

Contribution of bacteria to redox potential (E_h) measurements in sediments

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Received: 25 October 2011 / Accepted: 14 June 2012 / Published online: 11 July 2012
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Abstract Increasing evidence suggests that bacteria are capable of creating specific redox conditions which are visible as species-specific continuous redox potential (E_h) measurements. It has been demonstrated that continuous measurements of E_h are valuable for tracking bacterial metabolic activities of bacterial species in liquid cultures. However, it remains uncertain whether this phenomenon is widespread among bacterial species and whether E_h measurements reflect similar mechanisms in more complex systems such as soils and sediments. The purpose of this study is therefore to evaluate whether bacteria that naturally occur in sediments have the capacity to control E_h and assess the relative partitioning of biological processes involved in E_h in natural sediments. To this end, continuous E_h measurements are linked to growth of bacteria in liquid cultures and bacterial metabolic activity in aquatic sediment microcosms containing the bioturbator *Tubifex* spp., in which we evaluate bacterial partitioning in microcosms treated with the bactericide formalin. The tested bacterial species (*Micrococcus luteus*, *Paracoccus pantotrophus* and *Aminobacter aminovarans*) appeared to have specific stable E_h signals during linear-exponential growth phase, suggesting that these species are capable of exerting an extracellular control on E_h measurements, thereby supporting the notion that species-specific E_h

signals may be widespread among bacterial species. Formalin treatment reduced temporal variability of E_h in sediment microcosms. This outcome suggests that bacterial metabolism and inherent relative contributions of members of bacterial community principally determine development of E_h in sediment systems and that quantitation of sediment electrochemical properties may offer a potential indicator that characterizes bacterial processes.

Keywords Aquatic sediments · Extracellular · Microbial community · Redox potential · Species-specificity · *Tubifex* spp.

Introduction

Increasing evidence suggests that bacteria are capable of creating redox conditions which are visible as species-specific continuous E_h measurements (Brasca et al. 2007; Reichart et al. 2007; Michelon et al. 2010; Ignatova et al. 2010; Tachon et al. 2010). It has been demonstrated that continuous measurements of E_h are valuable for tracking bacterial metabolic activities in liquid cultures (e.g. Escalante-Minakata et al. 2009; Rosu et al. 2010), in which mixed bacterial assemblages may generate a composite redox potential where fluctuations reflect changes in relative metabolic activities of the different species within these assemblages (Escalante-Minakata et al. 2009; Rosu et al. 2010). This suggests the presence of an apparent redox niche, which is supported by the early observation of redox-taxis in a number of bacterial species (e.g. Grishanin et al. 1991; Bessalov et al. 1996). This phenomenon has been demonstrated for a limited number of bacterial species, but may be more widespread than previously anticipated (Rabaey et al. 2007; Reichart et al. 2007).

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In more complex systems such as soils and sediments, measurements of E_h are generally linked to concentrations of terminal electron acceptors involved in bacterial respiration (e.g. oxygen, nitrate, manganese et cetera). In this, E_h is formally defined by the Nernst equation and thereby carries an erroneous assumption that redox reactions in a system are reversible, complete and in equilibrium, while many reactions in nature are neither complete nor reversible. This approach does not consider the energetically flexible bacterial metabolites that serve as redox intermediates, while it has recently been demonstrated that bacterial-derived extracellular metabolites can dominate redox measurements under culture conditions (Xie et al. 2010). Numerous studies were not able to couple redox potential and concentrations of terminal electron acceptors in both soils and sediments, and uncertainties exist over the extent to which reactions participating in E_h are biologically mediated (Smayda 1990; Roden et al. 2004; Naudet and Revil 2005). It thus remains uncertain whether measurements of E_h in sediments accordingly reflect bacterial metabolism, suggesting that the relative contribution of bacteria in E_h measurements in sediment systems requires further evaluation.

The aim of this study is therefore to evaluate whether bacteria that naturally occur in sediments have the capacity to create species-specific E_h conditions and assess the relative partitioning of biological processes involved in E_h in sediments. To this end, continuous E_h measurements are linked to growth of bacteria in liquid cultures and bacterial metabolic activity in aquatic sediment microcosms.

