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Dissolved organic matter (DOM) enhances the competitiveness of weak exoelectrogens in a soil electroactive biofilm

Baoli Qin[1,2†], Yu Huang[1,3†], Tongxu Liu[1], Yundang Wu[1,4*] and Fangbai Li[1]

Abstract

Dissolved organic matter (DOM) as critical redox active soil carbon plays a crucial role in shuttling electrons between bacteria and solid electron acceptors, such as iron oxides. However, research on DOM as an electron shuttle has traditionally focused on its impact on typical iron-reducing bacteria, namely strong exoelectrogens, like *Geobacter*. Besides these strong exoelectrogens, there is a significant presence of weak exoelectrogens in the soil, but studies examining how DOM affects their survival and competitiveness are lacking. This study focused on exploring the influence of DOM on weak exoelectrogens like *Bacillus* in the soil. By utilizing soil-bioelectrochemical systems (s-BESs) to enrich soil electroactive microorganisms, it investigated the relationship between the abundance of strong and weak exoelectrogens under conditions rich in DOM and conditions lacking DOM. The results showed that in the rich DOM treatment, the abundance of *Geobacter* was relatively lower ($12 \pm 0.5\%$ vs. $41 \pm 3\%$), and there was a significant negative correlation between the abundance changes of 18 weak exoelectrogens and *Geobacter*. This suggests that DOM caused a decrease in the population of strong exoelectrogens (e.g., *Geobacter*) while simultaneously promoting the growth of weak exoelectrogens (e.g., *Bacillus* and *Sedimentibacter*). Based on this, we propose that DOM, acting as an electron shuttle, creates favorable ecological niches for the thriving and propagation of weak exoelectrogens, enhancing their competitiveness within the microbial community. This new understanding provides deeper insights into the significance of DOM electron shuttling in soil microbial ecology, and raises the question: is the role of weak exoelectrogens in soil iron cycling underestimated due to the existence of DOM?

Highlights

- s-BESs reveal the critical role of DOM in altering the electroactive microbial community.
- DOM boosts the abundance of weak exoelectrogens such as *Bacillus* and *Sedimentibacter* in soil biofilms.
- The abundance changes between strong and weak exoelectrogens show a negative correlation.
- This underscores the often-overlooked significance of weak exoelectrogens in soil.

Keywords Electron shuttle, *Geobacter*, Electroactive bacteria, Soil microbial community, Weak exoelectrogens
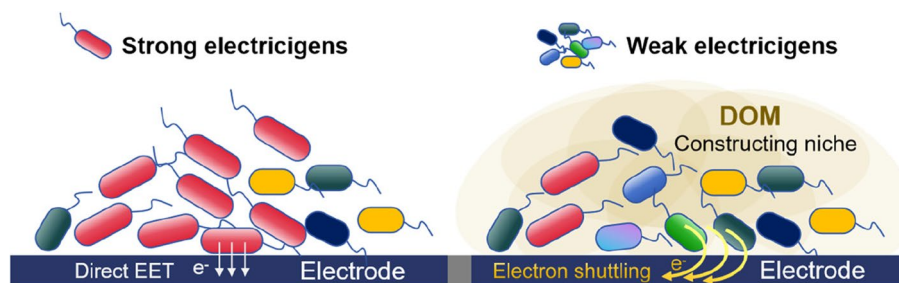
†Baoli Qin and Yu Huang contributed equally to these work.

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*Correspondence:
Yundang Wu
ydwu@soil.gd.cn
Full list of author information is available at the end of the article

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Graphical Abstract

1 Introduction

Extracellular electron transfer (EET) is known to be the key process of microbial anaerobic respiration (Kumar et al. 2017; Shi et al. 2016). Electrons were transferred from intracellular to extracellular, resulting in a reduction and dissolving of solid mineral (Lovley et al. 1987; Myers and Nealson 1988). Because of their electrochemical activity, these bacteria with EET capability were also classified as electroactive microorganisms (Logan et al. 2019). The microbial EET dramatically influenced the desorption and migration of toxic metals in soil (Brose and James 2010; Luan et al. 2014; Ohtsuka et al. 2013), and thus gave extracellular respiratory bacteria an important biogeochemical significance.

Dissolved organic matter (DOM) is critical redox active carbon in soil (Zsolnay 2003). In the past decades, the mechanism of how DOM played the role for shuttling electrons between bacteria and solid electron acceptor (e.g., iron oxides) has been revealed (Lovley et al. 1998; Lovley et al. 1996; Zhang and Weber 2009). The active groups on the surface of DOM, such as quinones, act as electroactive sites, accelerating microbial EET (Fimmen et al. 2007; Roden et al. 2010). However, research on DOM as an electron shuttle has traditionally focused on its impact on typical iron-reducing bacteria. These typical iron-reducing bacteria, such as *Geobacter*, are categorized as "strong exoelectrogens" (Doyle and Marsili 2018). Yet, in soil, there exists a high abundance of "weak exoelectrogens", such as *Bacillus* (Aiyer and Doyle 2022; Doyle and Marsili 2018; Eghtesadi et al. 2022). They may not have the ability to transfer electrons directly to extracellular solid electron acceptors, but they likely display a potential to transfer electrons to soluble electron shuttles, such as flavin (Light et al. 2018; Wu et al. 2014a). Even though their presence has been noted in several previous reported 16S rDNA results, researchers have not delved into investigating the role they play in the system, often overlooking their significance (Lovley and Holmes 2022).

