1   **Anaerobic methane oxidation driven by microbial reduction of natural organic matter**

2   **in a tropical wetland**

3

4   Edgardo I. Valenzuela[1], Alejandra Prieto-Davó[2], Nguyen E. López-Lozano[1], Alberto

5   Hernández-Eligio[3], Leticia Vega-Alvarado[4], Katy Juárez[3], Ana Sarahí García-González[5],

6   Mercedes G. López[5] & Francisco J. Cervantes[1,*]

7

8   1. División de Ciencias Ambientales, Instituto Potosino de Investigación Científica y

9   Tecnológica. Camino a la Presa San José 2055 Col. Lomas 4ª. Sección, San Luis Potosí,

10   SLP 78216 Mexico.

11   2. Facultad de Química, Unidad Sisal, Universidad Nacional Autónoma de México. Puerto

12   de Abrigo s/n, Sisal, Yucatán, 97356 Mexico.

13   3. Departamento de Ingeniería Celular y Biocatálisis, Instituto de Biotecnología,

14   Universidad Nacional Autónoma de México, Campus Morelos. Av. Universidad 2001,

15   Cuernavaca Morelos, 62210 Mexico.

16   4. Centro de Ciencias Aplicadas y Desarrollo Tecnológico, Universidad Nacional

17   Autónoma de México, Circuito exterior s/n, Ciudad Universitaria, Coyoacán, D.F., 04510

18   Mexico.

19   5. Departamento de Biotecnología y Bioquímica, Centro de Investigación y de Estudios

20   Avanzados del IPN, Unidad Irapuato, Irapuato, Mexico.

21

22   Running title: Anaerobic methane oxidation linked to humus reduction

23   Keywords: Anaerobic methane oxidation, humus, methanotrophy, wetlands

24   *Corresponding author: Francisco J. Cervantes, fjcervantes@ipicyt.edu.mx

Abstract

Wetlands constitute the main natural source of methane on Earth due to their high content

of natural organic matter (NOM), but key drivers such as electron acceptors supporting

methanotrophic activities in these habitats are poorly understood. We performed anoxic

incubations using freshly collected sediment along with water samples harvested from a

tropical wetland, amended with ^{13}C-methane (0.67 atm) to test the capacity of its microbial

community to perform anaerobic methane oxidation (AOM) linked to the reduction of the

humic fraction of its NOM. Collected evidence demonstrates that electron-accepting

functional groups (*e.g.* quinones) present in NOM fueled AOM by serving as terminal

electron acceptor. Indeed, while sulfate reduction was the predominant process accounting

for up to 42.5% of the AOM activities, microbial reduction of NOM concomitantly

occurred. Furthermore, enrichment of wetland sediment with external NOM provided

complementary electron-accepting capacity, which reduction accounted for ~100 nmol ^{13}C-

CH_4 oxidized cm^{-3} d^{-1}. Spectroscopic evidence showed that quinone moieties were

heterogeneously distributed in the wetland sediment, and that their reduction occurred

during the course of AOM. Moreover, an enrichment derived from wetland sediments

performing AOM linked to NOM reduction stoichiometrically oxidized methane coupled to

the reduction of the humic analogue, anthraquinone-2,6-disulfonate. Microbial populations

potentially involved in AOM coupled to microbial reduction of NOM were dominated by

44   divergent biota from putative AOM-associated archaea. We estimate that this microbial

45   process could potentially contribute to the suppression of up to 114 Tg CH_4 yr^{-1} in coastal

46   wetlands and more than 1,300 Tg yr^{-1} considering the global wetland area.

47

48   **Importance**

49   Identifying key processes governing methane emissions from natural systems is of major

50   importance considering the global warming effects triggered by this greenhouse gas. AOM

51   coupled to the microbial reduction of distinct electron acceptors plays a pivotal role in

52   mitigating methane emissions from ecosystems. Given their high organic content, wetlands

53   constitute the largest natural source of atmospheric methane. Nevertheless, processes

54   controlling methane emissions in these environments are poorly understood. Here we

55   provide tracer analysis with $^{13}CH_4$ and spectroscopic evidence revealing that AOM linked

56   to the microbial reduction of redox functional groups in natural organic matter (NOM)

57   prevails in a tropical wetland. We suggest that microbial reduction of NOM may largely

58   contribute to suppress methane emissions from tropical wetlands. This is a novel avenue

59   within the carbon cycle in which slowly decaying NOM (*e.g.* humic fraction) in

60   organotrophic environments fuels AOM by serving as terminal electron acceptor.

61

62

63

64

65

Introduction

Microbial processes produce and consume methane (CH_4) in anoxic sediments playing a

crucial role in regulating Earth´s climate. Virtually 90% of CH_4 produced from marine

environments is oxidized by microorganisms avoiding its release to the atmosphere (1).

Anaerobic oxidation of methane (AOM) associated with sulfate reduction was first

discovered in marine environments (2). More recently, AOM has also been linked to the

microbial reduction of nitrate (3, 4) and nitrite (5), as well as Fe(III) and Mn(IV) oxides (6–

8) in freshwater and marine environments. Wetlands are the largest natural source of CH_4

(9), contributing to about a third of global emissions (10), but key drivers, such as electron

acceptors fueling methanotrophic activities in these habitats, are poorly understood. CH_4

emissions from wetlands have been strongly responsive to climate in the past, and will

likely continue to be responsive to anthropogenic-driven climate change in the future,

predicting a large impact on global atmospheric CH_4 concentration (10). The traditional

assumption is that aerobic methanotrophy dominates wetlands' CH_4 cycling by oxidizing

an estimated 40 to 70% of gross CH_4 production in these ecosystems (11). Recent findings

(12) challenged this conjecture by providing evidence that AOM may consume up to 200

$Tg\ CH_4\ yr^{-1}$, decreasing their potential CH_4 emission by 50% in these habitats. Most AOM

activities observed in wetlands have been related to sulfate reduction (12, 13), but other

electron acceptors remain feasible. Natural organic matter (NOM), circumscribed to humic

substances (HS) in many studies (14), occurs at high concentrations in wetlands both in

soluble and solid phases (15). Recent evidence indicates that HS suppress methane

production in different ecosystems (16, 17), yet the mechanisms involved are still

enigmatic. HS can theoretically promote AOM as they can serve as terminal electron

89   acceptors for microbial respiration (18, 19) and have higher redox potential than sulfate

90   (20). However, compelling evidence demonstrating AOM driven by the microbial reduction

91   of NOM present in anoxic environments remains elusive (21, 22).

92   We aimed to document $^{13}CH_4$ anaerobic oxidation and the ongoing reduction of intrinsic

93   electron acceptors, including the electron accepting fraction of NOM, by the biota of

94   freshly sampled sediment from a coastal tropical wetland. We provide $^{13}CH_4$ tracer studies

95   and spectroscopic evidence demonstrating for the first time that AOM is linked to the

96   microbial reduction of redox functional groups present in the NOM of this tropical marsh.

