The distribution of active iron-cycling bacteria in marine and freshwater sediments is decoupled from geochemical gradients

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Summary
Microaerophilic, phototrophic and nitrate-reducing Fe(II)-oxidizers co-exist in coastal marine and littoral freshwater sediments. However, the in situ abundance, distribution and diversity of metabolically active Fe(II)-oxidizers remained largely unexplored. Here, we characterized the microbial community composition at the oxic-anoxic interface of littoral freshwater (Lake Constance, Germany) and coastal marine sediments (Kalø Vig and Norsminde Fjord, Denmark) using DNA-/RNA-based next-generation 16S rRNA (gene) amplicon sequencing. All three physiological groups of neutrophilic Fe(II)-oxidizing bacteria were found to be active in marine and freshwater sediments, revealing up to 0.2% anoxygenic photoferrotrophs (e.g., Rhodovulum iodosum (Wu et al., 2014), Rhodopseudomonas palustris sp. strain TIE-1 (Jiao et al., 2005), Rhodobacter ferrooxidans sp. strain SW2 (Ehrenreich and Widdel, 1994) and Chlorobium ferrooxydans (Heising et al., 1999), belong to purple sulfur, purple non-sulfur and green sulfur anoxygenic phototrophic bacteria respectively, and are characterized to be metabolically flexible (Widdel et al., 1993; Heising et al., 1999; Jiao et al., 2005). MFeOx mainly affiliate with Betaproteobacteria, e.g.,

Introduction
Iron (Fe) is an essential redox-active element and abundant in the environment (Canfield et al., 1993), typically found in the redox states of Fe(II) and Fe(III). In the biogeochemical Fe cycle, Fe is transformed between these two redox states via several abiotic and biotic processes (Melton et al., 2014b). Under neutral pH conditions, abiotic reactions include Fe(III) reduction by reduced sulfur species or humic substances and Fe(II) oxidation by oxygen, oxygen radicals, Mn(IV) oxides or reactive nitrogen species, such as nitrite or nitric oxide (Melton et al., 2014b). In addition, biotic processes include Fe(II) oxidation by phototrophic (PFeOx), microaerophilic (MFeOx) and nitrate-reducing Fe(II)-oxidizers (NRFeOx) (Widdel et al., 1993; Straub et al., 1996; Emerson and Moyer, 1997; Laufer et al., 2016), as well as Fe(III) reduction by dissimilatory Fe(III)-reducing bacteria (Lovley, 2013; Melton et al., 2014b). In marine and freshwater sediments, the biogeochemical Fe cycle is connected to carbon, nitrogen and sulfur cycles by Fe(II) oxidation and Fe(III) reduction processes (Canfield, 1989; Emerson and Moyer, 1997; Roden, 2004; Kappler and Straub, 2005; Li et al., 2012). Therefore, investigating microbially mediated Fe-cycling is crucial to understanding geochemical cycles in environmental systems.

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Gallionella spp. (Kucera and Wolfe, 1957; Hallbeck and Pedersen, 1991), Leptothrix spp. (Winogradsky, 1888; Fleming et al., 2011) and Sideroxydans spp. (Emerson and Moyer, 1997; Weiss et al., 2007) as well as with the class Zetaproteobacteria, represented by the genera Mariprofundus (Emerson et al., 2007) and Ghirosea (Mori et al., 2017). NRFeOx, e.g., Thiobacillus, Pseudomonas and H. felsii, couple the reduction of nitrate to the oxidation of Fe(II), which leads to the formation of gaseous products such as NO, N₂O and NO₂ (Straub et al., 1996). Fe(III)-reducing bacteria are phylogenetically diverse with members affiliating with Gamma-, Beta-, Epsilon-, Deltaproteobacteria and a few additional genera (e.g., Geothrix, Bacillus) (Lovley, 2013).

Microorganisms that oxidize Fe(II) and reduce Fe(III) have been reported from various Fe-rich environments (>1 wt % Fe) such as hot springs (Kasama and Murakami, 2001), ferrous hydrothermal vents (Emerson and Moyer, 2010; Edwards et al., 2011; Fitzsimmons et al., 2014), Fe seeps and springs (Blöthe and Roden, 2009; Hegler et al., 2012), rice paddy soils (Li et al., 2016; Peng et al., 2016), salt lake sediment (Emmerich et al., 2012), microbial mats in the arctic tundra and in close proximity of hydrothermal vents (Emerson et al., 2015) and ponds (Bruun et al., 2010). In contrast, iron-poor environments (with relatively low, i.e., micromolar, concentrations of Fe(II)) remained relatively unexplored, however, very recently, microbial Fe-cycling has also been described for environments with low Fe content (Laufer et al., 2016; Chiu et al., 2017).

General molecular assays for catabolic target genes of Fe(II)-oxidizing bacteria (as well as of Fe(III)-reducing bacteria) are lacking, due to mechanistic differences of microbial Fe(II) oxidation (Kato et al., 2015; Shi et al., 2016), and, thus, microbial community studies are currently needed to explore the in situ abundance, distribution and diversity of Fe(II)-oxidizers in the environment. A study, characterizing the microbial community based on 16S rRNA gene analysis in Baltic Sea sediments, showed that Fe(III)-reducing bacteria (e.g., DesulfofabaCar, GeobaCar and PelobaCar) were more abundant near the Fe(III)/Fe(II) redox boundary, while Fe(II)-oxidizing bacteria (e.g., Mariprofundus and Gallionsela) were detected in comparatively lower abundance (Reyes et al., 2016). Another DNA-based microbial community study of Baltic Sea sediments found Fe(III)-reducing bacteria of the orders Myxococcales and Desulfuromonadales positively correlating with total Fe in surface sediments (Edlund et al., 2008; Sinkko et al., 2011). In addition, a DNA-based microbial community study of the water column of Lake Cadagno, characterized by low Fe concentrations, detected Fe(II)-oxidizing bacteria, e.g., Chlorobium and Rhodococcus and Fe(III)-reducing bacteria, e.g., Rhodotherax and Geothrix (Berg et al., 2016).

