

Mangrove's species are weak isoprenoid emitters

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1 **Mangrove's species are weak isoprenoid emitters.**

Abstract

 Mangroves are ecosystems interfacing terrestrial and marine environments submitted to extreme abiotic factors (e.g. anoxia, flooding, salinity) producing stress on vegetation. Due to these stresses, we hypothesized that mangroves potentially emit biogenic volatile organic compound (BVOC), particularly isoprenoids as they are defense compounds. Despite mangroves cover only about 5% of the forest areas of the world, their emissions could impact air quality at the continental-ocean interface. As a result, it is important to fill the gap in the knowledge about BVOC emissions from the canopy of the major mangrove trees. The aim of this study was thus to screen isoprenoid emissions of the mangrove species. In this study, we sampled isoprenoid emissions of 15 species foliage among the 38 core species existing in the Indo-West Pacific (IWP) and the Atlantic Est Pacific (AEP) regions. Sampling was performed using a branch-bag dynamic enclosure system and analyzed with gas chromatography coupled to mass spectrometry. Our analysis showed that mangrove tree species are very low emitters suggesting that mangrove ecosystems would not strongly influence atmospheric chemistry and air quality.

Keywords

BVOC emissions; Isoprenoids; Mangroves; Flooded ecosystems; extreme environments

Highlights

- Isoprenoid emissions of 15 mangrove species were investigated.
- 41 Mangrove species studied are very low isoprenoid emitters.
- Mangrove forest weakly contribute to biosphere-atmosphere exchanges through BVOC.
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1. Introduction

 Vegetation (croplands, shrublands, woodlands etc) is the most important source of volatile organic 53 compound (VOC) as it accounts for 1015 $TgC.yr^{-1}$, about 90% of total biogenic emissions [\(Guenther](#page-13-0) *et al.* [1995\)](#page-13-0). VOCs gather a wide variety of chemical compounds having a high vapor pressure (at least 0.01 KPa) at ambient temperature. Among them, isoprenoids (including isoprene but also monoterpenes and, to a lesser extent, sesquiterpenes), as well as highly volatile compounds such as methanol, acetaldehyde, or formaldehyde, are mainly emitted (e.g. Saunier *et al*., 2017a). These compounds play a major environmental role on air quality and indirectly modulate climate conditions. Indeed, in the presence of sunlight and nitrogen oxides (NOx), biogenic VOCs (BVOCs) contribute to the formation of tropospheric ozone [\(Fehsenfeld](#page-13-1) *et al.* 1992) and secondary organic aerosols [\(Claeys](#page-13-2) *et al.* [2004;](#page-13-2) Ng *et al.* [2006\)](#page-14-0), which in turn act on cloud formation and albedo (Makkonen *et al*. 2012). Among BVOCs, isoprenoids are therefore key compounds for chemistry-model-transport and climate- change models that aim to forecast photo-chemical pollution episodes and their contribution in the future climate change [\(Makkonen](#page-14-1) *et al.* 2012). These two types of models rely on thousands of publications that describe, since the 1980s, BVOCs emissions from plant species [\(Keenan](#page-14-2) *et al.* 2009), their dependence on environmental factors such as light and temperature (Guenther *et al.* 1993). Since isoprenoid emissions are species-specific, it is crucial to investigate isoprenoid emissions from the main vegetal formations at the global scale. For this purpose, temperate environments (e.g. Mediterranean, continental, oceanic) have been widely studied in terms of BVOCs emissions (Owen *et al.* 2001; [Ormeno](#page-14-3) *et al.* 2007; [Saunier](#page-15-0) *et al.* 2017b), but plant species from extreme environments such as mangroves have been clearly neglected [\(Rinnan](#page-14-4) *et al.* 2014; [Exton](#page-13-3) *et al.* 2015).