Weak exoelectrogens often lack a typical EET chain on cell membrane, making them more reliant on electron shuttles (Light et al. 2019). Therefore, we hypothesize that the presence of DOM may enhance their competitiveness within the microbial community and stimulate them to exhibit strong electrochemical activity. Due to their diverse presence in soil (Harwood et al. 2013), their significance in processes like iron reduction has likely been underestimated.

Recent studies have provided further theoretical support for the above hypothesis. It is reported that Gram-positive bacteria, categorized as weak exoelectrogens, heavily rely on the shuttle process for extracellular respiration (Light et al. 2018). Additionally, *Shewanella*, though a typical electroactive microorganism, has dramatically weaker direct electron transfer compared to *Geobacter*. Our recent research shows that electron shuttles significantly boost *Shewanella* biofilm formation, enhancing its competitiveness (Qin et al. 2020; Wu et al. 2020). These reports supported the idea that shuttles may selectively enhance certain weak exoelectrogens. Furthermore, recent study shows that lipophilic shuttles can directly interact with microorganisms lacking outer membrane electron transfer proteins, aiding in their EET (Wu et al. 2023). This suggests that shuttles may have the potential to selectively increase the abundance of weak exoelectrogens with incomplete electron transfer chains, while DOM also contains some lipophilic components. In soil, microbial community structure is shaped by interactions between microbes and environmental factors. Based on the above analysis, the role of DOM as an electron shuttle in constructing niches suitable for weak exoelectrogens appears to be crucial yet remains unrevealed.

To study the impact of DOM on strong and weak exoelectrogens, we constructed a soil-based bioelectrochemical system (s-BES) (Wu et al. 2016) for precise electroactive microorganism enrichment from soil. We examined how DOM affects bioelectricity, biomass, and

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biofilm microbial communities by comparing conditions with rich DOM and conditions lacking DOM. Our main goal was to understand how DOM influences the dynamics of strong and weak exoelectrogens, shedding light on their competitive interactions. This study combines the electron shuttle theory with the interactions within soil microbial communities, revealing a new mechanism by which soil active carbon enhances the competitiveness of weak exoelectrogens. This new mechanism, with potential applications in soil microbial regulation—i.e., synthetic microbiome engineering—holds high significance in soil carbon research.

2 Materials and methods

2.1 Materials
The paddy soil was collected from a rice field located at the South China Botanical Garden in Guangzhou, Guangdong Province. Soil sample was sealed and stored in a refrigerator at $-4°C$ after removing the rice straw and roots. Physical and chemical properties of the soil samples were measured using standard methods (Margesin and Schinner 2005), and the results included pH (5.66), organic matters (11.5 g/kg), cation exchange capacity (11.2 cmol/kg), conductivity (10.1 ms/m), total Fe^{3+} (2.12 g/kg), total Mg^{2+} (1.04 g/kg), total Ca^{2+} (2.0 g/kg), total K^+ (6.1 g/kg), total nitrogen (1.3 g/kg), and total phosphorus (1.16 g/kg). The soil soluble components were removed from the paddy soil by a washing operation (Fig. 1). Milli-Q water (500 mL) and soil (50 g) were added to a 1 L of centrifuge tube, centrifuged (7000 g, 5 min) after being shaken at 180 rpm for 20 minutes, and then the supernatant which was rich in soluble substances was discarded. The above operation was repeated three times. Then a soil lack of DOM was obtained. The treatments using were defined as the treatments lack of DOM. To ensure that soil in system of high and low concentration of DOM undergo the same treatment, the soil in treatment rich in DOM was also shaken and centrifuged. The only difference was that the supernatant was not discarded. 30 mM of piperazine-N, N'-bis(2-ethanesulfonic

acid) (PIPES) was used as the buffer to control the pH. All solutions were prepared using Milli-Q deoxygenated ultrapure water (18 M V cm, Easy Pure'II RF/UV, USA).

2.2 BES setup and operation
The s-BES was equipped with three electrodes on the top of the serum bottle that held the soil suspension. A calomel electrode was used as a reference electrode. Two pieces of carbon cloth (2 cm × 2 cm) were used as a working electrode and a counter electrode (Zhu et al. 2023). 10 g prepared paddy soil, 30 mM lactate and 30 mM, 100 mL PIPES were included in the s-BES. Lactate was used as electron donor; PIPES was the pH buffer. The solution was purged with N_2 for 50 min before sealing to ensure an anaerobic environment. The s-BESs were then incubated at a constant temperature of 30°C in the water bath under a 441 mV (vs. SHE) of external potential. All of the experiments were conducted in biological triplicates.

2.3 Electrochemical characterization
A fixed potential of 441 mV (vs. SHE) was applied to the BES and controlled by the electrochemical workstation (Wu et al. 2014b). Cyclic voltammetry (CV) tests were conducted using a CHI660D potentiostat (Chenhua Co., Ltd., China). Low scan rate cyclic voltammetry (1 mV s^{-1}, -359 to $+441$ mV) (Marsili et al. 2008b) was performed at day 0, 6, 10, and 12. Differential pulse voltammetry (DPV) was performed under the following specific conditions: pulse height, 50 mV; pulse width, 300 ms; step height, 2 mV; step time, 500 ms; scan rate, 4 mV s^{-1} (Marsili et al. 2008a). In addition, CV and DPV tests for soil soluble components were also conducted.

