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

4

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14

15 **Abstract**

16

17 Bioaerosols are ubiquitous in the atmosphere and constitute ~30% of atmospheric aerosol particle mass in sizes >1 µm.
18 Bioaerosol components, such as bacteria, fungi and pollen, may affect the climate by acting as could-active particles, thus
19 having an effect on cloud and precipitation formation processes. In this study, size-segregated aerosol samples (<1.0, 1-
20 2.5, 2.5-10 and >10 µm) were collected in boreal forest (Hyytiälä, Finland) during one year and analyzed for free amino
21 acids (FAAs), DNA concentration and microorganism (bacteria, *Pseudomonas* and fungi). Measurements were performed
22 using tandem mass spectrometry, spectrophotometry and qPCR, respectively. Meteorological parameters and statistical
23 analysis were used to study their atmospheric implication for results. Distinct annual patterns of bioaerosol components
24 were observed, late spring and autumn being seasons of dominant occurrence. Elevated abundances of FAAs and bacteria
25 were observed during the local pollen season, whereas fungi were observed at highest level during autumn.
26 Meteorological parameters, such as air and soil temperature, radiation and rainfall were observed to possess close
27 relationship with bioaerosol abundances on an annual scale.

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40 **1 Introduction**

41

42 Bioaerosols are emitted directly from the biosphere into the atmosphere (Després et al., 2012;Fröhlich-Nowoisky et al.,
43 2016). Bioaerosol are released from multiple sources, such as soil, vegetation and oceans, and they include e.g. pollen,
44 plant fragments, spores, bacteria, algae and viruses. In recent years, the abundance and dispersal of microorganisms in
45 the atmosphere has attracted more and more interest, mainly due to the underestimation of their abundance and their
46 possible atmospheric impact (Jaenicke, 2005;Morris et al., 2011;Deguillaume et al., 2008;Burrows et al., 2009b;Estillore
47 et al., 2016). It is estimated that globally bioaerosols constitute ~30% of aerosol particles mass in the particles sized >1
48 µm in urban and rural air (Fröhlich-Nowoisky et al., 2016). This fraction can be much higher (~80%) in the tropical forest
49 areas (Pöschl et al., 2010;Elbert et al., 2007), and interestingly even up to 65% at boreal forest during pollen season
50 (Manninen et al., 2014). These high percentages provide the basis for assumptions that bioaerosols may play an important
51 role in the atmosphere by affecting cloud and precipitation formation processes by acting as cloud and ice nuclei (Huffman
52 et al., 2013;Burrows et al., 2009b;Burrows et al., 2009a; Després et al., 2012; Fröhlich-Nowoisky et al., 2016). Thus, to
53 clarify their atmospheric transport and ecosystem interactions, bioaerosols' chemical and microbial constituents need
54 more characterization and identification.

55

56 Bioaerosols have been studied by using a variety of techniques (Georgakopoulos et al., 2008), but cultivation and
57 microscopy have been frequently employed for the elucidation of microorganisms in aerosols (Manninen et al.,
58 2014;Després et al., 2012). Nowadays, molecular genetic analysis techniques, such as quantitative polymerase chain
59 reaction (qPCR) or next-generation sequencing (NGS), have gained ground, because they provide information not only
60 on the viable and cultivable cells, but also on uncultivable, dead or fragments of plant and animal cells (Després et al.,
61 2012). Alternative approach to determine particles of biological origin is based on chemical tracer techniques (Bauer et
62 al., 2008;Gosselin et al., 2016;Hock et al., 2008;Schneider et al., 2011;Zhang et al., 2010;Staton et al., 2015;Rathnayake
63 et al., 2017). The benefit of chemical tracers, such as carbohydrates, proteins/amino acids and lipids, is their applicability
64 to quantitative analysis, while their main disadvantage is disability to identify different biological species. Specific tracers
65 have been used to estimate the total amount of bioaerosols in the atmosphere (Gosselin et al., 2016;Hock et al.,
66 2008;Schneider et al., 2011;Zhu et al., 2015).

67

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

74

75 In this study size-segregated aerosol samples were collected in boreal forest during one year period. Aerosol samples
76 were analyzed for DNA concentration, microorganism-DNA (bacteria, *Pseudomonas* and fungi) and FAAs. The annual
77 concentration and size distribution variation of microorganisms and FAAs were investigated in detail in order to
78 understand their potential sources. Correlation study between the bioaerosol components and meteorological parameters

