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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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S1. Materials and reagents.

L-Tryptophan, L-Phenylalanine, L-Leucine, L-Isoleucine, L-Methionine, L-Tyrosine, L-Valine, L-Proline, L-Alanine, L-Threonine, Glycine, L-Glutamic acid, L-Aspartic acid, L-Glutamine and L-Lysine hydrochloride were purchased from Seikagaku Kogyo Co. (Tokyo, Japan). L-Histidine (purity ≥99%) and L-Arginine (purity 99%) were purchased from Merck (Darmstadt, Germany). L-Serine (purity ca. 99%) was purchased from Ega-Chemie (Steinheim, Germany) and D/L-Cysteine (purity 97%) was purchased from Aldrich-Chemie (Steinheim, Germany). Asparagine anhydrous (purity ≥98%) was purchased from Sigma Chemical Co (St. Louis, USA). L-Phenylalanine-3,3-d_2 (98 atom % D), Glycine-2,2-d_2 (98 atom % D) and L-Lysine-4,4,5,5-d_4 hydrochloride (98 atom % D, 98% (CP)) were purchased from Aldrich (St. Louis, USA) and these deuterated amino acids were used as internal standards (ISTD). Formic acid (purity 99%) was purchased from Acros Organics (Geel, Belgium) and acetic acid (purity ≥99%) was from Fluka Chemie GmbH (Buchs, Switzerland). Toluene (purity 100%) and *n*-hexane (purity 98%) were purchased from VWR Chemicals (Fontenay-sous-Bois, France). Acetonitrile (purity ≥99.9%) from Sigma-Aldrich (St. Louis, USA) and ultrapure water (Millipore DirectQ-UV, Billerica, MA, USA) were used for the HPLC analysis.

Primary standard solutions of amino acids and ISTDs were prepared separately by weighting and diluting the crystalline compounds in 0.1% formic acid. The primary solution concentrations were approximately 2000 µmol/L-8000 µmol/L. A working mixture standard solution containing 80 µmol/L of each amino acid was prepared in 0.1 % formic acid. A working ISTD mixture solution was prepared similarly. Standard solutions at concentrations of 8, 40, 80, 160, 240, 320 and 400 nmol/L of each amino acid were prepared by pipetting appropriate amount of working solution and diluting with 0.1 % formic acid. All the standard solutions were containing 100 nmol/L of each ISTD. Working standard solutions and calibration solutions were prepared each day of analysis. All the solutions were stored in the dark at 4 °C.

Table S1. Aerosol sampling dates and sampling volume. One sampling date was excluded from the data set A due to deviating sampling period length.

Set A			Set B		
Start	Stop	sampling volume (m^3)	Start	Stop	sampling volume (m^3)
31.1.2014 9:30	3.2.2014 8:40	133	3.2.2014 8:40	5.2.2014 9:04	92
21.2.2014 9:04	24.2.2014 9:44	124	25.2.2014 9:00	28.2.2014 10:10	121
7.3.2014 8:47	10.3.2014 8:43	119	10.3.2014 8:43	12.3.2014 8:45	80
21.3.2014 9:02	24.3.2014 9:20	132	24.3.2014 9:20	26.3.2014 8:25	86
4.4.2014 8:02	7.4.2014 7:58	133	7.4.2014 7:58	9.4.2014 7:39	92
~~16.4.2014 7:44~~	~~23.4.2014 8:15~~	~~300~~	23.4.2014 8:15	25.4.2014 7:35	84
7.5.2014 7:40	9.5.2014 7:54	80	9.5.2014 7:54	12.5.2014 7:50	125
21.5.2014 7:39	22.5.2014 7:30	93	22.5.2014 7:30	26.5.2014 9:12	144
4.6.2014 7:50	6.6.2014 8:03	93	6.6.2014 8:03	9.6.2014 7:46	138
25.6.2014 7:33	27.6.2014 7:47	79	27.6.2014 7:47	30.6.2014 8:13	118
9.7.2014 7:41	11.7.2014 7:58	89	11.7.2014 7:58	14.7.2014 6:48	128
30.7.2014 7:00	1.8.2014 6:56	96	1.8.2014 6:56	4.8.2014 7:09	142
8.8.2014 6:58	11.8.2014 7:04	141	11.8.2014 7:04	13.8.2014 6:55	92
22.8.2014 7:00	25.8.2014 7:04	122	25.8.2014 7:04	27.8.2014 7:04	83
8.9.2014 7:58	10.9.2014 8:00	82	10.9.2014 8:00	12.9.2014 8:15	84
19.9.2014 7:59	22.9.2014 8:05	119	22.9.2014 8:05	24.9.2014 8:25	76
29.9.2014 7:58	1.10.2014 8:01	81	1.10.2014 8:01	3.10.2014 7:10	83
24.10.2014 8:35	27.10.2014 9:27	114	27.10.2014 9:27	29.10.2014 9:02	79

S2. Determination of amino acids and method validation.

Aerosol sample extraction

Ultrasonic assisted extraction with 0.1% formic acid as solvent was used for the extraction of free amino acids from the collection substrates. All the tools used in the following process were cleaned with water and methanol under sonication and dried in an oven at 150 °C. Filter was removed from the petri dish with tweezers and placed into a test tube. Due to the grease used to cover the filters, there was a spot of grease left onto the surface of the petri dish. In order to recover all the particles, the grease spot was dissolved with hexane and the solute was pipetted into the test tube. Filter was spiked with 100 µL of isotopically labelled amino acid internal standard mixture solution. The sample was sonicated at room temperature for 15 minutes with 2 mL of 0.1% formic acid. Sonication was repeated twice with 2 mL and 1 mL of 0.1 % formic acid for 10 minutes each time. Finally, 1 mL of toluene was added into the extract solution in order to ensure partitioning of the grease into organic phase. The solution was vortexed and centrifuged. Aqueous layer was separated and filtered through a 0.45 µm filter (Merck Millipore Ltd., Tullagreen, Ireland) into a 5 mL volumetric flask. The samples were stored at 4 °C and analyzed within 72 hours. Blank filters were processed simultaneously with the real samples and used for blank correction.

Chromatographic analysis

The analyses were performed with an Agilent 1260 Infinity HPLC coupled to an Agilent 6420 triple quadrupole mass spectrometer (Agilent Technologies, USA). Electrospray ionization in positive mode was used. The column used was a SeQuant ZIC-cHILIC (100 x 2.1 mm, particle size 3 µm, 100 Å, Merck KGaA, Germany). The column temperature was maintained at 30 °C and the injection volume was 3 µL. Eluent A was 0.01% acetic acid in acetonitrile and eluent B was 0.01% acetic acid in ultrapure water. The optimized gradient program was as follows: 0-10 min, 20% eluent B; 10-20 min, 60% eluent B; 20-40 min, 60% eluent B; 40-45 min, 20% eluent B and equilibration for 25 min. All the mobile phase gradient changes were linear and the flow rate was 150 µL/min. Mass spectrometer (MS) source parameters were optimized and they were set as follows: drying gas temperature 350 °C, drying gas flow rate 12 L/min, nebulizer pressure 40 psi and capillary voltage +4000 V. The MS was operated in a dynamic multiple reaction monitoring (MRM) mode. Precursor ions and product ions were selected based on observations during method development. Fragmentor voltage, collision energy and cell accelerator voltage were optimized for each amino acid separately (Table S2). An example chromatogram of the analysis of amino acid standard solution is shown in Figure S1.

Table S2. Optimized dynamic multiple reaction monitoring (MRM) mode settings for each amino acid.

