Supplementary Materials

Algae 2025, 40(1): 67-79
https://doi.org/10.4490/algae.2025.40.2.11
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Supplementary Text S1. Additions to the materials and methods and results and discussion

MATERIALS AND METHODS

Chemicals and standards

Acetonitrile, 2-propanol, hexane and dichloromethane, were purchased from Merck (previously Sigma-Aldrich, Prague, Czech Republic). 1,2-Dipalmitoyl diacylglyceryltrimethylhomoserine, 1,2-dioleoyl-phosphatidylinositol (ammonium salt), monogalactosyldiacylglycerols (MGDG), digalactosyldiacylglycerols, 1,2-dioleoyl-phosphatidic acid (PA), 1,2-dioleoyl-phosphatidylcholine (PC), 1,2-dioleoyl-phosphatidylethanol-amine (PE), 1,2-dioleoyl-phosphatidylglycerol (PG) (Na-salt), and 1,2-dimyristoyl-phosphatidylserine (Na-salt), lyso-PG (16:0 lyso PG), lyso-PE (16:0 Lyso PE), lyso-PC (16:0 lyso PC), ceramide (*N*-palmitoyl-D-erythro-sphingosine), glucosylceramide (i.e., D-glucosyl-ß-1,1'-*N*-palmitoyl-D-erythro-sphingosine), hydroxyl-glucosyl-ceramide (i.e., *N*-[2'-(S)-hydroxypalmitoyl]-D-erythro-sphingosine), sphingosine (d18:1), triacylglycerol (TAG; i.e., 18:1/18:1/18:1-TAG) was purchased from Avanti Polar Lipids, Inc., Alabaster, AL, USA.

Hydrophilic interaction liquid chromatography

The high-performance liquid chromatography (HPLC) equipment consisted of a 1090 Win system, PV5 ternary pump and automatic injector (HP 1090 series; Hewlett Packard, Houston, TX, USA), and two Ascentis Express hydrophilic interaction liquid chromatography connected in series, 2.7 µm particle size, L × I.D. 150 mm × 4.6 mm Merck (formerly Supelco, Prague, Czech Republic) with an injection volume of 30 µL. HPLC was performed at a flow rate of 0.95 mL min^{-1} with a linear gradient from the mobile phase containing methanol : acetonitrile:aqueous 1 mM ammonium acetate (50 : 30 : 20, v/v/v) to methanol : acetonitrile:aqueous 1 mM ammonium acetate (10 : 70 : 20, v/v/v) for 60 min. The column temperature was 35°C and the re-equilibration period between runs was 30 min. The 10% HPLC flow was introduced into the electrospray ionization (ESI) source, and 90% of the flow containing fractions of lipid classes was collected manually. In order to have sufficient material, the fractionation was repeated up to five times, and the corresponding fractions were pooled, evaporated to dryness, and re-dissolved in an appropriate volume of the mobile phase. The system backpressure reached 1,000 bars during gradient analysis. The Orbitrap Elite (Thermo Fisher Scientific, Bremen, Germany) high-resolution hybrid mass spectrometer was used for the ESI-mass spectrometry (MS) analysis under the conditions described above.

http://e-algae.org pISSN: 1226-2617 eISSN: 2093-0860

RESULTS AND DISCUSSION

Triacylglycerols

The positive electrospray (ESI) mass spectrum of TAG usually contains a few abundant ions, e.g., the mass spectrum (Fig. 2) of TAG having ions $[M+NH_4]^+$ at *m/z* 842.7243, $[M+NH_4\text{-RCOOH-}NH_3]^+$ at *m/z* 571.4725 (DAG, 16:1/18:3), at *m/z* 547.4725 (DAG, 16:1/16:1), $[RCO+74]^+$ at *m/z* 335.2585 $[18:3]^+$, *m/z* 311.2584 $[16:1]^+$, $[RCO]^+$ at *m/z* 261.2215 (18:3), and at *m/z* 237.2215 (16:1). The structure of the majority represented by TAG, i.e., 16:1/16:1/18:3, was thus proved.