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79 was carried out. Our objective was to gain further information about the abundances of bioaerosols in different particle
80 size fractions at the boreal forest region and to understand better their biosphere-atmosphere interactions.
81
82 **2 Experimental section**
83
84 2.1 Materials and reagents
85
86 Detailed information on materials and reagents is presented in the Supporting Information (SI).
87
88 2.2 Aerosol sampling
89
90 The aerosol samples were collected in a Scots pine forest between February and October 2014 at the SMEAR II station
91 (Station for Measuring Forest Ecosystem-Atmosphere Relations) in Hyytiälä, Finland (Hari and Kulmala, 2005). A Dekati
92 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
93 canopy. The sampling flow rate was on average 30 L/min and the collection time was from one to four days (sampling
94 volume 76-144 m^3). The collection filters were 25 mm polycarbonate membranes (Whatman Nuclepore) for the three
95 largest particle size fractions. To prevent particles bouncing, membranes were smeared with diluted Apiezon L vacuum
96 grease. The smallest size fraction (<1.0 µm) was collected on a 47 mm Teflon filter (Gelman Sciences) with 2 µm pore
97 size. After sampling, the filters were dried and weighted. The sampling procedure is described in more details in Laakso
98 et al. (2003). After gravimetric analysis, the filters were placed inside a closed polystyrene petri dish, covered with
99 aluminum foil and stored at -20 °C.
100
101 Two sets of aerosol samples were collected in consecutive days, so that the sampling dates were close to each other (Table
102 S1). The first set of aerosol samples were analyzed for DNA and microorganisms (set A) and the other set for free amino
103 acids (set B). For molecular biological analysis only particles larger than 1 µm were selected because smaller fractions
104 were suspected to contain only virus particles and fragmented DNA.
105
106 2.3 Determination of amino acids
107
108 After ultrasonic assisted extraction, free amino acids were determined by liquid chromatography-tandem mass
109 spectrometry. Detailed information about the validation and quality control of the analytical method is presented in the
110 Supporting Information, Figure S1 and Tables S2-S4.
111
112 2.4 Extraction of DNA
113
114 Total nucleic acids were extracted from the collection filters with a commercial DNA extraction kit (PowerWater DNA
115 Isolation Kit, MoBio Laboratories, USA) with slight modification (see Supporting Information). The DNA concentration
116 and purity was measured spectrophotometrically at 260 nm and 280 nm with NanoDrop ND-1000 (Thermo Scientific,
117 USA). The DNA samples were stored at -20 °C prior to qPCR.
118

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119 2.5 DNA amplification

120

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

125

126 In the bacterial and *Pseudomonas* specific qPCR reactions, standard curves were generated with DNA extracted
127 *Pseudomonas fluorescens* H-27 (Hambi culture collection, University of Helsinki) and for the fungal specific qPCR, the
128 DNA from the newly whole genome sequenced *Phlebia radiata* FBCC43 (genome size 40.92 Mb, FBCC culture
129 collection, University of Helsinki) was used (Kuuskeri et al., 2016). When converting the copy number of the samples
130 into bacterial cells (/colony forming units), all bacteria were assumed to contain average of three gene copies of ribosomal
131 16S DNA per cell, and bacteria belonging to genus *Pseudomonas* was assumed to contain five gene copies of 16S rDNA
132 in a cell (Stoddard et al., 2014). According to Fröhlich-Nowoisky et al. (2009), majority of the fungal DNA detected
133 during summer and autumn are from fungal species belonging to Basidiomycota. Based on previous results (Fröhlich-
134 Nowoisky et al., 2012;Fröhlich-Nowoisky et al., 2009), we have assumed that most the fungal DNA collected on our
135 filters is also derived from basidiomycete fungi. Because only few 18S rDNA-regions of fungi are fully annotated, we
136 have selected the *P. radiata* as a model basidiomycete fungus and calculated the gene copy numbers to fungal cells based
137 on the assumption that all fungi in this experiment have approximately the same amount of 18S rDNA gene copies as this
138 fungus has (Kuuskeri et al., 2016).

