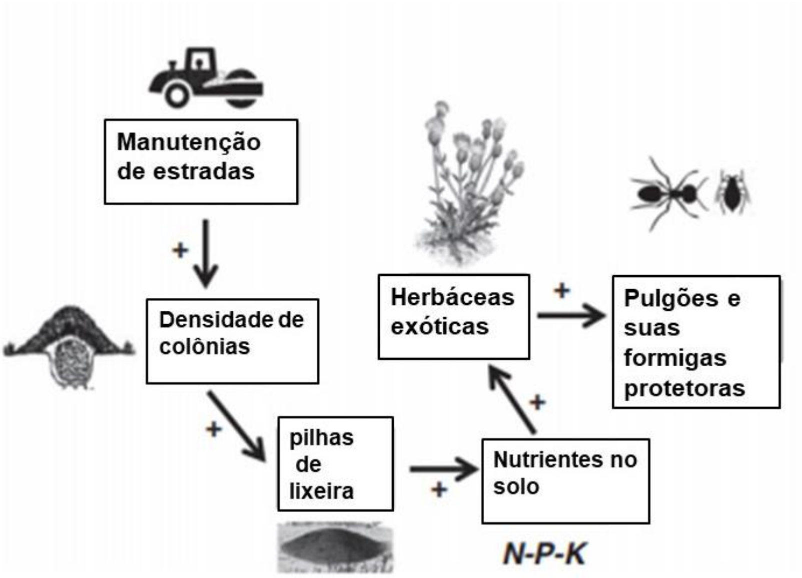

Figura 3. Fluxograma do efeito da perturbação antrópica na densidade de ninhos de LCA e no seu papel no enriquecimento do solo através do aumento das concentrações de nutrientes (Adaptado de Farji-Brener; Tadey; Lescano, 2017).

Em um estudo bastante elucidativo, Moutinho et al. (2003) mostraram que o efeito dos ninhos de formigas cortadeiras permanecem no solo mesmo após anos de inatividade da colônia em florestas secundárias. Nesse estudo, foi encontrado que as concentrações de C e N diminuem nos murundus dos ninhos em relação a solos controle, mas em compensação, as concentrações de Ca e Mg aumentam em profundidades abaixo de 1 metro no solo. Para o elemento potássio (K), por exemplo, esse aumento pode ser de mais de 10 vezes nos solos dos ninhos (MOUTINHO; NEPSTAD; DAVIDSON, 2003). A capacidade de troca catiônica (CTC) do solo parece aumentar abaixo da superfície dos solos de ninhos já que nutrientes mais disponíveis em forma de íons, como Mg, Ca e K, aumentam consideravelmente nesses solos (MOUTINHO; NEPSTAD; DAVIDSON, 2003). Esses achados indicam que solos enriquecidos pelos ninhos podem contribuir de forma positiva para a regeneração florestal em áreas de floresta secundária, aumentando o fitness das plantas já que a vegetação adulta ao redor dos ninhos acessam os nutrientes incrementados no solo (FARJI-BRENER; GHERMANDI, 2008; STERNBERG et al., 2007).

A diminuição de C e N nos murundus é explicada pela constante remoção de matéria orgânica da superfície ao mesmo tempo em que há o acúmulo de material mineral proveniente das camadas abaixo da superfície do solo, como parte da construção e manutenção dos ninhos (HÖLLDOBLER; WILSON, 1990). Portanto, essa diminuição pode não ter a ver com a perturbação aguda sofrida pela floresta. No entanto, o aumento da densidade de colônias associado com perturbação, como nas áreas de sucessão secundária, pode intensificar a diminuição de nutrientes como C e N nas camadas próximas a superfície do solo. Isso porque alterações dos solos dos murundus, com menos matéria orgânica, diminui a CTC, retendo menos nutrientes (MCKENZIE; JACQUIER; ISBELL, 2004). Essa relação é evidenciada pela menor concentração de C e N encontrada nos murundus (VERCHOT; MOUTINHO, P. R.; DAVIDSON, 2003). Dessa forma, contrário ao efeito positivo para plantas adultas, o recrutamento reduzido de plântulas nos murundus indica que os ninhos de formigas cortadeiras podem exercer efeito negativo para o ritmo da sucessão ecológica (MEYER et al., 2011a, 2013).

Além das propriedades químicas, as alterações promovidas pelas colônias incluem modificações das propriedades físicas do solo. Os solos dos ninhos podem apresentar menor resistência e menor densidade aparente, ao passo que apresentam maior porosidade (MOUTINHO; NEPSTAD; DAVIDSON, 2003). Sabe-se que o maior acúmulo de matéria orgânica nas camadas abaixo da superfície dos solos dos ninhos também afetam a física do solo, já que é um fator responsável pela diminuição da sua densidade aparente e aumento da porosidade (FARJI-BRENER; TADEY, 2009; RONQUIM, 2010). Dessa forma, além da incorporação direta de elementos nos solos, os ninhos ainda promovem a retenção de nutrientes através da modificação das propriedade físico-químicas (MOUTINHO; NEPSTAD; DAVIDSON, 2003; STERNBERG *et al.*, 2007; VERCHOT; MOUTINHO; DAVIDSON, 2003). No entanto pouco se sabe sobre como esses efeitos dos ninhos na física do solo podem ser alterados pelas perturbações antrópicas.

2.5. A Caatinga, uma floresta tropical seca no Brasil: efeitos das perturbações antrópicas crônicas sobre a floresta

A Caatinga é a uma floresta tropical seca que tem a maior parte de seu território dentro da região nordeste do Brasil, constituindo a mais vasta floresta seca do Globo (SILVA, Jose Maria Cardoso Da; LEAL; TABARELLI, 2017). Essa região é também o ecossistema seco mais densamente povoado (26 habitantes/km², MEDEIROS et al., 2012). A população da Caatinga é composta principalmente de famílias de baixa renda e por isso, muito dependentes de recursos naturais para subsistência (AB'SABER, 2000), fator que tem imposto uma pressão histórica sobre os recursos da floresta, tanto através do uso da terra (i.e. perturbação aguda), como através do uso de recursos da floresta (i.e. perturbação crônica). Por causa do processo de perturbação aguda, atualmente a Caatinga conserva apenas 33,4% da sua vegetação original (SILVA, Jose Maria Cardoso Da; LEAL; TABARELLI, 2017). Tanto as áreas com vegetação original como as já modificadas sofrem com os efeitos da perturbação crônica. Por exemplo, os efeitos da perturbação crônica são suficientes para causar a perda da biodiversidade e homogeneização das comunidades de plantas na Caatinga (RIBEIRO, Elâine M.S. et al., 2016; RIBEIRO, Elaine M .S. et al., 2015; RITO et al., 2017). Além disso, tanto a perturbação aguda como as diversas fontes de perturbação crônica são responsáveis pela de nutrientes nos solos da região (ALTHOFF et al., 2018; SANTANA et al., 2019; SCHULZ et al., 2016; TIESSEN; SAMPAIO; SALCEDO, 2001).

A Caatinga possui uma forte sazonalidade climática, com uma estação chuvosa e outra seca bem definidas, com um período de chuva menor que o período de seca (SAMPAIO, 1995). Os efeitos de perturbação na Caatinga podem ser mais severos por causa das possíveis interações com os fatores climáticos. A Caatinga, que já é semiárida, é um dos ecossistemas da Terra que pode sofrer com diminuição da precipitação em até 22%, juntamente com aumento de temperatura de até 6º na média, o que resulta em aumento de aridez. (MAGRIN et al., 2014). Um dos efeitos de interação entre perturbação e aridez, por exemplo, é a ruptura nos processos de ciclagem de nutrientes e balanço de elementos nos solos de regiões secas (SARDANS; PEÑUELAS; ESTIARTE, 2006; ST.CLAIR; LYNCH, 2010).

Assim como na maioria das florestas neotropicais, as formigas cortadeiras são organismos conspícuos na Caatinga, e tem aumentado suas populações em áreas sob perturbação humana (SIQUEIRA et al., 2017; TABARELLI et al., 2017) (**Figura 4**). Além disso, as colônias de formigas cortadeiras estão forrageando em taxas maiores na estação seca da Caatinga, quando há menos material vegetal fresco disponível (SIQUEIRA et al., 2018). Além disso, há regiões da Caatinga que apresentam diferentes níveis de precipitação, e áreas mais áridas podem sofrer com maior pressão de herbívoros comparado com áreas mais úmidas (ANDRADE et al., 2020). Assim, a interação entre o aumento da aridez e atividade de herbivoria pode exercer uma pressão ainda maior sobre a vegetação da floresta. Isso pode ser especialmente preocupante em um cenário de onde a aridez interaja com a perturbação antrópica, com aumento da densidade de colônias de formigas cortadeiras e aumento da herbivoria dessas colônias, bloqueando o avanço da regeneração florestal (KNOECHELMANN et al., 2020).

Assim como acontece com perturbação, a aridez também pode impactar negativamente os estoques de nutrientes na Caatinga (ALTHOFF et al., 2016). No entanto, o conhecimento sobre como os efeitos combinados de perturbação e aridez podem interferir nos efeitos das formigas cortadeiras nos solos da Caatinga também são escassos. Além disso, a maioria dos estudos que relacionam os efeitos que as formigas cortadeiras promovem nos solos em geral foram feitos com espécies do gênero Atta (Fabricius, 1805) (MOUTINHO; NEPSTAD; DAVIDSON, 2003; STERNBERG et al., 2007; SWANSON et al., 2019). Os efeitos produzidos por colônias do gênero Acromyrmex (Mayr, 1865) são menos entendidos, principalmente porque os estudos que tenta compreender esse efeito precisam acessar mais de um perfil do solo, e não apenas as lixeiras (mas veja FARJI-BRENER, Alejandro G.; TADEY, Mariana; LESCANO, 2017; FARJI-BRENER, Alejandro G.; WERENKRAUT, 2014).

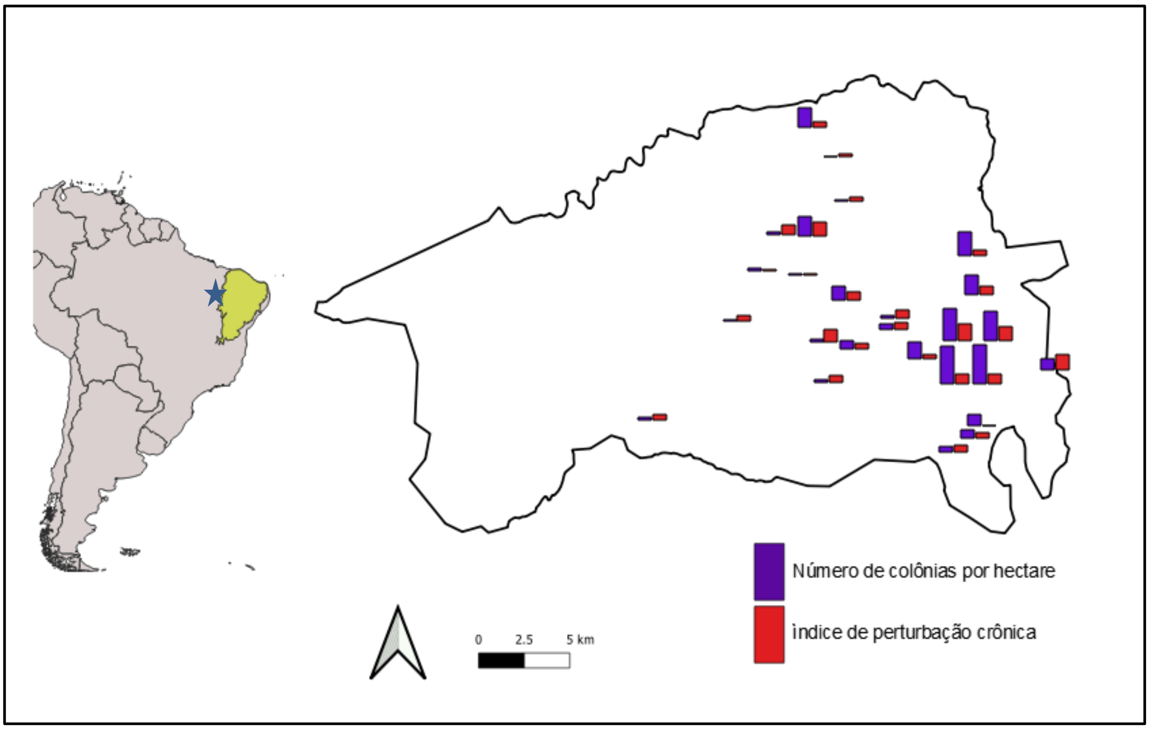

Figura 4. Densidade de colônias de formigas cortadeiras em áreas com diferentes níveis de perturbação antrópica crônica no Parque Nacional do Catimbau, no estado de Pernambuco. As espécies de formigas cortadeiras encontradas foram *Atta sexdens, Atta opaciceps*, *Acromyrmex balzani* e *Alcromrmex rugosus.* A densidade geral de colônias varia de 12,5 a 131, 2 colônias/ha, e perturbação crônica está padronizada em um índice contido em Arnan et al (2018).

que o enriquecimento do solo pelas colônias é resultado da incorporação do material forrageado nos solos dos ninhos, principalmente. Na Caatinga, a perturbação crônica é promotora de aumento das taxas de herbivoria de colônias do gênero *Atta*, assim como a estação seca também desencadeia esse efeito nessas colônias (SIQUEIRA et al., 2018). Essa atividade de forrageio das colônias produz um efeito negativo na regeneração da Caatinga porque retira grande quantidade de folhas da vegetação, deixando as áreas mais abertas (KNOECHELMANN et al., 2020). No entanto, os nutrientes contidos nessas folhas e incorporados nos solos podem afetar positivamente a regeneração florestal.

A partir de então, podemos investigar se a perturbação e aridez afetam as concentrações e os estoques de nutrientes incorporados nos solos dos ninhos. Para isso, é necessário investigar diferentes perfis do solo dos ninhos dispostos em áreas com diferentes níveis de perturbação, quantificando suas concentrações de diferentes nutrientes. A perturbação já foi reportada como responsável por redução de nutrientes nas lixeiras de colônias de formigas cortadeiras em áreas semiáridas (TADEY; FARJI-BRENER, 2007b). A terceira investigação é sobre como as plantas que crescem nos solos dos ninhos irão se desenvolver, mas levando em consideração o efeito da perturbação e da aridez nesse processo. Ou seja, o efeito imposto pela perturbação e aridez no desenvolvimento de plantas que crescem em solos dos ninhos de cortadeiras. Esse objeto de investigação é um contraponto ao efeito negativo que o forrageamento produz na regeneração florestal (veja acima) (KNOECHELMANN et al., 2020). Plantas se beneficiam das lixeiras dos ninhos mesmo em áreas perturbadas (FARJI-BRENER, Alejandro G.; GHERMANDI, 2008), mas não sabemos o real efeito da perturbação. Além disso, os efeitos de aridez nos solos são geralmente negativos (DELGADO-BAQUERIZO et al., 2013; MORENO-JIMÉNEZ et al., 2019), mas a interferência da aridez nos efeitos que as colônias aplicam nas plantas é discutida mais de forma anedótica (FARJI-BRENER, Alejandro G.; TADEY; LESCANO, 2017).

3 ARTIGO 1: INFLUENCE OF LEAF-CUTTING ANTS ON SOIL NUTRIENT STOCKS ACROSS HUMAN DISTURBANCE AND ARIDITY GRADIENTS IN CAATINGA DRY FOREST

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Influence of leaf-cutting ants on soil nutrient stocks across human disturbance and aridity gradients in Caatinga dry forest

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Abstract

The combined effects of human disturbance and climate change are the main reasons for biodiversity loss and homogenization of communities in natural ecosystems. Some organisms that profit and increase their population under these new conditions may change resources and influence the occurrence of other species. In this study we investigated the effects of human disturbance and aridity on the populations of two species of leaf-cutting ants (LCA), *Acromyrmex balzani* and *Acromyrmex rugosus*, and on their role in the soil nutrient enrichment. These organisms benefit from disturbances and constitute important agents of soil physical and chemical improvement. The study was conducted in Catimbau National Park, Pernambuco, Brazil, in plots located across gradients of disturbance and aridity. We quantified colony density and selected colonies along the gradients to investigate their biomass consumption rate. Finally, 24 nests (in 12 plots) were excavated to measure soil bulk density, fine root biomass and nutrient concentrations and stocks. Colony density was higher in more disturbed sites, while the consumption rate of the colonies was affected by season, being higher in the wet season. Bulk density and root biomass were affected by the nest and by soil depth, while nutrient concentrations and stocks showed different adverse results. Nutrient concentration and stocks were in general higher in nest soils compared to non-nest soils, but the interaction between nest and disturbance can increase the concentration and stocks of some elements. Aridity, however, seems to apply a more negative effect, being associated in most cases to the depletion of concentration and stocks of soil nutrients. Our study reconfirms that disturbance can promote the proliferation of LCA populations, and that disturbance and aridity can interact with the colonies, altering soil nutrient concentration and stocks.

Key-words – Nutrient concentrations, soil properties, seasonality dry tropical forest, ecosystem engineers, herbivory

1. Introduction

Human disturbances and climate change are primary drivers of biodiversity decline globally (PEREIRA, H. M. *et al.*, 2010; SALA *et al.*, 2000), and their effects are also a threat to soil systems and ecosystems functioning. Human disturbance constitute the main source of depletion in nutrient concentration and stocks in forest soils due to reduced litter deposition and nutrient cycling rates (CARDELÚS *et al.*, 2020; CHEN; LI, 2003; STYGER *et al.*, 2009). Similarly, nutrient content can shrinkage in soils under increasing aridity (BERDUGO *et al.*, 2020; LUO *et al.*, 2020). However, the combined effects of disturbance and climate change are more complex. Disturbance and climate can interact and modify differently the soil carbon stocks due to pH patterns in wet and dry lands (DLAMINI; CHIVENGE; CHAPLOT, 2016). These soil altered properties mediated by disturbance and climate change can suppress soil texture and fertility and turn to be the more determinant factor influencing plant growth rates (TOLEDO *et al.*, 2011).

Although being in general hostile, the new altered habitat conditions promoted by human disturbance and climate change are suitable for the proliferation of some few organisms. One of these organisms are the leaf-cutting ants (LCA, species from the genus *Atta* Fabribius, and *Acromyrmex* Mayr), ravenous herbivores of the neotropical region, being able to harvest ca. 70 to 500 kg of dry weight/colony/yr (HERZ, 2007; WIRTH *et al.*, 2003) in rain forests and ca. of 244 kg of dry weight/colony/yr (SIQUEIRA *et al.*, 2018) in dry forests. The LCA have been reported to proliferate across human-modified landscapes due to more nesting sites and relaxation of bottom-up and top-down controls found in disturbed areas (ALMEIDA; WIRTH; LEAL, I. R., 2008; FALCÃO *et al.*, 2011; FARJI-BRENER, A. G., 2001; LEAL, I. R.; WIRTH; TABARELLI, 2014; MEYER, Sebastian Tobias; LEAL, I., 2009; RAO, 2000; SIQUEIRA *et al.*, 2017; VASCONCELOS *et al.*, 2006; VIEIRA-NETO; VASCONCELOS; BRUNA, 2016). The LCA also increase their consumption and herbivory rate in disturbed sites and during drier periods (SIQUEIRA *et al.*, 2018; URBAS *et al.*, 2007), inflicting higher pressure upon vegetation and potentializing the effects of disturbance.

Due to the herbivory activity and nutrient transference from the biomass above ground to their fungus garden and refuse dumps, LCA are important agents of nutrient stocks and cycling in neotropical ecosystems (FARJI-BRENER, Alejandro G.; ILLES, 2000; MOUTINHO, P.; NEPSTAD; DAVIDSON, 2003; STERNBERG *et al.*, 2007). The soil nutrient accumulation due to nest activity can occur at depths greater than 2 m and can remain at high-levels compared to non-nest soils even when the nest is inactive (MOUTINHO, P.;

NEPSTAD; DAVIDSON, 2003). The effects of LCA on soil layers include (1) lower bulk density in nest areas compared with surrounding non-nest soils, and (2) increasing in Ca, Mg and K stocks (MOUTINHO, P.; NEPSTAD; DAVIDSON, 2003). The higher nutrient content incorporated in deeper soil layers in the nests could be an important resource for plants as they can be mineralized and absorbed by plant roots established nearby (STERNBERG *et al.*, 2007). The nutrient input on soil by LCA can be associated with the number of plant species harvested by colonies, which in turn is affected by disturbance (SCHULZ *et al.*, 2019; TADEY; FARJI-BRENER, Alejandro G., 2007). However, the extent to what disturbance influence the LCA nest effects on soil physical-chemical traits is poorly understood (SOUSA-SOUTO; SCHOEREDER; SCHAEFER, 2007; TABARELLI *et al.*, 2017; TADEY; FARJI-BRENER, 2007), and studies assessing this knowledge were mainly carried out in rain forests and savannas (FARJI-BRENER, Alejandro Gustavo; SILVA, J., 1995; SOSA; BRAZEIRO, 2010; VERCHOT; MOUTINHO, P. R.; DAVIDSON, 2003), lacking information in dry forests.

The herbivory rates of LCA can be higher during the dry season when associated to disturbance in dry forest (SIQUEIRA *et al.*, 2018), triggering an alert on the consequences of climate change on historically disturbed regions as the dry ecosystems of the neotropical region. Increasing aridity requires a special attention, because it is the main climate change effect projected for semiarid regions (MAGRIN *et al.*, 2014), and is reported to increase herbivory rates of different herbivore species in addition of LCA (ANDRADE, J.F. *et al.*, 2020). Additionally, aridity can reduce soil nutrient stocks in tropical dry forest, impacting forest resilience (ALTHOFF *et al.*, 2016). Another lack of knowledge is related to the LCA different species. Most information on herbivory rates, nutrient stocks and soil properties, come from *Atta* species, which produce bigger and more conspicuous nests (but see Sousa-Souto *et al.* 2012), thus little is known on the LCA species of the genus *Acromyrmex*. Although are not as big as *Atta* nests and have not high herbivory rates, *Acromyrmex* species can colonize areas with density of nests up to 30 nests per ha (TABARELLI *et al.*, 2017) and their effect on soils should not be negligible.

The Brazilian Caatinga is the largest and most diverse dry forest globally (SILVA, José Maria Cardoso Da; LEAL, I. R.; TABARELLI, 2017) and constitute an example of ecosystem where human disturbance and climate change are a source of forest degradation (TABARELLI; SCARANO; SILVA, J. M. C., 2017). The region has a dense human population (26 inhabitants/km², Medeiros et al., 2012), mostly composed of low-income families (AB'SABER, 2000) which are strongly dependent on natural resources for subsistence in a typical chronic anthropogenic disturbance (SINGH, 1998b). This dependency on natural resources is mainly related to the use of native vegetation for livestock grazing, firewood collection, exploitation of bark and leaves for medicinal use, hunting and slash-and-burn agriculture (ARNAN *et al.*, 2018; RIBEIRO, Elaine M.S. *et al.*, 2015; SOUZA, Danielle Gomes *et al.*, 2019). Caatinga dry forests also emerges among the top six ecosystems with the largest intrinsic vulnerability to climate variability, which is largely explained by its critical water balance (SEDDON *et al.*, 2016). The region is projected to receive about 22% less rainfall following an increase in temperature up to 6°C (i.e. increasing aridity) until the end of this century (MAGRIN *et al.*, 2014). Recent studies demonstrated that both human disturbance and climate changes promote impoverishment of taxonomic, phylogenetic and functional diversities and biotic homogenization of plant and ant communities, loss of plant-ant interactions and reduced of plant biomass and nutrient stocks (Arnan et al., 2018; Oliveira et al., 2019; Ribeiro-Neto et al., 2016; Ribeiro et al., 2015; Ribeiro et al., 2016; Ribeiro et al., 2019; Schulz et al., 2016), but their interaction are very complex (CÂMARA *et al.*, 2018; RITO *et al.*, 2017) and still poorly studied.

In this study we investigate how two LCA species, *Acromyrmex balzani* and *Acromyrmex rugosus*, respond to increasing chronic anthropogenic disturbance and aridity, and how their colonies influence soil physical properties and soil nutrient concentration and stocks in the Caatinga dry forest. As LCA of *Atta* genus benefit from human disturbance in Caatinga dry forest due to the proliferation of palatable vegetation as Euphorbiaceae species and herbs (SIQUEIRA *et al.*, 2018), we hypothesize that *Acromyrmex* species will also proliferate in disturbed and drier sites of Caatinga, harvesting more biomass and having stronger effects on soil nutrient stocks. Additionally, as LCA nests harvest plant items of a large surrounding area and implement this material in deep fungus chambers, they can constitute sink channels of soil nutrients that are leached through vertical macro pore spots (nest tunnels). Thus, we predict that in disturbed and drier sites, *Acromyrmex balzani* and *Acromyrmex rugosus* will have (1) higher colony densities, (2) higher biomass-base consumption rate per colony. We also predict that soil of the nests will show (3) higher nutrient concentration, (4) increased soil nutrient concentration and stocks with increasing depth and (5) higher fine root biomass than non-nest soils. Finally, we predict that in disturbed and drier sites, (6) the nest effects on soils will be stronger with higher.

2. Methods

2.1.Study site

This work was carried out in the Catimbau National Park (8º24'00' and 8º36'35' S; 37º0'30' and 37º1'40' W), located in the state of Pernambuco, northeastern Brazil (hereafter Catimbau). The climate is semiarid, with annual temperature averaging 23°C, and mean annual rainfall varying from 480 to 1100 mm, concentrated between March and July (Sociedade Nordestina de Ecologia 2002). Deep sandy soils are predominant in Catimbau (quartzite sands, 70% of area), but planosols and lithosols are also present (15% each one; Sociedade Nordestina de Ecologia 2002). Vegetation is dominated by low-statured trees with Fabaceae, Euphorbiaceae and Boraginaceae as the predominant plant families, while the ground layer is composed of Cactaceae, Bromeliaceae, Malvaceae, Asteraceae and Fabaceae (Rito et al. 2017a).

2.2.Study species

There are at least two *Acromyrmex* species in Catimbau: *A. balzani* (Emery, 1890) and *A. rugosus* (Smith, 1858) (Backé, 2015). These two species have different foraging habits: while *A. balzani* is a grass-cutter species, *A. rugosus* is a dicot-cutter species (P.E. Santos-Neto, personal observations, Camargo *et al.* 2006). *A. balzani* and *A. rugosus* can be very abundant in Catimbau, reaching up to 31 nests/ ha (Backé, 2015, Tabarelli *et al.* 2017).

2.3.Study design

For colony density, we selected 22 0.1-ha plots covering a wide range of chronic disturbance and mean annual rainfall levels, based on RapidEye satellite imagery and field observations. All plots were on sandy soil, had similar slope, and supported old-growth vegetation that had not experienced slash-and-burn agriculture for at least 50 years. Plots were separated by a minimum of 2 km and occurred within an area of 214.3 km^2 (RITO *et al.*, 2017). Disturbance level of each plot was first calculated as three distinct main sources of chronic anthropogenic disturbances in Catimbau, identified and quantified by (ARNAN *et al.*, 2018), which generated three indices: (1) People Pressure Index – The use of resources by people, calculated by indirect measures related to the ecological and social-ecological context, (2) Grazing Pressure Index – Disturbance type related to herbivore activity, trampling and other physical damage in the plots caused by cattle and goats, and (3) Wood Extraction Index – Disturbance type characterized by extraction of both live and dead wood for using as fuel, fence construction and handicraft production (Arnan et al. 2018). Each disturbance type was characterized by quantifying different disturbance metrics (see below) which were integrated in each index using the following formula:

$$I = \frac{\sum_{n=1}^{n} \frac{y_i - y_{\min}}{y_{max} - y_{min}}}{n} x\ 100$$

here *I* is the intensity of each disturbance type; *yi* is the observed value for a given disturbance metric in plot *i*; *ymin* is the minimum observed value for the disturbance metric across all plots; *ymax* is the maximum observed value for the disturbance metric across all plots; and n is the number of individual disturbance metrics incorporated in the index. Thus, this formula first standardizes the values of each component disturbance metric between 0 and 1, and so they are weighted equally. The overall index *I* varies from 0 (zero values for all component metrics) to 100 (maximum values of all component disturbance metrics). For full description of the method see Arnan et al. (2018).