The co-existence of all physiological types of Fe(II)-oxidizing and Fe(III)-reducing microorganisms in marine sediments was demonstrated by cultivation-dependent most probable number experiments (Laufer et al., 2016). However, the distribution of active Fe-metabolizing bacteria was neither identified nor localized in situ in littoral freshwater or coastal marine sediments. Moreover, it remained unknown if the distribution of active Fe-metabolizing bacteria in the environment follows, as expected, the geochemical gradients of oxygen, light and Fe(II) (Schmidt et al., 2010). Here, we analysed the distribution, abundance, diversity and co-existence of present and active Fe-cycling communities in six marine and two freshwater sediment cores using DNA- and RNA-based 16S rRNA (gene) analyses respectively, and explored the distribution patterns of active Fe-cycling bacteria with respect to geochemical gradients.

Results

Geochemical characterization of the sediments

The marine sediments from Norsminde Fjord and Kalø Vig and the freshwater sediment from Lake Constance were geochemically distinct (Table 1). In the marine and freshwater sediments, light and oxygen were only detected in the upper 3 mm. The highest values of dissolved organic carbon (DOC) were detected in the porewater from Lake Constance sediment (7.5 mg l⁻¹), while the porewater from Kalø Vig sediment contained only 3.5 mg l⁻¹ and from Norsminde Fjord sediment around 5 mg l⁻¹ of DOC. The concentrations of nitrate in the porewater of both marine sediments and in Lake Constance sediment were low, i.e., with maximum values of 18 µM in Kalø Vig, 21–65 µM in Norsminde Fjord, and 50–80 µM in Lake Constance. Dissolved Fe(II) concentrations in the porewater of the sediment were maximal at around 70 µM in Kalø Vig, 90 µM in Norsminde Fjord and 76 µM in Lake Constance sediments.

Microbial community characterization of the sediments

Absolute bacterial and archaeal 16S rRNA (gene) copy numbers remained relatively stable over the investigated sediment depth and among replicate sediment cores from Norsminde Fjord, Kalø Vig and, Lake Constance. Bacterial abundance was highest at 0–3 mm [0.4–6 × 10⁸ 16S rRNA (gene) copies per g wet sediment] compared to the deeper layers in both marine sediments, while the archaeal abundance was highest at deeper sediment layers [20–30 mm; 0.6–1 × 10⁸ 16S rRNA gene copies per g wet sediment] in both marine sediments (Supporting Information Figs. S1 and S2). In the freshwater sediment, the highest bacterial and archaeal abundances were found at 3–10 mm sediment depth [0.1–1 × 10⁸ bacterial 16S rRNA (gene) copies g per wet sediment; 0.5–1 × 10⁸ archaeal 16S rRNA gene copies g per wet sediment].

While diversity indices were similar among all investigated sediments (Supporting Information Table S1), each
field site had distinct microbial community structures. DNA and RNA-based analyses, comparing present and active microbial communities, revealed highly significant differences (Permanova, $P < 0.001$) at the two marine field sites and at the freshwater site respectively (Supporting Information Fig. S3, Tables S2 and S3). Highly significant differences (Permanova, $P < 0.001$) were also found between the active microbial communities at the freshwater site and those found at the two marine field sites (Supporting Information Fig. S3). All sediments were dominated by bacteria (98%) (Supporting Information Fig. S4). In the marine sediments, Proteobacteria dominated (Kalø Vig = DNA: 39%, RNA: 53%; Norsminde Fjord = DNA: 41%, RNA: 65%), including Gamma-, Delta-, Alpha- and Betaproteobacteria. In addition, Cyanobacteria (Kalø Vig = DNA: 9%, RNA: 18%; Norsminde Fjord = DNA: 3%, RNA: 5%) and Bacteroidetes (Kalø Vig = DNA: 24%, RNA: 22%; Norsminde Fjord = DNA: 24%, RNA: 18.5%) were present at the marine sites. Similar to the marine sediments, freshwater sediments at Lake Constance were dominated by Proteobacteria (DNA: 35%, RNA: 38%) which were affiliated with Gamma-, Delta-, Alpha- and Betaproteobacteria. In addition, Cyanobacteria (DNA: 19%, RNA: 28%) and Bacteroidetes (DNA: 15%, RNA: 13.5%) were abundant in the freshwater sediments.

Diversity and relative abundance of Fe-metabolizing microorganisms

All three physiological types of Fe(II)-oxidizing bacteria and known Fe(III)-reducing bacteria were detected in the marine and freshwater sediments (Figs. 1A and 2; Supporting Information Table S4), including Fe-metabolizing isolates obtained from the same field sites (Supporting Information Table S5). Fe-metabolizers were phylogenetically diverse, partly forming specific marine or freshwater habitat clusters (Fig. 2). Since active Fe-cycling bacteria were of particular interest, in-depth analyses were performed focusing on RNA-based findings. Analysing separate groups of Fe-metabolizers, the relative abundance of Fe(II)-oxidizers was lower (< 0.1%) compared to Fe(III)-reducers (< 4%) at the marine field sites (Fig. 1A), while Fe(II)-oxidizers were more abundant (< 2%) than Fe(III)-reducers (< 0.4%) at the freshwater site (Fig. 1B).

In the marine sediment, anoxicogenic PFeOx were of low relative abundance (< 0.1%; Fig. 1A). Compared to Kalø Vig, the anoxicogenic PFeOx Rhodopseudomonas and Chlorobium were detected in higher abundance in Norsminde Fjord sediment, particularly in the deeper sediment layers. MFeOx, e.g., Marinobacter and Gallionella, were also detected in low relative abundance (< 0.1%) at the two marine sites and were probably most active in the organic-rich Norsminde sediment, where abundances were highest in 20–30 mm sediment depth. *Mariprofundus* was found to be active at one sampling site, in the Norsminde Fjord sediment. *Hyphomonas* was active in the sediments of both marine field sites. Heterotrophic denitrifiers and NRFeOx showed relatively low abundance (< 0.1%) in all layers from both investigated marine sampling sites. Most abundant, active NRFeOx affiliated with *Thiobacillus* in Norsminde Fjord sediment. In addition, most abundant, active Fe(III)-reducers affiliated with *Desulfobulbus, Desulfo bacterium, Geobacter* and *Shewanella* in all marine sediment layers (Figs. 1A and 2).