Amino acid	Precursor ion	Product ion	Fragmentor voltage (V)	Collision energy (V)	Cell Accelerator Voltage (V)
Alanine (Ala)	90	44	70	10	3
Arginine (Arg)	175	70	80	15	3
Asparagine (Asn)	133	87	70	5	3
Aspartic acid (Asp)	134	74	75	15	3
Cysteine (Cys)	122	76	70	15	3
Glutamine (Gln)	147	130	70	5	3
Glutamic acid (Glu)	148	130	70	5	3
Glycine (Gly)	76	30	70	5	3
Glycine-d_2 (Gly-d_2)	78	32	70	10	3
Histidine (His)	156	110	80	15	3
Isoleucine+Leucine (Ile+Leu)	132	86	75	10	3
Lysine (Lys)	147	130	70	5	3
Lysine-d_4 (Lys- d_4)	151	134	70	5	3
Methionine (Met)	150	133	70	5	3
Phenylalanine (Phe)	166	120	80	10	3
Phenylalanine-d_2 (Phe-d_2)	168	122	80	10	3
Proline (Pro)	116	70	80	15	3
Serine (Ser)	106	60	70	10	3
Threonine (Thr)	120	74	75	10	3
Tryptophan (Trp)	205	188	80	5	3
Tyrosine (Tyr)	182	136	75	10	3
Valine (Val)	118	72	75	10	3

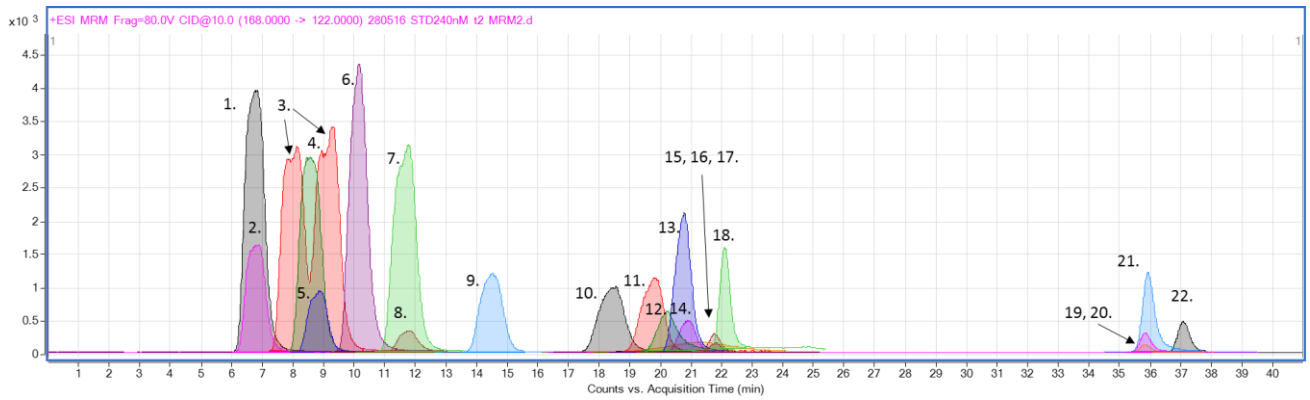

Figure S1. Dynamic multiple reaction monitoring chromatogram of analysis of 240 nmol/L standard solution containing 20 amino acids and three internal standards. Peak identification: 1. Phe, 2. Phe-d_2, 3. Ile+Leu, 4. Trp, 5. Met, 6. Pro, 7. Val, 8. Cys, 9. Tyr, 10. Thr, 11. Ala, 12. Glu, 13. Gln, 14.Asn, 15. Asp, 16. Gly, 17. Gly-d_2, 18. Ser, 19. Lys, 20. Lys-d_4, 21. His and 22. Arg.

Validation experiments and quality control

The amino acid concentrations were quantified by using internal standard method with three isotopically labelled amino acids. Prior to analysis of aerosol samples, linear calibration curves were prepared each time by analyzing standard solutions (8-400 nmol/L, two repetitions per standard). On average, six aerosol samples were analyzed by using a single calibration curve. This was done in order to minimize day-to-day signal variation. Most of the amino acid calibration curves were forced through origin, except for glycine and histidine the lowest point used was the LOQ value. There were few aerosol samples that had relatively high concentration levels of amino acids present, exceeding the normal calibration range. In order to quantify the amino acid concentrations in these samples, 700-50000 nmol/L standard solutions were analyzed and quadratic fit was applied.

The limit of quantification (LOQ) for the entire analytical procedure was determined by processing and analyzing multiple field blank filters (n=14). Equation LOQ=A+10*SD, where A=average peak area and SD=standard deviation, was used for the calculations of LOQ values. Some of the amino acids were absent in the blank samples and thus the LOQ was estimated by using signal-to-noise ratio of ten.

Different types of recovery experiments were done in order to: i) determine extraction recovery and ii) examine matrix effects. First, extraction recovery was determined by spiking four blank filters with 100 µL of 10 µmol/L amino acid mixture solution (c=200 nmol/L in solution) and the filters were processed in the same manner as real samples. These spiked blank filter recovery experiments were done in order to determine the recovery of amino acids from the filters, i.e. to test the extraction efficiency. The second type of recovery experiments were done with real samples, that were already extracted and analyzed. In these experiments, 195 µL of real sample was pipetted into a LC vial and 5 µL of amino acid mixture solution was added into the solution. These experiments were done with two different spiking concentrations (c=50 nmol/L and c=100 nmol/L in solution) and with four samples per concentration level. These recovery experiments, i.e. matrix matched recovery experiments, were done in order to simulate possible matrix effects.

The expanded measurement uncertainty (U) was estimated applying single laboratory validation Nordtest-approach.[1] The intermediate precision and recovery experiment results were used in the calculation of U by using the 95% confidence limit.

Validation results

All the relevant calibration curve information, such as calibration range, R^2 and ISTD for each amino acid, is shown in Table S3. The correlation coefficients of the linear calibration curves were generally above 0.9983. Linearity was evaluated based on residual analysis. Calibration curve slope stabilities were in the range 2.5-27.8% over a one month time period (10 separate calibration curves). In addition, the retention time variation was below 5% with all of the amino acids during a one month time period. Overall, the method stability was acceptable and the short analysis sequences ensured that the method performance was maintained accurate.

The LOQ for each amino acid and the intermediate precision close to the LOQ values are listed in Table S3. The LOQs varied between 4-160 nmol/L, corresponding to 0.03-1.18 ng/m^3 in atmospheric aerosol samples (estimated based on average sampling volume of 105 m^3). The intermediate precision was calculated from one month time period (22 repetitions per standard) and it is expressed as relative standard deviation. With most of the compounds, the intermediate precision was below 18%, but with tryptophan the intermediate precision was throughout the calibration range approximately 25%.

The different recovery experiments results are shown in Table S4. Some values are left blank because they were below the LOQ. The recovery from the spiked blank filters was around 100±20%. In the matrix spiking experiments, signal enhancement was observed with some of the compounds. The recovery was in the range of 88-120%, which was still considered to be acceptable. Based on both recovery experiments, it can be concluded that the extraction efficiency was sufficient and the matrix effects were not significantly affecting

the results. The expanded measurement uncertainty (U) with 95% confidence limit is shown in the far most right column of Table S4. The U varied between 31-58%, which was considered to be fit-for-purpose.

Table S3. Information about calibration curve, LOQ and intermediate precision.

Amino acid	Internal standard	Calibration curve slope	Slope stability [a] (RSD%)	Calibration curve range (nmol/L)	R^2	LOQ (nmol/L)	Intermediate precision[b] (RSD%)
Ala	Gly-d2	0.044	2.5	40-400	0.9998	20	16.2
Arg	Lys-d4	0.018	5.0	40-400	0.9983	30	9.3
Asn	Gly-d2	0.018	6.2	8-400	0.9998	8	11.6
Asp	Gly-d2	0.013	23.0	40-400	1.0000	20	17.4
Cys	Gly-d2	0.010	8.0	40-400	0.9991	20	17.9
Gln	Gly-d2	0.058	12.2	8-400	0.9993	5	15.5
Glu	Gly-d2	0.023	6.7	8-400	0.9998	8	11.5
Gly	Gly-d2	0.008	5.8	40-400	0.9999	40	10.2
His	Lys-d4	0.046	8.7	160-400	0.9999	160	11.3
Ile+Leu	Phe-d2	0.010	5.8	16-800	1.0000	10	7.3
Lys	Lys-d4	0.011	4.7	160-400	0.9998	130	12.5
Met	Phe-d2	0.002	4.8	8-400	0.9999	8	7.8
Phe	Phe-d2	0.010	1.7	8-400	1.0000	4	2.6
Pro	Phe-d2	0.009	3.7	8-400	0.9999	5	7.2
Ser	Gly-d2	0.035	6.2	80-400	1.0000	70	10.2
Thr	Gly-d2	0.040	5.8	40-400	0.9993	13	11.3
Trp	Phe-d2	0.004	27.8	8-400	0.9998	8	25.3
Tyr	Phe-d2	0.003	9.7	8-400	1.0000	7	9.1
Val	Phe-d2	0.009	5.2	8-400	1.0000	7	9.7

[a] One month time period, 10 separate calibration curves

[b] One month time period, 22 repetitions per standard

Table S4. Results from different recovery experiments and expanded measurement uncertainty (U).