 Mangroves are extreme ecosystems interfacing terrestrial and marine environments. These forests are key elements of tropical coastal ecosystems as they provide many ecosystem services such as limiting erosion (e.g. cyclone, wave) and saline intrusion [\(Brander](#page-13-2) *et al.* 2012, Hilmi *et al*., 2017), providing raw materials, food and habitat for fauna (e.g. migration, breeding, spawning), or being important carbon (C) sinks (Jakovac et al. 2020).They are found in two distinct regions: the Atlantic Est Pacific (AEP) and the Indo-West Pacific (IWP) [\(Spalding 2010\)](#page-15-1) and account for 147,359 km2 (127,925–168,895) in 2020 (Bunting et al., 2022). In both geographical areas, there are several common genera but no common species. To handle their particular changing environmental conditions (e.g. high salinity, flooding, anoxia), mangrove species have developed several specific defense mechanisms, including physical barriers (e.g. waxes, He *et al*. 2016) or chemical protections [\(e.g.](#page-14-5) [secondary metabolites, Naskar & Palit 2015\)](#page-14-5). Indeed, specialized compounds can be produced and/or emitted by plants to cope flooding and salinity as demonstrated for other species [\(Copolovici &](#page-13-4) [Niinemets 2016;](#page-13-4) [Yang](#page-15-2) *et al.* 2018). However, to our knowledge, only a few studies have investigated BVOC emissions from mangrove despite the large distribution of this unique ecosystem over the world. For instance, Barr *et al.* [\(2003\)](#page-13-5) focused on volatile compounds emitted by foliage of Red mangroves (*Rhizophora mangle* L.) in Florida, and found very low emissions of isoprene (< 0.1 μ g.g_{DW}⁻¹.h⁻¹). Despite these reported low emissions, mangrove trees could have a non-negligible effect on atmospheric processes since mangroves represent one of the most biomass-productive biomes on our planet and cover about 75% of the coasts and estuaries of tropical regions (Ellison and Farnworth, 2001; [FAO 2007\)](#page-13-6).

 The aim of this study was to perform for the first time a screening of isoprenoid emissions from 15 mangrove trees located in two geographical areas: the IWP (Vietnam) and the AEP (French Guyana) regions. We hypothesized that some mangrove species could be isoprenoid emitters in response to extreme conditions in mangrove ecosystems.

2. Materials and methods

2.1 Species

 From the 73 species recognized as "true" mangroves, we investigated 15 of the dominant mangrove species among the 38 core species (Spalding 2010): *Avicennia alba* Blume*, Avicennia germinans* (L.)L*., Avicennia marina* (Forsk) Vierh (Acanthaceae), *Aegiceras corniculatum* (L.) Blanco*, Aegiceras floridum* Roem. & Schult*.* (Myrsinaceae)*, Bruguiera gymnorrhiza* Lam*., Ceriops zippeliana* Blume*, Rhizophora apiculate* Salvoza*, Rhizophora racemose* (Hochr.) Salvoza*, Rhizophora stylosa* Griff*.* (Rhizophoraceae), *Laguncularia racemose* (L.) C.F. Gaertn*., Lumnitzera littorea* (Jack) Voigt*, Lumnitzera racemosa* Wild. (Combretaceae)*, Sonneratia alba* Sm*.* and *Sonneratia caseolaris* (L.) Engl. (Lythraceae). Due to the large surface area they occupy, these species could be the most important contributors to the biosphere atmosphere relationship.

 The 15 species were sampled in two distribution areas: 11 in the IWP (Vietnam) and 3 in the AEP (French Guyana) regions. In Vietnam, the experimental site was located in the botanical garden of Can Gio District (CGBG), Ho Chi Minh City in the South of Vietnam (10°30'N 106°51'E). The Can Gio mangrove is formed by the deltaic confluence of the Saigon, Dong Nai and Vam Co Rivers and it is the first mangrove biosphere reserve in Vietnam (UNESCO/MAB Project 2000). This estuary is subject to semi-diurnal tides, and the tidal elevation ranges from 2 to 4 m. The climate is monsoonal, with a dry season lasting from December to April and a wet season from May to November. In this site, the annual mean temperature is 27.4°C with the highest (37.6◦C) occurring in October and the lowest (21.7°C) in February. The annual average rainfall ranges from 13000-14000mm with highest monthly average in September (300-400mm). To the sampling site, not directly connected to the to the main estuarine channel, the salinity ranges from 16 to 23 (Vinh et al. 2020).