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### A Fe-cycling microorganisms in coastal marine sediment (Kalø Vig and Norsminde Fjord)

<table>
<thead>
<tr>
<th>Depth (mm)</th>
<th>Kalø Vig DNA</th>
<th>Kalø Vig RNA</th>
<th>Norsminde DNA</th>
<th>Norsminde RNA</th>
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</thead>
<tbody>
<tr>
<td>0-3</td>
<td>a) Rhodopseudomonas</td>
<td>b) Rhodobacter</td>
<td>c) Chlorobium</td>
<td></td>
</tr>
<tr>
<td>3-10</td>
<td>a) Thiomicrospira</td>
<td>d) Propionivibrio</td>
<td>e) Dechloromonas</td>
<td></td>
</tr>
<tr>
<td>10-20</td>
<td>a) Thiobacillus</td>
<td>e) Paracoccus</td>
<td>f) Pseudomonas</td>
<td></td>
</tr>
<tr>
<td>20-30</td>
<td>a) Thiomicrospira</td>
<td>d) Propionivibrio</td>
<td>e) Dechloromonas</td>
<td></td>
</tr>
</tbody>
</table>

### B Fe-cycling microorganisms in littoral freshwater sediment (Lake Constance)

<table>
<thead>
<tr>
<th>Depth (mm)</th>
<th>Lake Constance DNA</th>
<th>Lake Constance RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>a) Rhodopseudomonas</td>
<td>d) Propionivibrio</td>
</tr>
<tr>
<td>2-4</td>
<td>a) Rhodobacter</td>
<td>e) Pedomonibium</td>
</tr>
<tr>
<td>4-6</td>
<td>a) Rhodobacter</td>
<td>e) Pedomonibium</td>
</tr>
<tr>
<td>6-10</td>
<td>a) Rhodobacter</td>
<td>e) Pedomonibium</td>
</tr>
<tr>
<td>10-15</td>
<td>a) Rhodobacter</td>
<td>e) Pedomonibium</td>
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### C Cable bacteria in marine sediments

<table>
<thead>
<tr>
<th>Depth (mm)</th>
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<th>Kalø Vig RNA</th>
<th>Norsminde DNA</th>
<th>Norsminde RNA</th>
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<tbody>
<tr>
<td>0-3</td>
<td>a) Rhodopseudomonas</td>
<td>b) Rhodobacter</td>
<td>c) Chlorobium</td>
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<td>a) Rhodopseudomonas</td>
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<td>20-30</td>
<td>a) Rhodopseudomonas</td>
<td>b) Rhodobacter</td>
<td>c) Chlorobium</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Depth distribution and relative abundance of present and active Fe-metabolizing microorganisms in (A) coastal marine (Kalø Vig and Norsminde Fjord) and (B) littoral freshwater (Lake Constance) sediments.

A. Sediment layers: 0–3 mm, 3–10 mm, 10–20 mm, 20–30 mm; marine phototrophs from Kalø Vig and Norsminde Fjord (up to 0.2%; marine microaerophilic Fe(II)-oxidizers (up to 0.1%); marine NRFeOx (up to 0.2%); marine Fe(III)-reducers from Norsminde Fjord and Kalø Vig (up to 2.8%); cable bacteria in marine sediment (up to 2.5%).

B. Freshwater Fe(II)-oxidizers from Lake Constance (0–2 mm, 2–4 mm, 4–6 mm, 6–10 mm, 10–15 mm) (up to 2%); freshwater Fe(III)-reducers from Lake Constance (up to 0.4%).

C. Depth distribution and relative abundance of present and active cable bacteria in marine sediments (Kalø Vig and Norsminde Fjord). In Lake Constance sediment, the relative sequence abundance of cable bacteria is < 0.001% (data not shown).

* Crenothrix is most often associated with methanotrophy but there are also hints for Fe(II) oxidation.
In Lake Constance freshwater sediments, PFeOx and MFeOx were low abundant (< 0.1%; Fig. 1B). One exception was Crenothrix, which is most often associated with methanotrophy (Oswald et al., 2017) but there are also hints for Fe(II) oxidation (Rao et al., 2000; Emerson et al., 2010; Chen and Jiang, 2016; Demir, 2016).

Decoupling of active Fe-cycling bacteria from geochemical gradients

Fig. 2. Phylogenetic tree of most abundant (i.e., representing 30 most abundant OTUs respectively) Fe(II)-oxidizers and Fe(III)-reducers based on sequences obtained from Kalø Vig, Norsminde Fjord and Lake Constance sediments. Fe(II)-oxidizers are in green font, Fe(III)-reducers in red font, and bacteria which have the potential to oxidize and reduce Fe are in blue font. Recent Fe(II)-oxidizing isolated strains from Kalø Vig and Norsminde Fjord are in orange font. Symbols indicate sequences that appeared at specific field sites (C21–15 sequences). Boxes indicate marine sediment clusters (dark grey) and freshwater sediment clusters (light grey). *: Exceptions that they do not belong to the marked freshwater/marine site. #: Crenothrix is most often associated with methanotrophy but there are also hints for Fe(II) oxidation. A complete list of the taxa that were considered to harbour Fe-metabolizing strains can be found in the Supporting Information Table S4.

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was highly abundant (1.4%) compared to other Fe(II)-
oxidizers and was found in all investigated sediment layers.
The abundance of heterotrophic denitrifiers (e.g., Thioba-
cillus) and NRFeOx (e.g., Dechloromonas) were around
2% in the freshwater sediment respectively. Fe(III)-reduc-
ing bacteria affiliating with Geothrix, Geobacter and
Shewanella showed relatively high abundance (<0.4%) in
the freshwater sediment.

Diversity and relative abundance of cable bacteria
Specific clusters of sequences affiliating with cable bacte-
ria were detected at Norsminde Fjord, Kalø Vig and
Lake Constance. Described cable bacteria candidates are shown
(freshwater candidates in green font; marine candidates in blue, purple and
orange fonts, representing different species). The most abundant cable
bacteria sequence from our study (OTU0001), shown in red font, is
closely related to Electrothrix communis (purple font). Symbols
indicate sequences that appeared at specific field sites (≥ 1 sequences).
Boxes indicate marine sediment clusters (dark grey) and freshwater
sediment clusters (light grey). Grey clamps indicate sequences targeted
by probes DSB706 and ELF645, typically used to identify cable bacteria
via FISH. The solid lines of the clamps indicate a perfect probe match,
whereas the dotted lines indicate at least one mismatch.