139

140 2.6 Additional background data and back-trajectory analysis

141

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

149

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

155

156 2.7 Statistical analysis

157

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158 For analyzing the importance of the seasonality and the aerosol filter size, permutational multivariate analysis of variance
159 (PERMANOVA) was performed for the presence/absence transformed amino acid data. The PERMANOVA was
160 performed with the adonis-function of the vegan package (Oksanen et al., 2016) with 999 permutations, and the amino
161 acid data was set as response variables while sampling month and aerosol filter size were set as explanatory variables.
162 One-way analysis of variance (ANOVA) was performed to the fungal, bacterial and *Pseudomonas* gene copy numbers
163 individually with the aov-funcion from the stats package (R Core Team, 2016) in order to study the effect of seasonality
164 and aerosol filter size. Prior to performing ANOVA the normal distribution of each residual was checked individually
165 with shapiro.test-function, and all the gene copy number data was log-normalized. The linear correlations between the
166 FAA and microorganisms concentrations with meteorological variables were calculated using the rcorr-function of the
167 Hmisc package (Harrell, 2016) and visualized with the corrplot package (Wei and Simko, 2016) showing only
168 correlations with statistical significance ($p \leq 0.05$).
169
170
171 **3 Results and Discussion**
172
173 3.1 General characteristics
174
175 The average concentration and range of each component measured in different particle size fractions at the boreal forest
176 site is presented in Table 1 and Table S6. The lowest concentration levels of FAA and total DNA were measured during
177 winter and highest during late spring (Figures 1 and S2). The total amount of extracted DNA was highest from May to
178 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
179 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
180 concentrations was a maximum in spring and a secondary smaller maximum in autumn. Similarly, the highest PM
181 concentration in total particles was recorded during late spring and autumn (Figure S3); a pattern which has also been
182 previously observed at the same location (Laakso et al., 2003;Manninen et al., 2014). The measured DNA concentrations
183 agreed with those obtained for $PM_{2.5}$ samples at a rural mountain site in Germany, where the DNA concentration was in
184 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
185 as those observed in other rural and semi-urban locations (Zhang and Anastasio, 2003;Zhang et al., 2002;Samy et al.,
186 2011;Samy et al., 2013). For example, at two rural sites in US the concentration of FAAs in $PM_{2.5}$ samples was measured
187 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
188 (Zhang and Anastasio, 2003). To our knowledge, there are no studies covering DNA and FAA abundances at the boreal
189 forest region, thus comparison to previous results is not feasible.
190
191 Similar to the annual trend of total DNA and FAAs, the lowest concentration levels of microorganisms were detected
192 during winter and highest during spring and autumn. However, bacterial and fungal DNA reached their maximum levels
193 at different seasons; bacteria peaking in late spring (Figure 2a) and fungal DNA in late summer and autumn (Figure 3).
194 The bacterial DNA abundance in total particles was lower than 900 cells/m^3 during winter and early spring, whereas in
195 late spring the concentration peaked at 58731 cells/m^3. During summer and autumn, the concentration of bacterial DNA
196 was one magnitude lower. In contrast to the total number of bacteria, the highest amounts (>200 cells/m^3) of *Pseudomonas*
197 DNA were measured in late May, late June and October (Figure 2b). In rest of times, the concentration of bacteria

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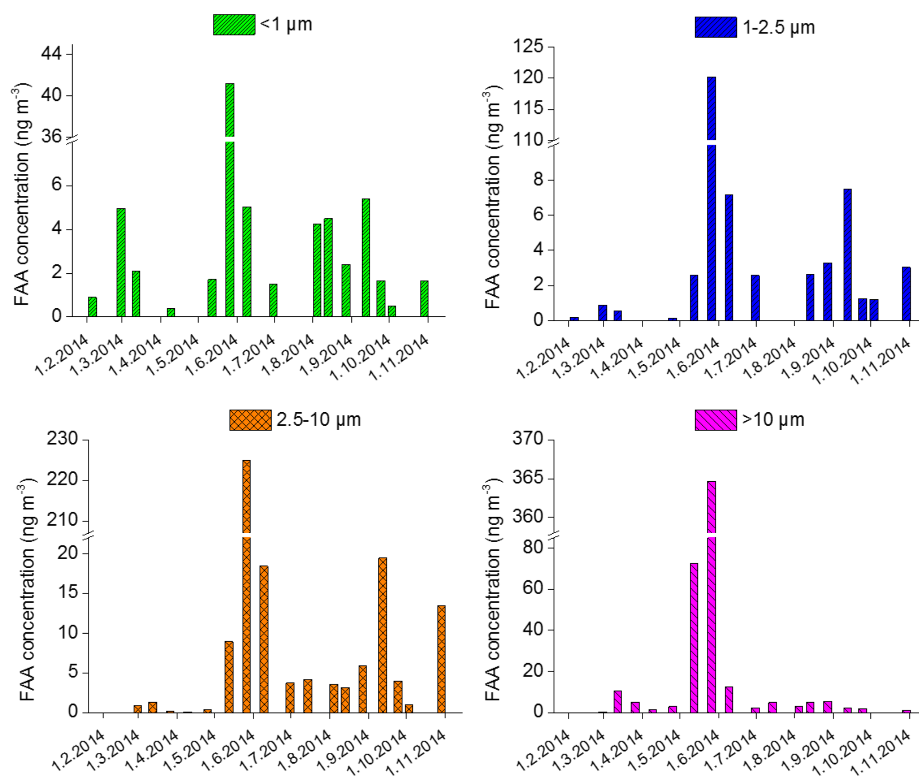
198 belonging to the genus *Pseudomonas* was much lower, and the highest concentration levels in total particles rarely
199 exceeded 50 cells/m^3. The fungal abundance was lower than 300 cells/m^3 during winter and early spring, whereas in late
200 spring and summer the concentrations started to increase and generally exceeded 6000 cells/m^3 in total particles. The
201 highest amounts of fungal DNA were measured in late June (30149 cells/m^3), in August (55839 cells/m^3) and in early
202 October (35050 cells/m^3). In general, the concentration levels of microorganisms vary seasonally and geographically, but
203 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
204 (Burrows et al., 2009b;Després et al., 2012;Spracklen and Heald, 2014). Our observations are consistent with the common
205 trend, when considering that low concentration levels are typically observed at rural locations.
206
207
208



209
210 Figure 1. Free amino acid concentrations over the sampling period in different particle size fractions (date format is
211 dd.mm.yyyy.). Note the different y-axis scales in panels.

