Characterization of free amino acids, bacteria and fungi in size-segregated atmospheric aerosols in boreal forest: seasonal patterns, abundances and size distributions

Aku Helin[1], Outi-Maaria Sietiö[2], Jussi Heinonsalo[2], Jaana Bäck[3], Marja-Liisa Riekkola[1] and Jevgeni Parshintsev[1]

The response to the Referees

We appreciate a lot the work done by the reviewers and wish to express our gratitude to their critical comments. The manuscript definitely benefits from the suggested corrections. Below, we give the detailed answers to the reviewer's suggestions, while text is corrected with track changes in the revised manuscript.

Referee 1

(1) Bioaerosols are emitted directly from the biosphere into the atmosphere (Després et al., 2012;Fröhlich-Nowoisky et al., 2016)". Although this statement is not conceptually wrong, one of the cited articles, i.e. Després et al., 2012 (Després, V.R., Alex Huffman, J., Burrows, S.M., Hoose, C., Safatov, A.S., Buryak, G., Fröhlich-Nowoisky, J., Elbert, W., Andreae, M.O., Pöschl, U., Jaenicke, R., 2012. Pri- mary biological aerosol particles in the atmosphere: a review. Tellus B 64, 1–. doi:10.3402/tellusb.v64i0.15598), recommends the use of the term " primary biological aerosol particles (PBAP)" instead of ". Therefore, I suggest the authors to check the definition presented Després et al., 2012 and consider its revision throughout the article.
(2) Both terms are used in the literature, but we agree with the Referee, that PBAP suits better here.
(3) Terms "Bioaerosols" was corrected to "Primary biological aerosol particles" and abbreviated as PBAP throughout the article.

(1) Throughout the manuscript, the authors mention several times that the sampling period was one year. However, in section 2.2, the sampling period was defined from February to October. Although cover all seasons, there are 3 months missing and the sampling period is in fact only 9 months. This information should be corrected in the manuscript.
(2) We agree.
(3) Corrected throughout the article.

(1) Line 203. The bacterial cells and fungal spores have the exact same concentration levels, or there is some mistake in this sentence?
(2) We reported a wide range of typical concentration levels for both fungal spores and bacterial cells based on the references cited in the text. Therefore, the values presented seem to be identical or in the same order of magnitude, and there is no mistake in the reported concentration ranges. However, we acknowledge that in reality, the concentration levels are seldom similar as mentioned in Line 202. The concentration levels vary depending on the geographical location, meteorological factors etc. Nevertheless, in our opinion, adding additional comments on varying microorganisms' levels is not relevant at this point.
(3) No changes were made to the manuscript.

(1) Line 339. The presence of particles enriched with FAAs from the sea bubble-bursting phenomena?

(2) In principle, it might be possible that there is some enrichment due to bubble-bursting phenomena, although this is not likely the case here. This was not the only sampling period during which the air-masses were passing the Baltic Sea region (e.g. during non-frozen sea periods, Line 391). When taking into account the presented evidence that supports the contribution of pollen, e.g. pragmatically considering that the filters were visibly yellow, it is not convenient to speculate that the vast peak in concentration levels would be caused by bubble-bursting phenomena.
(3) No changes were made or comments added to the manuscript.

(1) Line 342. Barbaro et al., (Barbaro, E., Zangrando, R., Vecchiato, M., Piazza, R., Cairns, W. R. L., Capodaglio, G., Barbante, C. and Gambaro, A.: Free amino acids in Antarctic aerosol: potential markers for the evolution and fate of marine aerosol, Atmos. Chem. Phys., 15(10), 5457–, doi:10.5194/acp-15-5457-2015, 2015) introduces an argument contradictory to that presented in this manuscript. According to Barbaro et al., (2015), the enrichment of aerosol samples in hydrophobic FAAs (e.g., methionine, cysteine and tryptophan) supports the assumption that long-range transport processes, as the different chemical and photochemical events that occur during long-range transport were faster for hydrophilic than for hydrophobic amino acids. Can authors comment on this contradiction?
(2) As far as we could interpret, there is no such contradiction present between our results and the ones presented by Barbaro et al. In their "hydropathy" index classification, only Met is included in hydrophobic amino acids, whereas Trp and Cys are not accounted for. Nowhere in their article is a statement that the presence of Met, Cys and Trp would be indicative of long-range transported aerosols. Oppositely, in another article by the same authors (Scalabrin et al., ACP 2012, doi: 10.5194/acp-12-10453-2012), they emphasize that Met is not typically observed in long-range transported aerosols. We considered that there is enough references in the current version of the manuscript to support our observations.
(3) No changes were made or comments added to the manuscript.

(1) In line 423, the authors state that is relatively strong evidence that cloud-active particles larger than $\sim$1 μm are biological in origin". In my opinion, to be a "strong evidence" needs to be better justified.
(2) We agree that evidence might not be strong.
(3) "Strong" changed to "some".

(1) In conclusion, after reading the Supporting Information, I believe that I understood the estimations made by the authors to reach the percentage of PM that should be of biological origin. However, since the two conversion factors used are subject to high uncertainties, in my opinion, these estimation uncertainties should be emphasized in the text of the manuscript, to avoid misleading the reader.
(2) We agree with the referee. However, detailed explanation on estimation uncertainty does not suit the "conclusion" part. Thus, we emphasized the SI and added "high uncertainty" to the text.
(3) The following sentences were modified: "Elevated PBAP abundances occur during the pollen season (Manninen et al., 2014), and based on our estimation even up to ~77% of total PM may be of biological origin (see SI for details). Even though our estimation is highly uncertain, the magnitude of biological cloud-active particles during this period may be atmospherically relevant (Diehl et al., 2002;Diehl et al., 2001;Pummer et al., 2012;Pummer et al., 2015)."

(1) In Supporting Information, section "Validation experiments and quality control "the authors state that of the amino acid calibration curves were forced through origin". Could you please justify this choice?

(2) The relevance of forcing through the zero was not dramatic on the results due to intercepts being close to zero anyway. However, in practice it was noticed that forcing through zero gave more realistic LOQ value, i.e. higher values than compared to not forcing through zero.
(3) No changes were made to the manuscript

Referee 2

(1) As already pointed out by referee #1, the use of the term primary biological aerosol particles (PBAPs) defined in detail in one of the references (Despres et al., 2012) may be considered instead of bioaerosols. At the end of page S21 in the supplementary information it is already in use without prior definition.
(2) We agree.
(3) Corrected throughout the article.

(1) line 97 word 9: weighted → weighed
(2) We agree.
(3) Corrected.

(1) line 121 end: primers pairs → primer pairs
(2) We agree.
(3) Corrected.

(1) line 586: Ozler → Özler
(2) We agree.
(3) Corrected.