	50 nM spiked sample[a]	100 nM spiked sample[b]	200 nM spiked blank[c]	
Amino acid	RR% (±sd)	RR% (±sd)	RR% (±sd)	U (%)
Ala	114±13	114±2	101±6	39
Arg	88±5	103±10	97±2	33
Asn	108±8	112±5	100±3	30
Asp	113±18	108±12	103±12	54
Cys	91±11	100±6	94±3	41
Gln	89±9	101±17	93±5	42
Glu	104±8	111±15	96±3	26
Gly	122±18	116±7	105±9	58
His	-	-	102±2	38
Ile+Leu	116±7	110±7	100±2	37
Lys	-	103±6	99±4	30
Met	119±8	115±7	97±1	43
Phe	115±8	112±4	99±2	34
Pro	112±11	113±7	98±4	35
Ser	-	120±10	109±18	48
Thr	115±10	116±5	96±4	40
Trp	110±8	99±6	82±8	56
Tyr	110±10	108±9	97±3	33
Val	112±8	111±8	100±3	31

[a] 195 µL sample+5 µL 2000 nmol/L standard solution (*N*=4)
[b] 195 µL sample+5 µL 4000 nmol/L standard solution (*N*=4)
[c] blank filter spiked (*N*=4)

S3. DNA extraction and DNA amplification.

The total nucleic acids extraction with PowerWater DNA Isolation Kit (MoBio Laboratories, USA) was performed according to the manufacturer's instructions with the following modifications. The aerosol filters were inserted into the 5 mL PowerWater Bead tube and 1 mL of lysis buffer was added. The samples were incubated in the lysis buffer at 65 °C for 1 h and then homogenized with horizontal vortexing for 5 min at maximum speed. The remaining steps were carried out according to the supplier´s protocol, and the DNA was eluted into 100 µL of elution buffer.

Blank filters (n=6) were processed and no contamination was observed in qPCR. DNA concentrations were below 0.282 ng/µL corresponding to 0.003 ng/m^3 in atmospheric aerosol samples (estimated based on average sampling volume of 105 m^3).

The qPCR reactions were carried out with Bio-Rad CFX96 iCycler on 96-well white polypropylene plates (Bio-Rad, USA). The qPCR reaction mixtures were done according to manufacturer's instructions. The bacterial and *Pseudomonas* genus specific reaction mixtures contained SsoAdvanced universal SYBR Green supermix (Bio-Rad, USA) at final concentration of 1x, 5 µL of template DNA, 250 nM of forward and reverse primers (Table S5). The reaction volume was adjusted to 20 µL with nuclease-free water. With fungal primers, the reaction mixture was otherwise the same, but the FF390 primer was in concentration of 250 nM and the FR1 primer in 200 nM. The qPCR reactions were conducted according to the manufacturer's protocol with combined annealing and extension (Table S5). For quantification, fluorescence was measured during the elongation step. From each DNA sample and standard, the three technical replicates were prepared, and from each mastermix three negative controls were analysed. The qPCR products were analysed in 1.5 % (w/v) agarose gel (BioTop) and visualized with 0.3 % (w/v) ethidium bromide (Sigma-Aldrich) under UV-light to ensure the correct amplicon length and the specificity of amplification (Table S5). Detection limits of the used qPCR method were calculated from the standard curves separately for each primer pair (Table S5). The genus *Pseudomonas* specific primers produced nonspecific amplicons when the amount of *Pseudomonas* DNA in the reaction was under detection limit (Table S5). These data points were set as below "detection limit" in *Pseudomonas* qPCR-results.

Table S5. Primer pairs used for qPCR assays; primer sequences and approximate amplicon lengths and detection limit.

Target group	Primer name	Sequence (5´->3´)	Approximate amplicon length (bp)	Combined annealing and extension conditions	Amplification efficiency (%)	Detection limit (cells of microorganism)	Reference
All Bacteria	Eub338f	ACT CCT ACG GGA GGC AGC AG	200	temp 55 °C time 30 s	96 %	20 (0.13 cells/m^3)	Fierer et al. 2005[2]
	Eub518r	ATT ACC GCG GCT GCT GG					
Genus: *Pseudomonas*	Eub338f	ACT CCT ACG GGA GGC AGC AG	150	temp 55 °C time 30 s	97 %	16 (0.15 cells/m^3)	Fierer et al. 2005[2]
	PseudoR	TCG GTA ACG TCA AAA CAG CAA AGT					Purohit et al. 2003[3]
All Fungi	FF390	CGA TAA CGA ACG AGA CCT	350	temp 60 °C time 45 s	89 %	6 (0.06 cells/m^3)	Vainio et al. 2000[4]
	FR1	AIC CAT TCA ATC GGT AIT					

Table S6. Concentration (ng/m^3) of each amino acid found in different particle size fractions. Average concentration (±standard deviation), detected range and detection frequency (d.f.) percentage are reported.

	<1 µm			1-2.5 µm			2.5-10 µm			>10 µm		
Amino acid	Average±sd	Range	d.f. (%)	Average±sd	Range	d.f. (%)	Average±sd	Range	d.f. (%)	Average±sd	Range	d.f. (%)
Ala	0.47±0.43	0.12–1.65	65	0.47±0.76	0.09–2.61	63	0.87±1.24	0.12–4.57	67	1.07±2.06	0.15–6.89	56
Arg	3.18±5.76	0.19–11.82	24	4.95±12.94	0.39–39.44	56	7.51±20.44	0.29–69.09	61	9.32±26.94	0.34–101.77	78
Asn	0.19±0.22	0.04–0.44	18	0.27±0.45	0.08–1.37	50	0.44±0.72	0.07–2.65	67	1.17±2.25	0.08–6.71	67
Asp	0.58±0.82	0.18–2.99	65	1.06±2.12	0.15–6.31	50	1.62±3.53	0.17–12.17	61	2.80±6.66	0.16–19.23	44
Cys	-	-	-	0.1	-	6	0.21	-	6	0.39	-	6
Gln	0.48±0.54	0.09–2.02	65	1.62±2.04	0.07–7.41	81	2.31±3.39	0.05–13.77	94	1.93±4.55	0.07–19.36	94
Glu	0.53±1.04	0.05–3.81	71	1.33±2.71	0.07–9.44	69	1.72±3.75	0.12–15.12	83	1.86±4.41	0.07–17.46	89
Gly	2.06±1.44	0.39–4.78	76	0.45±0.20	0.19–0.68	31	0.55±0.34	0.18–0.90	39	0.41±0.40	0.15–1.00	22
His	-	-	-	1.23	-	6	2.1	-	6	2.94±0.85	2.34–3.54	11
Ile+Leu	0.39±0.44	0.09–0.70	12	0.90±1.37	0.07–2.48	19	0.72±1.35	0.08–4.51	56	1.01±2.23	0.08–7.46	61
Lys	0.95	-	6	3.59	-	6	6.27	-	6	9.27	-	6
Met	0.05	-	6	0.35	-	6	0.67	-	6	0.68±0.54	0.30–1.07	11
Phe	0.11±0.09	0.05–0.18	12	0.25±0.38	0.03–0.82	25	0.28±0.45	0.04–1.53	56	0.36±0.73	0.03–2.35	56
Pro	1.02±3.04	0.03–9.68	59	3.57±11.35	0.03–37.80	69	5.37±19.83	0.07–79.67	89	11.72±36.79	0.04–151.83	94
Ser	0.74	-	6	2.08	-	6	1.63±1.25	0.29–3.66	33	1.77±2.42	0.31–5.36	22
Thr	0.18±0.21	0.08–0.65	41	0.74±1.04	0.06–1.94	19	0.68±1.10	0.10–3.53	50	0.88±1.82	0.10–5.34	44
Trp	0.14	-	6	0.61	-	6	0.50±0.62	0.06–0.93	11	0.68±0.77	0.07–1.55	17
Tyr	0.14±0.09	0.08–0.20	12	0.43±0.48	0.09–0.77	13	0.36±0.48	0.06–1.41	39	0.51±0.82	0.05–2.15	33
Val	0.23±0.25	0.05–0.41	12	0.46±0.65	0.04–1.20	19	0.36±0.65	0.05–2.26	61	0.50±1.05	0.04–3.51	61