Fig. 3. Phylogenetic tree of cable bacteria related sequences obtained from Kalø Vig, Norsminde Fjord and
Lake Constance. Described cable bacteria candidates are shown (freshwater candidates in green font;
marine candidates in blue, purple and orange fonts, representing different species). The most abundant cable
bacteria sequence from our study (OTU0001), shown in red font, is closely related to Electrothrix communis
(purple font). Symbols indicate sequences that appeared at specific field sites (≥ 1 sequences). Boxes indicate marine sediment clusters (dark grey) and freshwater sediment clusters (light grey). Grey clamps indicate sequences targeted by probes DSB706 and ELF645, typically used to identify cable bacteria via FISH. The solid lines of the clamps indicate a perfect probe match, whereas the dotted lines indicate at least one mismatch.

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hybridization (FISH) (Fig. 3) and, in addition, by light microscopy (Supporting Information Fig. S5). The most abundant OTU, closely related to cable bacteria, was present in all layers of the three investigated sediment cores from Kalø Vig (based on DNA and RNA). This OTU shared 99.6% and 99.2% gene sequence identity with Candidatus *Electrothrix communis* and Candidatus *Electrothrix japonica* respectively (Fig. 3), and had relative abundances of 1–4.5% (Fig. 1C). In contrast, in Lake Constance and Norsminde Fjord sediments only between 0.001% and 0.4% of the sequences were related to cable bacteria.

**Vertical distribution of microbial groups and correlation with geochemical parameters**

Vertical distribution patterns in Kalø Vig sediment revealed that 56% of the OTUs were more abundant in the deeper sediment layers, 17% were more abundant in the surface sediment layers and 27% were homogenously distributed. In Norsminde Fjord sediment, 76% of OTUs were more abundant in the deeper sediment layers, 8% were more abundant in upper sediment layers and 16% were homogenously distributed. In Lake Constance sediment, 50% of OTUs were more abundant in deeper sediment layers, 25% were more abundant in upper sediment layers and 25% were homogenously distributed among sediment depth. Most of the vertical distribution patterns of the microbial groups (between 75% and 85% of microbial taxa) could, therefore, be explained by sediment depth and geochemical parameters such as oxygen, nitrate, sulfate and light. For example, sulfate-reducing bacteria affiliating with *Desulfonema* correlated significantly (Permanova, *P* < 0.0001) with depth at the marine sites, showing highest abundance (RNA: up to 0.04%) at greater depths (20–30 mm) (Fig. 4A). Sequences affiliating with phototrophic *Merismopedia* (Cyanobacteria) in Norsminde Fjord sediment showed highest abundance (RNA: up to 5%) at shallow depths (0–3 mm), while they were found to be less abundant (RNA: 1%) at greater sediment depths (20–30 mm) (Fig. 4A). In addition, members of the genus *Pseudohaloea*, that are strictly aerobic and typically found widespread in saline environments, correlated (Permanova, *P* < 0.0001) with oxygen penetration, revealing highest abundances (RNA: up to 1.5%) at 0–3 mm with a steep decrease down to 3 cm sediment depth at both marine sampling sites (Fig. 4A).

In contrast, Fe(III)-reducers and Fe(II)-oxidizers did not follow geochemical gradients, i.e., they were homogenously distributed with sediment depth and they did not correlate with any of the measured geochemical parameters (Figs. 1 and 4B). In addition, cable bacteria were almost completely homogenously distributed among sediment depths (0.1%–4.5%; Fig. 1C) and showed a positive and significant (FDR ≤ 0.05) correlation with different parameters in Norsminde Fjord sediment (Supporting Information Table S6).

Summing up all Fe(II)-oxidizer groups (i.e., MFeOx, PFeOx and NRFeOx), a positive but not significant correlation to Fe(II) and Fe(III) concentrations was found (FDR > 0.5). Similar to the marine sampling sites, Fe(III)-reducers and Fe(II) oxidizers showed no correlation with geochemical data in Lake Constance sediment (Supporting Information Table S6, Fig. S6).

**Correlations between Fe-cycling bacteria and cable bacteria**

To investigate interrelationships between active Fe-cycling bacteria and other active microbial groups, RNA-based distribution patterns were further explored for positive and negative correlations using the Spearman’s rank (Supporting Information Table S6, Fig. S6). In the Kalø Vig sediment, a negative but not significant correlation between Fe(III)-reducers and Fe(II)-oxidizers was found (Supporting Information Table S6). Surprisingly, in all investigated sediment layers of Kalø Vig sediment, a positive and highly significant (FDR < 0.001) correlation was determined between cable bacteria and the group of Fe(III)-reducers. Further analysis showed positive correlations between cable bacteria and most of the individual genera of known Fe(III)-reducers such as *Desulfovibrio*, *Desulfococcus* and *Geobacter*, though not significant (FDR ≤ 0.8; Supporting Information Fig. S6). In addition, the correlation between cable bacteria and most of the single Fe(II)-oxidizing populations such as *Pedomicrobium* and *Hoeflea* was also positive in Kalø Vig sediment (FDR < 0.8). In the sediment from Norsminde Fjord, a positive correlation between Fe(III)-reducers and Fe(II)-oxidizers was found. Similar to the trends observed in the Kalø Vig results, a positive correlation between cable bacteria and Fe(III)-reducers was detected. In particular, the Fe(III)-reducers affiliating with *Desulfovibrio* and *Geobacter* showed a positive correlation (FDR = 0.12 and 0.42) with the cable bacteria. Furthermore, the correlation between cable bacteria and Fe(II)-oxidizers (i.e., the PFeOx *Chlorobium* and *Rhodopseudomonas*) was positive (FDR < 0.5). In Lake Constance freshwater sediment, a positive but not significant correlation between Fe(III)-reducers and Fe(II)-oxidizers was found (Supporting Information Table S6) but there was no correlation between cable bacteria and Fe(II)-oxidizers or Fe(III)-reducers respectively. In summary, significant (FDR < 0.05) correlations were found for Fe(III)-reducers and cable bacteria, particularly for the field sites with high abundances of active cable bacteria (i.e., Kalø Vig).

