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Abundant trimethylornithine lipids and specific gene sequences indicate Planctomycete importance at the oxic/anoxic interface in *Sphagnum*-dominated northern wetlands

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ABSTRACT

Northern wetlands make up a substantial terrestrial carbon sink, and are often dominated by decay-resistant *Sphagnum*-mosses. Recent studies have shown that Planctomycetes appear to be involved in degradation of *Sphagnum*-derived debris. Novel trimethylornithine (TMO) lipids have recently been characterized as abundant lipids in various *Sphagnum*-wetland planctomycete isolates, but their occurrence in the environment has not yet been confirmed. We applied a combined intact polar lipid (IPL) and molecular analysis of peat cores collected from two northern wetlands (Saxnäs Mosse, Sweden; Obukhovskoye, Russia) in order to investigate the preferred niche and abundance of TMO-producing planctomycetes. TMOs were present throughout the profiles of *Sphagnum* bogs but their concentration peaked at the oxic/anoxic interface, which coincided with a maximum abundance of Planctomycete-specific 16S rRNA gene sequences. The sequences detected at the oxic/anoxic interface were affiliated to the *Isosphaera* group, while sequences present in the anoxic peat layers were related to an uncultured planctomycete group. Pyrosequencing-based analysis identified Planctomycetes as the major bacterial group at the oxic/anoxic interface at the Obukhovskoye peat (54% of total 16S rRNA gene sequence reads) followed by Acidobacteria (19% reads), while in the Saxnäs Mosse peat Acidobacteria were dominant (46%), and Planctomycetes contributed to 6% of total reads. The detection of abundant TMO lipids in planctomycetes isolated from peat bogs and the lack of TMO production by cultures of Acidobacteria suggests that planctomycetes are the producers of TMOs in peat bogs. The higher accumulation of TMOs at the oxic/anoxic interface and the change in planctomycete community with depth suggest that these IPLs could be synthesized as a response to changing redox conditions in the oxic/anoxic interface.

INTRODUCTION

Peat-accumulating northern wetlands are important sinks for terrestrial carbon, making up one third of the global soil organic carbon pool (1-4). Nutrient-poor and acidic conditions, as well as low temperatures and decay resistant *Sphagnum*-moss dominated vegetation result in low rates of microbial decomposition of plant debris and net carbon sequestration in these ecosystems (5-12). However, carbon respiration has been shown to accelerate in subsurface peat due to climate warming in the subarctic (13) and in climate warming simulations (14). Additionally, the decomposition of organic matter in anoxic peat layers of northern wetlands is also a significant source of methane to the atmosphere (15-18). Permafrost melt has been shown to result in a net carbon release in northern tundra, including methane emission from thaw lakes (19-22). The microbial community responsible for decomposition of *Sphagnum*-derived litter is unique compared to other soil systems and important to the global carbon cycle (23-25). Further study is needed on the physiology of this microbial community to understand how it will respond to changing environmental conditions.

Planctomycetes have recently been observed to be abundant microbes in *Sphagnum*-dominated northern wetlands and appear to play a role in *Sphagnum* degradation (24, 26, 27). All currently described peat-inhabiting planctomycetes have the ability to degrade various heteropolysaccharides (28-32), but the addition of available nitrogen to cellulose-amended *Sphagnum* peat resulted in a decrease in the relative abundance of planctomycetes compared to the total microbial community (25). In a 16S rRNA gene pyrosequencing survey of a northern acidic *Sphagnum*-dominated wetland, Serkebaeva et al. (33) observed that planctomycetes contribute to higher percentage of bacterial 16S rRNA gene reads in the anoxic subsurface peat layer than in the surface. These studies suggest that wetland-inhabiting planctomycetes preferentially occupy anoxic niches of the peat and are also more suited to nutrient poor conditions probably contributing to the final stages of plant litter decomposition.

49 The functional role of planctomycetes in these ecosystems, however, remains poorly
50 understood.

51 Characterizing the cell membranes of bacteria is important in understanding how they
52 are adapted to their niches as their membranes come into contact with the environment (34,
53 35). Intact polar lipids (IPLs) are the building blocks of cell membranes consisting of a polar
54 head group connected to nonpolar core lipids. IPLs are thought to represent living biomass
55 and their molecular structures can be taxonomically and environmentally specific, making
56 them useful biomarkers (36, 37). Novel trimethylornithine lipids (TMOs) (Fig. S1) have
57 recently been characterized as abundant lipids in various isolates of *Sphagnum*-wetland
58 planctomycetes (38). Like ornithine lipids (OLs), TMOs are composed of a core containing
59 esterified normal and beta-hydroxy (βOH) fatty acid core lipids. The occurrence of TMOs in
60 the environment has not yet been confirmed. Here, we applied a combined approach including
61 IPL and molecular analysis of peat cores collected from two northern wetlands in order to
62 investigate the preferred niche and abundance of TMO-producing planctomycetes and shed
63 light on their potential role in the microbial community of this ecosystem.

MATERIALS AND METHODS

Sample collection

Acidic peat samples were collected from two *Sphagnum*-dominated ombrotrophic (receiving water and nutrients solely from atmospheric precipitation) peat bogs: Obukhovskoye bog, Yaroslavl region, European north Russia (58° 14'N, 38° 12'E; 25, 33) sampled at five depth intervals (5−10 cm, 10−20 cm, 20−30 cm, 30−40 cm, 40−50 cm); and Saxnäs Mosse raised bog near the village of Lidhult, SW Sweden (56° 51'20 78"N, 13° 27' 39.62"E; collected by Weijers et al. 39) sampled at two cm intervals throughout the 54 cm core (Fig. S2). *Sphagnum angustifolium* and *S. fuscum* were the predominant vegetation species in Obukhovskoye bog (25), while *S. magellanicum* and *S. papillosum* were most abundant in the Saxnäs Mosse bog (39). The pH was 4.0-4.2 throughout the 50-cm-depth peat core from the Obukhovskoye bog. The pH level in the Saxnäs Mosse core was not recorded, however, ombrotrophic bogs in central and northern Sweden typically range between 3.7 and 4.2 (40). The Obukhovskoye core water table reached 15 cm at its highest point, and was continuously water saturated and anoxic below 30 cm. The water table of the Saxnäs core ranged from 14 to 25 cm. The top 14 cm of the Saxnäs core consisted of non-decomposed vegetation, followed by 13 cm of more decomposed material, and finally the remaining core consisted of highly decomposed peat. Peat samples were stored at -20ºC until further analysis.

Reference culture of a peat-inhabiting planctomycete

The *Isosphaera*-like bacterium, strain PX4, which was isolated from just above the oxic/anoxic interface (15−20 cm) of the Obukhovskoye peat bog and is capable of growth in micro-oxic conditions (Kulichevskaya and Dedysh, unpublished), was also analyzed to compare its IPL composition with environmental samples. For lipid analyses, strain PX4 was grown in medium M31 containing (g per liter of distilled water): KH_2PO_4, 0.1; Hutner's basal salts (41), 20 ml; N-acetylglucosamine, 0.5; glucose, 0.5; yeast extract, 0.1; pH 5.8.

89 Cultivation under fully oxic conditions was performed in 500 ml flasks containing 200 ml
90 medium M31 with shaking at 120 rpm for 2 weeks at 20°C. Strain PX4 was then cultured in
91 triplicate in oxic and micro-oxic conditions to observe potential responses in IPL composition.
92 For cultivation in micro-oxic conditions, medium M31 was boiled for 10 min to remove
93 oxygen. After that, hermetically closed 500 ml flasks were filled with 450 ml medium M31,
94 inoculated with strain PX4 and incubated under static conditions for 2 weeks. Dissolved O_2
95 concentration was measured in cultivation flasks prior to inoculation by using Dissolved
96 Oxygen Meter sensION6 (Hach, USA). The respective dissolved oxygen concentrations were
97 7.0 and 1.5 mg O_2 in "oxic" and "micro-oxic" flasks, respectively. Culture biomass was
98 freeze dried and stored at -20ºC until further analysis.

99 ***IPL Extraction and Analysis***

100 Saxnäs Mosse peat samples were extracted and the IPLs were analysed by Peterse et al. (42).
101 Obukhovskoye peat samples and biomass of *Isosphaera*-like strain PX4 were freeze-dried and
102 ground to a powder with a mortar and pestle prior to extraction. Lipids were extracted from
103 the freeze-dried powdered peat by a modified Bligh and Dyer method (43, 44). A solvent
104 mixture (approximately 5 ml g^{-1} dry weight, dw, peat) of methanol (MeOH):dichloromethane
105 (DCM): potassium phosphate buffer at pH 7.4 (2:1:0.8, v/v/v) was added to ca. 0.3−1.3 g dry
106 weight of peat in a centrifuge tube and placed in an ultrasonic bath for 10 min. The extraction
107 was repeated twice more and the extracts were combined for each sample. DCM and
108 phosphate buffer were added to the combined extracts to yield a ratio of 1:1:0.9 (v/v/v) and
109 achieve separation of a DCM phase and an aqueous MeOH/phosphate buffer phase by
110 centrifugation at 2,500 rpm for 2 min. The DCM phase, containing the IPLs, was pipetted off
111 and passed over extracted cotton wool to remove any remaining particles and collected in a
112 glass tube. The aqueous phase was rinsed twice with DCM, the rinses were also passed over

113 extracted cotton wool and combined with the original DCM phase. The combined DCM phase
114 and rinses were dried under a N_2 flow and stored at -20°C until analysis.

115 Extracted IPLs from the Obukhovskoye core and *Isosphaera*-like strain PX4 were
116 analyzed by high-performance liquid chromatography–electrospray ionization-ion trap mass
117 spectrometry (HPLC-ESI/IT/MS) according to Sturt et al. (36), with some modifications (38).
118 An Agilent 1200 series high-performance liquid chromatograph (Agilent, San Jose, CA), with
119 thermostatted autoinjector was coupled to a Thermo LTQ XL linear ion trap mass
120 spectrometer with an Ion Max source and ESI probe (Thermo Scientific, Waltham, MA).
121 Chromatographic separation was performed on a Lichrosphere diol column (250 mm by 2.1
122 mm; 5-μm particles; Grace Alltech Associates Inc., Deerfield, IL). The MS scanning mass
123 range of *m/z* 400 to 2,000 in positive-ion mode, followed by data dependent dual-stage
124 tandem MS (MS^2), in which the four most abundant masses in the mass spectrum were
125 fragmented successively. Each MS^2 was followed by data-dependent, triple-stage tandem MS
126 (MS^3), wherein the base peak of the MS^2 spectrum was fragmented. IPL abundance was
127 assessed by integrating the HPLC-ESI/IT/MS base peak chromatogram area per gram of peat,
128 dry weight. Performance of the HPLC-ESI/IT/MS was monitored by regular injections of
129 platelet-activating factor (PAF) standard (1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-
130 phosphocholine). The absolute amount of IPLs in Obukhovskoye 30-40 cm peat was
131 measured using the PAF internal standard and 1,2-dipalmitoyl-*sn*-glycero-3-
132 phosphoethanolamine-N-methyl external standard. Student's t-tests were performed using the
133 GraphPad *t* test Calculator (GraphPad Software, Inc. La Jolla, CA) in order to identify
134 statistically significant differences in the fractional abundances of IPLs under different growth
135 conditions; p-values <0.05 were considered statistically significant.

