



Resource aromaticity affects bacterial community successions in response to different sources of dissolved organic matter

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ABSTRACT

Microbe-mediated transformation of dissolved organic matter (DOM) contributes substantially to the carbon dynamics and energy flow of aquatic ecosystems; yet, the temporal dynamics of bacterial communities in response to diverse DOM sources are scarcely known. Here, we supplied four distinct sources of DOM (algae-derived, macrophyte-derived, sewage-derived, and soil-derived) to the same bacterial community to track the effects of these DOM sources on the carbon processing and successional dynamics of bacterial communities. Although by the end of the incubation the proportion of bio-degraded DOM was significantly lower in the soil-derived DOM treatment than for the other sources, rapid initial metabolism of protein-like and aliphatic compounds and increasing aromaticity and humification degree of DOM during the incubation period were observed for all sources. The role of stochastic processes in governing the community assembly decreased substantially from 61.4% on the first day to 16.7% at the end of the incubation. Moreover, stronger deterministic selection and lower temporal turnover rate were observed for the soil-derived than the other DOM sources, indicating stronger environmental filtering by the more aromatic DOM. Significant correlations were also observed between the humification index (HIX) of DOM and bacterial community diversities, co-occurrence patterns, habitat niche breadths, and the contribution of deterministic ecological processes. In addition, we demonstrated that taxa with different abundance patterns all play crucial but different roles in the response to DOM variation. Our results indicate the importance of DOM aromaticity as a predictor of the outcome of different DOM sources on bacterial community dynamics.

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1. Introduction

Microbes play crucial roles in regulating biogeochemical systems (Banerjee et al., 2018; Jiao et al., 2010). The microbe-mediated transformation of dissolved organic matter (DOM) is essential to the carbon dynamics and energy flow of aquatic ecosystems

(Lapierre et al., 2013; Tranvik et al., 2009), in terms of trophic transfer, greenhouse gas emissions, and water quality changes (Bai et al., 2017; Repeta et al., 2016). This process is partly influenced by the physicochemical conditions in the water column, such as temperature, inorganic nutrient levels, and ultraviolet (UV) radiation intensity (Goldberg et al., 2017; Perez and Sommaruga, 2007), but is also dependent on the inherent quality of DOM showing variations in bioavailability and reactivity (Guillemette et al., 2016; Hur et al., 2011; Judd et al., 2006; Perez and Sommaruga, 2006).

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The bioavailability of DOM is primarily determined by its chemical composition and molecular size (Berggren and del Giorgio 2015; Berggren et al., 2010), which varies with its sources (Murphy et al., 2008; Song et al., 2019; Spencer et al., 2014). Generally, DOM in aquatic ecosystems comprises terrestrial DOM from soil runoff (allochthonous sources) and autochthonous sources derived from exudates of aquatic primary producers, including herbaceous plants and phytoplankton (Stedmon et al., 2007). Cyanobacteria forming blooms facilitated by lake eutrophication and climate warming can release large amounts of DOM (Zhang et al., 2009). Additionally, with increasing intensity of human activities, DOM of anthropogenic origin, such as discharge of sewage and industrial effluents, has also become an increasingly important contributor of DOM (Chen and Hur 2015; Liu et al., 2020). Autochthonous DOM usually enriched in protein and labile polysaccharides is considered more biolabile, whereas terrestrial DOM composed of more humic substances and structural polysaccharides such as cellulose is more recalcitrant to bacterial degradation (Guillemette et al., 2016; Perez and Sommaruga 2006). Furthermore, bacterial degradation of DOM in aquatic ecosystems occurs along a reactivity continuum (Asmala et al., 2018), where the most biolabile fractions are consumed first, followed by the semi-labile fractions, while the recalcitrant pools are persistent in aquatic ecosystems (Bai et al., 2017). For instance, several studies found that a portion of riverine terrestrial DOM is biolabile in the short term, being degraded within estuaries before it reaches off-shore waters (Sipler et al., 2017). Therefore, the interactions between DOM and bacterial communities may depend on the time scale of the biogeochemical reactions in the receiving aquatic ecosystems. However, despite intensive studies examining the association between bacterial communities and DOM composition (Amaral et al., 2016; Melo et al., 2020; Osterholz et al., 2016), little is known about how DOM sources affect microbial successional dynamics at different states of degradation.

Disentangling the mechanisms underlying the temporal dynamics of ecological communities is a central topic in community ecology and is also critical for the prediction of the responses of biodiversity, ecological functions, and services to environmental changes (Dini-Andreote et al., 2015; Zhou et al., 2014). It is generally accepted that the assembly of the microbial community is driven by both deterministic (niche-related theory) and stochastic processes, including diversification, selection, dispersal, and ecological drift (Guo et al., 2018; Zhou and Ning, 2017). Thus, in the context of successional trajectories, knowledge about the linkages between the relative importance of different ecological processes and the changes in DOM composition is required. Moreover, bacterial species of different abundance patterns, i.e. abundant and rare species, may react differently to environmental changes and contribute unevenly to community variations (Jiao et al., 2017; Liang et al., 2020). Specifically, rare species have been considered to be substantial contributors to the temporal variability of community structures (Lynch and Neufeld, 2015). The roles of abundant and rare species in response to different sources of DOM, however, need to be further elucidated to fully understand the temporal dynamics of bacterial community assembly.

We supplied four distinct sources of DOM (algae-derived, macrophyte-derived, sewage-derived, and soil-derived) to the same microbial community to track the effects of DOM sources on the carbon processing and successional dynamics of bacterial communities. DOM chemical composition (using optical and molecular measurements), bacterial abundance, and community composition (using high-throughput sequencing) were characterized during the incubations. The objectives were to: (1) explore the temporal patterns of bacterial degradation of DOM from different sources; (2) examine the divergences of the bacterial communities, including abundant and rare subcommunities, fed by distinct DOM sources at

different stages of degradation; (3) reveal how differences in DOM composition cause shifts in the ecological processes underlying the community assembly. We hypothesized that DOM source-induced environmental filtering imposed to bacterial communities increases as succession proceeds due to decreasing resource bioavailability, being faster for the more aromatic DOM sources.

2. Material and methods

2.1. Collection of DOM sources and experimental set-up

Information about the collection of solutions from four sources (algae-, macrophyte-, sewage-, and soil-derived) is provided in the Supplementary Material. Solutions were subjected to stepwise filtration in filters with a pore size ranging from 150 to 50 (nylon net) down to 2.7 (Whatman GF/D) and 0.22 μm (Millipore membrane). The media were prepared from the 0.22 μm filtrates of the four sources by diluting them to ~ 15 mg dissolved organic carbon (DOC) per liter (to sufficiently track temporal changes in DOM) using Milli-Q water. For the preparation of the inoculum, bacterial abundances of the GF/D-filtrates of the four DOM source solutions were firstly accessed by flow cytometry; the four GF/D-filtrates were then mixed following a 1:1:1:1 ratio of bacterial abundances to cover the species pool degrading the four sources of DOM; the inoculum was finally concentrated to $\sim 5 \times 10^7$ cells/mL.

