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Supporting Information for “High-resolution mapping of cellular distributions: imaging analysis of human tissue microstructures”

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1. Supporting method description

Identification of bacteriochlorophyll c species and related compounds

BChl c species were identified based on the fragment representing the characteristic macrocycle, with increasing number of methylations, putatively at positions 8 and 12 (Scheer, 1991). We tested macrocycle fragments 567.2966 (equivalent to a 8-Et, 12-Me configuration as observed in Cfx. aurantiacus), 581.3122, 595.3279 and 609.3430 corresponding, for example, to increasing methylations at the C-8 position and an ethyl group at the C-12 position, as described for Chl. thermophilum. The latter compounds are termed highly methylated BPheo c (mBPheo c) in the present study. Different combination of methylations can yield the same m/z and fragmentation pattern. However, considering the available BChl c profiles described in the literature (García Costas et al., 2012; Fages et al., 1990; Scheer, 1991), we tentatively identified the detected m/z with the macrocycles configurations described above. In addition to this general approach, we specifically, but unsuccessfully, searched for losses of a farnesyl group, given the abundance of farnesyl-esterified BChl.

As MSI lacks chromatographic separation and we did not employ MS2, we cannot distinguish BPheo with increasing number of methylations from BPheo with longer alcohol chains (e.g. C_{16}, C_{17}, C_{18}). Therefore, we did not assign m/z 818.5705, 832.5861, 846.6018 and 860.6174 to any particular BPheo c, but grouped the latter three as highly methylated BPheo c esterified to non-isoprenoidal alcohols (mBPheo c-non iso). The compound with m/z 818.5705 is not included in this group as our bulk analysis indicate the presence of both BPheo c esterified with a C_{18} alcohol and highly methylated BPheo c esterified to C_{17} and C_{16} alcohols.

2. Supporting tables and figures

Table S1. Response factors of lipid classes relative to the internal standard (1,2-dihenarachidoyl-sn-glycero-3-phosphocholine, PC-$DAG_{42:0}$, Avanti Lipids)

Lipid class	Response factor
Monoglycosyl lipids (1G)	1.20
Diglycosyl lipids (2G)	0.45
Sulfoquinovosyl (SQ) lipids	0.11
Betaine lipids (BL)	2.41
Phosphoatidylglycerol lipids (PG)	0.11
Phosphatidyl-N-methylethanolamine lipids (PME)	0.69

Phosphatidyl-N-ethanolamine lipids (PE)	0.32
Phosphatidylcholine lipids (PC)	0.79
Phosphatidylinositol lipids (PI)	0.11
Ornithine lipids (OL)	2.41

Table S2. Summary of biomarkers targeted by MSI. Tentative assignment is based on exact mass and on retention time and fragmentation pattern observed in the conventional biomarker analysis. Only the most abundant adduct detected in the average MSI spectrum was targeted.