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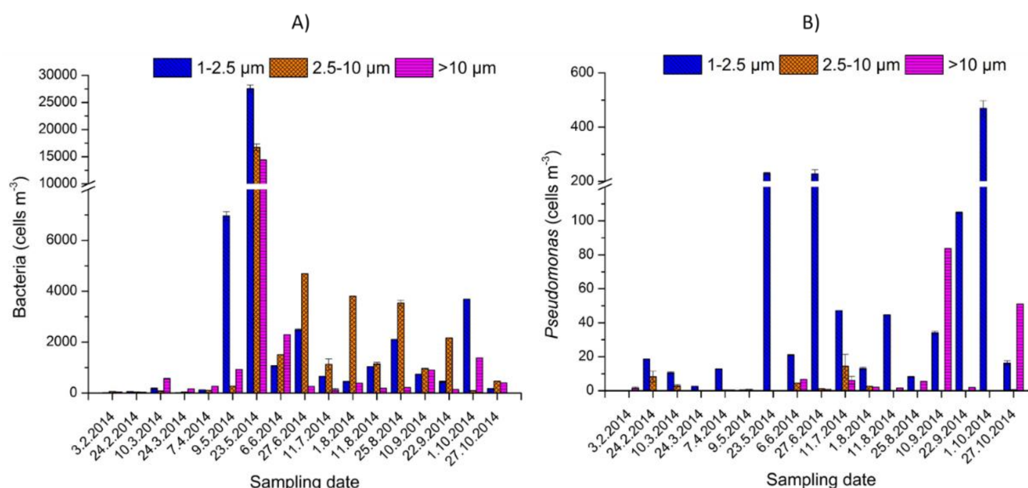
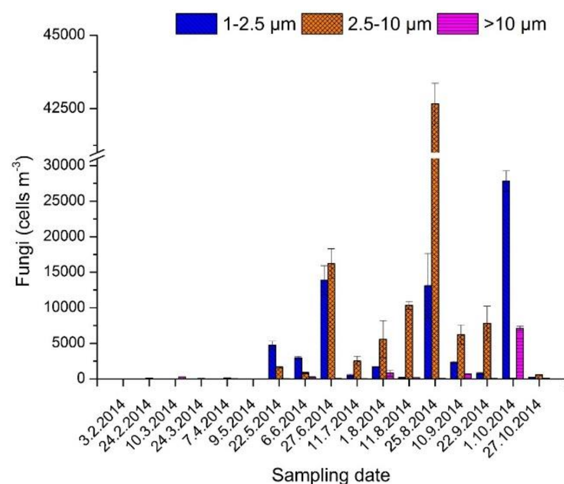


212

213 Figure 2. a) The amount of all bacterial cells over the sampling period, and b) cells of bacteria belonging to genus
214 *Pseudomonas*, detected with qPCR from the aerosol filters, specified by the particle size fractions (date format is
215 dd.mm.yyyy.).

216

217



218

219 Figure 3. The amount of fungal cells and spores over the sampling period, detected with qPCR from the filters of
220 different particle size fractions (date format is dd.mm.yyyy.).

