Supplementary Information for

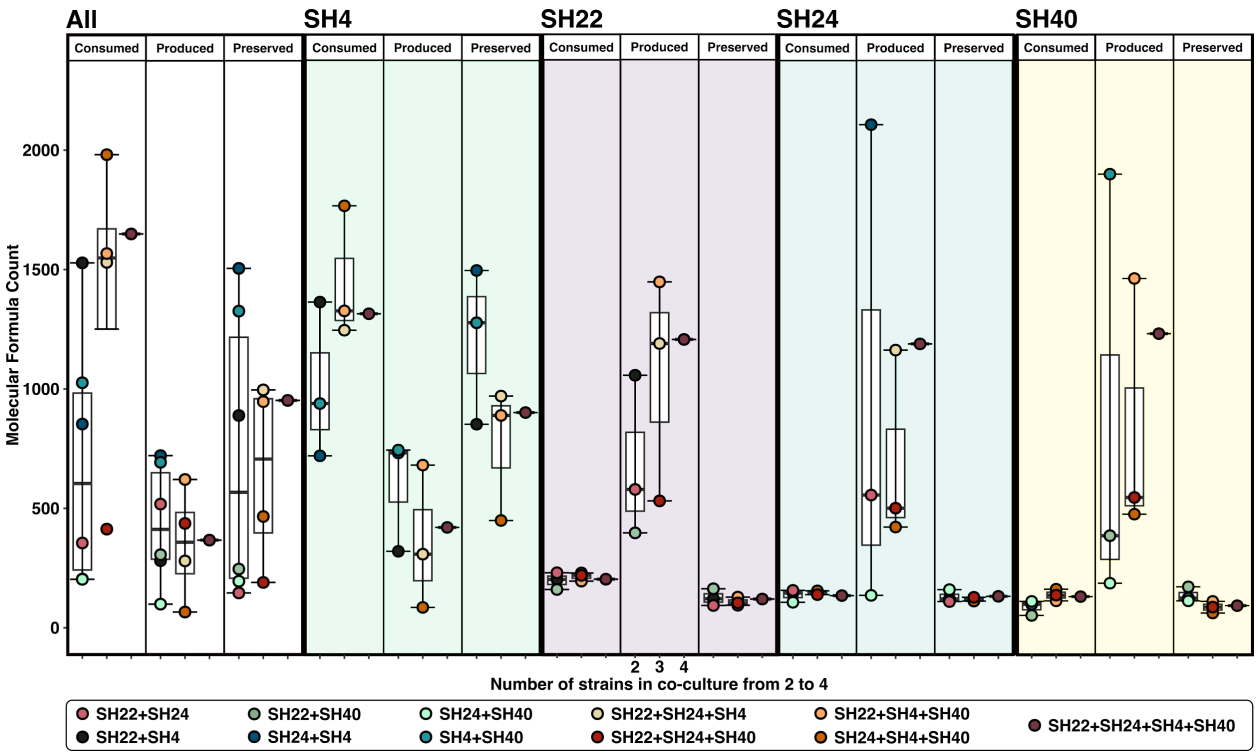
Bacterial interactions shape the molecular composition of dissolved organic matter

Jessika Füssel[1,*], Samuel Miller[2], Carlotta Hecker[3], Florian Trigodet[4,5], Leon Dlugosch[1], Thorsten Brinkhoff[1], Thorsten Dittmar[1,5,6], A. Murat Eren[1,2,4,5,6], Sinikka Lennartz[1,†,*], Carina Bunse[7,†,*]