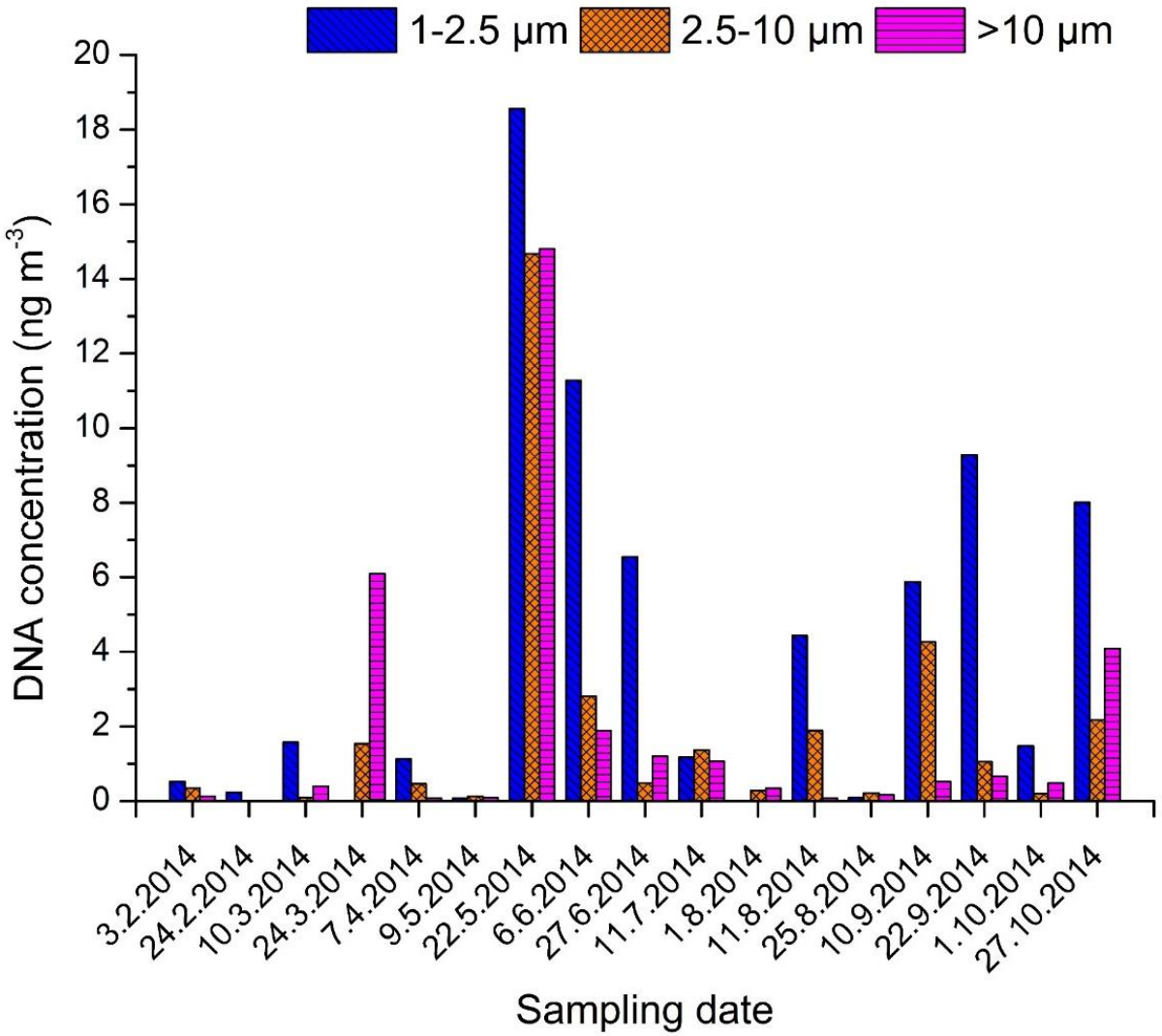

Figure S2. DNA concentration detected over the sampling period in different particle size fractions.

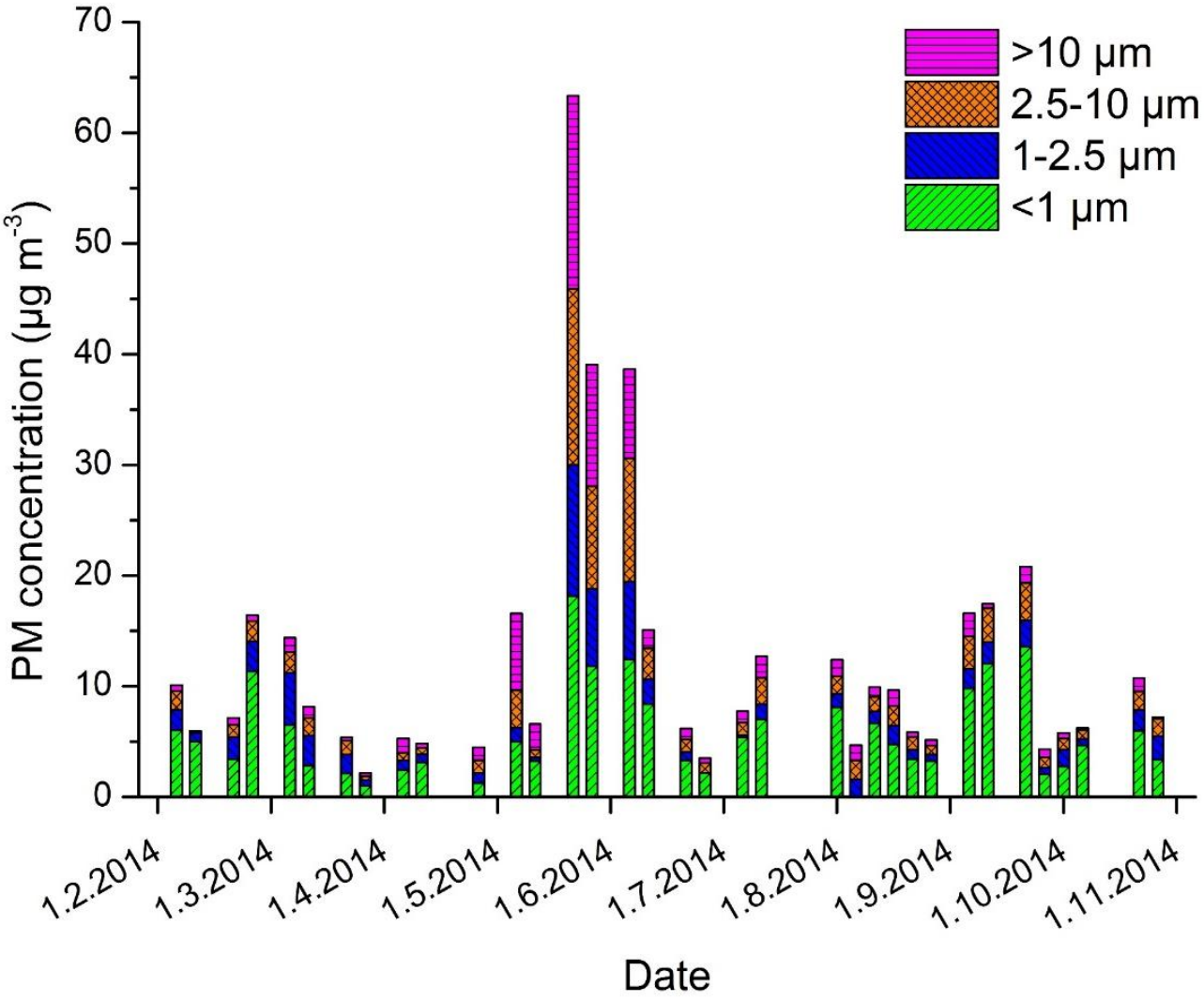

Figure S3. Particulate matter (PM) concentration over the sampling period in different particle size fractions.

