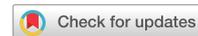




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Surprising chiral composition changes over the Amazon rainforest with height, time and season

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Many biogenic volatile organic compounds (BVOC) are chiral, existing in two mirror image forms called enantiomers. The most abundant atmospheric chiral BVOC is α -pinene ($C_{10}H_{16}$), whose enantiomeric ratio has been reported to be regiospecific. Here we show with measurements made on a 325 m tower in the Amazon rainforest that the enantiomeric ratio varies unexpectedly (by a factor of ten) with (+)- α -pinene dominating at canopy level and (–)- α -pinene at tower top. The ratio is independent of wind direction, speed and sunlight but shows diurnal temperature dependent enrichment in the (–)- α -pinene enantiomer at the lowest 80 m height. These effects cannot be caused by atmospheric reaction with oxidants, or aerosol uptake. The reversal of chiral ratio at 80 m reveals the presence of a potent uncharacterized local (+)- α -pinene rich source, possibly linked to herbivory and termites. These results suggest the presence of a strong uncharacterized BVOC source that is overlooked in current emission models.

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Each year an estimated 760 TgC of biogenic volatile organic compounds (BVOCs) enters the atmosphere, primarily from tropical forests¹. These compounds react rapidly with ambient OH radicals and O₃, thereby influencing the atmosphere's oxidation capacity for pollutants (e.g., CO) and greenhouse gases (e.g., CH₄). Furthermore, they are precursors for secondary organic aerosols (SOA), which influence the Earth's radiative budget. Many of the BVOC emitted are chiral, meaning that they exist in two non-superimposable mirror image forms. Although isoprene, the most strongly emitted BVOC is not chiral, most monoterpenes and sesquiterpenes that are known to be emitted from tropical forests^{2,3} exist as enantiomeric pairs. The enantiomers can be differentiated by how they interact with plane polarized light, (–) deflecting to the left and (+) deflecting to the right. However, all physical properties such as boiling point, exact mass, and reaction rate with atmospheric oxidants (OH, O₃, and NO₂) are identical. In almost all air chemistry studies, the enantiomers are not quantified separately and therefore inherently assumed to have similar source characteristics. Despite the similarity in structure, insects and plants can distinguish enantiomeric forms, and each may elicit different biological responses (e.g., pheromonal attraction or chemical defense repellent)^{4,5}.

The few available chirally speciated ambient measurements have shown a remarkable and as yet unexplained regiospecific behavior in the enantiomers of α -pinene. While measurements taken at a ground station in the boreal forest were all dominated by (+)- α -pinene, measurements taken by aircraft in the biodiverse tropical rainforest boundary layer were consistently richer in the (–)- α -pinene enantiomer⁶. Equally puzzling is that several ambient studies have reported a diurnal enhancement in the (–)- α -pinene enantiomer, indicating that the emissions of the enantiomers vary independently⁷. However, in apparent contradiction, laboratory studies on Mediterranean plants using branch cuvettes have shown that although increased light and temperature increase emissions of monoterpenes from plants, the enantiomeric emission ratio of some chiral monoterpene pairs from plant species were not changed⁸. Recent studies within a monoculture maritime pine forest have shown chiral signatures can vary between canopy, stem, and litter⁹. This suggests that accurate modelling of BVOC emissions will require chirally and compartment specific measurements in future.

In order to understand better the emissions of chiral BVOC compounds at the ecosystem scale, as well as their vertical and diel distribution, we performed measurements of chiral monoterpenes at several heights on the 325 m ATTO (Amazonian Tall Tower Observatory) tower facility. The ATTO tower is surrounded by

pristine rainforest (see Fig. 1), which extends over 500 km upwind of the site^{10,11}. Each day biogenic volatile organic compounds emitted at ground level are transported through vertical turbulent mixing up to the tower's summit at 325 m over timescales of circa 20–40 min by day¹². Generally, the emission rate of BVOC increases with sunlight and temperature¹³, and at ATTO a maximum mixing ratio is observed around 15:00 local time (UTC-4h) for isoprene and between 15:00 and 18:00 for monoterpenes¹⁴. Atmospheric OH radical concentrations and turbulent mixing peak at local noon so that BVOC concentration gradients from canopy to tower top are steepest around midday. The reaction of the monoterpenes with OH is not thought to be enantiomerically selective, so based on current understanding, we would expect the absolute concentration of the monoterpenes to decrease with height but the enantiomeric ratio to remain constant. Similarly, although the emission rates of the α -pinene enantiomers are expected to rise with increasing temperature during the day, the ratio of the enantiomers is expected to be conserved.

Here we show from about 600 dry and wet season measurements at four separate heights (40, 80, 150, and 320 m) that this is not the case and that large unexpected changes occur in the enantiomeric ratio both in the vertical extent and diel cycle. Possible explanations for these phenomena are discussed including abiotic and biotic factors driving the emission, inhomogeneous source distributions, selective uptake onto aerosol and hitherto unknown selective chiral chemistry.

Results

Measurements. Samples of ambient air were collected in central Amazonia during the dry and wet season campaigns (November 2017 and March 2018) at the ATTO field site (Amazonian Tall Tower Observatory) within the German-Brazilian project ATTO¹⁰. The field site is located about 150 km northeast of Manaus, Brazil, about 12 km northeast the Uatumã river, in the Uatumã Sustainable Development Reserve. The dominant ecosystem is a dense, non-flooded upland forest (terra firme), while ecosystems such as campinas (savanna on white-sand soils) and campinaranas (white-sand forest) prevail near to the river (Fig. 1). Air is usually transported from the East sector (NE~20%, ENE~27%, E~33%, ESE~19%, Supplementary Fig. 1), covering a footprint of continental area of $\sim 1.5 \times 10^6$ Km², whereby 54% is considered untouched¹¹. Meteorological conditions vary through the year according to the position of the inter-tropical convergence zone (ITCZ), with a cooler rainy season (February–May) and a hotter drier season (June–October)¹⁰. Gas measurements at ATTO

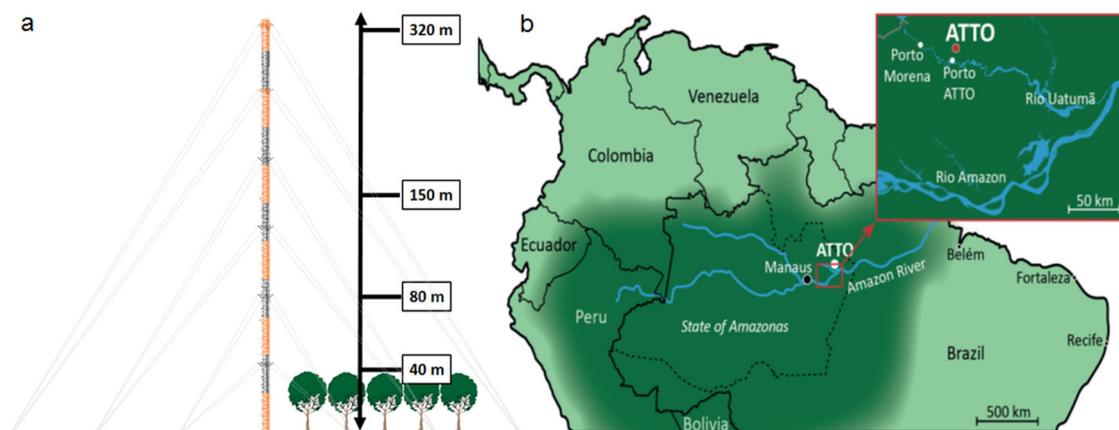


Fig. 1 Height and location of the ATTO tower. Composite figure showing the sampling heights chosen for the VOC measurements at the ATTO tower relative to the canopy height (a) and the location of the field site ATTO relative to the city of Manaus, the Amazon river and its affluent the Uatumã River (b). Map drawn by Iris Moebius, MPI-BGC.

