Supplementary methods

Shotgun lipidomics

The LTQ-Orbitrap Velos mass spectrometer (MS) manufactured by Thermo Fisher Scientific (San Jose CA, USA) was used for shotgun lipidomics profiling of total lipid extracts. This is a high-resolution hybrid MS equipped with a heated electrospray interface (H–ESI). It was operated in negative ionization mode. Flow injection analysis was used for sample introduction into the H–ESI ion source. Acetonitrile:water (50:50, v:v) was used at a flow rate of 150 µL min^{-1}. The MS scan range was performed in the Fourier transform (FT) mode and recorded within a window between 200–2000 Da.

Mass spectra were acquired with a target mass resolution of R = 110,000 at *m/z* 800 and the ion spray voltage was set at -2.5 kV in the negative ionization mode. Ionization mode used the following parameters: sheath gas flow, 18 arbitrary units (AU); auxiliary gas flow, 7 AU; ion source temperature, 250 °C; capillary temperature, 230 °C; capillary voltage, 50 V; and tube lens voltage, 170 V. Helium was used as a collision gas for collision-induced dissociation (CID) experiments. The CID normalization energy of 35% was used for the fragmentation of the precursor ions.

The tandem MS (MS/MS) product ions used for structure identification of specific compounds were detected in high resolution FT mode. The calibration of the MS spectrometer was performed using a Pierce LTQ Orbitrap negative ion calibration solution (Thermo Fisher Scientific). The internal lock mass was used in mass spectra acquisition, i.e., 255.2330 *m/z* $[M-H]^-$ palmitic acid in the negative ESI. The mass accuracy was better than 0.9 ppm. The chemical structures of the compounds were confirmed with the help of the LIPID MAPS® Lipidomics Gateway spectral database (http://www.lipidmaps.org/). Common chemicals, i.e., acetonitrile, 2-propanol, hexane, and dichloromethane, were purchased from Sigma-Aldrich, Ltd. (Prague, Czech Republic). Cardiolipin containing two oleic and two stearic fatty acid residues (used as an internal standard) also was purchased from Sigma-Aldrich.

Identification of the 76:12-cardiolipin molecular structure

Tandem mass spectrum of 76:12-CL (i.e., 20:4/20:5/18:1/18:2-CL, see Table S2 below for explanation of the abbreviations) was used to determine the structure of molecule (see Fig 1b, Fig S2 and Table S3 below). This CL had a mass of 1495.9653 Da for the $[M-H]^-$ ion. The probable structure and mass-to-charge (*m/z*) values of the diagnostic ions are shown in Table S3 and confirm the structure of four fatty acids in the molecule of 76:12-CL, i.e., 20:4ω6, 20:5ω3, 18:1ω9, and 18:2ω6. This structure also was supported by six ions of type **a**, **a+56**, **a+136**, **b**, **b+56**, and **b+136**. On the basis of the tandem mass spectrum, the ion at *m/z* 1193.7406 with total elemental composition $[C_{65}H_{111}O_{15}P_2]^-$, which is the base peak in tandem MS, was selected as a biomarker for further determination of the limits of quantification and detection (Table S1). This ion, designated as $[20:4/18:1/18:2]^-$ (Table S3) arises from neutral loss of the RCOOH group, in this case 20:5ω3 from $[M-H]^-$.

The use of a high-resolution instrument allowed us to distinguish the ions of two compounds that differed by only one double bond. For example, $[M-H+2]^-$ at *m/z* 1495.9560 with summary formula $C_{83}{}^{13}C_2H_{139}O_{17}P_2$ (20:5/20:5/18:1/18:2-CL + 2 Da), respectively $[M-H]^-$, at *m/z* 1495.9649 with the summary formula $C_{85}H_{141}O_{17}P_2$ (20:4/20:5/18:1/18:2-CL). These ions do not interfere with each other, and thus it was possible to use the compound 20:4/20:5/18:1/18:2-CL as a taxonomic marker.