Materials and methods

Redox potential development in culture media

In order to test whether organotrophic bacteria that naturally occur in aquatic sediments have species-specific effects on redox potential, the taxonomically and metabolically distinct bacterial species *Micrococcus luteus* (strictly aerobic), *Paracoccus pantotrophus* (facultative aerobic, denitrifier) and *Aminobacter aminovorans* (strictly aerobic) (obtained from the Netherlands Culture Collection of Bacteria, NCCB, originally sampled from aquatic sediments) were cultured in triplicate in brain heart broth (MERCK) and peptonised milk nutrient (Sigma) (ratio 100:15) with continuous stirring. Bacterial growth was monitored by measuring the absorbance at 600 nm (Shimadzu 1601-UV), and continuous redox potential development was measured every minute using permanently installed redox microelectrodes and dataloggers described recently (Vorenhout et al. 2011). After 40 h,

formalin was added (final concentration: 2 %) in order to disrupt bacterial activity and monitoring of E_h development continued.

Redox potential in aquatic sediments

Species-specificity of E_h conditions in subsurface sediment layers (1–2 mm depth) was evaluated (measured every minute) in sediment microcosms (Ø 25 mm) consisting of 17.5 g quartz. As organic matter, we used 3 g nettle powder (*Urtica dioica*) since this plant is easily degradable and often dominates the riparian zones of temperate aquatic systems. Treatments included inoculums of single bacterial species, in which sterile microcosms were used as negative control.

In order to test the relative partitioning of bacteria-mediated control on redox potential measurements, we reconsidered E_h in relation to bacterial activity in microcosms as described recently (Hunting and Van der Geest 2011). In brief, organic rich sediment was collected from Lake Drontmeer, The Netherlands (52°58'N, 5°50'E) for natural bacterial communities and organic matter. The collected sediment was frozen in order to kill fauna and thawed to allow for bacterial re-growth. Natural sediments were subsequently mixed with clean river sand (ignited quartz; grain size 0.5–1.0 mm; ratio 1:5). This mixture was homogenized and oxygenated prior to experimentation. Experiments were performed in a circular polyvinyl chloride core which allows for continuous monitoring of sediment without disturbance. The core (110 mm diameter, 250 mm height) has 7 gastight connectors for bacterial probes alongside the redox electrodes containing a Nuclepore polycarbonate membrane. The membrane acts as semi-permeable membrane (mesh size 5 µm) to sample porewater containing bacterial cells while retaining particles and flocs. Redox potential (E_h) was measured at various depths (0, 10, 20, 30, 40, 90 mm; every 15 min.) using permanently installed platinum electrodes and a calomel reference electrode (Vorenhout et al. 2004). Measurements of parameters in cores commenced immediately after homogenization, and continued toward the end of the experimental runs at fixed time-intervals over a period of 4–5 days. Sediments were allowed to reach steady state conditions (24–48 h) and were subsequently irrigated and reworked by the introduction of the aquatic oligochaete *Tubifex* spp. (approx. 500 individuals/m²). Bacterial activity was determined by sampling pore water (400 µL) alongside the platinum electrodes on a daily basis at fixed times and immediately assayed for bacterial activity as described below. The relative participation of bacterial activity on E_h development was assessed in bulk sediment mixed with dilute formaldehyde (final concentration 0.46 ± 0.1 %) in order to stop bacterial activity,



while preventing contribution of the formaldehyde/methanol redox couple. Sediment was spread over 0.5 m² trays to minimize depth (1–2 mm) for 48 h in a flow cabinet prior to experimentation to allow evaporation of uncomplexed formalin. Dilute formaldehyde was used as this appears the most suitable treatment to inhibit long term bacterial activity with minimal increase of dissolved nutrients (Tuominen et al. 1994) and preliminary runs revealed that *Tubifex* spp. could survive formalin treated conditions for at least 48 h.

