1   **Extracellular electron transfer may be an overlooked contribution to pelagic**

2   **respiration in humic-rich freshwater lakes**

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19 **ABSTRACT**

20 Humic lakes and ponds receive large amounts of terrestrial carbon and are important

21 components of the global carbon cycle, yet how their redox cycling influences the carbon

22 budget is not fully understood. Here we compared metagenomes obtained from a humic

23 bog and a clearwater eutrophic lake, and found a much larger number of genes that might

24 be involved in extracellular electron transfer (EET) for iron redox reactions and humic

25 substance (HS) reduction in the bog than in the clearwater lake, consistent with the much

26 higher iron and HS levels in the bog. These genes were particularly rich in the bog's

27 anoxic hypolimnion, and were found in diverse bacterial lineages, some of which are

28 relatives of known iron oxidizers or iron/HS reducers. We hypothesize that HS may be a

29 previously overlooked electron acceptor and EET-enabled redox cycling may be

30 important in pelagic respiration and greenhouse gas budget in humic-rich freshwater

31 lakes.

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34 **KEYWORDS**

35 Humic substances (HS); Iron (Fe); Humic lake; Extracellular electron transfer (EET);

36 Cyc2; Multiheme cytochrome *c* (MHC); Porin-cytochrome *c* complex (PCC); Redox

37 cycling

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41   **INTRODUCTION**

42   Inland lakes receive allochthonous carbon (C) fixed in their catchment areas, and

43   play an important role in the cycling of terrestrial C and affect global C budgets. Many

44   northern freshwater lakes are experiencing a "browning" process, and this trend may

45   continue with changes in precipitation patterns and atmospheric deposition chemistry (1-

46   3). A leading factor contributing to the brownification is the increasing inputs of

47   allochthonous dissolved organic C (DOC) (4). A major component of terrestrially derived

48   allochthonous DOC in freshwater is humic substances (HS), which are heterogeneous

49   mixtures of naturally occurring recalcitrant organic carbon derived from plant and animal

50   decay (5). Another factor contributing to surface water brownification is increasing iron

51   (Fe) inputs (6), which were positively correlated to the increasing organic C inputs (7, 8).

52   This correlation may be partly due to the complexation of Fe by organic matter, in

53   particular HS, as the complexation may increase Fe leaching from catchment soil and

54   maintain Fe in the water column instead of sedimentation within the receiving water body

55   (8, 9).

56   Understanding the roles of HS and Fe in freshwater lakes is critical to predict how

57   such ecosystems will respond to the browning process, and to more accurately dissect

58   overall lake "metabolism" (10, 11). Humic lakes feature intensively brown-colored

59   waters originating from high concentration in HS and Fe. As a redox-active element, Fe

60   plays a role in defining redox conditions and C cycling; yet the complex roles of HS have

61   not been fully recognized, despite its high concentrations in humic lakes. On one hand,

62   HS and the more labile low-molecular weight C derived from the photodegradation of HS

63   is an important C source and electron donor for heterotrophic respiration in humic lakes

64   (12, 13). On the other hand, HS has also been recognized as an electron acceptor through

65   the reduction of their quinone moieties (14, 15), and its electron accepting capacity

66   (EAC) is fully regenerable under recurrent anoxic conditions (16). However, most prior

67   research on the EAC of HS considered the impact on C-cycling in wetlands, sediments

68   and soils, rather than truly pelagic ecosystems (16-19). Recently, a study on a humic lake

69   showed that native organic matter with more oxidized quinone moieties and therefore

70   higher EAC favored freshwater bacterial growth and production under anoxic conditions,

71   and further suggested organic matter as an important electron acceptor in stratified lakes

72   with oxycline fluctuations (20). Despite this, the role of HS as an electron acceptor in

73   freshwater lakes has not been widely appreciated.

74   Theoretically, if HS is used to respire organic C, it has the potential to lower

75   methane emissions from lakes. The reduction potential distribution in HS suggests HS

76   reduction to be thermodynamically more favorable than methanogenesis in anoxic waters

77   (16). As the resulting competitive mitigation of methanogenesis was observed in peat

78   bogs and peat soils (21-23), a similar process is expected for pelagic respiration in lakes.

79   Therefore, we judge it timely to further explore the contribution of HS and Fe reduction

80   to pelagic respiration in freshwater lakes.

81   In humic lakes, light does not penetrate deep into the water column due to the

82   absorbance by HS and Fe. Therefore, humic lakes generally have a shallower

83   phototrophic (and therefore oxygenated) zone than clearwater lakes during stratification

84   (13), leaving a larger proportion of water column under anoxic conditions. Due to this

85   redox distribution and their high concentrations, HS and Fe may become important

86   terminal electron acceptors in humic lakes. Thus, here we present the hypothesis that HS

87   and Fe redox cycling is more significant in humic lakes than in clearwater lakes, and that

88   these redox processes may influence ecosystem-level C budgets (i.e. overall lake

89   metabolism). As a preliminary examination of this hypothesis, we studied two contrasting

90   temperate lakes, including a small humic lake, Trout Bog, in which the DOC is primarily

91   of terrestrial origin; and a large eutrophic clearwater lake, Lake Mendota, which has

92   much lower concentrations of HS and Fe than Trout Bog, with most of its DOC being

93   produced in-lake via photosynthesis. Detailed lake characteristics are listed in **Table S1**.

94   Three combined assemblies of time-series metagenome libraries previously obtained

95   from Lake Mendota epilimnion (ME), Trout Bog epilimnion (TE), and Trout Bog

96   hypolimnion (TH), respectively, and over 200 metagenome-assembled genomes (MAGs)

97   were recovered from these combined assemblies (21). We examined these metagenomes

98   and MAGs to identify genes involved in HS and Fe redox processes to compare their

99   distributions in the two contrasting lakes.