2.4 Biofilm morphology and quantification
The morphology of biofilms was examined on a scanning electron microscope (SEM). The electrode samples (1 cm × 1 cm) with biofilm were fixed in a 2.5% glutaraldehyde solution for 4 h, then washed in PBS solution,
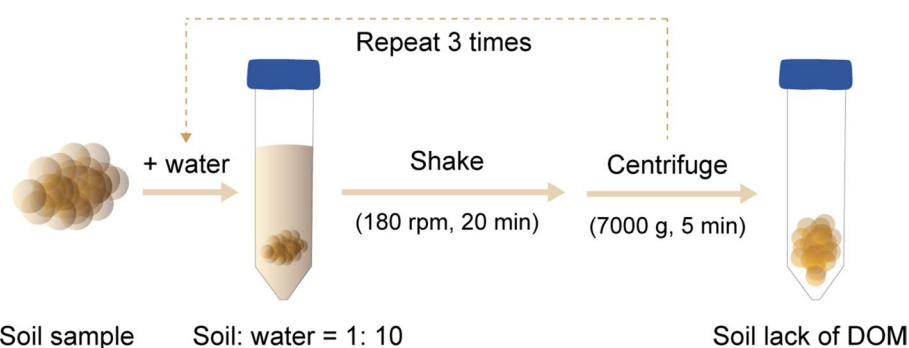

Fig. 1 Schematic diagram of soil pretreatment, used to remove soluble substances in the soil

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and dehydrated in a series of ethanol gradient solutions, and finally fixed in t-BuOH (Liu et al. 2019; Wu et al. 2016). The fixed samples were freeze-dried in a lyophilizer (Christ, Germany), then coated with evaporated platinum, and finally viewed by SEM (Phenom, Netherlands). The magnification of the SEM images for the biofilm is 5000 times. The biofilm sample was collected by cutting off the working electrodes. The total DNA of the biofilm was extracted using the PowerSoil DNA isolation kit (Mobio Laboratories, Inc., USA) and quantified with the Nanodrop 2000 (Thermo Scientific, USA). The DNA extraction procedure followed the protocol provided by the kit (Wu et al. 2020).

2.5 Microbial community analyzing
DNA of biofilm was stored in a refrigerator at $-80°C$ according with the preservation requirements for microbial samples. Illumina Miseq sequencing was conducted by Personalbio (Shanghai, China). QIIME software was used to analyze the bacterial α-diversity index of samples. The Chao1 index was the species richness index, which was used to estimate the number of OTUs in the community. Unweighted uniFrac nonmetric multidimensional scaling (NMDS) analysis using R software (version 3.4.4) was utilized to assess the differences in bacteria communities and their changes during two incubation processes. Cytoscape software (v. 3.2.1) was used to perform network analyses. The sequence data have been submitted to the NCBI Short Read Archive in Bioproject PRJNA1060427.

3 Results and discussion
3.1 Enrichment of electroactive biofilms from soil
To investigate the role of DOM in the formation of electroactive biofilms, electrodes with an applied positive voltage of 441 mV were immersed in soil solutions within BESs containing both low and high concentrations of DOM (Fig. 1). This allowed bacteria capable of EET in soil to potentially enrich on the electrode surface.

Figure 2 shows the comparison of bio-current production in s-BESs with low and high concentrations of DOM. The graph reveals that the current in both s-BESs began to increase around day 2 to day 3, and then it sharply increased, stabilizing at approximately 0.5 ~ 0.6 mA by day 5 to day 6. The increase in bio-current indicates that electroactive microorganisms have begun to grow on the electrode surface (Yee et al. 2020), signifying the selective enrichment of electroactive microorganisms on the electrode surface in the soil. However, the current in s-BESs rich in DOM continued to rise steadily after day 6, while the current in s-BESs lack of DOM remained relatively stable. By day 10, the current in the biofilm from s-BESs
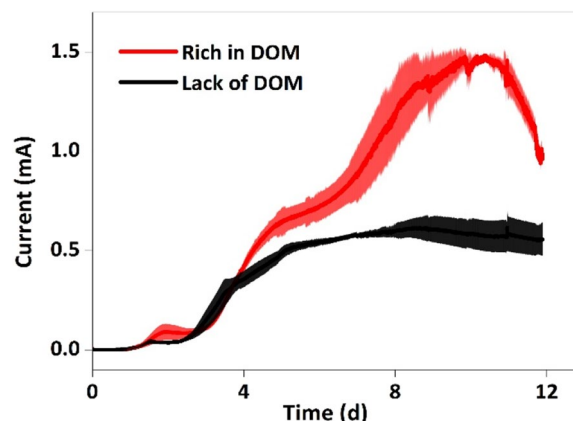

Fig. 2 Current generated by biofilms in the BESs rich in and lack of DOM. All current values are derived from the average of biological triplicates

rich in DOM was approximately 2.5 times higher than that in biofilm from s-BESs lack of DOM. According to previous understanding, this is primarily attributed to the role of DOM as the electron shuttle (Yang et al. 2021; Yuan et al. 2017), which enhances the EET of the microbial community. Subsequently, a decrease in current was observed at day 11, possibly due to the consumption of the carbon source (Zhao et al. 2009). The above results indicate that, through the construction of s-BESs, we successfully enriched electroactive microorganisms from the soil, and DOM had a significant impact on the bio-current generation of these microorganisms.

By the 12th day in this study, the current had begun to decline, indicating an impending depletion of the carbon source. To ensure that carbon source depletion would not compromise the results between the control and treatment groups, a 12-day study period was chosen for the biofilm study. Drawing from previous research, it has been established that a 12-day duration is sufficient for *Geobacter* to complete its growth on the electrode (Wang et al. 2024). Although longer cultivation periods may lead to further changes in microbial communities, 12 days fulfill the prerequisites for exploring the relationship between *Geobacter* and other weak exoelectrogens.

