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Herbivory and litter production in response to nutrient addition in "Terra Firme" forest in Central Amazon

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Manaus, Amazonas

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Sinopse:

O foco do trabalho é entender o papel das limitações nutricionais na produtividade do dossel, perdas herbívoras e investimentos em compostos secundários em escala ecossistêmica na Amazônia Central. Nossos resultados sugerem que a produção de serapilheira, folhas e herbivoria apresentaram influência com rápida resposta inicial à adição de nutrientes.

Palavras-chave: Amazônia, serapilheira, cicla gem de nutrientes, herbivoria, fertilização.

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Para minha momis, papis, queridos irmãos Harley’s

E minha anja Eliana,

sei que a senhora está me vendo e me apoiando.

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Resumo

Título: Herbivoria e produção de serapilheira em resposta a adição de nutrientes em floresta de terra firme não alagada na Amazônia Central.

O papel dos herbívoros na determinação da produtividade das florestas tropicais, balanço de carbono e ciclagem de nutrientes é incerto na Amazônia. O consumo de folhas por herbívoros e sua consequente excreção podem representar um fluxo adicional da serapilheira, muitas vezes não documentado, de nutrientes e carbono no ecossistema. O foco desta pesquisa foi compreender o papel das limitações nutricionais sobre a produtividade do dossel no nível do ecossistema, levando em consideração as perdas por herbivoria e investimento de folhas em compostos secundários em uma floresta de terra firme na Amazônia Central. O local do estudo foi no projeto AFEX/ PDBFF, um experimento em larga escala que visa compreender as respostas do ecossistema à limitação de nutrientes no solo. Os tratamentos consistiram na adição de nitrogênio (N), fósforo (P), cátions, N + P, N + cátions, P + cátions, N + P + cátions e controle. Os oito tratamentos foram distribuídos em quatro blocos, representando o desenho fatorial completo (n = 32 parcelas). A serapilheira foi coletada quinzenalmente por oito meses (julho/ 2017 e fevereiro/ 2018), utilizando cinco coletores de serapilheira suspensas a um metro de altura, com área de 0,25 m² cada, totalizando 160 coletores, distribuídos na área central da parcela de 30 x 30 m. Biomassa de serapilheira, área foliar e perda de área foliar, macro e micronutrientes, lignina, celulose e polifenóis foram determinados por análises químicas. Encontramos uma tendência para o aumento da produção de biomassa de serapilheira no tratamento com N + P durante a estação seca de julho a agosto/ 2017, e obteve maior input de nutrientes retornando aos solos. Houve um forte aumento nas concentrações de fósforo, magnésio, cálcio, potássio e manganês nas folhas de serapilheira em parcelas onde o fósforo foi adicionado. Em contraste com as fortes mudanças nas concentrações de nutrientes com a fertilização, não houve diferenças significativas na concentração de compostos de defesa. Como resultado, não houve diferenças significativas na porcentagem de área foliar perdida para herbívoros (variando de 9 a 10%) entre os tratamentos. Também não houve diferença significativa na quantidade de biomassa de herbivoria, porém houveram diferenças significativas de nutrientes de fezes de insetos, nos tratamentos com adição de P. Juntas, as respostas iniciais da produção de serapilheira fina sugerem mudanças rápidas nos inputs de nutrientes da serapilheira, com respostas mais lentas das defensas anti-herbivoria juntamente com herbivoria às adições de nutrientes.

Palavras-chave: Amazônia, serapilheira, ciclagem de nutrientes, herbivoria, fertilização.

Abstract

Title: Herbivory and litter production in response to nutrient addition in a *terra firme* forest in Central Amazonia.

The role of herbivores in determining tropical forest productivity, carbon balance and nutrient cycling is uncertain in the Amazon. Leaf consumption by herbivores and their consequent excretion may represent an additional, but often undocumented, flow of nutrients and carbon into the ecosystem. The focus of this research was to understand the role of nutritional limitations on canopy productivity at the ecosystem level, taking into account losses by herbivory and leaf investment in secondary compounds in a "terra firme" forest in Central Amazonia. The study site was the AFEX project, a large scale experiment that aims to understand ecosystem responses to soil nutrient limitation. The treatments consisted in the addition of nitrogen (N), phosphorus (P), cations, N + P, N + Cations, P + Cations, N + P + Cations and control plots. The eight treatments were distributed in four blocks, where each block contains eight plots representing the complete factorial design (n= 32 plots). Litter was sampled biweekly for eight months (between July/ 2017 and February/ 2018), using five suspended littertraps at one meter height, with an area of 0.25m² each, for a total of 160 collectors. Littertraps were distributed in the central 30 x 30 m area of each plot. Litterfall biomass, leaf area and leaf area loss, macro and micronutrient and lignin, cellulose and polyphenols were determined. We found a trend for increased production of leaf litter biomass in the N + P treatment during the dry season from July to August/2017 (dry season). This increase in N+P, translated through litterfall with more nutrients inputs returning to soils. There was a strong increase in phosphorus, magnesium, calcium, potassium and manganese concentrations in the litter leaves in plots where phosphorus was added. In contrast to the strong changes in nutrient concentrations with fertilization, there were no significant differences in the concentration of defense compounds. Furthermore, there were no significant differences in the percent leaf area lost to herbivores (varying from 9 to 10%) among the treatments. There was also no significant difference in the biomass of herbivore frass. However, there were significant differences of nutrients in the frass, mostly in treatments with P addition. Together, the intial responses of fine litterfall production suggest rapid changes in litter nutrient inputs, but slower responses of herbivores and anti-herbivore defenses to nutrient additions.

Key-words: aboveground productivity, Amazon, herbivory, fertilization, largescale experiment, litterfall, nutrient cycling, nutrient limitation, tropical forest.

1. Introdução

As florestas tropicais desempenham um papel fundamental no ciclo global do carbono (C) (Cramer et al., 2004), com a própria floresta amazônica respondendo por cerca de 25% da produtividade primária líquida global. Toda a bacia amazônica cobre 600 milhões de hectares, com 390 bilhões de árvores, que armazenam cerca de 120 petagramas de C (Pan et al., 2013; Fauset et al., 2015; Zhao e Running, 2011). Os solos ao longo da bacia amazônica variam muito em geologia e disponibilidade de nutrientes, com um claro gradiente de fertilidade aumentando de leste para oeste, esta variação pode afetar a produtividade florestal (Quesada et al., 2010, 2011, 2012).

A idade do solo e as condições climáticas quentes e úmidas da bacia Amazônica Central contribuem para a transformação do material de origem, gerando solos altamente intemperizados. A concentração de nutrientes derivados da rocha, como fósforo (P), cálcio (Ca), magnésio (Mg) e potássio (K), tendem a diminuir, tornando-se gradativamente menos disponíveis e lixiviados, limitando sua disponibilidade às plantas (Quesada et al. al., 2010; 2011). Em contraste, as entradas de nitrogênio (N) no solo ocorrem através da fixação atmosférica de N_2 pelos microrganismos do solo, resultando em acúmulo de N ao longo do tempo. Assim, em solos de florestas tropicais antigas, o N é encontrado em grandes quantidades em comparação com outros nutrientes do solo, sugerindo que as florestas tropicais da Amazônia não são limitadas pelo N (Quesada e Lloyd, 2016; Lambers et al., 2008) .

Em solos amazônicos de baixa fertilidade, a conservação eficiente e o ciclo de nutrientes é importante para a manutenção do funcionamento da floresta, que é em grande parte devido à decomposição da serapilheira (Vitousek , 1984). A produção e a rápida decomposição da serapilheira são processos críticos para o ciclo de nutrientes e a transferência de energia entre plantas e solo (Quesada et al., 2010; Sayer et al., 2012). Um ciclo de nutrientes rápido e eficiente também ajuda a reduzir a lixiviação, impedindo que a floresta em solos de baixa fertilidade se tornem ainda mais pobres em nutrientes (Went e Stark, 1968; Luizão , 1989).

A serapilheira é definida como a camada de matéria orgânica encontrada em diferentes estágios de decomposição, formada por folhas, flores, frutos e pequenos ramos (DAP <2 cm) (Luizão, 1989; Sayer, 2006). Portanto, a serapilheira é considerada o fornecedor de todos os elementos que as plantas precisam para o crescimento, funcionando como um sumidouro e como uma fonte de nutrientes nas florestas (Tobon et al., 2004; Brancalion et al., 2012; al., 2012).

A produção de serapilheira varia durante o ano com padrões sazonais de precipitação e é afetada por eventos de seca e chuvas (Wu et al., 2016). Segundo Wu (2016), a produção de serapilheira é baixa na Amazônia central durante a estação chuvosa (novembro a maio) e a produção de serapilheira principalmente nos meses secos (agosto e setembro) tem alta liberação de nova folha. O aumento da irradiância durante o período de baixa precipitação coincide com o pico de produção de novas folhas para diversas espécies, devido à resposta da vegetação ao estresse hídrico, uma vez que a perda de folhas reduz a perda de água pela transpiração (Martins e Rodrigues, 1999). Durante a estação seca, a capacidade fotossintética do dossel e a produtividade primária também aumentaram com a expansão da área foliar (Restrepo- Coupe et al., 2013 , Albert et al., 2018). No final da estação chuvosa, o declínio da taxa fotossintética ocorre devido à senescência foliar, onde os nutrientes são reabsorvidos ou remobilizados antes da abcisão das folhas, uma importante estratégia nos solos inférteis para a conservação de nutrientes (Huete et al., 2006 ; Vergutz et al. 2012).

A produtividade florestal é influenciada por fatores como a luz, a fenologia foliar, a disponibilidade de água e nutrientes (Restrepo- Coupe et al., 2013, Borchert et al., 2015). Para executar as várias funções, as plantas precisam de um conjunto de diferentes nutrientes. O nitrogênio é essencial no metabolismo vegetal, regulando a fotossíntese, a assimilação de carbono na enzima rubisco, a manutenção celular, um componente importante dos ácidos nucléicos, aminoácidos e proteínas, e alguns metabólitos secundários, bem como a degradação de substâncias (Taiz e Zeiger , 2009; Marenco e Lopes, 2011; Lawlor, 2002). Os cátions funcionam como ativadores enzimáticos nas plantas e também fazem parte da composição das membranas celulares. Mais especificamente, o cálcio participa da síntese dos tecidos da parede celular e auxilia nas divisões celulares (Taiz e Zeiger , 2009). O magnésio desempenha um papel importante na ativação de enzimas envolvidas na respiração, fotossíntese e síntese de DNA e RNA, sendo também parte da estrutura da clorofila (Taiz e Zeiger , 2009). O potássio é responsável pelo movimento eletroquímico e pela abertura das células estomáticas (Nunes et al., 2013).

Além disso, uma variedade de compostos orgânicos também são produzidos pelas plantas, chamados metabólitos secundários (terpenos e fenóis), e sua produção é influenciada pelos recursos disponíveis (Coley, 1985). Esses metabólitos, também conhecidos como compostos de defesa, podem interagir nas funções reprodutivas das plantas, atraindo polinizadores, afetando a competição planta-planta e atuando como defensivos químicos contra herbívoros e defesas estruturais diminuindo a palatabilidade. Esses dois tipos de compostos de defesa ocorrem na planta. variando de acordo com o

ambiente e idade da folha da planta (Coley et al., 1985; Endara e Coley, 2011 ; Zangerl et al., 2002).

Compostos de defesa da planta podem ser classificados como móvel e não-móvel e a sua abundância em folhas varia com a idade da planta e renovação do tecido (Coley et ai., 1985). As defesas móveis, como alcalóides, taninos e fenóis, exigem alto investimentometabólico , mas têm a vantagem de serem remobilizadas antes da senescência foliar (Chapin, 1980). Lignina e celulose são compostos estruturais de plantas que agem para aumentar a dureza foliar com a maturação, reduzindo a palatabilidade foliar de insetos herbívoros, mas estes são compostos não móveis e não podem ser remobilizados dentro da planta (Coley et al., 1985 ; Zangerl et al. al., 2002).

Outra característica importante da função foliar no crescimento das plantas e um importante indicador das estratégias de defesa das plantas é a massa foliar por unidade de área (LMA) (Lambers e Poorter , 1992; Grime, 2001; Westoby et al., 2002). O LMA está correlacionada com características fisiológicas e influenciada por recursos ambientais no campo (Wright et al., 2004). Quando os componentes das características da folha (por exemplo, compostos de defesa) estão em maior quantidade por alguma razão ambiental, a concentração por unidade seria duas vezes maior para todos os constituintes, dobrando a LMA (Poorter e Bergkotte , 1992; Van Arendonk e Poorter , 1994). Juntamente com as defesas secundárias, folhas com alta LMA parecem ter uma melhor defesa contra herbívoros e perigos físicos (Onoda et al., 2017). Mesmo pequenas concentrações de defesas secundárias que dificilmente afetam o LMA podem restringir significativamente o conjunto herbívoro capaz de se alimentar de certas folhas (Coley, 1983).

Herbivoria é o consumo de tecidos vegetais por animais. Existem diferentes tipos de herbivoria que podem variar entre mamíferos e insetos. Mamíferos maiores (como macacos e preguiças) representam 25% dos vertebrados herbívoros, e seu consumo é difícil de medir porque eles removem completamente ramos e folhas (Metcalfe et al., 2014). Os insetos, com cerca de 500.000 espécies, são considerados os principais herbívoros, pois representam cerca de 75% da herbivoria (Metcalfe et al., 2014, Herrera e Pellmyr , 2002, Thomanzini eThomanzini 2000, Novontny e Missa 2000). Na maioria dos ecossistemas, os tipos mais comuns de herbivoria podem ser identificados como sugadores foliares ou consumidores de tecido foliar (Hochuli, 2001). A herbivoria é maior em folhas jovens que crescem durante a estação seca, sendo o principal substrato para herbívoros (Coley e Barone 1996, Wu et al., 2016). O dano total na área foliar é de cerca de 12% na floresta tropical, com cerca de 0,0003-0,8% da superfície foliar sendo consumida por dia, dependendo da espécie arbórea (Coley, 1983; Coley e Barone, 1996). Em florestas montanhosas ao longo de um transecto entre a Amazônia e os Andes, a herbivoria tem mostrado afetar de 12 a 19% da produtividade das folhas (Metcalfe et al.,

2014). O principal fator que controlou a variação nas taxas de herbivoria com elevação (200 a 3400 manm .) Foi a diferença na temperatura e na concentração foliar de nutrientes entre as florestas (Metcalfe et al., 2014).

