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Peer Review File

Multi-omics and cultivation reveal laminarin-degrading PVC bacteria in the deep sea

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
REVIEW for:
Microbial metabolism of complex organic matter across diverse deep-sea ecosystems
Sun et al. utilized a multi-omics approach and provided a comprehensive overview of microbial metabolism of complex organic matter (COM) across deep-sea ecosystems. They found that deep-sea microbial communities have widespread genomic and transcriptomic potential for degrading complex organic matter, with the PVC superphylum showing specialized adaptations for polysaccharide metabolism, especially through obligate laminarin utilization via distinct enzymatic pathways. This enables their selective enrichment and isolation from diverse deep-sea environments. I praise the authors' integration of multi-omics approaches and pure-culture isolates to profile the taxonomic and metabolic potential/functions of the prokaryotic community in deep-sea microbiomes. Overall, this work is a valuable resource for the field and demonstrates the ubiquitous potential of deep-sea environments for COM degradation.
Still, I have some comments about the main ecological claims of the study. Specifically, the community composition of the 2018 sites was characterized using outsourced OTU sequencing, while the 2022 samples were processed with in-house workflows and different metagenomic extraction kits (Figure 1, Methods). CAZyme repertoires and expression are then compared across sites (Figures 2–3), informing conclusions about spatial-temporal heterogeneity and pervasive polysaccharide degradation. Differences in extraction kits and analytical pipelines between the 2018 and 2022 datasets could introduce batch effects that might confound comparisons of community composition and CAZyme repertoires. Different extraction methods have been shown to cause significant batch effects that distort community compositions across taxonomic levels, and variations in OTU versus ASV pipelines can also impact community profiles. Apparent differences in taxa or gene prevalence across sites or years may result from processing artifacts rather than true biological differences, which weakens interpretations about geochemical influences and broad CAZyme activity. To ensure valid cross-cohort comparisons, harmonized reprocessing or batch correction techniques specifically designed for microbiome data are necessary. I encourage the authors to address these methodological concerns and provide additional rationale for clarification.
The authors did not provide abundance- and rate-normalized estimates of the PVC superphylum's role in laminarin turnover or carbon flux compared to other taxa, even though they conclude they are “pivotal drivers”. In particular, the CAZyme expression heatmaps showed broad transcription across multiple phyla (Figure 3), and the isolates grow while depleting laminarin in laboratory conditions (Figure 5). The GH survey suggests GH16/GH2 are common in PVC genomes (Figure 7). These lines of evidence underpin the conclusion that PVC bacteria are pivotal in deep-sea carbon cycling. This is concerning, given that previous quantitative studies (e.g., Zhang et al., 2024; mSphere) have shown that Bacteroidetes dominate laminarin degradation during algal decay and that Flavobacteriaceae possess complete laminarin utilization pathways that are abundant in marine sediments (i.e., Kalenborn et al., 2024; Front. Microbiol.). Without taxon-resolved rate measurements (e.g., laminarinase activity partitioned by lineage or stable isotope probing), the relative impact of PVC cannot be distinguished from other highly active groups, which weakens the central ecological conclusion of the study. I suggest the authors strengthen their argument by explicitly referencing convergent lines of evidence to support their findings.
Moreover, the evidence for the claim centers on the repeated isolation of PVC superphylum bacteria after laminarin enrichment, including two strains from cold seep sediments and additional isolates from hydrothermal vent and seamount samples, as shown in the isolation figures and the phylogenetic placements. These isolate-level successes are compelling and suggest laminarin supports the growth of Planctomycetota and Lentisphaerota under the reported culture conditions. However, the figures presenting the isolation and phylogenetic trees (including the extended phylogeny for additional isolates) do not quantify how laminarin alters community composition during enrichment. Since the authors did not quantify

