

# Seasonal shifts in isoprenoid emission compositions from three hyperdominant tree species in central Amazonia

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## Abstract

Volatile isoprenoids regulate plant performance and atmospheric processes, and Amazon forests comprise the dominant source to the global atmosphere. Still, there is a poor understanding of how isoprenoid emission capacities vary in response to eco-physiological and environmental controls in Amazonian ecosystems. We measured isoprenoid emission capacities of Amazonian hyperdominant tree species—*Protium hebetatum*, *Eschweilera grandiflora*, *Eschweilera coriacea* — across seasons and along a topographic and edaphic environmental gradient in the central Amazon. From wet to dry season, both photosynthesis and isoprene emission capacities strongly declined, while emissions increased among the heavier isoprenoids—monoterpenes and sesquiterpenes. Plasticity across habitats was most evident in *P. hebetatum*, which emitted sesquiterpenes only in the dry season, at rates that significantly increased along the hydro-topographic gradient from white sands (shallow root water access) to upland (deep water table). We suggest that emission composition shifts are part of a plastic response to increasing abiotic stress (e.g., heat and drought) and reduced photosynthetic supply of substrates for isoprenoid synthesis. Our comprehensive measurements suggest that more emphasis should be placed on other isoprenoids besides isoprene in the context of abiotic stress responses. Shifting emission compositions have implications for atmospheric responses due to the strong variation in reactivity among isoprenoid compounds.

## 1. Introduction

Isoprenoids are volatile organic compounds (VOCs) that are emitted to the atmosphere mostly by plants. They have diverse functional roles at multiple scales, from cellular protection and defense at the foliar level, through chemical signaling within and among plants, up to the regulation of large-scale biogeochemical processes, such as the effect on atmospheric chemical composition and contribution to aerosol formation (Laothawornkitkul, Taylor, Paul & Hewitt 2009). Volatile isoprenoids are represented by isoprene (C<sub>5</sub>H<sub>8</sub>), monoterpenes (C<sub>10</sub>H<sub>16</sub>), and sesquiterpenes (C<sub>15</sub>H<sub>24</sub>), and their largest source is from tropical trees that contribute ca. 80% of global emissions (Guenther *et al.* 2012). With half of the world's tropical forests, Amazonia is recognized as the most important global source of isoprenoids to the atmosphere (Sindelarova *et al.* 2014). Since the early 1980s, multiple investigations have studied canopy flux, canopy concentrations, and

to a lesser degree, leaf-level emissions of isoprenoids. These studies have reported meaningful insights into the emission drivers and how these compounds are involved in subsequent atmospheric processes (Yáñez-Serrano *et al.* 2020). These combined findings contributed to developing and optimizing an isoprenoid emission model (Guenther *et al.* 2012). Despite this effort, emission estimates from tropical vegetation still carry a high uncertainty due to a poor understanding of the biological controls that determine the *capacity* of emission and its plasticity in response to ecological and environmental conditions, as well as of the environmental controls that determine *rates* of emissions (Alves *et al.* 2018).

The constitutive emission capacity of isoprenoids is determined by the emission at leaf standard conditions (1000  $\mu\text{mol m}^{-2}\text{s}^{-1}$  photosynthetically active radiation, 30 °C), and this is a physiological property that may vary plastically (across individuals) under different ecological and physiological conditions. Actual emission rates are a function of emission capacity and variation in light and temperature (Niinemets *et al.* 2011). This implies that, for modeling isoprenoid emissions, first it is necessary to quantify emission capacities across species, and their plasticity across individuals, to then quantify the emission variation driven by environmental factors such as light and temperature (Duhl, Helmig & Guenther 2008; Niinemets *et al.* 2011; Guenther *et al.* 2012). For several practical reasons, such as the remoteness and high plant species diversity of Amazonia, most studies so far have only investigated isoprenoid emission rates at the ecosystem level and how they vary with environmental factors. The majority of these studies measured canopy concentration or flux of isoprenoids, focusing on isoprene, known to be the strongest emitted compound (Eerdeken *et al.* 2009). Only a few studies measured monoterpenes, and very few studies quantified sesquiterpenes (Yáñez-Serrano *et al.* 2020).

A first attempt to address isoprene emission capacities at the leaf level and the upscaling to ecosystems was made by Harley *et al.* (2004). Knowing that not all plant species emit isoprene (Monson, Jones, Rosenstiel & Schnitzler 2013), the aforementioned study aimed at quantifying the isoprene emission capacity for multiple plant species from different Amazonian regions, and a method was created to impute the isoprene trait to other non-measured trees by using species identification and phylogenetic proximity; then, results were used to upscale the isoprene emission capacity to the ecosystem level based on the trees fraction of isoprene emitters. Subsequent work has further expanded the number of species measured (Jardine *et al.* 2020; Taylor *et al.* 2021). Recent work has derived more mechanistic approaches to scaling isoprene emission across the landscape by determining how the fraction of emitters relates to mean climate conditions (Taylor *et al.* 2018) due to differential performance between isoprene-emitting and non-emitting species (Taylor, Smith, Slot & Feeley 2019). These studies were important as they identified the emission capacity of isoprene, and in a few cases also of monoterpenes, and certainly contributed to the overall body of work from nearly 30 years of research on modeling isoprenoid emission in Amazonia.

Yet, it is also known that isoprenoid composition is conserved within plant species, but that these compounds' emission capacity may vary significantly within species and individuals with photosynthetic capacity, carbon and nutrient investment tradeoffs, habitat, and the environment (Harrison *et al.* 2013). This variability in emission capacities is an essential factor to explain why we observe seasonal variation in isoprenoid emission (see a synthesis of studies in Yanez-Serrano *et al.* 2020), which does not entirely follow the seasonal variation in solar radiation and temperature in central Amazonia (Alves *et al.* 2016, 2018).

Seasonal factors such as leaf demography and phenology are important drivers of variability in leaf emission capacities and the composition of emitted isoprenoids. During early leaf development, young leaves synthesize less isoprene and more monoterpenes and sesquiterpenes (Gershenson & Croteau 1991; Kuhn *et al.* 2004b), and the opposite occurs with leaf maturation (Alves, Harley, Goncalves, Silva & Jardine 2014). This shift in emission composition results from physiological and ecological factors, which cannot be explained by atmospheric observations and direct abiotic effects alone. Variation in leaf physiology with ontogeny scales up through leaf age distributions — with a higher proportion of young leaves during the dry season (Lopes *et al.* 2016; Wu *et al.* 2016) — to influence seasonal variation in isoprenoid emission capacities and total ecosystem emissions (Alves, Harley, Goncalves, Silva & Jardine 2014; Alves *et al.* 2016, 2018).