100   Due to the high molecular weight of HS and the poor solubility of Fe(III), these

101   electron acceptors are reduced extracellularly via a process called extracellular electron

102   transfer (EET). The reduced HS and Fe can be abiotically re-oxidized by oxygen under

103   oxic conditions. In addition, biological Fe(II) oxidation may also occur, which employs

104   EET due to the poor solubility of the reaction product, Fe(III). One form of

105   oxidoreductase in Fe redox EET processes involves outer-surface proteins, such as Cyc2,

106   a monoheme cytochrome *c* typically found in Fe(II) oxidizers (22-24), and multiheme *c*-

107   type cytochromes (MHCs) in Fe(III) reducers (25, 26). Another form of EET

108   oxidoreductase forms a "porin-cytochrome *c* protein complex" (PCC) (27), in which the

109   oxidoreductase, usually a MHC, is secreted to the periplasm and embedded into a porin

110   on the outer membrane to form the EET conduit. Most Fe(III) reducers can also reduce

111   HS (28, 29), and probably use the same EET systems to transfer electrons to HS. For

112   example, in *Geobacter sulfurreducens*, a number of outer membrane MHCs that are

113   important in the reduction of Fe(III) are able to reduce extracellular AQDS and HS (30),

114   and in *Shewanella oneidensis*, the porin and periplasmic MHC components of its Fe(III)-

115   reducing PCC are essential for AQDS and HS reduction (31, 32). These findings suggest

116   that reduction of the quinone moieties in HS is a non-specific redox process by EET

117   systems.

118   In this study, we searched for putative EET genes (including PCC, outer surface

119   MHCs not associated with PCC, and Cyc2) in MAGs and metagenomes to examine if

120   these genes are indeed more abundant in the humic bog than in the clearwater lake.

121   Method details on the identification and quantification of putative EET genes were

122   described in Supplemental Methods. All (meta)genome data are publicly available at the

123   Integrated Microbial Genomes & Microbiomes (IMG/M, https://img.jgi.doe.gov/m). The

124   IMG IDs for the ME, TE, and TH metagenomes are 3300002835, 3300000439, and

125   3300000553, respectively, and the IMG IDs for putative EET gene-containing MAGs

126   (together with details on these MAGs and putative EET genes in the three metagenomes)

127   are listed in **Tables S2** and **S3**.

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129

130   **RESULTS**

131   MHCs are important components of EET systems involved in Fe redox reactions

132   and HS reduction. In particular, MHC with large numbers of hemes may be able to form

133   molecular "wires" for conducting electrons from the periplasmic space across the outer

134   membrane (33, 34). We therefore estimated the normalized abundance of MHCs with at

135   least five heme-binding sites in the metagenomes. In general, TH had the highest

136   abundance of MHCs, followed by TE and ME, and such differences were even more

137   pronounced for MHCs with at least eight heme-binding sites (**Fig. 1A**). Some of these

138   MHCs are components of other redox enzyme complexes, such as the pentaheme and

139   hexaheme MHCs in alternative complex III (ACIII), and octaheme MHCs in tetrathionate

140   reductases and hydroxylamine oxidoreductases. Putative EET MHC components (i.e.

141   MHCs in PCC and outer surface MHCs not associated with PCC, as listed in **Table S2**)

142   were much more frequently found in MHCs with large heme binding sites (e.g. >9), and

143   these putative EET genes were more abundant in TH than TE, and nearly absent in the

144   ME metagenome (**Fig. 1B**). This may indicate that MHC-based EET potential was more

145   significant in the anoxic layer than in the oxic layer of the humic bog, and was minimal in

146   the oxic layer of the clearwater lake with low Fe and HS concentrations. Notably, the

147   largest number of heme-binding sites (i.e. 51) was found in an MHC component of a

148   putative PCC, encoded in an un-binned contig in the TE metagenome (**Table S2**).

149

150   **Porin-cytochrome *c* protein complex (PCC) genes**

151   The best studied PCC system, MtrABC (consisting of a porin, a periplasmic

152   decaheme Cyt *c*, and an extracellular decaheme Cyt *c*), was first identified in *S.*

153   *oneidensis* as being essential for Fe(III) reduction (35). Their homologous PCCs, PioAB

154   and MtoAB, which lack the extracellular MHC component, were suggested to be

155   involved in Fe(II) oxidation in the phototrophic *Rhodopseudomonas palustris* TIE-1 (36)

156   and the microaerophilic Fe(II) oxidizers in the family of *Gallionellaceae* (37-39),

157   respectively. The more recently discovered PCC proteins in *G. sulfurreducens* are not

158   homologous to MtrABC, but are also encoded in operons with genes encoding a porin

159   (OmbB), a periplasmic octaheme Cyt *c* (OmaB), and an outer-membrane dodecaheme

160   Cyt *c* (OmcB) (40). This suggests that multiple PCC systems evolved independently, and

161   may provide a clue to search for new types of PCC by examining genome-level

162   organization. For example, putative novel PCC genes not homologous to previously

163   identified PCCs were found in some Fe(II) oxidizer genomes by searching for the unique

164   genetic organization of porin- and periplasmic MHC-coding genes (41).