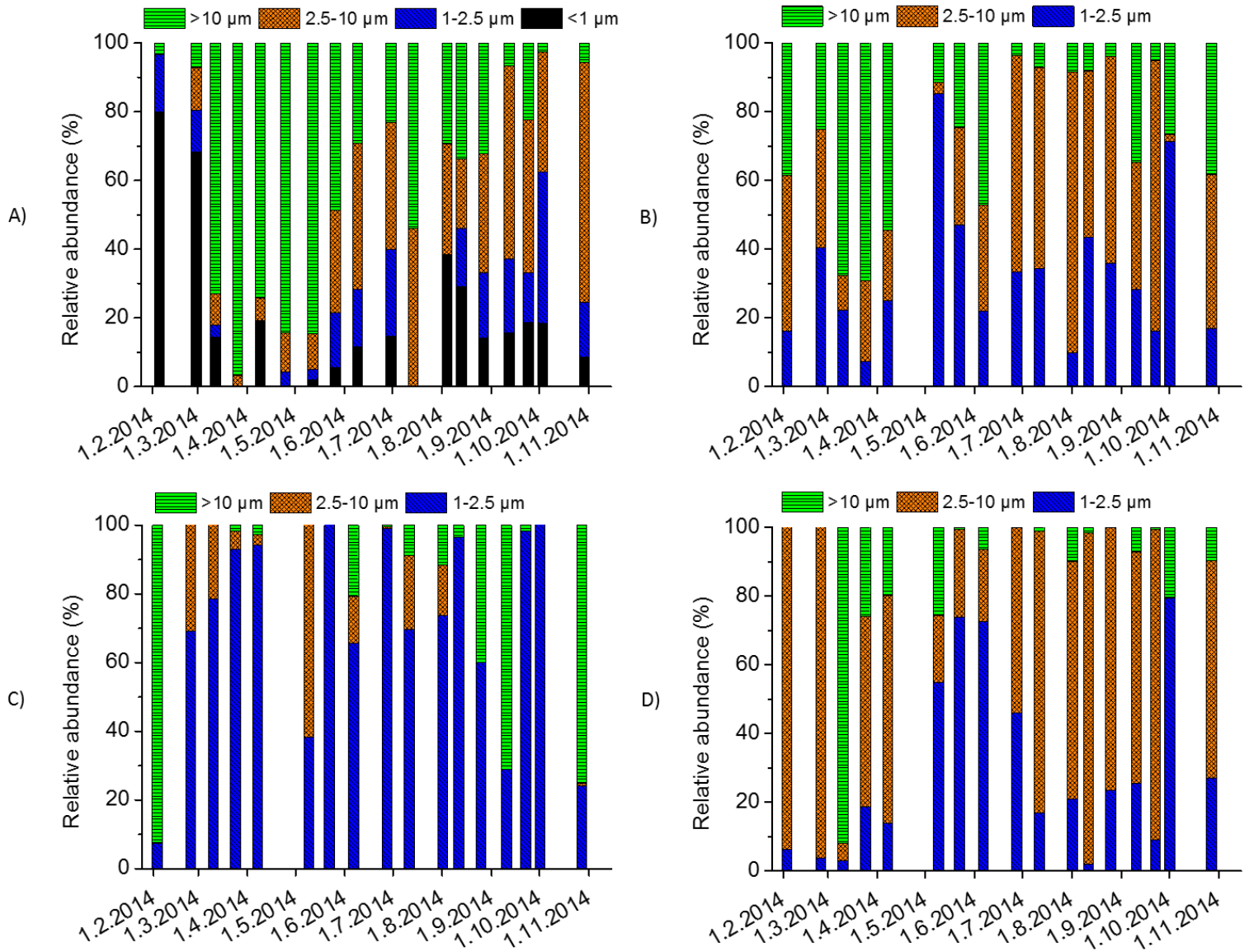

Figure S4. Relative abundance of a) free amino acids b) bacteria c) *Pseudomonas* and d) fungi in different particle size fractions.

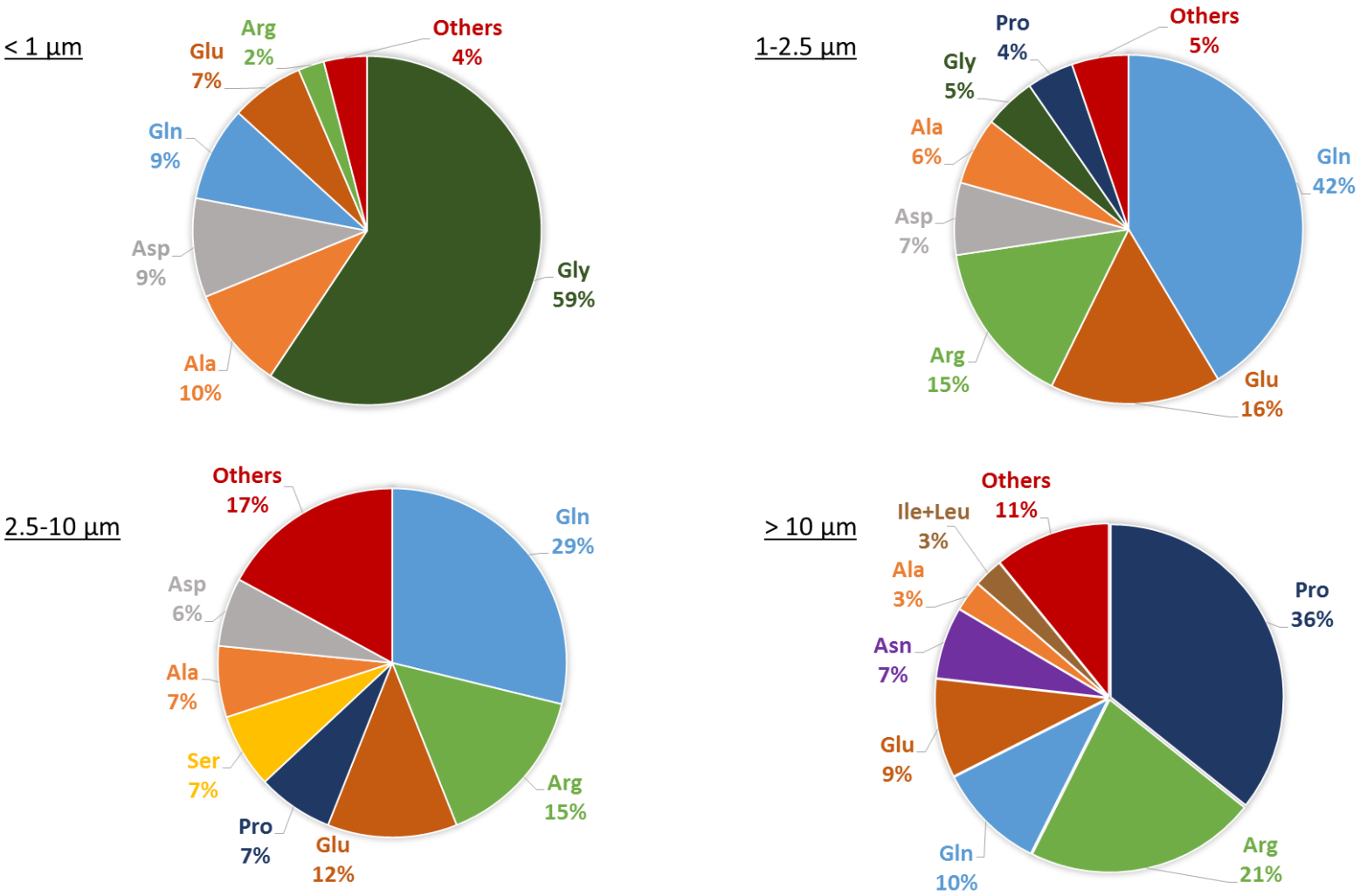

Figure 5. Amino acid relative concentration distribution in each size fraction (calculated as percentage of the cumulative amount). One pollen season sampling date was excluded from the calculations due to the dominance of proline and arginine.