Faced with these reports, we can infer that most of the studies in Amazonia were focused on emission *rates* —

canopy-level sensitivity concerning environmental factors and landscape-level sensitivity to leaf quantity and emitter fraction. Relatively little work has focused on mechanisms of variation in emission *capacity*, either within or between species, and these have focused exclusively on leaf age, and primarily on isoprene (Alves *et al.* 2014, 2016, 2018). There is still a lack of understanding of the different physiological roles of the other light-dependent isoprenoids, and, by measuring all of them across habitats and seasons within species, we can begin to infer conditions under which one or the other compound is favored and how this can be more accurately scaled to the ecosystem. Therefore, the determination of intraspecific variation in emission capacities of different isoprenoids is a critical knowledge gap that this study intends to address.

In this study, we present a uniquely comprehensive set of leaf-level isoprenoid emission measurements to characterize variation in emission capacities and chemical compositions within species, across habitats, and across seasons. We performed our measurements on trees of three hyperdominant species from central Amazonia — *Protium hebetatum*, *Eschweilera grandiflora*, and *Eschweilera coriacea* (ter Steege *et al.* 2013) — distributed along a topographic and edaphic environmental gradient at the Amazon Tall Tower Observatory (ATTO) site, during the wet and the dry seasons. Simultaneous measurement of photosynthesis and emissions allowed us to assess shifting isoprenoid investments in the context of the leaf carbon balance and the inferred availability of photosynthetic substrates for isoprenoid synthesis.

## 2. Methods

### 2.1. Study site

The study was conducted at the Amazon Tall Tower Observatory (ATTO) within the PELD-MAUA (PELD is the acronym in Portuguese for Long-term Ecological Research) experimental plots. This experimental site is in the Uatuma Sustainable Development Reserve (USDR), about 150 km northeast of the city of Manaus (S 02 08.9deg W 059 00.2deg), in central Amazonia (Fig. 1). The climate is tropical humid, with mean annual temperature and precipitation of 28°C and 2376 mm, respectively, and is marked by a pronounced rainy season from November to May and a drier season from June to October (Andreae *et al.* 2015). The reserve covers 4244 km<sup>2</sup> with a mosaic of dense non-flooded upland forest vegetation, dense non-flooded forest upon ancient river terraces, and shrubland/closed-canopy vegetation on white sands (Yanez-Serrano *et al.* 2015). These three forest types are characterized with differences in soil and vegetation attributes. In the upland forest, soils are classified as ferralsols, which are highly weathered and well-drained (Chauvel, Lucas & Boulet 1987). Soils in the white-sand forests are classified as arenosols, with characteristic properties of high water permeability, low water holding capacity, low specific heat capacity, and often low nutrient contents that are mostly held on organic matter (Quesada *et al.* 2011). Also, white-sand forests can be subject to extremes of flooding and drought at different times of the year. Through intense leaching, Fe, Al, Mg and other compounds are deposited in the lower layers of the soil forming a hard layer that can block water drainage. Thus, in the dry season the vegetation can suffer severe water deficit, and in the rainy season water-logged soils or even superficial inundation of the root system for months (Kubitzki 1989). Soils in the ancient river terrace forests are classified as alisols, which represent a more recent pedogenetic status compared to the ferralsols from the upland forest and therefore have greater capacity to supply nutrients, as it was observed with higher total phosphorus and higher total reserve bases (Andreae *et al.* 2015). However, some ancient river terrace soils show signs of anoxia (mottling) in deeper horizons, which may have an influence on forest structure (Quesada *et al.* 2012; Emilio *et al.* 2013) and dynamics (Cintra *et al.* 2013), and possibly restrict tree height and individual biomass storage (Martins *et al.* 2015) compared to upland forests. The vegetation across the three forest types presents differences in tree species richness, with the highest number of species on upland forest (137+5), followed by ancient river terrace forest (127+8) and white-sand forest (64+18) (Andreae *et al.* 2015). Carbon stocks in above-ground biomass followed the same pattern as species richness, by increasing from 79 ± 26 Mg ha<sup>-1</sup> in the white-sand and 101 ± 13 Mg ha<sup>-1</sup> in the ancient river terrace to a maximum of 170 ± 13 Mg ha<sup>-1</sup> in the upland forests. The ATTO site combines high alpha diversity with high beta diversity within a small geographic scale, where tree species diverge mostly to local edaphic conditions (Andreae *et al.* 2015).

### 2.2. Sampling

We sampled isoprenoid emissions from 30 trees across two permanent plots for each forest type: upland forest, white-sand forest and ancient river terrace forest. Plots were distributed at least one kilometer from each other along the ATTO access road (Fig. 1). Tree species were previously identified (with individual vouchers collected) and confirmed taxonomically. Two species were chosen from a preliminary selection based on (i) their abundance in the PELD MAUA tree species inventory (Andreae *et al.* 2015), (ii) their contribution to a wider distribution in central Amazonia (ter Steege *et al.* 2013; Fauset *et al.* 2015), and (iii) their occurrence in at least two of the forest types of the study experimental site. *Protium hebetatum* (Burseraceae) occurred across the three forest types: upland forest, white-sand forest and ancient river terrace forest. *Eschweilera grandiflora* (Lecythidaceae) was present in two forest types: upland forest and ancient river terrace forest. In addition, we selected the second most dominant species in stem abundance, biomass and productivity in central Amazonia - *Eschweilera coriacea* (Lecythidaceae) (ter Steege *et al.* 2013; Fauset *et al.* 2015), but this species was only present in the plots of white-sand forest. For each species we selected and sampled five trees with similar diameters in each forest type where the species occurred. Their height and position in the canopy varied with species life history. Leaf traits and isoprenoid emission were repeatedly sampled in May 2019 (wet season) and September 2019 (dry season). In addition, a smaller subset of samples were previously taken in December 2018 (dry-to-wet transition season). See table 1 for more details on the weather and climatic conditions for each campaign.