165   Nearly all MtrAB/MtoAB/PioAB homologs were recovered in Trout Bog, and

166   mostly from TH (Table S2). They are present in MAGs affiliated with the Proteobacteria,

167   including the Fe(II)-oxidizing *Gallionella* and *Ferrovum*, Fe(III)-reducing *Albidiferax*,

168   Fe(III)- and AQDS-reducing *Desulfobulbus* and genera not known for EET, such as

169   *Polynucleobacter*, *Desulfocapsa*, and *Methylobacter* (**Fig. 2**). Interestingly, among the 46

170   *Polynucleobacter* genomes available at IMG/M (https://img.jgi.doe.gov/m),

171   MtrAB/MtoAB/PioAB homologs were only found in *Polynucleobacter* recovered from a

172   wetland and two humic lakes (including Trout Bog and Lake Grosse Fuchskuhle located

173   in Brandenburg, Germany), suggesting that this PCC might be an acquired trait of some

174   *Polynucleobacter* spp. adapting to humic-rich environments.

175   Homologs of another studied PCC (represented by OmbB-OmaB-OmcB in

176   *Geobacter* spp.) were present in MAGs affiliated with relatives of known Fe(III) (and

177   HS) reducers, including *Geothrix*, *Ignavibacteriaceae* and *Geobacteraceae*, as well as in

178   *Methylobacter* (**Fig. 2**).

179   Based on the unique genetic organization of PCC-encoding genes, we found a

180   number of putative PCC that do not share a significant sequence homology with known

181   PCCs, probably representing novel PCC types. These putative PCC genes were present in

182   Fe(III) (and HS) reducers (*Geothrix*, *Albidiferax*, and *Geobacteraceae*) and bacteria not

183   known for EET, including *Methylotenera*, *Methylobacter*, *Methyloversatilis*, and a

184   number of Bacteroidetes and Verrucomicrobia (**Fig. 2**). Among them, Verrucomicrobia

185   with putative PCC genes were previously found in humic-rich environments, such as

186   soils and lake sediment, in addition to the Verrucomicrobia MAGs from Trout Bog (42).

187

188   **Outer surface MHCs not associated with PCC**

189   Outer surface MHCs that are not PCC components may also be involved in EET.

190   Examples include the OmcE, OmcS, and OmcZ in *G. sulfurreducens* (25, 26), outer

191   surface MHCs in Gram-positive Fe(III)- and AQDS-reducing Firmicutes (43), and MHCs

192   in deltaproteobacterial sulfate-reducing bacteria that may be responsible for EET with its

193   anaerobic CH_4-oxidizing archaeal syntrophic partner (44).

194   Here, we found a number of non-PCC-associated outer surface MHCs in the

195   metagenomes (**Table S2**) and MAGs, including Fe(III)- (and HS-) reducing taxa

196   (*Albidiferax*, *Geothrix*, *Desulfobulbus*, *Ignavibacteriaceae* and *Geobacteraceae*), and

197   several members in the Bacteroidetes and Verrucomicrobia phyla (**Fig. 2**). In particular,

198   seven genes predicted to encode MHCs located on the cell wall were found in a Gram-

199   positive actinobacterial MAG classified to *Solirubrobacterales* from TH, and four of

200   these genes are located in the same gene cluster with up to 15 heme-binding sites in a

201   single MHC (**Table S2**), probably involved in electron transfer on the cell wall.

202

Cytochrome Cyc2

204   Cyc2 is an outer membrane *c*-type cytochrome with one heme-binding motif in

205   the N-terminus and a predicted porin structure at the C-terminus, and was therefore

206   proposed as a fused PCC (45). Cyc2 was originally identified as the Fe(II) oxidase in

207   acidiphilic *Acidithiobacillus ferrooxidans* (23) with distant homologs later found in

208   neutrophilic microaerobic *Mariprofundus* spp. (22) and some other neutrophilic Fe(II)

209   oxidizers (24, 41).

210   Similar to EET MHC genes, the normalized abundance of total Cyc2-like genes

211   was much higher in the TH than in the TE metagenome, and Cyc2-like genes were

212   largely absent in the ME metagenome (**Fig. 1C**). Cyc2 homologs were present in 29

213   MAGs exclusively from Trout Bog (**Table S3**), including relatives of Fe(II)-oxidizing

214   genera (*Ferrovum* and *Gallionella*) and Fe(III)- reducing taxa (*Ignavibacteriaceae* and

215   *Albidiferax*), as well as bacteria not known for EET, including *Methylotenera*,

216   *Methylobacter*, *Pelodictyon*, and members in Bacteroidetes and Verrucomicrobia (**Fig.**

217   **2**).

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DISCUSSION

221   With the ongoing brownification of surface water due to increasing inputs of

222   terrestrial C and Fe on a large scale, elucidating the roles and contribution of HS and Fe

223   in redox and C cycling becomes even more relevant to C budgets at an ecosystem level.

224   Here we inspected EET genes/organisms involved in HS and Fe redox processes in two

225   freshwater lakes with contrasting HS and Fe levels to examine if these genes/organisms

226   were more abundant in the humic lake, particularly in its anoxic layer. All together, a

227   total of 103, 36, and 66 MAGs were recovered from the ME, TE, and TH metagenomes,

228   respectively. Among them, putative EET genes were found in 7, 12 and 31 MAGs from

229   ME, TE and TH, respectively (**Fig. 2**). Therefore, a larger fraction of MAGs might

230   encode EET function in Trout Bog, especially in its hypolimnion, than in Mendota. This,

231   together with the normalized abundance of putative EET genes in the three metagenomes

232   (**Fig. 1**), suggests that the genetic potential of EET was more significant in the anoxic

233   layer than in the oxic layer of the humic bog, and was the lowest in the oxic layer of the

234   clearwater lake. This distribution pattern is consistent with the availability of the

235   thermodynamically more favorable electron acceptor, i.e. oxygen, between the two layers

236   and the much higher concentrations of HS and Fe in the bog than in the clearwater lake.