97   Furthermore, we found evidence, based on 16S rRNA gene sequences, indicating that

98   microbial populations potentially involved in AOM coupled to microbial reduction of

99   NOM were dominated by divergent biota from putative AOM-associated microorganisms.

100

101   **Results**

102   **Kinetics of ^{13}C-methane oxidation and electron balances**

103   Exponential phase of AOM was observed in microcosms over the first 15 days of

104   incubation in the case of unamended sediment (free from external NOM addition). The

105   methanotrophic rate in this experimental treatment was ~1.34 μmol ^{13}C-methane oxidized

106   cm^{-3} d^{-1} (Fig. 1). At the end of the exponential phase, sulfate and Fe(III) reduction

107   accounted for 42.5% and 0.5% of ^{13}C-methane oxidized, respectively, while the role of

108   nitrate was marginal (Fig. 2 and Table S2). These unamended sediment microcosms

109   exhibited a reduction in intrinsic NOM during the course of AOM, which was expected due

110   to the high concentration of organic carbon in the tropical wetland, with the capacity to

111   accept electrons (Table S1, Fig. 2). Nevertheless, large perturbation caused by endogenous

112   NOM reduction in experimental controls lacking ^{13}C-methane obstructed accurate

113   assessment of AOM driven by this microbial process (Fig. 2). The large endogenous NOM

114   reduction observed in these control experiments may be explained by concomitant methane

115   production (and subsequent consumption) observed (Fig. S2), and by oxidation of labile

116   organic matter present in the sediment (Table S1). Supplementary incubations spiked with

117   the sulfate-reduction inhibitor, sodium molybdate (25 mM), showed decreased sulfate

118   reducing activities (~50%, Fig. 2), while AOM rates remained high when compared against

119   their non-inhibited counterparts (Fig. 1). Remarkably, when sulfate reduction was inhibited,

120   the reduction of intrinsic NOM was doubled (from 1.6±0.11 to 3.4±0.19 milli-electron

121   equivalents (meq) l^{-1}), implying that the reduction of redox functional groups in NOM was

122   promoted when the utilization of sulfate was impeded.

123   Further enrichment of wetland sediment with external NOM, in the form of HS derived

124   from *Pahokee Peat* (Florida Everglades, 2.5 g l^{-1}), provided complementary electron

125   accepting capacity, which significantly elicited AOM up to ~1.88 μmol ^{13}C-methane

126   oxidized cm^{-3} d^{-1} and extended the exponential phase to 20 days (Fig. 1). In this

127   experimental treatment, electron balances revealed a methanotrophic activity responsible of

128   ~100 nmol ^{13}C-CH_4 oxidized cm^{-3} d^{-1} linked to microbial reduction of NOM (including

129   both intrinsic and externally added as *Pahokee Peat* HS)**.** As hypothesized before,

130   consumption of intrinsically produced methane was confirmed by experimental controls

131   enriched with HS from *Pahokee Peat* and incubated in the absence of ^{13}C-methane, which

132   showed significant consumption of $^{12}CH_4$ (Fig. S2). This was also confirmed by increased

133   $^{12}CO_2$ production quantified, which was reflected on 2 to 4-fold lower enrichment of $^{13}CO_2$

134   in HS enriched incubations as compared to unamended controls (see $^{13}FCO_2$ values in Fig.

135   1). Reports (23, 24) indicate that methanotrophic microorganisms prefer to oxidize $^{12}CH_4$ as

136   compared to $^{13}CH_4$, which may partly explain our findings.

137   The role of sulfate reduction on AOM when wetland sediment was enriched with HS was

138   not possible to assess (Table S2) due to large endogenous sulfate reduction elicited by

139   degradation of the labile fraction of externally added NOM (Fig. 2), which also triggered

140   methanogenesis in these microcosms. Since no significant differences in iron reduction

141   were detected between microcosms with or without $^{13}CH_4$ addition, the only microbial

142   process clearly identified driving AOM in *Pahokee Peat* enriched sediments was the

143   microbial reduction of HS (Table S2).

144   **Spectroscopic evidence on presence and reduction of redox-functional groups in NOM**

145   Initial exploration of the solid phase NOM present in wetland sediment by micro-ATR-

146   FTIR spectra, revealed the presence of electron accepting moieties both in unamended and

147   in HS enriched wetland sediments. By mapping of acquisition points at 1650-1620 cm^{-1},

148   presence and heterogeneous distribution of quinone functional groups was evidenced in

149   sediments confirming the presence of non-soluble electron accepting moieties classically

150   attributed to humic-like materials (Fig. 3a and b). To further confirm this, we looked for

151   double bonded carbon and oxygen (C=O) by use of X-ray photoelectron spectra (XPS),

152   technique that supported the existence of quinone-like functional groups in unamended

153   sediment and furthermore, provided evidence of the reduction of these moieties by showing

154   the disappearance of the C=O signal from C1s and O1s high resolution spectra when

155   comparing signals from sediment analyzed before and after incubation with $^{13}CH_4$ in the

156 absence of external HS (Fig. 3c to f). Another missing signal after the AOM process was

157 that which corresponds to metallic oxides, evidenced by analysis of the O1s high resolution

158 spectra (Figure 3d and f), which may imply reduction of intrinsic iron oxides that supported

159 ~0.5% of methanotrophy according to electron balances (Table S2). Further analysis of the

160 liquid phase of pristine sediment microcosms also revealed the reduction of quinone-like

161 moieties during the course of AOM (Fig. 4). Initial samples exhibited a well-defined and

162 strong peak at 1690 cm^{-1} associated with quinone moieties, while reduced samples, at the

163 end of the incubation period, showed an increase in the signal related to phenolic groups

164 (1660 cm^{-1}). Additional signals of phenolic groups were detected after incubation with

165 $^{13}CH_4$ and *Pahokee Peat* by spectral signals detected around 2260-2500 cm^{-1} (25).

Microbial communities performing AOM

167 According to 16S rRNA gene sequences from wetland sediment samples performing AOM,

168 anaerobic methanotrophic archaea (ANME), which are traditionally linked to anaerobic

169 methanotrophy under sulfate-reducing (2, 26), Fe(III)-reducing (6, 8), and artificial electron

170 acceptor-reducing conditions (27), were barely detected in our experiments, with ANME-

171 1b and ANME-3 representing less than 0.5% and 0.2%, respectively, from the archaeal

172 community in all experimental treatments (Fig. 5). The only abundant *Euryarchaeota*

173 members detected were affiliated to an unclassified genus of the Marine Benthic Group D

174 family (MBGD and DHVEG-1), which accounted for 18 to 23% of the archaeal biota in all

175 treatments. Outside of the *Euryarchaeota* phylum, members from the newly named

176 *Bathyarchaeota* lineage (formerly known as Miscellaneous Crenarchaeotic Group) were

177 another cluster of microorganisms that remained in high percentages (from 8 to 14%) in all

178 treatments. Two genera from the *Thaumarchaeota* phylum, one belonging to the

8

179   pMC2A209 class, and the other from the Marine Benthic Group B (MBG-B) were also

180   consistently present in all sediment samples showing AOM, the latter one increasing its

181   proportion up to 12% when sulfate reduction was inhibited (Fig. 5). From the bacterial

182   counterpart, the most abundant bacteria in two of the treatments was a genus of

183   *Oceanimonas* from *Aeromonadaceae* family (*Gammaproteobacteria*), whose presence was

184   diminished when sulfate reduction was inhibited and when $^{13}CH_4$ was absent (Fig. S3),

185   suggesting that this microorganism might have been involved in sulfate-dependent AOM.

186   Other evident changes in the bacterial community included the increase of *Clostridia* and

187   *Bacilli* members when external NOM was supplied (Fig. S3), which agrees with their

188   capacity to reduce HS (28).

189   **AOM linked to AQDS reduction**

190   In order to confirm the capacity of the sediment biota to channel ^{13}C-methane derived

191   electrons to quinone groups, the humic analogue, anthraquinone-2,6-disulfonate (AQDS),

192   was added as an electron acceptor to the artificial basal medium for sediment enrichments.