136 ***DNA extraction, PCR amplification & Phylogenetic Analysis***

137 Peat samples collected at 10-12, 16-18, 22-24, 24-26, 28-30 and 40-42 cm of the Saxnäs
138 Mosse bog were defrosted on ice prior to extraction and water content removed by
139 centrifugation at 4,000×*g* 10 min before proceeding with the DNA extraction (quantification
140 values are given per gram of dry weight as remaining drained wet weight material from the
141 extraction was later freeze-dried and the correction applied). Peat samples of the
142 Obukhovskoye core at 5-10, 10-20, 20-30, 30-40 and 40-50 cm were extracted from freeze-
143 dried material. DNA was extracted with the DNA PowerSoil® Isolation kit (Mo Bio
144 Laboratories, Inc., Carlsbad, CA) with a final volume of 60 µl. Integrity and concentration of
145 the extracted DNA was tested by agarose gel electrophoresis and Nanodrop (Thermo
146 Scientific, Waltham, MA) quantification. Amplification of the 16S rRNA gene fragment from
147 members of the Planctomycetes was performed with the primer pair Pla352F/Pla920R (45)
148 with DNA extracted from the Saxnäs Mosse peat collected at 16-18, 22-24, and 40-42 cm
149 depth. Total bacterial 16S rRNA gene amplification was performed with the 341F/907R
150 primer pair (46, 47) with DNA extracted from the Saxnäs Mosse peat at 22-24 cm depth. PCR
151 reaction mixture was the following (final concentration): Q-solution 1× (PCR additive,
152 Qiagen); PCR buffer 1×; BSA (200 µg ml^{-1}); dNTPs (20 µM); primers (0.2 pmol $µl^{-1}$); $MgCl_2$
153 (1.5 mM); 1.25 U Taq polymerase (Qiagen, Valencia, CA, USA). PCR conditions for these
154 amplifications were the following: 95°C, 5 min; 30−35 × [95°C, 1 min; Tm (melting
155 temperature, see Table S1 for details), 1 min; 72°C, 1 min]; final extension 72°C, 5 min. PCR
156 products were gel purified (QIAquick gel purification kit, Qiagen, Valencia, CA, USA) and
157 cloned in the TOPO-TA cloning® kit from Invitrogen (Carlsbad, CA, USA) and transformed
158 in *E. coli* TOP10 cells following the manufacturer's recommendations. Recombinant clones
159 plasmid DNAs were purified and sequenced by Baseclear (Leiden, The Netherlands).
160 Sequences were analyzed for the presence of chimeras using the Bellerophon tool (http:

161 //greengenes.lbl.gov/). The phylogenetic affiliation of the partial planctomycete 16S rRNA
162 gene sequences was compared to release 119 of the Silva NR SSU Ref database (http://www.
163 arb-silva.de/; 48) using the ARB software package (49). Sequences were added to the
164 reference tree supplied by the Silva database using the ARB Parsimony tool. Partial
165 planctomycete 16S rRNA gene sequence data is deposited in the NCBI GenBank database
166 under accession numbers: KP161502−KP161600.

167 ***Quantitative PCR (qPCR) Analysis***

168 Quantitative PCR analyses were performed on a Bio-rad CFX96^{TM}Real-Time System/C1000
169 thermal cycler equipped with CFX Manager^{TM} Software. The copy numbers of total bacteria
170 and planctomycetes 16S rRNA genes were estimated by using the primers mentioned above.
171 The qPCR reactions were performed in triplicate with standard curves from 10^0 to 10^7
172 molecules per microliter. Standard curves were generated as described before (50). For the
173 general bacteria and planctomycetes 16S rRNA gene quantification 16S rRNA gene
174 fragments cloned from the 22-24 cm peat were used as standard (Acc. Number KP161600, for
175 bacteria; KP161571, for planctomycete). Gene copies were determined in triplicate on diluted
176 DNA extract. The reaction mixture (25 µl) contained 1U of Pico Maxx high-fidelity DNA
177 polymerase (Stratagene, Agilent Technologies, Santa Clara, CA, USA), 2.5 µl of 10× Pico
178 Maxx PCR buffer, 2.5 µl of 2.5 mmol l^{-1} of each dNTP, 0.5 µl BSA (20 mg ml^{-1}), 0.02 pmol
179 μl^{-1} of primers, 10,000 times diluted SYBR Green® (Life technologies, Carlsbad, CA, USA)
180 (optimized concentration), 0.5 µl of 50 mmol l^{-1} of $MgCl_2$ and ultra-pure sterile water. All
181 reactions were performed in iCycler iQ^{TM}96-well plates (Bio-Rad, Hercules CA, USA) with
182 optical tape (Bio-Rad). One µl of diluted environmental DNA was added to 24 µl of mix in
183 each well. Specificity of the reaction was tested with a gradient melting temperature assay.
184 The cycling conditions for the qPCR reaction were the following: 95°C, 4 min; 40–45 ×
185 [95°C, 30 s; Tm (see Table S1 for details), 40 s; 72°C, 30 s]; final extension 80°C, 25 s.

Specificity for qPCR reaction was tested on agarose gel electrophoresis and with a melting curve analysis (50–95°C; with a read every 0.5°C held for 1s between each read). Efficiencies and R^2 of the qPCR analysis are specified in Table S1.

PCR Amplicon Library Preparation for Pyrosequencing and Analysis

PCR reactions were performed with the universal (Bacteria and Archaea) primers S-D-Arch-0519-a-S-15 (5'-CAG CMG CCG CGG TAA-3') and S-D-Bact-785-a-A-21 (5'-GAC TAC HVG GGT ATC TAA TCC-3') (51) adapted for pyrosequencing by the addition of sequencing adapters and multiplex identifier (MID) sequences. Each 30 µl PCR reaction comprised 5× Phusion HF Buffer containing 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of each primer, 1 U Phusion High-Fidelity DNA Polymerase (Thermo Scientific, Pittsburgh, PA). The following PCR conditions were used: initial denaturation at 98°C for 30 s, followed by 25 cycles consisting of denaturation (98°C for 10 s), annealing (53°C 20s), and extension (72°C 30s) and a final extension step at 72°C for 7 min. To minimize PCR bias three individual reactions were performed per template. PCR products were pooled, loaded in a 0.8% agarose gel and purified using Qiagen Qiaquick gel extraction kit (Qiagen, Germany). PCR products were quantified with the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life technologies, Netherlands). Equimolar concentrations of the barcoded PCR products were pooled and sequenced on GS FLX Titanium platform (454 Life Sciences) by Macrogen Inc. Korea. Samples were analyzed using the QIIME pipeline (52). Raw sequences were demultiplexed and then quality-filtered with a minimum quality score of 25, length between 250−350 bp, and allowing maximum two errors in the barcode sequence. Taxonomy was assigned based on blast and the SILVA database (48, 53). Representative OTU sequences assigned to the Planctomycetes were extracted through classify.seqs and get.lineage in Mothur (54) by using the bacteria aligned sequence and taxonomy file from the SILVA SSURef database (v102), and then they were added to the guided tree of the release 119 of the Silva NR SSU Ref

211 database as specified above. The pyrosequencing reads (raw data) have been deposited in the

212 NCBI Sequence Read Archive under the study number SRP059351.

213 **RESULTS**

214 ***Distribution and abundance of IPLs in peat bogs***

215 The distribution of IPLs in the Saxnäs Mosse core was previously described by Peterse
216 et al. (42). Briefly, in the surface layers of both peat bogs, betaine IPLs were highly abundant
217 along with phosphatidylcholine lipids (PCs) and followed by TMOs (Table 1). Betaine lipids
218 became relatively less abundant starting at 16-18 cm in the Saxnäs Mosse core and at 10−20
219 cm in the Obukhovskoye core and continued to decline with increasing depth (Table 1). PCs
220 were highly to moderately abundant from 2 to 20 cm in the Saxnäs Mosse bog, more abundant
221 from 20 to 32 cm, and fluctuated as a moderate to low abundance IPL down the rest of the
222 core (Table 1). PCs were abundant throughout the Obukhovskoye bog. Phosphatidyl-
223 ethanolamines (PEs) and monomethylphosphatidylethanolamines (MMPEs) were low in
224 abundance or not detected at middle or deep part of both cores (Table 1).

225 Trimethylornithine (TMO) IPLs were observed throughout both the Saxnäs Mosse and
226 Obukhovskoye peat bogs in the oxic and anoxic layers (Fig. 1). The highest abundance of
227 TMO IPLs was detected at the oxic/anoxic interface in both cores around and just below the
228 water table low point (i.e. approx. 25 and 30 cm depth, respectively; Fig. 1). This was
229 particularly evident in the Saxnäs Mosse bog given the higher sampling resolution (Fig. 1A).
230 The oxic/anoxic interface is also the acrotelm (periodically water saturated oxic upper
231 layer)/catotelm (continuously water saturated, anoxic lower layer) interface, where the
232 transition occurs from living vegetation (acrotelm) to dead plant material (catotelm). At these
233 interface depths TMOs are the most abundant IPL for Saxnäs Mosse (24-26 cm) and second
234 most abundant for Obukhovskoye (30-40 cm) peat bogs, with PCs as the second most
235 abundant IPL in Saxnäs Mosse interface peat and slightly more abundant than TMOs in
236 Obukhovskoye interface peat (Table 1). The remaining IPLs identified at these depths were

237 betaine and PE in the Saxnäs Mosse peat bog, and betaine, MMPE and lyso-PC in the
238 Obukhovskoye peat bog (Fig. 2, Table 1).

239 As the abundance of TMOs changed down core, the fatty acid composition of TMO
240 lipids changed as well (Fig. 3). The most abundant TMO in the Saxnäs Mosse peat bog at the
241 oxic/anoxic interface (where total TMOs peaked; Fig. 1), contained predominantly (>60%)
242 C18:1 and βOH-C19:0 fatty acids (Fig. 3A). The C18:1, βOH-C19:0 TMO also peaked
243 (>40%) at the oxic/anoxic interface layer (30−40 cm) of the Obukhovskoye core, although not
244 as highly as in the Saxnäs Mosse core (Fig. 3). One other individual TMO peaked near the
245 oxic/anoxic interface of the Saxnäs Mosse core (C19:1, βOH-C19:0), but it only made up 5%
246 of total TMOs at the interface depth. Two individual TMOs (core lipids C19:2, βOH-C18:0
247 and C18:2, βOH-C18:0) peaked above and below the oxic/anoxic interface of the Saxnäs
248 Mosse core, and the most abundant individual TMOs in the near surface layers (i.e. C19:1,
249 βOH-C16:0 and C18:1, βOH-C18:0) declined with depth followed by minor peaks around the
250 oxic/anoxic interface. The C18:1, βOH-C18:0 TMO was the most consistently abundant from
251 top to bottom in both cores mostly ranging from 20 to 40% in the Saxnäs Mosse bog and 39
252 to 65% in the Obukhovskoye bog. Other TMOs not described above followed similar trends
253 in the Saxnäs Mosse core with peaks above and below the oxic/anoxic interface with much
254 lower contribution to total TMOs.