Lipid nomenclature

Lipid nomenclature was applied according to Liebisch et al. (2013).

Supplementary Figures

Fig. S1. *In vitro* culture of the arbuscular mycorrhizal (AM) fungus *Rhizophagus irregularis* in association with Ri-T-DNA transformed cichory root organ culture. The cultures were grown in Microbox containers (originally acquired from Combiness, Belgium, now available from SAC O_2, Deinze, Belgium), using a plastic floater (red edge visible in the photo) self-made of sterile filter tips stands and covered with nylon mesh with 42 µm pores (Silk & Progress, Brněnec, Czech Republic). Containers were filled with liquid MSR medium as described previously (Rozmoš et al 2022) with a fourfold increased phosphate concentration as compared to the original recipe to compensate for the phosphorus inputs normally provided with phytagel. Mycorrhizal or non-mycorrhizal cichory roots are placed onto the mesh, which prevents their ingrowth into the liquid medium. The AM fungal hyphae penetrate the mesh and proliferate in the liquid medium below the mesh, from which they can be recovered easily.

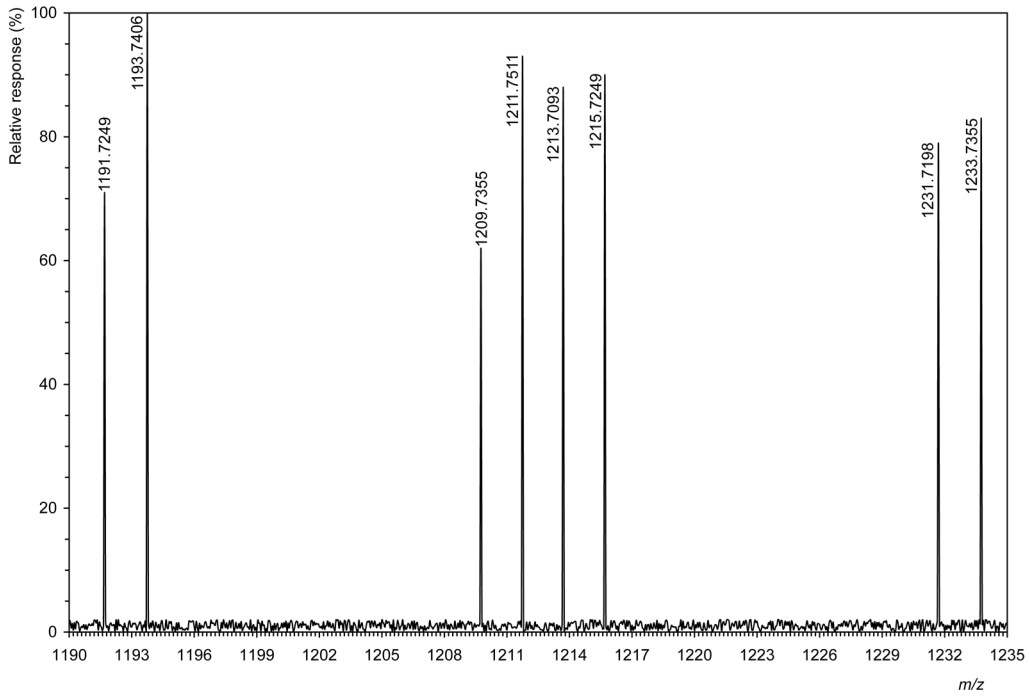


Fig. S2. Cutaway from Fig. 1b showing ions in the interval 1190–1235 Da. These ions are formed by loss of acyl chains as ketenes (RCH=C=O) and neutral loss of RCOOH groups from $[M-H]^-$ precursor ion.