Assignment	**Molecular formula**	**Adduct**	**m/z**
Chloropigments			
Pyropheo a	$C_{53}H_{72}N_4O_3$	$M+H^+$	813.568
Pheo a	$C_{55}H_{74}N_4O_5$	$M+H^+$	871.573
OH-Pheo a	$C_{55}H_{74}N_4O_6$	$M+H^+$	887.568
BPheo a	$C_{55}H_{76}N_4O_6$	$M+H^+$	889.584
BPheo c gg	$C_{54}H_{70}N_4O_4$	$M+H^+$	839.547
BPheo c phytol	$C_{54}H_{76}N_4O_4$	$M+H^+$	845.594
mBPheo c gg	$C_{55}H_{72}N_4O_4$	$M+H^+$	853.563
mBPheo c phytol	$C_{55}H_{78}N_4O_4$	$M+H^+$	859.610
2mBPheo c gg	$C_{56}H_{74}N_4O_4$	$M+H^+$	867.578
2mBPheo c phytol	$C_{56}H_{80}N_4O_4$	$M+H^+$	873.625
3mBPheo c gg	$C_{57}H_{76}N_4O_4$	$M+H^+$	881.594
3mBPheo c phytol	$C_{57}H_{82}N_4O_4$	$M+H^+$	887.641
BPheo c m/z 819	$C_{52}H_{74}N_4O_4$	$M+H^+$	819.578
BPheo c m/z 833	$C_{53}H_{76}N_4O_4$	$M+H^+$	833.594
BPheo c m/z 847	$C_{54}H_{78}N_4O_4$	$M+H^+$	847.610
BPheo c m/z 861	$C_{55}H_{80}N_4O_4$	$M+H^+$	861.625
Quinones			
$PQ_{9:9}$	$C_{53}H_{80}O_2$	M^+	748.615
$PQ_{9:8}$	$C_{53}H_{82}O_2$	M^+	750.631
$MK_{7:7}$	$C_{46}H_{64}O_2$	M^+	648.490
$MK_{8:8}$	$C_{51}H_{72}O_2$	M^+	716.553
$MK_{8:7}$	$C_{51}H_{74}O_2$	M^+	718.568
$MK_{9:9}$	$C_{56}H_{80}O_2$	M^+	784.615
$MK_{10:10}$	$C_{61}H_{88}O_2$	M^+	852.678
$MK_{11:11}$	$C_{66}H_{96}O_2$	M^+	920.740
$UQ_{8:8}$	$C_{49}H_{74}O_4$	M^+	726.558
$UQ_{8:7}$	$C_{49}H_{76}O_4$	M^+	728.574
$UQ_{10:10}$	$C_{59}H_{90}O_4$	M^+	862.683
$UQ_{10:9}$	$C_{59}H_{92}O_4$	M^+	864.699
Intact polar lipids			
$1G\text{-}DEG_{31:1}$	$C_{40}H_{78}O_8$	$M+Na^+$	709.559
$1G\text{-}DEG_{32:1}$	$C_{41}H_{80}O_8$	$M+Na^+$	723.575
$1G\text{-}DEG_{33:1}$	$C_{42}H_{82}O_8$	$M+Na^+$	737.590

1G-DEG$_{35:1}$	$C_{44}H_{86}O_8$	M+Na$^+$	765.621
1G-DEG$_{36:1}$	$C_{45}H_{88}O_8$	M+Na$^+$	779.637
1G-AEG$_{32:0}$	$C_{41}H_{80}O_9$	M+Na$^+$	739.569
1G-AEG$_{33:0}$	$C_{42}H_{82}O_9$	M+Na$^+$	753.585
1G-AEG$_{34:0}$	$C_{43}H_{84}O_9$	M+Na$^+$	767.601
1G-DAG$_{32:1}$	$C_{41}H_{76}O_{10}$	M+Na$^+$	751.533
1G-DAG$_{32:0}$	$C_{41}H_{78}O_{10}$	M+Na$^+$	753.549
1G-DAG$_{33:1}$	$C_{42}H_{78}O_{10}$	M+Na$^+$	765.549
1G-DAG$_{34:1}$	$C_{43}H_{80}O_{10}$	M+Na$^+$	779.564
1G-DAG$_{34:0}$	$C_{43}H_{82}O_{10}$	M+Na$^+$	781.580
1G-DAG$_{35:1}$	$C_{44}H_{82}O_{10}$	M+Na$^+$	793.580
1G-DAG$_{36:1}$	$C_{45}H_{84}O_{10}$	M+Na$^+$	807.596
2G-DEG$_{31:1}$	$C_{46}H_{88}O_{13}$	M+Na$^+$	871.612
2G-DEG$_{32:2}$	$C_{47}H_{88}O_{13}$	M+Na$^+$	883.612
2G-DEG$_{32:1}$	$C_{47}H_{90}O_{13}$	M+Na$^+$	885.627
2G-DEG$_{33:2}$	$C_{48}H_{90}O_{13}$	M+Na$^+$	897.627
2G-DEG$_{33:1}$	$C_{48}H_{92}O_{13}$	M+Na$^+$	899.643
2G-DEG$_{34:1}$	$C_{49}H_{94}O_{13}$	M+Na$^+$	913.659
2G-DEG$_{35:1}$	$C_{50}H_{96}O_{13}$	M+Na$^+$	927.674
2G-AEG$_{34:0}$	$C_{49}H_{94}O_{14}$	M+Na$^+$	929.654
2G-AEG$_{35:0}$	$C_{50}H_{96}O_{14}$	M+Na$^+$	943.669
2G-DAG$_{32:0}$	$C_{47}H_{88}O_{15}$	M+Na$^+$	983.664
2G-DAG$_{34:1}$	$C_{49}H_{90}O_{15}$	M+Na$^+$	969.648
2G-DAG$_{35:1}$	$C_{50}H_{92}O_{15}$	M+Na$^+$	967.633
2G-DAG$_{36:2}$	$C_{51}H_{92}O_{15}$	M+Na$^+$	955.633
2G-DAG$_{36:1}$	$C_{51}H_{94}O_{15}$	M+Na$^+$	941.617
2G-DAG$_{37:1}$	$C_{52}H_{96}O_{15}$	M+Na$^+$	915.602
1G-Cer$_{36:2}$	$C_{42}H_{79}N_1O_8$	M+H$^+$	726.588
1G-Cer$_{36:0}$	$C_{42}H_{83}N_1O_8$	M+H$^+$	730.619
1G-Cer$_{40:0}$	$C_{46}H_{91}N_1O_8$	M+H$^+$	786.682
1G-Cer$_{41:0}$	$C_{47}H_{93}N_1O_8$	M+H$^+$	800.697
1G-OH-Cer$_{38:1}$	$C_{44}H_{85}N_1O_9$	M+H$^+$	772.630
1G-OH-Cer$_{39:1}$	$C_{45}H_{87}N_1O_9$	M+H$^+$	786.645
BL-DEG$_{32:1}$	$C_{42}H_{83}N_1O_5$	M+H$^+$	682.634
BL-DEG$_{33:1}$	$C_{43}H_{85}N_1O_5$	M+H$^+$	696.650
BL-AEG$_{34:0}$	$C_{44}H_{87}N_1O_6$	M+H$^+$	726.661
BL-AEG$_{35:0}$	$C_{45}H_{89}N_1O_6$	M+H$^+$	740.676
BL-DAG$_{30:0}$	$C_{40}H_{77}N_1O_7$	M+H$^+$	684.577
BL-DAG$_{31:0}$	$C_{41}H_{79}N_1O_7$	M+H$^+$	698.593
BL-DAG$_{32:0}$	$C_{42}H_{81}N_1O_7$	M+H$^+$	712.609
BL-DAG$_{33:0}$	$C_{43}H_{83}N_1O_7$	M+H$^+$	726.624
BL-DAG$_{34:0}$	$C_{44}H_{85}N_1O_7$	M+H$^+$	740.640
BL-DAG$_{35:0}$	$C_{45}H_{87}N_1O_7$	M+H$^+$	754.656
TM-OL$_{35:0}$	$C_{43}H_{84}N_2O_5$	M+H$^+$	709.645
TM-OL$_{36:1}$	$C_{44}H_{84}N_2O_5$	M+H$^+$	721.645
TM-OL$_{36:0}$	$C_{44}H_{86}N_2O_5$	M+H$^+$	723.661