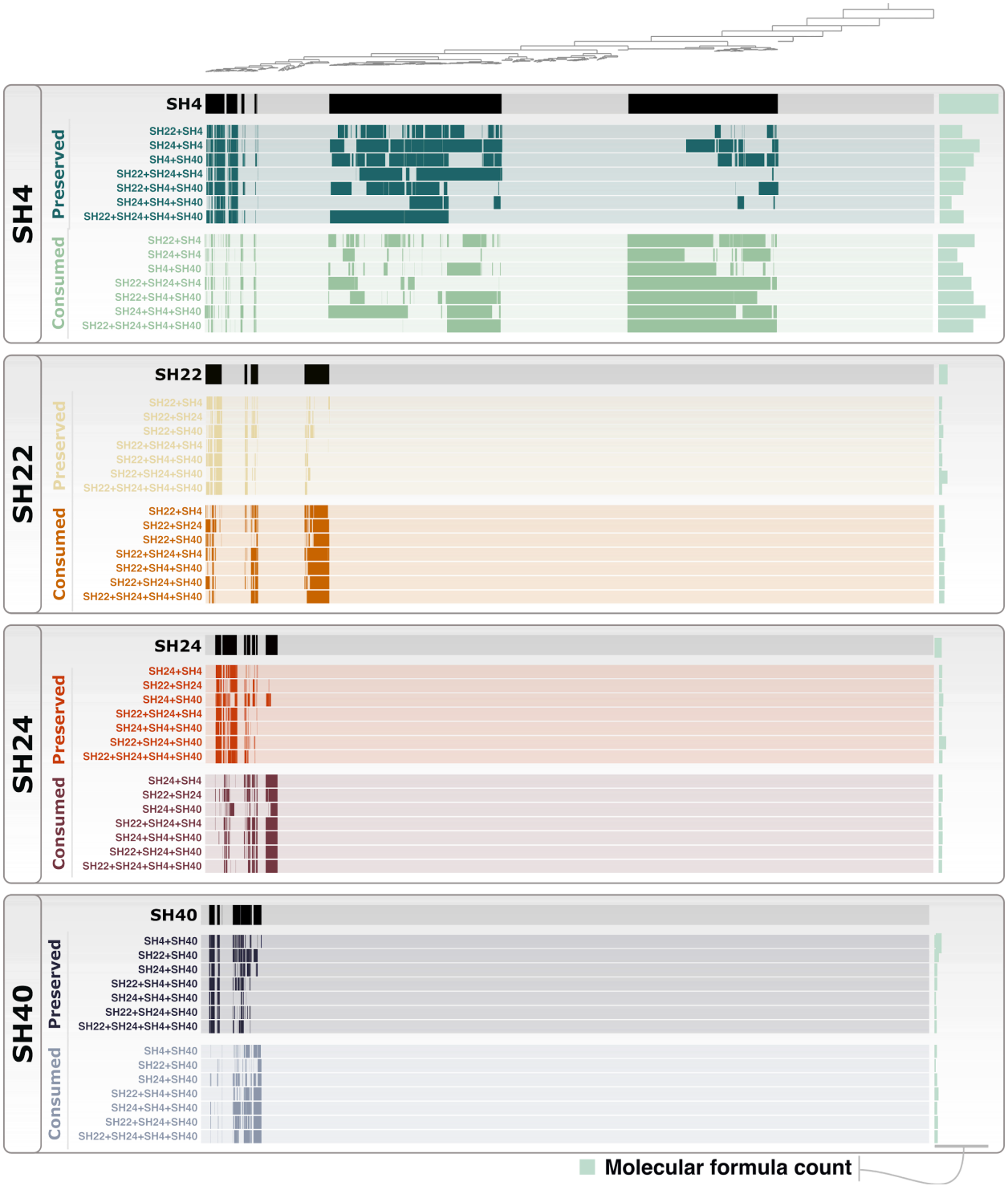
[1]University of Oldenburg, Institute for Chemistry and Biology of the Marine Environment, Oldenburg, Germany; [2]Josephine Bay Paul Center, Marine Biological Laboratory, Woods Hole, Massachusetts, USA; [3]Leibniz Institute for Natural Product Research and Infection Biology (Leibniz-HKI), Jena, Germany; [4]Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, Germany; [5]Helmholtz Institute for Functional Marine Biodiversity at the University of Oldenburg (HIFMB), Germany; [6]Max Planck Institute for Marine Microbiology, Bremen, Germany; [7]University of Gothenburg, Department of Marine Sciences, Gothenburg, Sweden.

[†] These authors contributed equally.

