Supplement of Biogeosciences, 18, 4873–4887, 2021
https://doi.org/10.5194/bg-18-4873-2021-supplement

Biogeosciences Open Access EGU

Supplement of

Bioaerosols in the Amazon rain forest: temporal variations and vertical profiles of Eukarya, Bacteria, and Archaea

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S1 Supporting Text

S1.1 Measurement location: The Amazon Tall Tower Observatory

Aerosol sampling and measurements were conducted at the Amazon Tall Tower Observatory (ATTO, 2°08.602′ S, 59°00.033′ W, 130 m.s.l.), a research site in the Amazonian tropical rainforest, 150 km northeast of the city of Manaus, Brazil (Andreae et al., 2015). Two 80 m towers, a tall tower of 325 m height and several containers at ground level are equipped with various instruments monitoring e.g. greenhouse gases and aerosols. A detailed site description and instrumental setup is presented in (Andreae et al., 2015). The Amazon rain forest is characterized by a pronounced seasonality. During the wet season, from February to May, conditions at this location can be described as near-pristine. At that time, winds are coming from northeast. Thus, air masses are traveling across large areas of undisturbed terra firme forest before reaching the site (Pöhlker et al., 2019). The map in Figure S1 displays the location of the ATTO site and 3-day backward trajectories. The trajectories prove, that typical wet season conditions prevailed during the FISH-sampling period. Air masses mainly traveled from northeastern direction across untouched rain forest towards the site. Moreover, the modelled precipitation rates show several strong rain events above the forest leading to particle scavenging. Consequently, the air masses which were sampled at ATTO in February and March 2018, are assumed to be unaffected by any anthropogenic activities. Moreover, dust plumes that originate in Africa typically during the wet season and influence aerosol mixtures and properties in the Amazon basin, did not reach the site at that time (Swap et al., 1992; Moran-Zuloaga et al., 2018). Accordingly, during the wet season FISH sampling, conditions are temporarily referred to as clean, green ocean conditions, characterized by natural biosphere atmosphere exchange only. As visualized in the time series (Figure S2), precipitation, relative humidity, and temperature during sampling conform to the mean values calculated for the past 36 years. A medium strong La Niña period apparently did not have major impact on climatic conditions in the ATTO foot print region.

S1.2 Bioaerosol emission in the Amazon

Bioaerosols are defined as liquid or solid airborne particles of biological origin, which were released directly from their sources into the air (Després et al., 2012). The Amazon rain forest is thought to be the most species-rich freshwater and terrestrial ecosystem in the world. Consequently, there are manifold potential bioaerosol sources including water and soil surfaces, animals, plants, microbial surface communities, and decaying biomaterial. (Artaxo et al., 1990; Fröhlich-Nowoisky et al., 2016; Löbs et al., 2020). Their emission can be effected by means of active processes (e.g. fungal spore release based on osmotic pressure changes; Pringle et al., 2005; Trail et al., 2005; Elbert et al., 2007) or passive mechanisms (e.g. air currents or rain splash; Jones and Harrison, 2004; Joung et al., 2017). Bioaerosol emission rates in the Amazon are therefore dependent on factors such as relative humidity and precipitation, air temperature and wind speed (Fröhlich-Nowoisky et al., 2016, and references therein). Previous studies investigated bioaerosols in tropical (and boreal) rainforests by use of various instrumentations and analysis techniques. The obtained number concentrations vary according to the different detection methods and sampling location (Table S3; Šantl-Temkiv et al., 2019, Artaxo 2020, under revision).

S1.3 Bioaerosols' role in the Amazon rain forest

From an ecological point of view, bioaerosols play an essential role in the reproduction and biogeographic distribution within the Amazonian ecosystem. Moreover, they are supposed to influence its hydrological cycle by acting as ice nuclei (IN) or giant cloud condensation nuclei (GCCN; Pöschl et al., 2010; Tobo et al., 2013; Artaxo, 2020, under revision). Still, the effect aerosols in general and bioaerosols in particular have on regional and global atmospheric processes is still under discussion and one of the major uncertainties in understanding the climate system (Ariya et al., 2009; Pöschl et al., 2010). Previous studies reported bioaerosol concentrations to range between ~10^4 and ~10^6 m^{-3} accounting for the majority of coarse mode

aerosols in the Amazon under pristine conditions (Table S3; Graham et al., 2003; Huffman et al., 2012; Whitehead et al., 2016; Moran-Zuloaga et al., 2018). Exhibiting strong diurnal cycles, fungal spores were reported to be the most frequent bioaerosol type (Elbert et al., 2007; Souza et al., 2019). Bacteria in contrast, are found to be released in lower concentrations (in the understory; Souza et al., 2019). As IN particle abundance was suggested as limiting factor for cloud ice formation in the Amazon, bioaerosol number concentrations and especially their vertical diffusion are highly important to advance modelling studies (Pöschl et al., 2010). Thus, quantification and identification of bioaerosol abundances and cycling in the Amazon is key to shed light on their role in the pre-industrial biosphere-atmosphere interaction.

