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Title: Potential syntrophic associations in anaerobic Naphthenic Acids biodegrading consortia inferred with microbial interactome networks

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Keywords: Anaerobic microcosms; Indigenous microorganisms; Interactome network; Microbial function; Electron acceptor

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Abstract: Naphthenic acids (NAs) can be syntrophically metabolized by indigenous microbial communities in pristine sediments beneath oil sands tailings ponds. Syntrophy is an essential determinant of the microbial interactome, however, the interactome network in anaerobic NAs-degrading consortia has not been previously addressed due to complexity and resistance of NAs. To evaluate the impact of electron acceptors on topology of interactome networks, we inferred two microbial interactome networks for anaerobic NAs-degrading consortia under nitrate- and sulfate-reducing conditions. The complexity of the network was higher under sulfate-reducing conditions than nitrate-reducing conditions. Differences in the taxonomic composition between the two modules implies that different potential syntrophic interactions exist in each network. We inferred the presence of the same syntrophic microorganisms, from genera Bellilinea, Longilinea, and Litorilinea, initiating the metabolism in both networks, but within each network, we predicted unique syntrophic associations that have not been reported. Electron acceptor has a large effect on the interactome networks for anaerobic NAs-degrading consortia, offers insight into an unrecognized dimension of these consortia. These results provide a novel approach for exploring potential syntrophic relationships in biodegrading processes to help cost-effectively remove NAs in oil sands tailings ponds.

*Novelty Statement (maximum limit:100 words)

Novelty Statement

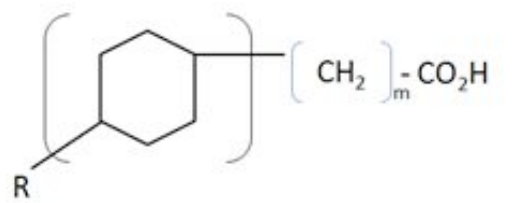
Syntrophic processes make essential contribution in anaerobic biodegradation of NAs from sediments of oil sands tailings ponds (OSTP). This study inferred two microbial interactome networks for anaerobic NA-degrading consortia enriched from sediments of OSTP under nitrate- and sulfate-reducing conditions, respectively. Specifically, within each network, unique syntrophic associations were predicted that have not been reported previously. Our results demonstrate that electron acceptor has a large effect on the microbial interactome networks for anaerobic NA-degrading consortia, offer vital insight into an unrecognized dimension of these consortia, and provide a novel approach for exploring potential syntrophic relationships.

Highlights

- Inferred microbial interactome networks in anaerobic NA-degrading consortia
- Sulfate-reducing networks were more complexity than nitrate-reducing networks
    - Differences between microbial networks can be ascribed to electron acceptors
    - Predicted potential syntrophic associations in anaerobic NA-degrading consortia

Graphical Abstract

Naphthenic Acids



NO_3^-

Groundwater

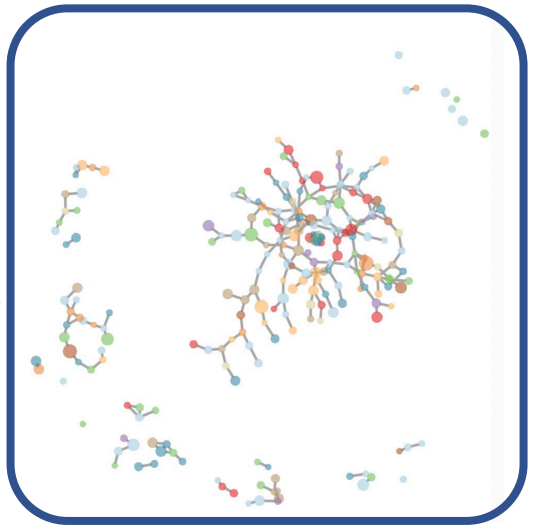
Sediment

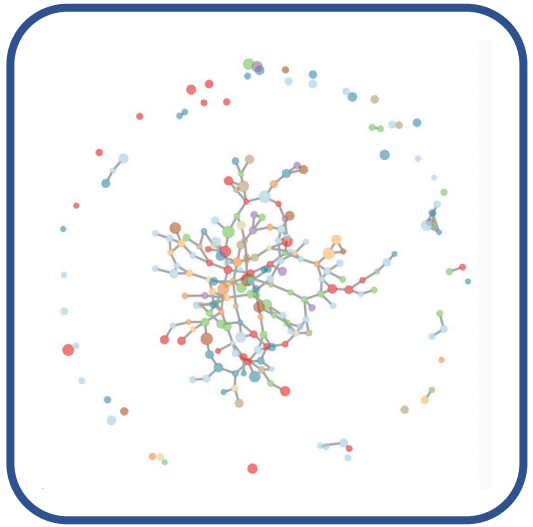
SO_4^{2-}

Groundwater

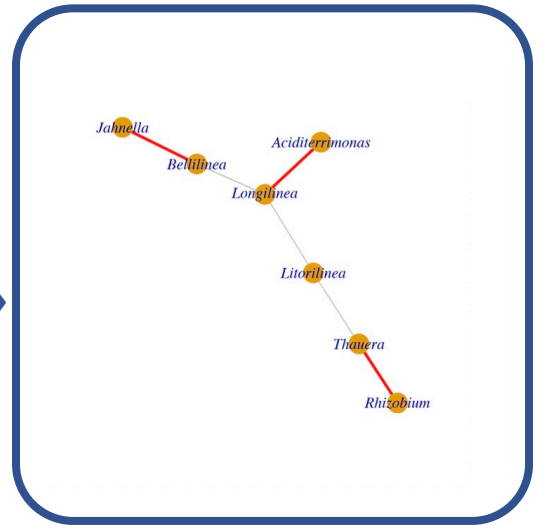
Sediment

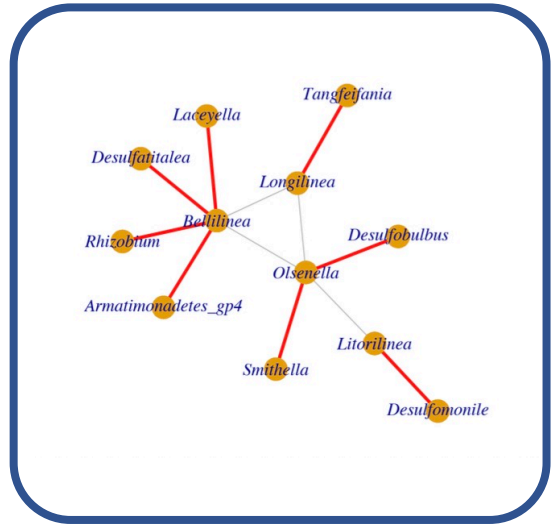
Interactome Microbial Network





Potential Syntrophic Associations





ABSTRACT

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Potential Syntrophic Associations in Anaerobic Naphthenic Acids Biodegrading Consortia Inferred with Microbial Interactome Networks