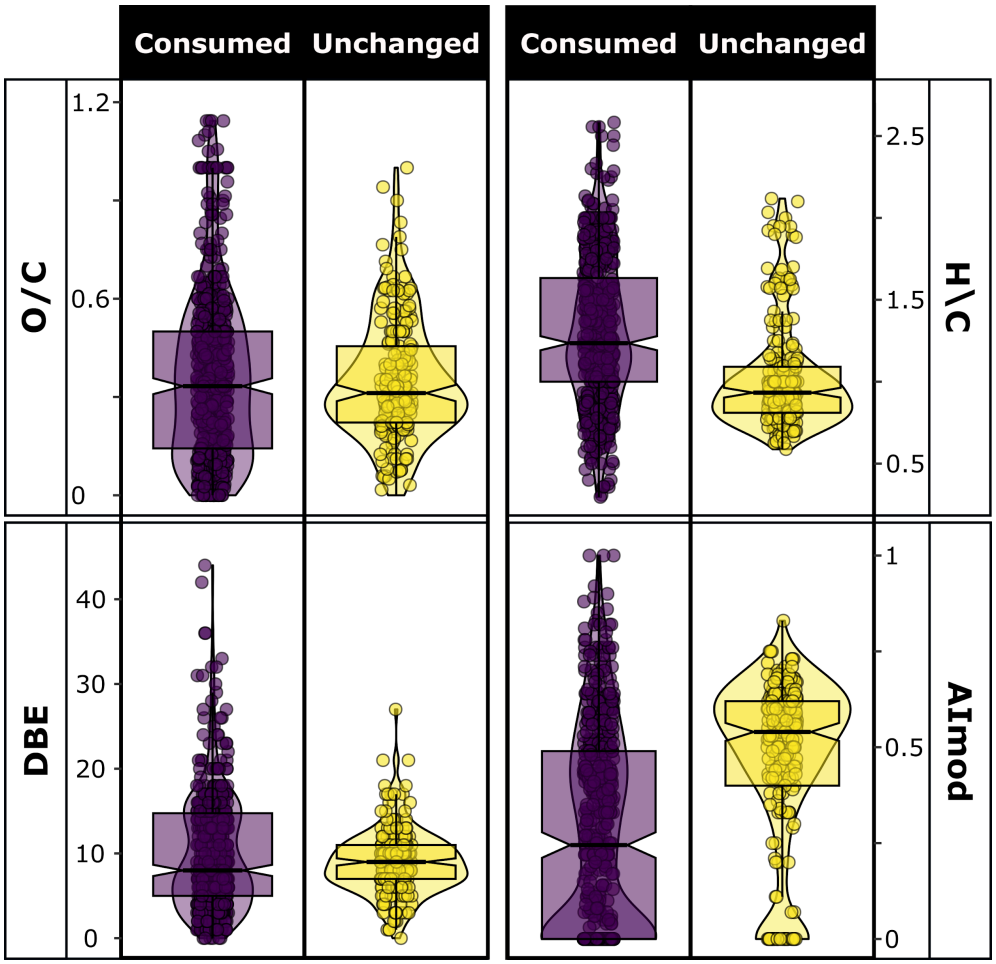
[*] Correspondence: jessika.fuessel@uol.de, sinikka.lennartz@uol.de, and carina.bunse@gu.se