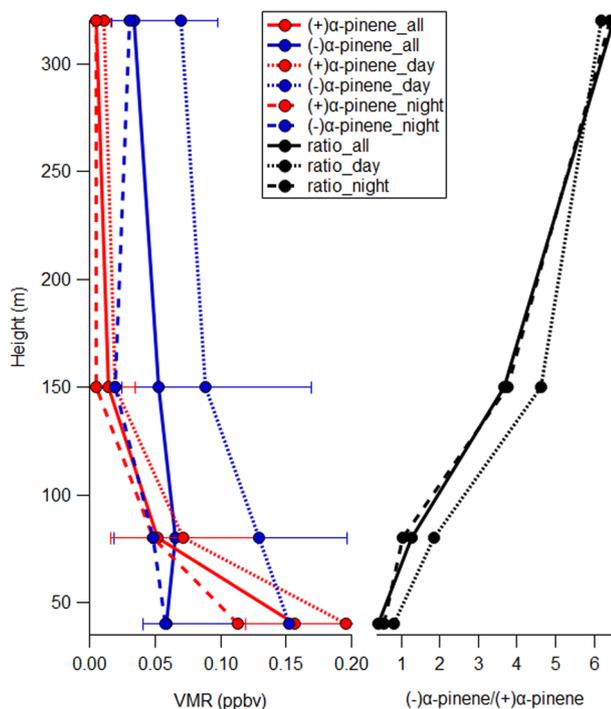


Fig. 2 Vertical profile of (–)- α -pinene and (+)- α -pinene volume mixing ratio measured at 40, 80, 150, and 320 m along the ATTO tower.

Sampling was conducted every 3 h for 10 days during the dry season 2017. Data points are median values of the measurements taken. Daytime values refer to samples taken between 8:00 and 17:00, while nighttime values refer to samples taken between 20:00 and 5:00 (all times are local). Sunrise is usually around 5:00–6:00 and sunset around 17:00–18:00. Error bars are 25 and 75 percentiles of the measurements (at 40 m the 75 percentile is 0.39 ppbv and 0.29 ppbv for (+)- α -pinene and (–)- α -pinene, respectively). The right panel shows the chiral ratio (here as (–)- α -pinene/(+)- α -pinene) median values for the same sampling heights. In the 1:1 ratio the mixture is racemic.

are performed from two main towers, the INSTANT tower (80 m height, 150 m distant from ATTO tower, coordinates: S 02° 08.6470' W 58°59.9920') and the ATTO tower (325 m, coordinates: S 02°08.7520' W 59°00.3350'). Air samples were collected from the ATTO tower on sorbent tubes with automatic samplers¹⁵ installed at four different heights on the tower: 40 m (top of the canopy), 80, 150, and 320 m (Fig. 1). Sampling was programmed to occur at the same time of the day for the different heights, every 3 h, eight times a day, for the whole campaign time (10 days dry season, 15 days wet season). During the wet seasons sampling was restricted to a maximum of 150 m for safety reasons based on the time needed to vacate the tower during a rainstorm. Full experimental details of the analysis are given in the method section. Online measurements of VOCs through PTR-MS (Proton transfer reaction-mass spectrometer) and OH reactivity^{16,17} occurred simultaneously during March 2018. Meteorological parameters including, wind speed, wind direction, rainfall, temperature, humidity, and photosynthetically active radiation were monitored continuously from the nearby INSTANT tower. See Methods for comprehensive details about the measurements.

Vertical gradient. Figure 2 presents the atmospheric volume mixing ratios of the two enantiomers of α -pinene at four heights as measured during November 2017 (here referred as dry season) from the ATTO tower. Values are represented as campaign median values (solid line) and median values for daytime (8:00–17:00 local

time, UTC-4h) and nighttime (20:00–5:00) (pointed and dashed lines). Mixing ratios for the two α -pinene enantiomers ranged between the instrumental limit of detection (ca. 1 pptv) and the maximum median value 0.41 ± 0.09 for the (+) isomer and the maximum median value 0.34 ± 0.07 ppbv for the (–) isomer (1σ of all campaign data). α -pinene was the most abundant chiral VOC measured at ATTO.

On the vertical profile (full line in Fig. 2), the (+)- α -pinene mixing ratio was 0.16 ± 0.32 ppbv (median value \pm standard deviation of dataset) at the lowest sampling height (40 m), decreasing to 0.005 ± 0.007 ppbv at the top level (320 m). The median concentration of (–)- α -pinene decreased less steeply varying from 0.06 ± 0.18 ppbv at 40 m to 0.03 ± 0.03 ppbv at 320 m (median values \pm standard deviation of dataset). Similar levels of these enantiomers were measured from an aircraft over the tropical rainforest of Suriname at ca. 300 m height above canopy⁶. Lower mixing ratios for α -pinene enantiomers have been reported from higher latitude sites: 0.06 ± 0.015 ppbv (+)- α -pinene and 0.020 ± 0.005 ppbv (–)- α -pinene were measured on average at Hyytiälä in the Finnish boreal forest⁶ and 0.004 ± 0.003 ppbv (+)- α -pinene and 0.004 ± 0.003 (–)- α -pinene in a temperate forest in Spain during winter¹⁸. Higher emission rates of monoterpenes are expected in the tropics for several reasons, including higher ambient temperature^{13,19} and higher leaf area index.

Assuming that the ground-based chiral sources emit equal proportions of the two enantiomers, we may expect a priori that although the absolute mixing ratios decrease with increasing height due to oxidation chemistry and mixing, the enantiomeric ratio will remain constant from the point of emission at the canopy to the top of the tower. This assumes the surrounding fetch region is homogenous and uniform, no atmospheric chirally selective oxidant is known, and no chirally selective uptake process is known to occur in the rainforest. However, as shown in Fig. 2, (–)- α -pinene/(+)- α -pinene between the 40 m and 320 m sampling points varies from 0.38 (40 m) to 6.5 (320 m), corresponding to a substantial change in the enantiomeric ratio (94%). Interestingly, the ratio at 320 m is dominated by (–)- α -pinene, consistent with the aircraft data obtained at approximately the same height over another area of tropical rainforest. Surprisingly, at the 40 m level the ratio favours (+)- α -pinene, which was consistent with the ground-based data taken in the boreal forest. While Williams et al.⁶ interpreted the (–)- α -pinene dominated tropical forest dataset (airborne) and the (+)- α -pinene dominated boreal dataset (ground based) as regiospecific chiral signatures, the tower data reported here show that these ratios may also be due to changes in the vertical profile. The question is therefore what processes can cause this striking change in enantiomeric ratio with height.