237   It was not surprising to find putative EET genes in relatives of bacteria that are

238   known to be capable of Fe redox reactions and HS reduction in anoxic lake waters.

239   However, finding putative EET genes in taxa not known for EET functions is intriguing.

240   Like many known EET organisms, some of these bacteria (e.g. Bacteroidetes and

241   Verrucomicrobia) contain multiple sets of putative EET genes. In particular, some

242   *Methylotenera* and *Methylobacter* contain both Cyc2 and putative PCC genes. If these

243   methylotrophs are indeed capable of EET, this may enable insoluble or high-molecular

244   weight substrates, such as Fe(III) and HS, to be used as an electron acceptor to oxidize

245   the methyl-group in methanol and methylamine. Such EET processes, if they occur,

246   could allow methylotrophs to survive in the anoxic layer, and this agrees with the

247   recovery of *Methylotenera* and *Methylobacter* MAGs in the largely anoxic hypolimnion

248   of Trout Bog.

249   HS have until now usually been more regarded as an electron donor and C source

250   in freshwater lakes, and not as an electron acceptor. However, evidence for the role as an

251   electron acceptor was recently documented in another peat bog lake (20). In the current

252   study, we measured the electron accepting capacity (EAC) of HS in the epilimnion and

253   hypolimnion water of Trout Bog according to Kappler et al. (17), and their EAC was

254   0.115 and 0.128 mM, respectively (See Supplemental Methods for the determination of

255   lake water EAC). Notably, these values are an order of magnitude higher than the EAC of

256   Fe (~0.01 mM) in Trout Bog. Therefore, HS may be a significant, but previously

257   overlooked source of electron acceptors in this bog system.

258   Due to its high EAC and concentration, HS may play an important role in the

259   redox cycling in Trout Bog. On one hand, HS facilitates Fe redox reactions by shuttling

260   electrons from Fe(III)-reducers to Fe(III) in heterotrophic respiration (46). On the other

261   hand, HS may be directly used as an electron acceptor to respire the more labile organic

262   C (**Fig. 3**). The anaerobic respiration of organic C with Fe(III) and HS are both

263   thermodynamically more favorable than methanogenesis, therefore promoting the

264   transformation of organic C towards CO_2, not CH_4. This may lower the overall global

265   warming potential of greenhouse gas emissions from humic lakes, as CH_4 is a much more

266   potent greenhouse gas than CO_2. Because of lake seasonal mixing and more frequent

267   micro-mixing, such as wind-driven turbulence and convectively derived diurnal oxycline

268   fluctuations (20, 47), reduced HS and Fe can be re-oxidized through mixing-introduced

269   oxygenation to regenerate their EAC, which makes these anaerobic respiration processes

270   sustainable in the anoxic layer (**Fig. 3**). In these redox processes, oxygen is the ultimate

271   electron acceptor, and Fe and HS "recharge" the EAC with oxygen for subsequent use

272   when oxygen becomes unavailable in stratified hypolimnia. Hypothetically, such

273   recharging process would increase the effective EAC of humic water and shunt more

274   organic C to anaerobic respiration. Therefore, we hypothesize that HS may be a

275   previously overlooked electron acceptor and EET may be an important contribution to

276   pelagic respiration in humic-rich freshwater lakes. Coupled with C metabolism, EET-

277   enabled HS and Fe redox dynamics can significantly influence C cycling and greenhouse

278   gas emission in humic lakes that experience recurrent oxic-anoxic conditions. The

279   overrepresentation of EET genes/organisms potentially involved in HS and Fe redox

280   processes in the humic lake strongly support this hypothesis, given that the energetic

281   advantage such organisms can obtain stays marginal when powerful recharge

282   mechanisms at the oxic/anoxic interface are lacking. Yet further combined

283   biogeochemical, hydrodynamic, genomic and transcriptomic studies are required to test

284   our hypothesis and reveal organisms and genes actually involved *in-situ*.

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287   **CONFLICT OF INTEREST**

288   The authors declare no conflict of interest.

289

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339   **REFERENCE**
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341   1.   **Roulet N, Moore TR**. 2006. Browning the waters. Nature **444**:283.
342   2.   **Monteith DT, Stoddard JL, Evans CD, de Wit HA, Forsius M, Høgåsen T,**
343   **Wilander A, Skjelkvåle BL, Jeffries DS, Vuorenmaa J, Keller B, Kopácek J,**
344   **Vesely J**. 2007. Dissolved organic carbon trends resulting from changes in
345   atmospheric deposition chemistry. Nature **450**:537.
346   3.   **Tranvik LJ, Cole JJ, Prairie YT**. 2018. The study of carbon in inland waters—
347   from isolated ecosystems to players in the global carbon cycle. Limnology and
348   Oceanography Letters **3**:41-48.
349   4.   **Freeman C, Evans CD, Monteith DT, Reynolds B, Fenner N**. 2001. Export of
350   organic carbon from peat soils. Nature **412**:785-785.
351   5.   **MacCarthy P, Malcolm R, Clapp C, Bloom P**. 1990. An introduction to soil
352   humic substances. Humic Substances in Soil and Crop Sciences: Selected
353   Readings:1-12.
354   6.   **Kritzberg E, Ekström S**. 2012. Increasing iron concentrations in surface waters-
355   a factor behind brownification? Biogeosciences **9**:1465.
356   7.   **Neal C, Lofts S, Evans C, Reynolds B, Tipping E, Neal M**. 2008. Increasing
357   iron concentrations in UK upland waters. Aquatic Geochemistry **14**:263-288.
358   8.   **Sarkkola S, Nieminen M, Koivusalo H, Lauren A, Kortelainen P, Mattsson**
359   **T, Palviainen M, Piirainen S, Starr M, Finer L**. 2013. Iron concentrations are
360   increasing in surface waters from forested headwater catchments in eastern
361   Finland. Sci Total Environ **463-464**:683-9.
362   9.   **Maranger R, Canham CD, Pace ML, Papaik MJ**. 2006. A spatially explicit
363   model of iron loading to lakes. Limnology and Oceanography **51**:247-256.
364   10.   **Hanson PC, Pace ML, Carpenter SR, Cole JJ, Stanley EH**. 2015. Integrating
365   landscape carbon cycling: research needs for resolving organic carbon budgets of
366   lakes. Ecosystems **18**:363-375.