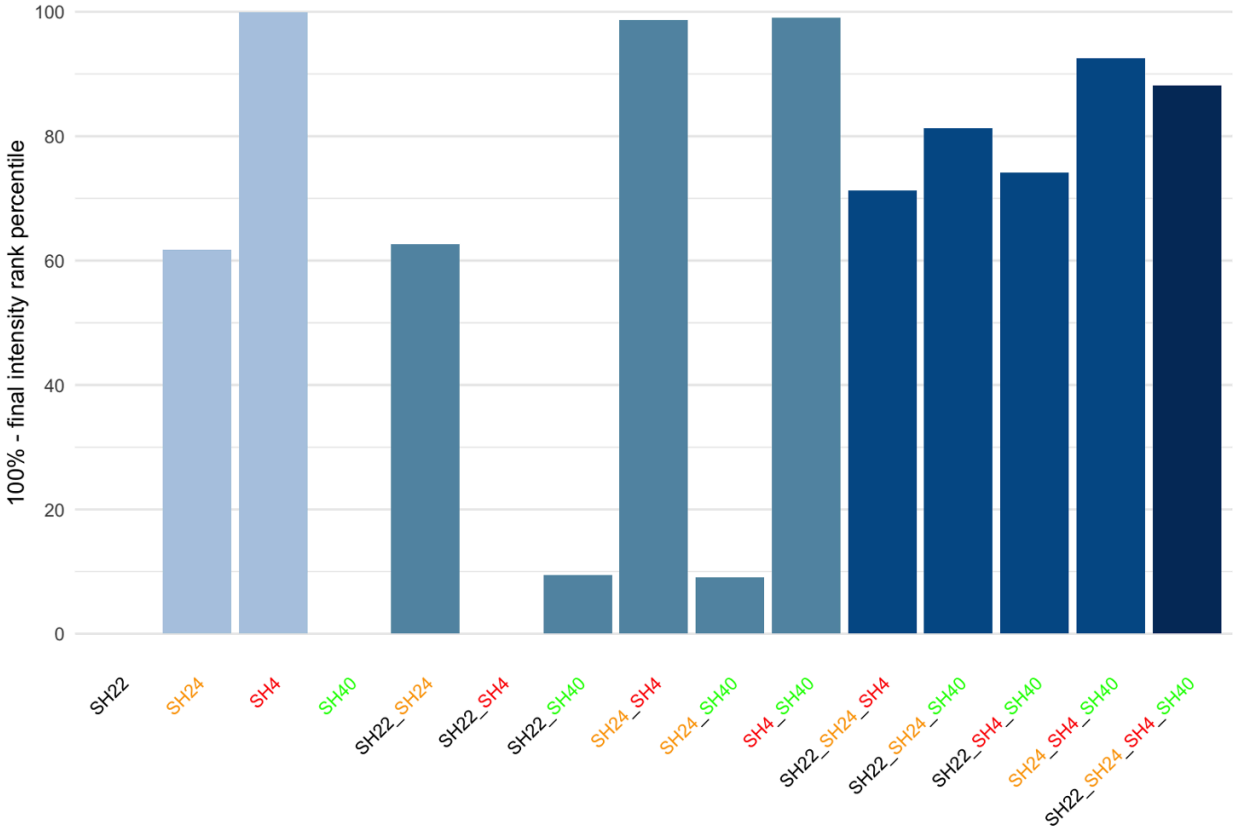
SI Figure 1: *Number of consumed, produced and unchanged molecular formulas (a) when considering the contribution of all isolates in a microbial consortium (b) when considering molecular formulas that occurred in the pure culture of SH4, (c) in the pure culture of SH22, (d) in the pure culture of SH24 and (e) in the pure culture of SH40. Note that the "produced" molecular formulas in b, c, d and e can represent either compounds that were produced by any of the other members of the microbial consortium and remained unchanged or compounds that newly emerged in the co-cultures.*



SI Figure 2: *Preservation and consumption across microbial consortia of molecular formulas observed in pure cultures of SH4, SH22, SH24 and SH40. Molecular formula clustering is based on distribution across samples. The light green bars to the right show the number of molecular formulas associated with each fraction.*



SI Figure 3: *Molecular indices of intrinsic stability including O/C and H/C ratios, double bond equivalents (DBE) and the modified aromaticity index (AI_{mod}) of compounds consumed (purple) or preserved (yellow) in over seven different microbial consortia.*



SI Figure 4: *Rank abundances of the molecular formula putatively identified as 3-hydroxyisobutyrate in final samples of cultures taken at 255h. The blue shade of bars indicates the number of strains in each culture, with pure cultures colored the lightest blue and co-cultures with more strains colored progressively darker shades. Colors of strain names in culture labels reflect the growth stage of the strain in pure culture at 255h. SH22, colored black, displayed minimal growth to apparent stationary phase. SH24, colored orange, was in late log phase. SH4, colored red, was in decline phase. SH22, colored green, was in mid-log phase.*

Accumulation of 3-hydroxybutanoate indicates metabolic state of pure and co-cultures

