SUPPORTING INFORMATION.

Rivas-Ubach et al., 2016

Supplementary text. Sonication optimization test.

For the sonication time optimization, the 18 test-filters (sampled for 2 consecutive days) were cut by 2 equal parts (n = 36) and each piece followed the procedures explained above but varying the sonication time (12 pieces were sonicated for 10 minutes, 12 pieces for 15 minutes and 12 pieces for 20 minutes). After testing different sonication times on the test-filters from the additional sampling, the results showed that more than 10 minutes of sonication did not increase the concentration of the extracts.

To test for differences among different sonication times during the extraction of polar and semi-polar metabolites, separately we performed PERMANOVAs for the metabolomic fingerprints obtained by LC-MS and GC-MS. PERMANOVAs were performed with sonication time as fixed factor for each of the sampling rounds of two days to avoid any possible variability from different days (Tables S7 and S8). PERMANOVAs were conducted using the Bray Curtis distance and setting the permutations at 10000.

PERMANOVA performed for each of the sampled days with "sonication time" as dependent categorical variable showed that sonication time did not significantly vary the relative abundances of the extracts in any of the sampled days for datasets generated by LC-MS ($P > 0.05$) (Table S7). Same PERMANOVAs applied to the GC-MS datasets did not show differences between sonication times ($P > 0.05$) with the exception of the test corresponding to 5^{th} and 6^{th} June 2015 ($P = 0.02$) (Table S8).

Because we did not detect significant variation in the concentration of the detected ions among different sonication times (Tables S7 and S8), we considered that 10 minutes of sonication was enough to extract the metabolites in methanol/water (80:20) and get the metabolomic fingerprints of the aerosols.

Table S1. Description of the processes and parameters applied to LC-MS chromatograms with MZMine 2.17 (Pluskal et al., 2010) to obtain the metabolomic fingerprintings of the aerosol extracts from both positive and negative ionization modes. All LC-MS fingerprints including seasonal sampling and additional sampling for sonication test were obtained with the same parameters as shown in the table.

		(+H) Chromatograms		(-H) Chromatograms
1	**Baseline correction – RollingBall baseline corrector**			
	Chromatogram type	TIC		TIC
	Use m/z bins	No		No
	wm	10		12
	ws	8		8
2	**Mass detection** (exact Mass)			
	Noise level	7×10^4		5×10^3
3	**Chromatogram builder**			
	Minimum time span	0.03		0.03
	Minimum height	1×10^4		1×10^3
	m/z tolerance	0.0005		0.0005
4	**Smoothing**			
	Filter width	5		5
5	**Chromatogram deconvolution (local minimum search)**			
	Chromatographic threshold	30%		30%
	Search minimum in RT range (min)	0.1		0.1
	Minimum relative height	5%		5%
	Minimum absolute height	1000		1000
	Minimum ratio of peak top/edge	2		2
	Peak duration range	0-0.5		0-0.5
6	**Chromatogram alignment (join alignment)**			
	m/z tolerance	0.0005		0.0005
	Weight for m/z	80		80
	RT tolerance	0.3		0.3
	Weight for RT	20		20
7	**Gap filling (Peak Finder)**			
	Intensity tolerance	30%		30%
	m/z tolerance	0.0005		0.0005
	Retention time tolerance	0.3		0.3
	RT correction	Yes		Yes
8	**Metabolite Assignation**			
	m/z tolerance	0.0005		0.0005
	RT tolerance	0.3		0.3
9	**Data Exported**	Peak Area		Peak Area
10	**Ions excluded from database**	81.519 84.079 102.032 140.000 146.018 158.995 180.97 181.027 200.022		59.014 119.036 141.018 155.003 217.003 223.020 293.179 311.169