### 2.3. Isoprenoid emission

Leaf-level gas fluxes were measured on site from one leaf per tree and repeated in the same tree across seasons. Measurements were made using a commercial, portable gas exchange system with infrared gas analyzer (IRGA), the LI6400XT (LiCor, U.S.A.). A hydrocarbon filter (Restek Pure Chromatography, Restek Corporations, U.S.A.) was installed at the air inlet of the IRGA to remove hydrocarbons from incoming ambient air. All tubing in contact with the sampling air was PTFE (a material that is non-reactive to hydrocarbons). Before each measurement, a blank was sampled from the empty leaf chamber (see more details on blank samples in Table S1, Supporting Information). One leaf was separately enclosed in the leaf chamber with standard conditions — photosynthetic photon flux density (PPFD) at 1000  $\mu\text{mol m}^{-2}\text{s}^{-1}$  and leaf temperature at 30°C — until net assimilation ( $A_n$ ), stomatal conductance ( $g_s$ ) and internal  $\text{CO}_2$  concentration ( $C_i$ ) were stable. The stability criterion for measurements was assigned as up to one standard deviation of the mean  $A_n$ . The flow rate of air going into the leaf chamber was 400  $\mu\text{mol s}^{-1}$ ,  $\text{CO}_2$  and  $\text{H}_2\text{O}$  concentrations were 400  $\mu\text{mol mol}^{-1}$  and 21  $\text{mmol mol}^{-1}$  (relative humidity of  $\sim 60\%$ ), respectively. Air exiting the IRGA leaf chamber was routed to flow through adsorbent cartridges (stainless silico steel tubes filled with Tenax TA and Carbograph 5 TD adsorbents) at a rate of 200 sccm for 10 min, which resulted in 2 L air samples for isoprenoid chemical analysis. The isoprenoids accumulated in the adsorbent cartridges were determined subsequently by laboratory analysis. Samples from December 2018 and May 2019 were analysed in the State University of Amazonas (UEA, Brazil), and samples from September 2019 were analysed in the Max Planck Institute for Chemistry (MPIC, Germany).

In UEA, cartridges were analyzed with a thermal desorption system (TD; Markes International, U.K.) interfaced with gas chromatograph-mass spectrometer and flame ionization detectors (GC-MS-FID; 7890B-GC and 5977A-MSD series, Agilent Technologies, U.S.A.). The cartridges were loaded in the TD automatic sampler (TD-100, Markes International, U.K.), which connects to the thermal desorption system. Then, samples were dried by purging for 5 min with 50 sccm of ultrahigh-purity helium (all flow vented out of the split vent) and transferred (300°C for 10 min with 50 sccm of ultrapure nitrogen) to the thermal desorption cold trap held at -10°C (Unity Series 1, Markes International, U.K.). During GC injection, the trap was heated to 300°C for 3 min while backflushing with carrier gas (helium) at a flow rate of 6.0 sccm directed into the column (Agilent HP-5, 5% phenyl methyl siloxane capillary, 30.0 m x 320  $\mu\text{m}$  x 0.25  $\mu\text{m}$ ). The oven ramp temperature was programmed with an initial hold of 6 min at 27°C, followed by an increase to 85°C at 6°C  $\text{min}^{-1}$ , followed by a hold at 200 °C for 6 min. We confirmed the identification of emitted isoprenoids from the samples by comparison of retention time with a solution of authentic liquid standards in methanol (Sigma-Aldrich, U.S.A.) and comparison to the library of the National Institute of Standards and Technology (NIST). The GC-MS-FID was calibrated at least three times before the analysis of the sample

cartridges; with calibration curves that were generated by injecting different amounts of gas standard (27 biogenic VOCs gas mixture, by Apel & Riemer Environmental Inc., U.S.A.) into separate cartridges, a mean correlation coefficient  $\geq 0.98$  was obtained, and the LOD quantified as 27 pptv.

In MPIC, the cartridges were analyzed through thermal-desorption gas chromatography time of flight mass spectrometry (TD-GC-TOF-MS, Bench ToF Tandem Ionisation from Markes International, U.K.). The analysis consisted of three main steps: desorption of the analytes from the cartridges (TD), separation of the analytes through gas chromatography (GC), and quantification and identification of the analytes through time-of-flight mass spectrometry (ToF-MS). Samples were first dried by purging them for 5 min with a flow of ultrapure  $N_2$  at  $50 \text{ ml min}^{-1}$ , then transferred to the thermal-desorption unit. Thermal-desorption was carried out in two stages - tube desorption and trap desorption, both performed at  $250^\circ\text{C}$  for 10 minutes through a TD 100xr (Markes International, U.K.). The desorbed components were carried in a flow of Helium into the GC column (dimethyl T.B.S.  $\beta$ -cyclodextrin  $0.15\mu\text{m}$ ,  $0.15\text{mm ID}$ ,  $25\text{m L}$ , from MEGA, Italy). The temperature ramp consisted of an initial 5 min at  $40^\circ\text{C}$ , after which the temperature was increased at a rate of  $1.5^\circ\text{C/min}$  from  $40^\circ\text{C}$  to  $150^\circ\text{C}$ , and further increased at a rate of  $30^\circ\text{C/min}$  from  $150^\circ\text{C}$  to  $200^\circ\text{C}$ . After the GC column, the analytes were fragmented through electron impact ionization at  $-70 \text{ eV}$  in the ToF. Identification was obtained by comparing the MS spectra with the MS NIST library for the same ionization energy and by injection of gas mixtures (162 VOCs gas mixture and 25 biogenic VOCs gas mixture, by Apel & Riemer Environmental Inc., U.S.A.) and liquid standards. The obtained chromatograms were integrated with TOF-DS (Markes International, U.K.). Gas standard cartridges were used to calibrate the instrument, determine the precision and LOD of the analysis, which was quantified as 23% and  $\sim 1$  pptv, respectively. More information on the material and method used can be found in Zannoni *et al.* (2020).

For the final flux calculation, isoprenoid concentrations were determined using the sample volume that was passed through each cartridge. This volume is the integration of the mass flow rate measured and controlled by the pump used to suck the air coming out from the IRGA leaf chamber. Once the volume mixing ratios of isoprenoids (ppbv) were obtained, leaf emission fluxes were determined using the equation ( $F = R \text{ ppbv} \times Q/A$ ), where  $F$  ( $\text{nmol m}^{-2}\text{s}^{-1}$ ) is leaf flux of isoprenoid emission;  $R$  (ppbv) is isoprenoid concentration of the sample;  $Q$  is flow rate of air into the leaf chamber ( $400 \times 10^{-6} \text{ mol s}^{-1}$ ); and  $A$  is the area of leaf within the chamber ( $0.06 \text{ m}^2$ ). In order to calculate isoprenoid emission on a mass basis, we measured Leaf Mass per Area (LMA). LMA was calculated as the ratio of leaf dry weight to leaf area. We did not include petioles in the LMA calculation since they can be quite large for rainforest species and are usually more related to leaf positioning rather than biomass efficiency (Poorter, Castilho, Schiatti, Oliveira & Costa 2018). With LMA, isoprenoid emissions were then calculated to  $\mu\text{gC g}^{-1} \text{ h}^{-1}$ .