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ABSTRACT

Naphthenic acids (NAs) can be syntrophically metabolized by indigenous microbial communities in pristine sediments beneath oil sands tailings ponds. Syntrophy is an essential determinant of the microbial interactome, however, the interactome network in anaerobic NAs-degrading consortia has not been previously addressed due to complexity and resistance of NAs. To evaluate the impact of electron acceptors on topology of interactome networks, we inferred two microbial interactome networks for anaerobic NAs-degrading consortia under nitrate- and sulfate-reducing conditions. The complexity of the network was higher under sulfate-reducing conditions than nitrate-reducing conditions. Differences in the taxonomic composition between the two modules implies that different potential syntrophic interactions exist in each network. We inferred the presence of the same syntrophic microorganisms, from genera *Bellilinea*, *Longilinea*, and *Litorilinea*, initiating the metabolism in both networks, but within each network, we predicted unique syntrophic associations that have not been reported. Electron acceptor has a large effect on the interactome networks for anaerobic NAs-degrading consortia, offers insight into an unrecognized dimension of these consortia. These results provide a novel approach for exploring potential syntrophic relationships in biodegrading processes to help cost-effectively remove NAs in oil sands tailings ponds.

Keywords: Anaerobic microcosms; Indigenous microorganisms; Interactome network; Microbial function; Electron acceptor

1. Introduction

Naphthenic acids (NAs) are a complex mixture of carboxylic acids that occur naturally in petroleum [1]. NAs can cause engineering and production difficulties through corrosion of refinery plant and deposition as salts in pipelines [2]. NAs also cause environmental problems because of their toxicity, recalcitrance, and persistence. NAs may persist in the environment for many years, especially in aged wastewater. NAs concentrations in wastewaters can remained > *19 mg* L^{-1} even after several decades [3]. NAs have been identified as the main component responsible for the acute toxicity in produced waters in the oil sands tailing ponds (OSTP) in northeastern Alberta, Canada [4]. The ponds are estimated to exceed a billion cubic meters by 2025 and are already visible from space [5].

Bioremediation is an attractive option for reducing the toxicity of NAs wastes[6]. The anaerobic degradation of simple single-ringed surrogate NAs has been reported under sulfate-, nitrate-, and iron-reducing conditions [7,8] . Syntrophy is a beneficial metabolic process occurring between organisms, where a given compound can be only be degraded by one organism when a second organism consumes the intermediate products and keeps them at a low concentrations [9]. Syntrophic metabolism of hydrocarbons is common in methanogenic ecosystems where electron acceptors are limited or absent [10]. In syntrophic environments, the syntrophic organisms initially degrade hydrocarbons to intermediates such as acetate, formate, or H_2. Methanogens consume the intermediates and keep them at low concentrations to make the initial anaerobic oxidation energetically favorable. However, it is hard to figure out syntrophic relationships in the communities without any reference information. The high proportion of microbial “dark matters”, the uncultured environmental microbes, also hinder discovering syntrophic relationships.

Genomic analysis have shown that syntrophic interactions are essential for anaerobic biodegradation of hydrocarbons [11], and a recent review comprehensively describes the underlying principles of syntrophic hydrocarbon degradation [9]: the initial fermentative organism degrades the hydrocarbons with reactions that are not thermodynamically favorable, and produces intermediates such as acetate, formate, and/or H_2 that are consumed by methanogens [9]. Recent studies have also shown that benzene is syntrophically degraded in the presence of electron acceptors such as nitrate [12], ferric iron [13], and sulfate [14]. For example, methane from gas hydrates in ocean sediments is consumed by anaerobic methane-oxidizing archaea that associate with sulfate-reducing Deltaproteobacteria [15]. Hermann et al.[16] found that *Cryptanaerobacter* degraded benzene into intermediates such as acetate/H_2, which were then consumed by sulfate reducers. van der Zaan et al.[12] used stable isotope probing (SIP) with $^{13}C_6$-benzene to identify that the Peptococcaceae degraded benzene to H_2/acetate that was then consumed by the Betaproteobacteria, with both processes coupled to the nitrate reduction. Syntrophy also occurs in methanogenic cultures which can degrade surrogate NAs [7] . Since degradation of complex organics is generally more efficient under nitrate- and sulfate-reducing conditions, understanding the syntrophic interactions under nitrate- and sulfate-reducing conditions is critical for understanding anaerobic degradation of NAs.

The microbial interactome represents all of the interactions taking place among all community members. A recent study highlighted the critical role of microbial interactions in shaping microbial communities structure [18]. Interactome network inferred from abundance data can allow better understanding of community composition and function [19], and is a promising approach to explore microbial interactions in complex environments such as gastrointestinal tracts [20], bioreactors [21], soils [22,23], and oceans [19]. The complexity of

these networks provides a new perspective on the structure of the microbial communities and adds to our understanding of microbial ecology [24]. However, to the best of our knowledge, the microbial interactome network and potential syntrophic relationships in the anaerobic NAs-degradation processes has not been previously addressed due to extremely slow degradation rates.

This study inferred the microbial interactome networks for anaerobic NAs-degrading consortia enriched from sediments underlying OSTP under either nitrate- or sulfate-reducing conditions to: (i) determine if the electron acceptor affects the topological patterns of interactome networks and (ii) predict syntrophic interactions involved in anaerobic NAs-degradation.

2. Materials and methods

2.1 Naphthenic acid substrates

A stock solution of acid extracted organics (AEO) was prepared by extracting acidified (pH<2) oil sands processing-affected water (OSPW) with dichloromethane as previously described [25]. The Merichem NAs were NA mixtures obtained from Merichem Chemicals and Refinery Services LLC. (Houston, Texas).

2.2 Microcosm establishment

Sediments were collected from the Northwest edge of the Suncor Energy Inc.'s South Tailings Pond, situated approximately 35 km north of Fort McMurray, Alberta, Canada [26]. The clay and sand sediments were sampled from 6.1-6.4 m below ground surface (mbgs) and 37.7-38.7 mgbs, respectively [28]. Nitrate- and sulfate-reducing consortia were enriched from either clay or sand sediments amended with either acid extractable organics (AEO) or Merichem NAs (Merichem Chemicals and Refinery Services LLC.; Houston, Texas) [2]. A stock solution of

AEO was prepared by extracting acidified (pH<2) oil sands process-affected water with dichloromethane as previously described [27].

Microcosms were established from enrichments that were actively depleting NAs and electron acceptors. The microcosms consisted of 50 mL of original sediments inoculated into 450 mL of groundwater from the same site. Electron acceptors (7 mmol L^{-1} sodium nitrate or 14 mmol L^{-1} sodium sulfate) and NA substrates (100 mmol L^{-1} Merichem NAs or 100 mmol L^{-1} AEO NAs) were added individually to each incubation from sterile anoxic stock solutions using N_2-flushed syringes. The nitrate and sulfate concentrations in all enrichments were monitored monthly and maintained at 7 and 14 mmol L^{-1}, respectively. Each treatment was performed in triplicate. Autoclaved sterile controls were treated under 121 ˚C for 30 min for all experiments. The sterility was approved by both the methane production and DNA extraction. The procedural blanks for both physiochemical analysis and DNA extraction were performed during experiments.