TM-OL$_{37:1}$	$C_{45}H_{86}N_2O_5$	M+H$^+$	735.661
TM-OL$_{37:0}$	$C_{45}H_{88}N_2O_5$	M+H$^+$	737.677
TM-OL$_{38:1}$	$C_{46}H_{88}N_2O_5$	M+H$^+$	749.677
PG-DAG$_{34:1}$	$C_{40}H_{77}O_{10}P_1$	M+Na$^+$	771.515
PG-DAG$_{34:0}$	$C_{40}H_{79}O_{10}P_1$	M+Na$^+$	773.530
PG-DAG$_{35:1}$	$C_{41}H_{79}O_{10}P_1$	M+Na$^+$	785.530
PME-DAG$_{30:0}$	$C_{36}H_{72}N_1O_8P_1$	M+H$^+$	678.507
PME-DAG$_{31:0}$	$C_{37}H_{74}N_1O_8P_1$	M+H$^+$	692.522
PME-DAG$_{32:0}$	$C_{38}H_{76}N_1O_8P_1$	M+H$^+$	706.538
PE-DAG$_{32:1}$	$C_{37}H_{72}N_1O_8P_1$	M+H$^+$	690.507
PE-DAG$_{32:0}$	$C_{37}H_{74}N_1O_8P_1$	M+H$^+$	692.522
PE-DAG$_{34:1}$	$C_{39}H_{76}N_1O_8P_1$	M+H$^+$	718.538
PC-DAG$_{37:0}$	$C_{45}H_{90}N_1O_8P_1$	M+H$^+$	804.648
PC-DAG$_{38:0}$	$C_{46}H_{92}N_1O_8P_1$	M+H$^+$	818.663
PI-AEG$_{41:2}$	$C_{50}H_{95}O_{12}P_1$	M+Na$^+$	941.645
PI-AEG$_{42:2}$	$C_{51}H_{97}O_{12}P_1$	M+Na$^+$	955.661
SQ-DEG$_{32:1}$	$C_{41}H_{80}O_{10}S_1$	M+Na$^+$	787.536
SQ-DEG$_{33:1}$	$C_{42}H_{82}O_{10}S_1$	M+Na$^+$	801.552
SQ-DEG$_{34:1}$	$C_{43}H_{84}O_{10}S_1$	M+Na$^+$	815.568
SQ-DAG$_{32:0}$	$C_{41}H_{78}O_{12}S_1$	M+Na$^+$	817.511
SQ-DAG$_{34:1}$	$C_{43}H_{80}O_{12}S_1$	M+Na$^+$	843.526
SQ-DAG$_{34:0}$	$C_{43}H_{82}O_{12}S_1$	M+Na$^+$	845.542
SQ-DAG$_{35:1}$	$C_{44}H_{82}O_{12}S_1$	M+Na$^+$	857.542
SQ-DAG$_{36:0}$	$C_{45}H_{86}O_{12}S_1$	M+Na$^+$	873.573