255 ***IPLs of planctomycete strain Isosphaera sp. PX4***

256 The classes of IPLs identified in the *Isosphaera*-like strain PX4 lipid extract were
257 similar to the IPLs identified at the oxic/anoxic interface in both peat bogs, particularly the
258 Obukhovskoye bog (Fig. 2). PCs were the most abundant IPLs detected in strain PX4 lipid
259 extract, followed by TMOs and phosphatidylglycerols (PGs) (Fig. 2C). By far the most
260 abundant TMO core lipid fatty acids in the PX4 strain were C18:1, βOH-C18:0, which were

also among the major TMO core lipids identified in both the Saxnäs Mosse and Obukhovskoye peat cores (Fig. 3). There was a statistically significant increase in the ratio of TMO/(PC + PG) IPLs in PX4 cultures grown in micro-oxic conditions compared to oxic conditions (Fig. 4A). There was also a statistically significant increase in the relative abundance of TMO containing fatty acids C19:1 and βOH-C18:0 in biomass of strain PX4 grown in micro-oxic conditions compared to oxic conditions (Fig. 4B).

Bacterial diversity by 16S rRNA gene amplicon pyrosequencing analysis

In order to determine the microbial diversity, partial 16S rRNA gene sequences were retrieved by pyrosequencing of the material sampled from 10-12, 16-18, 22-24, 28-30 and 40-42 cm depth from the Saxnäs peat bog, and at 5-10, 10-20, 20-30, 30-40 and 40-50 cm depth from the Obukhovskoye core. In the Saxnäs peat bog between 12 and 30 cm depth, bacterial 16S rRNA gene sequence reads comprised ca. 96% of the total reads, while in the deepest interval studied (40-42 cm depth) 52% of the reads were attributed to Bacteria and 48% to Archaea, specifically from the Thaumarchaeota terrestrial group (37%), and the Miscellaneous Crenarchaeotic Group (8.5%) (Fig. 5A, Table S2). Acidobacterial 16S rRNA gene sequences contributed on average 49% of the total reads in all depth intervals analyzed (Fig. 5A, Table S2) and fell in the Acidobacteria subgroups 1, 2, and 13 (Fig. S3). Other bacterial 16S rRNA gene sequences attributed to *Isosphaera*-like planctomycetes, other planctomycetes, Alphaproteobacterial families Rhizobiales and Rhodospirillales, Deltaproteobacterial genus *Syntrophobacter* and phylum Verrucomicrobia contributed on average with 2−10% to the total reads (Fig. 5A, Table S2). At the oxic/anoxic interface (22-24 cm depth) of the Saxnäs peat bog, total gene reads were distributed as it follows: Acidobacteria, 55%; Planctomycetes *Isosphaeraceae*, 6%; Rhizobiales, 3.5%; Rhodospirillales, 0.7%; *Synthrophobacter*, 8%; Verrucomicrobia, 13% (Fig. 5A, Table S2).

285 In the Obukhovskoye peat bog, Acidobacteria 16S rRNA gene reads contributed 27%
286 of the total reads on average throughout the peat, followed by Actinobacteria reads (22%),
287 *Isosphaera*-like planctomycetes (16%), Alphaproteobacteria Rhizobiales (15%), other
288 planctomycetes (4%), and Verrucomicrobia (2%) (Fig. 5B, Table S2). At the oxic/anoxic
289 interface of the Obukhovskoye peat bog (maximum abundance of TMOs 30-40 cm depth), a
290 substantial change in the relative abundance of bacterial reads was observed with respect to
291 the distribution observed above (20-30 cm) and below the interface (40-50 cm), with the
292 *Isosphaera*-like planctomycetes 16S rRNA gene reads forming 53% of the total (Fig. 5B,
293 Table S2).
294 Planctomycete and total bacteria 16S rRNA gene abundances were quantified by
295 quantitative PCR in seven peat intervals between 10-42 cm depth of the Saxnäs Mosse core,
296 and in the 5 intervals between 5-50 cm in the Obukhovskoye peat bog. In the Saxnäs Mosse
297 peat, planctomycete 16S rRNA gene abundance ranged from 3.7×10^5–2.3×10^7 copies per
298 gram of dry weight peat, with a maximum at the oxic/anoxic interface (average 2.2×10^7
299 copies g^{-1}), and a minimum at the deepest interval studied (i.e. 40-42 cm) (Fig. 6A). Bacterial
300 16S rRNA gene abundance was between 3.8×10^7–5.9×10^8 copies per gram of peat. Like in
301 the case of planctomycete 16S rRNA gene, bacterial 16S rRNA gene abundance also showed
302 maximum values at the oxic/anoxic interface (average 5.1×10^8 copies g^{-1}) and a minimum at
303 the deepest interval studied (3.8×10^7 copies g^{-1}; Fig. 6A). In the Obukhovskoye peat,
304 planctomycete 16S rRNA gene abundance ranged from 6×10^5 copies g^{-1} of dry weight at 10-
305 20 cm to a maximum of 3×10^7 copies g^{-1} at the oxic/anoxic interface (maximum abundance
306 of TMOs) at 30-40 cm depth (Fig. 6B). Bacterial 16S rRNA gene abundance increased
307 approximately 5-fold at 30-40 cm with respect to the uppermost layers. The maximum of
308 bacterial 16S rRNA gene copies was detected between 40-50 cm depth (2.4×10^8 copies g^{-1}
309 dry weight).

310 Planctomycete 16S rRNA gene fragments were amplified from the Saxnäs Mosse peat
311 bog intervals 16-18 cm (acrotelm), 22-24 cm (oxic/anoxic interface), and 40-42 cm
312 (catotelm), cloned and the obtained sequences included in a phylogenetic tree together with
313 the planctomycete reads retrieved by means of pyrosequencing analysis both in the Saxnäs
314 Mosse and the Obukhovskoye peat bogs (Fig. 7). Approximately 93% (38 out of 41 clones) of
315 the planctomycete sequences obtained from the Saxnäs Mosse peat oxic/anoxic interface
316 belonged to the phylogenetic lineage defined by the genus *Isosphaera* (Fig. 7A). Some of the
317 sequences included in this lineage have previously been retrieved from peat bogs, mainly
318 from the oxic peat layer of the ombrotrophic bog Obukhovskoye (in bold, Fig.7), and from the
319 oligo-mesotrophic bog Bakchar (Fig. 7A; 27). The *Isosphaera* lineage also contained 37% of
320 the sequences retrieved from the Saxnäs Mosse peat layer at 16-18 cm depth. On the other
321 hand, all sequences obtained from the Saxnäs Mosse peat at 40-42 cm depth (n = 29 clones)
322 fall in a subcluster (named here subcluster-1, Fig. 7C) that is part of a lineage of uncultured
323 planctomycete 16S rRNA gene sequences containing sequences previously retrieved from the
324 anoxic peat layer of the Bakchar bog (27), in addition to many other environmental sequences
325 (Fig. 7C). Approximately 63% of the sequences obtained from the Saxnäs Mosse peat at 16-
326 18 and 7% of those obtained at 22-24 cm depth also group in this subcluster-1. Most of the
327 representative reads of the pyrosequencing analysis from the Saxnäs Mosse peat layers 10-12,
328 16-18 and 28-30 cm fell in the subcluster-1 (Fig. 7C), while the reads from 22-24 cm depth
329 were part of the *Isosphaera* cluster (Fig. 7A). Pyrosequencing reads of the Obukhovskoye
330 peat layers 30-40 cm and 40-50 cm were also found in the *Isosphaera* cluster, while reads
331 from the 5-10 cm sample were closely related to the *Singulisphaera* group together with some
332 reads of the Saxnäs Mosse peat sample 10-12 cm, and previously detected sequences from
333 oxic parts of the Obukhovskoye and Bakchar peat. Pyrosequencing reads of the
334 Obukhovskoye peat samples 10-20, 30-40 and 40-50 cm also fall in subcluster-1 (Fig. 7C).

335 **DISCUSSION**

336 ***Abundance of* Isosphaera*-like planctomycetes at the oxic/anoxic interface of the***

337 ***Obukhovskoye and Saxnäs Mosse bogs***

338 A recent study estimated the bacterial diversity in the surface and subsurface layers of
339 the acidic *Sphagnum*-dominated Obukhovskoye peat bog (33), concluding that Acidobacteria,
340 Proteobacteria, Actinobacteria and Planctomycetes were the dominant phylum-level groups in
341 both the oxic and anoxic zones of the peat. In our study, the percentage of Obukhovskoye peat
342 bog reads attributed to Acidobacteria, Planctomycetes, Actinobacteria and Rhizobiales were
343 on average similar (15-27% of the total reads; Table S2) but it revealed that 16S rRNA reads
344 attributed to Planctomycetes dominated at the oxic/anoxic interface (i.e. 53% reads at 30-40
345 cm; Fig. 5B). In the Saxnäs Mosse peat bog, we detected members of the Acidobacteria,
346 Proteobacteria, Planctomycetes and Verrucomicrobia as dominant groups with Acidobacteria
347 being represented on average by 50% of the total 16S rRNA gene reads (Fig. 5A). At this
348 location the percentage of 16S rRNA gene reads attributed to Planctomycetes was also highest
349 in the oxic/anoxic interface (i.e. 7% reads at 24-30 cm).

350 Total bacterial and planctomycete cell numbers were estimated assuming that the
351 average 16S rRNA copy number per bacterial cell is 3.6 (55), and that planctomycetes have
352 an average of 2.5 copies of 16S rRNA gene per genome (56). Based on the copy numbers
353 (Fig. 6), the maximum abundance of planctomycetes in the Saxnäs Mosse core was 2.2×10^7
354 cells per gram of dry weight in the oxic/anoxic interface, with planctomycetes making up
355 approximately 6% of the total bacterial cells. These values are comparable with those reported
356 for diverse *Sphagnum* peat bogs in Russia by Ivanova and Dedysh (27) and in good agreement
357 with the pyrosequencing data indicating that planctomycetes comprised 6% of the total reads
358 in the oxic/anoxic interface. In the Obukhovskoye peat bog maximum abundance of
359 planctomycetes at 30-40 cm depth was estimated to be 1.3×10^7 cells per gram of dry weight

according to the same calculations, with planctomycetes accounting for 27% of the total bacterial cells.

Clone libraries and pyrosequencing indicated that at the oxic/anoxic interface of both the Saxnäs Mosse (24-26 cm) and the Obukhovskoye peat bog (30-40 cm) there is also a marked change in the phylogenetic affiliation of the planctomycetes; i.e. almost all 16S rRNA gene reads and clone sequences were closely related to members of the *Isosphaeraceae*, whereas most other gene sequences recovered from different intervals were included in an uncultured planctomycete group (subcluster-1 as shown in Fig. 7). Sequences of the *Isosphaera* group have previously been detected both in the oxic and anoxic part of the Obukhovskoye peat bog (33). The only currently described member of the *Isosphaera* group (i.e. *Isosphaera pallida*) is aerobic, but the retrieval of environmental sequences from anoxic layers of the peat affiliated to this group suggests that other uncultured *Isosphaera* species may be adapted to a microaerophilic or facultative anaerobic lifestyle. This would represent an advantage for this specialized planctomycete group to rapidly adapt to changing oxic/anoxic interfaces in peat bog systems. *I. pallida* is the type species of the genus *Isosphaera* (57, 58), but since *I. pallida* is a thermophilic planctomycete, it is deemed to be not relevant to northern wetlands. However, an *Isosphaera*-like bacterium, strain PX4, which was recently isolated from just above the oxic/anoxic interface of the Obukhovskoye peat bog and capable of growth at micro-oxic conditions (Kulichevskaya and Dedysh, unpublished), is phylogenetically related to the planctomycetes detected at the oxic/anoxic interface of the Saxnäs Mosse peat bog (See Fig. 7A, marked with triangle). Strain PX4 possesses a hydrolytic potential and is likely to be involved in the process of biopolymer degradation in peat (59).