S1.4 Bioaerosol analysis methods

Environmental bioaerosol populations comprise highly complex and diverse particle mixtures. As a consequence, the choice of analysis method is not trivial and has to be made carefully to avoid biases caused by e.g., differences in particle size, metabolic state, or physical and chemical properties. A long time, cultivation was the method of choice for bioaerosol analysis. Since less than 1 % of all bioaerosols are assumed to be culturable, this technique was more and more superseded by new analytical methods based on DNA analysis or real-time autofluorescence detection (Amann et al., 1995; King et al., 2020). To find the most suitable analysis technique, the main investigation target has to be defined first. We suggest the categorization into three objectives: 1. quantification, 2. identification, and 3. qualitative analysis of bioaerosols. Online autofluorescence detectors such as the Wideband Integrated Bioaerosol Sensor (WIBS, Droplet Measurement Technologies) and Spectral Intensity Bioaerosol Sensor (SIBS, Droplet Measurement Technologies) are especially useful for long-term quantification of bioaerosols, as no time-consuming laboratory sample analysis is needed (Könemann et al., 2019; Huffman et al., 2020). Furthermore, data is generated in comparably high time resolution. Nevertheless, data provided by these detectors has to be evaluated

carefully, as bioaerosol number concentrations based on autofluorescence detection are prone to biases caused by bioaerosols' diverse autofluorescence intensities and wavelength range as well as interferences with autofluorescence from inorganic aerosols (e.g., Pöschl et al., 2010; Huffman et al., 2010; Savage and Huffman, 2018). In contrast, methods based on DNA analysis are focused on the specific identification of bioaerosols. By now, a broad range of tools are used, such as metagenome sequencing, metabarcoding, rRNA sequencing, or qPCR. The different techniques have in common that the taxonomic resolution is determined by the choice of the target sequence. Beyond taxonomic classification, microbial abundances can be quantified indirectly, e.g., by calculating microbial cell numbers out of detected gene copy numbers. These calculations are statistically robust, as DNA analysis requires high load of biological sample material on one hand, and automated high-throughput instruments enable a quick analysis of large sample numbers on the other hand. However, they can be biased by multiple copy numbers of marker genes. With the microscopic analysis of FISH, we combine the quantification and identification with a qualitative analysis leading to the following advantages: i) The quantification is based on direct enumeration of fluorescent cell signals and therefore assumed to be very accurate. ii) A countercheck with DAPI staining provides additional safety. iii) Particle loss during laboratory analysis is assumed to be minimized, as bioaerosols are identified directly on the filter as collection medium. In contrast, for qPCR or flow cytometric analysis a re-suspension into liquid and a cell concentration is required after bioaerosol filtration, which enhances the chance of particle loss, especially in terms of charged and/or hydrophobic bioaerosols such as certain molds (Lin et al., 1999). Bioaerosol collection directly into liquid (e.g., impingement) could solve this issue. However, for longterm sample collections as conducted for this study varying collection efficiency due to liquid evaporation over time and therefore changes in chemical composition (e.g., pH or fixative concentration)

as well as microbial growth within the liquid can play a role and have to be considered (Lin et al., 1997).

The microscopic analysis of FISH treated samples also bears drawbacks such as the time consuming manual enumeration of fluorescent single particles. Here, automated image generation and software based particle detection or sample analysis with flow cytometry could improve the analysis by speeding up the process. The application of these two techniques is dependent on the careful characterization of sample's properties such as aerosol mixing state and diversity, sample purity or abundance of interfering materials.