Monogalactosyldiacylglycerols

In the positive-ion tandem ESI spectrum, MGDG (Fig. 3) exhibits ion $[M+NH_4]^+$ at 792.5630 Da, which produces two intense fragments $[M+NH_4\text{-RiCOOH}]^+$ resulting from the loss of neutral group (free acids) either from the *sn*-1 (i = 1) or the *sn*-2 position (i = 2) of glycerol. The base peak is just an ion at *m/z* 512.3225 ($M+NH_4$-18:4), and the second very abundant ion of the type ($M+NH_4$-18:2) was identified at *m/z* 516.3538. Ion of the type $[M+NH_4\text{-Gal}]^+$ at *m/z* 629.5023 arises by elimination of a monosaccharide (galactose) from the ion $[M+NH_4]^+$. The very intense peak in the spectrum of MGDG is an ion having a value of 238.1292 Da ($[C_9H_{16}O_6+NH_4]^+$), which arises from the galactosyl group head and is characteristic for MGDG. Ions at *m/z* 259.2058 and *m/z* 263.2377, respectively, which belong to two acyls, were also identified as minor.

Phosphatidylglycerols

Similarly, in the product ion spectrum $[M+NH_4]^+$ of 16:1/18:3-PG at *m/z* 760.5134 (Fig. 4) dominated ($[M+NH_4\text{-}(HO)_2P(O)OCH_2CH(OH)CH_2OH]^+$) the ion at *m/z* 571.4728 resulting a loss of phosphoglycerol. Fatty acyl substituents can be determined based on acyl ions (RCO^+) at *m/z* 261.2215 (18:3) and 237.2214 (16:1) and ions at *m/z* 335.2587 and 311.2586 resulting in further dissociation *m/z* 571.4728, i.e., the loss of $R'_1CH=C=O$ and / or $R'_2CH=C=O$. The position of the fatty acyl substituents on the glycerol backbone was determined on the differences in abundances of the R_2CO^+ ion at *m/z* 261.2215, which is significantly more abundant than the R_1CO^+ ion at *m/z* 237.2214 in tandem MS. Thus, the position of fatty acid substituents on the glycerol skeleton can be determined (Hsu and Turk 2001).

REFERENCES

Hsu, F.-F. & Turk, J. 2001. Studies on phosphatidylglycerol with triple quadrupole tandem mass spectrometry with electrospray ionization: fragmentation processes and structural characterization. J. Am. Soc. Mass Spectrom. 12:1036–1043. doi.org/10.1016/S1044-0305(01)00285-9

Supplementary Table S1. List of lipid species from snow algae analysed by ESI-MS in positive ion mode. Background colour of the sample codes indicates the type of the snow algal bloom (red, orange and green, respectively). For sample codes, see Table 1 (view the website https://www.e-algae.org).