As plantas também podem adotar mecanismos para evitar perdas de nutrientes da herbivoria, tais como rápida expansão da folha e maturação do cloroplasto durante a expansão, produção de folhas síncronas, compostos de defesa e várias formas de mutualismo e antagonismo (Coley e Barone, 1996). Um estudo de Werner e Homeier (2015) em uma floresta tropical de altitude mostrou que a quantidade de N e P nas folhas, pode influenciar a área foliar removida pela herbivoria. Além disso, é importante observar que o manejo de nutrientes e gradientes ambientais indicam que as concentrações de nutrientes do solo em florestas tropicais podem influenciar os níveis de nutrientes nos tecidos vegetais (Sayers et al., 2012, Sullivan et al., 2014; Coley e Kursar , 1996; Throop e Lerdau , 2004). Além disso, o experimento de fertilização no Panamá mostrou que as taxas de herbivoria diferiam dependendo do nutriente que foi adicionado (Santiago et al., 2012). Mais herbivoria foi observada com adição de P e K, quando comparado ao N, sugerindo que o N não foi um nutriente limitante para herbívoros neste local.

Muitos herbívoros preferem novos tecidos foliares por reduzir as concentrações de dureza folha e nutrientes de N, P, Mg, K, a água, o conteúdo de hidrato de carbono s, em comparação com as folhas maduras, e mudanças na fenologia folha pode afetar as interacções de insectos de plantas (Coley, 1980; 2005). Quantidade de grau superior H de polifenóis na folha está relacionada a menores taxas de herbivoria (Markow et al., 1999). A concentração de N na folha pode servir como um bom preditor de herbivoria, uma vez que existe uma forte relação positiva entre a concentração de N e o ciclo de vida dos insetos na planta. e Lerdau , 2004. Além de N que aumenta a palatabilidade das folhas para insetos, outros nutrientes também são importantes, como P, K e Mg, que são abundantes em tecidos de insetos (Mattson e Scriber, 1987; Clancy e King, 1993).

Os herbívoros de insetos também podem afetar o ciclo de nutrientes no solo com deposição de fezes de inseto nos insumos de superfície do solo (Hunter, 2001, Riesley e Crossley, 1988, Hunter et al., 2003). Alguns estudos que descrevem a importância deexcrementos de insectos no ciclo dos nutrientes (Bardgett e Wardle, 2003) mostram que a deposição dos excrementos resultou num aumento da mineralização N (Lightfoot e Whitford, 1990; Reynolds et ai, 2000), o aumento da imobilização microbiana de N em microcosmos e experimentos de campo (Lovett e Ruesink , 1995).

Houve apenas quatro experimentos em grande escala que tentam entender o efeito da limitação nutricional no ciclo do carbono em florestas tropicais de florestas maduras: Bornéu (Mirmanto et al. 1999), Camarões (Newberry et al 2002), Panamá (Wright et al. al. 2011) e Costa Rica (Alvarez-Clare et al., 2013). Mesmo com as adições de nutrientes, as respostas de crescimento do tronco de árvore são relativamente baixas (Mirmanto et

al., 1999, Wright et al., 2011, 2018, Alvarez-Clare et al 2013), uma possível explicação para respostas fracas de crescimento às adições de nutrientes é aumentada pressão de pragas nas parcelas fertilizadas (Campo e Dirzo 2003, Andersen et al., 2010, Santiago et al., 2012, Wright et al., 2018).

Um estudo de Werner e Homeier (2015) em uma floresta tropical de altitude mostrou que a quantidade de N e P nas folhas, pode influenciar a área foliar removida pela herbivoria. Além disso, é importante observar que o manejo de nutrientes e gradientes ambientais indicam que as concentrações de nutrientes do solo em florestas tropicais podem influenciar os níveis de nutrientes nos tecidos vegetais (Sayers et al., 2012, Sullivan et al., 2014; Coley e Kursar , 1996; Throop e Lerdau , 2004). Além disso, o experimento de fertilização no Panamá mostrou que as taxas de herbivoria diferiam dependendo do nutriente que foi adicionado (Santiago et al., 2012). Mais herbivoria foi observada com adição de P e K, quando comparado ao N, sugerindo que o N não foi um nutriente limitante para herbívoros neste local.

A fertilização geralmente aumenta o conteúdo de nutrientes dos tecidos vegetais (Sayer et al., 2012), potencialmente tornando as folhas suscetíveis à herbivoria. De fato, vários estudos mostraram aumento nas taxas de herbivoria com o aumento de nutrientes foliares para plântulas crescendo em parcelas fertilizadas (Andersen et al., 2010, Santiago et al., 2012). No entanto, não se sabe se o aumento herbivoria com adição de nutrientes pode afetar um dossel nas taxas de crescimento de árvores ou como efeitos herbivoria adaptar-se ao nível do ecossistema (Metcalfe et al., 2014). Aqui, tentamos responder à questão de saber se a herbivoria no nível do ecossistema muda com a adição de nutrientes e, portanto, possivelmente medeia o crescimento das árvores, concentrando-se na produção de dossel e na herbivoria.

Este estudo examinou a limitação de nutrientes em um local de baixa fertilidade do solo na Amazônia Central, quantificando a função florestal e a produtividade em resposta à fertilização. N, P, cátions (Ca, Mg e K) e o experimento de fertilização da Amazônia (AFEX)foram instalados em uma floresta madura em um dos tipos de solo mais difundido em toda a Amazônia e são o primeiro experimento em grande escala. Dada a baixa disponibilidade de P e cátions na Amazônia Central, este experimento está preparado para melhorar nossa compreensão da limitação de nutrientes em toda a Amazônia.

1.1. Introduction

Tropical forests play a key role in the global carbon cycle (C) (Cramer et al., 2004), with the Amazon rainforest itself accounting for about 25% of global net primary productivity. The entire Amazon basin covers 600 million hectares with 390 billion trees, which stores about 120 petagrams of C (Pan et al., 2013, Fauset et al., 2015, Zhao and Running, 2011). Soils along the Amazon basin vary widely in geology and nutrient availability, with a clear fertility gradient increasing from east to west, this variation could affect forest productivity (Quesada et al., 2010, 2011, 2012).

Soil age and hot and humid climatic conditions of the Central Amazon basin contribute to the transformation of the source material, generating highly weathered soils. The concentration of nutrients derived from the rock, such as phosphorus (P), calcium (Ca), magnesium (Mg) and potassium (K), tend to decrease, becoming gradually less available and leached, limiting their availability to plants (Quesada et al., 2010; 2011). In contrast, nitrogen (N) inputs in the soil occur through the atmospheric fixation of N_2 by soil microorganisms, resulting in accumulation of N over time. Thus, in ancient tropical forest soils, N is found in large amounts compared to other soil nutrients, suggesting that Amazonian rainforests are not limited by N (Quesada and Lloyd, 2016; Lambers et al., 2008).

In low fertility Amazonian soils, efficient conservation and cycling of nutrients is important for the maintenance of forest functioning, which is largely due to the decomposition of the litter (Vitousek, 1984). The production and rapid decomposition of litter are critical processes for nutrient cycling and energy transfer between plants and soil (Quesada et al., 2010; Sayer et al., 2012). A fast and efficient nutrient cycle also helps reduce leaching, preventing forest in low fertility soils from becoming even poorer in nutrients (Went and Stark, 1968; Luizão, 1989).

The litter is defined as the layer of organic matter found in different stages of decomposition, formed by leaves, flowers, fruits and small branches (DAP <2 cm) (Luizão, 1989; Sayer, 2006). Therefore, litterfall is considered to be the supplier of all the elements that plants need for growth, functioning both as a sink and as a source of nutrients in forests (Tobon et al., 2004; Brancalion et al., 2012; al., 2012).

Litter production varies during the year with seasonal precipitation patterns and are affected by drought and rainfall events (Wu et al., 2016). According to Wu (2016), litter production is low in central Amazonia during the rainy season (November to May) and litter production mainly in the dry months (August and September) has high release of new leaf. The increase in irradiance during the period of low rainfall coincides with the peak production of new leaves for several species, due to the vegetation response to water stress, since leaf loss reduces

water loss through transpiration (Martins and Rodrigues , 1999). During the dry season, the photosynthetic capacity of the canopy and primary productivity also increased with leaf area expansion (Restrepo-Coupe et al., 2013, Albert et al., 2018). At the end of the rainy season, the decline in the photosynthetic rate occurs due to leaf senescence, where nutrients are reabsorbed or remobilized before leaf excision, an important strategy in infertile soils for nutrient conservation (Huete et al., 2006; Vergutz et al. 2012).

Forest productivity is influenced by factors such as light, foliar phenology, water availability and nutrients (Restrepo-Coupe et al., 2013, Borchert et al., 2015). To perform the various functions, plants need a set of different nutrients. Nitrogen is essential in plant metabolism, regulating photosynthesis, carbon assimilation in the rubisco enzyme, cell maintenance, an important component of nucleic acids, amino acids and proteins, and some secondary metabolites, as well as degradation of substances (Taiz and Zeiger, 2009; Marenco and Lopes, 2011; Lawlor, 2002). Cations function as enzyme activators in plants are also part of the composition of cell membranes. More specifically, calcium participates in the synthesis of cellular wall tissues and assists in cell divisions (Taiz and Zeiger, 2009). Magnesium plays an important role in the activation of enzymes involved in respiration, photosynthesis and synthesis of DNA and RNA, being also part of the structure of chlorophyll (Taiz and Zeiger, 2009). Potassium is responsible for the electrochemical movement and stomatal cell opening (Nunes et al., 2013).

In addition, a variety of organic compounds are also produced by plants, called secondary metabolites (terpenes and phenols), and their production is influenced by available resources (Coley, 1985). Such metabolites, also known as defense compounds, could interact in the reproductive functions of plants, attracting pollinators, affecting plant-plant competition and acting as chemical defenses against herbivores, and structural defenses decreasing palatability These two types of defense compounds occur in the plant, varying according to the environment and age of the plant leaf (Coley et al., 1985; Endara and Coley, 2011; Zangerl et al., 2002).

Plant defense compounds can be classified as mobile and non-mobile and their abundance in leaves varies with plant age and tissue turnover (Coley et al., 1985). Mobile defenses, such as alkaloids, tannins and phenols, require high metabolic investment, but have the advantage of being remobilized prior to foliar senescence (Chapin, 1980). Lignin and cellulose are structural compounds of plants that act to increase foliar toughness with maturation, reducing the foliar palatability of herbivorous insects, but these are non - mobile compounds and can not be remobilized within the plant (Coley et al., 1985; Zangerl et al., 2002).

Another important feature of leaf function on plant growth and an important indicator of plant defense strategies is leaf mass per unit area (LMA) (Lambers and Poorter, 1992; Grime, 2001; Westoby et al., 2002). LMA is correlated with physiological characteristics and influenced by environmental resources in the field (Wright et al., 2004). When components of leaf

characteristics (eg defense compounds) are in greater quantity for some environmental reason, the concentration per unit would be twice as large for all constituents, doubling the LMA (Poorter and Bergkotte, 1992; Van Arendonk and Poorter , 1994). Together with the secondary defenses, leaves with high LMA seem to have a better defense against herbivores and physical hazards (Onoda et al., 2017). Even small concentrations of secondary defenses that hardly affect LMA may significantly restrict the herbivorous set capable of feeding on certain leaves (Coley, 1983).

Herbivory is the consumption of plant tissues by animals. There are different types of herbivory that can vary between mammals and insects. Larger mammals (such as monkeys and sloths) represent 25% of herbivorous vertebrates, and their consumption is difficult to measure because they completely remove branches and leaves (Metcalfe et al., 2014). The insects, with about 500,000 species, are considered the main herbivores because they account for about 75% of the herbivory (Metcalfe et al., 2014, Herrera and Pellmyr, 2002, Thomanzini and Thomanzini 2000, Novontny and Missa 2000). In most ecosystems, the most common types of herbivory can be identified as suckers and leaf strippers or consumers of leaf tissue (Hochuli, 2001). The herbivory is larger in young leaves growing during the dry season, being the main substrate for herbivores (Coley and Barone 1996, Wu et al., 2016). The total leaf area damage is about 12% in the rainforest, with about 0.0003-0.8% of the leaf surface being consumed per day, depending on the tree species (Coley, 1983; Coley and Barone, 1996). In mountainous forests along an Amazon-Andes transect, herbivory has been shown to affect 12 to 19% of leaf productivity (Metcalfe et al., 2014). The main factor that controlled the variation in herbivory rates with elevation (200 to 3400 m.a.n.m.) was the difference in temperature and foliar P concentration of nutrients among forests (Metcalfe et al., 2014).

Plants can also adopt mechanisms to avoid nutrient losses from herbivory, such as rapid leaf expansion and chloroplast maturation during expansion, synchronous leaf production, defense compounds and various forms of mutualism and antagonism (Coley and Barone, 1996). A study by Werner and Homeier (2015) in a tropical montane rainforest showed that the amount of N and P in leaves, can influence the leaf area removed by herbivory. In addition, it is important to note that nutrient handling and environmental gradients indicate that soil nutrient concentrations in tropical forests can influence the levels of nutrients in plant tissues (Sayers et al., 2012, Sullivan et al., 2014; Coley and Kursar, 1996; Throop and Lerdau, 2004). Additionally, the fertilization experiment in Panama showed that herbivory rates differed depending on the nutrient that was added (Santiago et al., 2012). More herbivory was observed with P and K addition, when compared to N, suggesting that N was not a limiting nutrient for herbivores at this site.

Many herbivores prefer new leaf tissues due to lower leaf toughness and nutrients concentrations of N, P, Mg, K, water, carbohydrates content, compared with mature leaves, and changes in leaf phenology may affect insect-plant interactions (Coley, 1980; 2005). Higher amount of polyphenols in the leaf is related to lower herbivory rates ((Markow et al., 1999). Leaf

N concentration can serve as a good predictor of herbivory, since there is a strong positive relationship between the N concentration and the life cycle of insects in the plant (Throop and Lerdau, 2004). In addition to N which increases leaf palatability for insects, other nutrients are also important, such as P, K and Mg which are abundant in insect tissues (Mattson and Scriber, 1987; Clancy and King, 1993).

Insect herbivores can also affect soil nutrient cycling with frass (insect feces) deposition in surface soil inputs (Hunter, 2001, Riesley and Crossley, 1988, Hunter et al., 2003). A few studies that discuss the importance of insect frass in nutrient cycling (Bardgett and Wardle, 2003) show that frass deposition has resulted in increased N mineralization (Lightfoot and Whitford, 1990; Reynolds et al., 2000), increased microbial immobilization of N in microcosm and field experiments (Lovett and Ruesink, 1995).

There have only been four large-scale experimental tests that attempt to understand the effect of nutritional limitation on the carbon cycle in mature lowland tropical forests: Borneo (Mirmanto et al. 1999), Cameroon (Newberry et al 2002), Panama (Wright et al. 2011), and Costa Rica (Alvarez-Clare et al., 2013). Even with the nutrient additions the tree trunk growth responses are relatively weak (Mirmanto et al., 1999, Wright et al., 2011, 2018, Alvarez-Clare et al 2013), a possible explanation for weak growth responses to nutrient additions is increased pest pressure in the fertilized plots (Campo and Dirzo 2003, Andersen et al., 2010, Santiago et al., 2012, Wright et al., 2018).