The Exploitation of Non-Timber Forest Products Index was calculated based on four indirect measures of accessibility to our plots as surrogates of intensity of harvesting of non-timber products: proximity to the nearest house, proximity to the nearest village, proximity to the nearest road (the geographic measures) and number of people living in the houses (a socio-ecological context) with influence in the plots (Arnan et al. 2018b). Grazing pressure index was quantified in the field by measuring goat-trail length and counting goat and cattle dung; the two measures of goat activity were combined to obtain a single measure for goats by means of PCA. Both variables were highly and positively correlated with the first PCA axis which explained 88% of the variance. The grazing pressure index was then calculated as the sum of goat usage and cattle dung using the formula described above. The wood extraction index was measured by direct counting stem cuts and litter collection. The field measurements were then added using the above formula. The three indices were not correlated, which underscores that they are independent and quantify different forms of anthropogenic disturbance. The three disturbance pressure indices were then integrated into a single global multi-metric index (hereafter, disturbance index), using the same formula described above. A more detailed description of the method for calculating each index is provided in Arnan et al. (2018b).

To estimate mean annual rainfall levels of each plot we used water deficit, which represents the potential additional evaporative demand not met by available water based on energy input and precipitation (Lutz et al. 2010). Water deficit was calculated based on 30-

arc-seconds (1 km) resolution maps of long-term average annual and actual evapotranspiration (CGIAR-CSI's Global Aridity and PET Database and Global High-Resolution Soil-Water Balance database, Trabucco and Zomer 2009, 2010). These maps were generated using temperature and precipitation data from WorldClim global climate data repository (www.worldclim.org). For each plot, the difference between potential and actual evapotranspiration was calculated to obtain water deficit values (ranging from 658 to 1086 mm/yr, a variation of 40% of DH in less than 30 km of extension). All measures were performed with ArcGIS 10.1 (ESRI 2012).

2.4. Colony density

To address the colony density of the two *Acromyrmex* species, we roamed four transects of 4 x100 m (1600 m^2) in each plot, totalizing 35,200 m^2 coursed. We started at the center of the plot and going in the four opposite directions separated from each other by an angle of 90º at the starting point. For determine the transect length and direction we used the GPS tracking function (Global Positioning System, Garmin e-Trex 20), which result in an estimated resolution better than 3 m. Using the GPS device, we marked every colony encountered along the transect. When a trail was found, it was followed until the nest entrance and then we returned to the transect. The colony density was calculated by dividing the number of nests found in the total area sampled per plot (1600 m^2) by 0.16, thus, obtaining nest density per hectare.

2.5. Colony consumption rate

To quantify the effects of disturbance and precipitation on the colony consumption rates and on the soil nutrient concentration and stocks mediated by the colonies, we used a subset of eight plots, disposed of such a way that they include intermediate and the end values of the gradients. We marked 32 active colonies of *A. balzani* and 21 of *A. rugosus* disposed along these eight plots. We sampled the focal colonies from May to October. To avoid overfitting the models in the statistical analysis, we categorized the areas across the gradients into two level of each explanatory variable. Thus, we took all the areas with disturbance index varying between 0 and 20 and categorized them as "conserved areas"; areas with disturbance index higher than 30 as "disturbed areas"; areas with water deficit values varying between

600 and 800 as "rainy areas"; and areas with water deficit values higher than 800 as "arid areas".

We sampled all ants carrying plant fragments (leaves, flowers, stalks and seeds) at 5-min intervals during the peak of daily activity on the trail (URBAS *et al.*, 2007). Plant fragments were packed in plastic bags and then the fragments were placed in a stove and dried to a constant weight at approximately 70°C for dry weight determination. All ants were released back to the trail. Based on fragments weight, we calculated the biomass-based consumption rate by predicting daily totals from previously established regression equations relating 24-h counts of leaf fragments (F_{24d}) to the respective 5-min counts at the daily foraging peak (F_{5d}) during the two seasons; dry season totals: $F_{24d} = 5.7114 + (- 1.1826) x F_{5d} + 0.0717 x F_{5d}^2 + (- 0.001) x F_{5d}^3$; ($R^2 = 0.7353$, P = <0.001, N = 12); rainy season: $F_{24w} = 3.8229 (- 0.9730) x F_{5w} + 0.0890 x F_{5w}^2 +(- 0.002) x F_{5w}^3$; ($R^2 = 0.6855$, P < 0.001, N = 12) for *A. Balzani*; and dry season totals: $F_{24d} = 36.7932 + (- 7.595) x F_{5d} + 0.3926 x F_{5d}^2 + (- 0.002) x F_{5d}^3$; ($R^2 = 0.8499$, P = <0.001, N = 5); rainy season: $F_{24w} = 43.5486 (- 10.2141) x F_{5w} + 0.6575 x F_{5w}^2 +(- 0.010) x F_{5w}^3$; ($R^2 = 0.8587$, P < 0.001, N = 5) for *A. rugosus*. Then, to obtain the biomass-based consumption rates per day, we replaced the respective value of number of fragments in 5 min by the average area or biomass calculated for the whole samples.

2.6. Soil physical-chemical properties and root biomass

Soil physical and chemical properties and soil fine plant root biomass were evaluated in 12 colonies of *A. balzani* and 12 of *A. rugosus* (i.e. one colony of each specie per plot) in the same 12 plots described above. For each colony we manually excavated a shaft (1m x 2m x 2 m deep) at the center of the nest in a way that the shaft cuts the center profile until 2 m depth (nest soil). We also excavated another shaft 15 m from the edge of the nest, in a randomly selected direction (non-nest soil), which was considered as a non-nest control area. For each shaft dug at the nest center, we measured the area of intersection between the shaft wall and the subterranean chambers of the nests, and classified them as fungal garden, refuse chamber, loose soil chamber, or empty chamber. We sampled two intact soil cores to measure soil bulk density at five different depths: 0–10, 40–50, 90–100, 140–150, and 190–200 cm, in all nests and non-nests excavated sites. At each depth, the two soil core samples were taken 20 cm apart horizontally, considering the center of the shaft (oriented by the center of the nest before beginning the excavations) as a reference point. The soil core samples were taken using a PVC ring of 200 cm^3, stored in paper bags and dried at 70º C for 24 h. The soil bulk density was calculated by dividing the dry biomass of the soil amount in each core sample by its volume, adding the results and dividing by two to have an average of the soil bulk density for each depth.

We collected 1000 cm^3 of soil volume in the center of the shaft at the same depths used for the bulk density soil samples (i.e. 0–10, 40–50, 90–100, 140–150, and 190–200 cm) to quantify nutrient concentration and stocks, and the fine root biomass at each depth. For this, we used the 200 cm^3 PVC ring, colleting five times the ring quantity. The ring was cleaned using 70% alcohol and water every time we changed treatment sampling (i.e. nest to non-nest and vice-versa). First, we measured the mass and vertical distribution of fine roots (diameter <.2 mm) in all the nests and non-nests samples by separating the roots manually and using a plastic sieve (0.8 mm diameter) in each 1000 cm^3 soil sample. The sieve was also cleaned using 70% alcohol and water every time we changed samplings. The roots were then stored in paper bags, dried to constant mass at 70ºC and weighed.

After sorting out the roots, the soil samples (ca 400 g) were than dried at 70° C by minimum 24 h, identified and stored in clean plastic bags. The dry soil samples were first grind using agate crucibles and pestles in laboratory and sieved with a fine fabric mesh (ca 0.01 mm diameter). The soil powder obtained was then weighted in one gram per sample and was packed into a polyethylene pill of 20 mm internal diameter and covered with 6-µm-thick polypropylene film (Mylar®). To quantify nutrient concentration in each soil samples, we used an energy dispersive X-ray fluorescence spectrometer, model EDX 720 from Shimadzu, which consists of a rhodium tube for generating X-rays, a sealed chamber for sample analysis in a vacuum atmosphere, and a Si(Li) detector to measure the induced radiation. The element concentration outcome of the analyzer gives the concentration in ppm of several elements, of which we selected: magnesium, potassium (soil major elements), iron, manganese, zinc, and silicon (soil trace elements) to investigate their concentrations in the soil. We also quantified soil organic carbon and soil total nitrogen using an elemental analyzer EuroVector (EA3000) coupled to an isotopic ratio mass spectrometer *Denta V Advantage* (Thermo Scientific). We used the elemental analyzer configured with a CHN reactor filled with chromium oxide, reduced copper wires and silver cobalt oxide, a water adsorption trap (magnesium cobalt) and a chromatographic separation column. Then, we quantified the masses of soil organic carbon (SOC) and total nitrogen in the soil samples by adjusting an analytical curve ($R \geq 0.995$), using the sediment B2151 as reference (*Elemental microanalysis* N = 0.52%; C = 7.45%; δ 13C = -28.85 ‰; δ15N = + 4.32 ‰).

We transformed the concentration of each nutrient quantified from ppm to g/kg by simply dividing each concentration value by 1000. Additionally, we calculated soil nutrient stocks by multiplying each nutrient concentration by the soil bulk density in the different soil layers, by the soil depth in meters and by one hectare of area in m^2 (*nutrient concentration * bulk density at soil layer * soil depth * 10,000*). Then we sum up the different results of nutrient stock for each soil layer until two meters depth obtaining the nutrient stock of each element in one hectare of area of two meters depth.

2.7. Nutrient concentration in plant material harvested

We also quantified nutrient concentration in the plant material collected by the ants to feed the fungus garden. For that, a similar process conducted to the soil samples was taken for the plants. After weighted, the harvested material was dried at 50-60° C by 48 h, identified and stored in clean plastic bags. The dry samples were first grind using a planetary ball mill (PM 200, Retsche ®) for 10, 15 or 20 minutes depending on the material hardness. We obtained a plant loose powder that was weighted in one gram per sample and packed into a polyethylene pill of 20 mm internal diameter and covered with 6-µm-thick polypropylene film (Mylar®). We put the pills in an energy-dispersive X-ray fluorescence spectrometer, model EDX 720 from Shimadzu, which consists of a rhodium tube for generating X-rays, a sealed chamber for sample analysis in a vacuum atmosphere, and a Si(Li) detector to measure the induced radiation. We selected magnesium, phosphorus, potassium, iron, manganese, and calcium as nutrients to investigate their concentrations in the plant material. We also quantified total carbon and nitrogen in the plant material with an elemental analyzer EuroVector (EA3000) with the same configuration as made for soil samples (see above).

2.7. Statistical analysis

To test the hypothesis that increasing chronic disturbance and aridity lead to an increasing in *A. rugosus* and *A. balzani* colony densities, we used Generalized Linear Models (GLM) with disturbance index and water deficit as independent continuous variables and colony density in each plot as response variable. For that, we used a backward-selection procedure to choose the best-fit models according to the lowest Akaike information criterion with a correction for small sample sizes (AICc). For each ant species, we built a starting model that included disturbance index and water deficit as main independent variables and the interactions between them (disturbance index x water deficit). We applied a standardization in the starting model to have comparable estimates and confidence intervals for the independent variables using the function *standardize* in the package *arm* (Gelman & Su, 2020). The best-fit models were those selected based on AICc weights, with AICc delta (AICc difference) of <2 (Burnham & Anderson, 2002). We then applied model averaging to make inferences about how chronic disturbance and aridity influenced response variables. First, for each explanatory variable, we averaged coefficients and 95% confidence intervals across the best-fit models. Second, we considered explanatory variables as having a significant effect on response variables when the 95% confidence intervals did not include 0 (GRUEBER *et al.*, 2011). We also calculated the relative variable importance (RVI) for the explanatory variables that fit in a best-fit model. Model selection and averaging were carried out using MuMin package (Bartón, 2018) in the programming environment *R* (R CORE TEAM, 2019).

To test the influence of disturbance and aridity on the consumption rate based on biomass harvested by the ants we used Generalized Linear Mixed Models (GLMM) with disturbance and aridity levels as explanatory fixed factors, plot as random factor, and biomass of the harvested material per day as response variable. These models were fitted using *Lme4* and *lmerTest* packages in R (BATES *et al.*, 2015; KUZNETSOVA; BROCKHOFF; CHRISTENSEN, R, H, 2017). We also applied model selection and calculated relative importance of those variables that fit in a best fit model. Model selection and averaging were carried out using MuMin package (Bartón, 2018) in the programming environment *R* (R CORE TEAM, 2019).

For soil properties (soil bulk density, soil nutrient concentration and stocks, and soil fine root biomass) we first searched for the effects of depth (10, 50, 100, 150 and 200 cm) and treatment (nest and non-nest) on the response variables. For this, we applied Generalized Linear Mixed Models (GLMM) using treatment and depth as fixed factors, and plot as random factor for each ant species separately. We tested the main effects of each explanatory variable and the interactions between them on the soil physical and chemical properties and soil fine root biomass. Thus, we investigated the effects of disturbance and aridity on the soil bulk density, soil nutrient concentrations, soil carbon and nitrogen ratios (hereafter C/N ratio), soil nutrient stocks and soil fine root biomass. For this, we also applied Generalized Linear Mixed Models (GLMM) using disturbance index and water deficit, and their interactions with treatment as the fixed factors, as well as plot as the random factor. Finally, based on the plant material collected for the consumption rate, we also investigated the amount of the nutrient stocks carried into the nest across the areas, using GLMM with disturbance and water deficit as fixed factors and ant species as random factor, as well as each nutrient stock (C, N, Mg, K, P, Mn, Fe and Ca) as response variables. All these analyses were carried out with the packages *Lme4* and *lmerTest* in R (BATES *et al.*, 2015; KUZNETSOVA; BROCKHOFF; CHRISTENSEN, R, H, 2017).

3. Results

The colony density varied from 0 to 43.7 nests/ha for *A. balzani* (17 ± 20.1, mean ± SD) and from 0 to 81 nests/ha for *A. rugosus* (20.7 ± 23.7). According to the model selection, the colony density of *A. balzani* retained only disturbance index as explanatory variable in a best fit model (Table 1, Figure 1A). Disturbance index and water deficit were selected in the best fit models for colony density of *A. rugosus*, however, for water deficit the confidence interval included zero (Table 1, Figure 1B).

Biomass harvested daily by *A. balzani* colonies varied from 0 to 4.81 g of dry weight per day (1.22 ±1.16 g of dry weight per day) (Table 1), and from 5.6 to 74 g (22.5 ±16.04 g of dry weight per day) in colonies of *A. rugosus*, (Table 1). Water deficit and Disturbance index together with water deficit were selected in the best fit models for biomass loaded daily by *A. balxani* and *A. rugosus* colonies, respectively, however, their confidence intervals included zero, and thus we considered that there was no considerable effect of the explanatory variables on the biomass loaded by the colonies (Table 1).

Soil bulk density increased with depth in nest and non-nest soils of the two ant species (Table S1, Figure 2). Nest and non-nest soils of *A. rugosus* showed significantly different values of soils bulk density, while nest and non-nest soils of *A. balzani* did not show significant effect in the soil bulk density (Table S1, Figure 2a, b). The fine root biomass by soil volume was affected by treatment, being two times higher in nest soils (ranging from 0 to 1.1 mg/cm^3, 0.2 ± 0.22 mg/cm^3) than in non-nest soils (ranging from 0.003 to 0.69 mg/cm^3, 0.106 ± 0.108 mg/cm^3) for *A. balzani* colonies. There was also an effect of the interaction between treatment and depth for *A. balzani* colonies (Table S1, Figure 2c). For *A. rugosus* the fine root biomass by soil volume was affected by treatment, being almost three times higher in nest soils (ranging from 0.002 to 3.73 mg/cm^3, 0.38 ± 0.56 mg/cm^3) compared to non-nest soils (ranging from 0.01 to 0.9 mg/cm^3 0.16 ± 0.18 mg/cm^3), suffering an effect of the interaction between treatment and depth (Table S1, Figure 2d). The analysis on the nutrient in the plant material harvested by the colonies of *A. rugosus* showed that that calcium stock caried into the nest was significantly higher in disturbed than in preserved sites (Table S2). The remained elements were not associated with disturbance index or water deficit neither.

The analysis of the influence of depth and treatment showed that these two factors were determinant in the soil nutrient concentration of three major elements: nitrogen, magnesium, and phosphorus. In *A. balzani* soils, the total phosphorus concentration decreased with depth in nest but increased in non-nest soils, while the total magnesium concentration increased with depth without effect of the nest (Table S3, Figure 3 a, b). None of the trace elements and the C/N ratio varied significantly among treatment and depths (Table S3). In *A. rugosus* soils, the concentration of nitrogen was higher in the nest compared to non-nest soils, and showed an effect of the interaction between treatment and depth, being higher in nest than non-nest soils near the soil surface but inverting this pattern at 2 meters deep (Table S4, Figure 3c). The concentration of magnesium also showed higher values in the nest compared to non-nest soils of *A. rugosus*, in this case, without effect of depth (Table S4, Figure 3d). For the trace elements, iron concentration increased with depth, without effect of the nests, while silicon concentration was lower in nest compared to non-nest soils, without effect of depth (Table S4, Figure S13 a, b).

The analysis on the influence of disturbance and aridity on the soil bulk density and fine root biomass in nest and non-nest soils of *A. balzani* showed that disturbance and aridity did not affect soil bulk density and soil fine root biomass (Table S5), while the found before result of the effect of treatment, with lower bulk density and higher fine root biomass in nest than in non-nest soils, was repeated (Table S5). Similarly, the influence of disturbance and aridity on the soil bulk density and fine root biomass in nest and non-nest soils of *A. rugosus* showed that disturbance and aridity did not affect soil bulk density and soil fine root biomass (Table S6), while again, the result of lower bulk density and higher fine root biomass in nest than in non-nest soils, was repeated (Table S6).

The analysis on the influence of disturbance and aridity on the soil nutrient concentration in nest and non-nest soils of *A. balzani* showed that SOC concentration was positively associated with nest soils, negatively associated with increasing disturbance, and was associated with the interaction between disturbance index and water deficit, higher at high water deficit and moderate disturbance index (Table S7; Figure 4a). The total soil potassium concentration was negatively associated with increasing disturbance, positively associated with increasing water deficit, and associated with the interaction between disturbance and water deficit, being higher at sites with high water deficit and decreasing as disturbance index increases (Table S7, Figure 4b). The soil total iron concentration was negatively associated with water deficit, and also associated with the interaction between disturbance and water deficit, increasing with increasing disturbance but decreased water deficit (Table S8, Figure 4c).

The total soil phosphorus concentration was negatively associated with water deficit (Table S7, Figure 4d). The concentration of zinc was first associated with the interaction between disturbance and treatment, decreasing rapidly in non-nest soils as disturbance increases but being uniform in nest soils (Table S8, Figure S14a); and second, associated with the interaction between water deficit and treatment, decreasing in nest and increasing in non-nest soils as water deficit increases (Table S8, Figure S14b). The total silicon concentration in the soil was positively associated with water deficit (Table S8, Figure S14c). The concentration of manganese was in general higher in non-nest soils but increased in nest soils as disturbance and water deficit increase (Table S8, Figure S14d). The other elements were not significantly associated with none of the predictor variables.

For *A. rugosus* the concentration of total soil nitrogen and iron were negatively associated with water deficit, without effect of treatment (Table S9, Figure 4e,f). On the other hand, total silicon concentration was positively associated with water deficit and was higher in non-nest soils than in nest soils (Table S10, Figure 4g). Total iron concentration was positively associated with disturbance index (Table S10, Figure 4h). The total magnesium concentration was consistently affected by the nests only, being higher in non-nest than in nest soils (Table S9, Figure 3d). The other elements were not significantly associated with none of the predictors.

Regarding the effects of disturbance and aridity on the soil nutrient stocks in nest and non-nest soils of *A. balzani*, stock of SOC was higher in nest soils than in non-nest soils and was negatively associated with disturbance (Table S11, Figure 5a). The stock of nitrogen was associated with the interaction between treatment and disturbance, and with the interaction between treatment, disturbance and water deficit, increasing the stock values of nest soils as disturbance index and water deficit increase (Table S11, Figure 5b). Total soil potassium stocks were associated with the interaction between disturbance index and water deficit, decreasing with disturbance and increasing with water deficit (Table S11, Figure 5c). Total phosphorus stocks were associated with the interaction between disturbance index and water deficit, increasing as disturbance index increases and water deficit decreases (Table S11, Figure 5d). Total manganese stocks in the soil increased with disturbance and water deficit, being higher in nest than non-nest soils at high disturbance and water deficit levels (Table S12, Figure S15a). Total zinc stocks tended to increase at high water deficit but low disturbance levels, being higher in nest than non-nest soils at low water deficit (Table S12, Figure S15b). Total iron stocks increased at high disturbance and low water deficit (Table S12, Figure S15c). The other elements were not significantly associated with none of the predictors.

The effects of disturbance and water deficit on the soil element stocks in nest and non-nest soils of *A. rugosus* showed that SOC stock was higher in nest than non-nest soils (Table S13). The stocks of SOC were also associated with the interaction between treatment and disturbance, decreasing rapidly with increasing disturbance in nest soils, but not in non-nest soils (Table S13, Figure 6a); it was also associated with the interaction between treatment, disturbance index and water deficit, decreasing as disturbance index and water deficit increase, with a more pronounced decrease in nest soils compared to non-nest soils (Table S13, Figure 6a). The stock of nitrogen was negatively associated with water deficit, without association with treatment (Table S13, Figure 6b). Magnesium stock was associated with treatment, being higher in non-nest than in nest soils; it was also associated with the interaction between treatment and disturbance, increasing more rapidly in nest than non-nest soils at higher disturbance index levels, and with the interaction between treatment and water deficit, increasing more rapidly at higher levels of water deficit (Table S13, Figure 6c). The stock of total potassium was positively associated with water deficit and higher in nest than non-nest soils (Table S13, Figure 8d). The total manganese stocks were higher in non-nest soils and were associated with the interaction between disturbance index and water deficit, increasing as both disturbance and water deficit increased (Table S14, Figure S15d). Total iron stocks were associated with treatment, being also higher in non-nest soils, and associated with the interaction between disturbance water deficit, increasing at high disturbance and low water deficit levels (Table S14, Figure S15e). The stocks of the remaining elements were not associated with treatment, disturbance index or water deficit (Tables S13 and S12).

4. Discussion

Our results show that *Acromyrmex* species benefit from human disturbance and their nests alter soil physical and chemical properties, increasing nutrient concentration and enhancing plant root biomass across different depths in the soil. More precisely, the nest soils present lower soil bulk density, higher plant root biomass, higher nitrogen concentration and lower magnesium concentration than non-nest soils. However, these effects are dependent on the ant species, being more pronounced in *A. rugosus* than in *A. balzani*. The effects of chronic disturbance, aridity, and their interaction with the alteration on soil properties promoted by colonies may be complex, but, in general, as several soil nutrients remain higher in nest than non-nest soils at moderate to high disturbance levels, disturbance can potentialize the nest effects, widening the difference in the nutrient content in these two soil spots. All these findings show that LCA nests may increment the soil nutrient stocks, mainly SOC, total nitrogen and total potassium, having their effects on soil potentialized by human activities. At the same time, the separate effect of increasing aridity is consistently deleterious to soil nutrient concentration and stocks.

Our findings on colony density of the two *Acromyrmex* species studied reinforce that chronic disturbance have a propulsive effect in the populations of LCA, as a wide range of studies have reported for acute disturbance and with *Atta* species (SIQUEIRA *et al.*, 2017; VASCONCELOS *et al.*, 2006; VIEIRA-NETO; VASCONCELOS; BRUNA, 2016; WIRTH *et al.*, 2007). Surprisingly though, disturbance and aridity do not seem to alter the biomass loaded by each colony, despite disturbance related to landscape alterations being a factor that enhance colony activity and biomass harvested (BUSTAMANTE; AMARILLO-SUÁREZ; WIRTH, 2020; SIQUEIRA *et al.*, 2018). Soil physical alterations promoted by LCA, as the reduced soil bulk density found in our study, are well known (MOUTINHO, P.; NEPSTAD; DAVIDSON, 2003). The knowledge about LCA nests promoting soil nutrient enrichment, as increased SOC and N concentrations around the nests, are also well described (FARJI-BRENER, Alejandro G.; WERENKRAUT, 2014; FARJI-BRENER, Alejandro Gustavo; SILVA, J., 1995; HUDSON *et al.*, 2009; MOUTINHO, P.; NEPSTAD; DAVIDSON, 2003; WIRTH *et al.*, 2003), although they can also cause depletion of C and N in the topsoil of nest mounds when colony refuse dumps are internal (MEYER, Sebastian T. *et al.*, 2013). However, the depletion of magnesium concentration found in nest soils of *A. rugosus* diverges from results of studies with *Atta* spp. nests (FARJI-BRENER, Alejandro Gustavo; SILVA, J., 1995; MOUTINHO, P.; NEPSTAD; DAVIDSON, 2003).

Few studies, however, have assessed how the increment in the soil nutrient concentration and stocks promoted by LCA is influenced by human disturbance and aridity. One of these studies found that human disturbance can reduce the nutrient content of LCA nests by reducing available food-plants richness (FARJI-BRENER, Alejandro G.; TADEY; LESCANO, 2017). These findings partially agree with our results, since chronic disturbance is associated with reduced SOC and potassium in *A. balzani* and *A. rugosus* nests and non-nest soils, although moderate disturbance may increase nutrient content in nest soil. When considering the effects of disturbance and aridity separated from the nest effects, our results agree with the idea of disturbance and aridity, isolated and combined, generate mostly negative effects to soil systems (GAITÁN *et al.*, 2018). When nest soils interact with disturbance and aridity, the outcomes seem to go in the direction of disturbance and aridity potentialize the effects of nests, as seem for ecosystem engineers (DECKER; ELDRIDGE; GIBB, 2019; FARJI-BRENER, Alejandro G.; TADEY; LESCANO, 2017; LEAL, I. R.; WIRTH; TABARELLI, 2014).

Although chronic disturbance does not consist of clear-cut of vegetation, their effects seem to be strong enough to trigger an increase in LCA populations. This can be explained by (1) reduced plant stem abundance with chronic disturbance and increasing in intensity of chronic disturbance in small remnants of Caatinga vegetation (Antongiovanni et al., 2020; Ribeiro et al., 2015); together with (2) the proliferation of more palatable plants in disturbed areas, as Euphorbiaceae and Fabaceae species (RIBEIRO, Elaine M.S. *et al.*, 2015; RITO; TABARELLI; LEAL, I. R., 2017), which are largely used by LCA. However, our results indicate that the conditions imposed by disturbance and aridity do not alter the amount of plant material harvested per colonies of both species. The colonies can reach an equilibrium in their harvesting rates across areas with different disturbance levels due the reduced palatable plant in more conserved areas and competition for food in more disturbed areas. The soil enrichment mediated by LCA is mainly driven by the accumulation of organic matter through deposition of refuse dumps (FARJI-BRENER, Alejandro G.; GHERMANDI, 2008; FARJI-BRENER, Alejandro Gustavo; SILVA, J., 1995; HUDSON *et al.*, 2009; STERNBERG *et al.*, 2007). This explains higher SOC concentration and stocks in nests of *A. balzani*, since their colonies always show a single external refuse dump close to the nest. For *A. rugosus*, however, refuse dumps are more often found internally, deep in the soil and irregularly aligned to the main nest shaft (VERZA *et al.*, 2020).