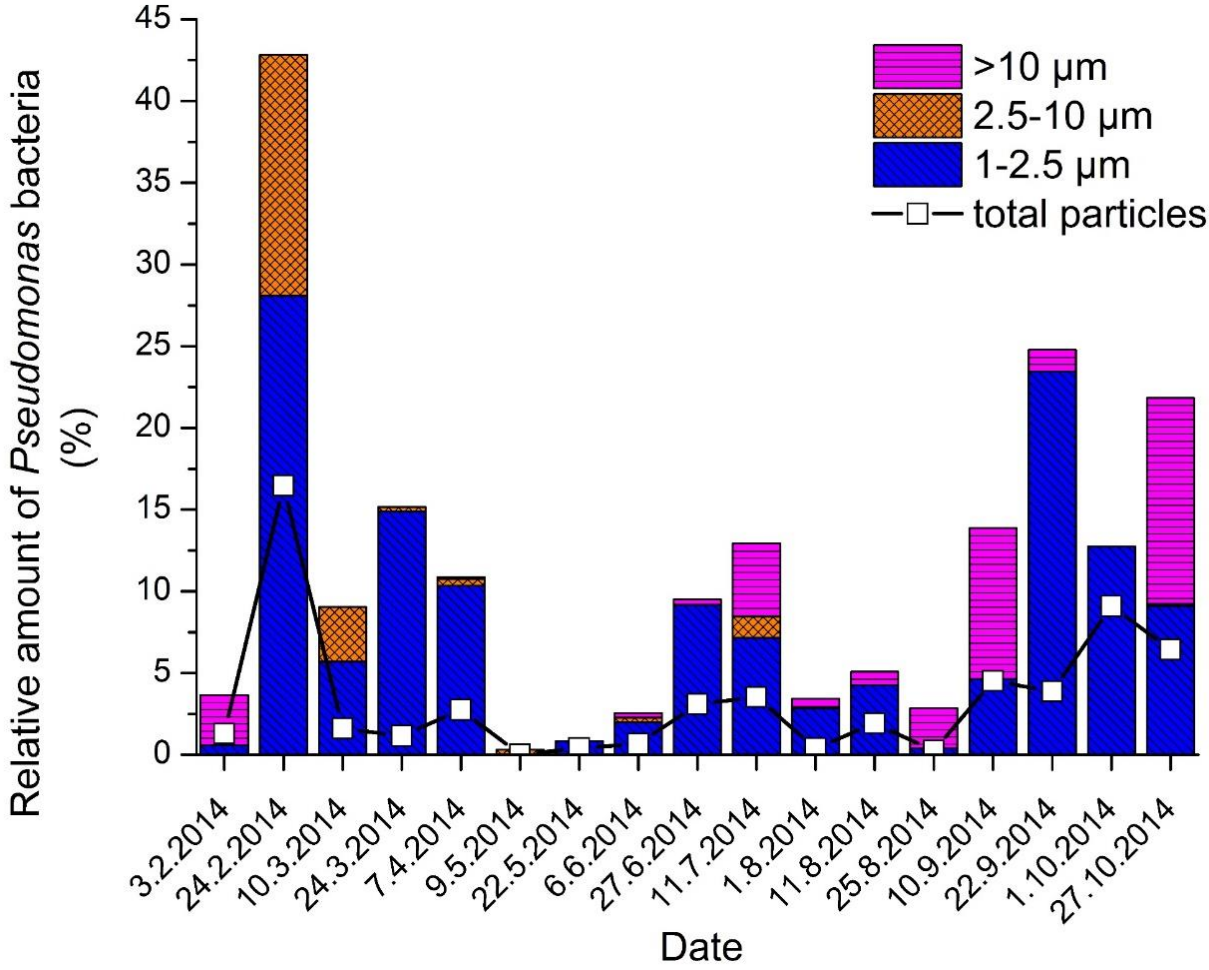

Figure S6. Relative amount of bacteria belonging to genus *Pseudomonas* compared against all the bacteria detected in different size fractions and in total particles.

S4. Results from correlation analysis.

As discussed in the article, our meteorological variables correlation results are showing long-term effects of ~~bioaerosols~~ PBAP due to the time resolution of sampling. Note, that in case of correlation between individual amino acids, the results may not be representative, despite statistical significance. This is due to the fact that some amino acids were present in the same limited number of samples (detection frequency, Table S6). Thus, herein we will focus mainly on the total FAA and microorganism correlation results.

A general trend observed in the correlation results in different size fractions were the positive correlation between FAA and microorganisms with soil and air temperature (Figure S7 and Figure S8). In the case of FAA, air temperature showed positive correlation in all the size fractions, and soil temperature showed statistically significant correlation in all the size fractions except in the largest size fraction (>10 µm). Fungal DNA concentration correlated positively with air and soil temperature in all the size fractions, whereas bacterial DNA concentration did not correlate with these parameters in the largest size fraction (>10 µm). Oppositely, *Pseudomonas* showed positive correlation between soil temperature only in the largest size fraction (>10 µm), but not in the smallest size fractions (1-2.5 µm and 2.5-10 µm). All of the before mentioned meteorological variables are closely related to local biological activity and seasonality. High concentration levels of FAA and microorganisms were generally observed in spring-autumn; seasons which are typically related to increased biological activity.

Negative correlation between microorganisms' concentrations with wind speed in the small size fractions (particles below 10 µm) was observed. Microorganisms may be removed from plant surfaces by higher wind speed, however, at the same time dilution and/or deposition may lower the concentration levels in the near-surface atmosphere.[5] Wind speed was higher during cold months and lower during warm months, opposite to trend in microorganism abundances observed in this study. By our opinion, low time resolution of sampling is responsible for some correlation, which is a clear drawback of our study.

