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A screening study of leaf terpene emissions of 43 rainforest species in Danum Valley Conservation Area (Borneo) and their relationships with chemical and morphological leaf traits

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Abstract

We have conducted a screening study of leaf terpene emissions for 43 rainforest woody species of Borneo. To the best of our knowledge, this study reports for first time the terpene emission capacity of 43 species belonging to 22 genera of rainforest woody plant species. We have used a general lineal model (GLM) with phylogenetic control by the phylogenetic distance matrix when necessary. The proportion of the species that emitted terpenes in this set of Borneo woody species was 95% and the species average total terpene emissions of emitting species were 0.04-11.6 μg g^{-1} h^{-1}, which is in the range of the reported emissions in similar screening studies conducted in other biomes. Altogether, 85 terpene compounds were detected, and 11 common mono and sesquiterpenes were identified and quantified. Only two of the terpenes, ocimene and γ-terpinene, of the 11 determined compounds showed a phylogenetic signal. No significant relationships were found between the terpene emissions and the physiological, chemical and morphological foliar traits and the data also showed a lock of constant applicability of the "excess carbon" hypothesis for this set of species. This evidence suggests multiple and diverse factors and conditions driving plant chemistry in the tropical forests.

Keywords: Herbivory, LMA, nitrogen, phosphorus, terpene emissions, trace elements, tropical forest.

Introduction

Protection, defense and infochemical function have been highlighted as possible physiological and ecological roles of terpenes (Llusia and Peñuelas 2001; Wheeler et al. 2002; Peñuelas and Llusia 2003; Peñuelas and Llusia 2004). Examples of these roles are photoprotection (Peñuelas and Munne-Bosch 2005), thermotolerance (Sharkey and Singsaas 1995; Peñuelas and Llusia 2001; Peñuelas and Llusia 2002; Peñuelas et al. 2005; Copolovici et al. 2005), protection against drought stress (Kainulainen et al. 1992; Llusia and Peñuelas 1998) and antioxidative capacity whereby terpenes protect photosynthetic membranes against peroxidation and reactive oxygen species such as singlet oxygen (Loreto and Velikova 2001; Peñuelas and Llusia 2002; Loreto et al. 2004; Munne-Bosch et al. 2004; Llusia et al. 2005). Among plant chemical defenses, terpenes have been shown to have direct and indirect roles in protecting plants against herbivory (Llusia and Peñuelas 2001; Cornara et al. 2001; Peñuelas and Llusia 2004; Owen and Peñuelas 2005; Mumm and Hilker 2006) and allelopathic function (Kaligaric et al. 2011). Finally, leaf volatile terpenes are not only direct chemical defences, but also indirect defences through their emission as relevant infochemicals (Dicke et al. 1991; Vet and Dicke 1992; Steidle and van Loon , 2003; Harmel et al. 2007; Sampedro et al. 2010; Gols et al. , 2011).

The emissions of terpenes have significant effects on atmospheric chemistry and climate. VOCs interact with atmospheric radicals influencing the oxidative capacity of the troposphere (concentration of the hydroxyl radicals) and, therefore, the concentration and distribution of other environmentally important trace gases (Thompson 1992; Chameides et al. 1988). In addition, the volatile compounds formed during the degradation of VOCs are able to increment existing particles or lead to the formation of new secondary organic aerosol (SOA) particles. These particles affect the chemistry of the atmosphere and the radiation balance of the earth (Brasseur et al. 1999). And finally, VOCs in combination with a sufficient level of nitrogen oxide concentrations can lead to ozone production and other photooxidants (Trainer et al. 1987; Fehsenfeld et al. 1992; Hewitt et al. 2011).

Changes in nutrient availability and use can affect terpene production and emission. Higher nitrogen availability is usually expected to be translated into higher terpene production and emission, as a result of increased carbon fixation and activity of the limiting enzymes (Harley et al. 1994; Litvak et al. 1996; King et al. 2004). However, recent studies have observed a decrease in terpene emissions in *Phragmites australis* at high levels of phosphorus supply (Fares et al. 2008). A negative relationship has also been found between the concentration of N and P_E (extractable phosphorus) and terpene emissions in *Pinus*

halepensis (Blanch et al. 2007). However, Ormeno et al. 2007 observed no relationship between terpene emissions and phosphorus supply) and Ormeño and Fernandez (2012) reported different effects depending on the abiotic or biotic factors. . In fact, a lower production of terpenes as carbon based secondary compounds (CBSC) under higher nutrient availabilities can be expected from the CBSC source-sink or "excess carbon" hypotheses. This is based on the assumption of higher allocation to defensive and storage carbon-based-secondary-compounds when fixed CO_2 is in "excess" because it cannot be processed for growth, i.e. when carbon sources exceed carbon sinks (Loomis 1932; Bryant et al. 1983; Herms and Mattson 1992; Peñuelas and Estiarte 1998). However, in this study all sampled leaves belonged to plants grown in the Danum Valley Conservation Area Field Centre, thus under similar soil nutrient availability for each species. What could be different among species was the nutrient "uptake and use" by plants. Then, the "excess carbon" hypotheses was tested on the basis of the competition for nutrient uptake among the different plant species under conditions of potential soil P and N deficiencies.