Lower bulk density, and higher soil carbon and nitrogen concentrations explain the proliferation of fine roots in the nest soil of both LCA species. However, when comparing the two species, fine root biomass observed in *A rugosus* nest soils had around twice biomass than in *A. balzani*, which can be a result of internal refuse dump and higher nitrogen concentration found only in *A. rugosus* nest soils. We need to consider that the accumulation of organic matter in ant nest is a result of plant material collected from colony foraging areas, which are much larger than nests (URBAS *et al.*, 2007). LCA nests concentrates in a small soil volume the nutrient stocks imported from a larger area, and although the nest does not constitute a sink of nutrient (only Mg increased concentration with depth), plants growing nearby the nests need to compete for a relatively small nutrient-rich soil spot. The nest-concentrated nutrient content is probably used by the enhanced fine root web before it reaches 1.5-m depth in the nest soils, process that may include water, as evidenced in *Atta* nests (MOUTINHO, P.; NEPSTAD; DAVIDSON, 2003). This explains the lower P and N concentrations in nest soils deeper than 1.5-m compared to non-nest soils.

We expected that disturbance and aridity potentialize the effect of LCA nests by the proliferation of less defended and more palatable plants (FARJI-BRENER, Alejandro G.; TADEY, 2009; FARJI-BRENER, Alejandro G.; TADEY; LESCANO, 2017; RIBEIRO-NETO *et al.*, 2016; RIBEIRO, Elaine M.S. *et al.*, 2016; RITO; TABARELLI; LEAL, I. R., 2017). However, we did not find differences in the biomass harvested per colony nor in the concentration and stocks of all elements but calcio examined in plant material collected by colonies across the disturbance and aridity gradients. This indicate that nest effects are a result of accumulation of plant material collected along the colony life, which increase organic matter content, increasing root absorption and decreasing mineralization and retention of nutrients in nest soils, especially in less disturbed and arid areas. Additionally, the nutrient content in non-nest soils from disturbed areas tend to suffer depletion compared to non-nest soils from more conserved areas, as the cases of SOC and N stocks in non-nest soils, as chronic disturbance deteriorates SOC (SCHULZ *et al.*, 2016).

Alterations in soils attributes along disturbance and aridity gradients are also dependent on the LCA species. While for *A. balzani* nests disturbance and aridity amplify the difference between nest and non-nest soils by increasing concentration and stocks of SOC and total manganese and zinc (i.e. potentialize a positive nest effect), in *A. rugosus* nests there is the opposite effect. The nests and non-nest soils of *A. rugosus* are affected by disturbance via reduced total iron and SOC (i.e. potentialize a negative nest effect), while aridity effects are more isolated from the nest and disturbance effects and are consistently negative, reducing total SOC, total iron and total nitrogen stocks The strongest effect on magnesium stock seems to be a result of increased fine root biomass in nest soil layers (ESPELETA; CLARK, 2007) together with reduced soil bulk density (MULLINS; EDWARDS, 1987). For potassium in *A.*

rugosus nest, aridity did not enlarge the nest effect but lead nest and non-nest to a potassium stock convergence, narrowing the difference between them. Rock-derived nutrients, as potassium, are leached at higher rate with increased precipitation (AUSTIN; VITOUSEK, 1998), and thus turn to be less abundant. However, nests soils of *A. rugosus* still keep potassium at higher stocks.

In synthesis, *Acromyrmex* LCA profits from human disturbance and their effects on soil nutrients are largely positive and can be determinant to plant nutrient uptake, confirming our first hypothesis. The biomass harvested per colony does not change in function of disturbance and aridity, nor the nests are sink of nutrients, contrary to what we expected for our second hypothesis. Nest effects are primarily positive for soil nutrient enrichment, corroborating our third hypothesis, but without being a nutrient sink, contrary to what we expected for the fourth hypothesis. Human disturbance and aridity influence nest effects in a complex way and need to be examined carefully. In general, disturbance and aridity can amplify positive nest effects, increasing concentration and stocks of certain elements in nest soils probably due to higher productivity and lower mineralization of nutrients related to accumulation of organic matter from refuse dumps. All these findings indicate that the greater effect of nests in disturbed and arid sites can benefit a selected group of plant species, increasing their fitness and populations. On the other hand, the isolated effects of aridity can cause convergence of nest and non-nest soils regarding some nutrient content, nullifying the nest effect, whereas promote proliferation of less defended and more palatable plant-food for LCA (DIRZO, R.; BOEGE, 2008), negative effect. Our finding added more information on the role of LCA in dry forest, however, we stress that further studies is needed in order to evaluate the plant nutrient uptake in these areas and the direction of the nest effects to plants.

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88881.030482/2013-01) and Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE processes APQ-0738-2.05/12).

Table 1. Results of the best-fit models (ΔAICc < 2) examining the influences of chronic anthropogenic disturbance and water deficit on the colony density and biomass harvesting of plant material per day of two LCA species, *A. balzani* and *A. rugosus*, in Catimbau National Park, Pernambuco, Brazil. For each variable retained in the best-fit model, we have indicated the averaged coefficient (β), the unconditional standard error (SE), the 95% confidence intervals (95% CI) and the relative variable importance (RVI). Significant variables (according to 95% confidence intervals) are in bold. We excluded from the table variables that did not appear in the subset of best-fit models.

			95% CI			
Colony density	**β**	**SE**	**lower**	**upper**	***p*-value**	**RVI**
A. balzani						
Disturbance index	0.592	0.265	0.044	1.140	**0.034**	0.76
A. rugosus						
Disturbance index	19.874	8.438	1.423	38.323	**0.034**	0.79
Water Deficit	-13.670	8.070	-31.43	4.091	0.131	0.32
Biomass loaded daily	**β**	**SE**	**lower**	**upper**	***p*-value**	**RVI**
A. balzani						
Water Deficit	-0.418	0.287	-0.997	0.237	0.154	0.44
A. rugosus						
Disturbance index	-6.740	5.252	-17.3	3.83	0.208	0.26
Water Deficit	8.906	4.768	-0.74	18.5	0.070	0.68

Legend of figures

Figure 1. Colony densities of two species of LCA: (A) *A. balzani* and (B) *A. rugosus* across an anthropogenic chronic disturbance gradient in Catimbau National Park, Pernambuco, Brazil.

Figure 2. Distribution of soil bulk density (A and B) and plant fine root biomass per soil volume ($g.kg^{-1}$) (C and D) at different depths of nest and non-nest soils of *Acromyrmex balzani* and *Acromyrmex rugosus* colonies, in Catimbau National Park, Pernambuco, Brazil.

Figure 3. Effects of depth in the concentration of (A) phosphorus and (B) magnesium in nest and non-nest soils of *Acromyrmex balzani* colonies. Effects of treatment and depth, as well as the interaction between these two factors on the concentration of (C) nitrogen in the soils of *Acromyrmex rugosus* colonies. Main effects of treatment and depth on the concentration of (D) magnesium in soil and non-nest soils of *Acromyrmex rugosus* colonies, in Catimbau National Park, Pernambuco, Brazil.

Figure 4. Effects of disturbance index, water deficit, and their interactions in the concentration of different elements in nest and non-nest soils (treatment) of *Acromyrmex balzani* colonies: (a) effects of treatment, disturbance and the interaction between disturbance and water deficit on the carbon concentration; (b) effects of disturbance, water deficit and the interaction between these two factors on the concentration of potassium in the soil; (c) effects of water deficit and the interaction with disturbance on the concentration of iron in the soil; (d) negative effect of water deficit on the phosphorus concentration in the soil. Effects of water disturbance and water deficit in the concentration of different elements in nest and non-nest soils (treatment) of *Acromyrmex rugosus* colonies: negative effect of water deficit on the (e) nitrogen and (f) iron concentrations in the soil; (g) positive effect of water deficit and non-nest soils on the silicon concentration in the soils; (h) positive effect of disturbance on the iron concentration in the soil. The samples were taken in 12 plots across a chronic disturbance and aridity gradients in Catimbau National Park, Pernambuco, Brazil.

Figure 5. Effects of disturbance index, water deficit and their interactions on the stocks of different elements in nest and non-nest soils of *Acromyrmex balzani* colonies: (a) separate

effects of treatment and disturbance index on the SOC stocks; (b) effect of the interaction between treatment, disturbance index and water deficit on the total nitrogen stocks in the soils; (c) effect of the interactions between disturbance index and water deficit on the potassium stocks in the soil; (d) effect of the interaction between disturbance index and water deficit on the total phosphorus stock in the soils. The soil samples were taken in 12 plots across a chronic disturbance and aridity gradients in Catimbau National Park, Pernambuco, Brazil.

Figure 6. Effects of disturbance index, water deficit, and their interactions in the stock of different elements in nest and non-nest soils (treatment) of *Acromyrmex rugosus* colonies: (a) effect of the interaction between treatment, disturbance and water deficit on the stock of SOC; (b) negative effect of water deficit on the total nitrogen stocks in the soil; (c) separate effect of treatment, and the effect of the interaction between disturbance index and water deficit on the total magnesium stocks in the soil: (d) positive effect of nest and water deficit on the total potassium stocks in the soil. The samples were taken in 12 plots across a chronic disturbance and aridity gradients in Catimbau National Park, Pernambuco, Brazil.

Figure 1.

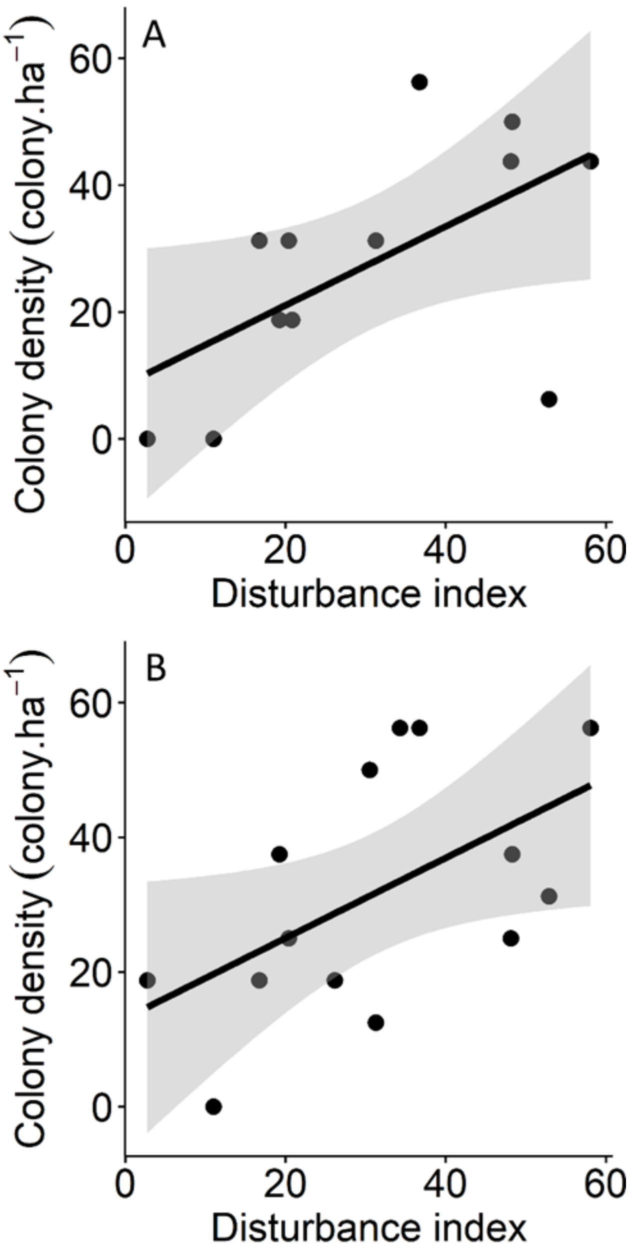
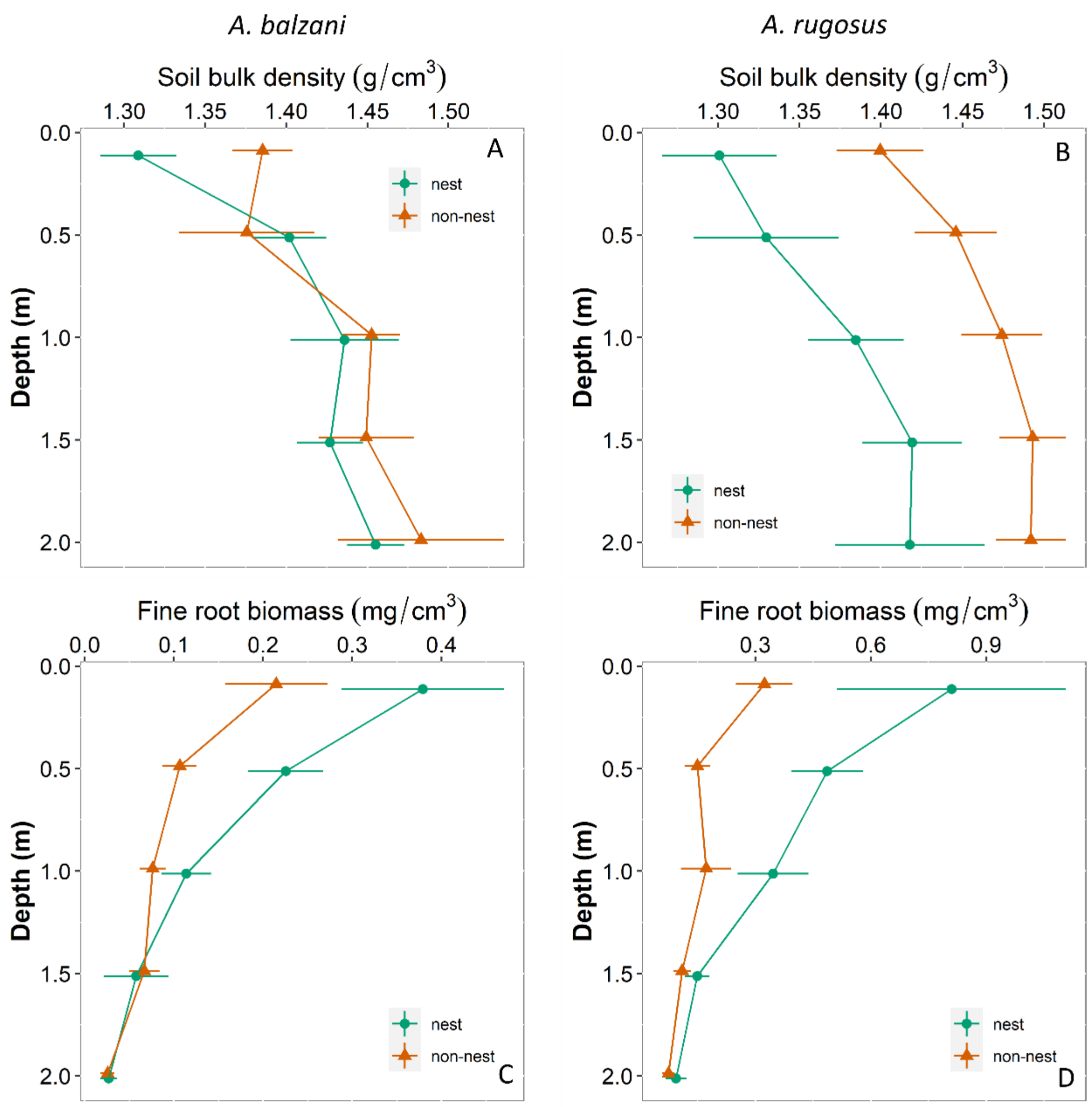

A
Colony density (colony.ha^{-1})
60
40
20
0
0
20
40
60
Disturbance index
B
Colony density (colony.ha^{-1})
60
40
20
0
0
20
40
60
Disturbance index

Figure 2.



A. balzani
A. rugosus
Soil bulk density (g/cm3)
1.30
1.35
1.40
1.45
1.50
Depth (m)
0.0
0.5
1.0
1.5
2.0
A
B
nest
non-nest
Fine root biomass (mg/cm3)
0.0
0.1
0.2
0.3
0.4
0.3
0.6
0.9
C
D

Figure 3

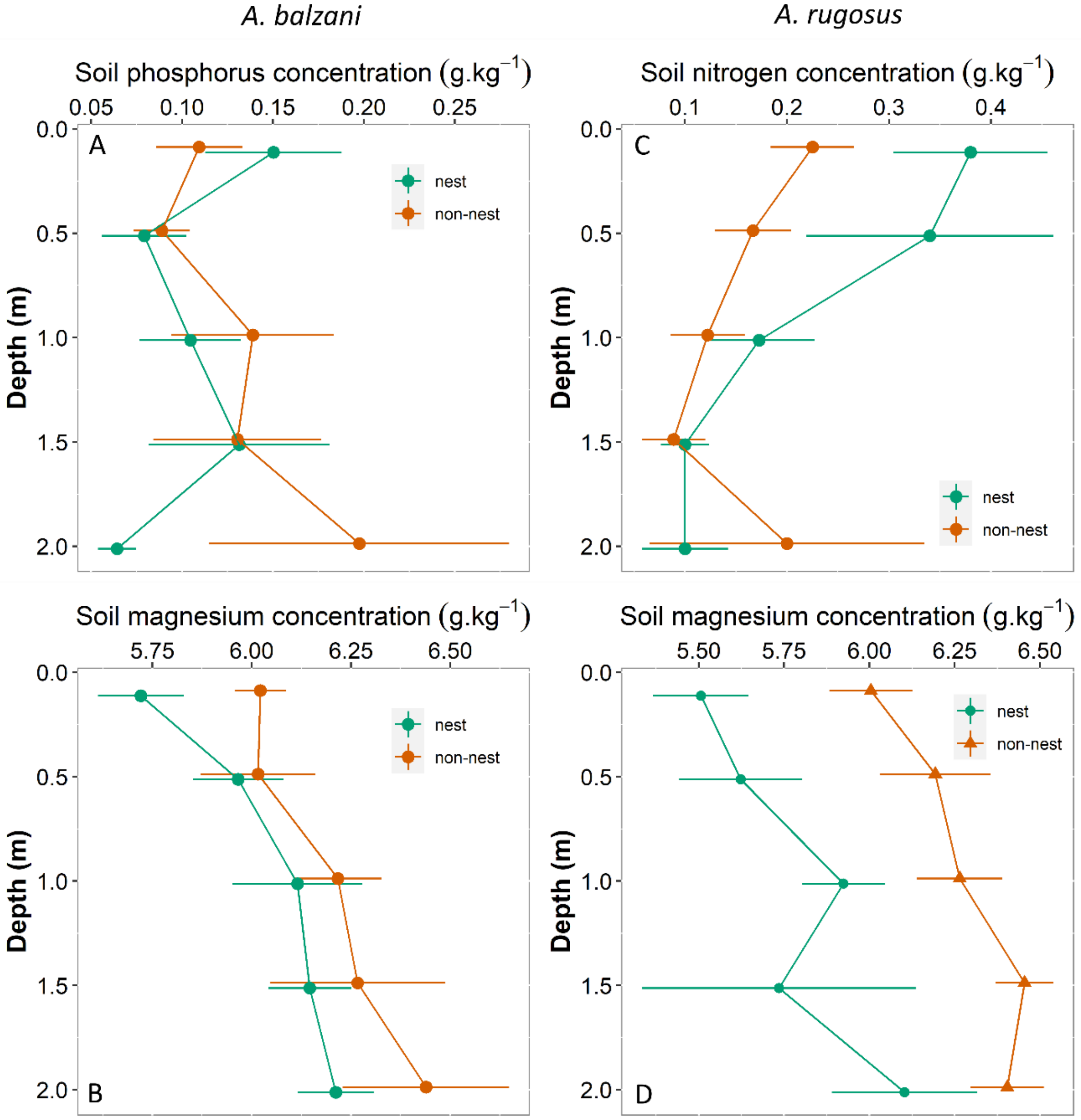

A. balzani
A. rugosus
Soil phosphorus concentration (g.kg−1)
0.05
0.10
0.15
0.20
0.25
Depth (m)
0.0
0.5
1.0
1.5
2.0
A
nest
non-nest
Soil nitrogen concentration (g.kg−1)
0.1
0.2
0.3
0.4
C
Soil magnesium concentration (g.kg−1)
5.75
6.00
6.25
6.50
B
5.50
D

Figure 4.

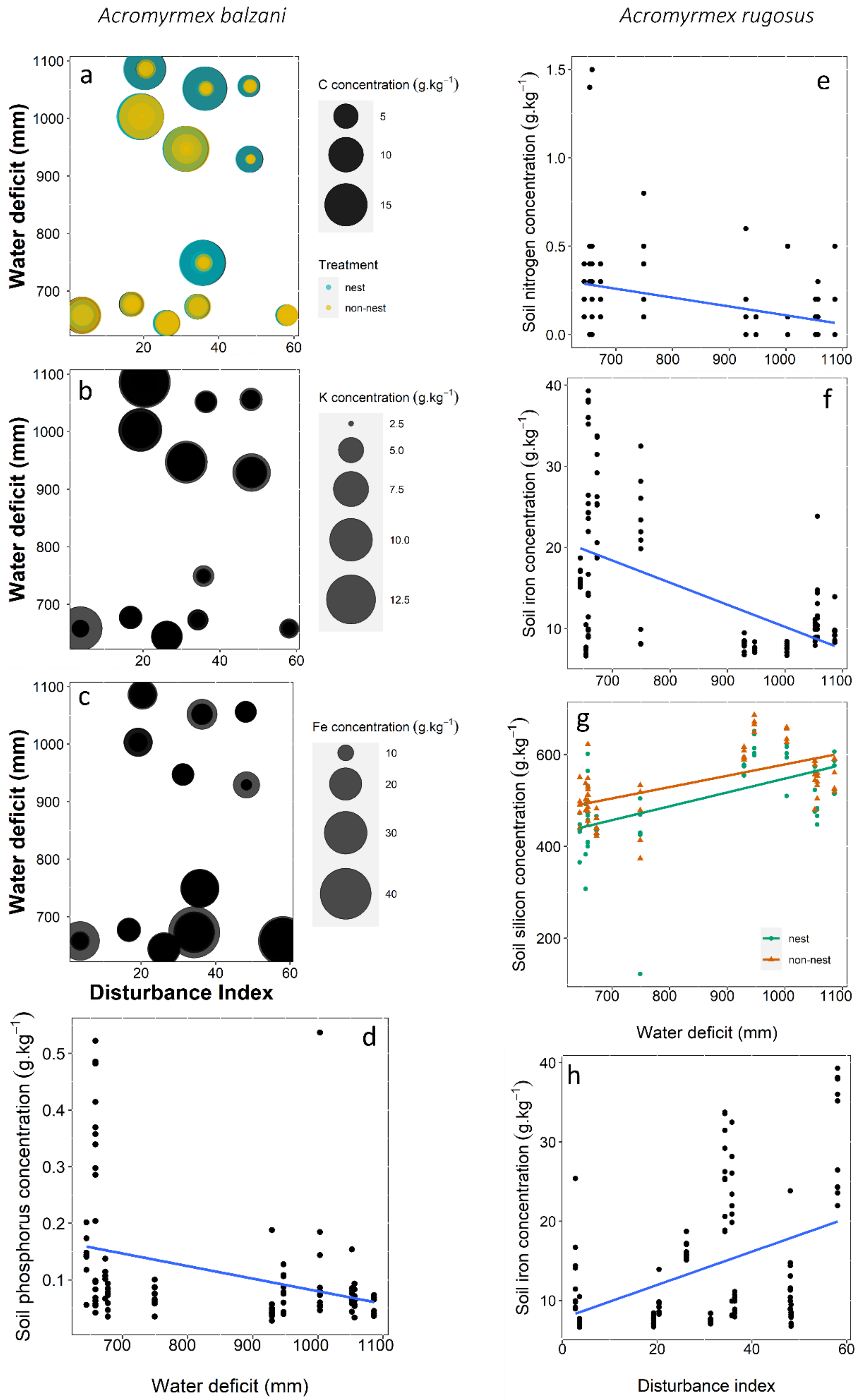

Acromyrmex balzani
Acromyrmex rugosus
a
b
c
d
e
f
g
h
Water deficit (mm)
Disturbance Index
C concentration (g.kg−1)
5
10
15
Treatment
nest
non-nest
K concentration (g.kg−1)
2.5
5.0
7.5
10.0
12.5
Fe concentration (g.kg−1)
10
20
30
40
Soil phosphorus concentration (g.kg−1)
Soil nitrogen concentration (g.kg−1)
Soil iron concentration (g.kg−1)
Soil silicon concentration (g.kg−1)
Disturbance index

Figure 5

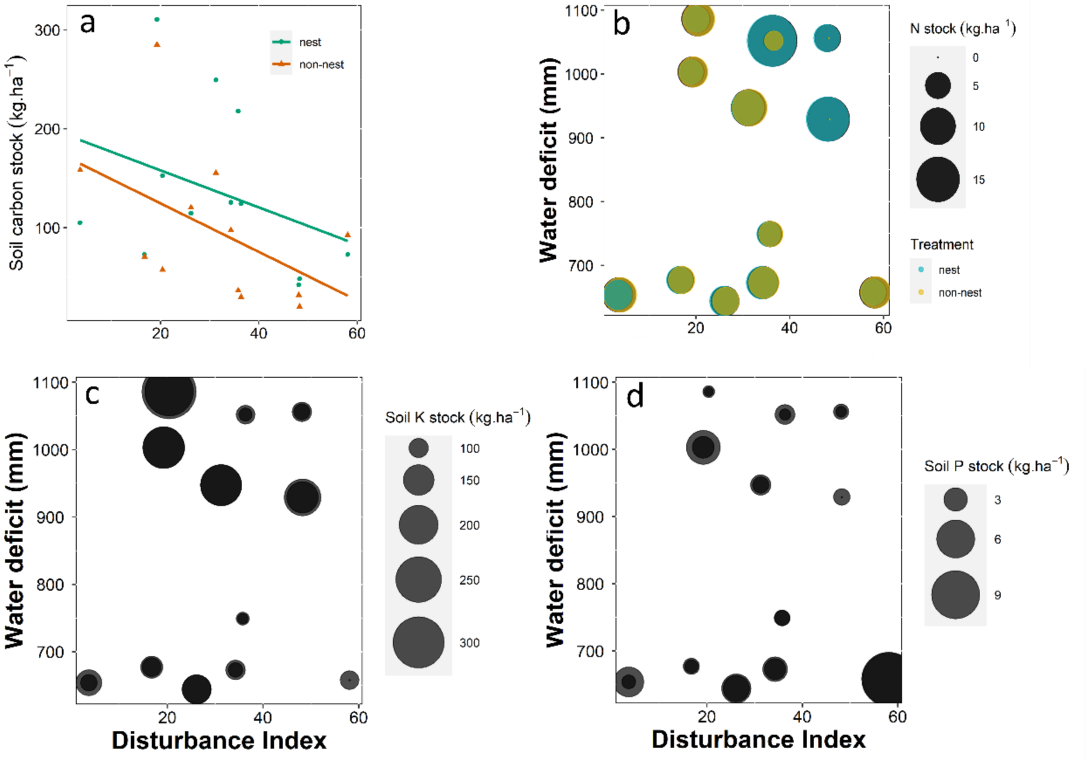
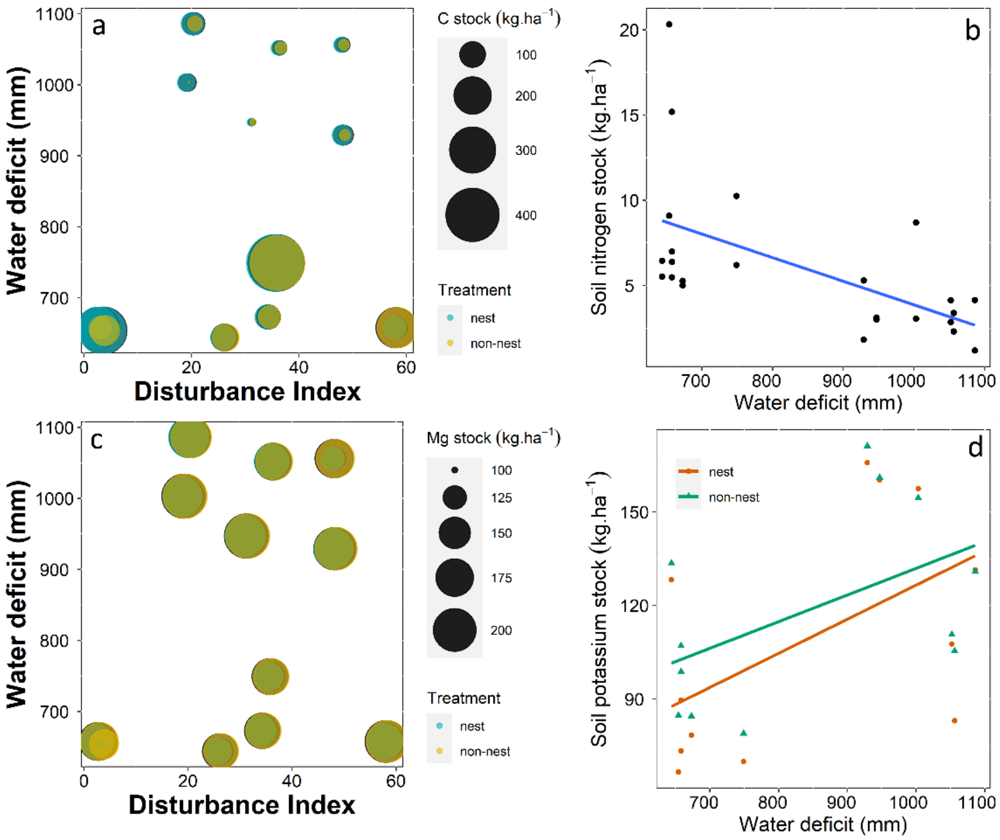

a
Soil carbon stock (kg.ha⁻¹)
nest
non-nest
b
Water deficit (mm)
N stock (kg.ha⁻¹)
Treatment
nest
non-nest
c
Water deficit (mm)
Disturbance Index
Soil K stock (kg.ha⁻¹)
d
Water deficit (mm)
Disturbance Index
Soil P stock (kg.ha⁻¹)

Figure 6



a
b
c
d
Water deficit (mm)
Disturbance Index
C stock (kg.ha⁻¹)
100
200
300
400
Treatment
nest
non-nest
Soil nitrogen stock (kg.ha⁻¹)
Mg stock (kg.ha⁻¹)
100
125
150
175
200
Soil potassium stock (kg.ha⁻¹)

Supplementary Information

Influence of leaf-cutting ants on soil nutrient stocks across human disturbance and aridity gradients in Caatinga dry forest

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Table S1. Effects of treatment (nest and non-nest soils) and depth, as well as the interactions between them on the soil bulk density and plant fine root biomass in the soils of nests of the leaf-cutting ant *A. balzani*, in the Catimbau National Park, Pernambuco, Brazil.