367   11.   **Hotchkiss E, Sadro S, Hanson P**. 2018. Toward a more integrative perspective
368   on carbon metabolism across lentic and lotic inland waters. Limnology and
369   Oceanography Letters **3**:57-63.
370   12.   **Bertilsson S, Stefan LJ**. 1998. Photochemically produced carboxylic acids as
371   substrates for freshwater bacterioplankton>. Limnology and Oceanography
372   **43**:885-895.
373   13.   **Houser JN, Bade DL, Cole JJ, Pace ML**. 2003. The dual influences of dissolved
374   organic carbon on hypolimnetic metabolism: organic substrate and photosynthetic
375   reduction. Biogeochemistry **64**:247-269.
376   14.   **Scott DT, McKnight DM, Blunt-Harris EL, Kolesar SE, Lovley DR**. 1998.
377   Quinone Moieties Act as Electron Acceptors in the Reduction of Humic
378   Substances by Humics-Reducing Microorganisms. Environmental Science &
379   Technology **32**:2984-2989.
380   15.   **Lovley DR, Coates JD, Blunt-Harris EL, Phillips EJP, Woodward JC**. 1996.
381   Humic substances as electron acceptors for microbial respiration. Nature **382**:445.
382   16.   **Klupfel L, Piepenbrock A, Kappler A, Sander M**. 2014. Humic substances as
383   fully regenerable electron acceptors in recurrently anoxic environments. Nature
384   Geosci **7**:195-200.
385   17.   **Kappler A, Benz M, Schink B, Brune A**. 2004. Electron shuttling via humic
386   acids in microbial iron(III) reduction in a freshwater sediment. FEMS Microbiol
387   Ecol **47**:85-92.
388   18.   **Lau MP, Sander M, Gelbrecht J, Hupfer M**. 2016. Spatiotemporal redox
389   dynamics in a freshwater lake sediment under alternating oxygen availabilities:
390   combined analyses of dissolved and particulate electron acceptors. Environmental
391   Chemistry **13**:826-837.
392   19.   **Roden EE, Kappler A, Bauer I, Jiang J, Paul A, Stoesser R, Konishi H, Xu
393   H**. 2010. Extracellular electron transfer through microbial reduction of solid-
394   phase humic substances. Nature geoscience **3**:417-421.
395   20.   **Lau MP, Hupfer M, Grossart HP**. 2017. Reduction-oxidation cycles of organic
396   matter increase bacterial activity in the pelagic oxycline. Environ Microbiol Rep
397   **9**:257-267.
398   21.   **Bendall ML, Stevens SLR, Chan L-K, Malfatti S, Schwientek P, Tremblay J,
399   Schackwitz W, Martin J, Pati A, Bushnell B, Froula J, Kang D, Tringe SG,
400   Bertilsson S, Moran MA, Shade A, Newton RJ, McMahon KD, Malmstrom
401   RR**. 2016. Genome-wide selective sweeps and gene-specific sweeps in natural
402   bacterial populations. ISME J doi:10.1038/ismej.2015.241.
403   22.   **Barco RA, Emerson D, Sylvan JB, Orcutt BN, Jacobson Meyers ME,
404   Ramirez GA, Zhong JD, Edwards KJ**. 2015. New insight into microbial iron
405   oxidation as revealed by the proteomic profile of an obligate iron-oxidizing
406   chemolithoautotroph. Appl Environ Microbiol **81**:5927-37.
407   23.   **Castelle C, Guiral M, Malarte G, Ledgham F, Leroy G, Brugna M, Giudici-
408   Orticoni MT**. 2008. A new iron-oxidizing/O_2-reducing supercomplex spanning
409   both inner and outer membranes, isolated from the extreme acidophile
410   *Acidithiobacillus ferrooxidans*. J Biol Chem **283**:25803-11.

24. **Kato S, Ohkuma M, Powell DH, Krepski ST, Oshima K, Hattori M, Shapiro N, Woyke T, Chan CS**. 2015. Comparative genomic insights into ecophysiology of neutrophilic, microaerophilic iron oxidizing bacteria. Front Microbiol **6**:1265.

25. **Leang C, Coppi MV, Lovley DR**. 2003. OmcB, a c-type polyheme cytochrome, involved in Fe(III) reduction in Geobacter sulfurreducens. J Bacteriol **185**:2096-103.

26. **Mehta T, Coppi MV, Childers SE, Lovley DR**. 2005. Outer membrane c-type cytochromes required for Fe(III) and Mn(IV) oxide reduction in *Geobacter sulfurreducens*. Appl Environ Microbiol **71**:8634-41.

27. **Richardson DJ, Butt JN, Fredrickson JK, Zachara JM, Shi L, Edwards MJ, White G, Baiden N, Gates AJ, Marritt SJ, Clarke TA**. 2012. The 'porin-cytochrome' model for microbe-to-mineral electron transfer. Mol Microbiol **85**:201-12.

28. **Piepenbrock A, Behrens S, Kappler A**. 2014. Comparison of Humic Substance- and Fe(III)-Reducing Microbial Communities in Anoxic Aquifers. Geomicrobiology Journal **31**:917-928.