**Discussion**

**Significance of neutrophilic Fe-cycling bacteria in low-iron freshwater and marine sediments**

The low-iron field sites from our study are widely representative for typical marine and freshwater habitats. At all field
sites, diverse, active Fe-cycling bacteria (MFeOx, PFeOx, NRFeOx and Fe(III)-reducing bacteria) were identified based on taxonomy. However, using taxonomy to infer metabolic functions is challenging, thus, some of the Fe-metabolizing taxa identified in this study may perform different metabolic functions in situ. Comparing the relative abundance among Fe-cycling bacteria at our field sites, active Fe(III)-reducers were more abundant but might be overestimated because of their metabolic flexibility (iron and sulfate reduction), while less abundant active Fe(II)-oxidizers might be underestimated since many Fe(II)-oxidizers might currently be uncultured and, therefore, remain unexplored. Albeit detected in low abundance, as also previously shown (Berg et al., 2016; Laufer et al., 2016), Fe-metabolizers can have a substantial impact on important biogeochemical cycles, such as carbon, sulfur and nitrogen, thereby influencing the fate of contaminants and greenhouse gases (Canfield, 1989; Borch et al., 2010; Picardal, 2012; Buchwald et al., 2016). Furthermore, Fe-metabolizers might be actively involved in 'cryptic element cycles', which may lead to a rapid turnover (Kappler and Bryce, 2017), indicating that Fe(II)-oxidizers and Fe(III)-reducers are crucial in low-iron environments.

Neutrophilic Fe-cycling bacteria were decoupled from geochemical gradients

Based on the microbial redox 'tower', aerobic respiration, denitrification, manganese(IV) reduction, Fe(III) reduction, sulfate reduction and methanogenesis are thought to proceed in a sequence, determined by thermodynamics, i.e., the energy yield of the different reactions (Richards, 1965; Froelich et al., 1979; Orcutt et al., 2011), creating typical geochemical gradients. However, in the environment, different metabolic processes were found to be co-occurring in the same sediment zone (Chen et al., 2017a; Giling et al., 2017). At our field sites, the vertical distribution of most active microbial groups correlated with measured geochemical gradients, while active Fe-metabolizing bacteria were decoupled from these gradients, which was highly unexpected. Since, our marine and freshwater field sites provided favourable conditions for all three metabolic

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**Fig. 4.** A. Relative sequence distribution of selected microbial taxa in marine sediment at Norsminde Fjord. Selected bacterial distribution patterns following sediment depth and geochemical parameters, such as sulfate, light and oxygen. Relative sequence abundances of the 16S rRNA (gene) are shown based on DNA (blue circles) and RNA (red circles) analyses respectively.

B. Schematic illustration of relative sequence distribution of Fe(II)-oxidizers and Fe(III)-reducers in marine and freshwater sediment; PFeOx in orange, MFeOx in blue, NRFeOx in green and Fe(III)-reducers in red colour.
types of Fe(II)-oxidizers and of Fe(III)-reducers, it was hypothesized that their in situ abundances, especially of active populations (i.e., based on RNA sequences), would follow gradients of light, oxygen, nitrate and iron (Fig. 4). In contrast, we found homogeneously distributed abundances of present and active Fe(II)-oxidizers and Fe(III)-reducers in marine and freshwater sediments (Figs. 1 and 4). Our findings are supported by a previous study which demonstrated homogeneously distributed abundances of Fe(II)-oxidizers based on cultivation-independent most probable number experiments (Laufer et al., 2016) and of Zetaproteobacteria based on qPCR assays (Laufer et al., 2016). While the actual reason for an uncoupling of Fe-cycling bacteria and geochemical gradients remains currently unresolved, there are several possible explanations, including physical mixing, motility, dormancy, microchirons, metabolic flexibility, or interactions with cable bacteria.

Physical mixing might be caused by macrofauna (e.g., polychaetes and crustaceans), tidal activity, wind, or human activity (Laufer, 2016; Chen et al., 2017b) and might impact the distribution of Fe(II)-oxidizers and Fe(III)-reducers. Bioturbation by burrowing fauna is well known from the upper 6–8 cm of Aarhus Bay sediments (Thamdrup et al., 1994). In addition, a storm event in Norsminde Fjord showed a strong influence on geochemical gradients (e.g., oxygen, pH, redox potential and sulfide) (Laufer, 2016). However, in our study, the majority of microbial groups followed geochemical gradients and, thus, physical mixing might be a minor reasons for the homogeneous distribution of Fe-metabolizers.

Motile microorganisms might move towards favourable conditions (Thar and Fenchel, 2005; Krepski et al., 2012) or microorganisms might enter a dormant state with a slow metabolism, without growth and cell division, until conditions become favourable. To date, only certain Fe(II)-oxidizers and Fe(III)-reducers were found to be motile, e.g., Shewanella oneidensis, Rhodopseudomonas palustris and Maripropfundus ferrooxidans (Jiao et al., 2005; Wu et al., 2011; Laufer et al., 2017). It remains further unknown to which extent Fe(II)-oxidizers and Fe(III)-reducers switch into a dormant mode or enter a dormant state in the environment.

Metabolic flexibility is well-known for NRFeOx, PFeOx and Fe(III)-reducers expanding their possible niches. For example, NRFeOx might be able to perform microaerophilic Fe(II) oxidation or even growth with oxygen on organic carbon (Benz et al., 1998; Edwards et al., 2003; Weber et al., 2006b). Furthermore, PFeOx may grow heterotrophically under oxic conditions (Straub et al., 1999; Jiao et al., 2005; Melton et al., 2014a; Thompson et al., 2017), live chemolithoautotrophically on hydrogen or reduced sulfur species, or phototrophically on organic carbon (Widdel et al., 1993; Heising et al., 1999; Straub et al., 1999; Thompson et al., 2017). Some Fe(II)-reducers may grow heterotrophically with oxygen, while others can live on fermentation (Lovley, 2006; Klueglein et al., 2014; Ehrlich et al., 2015). In addition, Fe(III)-reducers may oxidize Fe(II) with nitrate (Finneran et al., 2002; Weber et al., 2006a) or perform sulfate reduction. Therefore, it is possible that potential Fe-cycling microorganisms in the environment are metabolically flexible and capable of utilizing electron acceptors or donors, in addition or instead of Fe(III) and Fe(II).

Since active Fe-metabolizing microorganisms were found to co-exist, there are opportunities for their interaction and competition with each other, for example, to obtain electrons from Fe(II) or to use Fe(III) as electron acceptor (Melton et al., 2012). Furthermore, most microorganisms may live in microniches, where they have access to electron acceptors that might be below detection of our analysis (Jørgensen, 1977; Lehto et al., 2014). This in turn may cause ecological niches to overlap, leading to competition or cooperation of Fe-metabolizers.