RT, retention time; m/z, mass to charge ratio

Table S2. Retention time (RT) and mass to charge ratio (m/z) of the deconvoluted ions in both negative and positive ionization modes assigned to metabolites with MZmine v.2.17 for LC-MS chromatograms. The assignment of the metabolites was based on the exact mass and RT of standards. RT, m/z and peak range of the standards are shown in the table. Malic acid typically present two peaks for the chromatographic method used. Ions representing fragments of the molecular compound are marked. Error of m/z and RT of assigned ions to metabolites respect the m/z and RT of standars are shown.

		m/z and RT of each ion assigned in MZmine v.2.17			m/z, RT, Peak range of ions from standards measured in the LC-MS Orbitrap system				Error of m/z and RT (deconvoluted ions vs. Standard ions)		
Ionization mode	Hits	Name	m/z	RT	m/z	RT	Fragment	Double peak	Absolutem /z	m/z (ppm)	RT
-H	30	a.ketoglutaric acid	145.01569	1.81	145.01495	1.65			0.00074	5.12	0.16
+H	30	Acacetin	285.07461	16.79	285.07553	16.85			-0.00092	-3.22	-0.06
-H	30	Adonitol (Ribitol)	151.06272	1.36	151.06195	1.42			0.00077	5.12	-0.06
-H	30	Citric acid	191.02057	1.78	191.01945	1.75			0.00112	5.86	0.03
-H	30	Coumaric acid	163.04150	11.15	163.04065	11.3			0.00085	5.24	-0.15
-H	30	δ-tocopherol	401.12877	1.32	401.12906	1.35			-0.00029	-0.72	-0.03
+H	30	Glutamine	147.07610	1.54	147.07630	1.46			-0.00020	-1.36	0.08
+H	30	Glutamine	130.04889	1.75	130.04900	1.46	Yes		-0.00011	-0.86	0.29
-H	30	Hexoses	179.05610	1.33	179.05595	1.43			0.00015	0.84	-0.10
+H	23	Isoleucine	86.09582	1.74	86.09600	1.7	Yes		-0.00018	-2.07	0.04
+H	30	Isoleucine	132.10199	1.74	132.10190	1.7			0.00009	0.69	0.04
+H	30	Leucine	132.10086	1.92	132.10160	1.81			-0.00074	-5.64	0.11
-H	30	Malic acid	133.01570	1.40	133.01560	1.51		Yes	0.00010	0.73	-0.11
-H	30	Malic acid	115.00522	1.62	115.00490	1.51	Yes	Yes	0.00032	2.81	0.11
-H	30	Malic acid	133.01570	1.77	133.01560	1.71		Yes	0.00010	0.79	0.06
-H	30	Malic acid	115.00527	1.85	115.00490	1.71	Yes	Yes	0.00037	3.22	0.14
+H	23	Phenilalanine	166.08657	1.86	166.08640	1.91			0.00017	1.01	-0.05
+H	30	Proline	116.06988	1.61	116.07030	1.49			-0.00042	-3.59	0.12
+H	30	Proline	116.06990	1.65	116.07030	1.49			-0.00040	-3.46	0.16
-H	30	Shikimic acid	173.04573	1.70	173.04553	1.63			0.00020	1.16	0.07
-H	30	Sorbitol - Mannitol	181.07308	1.36	181.07222	1.4			0.00086	4.76	-0.04
+H	30	Threonine	120.06499	1.63	120.06500	1.43			-0.00001	-0.08	0.20
+H	30	Tyrosine	182.08139	1.74	182.08140	1.77			-0.00001	-0.03	-0.03
+H	30	Valine	118.08581	1.35	118.08610	1.53			-0.00029	-2.44	-0.18

RT, retention time
m/z, mass to charge ratio
ppm, parts per million
Hits, number of samples where the metabolite was detected

Table S3. Description of the processes and parameters applied to GC-MS chromatograms with MZMine 2.17 to obtain the metabolomic fingerprintings for the sonication test.