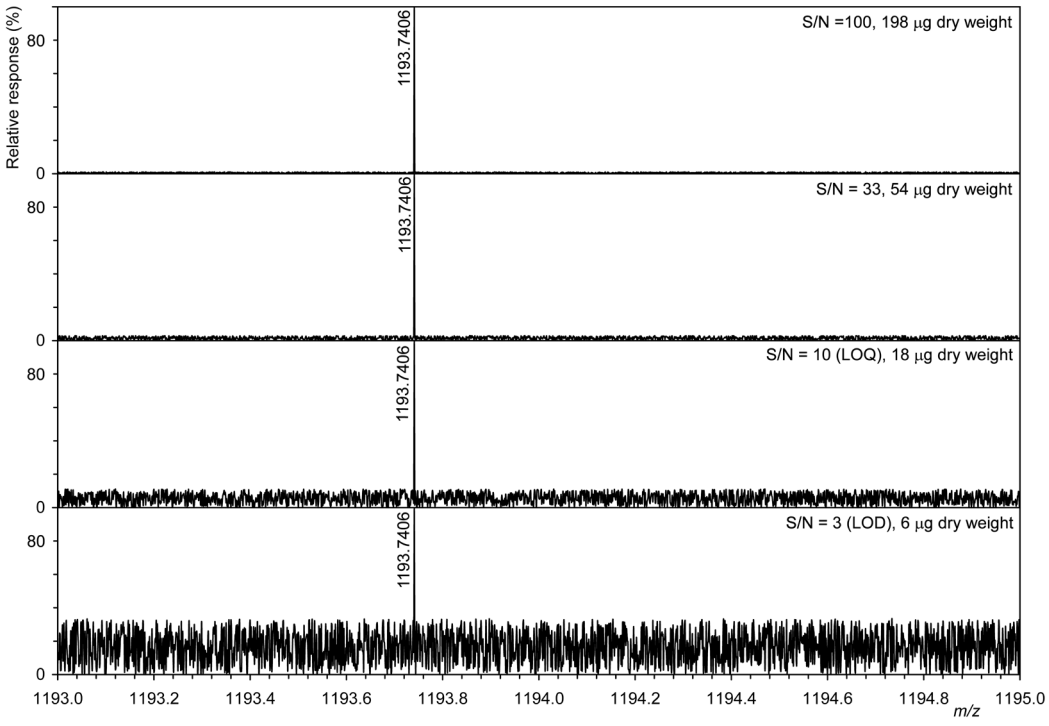


Fig. S3. Estimation of the limits of detection (LOD) and limit of quantification (LOQ) of the 76:12-cardiolipin by serial dilution of the lipids extracted from the pure *Rhizophagus* biomass, and monitoring of its base peak by tandem mass spectrometry.

Supplementary Tables

Table S1 Quantification of the AM fungus and the novel cardiolipin (CL) biomarker in the different samples, and determination of the minimum amount of biomass needed to reach limit of detection (LOD) and limit of quantitation (LOQ) for the particular CL.

Sample	Mycorrhizal status of roots	Root colonization assessed by microscopy after staining with Trypan Blue			qPCR quantification of *Rhizophagus* mtLSU	Fatty acid (16:1ω5) concentration - WCFA (µmol C/mg biomass)	Biomass used for extraction of lipids (mg dry weight)	concentration of 76:12-cardiolipin (% of the dry biomass weight)	LOD of 76:12-CL, S/N ratio = 3 (pg)	Biomass required to reach LOD (µg)	LOQ of 76:12-CL, S/N ratio = 10 (pg)	Biomass required to reach LOQ (µg)
		H%	A%	V%	mt5 copies mg^{-1}							
Cichorium + *Rhizophagus*	M	74	37	18	245956	0.322	11.3	0.00098	186	19	539	55
Allium + *Rhizophagus*	M	76	60	31	75546	0.343	12.8	0.00123	283	23	812	66
Andropogon + *Rhizophagus*	M	75	52	24	19072	0.276	13.5	0.00101	172	17	495	49
Cichorium	NM	n.d.	n.d.	n.d.	n.d.	0.054	16.2	n.d.	n.a.	n.a.	n.a.	n.a.
Allium	NM	n.d.	n.d.	n.d.	n.d.	0.034	13.6	n.d.	n.a.	n.a.	n.a.	n.a.
Andropogon	NM	n.d.	n.d.	n.d.	n.d.	0.121	19.6	n.d.	n.a.	n.a.	n.a.	n.a.
Rhizophagus pure biomass	n.a.	n.a.	n.a.	n.a.	94916	13.519	15.1	0.00315	189	6	567	18