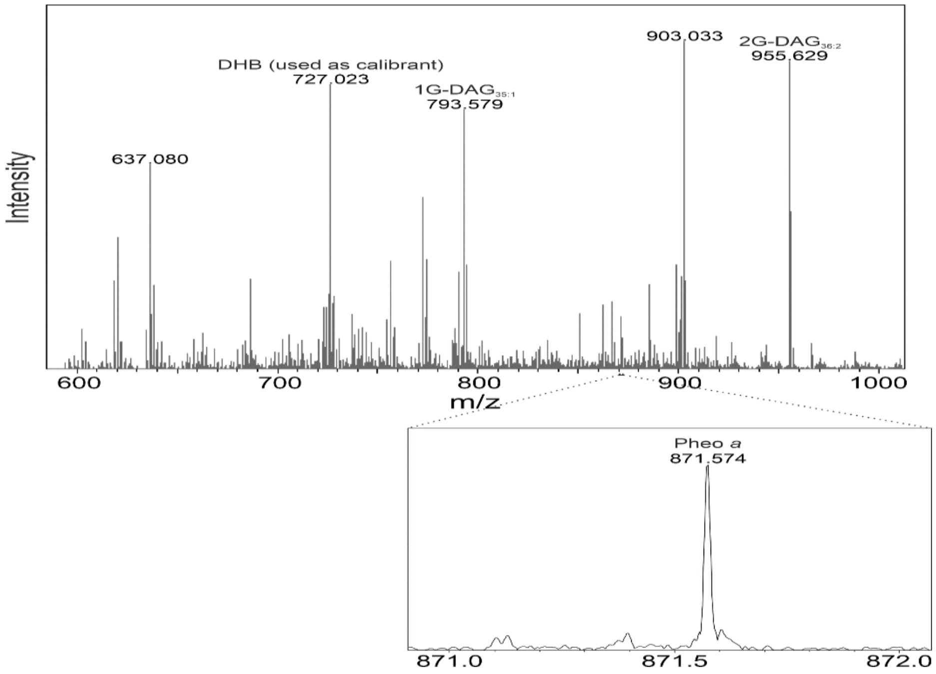

Figure S1. Single mass spectrum acquired by MSI with 75-µm resolution. m/z values are exemplarily provided for the five most intense peaks. Three of them could be assigned to targeted molecular biomarkers and the DHB-cluster employed for calibration. Zoom-in shows the mass spectrum at m/z 871 and the peak assigned to Pheo a.