			(-H) Chromatograms
1	**Baseline correction – RollingBall baseline corrector**		
	Chromatogram type		TIC
	Use m/z bins		No
	wm		12
	ws		8
2	**Mass detection** (exact Mass)		
	Noise level		5×10^3
3	**Chromatogram builder**		
	Minimum time span		0.03
	Minimum height		1×10^3
	m/z tolerance		0.0005
4	**Smoothing**		
	Filter width		5
5	**Chromatogram deconvolution (local minimum search)**		
	Chromatographic threshold		30%
	Search minimum in RT range (min)		0.1
	Minimum relative height		5%
	Minimum absolute height		1000
	Minimum ratio of peak top/edge		2
	Peak duration range		0-0.5
6	**Chromatogram alignment (join alignment)**		
	m/z tolerance		0.0005
	Weight for m/z		80
	RT tolerance		0.3
	Weight for RT		20
7	**Gap filling (Peak Finder)**		
	Intensity tolerance		30%
	m/z tolerance		0.0005
	Retention time tolerance		0.3
	RT correction		Yes
8	**Metabolite Assignation**		
	m/z tolerance		0.0005
	RT tolerance		0.3

RT, retention time; m/z, mass to charge ratio

Table S4. Description of the parameters applied to GC-MS chromatograms with Metabolite Detector 2.5 for the obtaining of the spring and summer metabolomic profilings.

Tool settings		
Centroid	Threshold begin	10
	Peak threshold end	-5
	Maximal baseline	30
	FWHM	0.1
Deconvolution	Peak threshold	10
	Minimum peak height	10
	Deconvolution width (scans)	8
Identification	Max RI difference	20
	Cutoff score	0.6
	Pure/Impure	0.6
	Scaled lib	Yes
	Combined score	Yes
Quantification	Minimal distance	0.5
	Minimal required quality index	1
	Exclude	72.5 to 73.5 146.5 to 147.5
Batch quantification Settings		
Compound matching	ARI	20
	Pure/Impure	0.6
	Req. Score	0.8
	RI+Spec	OK
Identification	ARI	20
	Pure/Impure	0.6
	RI+Spec	OK
Other settings	Compound reproducibility	0
	Max. Peak drisc. index	100
	S/N	15
	Number of ions	4
	Extended SIC Scan	Yes

Table S5. Score, retention index (RI), retention time (RT) and signal to noise ratio (S/N) of matched metabolites in GC-MS chromatograms processed with Metabolite Detector 2.5. The number of hits found after chromatogram alignment is indicated. Metabolites matches in less than 70% of the samples were not considered for the study case.

	Score	Quantification Ions	Avg. RI	Avg. RT (Min)	Avg. S/N	Hits	Considered for the study case
palmitic acid	0.95	75 117 313	1717.8	18.89	354.51	30	Yes
D-trehalose	0.83	191 361 362	2478.0	25.17	238.80	30	Yes
stearic acid	0.94	75 117 341	1913.6	20.69	315.26	30	Yes
linoleic acid	0.86	55 67 75	1885.0	20.44	49.92	30	Yes
oleic acid	0.91	55 75 129	1896.1	20.54	20.11	30	Yes
fumaric acid	0.97	73 147 245	1023.7	10.93	31.80	30	Yes
glyceric acid	0.80	73 189 292	1015.1	10.82	62.23	30	Yes
caprylic acid	0.91	75 131 201	938.9	9.78	25.08	30	Yes
capric acid	0.88	55 117 229	1133.8	12.36	30.84	30	Yes
D-glucose	0.80	160 205 319	1602.3	17.77	193.74	30	Yes
D-galactose	0.82	73 205 319	1622.9	17.97	32.98	30	Yes
uracil	0.85	99 131 241	1021.5	10.90	39.39	30	Yes
arachidic acid	0.93	75 132 369	2109.3	22.36	33.91	30	Yes
heptadecanoic acid	0.94	117 327 328	1813.7	19.81	48.14	30	Yes
maltose	0.82	73 204 217	2418.2	24.74	7.52	27	Yes
3-hydroxybutyric acid	0.87	73 117 147	839.2	8.41	9.64	6	No
glycerol	0.80	73 133 205	957.1	10.03	70.75	5	No
L-homoserine	0.81	73 174 218	1122.1	12.21	2.93	4	No
L-serine	0.84	73 204 218	1045.7	11.22	18.84	3	No
1-indanol	0.87	156 205 206	1030.5	11.02	0.72	2	No
4-hydroxypyridine	0.86	73 152 167	832.6	8.32	0.77	2	No

