Redox-active humics support interspecies syntrophy and shift microbial community

ZHENG Yue[1,2], KAPPLER Andreas[3], XIAO Yong[1], YANG Fan[1,2],
MAHADEVA Gurumurthy Dummi[1] & ZHAO Feng[1*]

[1]CAS Key Laboratory of Urban Pollutant Conversion, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China;
[2]University of Chinese Academy of Sciences, Beijing 100049, China;
[3]Geomicrobiology, Center for Applied Geosciences, University of Tuebingen, Tuebingen 72076, Germany

The network of microbial electron transfer can establish a syntrophic association of microbes by connecting interspecies metabolisms, and a variety of redox-active shuttles in environment have been proved to accelerate the electron flow in a microbial community. Using humic substances as models, we investigated how different redox-active shuttles with different electrochemical properties influence interspecies electron transfer, and affect the shift of microbial communities. The co-culture of two species was constructed with supplements of humics, and the electron transfer between these two strains was found to be linked by humic acid with a wider window of redox potential and multi-peaks of redox reactions. Based on the shift of microbial composition, the humic substances with a wide potential window and multi-peaks of redox reactions for accepting and donating electrons could increase the biodiversity (Chao 1 and phylogenetic diversity) with a large extent. The mechanism by which redox-active shuttles mediate the microbial electron transfer network could facilitate our understanding of syntrophic interactions between microbes.

humic acid, fulvic acid, redox-active shuttle, microbial diversity, interspecies electron transfer

1 Introduction

One of essential strategies of microbial metabolism is the redox process based on electron transfer. Extracellular electron transfer contributes to microbial energy metabolism, which works in bioelectrochemical systems e.g. microbial fuel cells, microbial electrolysis cells and geochemical process. Microbial metabolisms proceed close to the corresponding thermodynamic redox niches [1] they reside in. The availability of various electron acceptors and donors provides different living conditions to microbes, resulting in diverse ways of microbial energy metabolism. Different kinds of microbial energy metabolisms were associated through interspecies electron transfer in microbial communities, which coupled the biogeochemical process of different elements. For example, the methane oxidation could be driven by the electrical connection between methanotrophic archaea and sulfate reducing bacteria through microbial conductive nanowires [2]. Moreover, although different kinds of microbes were spatially separated, their respective biogeochemical redox processes could also be connected through extracellular electron transfer [3]. Sulfide oxidation in a deeper zone was shown to be coupled with oxygen reduction at the surface based on the conductive filamentous bacteria in marine sediments [4]. The electron transfer network among several kinds of species has been proved to have a key role in the environmental function of microbial consortia [5].

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*Corresponding author (email: fzhao@iue.ac.cn)

As to the interspecies electron transfer, several endogenous ways, including outer-membrane proteins, extracellular appendages and cell-secreted molecules [6–9], were suggested to carry electrons between species. On the other hand, some of the environmental redox-active mediators, such as sulfur compounds [10,11], organic compounds [12] and iron oxides [13] could also work as exogenous shuttles for indirect electron transfer between cells [14]. Microbes living in communities normally take advantage of the metabolic abilities of their syntrophic partners to digest substrates that cannot be utilized by either one of them alone [15], but there are energy barriers between two or more microbes, in which their respective energy metabolisms cannot be connected without exogenous electron shuttles [16]. Different electron shuttles, varying in their redox properties, provide diverse possibilities of interspecies electron transfer. However, it is not fully clear how the syntrophic interaction was mediated by redox-active shuttles in interspecies electron transfer, and how the composition of a microbial community was further shaped.

Among various environmental mediators, humic substances, with an estimated total of 1600×10^{15} g C, are regarded as the main carbon reservoir existing in both aquatic and terrestrial environment [17]. Humic substances are formed through the degradation of bio-precursors, and they play an important role in biogeochemical process [18] and pollutant treatment [19]. Humic substances have been reported to involve extracellular electron transfer in kinds of environments [14,20–24]. Quinonoid humics could also work in the interspecies electron transfer [25]. Thus, in this work, humic substances with redox-active functional groups [26] are selected as the representative electron shuttles for analysis.