Analytical techniques

Dissolved oxygen concentrations were monitored using an oxygen micro-sensor (Unisense OX25) connected to a picoammeter (Unisense, PA2000). Bacterial activity was analyzed by dehydrogenase enzyme activity via the reduction of 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT) to formazan (INTF), since INT reduction proved an accurate assay of electron transfer system activities (ETSA) under both anaerobic and aerobic conditions (Smith and Mcfeters 1997; Hunting et al. 2010). Bacterial cells were simultaneously sampled alongside the platinum electrodes (400 μ L porewater sample). The samples were vortexed and incubated at 20 °C for 30 min. Bacterial cell integrity was subsequently disrupted by 5 min sonication at room temperature (Branson, 1510). An aqueous INT solution (200 μ L; 6.2×10^{-2} M) was added and the samples were mixed and incubated for 1 h at 20 °C under dark room conditions. Enzymatic activity was stopped by adding 500 μ L of acetone and measured spectrophotometrically at 490 nm (Shimadzu, 1601-UV). An INT formazan standard was used for calibration, in which two protons equal the transfer of a single electron. Controls treated with formalin were included to correct for abiotic reduction of INT.

Results and discussion

Monospecific cultures and microcosms

Continuous redox potential (E_h) development of the individual bacterial species and their corresponding growth are presented in Fig. 1a–d. E_h becomes stable during continuous and exponential growth phase, but becomes variable at the onset of stationary bacterial growth (Fig. 1a–d, left panel).

Redox values of individual species during exponential growth and the corresponding standard deviation of the replicates are shown in Table 1. The redox measurements were similar in both liquid cultures and mono-specific sediment microcosms, although sediment microcosm

appeared more variable among replicates compared to liquid cultures. However, the reported variations correspond well with species-specific E_h variations as described previously (Brasca et al. 2007). Subsequent addition of formalin in order to stop bacterial metabolism at the progressive stage of bacterial growth reduced variability in E_h measurements (Fig. 1a–c, right panel). This outcome demonstrates the capacity of the tested bacteria to mediate E_h measurements.

Sediment microcosms with natural bacterial communities

Bacterial activity was generally most pronounced at 1 cm depth and often decreased with increasing depth in deeper layers of the sediment, yet occasionally peaked at a depth of 4 or 9 cm. Incubation of sediment with dilute formaldehyde decreased the bacterial activity to below the limits of detection (Fig. 2b). At the end of the experiment, bacterial activity was detected at the surface layers of the sediment microcosms (Fig. 2b), coinciding with reducing activity at the surface layer (Fig. 4b).

Oxygen generally penetrated to 10 mm depth with highest concentrations at the sediment surface (237 μ M/L \pm 1), although rapidly decreasing after a few millimeters (Fig. 3a). Addition of *Tubifex* spp. at 48 h resulted in a delayed increased penetration of dissolved oxygen (Fig. 3a). Treatments with dilute formaldehyde did not differ, as oxygen penetration depth also increased after the addition of *Tubifex* spp. (Fig. 3b).

Redox potential (E_h) ranged from –470 to 340 mV in depth and showed some variation, especially after the introduction of *Tubifex* spp. (Fig. 4a). E_h in sterilized sediments ranged from –80 to 10 and showed minimal variation over depth which was constant over time (Fig. 4b). At the end of the experimental period, reducing activity was visible at the surface layers (Fig. 4b). Overall, these results suggest a dominant partitioning of bacterial activity in the dynamics of E_h (cf. Figs. 2 and 4), and that measurements of E_h did not co-evolve with oxygen measurements (cf. Figs. 3 and 4).