Diel profile. Figure 3 shows the diel profile of the α -pinene enantiomer mixing ratios at 40, 80, 150, and 320 m (median values) together with the photosynthetic active radiation (PAR) and temperature monitored at 80 m on the INSTANT tower. The (–)- α -pinene and (+)- α -pinene mixing ratios both have a diurnal maximum, the highest concentrations broadly following PAR and temperature. Each enantiomer shows consistent temporal trends at the heights 80, 150, and 320 m, with shifted maxima due to the vertical transport time; however, these trends are not the same for each enantiomer. While (–)- α -pinene shows two peaks at 08:00 and 14:00 (local time, UTC-4h) at 80 m, (+)- α -pinene shows one peak at 08:00 at the same height. The mixing ratios at 40 m show markedly different behavior to the other heights. At the lowest level, both enantiomers show the same temporal trend with their

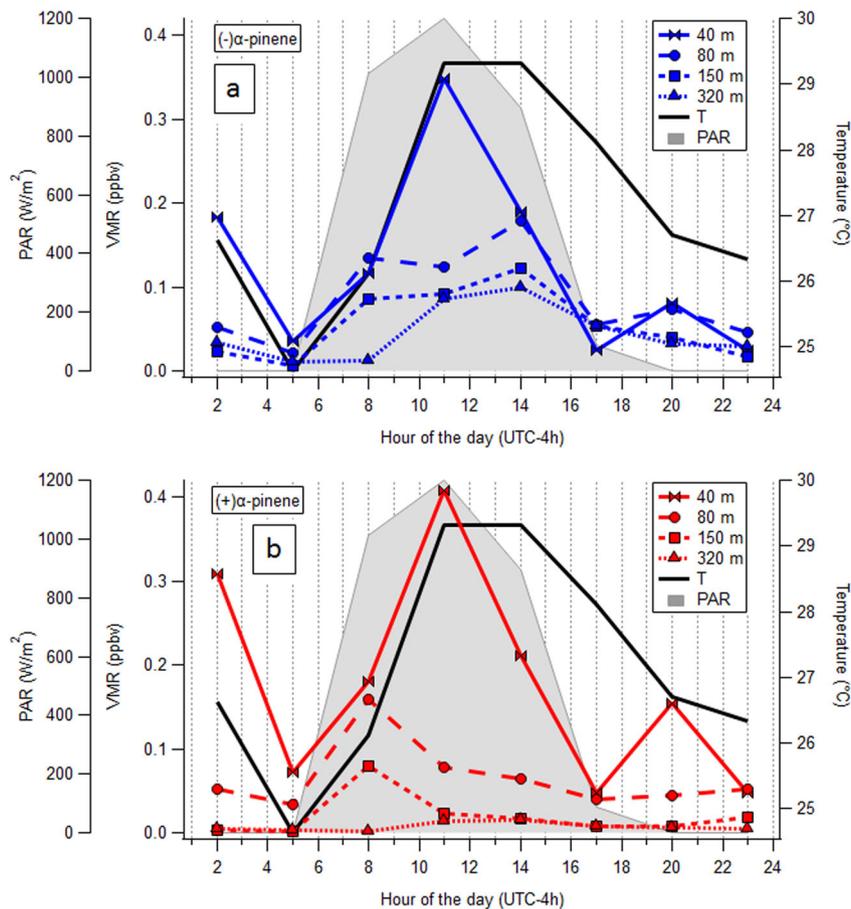


Fig. 3 Median values of (–)- α -pinene (A) and (+)- α -pinene (B) volume mixing ratios measured during the dry season. Incoming photosynthetic active radiation (PAR) and air temperature measured at 80 m at the neighbouring instant tower are shown with the gray shading and black line. Error bars indicating the 25 and 75 percentiles of the measurements taken at 80 m are shown in Fig. 7.

maxima peaking with PAR at 11:00. These diel results suggest that the 40 m height is impacted by other sources than the 80, 150, and 320 m heights. Possibly this includes the understory vegetation and soil around the base of the ATTO tower, which may affect the 40 m data via the cleared area around the base of the tower, meaning that the 80 m data are more representative of the surrounding rainforest.

The relative abundance of the two enantiomeric configurations is further investigated in Fig. 4. Here, we show how the relative chiral abundance varies for each sample taken during November 2017 across the investigated heights. The cases of 40, 150, and 320 m are striking. At these heights, each collected air sample was consistently higher either in the (+) enantiomer (40 m), or the (–) enantiomer (150 and 320 m). However, this is not the case for the 80 m height. At this level, both configurations exist in approximately a ratio of 1:1, and dependent on the time of the day. Indeed, the chiral ratio at 80 m appears to be driven by both the air temperature (Fig. 3) and changes in the mixing layer height; with the result that (+)- α -pinene is dominant during night and early morning, and (–)- α -pinene is dominant in the afternoon.

Seasonality. The chiral relative abundance measured at 80 and 150 m during the wet season (March 2018) is reported in Fig. 5. The samples show little variation in chiral abundance at 80 m, while more variability was observed at 150 m. Overall, except for a few samples, at the two heights (–)- α -pinene was the predominant enantiomer.

Temperature dependence of the emissions. The temperature dependency of (–)- α -pinene and (+)- α -pinene was investigated over the 80 m wet season dataset (96 cartridges samples) following the parametrization for mixing ratios adopted by Hellen et al.²⁰ and originally proposed by Guenther et al.¹⁹ for the emission rates, whereby $\text{VMR} = A \exp(\beta(T - T_s))$, with A being the intercept, β the temperature sensitivity, T the ambient temperature, and T_s the standard temperature (Fig. 6). Here, the two mirror images of the same molecule show a different behavior. Specifically, the volume mixing ratios increased with temperature with a factor of 0.30 and 0.28, with R^2 of the correlation of 0.58 and 0.52 for (–)- α -pinene and (+)- α -pinene, respectively. Temperature during March 2018 varied between 23 and 28 °C, while during November 2017 was between 24 and 29 °C. The divergent behavior reported here for (–)- α -pinene and (+)- α -pinene compare well the one reported in summertime in the boreal forest for α -pinene and β -pinene, respectively²⁰. However, Hellen et al.²⁰, also reported seasonal changes of such sensitivities in the boreal forest; so that the sensitivities obtained in this work may be a lower limit for temperature driven emissions in the tropical forest. Reporting ambient mixing ratios and not leaf-level concentrations does not disentangle temperature from other atmospheric factors such as atmospheric stability and boundary layer height, which also affect mixing ratios in air. Therefore, 80 m wet season data were preferred to 80 m dry season data (more stability in chiral ratio and larger number of samples collected). Leaf-level experiments in the forest are needed in order to obtain temperature normalization coefficients to be used in emission models. Emission models do not currently distinguish between

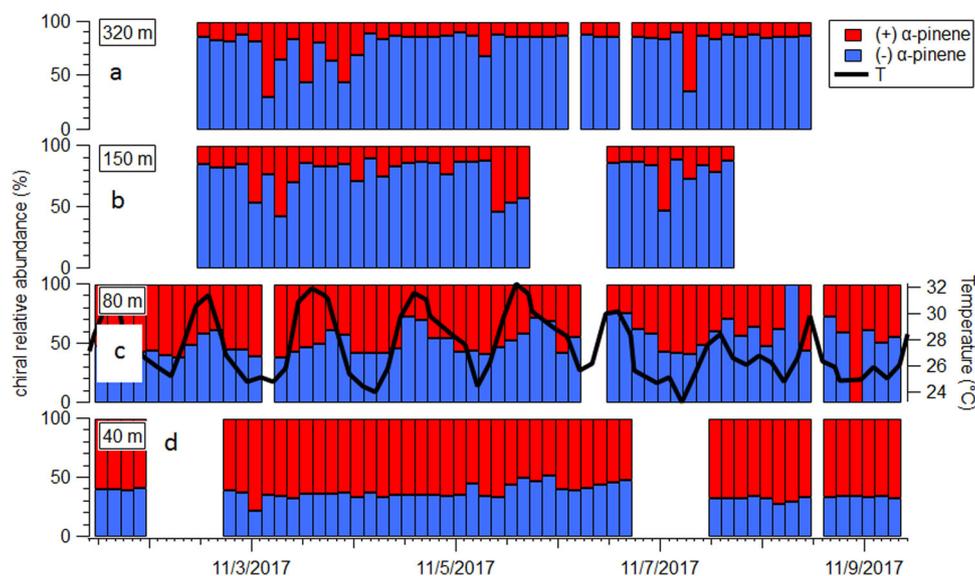


Fig. 4 Relative percentage of (–)- α -pinene and (+)- α -pinene air mixing ratios measured at 40, 80, 150, and 320 m up the ATTO tower during the dry season in 2017. Each bar corresponds to a sample collected from the tower. Temperature is measured at 80 m on the neighboring INSTANT tower. Diel profile of the chiral ratios measured at each height with the standard deviation of the dataset shown in Supplementary Fig. 3.