The objective of this study was to evaluate the potential effects of the electron transfer network of interspecies driven by redox-active humics. The role of humics was discussed based on three types with different redox-active properties: Firstly, the shuttle role of humics was explored by the co-culture to illustrate how redox-active shuttle mediated the interspecies electron transfer. Secondly, the syntrophic metabolism between interspecies was reflected by the microbial diversity with different humics. Standing on the level of microbial ecology, this study on the syntrophic interaction by redox-active shuttles would broaden our understanding of the complicated community from the perspective of biodiversity, and further strengthen the function of the microbial community.

2   Materials and methods

2.1   Preparation of humic substances

Humic acid (HA), fulvic acid (FA) and Anthraquinone-2,6-disulfonate (AQDS) were purchased from Aladdin, Jianglai (Shanghai) and Sigma-Aldrich, respectively. The HA was purified as follows: insoluble humin was removed by collecting the supernatant of HA dissolved in 0.1 M NaOH. Next, the supernatant was acidified to pH 2.0 with 1 M HCl to collect the precipitate. The precipitate was washed several times with a 5 M HCl-5% Hydrogen Fluoride to remove the remaining inorganic solids, and then washed with 1.8 M HCl to minimize the Fe content. Finally, the residue was thoroughly washed with Milli-Q water to remove chloride ions, and gently freeze-dried. The preparation was conducted under nitrogen atmosphere.

2.2   Electrochemical and structure analysis of humics

Cyclic voltammetry measurements were performed using an electrochemical workstation (CHI660D, Chenhua Co. Ltd, China) with a three-electrode system, including the working electrode of glass carbon, counter electrode of Pt wire, and reference electrode of Ag/AgCl (+197 mV vs standard hydrogen electrode). All potentials are reported versus the saturated Ag/AgCl reference electrode in this article. The measurements were conducted within the potential range of –0.8 to +0.8 V (vs Ag/AgCl) at a scan rate of 10 mV s^{-1} at 30°C under anoxic conditions. 30 mg L^{-1} of three humic substances were separately dissolved in the electrolyte of 50 mM phosphate buffer (pH 7.0), and the solutions were purged and saturated by N_2 for one hour to eliminate oxygen. All tests were conducted in darkness, considering the properties of humic substances would be affected by light [27].

Humic substances were mixed with KBr at a ratio of 1:100 and compacted to form pellets for Fourier Transform Infrared Spectroscopy (FTIR) analysis. The FTIR spectra from 4000 to 400 cm^{-1} were recorded using a Nicolet iS10 spectrophotometer (Thermo Nicolet, USA). X-ray photoelectron spectra (XPS) were measured by a Thermo VG Multilab 2000 spectrometer (Thermo Fisher Scientific Inc., USA) equipped with a monochromatic Al Kα radiation source at room temperature.

2.3   Setup of co-culture with humics

Geobacter sulfurreducens and *Thiobacillus denitrificans* were selected for the co-culture, and there is no direct electron transfer between *G. sulfurreducens* and *T. denitrificans* with acetate as the electron donor and nitrate as the electron acceptor [13]. *G. sulfurreducens* DSM12127 was obtained from German Collection of Microorganisms, which was isolated from surface sediments, Norman [28]. *T. denitrificans* ATCC25259 was obtained from American Type Culture Collection, which was isolated from soil [29].

The basic medium (10 mM NH$_4$Cl, 1 mM KH$_2$PO$_4$, 0.5 mM MgCl$_2$, 0.5 mM CaCl$_2$, 0.05% Yeast extract,