There is scarce information on terpene emissions of tropical plants species, particularly for Borneo (Hewitt et al. 2009; Misztal et al. 2010) and even less on their relationships with nutrients. Nutrient availability is limiting for woody plant productivity in Borneo rainforest (Paoli et al. 2005; Paoli 2006). Some areas are N-limited and others are P-limited (Kitayama et al. 2000; Nomura and Kikuzawa 2003; Paoli et al. 2005) due to the substrate variability from sedimentary to ultrabasic rocks (Kitayama et al. 2002). It is most likely that P is the nutrient limiting plant growth (Brearley et al. 2007). As far as we know, no screening studies of terpene emissions in Borneo rainforest species have been reported.

The aims of this study were (i) to screen the terpene emission of a large set of species of the Borneo flora, including some species that have not been previously analyzed, concentrating on the most common species in the ecosystems of interest, and (ii) to test whether the terpene emission rates fit the "excess carbon" hypotheses.

Materials and methods

Field site

The field screening campaign was conducted in the Danum Valley Conservation Area Field Centre, located at 117° 48.75' E and 5° 01' N on the east coast of the Malaysian state of Sabah, Borneo Island.

The station lies on the edge of the 438 km^2 Danum Valley Conservation Area (Class I protected rain forest) which itself lies within the Ulu Segama Forest Reserve, as part of the ca. 10000 km^2 Yayasan Sabah Forestry Concession. Danum Valley Conservation Area is the largest remaining area of undisturbed lowland dipterocarp forest in Sabah. Dipterocarp trees dominate the forest around Danum Valley Conservation Area Field Centre with the canopy in places reaching a height of over 70 metres. 90% of the Conservation Area is classified as lowland dipterocarp forest with the remaining 10% being low canopy, sub-montane forest mainly at Mt. Danum in the heart of the Conservation Area. The climate at Danum is equatorial with a mean annual temperature of 26.8 ºC. Temperatures in excess of 34 ºC are rare, occurring only during prolonged dry periods. Minimum temperatures rarely fall below 19 ºC. Mean relative humidity at 14.00 hours averages 78% and 95% at 08.00 hours. Mean annual rainfall (1985-2006) is 2825 mm. During the sampling period, from May 5, 2008 to June 3, 2008, the temperature ranged between 28.9 ºC to 30.9 ºC. Generally, the weather was sunny during the morning until late afternoon and cloudy and rainy at the end of the day. The relative humidity was around 80%.

Species studied and sampling procedure

A total of 43 common species were sampled (Figure 1). Species nomenclature follows the local floras (Whitmore 1972; Soepadmo et al. 2004). Plant sampling was conducted in medium to large forest gaps (10-100 m diameter). In all cases, even-aged well developed less than one-year old but already mature and non-senescent, sun-oriented leaves were sampled at least from three individual plants for given species. The plants were selected at random, with the condition that plants from given species were at least 100 m apart. From each plant, foliage branchlets were randomly sampled from the tips of the branches with an extensible pruning pole. Generally, 20 or more of these leaves were sampled from each plant, except for larger-leaved species carrying a small number of leaves such as *Macaranga gigantea* with average (± SE) leaf area (S_A) of 2600 ± 210 cm^2, for which we sampled 8-11 leaves, and *Artocarpus anisophyllus* (S_A = 3220 ± 260 cm^2, 7-10 leaves). Leaves sampled were sealed in plastic bags with wet filter paper and immediately (few minutes) transported to the laboratory in the Danum Valley Conservation Area field centre and processed as described in (Peñuelas et al. 2011). While this sampling method may induce stress-related production and emission of terpenes (Piesik et al. 2011; Raghava et al. 2010; Opitz et al. 2008; Banchio et al. 2005; Wang and Lincoln 2004; Funk et al. 1999) the