#### 2.4. Emission Metrics and Statistical Analysis

Isoprenoid emission and photosynthetic rates were analyzed in common units of  $\mu\text{gC g}^{-1} \text{ h}^{-1}$ . This enabled us to produce integrated metrics related to the leaf carbon balance. We analyzed total isoprenoid emissions both as the sum of all C emitted in the form of isoprenoids, and as a percentage of photosynthetic C assimilation rates. The three compound classes vary in mass (5C isoprene, 10C monoterpenes, 15C sesquiterpenes). We developed an ‘isoprenoid mass investment’ metric to assess the partitioning of C among the different classes of emitted isoprenoids, calculated by multiplying the emission rates of each compound (in  $\mu\text{gC g}^{-1} \text{ h}^{-1}$ ) by their respective masses (number of C atoms), and dividing by the combined total emission rate (i.e. the emission-rate weighted mean mass of emitted compounds). The mass investment metric reflects how much carbon is allocated to lighter versus heavier compounds. For example, the average mass investment of pure isoprene emission is 5C. Since sesquiterpenes contain three times as much C as isoprene, a 2:1 molar ratio of sesquiterpenes to isoprene yields an average mass investment of  $(2(15\text{C} \times 3) + 1(5\text{C} \times 1)) / 7 = 13.6\text{C}$ . Variation in metrics among habitats within species and seasons was analyzed by Tukey Honestly Significant Difference (HSD) test ( $\alpha = 0.05$ ). Variation between dry and wet seasons was analyzed by paired t-test ( $\alpha = 0.05$ ) on measurements taken from the same individuals in both seasons.

### 3. Results

Here we present results of isoprenoid emission capacity (including isoprene, monoterpenes, and sesquiterpenes), photosynthesis, and carbon allocation strategies across habitats and seasons for three tree species – *P. hebetatum*, *E. coriacea* and *E. grandiflora*.

### 3.1. Isoprenoid emission variation among habitats and seasons

Isoprenoid emissions and their habitat and seasonal associations varied among plant species (Fig. 2, Table 2). Isoprene emissions were mostly indistinguishable among habitats, except that emission capacities were higher from *P. hebetatum* in the ancient river terrace (AR) and upland (Up) forest habitats than white-sand (WS) forest in the dry season. Isoprene emission capacities were generally lower in the dry than wet season (Fig. 2), though only significantly so for *P. hebetatum* in two habitats. When aggregating all trees together, isoprene emissions were significantly lower in the dry than wet season (a factor of 0.33, paired T-test  $p < 0.05$ , Fig. S1, Supporting Information). Among the subset of trees sampled during the dry-to-wet transition season (December 2018), isoprene emissions were significantly higher than in the dry or wet seasons (Tukey HSD,  $p < 0.05$ ), averaging 3.3 and 2.4 times higher than wet season emissions for *E. coriacea* ( $n = 5$  trees) and *P. hebetatum* ( $n = 3$  trees) (Fig. S2).

Monoterpene emission capacities were highly variable in magnitude and chemical diversity among individuals within species, even in the same habitat and season (Table 3). Total monoterpene emission capacities were mostly indistinguishable among habitats, except for higher rates from *E. grandiflora* in AR than Up in the wet season, tracking patterns of isoprene emission. The species that emitted most monoterpenes was *P. hebetatum*, with emissions frequently exceeding  $15 \mu\text{gC g}^{-1} \text{h}^{-1}$ . Comparing monoterpene emission capacities between seasons, we observed that *E. coriacea* only emitted monoterpenes during the dry season. No significant seasonal differences were detected within habitats (Fig. 2) or when aggregating by trees and species (Fig. S1). However, the number of chemical species of monoterpenes increased from the wet season to the dry season, both in WS and AR habitats (Table 3). During the dry-to-wet transition season, no monoterpene emissions were detected from *E. coriacea* or *P. hebetatum*.

Sesquiterpene emissions were only detected from *P. hebetatum*, reaching rates comparable to isoprene (when analyzed in units of C emitted, but not moles emitted). These emissions only occurred during the dry season, and significantly increased from WS to AR and Up habitats (Fig. 2). No sesquiterpene emissions were detected during the dry-to-wet transition season. For the chemical species of monoterpenes and sesquiterpenes, see Table 3.

### 3.2. Photosynthesis and carbon investment in isoprenoid emissions

Photosynthesis significantly decreased in most species and habitats during the dry season, while trends varied among species in total isoprenoid emissions and their associated carbon investments (Fig. 3). Photosynthesis was significantly lower in the dry than wet season in most habitats for *E. grandiflora* and *P. hebetatum*, but not for *E. coriacea*. Total C emitted in the form of isoprenoids varied among habitats, being significantly greater from *E. grandiflora* in AR than Up habitats in the dry season, and greater from *P. hebetatum* in AR and Up than WS habitats in the wet season (mainly due to the trend in sesquiterpene emissions shown in Fig. 2). Total C investment in emissions as a percent of photosynthesis was mostly  $< 2\%$  and not distinguishable between habitats in either season, though it reached an average 6.1% in *P. hebetatum* in the Up habitat in the dry season. In *P. hebetatum*, isoprenoid mass investment was significantly greater in AR and Up than WS habitats in both wet season (attributable to greater monoterpene emissions, Fig. 2) and dry season (attributable to greater sesquiterpene emissions, Fig. 2), and was significantly greater in the dry than wet season in AR and Up habitats (Fig. 3).