193   AQDS reduction and methane consumption were observed since the first enrichment cycle,

194   although no clear relationship between net methane consumption and AH_2QDS production

195   was observed due to high concentrations of intrinsic electron donors and acceptors (data not

196   shown). Nevertheless, during the third incubation cycle, net AOM was observed within 11

197   days, which corresponded to a final ratio of oxidized methane/reduced AQDS of 1:4.7

198   corrected for endogenous controls, which is very close to the stoichiometric 1:4 according

199   to the following equation (Fig. 6a):

$$CH_4 + 4AQDS + 2H_2O \rightarrow CO_2 + 4AH_2QDS$$

9
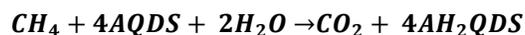
200 **Gibbs free energy ($\Delta G^{o\prime}$) = -43.2 kJ mol^{-1}**

201 Analysis of 16S rRNA gene sequences from enriched sediment sampled at the end of the

202 third cycle of AQDS-dependent AOM activity (Fig. S1) displayed a significant decrease on

203 the diversity of the microbial community evidenced by a decrease in Shannon index, from

204 5.52 in freshly sampled sediment to 3.56 after enrichment with CH_4 and AQDS. Significant

205 increments and decreases of specific groups of archaea and bacteria did occur in this

206 enrichment (Fig. 6b and c). From the archaeal fraction, the pMC2A209 class from the

207 *Thaumarchaeota* and the *Methanosaeta* genera were archaeal clusters that significantly

208 increased their presence in the AQDS enrichment (34% and 23%, respectively). Also in the

209 AQDS enrichment, the *Bathyarchaeota* phylum previously detected in wetland sediments,

210 both in the presence and in the absence of external NOM, significantly increased its

211 proportion in the archaeal community (around 10% respect to the original composition),

212 suggesting potential metabolic arrangements to thrive under AQDS-dependent AOM

213 conditions (Fig. 6b). Humus-reducing bacteria that proliferated throughout the five months

214 of enrichment included genera from the *Desulfuromonadales* (29, 30), *Clostridiales* (14,

215 28) and *Propionibacteriales* (31) orders in 27%, 7%, and 12%, respectively, with respect

216 to the original composition (Fig. S3).

217

218 **Discussion**

219 **NOM as terminal electron acceptor fueling AOM in wetland sediment.** Although the

220 complex composition of the studied wetland sediment challenged efforts to elucidate the

221 microbial processes responsible for the high methanotrophic activities quantified, the

222   present study provides multiple lines of evidence demonstrating that electron-accepting

223   functional groups present in its NOM fueled AOM by serving as terminal electron acceptor.

224   Indeed, while sulfate reduction was the predominant process accounting for up to 42.5% of

225   AOM activities, microbial reduction of NOM concomitantly occurred. Furthermore,

226   enrichment of wetland sediment with external NOM, as *Pahokee Peat* HS, significantly

227   promoted AOM with a quantified amount of ~100 nmol ^{13}C-CH$_4$ oxidized cm^{-3} d^{-1}

228   attributed to this microbial process. Spectroscopic evidence also demonstrated that quinone

229   moieties, main redox functional groups in HS (19), were heterogeneously distributed in the

230   studied wetland sediment and that their reduction occurred during the course of AOM.

231   Moreover, an enrichment derived from wetland sediments performing AOM linked to

232   NOM reduction stoichiometrically oxidized methane coupled to AQDS. Sediment

233   incubations performed in the presence of the sulfate reduction inhibitor, molybdate, further

234   confirmed the role of HS in AOM. Certainly, even though sulfate-reducing activities

235   significantly decreased in the presence of molybdate, AOM activities remained high, while

236   microbial reduction of NOM was doubled under these conditions. These interesting

237   findings suggest that methanotrophic microorganisms performing sulfate-dependent AOM

238   might have directed electrons derived from AOM towards NOM when sulfate reduction

239   became blocked as has been suggested based on experiments performed under artificial

240   conditions (27).

241   **Microbial communities in wetland sediments performing AOM.** Archaeal clusters

242   consistently found in wetland sediment incubations performing AOM included members

243   from the MBG-D family, which have already been proposed as players in metal-dependent

244   AOM (6), thus their presence agrees with evidence indicating AOM linked to iron

11

245   reduction observed in some experimental controls (Table S2). Additionally, these

246   microorganisms were not found in the AQDS enrichment, probably due to depletion of

247   intrinsic ferric iron throughout the incubation cycles. Archaea constantly present amongst

248   fresh sediment incubation and AQDS enrichment were those from the pMC2A209 class

249   and the *Bathyarchaeota* phylum. To our knowledge, the pMC2A209 class of archaea has

250   not been related to AOM, but its close partners from the MBG-B class have been

251   consistently found in environments in which AOM occurs (32–35). In fact,

252   *Thaumarchaeota* members, including the MBG-B, have been found in consortia

253   performing AOM in the absence of ANME clades (36). Interestingly, the pMC2A209

254   cluster seemed to duplicate its proportion up to 12% when sulfate reduction was inhibited

255   (by molybdate), which might suggest that the impediment of sulfate reduction enhanced its

256   activity promoting AOM coupled to NOM reduction. Respect to the *Bathyarchaeota*

257   phylum, increasing evidence suggests that this lineage might be involved in the methane

258   cycle. Recently, it has been demonstrated that this cluster possesses the necessary genetic

259   elements to express the enzymatic machinery required for methane production, and

260   potentially methane consumption (37). Additionally, Saxton and colleagues have found

261   abundant *Bathyarchaeota* representation in a fulvic acids rich deep sediment that oxidizes

262   methane uncoupled from sulfate reduction (22). Unexpectedly, a very low percentage

263   within the archaeal population was identified as members from the ANME type archaea,

264   even though it would be expected to find ANME-2 members since it is the only ANME

265   subgroup with proven capability to derive electrons extracellularly towards humus and its

266   analogues under artificial conditions (27). Our microcosms, both in fresh sediment as well

267   as in the AQDS long-term enrichment, showed a barely detectable number of copies of

12

268   ANME-1b and ANME-3 sequences retrieved by the methodology employed, suggesting a

269   low presence of ANME microorganisms in the ecosystem studied.

270   Regarding the bacterial composition, while *Clostridia*, *Bacilli* and *Gammaproteobacteria*

271   were significantly represented within the fresh sediment performing AOM (Fig. S3), the

272   AQDS enrichment (Fig. 6) exhibited the most significant increase in *Deltaproteobacteria*

273   of the *Desulfuromonadales* order, which includes several humus-reducing microorganisms

274   (14). Since a wide diversity of microorganisms have been proven to reduce humus

275   analogues or HS, we do not rule out that diverse bacterial clusters could have participated

276   in partnership with detected archaea to jointly performed AOM coupled to NOM reduction.

277   Nevertheless, humus-reducing bacteria possess metabolic versatility and capability to

278   reduce miscellaneous electron acceptors, which makes it difficult to come to conclusions

279   about their participation in our experiments. Further investigation must be done to unravel

280   the potential involvement of humus-reducing bacteria in AOM.

281   **Ecological significance.** To our knowledge this is the first report of AOM coupled to

282   microbial reduction of NOM, which constitutes a missing link within the carbon cycle. HS

283   frequently contribute up to 80% of soil NOM and up to 50% of dissolved NOM in aquatic

284   environments. While the labile fraction of NOM promotes methanogenesis in anaerobic

285   environments, the slowly decomposing humic portion may serve as an important barricade

286   to prevent methane emissions in organotrophic ecosystems by serving as terminal electron

287   acceptor driving AOM (Fig. 7). As an example, considering the maximum AOM driven by

288   microbial reduction of NOM measured in humic enriched sediments, and the global area of

289   coastal wetlands (38, 39), we approximate that this microbial process consumes up to 114

290   Tg CH_4 yr^{-1}. Considering the global wetland area (10), we anticipate methane suppression

291 of more than 1,300 Tg yr^{-1} (see Supplemental Material for details). Accordingly, NOM-

292 driven AOM may be more prominent in organotrophic sites with poor sulfate content, such

293 as peatlands, swamps and organotrophic lakes. This premise is supported by suppression of

294 methanogenesis by HS observed in different ecosystems (16, 17) and by the widespread

295 AOM activity reported across many peatland types (40–42). The potential role of HS is

296 further emphasized because their electron accepting capacity is fully recycled in recurrently

297 anoxic environments. Thus, the suppression of methanogenesis by HS estimated to be of

298 the order of 190,000 mol CH_4 km^{-2} yr^{-1} may be much larger than previously considered

299 (43).