142 photosynthesis rates of the sampled leaves indicated that the leaves were healthy (Peñuelas et al. 2013),
143 and the method facilitated rapid sampling of sunlit leaves, which cannot be achieved by other methods in
144 tropical locations with limited access to very few high canopy tree species.
145
146 *Leaf photosynthetic capacity and morphological analyses*
147
148 Foliar photosynthetic capacity was measured at a quantum flux density of 1000 μmol m^{-2} s^{-1} and leaf
149 temperature of 30 °C under ambient CO_2 concentration of 385 μmol mol^{-1}, using branchlets that had been
150 re-cut under water and stabilized at room temperature of 25-28 °C for one day. An ADC pro (LCpro+
151 Portable Photosynthesis System, ADC BioScientific Ltd. Hoddesdon, Herts, EN11 0DB) gas exchange
152 system was used (Peñuelas et al. 2013).
153 Leaves sampled for morphological analyses was handled in the same manner as described above..
154 Briefly, leaf area and leaf shape indices were determined by digital photographs taken with a Nikon
155 Coolpix 990 camera (Nikon Corporation, Tokyo, Japan) from a distance of 1.4-2 m depending on leaf
156 size. Objects of known area were photographed together with the foliage, and each digital photograph was
157 calibrated separately to obtain an appropriate pixel to cm conversion ratio. UTHSCSA Imagetool
158 2.00alpha software (C. Donald Wilcox, S. Brent Dove, W. Doss McDavid and David B. Greer,
159 Department of Dental Diagnostic Science, The University of Texas Health Science Center, San Antonio,
160 TX, USA; ddsdx.uthscsa.edu) was employed to measure foliage area, perimeter, roundness and foliage
161 compactness for each leaf (Niinemets et al. 2003).
162 After leaf fresh mass (using a precision balance) and area determination, the samples were dried
163 in an oven at 70 ºC for at least 48 hours, and dry mass of individual leaves was determined. From these
164 measurements, we calculated leaf dry mass per unit area (LMA, g m^{-2}) and leaf dry to fresh mass ratio.
165
166 *Leaf chemical analyses*
167
168 Dried plant material was ground by a CYCLOTEC 1093 sample homogenizer (Foss Tecator, Höganäs,
169 Sweden). The analytical processes for elemental analyses were described in Peñuelas et al. (1994).
170 Briefly, for C and N analyses, 1-2 mg of pulverized dried sample was mixed with the oxidant 2 mg of
171 V_2O_5. C and N contents were determined by combustion coupled to gas chromatography using a Thermo

172 Electron Gas Chromatograph model NA 2100 (C.E. instruments-Thermo Electron, Milan, Italy). For
173 analyses of other elements, dried and ground samples were digested with concentrated HNO_3 and H_2O_2
174 (30%, p/v) (MERCK, Darmstadt, Germany) in a microwave oven. To assess the accuracy of digestion and
175 the analytical biomass procedures, standard certified biomass (NIST 1573a, tomato leaf, NIST,
176 Gaitherburg, MD) was used. After digestion, the contents of As, Cd, Cr, Cu, Mo, Ni, Pb, V and Zn were
177 determined using ICP-MS (Inductively Coupled Plasma Mass Spectrometry) and Ca, Fe, K, Mg, Mn, S,
178 Na and P were determined using ICP-OES (Inductively Coupled Plasma Optic Emission Spectrometry).
179 The phenolics (Ph) concentrations of leaves were measured by using an improved Folin-
180 Ciocalteu Assay (Singleton and Rossi 1965; Marigo 1973) which used a blank of
181 polyvinylpolypyrrolidone (PVPP). An Helios Alpha spectrophotometer (Thermo Spectronic, Cambridge,
182 UK) was used to the determination the absorbance of the samples A and B (at 760 nm), with gallic acid
183 as the standard for calibration.
184 Total soluble tannins (Tan) were extracted from 20 mg of leaf powder with 12 ml of 70%
185 acetone. After centrifugation, the extract was assayed with the butanol/HCl method (Porter et al. 1986),
186 modified as in (Makkar and Goodchild 1996). The absorbance was measured at 550 nm by
187 spectrophotometer Helios Alpha (Thermo Spectronic, Cambridge, UK). Non-heated replicate tubes for
188 each extract were used as anthocyanin blank and their absorbance values subtracted from the absorbance
189 of the heated tubes (Porter et al. 1986). The Tta content on a dry weight basis was estimated by using a 1-
190 cm-wide cuvette (Porter et al. 1986, Makkar and Goodchild 1996). Tan analyses were conducted in
191 triplicate. For additional details on the analytical procedures, see Peñuelas, Sardans, Llusia, Owen,
192 Carnicer, et al. (2010a).
193
194 *Terpene emissions*
195
196 Three different plants were sampled for each of the species studied. Terpene sampling for each one of
197 them was conducted using the above described gas exchange system (ADC, LCpro+, Hoddeson,
198 Hertfordshire, UK) at a quantum flux density of 1000 μmol m^{-2} s^{-1} and chamber temperature of 30 ºC. A
199 whole leaf for large-leaved species or 2-3 leaves or an entire branchlet for small-leaved species was
200 enclosed in a clip-on gas-exchange cuvette of 35 cm^2 and 175 cm^3. Air flow through the dynamic cuvette
201 was around 500 ml min^{-1}. Exhaust air of the cuvette was pumped downstream through a glass tube (8 cm