2.3 Microbial community assessment

At 0, 163, and 331 days of incubation, representing different stages of microbial activities in the microcosms [28], we filtered the subsampled liquid with a 0.22 μm PVDF Millipore filter membrane for DNA extraction. The V3-V4 region of 16S rDNA was amplified and barcoded with primer set F515 and R806 [27], and sequenced using the Illumina MiSeq platform at the Molecular Biology Service Unit, University of Alberta. All samples generated a total of 6,631,481 sequences. After removing low quality sequences (expected error threshold=1), singletons, and chimeras, we obtained 5,618,515 sequences and defined 1165 operational taxonomic units (OTUs) with cutoff of 97% similarity and assigned to taxa using the UPARSE

pipeline. The sequences were deposited in the sequence read archive (SRA) database with accession number SRR5690441.

2.4 Interactome network inference

Interactome networks were constructed based on a maximal information coefficient (MIC) calculated with the minerva package in R [29]. To reduce rare operational taxonomic units (OTUs) in the dataset, we removed OTUs with relative abundances less than 0.01%. The nodes in this network represent OTUs and environmental variables. We adjusted all *p*-values for multiple tests using the Benjamini and Hochberg FDR controlling procedure in the multtest package of R. The direct correlation dependencies were distinguished using the network deconvolution method [30]. The co-occurrence networks for clay and sand sediments were constructed separately based on MIC and FDR adjusted *p*-values. The MIC thresholds, determined by Random matrix theory (RMT) method [31], were 0.88 and 0.89 for nitrate and sulfate networks, respectively. Network properties were calculated with the igraph package in the R program [32].

2.5 Module detection and keystone node identification

We identified modules (group of nodes that are highly connected within the group with few connections outside the group) for nitrate- and sulfate-reducing networks using the greedy modularity optimization method (igraph: cluster_fast_greedy). The connectivity of each node was determined based on its within-module connectivity (K_{within}) and among-module connectivity (K_{out}; WGCNA: intramodularConnectivity). Node topologies were organized into four categories: network hubs, module hubs, articulation nodes, and peripherals. Subnetworks for each sample were generated based on the group of OTUs occurring in each sample. The network level topological properties were calculated with the igraph package.

2.6 Statistical analysis

All statistical analyses were conducted using R version 3.4.0 (www.r-project.org). The NAs-associated nodes in nitrate- and sulfate-reducing networks were identified using random forest models. Phylogenetic diversity was calculated with the phyloseq package. Pearson's correlations were used to determine the relationships between topological features of subnetworks and geochemical properties or phylogenetic diversity.

3. Results

3.1 Interactome network topological characteristics

To identify potential microbe-microbe interactions in anaerobic NAs-degrading consortia, we constructed interactome networks for the microbial succession in the nitrate- (Fig. 1a) and sulfate-reducing (Fig. 1b) microcosms over 331 days of incubation. Both nitrate- and sulfate-reducing interactome networks exhibited non-random characteristics, as indicated by scale-free features (Fig. S1) and by comparison with the topological features of random networks generated using the same numbers of vertices and edges of both networks (Fig. S2a, b, Table 1). Both interactome networks had shorter diameters and mean path lengths, fewer neighbors, and larger transitivity than the corresponding random networks (Table 1). Network topological matrices (Table 1) and network associations across classes (Fig. 1c, d) showed that microbial association patterns were different in the nitrate- and sulfate-reducing networks. The link number in the nitrate-reducing network was larger than that in the sulfate-reducing network, but the giant subnetwork in the sulfate-reducing network was larger than that in the nitrate-reducing network (Fig. 1, Table 1). The nitrate-reducing network had longer diameter and mean path length, as well as smaller centrality than the sulfate-reducing network (Table 1). The associating patterns across dominant classes were different between the two networks (Fig. 1b, d). The numbers of

links associated with nodes assigned to Alphaproteobacteria and Betaproteobacteria in the nitrate-reducing network were greater than in the sulfate-reducing network. Conversely, the numbers of links associated with nodes assigned to *Anaerolinaea* and *Actinobacteria* in the nitrate-reducing network were smaller than in the sulfate-reducing network.

3.2 Keystone nodes in nitrate- and sulfate-reducing networks

We classified nodes into four categories based on their within-module connectivity (K_{within}) and among-module connectivity (K_{out}) values: peripherals, articulation nodes, module hubs, and network hubs (Fig. 2). The nitrate-reducing network had 12 module hub nodes and 7 articulation nodes. The sulfate-reducing network had 15 module hub nodes and 7 articulation nodes. No network hubs were detected in both of the networks, as no single node had $K_{within}>3$ and $K_{out}>1.5$. The degree of nodes in both networks was not correlated with the relative abundance of corresponding OTUs (Fig. S3). Most of the module hubs and articulation nodes had low relative abundance (<0.5%), except module hubs OTU1 (19.8%) in the nitrate-reducing network, OTU1310 (3.4%) and OTU910 (3.8%) in the sulfate-reducing network, and articulation node OTU119 (4.3%) in the sulfate-reducing network (Table S1-S2).

3.3 Impact of geochemical features and phylogenetic diversity on topological features

We inferred subnetworks for each sample based on OTU-occurrence (Fig. S4-S11). The geochemical characteristics impacted different topological features in the subnetworks for nitrate- (Fig. 3a, Fig. S12-S17) and sulfate-reducing microcosms (Fig. 3b, Fig. S18-S22). In the subnetworks for the nitrate-reducing microcosms, NA concentrations were positively correlated with mean neighbors, methane concentrations were positively correlated with diameter (Fig. 3a), nitrate concentrations were positively correlated with mean path length, mean neighbors, and centralization, dissolved organic carbon (DOC) concentrations were positively correlated with

transitivity, and nitrate and nitrite were negatively correlated with transitivity (Fig. 3a). In the subnetworks for the sulfate-reducing microcosms, methane concentrations were negatively correlated with vertex number and edge number, the DOC concentrations were negatively correlated with diameter and mean path length, and sulfate concentrations were negatively correlated with vertex number, edge number, and mean neighbors (Fig. 3b).

Phylogenetic diversity was positively correlated with vertex number, edge number, and mean degree in subnetworks for both nitrate- (Fig. 3a, Fig. S23) and sulfate-reducing microcosms (Fig. 3b, Fig. S24), but the correlation coefficient values for sulfate-reducing microcosms were higher than for nitrate-reducing microcosms. The phylogenetic diversity was positively correlated with diameter and mean path length in nitrate-reducing microcosms and was positively correlated with mean neighbors in sulfate-reducing microcosms.

3.4 Modularity in interactome networks

We focused on the modules with at least 5 nodes, which were assemblages with strong associations in microbial communities. The nitrate-reducing interactome network had 17 modules, 12 of which were connected with links among modules (Fig. 4a). The sulfate-reducing interactome network had 13 modules, all of which were connected with links among modules (Fig. 4b). The modularity coefficient of the nitrate-reducing interactome network (0.82) was greater than that of the sulfate-reducing interactome network (0.78). Given the intense association among the nodes in the same module, we postulate that nodes in the same module have potential syntrophic relationships. The taxonomic composition of modules varied within both networks, suggesting different potential syntrophic associations in different modules (Fig. 4c, Table S1-S2).