 The second experimental site was located in French Guyana, along the Mahury River (04° 51.4429'N 052°15.8035'W). In this site, the climate is humid equatorial climate with 2 wet seasons and 2 dry seasons. French Guiana coast is mesotidal with semi-diurnal tides (Ray et al. 2020) with a tide elevation ranged from 0.92 m and 3.06 m in this site. The average annual temperature is 26.5 °C with 124 a thermal amplitude of 3 \degree C at the most between the hottest and the coldest month and the average 125 annual rainfall ranges from 2500 to 3000 mm y^{-1} . In the site the salinity ranges from 16 to 26 (Marchand et al. 2004).

 Samplings were performed in the dry season during 2 field campaigns in April 2019 in Vietnam and October 2019 in French Guyana. The environmental conditions in terms of temperature (°C), relative humidity (%) occurring during the field campaigns were recorded using a HOBO device (Onset, USA). Photosynthetically Active Radiation (PAR) was measured using a quantum sensor (PAR‐ SA 131 190®, LI- COR, USA) (Table 1).

2.2 Isoprenoid sampling

 For each species, five mature trees (2-5m) were sampled during each field campaign and sampling site (only exception: 6 *Avicenia alba* were sampled), for a total of 76 trees sampled (see Table 2 for details). The sampling procedure was described in detail in [Saunier](#page-15-0) *et al.* (2017). On each sampled tree, one branch sun-exposed and without flower and having about 6 leaves was enclosed in a polytetrafluoroethynele (PTFE) chamber of about 10 L. A continuous atmospheric air flow, filtered 139 with VOC and ozone scrubbers, flowed through the chamber at 1 L.min⁻¹. After a 30 min system 140 stabilization period, air from the enclosure was collected during 30 min at 0.2 L.min⁻¹ on cartridges (two beds of adsorbants: tenax and cartotrap B), one for isoprene and one for other isoprenoids. Inlet and outlet air flows were controlled by mass flow controllers (Porter Instrument, USA). Cartridges were then stored at -20°C until analysis (portable freezer in the field and classical one in the laboratory). Samplings were performed between 10 h and 15 h (local time) on fully developed leaves. Twice a day, blanks were performed to control inlet air quality.

 One test was performed in April 2019 to check the reliability of our protocol of transport and storage. According to this test, no significant difference was detected between an analysis right after the sampling or after a period of transport/storage (Fig. S1 in supplementary files).

2.3 GC-MS analyses

 Cartridge analyses were conducted by gas chromatography (model 6890N, Agilent technologies, USA) coupled to mass spectrometry (model 5973, Agilent Technologies, USA). VOC were extracted using thermodesorption system (TDS3/CIS, Gerstel, Germany) using helium as carrier gas (50 $\,$ mL.min⁻¹) at 250 °C during 10 min. The extracted compounds were concentrated on-line by cryo-155 trapping at -50 °C using liquid nitrogen. The sample was then injected by rapid heating to 250 °C at 10°C/min. Initial oven temperature was 40 degrees hold for 5 minutes and then increasing by 3 degrees per minute to 250 °C maintained for 15 min.

 Two distinct chromatographic methods were used, one to detect and quantify isoprene (C5) and another one to analyze C10-C15 compounds. Isoprene was analysed using a capillary Al/KCl column (Agilent Technologies, USA; 30 m x 0.25 mm x 5 *µ*m) as described in [Saunier](#page-15-0) *et al.* (2017b) whereas 161 other isoprenoids were separated on a HP-5MS column (Agilent Technology, USA; 30 m x 0.25 mm x 0.25 *µ*m) as shown in [Ormeño](#page-14-6) *et al.* (2007). For both analyses helium was the carrier gas at a constant 163 flow rate of 1 mL.min⁻¹. Isoprenoids were identified based on the co-injection of analytical standards 164 (Sigma-Aldrich, Germany), the Kovàts' index calculation through the injection of alkanes (C_8-C_{20}) as well as the comparison of mass spectra to the NIST library (2011). BVOC emission rate (ER) were calculated according to [Saunier](#page-15-0) *et al.* (2017).

2.4 Statistical analyses

 The statistical analyses were performed with R software (version 4.2.1). After having checked the normality of the data set, one way-ANOVA or Kruskal-Wallis tests were performed for isoprene emissions and other BVOC emissions according to the species. Dunn post-hoc tests were performed to detect the species with statical differences in their emissions.