pre- and post-enrichment community composition to demonstrate that laminarin selectively increases PVC abundance relative to other taxa (community-level sequencing before and after laminarin addition), the observed isolate recovery (shown in the isolation and phylogeny figures) cannot distinguish substrate selectivity from subsequent purification bias. Demonstrating fold enrichment of PVC (e.g., via 16S rRNA profiling or metagenomics) could have been insightful for substantiating that laminarin preferentially selects PVC in mixed communities. Hence, the isolation outcomes show feasibility but not quantifiable selectivity.
In this context, the prevalence estimates for catabolic genes are not adjusted for MAG completeness/contamination, nor validated by read-based profiling, which risks biased presence/absence calls that underpin the majority inference. In particular, prevalence is computed at the bin level without modeling detection probability as a function of completeness (false negatives) or contamination/chimerism (false positives) (Figure 2), even though many bins are of medium quality where such effects are common (Figure 1b). Without a read-based cross-check and quality-stratified analyses using established protocols, the fraction exceeding 50% could be misestimated, affecting the core threshold logic supporting the claim. I suggest that the authors either perform a quality-stratified prevalence analysis using the reported completeness/contamination metrics (≥90% vs. ≥50% thresholds) and recompute the Figure 2 prevalence for each gene class, or acknowledge the computational limitation of the current parameterization used for the MAGs analysis.
The metatranscriptomic heatmap (Figure 3) showed high log2-transformed TPM values for multiple CAZyme families across many genomes, supported by site-level details in Supplementary Data 8-13. This provides strong evidence that carbohydrate-related genes are transcriptionally active in deep-sea sediments and aligns with the claim's focus on active pathways. However, the figures and text do not connect these expression profiles with direct measurements of extracellular enzyme activity or substrate turnover. The authors did not directly measure extracellular polysaccharide hydrolysis rates or secreted enzyme activities to confirm that the observed CAZyme transcripts indicate active degradation. Without activity measurements, the conclusion that pathways are actively breaking down environmental polysaccharides remains inferential rather than proven, potentially overstating the link between mRNA levels and functional turnover. Additionally, the metatranscriptomic expression analysis relies solely on TPM-based heatmaps without cross-sample normalization or replicate-level statistical inference, leaving the 'high expression' designation statistically unsupported. The lack of confidence intervals or tests against reference gene sets weakens the inference that these pathways are broadly active. Hence, I suggest the authors justify the current findings by grounding the inference in observed patterns and isolate data.
Nonetheless, the manuscript is well-written and thoroughly references the relevant literature. I encourage the authors to address the issues mentioned earlier and clarify or adjust the main claims highlighted in the study. Additionally, I have a few minor comments and some further suggestions to improve the manuscript. Below are my additional comments and suggestions for each section.
Abstract:
Line 28: I suggest the authors refer to "the deep-sea microbiome" rather than "deep-sea microbes," as the assessment focuses on community-level functional potential rather than individual taxa.
Line 39: The phrase "genomic and transcriptomic potential for COM metabolism" already conveys functional capacity. The latter reference to "robust degradation capacity" is somewhat redundant and could be streamlined for clarity and conciseness.
Line 39-40: GH16 and GH2 are not "distinct enzymes" but glycoside hydrolase families; better to phrase accordingly.
Introduction:
The introduction section was well-written. However, I still recommend improving/revising specific presentations, as they could create a more direct narrative and make it easier to read.
Lines 93-111: I recommend revising this section to improve narrative flow and reduce density. Several sentences are long and contain multiple ideas, which may obscure the central message. Streamlining the presentation, for example, by more clearly separating ecological distribution, deep-sea relevance, and culturing limitations, would help create a more direct and readable narrative. Additionally, some details (e.g., listing multiple environments or phyla) could be condensed without loss of meaning.
Methods:
Line 497-506: This section provides the necessary sampling information, but the presentation is dense and could be streamlined for clarity. The repeated listing of sites and years makes the paragraph difficult to follow. I recommend reorganizing the description to clearly separate the 2018 and 2022 expeditions, avoiding redundancy in site names, and presenting coordinates and depths in a more concise, tabular, or grouped format. Additionally, there appears to be a typographical error in "a2022 Site F cold seep 2." Clarifying the sampling strategy (e.g., rationale for site selection or differences between seep, vent, and seamount sites) would further strengthen this section.
Lines 507-524: The description of amplicon and metagenomic sequencing is currently too brief and lacks essential information about the downstream data-processing steps. Please provide additional methodological details, even if referencing previous studies. For example, clarify how raw reads were quality-filtered, how contigs were assembled (individually per sample or co-assembled by site/year), how bins were generated and refined, and which tools/parameters were used for taxonomic and functional annotation. While citing prior work is acceptable, the manuscript should still include enough information for readers to understand the analytical workflow and ensure reproducibility.
Lines 563: Did the authors explore model tree optimization methods? What model was used for the tree?
Data availability:
I suggest that the authors also deposit the codes, scripts, and workflows used in the analyses (e.g., for amplicon processing, metagenomic assembly/binning, and transcriptomic analyses) in a public repository such as GitHub or Figshare. Providing these resources would greatly enhance transparency and reproducibility.