A study by Werner and Homeier (2015) in a tropical montane rainforest showed that the amount of N and P in leaves, can influence the leaf area removed by herbivory. In addition, it is important to note that nutrient handling and environmental gradients indicate that soil nutrient concentrations in tropical forests can influence the levels of nutrients in plant tissues (Sayers et al., 2012, Sullivan et al., 2014; Coley and Kursar, 1996; Throop and Lerdau, 2004). Additionally, the fertilization experiment in Panama showed that herbivory rates differed depending on the nutrient that was added (Santiago et al., 2012). More herbivory was observed with P and K addition, when compared to N, suggesting that N was not a limiting nutrient for herbivores at this site

Fertilization generally increases nutrient content of plant tissues (Sayer et al., 2012), potentially making leaves susceptible to herbivory. Indeed, several studies have shown increased herbivory rates with increased leaf nutrients for seedlings growing in fertilized plots (Andersen et al., 2010, Santiago et al., 2012). However, it is uncertain whether the increased herbivory with nutrient addition can affect canopy tree growth rates or how herbivory effects scale up to the ecosystem level (Metcalfe et al., 2014). Here, we attempt to answer the question of whether ecosystem-level herbivory changes with nutrient addition, and therefore possibly mediates tree growth, by focusing on canopy production and herbivory.

This study examined nutrient limitation in a low soil fertility site in Central Amazonia by quantifying forest function and productivity in response to fertilization. N, P, Cations (Ca, Mg and K) and the Amazonian Fertilization Experiment (AFEX) were installed in a mature forest on one of the most widespread soil types across Amazonia and are the first large-scale experiment. Given the low availability of P and cations in Central Amazonia, this experiment is poised to enhance our understanding of nutrient limitation across Amazonia.

2. Objective

2.1. General Objective

Understand the role of nutritional limitation on canopy productivity, herbivory loss and investment in secondary compounds in terra firme forests in Central Amazonia, at the ecosystem scale.

2.2. Specific Objectives

1- Determine the response of fine litterfall productivity to nutrient addition;

2- Determine the response of leaf traits, including LMA, the concentration of macro and micronutrients as well as secondary compounds to fertilization;

3- Determine the effect of nutrient addition on leaf herbivory at the ecosystem level and the feedback of herbivore nutritional contribution across the different treatments;

4- Determine the relationships between litter production, investment in secondary compounds, concentration of nutrients and levels of herbivory.

3. Hypotheses

H1: If nutrient additions increase leaf quality, then leaf turnover expected to increase which will result in higher leaf litter productivity in fertilized compared to control treatments;

H2: If nutrient additions increase leaf quality, then leaf litter nutrient content will increase, whereas LMA and the investment in defense compounds are expected to decrease, resulting in greater palatability, and decreased leaf toughness;

H3: If nutrient additions increase leaf quality, and leaf quality determines both litter production and herbivory levels, a positive correlation between litter production and herbivory levels is expected.

4. Methods

4.1. Location and characterization of the study area

The study took place in the ZF-3 Reserve area called "Km 41", within the AFEX project area (Figure 1) at the Biological Dynamics of Forest Fragments Project (BDFFP/ INPA) located approximately 80 km north of Manaus/Amazonas/Brazil (02° 25' 00" S; 59° 43' 00' W). The climate in the region is characterized by small seasonal variation in air temperature, with an average of 26.7 °C. Mean annual rainfall ranges from 1900 mm to 3500 mm, with a peak in April (> 300 mm/month) and a dry period (<100 mm/month) between June and October (Laurance et al., 2010b). The relative air humidity reaches a minimum value of 75% in August and a maximum of 92% in April during the rainy season (Araújo et al., 2002).

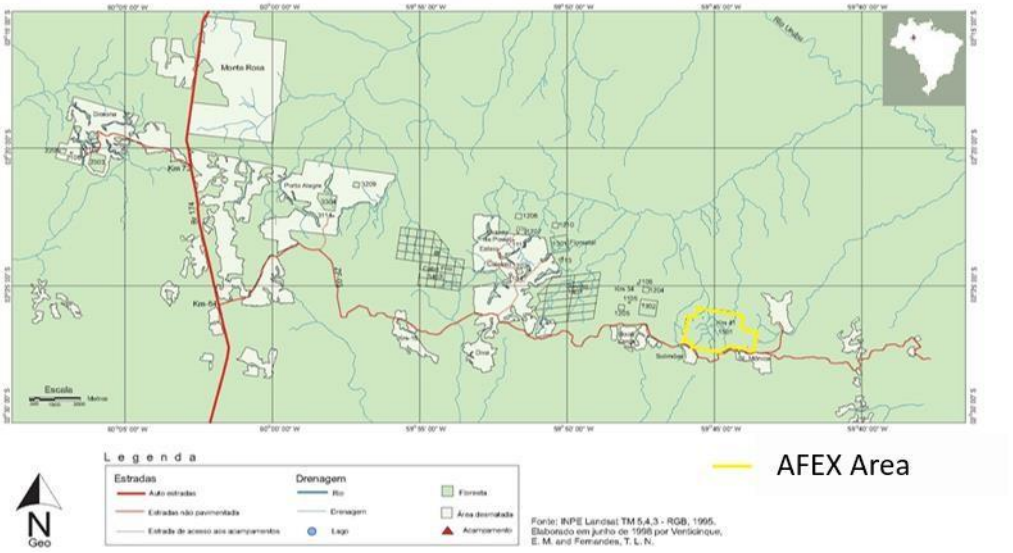

FIGURE 1. Map of BDFFP (Biological Dynamics of Forest Fragments Project) research area. Yellow area indicates the location of the AFEX research site, named forest reserve "Km 41". Source: E.M. e Fernandes T.L.N.

The area is composed of plateaus varying in altitude from 80 to 160 m a.s.l., steep slopes and valleys (Laurance et al., 2018). All the plots in the AFEX project area are however, located on plateaus. The soils of the region are clayey, classified as red yellow podzolic alic and yellow latosol alic by the FAO/UNESCO system (Ranzani, 1980; Laurence et al., 1999). They are considered very weathered, acidic and nutrient-poor soils, classified by the World Reference Base (WRB) as geric ferrasols (Chauvel 1982; Quesada et al., 2010, 2011). The vegetation is classified as "terra firme" non-flooded dense

ombrophilous forest (Laurance et al. 2018). The average height of the canopy varies from 30-37 meters with occasional emergent trees that can reach 55 meters (Oliveira and Mori, 1999). The area has an estimated minimum of 280 tree species ha^{-1} distributed in 53 families. Plant families with higher density in the area are Lecythidaceae, Fabaceae, Sapotaceae and Burseraceae. (Rankin–de-Merona et al., 1992).

AFEX project started in May 2017 as a full factorial experiment with the additions of Nitrogen (N), Phosphorus (P) and Cations and their combinations. The experiment has eight treatments (TABLE 1) and four replicates per treatment, resulting in a total of four (4) independent blocks (at least 250 m distance between each other) and thirty-two (32) plots, 50 m apart from each other (FIGURE 2). These treatments allow the analysis of potential limitations of the different essential elements on plant growth (canopy productivity), nutrient content of plant tissues and herbivory. Nutrients were added to the plots manually, with dry granules applied to the soil surface three times a year, avoiding the main dry season. Each plot measures 50 m x 50 m where nutrients are applied, with the main measurements limited to the central plot area (30 m x 30 m) (FIGURE 3), thus aiming to maximize the probability that the litter sampled belonged to trees inside the plots. Nutrients were added at the following rates: 125 kg ha^{-1} $year^{-1}$ of N as urea, 50 kg ha^{-1} $year^{-1}$ of P as triple superphosphate, 50 kg ha^{-1} $year^{-1}$ of Ca and 20 kg ha^{-1} $year^{-1}$ Mg as dolomitic limestone and 50 kg ha^{-1} $year^{-1}$ of K as potassium chloride.

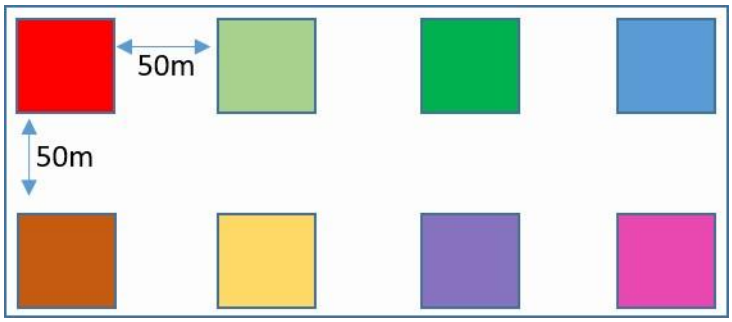

FIGURE 2. Example of the position diagram of plots inside one block with trails between the eight plots in blue line, and the treatments.

TABLE 1. List of treatments names in the factorial design used in AFEX fertilization.

Treatments used in AFEX fertilization.	
Control	Cations
Nitrogen (N)	Nitrogen (N) + Cations
Phosphorus (P)	Phosphorus (P) + Cations
Nitrogen (N) + Phosphorus (P)	Nitrogen (N) + Phosphorus (P) + Cations

TABLE 2. Concentrations of the nutrients Nitrogen, Phosphorus and Cations in the treatments

Concentrations of nutrients in treatments.	
Nitrogen (N)	125 kg ha $^{-1}$ year $^{-1}$, as urea
Phosphorus (P)	50 kg ha $^{-1}$ year $^{-1}$ in the form of triple superphosphate
Cations	Ca+ 50 e Mg +, 20 kg ha^{-1} year^{-1} dolomitic limestone
	K+, 50 kg ha $^{-1}$ year $^{-1}$ as potassium chloride

For the determination of litter production, litter traps were built using plastic tubes, with dimensions of 50 cm x 50 cm, occupying an area of 0.25 m^2 and one meter above the ground level. Five litter traps were installed at five (5) locations within the central area of each plot to insure litter reaching the trap was produced within the experimental plot area (FIGURE 3). To capture senesced leaves fallen from the canopy, we used polyethylene screens of 1 mm mesh. The installation of the littertraps occurred in the first week of July 2017, and the first collection was mid July. Samples were collected biweekly for eight months, making a total of 13 census collections ending in February 2018.

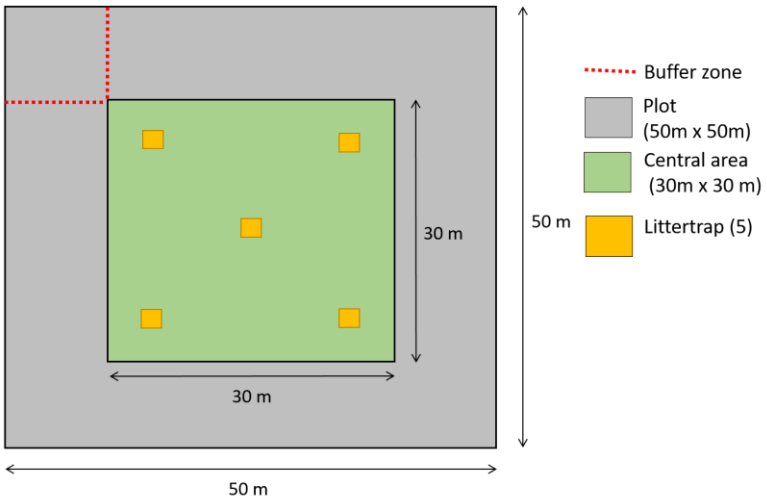

FIGURE 3.Picture of the plot design of 50 m² in grey, with the buffer zone of 10 m in red dotted lines, the central area inside the plot with 30m² in green, and distribution of the five litter traps in yellow.

FIGURE 4.a) Littertrap installed in the plot; b) litterfall collection in one of the five littertraps in the center plot area. Source: Andersen, K and Moraes, A.C.M.

4.3. Calculations

4.3.1. Litterfall biomass

After every collection, fine litter was sorted at INPA and separated into different fractions: leaves, twigs and thin branches (woody material) with a diameter <2 cm, reproductive material (flowers, fruits and seeds), residues (other fractions not identified) and insect frass (Pauletto, 2006; Luizão, 1982; 1991). The material was then dried at 65° C, weighed using a precision analytical balance (0.01 g) to obtain dry biomass ($g.kg^{-1}$) and stored for further chemical analyses.

4.3.2. Leaf Mass Area (LMA)

Litterfall fresh leaves from littertrap were scanned to determine area (m^2) and based on their corresponding dried biomass ($g.kg^{-1}$) leaf dry mass per area (LMA= g/m^2) was calculated. We used flat leaves that could be scanned in the field (Witkowski and Lamont, 1991) since they were more moist and malleable, making it easier to get the entire leaf area. Images were obtained using Canon CanoScan LiDE 120 scanner as shown in Figure 5. Six set of images were obtained from leaves sampled in August, October and November 2017.

4.3.3. Leaf area lost by herbivory

Litter from all treatments was collected and scanned the same way as for determining LMA (4.3.2.). The analysis was made in two ways according to time and logistics available. Between August and October 2017 (peak of litterfall production – 5 biweekly censuses), leaves from one littertrap per plot were analysed each time (32 littertraps total). In November, during a period of lower litter production, we were able to analyze all 160 littertraps for a single biweekly cenus. All these results were combined for an overall estimate of herbivory that likely represents realized herbivory throughout much of the year (not including peak leaf expansion periods). Images of leaf area and leaf area lost were analyzed with the image processing software ImageJ (Metcalfe et al., 2014, Rasband, 2012). To estimate leaf area (cm^2), images were formatted to represent leaves without herbivory by a gap filling process (FIGURE 6). The total amount of leaves analyzed was 3493 leaves in 1070 images.