Characterization of free amino acids, bacteria and fungi in size-segregated atmospheric aerosols in boreal forest: seasonal patterns, abundances and size distributions

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Abstract

Primary biological aerosol particles (PBAP)~~Bioaerosols~~ are ubiquitous in the atmosphere and constitute ~30% of atmospheric aerosol particle mass in sizes >1 um. ~~Bioaerosol~~ PBAP components, such as bacteria, fungi and pollen, may affect the climate by acting as could-active particles, thus having an effect on cloud and precipitation formation processes. In this study, size-segregated aerosol samples (<1.0, 1-2.5, 2.5-10 and >10 µm) were collected in boreal forest (Hyytiälä, Finland) during ~~one year~~a nine month period covering all seasons and analyzed for free amino acids (FAAs), DNA concentration and microorganism (bacteria, *Pseudomonas* and fungi). Measurements were performed using tandem mass spectrometry, spectrophotometry and qPCR, respectively. Meteorological parameters and statistical analysis were used to study their atmospheric implication for results. Distinct annual patterns of PBAP~~bioaerosol~~ components were observed, late spring and autumn being seasons of dominant occurrence. Elevated abundances of FAAs and bacteria were observed during the local pollen season, whereas fungi were observed at highest level during autumn. Meteorological parameters, such as air and soil temperature, radiation and rainfall were observed to possess close relationship with PBAP~~bioaerosol~~ abundances on an annual scale. ~~Based on our findings, bioaerosols have seemingly an important effect on cloud formation processes at the boreal forest region depending on the season.~~

1 Introduction

Primary biological aerosol particles (PBAP)~~Bioaerosols~~ are emitted directly from the biosphere into the atmosphere (Després et al., 2012;Fröhlich-Nowoisky et al., 2016). PBAP~~Bioaerosol~~ are released from multiple sources, such as soil, vegetation and oceans, and they include e.g. pollen, plant fragments, spores, bacteria, algae and viruses. In recent years, the abundance and dispersal of microorganisms in the atmosphere has attracted more and more interest, mainly due to the underestimation of their abundance and their possible atmospheric impact (Jaenicke, 2005;Morris et al., 2011;Deguillaume et al., 2008;Burrows et al., 2009b;Estillore et al., 2016). It is estimated that globally PBAP~~bioaerosols~~ constitute ~30% of aerosol particles mass in the particles sized >1 µm in urban and rural air (Fröhlich-Nowoisky et al., 2016). This fraction can be much higher (~80%) in the tropical forest areas (Pöschl et al., 2010;Elbert et al., 2007), and interestingly even up to 65% at boreal forest during pollen season (Manninen et al., 2014). These high percentages provide the basis for assumptions that PBAP~~bioaerosols~~ may play an important role in the atmosphere by affecting cloud and precipitation formation processes by acting as cloud and ice nuclei (Huffman et al., 2013;Burrows et al., 2009b;Burrows et al., 2009a; Després et al., 2012; Fröhlich-Nowoisky et al., 2016). Thus, to clarify their atmospheric transport and ecosystem interactions, PBAP~~bioaerosols~~' chemical and microbial constituents need more characterization and identification.

PBAP~~Bioaerosols~~ have been studied by using a variety of techniques (Georgakopoulos et al., 2008), but cultivation and microscopy have been frequently employed for the elucidation of microorganisms in aerosols (Manninen et al., 2014;Després et al., 2012). Nowadays, molecular genetic analysis techniques, such as quantitative polymerase chain reaction (qPCR) or next-generation sequencing (NGS), have gained ground, because they provide information not only on the viable and cultivable cells, but also on uncultivable, dead or fragments of plant and animal cells (Després et al., 2012). Alternative approach to determine particles of biological origin is based on chemical tracer techniques (Bauer et al., 2008;Gosselin et al., 2016;Hock et al., 2008;Schneider et al., 2011;Zhang et al., 2010;Staton et al., 2015;Rathnayake et al., 2017). The benefit of chemical tracers, such as carbohydrates, proteins/amino acids and lipids, is their applicability to quantitative analysis, while their main disadvantage is disability to identify different biological species. Specific tracers have been used to estimate the total amount of PBAP~~bioaerosols~~ in the atmosphere (Gosselin et al., 2016;Hock et al., 2008;Schneider et al., 2011;Zhu et al., 2015).

Biologically-derived amino acids are non-volatile and hygroscopic compounds that are mainly found in the condensed phases in the atmosphere (Matos et al., 2016;Samy et al., 2013;Zhang and Anastasio, 2003). They are present in aerosols either in the combined form (proteins and peptides) or as free amino acids (FAAs), and they are emitted either from biogenic sources or formed from the degradation of proteinaceous material (Milne and Zika, 1993;Matos et al., 2016). Due to their close relationship with PBAP~~bioaerosols~~, amino acids can be used as biomarker for an overall estimation of biomass in aerosols (Hock et al., 2008;Schneider et al., 2011).

In this study size-segregated aerosol samples were collected in boreal forest during ~~one year~~a nine month period. Aerosol samples were analyzed for DNA concentration, microorganism-DNA (bacteria, *Pseudomonas* and fungi) and FAAs. The annual concentration and size distribution variation of microorganisms and FAAs were investigated in detail in order to

understand their potential sources. Correlation study between the PBAP~~bioaerosol~~ components and meteorological parameters was carried out. Our objective was to gain further information about the abundances of PBAP~~bioaerosols~~ in different particle size fractions at the boreal forest region and to understand better their biosphere-atmosphere interactions.

2 Experimental section

2.1 Materials and reagents

Detailed information on materials and reagents is presented in the Supporting Information (SI).

2.2 Aerosol sampling

The aerosol samples were collected in a Scots pine forest between February and October 2014 at the SMEAR II station (Station for Measuring Forest Ecosystem-Atmosphere Relations) in Hyytiälä, Finland (Hari and Kulmala, 2005). A Dekati PM10-impactor was used for the sampling of four particle size fractions (<1.0, 1-2.5, 2.5-10 and >10 µm) below the canopy. The sampling flow rate was on average 30 L/min and the collection time was from one to four days (sampling volume 76-144 m^3). The collection filters were 25 mm polycarbonate membranes (Whatman Nuclepore) for the three largest particle size fractions. To prevent particles bouncing, membranes were smeared with diluted Apiezon L vacuum grease. The smallest size fraction (<1.0 µm) was collected on a 47 mm Teflon filter (Gelman Sciences) with 2 µm pore size. After sampling, the filters were dried and weigh~~t~~ed. The sampling procedure is described in more details in Laakso et al. (2003). After gravimetric analysis, the filters were placed inside a closed polystyrene petri dish, covered with aluminum foil and stored at -20 °C.