383 ***TMO IPL production by Isosphaera-like planctomycetes***

384 TMO IPL abundance and Planctomycete 16S rRNA sequences both peaked at the
385 oxic/anoxic interface of the Obukhovskoye and Saxnäs Mosse peat bogs (Fig. 6). In addition,
386 total bacterial abundance was also highest in those niches suggesting that it is a hotspot for
387 microbial activity, where Planctomycetes play an important role. To date, Planctomycetes are
388 the only known TMO IPL producers in culture, including the species *Singulisphaera*
389 *acidiphila* and *S. rosea* (38), which were isolated from Russian northern wetlands (29, 31) and
390 are related to the *Isosphaera* group (Fig. 7). This suggests that the maximum TMO IPL
391 abundance detected in the oxic/anoxic interfaces of two northern wetlands peat bogs may be
392 attributed to *Isosphaera*-like Planctomycetes thriving at the oxic/anoxic interface.
393 Acidobacteria are more abundant at the oxic/anoxic interface of the Saxnäs Mosse peat bog
394 (Fig. 5), and could potentially also be a source of TMO IPLs, however, previous studies
395 analyzed the IPL composition of many Acidobacterial species falling in subgroups 1, 3, 4, and
396 23 (60–62) and did not detect TMO IPLs. The 16S rRNA gene sequences retrieved in our
397 analysis of the Saxnäs peat bog were closely related to Acidobacteria subgroups 1, 2 and 13
398 (Fig. S3), and specifically those included in subgroup 1 were closely related to previously
399 tested strains with no TMO production capability. In addition, the percentage of reads
400 attributed to Acidobacteria decreased 2-fold (46 to 19%), and planctomycetes make up to
401 54% of the reads at 30-40 cm depth in the Obukhovskoye peat, where maximum abundance
402 of TMO was also detected. This evidence suggests that Acidobacteria are not TMO producers
403 but rather *Isosphaera*-like Planctomycetes are the most likely source of TMO lipids in this
404 setting. This also applies to the Verrucomicrobia, which made up 13% of the total bacterial
405 reads in the oxic/anoxic interface of the Saxnäs Mosse peat bog (Fig. 5A, Table S2), but only
406 1.2% of the total reads in the Obukhovskoye peat at the peak of TMO depth. The *Isosphaera*-
407 like strain PX4, which is closely related to the planctomycete 16S rRNA gene reads found at

408 the oxic/anoxic interface of the Saxnäs Mosse and Obukhovskoye peat (Fig. 7A), contains
409 TMO IPLs in high abundance (Fig. 2C), further supporting that the peak in TMO IPLs at the
410 oxic/anoxic interface of the two peat bogs is due to the abundance of *Isosphaera*-like
411 Planctomycetes (Fig. 6).
412 Although TMO IPLs peak at the oxic/anoxic interface, these lipids can still be detected
413 throughout the two peat bog cores (Fig. 1). Some of the most abundant Saxnäs Mosse and
414 Obukhovskoye TMO core lipids (C19:1, βOH-C16:0; C18:1, βOH-C19:0; C19:2, βOH-
415 C18:0; C18:0, βOH-C16:1) have not yet been observed in planctomycete cultures, and may be
416 produced by uncultured species or result from adaptation to specific conditions in the peat. In
417 addition, the most abundant TMO lipid at the oxic/anoxic interface of the Saxnäs Mosse bog
418 (i.e. comprised of the C18:1 and βOH-C19:0 fatty acids) is likely derived from the
419 *Isosphaera*-related species since it clearly peaks at the oxic/anoxic interface of the peat (Fig.
420 3). Remarkably, the most abundant TMOs in northern wetland planctomycete species,
421 including the *Isosphaera*-like strain PX4, do not contain the C18:1/βOH-C19:0 TMO in high
422 abundance (Table 2). Apparently, the *Isosphaera*-like species thriving at the the oxic/anoxic
423 interface of the Saxnäs Mosse and Obukhovskoye peat bogs have a different TMO
424 composition, which would be in line with the large variation in fatty acid composition of
425 TMOs in planctomycetes (Table 2). Many of the other TMO lipids identified in the two peat
426 cores (Fig. 3) have also been detected in cultured northern wetland planctomycetes (Table 2).
427 Variations in the relative abundance of these TMOs (Fig. 3) are likely related to the changing
428 composition of planctomycetes, which is evident from the genetic analyses (Fig. 7).
429 There are multiple lines of evidence supporting TMO production by members of the
430 *Isosphaera* group, yet there is an apparent disproportionate contribution of TMOs to total
431 IPLs *vs*. planctomycete to bacteria proportion at the oxic/anoxic interface of the Saxnäs
432 Mosse peat (Fig. S4). The relative abundance of TMOs makes up approximately 50% of total

IPLs in the Saxnäs Mosse oxic/anoxic interface, but planctomycete cells only accounted for 6.6% of total bacterial cells at the same depth (24-26 cm; Fig. S4, Table S3; cell amounts based on 16S rRNA to cell conversion calculations described above). Conversely, TMOs make up 22% of total IPLs in the Obukhovskoye oxic/anoxic interface and planctomycetes make up 27% of total bacterial cells (Fig. S4, Table S3). The difference between the calculated percentage of planctomycete cells and the percentage of total bacterial 16S rRNA gene reads is probably due to differences in 16S rRNA gene copy number in the bacterial and planctomycete groups present in this depth of the two locations, inducing biases in the calculation of percentages of cells. In addition, we should also consider the possibility that PCR-biases are introduced by the primers used for the quantification of 16S rRNA gene copies of bacteria and planctomycetes. The disproportionate amount of TMOs *vs.* planctomycete cells at the Saxnäs Mosse oxic/anoxic interface (Fig. S4) could be due to differences in the abundance of various microbial groups (Acidobacteria, Planctomycetaceae *Isosphaera*, etc.) in comparison with the Obukhovskoye bog (Fig. 5). Difficulty in extracting the membrane lipids of Acidobacteria (60, 61), which represent 30-60% of all pyrosequencing reads in Saxnäs Mosse peat (Fig. 5A), could also result in underrepresentation of Acidobacteria IPL contribution.

Purpose for TMO production

The high relative abundance of TMOs and total bacterial cells at the oxic/anoxic interface suggests that there is some functional role of TMOs at this specific niche. Ornithine lipids (OLs) are relatively common among bacteria, approximately 50% of known bacterial species have the capability to produce ornithine lipids (63, 64). In certain bacteria OLs can be produced in response to phosphorus limitation (65, 66), or modified in response to temperature or acid stress (67–69). TMOs are essentially modified OLs (38, 70), the addition of three methyl groups to the terminal nitrogen of TMOs results in a quaternary amine

functional group, which is positively charged making the lipid more polar and giving it a more cylindrical shape than conically shaped OLs, as observed in the methylation of conical PEs to yield cylindrical polar PCs. The increased relative abundance of TMOs compared to PCs and PGs, and the increased relative abundance of TMOs with C19:1 and βOH-18:0 fatty acids in PX4 strain cultures under micro-oxic growth conditions (Fig. 4) suggests that TMOs are used in response to low oxygen levels. As we hypothesized earlier (38), TMOs could be produced by northern wetland planctomycetes in order to provide greater membrane stability in rain fed, acidic, low nutrient conditions without using scarce phosphate. The high abundance of TMOs at the oxic/anoxic interface and increased relative TMO production in micro-oxic PX4 cultures indicates that there may be another niche specific function of these lipids that is potentially linked to microaerophilic conditions and/or organic matter degradation.

Conclusions

This study represents the first observation of TMOs in the environment. Initially discovered in northern wetland microbial isolates (38), it is now clear that TMOs are an important membrane lipid of microorganisms in north European ombrotrophic bog ecosystems, and possibly an adaptation to the unique environmental conditions found at the oxic/anoxic interface. It still remains to be determined if these lipids are present in different types of peats, or other ecosystems. Future environmental and culture-based studies will be needed to tackle these questions.

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661 **TABLES**

662 **Table 1:** Relative abundance of the most prevalent IPLs and the biopolymer
663 polyhydroxybutyrate in the (A) Saxnäs Mosse and; (B) Obukhovskoye bogs; (C) IPL absolute
664 abundance in Obukhovskoye bog 30-40 cm peat (μmol/g dw). Key: TMO =
665 trimethylornithine; PC = phosphatidylcholine; PHB = polyhydroxybutyrate; MMPE =
666 monomethylphosphatidylethanolamine; PE = phosphatidylethanolamine.
667

(A) Saxnäs Mosse

Depth (cm)	TMO	PC	Betaine	PHB	MMPE	PE
2-4	16.7	12.9	30.7	2.7	-	-
4-6	15.6	11.2	35.4	2.5	-	-
6-8	14.1	8.1	22.0	4.9	-	-
10-12	12.5	13.1	13.4	4.0	5.6	-
12-14	20.1	8.8	11.5	2.5	7.0	-
14-16	18.4	8.5	13.5	1.5	8.1	-
16-18	34.8	5.9	14.6	-	5.1	-
18-20	36.9	4.1	11.2	-	-	-
20-22	35.7	10.9	3.7	-	-	-
22-24	45.4	15.6	2.7	-	-	-
24-26	50.6	15.9	1.2	-	-	1.6
30-32	30.2	10.2	1.5	-	-	3.0
32-34	12.4	2.7	1.4	-	-	3.1
34-36	4.8	1.0	1.3	-	-	3.6
36-38	5.2	0.4	1.2	-	-	4.2
42-44	5.1	12.5	3.2	-	-	7.5
44-46	3.5	10.0	1.4	-	-	6.6
46-48	2.6	7.7	0.5	-	-	7.7
48-50	3.7	10.6	1.3	-	-	5.7
50-52	2.5	9.1	1.7	-	-	6.5
52-54	3.3	9.5	*	-	-	6.9

(B) Obukhovskoye

Depth (cm)	TMO	PC	Betaine	PHB	MMPE	PE
5-10	6.1	23.5	14.9	15.2	0.7	1.3
10-20	2.3	13.5	3.2	19.1	5.5	1.4
20-30	1.9	16.1	2.2	6.2	5.7	2.0
30-40	22.6	23.8	0.7	-	1.9	-
40-50	2.3	22.3	-	-	-	-

(C) Obukhovskoye peat IPL abundance (μmol/g dw)

Depth (cm)	TMO	PC	Betaine	MMPE	Lyso PC
30-40	0.131	0.139	0.004	0.022	0.010

Note: Ionization and apparent abundance can differ between different types of IPLs, thus the observed abundances are relative and not absolute.

668 **Table 2:** Relative abundance[a] (in % of total TMO abundance) of the main TMO lipids of northern wetland planctomycetes. *S. acidiphila*, *S.*

669 *rosea*, *T. sphagniphila*, and *Gemmata*-like SP5 strain TMO abundances from Moore et al., 2013 (38).

β-hydroxy fatty acid	β-16:0								β-17:0		β-18:0								β-20:0	
Regular fatty acid	14:0	16:0	16:1	16:0-OH	18:0	18:1	18:2	20:1	16:1	18:1	16:0	16:1	16:2	18:1	18:2	19:0	19:1	20:1	18:1	20:1
Isosphaera-like PX4 strain					3.8	6.8								81.9		2.2	5.3			
Singulisphaera acidiphila											6.3			61.8	29.2				2.6	
Singulisphaera rosea						1.4	2.2					2.8	1.5	42.4	49.7					
Telmatocola sphagniphila			20.9			12.1			23.0	20.2		9.9		13.8						
Gemmata-like SP5 strain	2.6	26.8		5.8		6.7		47.1										8.5		2.5

[a]IPL and TMO abundances were assessed by integrating the HPLC-ESI/IT/MS base peak chromatogram area.