1 mL L^{-1} vitamin solution, 1 mL L^{-1} trace element solution and 0.5 mL L^{-1} selenite-tungstate solution) was prepared as described in the section of "construction of microbial consortia". The corresponding electron donor and acceptor (10 mM Na-acetate and 40 mM Na-fumarate for *G. sulfurreducens*, or 20 mM Na$_2$S$_2$O$_3$ and 20 mM NaNO$_3$ for *T. denitrificans*) were added into the basic medium for enrichment culture. *G. sulfurreducens* and *T. denitrificans* were precultivated to early stationary phases under a N$_2$/CO$_2$ (v/v, 80/20) atmosphere at 30°C without shaking [13], followed by collecting the respective cells by centrifugation (7000 r/min, 5 min), washing twice, and suspending them in the fresh basic medium. Next, 6% of the two kinds of cell suspension was inoculated into the bottle containing 25 mL basic medium supplemented with 20 mM acetate and 30 mM nitrate for co-culture. At the same time, the cell suspension of *G. sulfurreducens* or *T. denitrificans* was separately inoculated into the basic medium also containing 20 mM acetate and 30 mM nitrate for single-culture. HA, FA and AQDS were separately added into co-culture and single-culture at a final concentration of 15 mg L^{-1}. All cultures with different additions were repeated three times, and incubated at 30°C under an atmosphere of N$_2$/CO$_2$ (v/v, 80/20).

Before metabolism analysis, samples were collected in a glovebox and stored anoxically at 4°C. Nitrate, nitrite and ammonia were measured by a flow injection analysis (FIA) system (3-Quattro; Bran & Lubbe, Germany). Acetate was quantified by HPLC (class VP with RID 10 A and DAD SPM 10A VP detectors [Shimadzu, Japan]; the precolumn was a Microguard cation H cartridge; the main column was an Aminex HPX-87H ion exclusion column [300 mm by 7.8 mm] [Bio-Rad, Austria]; eluent, 5 mM H$_2$SO$_4$ in MQ water). For quantification of cell growth, the optical density (OD) was measured at 600 nm (SPEKOL 1300, Analytik Jena).

2.4 Construction and analysis of microbial consortia with humics

The microbial consortia were cultivated in 25 mL of anaerobic medium supplemented with 20 mM of sodium acetate as the electron donor and 30 mM of sodium nitrate as the electron acceptor. The anaerobic medium (10 mM NH$_4$Cl, 1 mM KH$_2$PO$_4$, 0.5 mM MgCl$_2$, 0.5 mM CaCl$_2$, 0.05% Yeast extract) was prepared in a Widdel flask and flushed with N$_2$/CO$_2$ (v/v, 80/20) after autoclaving. Vitamin solution (1 mL L^{-1}), trace element solution (1 mL L^{-1}) and selenite-tungstate solution (0.5 mL L^{-1}) were supplemented from sterilized oxygen-free stocks [30]. NaHCO$_3$ (5 mM) and Hepes (30 mM) were used as the primary buffer, and the pH was adjusted to 7.0 with HCl or NaCO$_3$. Fifty milligrams (wet weight) of paddy soil collected from Shenyang, China (41°30.94′N, 123°21.155′E) was inoculated as a source of microbial consortia. Either HA, FA or AQDS was supple-

mented from sterilized oxygen-free stocks to reach the final concentration of 15 mg L^{-1}. The cultures were incubated at 30°C under an atmosphere of N$_2$/CO$_2$ (v/v, 80/20). Ten percent of the cultures were transferred into the fresh medium every 2–3 days at the logarithmic phase. All cultures with different additions were carried out in triplicate.

After ten times of transfer incubation, the genomic DNA of each culture was extracted and purified by FastDNATM SPIN KIT (MP Biomedicals LLC., USA). The purified DNA was PCR amplified with a set of primers (341F/806R) targeting the V3-4 region of 16S rRNAs in a 30 μL PCR reaction system. The specific 6 bp barcode was added to the forward primer to identify different samples as previously reported [31]. The purity and integrity of the PCR products were checked by agarose gel electrophoresis analysis and NanoDrop ND2000 (Thermo Fisher Scientific Inc., USA). After quantification by QuantiFluorTM-ST (Promega Biotech Co., USA), DNA libraries were sequenced using a Hi-Seq platform (Illumina Inc., USA) with a paired-end 250 strategy.

