INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA - INPA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DE FLORESTAS TROPICAIS

COMPOSTOS ORGÂNICOS VOLÁTEIS BIOGÊNICOS EMITIDOS A PARTIR DA DECOMPOSIÇÃO DE FOLHAS DE SERAPILHEIRA NA AMAZÔNIA CENTRAL

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Manaus, Amazonas

Março, 2022

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Dissertação apresentada ao Instituto Nacional de Pesquisas da Amazônia como parte dos requisitos para obtenção do título de Mestre em Ciências de Florestas Tropicais.

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ATA DE DEFESA PÚBLICA DE DISSERTAÇÃO
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Ata da Defesa Remota de **Murielli Garcia Caetano**, ocorrido no dia 30/03/2022, via Plataforma de Videoconferência Zoom.

Aos trinta dias de março do ano de 2022, às 8h (horário de Manaus/AM), realizou-se a Defesa Pública de Dissertação de **MURIELLI GARCIA CAETANO**, aluna do Programa de Pós-Graduação *Stricto sensu* em Ciências de Florestas Tropicais, intitulada "**Compostos orgânicos voláteis biogênicos emitidos a partir da decomposição de folhas de serapilheira na Amazônia Central**", sob a orientação da Profa. Dra. Juliana Schietti de Almeida (UFAM) e coorientação da Profa. Dra. Eliane Gomes-Alves (Max Planck Institute for Biogeochemistry), em conformidade com o Art. 52 do Regimento Geral da Pós-Graduação do Instituto Nacional de Pesquisas da Amazônia (MCTI/INPA) e Art. 67 do Regimento Interno do Programa de Pós-Graduação em Ciências de Florestas Tropicais, como parte das atividades para conclusão e obtenção do Título de Mestre em Ciências de Florestas Tropicais. A **Banca Examinadora** foi constituída pelos seguintes membros titulares: Lucia Fuchslueger (University of Vienna), Ana Maria Yañez-Serrano (Institute of Environmental Diagnosis and Water Research - IDAEA) e Tyeen Colligan Taylor (University of Michigan). O Presidente da Banca Examinadora deu início à Seção e informou os procedimentos do exame. A aluna fez uma exposição do seu estudo, e ao término foi arguida oralmente pelos membros da comissão. Após as arguições, os membros da banca se reuniram para avaliação e chegaram ao seguinte parecer:

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Ana Maria Yañez-Serrano	**(X) Aprovou**	**( ) Reprovou**	
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Menção: (x) "Com Distinção" ( ) "Com Louvor" ( ) "Com Distinção e Louvor"

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RESUMO

É conhecido que uma ampla diversidade química de compostos orgânicos voláteis biogênicos (BVOCs – sigla em inglês) pode ser emitida pela serapilheira em decomposição em diferentes regiões, e que estes compostos possuem diversas funções dentro do ecossistema, mediando importantes interações entre solo-planta-atmosfera. No entanto, o que não sabemos é a contribuição desse compartimento em solos tropicais, especialmente dentro da Amazônia, floresta esta responsável pela maior emissão de BVOCs para atmosfera. Aqui, nós investigamos a emissão de BVOCs em um experimento de decomposição foliar com *litterbags* em uma área de terra firme na Amazônia central. Inicialmente, caracterizamos a emissão buscando entender a magnitude e diversidade química de BVOCs emitidos pelas folhas em diferentes tempos de decomposição; após, relacionamos a emissão com a dinâmica de microrganismos e a qualidade da serapilheira. Foram observados 21 BVOCs (oxigenados e isoprenóides) ao longo de 229 dias de decomposição foliar. O início do processo de decomposição da serapilheira demonstrou ser mais importante na emissão de BVOCs que estádios tardios, tanto em magnitude quanto em diversidade química, o que provavelmente foi relacionado a quantidade de massa foliar disponível. A biomassa e atividade microbiana e qualidade da serapilheira, não apresentaram correlações significativas com concentração de BVOCs, quando considerado todo o estudo de decomposição, exceto para sesquiterpenos e conteúdo de água na folha (LWC, %). Embora os resultados deste estudo não possam ser generalizados e sejam ainda preliminares para a compreensão dos processos que controlam as trocas de BVOC entre solo-serapilheira-atmosfera, ele fornece uma caracterização dos compostos que podem ser encontrados abaixo do dossel durante a decomposição foliar na Amazônia central.

ABSTRACT

It is known that a wide chemical diversity of biogenic volatile organic compounds (BVOCs) can be emitted by leaf-litter in different regions and that these compounds have diverse functions within the ecosystem, mediating important soil-plant-atmosphere interactions. However, we do not know the contribution of this compartment in tropical soils, especially in the Amazon, which is responsible for the largest emissions of BVOCs to the global atmosphere. Here, we investigate the emission of BVOCs in a litterbag experiment of leaf decomposition in the *terra firme* area of central Amazonia. Initially, we characterized emission seeking to understand the magnitude and chemical diversity of BVOCs emitted by leaf-litter across the decomposition process; then, we related emission to microorganism dynamics and litter characteristics. Twenty-one BVOCs (oxygenated and isoprenoids) were observed over 229 days of leaf-litter decomposition. The early stages of leaf-litter decomposition proved to be more important in the emission of BVOCs than late stages, both in magnitude and chemical diversity, which was probably related to the amount of leaf mass available. Microbial biomass and activity, and leaf-litter characteristics, showed no significant correlations with BVOC concentration when considering the whole decomposition study, except for sesquiterpenes and leaf water content (LWC, %). Although the results of this study cannot be generalized and are still very preliminary towards understanding the processes that control BVOC exchanges between soil-litter-atmosphere, they provide a characterization of the compounds that can be found below the canopy during leaf decomposition in central Amazonia.

SUMARIO

LISTA DE TABELAS

LISTA DE FIGURAS

coloridos representam a pontuação média da PCA relacionado a cada tempo de decomposição. Os nomes completos das variáveis são apresentados na Tabela 4.

Figure S1. Esquema da configuração da calibração do PTR-MS.

Figure S2. Média (%) de umidade do solo e conteúdo de água foliar (LWC) durante o experimento de decomposição de serapilheira. As barras representam o erro padrão.

Figure S3. Correlação de Pearson entre conteúdo de água foliar (LWC) e concentração média de sesquiterpenos ($\mu g\ g^{-1}_{\text{massa seca}}$) (n= 10).

INTRODUÇÃO GERAL

As florestas tropicais ocupam cerca de 11.6 milhões de km^2 da superfície terrestre (Skole & Tucker, 1993), possuindo grande importância na manutenção da biodiversidade (Myers et al., 2000), no ciclo hidrológico, atuam como um grande sumidouro de carbono (Malhi et al., 2008; Pan et al., 2011) e são responsáveis por mais da metade da emissão global de compostos orgânicos voláteis biogênicos (BVOCs – sigla em inglês) importantes na regulação do clima (Guenther et al., 2012).

Por definição, BVOCs são compostos emitidos de forma natural, com pressão de vapor maior que 10 Pa a 25°C, baixo peso molecular (<300 Da) e cadeia constituída por 15 átomos de carbono ou menos (Dudareva et al. 2006; Williams & Koppman 2007). Além da regulação do clima, a emissão de BVOCs pela vegetação desempenha um papel crucial em funções fisiológicas e ecológicas, atuando como compostos de sinalização para mediação da comunicação dentro da planta e entre plantas, comunicação com microrganismos, na defesa contra o ataque de herbívoros, na proteção contra altas temperaturas ou outros estresses oxidativos; e em processos do ecossistema, como nos ciclos biogeoquímicos (Kesselmeier & Staudt, 1999; Heil & Silva Bueno, 2007; Kessler et al., 2008; Brilli et al., 2009; Fineschi & Loreto, 2012; Peñuelas et al., 2014).

A vegetação é fonte principal de uma ampla gama de BVOCs, incluindo diferentes classes químicas como isoprenóides, alcenos, álcoois, aldeídos e cetonas (Guenther et al., 2012). Dentro dessas classes, os BVOCs mais emitidos e estudados são os isoprenóides, como isopreno (C_5H_8) representando cerca de 70% do fluxo total de BVOCs, seguido pelos monoterpenos ($C_{10}H_{16}$) que contribuem com 11% e sesquiterpenos ($C_{15}H_{24}$) com 2,5 % das emissões globais (Sindelarova et al., 2014; Wang et al., 2018). Monoterpenos e sesquiterpenos possuem papel importante na química atmosférica regional, devido o potencial de produção de aerossóis orgânicos secundários e a influência na capacidade oxidativa da atmosfera a partir destes compostos (Guenther et al., 2011).

Hellén et al. (2018) encontraram que sesquiterpenos são os principais contribuintes na produção de produtos de oxidação abaixo do dossel em uma floresta boreal; e Bourtsoukidis et al., (2018) indicaram que a magnitude das emissões de sesquiterpenos de solos da Amazônia são comparáveis com emissões de dossel. Com isso, mesmo que a

vegetação seja considerada a maior fonte de BVOCs nos ecossistemas terrestres, altas taxas de emissões de BVOCs pelo solo já foram observadas (Gray et al., 2010; Isidorov et al., 2010; Leff & Fierer, 2008).

Na interface solo e serapilheira, os BVOCs são capazes de reduzir a atividade enzimática, a nitrificação e a mineralização, afetar a dinâmica populacional dos organismos do solo, e aumentar o crescimento de comunidades de fungos ou raízes (Asensio et al., 2012; Peñuelas et al., 2014). Estes compostos são produzidos, consumidos ou transformados por processos físicos e químicos - como oxidação e volatilização, ou processos biológicos - como a decomposição microbiana (Mäki et al., 2017). Pelos decompositores, os BVOCs são liberados como produtos metabólicos secundários (Peñuelas et al., 2014), resultantes de atividades enzimáticas (Insam & Seewald, 2010) e fatores abióticos (Asensio et al., 2008), o que faz com que as emissões por serapilheira possam ser até 15 vezes superiores às liberadas pelo solo (Leff & Fierer, 2008). No entanto, a magnitude de emissão e diversidade de BVOCs emitidos pela serapilheira depende das características da mesma e do processo de decomposição.

De forma geral, a serapilheira é um componente florestal constituído por mais de 70% de folhas (Robertson & Paul 1999), galhos, frutos, casca e flores depositadas no chão da floresta, responsável pela transferência de nutrientes da vegetação para o solo em sua decomposição (Vital et al., 2004; Lopes et al., 2010) e devolução de C para atmosfera, como CO_2, através da respiração de microrganismos e animais do solo (Krishna & Mohan, 2017). Em solos amazônicos de baixa fertilidade (Quesada et al., 2011), a ciclagem de nutrientes e a conservação, através da decomposição da serapilheira, são determinantes na manutenção e funcionamento da floresta (Vitousek, 1984), além de reduzir a lixiviação, o que faria com que florestas tropicais de baixa fertilidade se tornassem ainda mais pobres em nutrientes (Vitousek & Sanford, 1986). Dessa forma, a serapilheira é considerada uma fonte e sumidouro de nutrientes, fornecendo todos os elementos para o crescimento das plantas (Tobón et al., 2004; Brancalion et al., 2012).