Two sets of aerosol samples were collected in consecutive days, so that the sampling dates were close to each other (Table S1). The first set of aerosol samples were analyzed for DNA and microorganisms (set A) and the other set for free amino acids (set B). For molecular biological analysis only particles larger than 1 µm were selected because smaller fractions were suspected to contain only virus particles and fragmented DNA.

2.3 Determination of amino acids

After ultrasonic assisted extraction, free amino acids were determined by liquid chromatography-tandem mass spectrometry. Detailed information about the validation and quality control of the analytical method is presented in the Supporting Information, Figure S1 and Tables S2-S4.

2.4 Extraction of DNA

Total nucleic acids were extracted from the collection filters with a commercial DNA extraction kit (PowerWater DNA Isolation Kit, MoBio Laboratories, USA) with slight modification (see Supporting Information). The DNA concentration and purity was measured spectrophotometrically at 260 nm and 280 nm with NanoDrop ND-1000 (Thermo Scientific, USA). The DNA samples were stored at -20 °C prior to qPCR.

2.5 DNA amplification

The bacterial and fungal DNA amounts of the filter samples were quantified with qPCR using target specific primers pairs, Eub338F and Eub518R, and FF390 and FR1 (Table S5) (Fierer et al., 2005;Vainio and Hantula, 2000). In addition, genus specific primers, Eub338F and PseudoR, were utilized to detect the bacteria belonging to the genus *Pseudomonas* (Purohit et al., 2003).

In the bacterial and *Pseudomonas* specific qPCR reactions, standard curves were generated with DNA extracted *Pseudomonas fluorescens* H-27 (Hambi culture collection, University of Helsinki) and for the fungal specific qPCR, the DNA from the newly whole genome sequenced *Phlebia radiata* FBCC43 (genome size 40.92 Mb, FBCC culture collection, University of Helsinki) was used (Kuuskeri et al., 2016). When converting the copy number of the samples into bacterial cells (/colony forming units), all bacteria were assumed to contain average of three gene copies of ribosomal 16S DNA per cell, and bacteria belonging to genus *Pseudomonas* was assumed to contain five gene copies of 16S rDNA in a cell (Stoddard et al., 2014). According to Fröhlich-Nowoisky et al. (2009), majority of the fungal DNA detected during summer and autumn are from fungal species belonging to Basidiomycota. Based on previous results (Fröhlich-Nowoisky et al., 2012;Fröhlich-Nowoisky et al., 2009), we have assumed that most the fungal DNA collected on our filters is also derived from basidiomycete fungi. Because only few 18S rDNA-regions of fungi are fully annotated, we have selected the *P. radiata* as a model basidiomycete fungus and calculated the gene copy numbers to fungal cells based on the assumption that all fungi in this experiment have approximately the same amount of 18S rDNA gene copies as this fungus has (Kuuskeri et al., 2016).

2.6 Additional background data and back-trajectory analysis

Meteorological variables, gas fluxes and atmospheric gases are continuously measured at the SMEAR II and the data is available from AVAA-portal (Junninen et al., 2009). Half-hourly averaged data from the portal was further averaged according to each sampling time period by using arithmetic mean. These averaged values were used in statistical analyses. The selected variables were air (AT) and soil surface temperature (SST), soil surface water content (SSWC), wind speed (WS) and direction (WD), gross primary production (GPP), CO, CO_2 and ozone concentration, photosynthetically active radiation (PAR), UV-A and UV-B radiation and relative humidity (RH). In addition, rainfall was averaged according to different time periods as follows: 72 h before sampling (BSR), during sampling (DSR) and 168 h after sampling (ASR).

Backward air mass trajectories were calculated using the HYSPLIT (Hybrid Single-Paricle Lagrangian Integrated Trajectory) transport and dispersion model from NOAA Air Resources Laboratory to estimate the origin and transport route of aerosol particles (Draxler and Hess, 1998;Stein et al., 2015;Rolph, 2003). For the calculations, meteorological data from the Global Data Assimilation System (GDAS, 1 degree, global, 2006-present) was used. The backward air mass trajectories were modelled 48 hours back time during the whole period of sampling, using a resolution of 12-hours.

2.7 Statistical analysis

For analyzing the importance of the seasonality and the aerosol filter size, permutational multivariate analysis of variance (PERMANOVA) was performed for the presence/absence transformed amino acid data. The PERMANOVA was performed with the adonis-function of the vegan package (Oksanen et al., 2016) with 999 permutations, and the amino acid data was set as response variables while sampling month and aerosol filter size were set as explanatory variables. One-way analysis of variance (ANOVA) was performed to the fungal, bacterial and *Pseudomonas* gene copy numbers individually with the aov-funcion from the stats package (R Core Team, 2016) in order to study the effect of seasonality and aerosol filter size. Prior to performing ANOVA the normal distribution of each residual was checked individually with shapiro.test-function, and all the gene copy number data was log-normalized. The Spearman´s ~~linear~~ correlations between the FAA and microorganisms concentrations with meteorological variables were calculated using the rcorr-function of the Hmisc package (Harrell, 2016) and visualized with the corrplot package (Wei and Simko, 2016) showing only correlations with statistical significance ($p \leq 0.05$).

3 Results and Discussion

3.1 General characteristics

The average concentration and range of each component measured in different particle size fractions at the boreal forest site is presented in Table 1 and Table S6. The lowest concentration levels of FAA and total DNA were measured during winter and highest during late spring (Figures 1 and S2). The total amount of extracted DNA was highest from May to June (up to 48 ng/m^3) and from September to October (up to 14 ng/m^3). The FAA concentration in total particles was highest in late May (up to 751 ng/m^3) and in September (up to 35 ng/m^3). A common trend in both total DNA and FAA concentrations was a maximum in spring and a secondary smaller maximum in autumn. Similarly, the highest PM concentration in total particles was recorded during late spring and autumn (Figure S3); a pattern which has also been previously observed at the same location (Laakso et al., 2003;Manninen et al., 2014). The measured DNA concentrations agreed with those obtained for $PM_{2.5}$ samples at a rural mountain site in Germany, where the DNA concentration was in the range 1.7-4.2 ng/m^3 (Després et al., 2007). Also the observed FAA concentrations were in the same order of magnitude as those observed in other rural and semi-urban locations (Zhang and Anastasio, 2003;Zhang et al., 2002;Samy et al., 2011;Samy et al., 2013). For example, at two rural sites in US the concentration of FAAs in $PM_{2.5}$ samples was measured to be 22±9 ng/m^3 during summer (Samy et al., 2011), and 59±49 ng/m^3 (range 9-236 ng/m^3) during one year study period (Zhang and Anastasio, 2003). To our knowledge, there are no studies covering DNA and FAA abundances at the boreal forest region, thus comparison to previous results is not feasible.