The experiment was conducted in October 2018 in which the same inoculum was exposed to four different media derived artificially from the DOM sources. Each medium was distributed among two sets of 100 mL brown glass bottles. One set of bottles only received 100 mL medium and served as control of DOM biodegradation. The other set of bottles received 100 mL medium and 2 mL inoculum (final concentration: $\sim 1 \times 10^6$ cells/mL), as well as a nutrient amendment, thereby increasing the ambient concentration by 80 μM NH_4^+ and 10 μM PO_4^{3-} to avoid nutrient limitation (Vonk et al., 2015). In addition, another set of bottles received 100 mL Milli-Q water and 2 mL inoculum and served as control of bacterial abundance. All bottles were acid-soaked and rinsed several times with Milli-Q water and corresponding medium. All samples were incubated at room temperature (20 ± 2 °C) for 28 days to ensure adequate degradation (Vonk et al., 2015) and were loosely capped and shaken daily to supply adequate oxygen. Samples for bacterial abundance, DOC concentration, UV-visible absorbance, and fluorescence measurements were collected on day 0 (the beginning of the experiment), 0.25, 0.5, 0.75, 1, 2, 4, 7, 10, 14, 20, and 28. Samples for Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), were taken on day 0, 7, and 28. Bacterial community was sampled on day 0, 1, 7, and 28. Samples for bacterial community were additionally taken from the four original solutions (the respective GF/D-filtrates) and was referred as "Origin" for downstream bacterial community analyses. There were more than 36 bottles incubated simultaneously for each treatment and control; at each sampling time point, three bottles of 100 mL incubated samples were taken (as three independent triplicates), and in each of those we measured bacterial abundance (1 mL original samples), bacterial community (0.22 μm membranes passed by ~ 90 mL original samples), DOC concentration (10 mL GF/F-filtrate), UV-visible absorbance (20 mL 0.22 μm filtrate), and fluorescence (5 mL 0.22 μm filtrate). The remaining 0.22 μm filtrate of the triplicates ($\sim 65 \times 3$ mL) were mixed for the measurement of FT-ICR MS, and the volume for DOM extraction was adjusted based on DOC concentration to ensure a similar load of DOC with each cartridge (a target mass of 60 μg C) (Dittmar et al., 2008; Kellerman et al., 2014). All glass- and plasticware were used for incubations and sampling, including pipette tips and tubes, were acid-soaked and rinsed with Milli-Q water and

combusted or heat-sterilized (120 °C) to remove potential contaminants.

2.2. CDOM absorbance, fluorescence, and parallel factor analysis (PARAFAC)

Detailed information about DOC, DOM absorbance, and fluorescence measurements can be found in the Supplementary Material. CDOM spectral slope ($S_{275-295}$) increases with decreasing CDOM molecular size (Helms et al., 2008). The humification index (HIX) increases with increasing aromaticity of DOM (Huguet et al., 2009). PARAFAC modeling was conducted with the drEEM toolbox (Murphy et al., 2013). A six-component model was validated by split-half validation analysis and random initialization analysis along with the analysis of residuals (Murphy et al., 2013; Stedmon and Bro, 2008) in our study (Fig. S1).

2.3. FT-ICR MS measurement

A total of 12 samples (4 treatments $\times$ 3 time points) were measured for FT-ICR MS. Electrospray ionization coupled with ultrahigh resolution mass spectrometry was used to resolve the molecular composition of DOM, and samples were measured on a 15 T FT-ICR MS (Bruker Daltonics, USA) at the Korea Basic Science Institute, Ochang, Korea. The mass accuracy of the assigned formulae did not exceed 0.3 ppm after internal calibration using Data Analysis software V4.2, Bruker Daltonik GmbH. Molecular formulae were assigned to signals $\geq 6\sigma$ root mean square baseline noise in MATLAB R2015b, and 18,877 formulae were assigned within the molecular bounds of $C_{4-60}H_{4-80}O_{1-30}N_{0-4}S_{0-2}$. Information about sample pre-treatments, formula assignment, and molecular formulae classification is detailed in the Supplementary Material.

2.4. Bacterial abundance and community composition

One milliliter of original water was fixed immediately with 60 μ L formaldehyde and kept at 4 °C until bacterial abundance was assessed by flow cytometry following the approaches detailed in (Gong et al., 2017). The V4 region of the bacterial 16S rRNA gene was amplified using the primers 515F (5'-GTGYCAGCMGCCGCGTAA-3') and 806R (5'-GGACTACN VGGGTWCTAAT-3'). Detailed procedures for DNA extraction, PCR amplification and purification and sequence processing are provided in the Supplementary Material. All the sequences used in this study are available from the NCBI Sequence Read Archive (SRA) under accession number PRJNA665963. All generated amplicon sequence variants (ASVs) were categorized into four groups (Xue et al., 2018) – abundant taxa (AT): relative abundance $\geq 0.01\%$ in all samples and $\geq 1\%$ in at least one sample; rare taxa (RT): relative abundance $< 0.01\%$ in at least one sample but never $\geq 1\%$ in any sample; moderate taxa (MT): relative abundance ranging from 0.01% to 1% in all samples; and conditionally rare and abundant taxa (CRAT): relative abundance ranging from rare ($< 0.01\%$) to abundant ($\geq 1\%$).

2.5. Diversity analyses

Alpha-diversity was assessed using Chao1, Pielou's evenness, the Shannon and Simpson indices, and phylogenetic diversity (Faith, 2006). Beta-diversity was estimated using both taxonomic and phylogenetic distances (Jaccard, Bray-Curtis, Unweighted and Weighted UniFrac (Lozupone and Knight, 2005)) and visualized using non-metric multidimensional scaling (NMDS). Two-way analysis of variance (two-way ANOVA), together with Tukey's post-hoc test, and permutational multivariate analysis of variance (with

Adonis function) were performed to examine differences in alpha-diversity and community composition among experimental treatments and over time. The temporal turnover rate of the bacterial communities was calculated as the slope of the bacterial time-decay relationship. The time-decay relationship was assessed by applying a linear regression between logarithmic beta-similarities and logarithmic temporal distance using the following equation: $\ln(S_s) = \text{constant} - w \ln(T)$, where S_s is the pairwise similarity in the community, T is the time interval and w is a measure of the rate of species turnover (Liang et al., 2020). Similarity Percentage (SIMPER) analysis was performed to assess which subcommunity primarily contributed to the community dissimilarity over time using the “vegan” package in R.

2.6. Indicator ASVs and habitat niche breadth

Indicator species analysis was applied to identify bacterial taxa preferentially associated with a given environment or pair of environments. Indicator ASVs for experimental treatments were identified with a statistic value > 0.65 and a p -value < 0.05 , assessed after 999 permutations using the “indicspecies” R package (Cáceres and Legendre, 2009). Habitat niche breadth was estimated as the average of Levins' niche breadth values of all members occurring in one community, where lower values were expected to indicate metabolically less flexible communities (Jiao et al., 2020; Levins, 1968).

2.7. Co-occurrence network analysis

To examine how DOM sources affect co-occurrence patterns of bacterial communities, network analysis was constructed by calculating pairwise Spearman rank correlations (Spearman's rho). ASVs were selected by occurrence frequency in all samples ($> 20\%$) to simplify the dataset. Valid co-occurrences with $|\text{rho}| > 0.7$ and adjusted p -value < 0.05 were incorporated into networks. Network properties calculation, module detection, and visualization were conducted using the interactive platform Gephi (WebAtlas, Paris, France). Eigengene analysis (Langfelder and Horvath, 2007) is a useful tool to study the links between modules (a highly interacted group of ASVs) and DOM related parameters for network modules. Eigengene values of modules were calculated using the “WGCNA” R package (Langfelder and Horvath, 2008). To reveal how bacterial members of each module associated with DOM molecular composition, Spearman's rho was calculated between module-based eigengenes and the relative abundances of individual molecular formulae with occurrence frequency $> 30\%$ of all 12 samples (10,007 formulae) (Drake et al., 2019; Kellerman et al., 2014). Since no replicates were measured for FT-ICR MS, module-based eigengenes were calculated as the mean values of three replicates. Spearman's rho was then visualized in a heatmap and a van Krevelen diagram (Kim et al., 2003) for each module, and only formulae with Spearman's rho > 0.4 were presented in the van Krevelen diagram to simplify the plot.