Este processo chave na ciclagem de nutrientes e fluxo terrestre de C para a atmosfera, que é a decomposição, é influenciado principalmente pelo clima e qualidade ou características da serapilheira (Swift et al. 1979). A qualidade refere-se às características químicas, físicas, morfológicas e estruturais da serapilheira, que influenciam na suscetibilidade do

material à decomposição (Krishna & Mohan, 2017). Uma serapilheira contendo altas concentrações de compostos estruturais, como lignina ou fenólicos, possui menor taxa de decomposição, pois estes compostos não são processados rapidamente. Por outro lado, uma serapilheira rica em nutrientes pode ser facilmente metabolizada por microrganismos, aumentando a velocidade da decomposição (Karberg et al., 2008). A determinação da biomassa microbiana nesses casos se torna extremamente relevante, visto que os microrganismos, em suas atividades metabólicas, são responsáveis pela ciclagem de nutrientes e energia, constituindo a parte viva da matéria orgânica menor que 5.000 μm^3, composta de bactérias, actinobactérias, fungos e algas (Jenkinson & Ladd, 1981). Como resultado, a atividade destes microrganismos na decomposição de serapilheira contribui para a emissão e consumo de BVOCs.

Devido à falta de conhecimento sobre a troca de BVOCs pelo solo e serapilheira das florestas, as emissões abaixo do dossel não são incluídas nos modelos de emissão global, existindo assim uma grande lacuna de conhecimento sobre a quantidade e caracterização de BVOCs emitidos, e sobre quais são os fatores ambientais e os processos biológicos que regulam esses fluxos nos ecossistemas. No entanto, vale ressaltar, que esse tipo de estudo não possui uma metodologia consolidada, dificultando as comparações e estimativa de emissões de serapilheira. Por exemplo, (i) o momento de coleta do material - coleta da folha diretamente na árvore (antes da abscisão) ou então diretamente do chão da floresta sem conhecimento do tempo em que este material esteve em contato com o solo (Chomel et al., 2016) – (ii) e o pré-tratamento e processamento das amostras (ex. picar ou não as folhas) variam entre estudos (Gray et al., 2010). Tudo isso dificulta a inserção deste compartimento em modelos de estimativas BVOCs, especialmente em grande escala como a global.

Neste contexto, estudos como estes se fazem necessários, pois o conhecimento da magnitude e diversidade química de BVOCs emitidos por serapilheira na Amazônia pode contribuir para estudos de metabolômica, que por sua vez usam BVOCs como indicadores de processos biogeoquímicos; para o entendimento da capacidade oxidativa e composição química da atmosfera, uma vez que já foi demonstrado que a reatividade do ar na Amazônia é pouco compreendida devido ao desconhecimento da diversidade de BVOCs emitidos abaixo do dossel (Nölscher et al., 2016); e, em última escala, o entendimento de

tais processos é muito necessário para a compreensão do funcionamento das florestas e a retroalimentação com o clima.

OBJETIVOS

3.1 Objetivo geral

Avaliar a emissão de compostos orgânicos voláteis biogênicos (BVOCs) ao longo do processo de decomposição de folhas de serapilheira em terra firme na Amazônia Central.

3.2 Objetivos específicos

1. Caracterizar os BVOCs emitidos e as características da serapilheira foliar ao longo da decomposição.
2. Avaliar como a dinâmica dos microrganismos influencia na emissão de BVOCs.
3. Analisar as interações entre BVOCs e características da serapilheira no processo de decomposição.

CAPÍTULO 1

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Biogenic volatile organic compound (BVOC) emissions from decomposing leaf-litter in central Amazonia

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Abstract

It is known that a wide chemical diversity of biogenic volatile organic compounds (BVOCs) can be emitted by leaf-litter in different regions and that these compounds have diverse functions within the ecosystem, mediating important soil-plant-atmosphere interactions. However, we do not know the contribution of this compartment in tropical soils, especially in the Amazon, which is responsible for the largest emissions of BVOCs to the global atmosphere. Here, we investigate the emission of BVOCs in a litterbag experiment of leaf decomposition in the *terra firme* area of central Amazonia. Initially, we characterized emission seeking to understand the magnitude and chemical diversity of BVOCs emitted by leaves across the decomposition process; then, we related emission to microorganism dynamics and litter characteristics. Twenty-one BVOCs (oxygenated and isoprenoids) were observed over 229 days of leaf decomposition. The early stages of leaf-litter decomposition proved to be more important in the emission of BVOCs than late stages, both in magnitude and chemical diversity, which was probably related to the amount of leaf mass available. Microbial biomass and activity, and litter characteristics, showed no significant correlations with BVOC concentration when considering the whole decomposition study, except for sesquiterpenes and leaf water content (LWC, %). Although the results of this study cannot be generalized and are still very preliminary towards understanding the processes that control BVOC exchanges between soil-litter-

atmosphere, they provide a characterization of the compounds that can be found below the canopy during leaf decomposition in central Amazonia.

1 Introduction

The decomposition of litter in tropical soils is a key mechanism for forest subsistence because this process contributes to carbon (C) reallocation in the system, nutrient cycling (Vitousek, 1984), and emission of biogenic volatile organic compounds (BVOCs), which is still poorly investigated in tropical soils (Bourtsoukidis et al., 2018; Llusià et al., 2022). These compounds, in turn, have numerous important functions in soil ecology, in interactions between decomposer microorganisms, and ultimately in reactions at the atmosphere level (Peñuelas et al., 2014; Tang et al., 2019).

From the leaf-litter, BVOCs can be emitted through the metabolic activity of microorganisms acting on decomposition or by diffusion of stored compounds driven by physical processes, such as leaf breakage or leaching (Tang et al., 2019). There is evidence to suggest that different classes of microorganisms produce distinct types and amounts of BVOCs (Isidorov & Jdanova, 2002; Smolander et al., 2006). This may be caused by soil type; microbial composition and biomass; temperature and moisture (Greenberg et al., 2012; Leff & Fierer, 2008); material type (e.g. leaf, twig); stage of decomposition (Gray et al., 2010); and nutrient content, which is a factor that directly conditions biological activity and therefore BVOC synthesis (Llusià et al., 2022).

Some studies characterizing BVOC emission by leaf-litter with different forest species and their emission rates under different environmental conditions, especially in boreal forests (Aaltonen et al., 2011; Hellén et al., 2006), coniferous forests (Greenberg et al., 2012; Isidorov et al., 2010), and with Mediterranean species (Asensio et al. 2012; Viros et al. 2020), have already been performed and indicated functional roles for BVOC emission by leaf-litter, such as ecological interactions and responses to environmental stresses. Among the roles that some compounds emitted by leaf-litter play, Santonja et al. (2019) found that emissions of β-caryophyllene (sesquiterpene) from *Pinus halepensis* litter negatively affected the development of neighboring herbaceous plants, influencing ecosystem diversity. McBride et al. (2020) studied the effects of BVOCs on the soil microbial community during litter decomposition by ^{13}C screening, and the results indicated that BVOCs released from the litter contributed all the measured C in the soil

and that this could alter the composition of the soil bacterial and fungal community, influencing C dynamics. In addition, some terpenes emitted by the litter have high reactivity with the hydroxyl radical (OH) in the atmosphere and may represent an active component in determining the oxidative capacity of the atmosphere that is still poorly understood (Viros et al, 2020; Praplan et al., 2019). Thus, BVOCs emitted from the litter can impact the soil food web, alter community ecology, slow, or accelerate the litter decomposition process, and influence chemical composition and oxidative capacity of the atmosphere.

Amazon is the largest tropical forest in the world (Skole & Tucker, 1993) and is therefore considered the largest global source of BVOCs from vegetation to the atmosphere (Guenther et al. 2012), but studies that quantify emissions below the canopy are missing and therefore the contribution of other ecosystem components - such as litter- is not considered in modeling global estimates. In this sense, the Amazonian BVOC budget and its related processes may be underestimated, since the only study conducted in the Amazon to date reported that sesquiterpene emissions below the canopy are comparable to canopy emissions during the dry season (Bourtsoukids et al. 2018) and that this was primarily caused by decomposing litter.

Thus, here we propose to add another step to understand the processes related to emissions below the canopy. To this end, we investigated the BVOCs that were emitted during the decomposition of leaf-litter and their relationship with leaf-litter characteristics and microbial dynamics, in an upland (*terra firme*) area in central Amazonia. Thus, the hypotheses follow that: (1) early stages with higher remaining leaf mass have higher magnitude and chemical diversity of BVOCs; (2) higher biomass and microbial activity promote higher BVOC emission; and finally, (3) the characteristics of the litter (nutrient, structural compound, and water content) influence BVOC emissions.

2 Material and methods

2.1 Site description

Our study was conducted in the Uatumã Sustainable Development Reserve (RDSU), Amazon Tall Tower Observatory (ATTO) site (S 02 08.9° W 059 00.2°- Fig. 1a), located

northeast of the municipality of Manaus (central Amazonia), Amazonas State, Brazil (Andreae et al., 2015). The entire RDSU is divided by the Uatumã River, with plateaus and slopes predominantly classified by acrisols and ferralsols (Quesada et al., 2011). The mean annual temperature is 26.4 °C and the local mean precipitation is 2382.2 mm year^{-1}, presenting a distinct seasonality between the dry (July-October) and rainy (February-June) seasons (Löbs et al., 2020; Botía et al., 2022).

2.2 Sampling design

In an upland area (*terra firme*), a 720-meter transect was installed for the distribution of 10 litterfall collectors, at least 40 meters apart (Fig. 1b). The collectors were made of nylon (1 x 1 m) and placed 50 cm from the ground. Leaves of litter were collected from October 2019 to February 2020, every 15 days, to collect fresh and less degraded leaves. After each collection, leaves were dried in a 65°C oven for 72 hours and then stored until the start of the decomposition experiment.