Similar to the annual trend of total DNA and FAAs, the lowest concentration levels of microorganisms were detected during winter and highest during spring and autumn. However, bacterial and fungal DNA reached their maximum levels at different seasons; bacteria peaking in late spring (Figure 2a) and fungal DNA in late summer and autumn (Figure 3). The bacterial DNA abundance in total particles was lower than 900 cells/m^3 during winter and early spring, whereas in late spring the concentration peaked at 58731 cells/m^3. During summer and autumn, the concentration of bacterial DNA was one magnitude lower. In contrast to the total number of bacteria, the highest amounts (>200 cells/m^3) of *Pseudomonas* DNA were measured in late May, late June and October (Figure 2b). In rest of times, the concentration of bacteria

belonging to the genus *Pseudomonas* was much lower, and the highest concentration levels in total particles rarely exceeded 50 cells/m^3. The fungal abundance was lower than 300 cells/m^3 during winter and early spring, whereas in late spring and summer the concentrations started to increase and generally exceeded 6000 cells/m^3 in total particles. The highest amounts of fungal DNA were measured in late June (30149 cells/m^3), in August (55839 cells/m^3) and in early October (35050 cells/m^3). In general, the concentration levels of microorganisms vary seasonally and geographically, but are estimated to be in the level of ~10^4-10^5 m^{-3} and ~10^4-10^5 m^{-3} for bacterial cells and fungal spores, respectively (Burrows et al., 2009b;Després et al., 2012;Spracklen and Heald, 2014). Our observations are consistent with the common trend, when considering that low concentration levels are typically observed at rural locations.

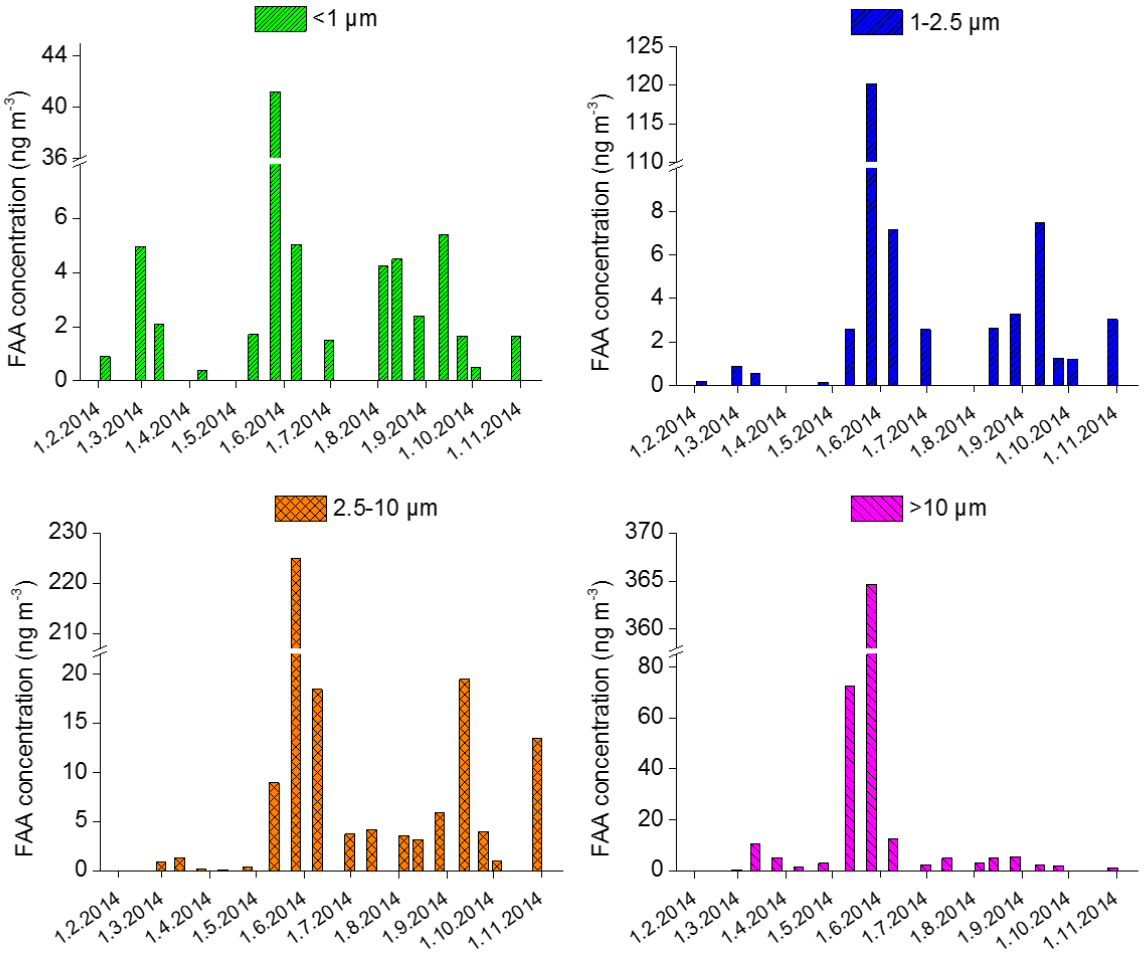

Figure 1. Free amino acid concentrations over the sampling period in different particle size fractions (date format is dd.mm.yyyy.). Note the different y-axis scales in panels.