When aggregated across forest habitats and species, seasonal variation was strongest in photosynthesis and isoprenoid mass investment, and less pronounced in total isoprenoid emissions and carbon emitted as a percent of photosynthesis (Fig. 4). Statistical significance reflects paired t-tests for individuals measured in both seasons ( $\alpha = 0.05$ ), while Figure 4 visualizes the dry season values as a factor of wet season values measured from the same individuals ('dry/wet factor'). Photosynthesis was non-significantly lower in the dry season in *E. coriacea* (mean dry/wet factor = 0.94), significantly lower in *E. grandiflora* (mean

dry/wet factor = 0.73) and *P. hebetatum* (mean dry/wet factor = 0.43), and in all species combined (mean dry/wet factor = 0.62). Total emission was non-significantly higher in the dry season (mean dry/wet factor for all species combined = 1.47), even though emissions were strongly suppressed in many individual trees, except in *E. coriacea* which showed significantly higher emissions (mean dry/wet factor = 2.97). Carbon emitted as a percent of photosynthesis was non-significantly higher in the dry season overall (mean dry/wet factor for all species combined = 3.22) but highly variable among trees, except in *E. coriacea* which showed a significantly higher percentage (mean dry/wet factor = 3.43). Isoprenoid emission mass investment in the dry compared to wet season was non-significantly greater in *E. coriacea* (mean dry/wet factor = 1.41) and *E. grandiflora* (mean dry/wet factor = 1.07), significantly greater in *P. hebetatum* (mean dry/wet factor = 1.65), and significantly greater in all species combined (mean dry/wet factor = 1.41). Total emissions were positively correlated with photosynthesis in *E. grandiflora* (linear regression on all measurements combined,  $p < 0.01$ ,  $r^2 = 0.37$ ), while there was no detectable correlation in the other two species (Fig. S3).

#### 4. Discussion

Past studies have examined constitutive leaf isoprenoid emission from tropical trees with a focus on isoprene (e.g. Keller & Lerdaу 1999; Harley *et al.* 2004; Kuhn *et al.* 2004b; Pegoraro, Rey, Abrell, Haren & Lin 2006; Alves, Harley, Gonçalves, Silva & Jardine 2014; Jardine *et al.* 2014, 2016; Taylor, Smith, Slot & Feeley 2019), followed by monoterpenes (e.g. Kuhn *et al.* 2004a; Jardine *et al.* 2017, 2020). These past studies offered meaningful insights on how isoprene and monoterpenes respond to light, temperature and drought, and how they might be related to plant stress tolerance. Still, constitutive sesquiterpene emission has only been shown from tropical species in one study in Borneo (Llusia, Sardans, Niinemets, Owen & Peñuelas 2014), and therefore most of our scarce knowledge on sesquiterpenes produced by tropical species comes from the content of these compounds either in leaf or resins (e.g. Salazar *et al.* 2018), which only configures the potential for induced emissions. In this study, we observed that Amazonian hyperdominant tree species emit isoprene, monoterpenes and sesquiterpenes in a light-dependent manner (constitutively), and that the amount and proportion of emissions change seasonally. The plasticity of emission capacity and chemical composition of isoprenoids emitted by trees distributed across different forest types, their seasonal behavior, and the significance of our findings are discussed in the following sections.

##### 4.1. Isoprenoid emission capacity from hyperdominant tree species distributed along a topographic and edaphic environmental gradient

We found that on average, isoprene emission capacities did not significantly vary across tree populations of *E. grandiflora* in different habitats, but did vary among populations of *P. hebetatum*. Although it is known that the isoprene trait is conserved within plant species, emission quantities may vary significantly with photosynthetic capacity, carbon and nutrient investment tradeoffs, habitat and the environment (Harrison *et al.* 2013). The species investigated here occur in forest types that vary in edaphic properties, soil water holding capacity, species richness and below- and above-ground biomass (Andreae *et al.* 2015). These changes in soil and vegetation attributes can influence plant performance and, in intraspecific processes, it is common to observe increases in both plant growth and defense secondary metabolites as resource availability increases (e.g. greater nutrient availability in uplands) (Agrawal 2020). Yet, although we observed the highest isoprene emission from *P. hebetatum* at upland forest, this was not observed in *E. grandiflora* and that was probably due to the large variability within individuals at the same forest type.

The plant intraspecific variability was even more pronounced within emissions of monoterpenes – either in diversity of chemical compounds or in total amount of monoterpenes – when comparing individuals distributed in the different forest types. This group of compounds is known to have two different processes of emission. After being synthesized, many monoterpenes are commonly stored in leaves and their inducible emission results mostly from biotic stress; nonetheless, when plant species lack storage structures, most hydrophobic monoterpenes that were synthesized can accumulate in the leaf lipid phase and be constitutively emitted to the atmosphere (Ormeño, Goldstein & Niinemets 2011). Although the functional role of the constitutive emission of monoterpenes has been reported as being similar to isoprene emission (Loreto *et al.* 1996) – namely, leaf thermal protection and leaf excess energy dissipation (Rosenstiel, Ebbets, Khatri, Fall

& Monson 2004; Sanadze 2004; Sharkey & Monson 2017) – the main functional role of monoterpenes is attributed to plant defense against pathogens and herbivores (Fineschi & Loreto 2012), and that may result in better success in plant competition and colonization (Salazar *et al.* 2018). Thus, the high variability of monoterpene emission within species may be related to a plant defense strategy.

In contrast to isoprene and monoterpenes, sesquiterpene emission was only observed from *P. hebetatum*, from trees in all three forest types. To the best of our knowledge, this is the first time that constitutive emission of sesquiterpenes has been observed for an Amazonian tree species that also presented variation across populations. *P. hebetatum* was measured for isoprenoid emission in another Amazonian forest site, but the authors only found emission of isoprene and the monoterpene *cis*- $\beta$ -ocimene (Jardine *et al.* 2020). *Protium* is a genus that is widespread across the whole Amazon basin (ter Steege *et al.* 2013; Fauset *et al.* 2015) and it has been hypothesized that its great abundance is probably related to its high diversity of chemical defenses, which provides a strategy against a large number of enemies that consistently attack plant species (Salazar *et al.* 2018). This hypothesis is indeed reinforced by the fact that *P. hebetatum* occurs in all three forest types of this study, in contrast to the other two hyperdominant species in our study, which occurred in only one or two forest types. Furthermore, given the fact that the upland forest has the highest plant species richness in this experimental site (Andreae *et al.* 2015), a high demand for chemical compounds for herbivory defense is expected in this environment. Habitats with high plant richness are commonly followed by high richness of herbivores, meaning that constant and high potential plant losses to herbivores are diminished with high investments in defenses (Bixenmann, Coley, Weinhold & Kursar 2016). This could explain why emission rates and chemical diversity of sesquiterpenes peaked in the upland forest (Table 3).