Implications

This study considered high resolution temporal redox potential measurements in relation to bacterial growth and redox activity in liquid culture conditions and sediment systems. In liquid cultures, redox potential measurements slowly developed during the initial stages (lag phase) of bacterial growth and became stable during the continuous and exponential growth phase of the tested organotrophic bacterial species, suggesting that bacteria extracellularly mediated E_h measurements during continuous growth and



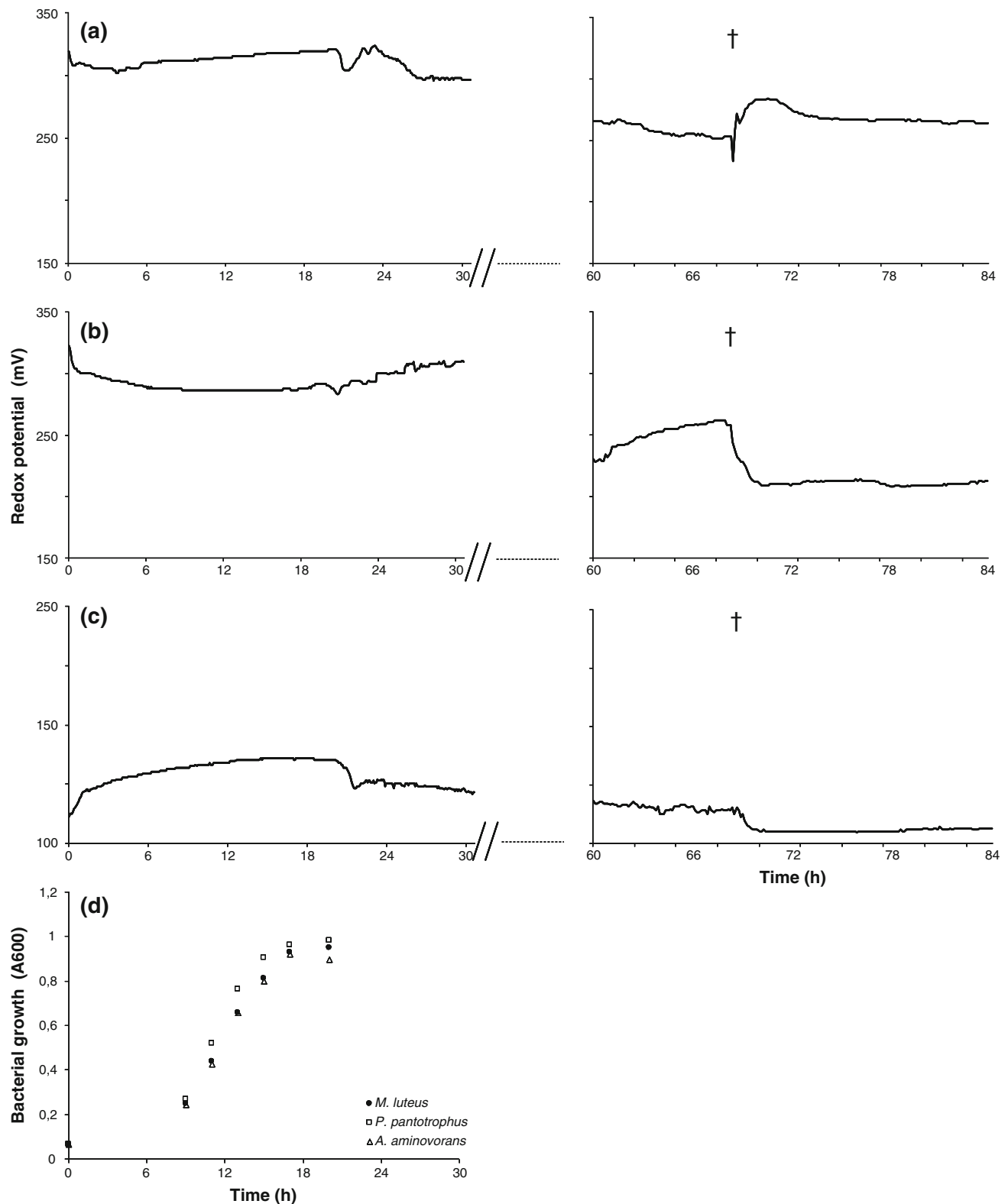


Fig. 1 Development of redox potential E_h during growth of the organotrophic bacteria **a** *M. luteus*, **b** *P. panthotrophus*, **c** *A. aminovorans* in culture conditions. Development of E_h can be compared to bacterial growth (**d**). One of the replicates is shown for clarity. It is evident that E_h measurements become stable during continuous and

exponential growth of bacteria, and becomes variable during stationary growth. Dagger symbol indicates addition of formalin resulting in stabilization of E_h measurements, thereby reflecting relative partitioning of bacteria-mediated processes in measurements of E_h