After the raw sequencing reads were produced, the paired-end reads were merged by Fast Length Adjustment of SHort reads (FLASH version 1.2.9). Next, bioinformatics analysis was conducted using the Quantitative Insights Into Microbial Ecology pipeline (QIIME version 1.9.1) as previously described [32]. The combined data were processed according to the following major principles: (1) check the completeness of the barcodes and primers; (2) remove low-quality reads (shorter than 300 bp or less than 25 of the quality score); and (3) remove reads comprising chimera. Next, the effective reads were classified for each sample with the corresponding barcode. Only the sequences with 97% identity were divided into operational taxonomic units (OTUs) for further analysis, and the centroid sequence from each OTU was selected as the representative sequence. After alignment by PyNAST, the representative sequences were assigned with the Uclust method for the taxonomic classification. For fair comparison, 70338 sequences per sample were randomly selected to calculate the α-diversity (Chao1 index and phylogenetic diversity index) in each sample and compare the β-diversity (principal coordinate analysis) between samples by UniFrac metric. Differentially expressed OTUs were identified and selected between each pair of groups with different additions, and further clustered for taxonomic classification at the phylum and class levels (relative abundances were presented as the means of three independent experiments).

3 Results

3.1 Redox properties of humics

Different types of humics with different functional groups results in different redox properties, which may show dif-

ferent effects on microbial electron transfer. Hence, two humic substances (HA and FA) and the humic analogue AQDS were firstly tested using voltammetry in this study. From the perspective of electron transfer, HA, FA and AQDS responded differently to the electrode (Figure 1(a)). The redox potential window of electron shuttles was defined as the span between the oxidation and the reduction potentials. The oxidation peak and reduction peak of FA were both in the positive potential area (oxidation potential +0.19 V, reduction potential +0.11 V), while the redox potential of AQDS appeared in the negative potential area, in which the corresponding oxidation potential was –0.27 V and the reduction potential was found at –0.45 V. As to HA, the peak potential of oxidation located around –0.34 V and +0.15 V respectively, and that of reduction could reach about –0.49 V. The more redox reactions of HA provide more possibilities for the extracellular electron transfer, while the redox potential window of HA is wider than that of FA and AQDS.

According to previous studies, the redox reversibility of humic substances was achieved due to the electron transfer between quinone, p-semiquinone, and hydroquinone [33]. The FTIR peak of 1653–1657 cm^{-1} was recorded as C=O, and the response in HA and AQDS was stronger than that in FA (Figure S1). This result was consistent with the information of X-ray photoelectron spectroscopy (Figure 1 (b)), in which the ratio of C=O was about 4:9:12 in FA, AQDS, and HA. Quinone has known as the key functional groups for humic substances, which serve as the electron transfer moieties for microbial electron transfer. According to a recent work, C=O of electron shuttles would contribute to the extracellular electron transfer [34].

3.2 Interspecies electron transfer of co-culture with humics

For verifying the proposed humic types effecting on microbial electron transfer, a co-culture was designed using two anaerobic bacteria: *G. sulfurreducens* and *T. denitrificans*. *G. sulfurreducens* could select acetate as electron donor, but could not utilize nitrate as electron acceptor, and *T. denitrificans* could metabolize nitrate but not acetate [27]. The co-culture of these two bacteria would fail to grow in the presence of only acetate and nitrate. Acetate and nitrate were used as electron donor and acceptor respectively, and HA, FA, AQDS were supplied as electron shuttles for co-culture.

According to the optical density (Figure S2), the growth of co-culture with added HA was observed, while there was no change in the other system with NA, FA and AQDS. Based on the changes in substrates and metabolites, the acetate and nitrate concentration decreased in the co-culture, concomitant with an increase in the nitrite and ammonium concentration at an approximate stoichiometry of $8 \times \delta C_{\text{acetate}} \approx 2 \times \delta C_{\text{nitrite}} + 8 \times \delta C_{\text{ammonium}}$ when supplemented with HA
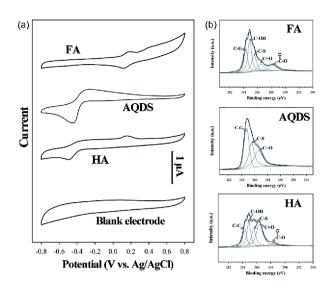

Figure 1   (a) The cyclic voltammetry of three different humics (HA, FA and AQDS) (working electrode: glassy carbon, count electrode: Pt wire, reference electrode: Ag/AgCl, pH: 7.0); (b) X-ray photoelectron spectroscopy of HA, FA and AQDS (HA: humic acid, FA: fulvic acid, AQDS: Anthraquinone-2,6-disulfonate).