The number of nodes in the module within each subnetwork varied in both the nitrate- and sulfate-reducing interactome networks (Fig. 5). In the subnetworks for nitrate-reducing microcosms, adding AEO caused modules dominated by Actinobacteria (module 2), Betaproteobacteria (module 10), and Deltaproteobacteria (module 11) to be more prevalent (Fig. 5a-b). Nitrate-amended microcosms with added Merichem NAs had modules dominated by Actinobacteria (module 3 and 6) (Fig. 5c-d), and microcosms with added clay sediments had modules dominated by Clostridia (module 1) (Fig. 5a, c). Nitrate-amended microcosms with added sand sediments had modules dominated by Anaerolineae, Betaproteobacteria, and Clostridia (Fig. 5b, d). In the subnetworks for sulfate-reducing microcosms, adding AEO caused the prevalence of modules dominated by Actinobacteria (module 1), Clostridia (module 5), Bacteroidia (module 10), and Deltaproteobacteria (module 12) (Fig. 5e, f). Sulfate-amended microcosms with added Merichem NAs had modules dominated by Clostridia and Deltaproteobacteria (module 7) (Fig. 5g, h), and microcosms with added clay till sediments had a module dominated by Anaerolineae (module 2) (Fig. 5e, g).

We then identified potential syntrophic associations by picking out the generic pairs that occur more than once in either nitrate- or sulfate-reducing networks. These potential syntrophic associations might play a critical role in the metabolic processes within these networks. The genera *Bellilinea, Longilinea,* and *Litorilinea* were identified as interior nodes in both networks. However, the peripheral nodes in the two networks were different in the potential syntrophic networks for the nitrate- (Fig. 6a) and sulfate-reducing (Fig. 6b) microcosms. We propose that these interior nodes represent syntrophic microorganisms which initiate the metabolic reactions, and the peripheral nodes represent microorganisms metabolizing intermediate compounds coupled with either nitrate reduction or sulfate reduction. In the nitrate-reducing microcosms, the

inferred potential syntrophic associations included links from *Bellilinea* to *Jahnella*, from *Longtilinea* to *Aciditerrimonas*, and from *Thauera* to *Rhizobium*. In the sulfate-reducing microcosms, the inferred potential syntrophic associations included links from *Litorilinea* to *Desulfomonile*, from *Longilinea* to *Tangfeifania*, from *Olsenella* to *Smithella* and *Desulfobulbus*, and from *Bellilinea* to *Laceyella, Desulfatitalea, Rhizobium,* and *Armatimonadetes* gp4.

4. Discussion

This study showed that anaerobic NAs-degrading consortia amended with different electron acceptors had distinct microbial interactomes. The taxonomic compositions in the different modules implied that there are different potential syntrophic assemblies. The variation of module sizes induced by sediment type and NA source indicated that different treatments affect the potential syntrophic interactions. We identified the same genera initiating the metabolism of organic compounds in both nitrate- and sulfate-reducing potential syntrophic networks, but identified different genera involved in metabolizing the intermediate compounds in each of these networks. The network analysis inferred unique syntrophic associations that have not been reported previously.

Sulfate-reducing networks had more complex connectivity than nitrate-reducing networks, the latter having a larger subnetwork of shorter diameter with less links, indicating that syntrophic metabolism may play a greater role in the biodegradation process. The higher connectivity of the sulfate-reducing networks may indicate the presence of more syntrophic associations (Fig. 6), and is consistent with the reported prevalence of syntrophic metabolism under sulfate-reducing environments in wetland sediments [33] and acid sulfate soil associated with sulfidic sediments [15]. On the other hand, changes in environmental properties are important drivers of changes in ecological networks in both macro- and microbiological

communities [34]. For example, the complexity of the interactome network in the rhizosphere was induced by higher organic carbon input from plant roots [24]. Similarly, the DOC concentrations in the sulfate-reducing microcosms were higher than in the nitrate-reducing microcosms and may have contributed to the increased connectivity and complexity of the interactome network in the sulfate-reducing microcosms. The correlation between network topological properties and biogeochemical features (Fig. 3) also supported the impacts of DOC on the diameter of the sulfate-reducing network.

Different association profiles between nitrate- and sulfate-reducing network can be ascribed to the influence of electron acceptors on microbial compositions and microbial interactions. More links associated with Alphaproteobacteria and Betaproteobacteria nodes in the nitrate-reducing network suggested that these taxa play critical roles in a nitrate-reducing environment. A metatranscriptome study suggested that nitrate reduction was performed predominantly by Alpha- and Betaproteobacteria in sub-seafloor sediment [35], and a DNA-SIP analysis indicated that Betaproteobacteria play a role in syntrophic benzene degradation coupled to nitrate reduction [12]. Sulfate-reducing microcosms fostered associations for Anaerolineae and Actinobacteria, which often dominate in sulfate-reducing environments [14,15,33]. Anaerolinaea are known to be 'semi-syntrophic' organisms, degrading carbohydrates cooperatively with hydrogenotrophic methanogens in methanogenic bioreactors [36]. Actinobacteria have been identified as the primary syntrophs involved in anaerobic benzene degradation under iron-reducing conditions [13].

Within both networks, we identified a number of keystone nodes, including highly-connected hubs within a module and articulation nodes connecting different modules. The taxa represented by these keystone notes may be very important in maintaining network structure.

283 The taxa represented by these keystone notes may be very important in maintaining network
284 structure [24]. Synergistetes, a typically syntrophic taxon, was identified as a module hub and
285 articulation node, confirming the important role of syntrophic metabolism in both nitrate- and
286 sulfate-reducing environments. The detection of different module hubs in the different cultures
287 supports the theory that keystone species play critical roles only under certain conditions [37].
288 Identifying modules in networks is important to comprehensively understand microbial
289 interactions [24]. Network modules represent closely associated functions in metabolic processes
290 and niche sharing [38], so the higher modularity value in the nitrate-reducing network indicates
291 close microbial interactions such as syntrophy. Module sizes varied in subnetworks for different
292 treatments, such as NA components and sediment texture, demonstrating that these treatments
293 changed the interactions within the microbial communities. The different impacts of those
294 treatments in nitrate- and sulfate-reducing networks suggest that cultures with different electron
295 acceptors might have different microbial communities, which are affected differently by the
296 various treatments. Alternatively, the modules may have responded differently in the two
297 networks because of functional redundancy of different taxa, where different microbes play the
298 same functional role in each network [39].
299 The same genera in the class Anaerolineae were inferred to initially metabolize NAs
300 under both nitrate- and sulfate-reducing conditions. Anaerolineae has been shown to ferment
301 various sugars and grow better in the presence of H_2-consuming methanogens, suggesting
302 syntrophic metabolism [40]. The genera which were identified in the present study to consume
303 intermediates under nitrate-reducing condition, including *Jahnella, Aciditerrimonas*, and
304 *Rhizobium*, are all identified as denitrifiers [41]. Although *Thauera* and *Rhizobium* have already
305 been found to co-occur in previous studies [42,43], this study is the first indication of a