Reviewer #2

(Remarks to the Author)
The manuscript submitted by Zheng et al. proposes an integrated multi -omics study to demonstrate the role of members of

the PVC superphylum (Planctomycetota-Verrucomicrobiota-Chlamydiota) into the degradation of complex organic matter in the deep-sea sediments. Their study focusses on the catabolism of Laminarin, a conspicuous glycan produced by macro and micro-algae that significantly contribute to the pool of particulate organic carbon in the oceans.
The methods used by the authors are particularly well suited, as they approach this issue with multiple methods (metagenomics, metatranscriptomics, cultivation and strain isolation, genomics, transcriptomics). Together, their results converge towards a central role of PVC members in the mineralization of complex organic molecules in the marine environment.
The methods and results interpretations are sound and their demonstration is compelling. Below, I list some remarques that may be addressed by the authors to improve the manuscript and facilitate the comprehension by a general readership and data retrieval and sharing for reproducibility.
- Title: I find the title much too broad and not corresponding to the actual results of the work. I suggest a title more focused on the PVC and laminarin. Organic matter complexity and reactivity is a very broad topic in marine microbiology, and the present study only partially addresses this issue. It would better fit the general conclusion “Collectively, these findings delineate specialized adaptations within the PVC superphylum for polysaccharide degradation, significantly expanding our understanding of deep-sea microbial roles in global carbon cycling” in the abstract
- Figure 1: Reading “geographic distribution” in the caption, I was looking for MAG distribution in the figure. I would suggest “sample location and phylogenetic analysis of MAGs”
- L. 172: Authors mention 316 MAGs reconstruction from 7 metagenomes. However, they then mention the same number of MAGs after clustering (ANI95%). There must be an error here: numbers of MAG pre clustering should be higher than post clustering.
- L. 189: The sentence: “which exclusively the high abundance in hydrothermal vent sediments” doesn’t make sense.
- L. 279: “of this understudied the PVC superphylum”. Remove the?
- L. 291: It would help if you would just mention that you sequenced the genome of these isolates early in this paragraph.
- L. 337: “Strain WC36 formed a distinct, deeply branching lineage that occupied a position equidistant from both the order Lentisphaerales and the order Victivallales Given the taxonomic threshold for order delineation (typically around 83.55%)46, the significant phylogenetic distance and distinct branching pattern observed for strain WC36 suggest that it may represent a novel order within the class Lentisphaeria.”. I somehow disagree with this since the WC36 branch has bootstrap values under 80%. It means that there is considerable uncertainity regarding its phylogenetic placement in the current tree. This could easily be fixed with a phylogenomic approach, using tools from GTDB-tk for instance. It surprising that authors did not use these approach given the availability of whole genome sequences.
- L. 439: “Our analysis encompassed 171 pure cultures of bacteria” lead the reader to think that thay are part of this study. Maybe use “171 available genomes in the X database” instead?
- L 492: The manuscript really misses a sampling material and method section: How were the sediment recovered? Which sediment horizons were sampled?
- L: 512: OTU instead of OUT
- L. 518: authors refer to a bioarxiv for their method description, meaning that the said methods have not been reviewed yet. Although I generally agree with methods described in this pre-print, I think it would be more appropriate to describe the methods in full here, unless the archive is now published.
- In general, I find difficult to follow the methods section due to many references to other articles. A minimum of information would facilitate the reading.
- Also, authors mention analysis on strain genomes, but do not mention the genome sequencing in the results nor in the material and methods. A section “genome sequencing is needed here”
- L. 562: When aligning 16S, using a 2d aware rRNA aligner such as SINA is generally a better option.
- L. 589: This section seems to lack accession numbers for metagenome reads and metatranscriptome reads. These must be available to the scientific community for publication.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I am happy to say that the authors have carefully and comprehensively addressed my major concerns. In particular, I appreciate that they harmonized analyses across datasets and removed overstated ecological claims. Also, the added community-level enrichment data substantially improved the statistical grounding and transparency of their interpretations. The revised manuscript now presents a well-supported and appropriately cautious assessment of PVC bacteria as contributors, rather than dominant drivers, to laminarin degradation in deep-sea sediments.