(Figure 2). This result suggested that electrons were transferred from *G. sulfurreducens* to *T. denitrificans*, facilitating the coupling of acetate oxidation by *G. sulfurreducens* ($CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^-$) to nitrate reduction by *T. denitrificans* ($NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$; $NO_2^- + 8H^+ + 6e^- \rightarrow NH_4^+ + 2H_2O$). The changes were not observed in the systems with NA, FA and AQDS, which are consistent with the results of optical densities.

In the setup with HA, the nitrate concentration did not decrease in the single-culture of *G. sulfurreducens*, nor was the acetate used in the single-culture of *T. denitrificans* (Figure S3). The acetate and nitrate were metabolized by the co-culture of *G. sulfurreducens* and *T. denitrificans*, which supported that HA can act as the electron shuttle for *G. sulfurreducens* and *T. denitrificans*. As shown in Figure 2(b), the electron transfer pathway was proposed as follows: HA acts as electron shuttles to connect the electron transfer between *G. sulfurreducens* and *T. denitrificans*, coupling acetate oxidation and nitrate reduction. From aforementioned results, it can be deduced that the humics with a wider redox potential window and multi-peaks of redox reactionss would be more likely to link the electron exchange between interspecies. As shown in Figure 3, the role of redox-active shuttles between microbes could be described as an electron bridge. A longer electron bridge (a wider redox potential window and multi-peaks of redox reactions) could cover a greater distance (an energy barrier between microbes).

3.3 Shifts of microbial composition driven by humics

From the perspective of microbial ecology, the interspecies relationship regulated by redox-active humics was further
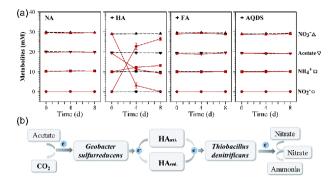
Figure 2   (Color online) (a) The metabolic dynamics in the co-cultures of *G. sulfurreducens* and *T. denitrificans* (solid lines), and their abiotic control (dashed lines) with or without different redox shuttles (NA: no addition, HA: humic acid, FA: fulvic acid, AQDS: Anthraquinone-2,6-disulfonate). Data points indicate means of duplicate samples, and error bars represent standard deviations. (b) The proposed electron transfer pathway for the co-culture of *G. sulfurreducens* and *T. denitrificans* with HA.

Figure 3   (Color online) The proposed mechanism of redox shuttles as the electron bridge for interspecies electron transfer.

investigated by microbial communities with more diversity. Anaerobic cultures were inoculated with paddy soil, and supplemented with acetate and nitrate as electron donor and acceptor, respectively. Two different humic substances (HA and FA) and the humic analogue AQDS were independently added into the cultures, and the cultures without adding shuttles (NA) were incubated as control. Before the exhaustion of the electron donor and acceptor, the culture was transferred into fresh medium. After 10 transfers, the microbial community of the mixed culture reached a relatively stable state. These microbial communities were then investigated through amplicon sequencing, and a total of 3183314 effective sequences with an average length of 463.9 bp were obtained from the 16S rRNA gene V3-V4 region of these samples. To compare the different samples at the same sequencing depth, 70338 sequences were extracted from each sample randomly. Although the rarefaction curves did not reach a plateau at 70338 sequences, the Good's coverage was up to $85.52 \pm 2.63\%$, suggesting that the 70338 sequences were enough to reflect the profile of the microbial communities.