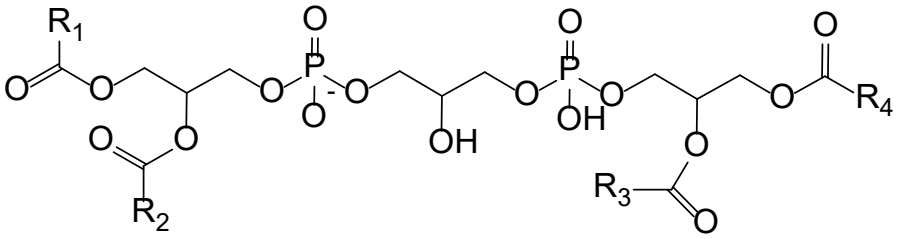
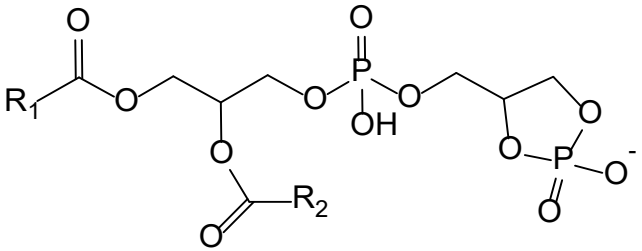
NM: non-mycorrhizal, H%: root length colonized by AM fungal hyphae, A%: root length colonized by arbuscules, V%: root length colonized by vesicles, n.a.: not applicable, n.d.: not detected, qPCR: quantitative real-time PCR, mtLSU: mitochondrial large ribosomal subunit gene, WCFA: whole cell fatty acids - lipids extracted but not fractionated and fatty acid transmethylated before the analysis, LOD: limit of detection, LOQ: limit of quantitation, S/N: signal to noise ratio

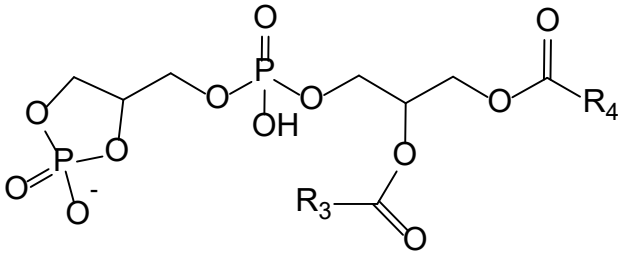
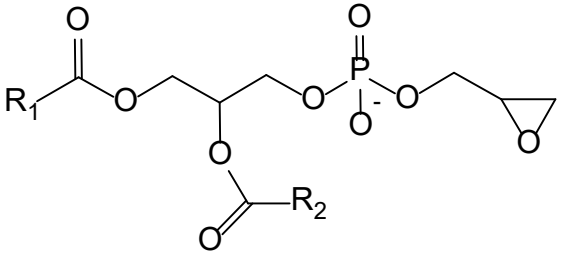
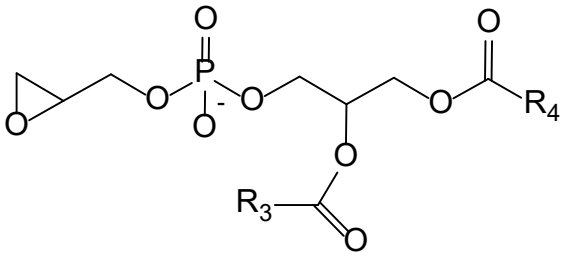
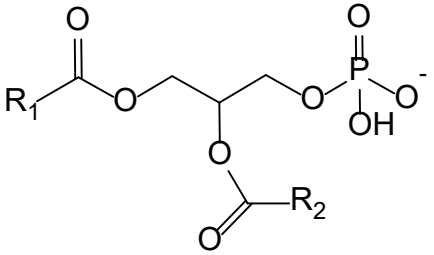
Table S2 Abbreviations of fatty acids referred to in this study