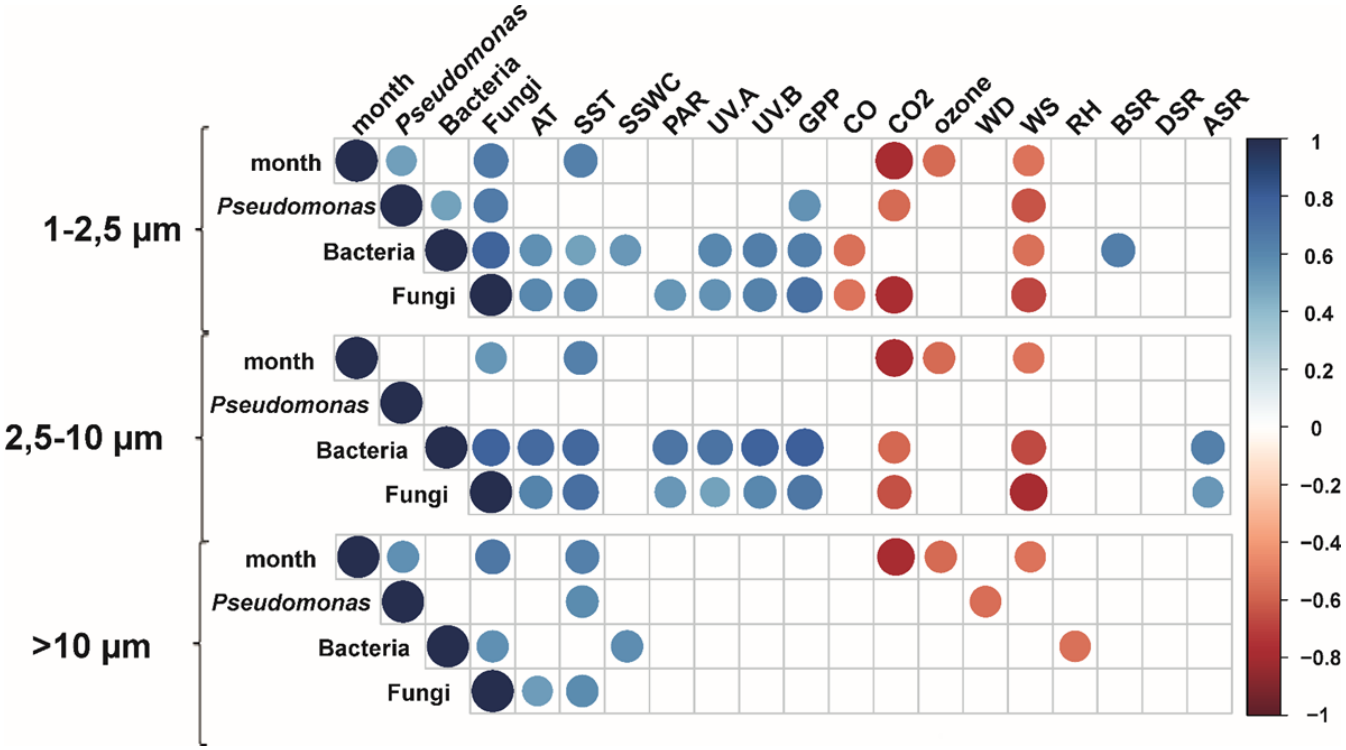

Figure S7. Illustration of correlation diagram presenting Spearman correlations between microorganisms with meteorological variables. The colour scale indicates negative/positive correlation and the size of the ball statistical significance level. The smallest ball is representative of p-value less than 0.05. The larger the ball is, the smaller the corresponding p-value is. Meteorological parameters were: AT-air temperature; SST-soil surface temperature; SSWC-soil surface water content; WS-wind speed; WD-wind direction; GPP-gross primary production; CO; CO_2; 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.

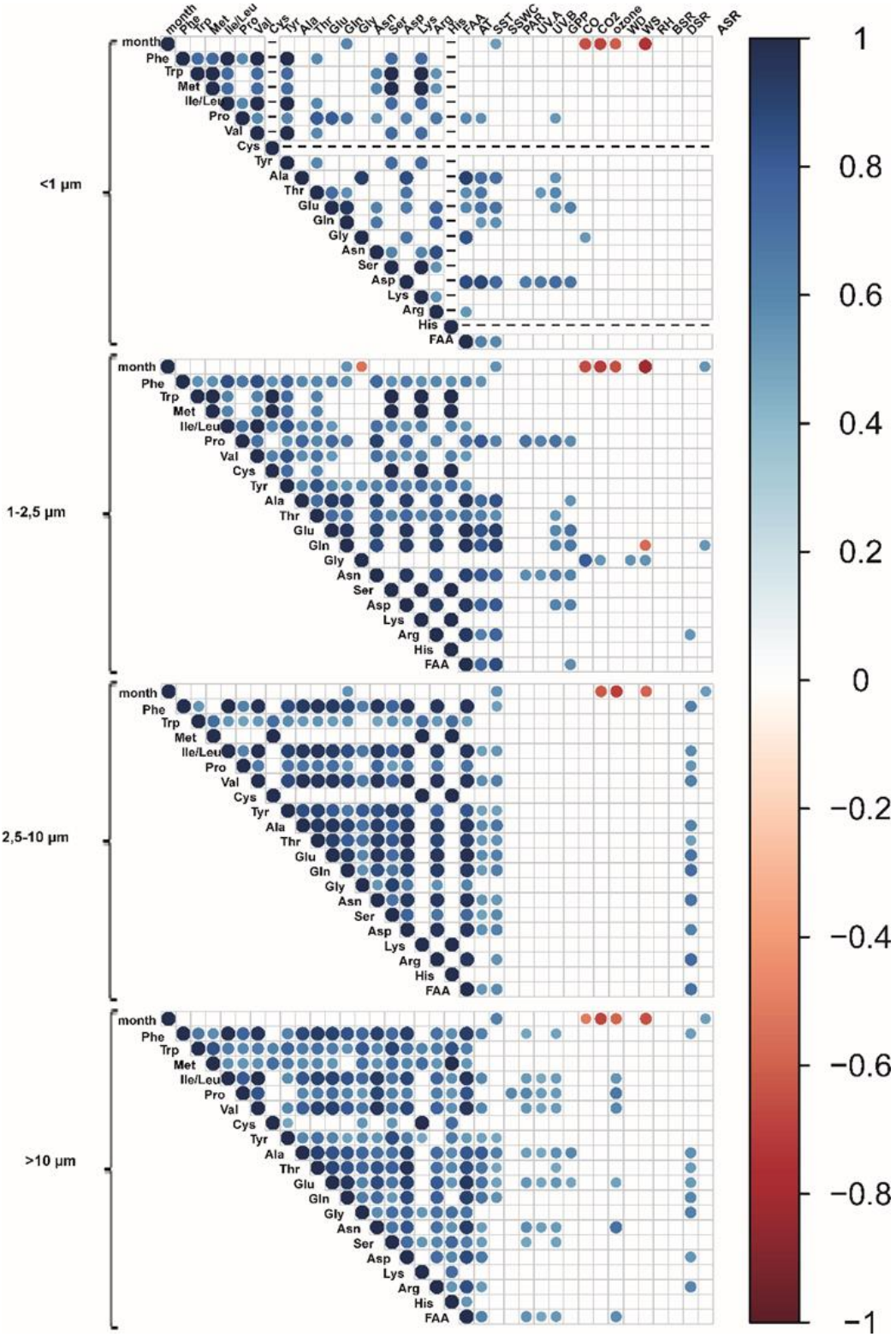

Figure S8. Illustration of correlation diagram presenting Spearman correlations between free amino acids with meteorological variables. The colour indicates negative/positive correlation and the size of the ball statistical significance. The smallest ball is representative of p-value less than 0.05. The larger the ball is, the smaller the corresponding p-value is. Abbreviations of meteorological parameters are the same as in Figure S7.