2.8. Null model analysis

The taxonomic normalized stochasticity ratio (tNST), developed by Ning et al. (2019), was calculated based on Bray-Curtis distance and used to estimate the ecological stochasticity, i.e. the relative importance of stochastic processes, with 50% as the cut-off between more deterministic ($< 50\%$) and more stochastic ($> 50\%$) assemblies using the “NST” package in R. We calculated tNST for different groups of samples classified according to incubation time and DOM sources. Accordingly, Bray-Curtis-based Raup-Crick (RC_{bray}) metrics, acting as a supplement to examine

whether communities were stochastically or deterministically assembled (Chase et al., 2011; Stegen et al., 2013), were also obtained using this package. Partial Mantel tests were performed to examine the linkage between Raup–Crick (RC_{bray}) metrics with changes in incubation time and HIX of DOM.

2.9. Other statistical analyses

Principal component analysis (PCA) was conducted based on the parameters related to the DOM optical and molecular compositions to examine the temporal changes in DOM composition. Spearman rank correlation analysis was also performed on the relative abundance of specific taxa and assigned formulae identified by FT-ICR MS to explore the coherence between bacterial species and DOM molecular signatures. All mentioned statistical analyses were carried out using R (v3.6.3).

3. Results

3.1. Variations of DOM characteristics and bacterial abundance

During the first day of incubation, DOC concentrations in the treatments with algae-, macrophyte-, sewage-, and soil-derived DOM decreased by $78.4 \pm 1.6\%$ from initial 12.6 ± 0.5 mg/L, $91.3 \pm 0.5\%$ from initial 17.3 ± 0.04 mg/L, $72.9 \pm 1.0\%$ from initial 21.8 ± 0.01 mg/L, and $23.6 \pm 1.3\%$ from initial 15.3 ± 0.2 mg/L, respectively, followed by relatively slow processing (Fig. 1a). DOC concentrations in the corresponding control samples were relatively stable and experienced a decline after 7 days likely due to colonization of residual or atmospheric bacteria. Accordingly, within one day bacterial abundances increased by more than 50% in the algae- and macrophyte-derived DOM treatments and 75% in sewage-derived DOM treatments from initially $\sim 1 \times 10^6$ cells/mL, and approximately 25% in the soil-derived DOM treatment (Fig. 1b). Bacterial abundances in the four treatments declined subsequently from day 2 to day 7 and reached a relatively stationary phase after day 7 (Fig. 1b). For all treatments, the humification index (HIX) increased after incubation, most pronouncedly for the soil-derived DOM treatment (Fig. 1c). Additionally, the spectral slope, $S_{275-295}$, decreased substantially for the treatments with algae, macrophyte, and sewage-derived DOM (Fig. S2), showing an increasing DOM molecular weight.

PARAFAC analysis identified six fluorescent components (Fig. S1). According to published models in the OpenFluor database (Murphy et al., 2014), the component C1 is humic-like DOM, which is associated with predominately terrestrial sources (Shutova et al., 2014); the component C2 shares spectral properties common to microbial humic-like substances (Cawley et al., 2012); the components C3 and C5 include tryptophan-like fluorophores (Stedmon and Markager, 2005); and the components C4 and C6 represent tyrosine-like fluorophores (Murphy et al., 2011). In the beginning of the experiment, the protein-like components C3–C6 were predominant in the algae, macrophyte, and sewage samples, and the humic-like components C1–C2 dominated the soil sample (Fig. 1; Fig. S3). In all treatments, the protein-like components experienced a notable decline during the experiment in comparison to the controls, most conspicuously during the first day. As for molecular composition, the number of assigned molecules for the soil-derived DOM was notably higher than the other sources (Table S1). Notable decreases of the abundance-weighted proportions of formulae categorized as sugar-like compounds, peptides, and aliphatics as well as increasing proportions of polycyclic and condensed aromatic, polyphenolic, and highly unsaturated and phenolic compounds at the end of the experiment were observed for all four treatments (Table S1; Fig. 2a). In the PCA analysis, all four treatments tended to become more aromatic over time

with decreasing PC1 scores, and the DOM compositions of the bio-degraded samples of the algae, macrophyte, and sewage sources became more similar to the soil-derived DOM composition (Table S1; Fig. 2a).

3.2. Successions of bacterial community composition and alpha-diversity

The subsampled dataset (29,500 reads per sample) consisted of 1209,500 reads and 3049 ASVs. Twenty-two ASVs (0.72%), representing 36.27% of all reads, were abundant taxa (AT) and persistent in all samples; 165 ASVs (5.41%) contributing 41.47% of all reads were conditionally rare and abundant taxa (CRAT); 2861 ASVs (93.93%), containing 22.13% of all reads, were rare taxa (RT); and only one ASV was moderate taxa (MT). AT belonged to the phyla of *Proteobacteria* (*Gammaproteobacteria* and *Betaproteobacteria*), *Cyanobacteria/Chloroplast*, and *Bacteroidetes*. After one day of incubation *Gammaproteobacteria*, *Betaproteobacteria* and *Bacteroidetes* became the most dominant bacteria, from day 1 to day 28, the relative abundance of *Gammaproteobacteria* gradually decreased, while the relative abundances of *Alphaproteobacteria*, *Planctomycetes*, *Verrucomicrobia*, and *Actinobacteria* increased (Fig. 2b).

In all four experimental treatments the analyzed alpha diversities of both the whole and the RT communities decreased within one day, which was followed by a sequent increase from day 1 to day 28 (Table S2; Table S3). These differences were significant both over time and among the four different DOM source treatments (Table S4). The effect of DOM sources was primarily due to the divergence of the soil-derived DOM treatment (Tukey's post-hoc test, $p < 0.05$), which exhibited the highest species richness and evenness (Table S2). Overall, from day 1 to day 28, the alpha-diversities of the whole, AT, RT, and CRAT communities were positively related to time, HIX, and the relative abundances of humic-like components C1–C2 and molecules classified as condensed aromatic compounds and highly unsaturated and phenolic compounds (Fig. S4). Additionally, the number of indicators was significantly higher in the soil-derived DOM (241 ASVs) than in the algae (8 ASVs), macrophyte (5 ASVs), and sewage (25 ASVs) treatments.

3.3. Beta-diversity and temporal turnover rate of bacterial communities

Taxonomic beta-diversity of all taxa, AT, RT, and CRAT differed significantly over time and among the four DOM source treatments (Fig. 3a; Table S5; Adonis's $p < 0.01$). Specifically, the NMDS1 and NMDS2 values of the samples during day 1 and day 28 were linearly significantly related to incubation time ($R^2 = 0.81$, $p < 0.01$) and HIX ($R^2 = 0.48$, $p < 0.01$) ($n = 12 \times 3$ replicates). The temporal turnover rates based on taxonomic diversity were all significantly lower in the soil-derived DOM treatment than in the other treatments, being most pronounced for the AT and CRAT subcommunities (Fig. 3b). The dissimilarities and temporal turnover rates of bacterial communities based on phylogenetic distance were substantially lower than for taxonomic diversity (Fig. S5), likely reflecting that the short experimental period (28 days) was not sufficient to allow distinct phylogenetic differentiations. Moreover, temporal changes in community composition were highest within the first day (day 0–day 1) compared with other periods (day 1–day 7, day 7–day 28) in all experimental treatments (Fig. 3c). Changes in community composition during day 0–day 7 were mainly attributed to changes in the AT subcommunity, while the contribution of the CRAT subcommunity to the changes was highest during day 7–day 28 (Fig. 3c).