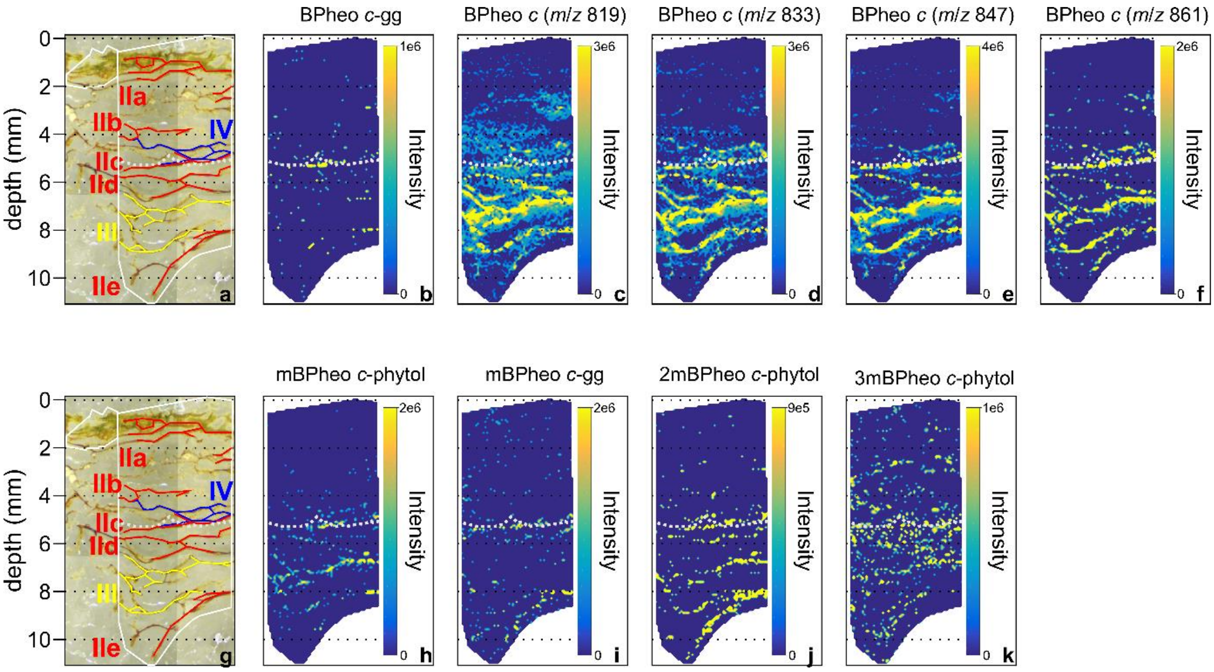

Figure S2. Images of the mat slice (a, g) and 75-µm resolution MSI of chloropigments which are not shown (b, c, h-k) or presented grouped in the main text: geranyl-geraniol (gg) esterified bacteriopheophytin c (b) and highly methylated bacteriopheopytin c esterified with non isoprenoidal (c-f) and isoprenoidal (h-k) alcohols. As the 95th quantile was 0 for panels (b), (h) and (i), the 99th quantile was chosen as the upper limit of the color scale. Distribution of the different groups of anoxygenic phototrophs (II-IV) is superimposed on the mat images.

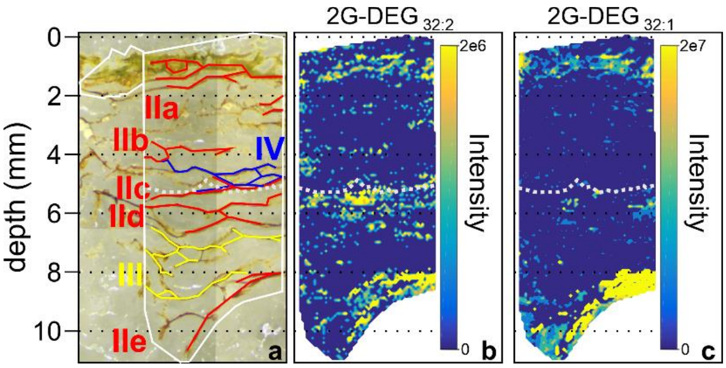

Figure S3. Images of the mat slice (a) and 75-µm resolution MSI of a mono- and diunsaturated 2G-DEG species (b, c).Distribution of the different groups of anoxygenic phototrophs (II-IV) is superimposed on the mat image.