	Acromyrmex balzani						*Acromyrmex rugosus*					
	Bulk density			**Fine root**			**Bulk density**			**Fine root**		
Predictors	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*
(Intercept)	1.37	1.32 – 1.42	**<0.001**	0.19	0.11 – 0.26	**<0.001**	1.42	1.36 – 1.48	**<0.001**	0.26	0.05 – 0.47	**0.016**
Treatment	-0.03	-0.09 – 0.02	0.229	0.17	0.08 – 0.26	**<0.001**	-0.11	-0.15 – -0.07	**<0.001**	0.47	0.28 – 0.66	**<0.001**
Depth	0.06	0.02 – 0.09	**0.001**	-0.09	-0.15 – -0.03	**0.002**	0.04	0.01 – 0.06	**0.004**	-0.06	-0.18 – 0.05	0.259
Treatment: Depth	0.01	-0.04 – 0.06	0.606	-0.11	-0.19 – -0.02	**0.011**	0.02	-0.02 – 0.06	0.290	-0.26	-0.41 – -0.10	**0.002**

Table S2. Effects of disturbance and water deficit on the stocks of eight elements (C, N, Mg, K, P, Mn, Fe and Ca) in the plant material collected by the workers of *A. balzani* and *A. rugosus* colonies, in the Catimbau National Park, Pernambuco, Brazil.

	C Stock			N Stock			Mg Stock			K stock		
Predictors	β	CI	p	β	CI	p	β	CI	p	β	CI	p
(Intercept)	8.52	-2.34 – 19.39	0.124	0.35	-0.26 – 0.96	0.26	0.05	-0.05 – 0.15	0.305	0.27	-0.08 – 0.63	0.129
Disturbance	-0.01	-0.09 – 0.07	0.748	0	-0.00 – 0.01	0.822	0	-0.00 – 0.00	0.422	0	-0.00 – 0.00	0.622
Water deficit	0	-0.01 – 0.00	0.343	0	-0.00 – 0.00	0.568	0	-0.00 – 0.00	0.547	0	-0.00 – 0.00	0.382
	P Stock			Mn Stock			Fe Stock			Ca stock		
Predictors	β	CI	p	β	CI	p	β	CI	p	β	CI	p
(Intercept)	0.03	-0.01 – 0.07	0.206	0	-0.01 – 0.01	0.893	0	-0.01 – 0.01	0.483	-0.03	-0.48 – 0.42	0.895
Disturbance	0	-0.00 – 0.00	0.925	0	-0.00 – 0.00	0.949	0	-0.00 – 0.00	0.265	0	0.00 – 0.01	**0.04**
Water deficit	0	-0.00 – 0.00	0.459	0	-0.00 – 0.00	0.443	0	-0.00 – 0.00	0.676	0	-0.00 – 0.00	0.692

Table S3. Effects of treatment (nest and non-nest soils) and depth, as well as the interactions between them on the concentration of five major elements (C, N, Mg, K and P), four trace elements and the carbon/nitrogen ratio (Mn, Fe, Zn, Si and C/N) in the soils of nests of the leaf-cutting ant *A. balzani*, in the Catimbau National Park, Pernambuco, Brazil.

Major elements		**C**			**N**			**Mg**			**K**			**P**	
Predictors	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*
(Intercept)	3.23	0.54 – 5.91	**0.018**	0.31	0.21 – 0.42	**<0.001**	5.94	5.71 – 6.16	**<0.001**	4.79	3.20 – 6.38	**<0.001**	0.09	0.03 – 0.15	**0.006**
Treatment	3.05	-0.21 – 6.32	0.067	0.03	-0.11 – 0.18	0.677	-0.16	-0.41 – 0.09	0.211	0.25	-0.69 – 1.20	0.598	0.03	-0.03 – 0.08	0.301
Depth	0.33	-1.93 – 2.60	0.772	-0.06	-0.16 – 0.04	0.205	0.25	0.09 – 0.40	**0.003**	0.45	-0.15 – 1.06	0.142	0.04	0.00 – 0.07	**0.037**
Treatment:Depth	-1.62	-4.67 – 1.42	0.296	-0.04	-0.18 – 0.09	0.530	0.02	-0.21 – 0.24	0.884	-0.38	-1.22 – 0.46	0.375	-0.05	-0.09 – 0.00	0.062

Trace elements		**Mn**			**Fe**			**Zn**			**Si**			**C/N**	
Predictors	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*
(Intercept)	0.16	0.14 – 0.18	<0.001	14.03	9.67 – 18.38	<0.001	11.38	9.30 – 13.46	<0.001	510.20	476.05 – 544.34	<0.001	18.85	-14.41 – 52.12	0.267
Treatment	0.01	-0.02 – 0.04	0.586	0.85	-2.05 – 3.74	0.566	1.70	-0.88 – 4.29	0.197	-20.95	-55.04 – 13.15	0.229	16.78	-23.93 – 57.50	0.419
Depth	-0.02	-0.03 – 0.00	0.058	1.04	-0.82 – 2.89	0.274	0.03	-1.61 – 1.67	0.972	13.47	-8.32 – 35.26	0.226	7.06	-21.09 – 35.20	0.623
Treatment:Depth	-0.01	-0.03 – 0.02	0.601	-0.23	-2.82 – 2.36	0.861	-0.37	-2.68 – 1.94	0.755	14.45	-16.04 – 44.93	0.353	-0.72	-38.27 – 36.84	0.970

Table S4. Effects of treatment (nest and non-nest soils) and depth, as well as the interactions between them on the concentration of five major elements (C, N, Mg, K and P), four trace elements and the carbon/nitrogen ratio (Mn, Fe, Zn, Si and C/N) in the soil of nests of the leaf-cutting ant *A. rugosus*,, in the Catimbau National Park, Pernambuco, Brazil.

Major elements	**C**			**N**			**Mg**			**K**			**P**		
Predictors	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*
(Intercept)	3.20	-0.23 – 6.64	0.067	0.18	0.06 – 0.30	**0.003**	6.05	5.74 – 6.37	**<0.001**	4.02	3.40 – 4.65	**<0.001**	0.09	0.06 – 0.11	**<0.001**
Treatment	2.28	-1.51 – 6.07	0.239	0.22	0.08 – 0.36	**0.002**	-0.54	-0.86 – -0.22	**0.001**	-0.08	-0.42 – 0.25	0.632	-0.00	-0.02 – 0.02	0.967
Depth	0.25	-1.99 – 2.49	0.824	-0.01	-0.09 – 0.07	0.802	0.18	-0.01 – 0.37	0.059	0.04	-0.16 – 0.24	0.679	0.00	-0.01 – 0.01	0.770
Treatment:Depth	-1.25	-4.40 – 1.90	0.438	-0.15	-0.27 – -0.04	**0.008**	0.05	-0.22 – 0.32	0.702	0.02	-0.26 – 0.30	0.899	0.01	-0.00 – 0.03	0.161

Trace elements	**Mn**			**Fe**			**Zn**			**Si**			**C_N**		
Predictors	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*
(Intercept)	0.14	0.13 – 0.16	**<0.001**	11.96	7.28 – 16.65	**<0.001**	10.33	8.78 – 11.88	**<0.001**	547.28	500.61 – 593.96	**<0.001**	22.56	3.41 – 41.70	**0.021**
Treatment	-0.01	-0.03 – 0.01	0.509	0.17	-2.42 – 2.75	0.900	0.79	-1.10 – 2.68	0.411	-39.01	-70.83 – -7.19	**0.016**	-15.59	-39.36 – 8.17	0.198
Depth	-0.00	-0.01 – 0.01	0.516	2.68	1.15 – 4.21	**0.001**	0.83	-0.28 – 1.95	0.142	-8.19	-27.01 – 10.63	0.394	-1.81	-17.29 – 13.68	0.819
Treatment:Depth	-0.00	-0.02 – 0.01	0.930	-1.12	-3.27 – 1.03	0.306	-0.52	-2.09 – 1.05	0.514	-0.12	-26.58 – 26.33	0.993	21.89	0.00 – 43.78	0.050

Table S5. Effects of treatment (nest and non-nest soils), disturbance index and water deficit, as well as the interactions between them on the soil bulk density and fine root biomass in soils of nests of the leaf-cutting ant *A. balzani*, in the Catimbau National Park, Pernambuco, Brazil.

	Bulk density			**Fine root biomass**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	1.412	1.38 – 1.44	**<0.001**	0.15	0.11 – 0.20	**<0.001**
Treatment	0.023	-0.00 – 0.05	0.076	-0.10	-0.17 – -0.03	**0.003**
Disturbance	-0.00	-0.07 – 0.07	0.929	-0.03	-0.14 – 0.07	0.563
Water deficit	0.00	-0.06 – 0.07	0.941	-0.03	-0.12 – 0.06	0.543
Treatment * Disturbance	0.06	-0.00 – 0.11	0.065	0.11	-0.03 – 0.26	0.132
Treatment * Water deficit	-0.03	-0.08 – 0.02	0.238	0.02	-0.11 – 0.15	0.757
Disturbance * Water deficit	-0.10	-0.23 – 0.04	0.155	0.01	-0.19 – 0.21	0.918
Treatment * Disturbance * Water deficit	-0.06	-0.18 – 0.05	0.264	0.13	-0.16 – 0.41	0.384

Table S6. Effects of treatment (nest and non-nest soils), disturbance index and water deficit, as well as the interactions between them on the soil bulk density and fine root biomass in soils of nests of the leaf-cutting ant *A. rugosus*, in the Catimbau National Park, Pernambuco, Brazil.

	Bulk density			**Fine root biomass**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	1.42	1.37 – 1.46	**<0.001**	0.28	0.09 – 0.47	**0.003**
Treatment	0.09	0.06 – 0.11	**<0.001**	-0.21	-0.34 – -0.09	**0.001**
Disturbance	-0.01	-0.11 – 0.09	0.866	-0.17	-0.60 – 0.26	0.435
Water deficit	0.08	-0.01 – 0.17	0.094	-0.09	-0.47 – 0.29	0.643
Treatment * Disturbance	0.05	-0.01 – 0.11	0.088	0.21	-0.07 – 0.48	0.140
Treatment * Water deficit	-0.02	-0.07 – 0.03	0.458	0.10	-0.14 – 0.35	0.413
Disturbance * Water deficit	-0.20	-0.39 – 0.00	0.065	0.34	-0.48 – 1.16	0.416
Treatment * Disturbance * Water deficit	0.13	0.00 – 0.24	0.066	-0.08	-0.61 – 0.45	0.760

Table S7. Effects of treatment (nest and non-nest soils), disturbance index and water deficit, as well as the interactions between them (treatment * disturbance index; treatment * water deficit) on the concentration of five major elements (C, N, Mg, K and P) in the soils of nests of the leaf-cutting ant *A. balzani*, in the Catimbau National Park, Pernambuco, Brazil.

		C			**N**			**Mg**			**K**			**P**	
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	4.41	3.27 – 5.54	**<0.001**	0.25	0.20 – 0.29	**<0.001**	6.09	5.95 – 6.23	**<0.001**	5.21	4.29 – 6.12	**<0.001**	0.12	0.08 – 0.15	**<0.001**
Treatment	-1.76	-3.29 – -0.23	**0.024**	-0.02	-0.10 – 0.06	0.650	-0.603	-1.97 – 0.77	0.388	0.17	-0.29 – 0.62	0.475	0.01	-0.01 – 0.04	0.397
Disturbance	-3.57	-6.11 – -1.03	**0.006**	0.01	-0.11 – 0.13	0.881	-0.10	-0.41 – 0.20	0.512	-2.82	-4.85 – -0.80	**0.006**	0.05	-0.03 – 0.13	0.276
Water deficit	0.76	-1.51 – 3.04	0.510	0.02	-0.07 – 0.11	0.678	0.17	-0.11 – 0.44	0.231	2.67	0.87 – 4.46	**0.004**	-0.09	-0.14 – -0.02	**0.046**
Treatment * Disturbance	0.21	-3.19 – 3.61	0.902	-0.21	-0.43 – 0.01	0.056	0.25	-0.03 – 0.53	0.083	-0.93	-1.95 – 0.09	0.073	-0.06	-0.11 – 0.01	0.087
Treatment * Water deficit	-1.93	-5.00 – 1.13	0.217	-0.12	-0.29 – 0.04	0.153	0.02	-0.23 – 0.27	0.879	-0.05	-0.97 – 0.86	0.911	0.00	-0.05 – 0.05	0.906
Disturbance * Water deficit	-5.46	-10.32 – -0.60	**0.028**	0.07	-0.16 – 0.29	0.553	-0.48	-1.07 – 0.11	0.108	-4.43	-8.28 – -0.58	**0.024**	-0.17	-0.331– -0.04	0.06
Treatment * Disturbance * Water deficit	3.22	-3.29 – 9.73	0.332	-0.39	-0.80 – 0.02	0.064	-0.18	-0.71 – 0.36	0.523	-0.86	-2.81 – 1.10	0.392	-0.01	-0.12 – 0.10	0.89

Table S8. Effects of treatment (nest and non-nest soils), disturbance index and water deficit, as well as the interactions between them (treatment * disturbance index; treatment * water deficit) on the concentration of four trace elements and the ration of carbon and nitrogen (Mn, Fe, Zn, Si and C/N) in the soils of nests of the leaf-cutting ant *A. balzani*, in the Catimbau National Park, Pernambuco, Brazil.

	Mn			**Fe**			**Zn**			**Si**			**C/N**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	0.14	0.13 – 0.15	**<0.001**	15.38	13.38 – 17.38	**<0.001**	12.32	11.27 – 13.38	**<0.001**	517.96	497.54 – 538.37	**<0.001**	31.39	17.22 – 45.57	**<0.001**
Treatment	0.00	-0.01 – 0.01	0.742	-0.38	-1.69 – 0.94	0.574	-0.96	-2.20 – 0.28	0.130	8.39	-7.17 – 23.96	0.290	-17.27	-40.38 – 5.85	0.143
Disturbance	0.01	-0.02 – 0.03	0.598	3.32	-1.13 – 7.76	0.144	-2.01	-4.36 – 0.34	0.093	-8.12	-53.50 – 37.27	0.726	-32.53	-68.97 – 3.91	0.080
Water deficit	0.02	-0.00 – 0.04	0.061	-7.69	-11.63 – -3.74	**<0.001**	0.87	-1.23 – 2.97	0.415	42.72	2.36 – 83.07	**0.038**	16.62	-11.8 – 45.08	0.252
Treatment * Disturbance	-0.02	-0.04 – -0.00	**0.034**	0.69	-2.23 – 3.60	0.643	-3.86	-6.62 – -1.11	**0.006**	32.53	-2.04 – 67.10	0.065	-2.07	-46.71 – 42.5	0.928
Treatment * Water deficit	-0.01	-0.03 – 0.01	0.507	-0.17	-2.80 – 2.46	0.898	3.49	1.00 – 5.98	**0.006**	-26.41	-57.61 – 4.78	0.097	-16.27	-49.34 – 16	0.335
Disturbance * Water deficit	-0.02	-0.06 – 0.02	0.412	-12.46	-20.93 – -3.99	**0.004**	-4.13	-8.62 – 0.37	0.072	81.15	-5.42 – 167.72	0.066	-58.85	-126.85 – 9.1	0.090
Treatment * Disturbance * Water deficit	-0.08	-0.12 – -0.04	**<0.001**	-3.56	-9.17 – 2.06	0.214	-1.38	-6.69 – 3.93	0.611	6.35	-60.25 – 72.96	0.852	11.96	-71.10 – 95.0	0.778

Table S9. Effects of treatment (nest and non-nest soils), disturbance index and water deficit, as well as the interactions between them (treatment * disturbance index; treatment * water deficit) on the concentration of five major elements (C, N, Mg, K and P) in the soils of nests of the leaf-cutting ant *A. rugosus*, in the Catimbau National Park, Pernambuco, Brazil.

	C			N			Mg			K			P		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	3.94	1.50 – 6.38	**0.002**	0.19	0.13 – 0.26	**<0.001**	6.04	5.83 – 6.25	**<0.001**	4.06	3.44 – 4.67	**<0.001**	0.09	0.07 – 0.12	**<0.001**
Treatment	-0.81	-2.61 – 0.98	0.374	-0.06	-0.14 – 0.02	0.166	0.46	0.28 – 0.64	**<0.001**	0.05	-0.11 – 0.22	0.524	-0.01	-0.02 – 0.00	0.084
Disturbance	-0.17	-5.69 – 5.34	0.951	-0.00	-0.16 – 0.16	0.998	-0.14	-0.61 – 0.33	0.553	-0.34	-1.73 – 1.05	0.633	0.03	-0.02 – 0.09	0.192
Water deficit	-4.32	-9.21 – 0.58	0.084	-0.18	-0.32 – -0.04	**0.010**	0.39	-0.02 – 0.80	0.065	0.97	-0.26 – 2.21	0.122	-0.03	-0.07 – 0.02	0.232
Treatment * Disturbance	3.11	-0.96 – 7.18	0.135	0.14	-0.06 – 0.34	0.166	0.23	-0.18 – 0.63	0.272	0.22	-0.16 – 0.60	0.251	-0.02	-0.05 – 0.01	0.108
Treatment * Water deficit	-0.54	-4.15 – 3.06	0.769	-0.09	-0.26 – 0.07	0.280	-0.16	-0.51 – 0.20	0.394	-0.26	-0.60 – 0.07	0.125	-0.01	-0.04 – 0.01	0.284
Disturbance * Water deficit	-0.54	-11.09 – 10.01	0.920	-0.05	-0.35 – 0.26	0.760	-0.87	-1.76 – 0.02	0.055	-0.31	-2.97 – 2.35	0.819	0.05	-0.05 – 0.15	0.321
Treatment * Disturbance * Water deficit	-5.74	-13.53 – 2.05	0.149	-0.13	-0.50 – 0.24	0.488	0.57	-0.21 – 1.34	0.151	0.24	-0.49 – 0.97	0.522	-0.05	-0.11 – 0.00	0.065

Table S10. Effects of treatment (nest and non-nest soils), disturbance index and water deficit, as well as the interactions between them (treatment * disturbance index; treatment * water deficit) on the concentration of four trace elements and the ration of carbon and nitrogen (Mn, Fe, Zn, Si and C/N) in the soils of nests of the leaf-cutting ant *A. rugosus*, in the Catimbau National Park, Pernambuco, Brazil.

		Mn			**Fe**			**Zn**			**Si**			**C/N**	
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	0.14	0.13 – 0.15	**<0.001**	14.65	12.29 – 17.01	**<0.001**	11.39	10.47 – 12.31	**<0.001**	521.48	487.75 – 555.21	**<0.001**	21.75	6.35 – 37.16	**0.006**
Treatment	0.01	-0.00 – 0.01	0.101	0.93	-0.55 – 2.41	0.217	-0.24	-1.24 – 0.76	0.638	38.36	22.88 – 53.83	**<0.001**	-4.02	-22.42 – 14.37	0.668
Disturbance	0.00	-0.01 – 0.02	0.684	6.17	0.84 – 11.51	**0.023**	-2.08	-4.16 – 0.00	0.051	-38.58	-114.80 – 37.65	0.321	1.09	-33.49 – 35.66	0.951
Water deficit	0.00	-0.02 – 0.02	0.965	-10.99	-15.73 – -6.25	**<0.001**	0.15	-1.70 – 2.00	0.874	104.55	36.86 – 172.23	**0.002**	-9.36	-39.95 – 21.24	0.549
Treatment * Disturbance	0.01	-0.00 – 0.03	0.155	1.87	-1.48 – 5.23	0.274	-0.37	-2.63 – 1.89	0.746	4.83	-30.26 – 39.91	0.787	1.56	-39.33 – 42.45	0.940
Treatment * Water deficit	-0.01	-0.02 – 0.01	0.291	0.20	-2.76 – 3.17	0.894	-0.44	-2.44 – 1.56	0.668	-19.01	-50.06 – 12.04	0.230	3.27	-32.94 – 39.49	0.859
Disturbance * Water deficit	-0.04	-0.08 – 0.0	0.051	-10.14	-20.35 – 0.07	0.052	-2.56	-6.54 – 1.43	0.208	-18.01	-163.78 – 127.77	0.809	-5.94	-71.48 – 59.60	0.859
Treatment * Disturbance * Water deficit	0.01	-0.02 – 0.05	0.390	0.52	-5.89 – 6.94	0.873	-0.81	-5.13 – 3.51	0.714	19.94	-47.17 – 87.05	0.560	3.49	-72.67 – 79.64	0.929

Table S11. Effects of treatment (nest and non-nest soils), disturbance index and water deficit, as well as the interactions between them (treatment * disturbance index; treatment * water deficit) on the stocks of five major elements (C, N, Mg, K and P) in the soils of nests of the leaf-cutting ant *A. balzani*, in the Catimbau National Park, Pernambuco, Brazil.

	C stock			**N stock**			**Mg stock**			**K stock**			**P stock**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	121.34	85.88 – 156.80	**<0.001**	6.68	5.18 – 8.18	**<0.001**	170.96	163.44 – 178.48	**<0.001**	146.70	119.81 – 173.60	**<0.001**	3.31	2.27 – 4.35	**<0.001**
Treatment	-42.29	-80.16 – -4.41	**0.029**	-1.68	-4.67 – 1.32	0.273	5.61	-5.66 – 16.89	0.329	7.70	-5.71 – 21.10	0.260	0.46	-0.09 – 1.02	0.101
Disturbance	-97.55	-175.86 – -19.23	**0.015**	-0.75	-4.06 – 2.57	0.659	-4.01	-20.62 – 12.59	0.636	-81.54	-140.94 – -22.14	**0.007**	1.33	-0.96 – 3.62	0.256
Water deficit	19.95	-52.48 – 92.38	0.589	0.60	-2.46 – 3.66	0.700	6.77	-8.59 – 22.12	0.388	81.34	26.41 – 136.27	**0.004**	-2.51	-4.63 – -0.39	**0.020**
Treatment * Disturbance	2.62	-81.03 – 86.27	0.951	-7.84	-14.46 – -1.22	**0.020**	16.79	-8.11 – 41.69	0.186	-23.21	-52.81 – 6.39	0.124	-1.14	-2.37 – 0.08	0.067
Treatment * Water deficit	-49.51	-126.88 – 27.85	0.210	-4.77	-10.90 – 1.35	0.127	-5.76	-28.79 – 17.26	0.624	-0.01	-27.39 – 27.37	0.999	-0.27	-1.40 – 0.86	0.640
Disturbance * Water deficit	-150.67	-304.70 – 3.35	0.055	-0.65	-7.16 – 5.86	0.845	-24.71	-57.37 – 7.95	0.138	-137.24	-254.06 – -20.42	**0.021**	-5.19	-9.70 – -0.68	**0.024**
Treatment * Disturbance) * Water deficit	67.19	-97.33 – 231.71	0.423	-14.64	-27.66 – -1.62	**0.028**	-8.61	-57.58 – 40.36	0.730	-33.16	-91.38 – 25.07	0.264	-0.72	-3.13 – 1.69	0.557

Table S12. Effects of treatment (nest and non-nest soils), disturbance index and water deficit, as well as the interactions between them (treatment * disturbance index; treatment * water deficit) on the stocks of four trace elements (Mn, Fe Zn and Si) in the soils of nests of the leaf-cutting ant *A. balzani*, in the Catimbau National Park, Pernambuco, Brazil.

	Mn stock			**Fe stock**			**Zn stock**			**Si stock**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	4.01	3.82 – 4.20	**<0.001**	433.68	377.01 – 490.35	**<0.001**	344.50	313.24 – 375.77	**<0.001**	14530.00	13680.67 – 15379.32	**<0.001**
Treatment	0.11	-0.22 – 0.45	0.514	-1.34	-53.10 – 50.42	0.960	-21.40	-50.31 – 7.51	0.147	337.67	-635.61 – 1310.94	0.497
Disturbance	0.18	-0.24 – 0.60	0.400	89.76	-35.41 – 214.93	0.160	-67.27	-136.32 – 1.78	0.056	-305.00	-2180.85 – 1570.84	0.750
Water deficit	0.49	0.10 – 0.88	**0.014**	-224.04	-339.80 – -108.28	**<0.001**	35.13	-28.73 – 98.99	0.281	1420.08	-314.81 – 3154.97	0.109
Treatment * Disturbance	-0.46	-1.20 – 0.29	0.229	50.94	-63.38 – 165.26	0.382	-86.69	-150.54 – -22.83	**0.008**	1742.05	-407.56 – 3891.65	0.112
Treatment * Water deficit	-0.28	-0.96 – 0.41	0.431	-23.27	-129.00 – 82.46	0.666	86.39	27.33 – 145.44	**0.004**	-1313.61	-3301.70 – 674.47	0.195
Disturbance * Water deficit	-0.66	-1.49 – 0.18	0.122	-386.14	-632.31 – -139.97	**0.002**	-149.71	-285.51 – -13.91	**0.031**	1428.65	-2260.69 – 5117.99	0.448
Treatment * Disturbance * Water deficit	-2.33	-3.79 – -0.87	**0.002**	-148.35	-373.19 – 76.49	0.196	-41.63	-167.22 – 83.95	0.516	98.45	-4129.32 – 4326.23	0.964

Table S13. Effects of treatment (nest and non-nest soils), disturbance index and water deficit, as well as the interactions between them (treatment * disturbance index; treatment * water deficit) on the stocks of five major elements (C, N, Mg, K, and P) in the soils of nests of the leaf-cutting ant *A. rugosus*, in the Catimbau National Park, Pernambuco, Brazil.