FIGURE LEGENDS

Figure 1. Trimethylornithine IPL (TMO), phosphatidylcholine IPL (PC), and betaine IPL relative abundances based on HPLC-ESI/IT/MS chromatogram base peak area down core in (A) Saxnäs Mosse peat and (B) Obukhovskoye peat.

Figure 2. HPLC-ESI/IT/MS base peak chromatogram of lipid extracts from (A) Saxnäs Mosse 24-26 cm peat; (B) Obukhovskoye 30-40 cm peat and (C) planctomycete *Isosphaera*-like PX4 strain isolated from Obukhovskoye Bog. Retention times of IPLs in the various chromatograms are shifted due to slightly different chromatographic conditions used at the time of analysis. The unknown component was characterized by non-fragmentable *m/z* 680, 668, and 656 peaks and reported before by Peterse et al. (2011). Key: PC = phosphatidylcholine, TMO = trimethylornithine, MMPE = monomethylphosphatidylethanolamine, PG = phosphatidylglycerol.

Figure 3. Relative contribution (in % of total TMO lipids) of the five most abundant TMO lipids down core in (A) Saxnäs Mosse and (B) Obukhovskoye peat bogs.

Figure 4. (A) Changes in *Isosphaera*-like strain PX4 IPL abundance grown at oxic and micro-oxic conditions based on HPLC-ESI/IT/MS chromatogram base peak area of (A) TMO/(PC + PG) ratio; (B) TMO with fatty acids C19:1, βOH-C18:0/(Total TMO + PC + PG). Student's t-test statistically significant differences between different growth conditions are represented by letters (a, b) over each bar. TMO = trimethylornithine; PC = phosphatidylcholine; PG = phosphatidylglycerol.

690 **Figure 5.** Percentage of total bacterial and archaeal reads attributed to different microbial
691 groups detected in the (A) Saxnäs Mosse peat bog at five different depths (10-42 cm depth),
692 and (B) in the Obukhovskoye peat (five depths from 5-50 cm depth) by 16S rRNA gene
693 amplicon pyrosequencing analysis.

694 **Figure 6.** Comparison of planctomycete and total bacterial 16S rRNA gene copy number per
695 gram in comparison with total TMO abundance down core in the (A) Saxnäs Mosse, and the
696 (B) Obukhovskoye peat bog. Dotted lines indicate water table high and low point.

697 **Figure 7.** Phylogenetic tree including the 16S rRNA gene sequences detected in the clone
698 libraries of the Saxnäs Mosse peat bog at 16-18, 22-24 and 40-42 cm, which are affiliated to
699 planctomycete 16S rRNA gene sequences. Planctomycete 16S rRNA gene representative
700 sequences obtained by pyrosequencing from the Saxnäs Mosse and the Obukhovskoye peat
701 bog sediments are also included: (A) sequences closely related to the *Isosphaera* cluster, (B)
702 sequences related to the *Singulisphaera* cluster, and (C) sequences closely related to a cluster
703 formed by uncultured planctomycetes. Scale bar indicates 0.10% estimated sequence
704 divergence. Accession number of the sequences and percentage of sequences detected at a
705 given depth by the clone libraries are indicated.

Figure 1

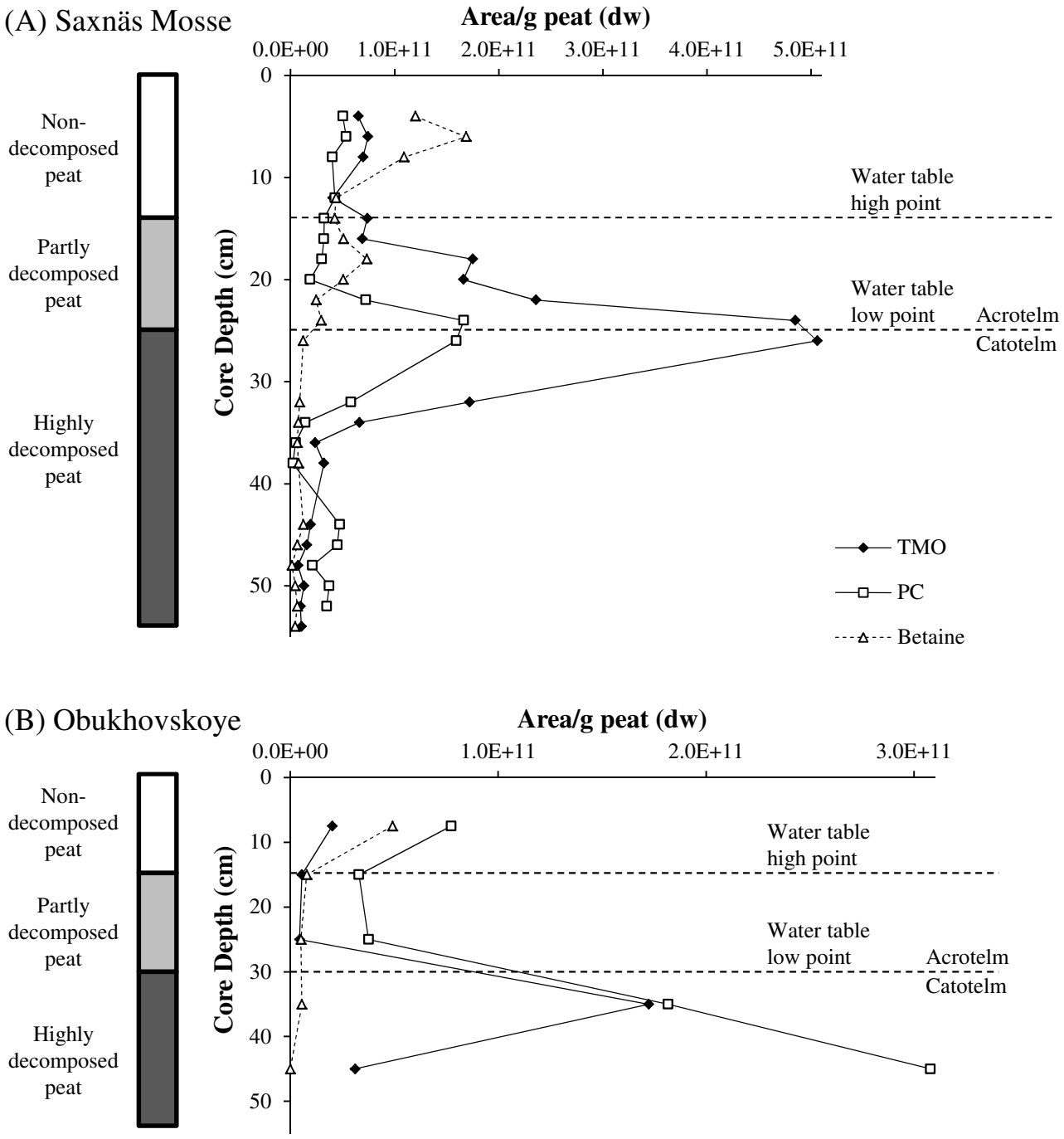
(A) Saxnäs Mosse
Area/g peat (dw)
0.0E+00
1.0E+11
2.0E+11
3.0E+11
4.0E+11
5.0E+11
Core Depth (cm)
0
10
20
30
40
50
Non-decomposed peat
Partly decomposed peat
Highly decomposed peat
Water table high point
Water table low point
Acrotelm
Catotelm
TMO
PC
Betaine
(B) Obukhovskoye
Area/g peat (dw)
0.0E+00
1.0E+11
2.0E+11
3.0E+11
Core Depth (cm)
0
10
20
30
40
50
Non-decomposed peat
Partly decomposed peat
Highly decomposed peat
Water table high point
Water table low point
Acrotelm
Catotelm

Figure 2

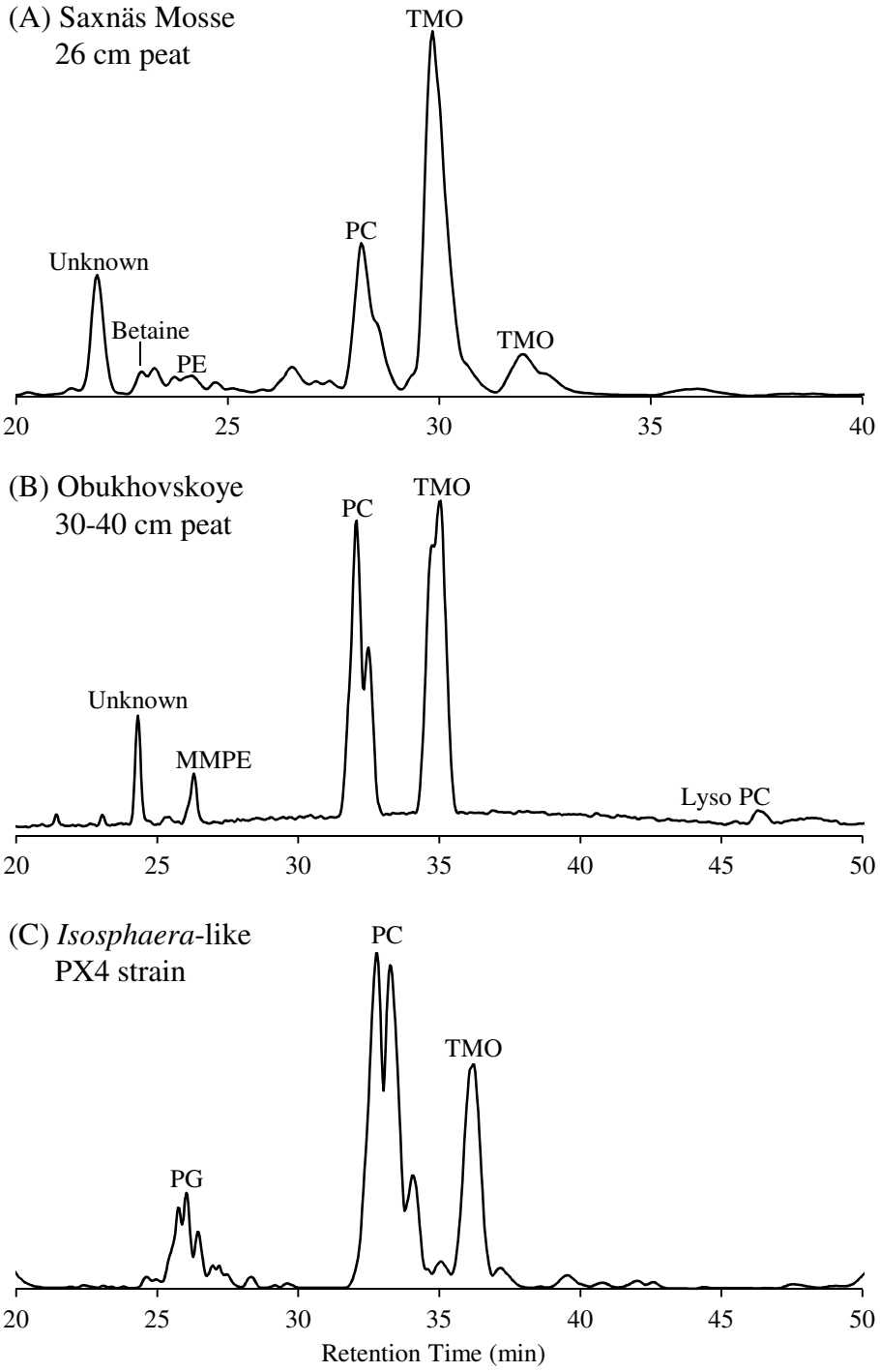
(A) Saxnäs Mosse
26 cm peat
TMO
PC
Unknown
Betaine
PE
TMO
20
25
30
35
40
(B) Obukhovskoye
30-40 cm peat
PC
TMO
Unknown
MMPE
Lyso PC
20
25
30
35
40
45
50
(C) *Isosphaera*-like
PX4 strain
PC
TMO
PG
20
25
30
35
40
45
50
Retention Time (min)