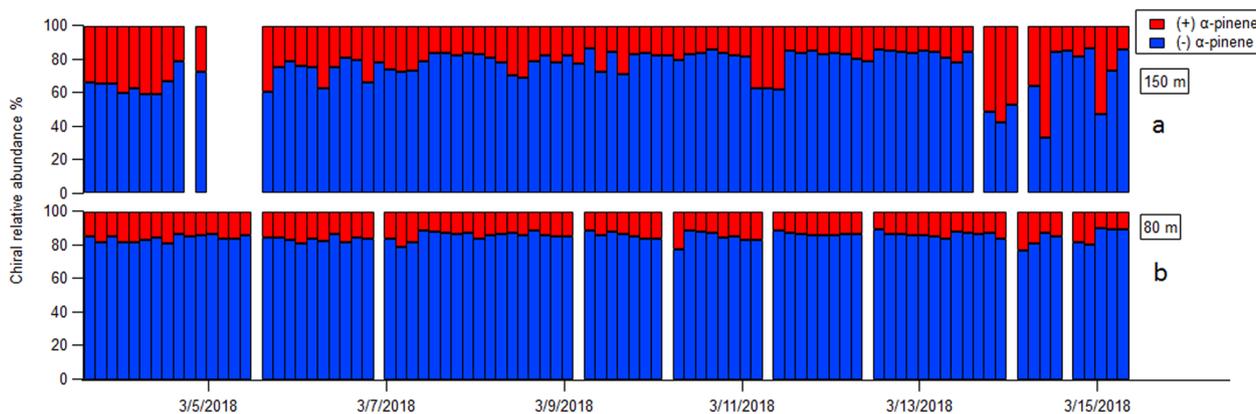


Fig. 5 Relative percentage of (–)- α -pinene and (+)- α -pinene air mixing ratios measured at 80 and 150 m up the ATTO tower during the wet season in 2018. Each bar corresponds to a sample collected from the tower. Diel profile of the chiral ratios measured at each height with the standard deviation of the dataset are shown in Supplementary Fig. 4.

chirally speciated compounds. The results shown here indicate that as the Earth warms over the coming decades the enantiomeric ratio of the pinene emissions will change, further impacting the ecosystem as many enantiomeric VOC are used for signaling and communication by plants and insects. The light dependency was also investigated, however, no significant correlation among mixing ratios and photosynthetic active radiation was found from our data. In Fig. 7 we examine the diel cycle of the pinene enantiomers with temperature, PAR and stomatal conductance as measured from four different trees between 20 and 32 m on 6/11/2017 (see Methods).

As the stomata first opened at dawn around 6:00 (local time) and at low ambient temperature (25 °C), an emission burst of both enantiomers occurs. After 9:00 stomatal conductance started decreasing, while the ambient temperature increased: here, the air mixing ratios of the two isomers followed distinct patterns. (–)- α -pinene, having a stronger temperature dependency continued to be released by plants, reaching a maximum concentration in the afternoon when temperature peaked. In contrast, the (+)- α -pinene concentration slowly decreased as stomatal conductance decreased. A second peak in the stomatal conductance

profile occurred at 13:00, within a period of steady 29 °C temperature; this point coincides with a larger (–)- α -pinene peak, possibly resulting from a combined effect of stomata opening and warmer air at 13:00 than at 9:00. Therefore, chiral concentrations measured in air at 80 m are driven by photosynthetic activity (which is dependent on temperature and associated with stomatal opening) driving the (–)- α -pinene emissions, explaining the afternoon concentration maximum. This indicates that (i) canopy emissions are a chirally selective source and that (ii) the canopy is the main terpenoid emission source at 80 m.

Possible existence of a chiral-selective sink. Thus far, we have interpreted the results in terms of changing emission signatures. However, another possibility is that a chirally selective sink process exists. In order to determine whether a selective chiral sink is also present we examined possible atmospheric processes that can potentially affect it. Firstly, any meteorological parameter (e.g., wind direction, wind speed, temperature, PAR, relative humidity, and rainfall) monitored at the site was inspected alongside the chiral ratio. Specifically, no dependence to the chiral ratio was observed for changes of wind direction, wind speed and

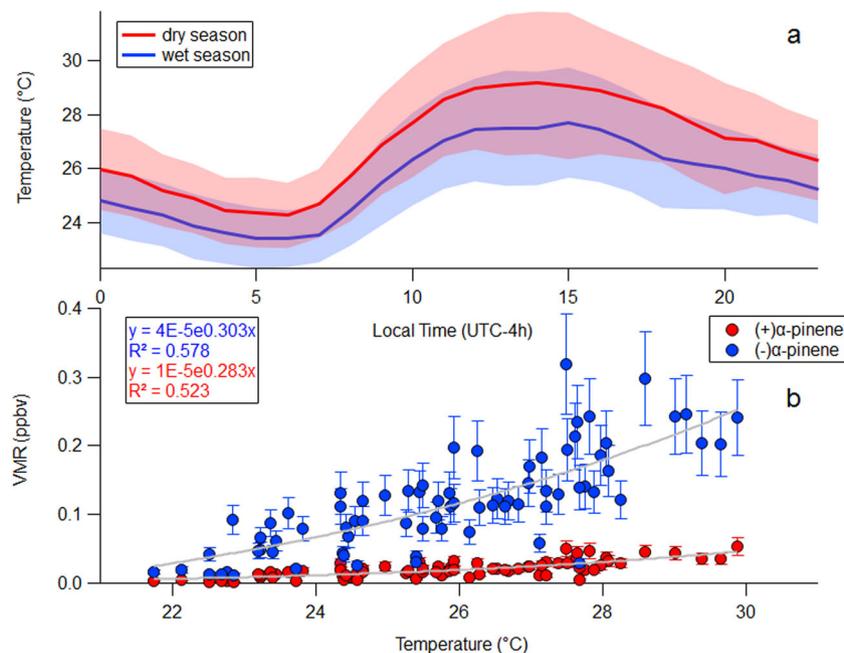


Fig. 6 Temperature dependency of (–)- α -pinene and (+)- α -pinene volume mixing ratios at 80 m during the wet season. Equations represent the exponential fit to (–)- α -pinene versus T and (+)- α -pinene vs T in the form: $\text{VMR} = A\exp\beta(T-T_s)$, where A is the intercept and β the temperature sensitivity factor. Panel **a** shows the air temperature registered during the dry season (November 2017) and wet season (March 2018) of sampling. Error bars in panel **b** represent the measurement uncertainty (23%) while shaded areas in panel **a** represent the standard deviation of temperature dataset. Dry season mixing ratios against temperature are shown in Supplementary Fig. 6.