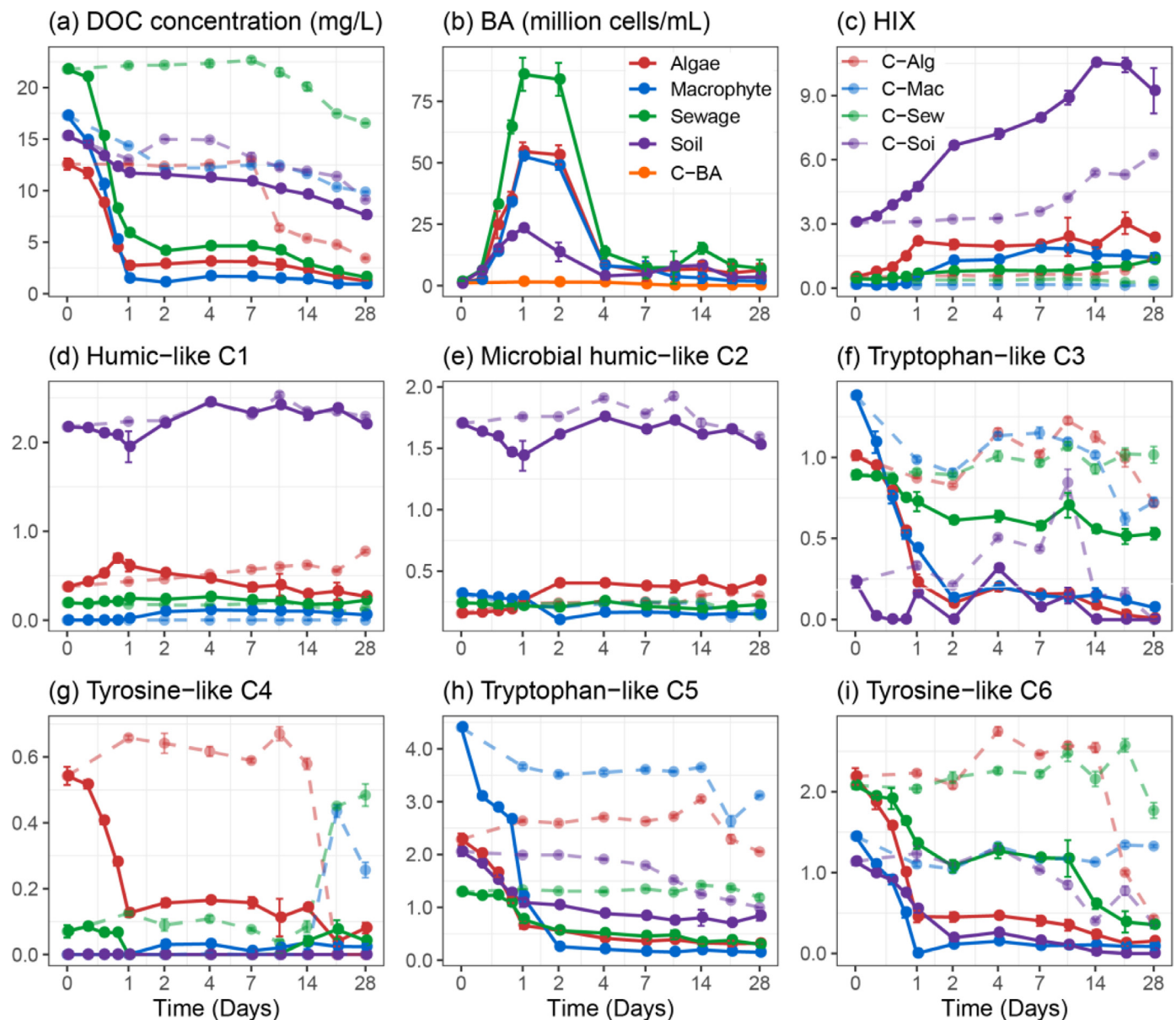


Fig. 1. Net changes in DOC concentration (a), bacterial abundance (b), humification index HIX (c), and fluorescent intensities (Raman Unit) of components identified by PARAFAC analysis (d-i) during the incubation period (total 28 days). The first axis (Days) is log-transformed. C-BA represents the control data for changes in bacterial abundances (bacterial inoculum + Milli-Q water); C-Alg, C-Mac, C-Sew, and C-Soi represent control treatments for bacterial degradation of algae-, macrophyte-, sewage-, and soil-derived DOM (no bacterial inoculum added), respectively. Invisible error bars (standard deviation) indicate that lengths are smaller than the symbols.

3.4. Co-occurrence networks of bacterial communities

The correlation-based network was composed of 622 nodes (ASVs) and 2582 edges (correlations) (Fig. 4). *Alphaproteobacteria*, *Bacteroidetes*, *Gammaproteobacteria*, *Betaproteobacteria*, and *Verrucomicrobia* were the main clades in the network (Fig. 4a). Some ASVs belonging to the same clade tended to co-occur and formed modular structures (Fig. 4a, c). For instance, module 2 and module 4 mainly consisted of *Alphaproteobacteria* and *Gammaproteobacteria*, respectively (Fig. 4a, c). This finding indicates that taxonomic relatedness plays a crucial role in determining network modular structure. Negative correlations (19.0%) were mainly observed between *Gammaproteobacteria* and *Alphaproteobacteria* and *Bacteroidetes*. Keystone taxa for maintaining the structure and function of bacterial communities, were identified based on high mean degree, high closeness centrality, and low betweenness centrality

(Banerjee et al., 2018). The top fifteen keystone taxa belonged to *Alphaproteobacteria* (5 CRAT and 3 RT), *Gammaproteobacteria* (3 AT and 2 RT), and *Bacteroidetes* (2 CRAT) and included the genera of *Acinetobacter*, *Reyranella*, and *Pedobacter* (Table S6).

Eigengene analysis was performed to calculate eigengenes of large modules (number of nodes > 30) in different groups of samples (Fig. 4d). The module-based eigengenes of module 2, module 4, and module 5 were only significantly different over time (ANOVA $p < 0.05$, Fig. 4d), suggesting that members of these modules mainly showed temporal variation. However, module 1 displayed significant differences over time and among the DOM treatments, while module-based eigengenes of module 3 and module 6 were only significantly different among treatments, and these differences were largely caused by the differences between the soil-derived DOM treatment and the other treatments on day 7 and day 28 (Fig. 4d).