300

301 **Materials and Methods**

302 **Sediment sampling and characterization**

303 Sediment cores were collected from the tropical marsh *Sisal,* located in Yucatán Peninsula,

304 south-eastern Mexico (21°09'26''N, 90°03'09''W) in January 2016. Sediment cores with a

305 depth of 15 cm were collected under a water column of approximately 70 cm. Water

306 samples were also collected from the area of sediment sampling points to be used as liquid

307 medium in anaerobic incubations. All sediment and water samples were sealed in hermetic

308 flasks and were maintained in ice until arrival to the laboratory. Upon arrival, all sampled

309 materials were stored at 4°C in a dark room until analysis and incubation. Sediment cores

310 were opened and homogenized within an anaerobic chamber (atmosphere composed of

311 N_2/H_2 (95%/5%, v/v)) before characterization and incubation. No amendments (addition of

312 chemicals, washing or exposure to air) were allowed on sediment and water samples in

14

313   order to reflect the actual conditions prevailing *in situ* as closely as possible.

314   Characterization of water and sediment samples is described in Table S1.

315

316   **Sediment incubations.** Water samples collected from sediment sampling points were

317   thoroughly mixed before amendment with HS (2.5 g l^{-1}) by magnetic stirring. *Pahokee Peat*

318   (Florida, Everglades) HS, purchased from the International Humic Substances Society,

319   were employed as external NOM in sediment incubations. Humic-enriched water was

320   flushed with N_2 to blow away any dissolved oxygen. Portions of 15 ml were then

321   distributed in 25-ml serological flasks. Sediment containers were opened inside an

322   anaerobic chamber. Portions of 2.5 ml of wet sediment previously homogenized were then

323   inoculated into each serological bottle. After sealing all bottles with rubber stoppers and

324   aluminum rings inside the anaerobic chamber, they were flushed with N_2. Once anaerobic

325   conditions were established, 5 ml of ^{13}C-labeled methane were injected into each vial to

326   reach a $^{13}CH_4$ partial pressure of 0.67 atm in a headspace of 7.5 ml. Controls incubated in

327   the absence of external HS were also prepared by following an identical protocol. Killed

328   controls included chloroform at a concentration of 10% (v/v) to annihilate any microbial

329   activity. Additional incubations were executed in the presence of the sulfate-reduction

330   inhibitor, molybdate (25 mM), in the presence and in the absence of external NOM. All

331   incubation bottles were statically placed in a dark room at 28 °C (temperature prevailing at

332   *Sisal* wetland at the sampling time). The pH remained at 7.5±0.05 throughout all

333   incubations.

334   **Enrichment incubations with AQDS.** Incubations were commenced by inoculating 120-

335   ml serological bottles with 10 g of volatile suspended solids (VSS) l^{-1} of Sisal sediment.

15

336   Prior inoculation, portions of 60 ml of artificial medium were distributed into the

337   incubation bottles and flushed for 15 min with a mixture of $N_2:CO_2$ (80%/20%, v/v) for

338   stripping any dissolved oxygen from the medium. AQDS (>98.0% purity, TCI AMERICA

339   Chemicals) was added at a concentration of 10 mM as terminal electron acceptor along

340   with the following basal medium components (g l^{-1}): $NaHCO_3$ (5), NH_4Cl (0.3), K_2HPO_4

341   (0.2), $MgCl_2 \cdot 6H_2O$ (0.03) and $CaCl_2$ (0.1). Trace elements were included in the medium by

342   adding 1 ml l^{-1} of a solution with the following composition (mg l^{-1}): $FeCl_2 \cdot 4H_2O$ (2,000),

343   H_2BO_3 (50), $ZnCl_2$ (50), $CuCl_2 \cdot 6H_2O$ (90), $MnCl_2 \cdot 4H_2O$ (500), $AlCl_3 \cdot 6H_2O$ (90),

344     $CoCl_2 \cdot 6H_2O$ (2000), $NiCl \cdot 6H_2O$ (920), $Na_2SeO \cdot 5H_2O$ (162), $(NH_4)_6Mo_7O_{24}$ (500), EDTA

345   (1,000), $Na_2WO_4 \cdot H_2O$ (100) and 1 ml l^{-1} of HCl at 36%. The final pH of the medium was

346   7.2 and no changes were observed throughout the incubation time. Once inoculation took

347   place, microcosms were sealed with rubber stoppers and aluminum rings, and then flushed

348   with the same $N_2:CO_2$ mixture. After anoxic conditions were established, 1 ml of sodium

349   sulfide stock solution was injected into each vial to reach a sulfide concentration of 0.1 g l^{-1}

350   in order to consume any traces of dissolved oxygen. Methane was provided into the

351   microcosms by injecting 30 ml of CH_4 (99.9% purity, Praxair) reaching a partial pressure of

352   methane of 0.54 atm. Subsequent incubations were performed after AQDS was reduced

353   (converted to AH_2QDS) coupled to anaerobic oxidation of methane (AOM). A new set of

354   bottles containing basal medium with AQDS (10 mM) were inoculated within an anaerobic

355   chamber by transferring 10 ml of slurry (sediment and medium) taken from previous

356   incubations (Fig. S1). The following incubations were completed under the same

357   experimental conditions.

358   **Analytical techniques**

359   **Isotopic carbon dioxide and methane measurements.** Ions 16 ($^{12}CH_4$), 17 ($^{13}CH_4$), 44

360   ($^{12}CO_2$) and 45 ($^{13}CO_2$) were detected and quantified in a Gas Chromatograph Agilent

361   Technologies 7890A coupled to a Mass Spectrometer (detector) Agilent Technologies

362   5975C, the ionization was achieved by electronic impact and quadrupole analyzer. For the

363   analysis, a capillary column Agilent Technologies HP-PLOT/Q with a stationary phase of

364   poly-styrene-di-vinyl-benzene (30 m × 0.320 mm × 20 μm) was employed as stationary

365   phase using helium as carrier gas. The chromatographic method was as follows: the starting

366   temperature was 70 ^{o}C which was held for 3 min, and then a ramp with an increase of 20 ^{o}C

367   per min was implemented until 250 ^{o}C was reached and maintained for 1 min. The total

368   time of the run had a duration of 13 min. The temperature of the injection port was 250 ^{o}C.

369   The injection volume was 20 μl and there was only one replicate of injection per bottle. The

370   gas injected into the GC was taken directly from the headspace of the incubations and

371   immediately injected in to the GC port. Methane calibration curves were made by injection

372   of different methane (99.9% of purity) volumes into serological bottles under the same

373   experimental conditions (atmosphere composition, pressure, temperature, and liquid

374   volume). $^{12}CO_2$ and $^{13}CO_2$ curves were made using different dried sodium bicarbonate

375   (99% purity, Sigma Aldrich) and sodium ^{13}C-labelled carbonate (99 atom %^{13}C, Sigma

376   Aldrich) concentrations, respectively, in serological bottles which contained the same

377   volume of wetland sediment and water used in incubations. Standards were incubated at

378   room temperature for 12 hours until equilibrium with the gaseous phase was reached. The

379   linear regression analysis of obtained measurements had a co-relation coefficient higher

380   than 0.97. $^{13}CO_2$ production rates were based on the maximum slope observed on linear

381   regressions considering at least three sampling points.

17

Methane quantification in AQDS enrichment. Net methane consumption was assessed in

terms of methane concentration measurements in the headspace of microcosms. These

measurements were carried out by injecting 100 µl of gas samples from the headspace of

incubation bottles into a gas chromatograph (Agilent Technologies 6890M) equipped with

a thermal conductivity detector, and a Hayesep D (Alltech, Deerfield, Illinois, USA)

column with the following dimensions: 3.048 m × 3.185 m × 2.16 mm. Helium was

employed as carrier gas at a flux of 12 ml min^{-1}. The temperature of injection port, oven

and detector was 250, 60 and 250 ^{o}C, respectively. Calibration curves were made for each

reaction volume used by injecting different methane concentrations into serological bottles

under the same experimental conditions at which microcosms were performed (atmosphere

composition, pressure, temperature, and liquid volume).