long and 0.3 cm internal diameter) manually filled with terpene adsorbents Carbopack B, Carboxen 1003, and Carbopack Y (Supelco, Bellefonte, Pennsylvania) separated by plugs of quartz wool. Samples were taken using a Q_{max} air sampling pump (Supelco, Bellefonte, Pennsylvania). The hydrophobic properties of activated carbon minimized sample displacement by water. In these tubes, terpenes did not undergo chemical transformations as checked against trapped standards (α-pinene, β-pinene, camphene, myrcene, *p*-cymene, limonene, sabinene, camphor, α-humulene and dodecane). Prior to use for terpene sampling, these tubes were conditioned for 15 min at 350 ºC with a stream of purified helium. The sampling time was 10 min, and the flow was around 230 mL/min depending on the glass tube adsorbent and quartz wool packing. The trapping and desorption efficiency of liquid and volatilized standards such as α-pinene, β-pinene or limonene was 99 %. Blank air sampling on tubes was conducted for 10 minutes immediately before and after each measurement without the plants in the cuvettes. The glass tubes were stored in a portable fridge at 4 ºC and taken to the laboratory. In the laboratory the tubes were stored at -28 ºC until the analysis. Analyses of the replicate samples immediately and after 6 months storage indicated no detectable changes in terpene amounts after storage of the tubes. In calculations of the terpene emission rates, terpene contents in the blank samples measured without the plants were subtracted from the samples measured with the plants.

Terpene analyses were performed by using a GC-MS system (Hewlett Packard HP59822B, Palo Alto, CA, USA). The monoterpenes trapped in the tubes were processed with an automatic sample processor (Combi PAL, FOCUS-ATAS GL International BV 5500 AA Veldhoven, The Netherlands) and desorbed using an OPTIC3 injector (ATAS GL International BV 5500 AA Veldhoven, The Netherlands) into a 30m x 0.25mm x 0.25µm film thickness capillary column (HP-5, Crosslinked 5% pH Me Silicone; Supelco Inc.). The injector temperature (60 ºC) was increased at 16 ºC s^{-1} to 300 ºC. The sample was injected with a Helium flow of 0.7 mL min^{-1} and cryofocused at -20 ºC for 2 min. After this time, the cryotrap was heated rapidly to 250 ºC. Helium flow into the capillary column was 0.7 mL min^{-1}. After the sample injection, the initial temperature (40 °C) was increased at 30 ºC min^{-1} up to 60 ºC, and thereafter at 10 ºC min^{-1} up to 150 ºC. This temperature was maintained for 3 min, and thereafter increased at 70 ºC min^{-1} up to 250 ºC, and maintained for another 5 min. Total run time was 23 min with a solvent delay of 4 min. The MS detection system was opperating in SIM mode.

The identification of monoterpenes and sesquiterpenes was conducted by comparing the retention times with standards from Fluka (Buchs, Switzerland), and the fractionation mass spectra with standards,

232 literature spectra, and the mass spectra library wiley7n. Terpene concentrations were determined from
233 calibration curves. The calibration curves for common monoterpenes, α-pinene, Δ^3-carene, β-pinene, β-
234 myrcene, *p*-cymene, limonene and sabinene, and common sesquiterpenes such as α-humulene were
235 determined once every five analyses using four different terpene concentrations. The calibration curves
236 were always highly significant ($r^2 > 0.99$ for the relationships between the signal and terpene
237 concentration). The other monoterpenes and sesquiterpenes were calibrated using these calibration curves
238 of the most common mono and sesquiterpenes. The most abundant terpenes had very similar sensitivity
239 with differences less than 5% among the calibration factors. The quantification of the peaks was
240 conducted using the fractionation product with mass 93.
241
242 *Phylogenetic and statistical analyses*
243
244 The program Phylomatic Webb and Donoghue was used to build a phylogenetic tree for the species
245 studied. The phylogeny was transformed into a PDI document of the phylogenetic distances with
246 PDTREE 5.0 module (University of California, Riverside, CA). Then, the PDDIST module (University of
247 Wisconsin, Madison, WI) was used to create the distance matrices in ASCII format. The phylogenetic
248 signal Blomberg and Garland (2002) was calculated for all the leaf variables analysed employing Matlab
249 7.6.0 with the PHYSIG module (Blomberg et al. 2003). A *k* statistic was calculated which indicates the
250 amount of signal in the emission trait relative to what would be expected for the specified phylogenetic
251 tree (topology and branch lengths) given a Brownian motion model of evolution. If $k = 1$, then the
252 specific emission trait has exactly the amount of signal expected for the given phylogenetic tree, whereas
253 values greater than one indicate more signal than expected and values less than one indicate less signal
254 than the expected. To determine whether the observed phylogenetic signal was statistically significant, the
255 actual data was compared with the values obtained after the data had been permuted randomly across the
256 tips of the tree without the phylogenetic relationships. With this aim, 1000 random datasets were
257 simulated under the Brownian motion assumption (Garland et al. 1993; Blomberg et al. 2003). Thus, the *k*
258 statistic and the probability of error in rejecting the phylogenetic signal (*P*) were determined according to
259 (Blomberg et al. 2003). Thereafter the variables with $P > 0.10$ were analyzed by an ordinary General
260 linear model (GLM) without the phylogenetic distances matrix. The variables with $0.10 > P > 0.05$ were
261 analyzed by an ordinary GLM without and with phylogenetic distance matrix, and the model with a lower

Akaike information criterion (AIC) was selected. Finally, the variables with a $P < 0.05$ were directly analyzed by a GLM using phylogenetic distance tree matrix. We conducted these GLM analyses with all the leaf traits rates of emission of terpenes as dependent variables. In the case of variables with a significant phylogenetic signal, phylogenetic distances were also included as a continuous independent factor. To conduct these analyses we used Matlab 7.6.0 with REGRESSIONV2 module (Lavin et al. 2008).