Still, I have minor issues, primarily related to clarity. For example, I suggest further tempering of statements like "highlight the crucial role of the PVC superphylum bacteria in the marine carbon cycle", to "potentially important role" or "previously underappreciated role", to keep the wordings aligned with the stated limitations. Please check the manuscript for similar statements. Also, the figures read as compacted. I suggest for some in-text information to be reduced further, and specifically for Figure 7, adding significance markers (e.g., $p < 0.05$) would be helpful for easier interpretation.

Hence, I only recommend minor editorial revisions to improve readability and figure clarity, but no additional analyses are required, and I happily recommend the study for acceptance after these minor corrections.

Reviewer #2

(Remarks to the Author)
I congratulate the authors for extensively addressing the multiple points raised by the two reviewers. While reading the new version, I however found a few sections for which a few changes would be interesting to better align the claims and the messages of the manuscript with the experimental work carried by the authors.
L56-70: In this part of the introduction, authors justify their work with the argument that deep subseafloor life is based in part on the degradation of recalcitrant organic matter. But later, the authors mention benthic communities and COM, and then move on to Laminarin as a part of COM. This is a little confusing and suggest that Laminarin is an important contribution to this recalcitrant organic matter and is buried in the deep subseafloor. I didn't find any evidence in the cited literature that Laminarin is recalcitrant, nor that it is present in the subseafloor. In addition, samples of this study come from benthic surface. I think this part of the intro may be slightly reorganized or maybe removed
L. 115-118: similar remark as for L.56-70
L. 168-181: This paragraph describes differences in Archaeal distribution between cold seeps and hydrothermal vents. I wouldn't describe these as "striking", but rather expected instead. These are two very different ecosystems (energetic metabolism, chemical environment, temperature…). I don't see how this section supports the main message of the article and would encourage the authors to remove or rewrite it.
L. 212: eDNA generally refers to environmental DNA, not necessarily extracellular. Maybe consider removing?
L. 182-233: this section describes the catabolic potential of different members of the community for which MAGs were reconstructed. My opinion is that it is hard to establish general rules such as "This genomic deficit suggests a limited capacity for the direct extracellular hydrolysis and utilization of complex polysaccharides by deep-sea archaea." with a limited number of MAGs (238 Bac and 45 Arch). For comparison, other studies of the deep sea environment have collected one order of magnitude more MAGS to discuss metabolic potential, see for instance (Lu et al. 2024; St. John et Reysenbach 2026; Schauberger et al. 2024)
L. 235: the sentence suggest that all polysaccharides derive from cell wall and are recalcitrant. I would remove the coma after "polysaccharides," to prevent this misinterpretation
L. 291-303: Here, authors use 16S rRNA genes (v3-v4) sequences to discuss is their isolates are new species. Why not using ANI % as this is a superior metric to delineate new species?
L. 385: Here, authors argue that "this broad distribution of laminarin-degrading genes highlights the crucial role of the PVC superphylum bacteria in the marine carbon cycle and underscores their potential importance in the breakdown of algal biomass, a major component of oceanic organic matter" However, this conclusion is based on the distribution on laminarin degradation genes only in the PVC superphylum. If these genes are present in other phyla, the PVC role may not be so central. Authors should probably show that GH16 and GH2 are not significantly present in genomes from other phyla to support this claim.

Version 2:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
I once more congratulate the authors for carefully addressing reviwer's comments. After reading this last version, I believe this manuscript is ready for publication. I only found a small typo L170 where a sentence needs revision:"in MAGs from gas hydrate-bearing subseafloor and the Helgoland mud are"

Responses to Reviewers

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

Comment 1: Sun et al. utilized a multi-omics approach and provided a comprehensive overview of microbial metabolism of complex organic matter (COM) across deep-sea ecosystems. They found that deep-sea microbial communities have widespread genomic and transcriptomic potential for degrading complex organic matter, with the PVC superphylum showing specialized adaptations for polysaccharide metabolism, especially through obligate laminarin utilization via distinct enzymatic pathways. This enables their selective enrichment and isolation from diverse deep-sea environments. I praise the authors' integration of multi-omics approaches and pure-culture isolates to profile the taxonomic and metabolic potential/functions of the prokaryotic community in deep-sea microbiomes. Overall, this work is a valuable resource for the field and demonstrates the ubiquitous potential of deep-sea environments for COM degradation.。