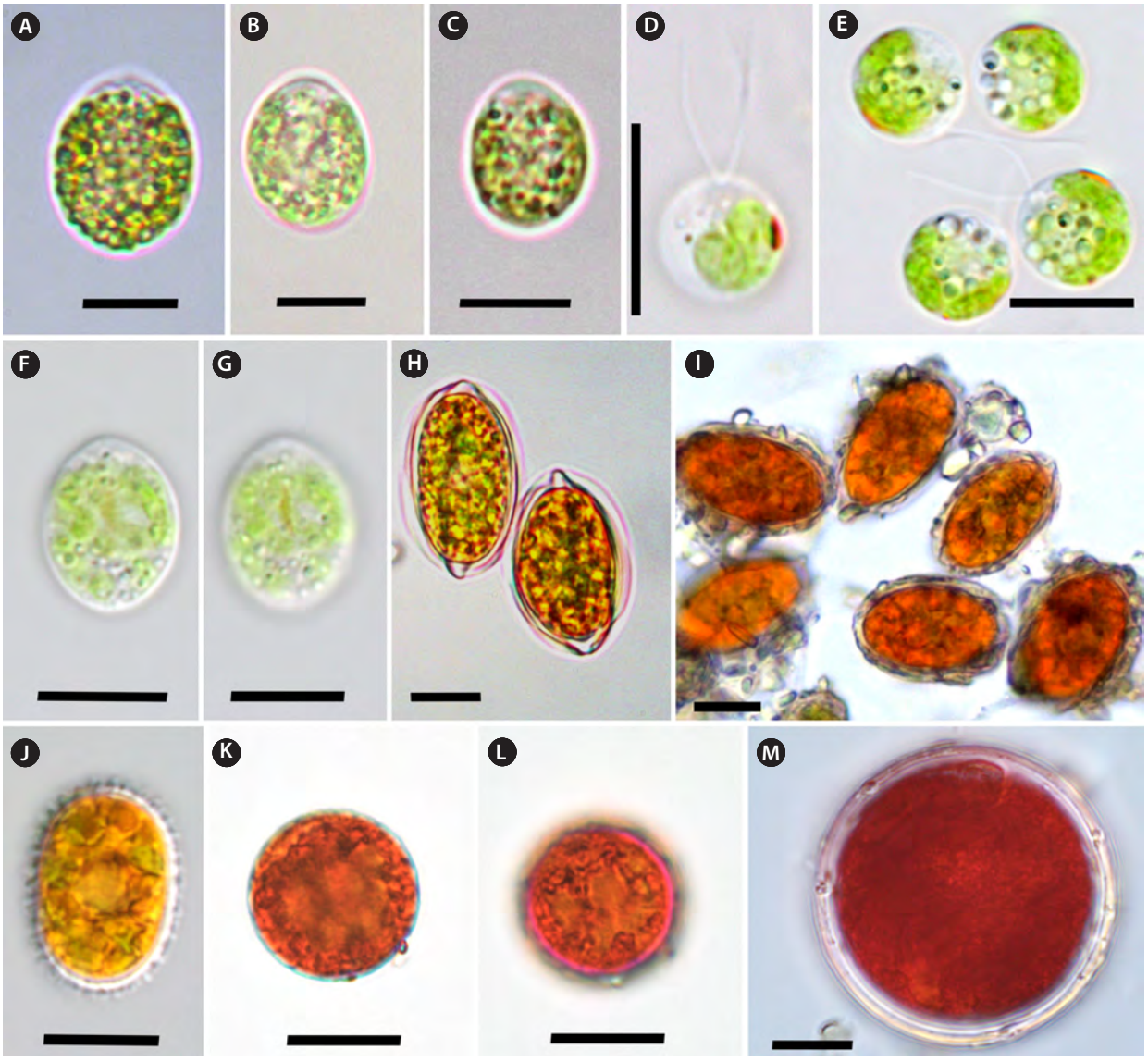
Supplementary Table S2. The description of the peaks in Fig. 4

Peak	Standard
1	Hydrocarbon
2	Squalen
3	Sterol ester
4	Triacylglycerol
5	Diacylglycerol
6	Monogalactosyldiacylglycerol
7	Free fatty acid
8	Ergosterol
9	Sulfoquinovosyldiacylglycerol
10	Diacylglycerylhydroxymethyl trimethylalanine/diacylglyceryltrimethylhomoserine
11	Phosphatidylglycerol
12	Digalactosyldiacylglycerol
13	Phosphatidylcholine
14	Phytosphingosine
15	Phosphatidylethanolamine
16	Lysophosphatidylcholine
17	Phosphatidylinositol
18	Phosphatidic acid
19	Phosphatidylserine
20	Phosphatidylinositolphosphate
21	Lysophosphatidic acid
22	Glucosylceramide
23	Glycosylinositol phosphoceramide
24	Glycosylinositol phosphoceramide
25	Glycosylinositol phosphoceramide
26	Unknown