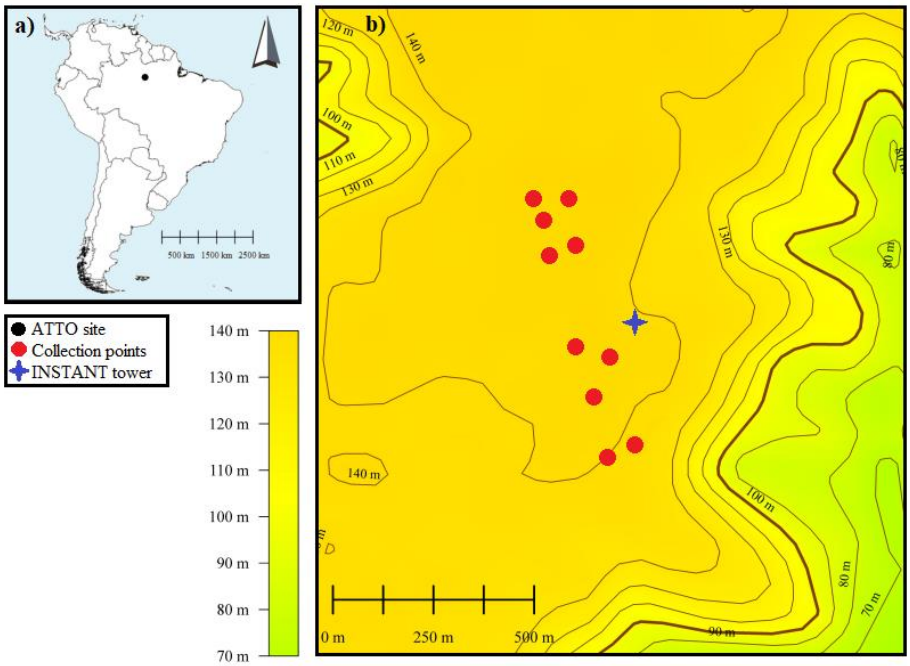

Figure 1. Location of the **(a)** ATTO site and the **(b)** transect where the decomposition experiment was set up.

In October 2020, the decomposition experiment was set up. Each sample from its respective collector was packed into litterbags (20 x 25 cm mesh size of 1.5 mm) for decomposition, where each litterbag held 10 ± 0.01 g of leaf-litter. In total, 15 litterbags

were distributed at each sampling point. Thus, the collections occurred after 20, 38, 51, 194, and 229 days from the installation of the experiment. In each campaign, 3 bags per sampling point were collected, totalizing 30 samples per campaign. In each campaign, the soil moisture was measured at the time of removal of the litterbags. Table 1 shows the values of litter content at the initial stage.

Table 1. The initial chemical composition of the leaf-litter used for the decomposition experiment, values are shown as means and ± standard error (n=10).

Leaf-litter trait	Amount
Total Ca^{+2} (g kg^{-1})	4.93 ± 0.35
Total Mg^{+2} (g kg^{-1})	1.75 ± 0.08
Total K^{+} (g kg^{-1})	1.50 ± 0.10
Total P (g kg^{-1})	0.25 ± 0.01
Lignin (%)	41.62 ± 1.35
Cellulose (%)	24.06 ± 0.76
Phenols (%)	0.008 ± 0.00

2.3 Laboratory incubation

The system consisted of 3-liter static glass (inert material) chambers. The litterbags, once collected in the field, were packed separately in plastic bags; then, in the laboratory, they were opened and roots, soil, or other debris between the leaves were removed with tweezers. Then, the material was weighed (initial wet weight) and incubated in the chambers in a temperature-controlled BOD (Biochemical Oxygen Demand) (25°C) for 24 hours. This temperature inside the BOD represents the average value of the soil temperature in the study area (data not shown).

Before incubation, BVOCs were sampled at time 0 (T0), and after 24 hours (T24) the sampling was repeated, following the same protocol as Asensio et al. (2012). Before and after the T0 measurement, chambers were opened for 1-2 minutes to equilibrate the concentration with ambient air (flushing). This incubation methodology optimizes the concentration of BVOCs in the headspace and allows for comparison between the samples since the temperature during incubation remained constant. A blank chamber sample - from an empty chamber - was taken at the beginning of each period (T0 and

T24) and all tubing was made of inert material (PTFE). Between samplings, the flasks were washed with distilled water and subjected to an oven at 50°C. Figure 2 represents the sequence of the experiment, from the installation of the collectors and litterbag to the incubation and measurement of BVOCs.

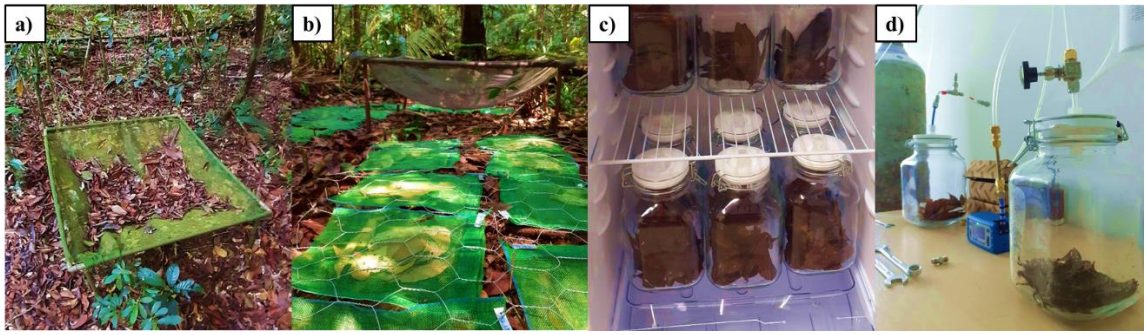

Figure 2. The sequence of activities in the decomposition experiment **(a)** litter trap; **(b)** decomposing litterbags; **(c)** incubation in the BOD; **(d)** BVOC measurements.

2.4 BVOC measurements and analysis

The concentration of the compounds emitted by leaf-litter was detected by two methods. The first one was the air sampling with the incubation chamber connected to a proton transfer reaction mass spectrometer (PTR-MS, IONICON, Austria). The PTR-MS was operated in standard conditions with a drift tube voltage of 600 V, drift tube pressure of 2.2 mbar and E/N 120. During each PTR-MS measurement cycle, the following mass-to-charge ratios (m/z) were monitored: 21 ($H_3{}^{18}O^+$), 32 ($O_2{}^+$) and 37 (H_2O-H_3O^+) with a dwell time of 500ms each; 33 (methanol), 31 (formaldehyde), 42 (acetonitrile), 45 (acetaldehyde), 47 (ethanol), 59 (acetone), 63 (dimethyl sulfide) and 69 (isoprene) with a dwell time of 1s each. BVOC emission was measured at a flow rate of 50 ml min^{-1} for 10 minutes. Humidity-dependent calibrations (using water bubbled nitrogen to dilute the standard gas, and simulating the humidity close to the ambient condition, Fig. S1) were performed with a certified standard gas provided by Apel-Riemer Environmental, Inc (Table S1), before each campaign. The concentrations of BVOCs were calculated from the calibration curves and determined by the difference between T24 and T0, then converted to micrograms (considering chamber volume and molar mass) and normalized by the weight of the leaf material.

The second method occurred only at T24. After collection with PTR-MS, adsorbent cartridges (stainless steel tubes filled with Tenax TA and Carbograph 5 TD) were

collected to identify the isoprenoids at a flow rate of 50 ml min^{-1} for 10 minutes, for subsequent detection in TD-GC-TOFMS. All cartridges were stored in a climate-controlled room, transported to the laboratory, and analyzed within three months.

In the laboratory, the compounds were first desorbed from the sampling tube by the TD-GC-TOF-MS equipment. Desorption took place in two sequential steps, both carried out at 250 °C for 10 min. The desorbed compounds were swept in a stream of Helium into the separation column housed in the gas chromatograph. The column was based on dimethyl TBS β-cyclodextrin (0.15 µm, 0.15 mm ID, 25 mL, from MEGA, Italy), separating compounds according to volatility enantiomeric configuration. The detection was performed by a Time-of-Flight mass spectrometer, which fragments the compounds by electron impact ionization at -70 eV to quantify and identify chemical species. Identification of the main chemical compounds was achieved by comparing the mass spectra with the mass library (NIST library) and by injecting a standard gas mixture (162 VOCs supplied by Apel Riemer, USA) and by using liquid standards. The peak areas of the chromatograms were integrated using the TOF-DS software provided with the instrument and custom IDL software for peak integration. Spectral matches were considered excellent for match factors above 900 and good for match factors between 800 and 900. The limit of detection (LOD) was ~1 pptv and method uncertainty is 23%. More details on the analytical method are available from Zannoni et al. (2020a) and Zannoni et al. (2020b).

2.5 CO_2 and CH_4 measurements

The production of carbon dioxide (CO_2) and methane (CH_4) was measured as a proxy for microbial activity and was determined using a Los Gatos (LG) Ultraportable Greenhouse Gas Analyzer. The LG-analyzer has a fixed flow rate of 300 ml min^{-1} and was set to a measurement frequency of 10s. At T0, when the chambers were flushed with outside air before closing, the LG-analyzer was measuring the outside air continuously, from which T0 was determined. At T24, after the measurement of the BVOCs, the LG-analyzer was connected directly to the chamber, and concentrations were measured for 2 minutes. The production was calculated by subtracting the concentrations at T0 from those at T24, converting it to micrograms (considering chamber volume and molar mass), and then by

dividing it by the weight of the leaf material. Subsequently, leaf-litter samples were sent for chemical and physical analysis.

2.6 Physical and chemical traits of litter

2.6.1 Microbial biomass

For the determination of microbial Carbon, Nitrogen, and Phosphorus (C, N, and P), a fresh 2g subsample was used from each litterbag. These were separated into fumigated (1g) and non-fumigated (1g) extracts. The fumigated samples were left for 24 hours with chloroform and then divided into two sub-samples (0.5 g). For the first, 50 mL of KCl (Potassium Chloride) was added where total C and N were extracted and in the second sub-sample, 50 mL of $NaHCO_3$ (Sodium Bicarbonate) was added for total P extraction. Following the same extraction protocol, the second set of samples was prepared for direct extraction without going through the 24-hour fumigation period. Microbial C, N, and P content was estimated in fumigated and non-fumigated extracts from the difference in organic C, N, and total P measured by a TOC/TN analyzer (Jenkinson et al., 2004).

2.6.2 Litter traits

Physical characteristics include dry mass content (DMC, %) and leaf water content (LWC, %). After drying and weighing the sample, the remaining mass was obtained by taking the initial dry mass (DM*i*) and the final dry mass (DM*f*) of each collection, according to Eq. (1) and to obtain the LWC (%), the dry mass by the initial wet mass was divided.

$$DMC\% = \left(\frac{DMf}{DMi}\right).100$$

Analysis of chemical characteristics includes total nutrient concentration: phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg); and structural compounds: lignin, cellulose, and phenols. The total concentration of nutrients was analyzed after digesting samples with a nitro-perchloric acid solution (Malavolta et al. 1989). Total P was determined by colorimetry (Murphy and Riley, 1962; Olsen and Sommers, 1982) and read on a UV spectrophotometer (Model 1240, Shimadzu, Kyoto, Japan). K, Ca, and Mg concentrations were measured by atomic absorption spectrophotometry (AAS, 1100 B, Perkin Elmer, Ueberlingen, Germany) as Anderson & Ingram (1993) described. The

cellulose and lignin content were quantified as Van Soest (1963) and the total polyphenol content (simple phenols and hydrolyzable tannins) was analyzed by the Folin Denis method (Coley, 1983). For these analyses, a composite sample of the three litterbags per point/time was taken, dried in an oven at 65°C for 72 hours, and ground.