#### 4.2. Investment of C in isoprenoid emissions shifts to heavier compounds when photosynthesis is reduced in the dry season

From the wet to dry season, we found a general trend of decreasing photosynthesis rates, coupled with relatively constant total isoprenoid emission rates (Fig. 4). This combination led to a three-fold average increase in C allocation to isoprenoid emissions relative to photosynthesis (though this was variable and non-significant, Fig. 4). Even so, the C sink to isoprenoid emissions was less than 2% of photosynthesis in most plants, except in strongly sesquiterpene-emitting *P. hebetatum*, whose emissions averaged 6.1% of photosynthesis (occasionally > 10%) in the upland in the dry season. Associated with this increasing investment in emissions, plants also allocated more C toward heavier isoprenoid compounds (Fig. 4). Increasing ‘isoprenoid mass investment’ was a result of reduced isoprene emissions and increased monoterpene and sesquiterpene emissions in the dry season (Figs. S2).

Previous studies indicated that seasonality in isoprenoid emissions from Amazonia is controlled by changes in solar radiation, temperature and leaf demography and phenology (Kuhn *et al.* 2004b; Alves *et al.* 2016, 2018). It is well known that the central Amazon dry season reaches maxima in solar radiation, temperature and leaf flushing, and the latter translates into a higher proportion of canopy young leaves during this season (Lopes *et al.* 2016; Wu *et al.* 2016; Gonçalves *et al.* 2020). In terms of changes in chemical composition of isoprenoid emission capacities, leaf age is an important factor because the synthesis of these compounds changes with leaf ontogeny. Isoprene and monoterpenes are produced from dimethylallyl diphosphate (DMADP), which is catalyzed by isoprene synthase and monoterpene synthases, in the methylerythritol 4-phosphate (MEP) pathway in chloroplasts. The DMADP pool size varies with leaf ontogeny, meaning that in early developmental stages, leaves have low DMADP – due to high demand for leaf growth and greening, and it increases with leaf maturation. A low DMADP pool in young leaves may be responsible for a down-regulation in the isoprene synthesis and up-regulation in the monoterpene synthesis; this might be in part explained by the fact that the enzymes involved in the monoterpene synthesis pathway have higher affinity for DMADP, implying that these enzymes achieve maximum catalytic efficiency already at low DMADP concentration, which favors the synthesis of monoterpenes rather than isoprene in young leaves (Kuhn *et al.* 2004b). As constitutive emission of monoterpenes and isoprene has been suggested to have similar functional roles to protect plants against high temperatures and oxidants (Loreto *et al.* 1996), when these stresses – typical of the Amazon dry season – are imposed to young leaves and isoprene synthesis is constrained by enzyme deficits

or low DMADP concentration (Kuhn *et al.* 2004b), the production of monoterpenes might be preferable and then confer the high demand for leaf protection.

Additionally, the plasticity in emission capacities and chemical compositions may not exclusively be a response to abiotic stress, but they may signal up-regulation of defenses against herbivores and pathogens, to which the plant is more susceptible under abiotic stress (Monson, Weraduwege, Rosenkranz, Schnitzler & Sharkey 2021). Monoterpenes and sesquiterpenes have long been recognized as important compounds for plant defense against herbivores and pathogens (Gershenzon & Dudareva 2007). Young leaves have more soft tissues and less physical and structural defenses that make them more palatable and attractive to predators than mature leaves. In this sense, the increase in emitted monoterpenes and sesquiterpenes (in *P. hebetatum*) during the dry season might also be interpreted as an increase in the demand for defense by new leaf tissues of the young leaves.

A higher proportion of young leaves during the dry season is clearly seen in the study site presented here (Lopes *et al.* 2016; Gonçalves *et al.* 2020). However, it was also observed that approximately 30% of trees do not have a massive leaf flushing in any time of the year, meaning that they are possibly flushing leaves continuously and therefore do not have a distinct seasonal leaf age distribution (Lopes *et al.* 2016). As the pattern of leaf phenology is plant species-specific and we do not have information on the leaf age for the individuals that we performed our measurements with, we cannot exclude other reasons for the shift in composition of isoprenoid emission capacities rather than leaf age. If significant changes in leaf age is unaccountable (i.e. specifically for these tree species and in the moments that our measurements were performed), then what might have been playing a role is the leaf physiological response to the environment, which is also affected by the environmental conditions previous to measurements, and not only to the standard conditions imposed to leaves during measurements.

The dry and wet season environment in central Amazonia is different for the leaf - the wet season imposes more light-limitation and the dry season imposes more vapor pressure deficit and higher temperature limitation to photosynthesis (Wu *et al.* 2017). The limitations imposed during the dry season are more stressful for plants, which might require more or different defense mechanisms. Recent work on the integration of isoprene, monoterpenes, sesquiterpenes and other secondary metabolites in signaling networks that mediate growth-defense tradeoffs (Monson, Weraduwege, Rosenkranz, Schnitzler & Sharkey 2021), suggests that plasticity and changes in emission composition (as can be observed seasonally and/or across habitats) could enhance plant chemical defense and therefore confer to the physiological system more resistance against different types and levels of stress - e.g., reducing isoprene and increasing monoterpene and sesquiterpene in the Amazon dry season might represent a switch in plant gas composition in order to cope with stresses such as heat and drought, and to prime the plant to associated biotic stresses.

While the dry-season increase in ‘isoprenoid mass investment’ was most significant for *P. hebetatum*, it was mirrored by non-significant trends within most species and habitats (Fig. 3). We devised the ‘isoprenoid mass investment’ metric to assess whether changing environmental conditions are associated with shifts in C allocation to emissions along the spectrum from lighter to heavier isoprenoids. Many light dependent isoprenoids are evidenced to play related metabolic-support roles as components of cellular signalling networks and antioxidant defenses (Vickers, Gershenzon, Lerdau & Loreto 2009; Riedlmeier *et al.* 2017; Frank *et al.* 2021; Zuo *et al.* 2019; Monson, Weraduwege, Rosenkranz, Schnitzler & Sharkey 202; Harrison *et al.* 2013). While the diversity of specific compounds is high, there are general trends along the mass gradient from 5C isoprene through 10C monoterpenes to 15C sesquiterpenes. Notably, along the low-to-high mass gradient, volatile isoprenoid production has a decreasing dependency on photosynthesis. Compared to monoterpene synthase enzymes, isoprene synthase has a low reaction affinity for the primary isoprenoid substrate DMADP (Harrison *et al.* 2013; Kuhn *et al.* 2004b). Isoprene therefore requires a larger substrate pool in the chloroplast that is derived from photosynthesis (Sharkey & Monson 2014). This variation in emission capacity with variation in the supply of photosynthetically derived substrates is likely an explanation for why species either emit monoterpenes or isoprene at high rates, but not both (Harrison *et al.* 2013). Sesquiterpene biosynthesis is at least partially localized to the cytosol and use Isopentenyl Pyrophosphate (IPP) and DMADP from the