	C stock			**N stock**			**Mg stock**			**K stock**			**P stock**		
Predictors	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*
(Intercept)	116.40	48.97 – 183.83	**0.001**	6.12	4.33 – 7.90	**<0.001**	171.23	160.36 – 182.09	**<0.001**	115.10	95.52 – 134.68	**<0.001**	2.62	2.00 – 3.24	**<0.001**
Treatment	-21.73	-40.62 – -2.84	**0.024**	-1.57	-3.87 – 0.73	0.180	23.16	15.11 – 31.22	**<0.001**	8.26	3.61 – 12.90	**<0.001**	-0.16	-0.50 – 0.18	0.355
Disturbance	-9.77	-166.05 – 146.5	0.902	-1.02	-5.16 – 3.12	0.630	-4.42	-29.61 – 20.77	0.731	-10.64	-56.02 – 34.74	0.646	1.02	-0.41 – 2.45	0.162
Water deficit	-128.3	-265.47 – 8.72	0.066	-4.85	-8.48 – -1.22	**0.009**	22.22	0.13 – 44.32	**0.049**	36.10	-3.71 – 75.91	0.076	-0.80	-2.05 – 0.46	0.213
Treatment * Disturbance	99.46	55.68 – 143.24	**<0.001**	4.43	-0.89 – 9.75	0.103	11.68	-6.99 – 30.35	0.220	9.83	-0.94 – 20.60	0.074	-0.46	-1.25 – 0.32	0.250
Treatment * Water deficit	-15.17	-53.58 – 23.23	0.439	-2.47	-7.14 – 2.20	0.300	-7.97	-24.35 – 8.41	0.340	-9.79	-19.24 – -0.34	**0.042**	-0.23	-0.92 – 0.46	0.515
Disturbance * Water deficit	-15.20	-325.53 – 295.12	0.924	-1.30	-9.52 – 6.92	0.756	-50.91	-100.93 – -0.90	**0.046**	-24.79	-114.90 – 65.32	0.590	0.88	-1.96 – 3.72	0.545
Treatment * Disturbance) * Water deficit	-170.55	-257.48 – -83.62	**<0.001**	-4.78	-15.34 – 5.79	0.375	30.39	-6.68 – 67.46	0.108	17.73	-3.65 – 39.12	0.104	-0.78	-2.34 – 0.79	0.330

Table S14. Effects of treatment (nest and non-nest soils), disturbance index and water deficit, as well as the interactions between them (treatment * disturbance index; treatment * water deficit) on the stocks of four trace elements (Mn, Fe, Zn and Si) in the soils of nests of the leaf-cutting ant *A. rugosus*, in the Catimbau National Park, Pernambuco, Brazil.

	Mn stock			**Fe stock**			**Zn stock**			**Si stock**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	3.87	3.55 – 4.19	**<0.001**	412.23	347.88 – 476.57	**<0.001**	322.04	292.44 – 351.63	**<0.001**	19572.22	5429.15 – 33715.29	**0.007**
Treatment	0.39	0.07 – 0.70	**0.015**	51.43	17.76 – 85.10	**0.003**	10.38	-25.31 – 46.06	0.569	-8025.13	-34975.08 – 18924.82	0.559
Disturbance	0.10	-0.63 – 0.83	0.788	191.60	42.48 – 340.73	**0.012**	-61.45	-130.05 – 7.14	0.079	19300.39	-13478.19 – 52078.98	0.248
Water deficit	0.25	-0.39 – 0.89	0.449	-291.48	-422.30 – -160.67	**<0.001**	30.32	-29.85 – 90.50	0.323	7624.45	-21130.21 – 36379.11	0.603
Treatment * Disturbance	0.43	-0.29 – 1.16	0.243	80.30	2.27 – 158.32	**0.044**	2.94	-79.76 – 85.64	0.944	-40916.88	-103377.23 – 21543.48	0.199
Treatment * Water deficit	-0.36	-1.00 – 0.28	0.270	-22.37	-90.81 – 46.08	0.522	-14.18	-86.72 – 58.37	0.702	-7517.22	-62309.89 – 47275.46	0.788
Disturbance * Water deficit	-1.94	-3.40 – -0.48	**0.009**	-355.30	-651.40 – -59.19	**0.019**	-132.85	-269.05 – 3.36	0.056	27039.00	-38047.73 – 92125.72	0.416
Treatment * Disturbance) * Water deficit	0.57	-0.87 – 2.01	0.437	16.75	-138.19 – 171.68	0.832	-5.16	-169.36 – 159.05	0.951	-56882.06	-180906.31 – 67142.20	0.369

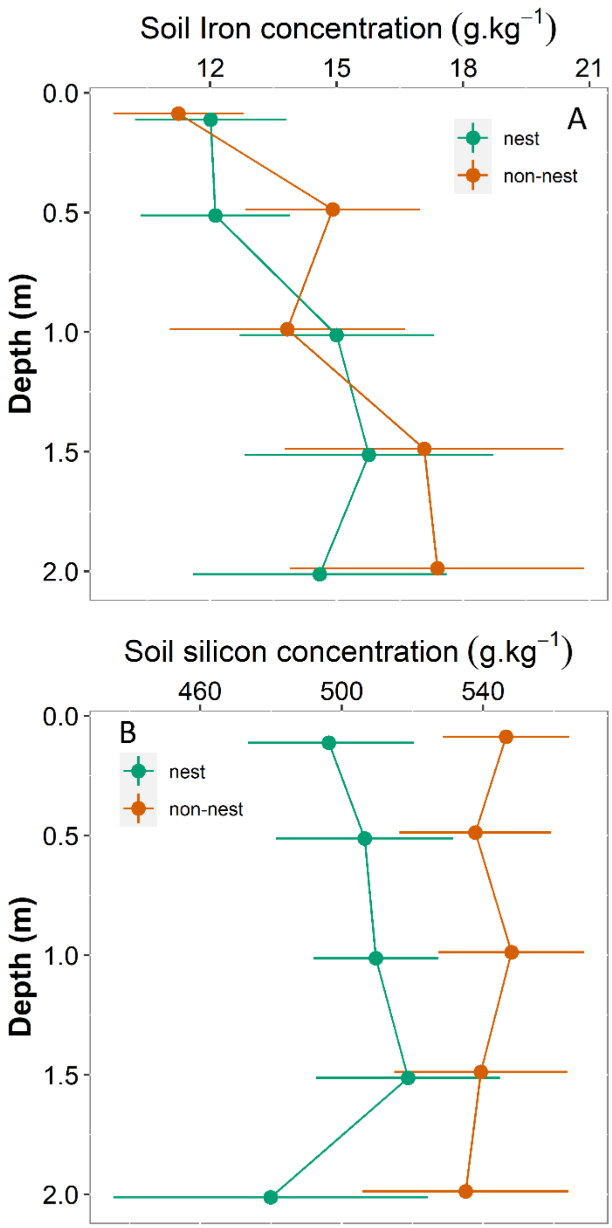

Figure S13. Effects of depth in (A) iron concentration in nest and non-nest soils of *Acromyrmex rugosus* colonies; and (B) effects of treatment on the silicon concentration of soils of *Acromyrmex rugosus* colonies situated in 12 plots across a chronic disturbance and aridity gradients in Catimbau National Park, Pernambuco, Brazil.

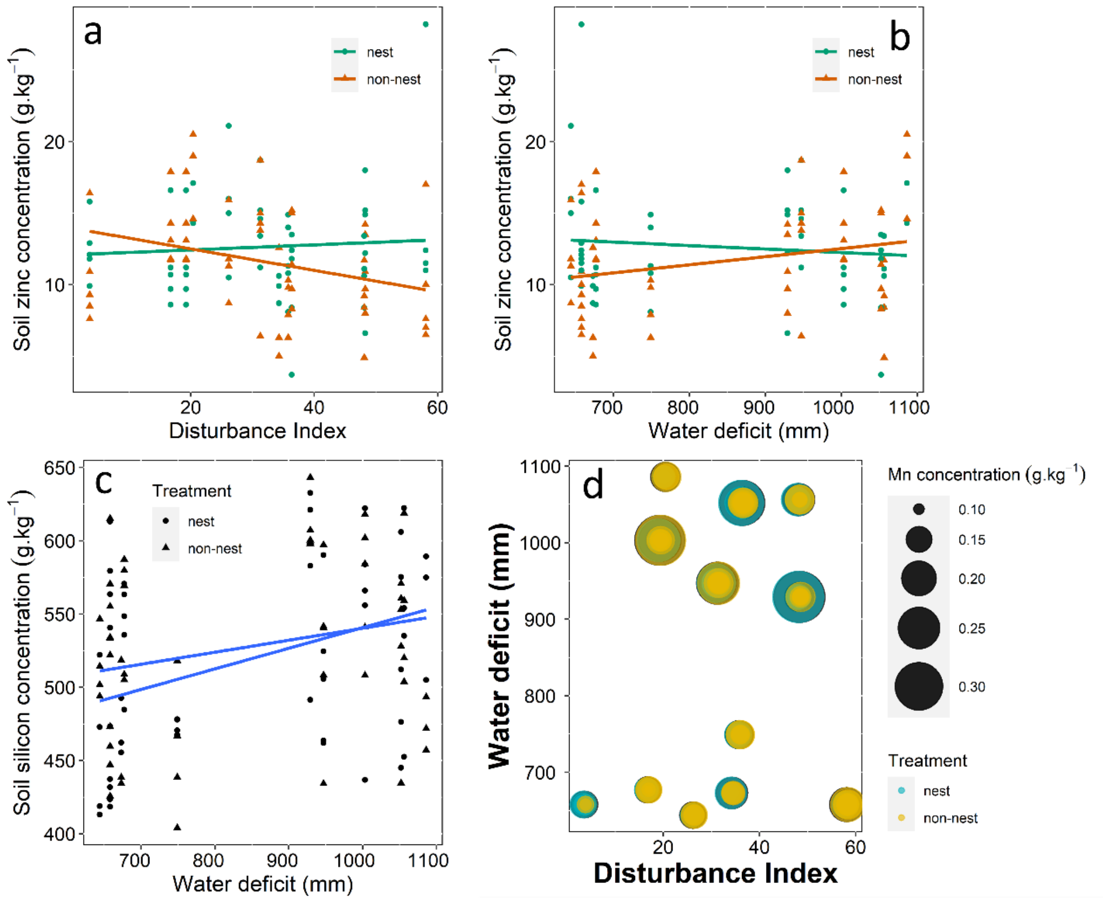

Figure S14. Effects of (a) disturbance index and the interaction with treatment on the total soil zinc concentration; (b) effects of water deficit and the interaction with treatment on the total soil zinc concentration; (c) positive effect of water deficit on the silicon concentration; and (d) effect of the interaction between treatment, disturbance index and water deficit on the total manganese concentration in soil The treatment consists of nest and non-nest soils of *Acromyrmex balzani* colonies situated in 12 plots across a chronic disturbance and aridity gradients in Catimbau National Park, Pernambuco, Brazil.

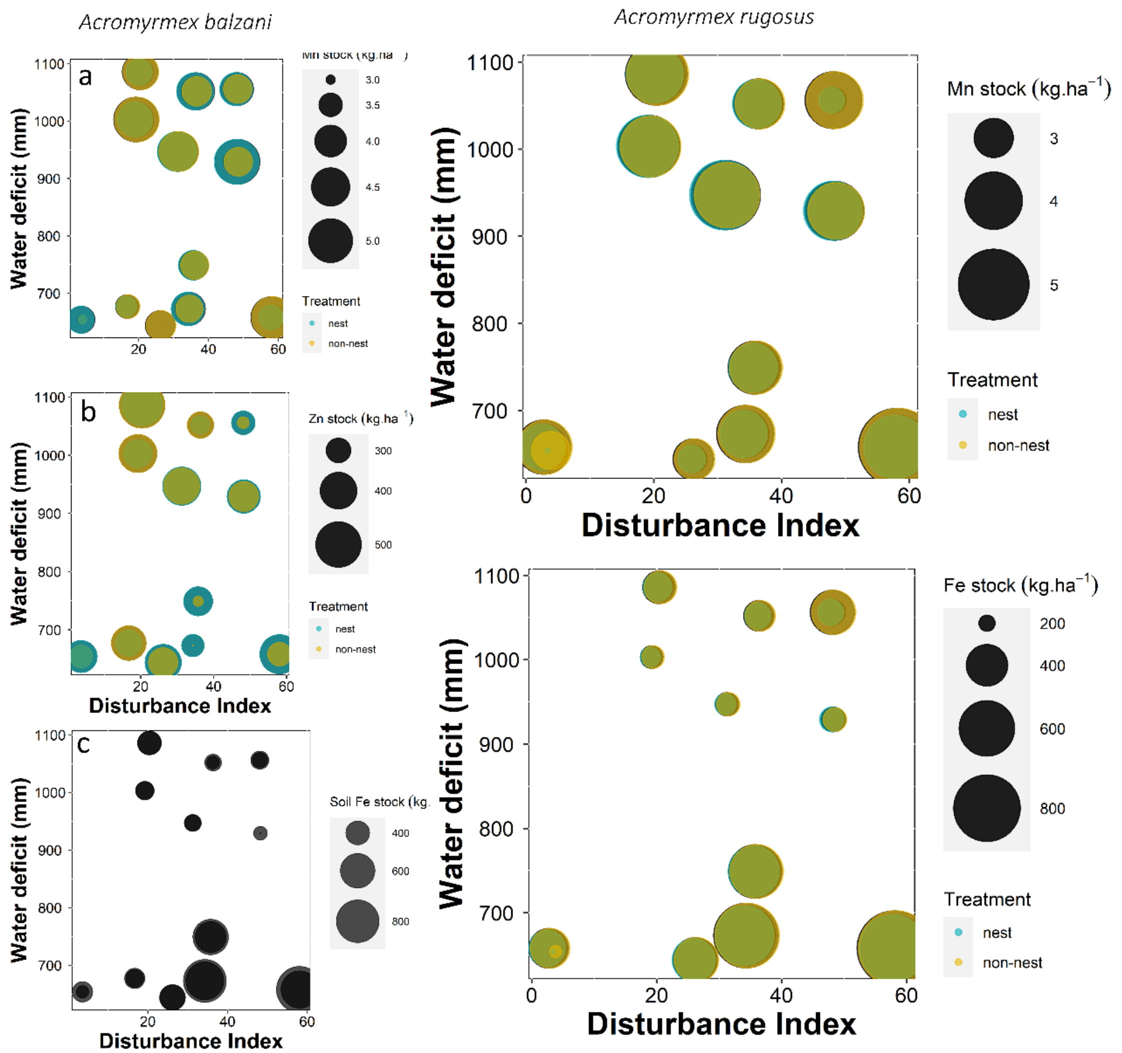

Figure S15. Effects of (a) the interaction between treatment, disturbance index and water deficit on total manganese stocks; (b) effects of water deficit and the interaction with treatment on the total soil zinc concentration; (c) effect of the interaction between disturbance and water deficit on the iron stocks in the soils of *Acromyrmex balzani* nests. Effect of (d) treatment and the interaction between disturbance index and water deficit on the total manganese concentration in soil; (e) separate effect of treatment, and the interaction between disturbance index and water deficit on the total soil iron stocks in nest soils of *Acromyrmex rugosus* colonies. All the colonies were situated in 12 plots across a chronic disturbance and aridity gradients in Catimbau National Park, Pernambuco, Brazil.

4 ARTIGO 2: EFFECTS OF LEAF-CUTTING ANT NESTS ON PLANT GROWTH AND NUTRIENT UPTAKE IN CAATINGA DRY FOREST UNDER HUMAN PRESSURE AND INCREASING ARIDITY

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Effects of leaf-cutting ant nests on plant growth and nutrient uptake in Caatinga dry forest under human pressure and increasing aridity

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Abstract

The combined effects of human disturbance and climate change are the main causes of biodiversity loss and its provision of ecosystem function and services. However, some organisms as the leaf-cutting antes (LCA) can benefit and increase their population under these new conditions. LCA are ecosystem engineers because they change edaphic conditions affecting plant recruitment and performance. In this study, we investigated the effects of nest soils of the two LCA *Atta opaciceps* and *Acromyrmex balzani* in regenerating and old growth forest stands under different level of disturbance and aridity on seed germination, the seedling development and seedling nutrient intake. Our results indicated that influence of LCA soils on plant growth is more intense in regenerating forest stand than in old growth forests. More precisely, nest mound and refuse soils increased plant biomass in the soils from nest of the two LCA species, but this effect was enhanced for plants growing in refuse soils from regenerating forest stand after pasture and agriculture uses, while diminished for plants in old growth forest and even nest mound soils in these same areas. In old growth forest under different level chronic disturbance and rainfall the effects of LCA on plant growth and the nutrient intake varied more according to nest treatment than disturbance and aridity level. However, some major plant nutrients, as phosphorus and calcium can constitute the key for matching plant growth.

Key-words – Nutrient concentrations, soil properties, seasonality dry tropical forest, ecosystem engineers, herbivory

Introduction

Human disturbance and climate change have critical effects on soil systems because they modify soil physical and chemical properties (BARBHUIYA *et al.*, 2004; TIESSEN; SAMPAIO, E. V. S. B.; SALCEDO, I.H., 2001). The isolated and combined effects of human disturbance and climate change are strong enough to diminish soil water capacity, soil nutrient stocks and microbial biomass, while increasing bulk density, altering nutrient cycling and ecosystem functioning (BARBHUIYA *et al.*, 2004; FERRENBERG *et al.*, 2015; FRATERRIGO *et al.*, 2005; SCHULZ *et al.*, 2016). These alteration on soil properties due to human disturbance and climate change negatively impact plant recruitment, performance and forest recovering (GARCÍA-ORENES *et al.*, 2017). Even when soil properties are not directly altered, disturbance can cause a long-term effect by transferring forest carbon stock from the living biomass to the deadwood (ROZAK *et al.*, 2018). Similarly, climactic factors, as aridity, has main effects on plant growth rates, even overcoming the influence of disturbance and soil fertility (TOLEDO *et al.*, 2011).

Despite the negative effects of human disturbances and climate change, their new biotic and abiotic conditions may be suitable for the proliferation of some organisms. One of these organisms are the leaf-cutting ants (LCA, species from *Atta* and *Acromyrmex* genera), dominant herbivores of the neotropical region, which proliferate across human-modified landscapes (LEAL, I. R.; WIRTH; TABARELLI, 2014; MEYER, Sebastian Tobias; LEAL, I., 2009; SIQUEIRA *et al.*, 2017; VIEIRA-NETO; VASCONCELOS; BRUNA, 2016). LCA colonies cut leaves and harvest plant material to grow their fungus garden, the food source of the colonies, disposing the organic waste not digested by the fungus in internal nest chambers or in external piles (hereafter, refuse dumps). The refuse dumps are several times richer in nutrient content than the adjacent soils without influence of the nests (FARJI-BRENER, Alejandro G.; TADEY, 2009; HAINES, 1978; MOUTINHO, P.; NEPSTAD; DAVIDSON, 2003; TADEY; FARJI-BRENER, Alejandro G., 2007; WIRTH *et al.*, 2003). The nutrient content of refuse dumps are mineralized and released to be absorbed by plants nearby (FARJI-BRENER, Alejandro G.; GHERMANDI, 2008; FARJI-BRENER, Alejandro G.; WERENKRAUT, 2014; STERNBERG *et al.*, 2007), resulting in improved plant fitness and growth rates (Verchot et al. 2003; Farji-Brener and Ghermandi 2008; Sousa-Souto et al. 2007; Saha et al. 2012). However, LCA nest construction and maintenance also alter soil structure, with the reallocation of a huge amount of minerals from deeper soil layers to the soil surface (ALVARADO, A.; BERISH; PERALTA, 1981; SWANSON *et al.*, 2019). In contrast with the refuse dumps, the altered topsoil of the nest mound experiment reduced soil carbon and nitrogen concentrations due litter removal from the soil surface (MEYER, Sebastian T. *et al.*, 2013). Consequently, in cases where colonies deposit their refuse in internal chambers, seedlings growing directly on the nest mounds cannot access the nutrient in the deep dumps and experiment less fertile topsoil with reduced plant recruitment (MEYER, Sebastian T. *et al.*, 2011b, 2013).

LCA colonies have been reported to proliferate in both, areas that suffered with acute disturbance (i.e. replacement of the vegetation for several types of land use causing habitat loss and fragmentation), and areas under regimes of chronic disturbance (e.g. exploitation of non-timber forest products, wood extraction, livestock grazing that does not cause habitat loss and fragmentation) (TABARELLI *et al.*, 2017). Mechanisms for this proliferation include higher availability of open areas for nesting sites and relaxation of bottom-up (more palatable host plants) and top down (less natural enemies as parasites and predators) controls, resulting in increased colony densities (ALMEIDA; WIRTH; LEAL, I. R., 2008; FARJI-BRENER, A. G., 2001; RAO, 2000; URBAS *et al.*, 2007; VASCONCELOS *et al.*, 2006). Additionally, in disturbed areas there are higher plant consumption and herbivory rate per colony, potentially increasing the transformation of stand leaf crop in refuse dumps, i.e. higher transference of nutrient from vegetation to soil (BUSTAMANTE; AMARILLO-SUÁREZ; WIRTH, 2020; FARJI-BRENER, A. G., 2001; FARJI-BRENER, Alejandro G.; TADEY; LESCANO, 2017; SIQUEIRA *et al.*, 2017; URBAS *et al.*, 2007). Although LCA refuse dumps may increase in number and amount, disturbance may impoverish their nutrient content by increasing the proportion of recalcitrant material collected by colonies in disturbed areas, such as feces, leaflitter, cacti and other conservative/tough leaves, which the symbiotic fungus is not able to properly metabolize due to high contents of lignin and cellulose (in fibers and cell wall components) (ABRIL; BUCHER, 2004; SIQUEIRA *et al.*, 2018; TADEY; FARJI-BRENER, 2007). Similarly, aridity can potentialize disturbance effects in the input of soil nutrient by LCA colonies in xeric regions (FARJI-BRENER, Alejandro G.; TADEY; LESCANO, 2017). However, the effect of human disturbance and aridity mediating the influence of LCA on soil properties and plant recruitment/performance is still poorly understood in tropical dry areas, where disturbance and aridity constitute a rising threat (MAGRIN *et al.*, 2014; MILES *et al.*, 2006).

The Brazilian Caatinga is the largest and most diverse tropical dry forest worldwide (SILVA, Jose Maria Cardoso Da; LEAL, I. R.; TABARELLI, 2017). However, it is also the denser in terms of human population (26 inhabitants/km², Medeiros *et al.* 2012), which is composed mostly of low-income and highly dependent on natural resources families (AB'SABER, 2000). This dependence on natural resource have imposed a historical process of land use change (i.e. acute disturbance) and currently only 33.4% of its original area remains (SILVA, Jose Maria Cardoso Da; LEAL, I. R.; TABARELLI, 2017). Additionally, remaining vegetation is also threatened by chronic disturbance (SINGH, 1998b), mainly by the use of native vegetation for livestock grazing, firewood collection and exploitation of non-timber forest products (ARNAN *et al.*, 2018; RIBEIRO, Elaine M.S. *et al.*, 2015; SOUZA, Danielle Gomes *et al.*, 2019). In addition of causing biodiversity loss and homogenization (RIBEIRO, Elaine M.S. *et al.*, 2015, 2016; RITO *et al.*, 2017), both, acute and chronic disturbances are reported to cause depletion on nutrient stocks in the soils of Caatinga (ALTHOFF *et al.*, 2018; SANTANA *et al.*, 2019; SCHULZ *et al.*, 2016; TIESSEN; SAMPAIO, E. V. S. B.; SALCEDO, I.H., 2001). Caatinga will also face an increase in aridity; projections include a reduction of 22% in rainfall and an increasing in temperature up to 6°C until the end of this century in the region (MAGRIN *et al.*, 2014). The effects of increasing aridity on soils of Caatinga and other semi-arid regions of the world can be even more severe if associated with disturbance. For example, disturbance and aridity can disrupt nutrient balance and cycling in plant-soil systems in these regions (SARDANS, J.; PEÑUELAS, J.; ESTIARTE, 2006; ST.CLAIR; LYNCH, 2010). Although we know that soil nutrient depletion has primarily impact for plants, little is known on how different types of disturbance (i.e. acute and chronic types) affects LCA nest soils and their response in plant recruitment and performance.

In this study we investigate the effect of human disturbance and aridity on the recruitment and performance of three common pioneer plant species in Caatinga (RITO; TABARELLI; LEAL, I. R., 2017), *Cenostigma microphylla, Croton argyrofilloides* and *Croton heliotropiifolius*, growing on soil associated with nests (mounds and refuse dumps) of two LCA species, *Atta opaciceps* and *Acromyrmex balzani*, and soil not affected by ants. We hypothesize that seedlings will have a better performance growing refuse dump soils than nest mound and control soils and that the difference in plant performance will be more pronounced as disturbance and aridity increase due to the nutrient depletion in the soil matrix, contrasting with enriched nest soils by refuse dumps. Thus, we predict that soils from refuse dumps will present (1) greater seedlings establishment, (2) higher plant dry biomass and length, (3) higher leaf area and water content, and (4) higher nutrient concentration compared to nest mounds and non-nest soils, (5) the difference of seedling recruitment and performance growing in refuse dumps compared to nest mound and control soils will be more pronounced as disturbance and aridity increase.

Material and methods

Study site

This study was carried out in the Catimbau National Park (8º24'00' and 8º36'35' S; 37º0'30' and 37º1'40' W), located in the state of Pernambuco, northeastern Brazil (hereafter Catimbau). The climate is semiarid, with annual temperature averaging 23°C, and mean annual rainfall varying from 480 to 1100 mm, concentrated between March and July (Sociedade Nordestina de Ecologia 2002). Deep sandy soils are predominant in Catimbau (quartzite sands, 70% of area), but planosols and lithosols are also present (15% each one; Sociedade Nordestina de Ecologia 2002). Vegetation is dominated by low-statured trees with Fabaceae, Euphorbiaceae and Boraginaceae as the predominant plant families, while the ground layer is composed of Cactaceae, Bromeliaceae, Malvaceae, Asteraceae and Fabaceae (Rito et al. 2017a).

The Park was created in 2002 but remains occupied by low-income rural populations that depend on natural resources for their livelihoods, resulting in a mosaic of agriculture fields, pasturelands, secondary forest and old growth forest stands (RITO *et al.*, 2017). The main activities are raising of livestock (goats and cattle), firewood collection and exploitation of non-timber forest products (ARNAN *et al.*, 2018; CÂMARA *et al.*, 2018).

Disturbance and rainfall treatments

We selected forest stands under two different disturbance regimes: areas previously used as agriculture and pasture and that now are under regeneration process (i.e. regenerating forest stands) and areas that never experienced clearcutting but that suffer different intensities of human use as firewood collection, timber exploitation and livestock grazing (i.e. old growth forest stands under chronic disturbance). Regenerating forest stands were located at the Brejo Farm, which is part of the Catimbau National Park. Vegetation is a mosaic of livestock pasture, secondary forest and old-growth forest stands. Regenerating forest stands used in this study included areas with two different historical of land use (1) areas that suffered clearcutting, had been used for at least 30 years as pasture (*Digitaria bicornis*) for cattle and goat livestock and now are regenerating for 8-10 years (i.e. regenerating forest stands after pasture use) and (2) areas that suffered clearcutting, had been used for corn and bean plantation for 3-4 years and then were abandoned and are regenerating for 12 years (i.e. regenerating forest stands after agriculture use). As control we used patches of old growth forest with no historical of land use for at least 80 years (i.e. old growth forest stands). The information about the historical use of the areas were first collected from locals and from two housekeepers of the Brejo farm, with subsequent field recognition and validation through satellite images with time series. Annual rainfall level in Brejo farm is 640 mm and soils consist of quartzite sandy soils (SNE, 2002).