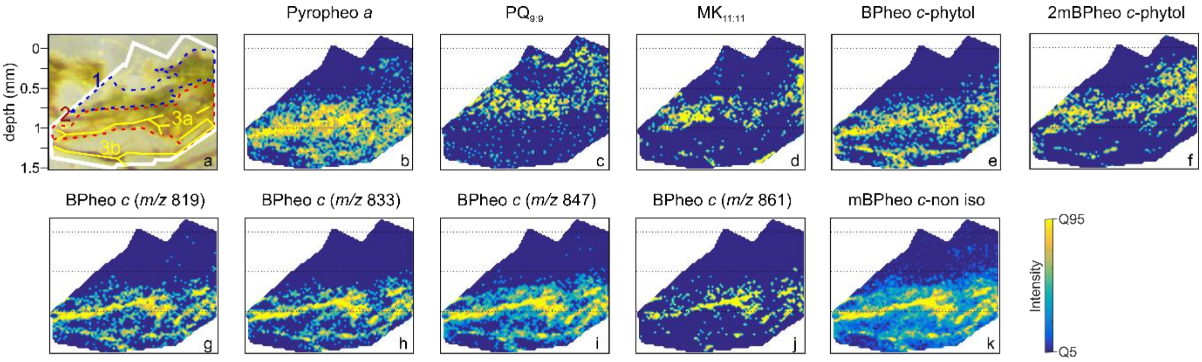

Figure S4. Zoom-in into the image of the mat slice (a) and 25-µm resolution MSI of quinones and chloropigments which are not shown (b, f, g) or presented grouped or as differences in the main text. Distribution of the different regions of chlorophototrophs in the green layer (1-3) is superimposed on the mat image.

3. Additional biomarker MSI not presented in the main text

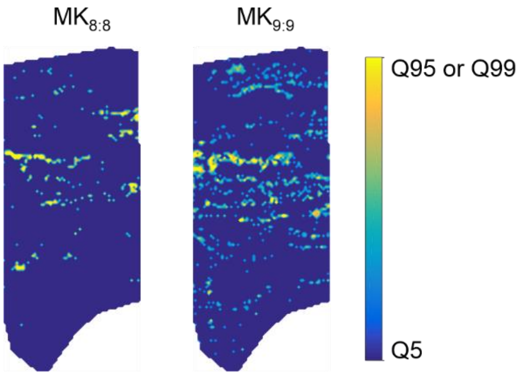

Figure S5. 75-µm resolution MSI of menaquinones not presented in the main figures. Lower limit of the color scale is the 5th quantile, upper limit is the 99th (MK8:8) or 95th (MK9:9) quantile