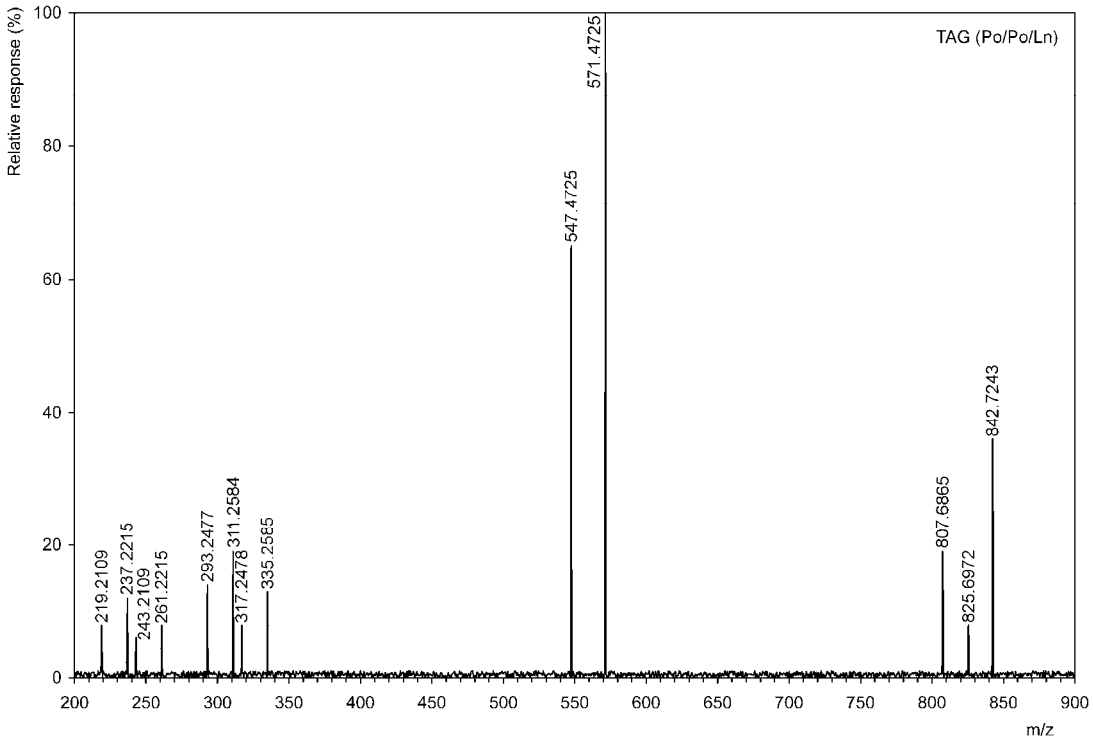
Supplementary Table S3. Relative proportion of dihydrosphingosine and phytosphingosine (main constituents of GIPs–glycosyl inositol phosphoryl ceramides) in total lipids as revealed by shot gun lipidomic analysis

Compound	Krk19-7	Jes19-3	Jes19-5	LP11	LP07	WP245	Jiz19-3	LP13	Krk19-2	WP235	WP242	WP266	WP244	Mil19-5
Dihydrosphingosine	1.59	2.25	1.94	1.45	1.03	1.41	1.41	1.23	1.54	10.80	13.30	12.92	5.70	6.39
Phytosphingosine	21.62	24.49	21.96	19.71	18.73	15.07	13.98	12.88	13.84	7.47	5.54	5.09	13.66	12.90

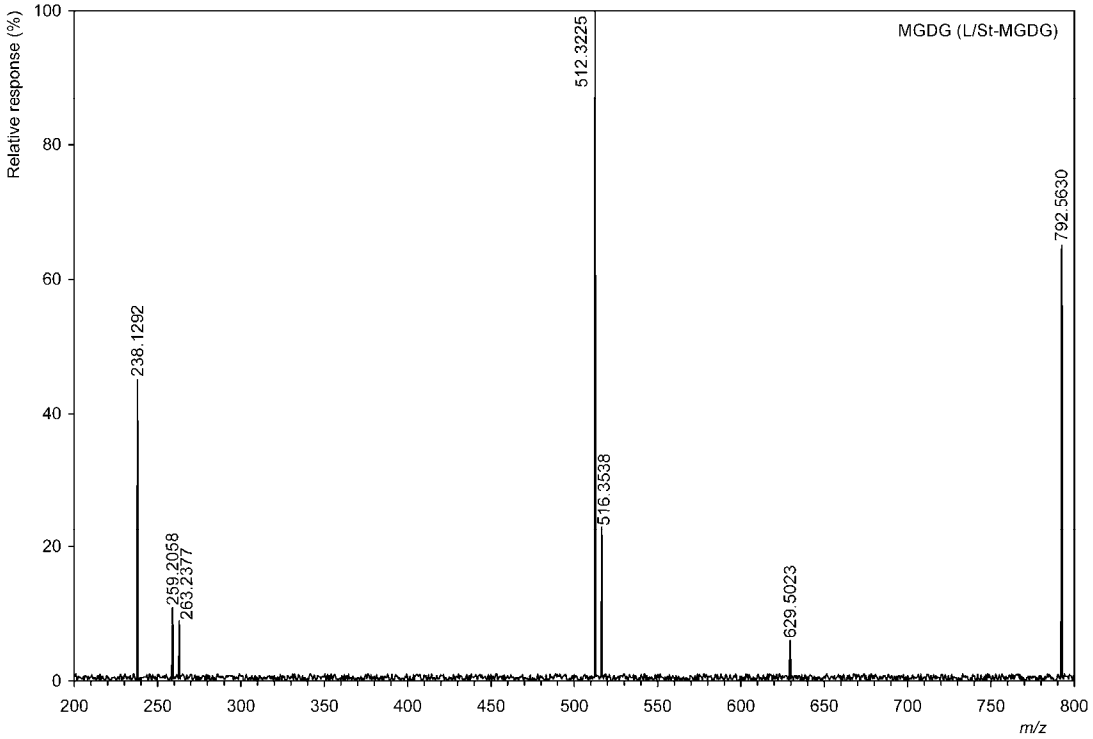
Background color of the sample codes indicates the type of the snow algal bloom (green, orange, and red, respectively).