3.2 Morphology and quantification of the enriched electroactive biofilm
The morphology of the two biofilms over time was further examined via SEM (Fig. 3a). It shows that a few of cells were attached on the carbon electrode at day 6. Besides microorganisms, there was also a small amount of material with a morphology resembling soil particles adhering to the electrode surface. Then, thick biofilm was formed at day 10, and it is clear to find that most of the
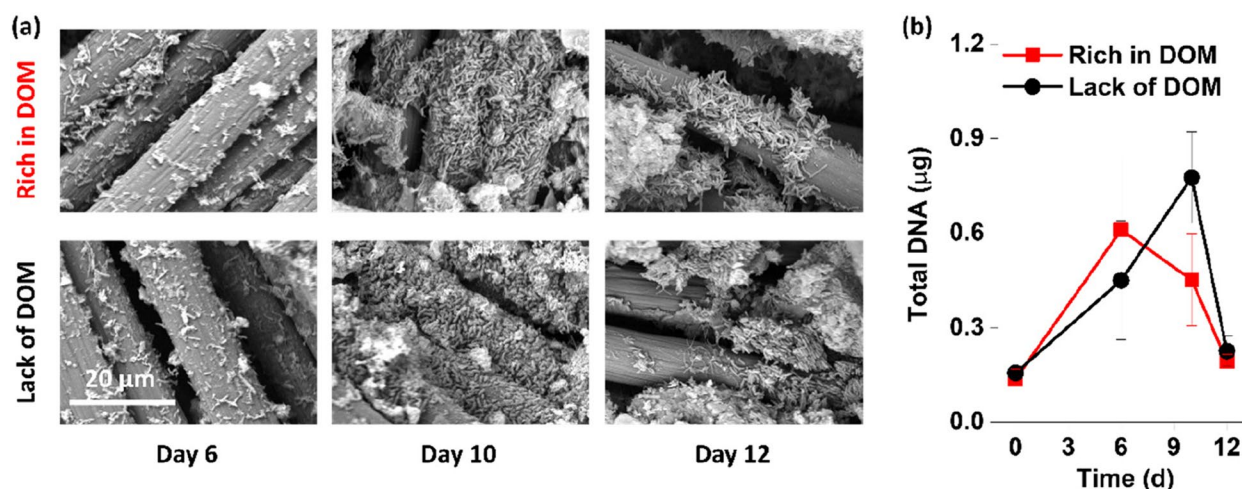
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Fig. 3 (**a**) SEM images of the electrodes with low (lack of DOM) and high (rich in DOM) concentration of DOM at the end of days 6, 10 and 12; (**b**) Concentration of biofilm total DNA on the electrodes over time

attached cells are rod-shape. The number of cells on the electrode visibly decreased at day 12, most likely due to the depletion of carbon sources, resulting in the detachment of cells from the biofilm (Flemming et al. 2016).

The biofilm was quantified by examining the total biofilm DNA over time. Figure 3b shows an increase in total biofilm DNA over time in both s-BESs, whether rich or lack of DOM. The total biofilm DNA reached its maximum at day 10 in the system with low DOM concentration, which was higher than that in the system with high DOM concentration. DOM did not increase the biofilm quantity, which differs from the phenomenon of electron shuttles accelerating the formation of electroactive biofilms in pure culture systems (Wu et al. 2020). This may be because that high concentration of DOM can act as an electron acceptor, supporting microbial growth in the solution, thereby delaying biofilm formation (Zhu et al. 2023). Subsequently, the DNA concentration decreased at day 12 in both systems, which is consistent with the results observed through SEM. Based on the trends in bio-current variations and total DNA changes in the biofilm, the period from day 0 to 10 is likely to represent the stages of biofilm attachment, reproduction, and maturation, while the period from day 10 to 12 should be associated with the dispersal stage. These results suggest that DOM has limited impact on the enrichment of soil electroactive biofilm in terms of biomass. This is probably because, although DOM may increase the growth rate of specific weak exoelectrogens as we hypothesized, some strong exoelectrogens may decrease due to competition for living spaces with these bacteria, which in turn makes

DOM have a limited impact on the total biomass of the soil biofilm.

3.3 Electrochemical analysis of the soil electroactive microorganisms

The CV (Fig. 4a) and DPV (Fig. 4b) were conducted at days 6, 10, and 12 to analyze the electrochemical properties of the enriched biofilms. The CV profile at day 6 appeared very similar between the treatments rich in and lack of DOM. Oxidized and reduced peaks were observed at approximately $+180\,mV$ and $-50\,mV$, respectively, indicating the presence of electroactive substances in the soil solution. This is consistent with previous findings that substances such as DOM in soil exhibit electrochemical activity, assisting microorganisms in electron shuttling (Aeschbacher et al. 2011).