Score, Score value obtained for each metabolite matching with the library.
Avg. RI, Average Retention Index
Avg. RT (min), Average Retention Time (minutes)
Avg. S/N, Average Signal to Noise
Hits, number of samples where the metabolite was detected

Table S6. Student t-tests for each of the assigned metabolites with season as the categorical tested factor. Mean (representing the peak area of ion chromatograms), standard error, statistic t and *P* value for each assigned metabolite are shown for each of the seasons.

LC-MS						
	Spring		**Summer**			
	Mean	**SE**	**Mean**	**SE**	**t**	***P***
a-ketoglutaric acid	48316.99	5099.042	25008.78	4212.44	3.52	0.00165
Acacetin	50809.67	13413.09	105584	35227.62	-1.45	0.17324
Adonitol	87878.02	12553.98	30032.83	4370.85	4.35	0.00049
Citric acid	1976793	179888.1	1574644	244477.2	1.32	0.19773
Coumaric acid	14540.78	3816.208	29699.1	6321.345	-2.05	0.08066
d-tocopherol	266205.7	110367.1	54500.64	8547.013	1.91	0.07784
Glutamine	2467791	1261357	1208597	250593.8	0.98	0.34903
Hexoses	179901.1	75768.74	37733.15	5521.39	1.87	0.08373
Isoleucine	179199.1	29111.56	355432.6	69281.29	-2.35	0.03339
Leucine	621572.5	84248.74	703402.2	152227.1	-0.47	0.64535
Malic acid	417618.5	120080.4	130458.8	36979.81	2.29	0.03681
Phenilalanine	185597.5	25034.1	346194.1	79907.79	-1.92	0.07526
Proline	305332.3	59165.33	151847.2	47975.5	2.01	0.05887
Shikimic acid	303799.8	75605.58	313967.9	108839.2	-0.08	0.93960
Sorbitol/Mannitol	368652.3	67777.98	106951.3	13302.26	3.79	0.00199
Threonine	1047720	178312.1	479188.3	98874.14	2.79	0.01386
Tyrosine	170745.7	45699.96	155459	33666.26	0.27	0.79024
Valine	49486.37	8290.428	63038.64	28060.53	-0.46	0.64978
GC-MS						
	Spring		**Summer**			
	Mean	**SE**	**Mean**	**SE**	**t**	**P**
Palmitic acid	10200493	421999	9742948	374890.5	0.81	0.42282
D-trehalose	8783896	2820577	3608147	344275.8	1.95	0.06154
Stearic acid	8111280	464659.7	7312959	207154.6	1.64	0.11238
Linoleic acid	625152.6	20056.59	582749.8	22021.17	1.41	0.17012
Oleic acid	407369	34730.99	350753.1	28752.97	1.27	0.21586
Fumaric acid	2736172	179111.4	3181144	163342.6	-1.84	0.07657
Glyceric acid	2594000	155801.8	2692241	215277	-0.36	0.72130
Caprylic acid	449961.4	91906.22	417070.9	51513.41	0.32	0.74945
Capric acid	281892.7	20643.83	314941.2	30600.84	-0.87	0.39218
D-glucose	6252214	1203738	2681638	338921.9	3.02	0.00528
D-galactose	2598398	435407.4	1023059	138967.8	3.64	0.00109
Maltose	212970	64761	142173	40001	0.94	0.35548
Uracil	960735	107724.2	1084987	118906.4	-0.77	0.45027
Arachidic acid	212423.2	27627.51	232219.6	35000.68	-0.44	0.66688
Heptadecanoic acid	440164.8	67015.78	435406.3	49581.21	0.06	0.95416

Table S7. PERMANOVA tables for sonication time of the LC-MS Orbitrap fingerprints of each of test samples.