Response: We thank you for the positive and encouraging assessment of our work. We are pleased that the reviewer appreciates our integration of multi-omics approaches with pure-culture isolation to profile the taxonomic and metabolic potential of deep-sea microbial communities. The recognition that our study serves as a valuable resource for the field is greatly encouraging.

We have carefully considered all the constructive comments provided and have revised the manuscript accordingly to strengthen the study. We believe these revisions have improved the clarity and rigor of our findings. We thank you again for the time and effort invested in evaluating our work.

Comment 2: Still, I have some comments about the main ecological claims of the study. Specifically, the community composition of the 2018 sites was characterized using outsourced OTU sequencing, while the 2022 samples were processed with

in-house workflows and different metagenomic extraction kits (Figure 1, Methods). CAZyme repertoires and expression are then compared across sites (Figures 2–3), informing conclusions about spatial-temporal heterogeneity and pervasive polysaccharide degradation. Differences in extraction kits and analytical pipelines between the 2018 and 2022 datasets could introduce batch effects that might confound comparisons of community composition and CAZyme repertoires. Different extraction methods have been shown to cause significant batch effects that distort community compositions across taxonomic levels, and variations in OTU versus ASV pipelines can also impact community profiles. Apparent differences in taxa or gene prevalence across sites or years may result from processing artifacts rather than true biological differences, which weakens interpretations about geochemical influences and broad CAZyme activity. To ensure valid cross-cohort comparisons, harmonized reprocessing or batch correction techniques specifically designed for microbiome data are necessary. I encourage the authors to address these methodological concerns and provide additional rationale for clarification.

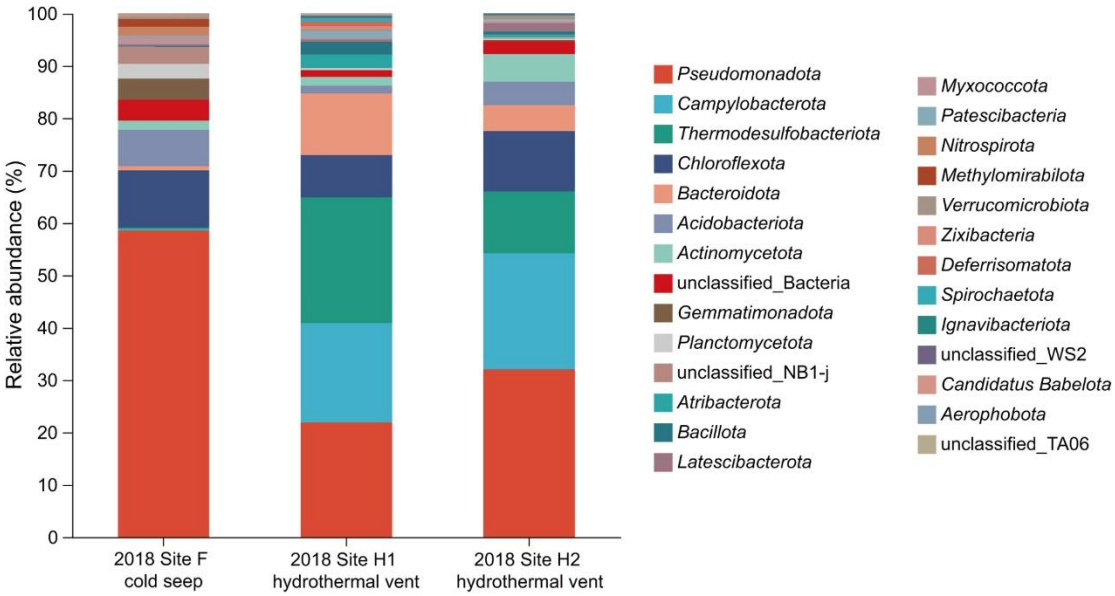
Response: We thank you for this insightful comment regarding potential batch effects arising from differences in sample processing between the 2018 and 2022 datasets. We agree that methodological consistency is essential for robust cross-cohort comparisons. To address this concern, we have reanalyzed all data using harmonized workflows, as detailed below.