Hypothetical interplay of Fe-metabolizing microorganisms and cable bacteria

Interaction between Fe-metabolizing bacteria with cable bacteria, i.e., multicellular structures which have the ability to transport electrons over a long distance in sediments (Nielsen et al., 2010; Pfeffer et al., 2012), might be another explanation for the homogenous distribution of Fe-cycling bacteria (Fig. 5A). Previous studies showed evidence for a potential influence of cable bacteria on Fe-cycling bacteria in sediments (Seitaj et al., 2015), however, their coexistence has not been studied so far. Here, we provide direct evidence for the co-existence of active cable bacteria and Fe-metabolizing bacteria in marine sediments (Fig. 5B), indicating that the activity of cable bacteria might be another reason for the observed decoupling of Fe-cycling microorganisms from geochemical gradients. Albeit third-party factors influencing the distribution patterns of microbial populations cannot be ruled out, the positive correlation of Fe-cycling bacteria with cable bacteria could be a first indicator of a causal interrelationship between both populations.

Several hypothetical scenarios could explain potential interrelationships between Fe-cycling bacteria and cable bacteria. It is known that cable bacteria oxidize sulfide with oxygen, but also use nitrate and nitrite as electron acceptors (Risgaard-Petersen et al., 2012; Marzocchi et al., 2014) (Fig. 6A). Furthermore, it was suggested that electrogenic oxidation processes, i.e., the coupling of the oxidation and the reduction step of a redox reaction via long-distance electron transport, seems to not only rely on sulfide but could also depend on other donors such as organic compounds, fatty acids, Fe(II), or methane. However, sulfate reduction rates might be sufficient to support
the activities of cable bacteria in these sediments (Nielsen and Risgaard-Petersen, 2015; Risgaard-Petersen et al., 2015). Therefore, if cable bacteria were able to take up electrons from Fe(II), thus oxidizing the Fe(II) to Fe(III), there might be potential competition with all three physiological types of Fe(II)-oxidizing bacteria (Fig. 6B). Furthermore, this may lead to a cooperation with Fe(III)-reducers that can re-reduce the Fe(III) produced by the cable bacteria, thus providing more Fe(II) for the cable bacteria. This potential stimulation of Fe(III)-reducers by the cable bacteria, however, may also fuel the activity of Fe(II)-oxidizers by the available Fe(II). And indeed, both Fe(II)-oxidizers and Fe(III)-reducers showed positive and significantly positive correlations with cable bacteria respectively, suggesting a possible cooperation and a potential for direct interspecies electron transfer (DIET) between Fe(II)-oxidizers, Fe(III)-reducers and cable bacteria (Rotaru et al., 2014) (Fig. 6B). This could lead to a localized cryptic Fe-cycle that requires relatively small amounts of Fe and that would not be observable by significant accumulations of Fe(III) oxyhydroxides (Kappler and Bryce, 2017), confirming our geochemical Fe data in the sediments.

Recently, it was mentioned that the long-distance electron transport by cable bacteria and a counter-ion diffusion in the marine sediment, is forming a kind of 'biogeobattery' (Revil et al., 2010; Risgaard-Petersen et al., 2012; Nielsen and Risgaard-Petersen, 2015). Such a biogeobattery system would allow the microbial community including the Fe-metabolizing microorganisms to be independent of substrate availability which indicates that Fe-oxidizing bacteria may donate electrons to the cable bacteria or Fe(III)-reducing bacteria may use the cables as an 'e–power line' to take up electrons (Fig. 6C). Therefore, the electrogenic multistep sulfur oxidation might be performed by a microbial consortium ('swarming e–community' surrounding the

Fig. 5. A. Interrelationships of biogeochemical cycles and microbial activities in marine and freshwater sediments. Geochemical gradients of light, oxygen, nitrate, Fe(II), Fe(III) and H2S are illustrated. The Fe cycle is mediated by phototrophic Fe(II)-oxidizers (PFeOx, yellow), microaerophilic Fe(III)-oxidizers (MFeOx, blue), nitrate-reducing Fe(II)-oxidizers (NRFeOx, green) and Fe(III)-reducing bacteria (FeRed, red) and coupled to anammox-bacteria performing Fe(III) reduction. The Fe cycle is linked to the nitrogen cycle and formation of the greenhouse gas N2O.

B. Relative sequence abundances of microorganisms in marine and freshwater sampling sites (averaged per physiological group, among sediment depths and replicate cores). Relative sequence abundance (RNA-based) of MFeOx: 0.07% in Kalo Vig (KV), 0.1% in Norsminde Fjord (NS), 2.3% in Lake Constance (LC); denitrifiers and potential NRFeOx: 0.1% (KV), 0.3% (NS), 2.2% (LC); PFeOx: 0.002% (KV), 0.2% (NS), 0.06% (LC); FeRed: 0.3% (KV), 1.2% (NS), 1.6% (LC); cable bacteria: 2.5% (KV), 0.8% (NS), 0.2% (LC).
cable bacteria) of Epsilon- and Gammaproteobacteria like Sulfurimonas, Sulforovum, Helio bacter, Arcobacter, Parabeggio atoa and Thiomicrospira (Vasquez-Cardenas et al., 2015; Lovley, 2017) from which the Fe-cycling bacteria might benefit through DIET. A recent study revealed that PFeOx (e.g., Rhodopseudomonas palustris TIE1) are able to use extracellular electron transfer for electron uptake (Bose et al., 2014; Byrne et al., 2015), supporting that Fe(II)-oxidizers might belong to the potential electrogenic community. We found that potential electrogenic Fe(III)-reducing microorganisms like Geobacter and Desulfovibrio producing Fe(II) for the potential metabolically flexible Fe(II)-oxidizing cable bacteria. Conversely, there could also be a cooperation of Fe(III)-reducing microorganisms like Geobacter and Desulfovibrio producing Fe(II) for the potential metabolically flexible Fe(II)-oxidizing cable bacteria.