221

222 3.2 Seasonal variation and size distribution of FAAs

223

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

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228 fractions (Figure S4a). In autumn they were mainly present in the particles below 10 µm, and accumulated especially in
229 the size fraction 2.5-10 µm (~51%). During autumn, there was a slight increase in the total FAA concentrations mainly
230 due to the increase in the size fraction 2.5-10 µm (Figure 1).
231
232 Similarly, in the size distribution of individual amino acids some clear tendencies were observed (Figure S5). The results
233 from the PERMANOVA analysis indicated that seasonal variation explained the observed variation across the whole
234 FAA data with statistical significance ($p<0.001$). In addition, the size of the aerosol filter was statistically significant
235 factor for explaining the observed variation across the whole amino acid data (PERMANOVA, $p<0.001$). Glycine was
236 the most dominant amino acid in the size fraction <1 µm, whereas in the larger size fractions its relative abundance was
237 much smaller (Figure S5). On average, glycine accounted 59% of the total FAAs in particles <1 µm, followed by alanine
238 (10%), aspartic acid (9%) and glutamine (9%). In the size fraction 1-2.5 µm, glutamine (42%), glutamic acid (16%) and
239 arginine (15%) were on average the most abundant amino acids. Similarly, in the size fraction 2.5-10 µm glutamine
240 (29%), arginine (15%) and glutamic acid (12%) were the dominant ones, accompanied by proline (7%), serine (7%) and
241 alanine (7%). In the largest size fraction >10 µm proline (36%), arginine (21%), glutamine (10%) and glutamic acid (9%)
242 were the most dominant ones. Our results are in agreement with several studies demonstrating that glycine, arginine,
243 serine, alanine, proline and glutamine are the most commonly abundant FAAs in aerosols (Barbaro et al., 2011;Barbaro
244 et al., 2015;Samy et al., 2011;Samy et al., 2013;Scalabrin et al., 2012;Violaki and Mihalopoulos, 2010;Zhang and
245 Anastasio, 2003), although the relative abundances vary depending on location, season and particle size fraction (Matos
246 et al., 2016).
247
248 3.3 Seasonal variation and size distribution of microorganisms
249
250 A distinct monthly variation in bacterial DNA abundance in different filter size fractions was observed (ANOVA, $p<0.01$).
251 During early spring, bacterial DNA was accumulated mainly in the size fraction >10 µm (~64%, Figure S4b). Oppositely,
252 in late spring the highest numbers of bacteria were detected in the size fraction 1-2.5 µm, whereas during summer, highest
253 numbers of bacteria were discovered in the size fraction 2.5-10 µm (Figure 2a). During summer, bacteria was mainly
254 present in the size faction 2.5-10 µm, which covered on average ~57% of the total bacteria amount. In general, most of
255 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
256 fraction >10 µm. However, on average the abundances did not differ significantly in different size fractions (ANOVA,
257 $p=0.494$). The size distribution observed is in line with that of Burrows et al. (2009b) who summarized that the median
258 aerodynamic diameter of particles containing bacteria is 4 µm at continental sites.
259
260 The abundance of *Pseudomonas* varied with statistically significance between different months (ANOVA, $p<0.05$) and
261 between different size fractions (ANOVA, $p<0.001$). *Pseudomonas* was mainly present (~70%) in the smallest particle
262 size fraction 1-2.5 µm throughout the year (Figures 2b and S4c). The accumulation of *Pseudomonas* in the smallest size
263 fraction is in line with the small aerodynamic size (<1 µm) of common *Pseudomonas* species (Chang et al., 2013;Möhler
264 et al., 2008;Pietsch et al., 2015). Overall, the relative amount of *Pseudomonas* from all bacteria was highest in the size
265 fraction 1-2.5 µm (Figure S6), being highest in late winter (28%), in early spring (15%) and in autumn (23%).
266 Interestingly, in February the relative amount of *Pseudomonas* DNA of the total bacterial DNA was 17% in total particles
267 (Figure S6).

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268

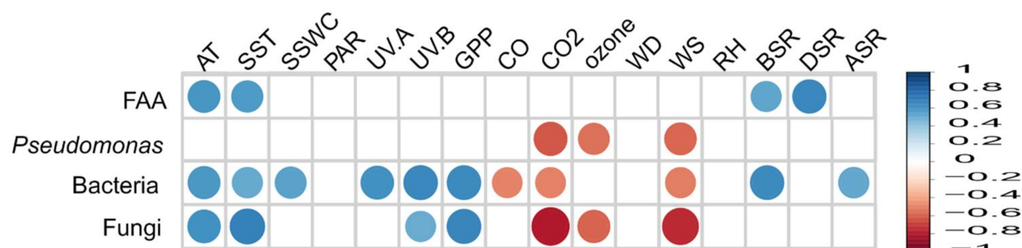
269 The amount of fungal DNA detected in different size fractions varied with statistical significance (ANOVA, $p<0.05$). The
270 fungal DNA was predominantly observed in the particle size fraction 2.5-10 µm, which accounted on average ~58% of
271 the total fungal concentration. The monthly variation in fungal DNA abundance was also statistically significant
272 (ANOVA, $p<0.001$). During May and early June, the fungal DNA was mainly accumulated in the size fraction 1-2.5 µm
273 (~67%, Figure S4d). In late summer, fungal DNA was detected at its highest level in the size fraction 2.5-10 µm (Figure
274 3), and in overall ~66% was accumulated in this size fraction during the summer months. Fungal spores are frequently
275 observed in aerosols in the size range of 2-10 µm (Després et al., 2012). Our observations are consistent with these
276 literature values.

277

278 3.4 Overview of meteorological factors and sources

279

280 The effect of local meteorological factors on the concentration levels of FAAs and microorganism was studied by means
281 of linear regression analysis and a summary of results is shown in Figure 4. Our results reflect rather long-term seasonal
282 effects (more details in Supporting Information), due to the time resolution of sampling. Thus, as expected, the key
283 meteorological factors explaining the observed concentration levels were air temperature, soil temperature, gross primary
284 production and radiation (Figures S7 and S8). In general, we believe that the positive correlation observed between FAAs
285 and microorganism abundances with the before mentioned meteorological variables are closely related to growing season
286 and seasonality, rather than being a decisive effect. These observations are in good agreement with previously reported
287 tendencies covering microorganism and different meteorological factors (Jones and Harrison, 2004;Burrows et al.,
288 2009b;Lighthart, 2000). Soil and vegetation have been previously suggested to be the predominant sources of
289 microorganisms in the atmosphere (Bowers et al., 2013), and our correlation results confirm these sources as discussed
290 below.