(1) Harmonization of 16S rRNA amplicon data: To ensure methodological consistency across all samples, we re-analyzed the three sediment samples collected in 2018 using the same ASV-based pipeline applied to the 2022 samples (Supplementary Figure 1). Specifically, we applied the DADA2 algorithm within the QIIME2 framework to generate amplicon sequence variants (ASVs) from all raw sequencing reads, thereby eliminating discrepancies between the OTU-based and ASV-based approaches originally used. The resulting community profiles are consistent with our initial observations, confirming that the reported spatial and temporal patterns reflect true biological variation rather than methodological artifacts.

(2) Harmonization of metagenomic data: All raw metagenomic sequencing reads were re-analyzed by Shanghai Biozeron Biological Technology Co., Ltd. using a standardized workflow. This included uniform quality control, assembly, gene prediction, and functional annotation across all samples. Figure 2 has been updated accordingly, and the revised analysis yields results consistent with our original findings. According to your suggestion, we have modified this related description as "Metagenomic sequencing of the seven deep-sea sediments yielded 238 high- to medium-quality (completeness ≥50%, contamination ≤10%) microbial metagenome-assembled genomes (MAGs)[39] after de novo assembly and binning. These 193 bacterial and 45 archaeal MAGs spanned 37 known and eight unclassified phyla (Fig. 1b and Supplementary Data 2). Bacterial MAGs were predominantly derived from dominant lineages, with significant representation from *Pseudomonadota* (n = 49), *Thermodesulfobacteriota* (n = 22), *Bacteroidota* (n = 20), *Chloroflexota* (n = 19), *Patescibacteriota* (n = 9), *Calditrichota* (n = 8), *Bipolaricaulota* (n = 4), *Planctomycetota* (n = 4), *Acidobacteriota* (n = 4), and *Nitrospinota* (n = 4). These dominant bacterial phyla prevailed in all seven deep-sea sediments analyzed, aligning with the amplicon sequencing results (Supplementary Figs. 1and 2). Within the Archaea domain, the 45 MAGs were predominantly derived from *Halobacteriota* (n = 12), *Asgardarchaeota* (n = 11), *Thermoplasmatota* (n = 7), *Nanobdellota* (n = 6), *Hydrothermarchaeota* (n = 4), *Thermoproteota* (n = 2), *Aenigmatarchaeota* (n = 1), along with two unclassified archaeal MAGs. While the identified archaeal lineages were widespread across the sampled deep-sea sediments, their distribution revealed a striking environmental niche specialization (Supplementary Data 2). Specifically, all detected *Halobacteriota* were recovered exclusively from cold seep sediments and were predominantly classified as anaerobic methanotrophic archaea, a distribution consistent with the high methane concentrations characteristic of these habitats[40,41]. In contrast, *Nanobdellota* MAGs displayed a highly restricted distribution, being recovered solely from the two hydrothermal vent sediment samples. This specialization aligns with previous reports

of *Nanobdellota* as a prominent lineage within the DPANN superphylum, which often exhibits high relative abundances exclusively in hydrothermal vent ecosystems[4,42].” in the revised manuscript (Lines 154-178).

(3) Data and code availability: To ensure transparency and reproducibility, all analysis pipelines, scripts, and workflows used in this study have been deposited in a public GitHub repository: https://github.com/zd200572/16s_bacteria_RNAseq_metgenome_metagenomic_pipelines. The raw sequencing data for all samples are available under the NCBI BioProject accession numbers provided in the Methods section.



Supplementary Figure 1 | Relative abundances of dominant prokaryotic communities in deep-sea cold seep and hydrothermal vent sediments (collected in 2018) at the phylum level.