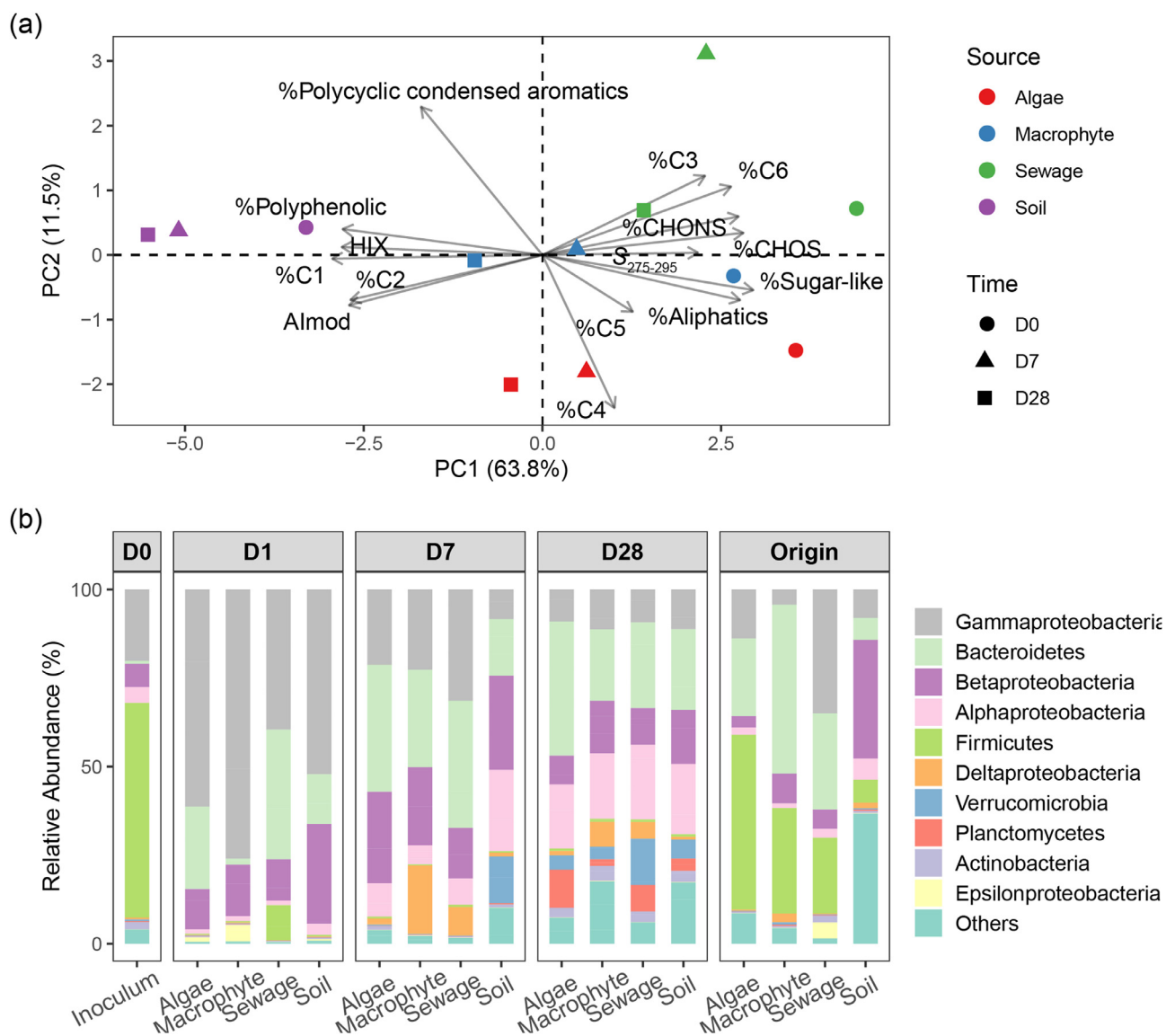


Fig. 2. (a) Principal component analysis (PCA) revealing changes in algae-, macrophyte-, sewage-, and soil-derived DOM composition during the 28-day incubation. The abundance-weighted proportions of formulae containing CHONS and CHOS and classified as sugar-like and aliphatics, together with CDM molecular size-related $S_{275-295}$ and the relative abundance of the protein-like components C3-C6, showed positive PC1 loadings. The abundance-weighted proportions of formulae classified as polycyclic condensed aromatics and polyphenolic and Al_{mod} (modified aromaticity index), as well as HIX (humification index) and the relative abundance of the humic-like components C1-C2, exhibited negative PC1 loadings. (b) Changes in the relative abundance of dominant phyla (relative abundance > 0.5%) of the bacterial communities in four treatments at different time scales. D0 represents the initial bacterial community (the inoculum), D1, D7, D28 represents day 1, day 7, and day 28, respectively. Origin represents the bacterial communities sampled from the four original solutions (the respective CF/D-filtrates) which were mixed for the preparation of the inoculum.

3.5. Associations between bacterial taxa and DOM composition

Modules in the network may represent niche partitioning and functioning differentiation in the bacterial communities (Rottgers and Faust, 2018). We found that module 1–3 and module 6 clustered together (Fig. S6) and overall showed positive correlations with highly unsaturated and phenolic compounds and polyphenolic compounds (Fig. 5), as well as humic-like components and HIX (Fig. S4). In contrast, module 4 was characterized by positive correlations with aliphatics and protein-like components (Fig. 5; Fig. S5). The relative abundances of identified keystone taxa were also positively related to more aromatic compounds except for the five *Gammaproteobacteria* keystone ASVs (Fig. S7). The associations between the molecules and the relative abundances of the top five indicators of the different DOM source treatments also reflected the effect of more aromatic and humic-like niches related to the soil-derived DOM (Fig. S7).

3.6. Ecological processes shaping bacterial community structures

The value of tNST, representing the relative importance of stochastic processes in governing taxonomic community structure, decreased substantially from day 1 (61.4%) to day 7 (40.6%) and day 28 (16.7%), and was most pronounced in the CRAT subcommunities (Fig. 6a). This coincided with the variation of Raup-Crick_{bray} in the whole community, which had a negative value closer to zero on day 1 and positive values closer to 1 on day 7 and day 28 (Fig. 6b). These results indicate increased importance of deterministic selection in governing the taxonomic community structure and more dissimilar communities over time. Moreover, the Raup-Crick_{bray} metric was significantly correlated with changes in incubation time (partial Mantel's correlation = 0.52, $p < 0.001$) and HIX (partial Mantel's correlation = 0.27, $p < 0.01$) ($n = 12 \times 3$ replicates). Deterministic processes dominated the assembly of the whole community in all treatments (tNST < 40%),

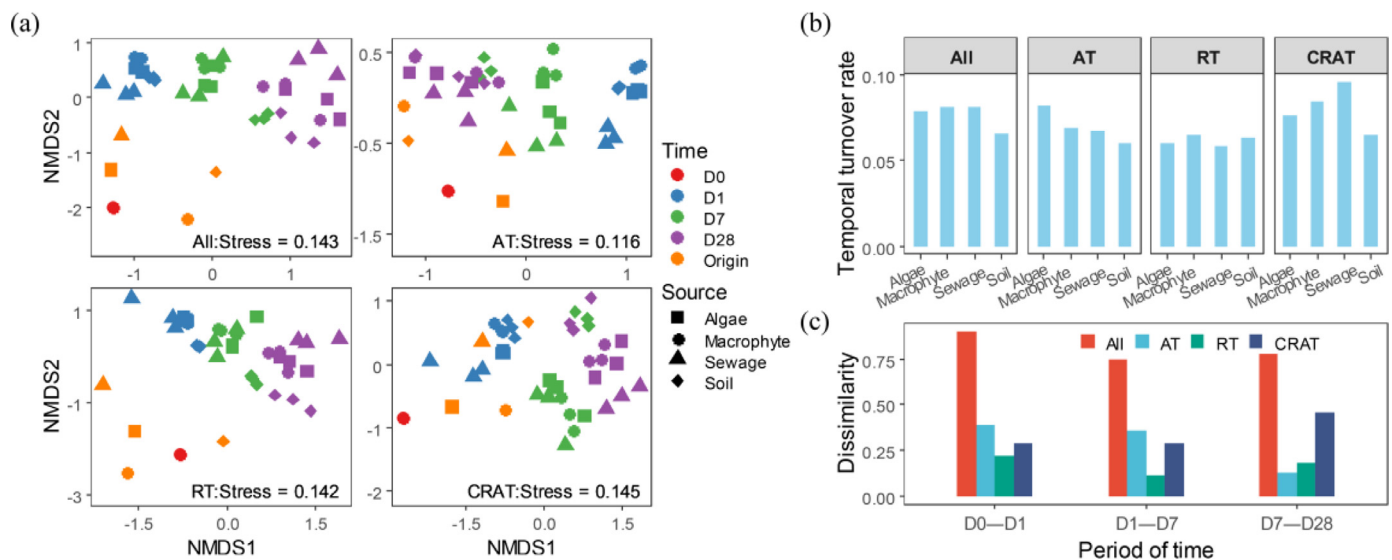


Fig. 3. (a) Non-metric multidimensional scaling (NMDS) ordination of bacterial communities of all taxa, abundant taxa (AT), rare taxa (RT), and conditionally rare and abundant taxa (CRAT) based on Bray–Curtis dissimilarities, showing the differences of bacterial communities over time and among four experimental treatments. (b) Temporal turnover rates based on Bray–Curtis distances of bacterial communities of all taxa, AT, RT, and CRAT in the four different DOM source treatments. Temporal turnover rates estimated as the slopes of the time-decay relationships using linear regressions between log-transformed community similarity and log-transformed temporal distance. (c) Contributions of each subcommunity to the community dissimilarities in different periods of time based on similarity Percentage (SIMPER) analysis using Bray–Curtis dissimilarities. D0 represents the initial bacterial community (the inoculum), D1, D7, D28 represents day 1, day 7, and day 28, respectively. Origin represents the bacterial communities sampled from the four original solutions (the respective GF/D-filtrates) which were mixed for the preparation of the inoculum.

and the relative importance of stochastic processes was lowest in the soil-derived treatment (tNST = 16.0%). The tNST value was overall lower in the CRAT subcommunity compared with AT and RT.