291

292 Figure 4. Summary of Spearman correlation results (total particles, $p<0.05$). The colour scale indicates positive/negative
293 correlation. Abbreviations: AT-air temperature; SST-soil surface temperature; SSWC-soil surface water content; WS-
294 wind speed; WD-wind direction; GPP-gross primary production; CO; CO2; ozone concentration; PAR-photosynthetically
295 active radiation; UV-A and UV-B radiation; RH-relative humidity; BSR-rainfall 72 h before sampling; DSR-rainfall
296 during sampling; ASR-rainfall 168 h after sampling.

297

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

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302 transport aerosols due to their relatively low reactivity and long half-lives in the atmospheric condensed phases (Barbaro
303 et al., 2011;Barbaro et al., 2015;Scalabrin et al., 2012;Samy et al., 2013). As the concentration levels of microorganisms
304 were low during winter (Figures 2 and 3), it is likely that the observed FAAs originated from distant sources. However,
305 FAAs and particularly glycine have been also associated with biomass burning emissions (Samy et al., 2013;Violaki and
306 Mihalopoulos, 2010;Zangrando et al., 2016). In Finland wood-burning, that is typical domestic heating system in winter,
307 is the most presumable source of over one third of the $PM_{2.5}$ emissions (Laakso et al., 2003;Saarnio et al., 2012). The
308 relative abundance of glycine in the size fraction 1-2.5 µm was 85% during winter, whereas in other seasons its
309 contribution was negligible. Thus, the observed glycine concentrations might be associated with wood-burning emissions,
310 coming from either local or distant sources. This conclusion is supported by the observed positive correlation between
311 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,
312 Figure S8). Furthermore, only glycine among the FAAs showed positive correlation with PM concentration in the size
313 fraction 1-2.5 µm (R=0.69, p<0.01, data not shown).
314
315 FAA and bacteria concentrations increased during the spring recovery and seemed to be influenced by the increase in
316 local biological activity. During early spring, FAAs and bacteria were mainly present in the largest size fractions (Figures
317 S4a and S4b), which may indicate the presence of pollen in aerosols, since microorganisms might be present on the
318 surface of pollen grains (Puc, 2003). Further, Manninen et al. (2014) observed pollen to be present at SMEAR II station
319 already before the local pollen season, most possible due to long-range transport from central Europe, where growing
320 season had already started. Interestingly though, during our sampling periods the back-trajectory analysis results showed
321 air-masses arriving primarily from Scandinavia and North Europe instead of central Europe. This could imply that the
322 larger sized particles are originating locally from some early pollinator rather than being long-range transported. In
323 addition, we observed an interesting phenomenon in early March when all FAAs, bacterial DNA and fungal DNA were
324 present mainly in the size fraction >10 µm. Then the corresponding relative abundances of their total amounts were 73%,
325 68% and 92%, respectively. During March sampling, the air temperature was almost constantly above zero degrees for
326 the first time in our campaign. The small increase observed in FAA and microorganism concentrations during this time
327 might indicate that a burst in bioaerosols emissions can occur when the temperature increases and snow starts to melt
328 revealing the ground vegetation and decomposed leaf litter for the first time after winter. Similar observations have been
329 previously made at the same site by Schumacher et al. (2013) in a study covering fluorescent biological aerosol particles
330 (FBAP), although they detected an increase in FBAP concentrations in November after first snowfall and snowmelt event.
331
332 The concentrations of FAAs and bacteria reached their maximum levels during the local pollen season peak in May
333 (Figures 1 and 2). Elevated amino acid concentrations have been observed during spring time also in other locations and
334 suspected to be influenced by pollen (Barbaro et al., 2011;Zhang et al., 2002). In our study, especially proline and arginine
335 concentrations increased during the pollen season peak. These amino acids have been shown to contribute significantly
336 to the total amino acid content of birch pollen (Ozler et al., 2009). In this study, the FAA concentrations increased in all
337 the size fractions (Figure 1), possibly due to rupture of relatively large (~30 µm) pollen grains (Taylor et al., 2004;Visez
338 et al., 2015), supported by visibly yellow impactor plates in all the size fractions. During the peak in FAA and bacteria
339 concentrations, the air-masses were arriving in Hyytiälä from Eastern Europe and Baltic Sea. Due to the absence of
340 pollinating species in the sea region, the observed high concentration levels of FAAs and bacteria could be mainly
341 explained by local sources in the boreal forest. This is corroborated by the presence of methionine, cysteine and tryptophan