Figure 3

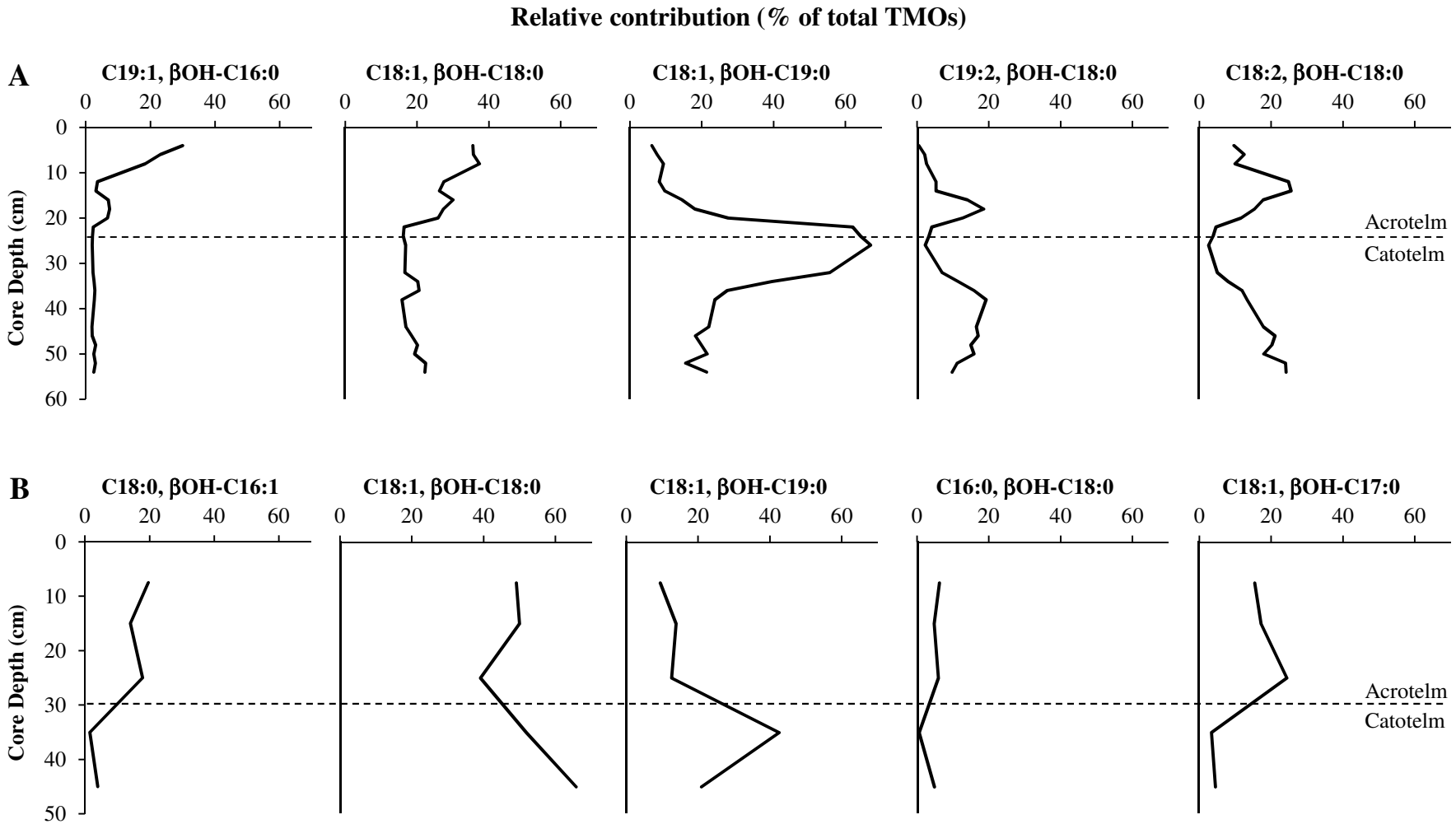
Relative contribution (% of total TMOs)
A
C19:1, βOH-C16:0
C18:1, βOH-C18:0
C18:1, βOH-C19:0
C19:2, βOH-C18:0
C18:2, βOH-C18:0
0 20 40 60
Core Depth (cm)
0 10 20 30 40 50 60
Acrotelm
Catotelm
B
C18:0, βOH-C16:1
C18:1, βOH-C18:0
C18:1, βOH-C19:0
C16:0, βOH-C18:0
C18:1, βOH-C17:0
0 20 40 60
Core Depth (cm)
0 10 20 30 40 50
Acrotelm
Catotelm

Figure 4

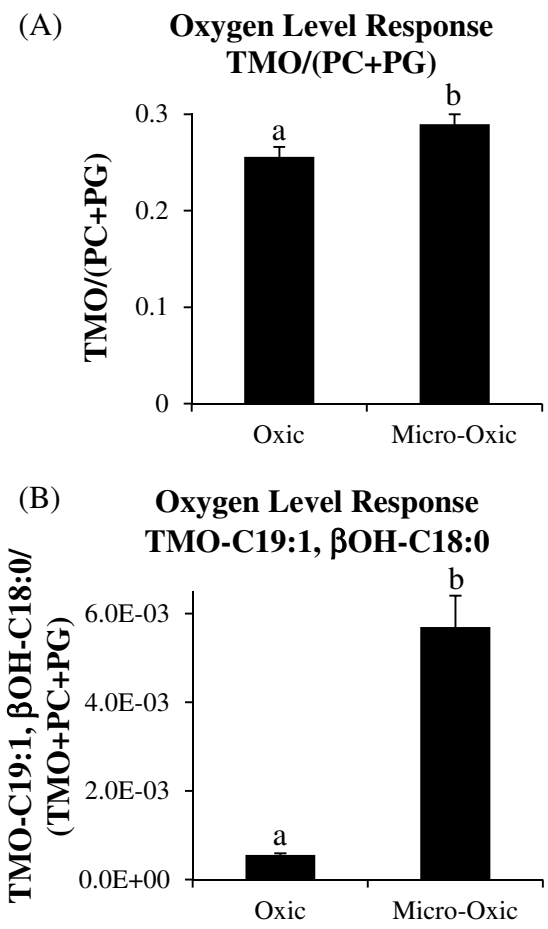
(A)
Oxygen Level Response
TMO/(PC+PG)
TMO/(PC+PG)
0.3
0.2
0.1
0
a
b
Oxic
Micro-Oxic
(B)
Oxygen Level Response
TMO-C19:1, βOH-C18:0
TMO-C19:1, βOH-C18:0/
(TMO+PC+PG)
6.0E-03
4.0E-03
2.0E-03
0.0E+00
a
b
Oxic
Micro-Oxic

Figure 5

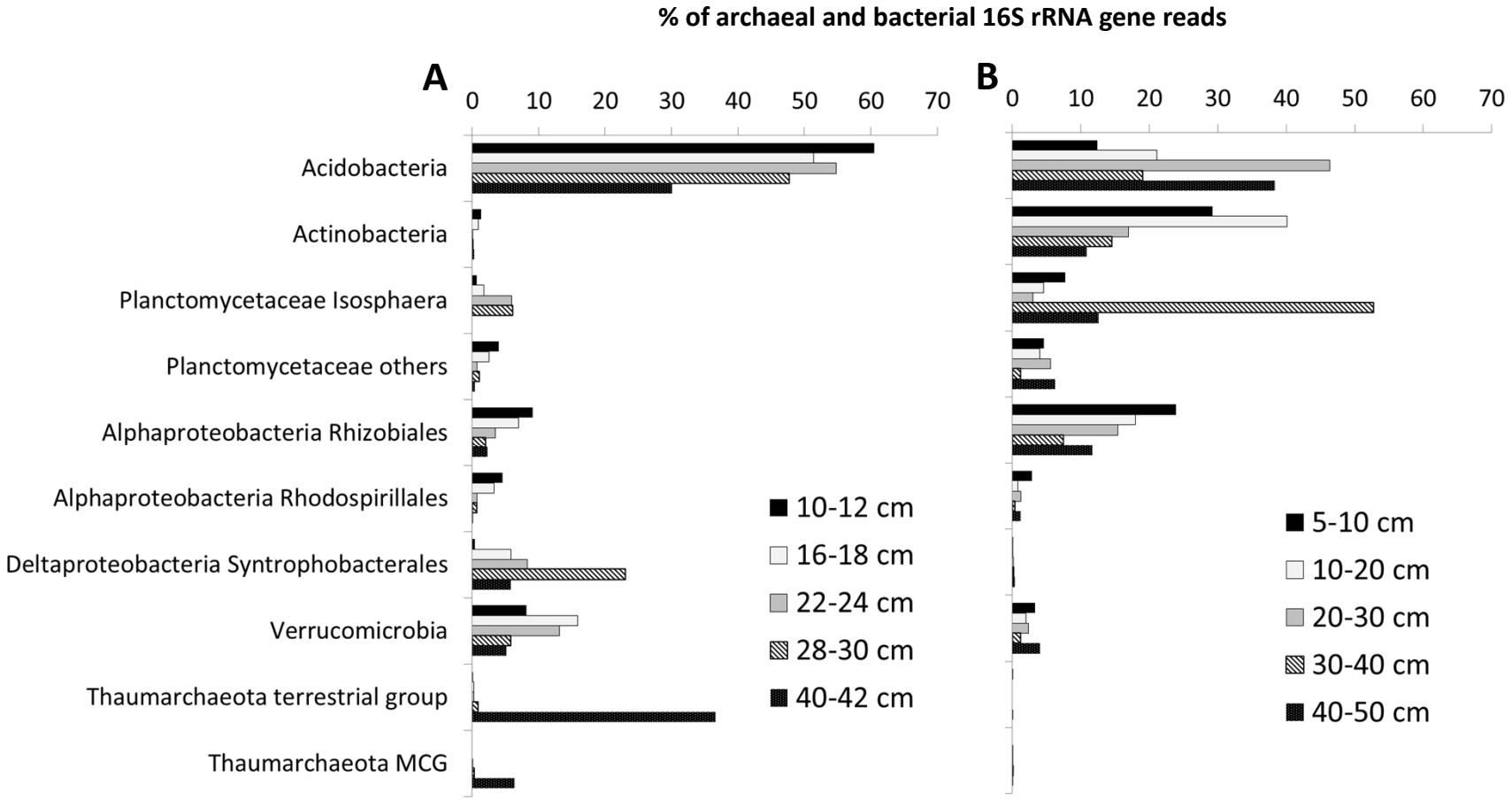
% of archaeal and bacterial 16S rRNA gene reads
A
B
0
10
20
30
40
50
60
70
Acidobacteria
Actinobacteria
Planctomycetaceae Isosphaera
Planctomycetaceae others
Alphaproteobacteria Rhizobiales
Alphaproteobacteria Rhodospirillales
Deltaproteobacteria Syntrophobacterales
Verrucomicrobia
Thaumarchaeota terrestrial group
Thaumarchaeota MCG
10-12 cm
16-18 cm
22-24 cm
28-30 cm
40-42 cm
5-10 cm
10-20 cm
20-30 cm
30-40 cm
40-50 cm