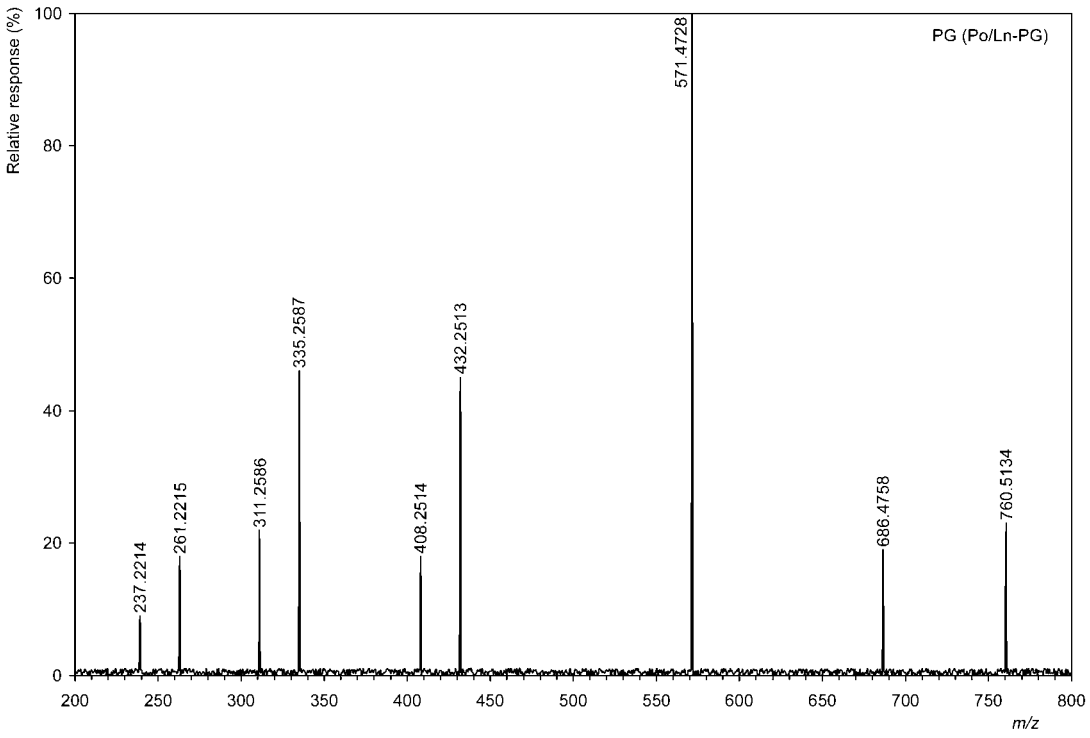
Supplementary Fig. S1. Dominant algal species in the studied snowfield samples. (A–G) Green snow taxa. (A) *Chloromonas* sp. 1 swarmer-like stages (Krk19-7). (B & C) *Chloromonas* sp. 2 swarmer-like stages (B, Jes19-3; C, Jes19-5). (D & E) *Chloromonas* cf. *miwae* flagellates (LP07). (F & G) *Chloromonas* cf. *muramotoi* swarmer-like stages (LP11). F, optical section; G, surface view showing red stigma. (H & I) Orange snow taxa. (H) *Chloromonas hindakii* cysts (Jiz19-3). (I) *Chloromonas nivalis* cysts (WP245). (J–M) Red snow taxa. (J) *Chloromonas brevispina* cyst (Mil19-5). (K & L) *Sanguina nivaloides* cyst (WP235). K, optical section; L, surface view. (M) *Chlainomonas* sp. cyst (WP244). Scale bars represent: A–M, 10 µm.