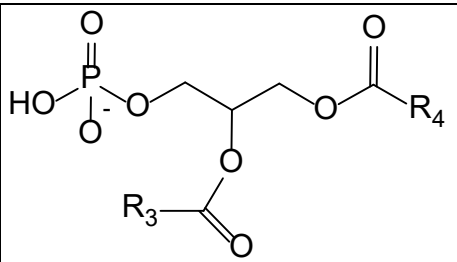
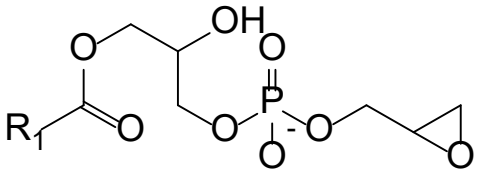
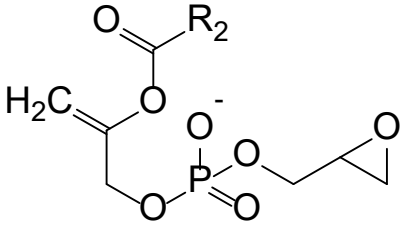
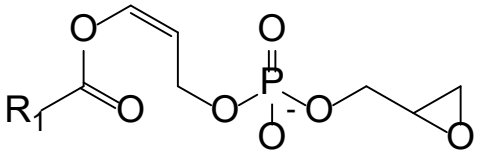
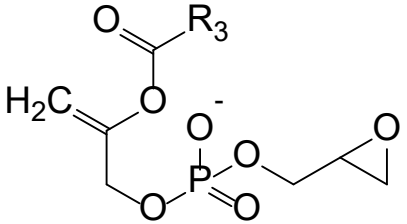
Abbreviation	Shorthand designation	Trivial name
16:0	16:0	Palmitic acid
16:1	16:1ω7 or 9-16:1[a]	Palmitoleic acid
16:1ω5	16:1ω5 or 11-16:1	Palmitvaccenic acid
18:0	18:0	Stearic acid
18:1	18:1ω9 or 9-18:1	Oleic acid
18:2	18:2ω6 or 9,12-18:2	Linoleic acid
18:3	18:3ω3 or 9,12,15-18:3	Linolenic acid
γ-18:3	18:3ω6 or 6,9,12-18:3	γ-Linolenic acid
20:4	20:4ω6 or 5,8,11,14-20:4	Arachidonic acid
20:5	20:5ω3 or 5,8,11,14,17-20:5	Eicosapentaenoic acid

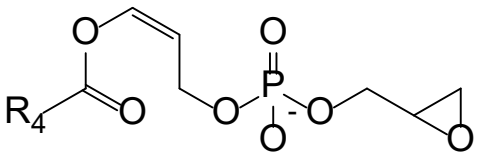
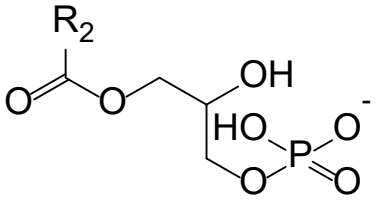
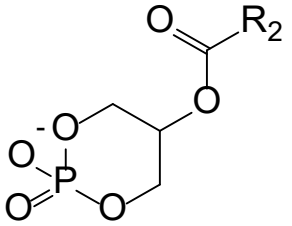
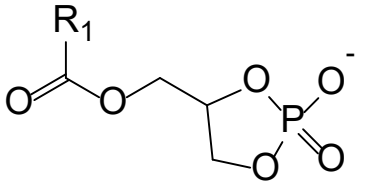
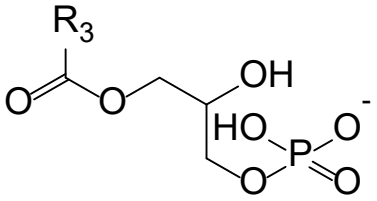
[a] All double bonds have a geometric cis (more recently Z) configuration.