Fig. 6. A. Hypothetical models of the potential metabolic flexibility of cable bacteria based on Risgaard-Petersen et al. (2015). Coupling of sulfide oxidation to cable bacteria (marked with an asterisk; * ) has been observed (Pfeffer et al., 2012). All other models are hypothetical. B. Hypothetical models of the potential connection and interaction of cable bacteria with Fe-cycling bacteria. If cable bacteria reduce nitrate as electron acceptor there might be a competition with nitrate-reducing Fe(II)-oxidizers (NRFeOx) bacteria. If cable bacteria have the metabolic flexibility to also oxidize Fe(II) there might be potential competition with all physiological types of Fe(II)-oxidizing microorganism. Conversely, there could also be a cooperation of Fe(III)-reducing microorganisms like Geobacter and Desulfovibrio producing Fe(II) for the potential metabolically flexible Fe(II)-oxidizing cable bacteria. C. Hypothetical models of the potential electrically connected microbial community in marine and freshwater sediments. In case of the absence of an electron acceptor, the ‘swarming e–community’ might donate electrons to the cable bacteria or they might use the cables as an ‘e–power line’ to take electrons. The significant positive correlation between some Fe-cycling bacteria and cable bacteria suggests a potential cooperation and network system in the marine and freshwater sediments. The current-forming microbial community might create a kind of ‘biogeobattery’. Such a biogeobattery system would allow the microbial community to be independent of substrate availability suggesting that the presence of all Fe-metabolizing microorganisms in all upper sediment layers might be based on the occurrence of sulfide-oxidizing cable bacteria communities. Cable bacteria might be essentially functioning as telephone cables in the sea floor. Therefore, the microbial communities might share information as well as energy through their electrical connections. CB indicates cable bacteria.
community members such as *Sulforovum* and *Helibacter* were among the most abundant genera in our marine sediments (Supporting Information Table S2), suggesting that Fe-metabolizing bacteria and additional community members may benefit from the electron transport by cable bacteria.

A possible additional mechanism by which Fe(II)-oxidizing bacteria might benefit from cable bacteria is that, in suboxic zones, the activity of cable bacteria promotes dissolution of Fe sulfides, leading to upwards Fe(II) diffusion and to the production and precipitation of Fe oxides in the oxic zone (Risgaard-Petersen et al., 2012; Rao et al., 2016; Sulu-Gambari et al., 2016) which may also form precipitates around the cable bacteria (personal communication with Nicole Geerlings, Utrecht University). The Fe precipitates around the cable bacteria may result in electrons not only flowing in the periplasm of the cable bacteria but also on the surface of the cables (Fig. 6C). This coating may lead to a rapid electron flow or a high-frequency electron transport on the cable bacteria surface (‘electrical skin effect’) (Lamb, 1883; Heaviside, 1885; Wheeler, 1942; Haines, 1959; Kim et al., 2017), suggesting a fast communication system within the sediments. Recently, it was proposed that electricity-generating cable bacteria are essentially functioning as telephone cables in the sea floor (Malkin and Meysman, 2015). Therefore, the microbial communities (including Fe-metabolizers) at our field sites might share information as well as energy through their electrical connections (Lovley, 2017) (Fig. 6C).

**Conclusions**

Our study demonstrated the co-existence of active Fe(II)-oxidizing and Fe(III)-reducing microorganisms in sediments that are widely representative of typical marine and freshwater environments. Metabolic flexibility of Fe-metabolizers, microniches in the sediments, and the activity of cable bacteria might be reasons for the observed uncoupling of the distribution of Fe-cycling bacteria from geochemical gradients. However, it remains to be explored whether cable bacteria might be, directly or indirectly, involved in Fe(II) oxidation or Fe(III) reduction and if Fe-metabolizing bacteria and other members of the microbial community interact with cable bacteria in marine and freshwater sediments.

**Experimental procedures**

**Site description, sampling procedure and geochemical analyses**

Marine sediment samples were taken in July 2015 from two field sites in the Aarhus Bay area (Denmark). Sediment from the shallow marine estuary Norsminde Fjord (NS), was collected at 0.5 m water depth near its narrow entrance from Aarhus Bay (N 56° 01.171'; E 010° 15.390'). Samples from the second marine field site, Kale Vag (KV) (N 56° 18.611'; E 010° 28.056'), a shallow lagoon with organic-poor sandy sediment, were collected at 0.5–1 m water depth. Freshwater sediment samples were taken in September 2015 from Ueberringsee close to the island of Mainau in the northwestern part of Lake Constance (LC) (N47° 41.710'; E9° 11.671') southern Germany, at 0.5 m water depth. All sediment samples from marine and freshwater sediments were collected with push-cores, 2.5 cm in diameter (cut-off 50 ml syringes). Push-cores were taken in close proximity to each other (~1 m) in triplicates or duplicates for marine and freshwater sediments respectively (Supporting Information Fig. S7), and preserved as described in the supporting information. Temperature, pH, salinity and oxygen concentration of the water column were analyzed in the field with a multimeter (WTW, Multi 3430). The geochemical gradients of oxygen, redox potential, pH and sulfide were directly measured on site at *in situ* temperatures at 0.5 mm depth intervals using microelectrodes (Unisense), while we used different microelectrodes for Aarhus Bay and Lake Constance sampling trips. All geochemical analyses are described in detail by Laufer and colleagues (2016).

**DNA/RNA extraction, DNA digestion, reverse transcription, quantitative PCR of 16S rRNA (genes) and 16S rRNA (gene) amplicon sequencing**

Total DNA and RNA was extracted using the PowerSoil® RNA and DNA isolation kit as directed by the manufacturer (MO BIO Laboratories, Carlsbad, CA, USA), with the following modifications: 0.8 g to 2 g sediment was used from each sediment slice; 5 min bead-beating; centrifugation steps at 4°C; and longer incubation times at −20°C (1.5–2.0 h). RNA and DNA were eluted in 50 µl 10 mM Tris buffer. DNA and RNA concentrations were determined using a Qubit® 2.0 Fluorometer with DNA and RNA HS kits (Life Technologies, Carlsbad, CA, USA). Subsequent DNA digestion and reverse transcription reactions were done using a Reverse Transcriptase (Invitrogen, Life Technologies), as described in the supporting information.

Quantitative PCR (qPCR) specific for the 16S rRNA (gene) of bacteria and archaea was performed using an iQ5 real-time PCR detection system (iQ5 optical system software, version 2.0, Bio-Rad). Plasmid standards, gene-specific qPCR primers, reaction mixtures and thermal programs are given in the supporting information.