Mevalonate pathway (MVA) (Sallaud et al. 2009), which further reduces their dependency on photosynthesis and DMADP pools in the chloroplast, relative to the smaller isoprenoids. This implies that seasonal changes in photosynthesis may affect isoprene and higher isoprenoid production, since the amount of DMADP available for isoprene synthesis is not only dependent on upstream flux through the MEP pathway, but also its downstream use for the production of higher compounds, such as monoterpenes and sesquiterpenes (Monson, Weraduwege, Rosenkranz, Schnitzler & Sharkey 2021), that can be demanded after distinct biotic and abiotic stresses over seasons (Harrison *et al.* 2013). In addition, the heavier compounds tend to be much more reactive, as evidenced for example by the typical atmospheric half-lives of isoprene (hours), monoterpenes (minutes), and sesquiterpenes (minutes) (Atkinson & Arey 2003). Higher reactivity during the short residence time of volatiles within the cell may allow for a stronger effect as signaling molecules or even as direct antioxidants at lower production rates.

We hypothesize that as photosynthesis declines, an investment shift toward emissions of heavier isoprenoid compounds may allow sustained metabolic support due to their increasing reactivity and decreasing dependency on photosynthesis for production. Consistent with this hypothesis, our results show that when photosynthesis was reduced under dry season conditions, there was a tendency among all species to shift emission compositions toward heavier compounds. Interestingly, the only species to emit detectable sesquiterpenes, *P. hebetatum*, emitted them only in the dry season, and showed significantly increasing emission rates along the soil hydrologic gradient from shallow (wetter) to deep (drier) water table depths (Fig. 2), suggesting an association between investment in sesquiterpenes and the seasonal drought conditions experienced by individual plants. We suggest that our hypothesis is consistent with our empirical data and general theory, but that further experimental physiology work is required to demonstrate emission rate tradeoffs, photosynthetic dependencies, and the relationship between emission rates and metabolic responses for specific isoprenoid compounds.

Although there is variability within and among the tree species studied here and it is not possible to generalize results from three species to a whole plant community, here we presented results of species that are widespread in the central Amazon and have meaningful contribution to plant biomass and productivity (Fauset *et al.* 2015). Furthermore, the trend of reduction in isoprene emission with proportional increase in monoterpene and sesquiterpene emission during the dry season has implications for atmospheric chemical and physical processes. As for aerosol formation, laboratory-determined secondary organic aerosol (SOA) yield from isoprene has been reported as < 6% (Kroll, Ng, Murphy, Flagan & Seinfeld 2005; Xu, Kollman, Song, Shilling & Ng 2014) or higher over forested regions when isoprene is emitted in much more quantities relative to other compounds (Carlton, Wiedinmyer & Kroll 2009). Nonetheless, if other isoprenoids are more emitted, the contribution to the SOA formation could be also higher, as shown by SOA yields of ~5-10% for monoterpenes (Griffin, Cocker, Flagan & Seinfeld 1999a; Griffin, Cocker, Seinfeld & Dabdub 1999b) and ~20-70% for sesquiterpenes (Hoffmann *et al.* 1997; Griffin *et al.* 1999b; Lee *et al.* 2006a b; Chen, Li, McKinney, Kuwata & Martin 2012; Jaoui, Kleindienst, Docherty, Lewandowski & Offenberg 2013).

In this light, it is important for future studies to consider a wider range of volatile organic compounds with their synergetic importance in plant ecophysiological processes and the subsequent impact on the atmosphere. Our results suggest that emissions of monoterpenes and sesquiterpenes might be higher than anticipated and indicate a seasonal change in the composition of the emitted isoprenoids. In fact, seasonal shifts in monoterpene composition have been already reported in ambient air (Jardine *et al.* 2015; Yáñez-Serrano *et al.* 2018); but sesquiterpenes might have been underestimated given their high reactivity with ozone and OH and thereby the difficulty to detect in ambient air (Yee *et al.* 2018), indicating that only leaf level measurements are likely to give us a true measure of forest sesquiterpene emissions. Even though our observations do not offer results to make a clear statement of how isoprene, monoterpenes and sesquiterpenes work together in the face of abiotic stress such as heat and drought during the dry season, they supports the recent findings that isoprene does not confer plant tolerance by itself, but rather it is likely part of an intricate network that involves other plant metabolic processes and therefore other compounds (Harvey, Li, Tjellström, Blanchard & Sharkey 2015; Monson, Weraduwege, Rosenkranz, Schnitzler & Sharkey 2021). This opens more questions in order to understand what are the processes regulating isoprenoid emission capacities

of Amazonian trees, and the need to consider seasonal shifts in isoprenoid composition in emission modeling.

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## Authors' contributions

Eliane Gomes Alves, Tyeen Taylor, Michelle Robin and Débora Pinheiro de Oliveira contributed to the development and sampling design of the study, the collection and the chemical analysis of isoprenoid samples, and to the statistical analysis of datasets. Juliana Schietti contributed to the development and sampling design of the study, and to the statistical analysis of datasets. Sérgio Duvoisin Júnior, Nora Zannoni, Jonathan Williams and Christoph Hartmann contributed to the chemical analysis of isoprenoid samples. José Francisco C. Gonçalves contributed to the collection of isoprenoid data. Jochen Schöngart, Florian Wittmann and Maria T. F. Piedade contributed with the dataset for the initial selection of species. All authors contributed to the writing of the manuscript.