4.3.3. Calculation of leaf mass and area missing by herbivory

Leaf area loss by herbivore was calculated as the proportion of the total leaf area without herbivory (AT in m²), the original leaf area collected in the field with herbivory (real area (AR in m²) generating by difference the proportion of leaf lost by herbivory (LH). The potential total leaf production in the canopy Mg ha^{-1} $year^{-1}$ (Pr) was calculated by dividing the known production of the litter of the collectors (PH) minus the proportion of biomass removed by herbivore by 1 – LH. To determine the effect of leaf herbivory on canopy productivity (Mg ha^{-1} $year^{-1}$) we calculated biomass consumed by herbivory Mg ha^{-1} $year^{-1}$ (Bh) (Metcalfe et al. 2014; Werner and Homeier 2015, Cárdenas et al. 2014).

$$LH\% = \frac{AT-AR}{AT} \qquad Pr = \frac{PH}{1-LH} \qquad Bh = Pr - PH$$

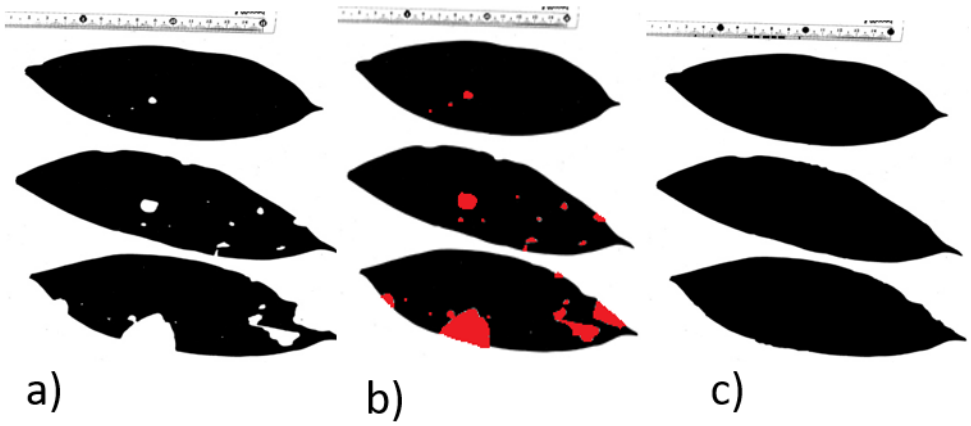

FIGURE 5. Example of leaves with and without herbivory used to calculate leaf area loss. a) is the scanned leaf original area measured (with herbivory); b) is the calculation of the area missing projected in red at the program Image J; c) is the leaf designed without herbivory. Source: K. Andersen e Moraes, A.C.M.

4.4. Chemical analysis

4.4.1. Macro and micro nutrients

To determine macro and micronutrients and secondary compounds, litter samples were bulked by plot using material collected during the months of August and September 2017. All analysis were conducted at INPA's soil and plants lab. After dried, samples were ground using a knife and/ or ball mill for nutritional analysis. Leaves and insect frass were analysed separately. Micronutrients, phosphorus and cations were analysed by nitroperchloric digestion protocol described by Malavolta et al. (1989). The bases cations (K, Ca and Mg) and micronutrients were determined by atomic absorption

spectrophotometry (AAS, 1100 B, Perkin-Elmer, Ueberlingen, Germany) (Anderson and Ingram, 1993). The total P concentrations were determined by colorimetry (Anderson and Ingram, 1993), and quantified by spectrophotometry (UV-120-01, Shimadzu, Kyoto, Japan). The C and N contents were determined using an automatic C and N analyzer (VARIO MAX CHN Element Analyzer) (Nelson and Sommers, 1996). Leaves were not identified by species and leaf characteristics represent means at plot level (Metcalfe et al., 2014).

4.4.6. Lignin and cellulose

Concentrations of lignin and cellulose in litter were determined by the ADF-sulfuric method (Van Soest 1963; Rowland and Roberts, 1994). The method consists in applying an acidic detergent solution (ADF), followed by the cellulose using H_2SO_4 72%, with lignin subsequently determined by weight loss on ignition 0 to 50° C in a muffle furnace.

4.4.7. Polyphenols

Total polyphenols (simple phenols and hydrolysable tannins) were determined following the method of Folin Denis (Coley, 1983). Extraction consists of homogenizing 1 g of leaves in 35 ml of 85% methanol solution, then the sample was diluted to the volume of 50 ml and put in a water bath (70°C) for 20 minutes. The concentration of polyphenols was determined by colorimetry (Anderson and Ingram, 1993), and quantified by spectrophotometry (UV-120-01, Shimadzu, Kyoto, Japan).

4.4.1. Calculations leaf litterfall nutrient input

Leaf litterfall nutrient biomass (LNB) was calculated by summing leaf biomass (g/m^2) from August and September (LBD). LBD was then converted to kg/ha^{-1} and multiplied by nutrient concentration in litter (LNut):

$$LNB = [LBD * 10] * LNut$$

4.4.2. Herbivory frass nutrient biomass input

To answer the fourth objective of determine the effect of the herbivory frass input in biomass ($kg/ ha^{-1}/ year^{-1}$) and nutrients on the treatments, we used the following equation for frass nutrient biomass input (FNB). Insect frass biomass (FBD)] was converted to kilogram per hectare and multiplyed by quantity of nutrients found in the frass (FNut).

$$FNB = [FBD * 10] * Fnut$$

4.5. Data analysis

The statistical program R version 3.4.4 (R core team, 2018) was used to test the effect of nutrient additions on the response variables studied here. We conducted a series of linear mixed models using package "lmer4" (Bates et al., 2015), considering block as a random factor. The fixed factor was used in two ways, first by treatments comparing the control against the other seven treatments. When the model was significant the Dunnett's test was applied to test treatments separately comparing with control, using the package "Multcomp" (Hothorn et al., 2008). The second approach was using the factorial experimental design to obtain the main effect of each nutrient and testing the interactions between the nutrients. Model simplification procedures were used to find the most parsimonious model based on AIC values. When the fixed effects were significant in the final model, a post-hoc tukey test was applied using the package "emmeans" (Lenth, 2018), to test the nutrient interaction effects. The model fit was verified to make sure the analysis met the model assumption, checking the normality, standardized residuals plot and density of the model residuals.

To determine the importance of leaf mechanisms against herbivory we tested the level of investment in secondary compounds with the availability of nutrients. More specifically, we tested the effect of lignin, cellulose, polyphenols, macro and micronutrients and specific leaf area on leaf biomass consumed by herbivory (Bh). We tested the effect of treatments on biomass consumed by herbivory (Bh), nutrient consumed by herbivory (Nh) and productivity of leaf litterfall biomass (PH). To determine the relationships between litter production, investment in secondary compounds, concentration of nutrients and levels of herbivory, we conducted correlation tests using the package "Hmisc" (Harrell, 2018) to obtain the Pearson Correlation Coefficient (r). If the test showed high correlation, we further explored the relationship using a Generalized Linear Mixed Model (GLMM), with the herbivore variables as response variables and the leaf litter variables as the fixed factors and block as the random factor. Finally, to better understand the multivariate relationships of leaf litter macro and micronutrients concentrations (P, Ca, Mg, K, Mn $g.kg^{-1}$), leaf mass area (LMA) and defense compounds (Lignin, Cellulose and Phenols) on herbivory responses we used a principal component analysis (PCA).

5. Results

5.1. Patterns of litterfall fractions with treatment

To answer the first objective, that is to determine the response of fine litterfall productivity to nutrient additions, we gathered information of mean values of fine litterfall biomass fractions, cumulative fine litterfal biomass, and production of fine litterfall over time. Over 8 months, with 13 field collections of fine litterfall biomass production, the values between treatments varied from 9.12±6.33 (P+Cations) to 10.73±8.81 (N+P) Mg ha^{-1} $year^{-1}$ (FIGURE 6). It was found a trend for differences in total litterfall between the treatments with higher production in the "N+P" treatment ($F_{7,24}$=2.32,P=0.067). The litterfall production of the different fractions did not differ significantly among the treatments. Leaf litterfall varied from 5.73±0.46 (N) to 7.59±0.82 (N+P) Mg ha^{-1} $year^{-1}$. Fine woody litterfall ranged from 1.48±0.17 (P+Cations) to 2.19±0.32 (N+P+Cations) Mg ha^{-1} $year^{-1}$. For reproductive material, productivity varied from 0.82±0.11 (P+Cations) to 1.6±0.24 (P) Mg ha^{-1} $year^{-1}$. Other residues varied from 0.08±0.01 (N+P+Cations) to 0.16±0.01 (P) Mg ha^{-1} $year^{-1}$. The insect frass fraction varied from 0.05±0.006 (N+Cations) to 0.08±0.009 (P+Cations) Mg ha^{-1} $year^{-1}$.

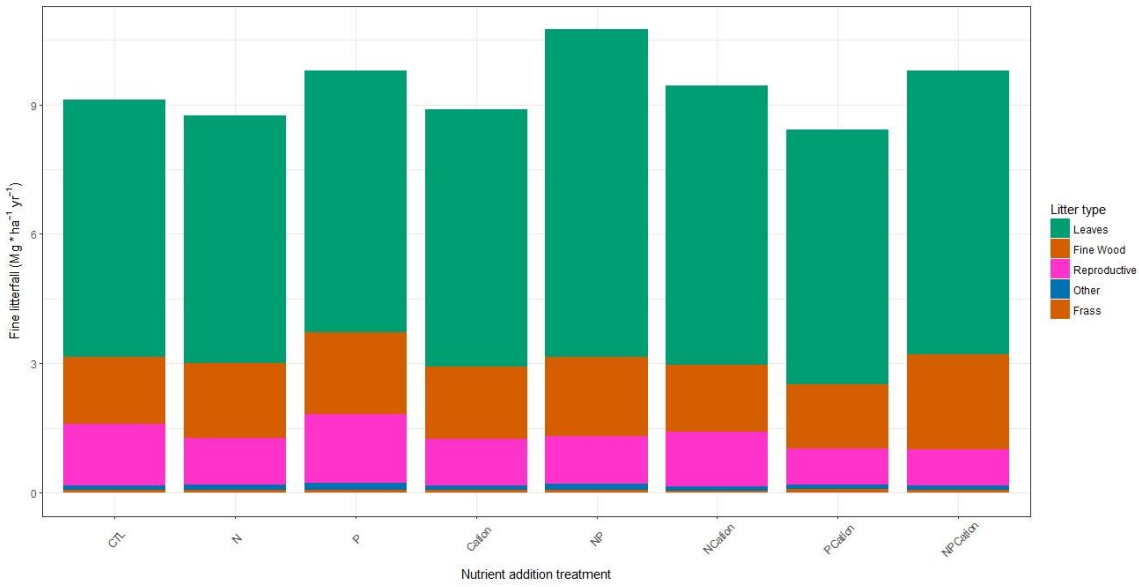

FIGURE 6. Mean fractions of fine litterfall Mg ha^{-1} $year^{-1}$ (leaves in green color, fine wood in orange, reproductive material in pink, other residues in blue, and frass in brown), by nutrient addition treatments of Control (CTL), Nitrogen (N), Phosphorus (P), Cations (Cation), N+P (NP), N+Cations (NCation), P+Cations (PCation), and N+P+Cations (NPCation).

Fine litterfall biomass production in all treatments showed a seasonal pattern, expected for Manaus region (FIGURE 8), with peak production occurring in the first week of September during the dry season. There were no significant differences among treatments through the sampling time. The "N+P" treatment showed the highest biomass

production and a slightly earlier peak in production in the dry season. Litter production declined from October 2017 until February 2018, during the transition from dry to wet season.

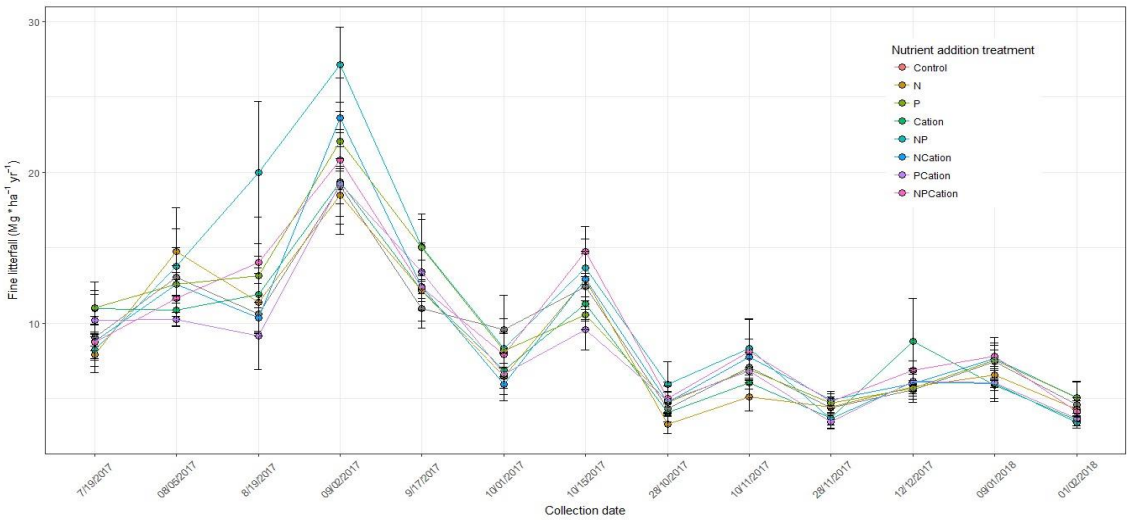

FIGURE 7. Fine litterfall (Mg ha^{-1} year^{-1}) with nutrient addition treatments of Control (CTL), Nitrogen (N), Phosphorus (P), Cations (Cation), N+P (NP), N+Cations (NCation), P+Cations (PCation), and N+P+Cations (NPCation). The x axis is counting the collection date over time (july/2017 – february/2018).

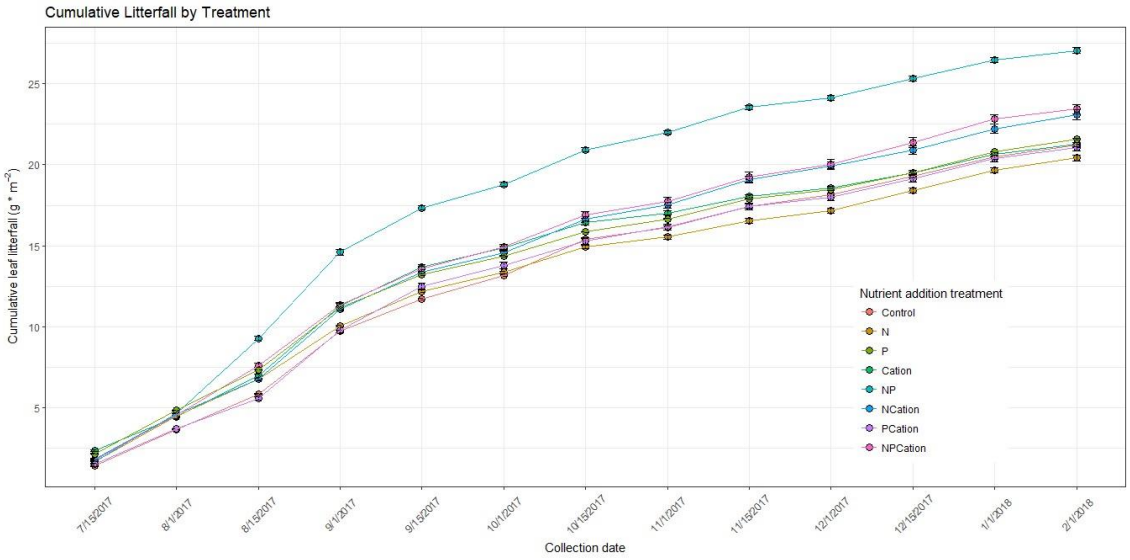

FIGURE 8. Cumulative leaf litterfall(g.m²) over time by treatments Control (CTL), Nitrogen (N), Phosphorus (P), Cations (Cation), N+P (NP), N+Cations (NCation), P+Cations (PCation), and N+P+Cations (NPCation).