Results and discussion

We detected foliar terpene emissions in 41 out of the 43 species studied. The 2 species in which we did not detect emissions were *Dipterocarpus appendiculata* and *Etlingera brevilabrum* (Table 1). This means that the 95 % of the studied species emitted terpenes in detectable concentrations (detection limit for our analytical method was 5 ng) (Table 1). We detected emissions of a total of 85 terpene compounds, but could positively identify and quantify only 11, the monoterpenes Camphene, Ocimene, α-Pinene, β-Pinene, Δ-3-Carene, β-Myrcene, γ-Terpinene, Sabinene and Limonene and the sesquiterpenes Junipene and β-Caryophyllene (Table 1).

To the best of our knowledge, this is the first report indicting the terpene emissions or non-emissions of the studied 43 species belonging to 21 genera, *Ardisia*, *Baccaurea*, *Barringtonia*, *Clidemia*, *Dillenia*, *Dimocarpus*, *Dryobalanops*, *Duabanga*, *Eusideroxylon*, *Fagraea*, *Hopea*, *Ludekia*, *Melastoma*, *Neonauclea*, *Octomeles*, *Parashorea*, *Pleiocarpidia*, *Poikilospermum*, *Semecarpus*, *Shorea* and *Uncaria*. Some species of the genus *Senna* (*Cassia*), *Senna fistula* and *Senna siamea* (Padhy and Varshney 2005), *Syzygium*, *Syzygium jambolanum* (Padhy and Varshney 2005), *Macaranga* and *Mallotus* (Cronn and Nutmagul 1982), *Syzygium* (Klinger et al. 2002; Padhy and Varshney 2005; Llusia et al. 2010) and *Diospyros* sp. (Guenther et al. 1994; Zhang et al. 2009) previously have been reported to emit terpenes.

The proportion of the species that emitted terpenes in this set of Borneo woody species (95.5%) is similar to the observed 100% of species emitting terpenes in a similar screening study in 18 different woody Mediterranean species conducted in the field (Owen et al. 1997). In a further study reporting emissions from 40 dominant Mediterranean species, 97.5% of species emitted terpenes (Owen et al. 2001).

There are also screening studies reporting similar percentage of terpene emitting species, e.g., 97% (*Casuarina equisetifolia*, *Grevillea robusta*, *Melaleuca quinquenervia, Lantana camara* and *Persea*

292 *americana*) (Llusia et al. 2010), and 71% (36 species were found to emit terpenes (4 high emitter; 28
293 moderate emitter; and 4 low-emitter) (Padhy and Varshney 2005) or 68% of 50 plant species sampled in
294 India (plantation forest of Haryana) emitted monoterpenes (Singh et al. 2011). These results suggest that
295 terpene emission might be very general in terrestrial plants and warrant and conducting further similar
296 screening studies throughout the world to further explore this supposition. In fact, terpene emission rates
297 are very variable. Large uncertainties derive from natural variability in individual plant health, herbivory
298 status, local soil moisture and nutrient status, local shading and microclimate, age of the plant, etc. (Llusia
299 et al. 2010; Niinemets et al. 2010a,b). For example, in *Macaranga* sp, Cronn and Nutmagul (1982)
300 reported emission rates of total VOCs of 44 μg g^{-1} h^{-1}, and for *Diospyros* sp, Guenther et al. (1994)
301 reported less than 0.1 μg g^{-1} h^{-1}.and 1 μg g^{-1} h^{-1} from *Mallotus* sp. Klinger et al. (2002) reported between
302 70 and 199 μg g^{-1} h^{-1} for *Syzygium* sp, whereas Padhy and Varshney (2005) reported 7.1 to 9.8 μg g^{-1} h^{-1}
303 (for α-Pinene 1.5 μg g^{-1} h^{-1}). Similarly, the range of total terpene emissions observed in this screening
304 study, 0.035-11.5 μg g^{-1} h^{-1}, is in the range of those reported by Owen et al. (1997) in 18 Mediterranean
305 woody species (from 0.1 to 20 μg g^{-1} h^{-1}).