As a less intense disturbance regime we selected forest stands that never suffered clearcutting, but are exploited by human populations for their livelihood (i.e. old growth forest stands under chronic disturbance). These stands were separated by a minimum of 2 km (within an area of 215 km^2) and support a low-stature (6-8 m) Caatinga dry forest over deep quartzite sand soils (ARNAN *et al.*, 2018; CÂMARA *et al.*, 2018) We used a disturbance multi-metric global index (ARNAN *et al.*, 2018) that integrate the three most common source of disturbance in the Catimbau region: livestock pressure (herbivory by goats and cattle), wood extraction (live and dead wood) and exploitation of non-timber forest products (poaching/hunting, medicinal plants, collection of food items for human consumption and livestock fodder) (ARNAN *et al.*, 2018; RITO *et al.*, 2017). To measure livestock grazing, we computed the length of goat trails and counted goat and cattle dung in the field. We combined the two measures of goat activity (trail length and dung frequency) to obtain a single measure for goats. For wood extraction we considered both live stems and coarse woody debris also in the field (ARNAN *et al.*, 2018). For exploitation of non-timber forest products, we used four measures of accessibility proximity to the nearest house, proximity to the nearest village, proximity to the nearest road and number of people living in the houses with influence in the plots, which were combined into a single disturbance metric representing human pressure. Then, the five disturbance indicators (cattle, goats, live-wood extraction, coarse woody debris extraction and human pressure) were integrated into a single chronic disturbance index, which ranged from 2 to 58 (from the lowest to the highest level of disturbance) among the forest stands (for methodological details, see Arnan et al. 2018).

These old growth forest stands under human pressure were distributed along a very steep rainfall gradient. Thus, we characterize the aridity level of each stand by computing water deficit, which represents the potential additional evaporative demand not met by available water based on energy input and rainfall (LUTZ; WAGTENDONK, VAN; FRANKLIN, 2010). Water deficit was calculated based on 30-arc-seconds (1 km) resolution maps of long-term average annual potential evapotranspiration and actual evapotranspiration (CGIAR-CSI's Global Aridity and potential evapotranspiration Database and Global High-Resolution Soil-Water Balance database Trabbuco & Zommer 2009, 2010). These maps were generated using temperature and precipitation data from WorldClim global climate data repository (FICK; HIJMANS, 2017).

Study species

The two different LCA species studied have different foraging habits: *Atta opaciceps* (Borgmeier, 1939) is a leaf-cutter ant, harvesting mostly dicots species, while *Acromyrmex balzani* (Emery, 1890) is a grass cutter ant (GONÇALVES, 1961). *Atta opaciceps* colonies are commonly found in areas with low vegetation cover, with a historical land use as livestock grazing and agriculture (SIQUEIRA *et al.*, 2017). Their nests cover an area of approximately 38 m² on average (SIQUEIRA *et al.*, 2018), and can reach a density up to 35.5 colonies ha^{-1} in Catimbau National Park (SIQUEIRA *et al.*, 2017) *Acromyrmex balzani* colonies are found in areas with low vegetation cover as well, but in areas that did not necessarily suffer clearcutting (Backé, 2015). *Acromyrmex balzani* nests are small compared to *Atta* spp. nests, covering a range of 0.17 m² on average, also showing high abundance and reaching a density of 31 colonies ha^{-1} in the Catimabu National Park (Backé, 2015, Tabarelli *et al.* 2017).

The four studied plant species, *Cenostigma microphyllum* ([Mart. ex G.Don] Gagnon & G.P.Lewis), *Croton argyrofilloides* (Müll.Arg.), *Croton heliotropiifolius* (Kunth), and *Trischidium mole* ([Benth.] H.E.Ireland), are abundant and widely distributed in the Catimbau National Park, being also associated to disturbed sites (RITO; TABARELLI; LEAL, I. R., 2017). Leaves of these species are frequently harvested by LCA colonies (Siqueira et al. 2018), including seeds have been registered being carried by *A. balzani* workers to the nest as food supply for the fungus garden (PSN, personal observation). Adult individuals are also frequently associated to nests of both LCA species (PSN, personal observation).

Experimental design

As the two LCA species present very different nest size and architecture, we adopted two different set ups. For *Atta opaciceps* we collected soil from three colonies located in the following habitats: (1) regenerating forest stands after pasture use, (2) regenerating forest stands after agriculture use, (3) old growth forest stands (i.e. control). All these nine colonies showed external refuse dumps and were least 500 m away from each other. We collected soils from four nest treatment: (1) nest mounds, (2) external refuse dumps, (3) 5 meters far from the center of the nest, and (4) 10 meters far from the center of the nest (i.e. control). For *Acromyrmex balza*ni we collected soil from at least two colonies located in the following habitats: (1) old growth forest stands with high level of chronic disturbance and aridity, (2) old growth forest stands with high level of chronic disturbance and low level of aridity, (3) old growth forest stands with low level of chronic disturbance and high level of aridity and (4) old growth forest stands with low level of aridity and chronic disturbance. Al these 12 colonies presented external refuse dumps and were distant at least 10 m from each other. Because *Acromyrmex balzani* nests are much smaller than *Atta opaciceps* nests, we sampled soils in three nest treatment: (1) nest mounds near the nest entrance, (2) external refuse dumps and (3) 10 meters away from the nest mound (i.e. control). For all colonies from the two LCA species we removed and discarded a soil layer of 5 centimeters of topsoil, and then collected soil samples from immediately below the layer removed. The soil samples were collected using plastic shovels and branches and taken to the experimental basement. The soils from each nest and from the same treatment were mixed for several hours and left to rest in plastic buckets for 48 hours. The soils were then reallocated on plastic trays where 20 seeds of each plant species were sown equidistant in four crossed quadrants in the trays. The trays were maintained under our experimental plant nursery in the Catimbau National Park study base, which is made of metal frame covered with white canvas without side cover. All the trays were kept together and totally under the roof of the nursery, receiving the same intensity of daily sunlight. The trays have 63.8 x 37.8 x 14 cm (ca. 0.33 m^3) and were watered every three days for five seconds each one (ca. 1.7 L of water). The soil collection, preparation and plant seeding were made in February 2018.

Seed collection, sowing and seeding measurements

We collected seeds from focal plant species (*Cenostigma microphylla, Croton argyrofilloides, Croton heliotropiifolius* and *Trischidium molle*) during the dry season between August and November 2017. To collect the seeds, we searched for the four focal plant species in the trails at different locations of the Catimbau National Park. We sow the seeds of all four species in each tray by burying them at 2 cm deep in the soils. Separated trays were used for the two different LCA species, nest treatments and habitats. Once the seeds were sow, we inspected the trays every day and considered germinated when the seed showed the cotyledon. No seed of the plant species *Trischidium molle* germinated. The seedlings were cultivated for 6 months and then were carefully removed from the trays for taking measurements. Some measurements were taken with the fresh plant material in the order as follows: (1) the aboveground plant individual length (until the first knot) and the belowground plant individual length; (2) the leaf weight, stem weight, and root weight for each plant individual. Then, we took photographs of all leaves of all individuals to measure leaf area, herbivory, and plant disease. The above (leaves and stems) and below (roots) ground plant parts were carefully separated and then dried in a drying oven for 24 hours at 70-80ºC. We measured the dry weight of: (3) leaf, stem, and root for each plant individual. The plant weights were taken with a precision scale (four decimal digits). The leaf area was calculated from the photographs using the ImageJ 1.52 software (Schneider *et al.* 2012). We also measured the leaf water content, as the difference between individual fresh leaf weight and dry leaf weight.

Nutrient content analysis

After all the measurements, the plant dry material was dried again in a drying oven for 24 hours at 100ºC. We select the leaves and stems to be grind. The material (leaves and stem together) were ground in a planetary ball mill (PM 200, Retsche ®) for 10, 15 or 20 minutes depending on the material hardness. The plant loose powder obtained was then weighted in one gram per sample and was packed into a polyethylene pill of 20 mm internal diameter and covered with 6-µm-thick polypropylene film (Mylar®). The equipment used in this study was the energydispersive X-ray fluorescence spectrometer, model EDX 720 from Shimadzu, which consists of a rhodium tube for generating X-rays, a sealed chamber for sample analysis in a vacuum atmosphere, and a Si(Li) detector to measure the induced radiation. We selected magnesium, phosphorus, potassium, iron, manganese, zinc, and calcium as micronutrients to investigate their concentrations in the plant material. We also quantified total carbon and nitrogen in the plant material. For that, we used an elemental analyzer EuroVector (EA3000) configured with an C-H-N reactor filled with chromium oxide, reduced cooper wires and silver cobalt oxide.

Statistical analysis

First, we tested if the number of seeds germinating responded to soil treatments and habitat types (i.e. for *Atta opaciceps*: regenerating forest stand after pasture, regenerating forest stand after agriculture and old-growth forest stands; for *Acromyrmex balzani*: old growth forest stands with high level of chronic disturbance and aridity, old growth forest stands with high level of chronic disturbance and low level of aridity, old growth forest stands with low level of chronic disturbance and high level of aridity and old growth forest stands with low level of aridity and chronic disturbance . For this, we applied a Pearson's Chi-Squared test with number of plants that germinated and grew in each treatment and habitat type. Subsequently, to test the effects soil treatments and habitat type on plant growth and nutrient uptake, we conducted generalized linear mixed models (GLMM) for the two different LCA species set ups (i.e. *Atta opaciceps* - nest mound soils, refuse soils, 5 meters-away soils, 10 meters-away soils from colonies located in regenerating forest stand after pasture, regenerating forest stand after agriculture and old-growth forest stands; *Acromyrmex balzani* - nest mound soils, refuse soils and 10 meters-away soils from colonies located in old growth forest stands with the two levels of chronic disturbance (high and low chronic disturbance) and aridity (high and low aridity) and their interactions (old growth forest with - high level of chronic disturbance and aridity, - high level of chronic disturbance and low level of aridity, - low level of chronic disturbance and high level of aridity, and - low level of aridity and chronic disturbance) as explanatory variables with plant species as random factor. We used total plant dry biomass, aboveground plant length, leaf area, water content and nutrient concentration in percentage in the plant as response variables in the two setups. All the tests were conducted with the packages lme4 and lmerTest in the R programming language environment (BATES *et al.*, 2015; KUZNETSOVA; BROCKHOFF; CHRISTENSEN, R, H, 2017; R CORE TEAM, 2019).

Results

Altogether, 191 seeds germinated in the soil treatments of all habitat types evaluated for *Atta opaciceps* colonies, belonging to the species *Cenostigma piramidalle* (137), *Croton argyrophylloides* (24), and *Croton heliotropiifolius* (20). The dry biomass per individual varied between 0.28 g to 18.06 g (2.92±2.73, mean ± SD) in *C. piramidalle*, 0.16 g to 5.09 g (1.34 ± 1.21) in *C. argyrophylloides*, and 0.44 g to 18.24 g (6.25 ± 5.15) in *C. heliotropiifolius*. Similarly, 215 seeds germinated in soil treatments of all habitat types evaluated for*A. balzani* colonies, belonging to the species *Cenostigma piramidalle* (152), *Croton argyrophylloides* (34), and *Croton heliotropiifolius* (29). The plant dry biomass per individual varied between 0.06 g to 19 g (2.29 ± 2.74) in *C. piramidalle*, 0.07 g to 8.59 g (2.14 ± 1.98) in *C. argyrophylloides*, and 1.02 g to 40.81 g (13.54 ± 10.96) in *C. heliotropiifolius*.

Effects of soil treatments and habitat types on seed germination and seedling development

The number of seeds germinated and established as seedlings in each *Atta opaciceps* soil treatment and habitat type did not differ significantly ($\chi = 8.53$, df =6, $p = 0.201$). Total plant dry biomass in *Atta opaciceps* colonies was significantly affected by soil treatment and the interaction between habitat type and soil treatment (Table 1). Thus, plants growing in soils of refuse soils showed greater biomass compared to nest mound soils in regenerating forest stands after both agriculture and pasture land uses. Additionally, plants growing in soils from regenerating forest stands after pasture showed greater biomass in refuse soils compared to 10- and 5-meters (Table 1, Figure 1A). No significant difference was observed across soil treatments in old growth forest stands (Table 1, Figure 1A). When separating the dry biomass by below and aboveground plant parts, the biomass of plant roots and plant stems + leaves were affected by soil treatment and the interaction between treatment and habitat type (Table 1). Thus, plants growing in refuse soils from regenerating forest stands after pasture showed higher root biomass compared to plants growing in soils from 10- and 5-meters treatments of all habitat types, being similar only with those plants growing in nest soils from regenerating forest stands after pasture and refuse soils from regenerating forest stands after agriculture use (Figure 1C). Similarly, plants growing in refuse soils from regenerating forest stands after pasture also showed higher shoots biomass than plants in 10-meters soils from regenerating forest stands after agriculture use and old growth forest stands and 5-meters from regenerating forest stands after agriculture, while plants growing in nest mound soils from agriculture showed lower shoots biomass compared to all other treatments and land use combinations (Figure 1E).

Similar to *Atta opaciceps*, the number of germinated seeds that established as seedlings in *Acromyrmex balzani* soil treatments and across habitat types was not significantly different (χ = 1.94, df =6, p = 0.924). Total plant dry biomass, as well as above and below ground biomass were affected only by soil treatment (Table 2). Thus, plant total biomass, and roots and stems + leaves biomass showed higher values in those plants growing in nest mound and refuse soils compared to those growing in 10-meters soils for all types of habitat (Figures 1B, D and F).

Concerning the length of aboveground parts, only soil treatment had a significant effect in plants growing in *Atta opaciceps* and *Acromyrmex balzani* nests (Table 1). Plants growing in refuse soils were significantly taller than plants growing in 10- and 5-meters and nest soils for *Atta opaciceps* nests (Table 1, Figure 2A), as well as taller in the soils from nest mound and refuse soils compared to 10-meters soils in *Acromyrmex balzani* soils (Table 2, Figure 2B). Soil treatment and the interaction between soil treatment and habitat type had a significant effect on the percentage of water in plants in *Atta opaciceps* nests (Table 1), with decreased water content in plants growing in nest mound soils from regenerating forest stands after agriculture and old growth forest stands compared to plants growing in 5-meters and refuse soils from old growth forest areas, and plants growing in 10- and 5-meters soils and nest soils from regenerating forest stands after agriculture (Figure 2C). Water content did not change significantly across soil treatments and habitat types in *Acromyrmex balzani* nest soils (Table 2, Figure 2D). The leaf area was affected by soil treatments and the interaction between soil treatments and habitat types in *Atta opaciceps* nests (Table 1), with higher values in plants growing in refuse soils from old growth forest stands and 5-meters soils from regenerating stands after pasture compared to those growing in 5-meters soils from regenerating stands after agriculture (Figure 2E). In the soils of *Acromyrmex balzani* nests, the leaf area was affected in a complex way by soil treatment, the interaction between disturbance and aridity and between disturbance, aridity, and treatment (Table 2). Thus, plants growing in (1) control soils from old growth forest stands with low level of aridity and chronic disturbance produced leaves with greater leaf area compared to control soils of all other groups (i.e. from old growth forest stands with high level of chronic disturbance and high/low levels of aridity and old growth forest stands with low level chronic disturbance and high level of aridity); (2) nest soils from old growth forest stands with high chronic disturbance and aridity produced plants with greater leaf area compared to nest soil from old growth forest stands with low chronic disturbance and aridity; (3) refuse soils produced plants with higher leaf area compared to 10-meters soils in all groups excepting old growth forest stands with low chronic disturbance and aridity; (4) within each group site, there was no difference between leaf area of plants growing in the refuse soils and nest mound soils (Figure 2F).

Effects of soil treatments and habitat types on the plant nutrient concentration

For Atta opaciceps colonies, plant nutrient concentration was affected by soil treatment, habitat type and the interaction between soil treatment and habitat type (Supplementary Information, Tables S1 and S2). The carbon concentration in plants growing in nest soils from old growth forest stands was higher compared to those plants growing in 10-meters soils from regenerating forest stands after pasture, but similar among plants growing in all other soil treatments (Figure 3A). Nitrogen concentration was lower in plants growing in nest mound soils and refuse soils compared to 5-meters soils of regenerating forest stand after pasture and old growth forest, but without this difference in regenerating forest after agriculture use (Table S1, Figure 3C). We also observed an increase of potassium concentration in plants from refuse soils and 5-meters soils of regenerating forest after pasture, being higher when compared to all treatments from regenerating forest after agriculture and 10-meters soils and nest soils from old growth forest, but similar to 5-meters and refuse soils of old growth forest (Table S1, Figure 3E). Phosphorus concentration in plants growing nests soils was significantly reduced in regenerating forest after agriculture compared to regenerating forest after pasture and old growth forest, with plants from 5-meters soils of regenerating forest after pasture showing 2 to 3 fold higher phosphorus concentration than any treatment of regenerating forest after agriculture (Table S2, Figure 3G). Calcium concentration in plants growing in nest soils did not show significant variation across soil treatments and habitat types (Table S1). Iron was higher in plants from nest mounds and refuse soils compared to 5- and 10-meters, as well as also higher in plants from nest mound from old growth forest compared to nest soils from regenerating forest after agriculture and pasture (Figure S3A). For manganese, higher concentrations were recorded in plants from nest mound in regenerating forest after agriculture compared to all other treatments from regenerating forest after pasture and from old growth forest; while zinc was present in lower concentration in plants growing in nest mound compared to 5-meters soils in all forest areas (Figure S3 B and C).

Similarly to *Atta opaciceps* colonies, nutrient concentration of plants growing in *Acromyrmex balzani* nests was affected by soil treatments, habitat types and their interactions (Tables S1 and S2). Plants growing in *Acromyrmex balzani* nest soils and refuse soils from old growth forest showed higher carbon concentration compared to those from 10-meters soils in old growth forest stands with low level chronic disturbance, an effect of disturbance only (Figure 3B). Plants growing in refuse soils from old growth forest stands with high level of aridity had higher nitrogen concentration than those from refuse soils from old growth forest stands with low level of aridity, an effect of aridity only (Table S1, Figure 3D), as well as plants growing in 10-meters soils from old growth forest stands with low level of chronic disturbance and aridity showed higher nitrogen concentration than those from 10-meters soil from those areas of old growth forest stands with high chronic disturbance (Figure 3D). In Potassium concentration was twice as high in plants growing in nest mound soils of old growth forest stands with high level of chronic disturbance and low level of aridity compared to plants growing in 10-meters soils of all soil groups (Figure 3F). Nest mound and refuse soils significantly produced plants with higher phosphorus concentration compared to 10-meters soils in old growth forest stands with high levels of chronic disturbance and aridity compared to the other soil treatments (Table S2, Figure 3H). Magnesium and iron concentrations were in general higher in those plants from nest mound soils compared to 10-meters soils (Table S2, Figure S3 D and E). For manganese, plants showed higher concentration growing in 10-meters soils compared to nest and refuse soils in general (Figure S3 F). Zinc concentration in plants did not show significant variation across treatments and land use types (Table S2). Calcium was higher in plants from refuse soils in old growth forest stand with high levels of chronic disturbance and aridity compared to all soil treatments from old growth forest stand with low level of chronic disturbance and high level of aridity and with low level of chronic disturbance and low level of aridity (Table S1, Figure S3G).

Discussion

Our study addressed the influence of historical land use, chronic disturbance, and aridity levels on the role of LCA colonies as soil improvers for plants, analyzing plant development and nutrient uptake. Our results reconfirm the role of LCA refuse dumps as plant growth enhancer, whilst indicate that depending on the habitat type, the soils of nest mound reduce plant growth, but not seed germination. More precisely, plants benefit from refuse soils of *Atta opaciceps* and from nest and refuse soils of *Acromyrmex balzani* nests, increasing their biomass, aboveground length, and leaf area. However, the role of LCA nest may be markedly influenced by the historical land use, while slightly altered by chronic disturbance and aridity. This means that historical land use promotes a deterioration of soil conditions in general, which amplifies *Atta opaciceps* refuse effects, widening the difference of plant size and biomass between plants growing in refuse and non-nest soils. In the other hand, the effects of chronic disturbance and aridity do not seem to be important drivers altering the influence of *Acromyrmex balzani* nests on plant growth. Finally, these results may emerge due land use for agriculture considerably reduce plant phosphorus concentration, especially in nest mound soils, while chronic disturbance can interact and reduce plant carbon and nitrogen concentration without reflecting in decreased plant growth.

Seedlings growing nearby leaf-cutting nests are reported to increased their leaf area, leaf biomass, root biomass, and germination rate, resulting in greater reproductive success, compared to those seedling growing in control soils (CERDA *et al.*, 2012; FARJI-BRENER, Alejandro G.; GHERMANDI, 2008; SANTOS, R. S.; MECENAS; SOUSA-SOUTO, 2019). Our results corroborate this idea by demonstrating that soils of *Atta opaciceps* refuse dumps and *Acromyrmex balzani* nest mounds and refuse dumps enhance plant growth and the concentration of some micronutrients in the plants. However, as we observed for reduced biomass of plants growing in nest mound of *Atta opaciceps*, seedlings may be harmed if growing directly in the nest mound soils (MEYER, Sebastian T. *et al.*, 2013). LCA nests are reported to effectively promote soil nutrient enrichment, via accumulation of refuse dumps, and make these nutrients available for plants (FARJI-BRENER, Alejandro G.; GHERMANDI, 2008; FARJI-BRENER, Alejandro G.; WERENKRAUT, 2014; MOUTINHO, P.; NEPSTAD; DAVIDSON, 2003; STERNBERG *et al.*, 2007). Our results indicate that the concentration of some plant trace elements is associated with nest mound and refuse soils of *Atta opaciceps* and *Acromyrmex balzani* nests, while for

Acromyrmex balzani, plant major elements, as calcium, have increased concentration plants growing in soils of refuse dumps.

Plant carbon, nitrogen, phosphorus and potassium concentration in general found higher in nest and refuse soils than in control soils, follow the idea of a positive effects of the nest in soil nutrient accumulation (FARJI-BRENER, Alejandro G.; WERENKRAUT, 2014; MOUTINHO, P.; NEPSTAD; DAVIDSON, 2003). However, as partially expected, LCA refuse soils can intermediate the effects of disturbance and increase plant establishment and biomass in areas newly disturbed (SANTOS, R. S.; MECENAS; SOUSA-SOUTO, 2019). Yet, depending on the disturbance type (i.e., forest regenerating after agriculture/pasture or old growth forest stand under chronic disturbance), the effects of nest mound can be negative or positive, for some plant major nutrients content. As land use (forest regenerating after agriculture/pasture) mainly potentialized the positive effects of soils under refuse dumps, this contradicts the idea of reduced nutrient content in refuse dumps mediated by disturbance (TADEY; FARJI-BRENER, Alejandro G., 2007), although the effects found here are seen in the plants not in the soils. Regenerating forests after land use present higher concentration of nutrient in the surface of LCA nest soils (FARJI-BRENER, Alejandro G.; WERENKRAUT, 2014; MOUTINHO, P.; NEPSTAD; DAVIDSON, 2003), and some plant trace elements can be found at higher concentrations in plants from LCA nest soils of newly burned areas (SOUSA-SOUTO; SCHOEREDER; SCHAEFER, 2007). Despite this, the net effects of disturbance as burning in plant micronutrients concentration in mainly negative (SOUSA-SOUTO; SCHOEREDER; SCHAEFER, 2007), while in our results, for acute disturbance, these differences between nest, refuse, and soils nearby the nest are highly variable.

Both, land use and chronic disturbance cause nutrient depletion in the soil (SANTANA *et al.*, 2019; SCHULZ *et al.*, 2016; TIESSEN; SAMPAIO, E. V. S. B.; SALCEDO, I.H., 2001), which may potentially reduce nutrient content in the refuse piles of leaf-cutting ants (TADEY; FARJI-BRENER, Alejandro G., 2007). The exportation of soil nutrient can be much higher in acute than in chronic disturbance regimes, mainly because some types of land use, as agriculture, remove the plant products from the producing area, and also cause drastic alterations in soil structure allowing nutrient leaching in dry forest (ARAÚJO-FILHO *et al.*, 2018; TIESSEN *et al.*, 1998). In the regenerating forest after land use, the nest mounds may be harmful to soils more than in areas of old growth forest under chronic disturbance, by leaching nutrients in higher rates than non-nest soils through bare soil and colony tunnels, making them unavailable for plants (LEHMANN; SCHROTH, 2003). This could be the mechanism behind less nutrient concentration in plants growing in nest mound soils of regenerating forest after agriculture and pasture. For the positive effects of the refuse dumps, the enriched soils with organic material may be higher at disturbed sites due to higher harvesting rates by LCA colonies of the genus *Atta* (SIQUEIRA *et al.*, 2018). This higher biomass is implemented in soils and accessed by the roots of plants growing nearby, enhancing plant biomass and plant nutrient concentraion (MOUTINHO, P.; NEPSTAD; DAVIDSON, 2003; STERNBERG *et al.*, 2007). This result reasserts the positive effect of the refuse soils but negative effect of the nest mound for seedlings (Meyer et al. 2011).

Our findings of chronic disturbance and nest effects are less pronounced, but at least for plant nitrogen concentration, a highly demanded nutrient, it accumulates more in plants growing in refuse soil from old growth forest stand with high levels of chronic disturbance. This result does not mean that chronic disturbance potentialize the nest effect, but rather indicates that ants can make this element scarcer in the surrounding non-nest soils. Chronic-related disturbances, as livestock in natural areas, reduce the nutrient content in LCA refuse dumps (FARJI-BRENER, Alejandro G.; TADEY; LESCANO, 2017; TADEY; FARJI-BRENER, Alejandro G., 2007), but this content may remain higher than in non-nest soils. Additionally, chronic disturbance promote soil SOC impoverishment in soils of dry fotrest (BARBHUIYA *et al.*, 2004; SCHULZ *et al.*, 2016), while can keep other elements at higher mineralization rates, as seen in plant calcium concentration. Similarly, aridity does not seem to be important affecting nest influence for plants, as could be expected since moisture enhance microbiota activity, and thus nutrient mineralization, in LCA refuses dumps (FERNANDEZ; FARJI-BRENER, Alejandro G.; SATTI, 2014). But it is important to highlight that our experiment was carried out with constant watering, what could mitigate the effects of aridity on the collected soils for plants.

High values of P and K in plant tissues growing in refuse and nest soils compared to non-nest soils from old growth forest stand under chronic disturbance, indicates that these elements elements can be mineralized and available in higher rates in the nest surroundings of *Acromyrmex balzani*. Soils from regenerating forests after land use, refuse soils increased K plant concentration, but nest decreased it, while for P plant concentration, nest and refuse were both negative. *Acromyrmex balzani* nests dispose their external refuse piles close to the small nest mounds, forming a zone of high soil microbiota activity and thus higher nutrient mineralization (FERNANDEZ; FARJI-BRENER, Alejandro G.; SATTI, 2014; SOUSA-SOUTO; CAROLINE; *et al.*, 2012). Contrarily, *Atta opaciceps* nests contains a huge mound and their refuse are more diluted in the nest soil. In the P-limited Caatinga soils (SALCEDO, I. H.; TIESSEN; SAMPAIO, E. V. S. B., 1997), the refuse dump represent a spot of high mineralization of limited nutrient in the soil matrix (FARJI-BRENER, Alejandro G.; WERENKRAUT, 2014; SOUSA-SOUTO; CAROLINE; *et al.*, 2012), but apparently, in the land use disturbance diminish refuse P enrichment. The higher plant biomass and length found in nest mound and refuse soils in both LCA species it is not followed by all or at least most of the nutrient concentration in the plant tissues. This relation allows us to identify some key elements, which are the ones with concentration in plant tissues from refuse and nest mound soils matching the higher plant size and biomass in these same soils. For *Atta opaciceps* nests, this element is K, while in *Acromyrmex balzani* this element is Ca, both being major plant nutrient, i.e. are found in higher concentration in plants. Soil physical properties should also be considered for explain plant growth. In the same study areas, two different LCA species from the genus *Acromyrmex* decreased soil bulk density, enhancing soil porosity and thus soil respiration (Santos-Neto et al. in preparation).