Generally, it is important to note that the large bioaerosol diversity imposes significant analytical challenges in terms of sound bioaerosol analysis. There is no general "method of choice" for bioaerosol analysis, but various different approaches, that have advantages and drawbacks. Accordingly, number and mass concentrations derived from different measurement techniques and sampling locations are comparable only within certain limits and similarities as well as deviations have to be evaluated carefully (see Table S3). We suppose that FISH, which was considered before but never established for environmental bioaerosol investigations, can advance the range of tools and techniques by combining the three major goals that are identification, enumerations and qualitative analysis.

S1.5 Airborne DNA Mass

Using number concentrations obtained by FISH, airborne DNA mass could be calculated. The factor 609.7 g mol^{-1} included in the equation is the average mass of a base pair in bound form:

$$m_{abp} = \frac{(m_{dAMP} + m_{dCMP} + m_{dGMP} + m_{dTMP})}{2} - (2 \cdot m_{H_2O})$$

$$= \frac{(331.22\,\frac{g}{mol} + 307.2\,\frac{g}{mol} + 347.22\,\frac{g}{mol} + 306.2\,\frac{g}{mol})}{2} - 2 \cdot 18\,\frac{g}{mol} = 609.7\,\frac{g}{mol}$$

m_{abp} = average mass of base pair in bound form

At 5 m height, 11.8 ± 4.63 ng m^{-3} (11.6 ± 4.53 ng m^{-3} eukaryotic and 0.29 ± 0.09 ng m^{-3} bacterial), at 60 m, 4.49 ± 1.14 ng m^{-3} (4.17 ± 0.99 ng m^{-3} eukaryotic, 0.26 ± 0.10 ng m^{-3} bacterial), and at 325 m, 1.20 ± 0.44 ng m^{-3} (1.07 ± 0.38 ng m^{-3} eukaryotic, and 0.12 ± 0.05 ng m^{-3} bacterial) DNA was calculated (Table S2). As explained before, archaeal numbers suffer from insufficient statistics and so do the respective calculated DNA masses. As a result, the standard deviation exceeds the mean archaeal number concentration.

As described in the article, these numbers are similar to DNA concentration found in other forested ecosystems. Nevertheless, Helin et al. (2017) found also samples with up to 48 ng m^{-3}, more than 4 times higher than measured in the Amazon during this project. The reason behind this is twofold: The boreal forest, which Helin et al. (2017) analyzed, mainly consists of pines and other vascular plants. As a result, bioaerosols are expected to include pollen with comparably big genome sizes. Furthermore, these pollen are emitted during a fairly short time period, specifically in spring, causing a temporal peak in bioaerosol load and consequently, in DNA concentration. When averaging DNA measurements in the boreal forest, Helin et al. (2017) found 8.60 ± 11.41 ng m^{-3}, which is well comparable to the DNA concentrations we calculated for the Amazon forest.