306 syntrophic association between *Bellilinea* and *Jahnella*, *Longtilinea* and *Aciditerrimonas*, and
307 *Thauera* and *Rhizobium* under nitrate-reducing conditions. Similarly, most of the genera which
308 could potentially consume intermediates under sulfate-reducing conditions were identified as
309 sulfate-reducing bacteria. Co-occurrence of potential syntrophic partners, such as *Olsenella* and
310 *Smithella* in anaerobic digester sludge [44], *Longilinea* and *Tangfeifania* in a upflow anaerobic
311 reactor [45], and *Bellilinea* and *Desulfatitalea* in a heavy oil reservoir [43], have been reported in
312 previous studies using different methods, but this study is the first to identify the syntrophic
313 interactions between them using network analysis.
314 Currently, our understanding of syntrophic processes is limited due to the few well-
315 defined co-cultures available [9]. The 'omics'-based approaches (metagenomics,
316 metatranscriptomics, and metaproteomics) can be useful to elucidate syntrophic interactions
317 between uncultured microorganisms [11]. The single amplified genome (SAG) approach can be
318 used to obtain genetic information on uncultured organisms, and can potentially be applied to
319 help understand syntrophic processes [21]. Given the extraordinarily high microbial diversity in
320 environmental samples, both 'omics' and SAG approaches need to be used with other processes
321 to identify the functional substrate degraders existing within the complex environmental
322 microbial communities [46].
323 The complexity of NAs in the environment prevents the application of SIP in identifying
324 NAs-degraders [7]. SIP and fluorescent *in situ* hybridization (FISH) are effectively used to label
325 functional taxa [9]. However, SIP needs suitable ^{13}C-labeled compounds, which do not exist for
326 NA. FISH needs known rRNA or functional gene sequences for to design an effective probe, and
327 is not able to find new syntrophic pathways and relationships. The interactome network provides

a new approach for exploring potential syntrophic relationships at the community level especially for unknown syntrophic relationships and uncultured species.

5. Conclusions

This study inferred the microbial interactome networks in anaerobic NAs degrading microcosms under either nitrate- or sulfate-reducing conditions. The difference in network complexity and association profiles among taxa in nitrate- and sulfate-reducing microcosms demonstrates that different electron acceptors lead to distinct microbial relationships. The relative abundance of keystone nodes was substantially low, highlighting the need to examine microbial interactions rather than only concerning microbial composition profiles. Network analysis also revealed that potential syntrophic associations were initial from genera *Bellilinea*, *Longilinea*, and *Litorilinea* in both interactome networks but interacted with different syntrophic taxa in the nitrate- and sulfate-reducing microcosms, respectively. These results indicate that network analysis can be used to reveal an unrecognized dimension of microbial community variation, and provide a novel approach for exploring potential syntrophic relationships to enhance petroleum wastes and other persistent organic pollutants biodegradation.

CRediT authorship contribution statement

Xiaofei Lv: Validation, Writing - Original Draft, Visualization. **Bin Ma**: Conceptualization, Methodology, Supervision. **Korris Lee**: Writing - Review & Editing. **Ania Ulrich**: Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure legends

Figure 1. Interactome networks for (a) nitrate-reducing microcosms and (b) sulfate-reducing microcosms; The associations among classes in the network for (c) nitrate-reducing microcosms and (d) sulfate-reducing microcosms.

Figure 2. The keystone nodes in the networks for nitrate- and sulfate-reducing microcosms. Module hubs represent nodes with $K_{within}>3$ and articulation nodes represent nodes with $K_{out}>1.5$.

Figure 3. Correlation matrices between network topological properties and geochemical properties in (a) nitrate- and (b) sulfate-reducing microcosms. The color and size of circles represent values of the Pearson correlation coefficient. The crosses represent non-significant correlations ($p>0.05$). PD=phylogenetic diversity; DOC=dissolved organic matters.

Figure 4. The modules of interactome networks for (a) nitrate- and (b) sulfate-reducing microcosms and (c) the taxonomic compositions of modules. The numbers indicate the specific module number.

Figure 5. The sizes of modules in subnetworks for (a-d) nitrate- and (e-h) sulfate-reducing microcosms with (a-b, e-f) AEO or (c-d, g-h) Merichem NAs, and (a, c, e, g) clay or (b, d, f, h) sandy sediments.

Figure 6. Potentially syntrophic associations in (a) nitrate- and (b) sulfate-reducing microcosms. The links represent generic pair associations occurring more than one time in nitrate- or sulfate-reducing networks. Red lines represent potential syntrophic associations between interior and peripheral nodes.

Tables

Table 1. Topological properties for the interactome networks for nitrate- and sulfate-reducing microcosms and their corresponding random Erdos-Renyi networks

Networks	Node number	Link number	Mean link number	Diameter	Mean path length	Mean neighbor number	Centrality	Transitivity
Nitrate-reducing network	239	226	0.95	19.0	8.38	2.21	0.076	0.14
Random network 1	239	226	0.95	22.0	9.81	2.42	0.100	0.00
Sulfate-reducing network	238	212	0.89	15.2	7.58	2.17	0.129	0.15
Random network 2	238	212	0.89	24.0	8.88	2.34	0.104	0.01

Associated content

Supporting Information.

The following files are available free of charge.

Figure S1-S24 (PDF).

Table S1. The module and taxon of nodes in the interactome network for nitrate-reducing microcosms (CSV).

Table S2. The module and taxon of nodes in the interactome network for sulfate-reducing microcosms (CSV).

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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CRediT authorship contribution statement

Xiaofei Lv: Validation, Writing - Original Draft, Visualization. **Bin Ma**: Conceptualization, Methodology, Supervision. **Korris Lee**: Writing - Review & Editing. **Ania Ulrich**: Supervision, Funding acquisition.