342 only in these aerosols samples. Particularly these amino acids are known to be highly reactive with short half-lives in the
343 atmospheric condensed phases (Scalabrin et al., 2012;Milne and Zika, 1993;McGregor and Anastasio, 2001). Thus, the
344 local pollen season likely explains our observations, although other factors may partly contribute. For example, high
345 ozone concentration and strong UV radiation were recorded during this time period, and in some studies ozone has been
346 demonstrated to promote the decomposition of protein and peptides into free amino acids increasing the FAA
347 concentrations (Samy et al., 2013;Mumford et al., 1972). Positive correlation was observed between FAA concentration
348 in the size fraction >10 µm with ozone (R=0.58, p<0.05, Figure S8), which was mainly attributed to coinciding peaks in
349 concentration levels during pollen season.
350
351 Opposite to FAA concentrations being highest in the largest size fractions, bacterial DNA abundance was highest in the
352 size fraction 1-2.5 µm during the pollen season peak. As spring proceeds, new foliage growth enables larger surface area
353 for epiphytic bacteria to occupy and grow on. Bacterial cells may be lifted from leaves into the air on pollen (Jones and
354 Harrison, 2004), which may explain the peak in bacterial abundances during this season. Bacteria may be present in the
355 air as individual cells, clump of cells or attached to other particles, such as pollen grains and leaf fragments. We propose
356 that during the local pollen season and under favourable meteorological conditions, it is possible to observe a significant
357 increase in bacterial concentrations. However, most likely the magnitude of this increase varies from year-to-year and is
358 also closely related to varying pollen abundances.
359
360 Although we assume that the elevated bacterial DNA abundances are mainly related to pollen and vegetation during
361 spring, other sources might contribute as well. For example, soil water content and bacteria concentration correlated
362 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
363 snow melts, the soil moisture increase enhances the bacterial/microbial growth (Burrows et al., 2009b). When spring
364 proceeds and air and soil temperatures increase, the relatively dry soil surface layer might enable the dispersal of bacteria
365 via soil resuspension. Similarly, some studies indicate that soil-derived bacteria dominate during spring time (Rathnayake
366 et al., 2017), while others indicate that soil sources dominate during late summer and fall (Bowers et al., 2013). In addition,
367 rainfall may promote the bacterial growth on vegetation surfaces, leading to increased population sizes, which may
368 become airborne following rainfall (Bigg et al., 2015). We observed positive correlation between bacteria concentration
369 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
370 previous reports related to the relationship between rainfall and bioaerosols (Huffman et al., 2013;Prenni et al.,
371 2013;Gosselin et al., 2016;Rathnayake et al., 2017;Morris et al., 2016;Bigg et al., 2015), our results corroborate the
372 positive effect of bacteria abundances following rainfall.
373
374 The abundance and relative size distribution of fungal DNA varied seasonally and started to increase in summer (Figure
375 3). The relative distribution of fungal DNA to different size fractions correlated with the relative humidity (R=-0.53,
376 *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
377 more fungi were accumulated in the smallest size fraction. Since fungal cells are typically larger than 2.5 µm, the observed
378 fungal DNA in the 1-2.5 µm fractions during early summer is probably originated from the spores of moulds or ruptured
379 cells (Reponen et al., 2001). In addition, the size of fungal spores has been found to depend on the relative humidity, i.e.
380 higher the relative humidity is the larger the spores are (Reponen et al., 1996;Madsen, 2012). The spores of basidiomycete
381 fungi are in general larger than those of ascomycete (Reponen et al., 2001;Manninen et al., 2014;Hussein et al.,

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382 2013;Fröhlich-Nowoisky et al., 2012), and the basidiomycete fungi are known to sporulate mainly during autumn when
383 the relative humidity is high (Kauserud et al., 2011). Further, in the previous study by Manninen et al. (2014) the spores
384 of Basidiomycota dominated the phylum-level distribution of fungal spores in the autumn. Our results where fungal DNA
385 accumulated in the 2.5-10 µm size fraction during autumn are in agreement with this study. Consequently, the annual size
386 distribution of fungi can be expected to be similar from year-to-year in the boreal forest.
387
388 Opposite to the trend of fungi, FAA and bacterial DNA concentrations decreased after the spring pollen season peak. It
389 is noteworthy that both bacterial and fungal DNA were accumulated during the mid-summer to early fall in the particle
390 size fraction 2.5-10 µm (Figure S4b and S4d). In summer, the air masses arrived Hyytiälä mainly from Scandinavia and
391 Baltic Sea regions. In early August the air masses reached Hyytiälä from a large forest fire region in Sweden. Interestingly
392 derived from our samples, glycine was detected in relatively high concentrations (~2.9 ng/m^3) in the size fraction <1 µm.
393 This observation supports the hypothesis that the presence of glycine is partially related to biomass burning emissions
394 and long-range transport. However, no distinct effects on the origin of air-masses were seen in the levels of FAAs or
395 microorganisms. Although long-range transport cannot be completely ruled out, the accumulation of bacteria and fungi
396 in the particle size fraction 2.5-10 µm suggest that primarily local forest or nearby sources affect the concentration levels
397 of microorganisms during summer. For example, leaf-associated bacteria are known to contribute to the total bacterial
398 amount during summer (Bowers et al., 2013). Statistically significant correlation was observed between gross primary
399 production (R=0.79, P<0.001, Figure S7) and photosynthetically active radiation (R=0.69, P<0.01, Figure S7) with
400 bacteria in the size fraction 2.5-10 µm, possibly indicating the importance of vegetation especially during summer and
401 growing season.
402
403 The abundance of *Pseudomonas* was detected at its highest level in early October in the size fraction 1-2.5 µm, when it
404 was 2-fold higher than in other months (Figure 2b). It is suggested that an increase in the *Pseudomonas syringae*
405 population size on vegetation may occur during or after rainfall (Hirano et al., 1996;Bigg et al., 2015;Morris et al., 2016).
406 Even though no correlation was observed between average rainfall recorded before or during sampling with *Pseudomonas*
407 on an annual scale, we identified interesting separate individual rainfall events. The maximum single rainfall event was
408 recorded within 72 h before the above mentioned early October sampling (Figure S9). As far as we could tell, this was
409 the main exception in meteorological conditions during the period studied, i.e. the only factor potentially explaining the
410 observed increase in *Pseudomonas* concentration. Further, the back-trajectory analysis results showed air-masses arriving
411 from Norwegian Sea and North Sea via the Scandinavian Mountains, and to our knowledge these areas do not contain
412 sources that could explain the increase in *Pseudomonas* concentration. When considering the other maximum rainfall
413 events recorded before sampling, two out of three of the summer-autumn maximum events coincided with high
414 *Pseudomonas* concentrations (Figure S9). These findings corroborate the evidence of a potential relationship between
415 rainfall and *Pseudomonas*. However, the lack of statistically significant correlation suggests that other factors are
416 contributing to observed variation in *Pseudomonas* abundance.
417
418 Overall, considering the effect of rainfall on the levels of bacteria, fungi and FAAs, some interesting patterns were
419 observed. As mentioned earlier, the bacterial DNA concentration in size fraction 1-2.5 µm correlated with rainfall
420 recorded prior to sampling. Further, we observed positive correlation between FAA concentration and rainfall during
421 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).