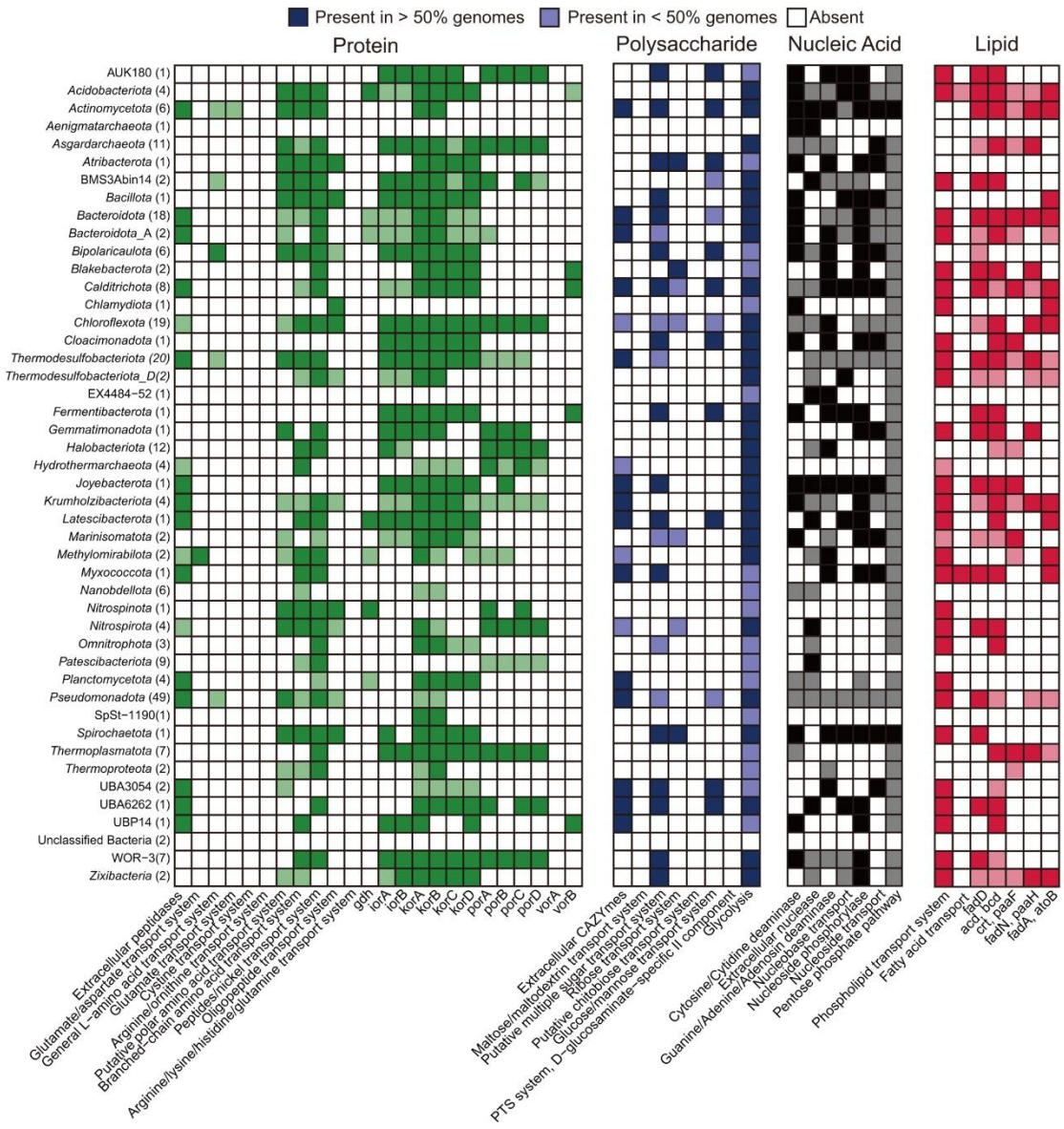


Figure 2 | Distribution of key catabolic genes across deep-sea MAGs reveals diverse degradation capabilities of complex organic matter. Key genes responsible for the catabolism of proteins, polysaccharides, nucleic acids, and lipids were detected in MAGs. Color intensity indicates gene prevalence within each cluster: dark shading denotes presence in >50% of MAGs, and light shading indicates presence in <50% of MAGs. The total number of MAGs per cluster is shown in parentheses. Pathway identification was performed with METABOLIC.