Although our experiments were carried out in controlled environment, we can precisely suggest that the influence of disturbance on the leaf-cutting ant nests of *Atta opaciceps* and *Acromyrmex balzani* can have several implications. However, we did not corroborate our first hypothesis since seedlings establishment did not vary due nest treatment or disturbance, while our second hypothesis was readily supported with nest and refuse dumps increasing plant biomass and length. The third and fourth hypothesis were partially, while the fifth was entirely confirmed. This means that all these processes can readily alter plant fitness in regenerating forest after land use, but unaffecting plants in old growth forest stand under chronic disturbance. In this sense, nests can create mosaics of vegetation islands, improving nutrient intake, increasing plant biomass, and helping in advancing the forest regeneration (FARJI-BRENER, Alejandro G.; GHERMANDI, 2008), but all these benefits can be delayed or even reversed when the regenerating forest was used for agriculture. We reconfirm the positive effect of nest soils, while argue that disturbance act in the plant nutrient assimilation, what can make plants more prone to deficiencies when other constraints are present. Although we did not find effect of aridity in the soil, but in natural conditions aridity necessarily plays an important role on plant development, and thus, nutrient deficiency can be pronounced in plant development.

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Table 1. Results of Linear Mixed Models testing the effects of land use type in the nest and non-nest soils (treatment) of the LCA species *Atta opaciceps* on the plant total biomass, plant root biomass, plant shoots biomass (upper table), plant aboveground length, plant water content and leaf area (lower table), in Catimbau National Park, Pernambuco, Brazil. We consider significant effects when their confidence intervals did not include zero.

	Total dry biomass			**Dry root biomass**			**Dry shoot biomass**		
Predictors	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p
(Intercept)	3.06	-0.30 – 6.41	0.074	1.24	0.04 – 2.44	**0.042**	1.83	-0.71 – 4.38	0.158
treatment – 5 m	0.45	-1.18 – 2.08	0.589	0.51	-0.50 – 1.52	0.318	-0.06	-0.85 – 0.73	0.878
treatment – Nest	-2.57	-4.68 – -0.46	**0.017**	-0.67	-1.96 – 0.63	0.314	-1.91	-2.93 – -0.89	**<0.001**
treatment – Refuse	2.9	0.74 – 5.06	**0.009**	1.95	0.62 – 3.29	**0.004**	0.95	-0.10 – 2.00	0.075
Pasture	0.32	-1.40 – 2.04	0.715	-0.04	-1.10 – 1.03	0.947	0.35	-0.49 – 1.18	0.412
Preserved	-0.1	-1.86 – 1.67	0.914	0	-1.10 – 1.09	0.993	-0.09	-0.94 – 0.77	0.839
Treatment – 5m * Pasture	-0.11	-2.51 – 2.29	0.928	-0.2	-1.69 – 1.28	0.792	0.11	-1.05 – 1.28	0.847
Treatment – Nest * Pasture	3.96	0.73 – 7.18	**0.016**	2.03	0.04 – 4.02	**0.046**	1.96	0.40 – 3.52	0.014
Treatment – Refuse * Pasture	1.88	-1.17 – 4.94	0.227	1.33	-0.56 – 3.21	0.168	0.56	-0.92 – 2.04	0.46
Treatment – 5 m * Preserved	-0.18	-2.64 – 2.28	0.885	-0.61	-2.13 – 0.90	0.427	0.44	-0.75 – 1.63	0.467
Treatment - Nest * Preserved	3.08	0.28 – 5.89	**0.031**	0.84	-0.89 – 2.56	0.343	2.26	0.90 – 3.62	0.001
Treatment – Refuse * Preserved	-1.05	-3.92 – 1.82	0.474	-1.21	-2.98 – 0.56	0.181	0.14	-1.25 – 1.53	0.84
	Shoot length			**Water content**			**Leaf area**		
Predictors	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p
(Intercept)	9.78	-0.21 – 19.76	0.055	39.23	31.4 – 47.0	**<0.001**	6.92	-1.59 – 15.43	0.111
Treatment – 5 m	-0.44	-3.35 – 2.47	0.766	0.85	-3.29 – 4.99	0.688	-0.65	-2.90 – 1.59	0.567
Treatment – Nest	-4.78	-8.54 – -1.02	**0.013**	-15.32	-20.6 – -9.9	**<0.001**	0.67	-2.26 – 3.60	0.653
Treatment – Refuse	2.56	-1.30 – 6.41	0.193	-1.83	-7.31 – 3.66	0.514	2.59	0.38 – 4.81	**0.022**
Pasture	2.38	-0.69 – 5.45	0.128	-1.53	-5.89 – 2.84	0.494	0.54	-1.72 – 2.80	0.638
Preserved	1.72	-1.42 – 4.87	0.283	-6.73	-11.2 – -2.2	**0.003**	0.38	-1.69 – 2.45	0.719
Treatment – 5m * Pasture	-0.06	-4.34 – 4.22	0.977	-4.83	-10.9 – 1.27	0.120	5.49	1.52 – 9.46	**0.007**
Treatment – Nest * Pasture	4.76	-0.98 – 10.51	0.104	11.81	3.63 – 19.9	**0.005**	-0.21	-4.34 – 3.91	0.92
Treatment – Refuse * Pasture	3.76	-1.69 – 9.20	0.176	-2.86	-10.6 – 4.89	0.470	0.09	-3.22 – 3.40	0.957
Treatment – 5 m * Preserved	0.13	-4.24 – 4.51	0.953	9.26	3.03 – 15.4	**0.004**	3.01	-0.39 – 6.42	0.083
Treatment - Nest * Preserved	3.61	-1.39 – 8.61	0.157	12.57	5.46 – 19.6	**0.001**	-0.9	-4.44 – 2.64	0.619
Treatment – Refuse * Preserved	-1.16	-6.26 – 3.95	0.657	11.29	4.0 – 18.56	**0.002**	0.28	-2.79 – 3.35	0.858

Table 2. Results of Linear Mixed Models testing the effects chronic disturbance and water deficit in the nest and non-nest soils (treatment) of the LCA *Acromyrmex bazalni* on the plant total biomass, plant root biomass and plant shoots biomass (upper table), plant aboveground length, plant water content and leaf area (lower table), in Catimbau National Park, Pernambuco, Brazil. We consider significant effects when their confidence intervals did not include zero.

	Total dry biomass			**Dry root biomass**			**Dry shoot biomass**		
Predictors	β	CI	p	β	CI	P	β	CI	p
(Intercept)	4.15	-3.96 – 12.26	0.316	1.25	-1.09 – 3.58	0.295	2.56	-3.22 – 8.34	0.386
Nest	5.46	2.88 – 8.03	**<0.001**	2.9	1.77 – 4.04	**<0.001**	2.55	0.89 – 4.21	**0.003**
Refuse	5	2.56 – 7.43	**<0.001**	2.37	1.30 – 3.44	**0.001**	2.62	1.06 – 4.19	**0.001**
Preserved	0.52	-1.75 – 2.79	0.656	0.19	-0.81 – 1.19	0.712	0.33	-1.13 – 1.79	0.661
Rainy	-0.32	-2.62 – 1.97	0.782	-0.21	-1.22 – 0.80	0.682	-0.11	-1.58 – 1.37	0.885
Nest * Preserved	-2.71	-6.74 – 1.33	0.188	-1.61	-3.39 – 0.17	0.077	-1.7	-3.58 – 0.18	0.077
Refuse * Preserved	-0.18	-4.07 – 3.71	0.928	-1.04	-2.29 – 0.21	0.103	-0.5	-2.32 – 1.33	0.594
Nest * Rainy	0.05	-4.19 – 4.29	0.981	-0.63	-1.92 – 0.66	0.337	-0.29	-2.18 – 1.59	0.759
Refuse * Rainy	0.22	-3.88 – 4.31	0.918	-0.47	-1.71 – 0.78	0.465	-0.82	-2.64 – 1.00	0.379
Preserved * Rainy	-0.85	-3.21 – 1.51	0.482	-0.22	-1.26 – 0.82	0.674	-0.62	-2.14 – 0.90	0.421
Nest * Preserved * Rainy	-2.02	-7.97 – 3.93	0.506	-0.70	-3.32 – 1.93	0.602	-1.30	-5.13 – 2.52	0.504
Refuse * Preserved * Rainy	-2.94	-8.69 – 2.80	0.315	-0.87	-3.40 – 1.66	0.501	-2.07	-5.76 – 1.62	0.272
	Shoot length			**Water content (log)**			**Leaf area**		
Predictors	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p
(Intercept)	13.18	-3.17 – 29.53	0.114	40.22	30.18 – 50.27	<0.001	1.3	-0.50 – 2.10	**0.001**
Treatment – Nest	5.76	1.91 – 9.61	**0.003**	-2.31	-6.57 – 1.95	0.288	0.53	0.38 – 0.67	**<0.001**
Treatment – Refuse	5.06	1.47 – 8.66	**0.006**	1.99	-1.98 – 5.97	0.326	0.60	0.44 – 0.75	**<0.001**
Preserved	-0.91	-3.83 – 2.00	0.539	-0.96	-4.84 – 2.92	0.627	0.07	-0.07 – 0.22	0.309
Rainy	-2.50	-5.45 – 0.44	0.096	-1.07	-5.02 – 2.87	0.594	-0.04	-0.23 – 0.14	0.633
Treatment – Nest * Preserved	-3.26	-7.02 – 0.50	0.089	-4.03	-9.77 – 1.70	0.168	0.02	-0.20 – 0.24	0.849

Treatment – Refuse * Preserved	-0.96	-4.59 – 2.68	0.607	1.45	-4.08 – 6.98	0.607	-0.00	-0.22 – 0.21	0.970
Treatment – Nest * Rainy	-0.06	-3.82 – 3.70	0.976	3.84	-2.18 – 9.87	0.211	0.10	-0.13 – 0.32	0.403
Treatment - Refuse * Rainy	-1.59	-5.22 – 2.05	0.392	0.04	-5.78 – 5.87	0.989	-0.12	-0.37 – 0.13	0.328
Preserved * Rainy	1.79	-1.24 – 4.83	0.247	0.74	-5.02 – 6.50	0.802	0.59	0.31 – 0.87	**<0.001**
Nest * Preserved * Rainy	0.27	-7.37 – 7.92	0.944	-1.41	-9.86 – 7.04	0.744	-0.80	-1.15 – -0.45	**<0.001**
Refuse * Preserved * Rainy	-2.66	-10.04 – 4.72	0.480	-0.56	-8.72 – 7.60	0.893	-0.53	-0.89 – -0.18	**0.003**

Legend of figures

Figure 1. Effects of land use (agriculture, pasture and conserved) and treatment (10 – and 5 - meters soils, nest mound soils and refuse soils) on the (A) total dry biomass, (C) plant dry root biomass, and (E) on the plant dry shoot biomass of plants growing in soils from nests of the LCA species *Atta opaciceps;* and effects of chronic disturbance, water deficit and treatment (control soils, nest mound soils and refuse dump soils) on the (B) total dry biomass, (D) plant dry root biomass, and (F) on the plant dry shoot biomass of plants growing in soils from nests of the LCA species *Acromyrmex balzani,* in the Catimbau National Park, Pernambuco, Brazil.

Figure 2. Effects of land use as regenerating forest after agriculture (agriculture), regenerating forest after pasture (pasture) and old growth forest stand (conserved), and treatment (10 – and 5 - meters soils, nest mound and refuse soils) on the (A) plant shoot length, (C) plant water content, and (E) on the leaf area of plants growing soils from nests of the LCA species *Atta opaciceps;* and effects of chronic disturbance and water deficit on old growth forest stands, and treatment (control soils, nest mound soils and refuse dump soils) on the (B) plant shoot length, (D) plant water content, and (F) on the leaf area of plants growing soils from nests of the LCA species *Acromyrmex balzani,* in the Catimbau National Park, Pernambuco, Brazil.

Figure 3. Effects of land use as regenerating forest after agriculture (agriculture), regenerating forest after pasture (pasture) and old growth forest stand (conserved), and treatment (10 – and 5 - meters soils, nest mound soils and refuse soils) on the plant concentration of the major elements (A) carbon, (C) nitrogen, (E) potassium and (G) phosphorus, of plants growing soils from nests of the LCA species *Atta opaciceps;* and effects of chronic disturbance and water deficit on old growth forest stands, and treatment (control soils, nest mound soils and refuse dump soils) on the plant concentration of (B) carbon, (D) nitrogen, (F) potassium, and (H) phosphorus of plants growing soils from nests of the LCA species *Acromyrmex balzani,* in the Catimbau National Park, Pernambuco, Brazil.

Figure 1

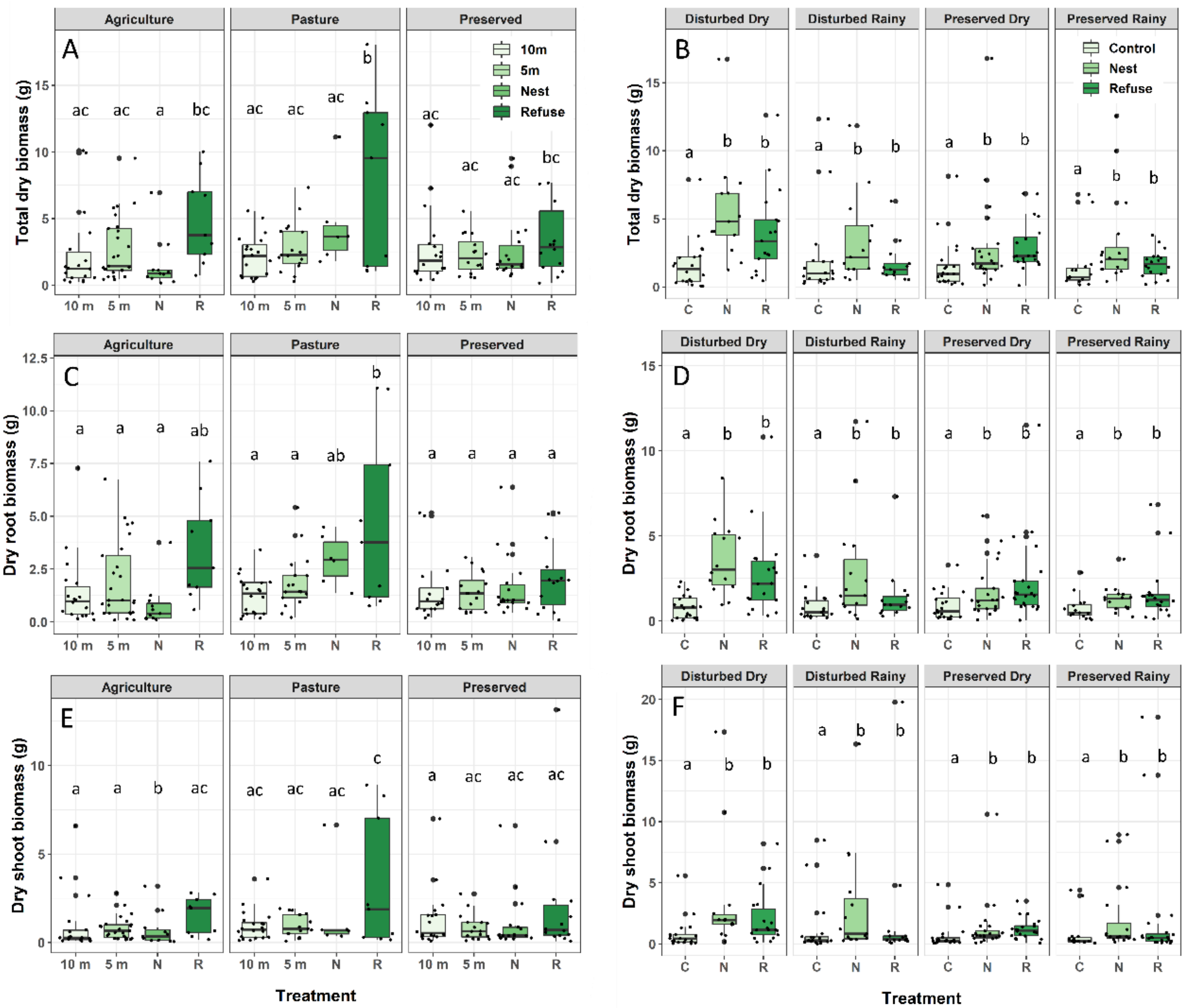

A
Agriculture
Pasture
Preserved
10m
5m
Nest
Refuse
Total dry biomass (g)
10 m
5 m
N
R
B
Disturbed Dry
Disturbed Rainy
Preserved Dry
Preserved Rainy
Control
Nest
Refuse
C
N
R
C
Dry root biomass (g)
D
E
Dry shoot biomass (g)
Treatment
F
Treatment

Figure 2

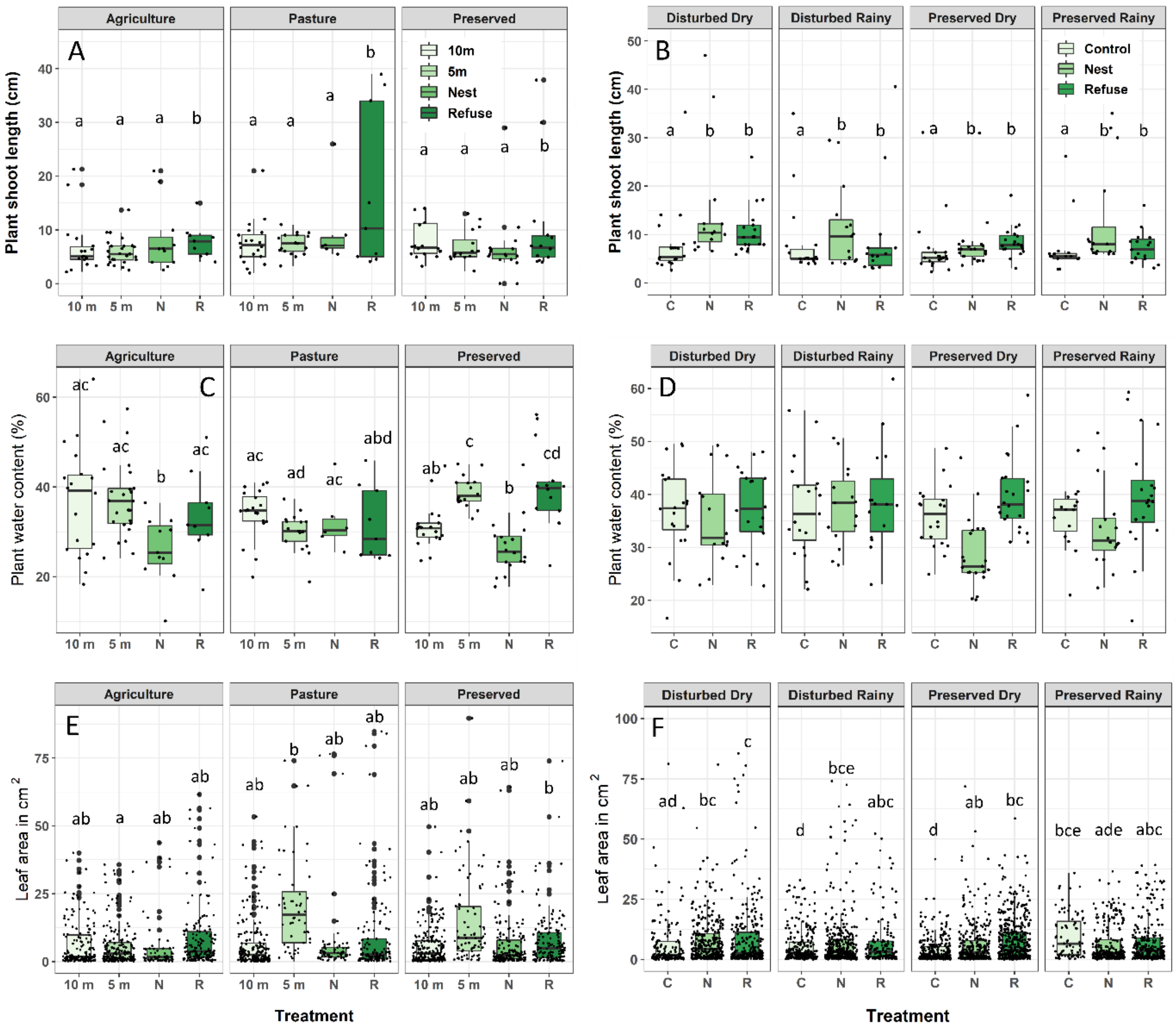
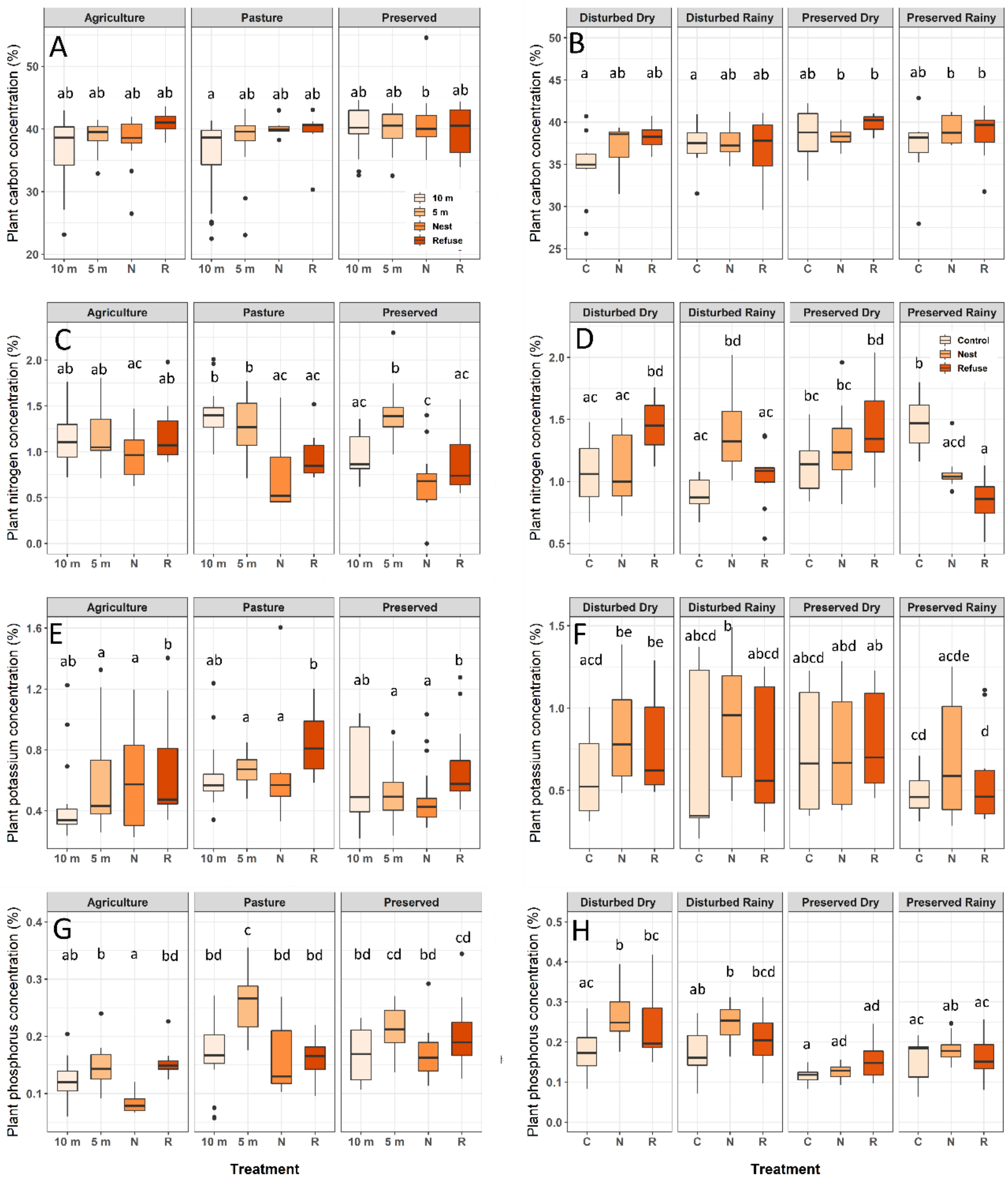

Agriculture
Pasture
Preserved
Disturbed Dry
Disturbed Rainy
Preserved Dry
Preserved Rainy
A
B
C
D
E
F
10m
5m
Nest
Refuse
Control
Plant shoot length (cm)
Plant water content (%)
Leaf area in cm 2
Treatment

Figure 3



Agriculture
Pasture
Preserved
Disturbed Dry
Disturbed Rainy
Preserved Dry
Preserved Rainy
A
B
C
D
E
F
G
H
Plant carbon concentration (%)
Plant nitrogen concentration (%)
Plant potassium concentration (%)
Plant phosphorus concentration (%)
10 m
5 m
Nest
Refuse
Control
Treatment

Supplementary Information

Effects of leaf-cutting ant nests under human disturbance and aridity on plant growth and nutrient uptake in Caatinga

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Table S1. Results of Linear Mixed Models testing the effects of land use in the nest and non-nest soils (treatment) of the LCA species *Atta opaciceps* on the plant concentration of carbon, nitrogen, magnesium, calcium and potassium (upper table); and the testing the effects chronic disturbance and water deficit in the nest and non-nest soils (treatment) of the LCA *Acromyrmex bazalni* on the plant concentration of carbon, nitrogen, magnesium, calcium and potassium (lower table), in Catimbau National Park, Pernambuco, Brazil. We consider significant effects when their confidence intervals did not include zero.