Determination of electron accepting functional groups in solid phase by XPS.

Sediment samples (solid fraction of microcosms) were dried under a constant nitrogen flow

after incubation with methane. Once sediments became dried, bottles were open inside an

anaerobic chamber with an atmosphere composed of N_2/H_2 (95%/5%, v/v) and were

triturated on an agate mortar. Samples were then kept under anaerobic conditions until

analysis in a X-Ray Photoelectron Spectroscopy Analyzer PHI VersaProbe II (Physical

Electronics, ULVAC-PHI). Two representative spectra were recorded per scanned sample.

Determination of electron accepting functional groups in solid phase by Micro-ATR-

FT-IR imaging. Micro-ATR-FT-IR images were collected from each sample with a

continuous scan spectrometer, Agilent 660 FT-IR interfaced to a 620 infrared microscope

with a 32 × 32 FPA detector and Ge ATR objective for micro-ATR. Each pixel obtains a

full IR spectrum or a total of 1024 spectra. Background spectra were collected from a clean

405   ATR crystal (i.e., without sample). The Ge crystal of the ATR microscope was lowered

406   onto the surface of each sample for a contact area of approximately 100×100 μm. Spectra

407   were collected by co-addition of 256 scans over a spectral range of 4000 to 900 cm^{-1}, at a

408   spectral resolution of 4 cm^{-1}. In all images, a color scale bar is set within the software to

409   reflect the relative concentration range, from low to high. Agilent Resolutions Pro was used

410   for data acquisition and analysis.

411   **Determination of electron accepting functional groups in liquid phase by high**

412   **resolution UV-Vis-NIR spectroscopy.** After each incubation cycle, liquid samples (1.5

413   ml) were taken in an anaerobic chamber with a disposable syringe and put into a quartz

414   cell, which was sealed with plastic film in order to keep anoxic conditions during

415   spectrometric analysis. Spectra were obtained in a Varian Cary 5000 UV–Vis (diffuse

416   reflectance) spectrophotometer, equipped with an integrating sphere.

417   **Nitrite and nitrate determinations.** Nitrite and nitrate concentrations were measured

418   according to spectrometric techniques established at Standard Methods (44). Nitrate

419   measurement is taken under acidic conditions at a wavelength of 275 nm and the value

420   obtained is corrected for dissolved organic matter which has its maximum absorbance at

421   220 nm. Nitrite forms a purple complex through a reaction with sulfanilamide and N-(1-

422   naphthyl) ethylene diamine, which presents its maximum absorbance at a wavelength of

423   543 nm. Samples were taken with a disposable syringe directly from the microcosms,

424   injected into sealed quartz cuvettes or glass tubes (depending on the required lecture

425   wavelength) and immediately taken to the spectrophotometer to avoid any reaction of the

426   sample with atmospheric oxygen.

427   **Sulfate and sulfide determinations.** Samples were extracted from microcosms and

428   immediately filtered through 0.22 μm nitrocellulose membranes. Filtered samples were

429   then diluted (1:10) with deionized water and processed in an Agilent Capillary

430   Electrophoresis System (Agilent Technologies) according to the methodology proposed by

431   Soga & Ross (45). Dissolved sulfide was measured by the spectrometric method proposed

432   by Cord-Ruwisch (46). Briefly, 100 μl of sample were taken and immediately mixed in

433   vortex with 4 ml of an acidic $CuSO_4$ solution. Absorbance at 480 nm was immediately

434   registered in a UV-VIS spectrophotometer (Thermo Spectronic) to avoid sulfide oxidation

435   before measurements.

436   **Humic substances reduction and ferrous iron measurements.** Quantification of the

437   reduction of electron-accepting functional groups in HS was performed according to

438   Lovley et al. (18). Slurry samples (~500 μl) were taken from microcosms with a disposable

439   syringe while bottles were being manually shaken inside an anaerobic chamber. A portion

440   of each sample (200 μl) was mixed with an equal volume of an acidic solution (HCl, 0.5 M)

441   and allowed to stand for 30 min, while the same volume of sample was reacted with ferric

442   citrate (20 mM) for 3 hours. After reaction with ferric citrate, samples were mildly re-

443   suspended in a vortex and 200 μl were left repose with the same volume of HCl solution for

444   30 min. Afterwards, each sample was centrifuged for 10 min at 10,000 g in a centrifuge

445   Spectrafuge 16M and 200 μl of supernatant were then recovered and reacted with a solution

446   0.2 g l^{-1} of 2,4,6-tris(2-pyridil)-1,3,5-triazine (ferrozine reagent). Ferrous iron produced due

447   to chemical reduction of ferric citrate by reduced functional groups in HS, forms a purple

448   complex along with ferrozine reagent, which has its maximum absorbance at 562 nm. The

449   ferrozine solution was buffered with HEPES (50 mM). Once centrifuged samples were

450   mixed with ferrozine solution, they were left reacting for 10 min before their measurement

451   in a spectrometer Thermo Scientific Genesis 10 UV located inside an anaerobic chamber.

452   All solutions employed in this determination were bubbled with N_2 for 30 min to ensure the

453   absence of dissolved oxygen.

454   **Total carbon (TC), total organic carbon (TOC) and total inorganic carbon (TIC)**

455   **measurements.** Water samples were filtered through 0.22 μm nitrocellulose membranes

456   and diluted with deionized water, while sediment samples were dried until constant weight.

457   Both liquid and solid samples were analyzed in a Total Organic Carbon analyzer Shimadzu

458   TOCVCS/TNM-1 equipped with a solids sampling port (SSM-5000A). Solid sample

459   processing time was 6 min at $900^{o}C$ using O_2 (500 ml min^{-1}) with a purity of 99.9% as

460   carrier gas, all samples were analyzed by triplicate.

461   **Total, volatile and fixed solids.** Total, fixed and volatile solids were measured by triplicate

462   according to Standard Methods procedure (44).

463   **Elemental composition.** Elemental composition of sediments was assessed by analyzing

464   acid-extracts from 2 g of wet sediment. In the case of iron and manganese measurements in

465   microcosms, supernatant samples were taken with disposable syringes, filtered and

466   acidified prior analysis. Samples were then analyzed by inductively coupled plasma optical

467   emission spectrometry (ICP-OES) in an equipment Varian 730-ES. The operational

468   conditions were: potency 1 kW, auxiliary flow: 1.5 l min^{-1}, net flow: 0.75 l min^{-1}, sample

469   taking delay: 30 s, and the number of measured replicates by sample was three. Argon was

470   employed as carrier gas.

471 **DNA extraction, PCR amplification and sequencing**. One microcosm for each selected

472 treatment was randomly chosen at the end of the incubation period (30 days for

473 experiments presented in Fig. 1, and 151 days for experiments depicted in Fig. 6). Before

474 DNA extraction, liquid medium was decanted and extracted from the serological bottles.

475 The total sediment was homogenized afterwards and a subsample of 0.5 g was taken to

476 proceed with DNA extraction. The remaining sediment and the other microcosms were

477 used for material characterization. The total DNA was extracted from sediment samples

478 using the Power Soil DNA extraction kit (MO BIO Laboratories, Carlsbad, CA, USA)

479 following the protocol described by the manufacturer. DNA isolated from each sample was

480 amplified using primers 341F and 785R, targeting the V3 and V4 regions of the 16S rRNA

481 gene fused with Illumina adapter overhang nucleotide sequences (47). The polymerase

482 chain reactions (PCRs) were performed in 50 μl reactions using Phusion Taq polymerase

483 (Thermo Scientific, USA) under the following conditions: denaturation at 98 °C for 60 s,

484 followed by 5 cycles of amplification at 98 °C for 60 s, 50 °C for 30 s and 72 °C for 30 s,

485 followed by 25 cycles of amplification at 98 °C for 60 s, 55 °C for 30 s and 72 °C for 30 s,

486 followed by a final extension of 72 °C for 5 min. Two independent PCR reactions were