Based on the unweighted principal coordinate analysis by UniFrac matrix (Figure S4), there was a relatively long distance between the inoculum samples and the samples fed

with humics, suggesting the obvious shifts of microbial communities resulting from the participation of humics. Additionally, three groups of samples fed with HA, FA and AQDS were clustered separately, which meant that humics varied in their functions due to the change of microbial composition. To estimate the effect of humics on phylogenetic distribution, the OTUs with a significant difference were selected through comparing OTUs between each pair of samples (Figure S5). These significantly different OTUs were classified into four groups based on the cluster analysis (Figure 4), abounding separately in HA, FA, AQDS and NA groups. Assigned to known taxonomies at the phylum and class levels, these OTUs consisted of 9 main phyla and 18 main classes (more than 1%). The OTUs abundant in NA samples mainly consisted of Firmicutes (68.39%) and Proteobacteria (15.02%). Although predominant OTUs were still Firmicutes and Proteobacteria in the samples of FA and AQDS groups, Proteobacteria (74.51% of FA samples and 77.49 of AQDS samples) turned into the top phylum, which was far more than Firmicutes (7.78% of FA samples and 5.90% of AQDS samples). Proteobacteria was suggested as the most abundant phylum in the community of soil systems, which were accompanied with abundant humic substances [35]. Compared with FA and AQDS samples, the taxonomic diversity of HA samples increased to 7 phyla (Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Firmicutes, Proteobacteria) and 15 classes (Holophagae, Acidimicrobiia, Actinobacteria, Saprospirae, Bacteroidia, Flavobacteriia, Sphingobacteriia, Anaerolineae, ML635J-21, Bacilli, Clostridia, Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Gammaproteobacteria), suggesting that HA enriched the taxonomic composition of the microbial community in this study.

At the OTU level, the distribution of OTU in each sample was illustrated in the alphabetical order of taxonomy (Figure S6). Both the number and evenness of OTUs (bright dots) in HA samples were obviously higher than those of others. According to statistical terms, Chao1 index and phylogenetic diversity were calculated by randomly selecting 70338 sequences and repeated 30 times per sample (Figure 5). These α-diversity indices were used for estimating and comparing the richness and diversity of microbial communities. Phylotype richness, measured as Chao1 index, was enhanced by the mediation of different humic shuttles to a different degree. HA samples were ranked highest in terms of Chao1 index, and about two times higher than the NA samples. Chao1 index of AQDS samples was less than that of HA samples, and that of FA samples was almost as low as that of NA samples. The trend of Chao1 index was consistent with that of phylogenetic diversity (PD index) in the systems. The difference of α-diversity indices indicated that humics contributed to mediate more species to grow together, but varied in their performance in enhancing the α-diversity.
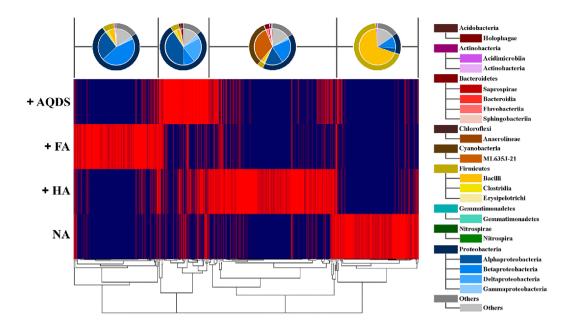
Figure 4 (Color online) Heatmap of significantly different OTUs among all samples. Based on the cluster analysis, the OTUs were classified into four groups, and the phylogenetic assignments were illustrated at the phylum and class levels (relative abundances were presented as the means of three independent experiments) (NA: no additions, HA: humic acid, FA: fulvic acid, AQDS: Anthraquinone-2,6-disulfonate).
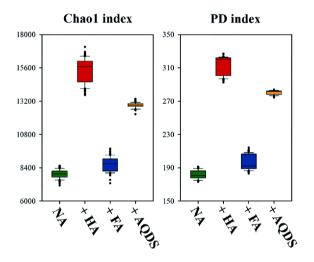

Figure 5 (Color online) The boxplot of Chao1 index and phylogenetic diversity index of different samples (NA: no addition, HA: humic acid, FA: fulvic acid, AQDS: Anthraquinone-2,6-disulfonate).