The cumulative leaf litterfall biomass (FIGURE 9) indicated that the treatment "N+P" had abscised leaves before the other treatments in the second week of August, making their biomass stand out and perhaps growing apart from the other treatments since then. In the middle of November the treatments "N+P+Cations" and "N+Cations"

appeared to start to slightly increase the amount of litterfall compared to the other treatments.

5.2. Leaf Mass Area

To answer the second objective to determinate foliar characteristics, like LMA, macro and micronutrients, and secondary compounds, we examined how , leaf mass area (g/m²), nutrient concentrations of leaves (g.kg^{-1}), leaf litter nutrient input (g.ha^{-1}), and defense compounds (%) change with fertilization.

The leaf mass area had significant interactions between Nitrogen and Cations ($F_{1,28}$=4.65,P=0.04*). Specifically, the leaves that received both N and Cations fertilizers (FIGURE 10a) had a higher leaf mass per unit leaf area, indicating the leaves got thicker (max = +N+Cations: 191.67±23.84 g.m^2; min = +N-Cations: 110,33±12.55 g.m²). The increase in LMA with added N and cations is particularly pronounced in comparasion to the other treatments (FIGURE 10b).

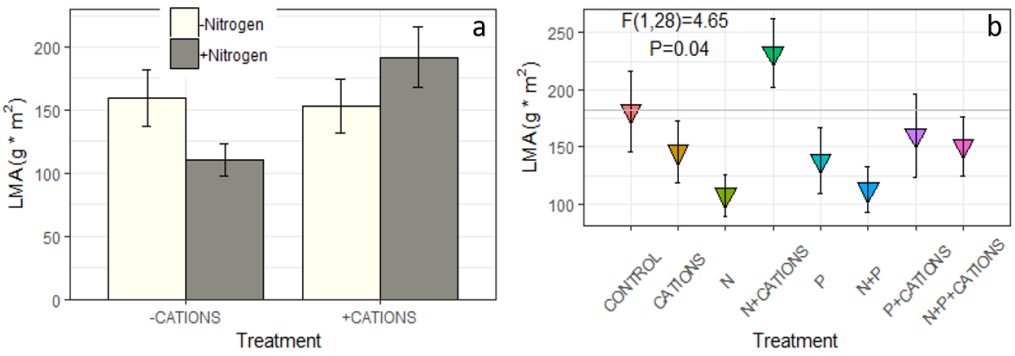

FIGURE 10. a show Leaf Mass Area (g.m²) with the factorial interaction of Nitrogen and Cations. Showing that the LMA had a significant response with the presence of both Nitrogen and Cations. Figure b show all treatments LMA pattern. Dotted line represent the mean LMA value in control plots. P<0.05.

5.3. Nutrient content in leaf litter

Mean leaf litter P content was significantly higher in the treatments containing P additions compared to those treatments without added P ($F_{1,29}$=5.23;P=0.03*; Fig. 11 a and b). Specifically, leaf litter P content was 38.1±2.71 when compared with Control 27.7±0 (FIGURE 11b, TABLE 3).

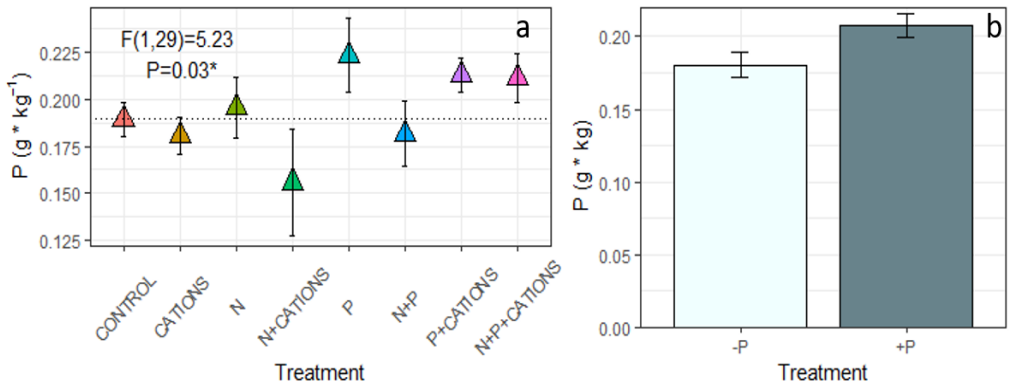

FIGURE 11. figure a is Leaf litter phosphorus concentration (g.kg^{-1}) with and without factorial P treatments, showing that phosphorus had a significant response in the presence of P treatments. FIGURE b shows the values of nutriet P with all treatments. Dotted line represent the mean leaf P content in control plots. P<0.05.

The other leaf litter nutrients Calcium (5.54±0.94 g.kg^{-1}), Magnesium (2.34±0.26 g.kg^{-1}), and Manganese (0.17±0.03 g.kg^{-1}), showed a pattern of increase in all treatments compared to the Control (FIGURE 12; TABLE 3). Although there were no significant differences in the mean concentration in the leaves for calcium ($F_{7,20}$=1.43;P=0.24) and magnesium ($F_{7,21}$=0.73;P=0.24) among the treatments. Manganese content increased (0.17±0.03 g.kg^{-1}) in all treatments compared to the Control, with the highest mean in the "P" treatment but was not significant ($F_{7,24}$=1.08; P=0.4) (FIGURE 12).

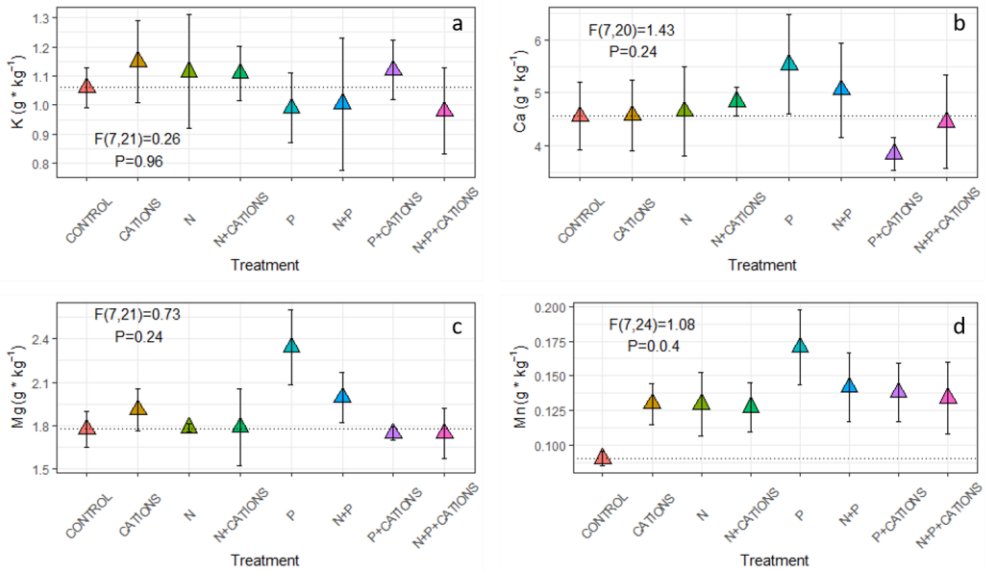

FIGURE 9. Leaf litter nutrient concentrations for (g.kg^{-1}) potassium (K) (a), Calcium (Ca) (b), Magnesium (Mg) (c), Manganese (Mn) d). The mean of control value in marked with black dotted line. P<0.05.

5.4. Leaf litter nutrient inputs

The leaf litter nutrient inputs had a strong response in the P+ treatments. Leaf litter phosphorus inputs were significantly higher in plots receiving P (+P; 34.77±1.55 g.ha^{-1})

compared to plots that did not receive it (–P; 27.6±1.77 g.ha^{-1}) ($F_{1,26}$=13.2; P=0.001**). The nutrient Manganese was higher in the treatments with P (+P; 24.49±2.08 g.ha^{-1}) in comparison with not receiving P (-P; 18.26±1.47 g.ha^{-1}), and was significant in factorial P ($F_{1,29}$=6.0; P=0.02*).

The leaf litter nutrients inputs of Calcium ($F_{7,21}$=2.74; P=0.03*) and Magnesium ($F_{7,24}$=3.89; P=0.005**) had a similar pattern, and are significantly different from control in the treatments N+P (Ca 1012±123 g.ha^{-1} and Mg 406±25 g.ha^{-1}) and P (Ca 974±193 g.ha^{-1} and Mg 407±59.2 g.ha^{-1}), with the highest mean values. Ca inputs increased significant in the N+P and +P treatments compared to the control. Potassium was the only nutrient that did not significant change in response of nutrient additions probably influenced by the high level of variation in K inputs, with the treatment N+P was the highest mean value (201±31.1 g.ha^{-1}) (FIGURE 12).

I also calculated the leaf nutrient input in kg.ha^{-1}yr^{-1} (TABLE 4), these results assume a constant value throught out the year based on dry season inputs. For P, the input varied from 1.56±0.21 kg.ha^{-1} (N+Cations) to 2.49±0.04 kg.ha^{-1} (N+P), showing that the mean. For Mg, the input varied from 16.6±1.76 kg.ha^{-1} (Control) to 27.5±1.5 kg.ha^{-1} (N+P). For Ca, varied from 37±3.94 kg.ha^{-1} (P+Cations) to 68.3±7.85 kg.ha^{-1} (N+P). For K input, varied 9.83±0.57 kg.ha^{-1} (Control) to 13.5±2.03 kg.ha^{-1} (N+P).

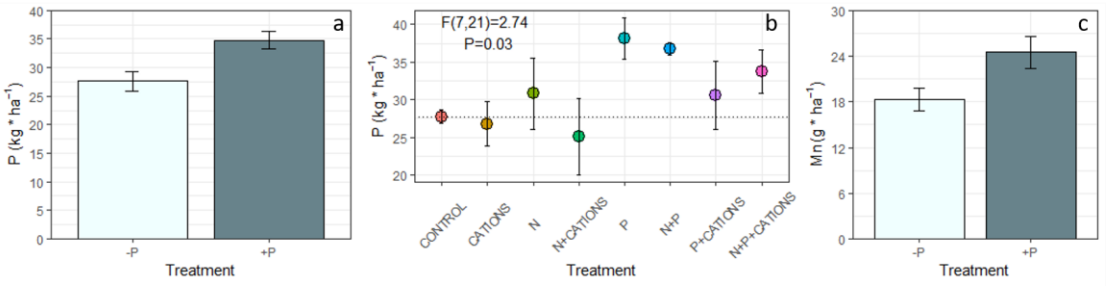

FIGURE 10. Amount of phosphorus (g.ha^{-1}) (figure a and b), phosphorus response in the presence of treatments P, and without the presence of Cations. Manganese (g.ha^{-1}) (figure c) response with factorial P treatment. P<0.05.

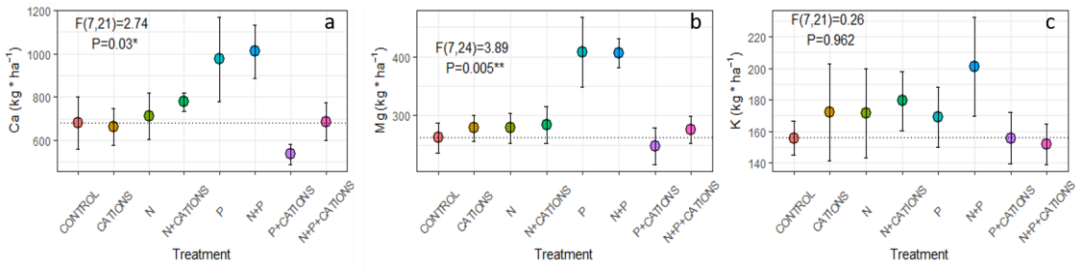

FIGURE 11. Quantity of litter nutrient inputs (g.ha^{-1}) of Calcium (Ca) (figure a), Magnesium (Mg) (figure b), Potassium (K) (figure c). The x axis show the treatments Cations in red, Control in yellow

(the mean value in marked with black dotted line), Nitrogen (N) in light green, N+Cations in dark green, N+P in light blue, N+P+Cations in turquoise blue, Phosphorus (P) in lilac, P+Cations in pink. P<0.05.

5.5. Defence Compounds

Defense compounds in the leaf litter did not differ significantly between the treatments. Lignin ($F_{7,21}$=0.89; P=0.52) in the treatment N+P (46.1±3.71%) had the highest fraction of lignin in their leaves, with a wide range of variation among the treatments. Cellulose ($F_{7,24}$=1.21; P=0.33), had the higher fraction in the treatment Cations (31.2±6.22%), again with a wide range of variation. The phenols ($F_{7,21}$=1.49; P=0.22), being a mobile defence, had very little fraction remaining on the leaves, with the highest value being at P+Cations (0.0123±0.002%), and the lowest from treatment P (0.002±0.002%). It can be seen that even though there was no significance, the percentage of phenols lowered considerably in comparison with the Control treatment.

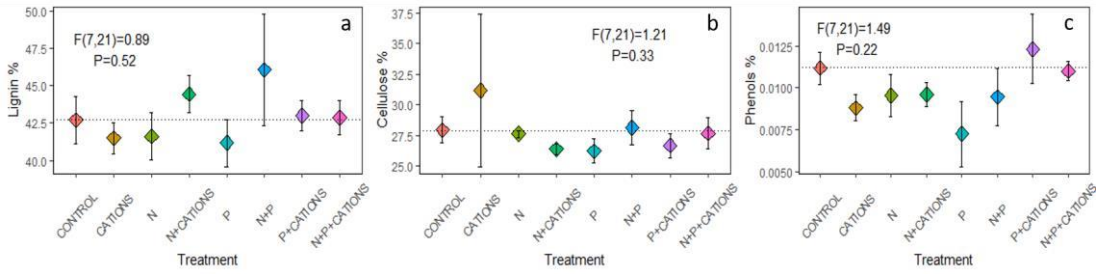

FIGURE 12.Percentage (%) of lignin (a), cellulose (b) and phenols (c) remaning in the leaf litterfall in all eight treatments. The x axis show the treatments Cations in red, Control in yellow (the mean value in marked with black dotted line), Nitrogen (N) in light green, N+Cations in dark green, N+P in light blue, N+P+Cations in turquoise blue, Phosphorus (P) in lilac , P+Cations in pink. P<0.05.

5.6. Leaf Area and Biomass consumed by Herbivory

In order to answer the third objective of determining the effect of nutrient additions on leaf herbivory, leaf area missing (m²), leaf mass missing (g.kg^{-1}), and leaf biomass missing(g.ha^{-1}) were measured.