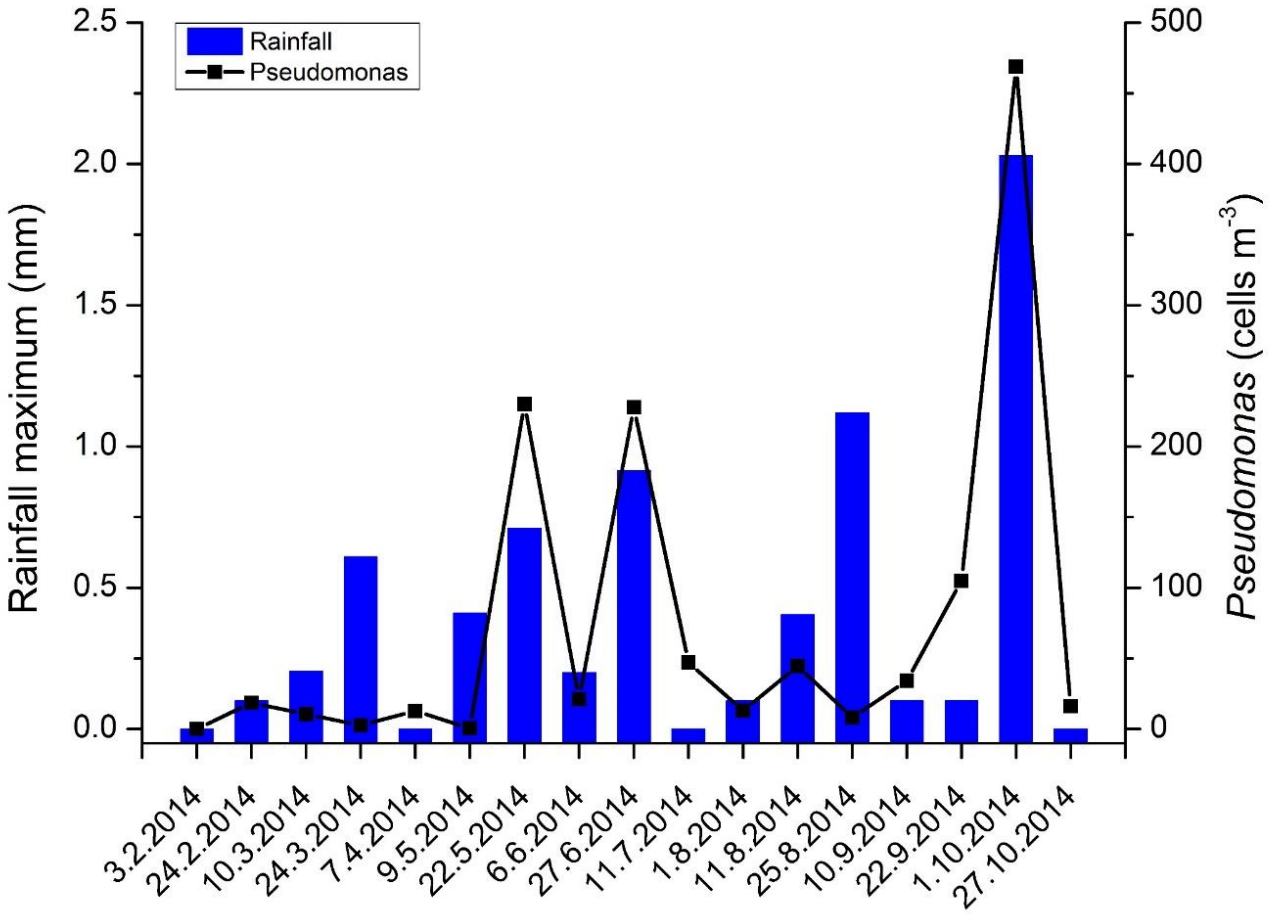

Figure S9. The pattern of *Pseudomonas* concentration in the size fraction 1-2.5 µm and maximum amount of rainfall recorded on a single 30 min event within 72 h before sampling.

S5. Estimation of biomass contribution to particulate matter in aerosols.

Contribution of FAA and DNA to PM

The relative amount of FAA mass to PM mass in total particles over the year was on average 0.33±0.45% (range 0.02-1.92%). The FAA concentration correlated positively with PM concentration in total particles ($R=0.54$, $p<0.05$). In different size fractions the percentage of FAA/PM (w/w-%) was: 0.07±0.08% (<1 µm), 0.36±0.48% (1-2.5 µm), 0.50±0.62% (2.5-10 µm) and 0.83±0.98% (>10 µm). The highest portion of FAA/PM (w/w-%) was observed in the size fraction >10 µm during the pollen season peak in May, when the FAA accounted for 3.32% of the PM. In general, the relative amount of FAA to PM was higher in the large sized particles (>2.5 µm) than in the small sized particles (<2.5 µm). Statistically significant correlation was observed between the FAA concentration with PM concentration in all of the size fractions except in the size fraction 1-2.5 µm ($R=0.53$, $p<0.05$ for <1 µm; $R=0.18$, $p=0.53$ for 1-2.5 µm; $R=0.54$, $p<0.05$ for 2.5-10 µm and $R=0.77$, $p<0.001$ for >10 µm).

The relative amount of DNA mass to PM mass in total particles over the year was on average 0.15±0.16% (range 0.01-0.47%). No statistically significant correlation was observed between DNA concentration with PM concentration in total particles. On average the relative amount of DNA/PM (w/w-%) in different size fractions was: 0.30±0.39% (1-2.5 µm), 0.10±0.13% (2.5-10 µm) and 0.22±0.47% (>10 µm). The relative amount of DNA/PM (w/w-%) was highest in total particles in late spring (0.42%), late summer (0.44%) and autumn (0.47%). Opposite to the trend observed with FAA, DNA showed bimodal relative amount distribution, namely being highest in the size fractions 1-2.5 µm and >10 µm and lowest in the size fraction 2.5-10 µm. DNA concentration and PM concentration did not correlate with statistically significance in any of the size fractions.

Estimation of biomass in aerosols

Amino acids have been previously used as an indicator for biomass in aerosols by using an estimation that on average ~10% of biomass is composed of proteins.[6, 7] In order to estimate the relative abundance of biomass in aerosols, we first converted FAA data into the form of combined amino acids (i.e. to proteins and peptides). FAA concentrations were multiplied by a factor of four based on previous studies conducted at rural and semiurban sites.[8-10] This correction factor is only a rough estimate and can be lower or higher depending on the season and size fraction.[10] Nevertheless, we assume it is suitable for our approximation purpose. After the conversion of FAA to proteins, we used the ~10% estimate to obtain the relative amount of biomass in aerosols.[6] This approximation leads to the following proportion of biomass/PM (w/w-%) in different size fractions: 3±3% (<1 µm), 14±19% (1-2.5 µm), 20±25% (2.5-10 µm) and 33±39% (>10 µm). In total particles, the corresponding biomass/PM (w/w-%) was over the year 13±20%, ranging from 1% to 77%. These results seem reasonable, if taking into account the general estimation that on average ~30% of PM mass is composed of PBAP.[11] However, the drawback of our estimation method is that the ratio of FAA to proteins can vary seasonally whereas we assume it to be constant.[10] Further, the relative amount of proteins in biomass varies greatly,[6, 7] yet we assume it to be constant. Thus, the uncertainty here can be significant (see also Hock et al. and Schneider et al.).[6, 7] However, our results indicate that the fraction of ~~bioaerosols~~ PBAP can be substantial during the local pollen season as suggested by Manninen et al.[12] Based on our estimation, the relative amount of biomass in total particles would be ~77%, comparable to the 65% proposed by Manninen et al.[12]

Another option to estimate the relative amount of biomass in aerosols would be to use the information obtained from the total DNA amount. In general, DNA amount is known to correlate with the microbial biomass.[13] However, estimating the proportion of DNA in biomass is challenging especially in case of aerosol samples due to the varying cell and genome sizes of putative PBAP-organisms, such as bacteria, fungi/fungal spores and plant cells/pollen.[13, 14] If assuming that 4% of biomass is composed of DNA,[15] the following ratio of biomass/PM (w/w-%) would be in different size fractions: 8±10% (1-2.5 µm), 3±10% (2.5-10 µm) and 6±12% (>10 µm). In total particles, the corresponding estimation method gave biomass/PM (w/w-%) ratio of 4±4% (range 0.2-12%). This DNA conversion to biomass gave even up to 6-fold smaller biomass/PM-ratio, when compared to the estimation derived from using FAA to estimate the biomass amount in aerosols. Even though the sampling times differed between DNA and FAA samples (Table S1), it is unlikely that this would result in

such a significant difference in biomass proportion on average, but is more likely to occur due to the variance of cell sizes of PBAPBs.

Collectively, these estimation methods presented here are prone to error, yet they give us a general estimate of the amount and seasonal variations of biomass in aerosols at the boreal forest site. By using the information obtained from both estimation methods, we obtained the following seasonal contribution of biomass in aerosols. The proportion of biomass is likely negligible during winter (~1% in total particles), whereas during spring and local pollen season the portion can be substantial (~11-77% in total particles). In addition, in late summer and autumn, the portion of biomass can be occasionally ~10% in total particles.

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