29. **Coates JD, Ellis DJ, Blunt-Harris EL, Gaw CV, Roden EE, Lovley DR**. 1998. Recovery of Humic-Reducing Bacteria from a Diversity of Environments. Applied and Environmental Microbiology **64**:1504-1509.

30. **Voordeckers JW, Kim BC, Izallalen M, Lovley DR**. 2010. Role of Geobacter sulfurreducens outer surface c-type cytochromes in reduction of soil humic acid and anthraquinone-2,6-disulfonate. Appl Environ Microbiol **76**:2371-5.

31. **Bucking C, Piepenbrock A, Kappler A, Gescher J**. 2012. Outer-membrane cytochrome-independent reduction of extracellular electron acceptors in Shewanella oneidensis. Microbiology **158**:2144-57.

32. **Shyu JB, Lies DP, Newman DK**. 2002. Protective role of tolC in efflux of the electron shuttle anthraquinone-2,6-disulfonate. J Bacteriol **184**:1806-10.

33. **Clarke TA, Edwards MJ, Gates AJ, Hall A, White GF, Bradley J, Reardon CL, Shi L, Beliaev AS, Marshall MJ, Wang Z, Watmough NJ, Fredrickson JK, Zachara JM, Butt JN, Richardson DJ**. 2011. Structure of a bacterial cell surface decaheme electron conduit. Proc Natl Acad Sci U S A **108**:9384-9.

34. **Bewley KD, Ellis KE, Firer-Sherwood MA, Elliott SJ**. 2013. Multi-heme proteins: Nature's electronic multi-purpose tool. Biochimica et Biophysica Acta (BBA) - Bioenergetics **1827**:938-948.

35. **Beliaev AS, Saffarini DA**. 1998. *Shewanella putrefaciens mtrB* encodes an outer membrane protein required for Fe(III) and Mn(IV) reduction. J Bacteriol **180**:6292-7.

36. **Jiao Y, Newman DK**. 2007. The *pio* operon is essential for phototrophic Fe(II) oxidation in *Rhodopseudomonas palustris* TIE-1. J Bacteriol **189**:1765-1773.

37. **Liu J, Wang Z, Belchik SM, Edwards MJ, Liu C, Kennedy DW, Merkley ED, Lipton MS, Butt JN, Richardson DJ, Zachara JM, Fredrickson JK, Rosso KM, Shi L**. 2012. Identification and characterization of MtoA: a decaheme c-type cytochrome of the neutrophilic Fe(II)-oxidizing bacterium *Sideroxydans lithotrophicus* ES-1. Front Microbiol **3**:37.

455 38. **Shi L, Rosso KM, Zachara JM, Fredrickson JK**. 2012. Mtr extracellular
456   electron-transfer pathways in Fe(III)-reducing or Fe(II)-oxidizing bacteria: a
457   genomic perspective. Biochem Soc Trans **40**:1261-7.
458 39. **Emerson D, Field EK, Chertkov O, Davenport KW, Goodwin L, Munk C,**
459   **Nolan M, Woyke T**. 2013. Comparative genomics of freshwater Fe-oxidizing
460   bacteria: implications for physiology, ecology, and systematics. Front Microbiol
461   **4**:254.
462 40. **Liu Y, Wang Z, Liu J, Levar C, Edwards MJ, Babauta JT, Kennedy DW, Shi**
463   **Z, Beyenal H, Bond DR, Clarke TA, Butt JN, Richardson DJ, Rosso KM,**
464   **Zachara JM, Fredrickson JK, Shi L**. 2014. A trans-outer membrane porin-
465   cytochrome protein complex for extracellular electron transfer by *Geobacter*
466   *sulfurreducens* PCA. Environ Microbiol Rep **6**:776-85.
467 41. **He S, Barco RA, Emerson D, Roden EE**. 2017. Comparative Genomic Analysis
468   of Neutrophilic Iron(II) Oxidizer Genomes for Candidate Genes in Extracellular
469   Electron Transfer. Frontiers in Microbiology **8**.
470 42. **He S, Stevens SLR, Chan L-K, Bertilsson S, Glavina del Rio T, Tringe SG,**
471   **Malmstrom RR, McMahon KD**. 2017. Ecophysiology of Freshwater
472   Verrucomicrobia Inferred from Metagenome-Assembled Genomes. mSphere **2**.
473 43. **Carlson HK, Iavarone AT, Gorur A, Yeo BS, Tran R, Melnyk RA, Mathies**
474   **RA, Auer M, Coates JD**. 2012. Surface multiheme c-type cytochromes from
475   Thermincola potens and implications for respiratory metal reduction by Gram-
476   positive bacteria. Proceedings of the National Academy of Sciences **109**:1702-
477   1707.
478 44. **Skennerton CT, Chourey K, Iyer R, Hettich RL, Tyson GW, Orphan VJ**.
479   2017. Methane-Fueled Syntrophy through Extracellular Electron Transfer:
480   Uncovering the Genomic Traits Conserved within Diverse Bacterial Partners of
481   Anaerobic Methanotrophic Archaea. mBio **8**.
482 45. **White GF, Edwards MJ, Gomez-Perez L, Richardson DJ, Butt JN, Clarke**
483   **TA**. 2016. Mechanisms of Bacterial Extracellular Electron Exchange. Adv
484   Microb Physiol **68**:87-138.
485 46. **Lovley DR, Fraga JL, Blunt-Harris EL, Hayes LA, Phillips EJP, Coates JD**.
486   1998. Humic Substances as a Mediator for Microbially Catalyzed Metal
487   Reduction. Acta hydrochimica et hydrobiologica **26**:152-157.
488 47. **Read JS, Hamilton DP, Desai AR, Rose KC, MacIntyre S, Lenters JD, Smyth**
489   **RL, Hanson PC, Cole JJ, Staehr PA, Rusak JA, Pierson DC, Brookes JD,**
490   **Laas A, Wu CH**. 2012. Lake-size dependency of wind shear and convection as
491   controls on gas exchange. Geophysical Research Letters **39**:n/a-n/a.
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497   **FIGURES**