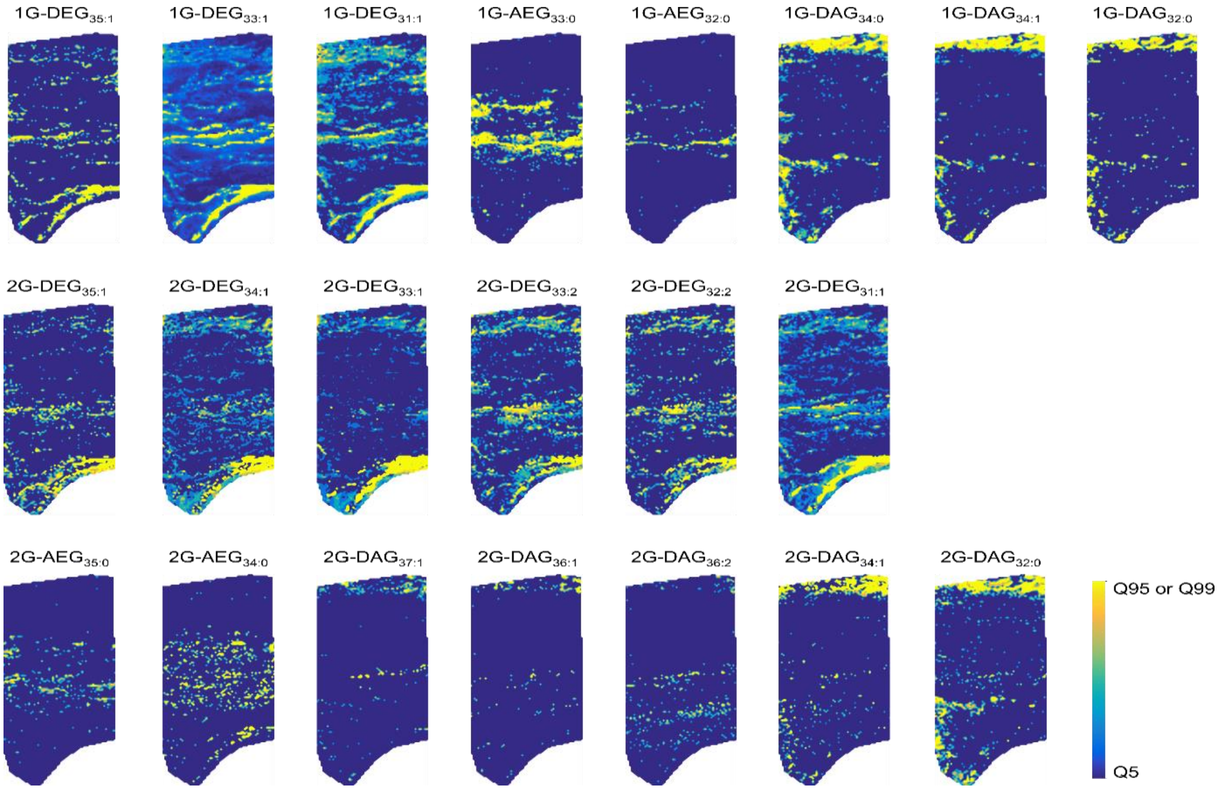

Figure S6. 75-μm resolution MSI of glycolipids not presented in the main figures. Lower limit of the color scale is the 5th quantile, upper limit is the 99th (1G-AEG$_{32:0}$, 2G-AEG$_{35:0}$, 2G-DAG$_{37:1}$, 2G-DAG$_{36:1}$, 2G-DAG$_{36:2}$) or 95th quantile.

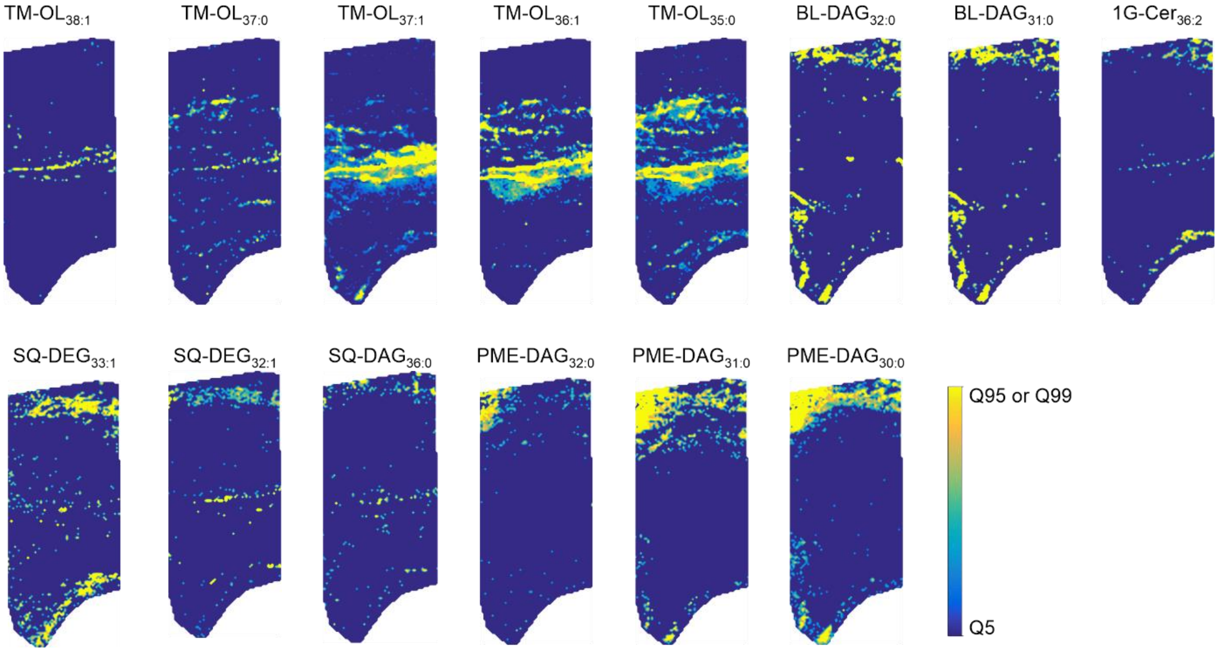

Figure S7. 75-μm resolution MSI of N-, P- or S-bearing lipids not presented in the main figures. Lower limit of the color scale is the 5th quantile, upper limit is the 99th (1G-Cer$_{36:2}$, TM-OL$_{38:1}$, TM-OL$_{37:0}$, SQ-DEG$_{32:1}$, SQ-DAG$_{36:0}$, PME-DAG$_{32:0}$) or 95th quantile.