2.7 Decomposition constant

The decomposition constant (k) was calculated considering the ratio between the values of remaining dry masses in 229 days of the experiment. The exponential model used is proposed by Thomas and Asakawa (1993), according to Eq. (2):

$$Pt = Pi\,.\,e^{-kt}$$

where, Pt: remaining dry weight of the sample after the time (t) in days; Pi: dry weight initially inserted into the litterbags at time zero (Pi=10g); k: describes the decomposition constant.

2.8 Statistical analysis

All analyses were performed using R Studio 4.1.2 software. To test the effect of time (independent variable) on BVOC concentration, mass loss, microorganism dynamics, and litter characteristics (response variables) we used linear mixed models (LMMs) from the lme4 package (Bates et al., 2015). We used time as a fixed factor, and as random factors, we tested "sampling unit", "time" and "soil moisture", the best model was determined by Akaike's information criterion (AIC). Then, for the models with significant results, we applied Tukey's posthoc using the emmeans package (Length, 2018), to determine the difference between the levels of the fixed factors. All model fits were checked for normality of the residuals. Then, to understand the interactions between the variables collected, we performed principal component analysis (PCA) and correlation tests with the Hmisc package (Harrell, 2018) to obtain Pearson's correlation coefficient.

3 Results

After 229 days of decomposition, a 39.1% loss of leaf mass was observed (Fig. 3) and this mass loss was significantly different over time ($F_{(5:45)}$ =118.3 P=<0.0001). The exponential model of the decomposition constant was well fitted (R^2 0.7370), resulting in a daily mass loss of 0.002 g day^{-1} or 0.730 g $year^{-1}$ (k), equivalent to 73% leaf mass loss per year.

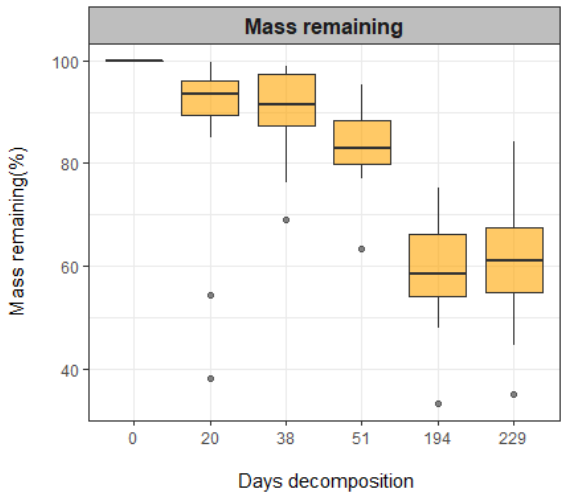

Figure 3. Leaf-litter remaining mass (in % of initial leaf-litter mass) throughout the leaf-litter decomposition experiment (n=10). Box plot midlines are medians, box edges are first and third quartiles, whiskers are the minimums and maximums.

Twenty-one BVOCs were identified during the 229 days of decomposition (Table 2). These were grouped into two chemical classes: isoprenoids (14) and oxygenated (7) (Fig. 4a, b). Within the isoprenoid class, we identified isoprene, monoterpenes (MNT), and sesquiterpenes (SQT). Methanol and acetaldehyde were the compounds observed with the highest concentration within the oxygenated class, whereas in the isoprenoid class, the concentration of isoprene and terpinen-4-ol (MNT) prevailed. Overall, the concentration ranged from -0.31 to 0.91 μg g^{-1} $_{(dry\ mass)}$, and the concentration of oxygenated compared to isoprenoids was 10 times higher.

Regarding the different decomposition periods, higher chemical diversity of BVOCs was observed at 20 and 38 days. In addition to higher diversity, we found a significantly higher concentration peak at 38 days for isoprenoids ($F_{(4:36)}$ =6.03 P= <0.0001) and oxygenated ($F_{(4:36)}$ =2.63 P= 0.0499) (Table 3). At 51 days of decomposition we observed negative concentrations, suggesting more consumption of BVOCs than emission, especially of methanol and isoprene. In the last campaign - considered the final stage of decomposition and the wet-to-dry transition season - we observed a higher diversity of monoterpenes in the samples.

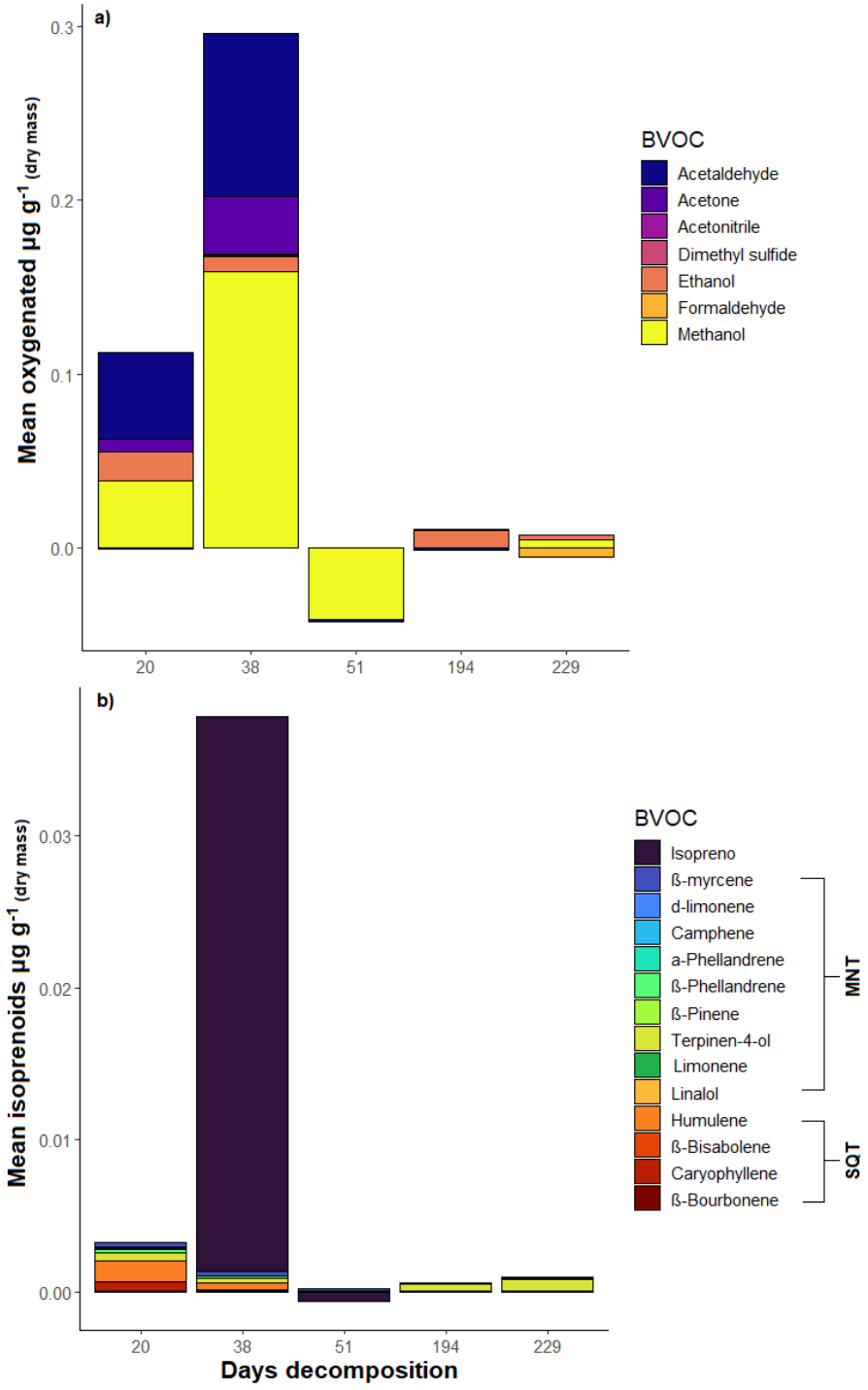

Figure 4. Average BVOC concentration (µg g^{-1} $_{dry\ mass}$) of **(a)** oxygenated and **(b)** isoprenoids during the decomposition of litter leaves (n=10). MNT= monoterpenes; SQT= sesquiterpenes.

Table 2. Average concentration (µg $g^{-1}_{dry\ mass}$) of BVOCs in the headspace during the leaf decomposition experiment (n=10). SE= standard error.

				12/11- dry-to-wet season		*29/11- dry-to-wet season*		*12/12- dry-to-wet season*		*02/05- wet season*		*07/07- wet-to-dry season*	
				20 days		**38 days**		**51 days**		**194 days**		**229 days**	
Compound	**m/z**	**formula**	**method**	**mean (µg g^{-1})**	**± SE**	**mean (µg g^{-1})**	**± SE**	**mean (µg g^{-1})**	**± SE**	**mean (µg g^{-1})**	**± SE**	**mean (µg g^{-1})**	**± SE**
Oxygenated													
Acetaldehyde	45	C_2H_4O	PTR-QMS	0.0499	0.0684	0.0934	0.0828	-0.0009	0.0019	-0.0011	0.0010	-	-
Acetone	59	C_3H_6O		0.0068	0.0033	0.0334	0.0143	-	-	-	-	-	-
Acetonitrile	42	CH_3CN		0.0001	0.0000	0.0004	0.0002	0.0000	0.0000	-	-	-	-
Dimethyl sulfide	63	C_2H_6S		-0.0004	0.0008	0.0007	0.0005	-0.0001	0.0001	0.0001	0.0000	0.0000	0.0000
Ethanol	47	C_2H_6O		0.0170	0.0047	0.0086	0.0018	-0.0002	0.0044	0.0103	0.0018	0.0028	0.0007
Formaldehyde	31	CH_2O		-	-	-	-	-	-	-	-	-0.0050	0.0068
Methanol	33	CH_4O		0.0384	0.0184	0.1593	0.0676	-0.0408	0.0370	0.0002	0.0001	0.0047	0.0024
Isoprenoids													
Isoprene	69	C_5H_8		-	-	0.0365	0.0141	-0.0006	0.0006	-	-	-	-
d_limonene	136	$C_{10}H_{16}$	TD-GC-TOF-MS	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Limonene	136	$C_{10}H_{16}$		0.0000	0.0000	-	-	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
ß-myrcene	136	$C_{10}H_{16}$		0.0003	0.0002	0.0003	0.0001	0.0001	0.0001	0.0001	0.0000	0.0001	0.0000
Camphene	136	$C_{10}H_{16}$		0.0001	0.0001	-	-	-	-	-	-	0.0001	0.0001
ß-phellandrene	136	$C_{10}H_{16}$		0.0002	0.0001	0.0001	0.0001	-	-	-	-	-	-
α-phellandrene	136	$C_{10}H_{16}$		-	-	0.0000	0.0000	-	-	-	-	-	-
ß-pinene	136	$C_{10}H_{16}$		-	-	-	-	-	-	-	-	0.0000	0.0000
Terpinen_4_ol	154	$C_{10}H_{18}O$		0.0006	0.0005	0.0004	0.0002	-	-	0.0004	0.0001	0.0008	0.0004
Linalool	154	$C_{10}H_{18}O$		-	-	0.0001	0.0000	0.0001	0.0000	-	-	-	-
Humulene	204	$C_{15}H_{24}$		0.0014	0.0008	0.0004	0.0003	-	-	0.0000	0.0000	0.0000	0.0000
ß-bisabolene	204	$C_{15}H_{24}$		0.0000	0.0000	-	-	-	-	-	-	-	-
Caryophyllene	204	$C_{15}H_{24}$		0.0006	0.0003	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
ß-bourbonene	204	$C_{15}H_{24}$		0.0001	0.0000	0.0001	0.0000	-	-	0.0000	0.0000	-	-