S2 Supplementary Figures and Tables

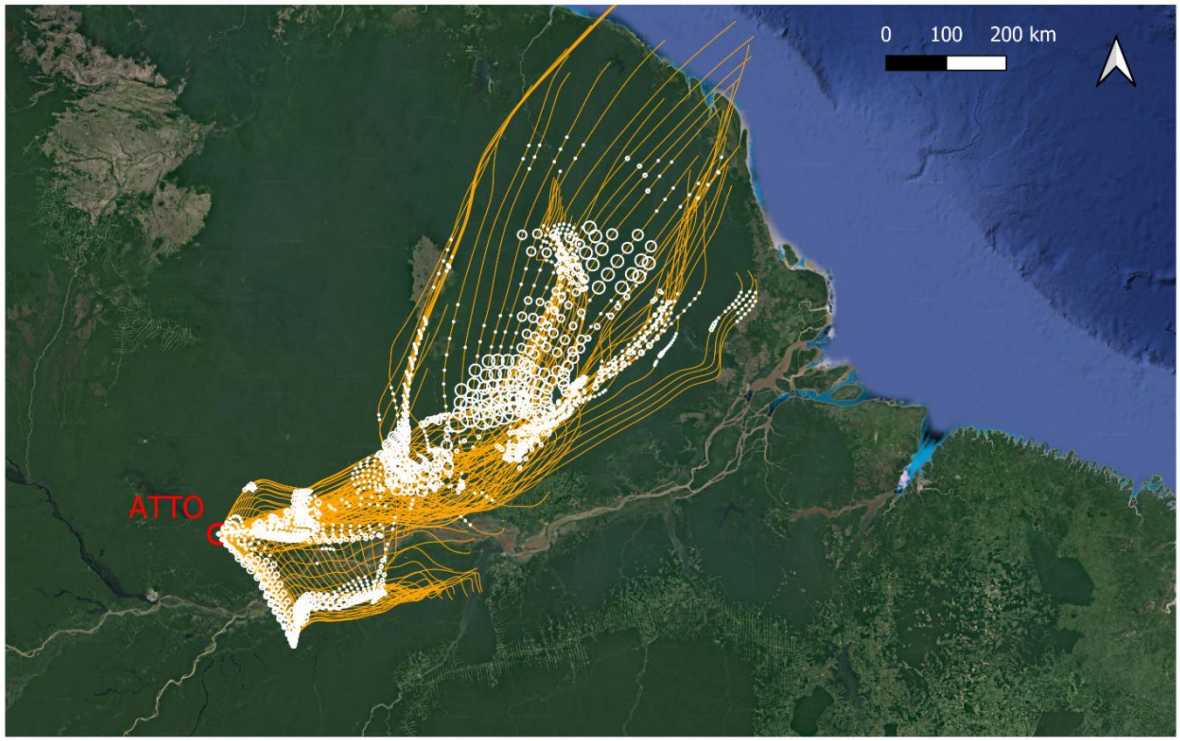

Figure S1. Map showing location of the ATTO site with ensemble of 3-day backward trajectories (yellow lines) using the Hybrid Single-Particle Lagrangian Integrated Trajectory model (HYSPLIT, NOAA-ARL) with meteorological input data from the Global Data Assimilation System (GDAS1, 1° resolution). Trajectories were started at 200 m above ground every hour during the sampling period of this study. White circles represent precipitation obtained through GDAS (HYSPLIT model) for every hourly data point of the individual trajectories. The size of white circles shows the extent of precipitation en route (ranging from 0 to ~11 mm). The map shows that the trajectories during the sampling period mostly moved over the relatively untouched rain forest areas north of the Amazon River with some direct influence from the Amazon River valley itself. Precipitation along the trajectories shows that the transported air masses experienced relatively strong rain-related scavenging.