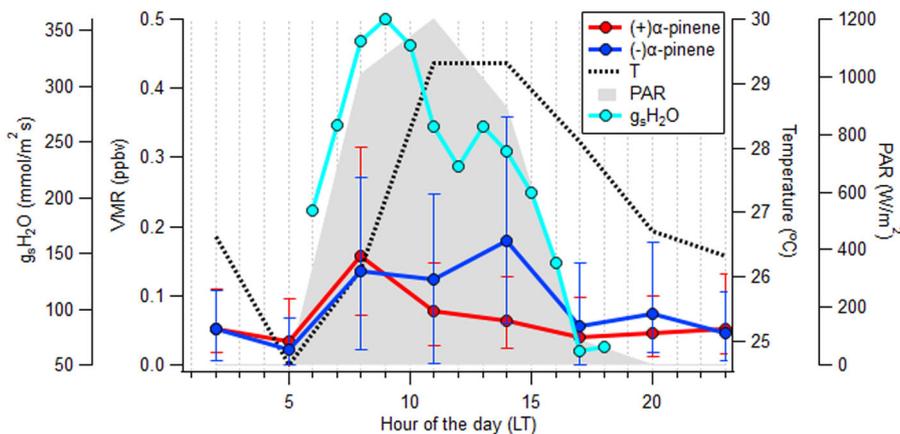


Fig. 7 Median values of (–)- α -pinene and (+)- α -pinene volume mixing ratios measured during the dry season campaign at 80 m. Incoming photosynthetic active radiation (PAR) and air temperature were measured at 80 m at the neighboring instant tower at the same time, stomatal conductance was measured on 6/11/2017 for four trees at 20 m and 32 m adjacent to the INSTANT tower. Error bars indicate 25 and 75 percentiles of the campaign data.

light. We calculated the depletion rate of the two compounds based on atmospheric chemistry from the dry and wet season data between datasets collected at 80, 150, and 320 m (see Methods). In all the investigated cases (day and night, dry and wet seasons), the depletion rates indicated an apparently faster atmospheric removal of the (+)- α -pinene. Therefore, we examined possible chemical and physical process that can potentially lead to such removal (e.g., chemical reactions and physical uptake). While a chiral pair of molecules will have the same reactivity towards an achiral molecule, the same chiral pair could potentially react at different rates with a chiral species. The prevalent atmospheric oxidants at ATTO are the hydroxyl radical (OH) and ozone, which are both achiral; therefore, their reactivity to α -pinene is expected to be the same for both chiral configurations. Nevertheless, we tested with our instrumentation and

sampling material whether the chiral pair reacted differently to OH and O_3 . Tests were performed in the laboratory using a glass flow reactor in which both OH and O_3 could be generated at various elevated levels²¹. At each oxidant level, the total concentration of α -pinene in the samples decreased, due to oxidation by O_3 and OH. However, the ratio of the two enantiomers remained unchanged (Supplementary Fig. 5). We hypothesized the existence of a chiral Criegee radical that selectively uptakes α -pinene, but rejected this potential chiral enrichment process as the reaction of ozone with pinene will always dominate over criegees under Amazon rainforest conditions. To our knowledge, no other gas phase chiral atmospheric oxidant is known. However, chiral molecules in aerosol samples have been reported in samples collected in the Amazon^{22,23} and Sweden²⁴. A simple test was therefore also conducted in the laboratory to check whether

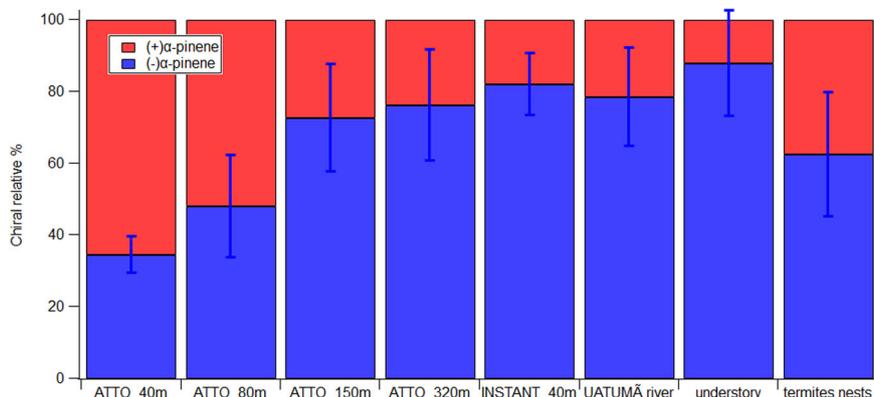


Fig. 8 Average relative abundance in percent of the (–)-α-pinene and (+)-α-pinene for different samples collected on or around the ATTO tower.

From left to right, the number of samples represented by each bar is 64, 64, 48, 48, 10, 12, 5, 12. Error bars represent the standard deviation of the abundance for all the samples taken. The towers ATTO and INSTANT are located at (S 02° 08.7520' W 59° 00.3350' and S 02° 08.6470' W 58° 59.9920', respectively). The Uatumã river samples were collected on a 5 km stretch of river upstream from the ATTO site. Understory samples were collected below the ATTO tower at 20–30 cm height from the forest floor. Termite nest sampling was conducted above two nests near to the ATTO site (see supplementary material).

particles collected in the field could selectively uptake one of the α-pinene enantiomers. Blank filters and 24-h-sampled aerosols filters collected at 40 m and 320 m during the same days of sampling of chiral VOC (see method) were placed in a filter holder and exposed to a constant flow of a racemic mixture of α-pinene. All lines were heated up to 25 °C and the racemic mixture was diluted in humidified (100%) synthetic air to resemble experimental conditions in the field. Sorbent tubes were collected before and after the filter samples and no differences in the racemic pinene mixture were observed.

Sampling from the surrounding area. Figure 8 shows the variability of the chiral ratio in the area surrounding the ATTO tower. Air samples were collected in the same fashion on sorbent tubes at 40 m on the INSTANT tower (670 m distant direction NE, during the dry season 2018) which unlike the ATTO tower is embedded into the forest structure without a surrounding cleared area. Samples were also taken from further afield, travelling by boat along the Uatumã river, for 12 km in a East-South East direction, upwind the ATTO tower (dry season 2018, Supplementary Fig. 1). This enabled an impression of the regional characteristics to be gained. Samples were also taken below the ATTO tower at 0.3 m height from the soil (wet season 2019) and above two termites nests built on trees (dry season 2019, Supplementary Fig. 10). It is evident from Fig. 8 that the chiral signature of α-pinene at ATTO is very generally consistent: with the (–)-α-pinene configuration dominating over the (+)-α-pinene basically in all the collected samples (error bars indicate the standard deviation across all samples taken). Only at the 40 m level of the ATTO tower and above the termites nest for a few samples by night was (+)-α-pinene the more abundant enantiomer. These results agree with what was previously stated for the vertical and diel profiles of the two molecules: 80, 150 and 320 m heights are consistent and fingerprint the canopy emissions, while 40 m is impacted by a different source than the canopy. The data shown in Fig. 8 highlight the differences between 40 m from the ATTO tower and 40 m from the INSTANT tower. Indeed, the INSTANT tower, being smaller and older, is fully assimilated into the forest structure, while the base of the ATTO tower remains clear with younger vegetation growing back. The cleared area around ATTO may promote insects populations feeding on fresh new leaves and consuming decaying cleared wood. The clearing will also enable an unnatural fast transport in the first 40 m, so that the forest floor and in-

canopy processes can significantly impact the air composition at 40 m on the ATTO tower.