***Declaration of Interest Statement**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure1

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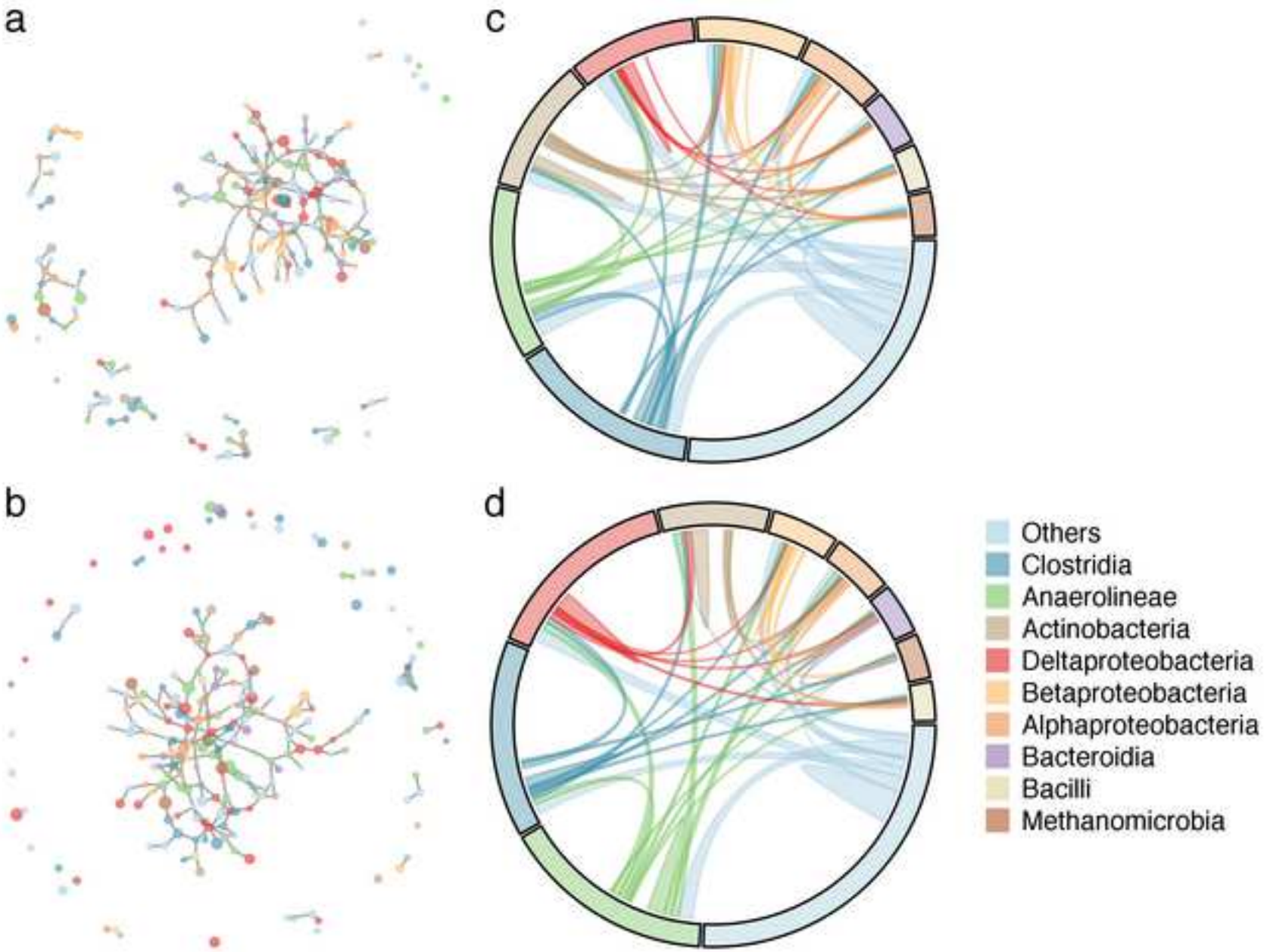

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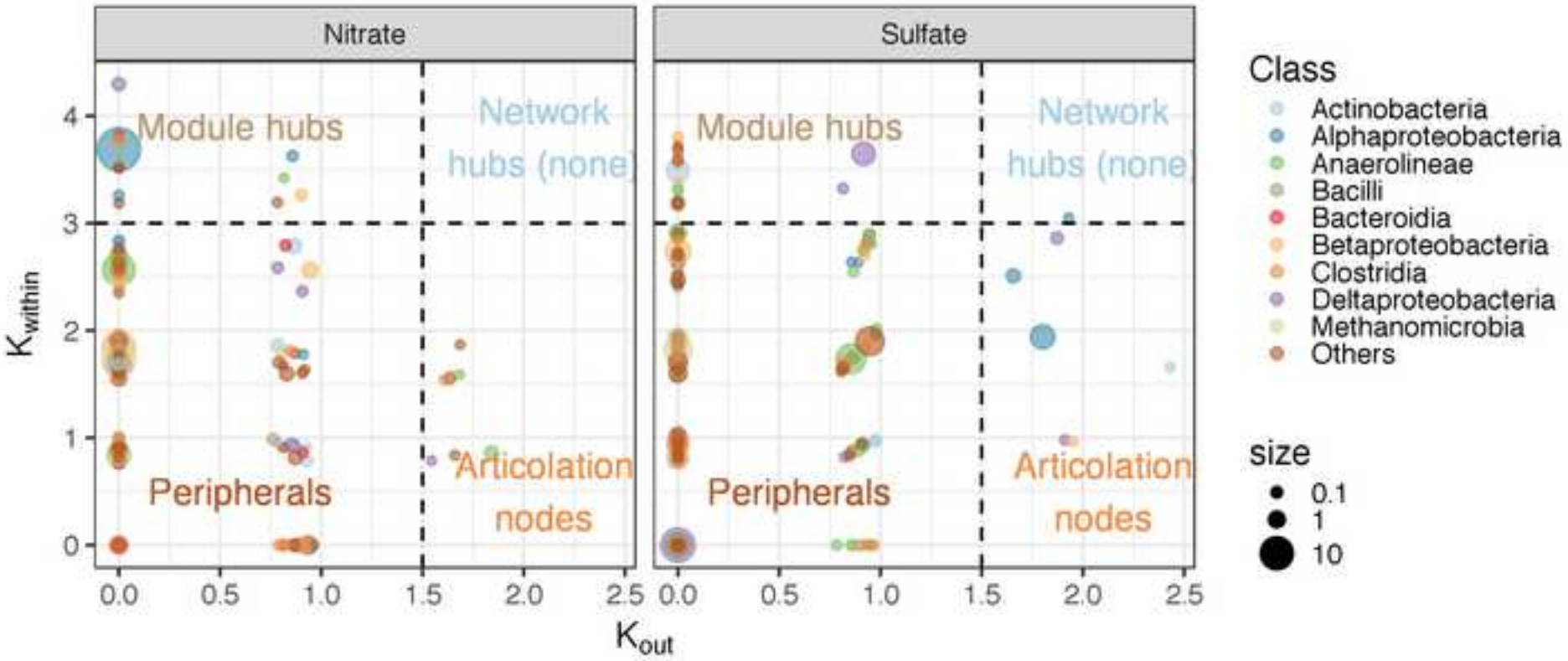