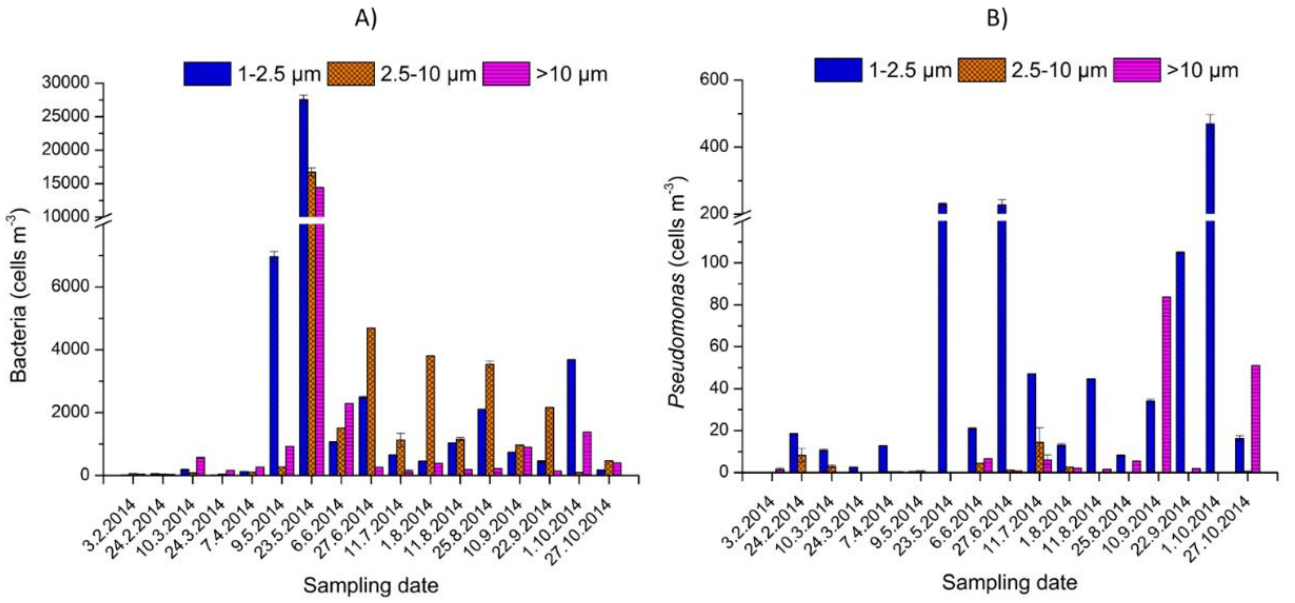

Figure 2. a) The amount of all bacterial cells over the sampling period, and b) cells of bacteria belonging to genus *Pseudomonas*, detected with qPCR from the aerosol filters, specified by the particle size fractions.

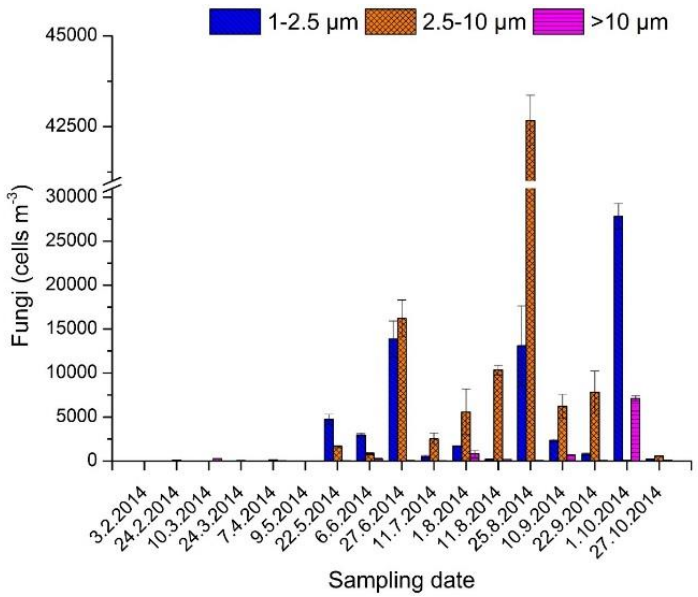

Figure 3. The amount of fungal cells and spores over the sampling period, detected with qPCR from the filters of different particle size fractions.

3.2 Seasonal variation and size distribution of FAAs

In winter the amino acids were mainly accumulated in the particle size fraction <1 µm (~74%, Figure S4a), whereas during spring the amino acids were mainly present in the largest particle size fraction >10 µm (~77%). The highest amount of FAAs were observed during late spring and early summer (Figure 1), when the local pollen season plumed. During summer, the concentrations of FAAs were relatively constant and seemingly FAAs evenly distributed in all the size fractions (Figure S4a). In autumn they were mainly present in the particles below 10 µm, and accumulated especially in

the size fraction 2.5-10 µm (~51%). During autumn, there was a slight increase in the total FAA concentrations mainly due to the increase in the size fraction 2.5-10 µm (Figure 1).

Similarly, in the size distribution of individual amino acids some clear tendencies were observed (Figure S5). The results from the PERMANOVA analysis indicated that seasonal variation explained the observed variation across the whole FAA data with statistical significance ($p<0.001$). In addition, the size of the aerosol filter was statistically significant factor for explaining the observed variation across the whole amino acid data (PERMANOVA, $p<0.001$). Glycine was the most dominant amino acid in the size fraction <1 µm, whereas in the larger size fractions its relative abundance was much smaller (Figure S5). On average, glycine accounted 59% of the total FAAs in particles <1 µm, followed by alanine (10%), aspartic acid (9%) and glutamine (9%). In the size fraction 1-2.5 µm, glutamine (42%), glutamic acid (16%) and arginine (15%) were on average the most abundant amino acids. Similarly, in the size fraction 2.5-10 µm glutamine (29%), arginine (15%) and glutamic acid (12%) were the dominant ones, accompanied by proline (7%), serine (7%) and alanine (7%). In the largest size fraction >10 µm proline (36%), arginine (21%), glutamine (10%) and glutamic acid (9%) were the most dominant ones. Our results are in agreement with several studies demonstrating that glycine, arginine, serine, alanine, proline and glutamine are the most commonly abundant FAAs in aerosols (Barbaro et al., 2011;Barbaro et al., 2015;Samy et al., 2011;Samy et al., 2013;Scalabrin et al., 2012;Violaki and Mihalopoulos, 2010;Zhang and Anastasio, 2003), although the relative abundances vary depending on location, season and particle size fraction (Matos et al., 2016).

3.3 Seasonal variation and size distribution of microorganisms

A distinct monthly variation in bacterial DNA abundance in different filter size fractions was observed (ANOVA, $p<0.01$). During early spring, bacterial DNA was accumulated mainly in the size fraction >10 µm (~64%, Figure S4b). Oppositely, in late spring the highest numbers of bacteria were detected in the size fraction 1-2.5 µm, whereas during summer, highest numbers of bacteria were discovered in the size fraction 2.5-10 µm (Figure 2a). During summer, bacteria was mainly present in the size faction 2.5-10 µm, which covered on average ~57% of the total bacteria amount. In general, most of the bacteria were observed in the size fractions 1-2.5 µm and 2.5-10 µm, and less bacterial cells were found in the size fraction >10 µm. However, on average the abundances did not differ significantly in different size fractions (ANOVA, $p=0.494$). The size distribution observed is in line with that of Burrows et al. (2009b) who summarized that the median aerodynamic diameter of particles containing bacteria is 4 µm at continental sites.

The abundance of *Pseudomonas* varied with statistically significance between different months (ANOVA, $p<0.05$) and between different size fractions (ANOVA, $p<0.001$). *Pseudomonas* was mainly present (~70%) in the smallest particle size fraction 1-2.5 µm throughout the year (Figures 2b and S4c). The accumulation of *Pseudomonas* in the smallest size fraction is in line with the small aerodynamic size (<1 µm) of common *Pseudomonas* species (Chang et al., 2013;Möhler et al., 2008;Pietsch et al., 2015). Overall, the relative amount of *Pseudomonas* from all bacteria was highest in the size fraction 1-2.5 µm (Figure S6), being highest in late winter (28%), in early spring (15%) and in autumn (23%). Interestingly, in February the relative amount of *Pseudomonas* DNA of the total bacterial DNA was 17% in total particles (Figure S6).