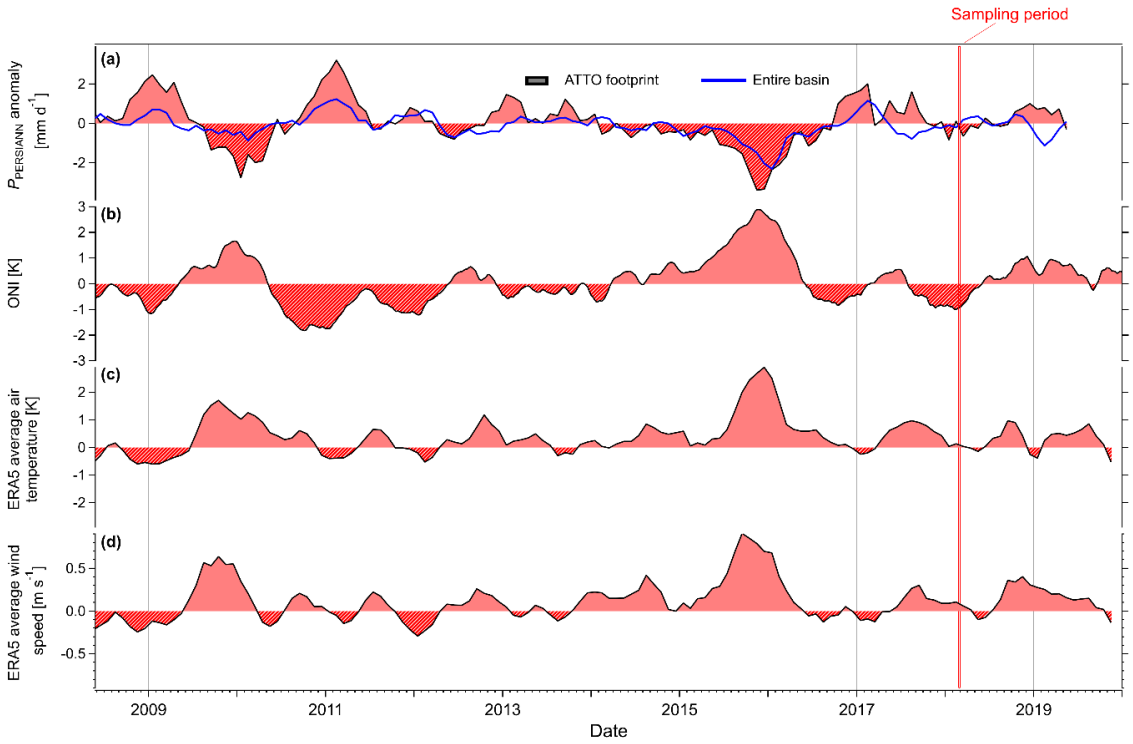

Figure S2. Time series of anomalies in precipitation, air temperature, and wind speed including the sampling period to characterize the overall atmospheric conditions. The analysis was conducted in close relation to the study by Pöhlker et al. (2019), where detailed information can be found. (a) Anomaly in precipitation rate P (from the Precipitation Estimation from Remotely Sensed Information using the Artificial Neural Networks for Climate Data Record, PERSIANN-CDR, data product, reference time frame 1983-01-01 to 2019-12-31) for two regions: (i) the core region of the ATTO footprint (i.e., contour line with largest 0.5 % of air mass residence times) and (ii) for the entire Amazon watershed region (blue line). (b) Oceanic Niño index (ONI) for comparison with precipitation anomaly (reference time frame 1982 to 2019), representing Pacific sea surface temperature variability and indicating El Niño vs. La Niña periods (i.e., El Niño influence is very strong for ONI > 2.0, strong for 2.0 > ONI > 1.5, medium for 1.5 > ONI > 1.0, and weak for ONI > 1.0. La Niña influence is strong for -2.0 < ONI < -1.5, medium for -1.5 < ONI < -1.0, and weak for ONI > -1.0.). (c) Anomaly in daily averaged air temperature (2 m height) from ERA5 reanalysis product (reference time frame 1979-01-02 to 2019-12-31). (d) Anomaly in daily averaged wind speed (10 m height) from ERA5 reanalysis product (reference time frame 1979-01-02 to 2019-12-31).

Table S1: Particle number concentrations obtained by FISH, relative fractions and raw counts (number of fluorescent signals identified under the microscope) for bioaerosols hybridized with a FISH probe or stained with DAPI. Presented are numbers from samples collected at three heights at 5 or 6 consecutive days during approximately 23 h sampling time per day.