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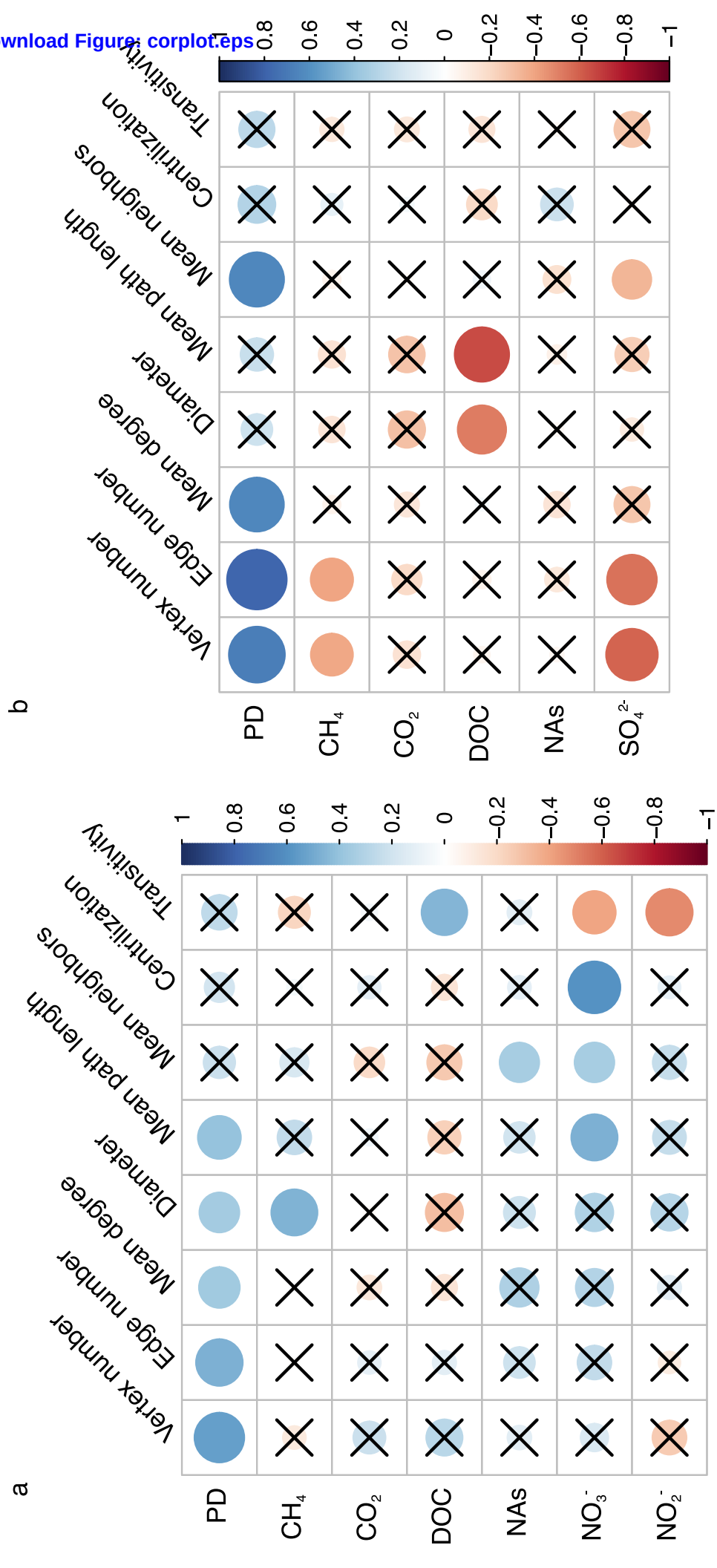

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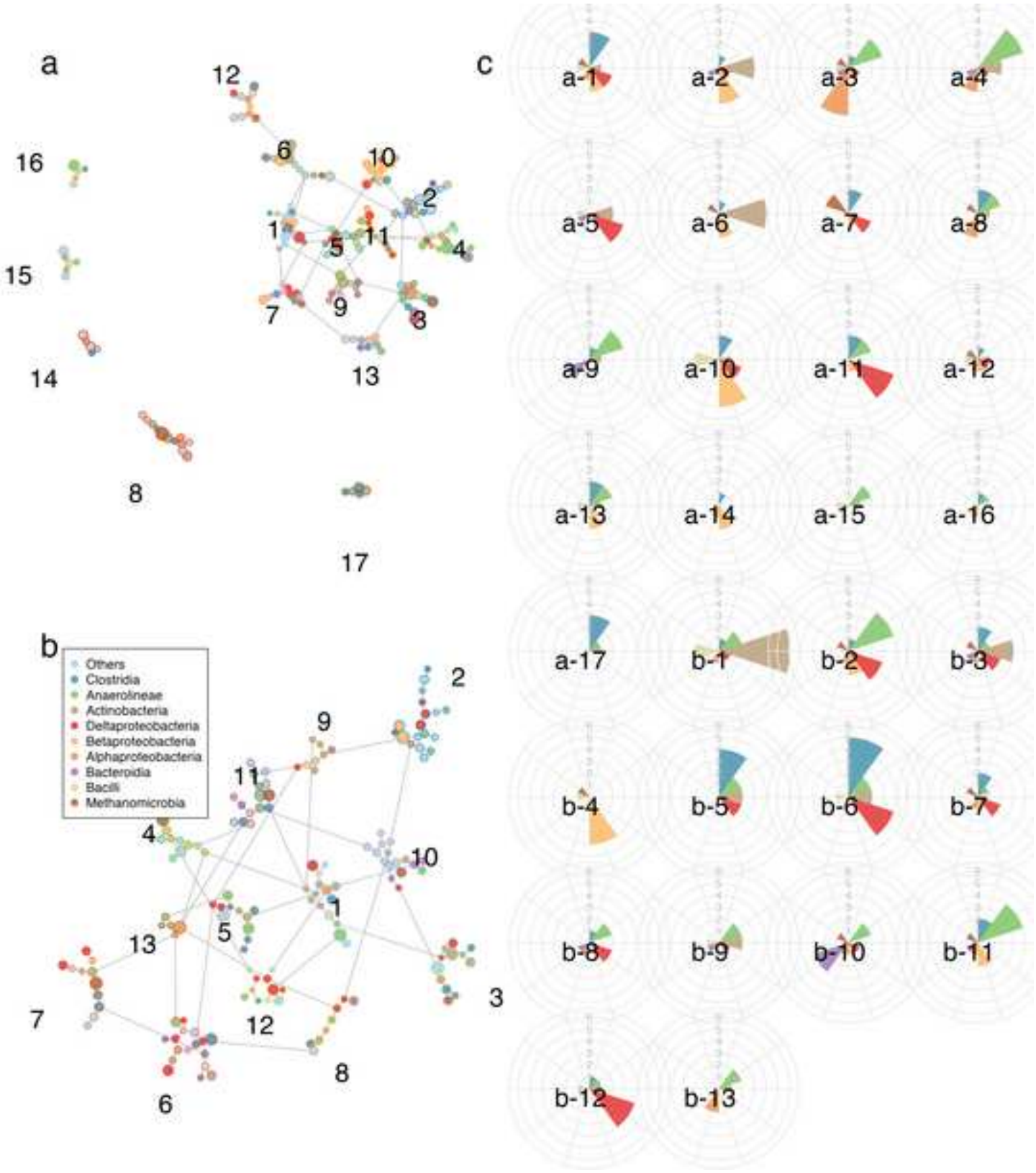