Subsequently, the CV curve gradually transformed into a sigmoidal profile characteristic of s-BES rich in DOM at days 10 and 12, resembling the shape of CVs of previously reported electroactive biofilms, indicating the formation of a mature biofilm (Logan et al. 2006; Wu et al. 2020). In contrast, the CV profile in s-BES lacking DOM showed minimal changes on days 6, 10, and 12, despite an increase in peak current, suggesting a relatively lower electron transfer efficiency. DPV subtracts the background current, allowing for a clearer view of the oxidation-reduction potentials of the two reactions. Two DPV peaks at approximately $-126\,mV$ and $+218\,mV$ were observed in the s-BESs rich in DOM at day 10, but no peak was obtained in s-BESs lack of DOM. This further highlighted that in the presence of DOM, the enriched
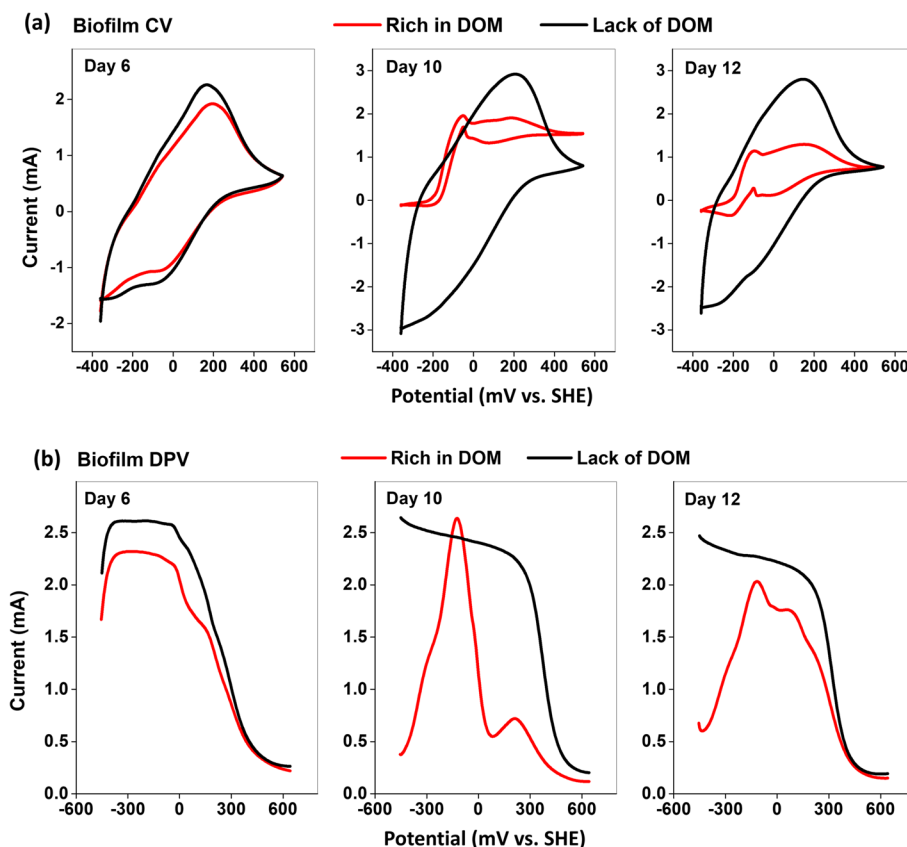
Fig. 4 (**a**) CVs and (**b**) DPVs of biofilms at day 6, 10 and 12 cultured with low and high concentration of DOM. Data are representative of the three replicates

electroactive biofilm demonstrates higher levels of electrochemical activity, making it more favorable for EET.

3.4 Analysis of microbial communities in the enriched biofilm

To further reveal the impact of DOM on strong and weak exoelectrogens, we analyzed the 16S rDNA of cells on the electrode on days 6, 10, and 12. The analysis of bacterial community composition in biofilms with high and low DOM concentrations revealed a total of 36 phyla (Fig. 5). The bacteria enriched on the electrode differed significantly from the bacterial community in the original soil samples (day 0). In the original soil, no specific bacterium accounted for more than 10% of the community. However, *Geobacter*, one of the most well-known strong exoelectrogens (Bond et al. 2012), was highly enriched on the electrode at day 6, constituting $83 \pm 2\%$ and $85 \pm 2\%$ of the community in the DOM-rich and DOM-lacking s-BESs, respectively. This indicates that in the early stages of the reaction, the electrode surface tends to preferentially enrich strong electron transfer bacteria.