The amount of fungal DNA detected in different size fractions varied with statistical significance (ANOVA, $p<0.05$). The fungal DNA was predominantly observed in the particle size fraction 2.5-10 µm, which accounted on average ~58% of the total fungal concentration. The monthly variation in fungal DNA abundance was also statistically significant (ANOVA, $p<0.001$). During May and early June, the fungal DNA was mainly accumulated in the size fraction 1-2.5 µm (~67%, Figure S4d). In late summer, fungal DNA was detected at its highest level in the size fraction 2.5-10 µm (Figure 3), and in overall ~66% was accumulated in this size fraction during the summer months. Fungal spores are frequently observed in aerosols in the size range of 2-10 µm (Després et al., 2012). Our observations are consistent with these literature values.

3.4 Overview of meteorological factors and sources

The effect of local meteorological factors on the concentration levels of FAAs and microorganism was studied by means of ~~linear regression~~correlation analysis and a summary of results is shown in Figure 4. Our results reflect rather long-term seasonal effects (more details in Supporting Information), due to the time resolution of sampling. Thus, as expected, the key meteorological factors explaining the observed concentration levels were air temperature, soil temperature, gross primary production and radiation (Figures S7 and S8). In general, we believe that the positive correlation observed between FAAs and microorganism abundances with the before mentioned meteorological variables are closely related to growing season and seasonality, rather than being a decisive effect. These observations are in good agreement with previously reported tendencies covering microorganism and different meteorological factors (Jones and Harrison, 2004;Burrows et al., 2009b;Lighthart, 2000). Soil and vegetation have been previously suggested to be the predominant sources of microorganisms in the atmosphere (Bowers et al., 2013), and our correlation results confirm these sources as discussed below.

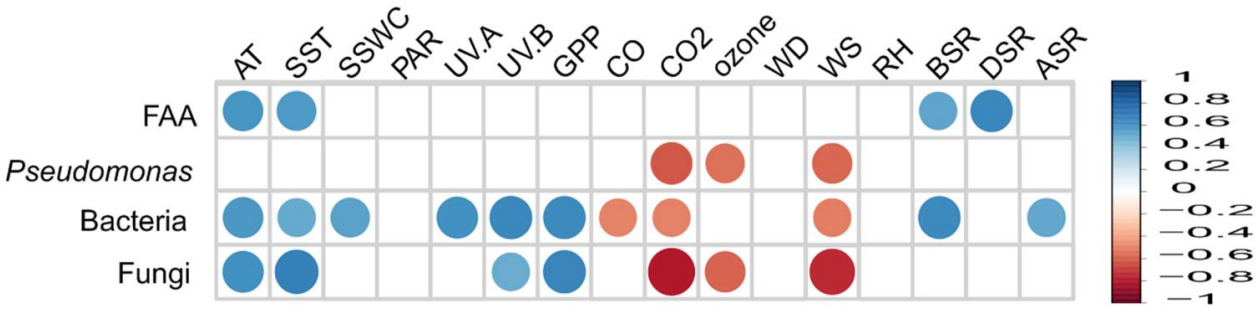

Figure 4. Summary of Spearman correlation results (total particles, $p<0.05$). The colour scale indicates positive/negative correlation ~~and the size of the ball statistical significance level. The larger the ball is, the smaller the p-value is (smallest ball is representative of $p<0.05$~~). Abbreviations: AT-air temperature; SST-soil surface temperature; SSWC-soil surface water content; WS-wind speed; WD-wind direction; GPP-gross primary production; CO; CO2; ozone concentration; PAR-photosynthetically active radiation; UV-A and UV-B radiation; RH-relative humidity; BSR-rainfall 72 h before sampling; DSR-rainfall during sampling; ASR-rainfall 168 h after sampling.~~Abbreviations are presented in Section 2.6.~~

As could be expected (Manninen et al., 2014;Schumacher et al., 2013), the lowest concentration levels of FAAs and microorganisms were detected during winter, when the air and soil temperatures were below 0 °C and the ground was covered by snow. In February, particles below 2.5 µm accounted for 89% of the total FAA concentration. In these samples, glycine and alanine were the dominant amino acids. Glycine and alanine have been exploited as markers for long-range

transport aerosols due to their relatively low reactivity and long half-lives in the atmospheric condensed phases (Barbaro et al., 2011;Barbaro et al., 2015;Scalabrin et al., 2012;Samy et al., 2013). As the concentration levels of microorganisms were low during winter (Figures 2 and 3), it is likely that the observed FAAs originated from distant sources. However, FAAs and particularly glycine have been also associated with biomass burning emissions (Samy et al., 2013;Violaki and Mihalopoulos, 2010;Zangrando et al., 2016). In Finland wood-burning, that is typical domestic heating system in winter, is the most presumable source of over one third of the $PM_{2.5}$ emissions (Laakso et al., 2003;Saarnio et al., 2012). The relative abundance of glycine in the size fraction 1-2.5 µm was 85% during winter, whereas in other seasons its contribution was negligible. Thus, the observed glycine concentrations might be associated with wood-burning emissions, coming from either local or distant sources. This conclusion is supported by the observed positive correlation between glycine with CO concentration in the size fractions <1 µm ($R=0.54$, $p<0.05$, Figure S8) and 1-2.5 µm ($R=0.80$, $p<0.001$, Figure S8). Furthermore, only glycine among the FAAs showed positive correlation with PM concentration in the size fraction 1-2.5 µm ($R=0.69$, $p<0.01$, data not shown).

FAA and bacteria concentrations increased during the spring recovery and seemed to be influenced by the increase in local biological activity. During early spring, FAAs and bacteria were mainly present in the largest size fractions (Figures S4a and S4b), which may indicate the presence of pollen in aerosols, since microorganisms might be present on the surface of pollen grains (Puc, 2003). Further, Manninen et al. (2014) observed pollen to be present at SMEAR II station already before the local pollen season, most possible due to long-range transport from central Europe, where growing season had already started. Interestingly though, during our sampling periods the back-trajectory analysis results showed air-masses arriving primarily from Scandinavia and North Europe instead of central Europe. This could imply that the larger sized particles are originating locally from some early pollinator rather than being long-range transported. In addition, we observed an interesting phenomenon in early March when all FAAs, bacterial DNA and fungal DNA were present mainly in the size fraction >10 µm. Then the corresponding relative abundances of their total amounts were 73%, 68% and 92%, respectively. During March sampling, the air temperature was almost constantly above zero degrees for the first time in our campaign. The small increase observed in FAA and microorganism concentrations during this time might indicate that a burst in PBAP~~bioaerosols~~ emissions can occur when the temperature increases and snow starts to melt revealing the ground vegetation and decomposed leaf litter for the first time after winter. Similar observations have been previously made at the same site by Schumacher et al. (2013) in a study covering fluorescent biological aerosol particles (FBAP), although they detected an increase in FBAP concentrations in November after first snowfall and snowmelt event.