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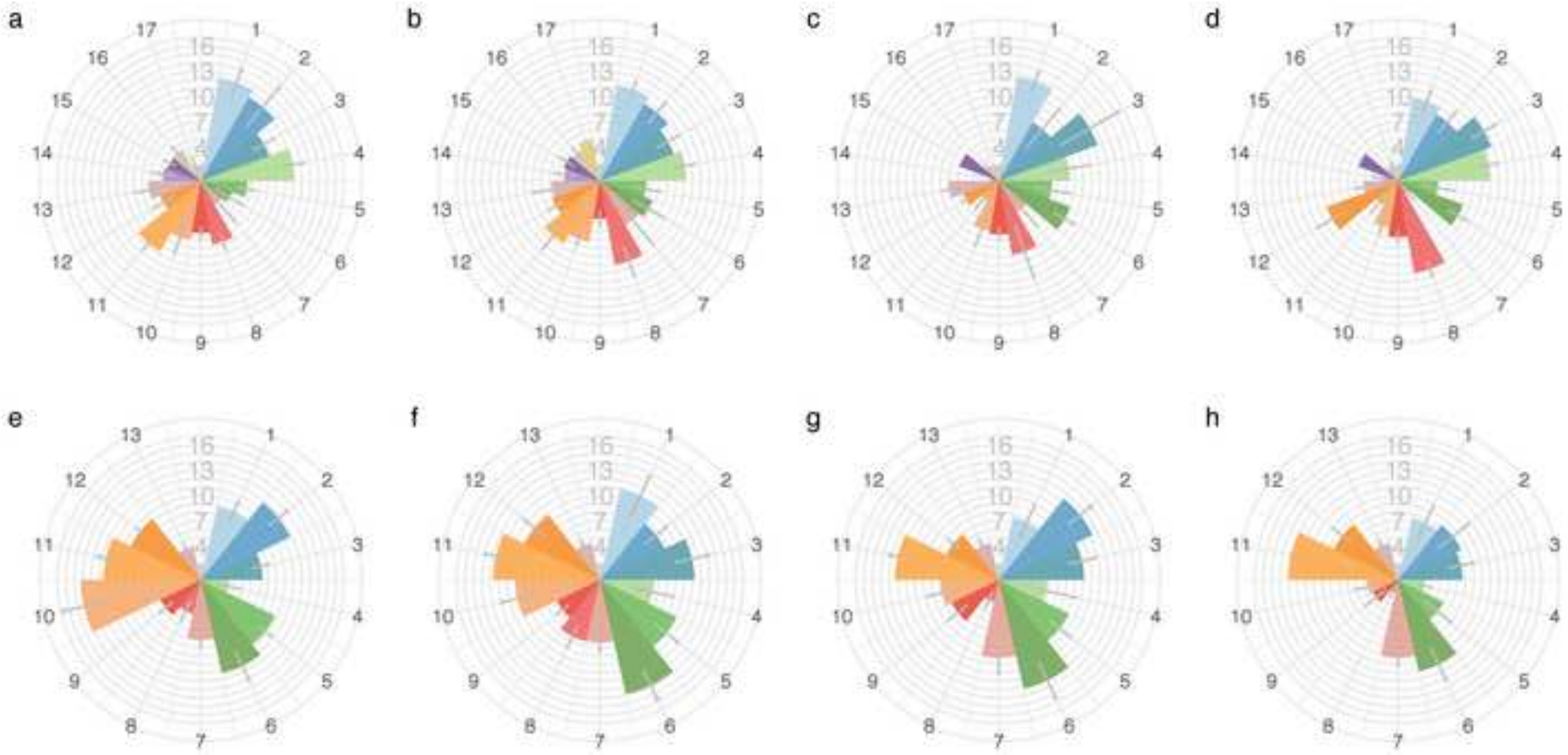

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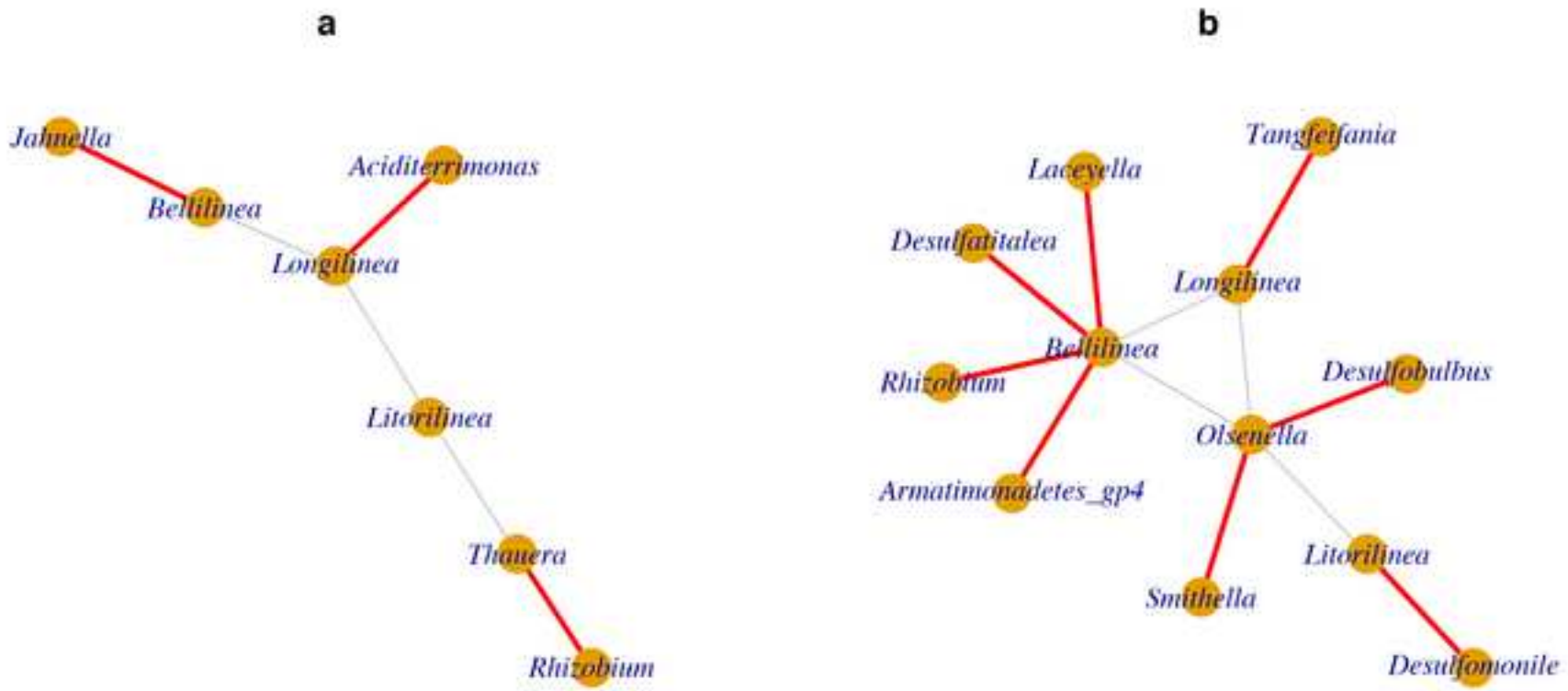

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