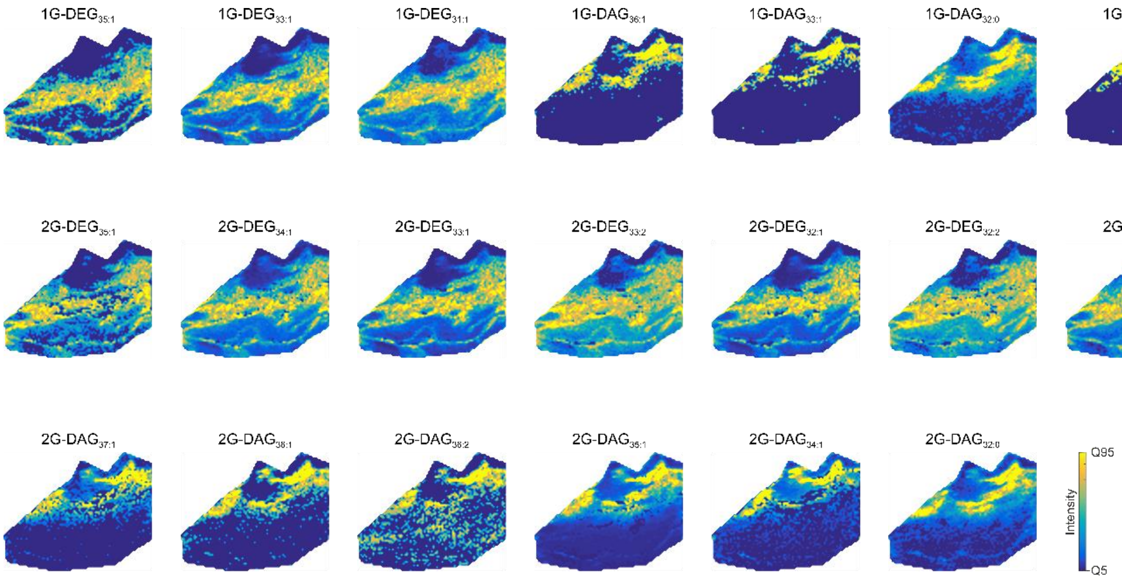

Figure S8. 25-μm resolution MSI of glycolipids not presented in the main figures. The color scale ranges between the 5th and 95th quantiles.

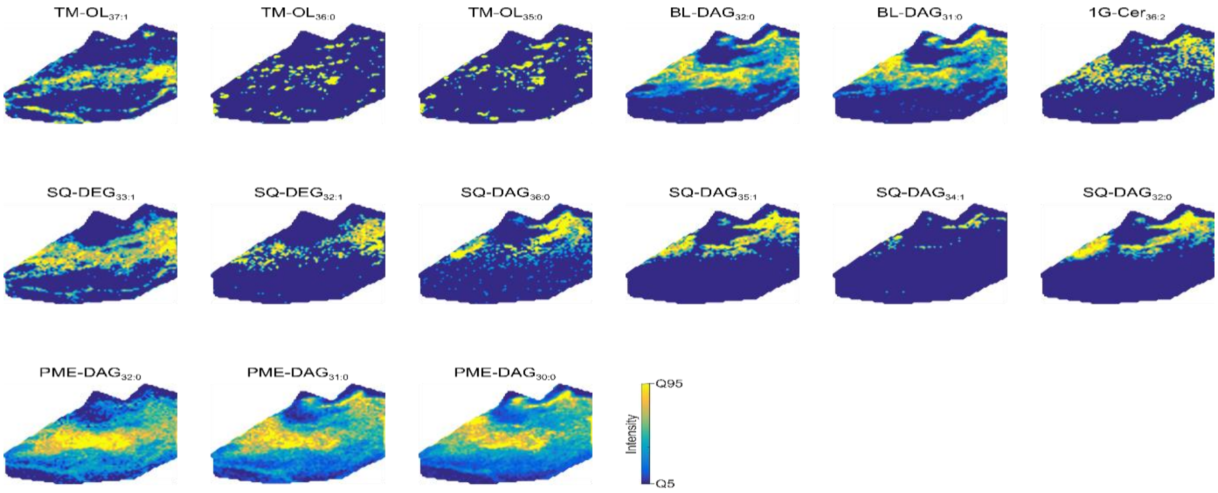

Figure S9. 25-μm resolution MSI of N-, P- or S-bearing lipids not presented in the main figures. The color scale ranges between the 5th and 95th quantiles.

References

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Scheer, H., 1991. Structure and occurence of chlorophylls, in: Scheer, H. (Ed.), Chlorophylls. CRC Press, pp. 3-30.