The values of habitat niche breadths of all taxa and RT were significantly higher on day 1 and day 7 than on day 28 and were significantly lower in the soil-derived DOM treatment than in the other treatments on day 7 and day 28 (Fig. 6c; Tukey's post-hoc test, $p < 0.001$), indicating more habitat specialists in the soil-derived DOM treatment on day 7 and day 28 (Jiao et al., 2020). The habitat niche breadths of RT and CRAT were negatively correlated with the incubation time and the proportion of microbial humic-like C2 (Fig. S4).

4. Discussion

Our study showed that DOM sources influenced the time-dependent successional patterns of bacterial community diversity and composition, and the influence was highly associated with aromaticity of the DOM. We further revealed that DOM source-induced deterministic selection of bacterial communities differs at different degradation stages, with community assembly processes shifting from more stochastic to more deterministic processes over time. Finally, we observed that taxa with different abundance patterns showed different reactive strategies, but all contained key-stone species in response to the variation of DOM during the experiment.

4.1. Temporal patterns of bacterial degradation of DOM from different sources

We demonstrated that the availability of different DOM sources, i.e. the proportion of the bio-consumed fraction, is inherently related to DOM composition – specifically, the abundances of protein-like components, sugar-like molecules, or aliphatics relative to humic-like components and polyphenolic compounds. Although the overall amount of bio-consumed DOM differed among

sources, similar temporal variation patterns were observed for all DOM sources during the experiment (Fig. 1; Fig. 2). The greatest changes in fluorescence protein-like components within the first day coincided with the greatest changes in DOC concentration across all sources, followed by relatively stable and slow processing (Fig. 1). This agrees with previous incubation experiments and suggests that highly bio-labile protein-like, sugar-like, and aliphatic compounds are involved in microbial metabolism and material cycles in the short term (Bai et al., 2017; D'Andrilli et al., 2019; Guillemette et al., 2013; Sipler et al., 2017). However, the proportion of biodegradable DOC in natural ecosystems can be lower than we observed since enriched DOM derived from various specific sources was used in the experiment (Vonk et al., 2015). Importantly, the initial structure of the bacterial community can play a crucial role in transforming DOM over time (Logue et al., 2016); therefore, to examine the effect of DOM sources on bacterial carbon processing and community dynamics, we incubated all cultures with the same bacterial community, covering the species pool degrading all four types of DOM. We further found that the bacterial processing of DOM contributed to increasing the aromaticity and humification degree of DOM across all sources (Fig. 2).

The shifts in DOC concentration and DOM composition over time coincided with the compositional changes in bacterial communities. Highly biolabile DOM boosted the bacterial abundance within the first day, while bacterial alpha-diversity decreased due to the prevalence of *Gammaproteobacteria*, *Betaproteobacteria*, and *Bacteroidetes* across all sources. These members have also been reported to respond to enrichment of other types of DOM, such as diatom-derived DOM (Landa et al., 2014), grass DOM (D'Andrilli et al., 2019), and virus-induced DOM from picocyanobacteria (Zhao et al., 2019), demonstrating that these types of bacteria react rapidly to biolabile DOM. For instance, *Gammaproteobacteria* are capable of utilizing amino acids, extracellular polysaccharides, and proteins (Blanchet et al., 2016; Zhao et al., 2019), and *Bacteroidetes* are able to decompose hydrolytic cellulose, starch polysaccharides, and carbohydrate (Bauer et al., 2006).

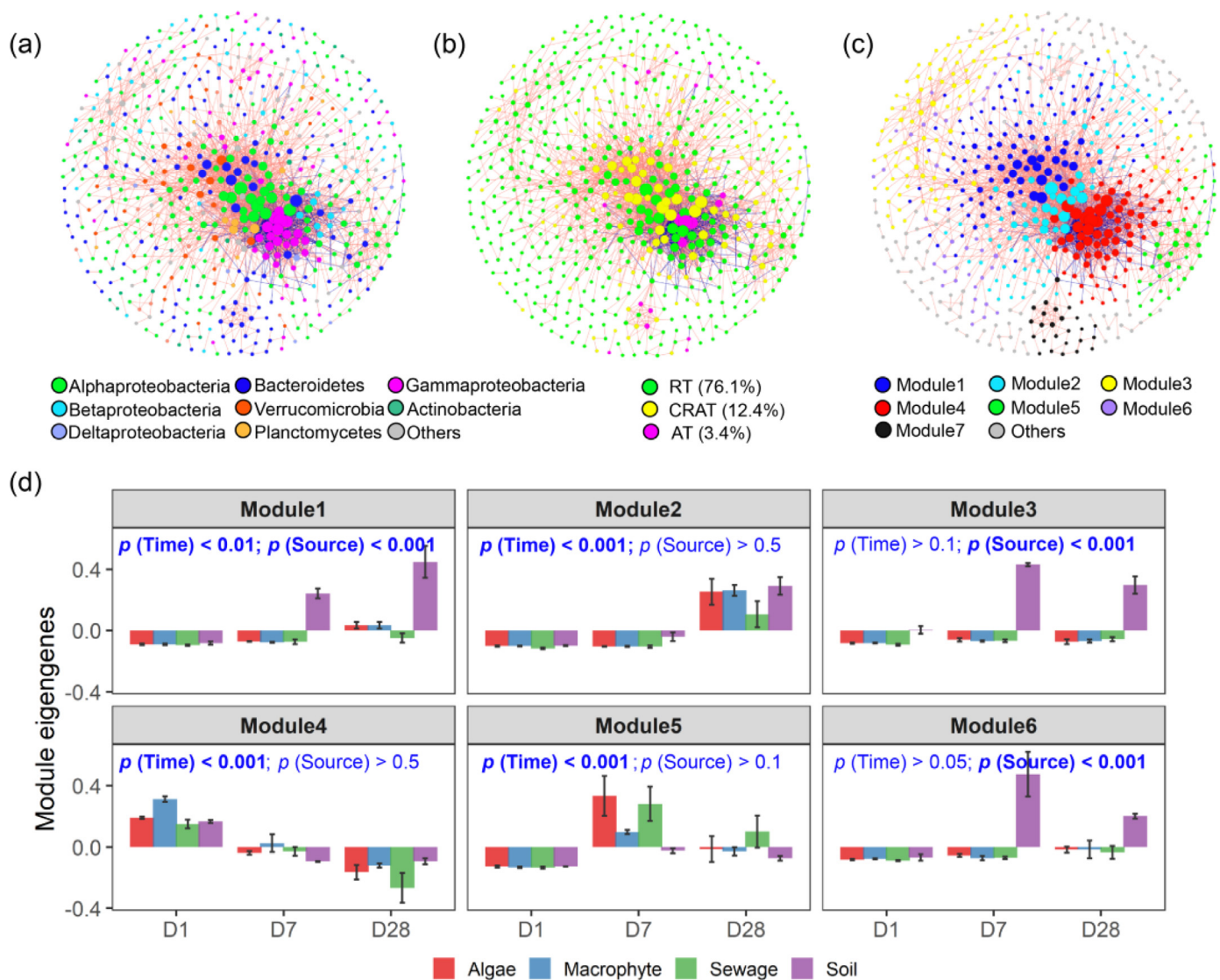


Fig. 4. Co-occurrence network of bacterial communities during the experiments coloured by corresponding phylum/class (a), taxa classification (b), and modules (c). Comparisons of the module-based eigengenes of different DOM source treatments during the experiments (d). Texts in the figure (d) show the p -value of ANOVA testing for differences in the module-based eigengenes over time (day 1, day 7, and day 28) and among the experimental treatments with four DOM sources. Eigengene analysis was performed to calculate module-based eigengenes of the different DOM source treatments during the experiments.