3. Results

174 The 15 studied mangrove species are low isoprenoid emitters $(< 1.200$ ng.gDW⁻¹.h⁻¹, Table 2). In details, species from the IWP region are the lower isoprene emitters with emissions rates ranging 176 between 0.3 and 1.6 ng.gDW⁻¹.h⁻¹ while species from the AEP region present a larger range of 177 isoprene emission rate with values ranging between 0.1 and 2.1 $ng.g_{DW}⁻¹ h⁻¹$. Isoprene emissions represent 0.3, 0.5 and 2.1% of total emissions for *A. germinans, L. racemosa* and *R. racemosa*, respectively.

180 Besides, those species are also low emitters of other BVOC $(200 - 1.2$ ng. $g^{-1}DW.h^{-1}$, Table 2 and 3) and the main BVOC detected are monoterpenes but also benzenoids as well as methyl salicylate. Sesquiterpene emissions are very low, closed to the detection limit of the analyzers.

 Other BVOC such as benzaldehyde, benzeneacetaldehyde, acetophenone or methyl salicylate contribute (Table 3) significantly to the total BVOC emission specifically for *A. germinans* and *L. racemosa* with 76 and 82% of the total emissions, respectively.

 Rhizophora racemosa is the highest emitter of the mangrove species with an average emission rate of 187 about 1200 ng.g⁻¹DW.h⁻¹. Contrary to the two other species from AEP region, *R. racemosa* emissions are dominated by monoterpenes which represent about 93% of its total emissions. The three dominant emitted monoterpenes of *R. racemosa* are α-pinene, sabinene and β-pinene.

4. Discussion

 The low isoprenoids emissions observed for the 15 mangrove species sampled in the two geographical regions (IWP and AEP) are in agreement with previous findings (Barr *et al.* 2003). In addition, 194 Guenther et al. (1994) reported values below 100 ngC.g^{-1.}h⁻¹ for emissions from the genera *Avicennia*, *Laguncularia* and *Rhizophora* in the United States of America, referring to the works of Zimmernan (1979) and Evans *et al.* (1982). Moreover, the dominant monoterpenes α-pinene and β-pinene detected here are also present in the study of Barr *et al*. (2003). Sabinene was detected in the essential oil of *Melaleuca cajuputi*, a species not directly related to the mangrove but often found in the back of mangroves (Sharif *et al*. 2009). The presence of monoterpenes but also benzenoids as well as methyl salicylate in emissions from mangrove species is not surprising as they are observed in many other biogenic emissions (Rodriguez-Saona *et al.* 2011; Gentner *et al.* 2014).

 The two geographic areas of mangroves have no species in common but share *Avicennia* L. and *Rhizophora* L., the most characteristic genera of mangrove systems worldwide (Robert *et al*., 2009). In our study, several species belonging to these two genera were sampled and show qualitatively different but always small emissions. These results agree with those of Lorto (2002) who showed that within the genus *Quercus*, that have a wide geographical distribution, non-emitting species and emitting species of isoprenoids (isoprene and/or terpenes) can be found.

 Although they share a common ecological niche and must adapt to similar environmental conditions, mangroves are nonetheless a genetically diverse group of flowering plants (Duke, 2002). This may explain the observed differences in their emission spectra, which are also driven by internal factors such as genetics (Peñuelas and Llusià, 2001).

 This emission rate from mangroves is low compared to the emission rates of most other tropical plant species. For instance, [Geron](#page-13-7) *et al.* (2002) performed a screening of BVOC from twenty species in a lowland wet forest located in Costa Rica. Half of these common species emitted isoprene at a rate 216 ranging from 10 to 126 μ g.gDW⁻¹.h⁻¹ or about 100 times higher than the isoprene emissions of the 15 217 mangrove species under study. The same trend is observed for the other BVOCs that are generally emitted by tropical species at higher rates than mangrove species [\(Singh](#page-15-3) *et al.* 2011; [Bracho-Nunez](#page-13-8) *et al.* [2013\)](#page-13-8).