Herbivory, presented as % leaf area lost, did not differ among the treatments ($F_{7,24}$=1.21; P=0.33). The fraction of leaf area missing ranged from 7.27 ± 0.69 % in the N+P treatment to 9.17± 0.6% in the N+Cations treatment. However, the leaf mass missing (g/kg) differed significantly ($F_{1,28}$=5.15, P=0.03), among the treatments with significantly higher mean value with added Nitrogen and Cations together (+N+Cations; 0.47±0.1 g.kg^{-1}), compared to plots that received only one or neither of these nutrients (Fig. 16C; +N-

Cations; 0.18±0.02 g.kg^{-1}). However, when scaling to hectares, this value is no longer significant ($F_{7,21}$=1.49, P=0.22). The treatment N+Cations (1.6±0.17 g.ha^{-1}), had the highest mean herbivory value, with the lowest being treatment P+Cations. Together, these results show that N+Cations was the treatment with more significance in all variables related to herbivory, probably because their higher mass and area originate from the same samples.

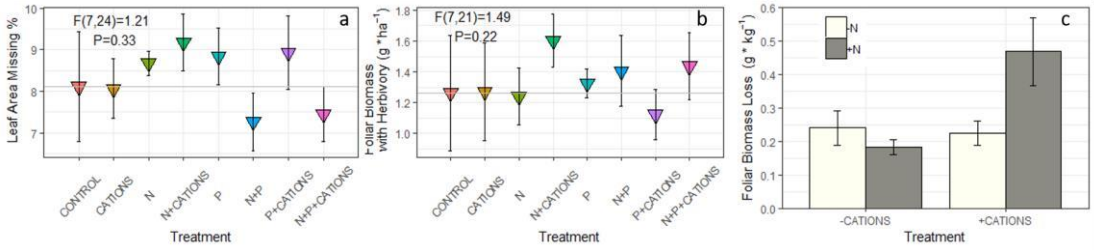

FIGURE 13. Leaf area missing (%) (a), Leaf Biomass Herbivory (kg.ha^{-1}) (b) in all 8 treatments in the x axis. Showing the control mean value in marked with black dotted line. P<0.05.

5.7. Insect Frass Biomass

To answer the fourth objective, that is determining the effect of the frass input in biomass (kg/ ha^{-1}/ year^{-1}) and its nutrient additions on each treatment, we collected insect frass (kg.ha^{-1}) and measured the nutrient contained in insect frass (g.kg^{-1}) and the frass nutrient input (g.ha^{-1}).

The cumulative insect frass did not differ among the treatments (TABLE 3, $F_{7,21}$=0.89; P=0.53). The cumulative insect frass biomass varied from 0.019±0.01 kg.ha^{-1} in the N+Cations treatment to 0.35±0.08 kg.ha^{-1} in the P+Cations treatment (FIGURE 16).

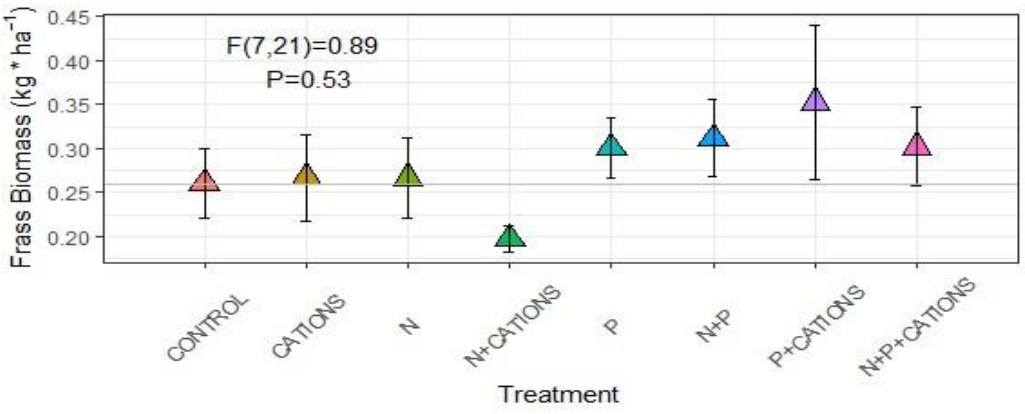

FIGURE 14. Insect frass biomass (kg.ha^{-1}), showing in all treatments, and the mean control value in marked with black line. P<0.05.

5.8. Insect Frass Nutrient content

Analyzing the nutrients of the insect frass, the nutrients that had significance using the factorial model were phosphorus ($F_{1,26}$=4.49; P=0.04*), and manganese ($F_{1,28}$=7.8; P=0.009**). For phosphorus in insect frass, the treatments that did not receive added Cations (-Cations; 0.63±0.035 g.kg^{-1}) were higher than those that received added Cations (+Cations; 0.54±0.06 g.kg^{-1}) this is where you include the LMM result.

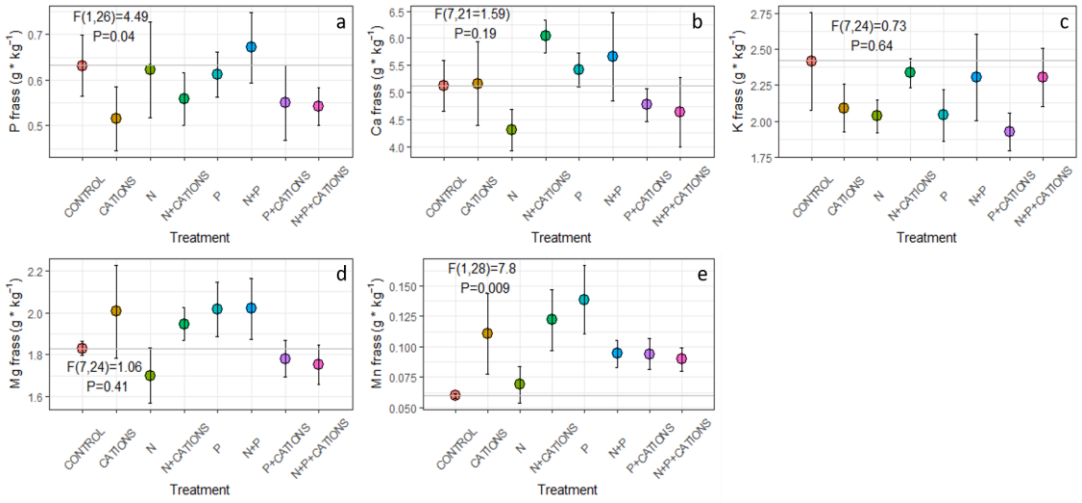

FIGURE 15. The Insect Frass Nutrient (g.kg^{-1}), showing in figure "a" the nutrient phosphorus, figure "b" the nutrient calcium, figure "c" the nutrient potassium, figure "d" magnesium and "e" manganese.. The x axis being all treatments, the mean control value in marked with black line. P<0.05.

The other frass nutrients had no significant difference from control in each treatment (TABLE 3). Magnesium ($F_{7,24}$=1.06; P=0.41) has the treatments N+P (2.02±0.14 g.kg^{-1}) and P (2.02±0.13 g.kg^{-1}) had the highest mean values, and the treatment N (1.7±0.13 g.kg^{-1}) with the lowest. Calcium ($F_{7,21}$=1.59; P=0.19) has the treatments N+P (6.04±0.3 g.kg^{-1}) with the highest value, and N (4.31±0.38 g.kg^{-1}) with the lowest mean value. All mean values for potassium had no significance ($F_{7,24}$=0.73; P=0.64), but they were all lower then control (TABLE 3), been the highest value for treatment N+Cation (2.34±0.09 g.kg^{-1}) and the lowest P+Cations (1.92±0.13 g.kg^{-1}).

5.9. Frass Biomass Nutrient dynamics

The nutrient input from insect frass responded strongly to the addition of P. This pattern differs from the frass nutrient content and frass biomass alone. There were significantly greater nutrient inputs from frass in the plots receiving P additions (+P) compared to plots that did not receive P additions (–P): This occurred for phosphorus (g.ha^{-1}) ($F_{1,29}$=5.24; P=0.03*), calcium (g.ha^{-1}) ($F_{1,26}$=5.43; P=0.02*), magnesium ($F_{1,29}$=5.87; P=0.02*) and manganese ($F_{1,29}$=4.79; P=0.03*), (FIGURE 18). The frass

nutrients inputs did not differ among treatments for potassium ($F_{7,24}$=0.61; P=0.73) (FIGURE 18).

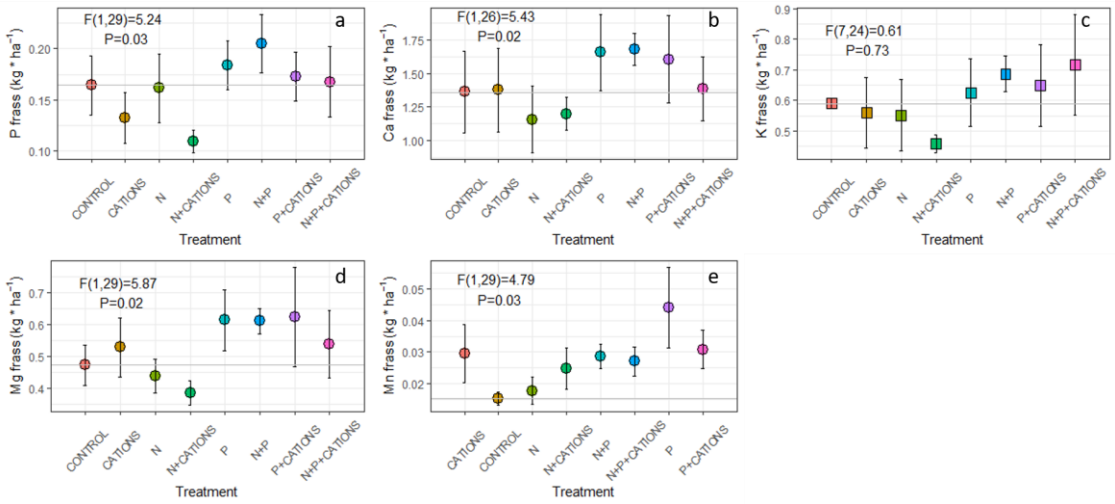

FIGURE 16. Quantity of P (a),Ca (b) , Mg (d), Mn (e) and K (c) in insect biomass frass (g.ha^{-1}) with and without P, and in all 8 treatments. The x axis being all treatments and the mean control value in marked with black line. P<0.05.

5.10. Interactions between leaf traits variables and herbivory frass

The result of the fifth objective of determination of the interrelations between litter production, investment in secondary compounds, concentration of nutrients and levels of herbivory. A Principle Component Analysis (PCA) (FIGURE 20), made with leaf traits variables shows they explain together 51.2% of the variation in the data. The first axis explain 34.9%. of the total variance and represents a leaf economics spectrum gradient, and the second explains an additional 16.3% of the variation. The leaf nutrients (g.kg^{-1}) phosphorus, mangnesium, calcium, potassium, manganese are separated from the defence compounds (%), lignin, cellulose, phenols and LMA (g.m²), showing the tradeoff between defense and nutrients in the leaf litterfall.

Leaf and insect frass nutrient inputs showed a very high significant positive correlation (Fig. 21). These were significant in phosphorus ($F_{1,30}$=7.86, P=0.008, R^2=0.35), magnesium ($F_{1,29}$=5.42, P=0.03, R^2=0.15), calcium ($F_{1,29}$=7.21, P=0.01, R^2=0.41), and potassium ($F_{1,27}$=4.8,P=0.04, R^2=0.15). The interaction between nutrient calcium (g.kg^{-1}) and phenols defense compounds showed a significant negative correlation ($F_{1,29}$=9.36, P=0.004), R^2=0.44.

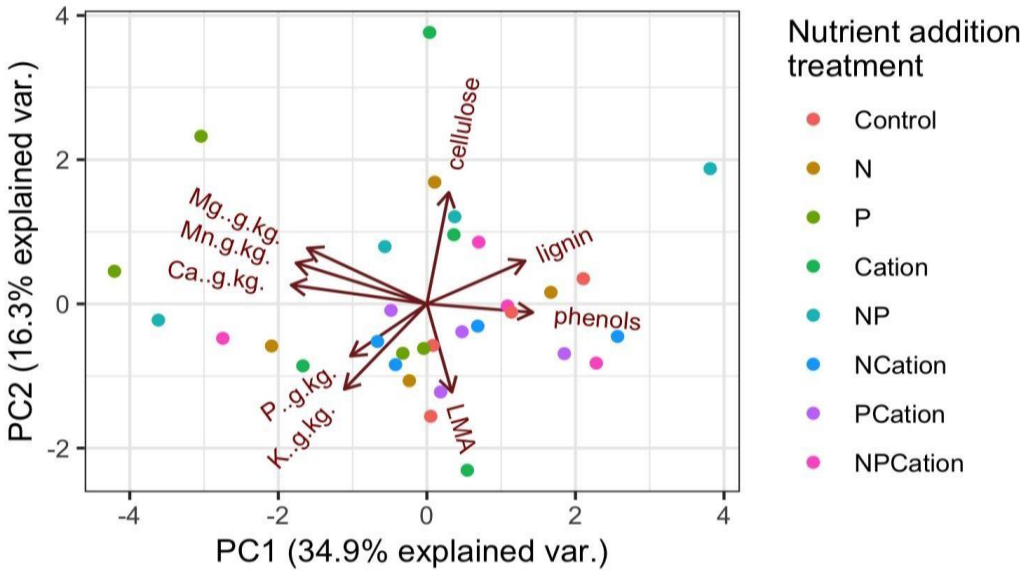

FIGURE 17. PCA on leaf trait variables (nutrients phosphorus, mangnesium, calcium, potassium, manganese (g.kg^{-1}), LMA (g.m^2), Cellulose, lignin and phenosl (%), based on means per plot. The two principal components accounted for 51.2% of the variation data. The PCA included all eight nutrient addition treatments of Control (red), Nitrogen (N) (yellow), Phosphorus (P) (light green), Cation (dark green), N+P (light blue), N+Cation (turquoise blue), P+Cation (lilac), N+P+Cation (pink).