Table 1 Average species-specific redox potentials based on the stable E_h period in liquid monocultures and sediment cores

Bacterial species	Average redox potential, E_h (mV) mean \pm SD	
	Liquid cultures	Sediment microcosm
Sterile control	304 \pm 24	270 \pm 15
<i>Micrococcus luteus</i>	332 \pm 30	308 \pm 38
<i>Paracoccus pantotrophus</i>	304 \pm 34	280 \pm 43
<i>Aminobacter aminovorans</i>	142 \pm 21	138 \pm 22

Provided are mean \pm SD ($n = 3$)

utilization of the available substrate. This corroborates redox development described for other bacterial species (e.g. Brasca et al. 2007; Reichart et al. 2007; Tachon et al. 2010). At the onset of the stationary growth phase, redox potential measurements became more variable. The stationary growth phase is typically characterized by a

balance between bacterial lysis and synthesis (Roszak and Colwell 1987), and therefore fluctuations in E_h likely reflect changes in culture media caused by the release of redox active compounds during bacterial lysis. This becomes evident when the bactericide formalin is added to the cultures, as this disrupted bacterial metabolism, synthesis and lysis, resulting in stable signals of E_h for a significant period. During this period, E_h differed between the different bacterial species. A mechanistic understanding of this observation remains uncertain and is subject for future research. Although we only used a limited number of (facultative) aerobic bacteria, these findings seem to suggest that bacteria that naturally occur in aquatic environment actively control redox conditions. This supports the notion that this phenomenon may be more widespread than previously anticipated (Reichart et al. 2007).

Electrical properties of bacterial communities are often attributed to extracellular electron shuttling by bacteria

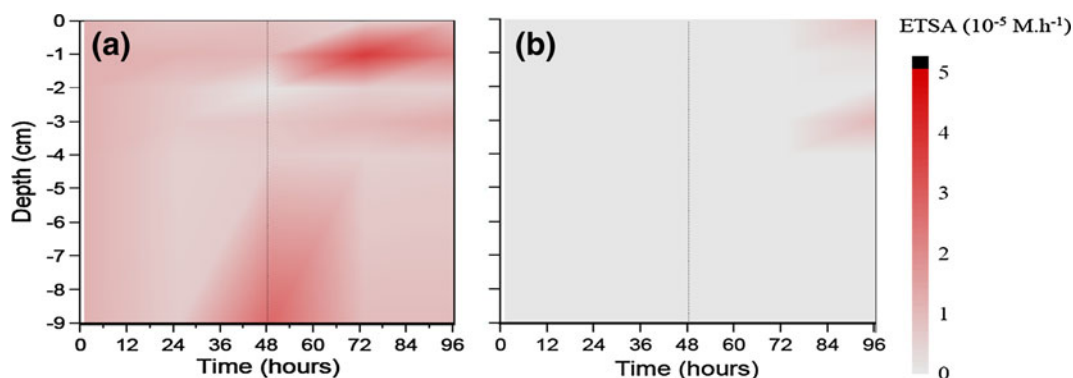


Fig. 2 Bacterial electron transfer activity/Dehydrogenase activity (ETSA) in **a** natural sediments and **b** sterilized sediments (0.48 % dilute formalin). Arrow indicates addition of the sediment reworking *Tubifex* spp. One representative replicate is shown