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422 Interestingly, bacterial DNA and fungal DNA abundances correlated positively in the size fraction 2.5-10 µm with rainfall
423 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
424 evidence that cloud-active particles larger than ~1 µm are biological in origin (Haga et al., 2014;Hassett et al., 2015;Mason
425 et al., 2016;Möhler et al., 2007), and in mixed-phase cloud conditions, bioaerosols may play an important role in triggering
426 rainfall. Collectively, the different correlations observed between rainfall and bioaerosol components suggest that a
427 potential feedback mechanism may persist at the boreal forest. This conclusion is based on the assumption that the
428 recorded rainfall events after sampling were produced at least partially on a local scale and that the meteorological factors
429 were favourable to formation of rainfall. We acknowledge, that the positive correlation observed between
430 microorganism's abundances with rainfall recorded following sampling, may be causal in nature. However, in light of the
431 recent findings, the possibility of a feedback mechanisms cannot be ignored (Bigg et al., 2015;Morris et al., 2016;Huffman
432 et al., 2013). Nonetheless, additional high time resolution and long-term measurements are needed to confirm the
433 observations presented in this research.
434
435 **4 Conclusions**
436
437 Considering the observations made in this and previous studies (Manninen et al., 2014;Schumacher et al., 2013), some
438 general conclusions related to bioaerosol abundances and size distribution at the boreal forest site can be drawn.
439 Correlation with meteorological parameters might indicate biosphere-atmosphere interactions through bioaerosols with
440 possible climate effects. In early spring, bioaerosol components are mainly accumulated in the size-fractions >10 µm.
441 According to our results, the spring pollen season has an impact on the pollen levels as well as on the bacterial abundances.
442 Elevated bioaerosol abundances occur during the pollen season (Manninen et al., 2014), and based on our estimation even
443 up to ~77% of total PM may be of biological origin (SI). Thus, the magnitude of biological cloud-active particles during
444 this period may be atmospherically relevant (Diehl et al., 2002;Diehl et al., 2001;Pummer et al., 2012;Pummer et al.,
445 2015). The importance of rainfall was observed in this study as well as to some extent in previous studies conducted at
446 the same site (Manninen et al., 2014;Schumacher et al., 2013). Positive correlation was observed between bioaerosol
447 component abundances with rainfall recorded before and during sampling as well as with rainfall recorded after sampling.
448 During late summer and autumn, the accumulation of bioaerosols in the size fraction 2.5-10 µm was evident (Figure S4).
449 In autumn the relative amount of biomass in PM was estimated to be around ~10% (SI). Modelling studies have suggested
450 that microorganisms may play an important role in the hydrological cycle in the boreal region(Sesartic et al., 2012;Sesartic
451 et al., 2013). However, the magnitude of upward lifting of microorganisms remains to be solved, and thus our results are
452 preliminary in nature and need to be confirmed.
453
454
455
456 **Author contribution**

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

460 **Competing interests**

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461 The authors declare that they have no conflict of interest.

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468

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666 Table 1. Average (±standard deviation) concentration and range of each component measured and the number of filter
667 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)