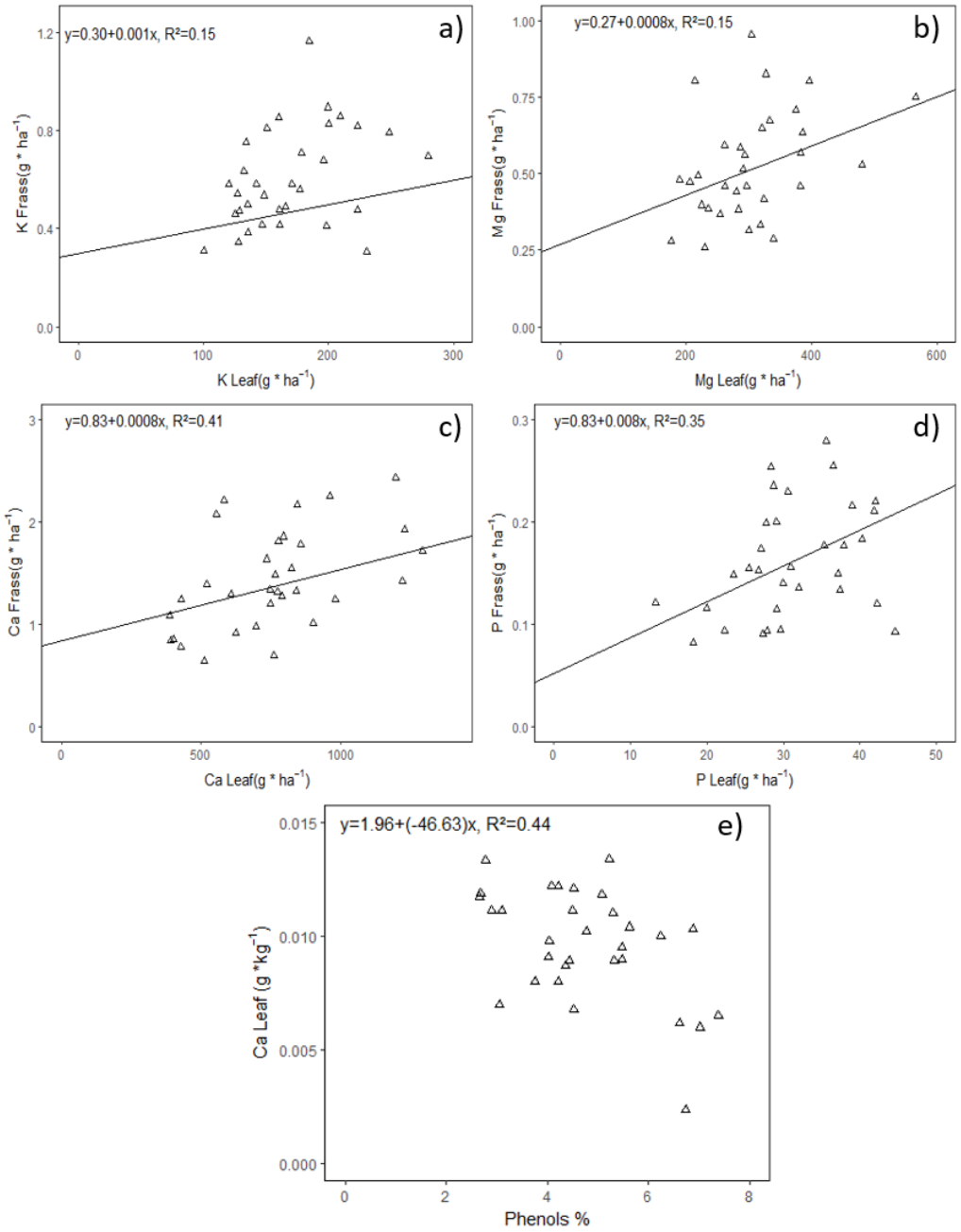

FIGURE 18. Simple correlation between concentration of the nutrients in insect frass biomass input (g.ha^{-1}) and leaf litter biomass input (g.ha^{-1}) of potassium (a), magnesium (b), calcium (c) and phosphorus (d) and figure e is the correlation between calcium (g.kg^{-1}) and phenols.

TABLE 3. Table of variables in treatments control. It contains the type of material, unit, number of repetitions (N), mean value, standard deviation (SD), standard error (SE), and confidence Interval (CI).

	Variable	Mean	SD	SE	CI 95%	N
Nutrient concentration in litter (g kg^{-1})	P	0.19	0.018	0.009	0.029	4
	Ca	4.56	1.27	0.637	2.028	4
	Mg	1.77	0.25	0.125	0.399	4
	K	1.06	0.136	0.068	0.216	4
	Mn	0.09	0.01	0.005	0.016	4
Nutrient concentration in frass (g kg^{-1})	P	0.63	0.13	0.066	0.211	4
	Ca	5.13	0.93	0.47	1.485	4
	Mg	1.83	0.07	0.034	0.109	4
	K	2.415	0.68	0.34	1.083	4
	Mn	0.06	0.004	002	0.006	4
Nutrient input from litter (g ha^{-1})	P	27.74	1.82	0.912	2.904	4
	Ca	680.09	239.4	119.7	380.94	4
	Mg	262.09	50.904	25.45	80.99	4
	K	155.72	21.68	10.84	34.504	4
	Mn	13.46	2.84	1.42	4.522	4
Nutrient input from frass (g ha^{-1})	P	0.16	0.06	0.03	0.092	4
	Ca	1.36	0.605	0.303	0.963	4
	Mg	0.47	0.126	0.063	0.200	4
	K	0.59	0.032	0.016	0.051	4
	Mn	0.015	0.004	0.002	0.007	4
Concentration of defense compounds in litter (%)	Cellulose	27.94	2.17	1.085	3.453	4
	Lignin	42.7	3.10	1.551	4.935	4
	Phenols	0.01	0.002	0.001	0.003	4
LH (%)		8.11	2.63	1.315	4.183	4
LMM_Bh (g kg^{-1})		1.26	0.75	0.374	1.192	4
				5.102	111.710	
LMA ($g.m^2$)		181.52	70.20			4
Litter biomass input (kg ha^{-1})		147.27	15.82	7.909	25.169	4
Frass biomass input (kg ha^{-1})		0.26	0.078	0.039	0.124	4

TABLE 4. Table of variables in all treatments. It contains the type of leaf nutrient input, unit ($kg.ha^{-1}yr^{-1}$), number of repetitions (N), mean value, standard deviation (SD), standard error (SE).

	Treatment	Mean	SD	SE	N
P ($kg.ha^{-1}yr^{-1}$)	CONTROL	1.75	0.0717	0.036	4
	CATIONS	1.83	0.477	0.238	4
	N	1.87	0.372	0.186	4
	N+CATIONS	1.56	0.420	0.210	4
	N+P	2.49	0.085	0.043	4
	N+P+CATIONS	2.23	0.501	0.250	4
	P	2.24	0.474	0.237	4
	P+CATIONS	2.12	0.706	0.353	4
Mg ($kg.ha^{-1}yr^{-1}$)	CONTROL	16.6	3.52	1.76	4
	CATIONS	18.9	2.70	1.35	4
	N	17.2	2.40	1.20	4
	N+CATIONS	18.1	3.34	1.67	4
	N+P	27.5	3.00	1.50	4
	N+P+CATIONS	18.2	3.72	1.86	4
	P	23.4	5.24	2.62	4
	P+CATIONS	17.3	5.29	2.64	4
Ca ($kg.ha^{-1}yr^{-1}$)	CONTROL	43.0	15.1	7.54	4
	CATIONS	44.4	8.1	4.05	4
	N	43.6	12.7	6.34	4
	N+CATIONS	50.0	5.20	2.60	4
	N+P	68.3	15.7	7.85	4
	N+P+CATIONS	45.3	12.5	6.23	4
	P	56.1	21.8	10.9	4
	P+CATIONS	37.0	7.88	3.94	4
K ($kg.ha^{-1}yr^{-1}$)	CONTROL	9.83	1.15	0.57	4
	CATIONS	11.8	4.94	2.47	4
	N	10.4	2.67	1.33	4
	N+CATIONS	11.4	1.45	0.72	4
	N+P	13.5	4.06	2.03	4
	N+P+CATIONS	10.0	1.81	0.91	4
	P	10.0	3.29	1.65	4
	P+CATIONS	10.7	2.46	1.23	4

6. Discussion

6.1. Short-term responses to fertilization

We investigated the short-term responses of litterfall production, nutrient inputs, and herbivory to nutrient addition in a Central Amazonian forest. We expected that nutrient additions would increase leaf quality resulting in an increase in leaf turnover rates, or increased leaf litterfall biomass. The leaf litter nutrient inputs (except K) had a strong significant response to the addition of P treatments, in the first three months postfertilization. In contrast, leaf litterfall biomass had a trend for increased biomass with the combined N + P treatment over the initial eight months post fertilization. With the increase of leaf litterfall nutrient concentrations, we expected an overall increase in leaf quality traits, such as a decrease in leaf mass per unit area (LMA) and defense compounds (greater palatability). In contrast to our expectations, we found that LMA increased significantly with added nitrogen and cations but defense compounds (lignin, cellulose and phenols) had no response to nutrient additions. Together, this suggests that leaf litter nutrient inputs are highly flexible in this low-nutrient tropical forest, whereas biomass and defense traits are more stable with longer lag times before changes are found.

We expected herbivory to increase with foliar nutrient content and overall greater palatability, but herbivory, as percentage leaf area lost, did not differ among the treatments. We did find increased leaf mass missing ($g.kg^{-1}$) with added nitrogen and cations, but this response disappeared when scaling to hectare level. To verify herbivore responses to nutrient addition we also quantified insect frass biomass and nutrient content, expecting that we could find an influence of nutrient addition. Similar to our ecosystem level herbivory estimates, cumulative insect frass did not differ between the treatments and control. However, there was a strong response of insect frass nutrient input with the addition of phosphorus. Together, the initial leaf litterfall and herbivore suggests that they had a rapid initial responses to nutrient addition.

6.2. Effect of nutrient addition in litterfall production

Fine litterfall productivity (mean value 9.12±6.33 Mg ha^{-1} $year^{-1}$) at our low phosphorus site was similar to other low nutrient tropical sites (Mirmanto et al 1999, Chave et al. 2010). The fine litterfall in this experiment had mean values in treatment N+P (10.73±8.81 Mg ha^{-1} $year^{-1}$) slightly above the rate found in different sites across Amazonian lowland and montane sites of 8.61±1.91 Mg ha^{-1} $year^{-1}$ (Chave et al., 2010; Vitousek, 1984; Heineman et al., 2015). Similarly, leaf litterfall production in our experimental site (5.73±0.46 (N) to 7.59±0.82 (N+P) Mg ha^{-1} $year^{-1}$) was slightly higher than the overall average of 5.6 Mg ha^{-1} $year^{-1}$ for *terra firme* forests across the Amazon (Chave et al. 2010).

Seasonality of litterfall production followed the pattern expected for the Brazilian Amazon region of Manaus (Wu et al., 2016). Peak litterfall production was the first week of september in all treatments, which follows patterns of other tropical forests with peak production during the dry season (Heineman et al.,2015). Plants produce a bigger flush of young leaves during the dry season, coinciding with lower damage rates by herbivores (Albert et al.2018). This might be considered a defense mechanism as well, because even though there are more palatable leaves, the higher amount of leaves might satiate the insects enough to inflict significantly less damage to trees than when leaves are produced in wet season (Aide, 1988;1991, Coley 1983). This suggests that leaf production occurring in the beginning of dry season may have a slight temporal escape from herbivores, as it also coincides with the period of lowest insect abundance, lowering damage rates in the dry season that (Wolda, 1978; Aide, 1988; 1991).

The cumulative leaf litterfall (FIGURE 9) indicated that the treatment N+P had higher production of leaves before the other treatments in the second week of august, suggesting that this treatment stimulated a coordinated early abscission, with probably a faster turnover than the other treatments. This result is similar to the fertilization experiment in Panama, with significantly higher litterfall production in treatments with N and P during the wet season after 2-3 years and increasing strength of this pattern for P with time (Sayer et al., 2012). After 11 years of fertilization, there were strong increases in annual litterfall production rates with added P (Wright et al. 2011). Thus, we expect that the trend for increased litterfall production with added N and P will eventually lead to increased annual production rates over longer periods. Similarly, fertilizations experiments in Malaysia, Costa Rica and Venezuela, did not find significant changes in litterfall production in the first year of the experiments (Tanner et al., 1992, Mirmanto et al., 1999, Alvarez-Clare et al. 2015), suggesting a lag time of at least one year to two years for increases in aboveground productivity for tropical forests.

Some studies suggest that the productivity obtained in littertraps could result in underestimation in litterfall production, because herbivory is more active in the most fertile forests soils, eating leaves before reaching the littertrap (Alvarez-Clare et al., 2013; Mirmanto et al., 1999; Newbery et al., 2002; Wright et al., 2011; 2018). The amount of missed litterfall is difficult to quantify, being an undocument flow in nutrients and carbon (Gentry and Emmons, 1987; Chave et al., 2010; Metcalfe et al. 2014). Below, I address this research question trying to fill this gap in the nutrient cycle by analyzing the amount of herbivory in the littertraps as an estimate for canopy level herbivory.

6.3. Effect of nutrient addition in foliar traits: LMA

We hypothesized that with nutrient addition, foliar characteristics would increase nutrient content of leaves, decreasing therefore LMA and the investment in defense compounds. We found that the LMA was higher in the treatments with cations and nitrogen, suggesting that fertilization most likely exacerbated the limitation of phosphorus. A higher LMA can be associated with thicker, thougher, and denser leaves, requiring greater force from insects to cut the leaves (Wright and Cannon, 2001). Higher LMA can also mean that the plant probably controls the timing of leaf death, investing in structural defense leaves tend to live longer periods, and with a greater physical resistance to herbivory to have a longer leaf lifespan (Poorter and Jong, 1999; Wright et al., 2004).

The treatments with P had a lower LMA ($g.m^2$) than found in Control, being close to significant results. We expect that is the future P can have significant influence in our results, probably because P, being naturally limiting in the amazon soils, became more available to the plant in those plots. With higher amount of P, the plant might lower their investment in defense mechanisms, making the concentration per unit dry mass probably lower, resulting in thinner leaves, with lower leaf lifespan, (Poorter et al., 2009; Kitajima et al., 2012). It may also relate with greater production of leaf litterfall in our treatment with the presence of P. Studies also show that P fertilization can change leaf density, making rapid changes to LMA in response to P nutrient supply to the soil (Hirose, 1987, Sardans et al., 2006).

6.4. Effect of nutrient addition in foliar traits: Litterfall nutrient concentrations

The strongest response to nutrient addition was an increase in leaf nutrient content and leaf litterfall inputs mainly affected by the addition of P (FIGURE 11, 12, 13, 14). These responses with phosphorus addition suggest that plants are indeed limited by this nutrient. The nutrient addition provides a greater proportion of the P and Cations available from the litter for plant uptake. Most P and Cations in natural forests can have a rapid cycling of nutrients in organic matter, showing that the forests are highly dependent of the production and decomposition of litterfall that result in nutrient inputs (Attiwill and Adams, 1993; Turner and Engelbrecht, 2011; Luizão et al., 1989)

The strong response of higher content of nutrients in the leaves following the treatments with phosphorus indicates that the plants at this site are P limited. The annual input for P was lower (1.56±0.21, N+Cations to 2.49±0.04 $kg.ha^{-1}yr^{-1}$ N+P) when compared P input for Central Amazon, 3.1 $kg.ha^{-1}yr^{-1}$ (Luizão et al.,1989). Phosphorus is a key nutrient in the metabolism of the cells, being responsible for providing energy to photosynthesis process and respiration by ATP (Taiz and Zeiger, 2009). Similar results occurred in fertilization experiments in Mexico, Panama and Australia, where the leaf

nutrient content raised with addition of nutrient P (Cordell et al. 2001; Bennet et al. 1996; Judd et al. 1996, Sayer et al., 2012). The addition of +P resulted in a very strong increase in foliar phosphorus concentration, much higher than where P is less limiting (Campo and Dirzo, 2003).