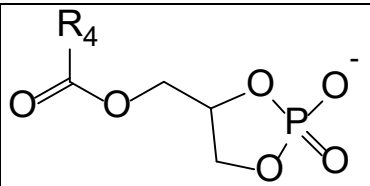
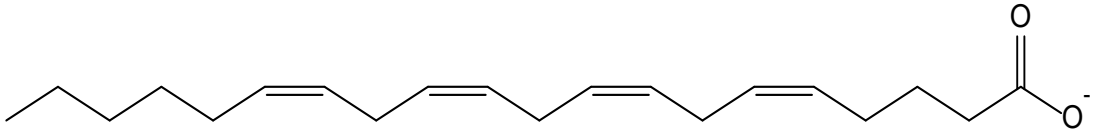
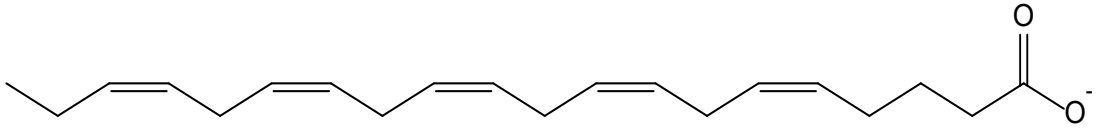
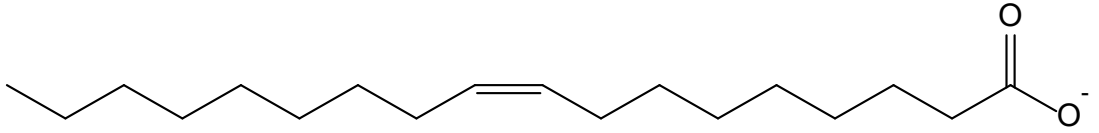
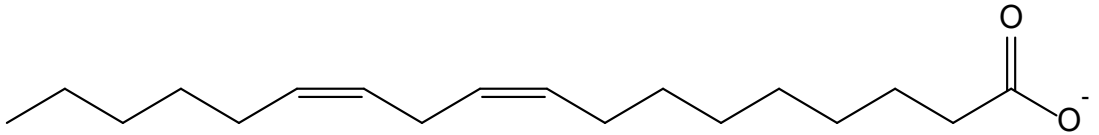
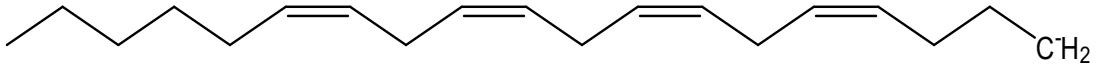
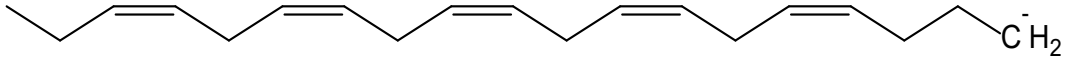
Table S3. Product ions for the 76:12-CL detected in the mycorrhizal root and biomass samples