height	day	Archaea [$\cdot 10^4$ m^{-3}]	A/DAPI [%]	particle counts	Bacteria [$\cdot 10^4$ m^{-3}]	B/DAPI [%]	particle counts	Eukarya [$\cdot 10^4$ m^{-3}]	E/DAPI [%]	particle counts	DAPI [$\cdot 10^4$ m^{-3}]	particle counts	probe/ DAPI
5 m	1	0.35 ± 0.52	0.7	7 / 987	2.5 ± 1.9	4.6	50 / 999	49 ± 33	91	1041 / 1284	54 ± 14	1864	96
	2	0.31 ± 0.44	0.6	9 / 1272	7.5 ± 2.1	15	236 / 1142	25 ± 2.9	51	1151 / 1628	50 ± 29	4395	67
	3	0.19 ± 0.32	0.3	6 / 1644	14.3 ± 2.3	24	455 / 1660	36 ± 2.7	61	1341 / 1914	59 ± 15	1971	85
	4	0.22 ± 0.37	0.4	7 / 1510	5.6 ± 1.7	10	168 / 1539	41 ± 18	75	1039 / 1341	54 ± 19	5663	86
	5	0.15 ± 0.24	0.3	3 / 979	5.3 ± 2.7	11	196 / 2076	40 ± 19	82	1403 / 1687	48 ± 29	6522	94
	mean (1-5)	0.25 ± 0.38	0.5		7.0 ± 2.1	13		38 ± 15	72		53 ± 21		86
60 m	1	1.02 ± 1.08	4.0	39 / 867	2.4 ± 2.2	9.3	67 / 714	13 ± 2.9	49	688 / 1031	26 ± 15	1968	62
	2	0.38 ± 0.48	2.4	32 / 1032	2.3 ± 1.0	15	157 / 986	10 ± 2.1	62	653 / 920	16 ± 8.2	1788	79
	3	0.52 ± 0.76	2.1	25 / 1233	5.4 ± 1.9	22	321 / 1328	13 ± 2.6	53	578 / 987	24 ± 7.7	1926	77
	4	2.30 ± 2.09	11	112 / 1069	7.7 ± 2.2	38	330 / 902	11 ± 3.5	56	434 / 815	20 ± 8.0	2290	105
	5	3.14 ± 2.43	12	120 / 1031	6.3 ± 3.2	24	352 / 1430	18 ± 2.7	66	995 / 1384	27 ± 9.4	1598	101
	6	0.21 ± 0.43	0.5	4 / 751	14.9 ± 4.8	39	347 / 890	18 ± 5.7	48	497 / 1032	38 ± 14	958	87
	mean (1-6)	1.26 ± 1.21	5.3		6.5 ± 2.5	24		14 ± 3.3	56		25 ± 10		85
325 m	1	0.11 ± 0.26	1.5	6 / 361	0.4 ± 0.6	6.1	24 / 329	3.3 ± 0.8	47	126 / 267	7.0 ± 2.2	672	54
	2	0.02 ± 0.07	0.4	2 / 649	2.6 ± 1.2	54	243 / 459	1.4 ± 0.7	29	87 / 284	4.8 ± 2.0	638	83
	3	0.09 ± 0.24	0.9	5 / 630	4.1 ± 1.6	39	255 / 674	4.6 ± 1.6	44	198 / 427	10 ± 3.0	688	85
	4	0.09 ± 0.25	0.4	5 / 1018	6.1 ± 2.2	25	405 / 1317	5.1 ± 1.9	21	188 / 716	24 ± 12	2503	46
	5	0.21 ± 0.24	1.6	11 / 667	1.7 ± 1.1	13	96 / 785	3.3 ± 1.3	25	158 / 651	13 ± 3.7	1058	39
	mean (1-5)	0.10 ± 0.21	0.9		3.0 ± 1.3	27		3.5 ± 1.2	33		12 ± 4.6		61