The nutrient K in the leaves did not have a significant effect on the treatments, having results similar to the control (FIGURE 12, 14). The annual input for K was lower (9.83±0.57 (Control) to 13.5±2.03 $kg.ha^{-1}yr^{-1}$ (N+P)) when compared K input for Central Amazon, 15 $kg.ha^{-1}yr^{-1}$ (Luizão et al.,1989). This could indicate low K uptake, that might be caused because of quantity of potassium added that might be prone to leaching losses (Hedin et al., 2003). Another possible explanation is that greater remobilization of K before leaf abscission occurred in response to low availability in the soil even with nutrient additions (Likens et al., 1994; Sayer et al., 2006b). A final possibility is that potassium is highly regulated and maintained within live plant tissues because K is responsible for a suite of crucial processes including stomatal regulation,plant respiration, mediating tree growth rates and affecting root biomass and turnover (Wright et a. 2018). This argument suggests high levels of remobilization and investments in K in other plant tissues during leaf senescence (Yavitt et al. 2010; Wright et al., 2011).

Calcium had significantly higher mean values in the P and N+P treatments compared with control when scaling up to $Mg/ha^{-1}/yr^{-1}$ (FIGURE 12, 14). Calcium, along with Mg, are key constituent of cellwalls and are less mobile in the plant and are not remobilized prior to leaf abscission, but cycled in litterfall. For Magnesium nutrient input was higher even in our control treatment (16.6±1.76 units) compared to previous values found for Central Amazonia 13.8 $kg.ha^{-1}yr^{-1}$. According to Parker (1983), the annual litterfall can contribute 80 to 90% of the Ca needed for plant growth, the annual input for Ca was higher (37±3.94 (P+Cations) to 68.3±7.85 (N+P)) when compared 36.7 Mg ha^{-1} $year^{-1}$ Ca (Luizão, 1989). Calcium is responsible for the synthesis of cell wall tissue, and growth with cell division, together with Magnesium, responsible for photosynthesis, respiration, DNA synthesis, (Vitousek, 1982; Taiz and Zeiger, 2009).

Manganese, an activator of a large range of enzymes, including proteins required for light induced water oxidation in photosynthesis (Broadley et al. 2012), was significantly greater in treatments with the presence of P, as well when scaling up to canopy productivity in Mg ha^{-1} $year^{-1}$ (FIGURE 13). This suggests that the manganese might be, with magnesium, affecting the amount of photosynthesis of the plants and enhancing the production of leaves as well (Lambers et al., 2015).

Results of nutrients in the leaves were similar to the Panama fertilization experiment with N+P+K, where the concentration of nutrients increased in the litterfall (Kaspari et al., 2008). This might indicate that changes in the proportion of nutrient

resorption is occurring prior to leaf absiscion, because we see an increased amount of nutrients in the leaf litterfall. Probably the plants do not see as a necessity of resorption before the senescence, because the addition of nutrients provides enough nutrients.

6.5. Effect of nutrient addition in foliar traits: Defense compounds

In contrast to the strong and rapid change in leaf litter nutrients, defense compounds (lignin, cellulose and phenols) in the leaf litter did not change in response to the treatments. Lignin and cellulose, had high variation, with no significant difference between the treatments. However, chemical analysis of the defense compounds was made two months after the fertilization. We suggest that the already older mature leaves that received the nutrient addition had the thick leaves with defense structure established before the onset of the experiment and were unable to change structural characteristics such as lignin or cellulose content (Kurokawa and Nakashizuka, 2008; Coley, 1983).

Total phenolic and condensed tannin content can be remobilized, and reduced in litterfall, being a defensive mechanism used in young leaves (Coley et al., 1985). Our results showed that the percentage for polyphenols had no significant difference between treatments but were slightly lower than control. This probably is due to the fact that the addition of nutrients in the plots may have influenced the amount of polyphenols lowering their values, because they are able to build more nutritious leaves as a defense mechanism that can lower the impact of herbivores (Chapin 1980). Other possibility is that, species that produce higher quality leaves may be more tolerant to that herbivory damage, because these plants will probably have resources to produce more leaves and lower the impact of these damages to the plant, and less investment in defense compounds (Cárdenas et al., 2014). To better understand anti-herbivore defense mechanisms, a longer study period of at least one year, with chemical analysis of both seasons is necessary.

6.6. Effect of Nutrient Addition in Leaf Herbivory

Approximately 60 to 80% of herbivory damage occur in the first weeks, when leaves are still young and expanding, since they have more nutrients in biomass and less defense compounds, making them more palatable to herbivores (Chapin, 1980; Endara and Coley, 2010). This suggests that the lifespan of leaves probably influenced our results because the leaves analyzed were expanded before the fertilization. In tropical forests with nutrient poor soils, nutrient content influences the levels of herbivory on leaves of rainforest plants (Coley and Kursar, 1996). With higher nutrient leaves, we expected to find increased levels of herbivory in the nutrient addition treatments. However, there was not an increase in the percentage of leaf area lost in the treatments receiving nutrients

compared to the control. Our results were slightly under average annual rates of leaf area removal (7.27 ± 0.69% (N+P) to 9.17± 0.6% (N+Cations)) found in other tropical forests (12 %), but were higher than for temperate broad-leaved forests (7.1%), and similar to seasonally dry tropical forests of Mexico (9.2%) (Dirzo and Domınguez, 1995; Coley and Barone 1996; Campo and Dirzo, 2003; Metcalfe et al., 2014).

When we calculate the leaf mass missing ($g.kg^{-1}$) using the % area missing and LMA ($g.m^2$), there was a significantly greater leaf mass missing with the addition of both nitrogen and cations compared to either nutrient alone or with neither. This result is likely driven by the change in leaf morphology with greater LMA in the N+cations treatment. Alternatively, the insects may accelerate their food intake to compensate for reduced leaf phosphorus content in leaves in treatments without P, to reach the resources that they need. The value of herbivory lower than average for tropical forest might be explained by the availability of nutrients previously limited in the sites, where herbivores do not need to eat as much in treatments with P because they feel satiated with the necessary nutrients more quickly (Coviella and Trumble, 1999).

6.7. Effect of nutrient addition in herbivory: Insect frass biomass and nutrient content.

We hypothesized that with nutrient addition, insect frass would increase in biomass and in frass nutrient content, with a higher amount of leaves eaten by herbivores and less investment in defense compounds. We found that the cumulative insect frass biomass did not differ between the treatments in comparison with control. The insect frass biomass samples were collected in dry season just two months after fertilization, suggesting that patterns of insect herbivory take longer timescales to change feeding patterns. Similarly, the nutrient addition did not yet impact the leaf strutuctural compounds, so the leaves were consistent of a forest with nutrient limitation (Moran and Hamilton, 1980) despite changes in foliar nutrient content.

The nutrients phosphorus, magnesium, calcium and manganese of the insect frass responded strongly to the addition of P. This suggests that insects, as well as their host plants, are limited by P. Studies also show that herbivory leads to reallocation of nutrients like N and P, where foliar nutrients would be resorbed by insects prior to leaf abscission resulting in nutrients transferred to soil pools via greenfall, frass deposition, and dead insect biomass (Lovett et al. 2002).

The amount of nutrients present in the frass can also indicate that the insects are egesting most of the eaten nutrients available in the leaves on the same plots. Other probability is that, like other studies show, insects select diets containing different nutrients, to compensate for feeding on diets probably deficient in proteins or

carbohydrates (Waldbauer and Friedman, 1991, Stadler, 2012). According to May and Killingbeck (1995), the insects assimilate most of the nutrients consumed in the leaves, like the gypsy moth larvae, that consume approximately 84% of the N is egested in frass, much of the defoliated foliar nutrient is diverted to insect frass (Grace, 1986; Hollinger, 1986; Risley and Crossley, 1993).

6.8. Effect of Nutrient Addition in the interactions between foliar traits and frass herbivory

We expected that the nutrient addition will shift leaf trait tradeoffs between the nutrients and defense compounds towards higher nutrient concentrations in leaves, with reduction of defense compounds, and reductions of leaf toughness, resulting in greater palatability and thus increasing herbivory levels. The PCA (FIGURE 20) showed that the leaf traits have tradeoffs between the nutrients and defense compounds and their influence on LMA. Since plants have limited resources and energy to choose in what to invest (growth or defense, for example) such investiments in conservative or acquisitive traits are fine tuned to environmental conditions. Similarly, Coley (1983) found that leaves with higher quantities of lignin and cellulose are negatively correlated with nutritional content. In forests with nutrient limited soil, plants invest less in growth and more in structural compounds, which can also result in a slow leaf turnover, and increasing leaf thickness (Coley, 1983, 1998; Campo and Dirzo, 2003).

The availability of nutrients has a direct effect on the amount and chemical composition of the leaves. Plants associated with fertile soils usually present leaves with rapid growth rates, higher nutrient content and lower LMA, which makes them more palatable and susceptible to herbivory, however, the higher leaf production associated with these characteristics imply gains in rates which compensates for the losses caused by herbivory. Another scenario is that phenols are also positively correlated with lignin and cellulose, and can be found along with nutrient content, because this mobile defense is more likely to be used in young leaves (Coley 1983, Campo and Dirzo 2003; Cardenas et al., Kurokawa and Nakashizuka, 2008; Sardans et al., 2006).

Leaf litter calcium content and structural compound phenols were negatively correlated. Calcium is important for the synthesis of cell wall tissue and growth and cell division, and therefore may be important in the structural defense of the plant (Taiz and zeiger, 2009). In contrasts, phenols are part of the mobile defenses (Coley, 1983; Vitousek, 1982; Taiz and Zeiger, 2009). This correlation is in agreement with Coley et al. (1983), suggesting that herbivory defense can occur in different scenarios varying on the trade off between structural defense and secondary mobile defenses.

Leaf litter phosphorus, calcium, magnesium and potassium contents and nutrient insect frass nutrient contents were positively correlated. These correlations suggests that the nutrients consumed by the insects are present in the frass as well. Considering that insects obey levels of vertical strata and horizontal distance, is presumed that the herbivory of leaves and egested frass occurred in the same plots. Studies report that insects have olfactory responses to plant, the volatiles phenols act over a short distance, varying from decimeters to 24 meters (Kennedy, 1977a; Nottingham, 1988; Rôttger, 1979; Jermy et al., 1988)

6.9. Limitations of the study

To obtain clearer responses over time the study should continue at least one year to examine stochasticity and stronger responses with time. Analysis of leaf carbohydrates and proteins as well as nutrient analysis of litterfall in the rainy season would give a clearer understanding of how palatability would influence herbivory, and their fluctuation through out the seasons, because natural variability in the level of herbivory is highly stochastic, making it difficult to find clear patterns. To understand the mechanism of defense and have an effective answer, more time of research is necessary.

7. Conclusion

Our results suggest that the litterfall production had a rapid initial response to nutrient addition, indicating that it may alter the mechanism of leaf turnover, accelerating senescence of leaves with treatment N+P. The leaf litterfall nutrient content changed significantly in two months of experiment, mostly in treatments with the presence of P, indicating that nutrient P limit the litterfall production. The nutrient content of herbivory frass also showed influence by nutrient addition with high significance on treatments with presence of P, indicating that P limits herbivory insects as well.

The LMA ($g.m^2$) presented a rapid response with a complex variation to addition of nutrient interactions. The nutrients availability influences other foliar traits, like defense compounds content, resulting in possible changes in dry mass and unit area. The analysis of defense compounds showed that the plants do not change structural defenses after foliar expansion, it is necessary a new chemical analysis with leaves flushed after fertilization.

The interactions between the nutrients of the leaves and the insect frass are significantly correlated and both have negative correlations with defense compounds. These foliar traits are influence by availability of nutrients on the soil, and can increase the leaf production with faster turnover, changing the nutrient and defense compounds trade offs, and probably influencing the palatability for herbivory.

With these preliminary responses, we expect this long-term experiment to increase the turnover of canopy productivity, decrease defense compounds, as well as LMA. Increasing nutrient uptake in leaves and palatability to herbivores, resulting in a possible increase in canopy biomass, and similarly to biomass and nutrients from insect frass, ultimately increasing the input of nutrient cycling.

7.1. Conclusão

Nossos resultados sugerem que a produção de serapilheira pode ter uma rápida resposta inicial à adição de nutrientes por fertilizantes, indicando que pode alterar o mecanismo de renovação foliar, acelerando a senescência das folhas com tratamento N + P. O teor de nutrientes na folha de serapilheira também mudou significativamente em três meses de experimento, principalmente em tratamentos com a presença de P, indicando que o nutriente P limita a produção de serapilheira como era esperado. O teor de nutrientes

das fezes de insetos de herbivoria mostrou influência pela adição de nutrientes com alta significância nos tratamentos com presença de P, indicando que o P também limita os insetos herbívoros.

O LMA ($g.m^2$) apresentou uma resposta rápida com uma variação complexa para adição de interações de nutrientes. A disponibilidade de nutrientes influencia outras características foliares, como o conteúdo dos compostos de defesa, resultando em possíveis alterações na massa seca e por unidade de área (LMA). A análise dos compostos de defesa mostrou que as plantas não alteram as defesas estruturais após a expansão foliar, sendo necessária uma nova análise química com folhas recentemente lançadas após a fertilização.

As interações entre os nutrientes das folhas e as fezes de insetos estão significativamente correlacionadas e ambas têm correlações negativas com os compostos de defesa. Estas características foliares são influenciadas pela disponibilidade de nutrientes no solo, podendo aumentar a produção de folhas com maior velocidade de troca, alterando as compensações dos nutrientes e compostos de defesa, e provavelmente influenciando a palatabilidade para herbivoria.

Tendo essas respostas preliminares, esperamos que esse experimento em longo prazo aumente o turnover da produtividade do dossel, diminua os compostos de defesa, assim como o LMA. Aumentando também a absorção de nutrientes nas folhas e a palatabilidade para herbívoros, resultando em um possível aumento na biomassa do dossel, e similarmente a biomassa e nutrientes de fezes de insetos, aumentando por fim o input da ciclagem de nutrientes.

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