The concentrations of FAAs and bacteria reached their maximum levels during the local pollen season peak in May (Figures 1 and 2). Elevated amino acid concentrations have been observed during spring time also in other locations and suspected to be influenced by pollen (Barbaro et al., 2011;Zhang et al., 2002). In our study, especially proline and arginine concentrations increased during the pollen season peak. These amino acids have been shown to contribute significantly to the total amino acid content of birch pollen (Ozler et al., 2009). In this study, the FAA concentrations increased in all the size fractions (Figure 1), possibly due to rupture of relatively large (~30 µm) pollen grains (Taylor et al., 2004;Visez et al., 2015), supported by visibly yellow impactor plates in all the size fractions. During the peak in FAA and bacteria concentrations, the air-masses were arriving in Hyytiälä from Eastern Europe and Baltic Sea. Due to the absence of pollinating species in the sea region, the observed high concentration levels of FAAs and bacteria could be mainly

explained by local sources in the boreal forest. This is corroborated by the presence of methionine, cysteine and tryptophan only in these aerosols samples. Particularly these amino acids are known to be highly reactive with short half-lives in the atmospheric condensed phases (Scalabrin et al., 2012;Milne and Zika, 1993;McGregor and Anastasio, 2001). Thus, the local pollen season likely explains our observations, although other factors may partly contribute. For example, high ozone concentration and strong UV radiation were recorded during this time period, and in some studies ozone has been demonstrated to promote the decomposition of protein and peptides into free amino acids increasing the FAA concentrations (Samy et al., 2013;Mumford et al., 1972). Positive correlation was observed between FAA concentration in the size fraction >10 µm with ozone ($R=0.58$, $p<0.05$, Figure S8), which was mainly attributed to coinciding peaks in concentration levels during pollen season.

Opposite to FAA concentrations being highest in the largest size fractions, bacterial DNA abundance was highest in the size fraction 1-2.5 µm during the pollen season peak. As spring proceeds, new foliage growth enables larger surface area for epiphytic bacteria to occupy and grow on. Bacterial cells may be lifted from leaves into the air on pollen (Jones and Harrison, 2004), which may explain the peak in bacterial abundances during this season. Bacteria may be present in the air as individual cells, clump of cells or attached to other particles, such as pollen grains and leaf fragments. We propose that during the local pollen season and under favourable meteorological conditions, it is possible to observe a significant increase in bacterial concentrations. However, most likely the magnitude of this increase varies from year-to-year and is also closely related to varying pollen abundances.

Although we assume that the elevated bacterial DNA abundances are mainly related to pollen and vegetation during spring, other sources might contribute as well. For example, soil water content and bacteria concentration correlated positively in the size fractions 1-2.5 µm ($R=0.53$, $p<0.05$, Figure S7) and >10 µm ($R=0.57$, $p<0.05$, Figure S7). After snow melts, the soil moisture increase enhances the bacterial/microbial growth (Burrows et al., 2009b). When spring proceeds and air and soil temperatures increase, the relatively dry soil surface layer might enable the dispersal of bacteria via soil resuspension. Similarly, some studies indicate that soil-derived bacteria dominate during spring time (Rathnayake et al., 2017), while others indicate that soil sources dominate during late summer and fall (Bowers et al., 2013). In addition, rainfall may promote the bacterial growth on vegetation surfaces, leading to increased population sizes, which may become airborne following rainfall (Bigg et al., 2015). We observed positive correlation between bacteria concentration in the size fraction 1-2.5 µm with rainfall recorded 72 h before the sampling ($R=0.64$, $p<0.01$, Figure S7). Based on previous reports related to the relationship between rainfall and PBAP~~bioaerosols~~ (Huffman et al., 2013;Prenni et al., 2013;Gosselin et al., 2016;Rathnayake et al., 2017;Morris et al., 2016;Bigg et al., 2015), our results corroborate the positive effect of bacteria abundances following rainfall.

The abundance and relative size distribution of fungal DNA varied seasonally and started to increase in summer (Figure 3). The relative distribution of fungal DNA to different size fractions correlated with the relative humidity ($R=-0.53$, $P=0.035$ for 1-2.5 µm and $R=0.45$, $P=0.081$ for 2.5-10 µm, data not shown). The lower the relative humidity was, the more fungi were accumulated in the smallest size fraction. Since fungal cells are typically larger than 2.5 µm, the observed fungal DNA in the 1-2.5 µm fractions during early summer is probably originated from the spores of moulds or ruptured cells (Reponen et al., 2001). In addition, the size of fungal spores has been found to depend on the relative humidity, i.e. higher the relative humidity is the larger the spores are (Reponen et al., 1996;Madsen, 2012). The spores of basidiomycete

fungi are in general larger than those of ascomycete (Reponen et al., 2001;Manninen et al., 2014;Hussein et al., 2013;Fröhlich-Nowoisky et al., 2012), and the basidiomycete fungi are known to sporulate mainly during autumn when the relative humidity is high (Kauserud et al., 2011). Further, in the previous study by Manninen et al. (2014) the spores of Basidiomycota dominated the phylum-level distribution of fungal spores in the autumn. Our results where fungal DNA accumulated in the 2.5-10 µm size fraction during autumn are in agreement with this study. Consequently, the annual size distribution of fungi can be expected to be similar from year-to-year in the boreal forest.

Opposite to the trend of fungi, FAA and bacterial DNA concentrations decreased after the spring pollen season peak. It is noteworthy that both bacterial and fungal DNA were accumulated during the mid-summer to early fall in the particle size fraction 2.5-10 µm (Figure S4b and S4d). In summer, the air masses arrived Hyytiälä mainly from Scandinavia and Baltic Sea regions. In early August the air masses reached Hyytiälä from a large forest fire region in Sweden. Interestingly derived from our samples, glycine was detected in relatively high concentrations (~2.9 ng/m^3) in the size fraction <1 µm. This observation supports the hypothesis that the presence of glycine is partially related to biomass burning emissions and long-range transport. However, no distinct effects on the origin of air-masses were seen in the levels of FAAs or microorganisms. Although long-range transport cannot be completely ruled out, the accumulation of bacteria and fungi in the particle size fraction 2.5-10 µm suggest that primarily local forest or nearby sources affect the concentration levels of microorganisms during summer. For example, leaf-associated bacteria are known to contribute to the total bacterial amount during summer (Bowers et al., 2013). Statistically significant correlation was observed between gross primary production ($R=0.79$, $P<0.001$, Figure S7) and photosynthetically active radiation ($R=0.69$, $P<0.01$, Figure S7) with bacteria in the size fraction 2.5-10 µm, possibly indicating the importance of vegetation especially during summer and growing season.