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## Tables

**Table 1:** Air temperature, relative humidity (RH), photosynthetic active radiation (PAR) and precipitation for the days of each intensive campaign and monthly average from 2013 to 2019

year	month	campaign days	season	air temp. (°C)	RH (%)	PAR ( $\mu\text{mol } \mu\text{-}^2 \text{s}^{-1}$ )	precipitation (mm)
2018	December	4 <sup>th</sup>	dry-to-wet transition	26.03 (1.9)	90.3 (8.3)	742.8 (555.0)	-
2019	May	from 1 <sup>st</sup> to 5 <sup>th</sup>	wet	25.8 (2.3)	94.8 (7.9)	732.2 (626.0)	23.6
2019	September	from 21 <sup>st</sup> to 24 <sup>th</sup>	dry	28.3 (3.1)	77.3 (12.8)	1060.6 (756.6)	-
2013-2019*	December		dry-to-wet transition	26.4 (2.8)	87.7 (12.6)	749.3 (574.0)	170.7 (79.1)
2013-2019*	May		wet	25.7 (2.4)	93.6 (8.7)	722.5 (584.4)	245.2 (73.9)
2013-2019*	September		dry	27.4 (3.3)	81.4 (14.3)	975.3 (680.2)	53.6 (21.9)

*Note:* Values within brackets represent one standard deviation of mean \* Monthly average of data provided by the weather station since its installation at the INSTANT tower

**Table 2:** Emission capacities of isoprene, total monoterpenes and total sesquiterpenes; and photosynthesis for the three forest types – white-sand forest (WS), ancient river terrace forest (ART) and upland forest (UP) – during the dry-to-wet transition season (December 2018), the wet season (May 2019) and the dry season (September 2019).

tree species	season	forest type	isoprene [ugC.g-1.h-1]	sum MT [ugC.g-1.h-1]	sum SQT [ugC.g-1.h-1]	photosynthesis [ugC.g-1.h-1]
<i>Eschweilera coriacea</i>	DWT	WS (n=5)	10.71 (1.75)			1897.45 (372.89)
	wet	WS (n=5)	3.30 (1.24)	0.75 (0.24)		2634.05 (692.45)
	dry	WS (n=5)	2.11 (0.58)	5.96 (4.85)		2283.18 (407.46)
<i>Eschweilera grandiflora</i>	wet	ART (n=5)	17.07 (10.37)	4.26 (1.19)		4053.90 (663.38)
		UP (n=5)	5.95 (2.87)	1.29 (0)		2429.67 (821.09)
	dry	ART (n=5)	2.89 (0.25)	2.74 (5.85)		2321.95 (800.14)
<i>Protium hebetatum</i>		UP (n=5)	2.10 (1.06)	0.04 (0.05)		1983.25 (707.86)
	wet	WS (n=5)	11.49 (4.48)	3.19 (2.63)		1977.45 (533.87)
		ART (n=5)	10.30 (7.14)	15.61 (19.28)		2065.45 (256.60)
		UP (n=5)	16.32 (5.69)	9.86 (10.56)		2195.56 (371.22)

tree species	season	forest type	isoprene	sum MT	sum SQT	photosynthesis
		WS ( <i>n</i> =5)	1.86 (0.46)	13.22 (10.16)	2.7	655.03 (487.07)
	<i>dry</i>	ART ( <i>n</i> =5)	3.90 (1.60)	7.20 (4.92)	7.32 (4.64)	1412.10 (349.11)
		UP ( <i>n</i> =5)	4.61 (1.18)	8.21 (6.76)	15.90 (3.16)	635.14 (281.49)

**Note:** Values in parenthesis represent one standard deviation of mean.

**Table 3:** Chemical species of monoterpenes and sesquiterpenes emitted by *Eschweilera coriacea*, *Eschweilera grandiflora* and *Protium hebetatum* measured in three forest types – white-sand forest (WS), ancient river terrace forest (ART) and upland forest (UP), during the wet season (May 2019) and the dry season (September 2019). Emissions are in  $\mu\text{gC g}^{-1}\text{h}^{-1}$

tree species	compound	wet season			
		WS	ART	UP	WS
<i>Eschweilera coriacea</i> *	$\alpha$ -Pinene	0.34; <i>n</i> =1			1.17; <i>n</i> =1
	$\beta$ -Pinene				0.36; <i>n</i> =1
	Camphene	0.53; <i>n</i> =1			0.13; <i>n</i> =1
	3-Carene				3.14; <i>n</i> =1
	Limonene				1.53 (1.54); <i>n</i> =2
	$\beta$ -Phellandrene	0.91; <i>n</i> =1			
	$\alpha$ -Terpinene				3.285 (1.92); <i>n</i> =2
<i>Eschweilera grandiflora</i> **	$\gamma$ -Terpinene	0.47; <i>n</i> =1			
	$\alpha$ -Pinene		0.24 (0.15); <i>n</i> =4		
	$\beta$ -Pinene				
	Camphene				
	3-Carene				
	D-Limonene		1.78 (0.15); <i>n</i> =5		
	Limonene				
	$\alpha$ -Phellandrene		0.26 (0.07); <i>n</i> =4		
	$\beta$ -Ocymene			1.29; <i>n</i> =1	
	<i>o</i> -Cymene		2.04 (0.65); <i>n</i> =5		
<i>Protium hebetatum</i>	$\gamma$ -Terpinene		0.49; <i>n</i> =1		
	$\alpha$ -Pinene	0.24; <i>n</i> =1	4.56 (7.21); <i>n</i> =4	0.41 (0.19); <i>n</i> =3	1.57 (0.15); <i>n</i> =2
	$\beta$ -Pinene		7.24 (4.05); <i>n</i> =2		0.67 (0.65); <i>n</i> =2
	Camphene	0.35 (0.01); <i>n</i> =2		0.16; <i>n</i> =1	0.18 (0.15); <i>n</i> =2
	3-Carene			5.27 (7.59); <i>n</i> =5	2.51 (2.46); <i>n</i> =2
	D-Limonene		6.59 (12.57); <i>n</i> =4	0.48 (0.63); <i>n</i> =3	
	Limonene				8.27 (3.75); <i>n</i> =2
	$\alpha$ -Phellandrene			0.26 (0.07); <i>n</i> =2	
	$\beta$ -Phellandrene		1.34; <i>n</i> =1		
	<i>o</i> -Cymene	4.32 (0.62); <i>n</i> =2		0.30 (0.20); <i>n</i> =3	
	$\alpha$ -Terpinene				0.01; <i>n</i> =1
	$\gamma$ -Terpinene			0.05; <i>n</i> =1	
	$\alpha$ -Cubene				
$\alpha$ -Copaene					
Caryophyllene				2.70; <i>n</i> =1	

Note: \* *Eschweilera coriacea* was only found in the white-sand forest type

\*\* *Eschweilera grandiflora* was not found in the white-sand forest type