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(a)   (b)   (c)
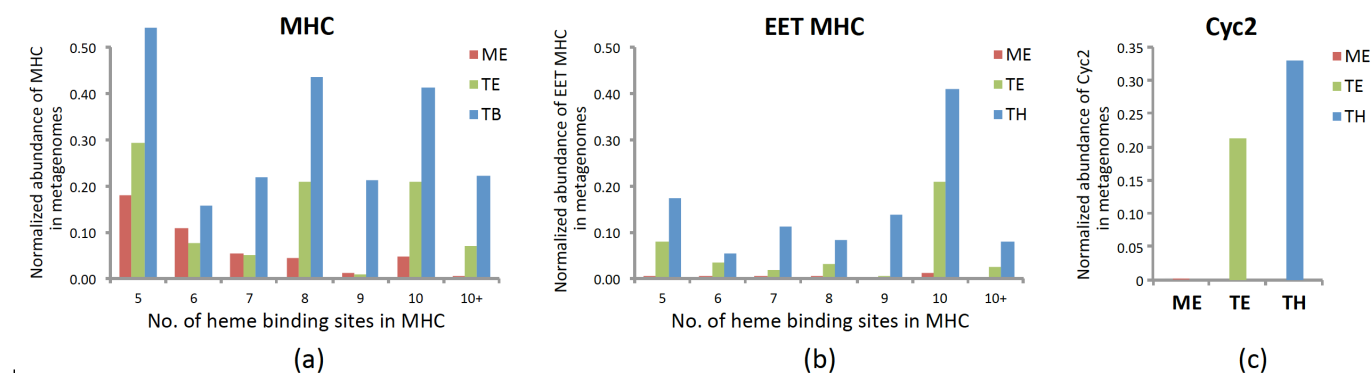
501   **Fig. 1.** Normalized abundance of multiheme *c*-type cytochromes (MHCs) (a), MHCs with

502   putative EET functions (i.e. MHCs in PCC and outer surface MHCs not associated with

503   PCC) (b), and Cytochrome Cyc2 homologs (c) found in metagenomes obtained from

504   Lake Mendota's epilimnion (ME), and Trout Bog's epilimnion (TE) and hypolimnion

505   (TH), respectively. In (a) and (b), normalized abundance was reported for MHCs with 5

506   to 10, and > 10 heme binding sites respectively. The normalized abundance was obtained

507   by mapping metagenome reads to assembled contigs and the read coverage was then

508   normalized by the average read coverage of single-copy conserved bacterial

509   housekeeping genes in the same metagenome. See Supplemental Methods for details on

510   the calculation of normalized abundance.