Following rapid consumption of highly biolabile DOM, bacterial abundances decreased close to the initial level on day 7. By contrast, bacterial diversity indices increased gradually from day 1 to day 28 across all sources, indicating that less bacteria, but more taxa participated in degrading less biolabile and more aromatic DOM molecules. Accordingly, bacteria that are competitive under resource-poor and aromatics-rich conditions, including *Alphaproteobacteria*, *Planctomycetes*, and *Actinobacteria* (Li et al., 2020; Liu et al., 2019) co-occurred in the network (Fig. 4). While we provide evidences for overall substrate preferences of different bacterial taxa, facilitating better understanding of changes of bacterial communities in responses to resource heterogeneity and environmental changes, we also acknowledge that our experiment occurred in a relatively closed environment, where the changes of bacterial community composition were highly influenced by the initial inoculum. Considering that natural ecosystems receive species pools from other sources and systems, the responses of bacterial community composition to DOM is likely more complex in nature.

4.2. Effects of DOM sources on the temporal dynamics of bacterial communities

We further revealed a notably decreased stochasticity ratio with incubation time (Fig. 6), supporting our hypothesis that the DOM source-induced deterministic selection of bacterial community assemblages increases as succession proceeds. Our results concur with macroecological theory, stating that stochasticity of community assembly increases with elevated resource supply (Chase 2003). In our study, at the early stage of degradation (within the first day), a fraction of DOM was readily available in all treatments, which reduced competitive pressures as reflected by exponential increase of the bacterial abundances and dominance of stochasticity (Dini-Andreote et al., 2015). As succession proceeded, the ratio of aromatic and less biolabile DOM increased causing resource depletion and limitation which manifested in the deterministic selection becoming progressively more important. Accordingly, we found substantial divergences of habitat niche breadths and bacterial co-occurrence patterns between the soil-derived DOM treatment and the other treatments on day

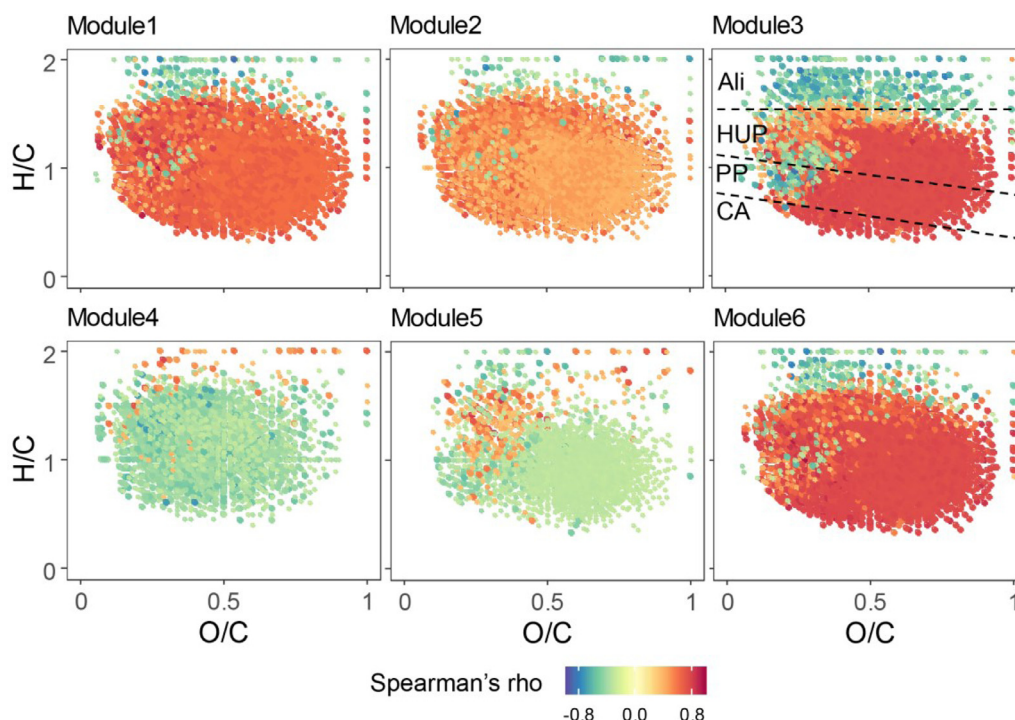


Fig. 5. van-Krevelen diagrams displaying the Spearman rank correlations (Spearman's rho) between module-based eigengenes and assigned molecules identified by ultrahigh-resolution mass spectrum (FT-ICR MS) ($n = 12$). Only formulae with Spearman's rho > 0.4 were presented in the van Krevelen diagram to simply the plot. Lines approximately delineate compound groups: Ali: aliphatics; HUP, highly unsaturated and phenolic compounds; PP, polyphenolic compounds; CA, condensed aromatic compounds.

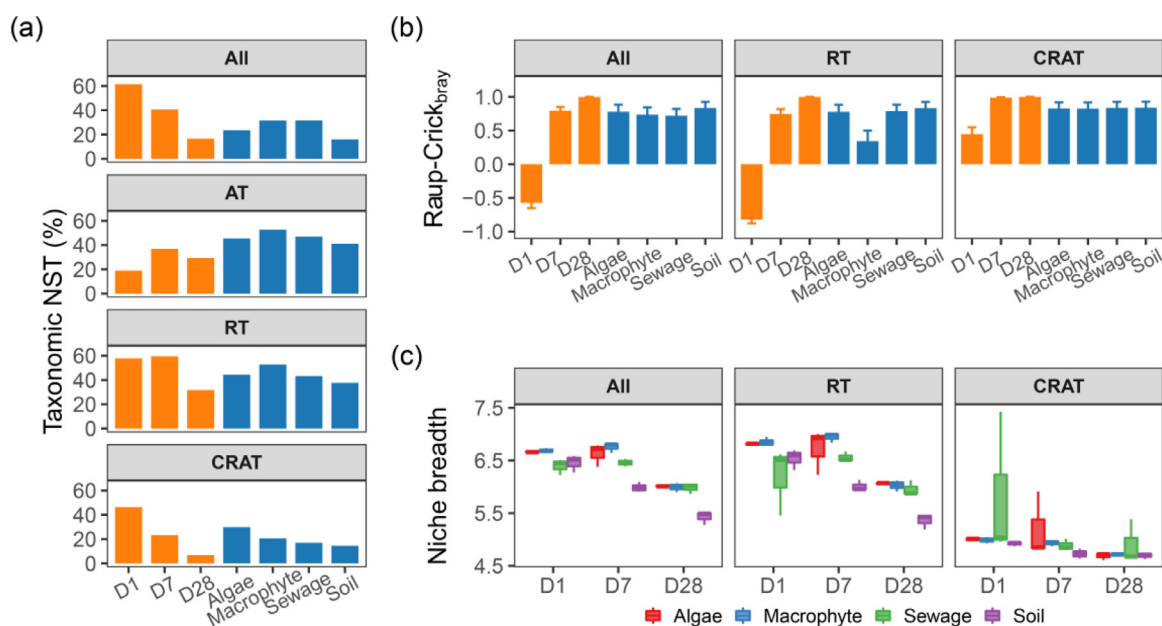


Fig. 6. Null model analyses revealing the taxonomic normalized stochasticity ratio (tNST) of bacterial community assemblages, i.e. the relative importance of stochastic processes in the community assembly, based on abundance-weighted Bray-Curtis distances during the incubation time and between the different DOM source treatments (a). Variation of modified Raup-Crick (Raup-Crick_{bray}, mean $\pm$ se) during the incubation time and between the different DOM source treatments (b), with a mean value close to 0 when stochastic processes dominate among communities; and a value approaching -1 when a community is deterministically assembled and more similar to each other than expected by chance; and a value close to $+1$ when deterministic processes favor dissimilar communities. Habitat niche breadths (mean $\pm$ sd) of all, RT, and CRAT in the different groups of samples (c). AT habitat niche breadths were identical among the different groups (value = 13.3) and thus not displayed. AT: abundant taxa; RT: rare taxa; CRAT: conditionally rare and abundant taxa.