In summary, the role of humic substances for microbes could be classified into three aspects: (1) used for metabolism. According to International Humic Substances Society (http://www.humicsubstances.org/), humic substances, as large ensembles of components, are recalcitrant with respect to biodegradation. Based on the absorbance measurement of supernatants before and after the mix-culture (Figure S7), there was no obvious change of humic substances. (2) affecting living conditions (e.g. pH). The pH of culture was maintained by Hepes buffer (30 mM), while the amount of humics is limited to change the pH of media. Thus, the different groups with humics provide fair living conditions for

microbes. (3) working as electron shuttles. Humic substances have been demonstrated as redox-active shuttles for microbes in different environments by different research groups. From the perspective of microbial electron transfer, the results of communities were consistent with the hypothesis of "electron bridge" in interspecies electron transfer. One of reasons for the shift of microbial community would be the interspecies electron transfer driven by humic substances.

4 Discussion

4.1 The redox-active shuttles in biogeochemical cycles

The biological cycling of basic elements is driven largely by microbial transformation of redox couples with different redox potentials [36,37]. Extracellular electron transfer, as the main microbial performance in biogeochemical cycles, could be classified into direct and indirect electron transfer. Although direct electron transfer, such as heme proteins and nanowires, could connect interspecies electron transfer within a relatively short distance [38], redox shuttling is one of the significant ways for transferring electrons across a long distance between cells. Exogenous shuttles (e.g. humic substances) distributed in various environments, along with a diversity of biogeochemical redox couples, lay the foundation for the diversity of microbial electron transfer.

4.2 Interspecies electron transfer facilitated by redox-active shuttles

In the electron transfer between cells, the role of electron

shuttles could be divided into redox-active mediation and electron-hopping mediation from the perspective of physical property (Figure S8). Specifically, redox-active shuttles are based on the redox reversibility of potential for accelerating microbial electron transfer, and examples for electron shuttles are sulfur compounds [10,11] and organic compounds containing quinone moieties [39]. Electron-hopping conduction occurs through electron hopping from one site to the next, such as the role of (semi)conductive minerals [13]. Despite no limitation in the potential range for minerals, the electron transfer just occurs when microbes are attached to the (semi)conductive interface [13]. Dissolved redox shuttles can be dispersed across a wide spatial range in environment, but their capacity of electron transfer is limited by the oxidation and reduction potential [40]. As to electron shuttles, the range of redox potential provides the chances to mediate the extracellular electron transfer. Our work has demonstrated that humic acid with a wider redox potential window and multi-peaks of redox reactions would link more electron transfers between interspecies, and increase the bio-diversity of the microbial community.

4.3 The function of microbial community mediated by electron shuttles

According to previous works, the microbial electron transfer mediated by electron shuttles could affect the function of a microbial community [41,42]. In microbial systems, there is a close relationship between microbial diversity and ecological function [43]. Therefore, the regulation of a microbial community would contribute to improve its function at the geochemical and environmental levels. Redox-active shuttles with different electrochemical properties could mediate the electron transfer between functional microbes, which provides a self-organization way to strengthen the function of the microbial community [44]. A better understanding of the network of interspecies electron transfer would be helpful for evaluating the mechanism of the synergic interaction between functional microbes in biogeochemical process and provide more theoretical supports for regulating and improving the biodegradation of environmental pollutants by the microbial cooperation.

5 Conclusion

In this study, we used redox-active humics with different electrochemical properties to demonstrate how electron shuttles influence the electron transfer network of interspecies, and shape the composition of microbial communities. We constructed a co-culture of two species (*G. sulfurreducens* and *T. denitrificans*) with different redox-active humics. Based on the results of interspecies electron

transfer, we proposed the mechanism of humics as electron bridge for interspecies electron transfer, as shown in the following scheme. The humics with a wider redox potential window and multi-redox potential for accepting and donating electrons would enable more microbes to exchange electrons. The enhanced interspecies syntrophy would increase the bio-diversity of microbial communities. Redox shuttles with different redox properties could mediate the electron transfer among different microbial cells, which would provide a self-organization way to strengthen the function of the microbial community.

Supporting Information

The supporting information is available online at tech.scichina.com and www.springerlink.com. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.

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