Plant stresses and tropical fauna. Plants release chemical compounds for signaling and communication^{25,26} and several compounds have been found to be released by plants under circumstances of abiotic and biotic stresses. For instance: GLVs (green leaf volatiles, C₆ oxygenated compounds, including aldehydes, esters, and alcohols) are reported to be released when plants undergo mechanical stress^{27,28} and when plants are exposed to drought stress, as happened in the Amazon region during the 2015 El Niño event²⁹. Indole was found to be a specific compound released when maize plants undergo biotic stress, specifically from herbivore attacks³⁰. Direct emissions of VOC as pheromones by some insects and animals have also been extensively studied^{31–35}. In our study, several VOCs reported as markers for abiotic and biotic stresses were considered and investigated alongside the chiral ratio observed during the dry and wet seasons. Hexanal (C₆H₁₂O), hexanone (C₆H₁₂O) and branched hexanols ((+)-2-ethylhexan-1-ol and (–)-2-ethylhexan-1-ol; C₈H₁₈O) were identified on certain samples and considered as markers for mechanical stress. None of these molecules showed a significant correlation with the chiral ratio reported at 40 m during the dry season. Nevertheless, as reported by Fall et al.²⁷ VOC emissions after plant wounding can undergo fast changes with time, which is not easily tracked with the time resolution of our instruments (sampling every 3 h). Interestingly, in both datasets taken during the dry and the wet seasons peaks of hexanal and branched hexanols do coincide with occasional inversions of chiral ratio recorded at any height (Supplementary Figs. 7, 8). These peaks occurred sporadically in time and height, which suggests that stochastic events of plants wounding (for example a falling tree) in some parts of the forest are detected as “chemical screams” by our instruments depending on its exact location, wind direction and wind speed at the time of release. This chirally specific mechanical stress signature was first observed in the boreal forest^{7,12} where larger concentration of (+)-α-pinene were detected when the wind was advected from a nearby sawmill. Although it is therefore conceivable that the abundance of (+)-α-pinene at 40 m is the result of damaged plants surrounding the tower, this is probably not the case for the following reasons: firstly, no falling trees were observed in the tower vicinity during the sampling days. Secondly hexanal and hexanol concentrations at 80 m were larger than at 40 m, and

thirdly sampling of the forest understory surrounding the ATTO tower reported larger concentrations of (–)- α -pinene (Fig. 8). Erb et al.³⁰ showed that when caterpillar regurgitant was applied to maize leaf surfaces five major VOCs families were induced in response with various time delays. These were GLVs, monoterpenes, homoterpenes, sesquiterpenes and aromatic compounds (including indole). We measured indole from our 40 m dry season samples and noticed that its temporal profile relative to (+)- α -pinene matched that found in the laboratory³⁰, with indole peaking first at 8:00, and (+)- α -pinene 3 h later (Supplementary Fig. 9). Further support for the (+)- α -pinene enhancement being related to herbivory, comes from herbivory rates being reported to be higher in tropical than temperate forests, more abundant on fresh and young leaves than mature ones, in dry tropical forests than in wet ones³⁶. Although a variety of other sources were reported for indole, including, natural plant flower oils, such as jasmine and daffodils, coal tar³⁷, animal faeces and bacterial strains³⁸, the temporal coincidence between indole and (+)- α -pinene suggests a contribution from this source.

Insects can also release chemical compounds, to signal their presence among the communities, for mating, defense and foraging. We considered one of the most abundant species of insects in Amazonia and existing in ATTO, namely termites. Interestingly, samples collected at the ATTO site in October 2019 above two termites nests showed generally low background values with slightly larger concentrations of (–)- α -pinene, with occasional much larger concentrations of (+)- α -pinene when the nest emissions were detected, together with ketones, carboxylic acids N-compounds and aromatics observed simultaneously (Fig. 8). Samples collected directly over a line of termite soldiers showed a 1:1 ratio of optical isomers (Supplementary Fig. 11).

Discussion

This study shows that all the collected air samples at the ATTO field site contain chiral species, and the most abundant chiral compound is the monoterpene α -pinene. Generally, the (–) optical isomer was found to be dominant, except at the lowermost sampling location of the ATTO tower (40 m) during the dry season. Williams et al.⁶ reported that air samples collected at 300 m above the tropical rainforest in Suriname were also chiral and dominated by the (–) configuration of α -pinene, which agrees with our results at 320 m during the dry season. However, this study highlights that the enantiomeric ratio of α -pinene is not constant as previously expected, instead it inverts over the vertical profile of the tower height and generates a diel profile at 80 m. Samples taken at 40 m appear decoupled from those above, indicating they are impacted by an additional large local source. Wet season samples, in contrast, showed little but not significant differences in height and time. These samples were used to investigate the temperature dependency of (–)- α -pinene and (+)- α -pinene, which helped relate the diel profiles of the two compounds in both seasons to temperature. Both pinene enantiomers increased rapidly in the air with the opening of the leaf stomata in the morning, but only (–)- α -pinene release is sustained across the day in parallel with raising air temperature. Therefore, we surmise that temperature drives the emission of (–)- α -pinene from the forest canopy, resulting in higher ratios relative to (+)- α -pinene in the afternoon when air temperature is higher. The (–)- α -pinene absolute concentration is then higher during the dry season for the same reason. The results presented here show that in the samples taken from the ATTO tower, 80 m is the lowest height that is primarily impacted by canopy emissions. Above 80 m, photochemical processes and air mixing reduce the absolute concentrations of (–)- α -pinene and (+)- α -pinene but these processes do not affect their relative ratio. As

both compounds were tested to react in the same way the observed relative loss rates here calculated are simply an effect of more forest influence on the air above 80 m: therefore no absolute loss of (+)- α -pinene occurred, and more (–)- α -pinene is released by the regional forest. When air temperature is cooler (nighttime and early morning) therefore when temperature is not the only driver of VOCs canopy emissions, other sources may dominate over canopy emissions. All air samples collected at 40 m and at 80 m during nighttime and early morning showed larger concentrations of (+)- α -pinene than (–)- α -pinene, which indicates that: (i) there was a large local source of (+)- α -pinene, (ii) daytime air mixing and rising temperature driven emissions determined the diel profile of the chiral ratio observed at 80 m, with the (+)- α -pinene concentration being larger during nighttime and early morning and the (–)- α -pinene concentration larger during the day. Potential local terpene sources including the forest floor, plants abiotic and biotic stresses and fauna emissions were taken into account. Understory sampling as well as previously conducted soil sampling at ATTO³ showed that the forest floor and soil are not large monoterpenes emitters, therefore they cannot explain the large variation reported with our vertical profile. Plants stresses, including mechanical wounding and fungal infection³⁹ can induce plants to release (+)- α -pinene in large concentrations, and tracers of these processes varied consistently with (+)- α -pinene at and above 80 m, in both seasons. However, the same tracers were not detected in higher concentration below 80 m, therefore it is unlikely that these processes can explain the large concentration and diel profile of (+)- α -pinene reported at 40 m. Therefore, we speculate that a further source, namely insects, that is not directly related or synchronous with canopy emissions affects the sampling at 40 m from the ATTO tower. Due to the tower construction, surrounding the tower and at the border with the path leading to the tower, there are fast growing plants (acquisitive plants), which continuously flush new leaves and have a higher N content, more palatable to insects^{40–42}. Induced emissions from plants due to insect wounding and feeding do not necessarily follow the same diel profile and temperature emission profile of the undisturbed canopy^{43,44} whose average fingerprint is what we detected at 320 m. Instead, such induced emissions can be local and stochastic, therefore not easy to spot in the field⁴⁵. Chiral pheromones have been extensively studied over a variety of insect species and animals, including beetles, moths, pollinators, and elephants; however, little has been reported about chiral terpenes. Lindström and Norin⁴⁶, and Valterova et al.⁴⁷, measured the chiral terpenes emitted by *Nasutitermes* termites soldiers as defensive secretions. They found that the chiral ratio of the emissions was inter and intraspecific and that (+)- α -pinene was produced in remarkably high enantiomeric purity by two subfamilies of the species. Interestingly, we found from samples collected at the ATTO site in October 2019 above two termites' nests that termites can overturn the ambient chiral ratio of α -pinene. Further sampling on isolated species will permit estimation of the emission rates of BVOC from termites and to assess their significance with respect to canopy emission. It can also elucidate whether the chiral inversion we see is due to the insects direct emissions or insect-plant interaction emissions or a combined effect of both. Termites have been studied for methane emissions⁴⁸ and PAHs⁴⁹, but little is known about other VOC emissions and in particular chiral VOCs. Here we show that they are potentially strong enough to overturn the ambient chiral ratio. They may have a significant impact on the local ecosystem since plants and animals rely on VOC odors for signaling and communication. Most importantly, from the chiral ratio we may deduce that the size of this additional local emission must be very large. If we assume that the regional canopy emission signature gives a pinene chiral ratio of