Nevertheless, the abundance of *Geobacter* decreased to $45 \pm 9\%$ and $76 \pm 8\%$ at day 10, and further dropped to $12 \pm 0.5\%$ and $41 \pm 3\%$ at day 12. The abundance of *Geobacter* significantly decreased with the involvement of DOM ($P_{\text{t-test}} < 0.01$), as shown in the results at day 10 and 12. It implies a microbial community regulatory role of DOM on strong exoelectrogens. Apart from typical strong electron transfer bacteria like *Geobacter*, Gram-positive weak exoelectrogens such as *Sedimentibacter*, *Clostridium*, etc., dominated the biofilm microbial community. Gram-positive bacteria were often considered to have weak extracellular electron transfer abilities (Aiyer et al. 2023; Aiyer and Doyle 2023; Aiyer and Doyle 2022; Doyle and Marsili 2018). However, the results of this study indicate an increase in the abundance of these bacteria in a DOM-rich culture medium, suggesting that DOM may have a favorable promoting effect on the extracellular respiration of some Gram-positive bacteria. In addition, although the *Geobacter* abundance on the electrode decreased over time in both systems with high and low concentrations of DOM, the bio-current production, as shown in Fig. 2, continued to increase. This indicates that other microorganisms also significantly contribute to the production of bio-current. This result aligns with our hypothesis, as the decrease in *Geobacter*
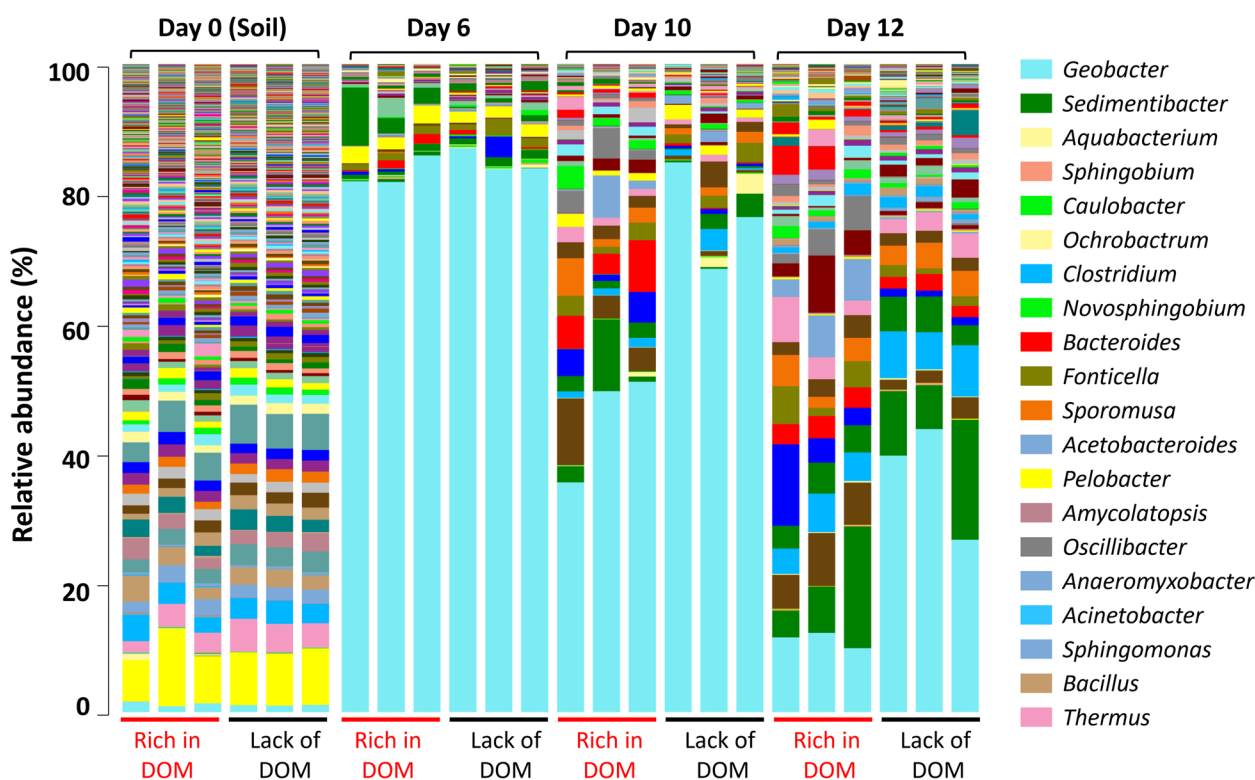
Fig. 5 Characterization of the bacterial community structure in the biofilm with high and low concentration of DOM. DNAs of biofilm were collected at the end of day 6, 10 and 12 by destructive sampling
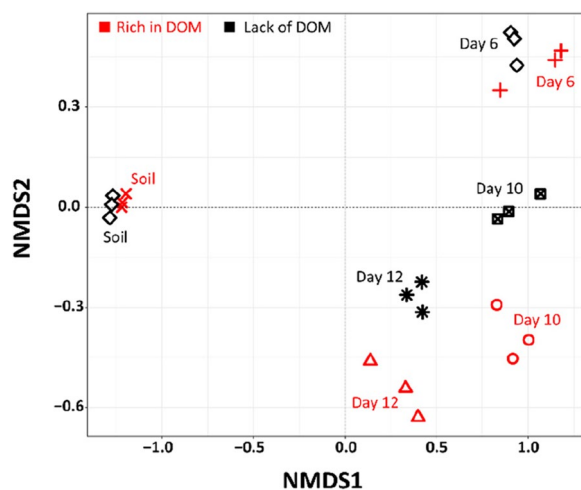

Fig. 6 Analysis of beta diversity in biofilms of s-BESs with high and low concentration of DOM. Bray–Curtis distance plotted as an NMDS plot

analysis in Fig. 6 demonstrated that the initial soil samples in s-BESs with and without DOM were quite similar. However, sample clusters in DOM-lacking and DOM-rich BESs began to separate on day 6. By day 10 and 12, the separation became even more pronounced. During the biofilm growth process, microorganisms can produce a considerable amount of extracellular polymers, which also fall under the category of organic matter. Although these extracellular polymers may also have further impact on the microbial community, the significant differences observed between the control and treatment groups in this study have already highlighted the crucial role of the original soil DOM in shaping microbial communities. These results show the significant differences in community composition between the two systems.