The abundance of *Pseudomonas* was detected at its highest level in early October in the size fraction 1-2.5 µm, when it was 2-fold higher than in other months (Figure 2b). It is suggested that an increase in the *Pseudomonas syringae* population size on vegetation may occur during or after rainfall (Hirano et al., 1996;Bigg et al., 2015;Morris et al., 2016). Even though no correlation was observed between average rainfall recorded before or during sampling with *Pseudomonas* on an annual scale, we identified interesting separate individual rainfall events. The maximum single rainfall event was recorded within 72 h before the above mentioned early October sampling (Figure S9). As far as we could tell, this was the main exception in meteorological conditions during the period studied, i.e. the only factor potentially explaining the observed increase in *Pseudomonas* concentration. Further, the back-trajectory analysis results showed air-masses arriving from Norwegian Sea and North Sea via the Scandinavian Mountains, and to our knowledge these areas do not contain sources that could explain the increase in *Pseudomonas* concentration. When considering the other maximum rainfall events recorded before sampling, two out of three of the summer-autumn maximum events coincided with high *Pseudomonas* concentrations (Figure S9). These findings corroborate the evidence of a potential relationship between rainfall and *Pseudomonas*. However, the lack of statistically significant correlation suggests that other factors are contributing to observed variation in *Pseudomonas* abundance.

Overall, considering the effect of rainfall on the levels of bacteria, fungi and FAAs, some interesting patterns were observed. As mentioned earlier, the bacterial DNA concentration in size fraction 1-2.5 µm correlated with rainfall recorded prior to sampling. Further, we observed positive correlation between FAA concentration and rainfall during

sampling in the size fractions 1-2.5 µm (R=0.53, p=0.0502, data not shown) and 2.5-10 µm (R=0.70, p<0.01, Figure S8). Interestingly, bacterial DNA and fungal DNA abundances correlated positively in the size fraction 2.5-10 µm with rainfall recorded 168 h after sampling (R=0.63, p<0.01 and R=0.53, p<0.05, respectively; Figure S7). There is ~~relatively strong~~ some evidence that cloud-active particles larger than ~1 µm are biological in origin (Haga et al., 2014;Hassett et al., 2015;Mason et al., 2016;Möhler et al., 2007), and in mixed-phase cloud conditions, PBAP~~bioaerosols~~ may play an important role in triggering rainfall. Collectively, the different correlations observed between rainfall and PBAP ~~bioaerosol~~ components suggest that a potential feedback mechanism may persist at the boreal forest. This conclusion is based on the assumption that the recorded rainfall events after sampling were produced at least partially on a local scale and that the meteorological factors were favourable to formation of rainfall. We acknowledge, that the positive correlation observed between microorganism's abundances with rainfall recorded following sampling, may be causal in nature. However, in light of the recent findings, the possibility of a feedback mechanisms cannot be ignored (Bigg et al., 2015;Morris et al., 2016;Huffman et al., 2013). Nonetheless, additional high time resolution and long-term measurements are needed to confirm the observations presented in this research.

4 Conclusions

Considering the observations made in this and previous studies (Manninen et al., 2014;Schumacher et al., 2013), some general conclusions related to PBAP~~bioaerosol~~ abundances and size distribution at the boreal forest site can be drawn. Correlation with meteorological parameters might indicate biosphere-atmosphere interactions through PBAP~~bioaerosols~~ with possible climate effects. In early spring, PBAP~~bioaerosol~~ components are mainly accumulated in the size-fractions >10 µm. According to our results, the spring pollen season has an impact on the pollen levels as well as on the bacterial abundances. Elevated PBAP~~bioaerosol~~ abundances occur during the pollen season (Manninen et al., 2014), and based on our estimation even up to ~77% of total PM may be of biological origin (see SI for details). Even though our estimation is highly uncertain, ~~Thus,~~ the magnitude of biological cloud-active particles during this period may be atmospherically relevant (Diehl et al., 2002;Diehl et al., 2001;Pummer et al., 2012;Pummer et al., 2015). The importance of rainfall was observed in this study as well as to some extent in previous studies conducted at the same site (Manninen et al., 2014;Schumacher et al., 2013). Positive correlation was observed between PBAP~~bioaerosol~~ component abundances with rainfall recorded before and during sampling as well as with rainfall recorded after sampling. During late summer and autumn, the accumulation of PBAP~~bioaerosols~~ in the size fraction 2.5-10 µm was evident (Figure S4). In autumn the relative amount of biomass in PM was estimated to be around ~10% (SI). ~~Considering bioaerosol properties as cloud active particles, we believe that the effect of bioaerosols on cloud formation processes at the boreal forest region may be important. For example, m~~Modelling studies have suggested that microorganisms may play an important role in the hydrological cycle in the boreal region (Sesartic et al., 2012;Sesartic et al., 2013). However, the magnitude of upward lifting of microorganisms remains to be solved, and thus our results are preliminary in nature and need to be confirmed.

Author contribution

A.Helin, O.-M. Sietiö, J. Heinonsalo, J. Bäck, M.-L. Riekkola, J. Parshintsev designed the experiments. A. Helin, O.-M. Sietiö, J. Parshintsev carried them out. A. Helin and O.-M. Sietiö performed the statistical analysis. A. Helin and J. Parshintsev prepared the manuscript with contributions from all co-authors.

Competing interests

The authors declare that they have no conflict of interest.

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Table 1. Average (±standard deviation) concentration and range of each component measured and the number of filter samples (n) analyzed in total.

Component	<1 µm	1-2.5 µm	2.5-10 µm	>10 µm	total particles
PM (µg/m^3)	5.9±4.1	2.1±2.3	2.4±3.2	2.1±3.5	12.3±12.2
n=138	(1.0-18.1)	(0.2-11.9)	(0.2-15.9)	(0.03-17.4)	(2.2-63.3)
FAA (ng/m^3)	5.22±10.11	10.95±31.54	18.45±53.55	27.62±85.71	57.91±174.17
n=69	(<LOQ-41.21)	(<LOQ-120.24)	(<LOQ-224.92)	(0.04-364.65)	(1.13-751.01)
DNA (ng/m^3)	-	5.16±5.20	1.99±3.47	2.18±3.76	8.60±11.41
n=51		(<LOQ-18.56)	(0.002-14.67)	(<LOQ-14.80)	(0.24-48.04)
Bacteria (cells/m^3)	-	2811±6619	2171±4032	1341±3424	6323±13748
n=51		(17-27551)	(56-16746)	(41-14434)	(137-58731)
Pseudomonas (cells/m^3)	-	74±125	4±5	13±26	86±122

n=51		(0.1-469)	(<LOQ-14)	(<LOQ-84)	(1-469)
Fungi (cells/m^3)	-	4022±7518	5579±10614	648±1809	10173±15622
n=51		(2-27838)	(9-42667)	(<LOQ-7129)	(27-55839)

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