10(−):1(+) (as measured at 320 m) then in order to bring the ratio to 1:1 an emission rate greater than that of the local canopy is required, even if the emission is enantiomerically pure in (+)- α -pinene. To be more quantitative an approximate calculation of the flux rate has been made assuming the termite nest behaves as an enclosure. An emission rate for (+)- α -pinene can be calculated considering the VMR of the samples representing the nest and the VMR of the samples representing background air, and using the mass of termites per nest as reported in Pequeño et al.⁵⁰. Upscaling this value using the nest population per hectare in the Amazon rainforest reported by Martius et al.⁵¹, gives an emission rate of 0.05 $\mu\text{gC g}^{-1} \text{h}^{-1}$. Simultaneous measurements of total monoterpenes and speciated monoterpenes during the wet season campaign of 2018 from the ATTO tower have shown that α -pinene constitutes ~80% of the sum of monoterpenes. Figure 5 shows that (+)- α -pinene is about 10–20% of the total α -pinene. Guenther et al.⁵² reported a 0.4 $\mu\text{gC g}^{-1} \text{h}^{-1}$ emission rate for total monoterpenes from rainforests, which corresponds, with the considerations above to a 0.06 $\mu\text{gC g}^{-1} \text{h}^{-1}$ emission rate of (+)- α -pinene from the rainforest. Our estimate from Amazonian termite nests therefore becomes important when compared to such a value. It should be emphasized that this result is approximate and further in-situ measurements of termite nests and isolated termites are required. As many atmospheric processes are determined by VOC emissions, including OH radical lifetimes and SOA formation, it is important to characterize animal related emissions in forests. These chiral measurements show that insects are an understudied and underestimated source of VOC. Termites are essential for plant turnover in tropical forests as they can recycle globally up to 1/3 of all existing biomass⁵³. Their population depends mostly on desiccation in modified forests⁵⁴, increases with deforestation⁵⁵ and works to mitigate drought⁵⁶, therefore we can expect the population to increase significantly in Amazonia with continued deforestation and climate warming.

In conclusion we report, to our knowledge for the first time, atmospheric mixing ratios of chirally speciated α -pinene from two seasons at different sampling heights above the tropical rainforest at the ATTO field site. The measured chiral ratio of α -pinene has an unexpected vertical, and diel variability; which is primarily temperature dependent at the ecosystem scale. The chiral ratio inverts between 40 and 80 m and further increases until 320 m; the 40 m air samples being dominated by the (+) optical isomer, and 80 m and above dominated by the (−) optical isomer. We investigated the processes that can potentially lead to the observed inversion of the chiral ratio, and excluded: reaction with a chiral oxidant/Criegee, uptake to chiral aerosols, selective photolysis, or chirally selective reaction with OH or O₃ are taking place. Other processes, such as mechanical damage stress and insects related emissions are suggested to be potential causes. These emissions must be greater than the emissions from the local canopy since they effect a reversal in the local (−) dominated chiral ratio. Current emission models consider one temperature parametrization as common to the chiral pair, however, we show here that each optical isomer has its own specific temperature dependency at the ecosystem scale, and some distinct specific sources. Therefore, in order to model monoterpene emissions accurately now and under future warmer drier conditions it is necessary to make chirally speciated measurements and to use them to implement separate enantiomeric emission inventories.

Methods

Air samples. Air samples were collected from the ATTO tower on sorbent tubes using custom-built automatic samplers¹⁵ installed at four different heights on the tower: 40 m (top of the canopy), 80, 150, and 320 m (Fig. 1). Simultaneous sampling across all the heights was programmed to occur at the same time of the day, every 3 h, eight times a day, for the whole campaign time (10 days during the dry

season, 15 days during the wet season). The sampling flow was held constant at 200 ml/min and the sampling time varied according to the season and ambient concentration of VOCs (10 min dry season, 15 min wet season). A quartz filter impregnated with a solution of 10% w/w sodium thiosulfate prepared in the laboratory was used to scrub ozone upstream the sampling flow. We used custom-built stainless steel cartridges (Silcosteel1 (Restek (USA) 89 × 5.33 mm I.D.) containing two sorbent beds composed of 130 mg of Carbograph 1 followed by 130 mg of Carbograph 5. The size of the Carbograph particles was in the range of 20–40 mesh. Carbographs 1 and 5 were provided by Lara s.r.l. (Rome, Italy), see Kesselmeier et al.⁵⁷ for more details. Samples and blanks were stored at room temperature in air conditioned containers, transported to the laboratory and analysed within three months. The analysis was performed in the laboratory with a Thermodesorption Gas Chromatograph system equipped with a Time of Flight Mass Spectrometer (TD-GC-TOF-MS, Bench ToF Tandem Ionisation from Markes International, UK). The TD-GC-TOF-MS first desorbs the analytes from the sampling tubes in two sequential stages, both performed at 250 °C for 10 min. The desorbed analytes are swept in a flow of He into the separating column housed in the gas chromatograph. The column is a dimethyl TBS β -cyclodextrin-based column (0.15 μm , 0.15 mm ID, 25 m L, from MEGA, Italy), which separates the analytes according to volatility and enantiomeric configuration (Supplementary Fig. 2). The separation method was specifically designed for the separation of chiral monoterpenes (C₁₀H₁₆) and sesquiterpenes (C₁₅H₂₄) and consists of an initial 5 min when the oven temperature was held at 40 °C, after which it was increased at a rate of 1.5 °C/min from 40 °C to 150 °C. Finally, the temperature was increased further at a rate of 30 °C/min from 150 °C to 200 °C. A chiral column is therefore required to separate the two enantiomers, standard columns for GC-MS and online PTR-MS do not allow enantiomeric separation. Detection was performed by a Time of Flight Mass Spectrometer, which fragments the analytes through electron impact ionization at −70 eV for quantification and identification of the chemical species. Identification of the main chemical compounds was obtained by comparing the MS spectra with the MS library for the same ionization energy (NIST library), by injection of a gas standard mixture (162 VOCs provided by Apel Riemer, USA) and by use of liquid standards. Chromatogram peak areas were integrated through TOF-DS software provided with the instrument and custom-built IDL software for peak integration⁵⁸. The limit of detection (LOD) of the instrument was quantified as three times the standard deviation of the measured signal within the retention time window where the compounds is eluting of measured blank cartridges. Blank cartridges were prepared by using a dilution system for filling cleaned cartridges with a standard gas (no flow in case of the blanks) diluted in synthetic air. The standard gas used is a certified concentrated mixture of 162 non-methane hydrocarbons including three couples of chiral terpenes (α -pinene, β -pinene, limonene). For the three couples the LOD estimated ranges between 0.1–4 pptv (α -pinene LOD = 2 pptv), therefore on average the LOD is ~1 pptv. Such values were confirmed by using standard cartridges with concentration used for determining the calibration curve. The analysis of the samples was performed in a workflow whereas every five samples analysed a standard cartridge (concentration of α -pinene ~0.3 ppbv) was also measured. The standard cartridges analysed in this way were used to determine the precision of the analysis (including any drift from the mass spectrometer) which was quantified as 22%. The accuracy of the analysis depends mainly on the uncertainty of the concentration of the standard used, therefore 5%. The deviation from the species calibration curve of the measured standard cartridges was within the 5%. The total uncertainty, here used as instrumental error, as propagation of calibration standard certified uncertainty (5%), uncertainties on dilution and sampling system used for preparing the standard cartridges (MFCs = 2% and 5%) and precision of the measured standards (22%) is 23%.