7 and day 28 but not on day 1. It is noteworthy that we manipulated the physicochemical conditions (e.g. temperature and nutrient supply) in all treatments to ensure that the deterministic selection could be mainly ascribed to the effect of DOM sources, but in reality, extreme physicochemical conditions may also alter the impact of resource properties on community assembly processes

(Dini-Andreote et al., 2015). The community assembly mechanisms have been thought to drive the relationships between microbial community and function (Curtis et al., 2013). For instance, increasing selection stress that drive the community assembly to a determinism stage will result in selection of efficient bacteria to handle the resources available, whereas high level of stochasticity de-

creases the function of microbial communities by increasing the proportion of non-functional taxa (Zhang et al., 2019). Our results thus imply that excessive enrichment of biolabile substrate for bacteria, such as eutrophication-induced algal bloom or sewage input, may cause instability in the aquatic microbial ecosystem functioning, though further studies are needed to elucidate this intrinsic mechanism.

Significant differences in the direction or magnitude of the effect of DOM sources were observed for bacterial diversity, community composition, and niche partitioning. The soil-derived DOM treatment had the highest alpha-diversity but the lowest taxonomic temporal turnover rate and habitat niche breadths, as well as distinct co-occurrence patterns of species groups. Positive correlations between bacterial richness and evenness with the number of molecules in the DOM pool have been documented recently (Muscarella et al., 2019). This may partly explain why soil-derived DOM had the highest bacterial alpha-diversity of the four DOM sources, since a significantly greater number of molecules provide more niches for species (Finke and Snyder 2008). On the other hand, soil-derived DOM are more humic-like and aromatic than algae-, macrophyte-, and sewage-derived DOM. More recalcitrant compounds may require enzymatic activities or transport systems at varying levels of complexity and thus be more selective, favoring species equipped with specific metabolic capabilities and more habitat specialists with narrow niche breadths (Landa et al., 2014). More recalcitrant compounds can also explain the lower temporal turnover rate in the soil-derived DOM treatment since the bacterial community structure tends to stabilize more quickly under the pressure of stronger selection, coinciding with more similar community structures on day 7 and day 28 in the soil-derived DOM treatment (Fig. 3). Moreover, the lowest value of the stochasticity ratio occurring in the soil-derived DOM treatment further supports that the less bioavailable compounds with a higher aromaticity and humification degree tend to be more selective for community assemblages. It is noteworthy that although the sewage-derived DOM is an anthropogenic source compared with the autochthonous sources derived from algae and macrophytes, all of these three sources were dominated by the protein-like compounds and had similar temporal turnover rates, habitat niche breadths, and co-occurrence patterns of species, suggesting a central role of DOM degradability in shaping bacterial community assemblages. In previous studies investigating the effect of environmental selection-induced determinism on bacterial communities, substrate quantity has often been in focus (Jiao et al., 2020), and our study showed that DOM quality is also of crucial importance and needs to be considered when searching for the relationships between environmental filtering and bacterial community structures.

Correlations between DOM molecules and bacterial taxa, describing the coherence between bacterial species and DOM molecular signatures, can be used to infer bacterial metabolic preferences and transformation of DOM (Muscarella et al., 2019; Zhao et al., 2019). In a network, species groups clustering in the same module are considered to share similar niches and perform similar functions (Rottjers and Faust, 2018), while keystone nodes are commonly considered as initiating components of networks that could drive community composition and function (Banerjee et al., 2018; Zhao et al., 2019). We found that the module-based eigengenes of module 1–3 and module 6, which increased over time or were the highest in the soil-derived DOM treatment, showed strong positive correlations with more aromatic compounds (Fig. 5). A similar correlation was also observed for the habitat specialists in the soil-derived DOM treatment and most keystone species except those belonging to *Gammaproteobacteria*, providing further evidence of the directional effect of increasing aromatic DOM on bacterial co-occurrence patterns, metabolic

profiles, and ecosystem functioning. Additionally, few correlations with aliphatics were detected, maybe due to the rapid metabolism of these materials (Zhao et al., 2019).

4.3. Different responses among bacterial taxa with different abundance patterns

The time-dependent successional patterns in community diversity and composition of AT, RT, and CRAT were significantly affected by DOM sources, but different reactive strategies were also detected among taxa with different abundance patterns. The prevalence of AT in the experimental treatments at the early stage of DOM degradation suggests that AT were cosmopolitan taxa and precursors reacting rapidly to complex compounds (Langenheder and Szekely, 2011). RT represented the majority of ASVs (93.9%) and mainly contributed to the changes in alpha-diversity, especially species richness, phylogenetic diversity, and habitat niche breadths over the course of the experiment. It is generally recognized that the rare biosphere with high species diversity has an important influence on the ability of a community to stabilize and maintain ecosystem function by acting as a functional cache or resource pool for responding to disturbance events (Jousset et al., 2017; Lynch and Neufeld 2015). Our study further showed that the highest proportion of all reads (41.5%) belonged to few CRAT ASVs (5.4%). We also found that the contribution of the CRAT subcommunity to the temporal changes in community composition was highest during day 7 – day 28, supporting the concept that rare species can serve as an active seed bank and pool of metabolic potential that can become dominant under favorable conditions (Lynch and Neufeld, 2015). Moreover, an overall lower taxonomic stochasticity ratio was observed for CRAT than for AT and RT, indicating a stronger deterministic selection imposing on members of the CRAT subcommunity. These results and those of previous studies demonstrate that rare bacteria, occasionally becoming very abundant, are important contributors to the temporal dynamics of the bacterial community in response to environmental changes (Nyirabuhoro et al., 2020; Shade et al., 2014). Importantly, the keystone species identified in the network analysis including both AT, RT, and CRAT suggest that taxa with different abundance patterns all played crucial roles in responding to the variation of DOM, thereby emphasizing the necessity of considering variations of species with distinct abundances, especially in the rare biosphere, to be able to better understand bacterial community dynamics in response to environmental changes at different spatiotemporal scales. Moreover, although RT have been generally considered to contribute little to the carbon flow due to their much lower biomass compared with that of AT (Pedros-Alio, 2012), our study highlights the important contribution of rare/conditionally rare taxa to the carbon flow of ecosystems especially the refractory carbon.

5. Conclusion

Our results revealed that the influence of DOM sources on time-dependent successions of the bacterial community was highly related to aromaticity of the DOM, which acts as an indicator of the temporal succession of DOM composition and bioavailability. Moreover, community assembly processes shifted from high stochasticity to high determinism as succession proceeded, indicating that deterministic selection was induced by more aromatic DOM. We thus propose that DOM aromaticity can be an efficient predictor in comparisons of the selective strength of DOM sources/composition on bacterial community dynamics. In addition, we demonstrated that taxa with different abundance patterns all play crucial but different roles in the response to DOM variation, and emphasized the importance of rare bacteria under re-

fractory DOM conditions. Our findings expand the knowledge of the interactions between microbes and DOM from the perspective of temporal dynamics. Considering the complexity of nature environments, the responses of microbial community structures and metabolic profiles to mixtures of different DOM sources need to be further explored.

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.watres.2020.116776](https://doi.org/10.1016/j.watres.2020.116776).

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