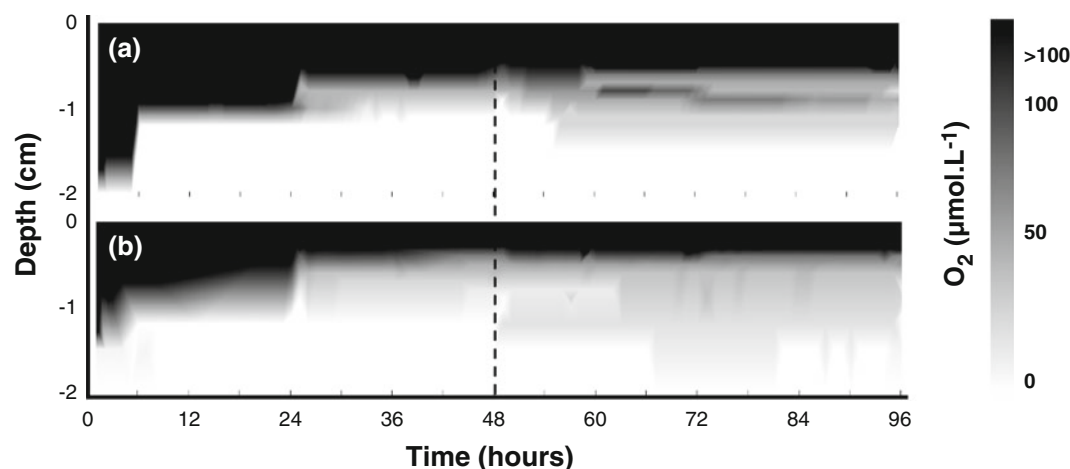


Fig. 3 Oxygen concentration in sediment microcosms in **a** natural sediments and **b** sediments treated with dilute (0.48 %) formalin. *Tubifex* spp. was added after 48 h (indicated with dotted line) and

resulted in an increased oxygen penetration depth in both treatments. One representative replicate is shown



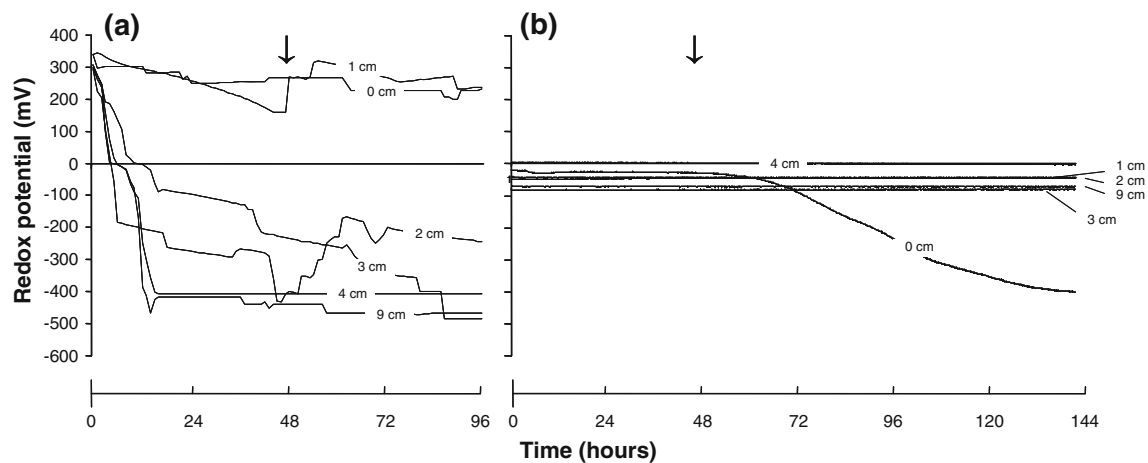


Fig. 4 Effect of sterilization on measurements of redox potential (E_h). Continuous E_h measurements in **a** untreated sediments and **b** sediments treated with dilute (0.48 %) formalin. Arrows indicate

addition of *Tubifex* spp. Absence of fluctuation in **b** reflects bacterial partitioning in E_h fluctuations in **a**. One replicate is shown for clarity