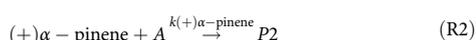
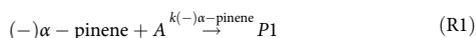
Termites sampling. Air samples were collected from two arboreal termites nests built next to the tower during September 2019. Two nests were identified with the help of a local and a small opening was made to confirm that they were populated. One nest was built on a tree that had no apparent damage, while the second nest was built on a tree that was broken, possibly by a lightning, and rotten. Their shape was hemi elliptic and their size was measured. A PTFE (1/4"OD) sampling line equipped with an ozone scrubber was placed outside the nests and samples were collected automatically on sampling tubes. The same material and analysis as for air sampling was used: same custom-built automatic sampler, same sampling tubes, same sampling flow (200 ml/min) and same samples volume (~2 L). Samples were collected every hour for 3 days, for a total of 75 samples. The same inlet was moved to an area apparently not affected by any source to take some background forest samples. At the end of the pilot experiment, nests were checked to be still populated and a few exemplars of termites were collected.

Filter samples. Aerosol filter samples were collected at various heights of the ATTO tower (40, 150, and 320 m) during the wet and dry season. The filter material was pure borosilicate glass bonded with PTFE (Pallflex® Emfab). The sampling time was roughly 12 h to ensure sufficient aerosol mass on the filter and to distinguish between night- and daytime. A preseparator (PM 2.5) was used prior to the filter holder to exclude aerosol particles with an aerodynamic diameter larger than 2.5 μm like fungi, spores, and pollen. The sampling flow was held constant at

38 L/min. Aerosol samples and blank filters were stored in a freezer before the analysis.

Stomatal conductance measurements. A diurnal course of leaf stomatal conductance (g_s) was performed during 6:00–18:00 during the dry season (November 2017). The g_s of leaves from four individual upper-canopy trees around the INSTANT tower was measured using a leaf porometer (SC-1 Leaf Porometer, Decagon, Pullman, WA). Thirty-nine leaves per tree ($n = 156$ leaves) were randomly chosen and tagged comprising three different regions of the crown in a way to better represent it. At every hour, a leaf from each tree crown region was measured, enabling to measure three leaves per hour for each tree. To avoid overestimations of g_s by the instrument due to the presence of water on the leaf surface (dew) in the early morning, leaves were all dried before each measurement using a piece of paper. Mean hourly values of g_s were calculated per tree. Lastly, mean vegetation values of g_s were calculated per hour.

Loss rate calculation. To determine the relative depletion rate of the chiral pair we considered the same relative approach used for determine rate constants of gas phase reactions based on kinetics analysis. Therefore, we assume that the two competitive reactions exist:



Where A competes with the chiral pair to lead P1 and P2, the respective products of the reactions R1 and R2. The chiral pair relative loss in time is then expressed as in Eqs. (1) and (2), whose ratio rearranged between the sampling heights gives Eq. (3):

$$\frac{-d[(-)\alpha\text{-pinene}]}{dt} = k[(-)\alpha\text{-pinene}] \times [A] \quad (1)$$

$$\frac{-d[(+)\alpha\text{-pinene}]}{dt} = k[(+)\alpha\text{-pinene}] \times [A] \quad (2)$$

$$\ln \frac{[(-)\alpha\text{-pinene}]_{80m}}{[(-)\alpha\text{-pinene}]_{320m}} = \frac{k_{(-)\alpha\text{-pinene}}}{k_{(+)\alpha\text{-pinene}}} \cdot \ln \frac{[(+)\alpha\text{-pinene}]_{80m}}{[(+)\alpha\text{-pinene}]_{320m}} \quad (3)$$

Chiral-selective sink. A small glass flow reactor was used to test reactions with α -pinene, with OH and ozone. Such reactor includes one arm to inject the compound of reference (α -pinene diluted in synthetic air), one arm where a Pen-ray Hg UV lamp is installed, one arm where dry/wet N_2 can be injected and outlets for pump and sampling/measuring devices (adapted from Sinha et al., 2008²¹). A known concentration (~1ppbv) of α -pinene from a certified racemic mixture diluted in synthetic air and dry N_2 was injected into the reactor and its concentration measured at the reactor outlet. Secondly, we generated O_3 in the reactor through photolysis of synthetic air by the UV lamp, and measured α -pinene at the reactor outlet. Lastly, we generated OH inside the reactor via photolysis of water vapor from the wet N_2 flow and measured α -pinene at the reactor outlet. Tubes with the resulting mixtures were collected at each sequential experimental stage at the reactor outlet in the same fashion as we did in the field. Therefore, same conditions and materials were used, including: same sampling flow and volume, same sorbent tubes, same procedure (O_3 scrubber upstream the sampling flow), and same off-line analysis.

For testing any uptake onto particles effect that may have happened, we considered blank and sampled (24 h) aerosol filters collected at 40 m and 320 m during the same days of VOC sampling in the dry season 2017. A small part of the filter (1/16) was cut and placed in a filter holder (as those used for holding the O_3 scrubber during VOC sampling) and installed downstream a diluted flow of the racemic α -pinene (~1ppbv). All lines and the filter holder were heated up to 25 °C and the α -pinene flow was diluted in 100% humidified synthetic air, to resemble conditions of temperature and humidity in the field. Air downstream the filter was collected on sorbent tubes at different stages: without filter, with blank filter, and with sampled (40 and 320 m) aerosols filters. Again, same conditions of sampling and analysis as for the field samples were used.

Data availability

Datasets reported in this manuscript are available at the ATTO project data repository at: <https://www.attodata.org/>. Dry and wet seasons datasets of (-)- α -pinene and (+)- α -pinene are accessible at: <https://doi.org/10.17871/atto.150.5.558>, <https://doi.org/10.17871/atto.151.3.559>, <https://doi.org/10.17871/atto.139.4.561>, <https://doi.org/10.17871/atto.143.5.560>.

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Author contributions

N.Z. conducted the sampling and analysis of VOCs, interpreted the results, and wrote the manuscript. D.L. helped sampling VOCs and conducted the filter sampling. P.A. measured the stomatal conductance. M.S. and A.A. provided the meteorological data. J.W. conceived and supervised the project. N.Z., D.L., P.A., T.H., and J.W. helped in the scientific discussion and revised the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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