 We can confirm that mangroves invest in isoprenoid emissions as chemical defences against the extreme environmental conditions that typically occur in their environment (e.g. flooding, salinity) but to a small extent. Indeed, mangroves have other strategies that ensure very good adaptation to these extreme conditions. They possess strategies such as osmolyte production which absorb salt as previously observed in *Avicennia* genus and other halophytes [\(Khan](#page-14-7) *et al.* 2016) [\(Slama](#page-15-4) *et al.* 2015). However, samplings were performed only on a short time. As a result, it cannot be excluded that we missed plant response to salinity and or flooding that could have caused emission puffs. For example, *Eucalyptus globulus*, a non-halophyte plant, exhibits puffs of isoprene only when salt stress is relieved [\(Loreto & Delfine 2000\)](#page-14-8). [Velikova](#page-15-5) *et al.* (2012) also observed important emissions of volatile isoprenoids from *Citru*s leaves, mainly limonene, during flooding with saline water.

 Moreover, it would be worth investigating the emission of highly volatile organic compounds such as acetaldehyde and methanol since mangroves face anoxic conditions that could lead to alcoholic fermentation processes and, as a result, ethanol could be produced and transformed to acetaldehyde 235 and to a lesser extent to acetic acid [\(Oikawa & Lerdau 2013\)](#page-14-9). More generally, plants can also emit methanol through growth process [\(Hüve](#page-14-10) *et al.* 2007) and acetone through acetoacetate decarboxylation [\(Fall 2003\)](#page-13-9). Acetaldehyde and acetic acid impact air quality by contributing to the atmospheric 238 oxidative capacity and forming toxic secondary pollutants such as formaldehyde (Oikawa & Lerdau [2013\)](#page-14-9). In addition, other halophytes have been shown to be a source of methyl halides and dimethylsulphoniopropionate (DMSP) (Otte *et al.* [2004;](#page-14-11) [Rhew & Mazéas 2010\)](#page-14-12). One experimental study conducted under greenhouse conditions also demonstrated that mangrove trees emit methyl halides with possible atmospheric impact [\(Manley](#page-14-0) *et al.* 2007). However, this study emphasizes the need for field experiments to check for the occurrence of these emissions from mangroves. Further investigations are thus required to explore if mangroves are a source of halide VOC.

4. Conclusion

 Our results show that mangrove trees are very low isoprenoid emitters. Only *R. racemosa* can be 248 regarded as a non-negligeable source though monoterpene emissions still low ~ 1000 ng.gDW.h⁻¹). Such results suggest that mangrove does not strongly affect local or regional atmospheric chemistry through BVOC emissions despite the large distribution of this ecosystem over the globe. However, despite the low-rate emission, the data from this study can be used in atmospheric chemistry models to study the impact of biogenic VOC emissions on air quality in areas where mangroves are present.

 It is still worthy to note that we only made punctual samplings and that continuous monitoring of isoprenoid emissions should be carried out to check for potential seasonal variations. Moreover, as far as our results showed important differences between plant species, it could be interesting to broaden the sampling design including other sites (African west coast and Caribbean Islands) as they include mangrove species that have not been investigated.

 It could be also interesting to focus on other compounds than isoprenoids such as highly volatile or halogenated compounds which might be tackled in future studies focused on BVOC emissions from mangroves.

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6. Author contributions:

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- 421 **Table 1:** Means (± SE) of temperatures (T, °C), relative humidity (RH, %) and Photosynthetic Active
- 422 Radiation (PAR, μ mol.m⁻².s⁻¹) between 10 h and 15 h (sampling time for BVOC, local time) during 423 each field campaign. PAR in March 2019 could not be calculated due to technical problems with the 424 HOBO sensor.

Table 2: Emission rates (ER, ng.gDW⁻¹.h⁻¹) of 15 mangrove species in terms of isoprene and other

426 BVOC (see Table 3 for details) detected with GC-MS, n.d.: no compound detected. Values are means

 \pm SE. Statistical test are presented for each BVOC type and site. Lower case letters denote post-hoc
428 test differences at 95% confidence level (Due to a problem during the analysis, we lost a series of

428 test differences at 95% confidence level (Due to a problem during the analysis, we lost a series of samples from French Guyana and therefore only have 4 samples with results) samples from French Guyana and therefore only have 4 samples with results)

430

435 $n.d. = not detected, E-DMNT = (E)-4,8-Dimethyl-1,3,7-nonatriene$