5th & 6th June 2015 (Test sample 1)	Df	Sum of Squares	Mean Square	F	*P*
Sonication time	1	0.052	0.052	0.92	0.40
Residuals	4	0.226	0.057	0.81	
Total	5	0.278	1		
7th & 8th June 2015 (Test sample 2)	Df	Sum of Squares	Mean Square	F	*P*
Sonication time	1	0.011	0.011	1.23	0.31
Residuals	4	0.036	0.009	0.77	
Total	5	0.046	1		
9th & 10th June 2015 (Test sample 3)	Df	Sum of Squares	Mean Square	F	*P*
Sonication time	1	0.018	0.018	1.04	0.49
Residuals	4	0.068	0.017	0.79	
Total	5	0.085	1		
11th & 12th June 2015 (Test sample 4)	Df	Sum of Squares	Mean Square	F	*P*
Sonication time	1	0.005	0.005	2.00	0.11
Residuals	4	0.010	0.002	0.67	
Total	5	0.015	1		
13th & 14th June 2015 (Test sample 5)	Df	Sum of Squares	Mean Square	F	*P*
Sonication time	1	0.004	0.004	0.15	0.91
Residuals	4	0.101	0.025	0.96	
Total	5	0.105	1		
15th & 16th June 2015 (Test sample 6)	Df	Sum of Squares	Mean Square	F	*P*
Sonication time	1	0.024	0.024	0.96	0.38
Residuals	4	0.099	0.025	0.81	
Total	5	0.122	1		

Table S8. PERMANOVA tables for sonication time of the GC-MS Orbitrap fingerprints of each of test samples.

5th & 6th June 2015 (Test sample 1)	Df	Sum of Squares	Mean Square	F	*P*
Sonication time	1	0.494	0.494	3.21	0.02
Residuals	4	0.616	0.154	0.55	
Total	5	0.494	0.494	3.21	
7th & 8th June 2015 (Test sample 2)	Df	Sum of Squares	Mean Square	F	*P*
Sonication time	1	0.171	0.171	1.38	0.27
Residuals	4	0.495	0.124	0.74	
Total	5	0.666	1		
9th & 10th June 2015 (Test sample 3)	Df	Sum of Squares	Mean Square	F	*P*
Sonication time	1	0.179	0.179	2.972	0.13
Residuals	4	0.240	0.060	0.57	
Total	5	0.419	1		
11th & 12th June 2015 (Test sample 4)	Df	Sum of Squares	Mean Square	F	*P*
Sonication time	1	0.303	0.303	6.29	0.07
Residuals	4	0.193	0.048	0.39	
Total	5	0.496	1		
13th & 14th June 2015 (Test sample 5)	Df	Sum of Squares	Mean Square	F	*P*
Sonication time	1	0.034	0.034	0.29	0.80
Residuals	4	0.465	0.116	0.93	
Total	5	0.500	1		
15th & 16th June 2015 (Test sample 7)	Df	Sum of Squares	Mean Square	F	*P*
Sonication time	1	0.282	0.282	2.38	0.16
Residuals	4	0.474	0.119	0.63	
Total	5	0.756	1		

Figure S1. Example of two chromatograms from 14 to 25 minutes of Methanol:Water (80:20) on the LC-MS Orbitrap instrument. Red chromatogram represents the mixture of solvents after 2 hours into a plastic tube. Blue chromatogram represents the solvents after 20 minutes sonication into a plastic tube.