A large proportion of the isoprenoids emitted from the leaf-litter samples could not be identified by TD-GC-TOFMS analyses (~67%), as many of the compounds were below the detection limit and/or identification was not confirmed by standard gas.

As for the mineralization of nutrients during decomposition, for Ca and Mg, no differences were observed in the first three times. But after 194 days, significantly lower values of Ca ($F_{(5:45)}$ =30.56 P= <0.0001) and Mg ($F_{(5:45)}$ =54.98 P= <0.0001) were observed (Table 3, Fig. 5a, b). As for P, an increase was observed over time, but not significantly ($F_{(5:45)}$ =0.56 P= 0.7254) (Table 3, Fig. 5c). On the other hand, the total K concentration was significantly different during decomposition ($F_{(5:45)}$ =74.81 P= <0.0001), showing a continuous loss of this nutrient since the beginning of the experiment (Table 3, Fig. 5d).

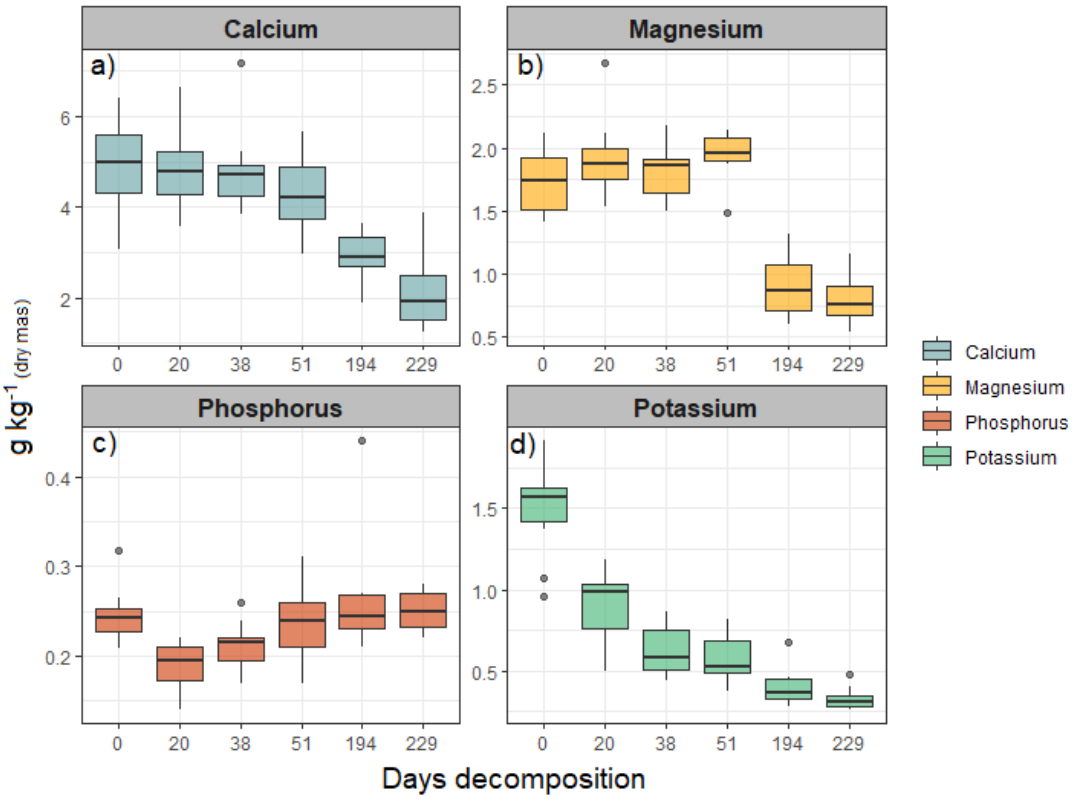

Figure 5. Nutrient concentration (g $kg^{-1}_{dry\ mass}$) **(a)** calcium **(b)** magnesium **(c)** phosphorus and **(d)** potassium in leaf-litter during decomposition *(n=10)*. Box plot midlines are medians, box edges are first and third quartiles, whiskers are the minimums and maximums.

Regarding the structural fractions present in the leaf-litter, we found no significant differences for lignin ($F_{(5:45)}$ =1.88 P= 0.1155) and cellulose ($F_{(5:45)}$ =1.21 P= 0.3193) during leaf decomposition (Table 3, Fig. 6a, b). For phenolics, we observed a significant loss after 38 days of decomposition ($F_{(5:54)}$ =14.56 P= <0.0001) throughout the experiment (Table 3, Fig. 6c).

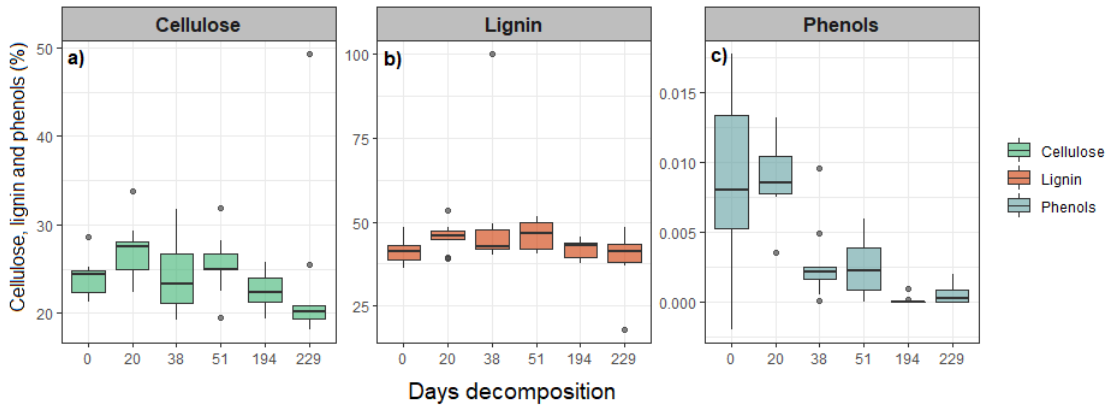

Figure 6. Percentage (%) of **(a)** cellulose, **(b)** lignin and **(c)** phenols in litter leaves during decomposition *(n=10)*. Box plot midlines are medians, box edges are first and third quartiles, whiskers are the minimums and maximums.

The biomass of microorganisms, i.e. the concentration of microbial C, N, and P immobilized by the microorganisms, varied between the different times. Overall, we observed a significantly higher concentration of microbial C at 51 days of decomposition and significantly lower at 38 days ($F_{(3:27)}$ =22.69 P= <0.0001) (Table 3, Fig. 7a). For microbial N we found differences between the first two and the final two campaigns ($F_{(3:27)}$ =16.59 P= <0.0001) (Table 3, Fig. 7b). As for microbial P, we observed a pattern of reduction along decomposition, however, not significant ($F_{(3:36)}$ =1.31 P= 0.2859) (Table 3, Fig. 7c). The microbial C, N, and P samples for the 229 decomposition campaign are still being analyzed in the laboratory at MPI-BGC.

Table 3. Effect of time (days of decomposition) on leaf-litter mass loss, BVOC concentration in the headspace, characteristics of the litter, microbial C-N-P, and CO_2, and CH_4 emission tested from linear mixed models (LMMs). For models that showed significant difference (Signif. Codes: *0.01; **0.001; ***0, ANOVA), a Tukey's posthoc was applied. Values are mean ± standard error (n=10). Different letters in superscript indicate statistically significant differences between days of decomposition (see further description in the method section).