3.5 Analysis of the relationship between strong and weak exoelectrogens

In order to further clarify the relationship between strong exoelectrogens and weak exoelectrogens in the enriched biofilm, we conducted a relevant network analysis of the changes in the microbial community (Fig. 7). The network was constructed by applying Spearman's correlation with a threshold greater than 0.6 and ensuring
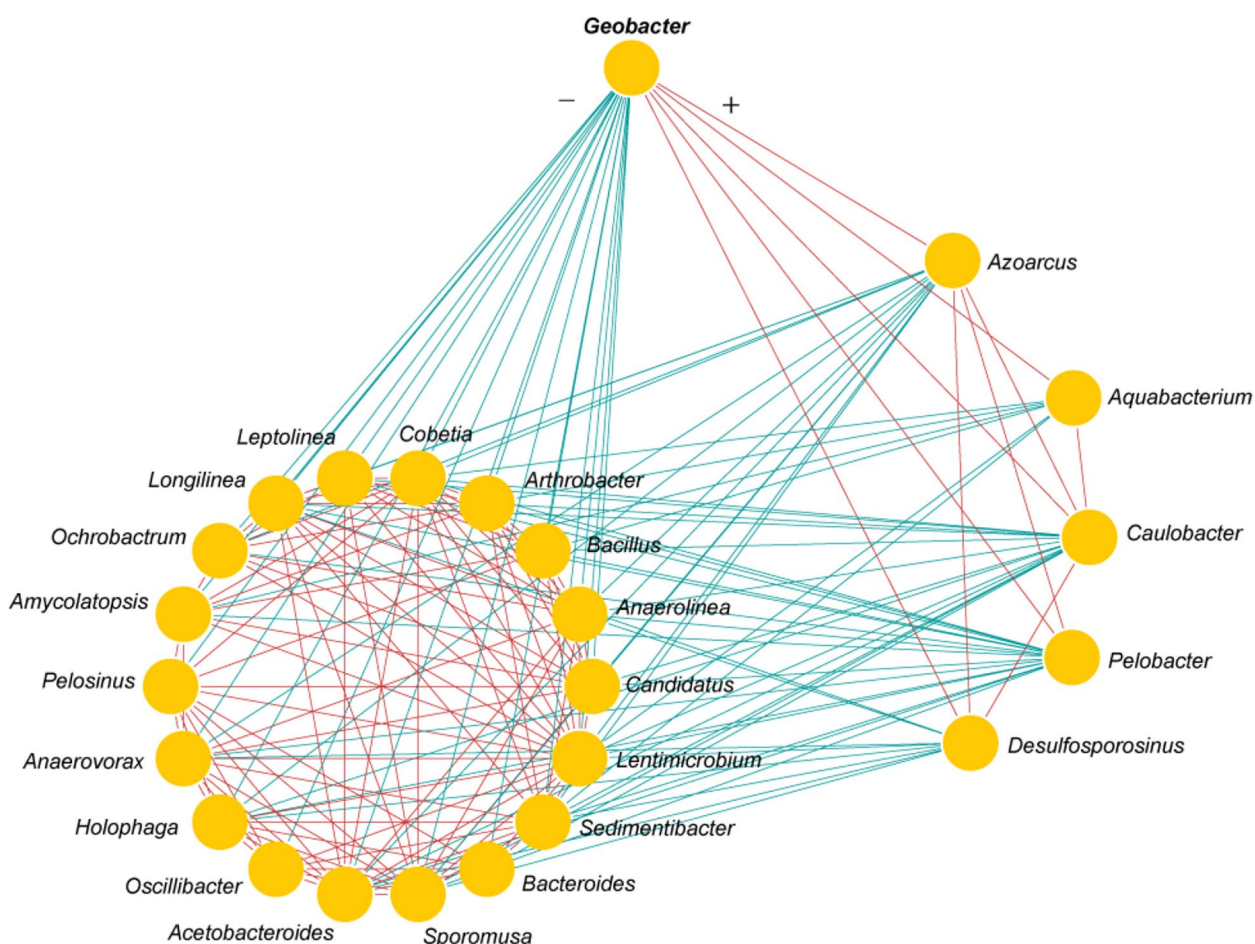
abundance may be due to the increase of other weak exoelectrogens.

The diversity analysis indicated a clear distinction between the biofilms in s-BESs with and without DOM. The non-metric multidimensional scaling (NMDS)

Fig. 7 The network analysis of the top 50 dominant genus abundance in all biofilm samples of day 6, 10 and 12. Each node represents an OTU, which signified a species. Each line connects two OTUs. A red line indicates a positive interaction between two individual nodes suggesting a mutualism or cooperation, while a green line indicates a negative interaction suggesting predation or competition

statistical significance with a *p*-value less than 0.05, using Cytoscape software (Shannon et al. 2003).

As shown in the network analysis in Fig. 7, five genera, namely *Azoarcus* (Benedek et al. 2016), *Aquabacterium* (Zhang et al. 2016), *Caulobacte*r (da Silva Neto et al. 2013), *Pelobacter* (Lovley et al. 1995), and *Desulfosporosinus* (Stanley and Southam 2018), exhibit a positive correlation with *Geobacter*. *Geobacter* is widely recognized for its ability to reduce iron, uranium, and sulfur, making it a strong exoelectrogen (Lovley and Holmes 2022). This implies that the abundance trends of these microorganisms are consistent with strong exoelectrogens. Among them, two genera (*Pelobacter* and *Desulfuromonas*) share a close phylogenetic relationship with *Geobacter* (Lovley et al. 1995). Furthermore, it has been reported that *Pelobacter* has been reported acts as a reducer of Fe(III), while *Desulfosporosinus* is a typical sulfur-reducing bacterium with the capacity to reduce U(VI) (Suzuki et al.

2004). In other words, they are all well-documented as typical metal-reducing bacteria. These findings strongly suggest that the abundance trends of some typical electroactive microorganisms are consistent with *Geobacter*.

However, Fig. 7 shows that 18 genera have negative correlation with Geobacter. Fifteen of them are Leptolinea, Longilinea, Ochrobactrum, Anaerovorax, Holophaga, Oscillibacter, Acetobacteroides, Sporomusa, Bacteroides, Sedimentibacter, Lentimicrobium, Candidatus_Accumulibacter, Anaerolinea, Arthrobacter, and Cobetia.