306 Only 2 terpene compounds, Ocimene and γ-Terpinene, of the 11 terpenes determined, presented a
307 phylogenetic signal (k = 1.15 and P = 0.03, and k = 1.31 and P = 0.03, respectively). There are few
308 similar studies testing the phylogenetic signal of terpene emissions in a broad set of plant species (Llusia
309 et al. 2010; Knudsen et al. 2006). In a set of 70 species of Hawaïi flora, comprising native and alien
310 plants, a phylogenetic signal was found only in one of the 15 different terpene compounds detected in
311 plant emissions (Llusia et al. 2010). Regarding terpene content, a review of 90 families and 38 orders of
312 high plants revealed a wide presence of the most common terpenes which were observed in 54-71%
313 (depending on the compound) of the families investigated (Knudsen et al. 2006). Terpene synthesis
314 enzymes are a mechanistically intriguing family of enzymes that catalyze complex, multi-step chain
315 reactions that are able of generating thousands of structurally diverse hydrocarbons of biological
316 importance. Although an evolutive genetic divergence of genes that encode for enzymes of terpene
317 synthesis has been observed in narrow phylogenetic groups (Bohlmann et al. 1998), a general
318 phylogenetic determinant for plant terpene emission is not clear (Welter et al. 2012). There are also
319 differences between terpene synthesis and terpene emission. The factors ruling terpene emission
320 (Peñuelas and Llusia 2001) might mask a phylogenetic control of terpene synthesis. However, the study
321 of the terpene content of these same tropical species in the same site (Sardans et al. 2013), also showed a

322 lack of phylogenetic signal. All these results suggest that terpene content and emission is a widespread
323 trait in this tropical forest that probably confers adaptative advantage in a very wide range of angiosperm
324 phylogeny.

325 Terpene emission was not correlated with the leaf concentration of any of the 20 different chemical
326 elements that were studied in this field campaign and that were reported in Peñuelas et al. (2013), in spite
327 of the large range of nutrient concentrations found among the 43 woody species studied (e.g. foliar N
328 concentrations ranged between 10 and 40 mg g^{-1}) that allowed testing the "Excess Carbon" hypothesis.
329 The absence of significant relationships between nutrients and emissions does not provide support for the
330 "Excess Carbon" hypothesis. Other previous studies have not either supported the "Excess Carbon"
331 hypothesis (King et al. 2004; Llusia et al. 2010).

332 The absence of significant relationships between nutrients and emissions does not provide support for
333 the "excess carbon" hypotheses. This absence of relationship seems to result from multiple factors
334 involved in the emissions that are different from those involved in the production, and from the very
335 diverse abiotic and biotic environments experienced by tropical plants. The absence of a clear relationship
336 between terpene emissions and physiological, chemical and morphological traits supports current
337 understanding of very diverse roles for terpene emissions, including plant protection against abiotic
338 stressors, plant defense against diverse in time biotic attacks, and signaling to attract pollinators and
339 predators and parasitoids of the herbivores (Peñuelas and Llusia 2003; Peñuelas and Staudt 2010), or
340 even may indicate that they do not necessarily have an immediate function, but that are inevitable
341 emissions of the plant metabolism (Peñuelas and Llusia 2004). Moreover, analysing other CBSCs (lignins
342 and tannins) in some plant species of Borneo, Kurokawa et al. (2004) concluded that, although the
343 resource conditions have the potential to change the quality and quantity of plant defenses, this hypothesis
344 is not sufficient to explain plant response to changing resources since different variables (variation of
345 resource changes among taxa, functional groups, habitats and investigated C-based compounds) play a
346 role in this plant response.

347 In any case, the emission rates here presented will be useful as emission factors for the modeling of
348 the emission rates of tropical forest in areas such as Borneo, which is of great interest regarding the
349 effects on atmospheric chemistry and climate (Trainer et al. 1987; Chameides et al. 1988; Fehsenfeld et
350 al. 1992; Thompson 1992; Brasseur et al. 1999; Peñuelas and Llusia 2003; Misztal et al. 2010; Peñuelas

and Staudt 2010), with the understanding of the uncertainties of the methodology, and with reference to further screening work on leaves intact on living plants.

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584 **Table 1.** Monoterpene and sesquiterpene compounds emitted by the species studied (in μg of terpenes g^{-1} h^{-1}; mean ± SE).