487 performed for each sample. The products were indexed using Illumina's 16S Metagenomic

488 Sequencing Library Preparation protocol and Nextera XT Index Kit v2 (Illumina, San

489 Diego CA). Libraries were deep sequenced with the Illumina MiSeq sequencer.

490 **Bioinformatics Analysis.** An analysis of 16S rRNA gene libraries was carried out using

491 Mothur open source software package (v 1.34.4) (48). The high quality sequence data were

492 analyzed for potential chimeric reads using the UCHIME algorithm. Sequences containing

493 homopolymer runs of 9 or more bases, those with more than one mismatch to the

494 sequencing primer and Q-value average below 25 were eliminated. Group membership was

495 determined prior to the trimming of the barcode and primer sequence. Sequences were

496 aligned against the SILVA 123 16S/18S rRNA gene template using the nearest alignment

497 space termination (NAST) algorithm, and trimmed for the optimal alignment region. A

498 pairwise distance matrix was calculated across the non-redundant sequence set, and reads

499 were clustered into operational taxonomic units (OTUs) at 3% distance using the furthest

500 neighbor method. The sequences and OTUs were categorized taxonomically using

501 Mothur's Bayesian classifier and the SILVA 123 reference set. The sequences obtained

502 have been submitted to NCBI GeneBank database.

Accession numbers

504 The accession numbers of sequences in this work were deposited in the GenBank sequence

505 read archive under the BioProject with SRP094593 accession number.

Acknowledgements

516 grateful for the use of infrastructure of the National laboratories LINAN and LANBAMA

517 at IPICYT, as well as USMB at UNAM.

518

519 **References**

520 1. **Reeburgh WS**. 2007. Oceanic methane biogeochemistry. Chem Rev **107**:486–513.

521 2. **Boetius A**, **Ravenschlag K**, **Schubert CJ**, **Rickert D**, **Widdel F**, **Gieseke A**, **Amann R**,
522   **Jørgensen BB**, **Witte U**, **Pfannkuche O**. 2000. A marine microbial consortium apparently
523   mediating anaerobic oxidation of methane. Nature **407**:623–626.

524 3. **Raghoebarsing A**, **Pol a**, **van de Pas-Schoonen K**, **Smolders A**, **Ettwig K**, **Rijpstra W**,
525   **Schouten S**, **Damste J**, **Op den Camp H**, **Jetten M**, **Strous M**. 2006. A microbial
526   consortium couples anaerobic methane oxidation to   denitrification RID B-9395-2011 RID
527   F-5114-2011 RID B-8834-2011. Nature **440**:918–921.

528 4. **Deutzmann JS**, **Stief P**, **Brandes J**, **Schink B**. 2014. Anaerobic methane oxidation coupled
529   to denitrification is the dominant methane sink in a deep lake. Proc Natl Acad Sci U S A
530   **111**:18273–18278.

531 5. **Ettwig KF**, **Butler MK**, **Le Paslier D**, **Pelletier E**, **Mangenot S**, **Kuypers MMM**,
532   **Schreiber F**, **Dutilh BE**, **Zedelius J**, **de Beer D**, **Gloerich J**, **Wessels HJCT**, **van Alen T**,
533   **Luesken F**, **Wu ML**, **van de Pas-Schoonen KT**, **Op den Camp HJM**, **Janssen-Megens**
534   **EM**, **Francoijs K-J**, **Stunnenberg H**, **Weissenbach J**, **Jetten MSM**, **Strous M**. 2010.
535   Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. Nature **464**:543–8.

536 6. **Beal EJ**, **House CH**, **Orphan VJ**. 2009. Manganese- and iron-dependent marine methane
537   oxidation. Science **325**:184–7.

538 7. **Egger M**, **Rasigraf O**, **Sapart CJ**, **Jilbert T**, **Jetten MSM**, **Röckmann T**, **Van Der Veen**

24

539   C, **Bânda N**, **Kartal B**, **Ettwig KF**, **Slomp CP**. 2015. Iron-mediated anaerobic oxidation of

540   methane in brackish coastal sediments. Environ Sci Technol **49**:277–283.

541 8. **Ettwig KF**, **Zhu B**, **Speth D**, **Keltjens JT**, **Jetten MSM**, **Kartal B**. 2016. Archaea catalyze

542   iron-dependent anaerobic oxidation of methane. Proc Natl Acad Sci **113**:12792–12796.

543 9. **Walter Anthony KM**, **Macintyre S**. 2016. Nocturnal escape route for marsh gas. Nature

544   **535**:363–365.

545 10. **Bridgham SD**, **Cadillo-Quiroz H**, **Keller JK**, **Zhuang Q**. 2013. Methane emissions from

546   wetlands: biogeochemical, microbial, and modeling perspectives from local to global scales.

547   Glob Chang Biol **19**:1325–46.

548 11. **Megonigal JP**, **Hines ME**, **Visscher PT**. 2004. Anaerobic Metabolism:Linkages to Trace

549   Gases and Aerobic Processes. Biogeochemistry 317–424.

550 12. **Segarra KE a.**, **Schubotz F**, **Samarkin V**, **Yoshinaga MY**, **Hinrichs K-U**, **Joye SB**. 2015.

551   High rates of anaerobic methane oxidation in freshwater wetlands reduce potential

552   atmospheric methane emissions. Nat Commun **6**:7477.

553 13. **Segarra KEA**, **Comerford C**, **Slaughter J**, **Joye SB**. 2013. Impact of electron acceptor

554   availability on the anaerobic oxidation of methane in coastal freshwater and brackish

555   wetland sediments. Geochim Cosmochim Acta **115**:15–30.

556 14. **Martinez CM**, **Alvarez LH**, **Celis LB**, **Cervantes FJ**. 2013. Humus-reducing

557   microorganisms and their valuable contribution in environmental processes. Appl Microbiol

558   Biotechnol **97**:10293–308.

559 15. **Lehmann J**, **Kleber M**. 2015. The contentious nature of soil organic matter. Nature **528**:60–

560   8.

561   16.   **Blodau C**, **Deppe M**. 2012. Humic acid addition lowers methane release in peats of the Mer
562   Bleue bog, Canada. Soil Biol Biochem **52**:96–98.

563   17.   **Miller KE**, **Lai CT**, **Friedman ES**, **Angenent LT**, **Lipson DA**. 2015. Methane suppression
564   by iron and humic acids in soils of the Arctic Coastal Plain. Soil Biol Biochem **83**:176–183.

565   18.   **Lovley DR**, **Coates JD**, **Blunt-Harris EL**, **Phillips EJP**, **Woodward JC**. 1996. Humic
566   Substances as Electron acceptors for Microbial Respiration. Nature.

567   19.   **Scott DT**, **Mcknight DM**, **Blunt-Harris EL**, **Kolesar SE**, **Lovley DR**. 1998. Quinone
568   moieties act as electron acceptors in the reduction of humic substances by humics-reducing
569   microorganisms. Environ Sci Technol **32**:2984–2989.

570   20.   **Straub KL**, **Benz M**, **Schink B**. 2000. Iron metabolism in anoxic environments at near
571   neutral pH. FEMS Microbiol Ecol **34**:181–186.

572   21.   **Timmers PH**, **Suarez-Zuluaga DA**, **van Rossem M**, **Diender M**, **Stams AJ**, **Plugge CM**.
573   2015. Anaerobic oxidation of methane associated with sulfate reduction in a natural
574   freshwater gas source. ISME J **10**:1–13.

575   22.   **Saxton MA**, **Samarkin VA**, **Schutte CA**, **Bowles MW**, **Madigan MT**, **Cadieux SB**, **Pratt
576   LM**, **Joye SB**. 2016. Biogeochemical and 16S rRNA gene sequence evidence supports a
577   novel mode of anaerobic methanotrophy in permanently ice-covered Lake Fryxell,
578   Antarctica. Limnol Oceanogr **61**:S119–S130.