Parameter	**Effect of the days of decomposition**	***Days of decomposition***					
		0	**20**	**38**	**51**	**194**	**229**
Litter remaining mass *(%)*	$F_{(5:45)}$=118.3 P=<0.0001***	100 ± 0,00^{a}	90.25 ± 2.38^{b}	91 ± 1.68^{b}	83.54 ± 1.51^{c}	59.87 ± 1.62^{d}	60.91 ± 2.96^{d}
Total Ca *(g kg^{-1})*	$F_{(5:45)}$=30.56 P= <0.0001***	4.93 ± 0.35^{a}	4.90 ± 0.33^{a}	4.82 ± 0.32^{a}	4.29 ± 0.28^{a}	2.91 ± 0.18^{b}	2.10 ± 0.27^{b}
Total Mg *(g kg^{-1})*	$F_{(5:45)}$=54.98 P= <0.0001***	1.75 ± 0.08^{a}	1.92 ± 0.10^{a}	1.81 ± 0.07^{a}	1.94 ± 0.06^{a}	0.90 ± 0.08^{b}	0.80 ± 0.07^{b}
Total P *(g kg^{-1})*	$F_{(5:45)}$=0.56 P= 0.7254	0.25 ± 0.01	0.19 ± 0.01	0.21 ± 0.00	0.23 ± 0.01	0.26 ± 0.02	0.25 ± 0.01
Total K *(g kg^{-1})*	$F_{(5:45)}$=74.81 P= <0.0001***	1.50 ± 0.10^{a}	0.90 ± 0.07^{b}	0.62 ± 0.05^{c}	0.57 ± 0.04^{cd}	0.40 ± 0.04^{de}	0.32 ± 0.02^{e}
Total Lignin *(%)*	$F_{(5:45)}$=1.88 P= 0.1155	41.62 ± 1.35	45.63 ± 1.43	49.44 ± 6.24	46.10 ± 1.54	41.87 ± 0.98	39.44 ± 2.88
Total Cellulose *(%)*	$F_{(5:45)}$=1.21 P= 0.3193	24.06 ± 0.76	27.14 ± 1.08	24.2 ± 1.42	25.46 ± 1.13	22.60 ± 0.69	23.29 ± 3.23
Total Phenols *(%)*	$F_{(5:54)}$=14.56 P= <0.0001***	0.008 ± 0.00^{a}	0.0090 ± 0.00^{a}	0.0027 ± 0.00^{b}	0.0024 ± 0.00^{b}	0.0001 ± 0.00^{b}	0.0005 ± 0.00^{b}
LWC (%)	$F_{(4:36)}$=34.06 P= <0.0001***	-	60.82 ± 5.20^{a}	45.76 ± 2.25^{b}	36.67 ± 1.39^{cd}	29.15 ± 1.28^{d}	41.91 ± 3.26^{bc}
Microbial C *(μg g^{-1})*	$F_{(3:27)}$=22.69 P= <0.0001***	-	2138 ± 376.58^{b}	1010 ± 213.88^{c}	3698 ± 330.93^{a}	2791 ± 200.05^{b}	-
Microbial N *(μg g^{-1})*	$F_{(3:27)}$=16.59 P= <0.0001***	-	290.37 ± 68.68^{b}	249.49 ± 42.63^{b}	607.61 ± 56.57^{a}	474.75 ± 50.80^{a}	-
Microbial P *(μg g^{-1})*	$F_{(3:36)}$=1.31 P= 0.2859	-	73.05 ± 19.38	54.95 ± 8.37	49.47 ± 10.32	43.87 ± 7.62	-
CO_2 *(μg g^{-1})*	$F_{(4:45)}$=0.67 P= 0.6119	-	1809.33±347.99	1685.08 ±123.78	1785.7 ± 85.26	2072.17 ± 162.68	1905.09 ± 164.46
CH_4 *(μg g^{-1})*	$F_{(4:45)}$=0.88 P= 0.4809	-	0.0894 ± 0.02	0.0755 ± 0.01	0.0020 ± 0.00	0.1193 ± 0.01	0.0983 ± 0.01
Total oxygenated *(μg g^{-1})*	$F_{(4:36)}$=2.63 P= 0.0499*	-	0.111 ± 0.10^{ab}	0.2957 ± 0.17^{a}	-0,0420 ± 0.04^{b}	0.0094 ± 0.00^{ab}	0.0024 ± 0.00^{ab}
Total isoprenoids *(μg g^{-1})*	$F_{(4:36)}$=6.03 P= <0.0001***	-	0.0032 ± 0.00^{b}	0.0378 ± 0.00^{a}	-0,0004 ± 0.00^{b}	0.0005 ± 0.00^{b}	0.0009 ± 0.00^{b}
Total monoterpenes *(μg g^{-1})*	$F_{(4:45)}$=0.78 P= 0.5417	-	0.0012 ± 0.00	0.0007 ± 0.00	0,0001 ± 0.00	0.0005 ± 0.00	0.0009 ± 0.00
Total sesquiterpenes *(μg g^{-1})*	$F_{(4:36)}$=2.85 P= 0.0374*	-	0.0019 ± 0.001^{a}	0.0005 ± 0.00^{ab}	0,0000 ± 0.00^{b}	0.0000 ± 0.00^{b}	0.0000 ± 0.00^{b}
Isoprene *(μg g^{-1})*	$F_{(4:45)}$=6.08 P= 0.0005***	-	-	0.0364 ± 0.01^{a}	-0.0005 ± 0.00^{b}	-	-

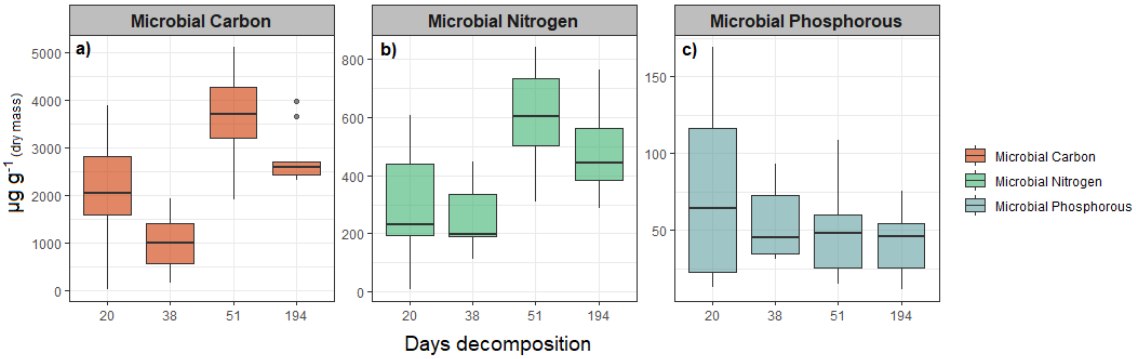

Figure 7. Mean of **(a)** C, **(b)** N and **(c)** microbial P (µg g^{-1} $_{dry\ mass}$) during decomposition of litter leaves (n=10). Box plot midlines are medians, box edges are first and third quartiles, whiskers are the minimums and maximums.

The headspace concentrations of CO_2 and CH_4 were analyzed as a proxy for the microbial activity of the microbial biomass presented in Figure 7. CH_4 ranged from 0 to 0.21 µg CH_4 g^{-1} $_{(dry\ mass)}$, with the lowest emission at 51 days, but not statistically significant ($F_{(4:45)}$ =0.88 P= 0.4809) (Table 3, Fig. 8a). CO_2 production ranged from 468.4 to 3461.9 µg CO_2 g^{-1} $_{(dry\ mass)}$, showing no significant difference between the different times ($F_{(4:45)}$ =0.67 P= 0.6119) (Table 3, Fig. 8b).

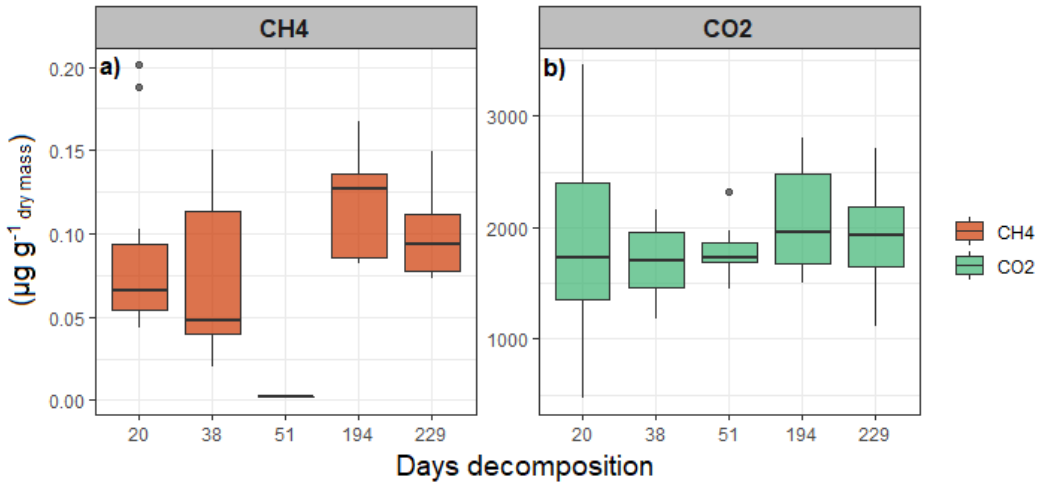

Figure 8. Average production of **(a)** CH_4 (µg CH_4 g^{-1} $_{dry\ mass}$) and **(b)** CO_2 (µg CO_2 g^{-1} $_{dry\ mass}$) during the decomposition of leaf-litter (n=10). Box plot midlines are medians, box edges are first and third quartiles, whiskers are the minimums and maximums.

A PCA performed with the litter characteristics, microorganism dynamics, and BVOC concentrations showed that together they explained 48% of the variance of the data (Fig.

9). The first axis, explaining 28.9% of the variance, suggested clustering of the litter characteristics, with a negative relationship with Ca, Mg, K, phenolics, cellulose, lignin, LWC, and LDM and positive with P. The second axis accounted for 19.1% of the total variance and suggested clustering of microorganism dynamics and BVOC emission, with a negative relationship with microbial C-N-P and CO_2, and positive with BVOCs and CH_4 concentrations. The variables correlated with PC1 and PC2 and the percentage of correlation values are presented in Table 4.

Figure 9 also shows a grouping between the first three campaigns and the characteristics related to the quality of the leaf-litter, demonstrating that these characteristics had a greater influence on the emission of BVOCs at the beginning of the decomposition than in the late stages. It is also important to note that the first three campaigns were grouped possibly because they were closer together temporally when compared to the last two campaigns. The difference between third and fourth campaign was 143 days.

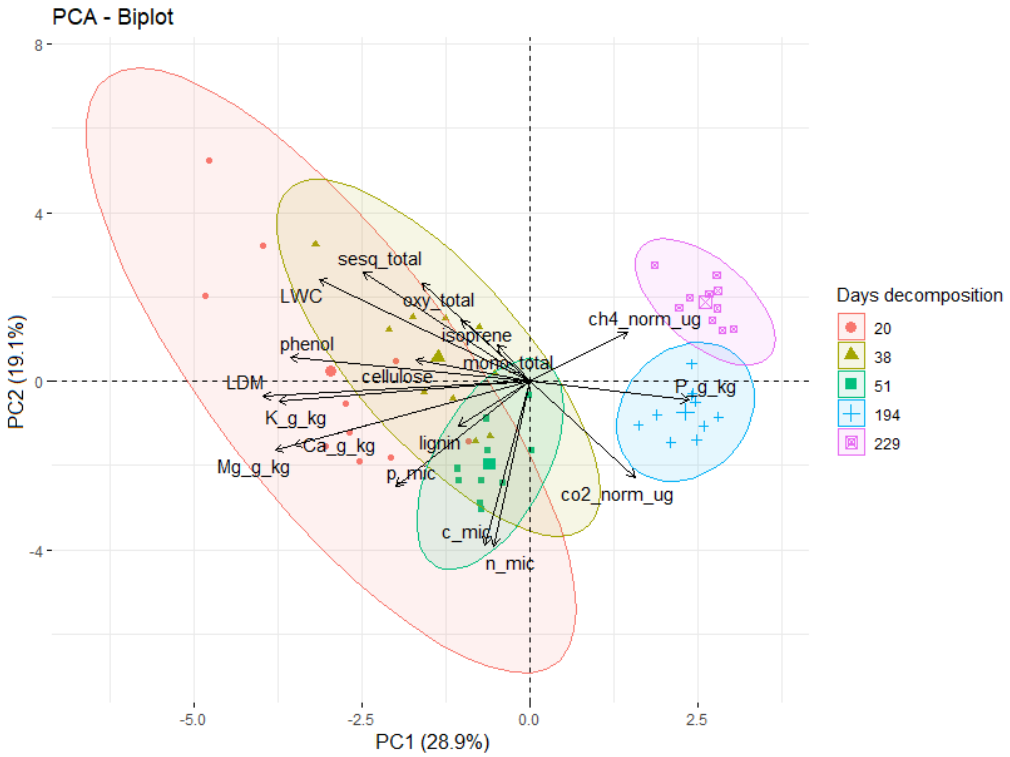

Figure 9. Principal component analysis with BVOC concentration, microorganism dynamics, and decomposing litter characteristics. The colored circles represent the average PCA score related to each decomposition time. The full variable names are presented in Table 4.