(e.g. Rabaey et al. 2007), and we suspect that a similar mechanism is responsible for the observed redox conditions in our study. A variety of mechanisms have been identified for extracellular electron shuttling and production of extracellular redox mediators in microbial communities. These include membrane bound pili, cytochromes and electron transport systems, and also the secretion of electron transport system related redox mediators such as cytochrome b/c, flavins and quinones in the extracellular matrix that either remain soluble or bind to electrodes (e.g. Rabaey et al. 2007; Lovley 2008; Marsili et al. 2008; Tachon et al. 2010; Juang et al. 2011). These compounds are produced in order to dispose respiratory electrons in the absence of terminal electron acceptors in the immediate environment, allowing electron shuttling to distant terminal electron acceptors (Rabaey et al. 2007; Xie et al. 2010). Since a number of redox mediators exist, it is difficult to predict which mechanisms would be potentially involved in our test systems, and this remains a central area for future research.

Bacteria were capable of exerting similar effects on redox potential in sediment microcosms as compared to liquid cultures. This suggests that bacteria are capable of creating apparent redox niches, irrespective of the medium. This result corroborates earlier findings of species-specific redox conditions and redox niche preferences as observed by the redox-tactic behavior of motile bacteria (e.g. Grishanin et al. 1991; Bessalov et al. 1996), but more importantly highlights the importance of redox niche construction. These phenomena are currently overlooked within microbial ecological theory and experimental design of studies manipulating bacterial assemblages.

In our natural sediment microcosms, bacterial activity and E_h evolved in a parallel fashion. The addition of the

sediment reworking oligochaete *Tubifex* spp. resulted in an increased oxygen penetration depth, increased redox potentials and bacterial activities in deeper layers of the sediment, as previously reported by (Davis 1974). Reduction of bacterial activity with dilute formalin washed out temporal fluctuation in E_h , including the momentum of sediment reworking by *Tubifex* spp., while oxygen penetration depth increased after the addition of *Tubifex* spp. This suggests that fluctuations in E_h measurements in untreated cores were principally mediated by shifts in the relative metabolic activity of members of the bacterial community. It has been demonstrated that microbial processes play a significant role in the electrolytic and interfacial geoelectrical properties of sediments (e.g. Atekwana et al. 2004). Previous reports on the contribution of bacterial activity on E_h measurements, however, remain contradictory, as changes in sediment E_h have been previously attributed to both biotic and abiotic influences (e.g. Smayda 1990; Roden et al. 2004; Naudet and Revil 2005). Measurements of E_h depend on concentrations of redox couples, but the contribution of redox couples to E_h highly depends on electrode affinity, exchange density and kinetics at the surface of electrodes, in which E_h measurements may be dominated by a single redox couple (Peiffer et al. 1992). Although oxygen is often considered to be a dominant redox active compound in the interpretation of E_h , an increased penetration depth of oxygen did not result in shifts in E_h . This corroborates the outcome of other studies that evaluated E_h in relation to removal of oxygen, suggesting that the reduction of oxidizing compounds other than oxygen can be important (Grenthe et al. 1992; Tachon et al. 2010). Taken together, measurements of E_h in complex natural systems seem to reflect the



metabolic activity of resident bacterial communities rather than concentrations of terminal electron acceptors in our microcosms. This complements previous studies that linked E_h to bacterial activity and community structure (Bertics and Ziebis 2009; Hunting and Van der Geest 2011; Hunting et al. 2012). Although it cannot be ruled out that E_h measurements may still be overruled by redox couples unrelated to bacterial activity, quantitation of the electrochemical properties of the sediment may offer a potential indicator of bacterial processes in sediment systems.

This study aimed to test whether bacteria that naturally occur in aquatic sediments are capable of mediating redox potential, E_h , and evaluate the relative contribution of bacteria-mediated activity on measurements of E_h in natural sediments. Our results indicate that bacteria mediate redox conditions in both liquid cultures and sediment microcosms, and that fluctuations in E_h can be minimized upon sterilization. This outcome illustrates the principal contribution of bacterial metabolism to redox potential measurements in more complex systems such as soils and sediments.

Acknowledgments The authors express their gratitude to the anonymous referees for their constructive comments on earlier drafts of this article.

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