	MAG	Phylum	Order	Family	Genus	MtrAB/MtoAB/PioAB	ombB-omaB-omcB	Other putative PCC	Outer surface MHC	Cyc2	Average of normalized abundance
ME	ME6031	Bacteroidetes	*Sphingobacteriales*	*Saprospiraceae*					+		0.0003
	ME9495	Bacteroidetes							+		0.0008
	ME12555	Planctomycetes						+			0.0006
	ME28789	Proteobacteria	*Rhodocyclales*	*Rhodocyclaceae*	*Methyloversatilis*			+			0.0006
	ME3293	Proteobacteria	*Methylococcales*	*Methylococcaceae*	*Methylobacter*		+				0.0027
	ME9104	Proteobacteria	*Xanthomonadales*					+			0.0016
	ME17476	Unclassified							+		0.0007
TE	* TE911	Acidobacteria	*Holophagales*	*Holophagaceae*	*Geothrix*		+	+			0.0235
	TE8052	Bacteroidetes	*Sphingobacteriales*					+			0.0035
	TE211	Chlorobi	*Chlorobiales*	*Chlorobiaceae*	*Pelodictyon*					+	0.0681
	TE2493	Chlorobi	*Chlorobiales*	*Chlorobiaceae*	*Pelodictyon*					+	0.0126
	* TE4548	Proteobacteria	*Burkholderiales*	*Comamonadaceae*	*Albidiferax*			+			0.0049
	** TE439	Proteobacteria	*Ferrovales*	*Ferrovaceae*	*Ferrovum*	+				+	0.0296
	TE2167	Proteobacteria	*Methylophilales*	*Methylophilaceae*	*Methylotenera*			+		+	0.0040
	TE5136	Proteobacteria	*Methylococcales*	*Methylococcaceae*	*Methylobacter*					+	0.0037
	TE8031	Proteobacteria	*Methylococcales*	*Methylococcaceae*	*Methylobacter*					+	0.0037
	TE2668	Proteobacteria								+	0.0084
	TE1800	Verrucomicrobia	*Opitutales*	Subdivision 4				+			0.0079
	TE4605	Verrucomicrobia	*Verrucomicrobiales*	Subdivision 1						+	0.0047
TH	* TH254	Acidobacteria	*Holophagales*	*Holophagaceae*	*Geothrix*		+	+	+		0.0430
	* TH3273	Acidobacteria	*Holophagales*	*Holophagaceae*	*Geothrix*		+	+			0.0047
	TH4565	Acidobacteria	*Holophagales*				+	+		+	0.0027
	TH3815	Actinobacteria	*Solirubrobacterales*						+		0.0038
	TH4848	Bacteroidetes	*Bacteroidales*	*Porphyromonadaceae*	*Paludibacter*			+	+		0.0015
	TH3311	Bacteroidetes	*Bacteroidales*	*Porphyromonadaceae*				+	+		0.0047
	TH2086	Bacteroidetes	*Bacteroidales*					+	+		0.0047
	TH6286	Bacteroidetes	*Bacteroidales*					+	+		0.0021
	TH4697	Bacteroidetes	*Flavobacteriales*	*Flavobacteriaceae*	*Flavobacterium*				+		0.0014
	TH111	Chlorobi	*Chlorobiales*	*Chlorobiaceae*	*Pelodictyon*					+	0.1162
	TH3520	Chlorobi	*Chlorobiales*	*Chlorobiaceae*	*Pelodictyon*					+	0.0053
	* TH5629	Ignavibacteria	*Ignavibacteriales*	*Ignavibacteriaceae*			+		+	+	0.0024
	TH5354	Ignavibacteria					+	+			0.0021
	TH6717	Proteobacteria	*Rhizobiales*							+	0.0019
	TH941	Proteobacteria	*Burkholderiales*	*Burkholderiaceae*	*Polynucleobacter*	+					0.0134
	* TH1542	Proteobacteria	*Burkholderiales*	*Comamonadaceae*	*Albidiferax*			+	+	+	0.0097
	* TH2159	Proteobacteria	*Burkholderiales*	*Comamonadaceae*	*Albidiferax*					+	0.0053
	* TH2160	Proteobacteria	*Burkholderiales*	*Comamonadaceae*	*Albidiferax*					+	0.0029
	* TH5891	Proteobacteria	*Burkholderiales*	*Comamonadaceae*	*Albidiferax*	+				+	0.0022
	** TH3415	Proteobacteria	*Gallionellales*	*Gallionellaceae*	*Gallionella*	+				+	0.0050
	TH545	Proteobacteria	*Methylophilales*	*Methylophilaceae*	*Methylotenera*			+		+	0.0222
	* TH2922	Proteobacteria	*Desulfobacterales*	*Desulfobulbaceae*	*Desulfobulbus*	+			+		0.0054
	TH433	Proteobacteria	*Desulfobacterales*	*Desulfobulbaceae*	*Desulfocapsa*	+					0.0282
	* TH4645	Proteobacteria	*Desulfuromonadales*	*Geobacteraceae*			+	+	+		0.0025
	TH1998	Proteobacteria	*Campylobacterales*	*Helicobacteraceae*	*Sulfurimonas*				+	+	0.0070
	TH1380	Proteobacteria	*Methylococcales*	*Methylococcaceae*	*Methylobacter*					+	0.0093
	TH3552	Proteobacteria	*Methylococcales*	*Methylococcaceae*	*Methylobacter*	+				+	0.0024
	TH2519	Verrucomicrobia	*Opitutales*	Subdivision 4				+			0.0050
	TH2747	Verrucomicrobia	*Verrucomicrobiales*	Subdivision 3				+			0.0021
	TH3004	Verrucomicrobia	*Verrucomicrobiales*	Subdivision 3				+	+		0.0045
	TH2746	Verrucomicrobia	*Verrucomicrobiales*	Subdivision 1				+	+		0.0032

Fig. 2. Occurrence of putative EET genes in MAGs and their normalized abundance in the metagenome as measured by mapping reads to assembled contigs for read coverage and normalizing by the average coverage of single-copy conserved bacterial housekeeping genes (see Supplemental Methods for details). * indicates MAGs with Fe(III) reducing relatives, and ** indicates MAGs with Fe(II) oxidizing relatives. The presence of putative EET genes was indicated with "+".
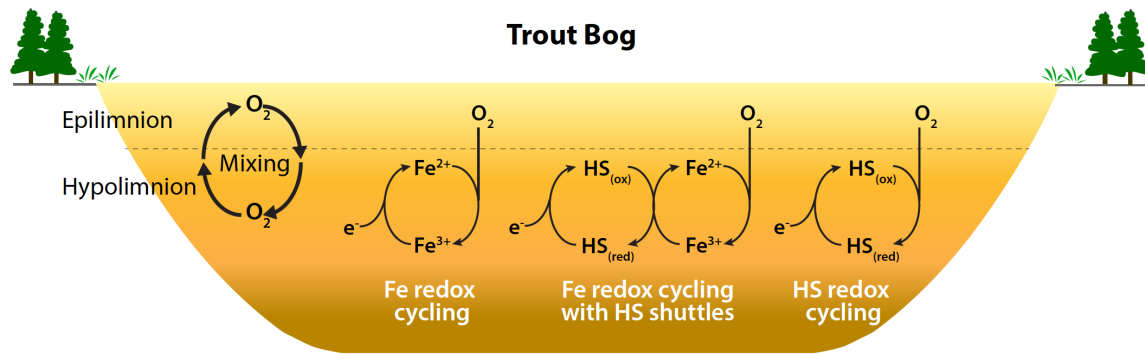
Fig. 3. Proposed roles of EET genes in facilitating redox cycling of Fe and HS in the bog. Oxygenation in the hypolimnion through seasonal mixing and more frequent micro-mixing (such as wind-driven turbulence and convectively derived diurnal oxycline fluctuations) regenerates the electron accepting capacity of reduced HS and Fe to enable these anaerobic respiration processes sustainable in the hypolimnion.

List of Supplemental Information

Supplemental Methods

Table S1. Lake characteristics

Table S2. List of MHCs that might be involved in EET in the metagenomes

Table S3. List of Cyc2-like genes in the metagenomes