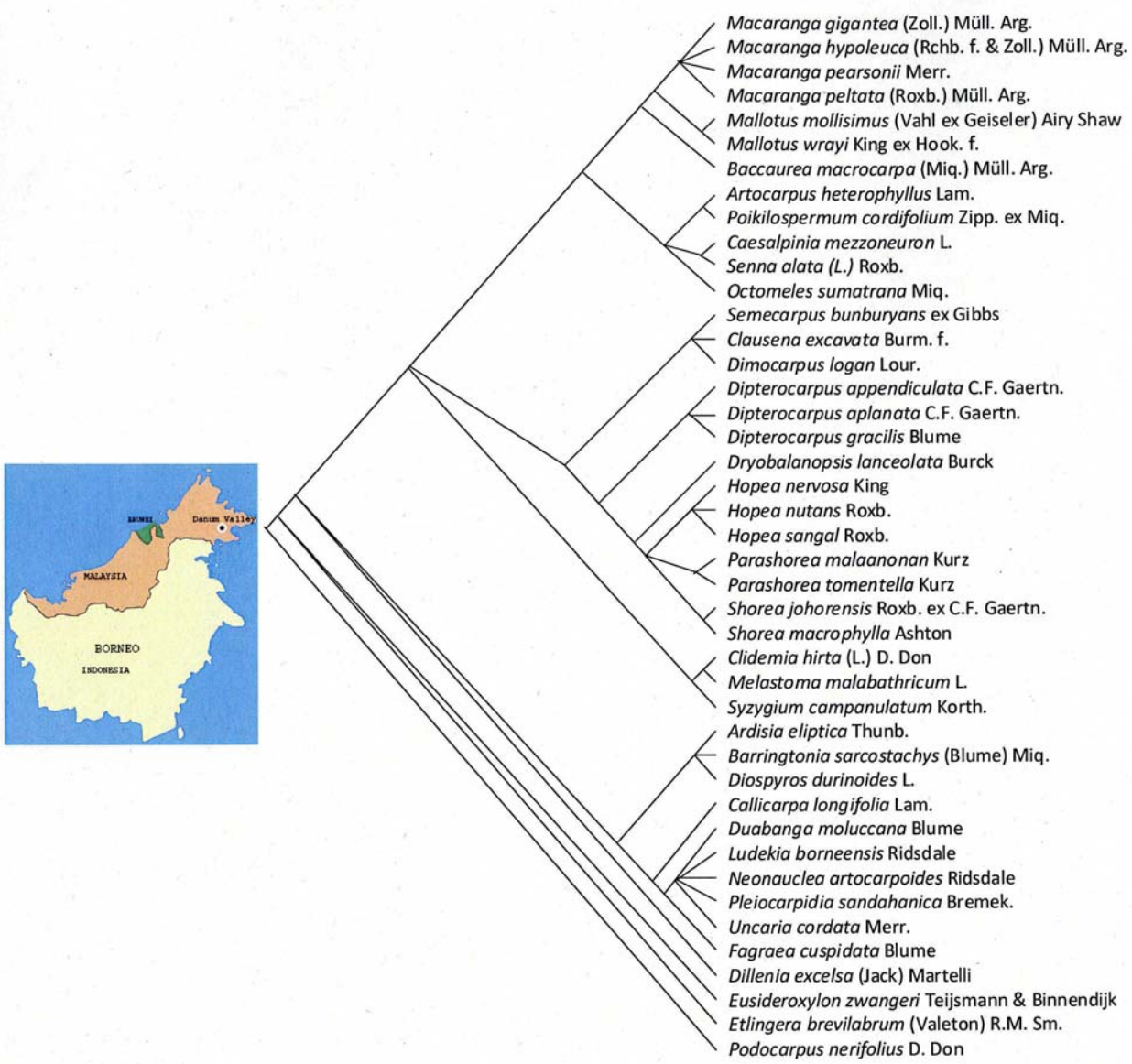
	Monoterpenes									Sesquiterpenes		Non identified terpenes	Total terpenes
Species	**α-Pinene**	**Camphene**	**β-Pinene**	**Limonene**	**Δ-3-Carene**	**β- Myrcene**	**Sabinene**	**Ocimene**	**γ-Terpinene**	**Junipene**	**β-Cariophyllene**		
Ardisia eliptica	0.025±0.012			0.355±0.273						0.034±0.025		0.412±0.319	0.826±0.753
Artocarpus heterophyllus	0.031±0.017		0.006±0.001	0.037±0.026	0.069±0.049	0.009±0.007				0.014±0.002		0.021±0.015	0.186±0.155
Baccaurea macrocarpa	1.033±0.668		0.002±0.002	0.493±0.327	0.009±0.006					0.059±0.036		1.017±0.685	2.613±2.415
Barringtonia sarcostachys	0.011±0.009			0.002±0.001	0.003±0.002	0.001±0.001						0.052±0.030	0.068±0.029
Caesalpinia mezzoneuron						0.009±0.008				0.002±0.002		0.029±0.015	0.040±0.024
Callicarpa longifolia	0.057±0.015			0.093±0.028	0.003±0.002					0.034±0.011		0.085±0.03	0.287±0.063
Clausena excavata	3.347±2.831		0.007±0.004	0.075±0.048	0.023±0.012	0.047±0.034	0.011±0.005			0.004±0.002		2.620±2.220	6.137±5.837
Clidemia hirta	1.980±1.600		0.014±0.012	2.320±1.870	1.078±0.880	0.713±0.572				0.004±0.003		0.030±0.012	6.156±5.994
Dillenia excelsa	0.029±0.012	0.015±0.012	0.006±0.003	0.086±0.036	0.009±0.008							0.048±0.006	0.198±0.089
Dimocarpus logan	0.047±0.019	0.002±0.001	0.005±0.001	0.049±0.019	0.087±0.045	0.005±0.003		0.025±0.014		0.003±0.001		0.012±0.007	0.235±0.153
Diospyros durinoides	0.428±0.193			1.300±0.830	0.012±0.010	0.501±0.409				0.170±0.131	0.007±0.006	1.830±1.260	4.643±2.283
Dipterocarpus aplanata	0.061±0.047		0.029±0.029	0.028±0.023		0.003±0.003				0.017±0.002		0.055±0.046	0.196±0.007
Dipterocarpus appendiculata													n.d.
Dipterocarpus gracilis	0.076±0.051		0.024±0.017	0.008±0.006	0.035±0.016								0.142±0.090
Dryobalanops lanceolata	0.057±0.040		0.021±0.017	0.021±0.013		0.015±0.012						0.057±0.046	0.170±0.157
Duabanga moluccana			0.056			0.029	0.034		0.088			0.016	0.25
Etlingera brevilabrum													n.d.
Eusideroxylon zwangeri	1.25		0.775	1.591		0.713		1.883	0.948	0.378			3.771±3.079
Fagraea cuspidata	1.163±0.927			0.138±0.113						0.034±0.013		1.090±0.710	2.426±1.958
Hopea nervosa	0.104±0.046			0.082±0.042	0.021±0.018	0.024±0.014	0.005±0.004	0.007±0.007		0.008±0.003		0.052±0.031	0.327±0.110