None of these genera are the reported typical extracellular metal-reducing bacteria. Another 3 genera which are also negatively correlated with *Geobacter* are *Bacillus* (Hobbie et al. 2012), *Amycolatopsis* (Zhang et al. 2019) and *Pelosinus* (Mosher et al. 2012). Although there are reports indicating that they can reduce iron, they are all fermentative iron-reducing bacteria, which differ from *Geobacter*, a respiratory

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iron-reducing genus. The EET ability of fermentative iron-reducing bacteria is significantly weaker in comparison to respiratory iron-reducing bacteria (Doyle and Marsili 2018). If we classify these fermentative iron-reducing bacteria and bacteria for which no iron-reducing function has been reported as weak exoelectrogens, the above results indicate that the decrease in *Geobacter* abundance is accompanied by an increase in the abundance of weak exoelectrogens. This is likely because DOM acts as a soluble electron shuttle, supporting the growth of weak electricity producers on the electrode and thereby increasing their abundance. Consequently, the rise in weak exoelectrogens might have overtaken a specific ecological niche, outcompeting *Geobacter* and reshaping the community structure.

3.6 Mechanism and environmental implications

Results in this study demonstrate that DOM notably boosts the growth and abundance of weak exoelectrogens within the biofilm. We propose that DOM, as electron shuttle, effectively provides these microorganisms with pathway for electron release, creating ecological niches for their survival and metabolism, ultimately enhancing their competitiveness within the microbial community.

Previous research has often emphasized the role of DOM in promoting electron transfer in typical electroactive microorganisms, like *Geobacter*, while overlooking the role of atypical iron-reducing bacteria in soil ecosystems. Many of these non-typical iron-reducing bacteria, found in natural environments, are considered "weak exoelectrogens" (Aiyer et al. 2023; Aiyer and Doyle 2023; Aiyer and Doyle 2022; Doyle and Marsili 2018). Due to the remarkable diversity of weak exoelectrogens, they hold significant ecological importance in nature (Doyle and Marsili 2018). They may not have the ability to directly transfer electrons to solid extracellular electron acceptors, but they are adept at transferring electrons to electron shuttles. In line with our previously reported findings in a pure culture system — that "electron shuttles promote microbial biofilm formation in iron-reducing bacteria that depend on shuttle processes (Wu et al. 2020) " and "lipophilic shuttles enhance extracellular electron transfer in bacteria lacking a complete extracellular electron transfer chain (Wu et al. 2023) "— this study further confirms the significant role of electron shuttling in the growth of weak exoelectrogens within the complex soil system. This research implies that the electron shuttling is an essential metabolic process for numerous microorganisms, which raises a question: if shuttles are critical for these microorganisms, how many of them owe their survival in rice paddies, sediments, and soils to the presence of DOM? Moreover, while typical iron-reducing bacteria exhibit strong iron-reducing

capabilities, due to the numerical advantages of weak exoelectrogens, who makes a more significant contribution to iron oxide reduction when DOM is present? This study raises awareness that these issues are worthy of further research.

Despite soil being viewed as a black box, we meticulously controlled variables to explore differences between DOM-rich and DOM-lacking treatments. Our results show a higher abundance of weak exoelectrogens in DOM-rich treatments, unveiling a negative correlation between specific strong and weak exoelectrogens. However, due to the complexity of the soil, future experiments involving multi-strain co-cultivation are recommended to better understand the role of DOM in microbial community regulation in a cultivation system without soil interference.

4 Conclusion

This study explored DOM's influence on the competitive dynamics of electroactive microorganisms in s-BESs. DOM significantly boosted bio-current generation in the biofilm, favoring weak exoelectrogens like *Bacillus* and *Sedimentibacter* while reducing strong exoelectrogens such as *Geobacter*. The observed negative correlation highlights DOM's critical role in favoring weak exoelectrogens. We suggest that DOM, acting as an electron shuttle, provides conducive ecological niches for the thriving and propagation of weak exoelectrogens, thus enhancing their competitiveness within the microbial community. Based on this, we proposed the viewpoint that the role of weak exoelectrogens on iron cycling in soil may be underestimated due to the existence of DOM. This offers new perspectives on soil electroactive microbial ecology, providing a theoretical basis for the regulation of microbial community structure in soil.

Abbreviations
CV	Cyclic voltammetry
DNA	Deoxyribonucleic acid
DOM	Dissolved organic matter
DPV	Differential pulse voltammetry
EET	Extracellular electron transfer
NMDS	Non-metric multidimensional scaling
OUT	Operational taxonomic units
PIPES	Piperazine-N, N'-bis(2-ethanesulfonic acid))
s-BESs	Soil-bioelectrochemical systems

Authors' contributions
Baoli Qin contributed to the investigation, data curation and writing the original draft. Yu Huang conducted the investigation and validation, reviewed and edited the draft, and prepared visualization. Tongxu Liu was responsible for the methodology and project administration. Yundang Wu supervised the

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Author details
[1]Guangdong Key Laboratory of Integrated Agro-environmental Pollution Control and Management, Institute of Eco-environmental and Soil Sciences, Guangdong Academy of Sciences, Guangzhou 510650, China. [2]Guangdong Key Laboratory of Environmental Pollution and Health, School of Environment, Jinan University, Guangzhou 510632, China. [3]College of Materials and Energy, South China Agricultural University, Guangzhou 510642, China. [4]National-Regional Joint Engineering Research Center for Soil Pollution Control and Remediation in South China, Guangzhou 510650, China.

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