Chemical Formula	Exact Mass	Abundance (relative %)	Ion Description	Structure
$C_{85}H_{141}O_{17}P_2^-$	1495.9653	49	**[M-H]$^-$**	
$C_{67}H_{111}O_{16}P_2^-$	1233.7355	83	**[20:5/20:4/18:1]$^-$**	Loss of acyl chain (18:2) as ketene (RCH=C=O) from [M-H]$^-$
$C_{67}H_{109}O_{16}P_2^-$	1231.7198	79	**[20:5/20:4/18:2]$^-$**	Loss of acyl chain (18:1) as ketene (RCH=C=O) from [M-H]$^-$
$C_{67}H_{109}O_{15}P_2^-$	1215.7249	90	**[20:5/20:4/18:1]$^-$**	Neutral loss of RCOOH group (18:2) from [M-H]$^-$
$C_{67}H_{107}O_{15}P_2^-$	1213.7093	88	**[20:5/20:4/18:2]$^-$**	Neutral loss of RCOOH group (18:1) from [M-H]$^-$
$C_{65}H_{113}O_{16}P_2^-$	1211.7511	93	**[20:4/18:1/18:2]$^-$**	Loss of acyl chain (20:5) as ketene (RCH=C=O) from [M-H]$^-$
$C_{65}H_{111}O_{16}P_2^-$	1209.7355	62	**[20:5/18:1/18:2]$^-$**	Loss of acyl chain (20:4) as ketene (RCH=C=O) from [M-H]$^-$
$C_{65}H_{111}O_{15}P_2^-$	1193.7406	100	**[20:4/18:1/18:2]$^-$**	Neutral loss of RCOOH group (20:5) from [M-H]$^-$
$C_{65}H_{109}O_{15}P_2^-$	1191.7249	71	**[20:5/18:1/18:2]$^-$**	Neutral loss of RCOOH group (20:4) from [M-H]$^-$
$C_{46}H_{71}O_{12}P_2^-$	877.4428	27	**a+136**	

$C_{42}H_{75}O_{12}P_2^-$	833.4743	31	**b+136**	
$C_{46}H_{70}O_9P^-$	797.4765	39	**a+56**	
$C_{42}H_{74}O_9P^-$	753.5080	44	**b+56**	
$C_{85}H_{140}O_{17}P_2^{2-}$	747.4790	8	**$[M-2H]^{2-}$**	
$C_{43}H_{66}O_8P^-$	741.4503	53	**a**	

$C_{39}H_{70}O_8P^-$	697.4818	48	**b**	
$C_{26}H_{40}O_8P^-$	511.2470	61		
$C_{26}H_{40}O_7P^-$	495.2519	38		
$C_{26}H_{38}O_7P^-$	493.2365	58		
$C_{24}H_{42}O_7P^-$	473.2678	41		

$C_{24}H_{40}O_7P^-$	471.2521	36		
$C_{23}H_{38}O_7P^-$	457.2363	33		
$C_{23}H_{36}O_6P^-$	439.2257	36		
$C_{23}H_{34}O_6P^-$	437.2102	44		
$C_{21}H_{40}O_7P^-$	435.2521	50		

$C_{21}H_{36}O_6P^-$	415.2258	47		
$C_{20}H_{31}O_2^-$	303.2331	66	**20:4**	
$C_{20}H_{29}O_2^-$	301.2176	75	**20:5**	
$C_{18}H_{33}O_2^-$	281.2488	64	**18:1**	
$C_{18}H_{31}O_2^-$	279.2332	71	**18:0**	
$C_{19}H_{31}^-$	259.2432	41	**20:4-CO_2**	
$C_{19}H_{29}^-$	257.2277	43	**20:5-CO_2**	

Supplementary references:

Liebisch G, Vizcaíno JA, Köfeler H, Trötzmüller M, Griffiths WJ, Schmitz G, Spener F, Wakelam MJO (2013) Shorthand notation for lipid structures derived from mass spectrometry. J Lipid Res 54:1523–1530. doi: 10.1194/jlr.M033506

Rozmoš M, Bukovská P, Hršelová H, Kotianová M, Dudáš M, Gančarčíková K, Jansa J (2022) Organic nitrogen utilization by an arbuscular mycorrhizal fungus is mediated by specific soil bacteria and a protist. ISME J 16:676-685. doi: 10.1038/s41396-021-01112-8