Table S2: Diel airborne DNA mass concentration calculated for bioaerosol classes at different heights. Mean bioaerosol number concentrations obtained by FISH were multiplied with the calculated mean DNA mass per cell.

sampling height	Archaea mean ± sdev [ng m^{-3}]	Bacteria mean ± sdev [ng m^{-3}]	Eukarya mean ± sdev [ng m^{-3}]
5 m	0.01 ± 0.02	0.29 ± 0.09	11.6 ± 4.53
60 m	0.05 ± 0.05	0.26 ± 0.10	4.17 ± 0.99
325 m	0.00 ± 0.01	0.12 ± 0.05	1.07 ± 0.38

Table S3: Overview table of bioaerosol number and mass concentrations found in tropical and boreal forest systems.

Campaign, location, year	Particle size [µm]	Instrument, technique	Season, time period, sampling conditions	N_F or N_{PBAP} [m^{-3}]	N_T [m^{-3}]	N_F / N_T [%]	N_{PBA} / N_T [%]	M_F [µg m^{-3}]	M_T [µg m^{-3}]	Reference
Tropical forests										
CLAIRE 2001, Balbina site, Amazon, Brazil, 2001	2 - 10	gravimetry and light microscopy	transition period (Jul):							Graham et al., 2003
			all particles, campaign av.	--	--	--	--	--	3.9 ± 1.4	
			night, pollen	--	103	--	--	--	--	
			day, pollen	--	1700	--	--	--	--	
			night, fern spores	--	0	--	--	--	--	
			day, fern spores	--	1100	--	--	--	--	
			night, fungi	--	$0.23 \cdot 10^6$	--	--	--	--	
			day, fungi	--	$0.024 \cdot 10^6$	--	--	--	--	
			night, algae	--	0	--	--	--	--	
			day, algae	--	<20	--	--	--	--	
			night, insect fragments	--	0	--	--	--	--	
			day, insect fragments	--	209	--	--	--	--	
AMAZE-08, ZF2 site, Amazon, Brazil, 2008	1 - 10	SEM	wet season	--	--	~40	~80	--	--	Pöschl et al., 2010
ACES-OP3 campaign, Malaysia, Borneo, Jun/Jul 2008	0.8 - 20	WIBS-3	below canopy, day	$(0.05\text{-}0.1)\cdot10^6$	--	--	--	--	--	Gabey et al., 2010
			below canopy, night	$(1\text{-}2.5)\cdot10^6$	--	--	--	--	--	
			below canopy, campaign av.	--	--	55	--	--	--	
			above canopy, day	$(0.05\text{-}0.1)\cdot10^6$	--	--	--	--	--	
			above canopy, night	$(0.2\text{-}0.4)\cdot10^6$	--	--	--	--	--	
			above canopy, campaign av.		--	28	--	--	--	
AMAZE-08, ZF2 site, Amazon, Brazil, 2008	1 - 20	UV-APS	wet season (Feb, Mar):							Huffman et al., 2012
			canopy height, campaign av.	$0.093 \cdot 10^6$	$0.55 \cdot 10^6$	26.3	--	0.85	2.49	
			canopy height, low dust	$0.099 \cdot 10^6$	$0.26 \cdot 10^6$	38.9	--	1.05	1.63	
			canopy height, high dust	$0.080 \cdot 10^6$	$0.93 \cdot 10^6$	14.0	--	0.92	3.89	
BUNIAACIC project, ZF2 tower, Amazon, Brazil, 2013	0.5 - 20	WIBS-3M	transition period (Jul):							Whitehead et al., 2016
			below canopy, day	$0.2 \cdot 10^6$	--	55-75	--	--	--	
			below canopy, night	$1.2 \cdot 10^6$	--	≥90	--	--	--	
			below canopy, campaign av.	$0.4 \pm 0.2 \cdot 10^6$	$0.46\pm0.3*10^6$	~85	--	--	--	
ATTO site, Amazon, Brazil 2014-2017	1 - 10	OPS	wet season, no/low dust	--	$0.42 \pm 0.34\cdot10^6$	--	--	--	$4.25 \pm 2.78 \cdot10^6$	(Moran-Zuloaga et al., 2018)
			wet season, high dust	--	$2.03 \pm 1.87 \cdot10^6$	--	--	--	$12.2 \pm 10.4 \cdot10^6$	
			transition periods	--	$0.81 \pm 0.75 \cdot10^6$	--	--	--	$6.19 \pm 4.62 \cdot10^6$	
			dry season	--	$1.15 \pm 0.81 \cdot10^6$	--	--	--	$8.48 \pm 3.88 \cdot10^6$	
Boreal forests										
SMEAR II station, Hyytiälä, Finland, 2009-2011	1 - 20	UV-APS	spring	$0.015 \pm 0.024\cdot10^6$	$0.43 \pm 0.53 \cdot10^6$	4.4	--	--	--	Schumacher et al., 2013
			summer	$0.046 \pm 0.048\cdot10^6$	$0.45 \pm 0.39 \cdot10^6$	13	--	--	--	
			fall	$0.027 \pm 0.032\cdot10^6$	$0.41 \pm 0.42 \cdot10^6$	9.8	--	--	--	
			winter	$0.004 \pm 0.005\cdot10^6$	$0.47 \pm 0.34 \cdot10^6$	1.1	--	--	--	
SMEAR II station, Hyytiälä , Finland, 2003-2004	<1 - >10	spore trap and impactor, light microscopy	spring 2003/ 2004	--	--	--	--	--	10.07/ 6.04	Manninen et al., 2014
			summer 203/ 2004	--	--	--	--	--	11.07/ 6.88	
			fall 2003/2004	--	--	--	--	--	5.74/ 6.41	
			winter 2003/ 2004	--	--	--	--	--	7.14/ 5.37	
SMEAR II station, Hyytiälä, Finland, 2014	<1->10	impactor, qPCR DNA analysis	Feb-Oct, bacteria	$0.0063 \pm 0.0137 \cdot10^6$	--	--	--	--		Helin et al., 2017
			Feb-Oct, fungi	$0.0102 \pm 0.0156 \cdot10^6$	--	--	--	--		
			all particles, campaign av.	--	--	--	--	--	12.3 ± 12.2	

Table S4: Chemicals and producing companies used for filter sample fixation, hybridization and microscopic visualization.

Chemical	product number	company
Acchromopeptidase	A3547-500KU	Sigma-Aldrich
Agarose LE	840001	Biozym
Blocking reagent	10447200	Roche
Citifluor AF1	17970-100	Citifluor
Diamidino-2-phenylindol-2HCl	18860.02	Serva
Ethanol	K928.5	Roth
Ethylendiaminetetraacetic acid	ED-100g	Sigma-Aldrich
Formaldehyde solution 37%	F8775-500mL	Sigma-Aldrich
Formamide	47671-1L-F	Roth
Lysozyme from chicken egg white	62970-5g-F	Sigma-Aldrich
Phosphate buffered saline 10x	79383-1L	Sigma-Aldrich
Sodium chloride	S7653-250g	Sigma-Aldrich
Sodium dodecyl sulfate	L3771-25g	Sigma-Aldrich
Tris HCl	15568-025	Invitrogen

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