Figure 6

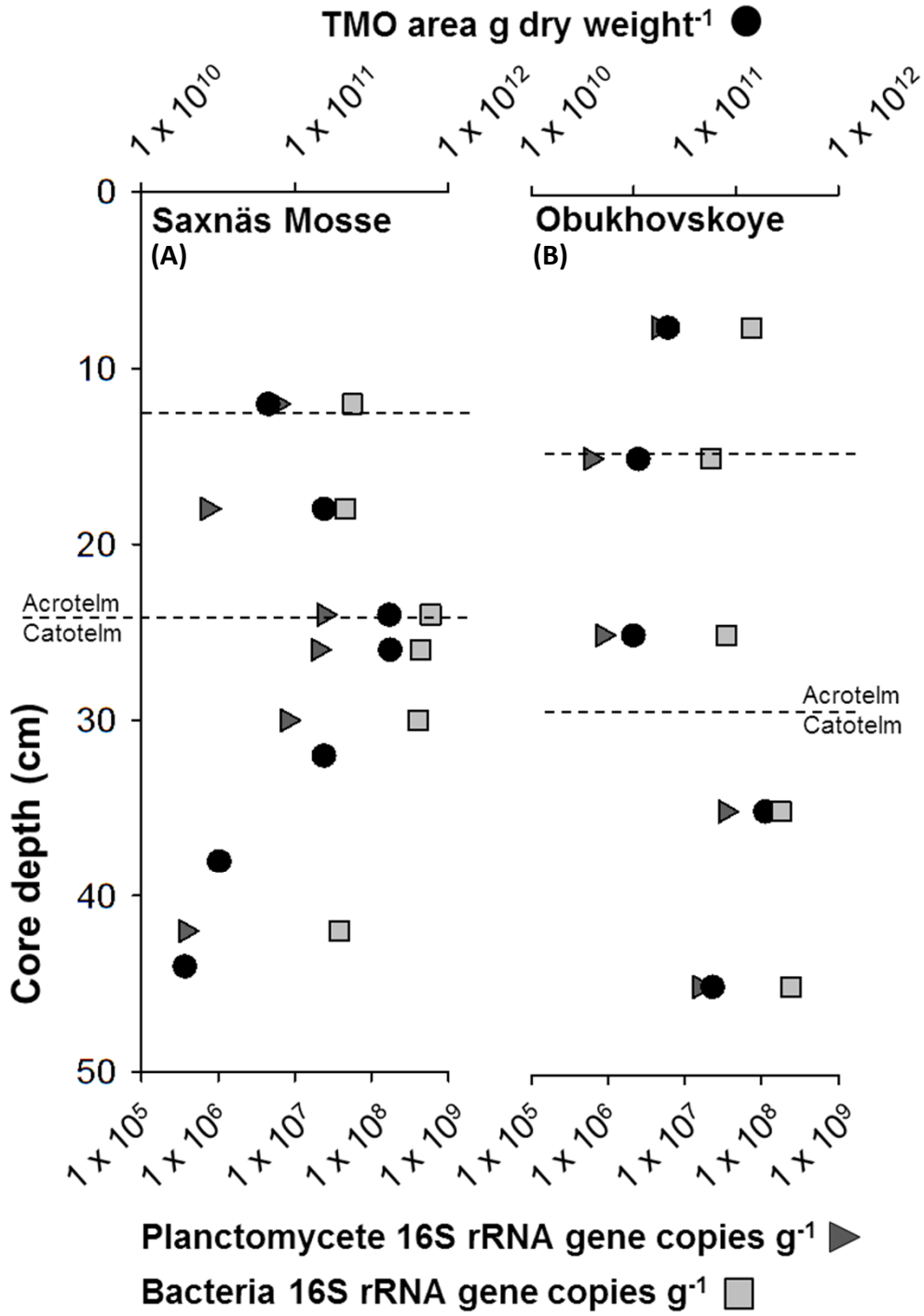
TMO area g dry weight-1
1 x 10^10
1 x 10^11
1 x 10^12
Saxnäs Mosse
(A)
Obukhovskoye
(B)
0
10
20
30
40
50
Acrotelm
Catotelm
Core depth (cm)
1 x 10^5
1 x 10^6
1 x 10^7
1 x 10^8
1 x 10^9
Planctomycete 16S rRNA gene copies g-1
Bacteria 16S rRNA gene copies g-1

Figure 7

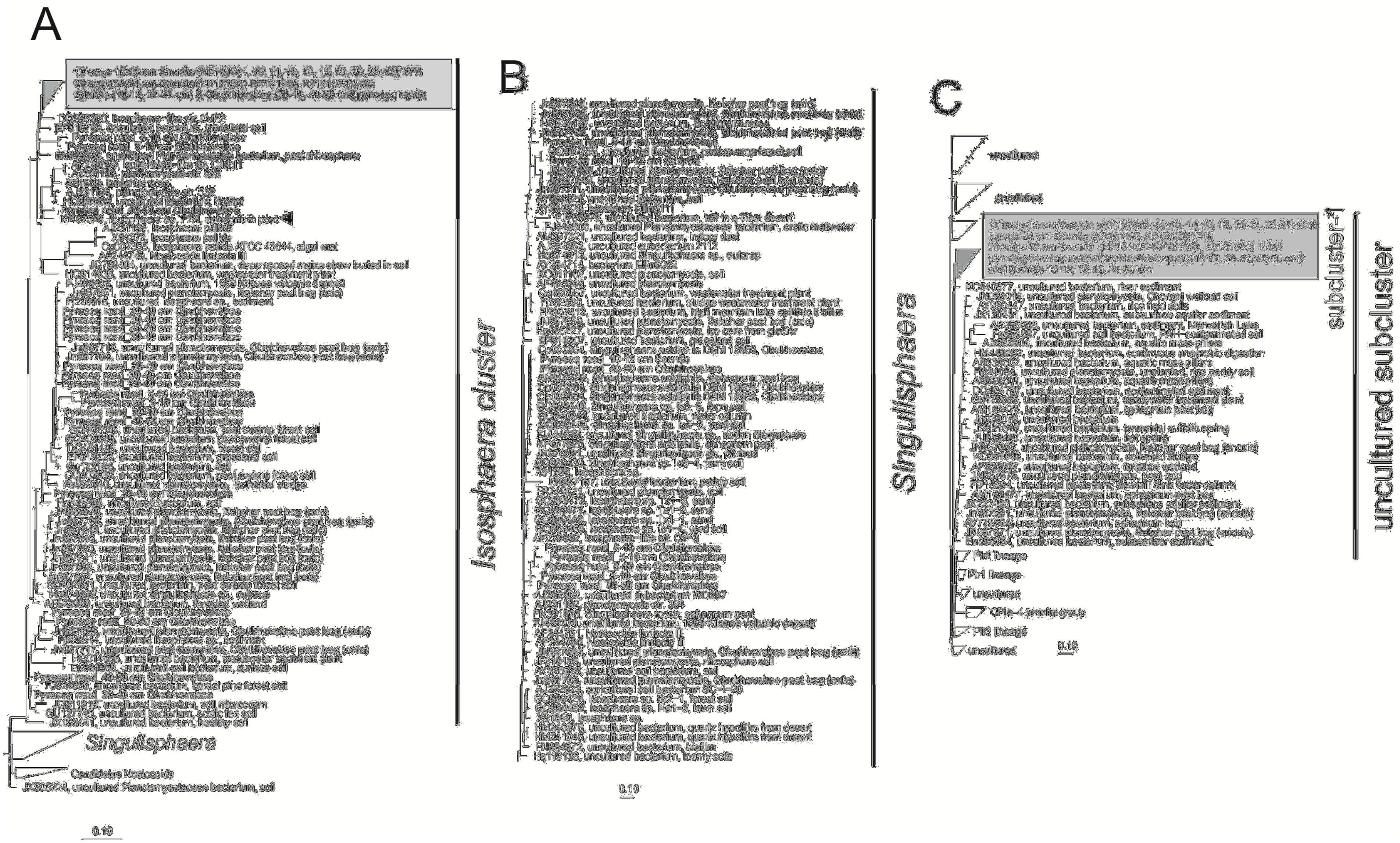
A
B
C
Isosphaera cluster
Singulisphaera
subcluster-1
uncultured subcluster
Candidatus Nostocoida
unculturd
Pir4 lineage
Pir1 lineage
OP3b-4 (marine group
Pir3 lineage
0.10

Table S1. Primer pairs described in the text and PCR conditions used in this study.

Assay	Target	Primer pair	Tm °C	Reference
PCR + cloning qPCR	General Bacteria 16S rRNA	341F (5'-CCTACGGGAGGCAGCAG-3') 907R (5'-CCGTCAATTCCTTTRAGTTT-3')	57 (58 qPCR*)	Muyzer *et al*., 1993 Lane *et al*., 1985
PCR + cloning qPCR	Planctomycetes 16S rRNA	Pla352F (5'-GGCTGCAGTCGAGRATCT-3') Pla920R (5'-TGTGTGAGCCCCCGTCAA-3')	58 (61 qPCR**)	Pollet *et al*., 2011

PCR conditions: 95°C 5 min; 40 × [95°C 1 min, Tm 40 s, 72°C 1 min]; 72°C 5 min.
qPCR conditions: 95°C 4 min; 40 × [95°C 30 s, Tm 40 s, 72°C 30 s]; 80°C 25 s.
*qPCR efficiency 100%; R_2=0.999
**qPCR efficiency 85%; R_2=0.998

Table S2. Distribution of percentage of reads of the 16S rRNA gene amplicon pyrosequencing in the Saxnäs Mosse and the Obukhovskoye peat bogs.

Saxnäs Mosse	**12 cm**	**18 cm**	**24 cm**	**30 cm**	**42 cm**
Acidobacteria	60.5	51.4	54.8	47.6	30.0
Planctomycetaceae *Isosphaera*	0.7	1.8	6.0	6.1	0.0
Planctomycetaceae others:	4.0	2.5	0.7	1.1	0.4
- *Gemmata*	0.0	0.1	0.0	0.0	0.0
- Pir4 lineage	0.0	0.0	0.1	0.0	0.0
- *Schlesneria*	0.2	0.0	0.0	0.0	0.0
- *Singulisphaera*	0.1	0.1	0.0	0.0	0.0
- *Zavarzinella*	0.3	0.0	0.0	0.0	0.0
- uncultured	3.4	2.3	0.6	1.1	0.4
Alphaproteobacteria Rhizobiales	9.1	7.0	3.5	2.0	2.3
Alphaproteobacteria Rhodospirillales	4.6	3.3	0.7	0.7	0.1
Deltaproteobacteria Syntrophobacterales	0.43	5.9	8.3	23.0	5.7
Verrucomicrobia	8.2	15.9	13.2	5.8	5.1
Thaumarchaeota terrestrial group	0.05	0.28	0.28	0.81	36.6
Thaumarchaeota MCG	0.00	0.00	0.03	0.3	6.3
Actinobacteria	1.4	1.0	0.05	0.06	0.3

Obukhovskoye	**5-10 cm**	**10-20 cm**	**20-30 cm**	**30-40 cm**	**40-50 cm**
Acidobacteria	12.4	21.1	46.4	19.0	38.3
Planctomycetaceae *Isosphaera*	7.7	4.6	3.0	52.7	12.6
Planctomycetaceae others:	4.6	4.0	5.6	1.2	6.2
- *Gemmata*	0.4	0.1	0.1	0.0	0.1
- *Planctomyces*	0.3	0.7	1.2	0.2	0.7
- *Schlesneria*	0.1	0.0	0.1	0.0	0.0
- *Singulisphaera*	0.8	0.5	0.2	0.1	3.8
- *Zavarzinella*	0.1	0.0	0.3	0.1	0.0
- uncultured	2.9	2.7	3.8	0.9	1.7
Alphaproteobacteria Rhizobiales	23.9	18.0	15.4	7.4	11.6
Alphaproteobacteria Rhodospirillales	2.9	0.8	1.2	0.3	1.2
Deltaproteobacteria Syntrophobacterales	0.0	0.0	0.2	0.2	0.3
Verrucomicrobia	3.3	2.0	2.4	1.2	4.0
Thaumarchaeota terrestrial group	0.0	0.0	0.0	0.0	0.1
Thaumarchaeota MCG	0.0	0.0	0.0	0.0	0.1
Actinobacteria	29.3	40.1	17.0	14.5	10.8

Table S3. Percentage of total bacterial reads attributed to members of the Planctomycetaceae family in the Saxnäs Mosse and the Obukhovskoye peat bogs.