The distribution of 3-hydroxybutanoate (3-HB) across cultures supports the putative assignment of this compound to the molecular formula and provides insight into processes that control the accumulation of a metabolite as DOM (Supplementary Figure 5). Many bacteria synthesize polyhydroxybutanoate (PHB), a carbon storage polymer, from 3-HB under conditions of carbon excess, and hydrolyze PHB back to 3-HB for maintenance metabolism as the carbon substrate is depleted. Diverse members of the *Roseobacter* clade have been found to accumulate significant PHB, especially when grown on glucose[1]. In SH4 pure culture, the 3-HB molecular feature had the second-highest normalized intensity of any formula in the final 255h DOM sample (Supplementary Figure 5). In SH24 pure culture, the normalized intensity of the 3-HB feature had a percentile rank of 38.2% among formulas in the 255h sample. The feature was not detected in SH22 and SH40 pure cultures. These relative abundances accord with the expected production of 3-HB by the cultures. SH4 was in decline phase at 255h, consistent with 3-HB mobilization under stress and release during cell death after significant PHB accumulation during efficient growth on glucose. At 255h, SH24 was in late log phase, indicating glucose depletion. Stressed cells at this stage may have been breaking down PHB reserves. SH22 barely grew and likely did not accumulate any PHB. In contrast, SH40 was in mid-log phase and was therefore unlikely to be degrading PHB.The 3-HB feature was also one of the most abundant in many co-cultures, with a rank of 1.3% in the co-culture of SH4+SH24 and 11.8% in the co-culture of all four strains. The co-cultures still in the log phase at 255h, SH22+SH40 and SH24+SH40, also had low intensities of the 3-HB feature (90.6% and 90.9% ranks, respectively), consistent with expected PHB accumulation rather than depletion.

The high level of 3-HB in many co-cultures despite its expected metabolic value to strains with an organic acid preference highlights the role of sources and sinks in maintaining DOM composition. Other molecular formulas identified as organic acids were consumed in co-culture. For example, the leucine/isoleucine feature had a rank of 8.1% in the 255 h sample of the SH4 pure culture, yet disappeared in all co-cultures. 3-HB was likely retained in DOM due to the strength of the source compared to the sink. Not only could 3-HB have been released by living and dead cells, but it also could have been generated by extracellular

depolymerization of PHB from dead cells[2]. Preservation of 3-HB despite consumption of other organic acids exemplifies the multifaceted effect of microbial interactions on the DOM pool.

Geochemical indices indicate higher intrinsic stability of consistently preserved molecular formulas

Chemical indices have been developed to classify molecular formulas according to their biogeochemical stability in aquatic and marine environments. A high degree of saturation and a low degree of oxygenation (H/C ratios >1.5 and low O/C ratios) often indicate relative lability towards biological degradation[3]. The presence of aromatic structures is often associated with relatively higher recalcitrance[4]. The modified aromaticity index (AI_{mod}) takes into account several characteristics and serves as an unequivocal indicator for the presence of aromatic cores (AI_{mod} >0.5) and condensed polyaromatic structures (AI_{mod}>0.67)[5]. Application of these indices to our bulk dataset did not show any association between produced, consumed or unchanged molecular formulas and their chemical characteristics (data not shown). In most cases, molecular formulas that were recalcitrant to one consortium were labile to another. However, when focusing on molecular formulas that were either consumed across all possible co-cultures, clear differences in H/C ratio and AI_{mod} emerge (SI Figure 5, SI Table 2g). Consistently consumed molecular formulas (539, 22.5% of all consumed molecular formulas) had higher median H/C (1.3±0.45) and lower AI_{mod} (0.27±0.27) values compared to consistently preserved (221, 10.7% or all preserved molecular formulas), with median H/C values of 1 ±0.33 and AI_{mod} of 0.54±0.14 (SI Figure 3). Even though these molecular formulae likely do not represent universally recalcitrant components, these differences demonstrate an influence of intrinsic properties on the bioavailability of molecules even within this small scale setup.

References

1. Xiao, N. & Jiao, N. Formation of polyhydroxyalkanoate in Aerobic anoxygenic phototrophic bacteria and its relationship to carbon source and light availability. *Appl. Environ. Microbiol.* **77**, 7445–7450 (2011).
2. Jendrossek, D. & Handrick, R. Microbial degradation of polyhydroxyalkanoates. *Annu. Rev.*

Microbiol. **56**, 403–432 (2002).

3. D'Andrilli, J., Cooper, W. T., Foreman, C. M. & Marshall, A. G. An ultrahigh-resolution mass spectrometry index to estimate natural organic matter lability. *Rapid Commun. Mass Spectrom.* **29**, 2385–2401 (2015).
4. Lechtenfeld, O. J. *et al.* Molecular transformation and degradation of refractory dissolved organic matter in the Atlantic and Southern Ocean. *Geochim. Cosmochim. Acta* **126**, 321–337 (2014).
5. Koch, B. P. & Dittmar, T. From mass to structure: an aromaticity index for high-resolution mass data of natural organic matter. *Rapid Commun. Mass Spectrom.* **20**, 926–932 (2006).