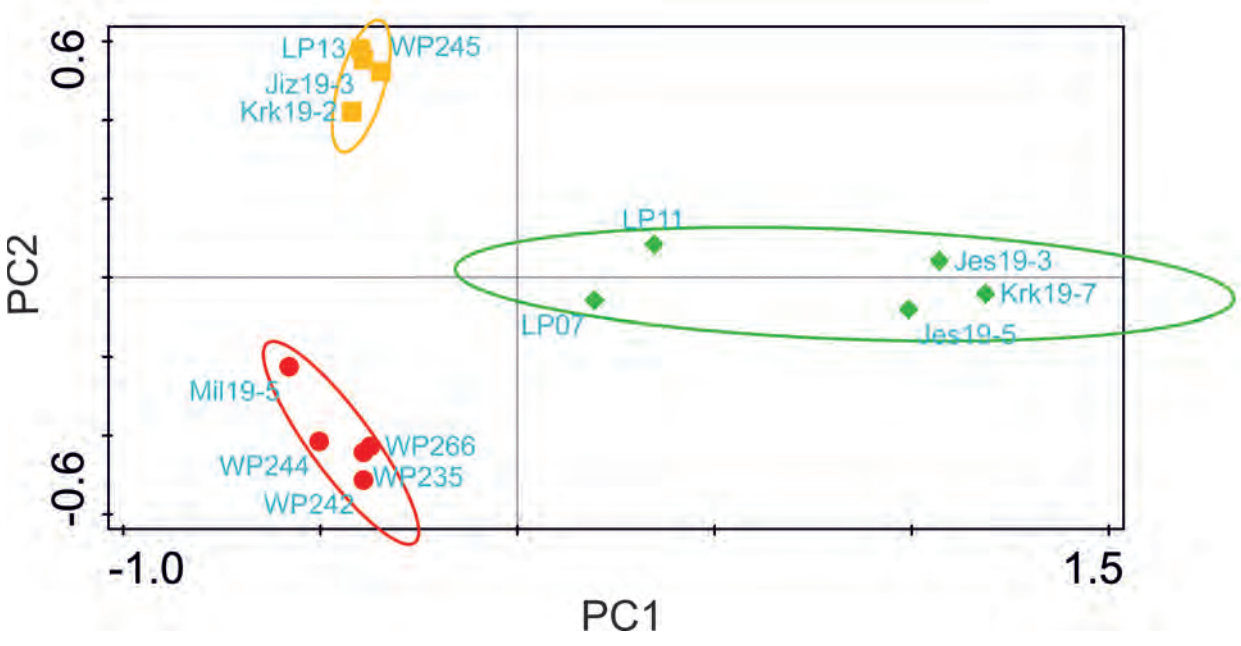
Supplementary Fig. S2. The collision-induced dissociation tandem mass spectrum of ion at *m/z* 842.7243 (triacylglycerol, TAG; Po/Po/Ln) from sample WP235.



Supplementary Fig. S3. The collision-induced dissociation tandem mass spectrum of ion at *m/z* 792.5630 (monogalactosyldiacylglycerol, MGDG; L/St/MGDG) from sample WP235.



Supplementary Fig. S4. The collision-induced dissociation tandem mass spectrum of ion at *m/z* 760.5134 (phosphatidylglycerol, PG; Po/Ln/PG) from the sample WP235 .



Supplementary Fig. S5. Principal component analysis based on all the identified lipid species in the snow blooms of different colour (red, orange and green). The first two canonical axes are plotted; the first axis explained 38.1% and the second 14.1% of the variability. For sample codes, see Table 1.