Table 4. Percentage correlation values extracted from Principal Component Analysis (PCA; Figure 9) and complete names.

PC1		PC2	
Name (Abv.)	**Correlation (%)**	**Name (Abv.)**	**Correlation (%)**
Phosphorus *(P_g_kg)*	22.8	Sesquiterpenes *(sesq_total)*	30.8
Lignin *(lignin)*	-10.3	Oxygenated *(oxy_total)*	27.6
Cellulose *(cellulose)*	-16.4	Isoprene *(isoprene)*	17.2
Phenolics *(phenol)*	-32.60	CH_4 *(ch4_norm_ug)*	13.8
Calcium *(Ca_g_kg)*	-32.77	Monoterpenes *(mono_total)*	10.4
Potassium *(K_g_kg)*	-33.92	CO_2 *(co2_norm_ug)*	-27.1
Magnesium *(Mg_g_kg)*	-35.79	Microbial P *(p_mic)*	-29.5
Leaf dry mass *(LDM)*	-36.92	Microbial C *(c_mic)*	-46.1
Leaf water content *(LWC)*	*-30.2*	Microbial N *(n_mic)*	-46.3

4 Discussion

We demonstrate that the emission of BVOCs during decomposition in a *terra firme* site of central Amazonia varies with time and the quality of the litter. The decomposition rate found in this study (k=0.72) is similar to that found in a similar forest similar (k=1.01, Martins et al., 2021). The highest concentrations of BVOCs were found in the first two campaigns (20 and 38 days), considered the initial stage of decomposition, and then decreased over time, corroborating our initial hypothesis. This higher concentration at the beginning and subsequent decline in BVOC release can be explained by two reasons, one linked to biological processes and the other to physical processes. First, microbial activity at this stage of decomposition may have been enhanced probably because the leaf-litter material had a higher quality, such as more leaf mass available and higher nutrient content (Leff & Fierer, 2008). However, our data did not clearly support this process since microbial biomass (Fig. 7) and microbial activity (Fig. 8) were not significantly higher in these initial campaigns, and, according to the PCA (Table 4, Fig. 9), these variables were inversely proportional to the maximum BVOC concentration. Second, since the leaf-litter was still at the beginning of the degradation process, possibly, those that had storage of MNT and SQT may have released these compounds by diffusion in this early decomposition (Tang et al., 2019). Although we are not aware of the species whose leaves were used in this experiment, thus we cannot infer whether they had storage structures

for isoprenoids, it is known that in the Amazon the genus *Protium* - a hyperdominant genus (ter Steege et al., 2013) - has species that store monoterpenes and sesquiterpenes (Salazar et al, 2018), and specifically at the ATTO site, of 91 individuals studied (including 31 locally dominant species), 24 possess store isoprenoids (Robin M., *in preparation*), meaning that it is possible that some of the BVOCs observed at the onset of decomposition may come from the diffusion of stored BVOCs from tree species. In addition, the high value of LWC at this early stage (Fig. S2) may have contributed to the maximum headspace concentration of BVOCs (Table 4, Fig. 9), especially for total SQT (r= 0.68; P= <0.0001, Fig. S3), which has been reported as positively correlated to soil and litter moisture (Bourtsoukidis et al., 2018).

It is important to emphasize the contribution of seasonality in the results of this study, because it is known that in the Amazon there is a greater deposition of leaves on the forest floor in the dry season, when most trees change leaves (Wu et al., 2016). Here we observed in 20 days of decomposition, whose period represents the dry-to-wet transition season, the highest concentration of total SQT (0.0022 $\mu g\ g^{-1}_{\text{dry mass}}$), corroborating Alves et al., (2016) who suggested higher SQT emission in this season, in response to the peak of litter stocks and the onset of rainfall in central Amazonia. Additionally, other studies conducted in Amazonia have identified higher terpene emissions in the dry season as a result of the higher accumulation of litter and higher levels of microbial activity (Bourtsoukidis et al., 2018; Llusià et al., 2022). Such compounds could have been released as a means to attract decomposer microorganisms in the soil (Austin et al. 2014).

Regarding the different compounds found in the decomposing litter, methanol, consistent with other studies (Asensio et al., 2008; Gray et al., 2010; Ramirez et al., 2010), was the most emitted compound (Table 3), and it is believed that this compound is predominantly produced via demethoxylation of pectin in cell walls and/or consumed by methylotrophic bacteria (Kramshøj et al., 2018; Tang et al., 2019). Isoprene was another compound observed in significant concentrations, and, although its production is primarily linked to photosynthesis (Kesselmeier and Staudt, 1999; Fernández-Martínez et al., 2018), some microorganisms in the soil can also produce isoprene to a lesser extent (Insam & Seewald, 2010; McGenity et al., 2018). Thus, the positive isoprene concentrations found at 38 days are likely coming from specific microorganisms that may have dominated the decomposer community.

Surprisingly, at 51 days of decomposition, concentrations indicating net consumption of BVOCs were observed, suggesting that such compounds served as an energy source for microorganisms since at this same time we found higher microbial C and N. This evidence of the litter acting as a sink for BVOCs, as indicated by the negative values, agrees with other studies (Asensio et al., 2012; Insam & Seewald, 2010; Ramirez et al., 2010) that highlighted the relevance of BVOCs as a source of available C for microorganisms in the litter and soil. Another interesting result in this same campaign is that we found the lowest production of CH_4 (0.0020 μg g^{-1} $_{dry\ mass}$), which may have resulted from a change in the decomposer community, as an example, a greater presence of methanotrophic organisms that are responsible for CH_4 oxidation (Walkiewicz et al., 2021). However, this would need to be confirmed by future studies with microbial community sequencing.

The other compounds found such as, acetone (Greenberg et al., 2012; Ramirez et al., 2010), acetaldehyde, ethanol (Gray et al., 2010), β-caryophyllene, α-pinene, d-limonene (Leff & Fierer, 2008), linalool, α-terpinene, camphene, myrcene, limonene (Viros et al., 2020) are BVOCs commonly found in litter from different forest types and species.

The relationship of nutrient dynamics with the emission of BVOCs from the leaf-litter, especially cations (Ca, Mg, and K), is not yet entirely clear. However, there is a theory related to the legacy of living leaf characteristics in dead leaves that would explain how these characteristics perpetuate after leaf senescence (Cornewll et al. 2008; Freschet et al., 2012) and could relate to BVOCs emissions (Peñuelas et al., 2014; Peñuelas & Staudt, 2010). Based on this assumption, it has already been observed that leaves with lower nutrient content store more MNT (Martinez-Fernandez et al., 2012), which could explain the negative relationship between P content and the headspace concentration of total monoterpenes and sesquiterpenes (Fig. 9). However, it is worth noting that an increase in P was observed during decomposition and, as reported by Krishna & Mohan (2017), the initial concentration of this nutrient is decreased due to leaching and may increase over time, as organic acids formed by microbial decomposition may accumulate to increase the concentration and thus, make phosphate more accessible to plants (Verhoef & Brussaard, 1990). As for the mineralization of Ca, K, and Mg, it is known that these are nutrients that are easily removed from the litter during decomposition (Anderson &

Ingran, 1983), as found in our study (Fig. 5a, b, d), and, therefore, the reduction in the availability of these nutrients accompanies the reduction in the emission of BVOCs.

In contrast to the rapid change of nutrients in the leaf-litter, structural compounds such as lignin and cellulose showed no difference over time, which can be explained by the fact that these compounds are resistant to decomposition (Klotzbucher et al. 2011). Berg & Staaf (1981) proposed a decomposition model that explains the loss of lignin in two phases: in the early phase, the loss of litter mass is controlled by easily degradable compounds and lignin is preserved. In the late phase of decomposition, the degradation of lignin and cellulose increases, which is then considered the main factor controlling leaf mass loss. Thus, it may be that 229 days of decomposition were not sufficient to initiate the loss of these structural compounds; only phenolics, which are also considered structural compounds, showed a significant loss during decomposition.

Although we found higher concentrations and chemical diversity of BVOCs at the beginning of decomposition, the last two campaigns also contributed to the emission of different compounds and the highest contribution of CO_2 and CH_4. For example, at 194 days the most emitted BVOC was ethanol (0.0103 μg $g_{\text{dry mass}}^{-1}$), coinciding with the highest soil moisture content of the experiment (Fig. S2). This compound is known to be typically produced under anaerobic conditions (Kramshøj et al., 2018), which could be explained by the higher amount of water in the 194-day samples. In addition, we found compounds that were not detected until 229 days of decomposition, such as formaldehyde and β-pinene (Table 3).

The analysis of microbial community structure was not performed in this work, but we believe that it is a crucial point to be understood in future work, because interactions mediated by BVOCs may result in functional traits that help microorganisms in the litter, even resulting in selective advantage for certain groups (Wheatley, 2002). Another aspect to consider is conducting field and laboratory studies with controlled factors to understand how different variables may influence litter emission, both with different species and in different environments. Finding these answers is important given the potential influence of BVOCs on soil ecology and the possibility of influence in above canopy.

5 Possible limitations and caveats

Overall, there was no significant correlation between BVOC concentration and: litter characteristics, microbial biomass, and microbial activity, when considering the whole decomposition experiment (data not shown). However, when analyzed individually, it was observed that different variables could be related to the concentration of BVOCs, which shows the variability among the samples and is not a mechanistic process to explain changes in emission. It is important to consider that this variability between samples could be a result of a methodological limitation, since it was an incubation and therefore does not necessarily reproduce natural conditions. Furthermore, it was not possible to make comparisons of BVOC concentrations with other studies, precisely because there is no standardization of methodology. Other studies, for example, measured BVOC fluxes with controlled conditions, or at different stages of decomposition, and investigated specific plant species. Also, since we have not performed continuous measurements, changes in weather conditions that influence the results could not be evaluated, for example, some unkown changes in weather conditions could have influenced the 51-day campaign and those are not assessed here. Therefore, our measurements do not allow for an in-depth investigation on BVOC emission changing with weather conditions and, therefore, it is not possible to make generalizations.