Saxnäs Mosse

Depth (cm)	% 16S rRNA reads Planctomycetaceae Isosphaera*	% 16S rRNA reads Planctomycetaceae others*	% 16S rRNA reads Planctomycetaceae TOTAL*	% Planctomycete cells**
10-12	0.7	4.0	4.7	15.1
16-18	1.8	2.5	4.3	2.3
22-24	6.0	0.7	6.7	5.8
28-30	6.1	1.1	7.2	2.8
40-42	0.0	0.4	0.4	1.4

Obukhovskoye

Depth (cm)	% 16S rRNA reads Planctomycetaceae Isosphaera*	% 16S rRNA reads Planctomycetaceae others*	% 16S rRNA reads Planctomycetaceae TOTAL*	% Planctomycete cells**
5-10	7.7	4.6	12.3	8.8
10-20	4.6	4.0	8.6	3.9
20-30	3.0	5.6	8.6	3.5
30-40	52.7	1.2	53.9	27.1
40-50	12.6	6.2	18.8	9.5

*Percentage of 16S rRNA gene reads obtained from the pyrosequencing analysis as shown in Table S2.
**Percentage of planctomycete cells respect to the total bacterial cells assuming that the average 16S rRNA copy number per bacterial cell is 3.6 (Schloss et al., 2009), and that planctomycetes have an average of 2.5 copies of 16S rRNA gene per genome (considering 2 copies present in *Pirellula marina* and *Planctomyces* species, and 3 copies in *Isosphaera pallida*; Ribosomal RNA Operon Copy Number Database; Klappenbach et al., 2001). See Fig. S4 for details.

Figure S1

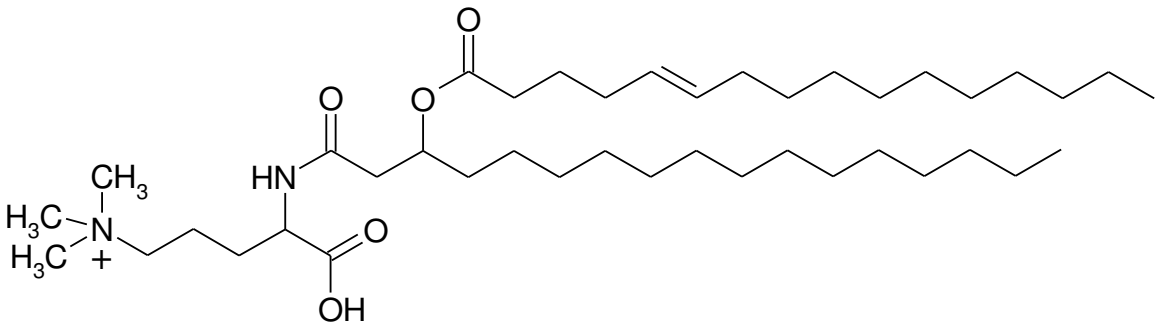

Fig. S1. Trimethylornithine (TMO) lipid structure.

Figure S2

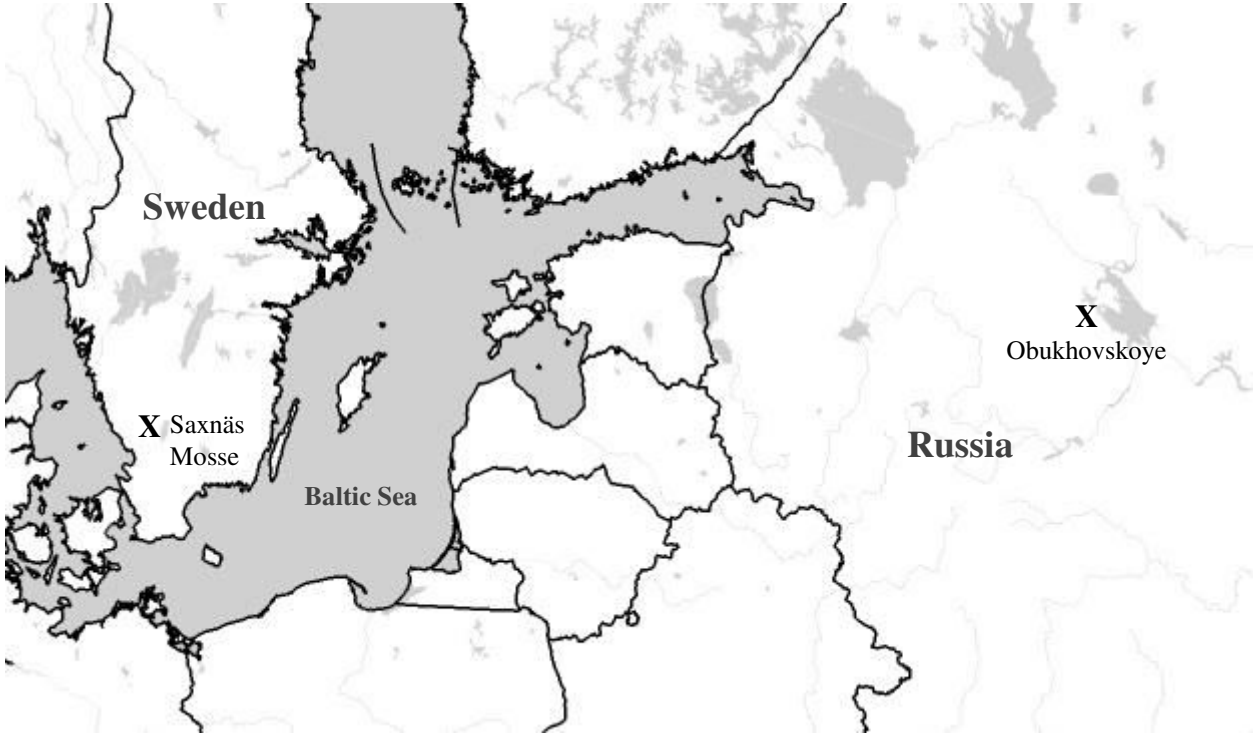

Fig. S2. Locations of peat sample collection in Russia (58° 14'N, 38° 12'E) and Sweden (56° 51' 20.78" N, 13° 27' 39.62" E).

Figure S3

Fig. S3. Phylogenetic tree including the representative pyrosequencing 16S rRNA gene reads (in bold) obtained from the Saxnäs Mosse peat bog samples and classified as Acidobacteria, and their closest relatives. Triangles indicate Acidobacteria strains or groups the lipid composition of which have been previously reported by Sinninghe Damsté et al., 2011.

Figure S4

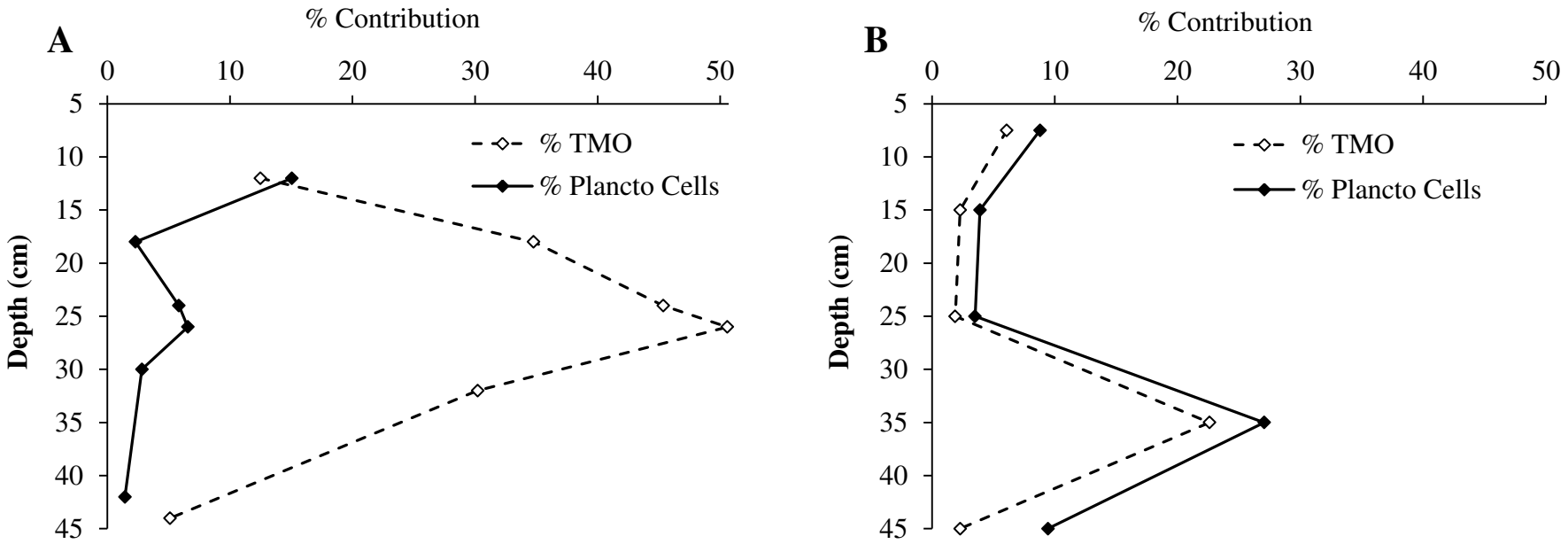

Fig. S4. Percent of total IPLs made up by trimethylornithine lipid (TMO) based on HPLC/MS base peak area; Planctomycete cells to total Bacterial cells based on the calculation of converting planctomycete and bacterial specific 16S rRNA gene copies to cell numbers in the (A) Saxnäs Mosse, and the (B) Obukhovskoye peat bogs. Total bacterial and planctomycete cell numbers were estimated assuming that the average 16S rRNA copy number per bacterial cell is 3.6 (Klappenbach et al., 2001), and that planctomycetes have an average of 2.5 copies of 16S rRNA gene per genome (considering 2 copies present in *Pirellula marina* and *Planctomyces* species, and 3 copies in *Isosphaera pallida*; Ribosomal RNA Operon Copy Number Database; Göker et al., 2011).

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