579   23.   **Alperin, M. J. RWS**. 1988. Carbon and hydrogen isotope fractionation resulting from
580   anaerobic methane oxidation. Biogeochem Cycles **2**:279–288.

581   24.   **Martens CS**, **Albert DB**, **Alperin MJ**. 1999. Stable isotope tracing of anaerobic methane
582   oxidation in the gassy sediments of Eckernforde Bay German Baltic Sea. Am J Sci **299**:
583   589–610.

584   25.   **Socrates G**. 2004. Infrared and Raman characteristic group frequenciesInfrared and Raman

585   characteristic group frequencies.

586   26.   **Orphan VJ**, **House CH**, **Hinrichs K-U**, **McKeegan KD**, **DeLong EF**. 2002. Multiple

587   archaeal groups mediate methane oxidation in anoxic cold seep sediments. Proc Natl Acad

588   Sci **99**:7663–7668.

589   27.   **Scheller S**, **Yu H**, **Chadwick GL**, **Mcglynn SE**. 2016. Artificial electron acceptors

590   decouple archaeal methane oxidation from sulfate reduction. Science (80-) **351**:703–707.

591   28.   **Xi B**, **Zhao X**, **He X**, **Huang C**, **Tan W**, **Gao R**, **Zhang H**, **Li D**. 2016. Successions and

592   diversity of humic-reducing microorganisms and their association with physical-chemical

593   parameters during composting. Bioresour Technol **219**:204–211.

594   29.   **Coates JD**, **Cole KA**, **Chakraborty R**, **Connor SMO**, **Achenbach LA**. 2002. Diversity

595   and ubiquity of bacteria capable of utilizing humic substances as electron donors for

596   anaerobic respiration diversity and ubiquity of bacteria capable of utilizing humic substances

597   as electron donors for anaerobic respiration. Appl Environ Microbiol **68**:2445–2452.

598   30.   **Lovley DR**, **Fraga JL**, **Blunt-Harris EL**, **Hayes LA**, **Phillips EJP**, **Coates JD**. 1998.

599   Humic substances as a mediator for microbially catalyzed metal reduction. Acta Hydrochim

600   Hydrobiol **26**:152–157.

601   31.   **Benz M**, **Schink B**, **Brune A**. 1998. Humic acid reduction by Propionibacterium

602   freudenreichii and other fermenting bacteria. Appl Environ Microbiol **64**:4507–4512.

603   32.   **Glass JB**, **Yu H**, **Steele J a**, **Dawson KS**, **Sun S**, **Chourey K**, **Pan C**, **Hettich RL**, **Orphan**

604   **VJ**. 2014. Geochemical, metagenomic and metaproteomic insights into trace metal

605   utilization by methane-oxidizing microbial consortia in sulphidic marine sediments. Environ

606   Microbiol **16**:1592–611.

607 33. **Knittel K**, **Lösekann T**, **Boetius A**, **Kort R**, **Amann R**, **Lo T**. 2005. Diversity and

608   Distribution of Methanotrophic Archaea at Cold Seeps Diversity and Distribution of

609   Methanotrophic Archaea at Cold Seeps †. Appl Environ Microbiol **71**:467–479.

610 34. **Schubert CJ**, **Vazquez F**, **L??sekann-Behrens T**, **Knittel K**, **Tonolla M**, **Boetius A**. 2011.

611   Evidence for anaerobic oxidation of methane in sediments of a freshwater system (Lago di

612   Cadagno). FEMS Microbiol Ecol **76**:26–38.

613 35. **Yoshinaga MY**, **Lazar CS**, **Elvert M**, **Lin YS**, **Zhu C**, **Heuer VB**, **Teske A**, **Hinrichs KU**.

614   2015. Possible roles of uncultured archaea in carbon cycling in methane-seep sediments.

615   Geochim Cosmochim Acta **164**:35–52.

616 36. **Biddle JF**, **Lipp JS**, **Lever MA**, **Lloyd KG**, **Sørensen KB**, **Anderson R**, **Fredricks HF**,

617   **Elvert M**, **Kelly TJ**, **Schrag DP**, **Sogin ML**, **Brenchley JE**, **Teske A**, **House CH**, **Hinrichs**

618   **K-U**. 2006. Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru.

619   Proc Natl Acad Sci U S A **103**:3846–3851.

620 37. **Evans PN**, **Parks DH**, **Chadwick GL**, **Robbins SJ**, **Orphan VJ**, **Golding SD**, **Tyson GW**.

621   2015. Methane metabolism in the archaeal phylum Bathyarchaeota revealed by genome-

622   centric metagenomics. Science **350**:434–8.

623 38. **Keller, Jason K. & Medvedeff C**, **A.** 2016. Soil organic matter, p. 165–188. *In* Wetland

624   soils: genesis, hydrology, landscapes, and classificationsVepraskas,. Taylor and Francis

625   Group.

626 39. **Pendleton L**, **Donato DC**, **Murray BC**, **Crooks S**, **Jenkins WA**, **Sifleet S**, **Craft C**,

627   **Fourqurean JW**, **Kauffman JB**, **Marbà N**, **Megonigal P**, **Pidgeon E**, **Herr D**, **Gordon D**,

628   **Baldera A**. 2012. Estimating Global "Blue Carbon" Emissions from Conversion and

629   Degradation of Vegetated Coastal Ecosystems. PLoS One **7**: e43542.

630   40.   **Gupta V**, **Smemo KA**, **Yavitt JB**, **Basiliko N**. 2012. Active Methanotrophs in Two

631   Contrasting North American Peatland Ecosystems Revealed Using DNA-SIP. Microb Ecol

632   **63**:438–445.

633   41.   **Smemo KA**, **Yavitt JB**. 2007. Evidence for Anaerobic CH $_4$ Oxidation in Freshwater

634   Peatlands. Geomicrobiol J **24**:583–597.

635   42.   **Smemo KA**, **Yavitt JB**. 2011. Anaerobic oxidation of methane: An underappreciated aspect

636   of methane cycling in peatland ecosystems? Biogeosciences **8**:779–793.

637   43.   **Klüpfel L**, **Piepenbrock A**, **Kappler A**, **Sander M**. 2014. Humic substances as fully

638   regenerable electron acceptors in recurrently anoxic environments. Nat Geosci **7**:195–200.

639   44.   **APHA/AWWA/WEF**. 2012. Standard Methods for the Examination of Water and

640   WastewaterStandard Methods.

641   45.   **Soga T**, **Ross GA**. 1999. Simultaneous determination of inorganic anions, organic acids and

642   metal cations by capillary electrophoresis. J Chromatogr A **834**:65–71.

643   46.   **Cord-Ruwisch R**. 1985. A quick method for the determination of dissolved and precipitated

644   sulfides in cultures of sulfate-reducing bacteria. J Microbiol Methods **4**:33–36.

645   47.   **Klindworth A**, **Pruesse E**, **Schweer T**, **Peplies J**, **Quast C**, **Horn M**, **Glöckner FO**. 2013.

646   Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-

647   generation sequencing-based diversity studies. Nucleic Acids Res **41**:1–11.

648   48.   **Schloss PD**, **Westcott SL**, **Ryabin T**, **Hall JR**, **Hartmann M**, **Hollister EB**, **Lesniewski**

649   **RA**, **Oakley BB**, **Parks DH**, **Robinson CJ**, **Sahl JW**, **Stres B**, **Thallinger GG**, **Van Horn**

650   **DJ**, **Weber CF**. 2009. Introducing mothur: Open-source, platform-independent, community-

651   supported software for describing and comparing microbial communities. Appl Environ

652   Microbiol **75**:7537–7541.

653

654 **FIGURE LEGENDS**

655 **Fig. 1. Anaerobic methane oxidation measured as $^{13}CO_2$ production in microcosms'**

656 **headspace and ^{13}C enrichment calculated as $^{13}F_{CO_2}$ ($^{13}CO_2/[^{13}CO_2 + ^{12}CO_2]$).** Panel a

657 displays the kinetics for incubations performed with unamended sediment. Panel b displays

658 the kinetics for incubations performed with sediment enriched with 2.5 g l^{-1} of external

659 NOM in the form of Pahokee Peat humic substances. Error bars represent the standard error

660 among replicates (n = 4, or 3*). SR-INH stands for sediment incubations performed with

661 molybdate (25 mM) in order to inhibit sulfate reduction. $^{13}CO_2$ production rates were based

662 on the maximum slope observed on linear regressions considering at least three sampling

663 points.