6 Conclusion

This study helped to characterize the BVOC exchange from decomposing leaf-litter in central Amazonia. The early stages of litter decomposition proved to be more important in the emission of BVOCs than later stages, both in magnitude and chemical diversity, which was probably related to the amount of leaf mass available. Microbial biomass and activity, and litter quality, did not show significant correlations with BVOC concentrations when considering the whole decomposition study, except for sesquiterpenes and LWC. Although the results of this study cannot be generalized and are still very preliminary towards understanding the processes that control BVOC exchanges between soil-litter-atmosphere, they provide a characterization of the compounds that can be found below the canopy during leaf decomposition in central Amazonia.

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SUPPLEMENTARY MATERIAL

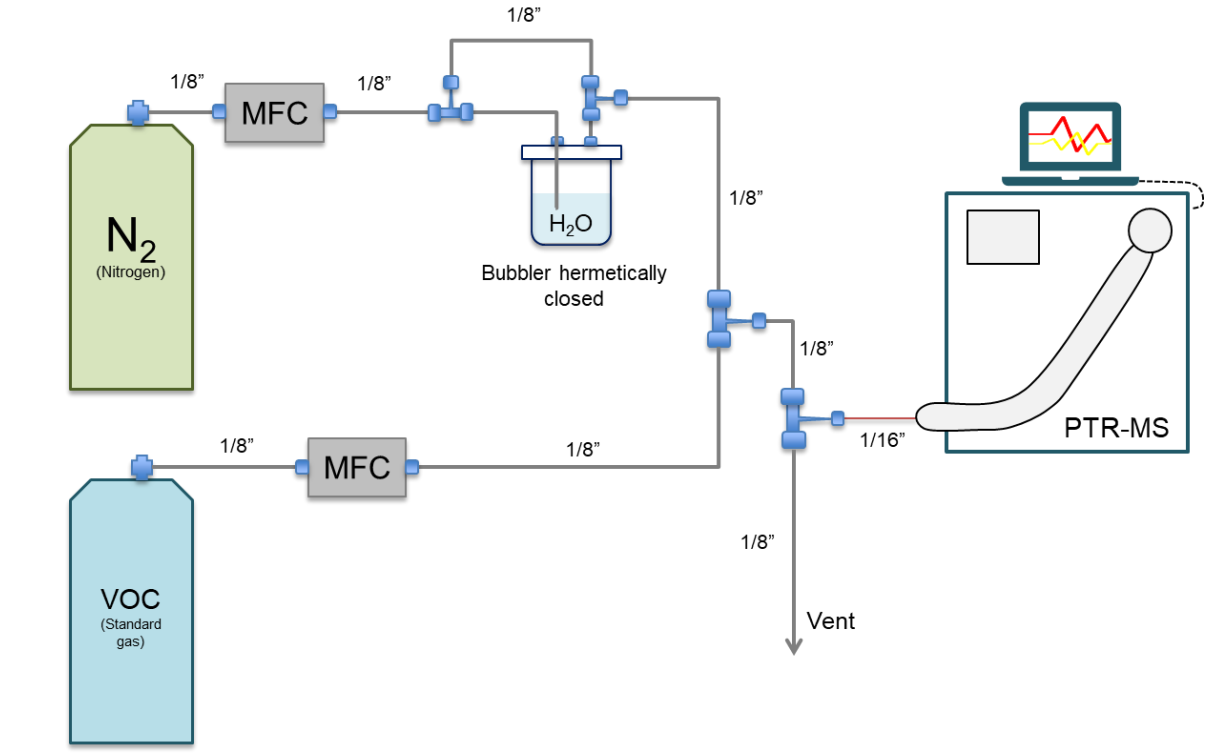

Figure S1. PTR-MS calibration setup scheme.

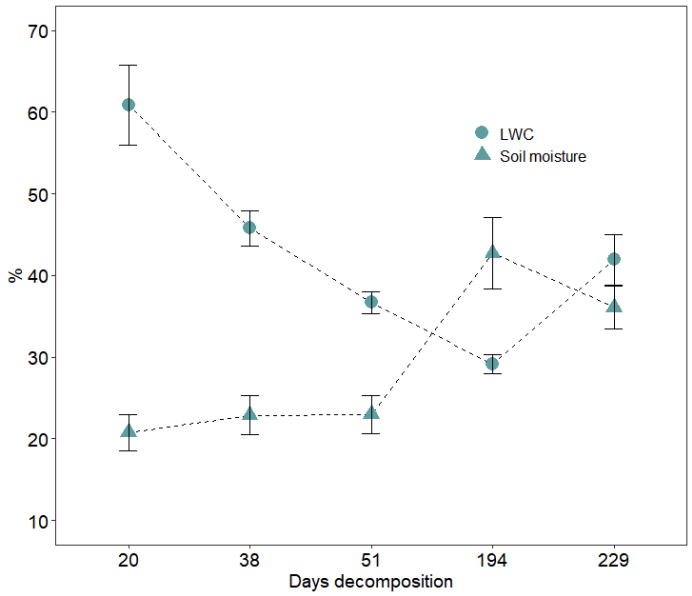

Figure S2. Mean (%) soil moisture and leaf water content (LWC) during the leaf-litter decomposition experiment. Bars represent standard error.

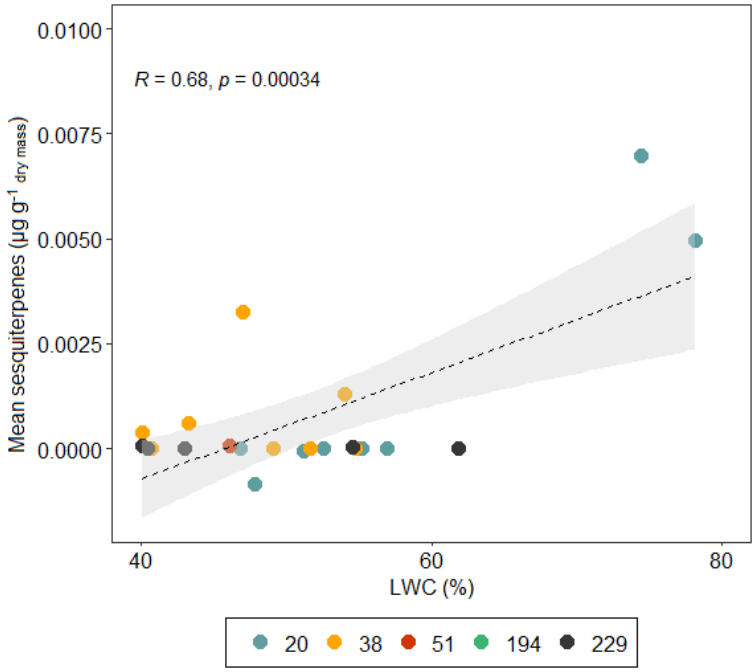

Figure S3. Pearson's correlation between leaf water content (LWC) and mean total sesquiterpene concentration (µg g^{-1} $_{dry\ mass}$) (n= 10).

Table S1. Composition and mixing ratios (ppbv) of gas standards used for quantification and identification of BVOCs.

Standard compound	CAS#	Concentration (ppbv)
Formaldehyde	50-00-0	1000
Methanol	67-56-1	500
Acetonitrile	75-05-8	500
Ethanol	67-17-5	500
Acetaldehyde	75-07-0	500
Acetone	67-64-1	500
Isoprene	78-79-5	500
Dimethyl sulfide	75-18-3	500
α-Pinene	80-56-8	500
β-Pinene	127-91-3	500
Camphene	79-92-5	500
α-Phellandrene	99-83-2	500
o-Cymene	527-84-4	500
Limonene	5989-27-5	500
3-Carene	13466-78-9	500
γ-Terpinene	99-85-4	500
β-Caryophyllene	87-44-5	500
α-Humulene	6753-98-6	500

CONSIDERAÇÕES FINAIS

A proposta do presente estudo foi entender a emissão de compostos orgânicos voláteis biogênicos durante o processo de decomposição de folhas de serapilheira em um sítio experimental na Amazônia Central. Inicialmente, caracterizamos tanto a emissão de BVOCs quanto a decomposição, que foi representada pela taxa de decomposição (*k*), características físicas (conteúdo de água foliar e massa seca foliar) e químicas (teor de nutrientes, lignina, celulose e fenólicos), e pela dinâmica dos microrganismos como emissão de CO_2 e CH_4 e biomassa microbiana. Posteriormente, avaliamos a relação da emissão de BVOCs com a decomposição da serapilheira foliar.

No capítulo 1, apresentamos os resultados de emissão de BVOCs e decomposição durante 229 dias de experimento. A caracterização da concentração dos BVOCs foi dividida em 2 grandes grupos: isoprenóides e oxigenados; e a concentração do grupo de oxigenados em relação aos isoprenóides foi 10 vezes maior. Observamos que a concentração de BVOCs variou conforme o tempo e a qualidade da serapilheira sendo que, o início da decomposição apresentou maiores concentrações em relação aos estádios mais tardios do processo, provavelmente relacionado a uma qualidade de serapilheira superior, compostos armazenados e maior atividade microbiana agindo na decomposição do material, além da importância da sazonalidade neste resultado, pois é conhecido que o período de transição da seca-chuvosa na Amazônia é quando o processo de decomposição é intensificado devido a maior deposição de folhas no chão da floresta no período seco; tal período coincidiu com o início deste experimento. Os resultados de concentração de BVOCs também demonstraram que os mesmos, possivelmente, serviram de fonte de energia para microrganismos em determinado estádio de decomposição, onde foi observado altas taxas de biomassa microbiana e consumo de BVOCs. De todas as características de serapilheira avaliadas, juntamente com a dinâmica dos microrganismos, não encontramos correlações significativas com concentração de BVOCs, exceto para conteúdo de água foliar e sesquiterpenos.

Embora os resultados deste estudo ainda sejam preliminares para entender processos que controlam as trocas de BVOCs entre solo-serapilheira-atmosfera, ele nos fornece uma caracterização inicial de que a decomposição pode ser uma fonte ou um sumidouro de BVOCs na Amazonia Central. Considerando que a maior parte da ciclagem de nutrientes e realocação de Carbono em florestas tropicais se dá através da decomposição da

serapilheira, acreditamos que mais estudos abordando esse compartimento seja de extrema importância para o entendimento de processos de emissão de BVOCs abaixo do dossel, como estudos de laboratório com temperatura e umidade controlada, estudos de campo em diferentes regiões, sequenciamento de microrganismos para entender a relação deles com emissão de BVOCs em microescala, estudos de metabolômica e, por fim, inserção da serapilheira em modelagens globais de emissão de BVOCs.