Hopea nutans	0.455±0.312			0.965±0.672	0.424±0.300					0.118±0.084	0.010±0.007	1.500±1.030	3.470±2.764
Hopea sangal	0.072			0.033		0.003				0.009		0.094	0.21
Ludekia borneensis	0.029±0.014		0.003±0.001	0.039±0.016	0.047±0.030	0.001±0.001				0.008±0.001		0.025±0.011	0.073±0.014
Macaranga gigantea	0.005±0.004		0.007±0.004	0.010±0.011	0.010±0.008	0.004±0.003		0.010±0.008		0.002±0.002		0.019±0.016	0.072±0.025
Macaranga hypoleuca	5.94	0.038	1.658	1.186	0.092	0.424	1.816	0.363		0.034		0.074	11.623
Macaranga pearsonii	0.028±0.007			0.057±0.001	0.085±0.060	0.002±0.002		0.133±0.094		0.018±0.004		0.018±0.007	0.341±0.031
Macaranga peltata	0.863				0.024	0.076	0.285	0.051				0.389	1.688
Mallotus mollisimus	0.010±0.006		0.001±0.001	0.013±0.009					0.006±0.005	0.005±0.004		0.001±0.001	0.035±0.029
Mallotus wrayi	0.040±0.031	0.015±0.012		0.030±0.023	0.004±0.003		0.011±0.009			0.046±0.035		0.096±0.077	0.266±0.250
Melastoma malabathricum	0.109		0.058	0.078		0.035				0.093	0.045	0.14	0.557
Neonauclea artocarpoides	0.013±0.004		0.004±0.002	0.033±0.004	0.004±0.003	0.002±0.001				0.003±0.002		0.015±0.006	0.150±0.075
Octomeles sumatrana	0.048±0.039		0.008±0.006	0.147±0.115	0.084±0.068	0.097±0.055				0.053±0.025		0.033±0.025	0.469±0.362
Parashorea malaanonan	0.130±0.088		0.043±0.035	0.849±0.596		0.017±0.008		0.013±0.011		0.092±0.052		0.096±0.053	1.177±0.709
Parashorea tomentella	0.832±0.552			0.258±0.149						0.009±0.006		0.002±0.002	1.101±0.801
Pleiocarpidia sandahanica	0.044±0.036			0.039±0.032	0.050±0.041	0.003±0.002				0.006±0.005		0.049±0.031	0.190±0.178
Podocarpus nerifolius	0.009±0.007		0.018±0.015	0.033±0.027	0.003±0.002	0.001±0.001				0.003±0.002		0.320±0.210	0.387±0.231
Poikilospermum cordifolium	0.3			1.046						0.553		3.653	5.553
Semecarpus bunburyans	0.527±0.242			0.304±0.215						0.143±0.101		0.856±0.152	1.829±0.261
Senna alata	0.002±0.001		0.003±0.002	0.015±0.010		0.001±0.001				0.009±0.004		0.010±0.008	0.039±0.033
Shorea johorensis	0.661±0.420		0.004±0.003	0.841±0.546	0.633±0.351					0.072±0.044		2.810±1.960	5.026±3.833
Shorea macrophylla	0.182			0.14		0.054				0.005		0.032	0.488
Syzygium campanulatum	0.039±0.030		0.045±0.037	0.068±0.041	0.030±0.026	0.042±0.037				0.001±0.001		0.083±0.039	0.309±0.241
Uncaria cordata	0.060±0.043		0.050±0.045	0.156±0.129	0.003±0.002	0.002±0.002				0.065±0.055		0.197±0.171	0.537±0.491

585 **n.d. not detected**

Legends to figures

Figure 1. Location of the study field site and the phylogenetic mega-tree for the species studied. See Methods section for details.



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