Microbial 16S rRNA (genes) were amplified using primers 515F and 806R (Caporaso et al., 2010) targeting the V4 region as described in the supporting information. The produced amplicons were purified using AMPure XP beads (Beckman Coulter, Brea, CA, USA) at a ratio of 0.8:1 (vv). Quality and quantity of the purified amplicons were determined using agarose gel electrophoresis and Nanodrop (NanoDrop 1000, Thermo Scientific, Waltham, MA, USA). Subsequent library preparation steps and sequencing were performed by IMGM Laboratories GmbH ( Martinsried, Germany) using an Illumina MiSeq sequencing system (Illumina, San Diego, CA, USA), as described in the supporting information. Primer-trimmed reads (accession number: SRP111913)
have been deposited in the NCBI Genbank database (bioproject: PRJNA393823).

Sequence analysis
Quality control of raw 16S rRNA read pairs was performed using Cutadapt v1.9 (Martin, 2011) and USEARCH v8.1.1812 (Edgar and Flyvbjerg, 2015), as described in the supporting information. Quality filtered sequences were imported in MOTHUR (1.35.1) (Schloss et al., 2009) and analysed according to Kozich et al. (2013), as described in the supporting information. A distance matrix was created and operational taxonomic units (OTUs) were clustered at 3% genetic distance using the average neighbour algorithm (Schloss et al., 2011). Sequences were classified using the Naive Bayesian Classifier (Wang et al., 2007) and the SILVA database (SSURef NR99 119). Random subsampling was performed to normalize the data set to the sample with the lowest number of reads (i.e., 25 796 reads). Rarefaction curves, diversity indices (Shannon diversity, Simpson diversity), richness (Chao1, ACE) and coverage estimators (Good's coverage) were calculated using MOTHUR (Supporting Information Table S1).

Phylogenetic analysis
16S rRNA (gene) sequences were aligned using SINA (Arb et al., 02012). Phylogenetic analyses of 16S rRNA (gene) sequences were conducted with the ARB software package (Ludwig et al., 2004) and the database SSURef NR99 119 from ARB SILVA (Pruesse et al., 2007). Phylogenetic trees were calculated by maximum-likelihood method (PhyML) using full-length sequences. Partial sequences, i.e., representative OTU sequences, were inserted into the tree by parsimony criteria without allowing changes in the overall tree topology. Sequences classified as cable bacteria were further analysed using the probe match function implemented in ARB. Probe matches were conducted for probes DBS706 (5′-ACCGGATATCTCCCGGAT-3′) (Loy et al., 2002; Lücker et al., 2007) and ELF645 (5′-CCTCTGATACTCAAGCACAAG-3′) (Pfeffer et al., 2012).

Statistical analysis
Statistically significant differences (P < .05; P < .001) among the relative sequence abundance of bacterial 16S rRNA (genes) were determined using STAMP (v2.0.8) (Parks et al., 2014) applying multiple group tests (PCoA and ANOVA), described in the supporting information. Sequencing data were further explored using the PRIMER software (version 7.0.11; Primer-E, Plymouth, UK) with the PERMANOVA+ add-on to compare DNA (present community) and RNA (active community), as described in the supporting information. Spearman's rank correlation coefficient and multiple testing correction (Supporting Information Table S6 and Fig. S6) between active microbial groups (e.g., RNA-based Fe-cycling microorganisms and cable bacteria sequences of OTU0001; see Fig. 3) and geochemical data were determined as described in the supporting information.

Acknowledgements
We thank Franziska Schädler for sharing unpublished information on freshwater Fe(II)-isolates as well as Karin Stögerer (deceased), Wiebke Ruschmeier, Dr. Pascal Weigold and Kalen Rasmussen for the help in the laboratory and for assistance in the field as well as Jesper T. Bjer (University of Aarhus) for light microscopy images. We are also grateful to Saskia Ruhgött, Dr. Jing He, Dr. Casey Bryce (University of Tübingen), Prof. Ralf Otte (University of Ulm) and Nicole Geerlings (University of Utrecht) for helpful discussions and comments. We additionally thank Prof. Ivan Berg (University of Münster) for providing archaeal DNA for the MOCK culture. This project was supported by the European Research Council under the European Union’s Seventh Framework Program (FP/2007–2013)/ERC Grant, agreement no. 307320-MICROFOX. S.K. is supported by an Emmy-Noether fellowship (grant # 326028733) from the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG).

Conflict of Interest
The authors declare no conflict of interest.

References


radicicola gen. nov. sp. nov., and Sideroxydans paludicola sp. nov. Geomicrobiol J 24: 559–570.


Supporting information

Additional supporting information may be found in the online version of this article at the publisher’s website:

**Fig. S1.** Absolute abundance of bacteria and archaea based on qPCR analysis specific for 16S rRNA genes (based on DNA).

**Fig. S2.** Absolute abundance of bacteria based on qPCR analysis specific for 16S rRNA (based on RNA).

**Fig. S3.** PCoA-Plots for the (A) marine and (B) freshwater sediment community showing clear differences of RNA and DNA sequence analyses and differences of the two marine sampling sites (Norsminde Fjord and Kalø Vig).

**Fig. S4.** Taxonomic identification of the microbial communities in Kale Vig, Norsminde Fjord and Lake Constance sediments based on 16S rRNA (gene) amplicon analysis comparing DNA- and RNA-based findings. Data were averaged among sediment depth layers and replicate cores.

**Fig. S5.** Microscopic images of cable bacteria from Aarhus sediment provided by Jesper T. Bjerg (Aarhus University).

**Fig. S6.** Comparison of Spearman’s rank correlation values of Fe-cycling bacteria and cable bacteria from Kale Vig and Norsminde Fjord.

**Fig. S7.** Sediment cores from Norsminde Fjord, Kalø Vig and Lake Constance.

**Table S1.** Microbial diversity indices for all sampling sites (Chao index, Shannon index, Simpson index).

**Table S2.** Most abundant genera of Norsminde Fjord, Kalø Vig and Lake Constance as well as implications for metabolic processes.

**Table S3.** Abundant present (DNA based) and active (RNA based) genera of Norsminde Fjord, Kalø Vig and Lake Constance sediment.

**Table S4.** Overview of iron-metabolizing microorganisms that were analysed in this study.

**Table S5.** Overview of iron-metabolizing isolates from (A) Kale Vig and Norsminde Fjord from Laufer et al., 2016, as well as from (B) Lake Constance sediment (personal communication with Franziska Schäder, University of Tübingen).

**Table S6.** Spearman correlation test with Fe(II)-oxidizers and Fe(III)-reducers from (A) Kale Vig, (B) Norsminde Fjord and (C) Lake Constance sediment.