664

665

666 **Fig. 2. Production of $^{13}CO_2$ and reduction of intrinsic or added electron acceptors at**

667 **the end of the exponential phase (20 days of incubation) in the absence (Panels a and**

668 **b) and in the presence (Panels c and d) of external NOM as HS from Pahokee Peat.**

669 SR-INH stands for controls amended with sulfate-reduction inhibitor, sodium molybdate

670 (25 mM). Error bars represent the standard error among replicates. $^{13}CO_2$ produced was

671 measured as described for Fig.1. Quantification of sulfate and nitrate reduction imply

672 decrease on their concentration at this sampling time, whereas Fe(III) reduction was

673 quantified in terms of the ferrous iron produced. Reduction of NOM and HS was

674 determined by the ferrozine technique.

675

676 **Fig. 3. Spectroscopic evidence of the presence of quinone moieties and their reduction**

677 **in wetland sediment samples**. Panels a and b depict the Micro-ATR-FTIR representative

678 spectra taken from imaged areas generated after processing quinone functional groups

679 (1650-1620 cm^{-1}) of sediment samples before incubation in the absence and in the presence,

680 respectively, of external NOM in the form of Pahokee Peat HS. Panels c and e portray XPS

681 high resolution profiles of C1s, while d and f represent O1s signal. Panels c and d belong to

682 sediment samples prior incubation, while panels e and f correspond to sediment samples

683 after incubation with ^{13}C-methane. Regions and components were corrected at 284.8 eV for

684 the C-C adventitious carbon A; B and G components belong to C-O bond (~286.6 and ~532

685 eV), C and H correspond to C=O functional group (~288.9 and ~533.3 eV), D belongs to –

686 COOH (~289.6), E is typical of the presence of carbonate (~291) and F suggests the

687 occurrence of a metallic oxide (~530).

688

689 **Fig. 4. High performance Ultraviolet-Visible-Near Infrared spectra obtained from**

690 **liquid samples before and after incubation with ^{13}CH$_4$.** Panels a and c depict spectra

691 obtained before incubation with ^{13}CH$_4$, while panels b and d show spectra obtained after

692 incubation with ^{13}CH$_4$.

693

694 **Fig. 5. Archaeal composition in wetland sediment samples performing AOM.** Most

695 abundant archaeal genera detected, based on 16S rRNA amplicon gene libraries, on

696   selected experimental treatments shown in Fig. 1 at the end of the incubation period (30

697   days).

698   **Fig. 6. AOM with AQDS as electron acceptor by an enrichment derived from wetland**

699   **sediment.** Panel a: Kinetics of methane consumption linked to AQDS reduction (to

700   AH_2QDS) observed during the last 11 days of the entire enrichment process lasting 151

701   days: filled squares (-■-) represent microcosms including CH_4 as electron donor and AQDS

702   as electron acceptor (complete experiments, n=3), open squares (-□-) represent controls

703   without electron acceptor provided (without AQDS control, n=3), solid circles (-●-)

704   represent CH_4 free microcosms (endogenous controls, n=3), and crosses (-×-) represent heat

705   killed controls (sterile controls, n=2). Error bars represent the standard error among

706   replicates. Panels b and c depict microbial community changes at the end of the enrichment

707   (151 days of incubation) at the phylum level based on Illumina sequencing of 16S rRNA

708   V3-V4 regions. Fresh sediment composition was used as a reference.

709

710   **Figure 7. Schematic representation of methane generation and consumption by**

711   **wetland sediment biota.** While a fraction of NOM may serve as electron acceptor to

712   support AOM (NOM-AOM) and decouple sulfate-reduction dependent AOM (SR-AOM),

713   depending on its chemical properties, a labile fraction of NOM could also be degraded

714   following the methanogenesis pathway by a fermenting and methanogenic fraction of the

715   consortia. Equilibrium between these three phenomena must be tightly dependent on

716   thermodynamic conditions, concentration of chemical species, and composition of

717   microbial community. *Anaerobic methanotrophic archaea are considered in a broader

718   perspective than ANME clades from *Euryarchaeota* phylum

719

a. Sediment

b. Pahokee Peat HS

a

%Transmittance

100 90 80 70 60 50 40

3367

2522

2162 2035 1978 1800 1637

1406

1008

872

712 631

b

100 90 80 70 60 50 40

3712

3369 2937

2827

2360 2161 2032 1978 1627

1407

1168

1008

872

713 661

4500 4000 3500 3000 2500 2000 1500 1000 500

Wavelength (cm⁻¹)

IR absorption images at the 1650-1620 cm⁻¹ range:

Y Pixel
30 25 20 15 10 5
5 10 15 20 25 30

Y Pixel
30 25 20 15 10 5
5 10 15 20 25 30
X Pixel

HIGH ... LOW

c
E C B A

d
G H F

e
D B A

f
G

Intensity (a. u.)

296 292 288 284 280   540 536 532 528 524

Binding Energy (eV)

%Transmittance

Sediment

HS enriched sediment

a

1690

b

1660

c

1690

d

2500-2260

1690

2400   2200   2000   1800   1600

Wavelength (cm^{-1})

Taxonomy			Treatment			
Phylum	Family	Genus	^{13}C-methane	HS enriched	HS + ^{13}CH4	HS + ^{13}C-methane + SR-INH
Diapherotrites	Unknown_Family	*Candidatus_Iainarchaeum*	0.0	1.1	0.0	1.9
Euryarchaeota	*Halobacteriaceae*	*Halomarina*	0.1	1.4	0.0	0.2
	ANME 1-b	unclassified (ANME 1-b)	0.0	0.1	0.5	0.0
	MHLsu47-B8A	unclassified (MHLsu47-B8A)	4.8	2.6	0.1	4.9
	Methanomicrobiaceae	*Methanoculleus*	0.0	1.2	0.0	0.0
		Methanogenium	0.2	0.5	1.6	0.4
		Methanomicrobium	2.4	2.4	2.8	2.0
	SMS-sludge-7	unclassified (SMS-sludge-7)	1.1	2.1	0.2	1.2
	Methanosaetaceae	*Methanosaeta*	4.0	1.5	3.2	1.3
	Methanosarcinaceae	ANME-3	0.2	0.0	0.0	0.0
	Unknown_Family	unclassified (Fe-A-9)	0.5	0.2	0.0	3.5
	AMOS4A-452-E11	unclassified (AMOS4A-452-E11)	1.4	0.6	0.1	0.9
	Marine_Benthic_Group_D_and_DHVEG-1	unclassified (MBGD and DHVEG-1)	22.6	18.9	20.6	17.7
	Marine_Group_III	unclassified (MG_III)	3.5	1.6	4.9	1.4
Bathyarchaeota	unclassified	unclassified (*Bathyarchaeota*)	12.8	7.5	14.0	10.0
SM1K20	unclassified	unclassified (SM1K20)	0.7	0.4	1.6	0.6
Thaumarchaeota	unclassified	unclassified (Group_C3)	3.9	2.6	2.7	2.1
	unclassified	unclassified (MBGB)	6.6	5.6	11.9	4.9
	unclassified	unclassified (pMC2A209)	19.3	16.3	14.9	12.5
Woesearchaeota_(DHVEG-6)	unclassified	unclassified (DHVEG-6)	11.7	26.2	15.6	25.0
	Others	Others	4.3	7.0	5.4	9.4

LOW ———— HIGH
Abundance code