At. opaciceps nests	C concentration			N concentration			Mg concentration			Ca concentration			K concentration		
Predictors	β	CI	p	β	CI	p	β	CI	p	β	CI	p	β	CI	p
(Intercept)	360.3	308 – 412.6	**0.001**	12.2	9.3 – 15.	**<0.001**	1.91	0.58 – 3.24	**0.005**	9.08	6.07 – 12.1	**<0.001**	6.97	3.42 – 10.53	**<0.001**
Pasture	-3.64	-26.6 – 19.3	0.757	2.15	0.49 – 3.8	**0.011**	-0.17	-0.45 – 0.11	0.245	0.48	-1.52 – 2.49	0.636	1.57	0.55 – 2.58	**0.002**
Preserved	27.06	3.20 – 50.92	**0.026**	-1.8	-3.5 – -0.08	**0.04**	-0.08	-0.37 – 0.20	0.571	1.65	-0.39 – 3.68	0.113	0.8	-0.22 – 1.83	0.126
5-meters	22.41	-0.04 – 44.8	0.056	0.24	-1.38 – 1.8	0.773	0.01	-0.26 – 0.29	0.92	1.41	-0.53 – 3.36	0.155	1.01	0.02 – 1.99	**0.045**
Nest	5.37	-20.6 – 31.3	0.685	-1.74	-3.6 – 0.12	0.067	-0.34	-0.67 – -0.02	**0.039**	-1.78	-4.09 – 0.54	0.132	-0.24	-1.41 – 0.94	0.694
Refuse	35.51	8.16 – 62.85	**0.011**	0.65	-1.3 – 2.62	0.514	-0.02	-0.34 – 0.31	0.926	-0.07	-2.38 – 2.25	0.955	1.7	0.53 – 2.87	**0.005**
Pasture * 5-meters	-14.61	-47.1 – 17.9	0.379	-0.47	-2.81 – 1.8	0.695	0.07	-0.33 – 0.47	0.727	-0.36	-3.18 – 2.46	0.801	0.58	-0.85 – 2.01	0.425
Preserved * 5-meters	-19.1	-52.6 – 14.3	0.262	4.5	2.09 – 6.91	**<0.001**	0	-0.40 – 0.39	0.983	-1.4	-4.23 – 1.43	0.333	-0.33	-1.77 – 1.10	0.65
Pasture * Nest	21.94	-18.5 – 62.4	0.288	-3.72	-6.6 – -0.8	**0.012**	0.55	0.06 – 1.03	**0.027**	1.66	-1.81 – 5.12	0.348	1.17	-0.58 – 2.93	0.19
Preserved * Nest	5.83	-29.7 – 41.3	0.748	-1.09	-3.64 – 1.4	0.405	0.23	-0.21 – 0.66	0.306	1.63	-1.44 – 4.70	0.299	-0.02	-1.58 – 1.54	0.982
Pasture * Refuse	-9.45	-48.9 – 30	0.639	-4.66	-7.5 – -1.8	**0.001**	-0.05	-0.52 – 0.42	0.833	-2.84	-6.17 – 0.48	0.094	-0.15	-1.84 – 1.53	0.857
Preserved * Refuse	-39.5	-76.6 – -2.4	**0.037**	-1.49	-4.16 – 1.1	0.273	-0.15	-0.59 – 0.29	0.498	-2.22	-5.35 – 0.92	0.166	-0.69	-2.28 – 0.89	0.393

Ac. balzani nests	C concentration			N concentration			Mg concentration			Ca concentration			K concentration		
Predictors	β	CI	p	β	CI	p	β	CI	p	β	CI	p	β	CI	p
(Intercept)	352.83	324.5 – 381.1	**0.001**	9.89	6.94 – 12.8	**<0.001**	1.88	0.30 – 3.47	**0.02**	10.08	7.66 – 12.50	**<0.001**	7.12	3.09 – 11.15	**0.001**
Preserved	22.58	5.41 – 39.75	**0.01**	1.34	-0.45 – 3.13	0.143	0.09	-0.27 – 0.44	0.635	-1.03	-3.25 – 1.19	0.363	0.59	-0.55 – 1.72	0.312
Nest	19.13	1.06 – 37.20	**0.038**	0.17	-1.61 – 1.95	0.851	0.42	-0.00 – 0.85	0.051	0.96	-1.06 – 2.99	0.352	2.32	1.12 – 3.51	**<0.001**
Refuse	32.76	13.87 – 51.6	**0.001**	3.53	1.63 – 5.42	**<0.001**	0.57	0.11 – 1.02	**0.014**	4.23	1.89 – 6.58	**<0.001**	2.39	1.14 – 3.63	**<0.001**
Rainy	5.97	-11.5 – 23.4	0.504	-0.79	-2.64 – 1.06	0.405	0.33	-0.03 – 0.69	0.074	-1.11	-3.07 – 0.85	0.267	0.56	-0.60 – 1.72	0.343
Preserved * Nest	-5.8	-27.06 – 15.46	0.593	0.33	-2.20 – 2.87	0.796	-0.56	-1.17 – 0.05	0.070	0.2	-2.19 – 2.60	0.869	-1.85	-3.77 – 0.08	0.060
Preserved * Refuse	-4.14	-25.61 – 17.33	0.706	-1.09	-3.68 – 1.50	0.411	-0.21	-0.66 – 0.23	0.344	-0.6	-3.00 – 1.80	0.625	-1.99	-3.96 – -0.03	**0.047**

Preserved * Rainy	-25.87	-57.06 – 5.33	0.104	3.94	1.24 – 6.65	**0.004**	-0.37	-0.99 – 0.25	0.236	-1.21	-4.56 – 2.14	0.480	-3.10	-5.15 – -1.05	**0.003**
Nest * Rainy	0.39	-20.91 – 21.69	0.971	4.05	1.48 – 6.61	**0.002**	0.05	-0.39 – 0.49	0.828	-0.42	-2.82 – 1.98	0.732	-0.39	-2.33 – 1.56	0.697
Refuse * Rainy	-21.1	-42.62 – 0.42	0.055	-2.71	-5.33 – -0.08	**0.044**	-0.5	-0.95 – -0.06	**0.026**	-6.80	-10.05 – -3.54	**<0.001**	-2.96	-4.96 – -0.97	**0.004**
Preserved * Nest * Rainy	15.50	-27.84 – 58.83	0.483	-8.36	-12.12 – -4.60	**<0.001**	-0.05	-0.96 – 0.86	0.916	1.36	-3.33 – 6.05	0.570	1.17	-1.70 – 4.05	0.424
Preserved * Refuse * Rainy	33.15	-9.90 – 76.19	0.131	-6.17	-9.90 – -2.43	**0.001**	0.32	-0.57 – 1.21	0.482	7.20	2.58 – 11.83	**0.002**	2.47	-0.36 – 5.30	0.087

Table S1. Results of Linear Mixed Models testing the effects of land use in the nest and non-nest soils (treatment) of the LCA species *Atta opaciceps* on the plant concentration of phosphorus, zinc, iron and manganese (upper table); and the testing the effects chronic disturbance and water deficit in the nest and non-nest soils (treatment) of the LCA *Acromyrmex bazalni* on the plant concentration of phosphorus, zinc, iron and manganese (lower table), in Catimbau National Park, Pernambuco, Brazil. We consider significant effects when their confidence intervals did not include zero.

At. opaciceps nests	P concentration			Zn concentration			Fe concentration			Mn concentration		
Predictors	β	CI	p	β	CI	p	β	CI	p	β	CI	p
(Intercept)	1.2	0.97 – 1.44	**<0.001**	0.06	0.05 – 0.07	**<0.001**	1.04	0.69 – 1.40	**<0.001**	0.27	0.04 – 0.50	**0.024**
Pasture	0.43	0.14 – 0.71	**0.003**	-0.03	-0.03 – -0.02	**<0.001**	-0.42	-0.62 – -0.23	**<0.001**	-0.05	-0.13 – 0.03	0.187
Preserved	0.46	0.17 – 0.76	**0.002**	0	-0.01 – 0.01	0.648	-0.21	-0.41 – -0.01	**0.044**	0.07	-0.01 – 0.15	0.109
5-meters	0.23	-0.05 – 0.51	0.11	0	-0.01 – 0.00	0.496	-0.32	-0.51 – -0.13	**0.001**	0.07	-0.00 – 0.15	0.065
Nest	-0.38	-0.71 – -0.05	**0.024**	-0.01	-0.02 – 0.00	0.056	0.04	-0.18 – 0.27	0.713	0.24	0.15 – 0.33	**<0.001**
Refuse	0.31	-0.03 – 0.64	0.071	-0.01	-0.02 – 0.00	0.062	0.19	-0.04 – 0.42	0.099	-0.07	-0.17 – 0.02	0.118
Pasture * 5-meters	0.64	0.24 – 1.04	**0.002**	0.01	-0.00 – 0.02	0.17	0.31	0.04 – 0.59	**0.026**	-0.02	-0.13 – 0.10	0.79
Preserved * 5-meters	0.16	-0.25 – 0.56	0.453	0	-0.01 – 0.01	0.514	0.31	0.04 – 0.59	**0.027**	-0.15	-0.26 – -0.03	**0.011**
Pasture * Nest	0.36	-0.14 – 0.85	0.158	0.01	-0.01 – 0.02	0.36	0.06	-0.28 – 0.40	0.713	-0.21	-0.35 – -0.07	**0.003**
Preserved * Nest	0.36	-0.08 – 0.79	0.107	0	-0.01 – 0.01	0.674	0.18	-0.12 – 0.48	0.246	-0.29	-0.41 – -0.17	**<0.001**
Pasture * Refuse	-0.37	-0.85 – 0.10	0.124	0.01	-0.01 – 0.02	0.279	0.14	-0.18 – 0.47	0.395	0.04	-0.09 – 0.17	0.573
Preserved * Refuse	0.02	-0.43 – 0.47	0.924	0.01	-0.01 – 0.02	0.344	-0.07	-0.38 – 0.24	0.648	-0.07	-0.19 – 0.06	0.306

Ac. balzani nests	P concentration			Zn concentration			Fe concentration			Mn concentration		
Predictors	β	CI	p	β	CI	p	β	CI	p	β	CI	p
(Intercept)	1.80	1.44 – 2.17	**<0.001**	0.06	0.04 – 0.07	**<0.001**	0.55	0.13 – 0.96	**0.010**	0.21	-0.01 – 0.43	0.063
Preserved	-0.64	-1.12 – -0.16	**0.009**	-0.00	-0.01 – 0.01	0.867	0.41	0.11 – 0.71	**0.007**	0.13	-0.02 – 0.25	**0.023**
Nest	0.83	0.35 – 1.30	**0.001**	0.01	-0.01 – 0.02	0.272	0.67	0.37 – 0.96	**<0.001**	-0.08	-0.18 – 0.03	0.14
Refuse	0.64	0.13 – 1.14	**0.014**	-0.01	-0.02 – 0.01	0.292	-0.02	-0.34 – 0.29	0.886	-0.03	-0.14 – 0.07	0.543
Rainy	-0.02	-0.51 – 0.48	0.943	0.00	-0.01 – 0.01	0.916	0.26	-0.05 – 0.57	0.105	0.03	-0.07 – 0.13	0.544
Preserved * Nest	-0.68	-1.35 – 0.00	0.051	-0.01	-0.03 – 0.01	0.454	-0.25	-0.67 – 0.17	0.250	0.03	-0.09 – 0.15	0.593
Preserved * Refuse	-0.23	-0.93 – 0.46	0.515	0.01	-0.01 – 0.03	0.275	-0.07	-0.51 – 0.36	0.737	-0.11	-0.23 – 0.01	0.063

Preserved * Rainy	0.39	-0.33 – 1.11	0.291	0.01	-0.01 – 0.03	0.354	-0.60	-1.06 – -0.15	**0.009**	0.06	-0.12 – 0.23	0.524
Nest * Rainy	-0.10	-0.79 – 0.59	0.776	0.00	-0.02 – 0.02	0.779	-0.82	-1.25 – -0.39	**<0.001**	0.01	-0.11 – 0.13	0.878
Refuse * Rainy	-0.41	-1.11 – 0.30	0.260	0.02	-0.00 – 0.03	0.105	0.27	-0.17 – 0.71	0.234	0.01	-0.12 – 0.13	0.935
Preserved * Nest * Rainy	0.25	-0.76 – 1.26	0.629	-0.00	-0.03 – 0.03	0.917	0.46	-0.18 – 1.09	0.156	0.18	-0.06 – 0.43	0.147
Preserved * Refuse * Rainy	0.14	-0.87 – 1.14	0.792	-0.02	-0.04 – 0.01	0.268	-0.17	-0.80 – 0.45	0.589	0.09	-0.15 – 0.33	0.464

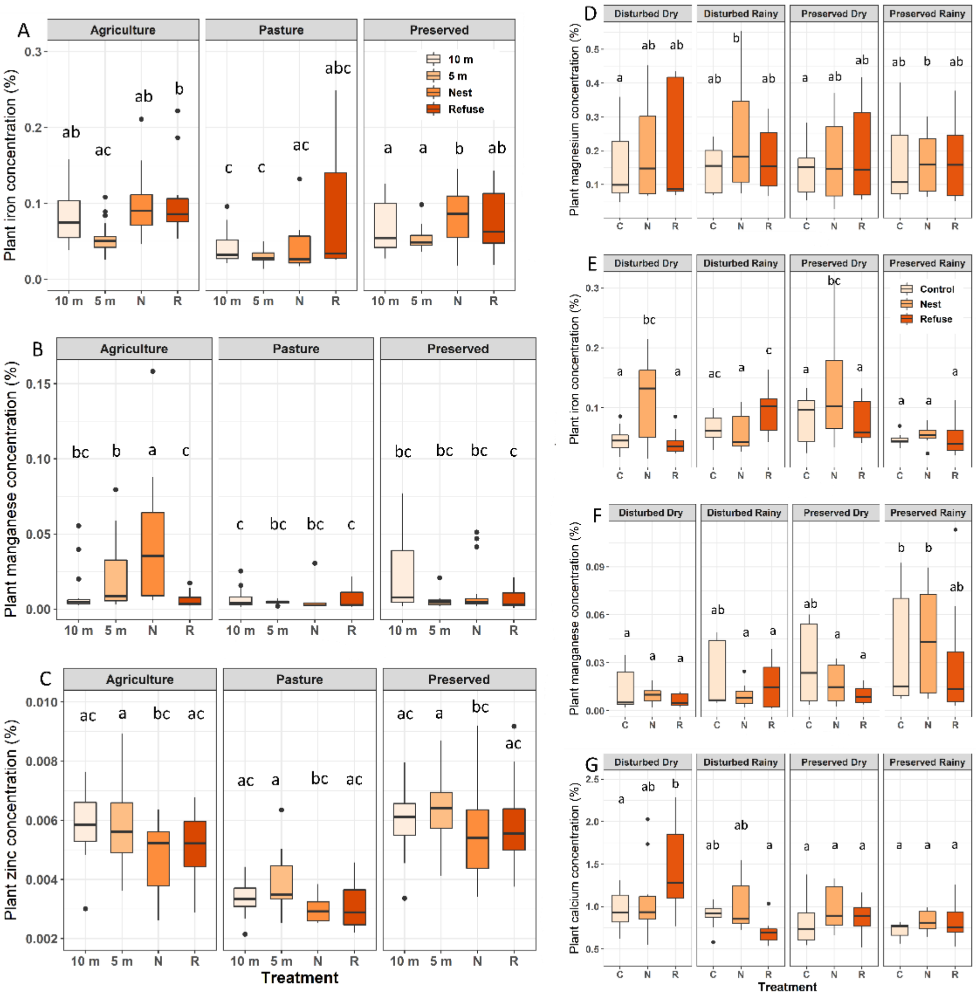

Figure S3. Effects of land use (agriculture, pasture and conserved) and treatment (10 – and 5 -meters soils, nest mound soils and refuse soils) on the plant concentration of the trace elements (A) iron, (B) manganese, (C) zinc of plants growing soils from nests of the LCA species *Atta opaciceps;* and effects of chronic disturbance, water deficit and treatment (control soils, nest mound soils and refuse dump soils) on the plant concentration of (D) magnesium, (E) iron, (F) manganese, and (G) calcium of plants growing soils from nests of the LCA species *Acromyrmex balzani*, in the Catimbau National Park, Pernambuco, Brazil.

5 CONCLUSÃO

Nossos resultados reconfirmam que as alterações ambientais causadas pelas perturbações humanas promovem o aumento das populações de formigas cortadeiras na Caatinga. Mas embora aumentem em densidade, as colônias mantêm suas taxas de forrageio constantes ao longo de áreas perturbadas e preservadas e secas e chuvosas. De forma similar, as mudanças físicas nos solos produzidas pelas colônias, como diminuição da densidade aparente do solo, são resultado de um efeito individual dos ninhos, sem influência de perturbação nem aridez. Já as mudanças edáficas químicas sofrem forte influência dos efeitos da perturbação e da aridez e podem ser muito complexas. No geral, os efeitos da perturbação podem potencializar os efeitos das colônias nos solos, aumentando a concentração de alguns nutrientes e diminuindo a concentração de outros, como já observado com efeitos de perturbação aguda (MOUTINHO, P.; NEPSTAD; DAVIDSON, 2003). A aridez, no entanto, parece exercer predominantemente um efeito negativo nas concentrações e nos estoques de nutrientes nos solos, com pouca relação com o efeito dos ninhos. A escassez de nutrientes nos solos relacionada com aridez é muito aguda o que pode tornar o efeito do ninho muito menos relevante em locais áridos. O aumento ou diminuição de nutrientes nos solos dos ninhos como um efeito das interações com perturbação, parecem ser resultado da atividade acumulada ao longo do tempo pelas colônias, já que a perturbação (ou aridez) não afetam as taxas de forrageio e nem a quantidade de nutrientes do material carregado. As mudanças edáficas promovidas pelas colônias refletem de forma positiva para as plantas, aumentando até duas vezes a biomassa de indivíduos crescendo em solos dos ninhos. Nesse caso, perturbação crônica e aridez não determinam as condições do solo para as plantas, mas perturbação aguda sim. A perturbação aguda é capaz de tornar o efeito do ninho nulo ou negativo para as plantas.

Em síntese, as colônias de cortadeiras do gênero *Acromyrmex* mantem as taxas de herbivoria de áreas mais conservadas e mais perturbadas. Como as áreas perturbadas tem menor disponibilidade de material vegetal para as colônias (SIQUEIRA *et al.*, 2018), isso pode representar uma maior pressão sobre a vegetação nessas áreas. O mesmo vale para áreas mais áridas onde a escassez de alimento força as colônias a aumentar suas área de forrageamento (SIQUEIRA *et al.*, 2018). As colônias não compensam esse potencial efeito negativo na vegetação de áreas perturbadas e áridas promovendo efeitos positivos no solo dessas áreas. Isso porque embora a quantidade de nutrientes do material carregado para as colônias não seja diferente de acordo com a área, esse material pode ser mais recalcitrante em áreas perturbadas e áridas (ABRIL; BUCHER, 2004). Dessa forma, (1) o material carregado pode ser de qualidade inferior e (2) estar sendo concentrado nas pequenas áreas restritas aos ninhos. Esses dois fatores podem contribuir para desacelerar a regeneração florestal (KNOECHELMANN *et al.*, 2020). Esses efeitos são mais severos para a vegetação crescendo em áreas que sofreram perda de cobertura vegetal, já que a diminuição de nutrientes é maior tanto nos solos sem influência do ninho (ARAÚJO-FILHO *et al.*, 2018; SANTANA *et al.*, 2019), como em solos sob influência dos ninhos (TADEY; FARJI-BRENER, 2007)

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ANEXO A - DECLARAÇÃO SOBRE PLÁGIO

Eu, **Pedro Elias Santos Neto**, autor da dissertação intitulada **"Efeito de perturbações antrópicas e níveis de precipitação nas interações entre plantas e insetos herbívoros na Caatinga”** defendida através do Programa de Pós-Graduação em Biologia Animal da Universidade Federal de Pernambuco, declaro que:

1. A pesquisa apresentada nesta dissertação, exceto onde especificado, representa minha pesquisa original.

2. Esta dissertação não foi submetida anteriormente para obtenção de nenhum grau em nenhuma outra instituição de ensino e pesquisa do país ou do exterior.

3. Esta dissertação não contem dados de terceiros, nem figuras, gráficos ou outras informações, a menos que devidamente especificado e devidamente creditado como sendo fornecido por outra pessoa.

4. Esta dissertação não contem material escrito por terceiros, a menos que devidamente especificado e creditado como sendo fornecido por outros pesquisadores. Onde material escrito por terceiros foi usado, eu:

4.1. Re-escrevi o texto, mas a informação passada foi devidamente referenciada.

4.2. Onde palavras exatas escritas por terceiros foram usadas, as mesmas foram marcadas no texto em itálico ou entre aspas e devidamente referenciadas.

5. Esta dissertação não contem texto, gráficos ou tabelas copiadas e coladas da internet, a menos que especificamente creditado, e a fonte original devidamente referenciada e datada na sessão de Referências Bibliográficas.

Recife ______ de _______________________ de __________.

____

Pedro Elias Santos Neto

ANEXO B – REGRAS DE SUBMISSÃO DA REVISTA “SOIL BIOLOGY AND BIOCHEMISTRY”

Introduction

This journal is a forum for research on soil organisms, their biochemical activities and their influence on the soil environment and plant growth. It publishes original work on quantitative, analytical and experimental aspects of such research. Soil biology and soil biochemistry cover many scientific disciplines but a single journal brings together the results and views of research workers working in a wide variety of research areas. The scope of this journal is wide and embraces accounts of original research on the biology, ecology and biochemical activities of all forms of life that exist in the soil environment. Some of the subjects which have proved to be prominent are the biological transformations of plant nutrients in soil, nitrogen fixation and denitrification, soil-borne phases of plant parasites, the ecological control of soil-borne pathogens, the influence of pesticides on soil organisms, the biochemistry of pesticide and pollution decomposition in soil, microbial aspects of soil pollution, the composition of soil populations, modelling of biological processes in soil systems, the biochemical activities of soil organisms, soil enzymes and the interactions of soil organisms with plants and the effects of tillage on soil organisms and soil biochemistry. **Sequence data** Papers dealing with amino acid sequences of proteins or with nucleotide sequences must carry a statement that the data have been deposited with an appropriate data bank, e.g., the European Molecular Biology Laboratory (EMBL) or GenBank Data Libraries. The data base accession number must be given at the end of the Materials and Methods section of the manuscript under the separate heading 'Accession numbers'. For example: Coordinates and structure factors have been deposited in the Protein Data Bank with accession number 2XYZ. Lengthy nucleotide sequences will be published only if, in the judgement of the Editorial Board, these results are of general interest and importance.

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3. Review articles*. Review articles are welcome but should be topical and involve critique and synthesis, and should not just comprise an overview or summary of extant literature. Before submission please contact the Review Paper Co-ordinators with an outline of your proposed review, via the SBB mailbox soilbb@elsevier.com

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*Review Articles For Soil Biology And Biochemistry

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The review might conclude with a set of hypotheses for future work that could be tested either using available technology or for which current technology could be improved.

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ANEXO C – REGRAS DE SUBMISSÃO DA REVISTA "PLANT AND SOIL"

Instructions for authors

Manuscript Submission

Manuscript Submission

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All figures and tables must be embedded in the manuscript text.

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Authors should avoid the following when preparing their manuscript for submission to Plant and Soil:

1. avoid use of inappropriate terminology (e.g., P2O5, K2O; 'bases' instead of 'cations') and non-SI units (e.g., miles, acres) – for more information, please refer to the Editorial "P2O5, K2O, CaO, MgO, and basic cations: pervasive use of references to molecules that do not exist in soil" (https://doi.org/10.1007/s11104-020-04593-2).

2. avoid fresh-weight root measurements when reporting the effect of plant growth-promoting (rhizo)bacteria on growth promotion of plants.

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International standards for authors

Plant and Soil endorses the following position statement on international standards for authors, developed at the 2nd World Conference on Research Integrity in Singapore in July 2010 and published on the website of the Committee on Publication Ethics

COPE

Wager E & Kleinert S (2011) Responsible research publication: international standards for authors. A position statement developed at the 2nd World Conference on Research Integrity, Singapore, July 22-24, 2010. Chapter 50 in: Mayer T & Steneck N (eds) Promoting Research Integrity in a Global Environment. Imperial College Press / World Scientific Publishing, Singapore (pp 309-16). (ISBN 978-981-4340-97-7)

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- Abstract

- Abbreviations

- Introduction

- Materials and Methods

- Results

- Discussion

- Acknowledgements

- References

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- Conclusion

For life science journals only (when applicable)

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Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

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- The Latin name(s) as well as authority (and, where appropriate, cultivar preceded by cv.) of investigated species must be mentioned both in the Abstract and in the Materials and Methods. Latin genus and species names should be italicised. Only SI units should be used.
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References

Citation

Cite references in the text in alphabetical order first, and chronological order second. Some examples:

1. Negotiation research spans many disciplines (Thompson 1990).

2. This result was later contradicted by Becker and Seligman (1996).

3. This effect has been widely studied (Abbott 2002; Barakat et al. 1995a, b; Kelso and Smith 1990; Medvec et al. 1999, 2000).

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- Journal article

  Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8

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  Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. https://doi.org/10.1007/s001090000086

- Book

  South J, Blass B (2001) The future of modern genomics. Blackwell, London

- Book chapter

  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

- Online document

  Doe J (1999) Title of subordinate document. In: The dictionary of substances and their effects. Royal Society of Chemistry. Available via DIALOG. http://www.rsc.org/dose/title of subordinate document.

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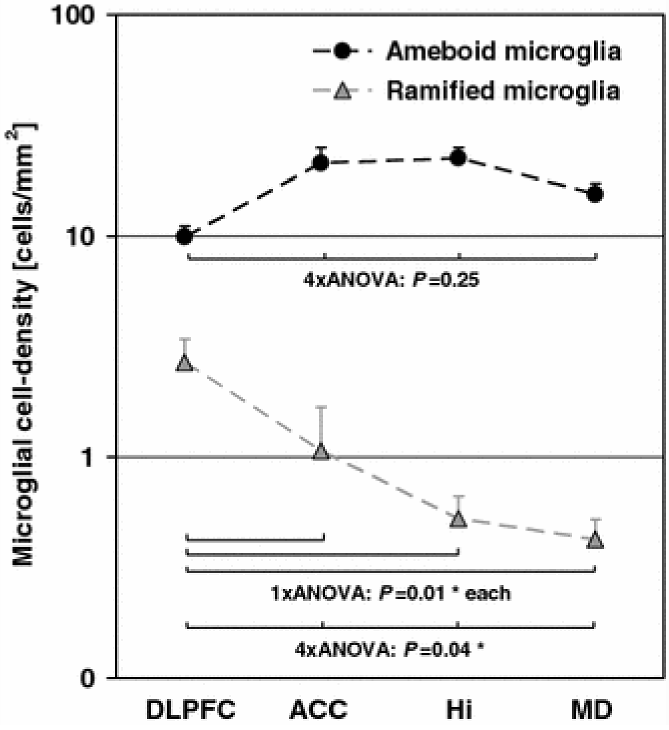

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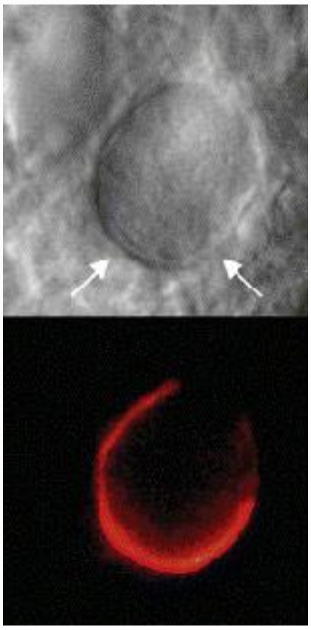

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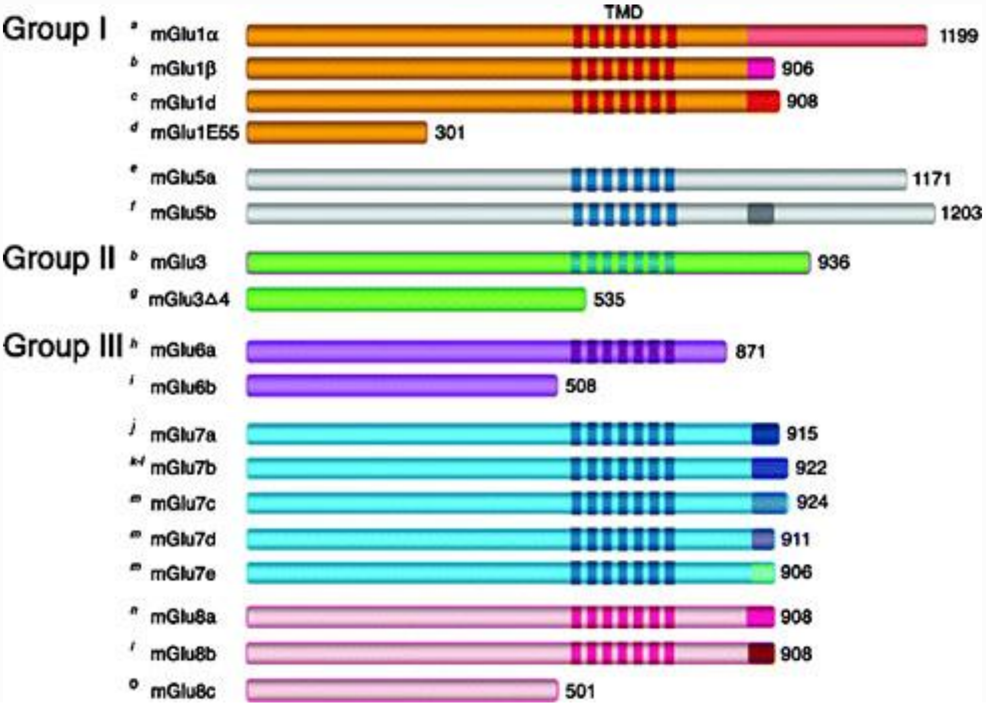

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