Comment 3: The authors did not provide abundance- and rate-normalized estimates of the PVC superphylum's role in laminarin turnover or carbon flux compared to other taxa, even though they conclude they are “pivotal drivers”. In particular, the CAZyme expression heatmaps showed broad transcription across multiple phyla (Figure 3), and the isolates grow while depleting laminarin in laboratory conditions (Figure 5). The GH survey suggests GH16/GH2 are common in PVC genomes (Figure 7). These lines

of evidence underpin the conclusion that PVC bacteria are pivotal in deep-sea carbon cycling. This is concerning, given that previous quantitative studies (e.g., Zhang et al., 2024; mSphere) have shown that Bacteroidetes dominate laminarin degradation during algal decay and that Flavobacteriaceae possess complete laminarin utilization pathways that are abundant in marine sediments (i.e., Kalenborn et al., 2024; Front. Microbiol.). Without taxon-resolved rate measurements (e.g., laminarinase activity partitioned by lineage or stable isotope probing), the relative impact of PVC cannot be distinguished from other highly active groups, which weakens the central ecological conclusion of the study. I suggest the authors strengthen their argument by explicitly referencing convergent lines of evidence to support their findings.

Response: We thank you for this thoughtful critique regarding the quantitative evidence for the ecological role of PVC bacteria in laminarin degradation. We agree that our original wording overstated the conclusions that could be drawn from our data. Given that no previous studies have demonstrated a contribution of PVC bacteria to polysaccharide degradation or carbon cycling in deep-sea environments, we adopted the prevailing view in the literature—that *Bacteroidota* dominate laminarin degradation in marine systems—as the appropriate framework for contextualizing our findings. We have revised the manuscript accordingly to address these concerns, particularly in light of previous studies demonstrating the importance of *Bacteroidota* in laminarin turnover.

The details are as follows: (1) **Removal of overstated claims:** We have removed the term "pivotal drivers" and similar overreaching statements throughout the manuscript. The central ecological conclusion has been reframed to reflect that PVC bacteria participate in laminarin degradation alongside other microbial lineages, rather than dominating this process. According to your suggestion, we have added this related description as “Collectively, these findings reveal that PVC bacteria—an overlooked group in laminarin degradation—possess specialized adaptations for polysaccharide breakdown and actively participate in laminarin turnover in deep-sea environments.” in the revised manuscript (Lines 45-47). (2) **Revised discussion of**

laminarin degradation: In the "Laminarin facilitates the isolation of deep-sea PVC superphylum bacteria" section, we have added this description as “Together with the established role of *Bacteroidota* in laminarin degradation[53,56,57], our findings suggest that laminarin turnover in deep-sea sediments is mediated by a diverse microbial consortium, with PVC bacteria contributing alongside other functionally specialized lineages. However, in the absence of quantitative rate measurements, their ecological contribution relative to other taxa, such as *Bacteroidota*, cannot be determined from the current data. Future studies using stable isotope probing or lineage-resolved enzyme assays will be needed to partition laminarin degradation rates among co-occurring microbial groups.” in the revised manuscript (Lines 421-428) to contextualize our findings within the broader literature and explicitly acknowledge the limitations of our study.

This revision achieves three goals: (1) it explicitly acknowledges the established role of *Bacteroidota* by citing the relevant literature (including studies suggested by the reviewer); (2) it positions PVC bacteria as contributors within a diverse consortium rather than dominant players; and (3) it clearly states the limitation regarding quantitative rate measurements and suggests future directions to address this gap.

Reference related to this response:

Kalenborn, S. *et al.* Genes for laminarin degradation are dispersed in the genomes of particle-associated *Maribacter* species. *Front Microbiol* **15**, 1393588, (2024).

Zhang, Y.-S. *et al.* Metagenomic insights into the dynamic degradation of brown algal polysaccharides by kelp-associated microbiota. *Applied and Environmental Microbiology* **90**, e02025-02023, (2024).

Krüger, K. *et al.* In marine *Bacteroidetes* the bulk of glycan degradation during algae blooms is mediated by few clades using a restricted set of genes. *The ISME Journal* **13**, 2800-2816, (2019).

Comment 4: Moreover, the evidence for the claim centers on the repeated isolation of PVC superphylum bacteria after laminarin enrichment, including two strains from cold seep sediments and additional isolates from hydrothermal vent and seamount samples, as shown in the isolation figures and the phylogenetic placements. These isolate-level successes are compelling and suggest laminarin supports the growth of

Planctomycetota and Lentisphaerota under the reported culture conditions. However, the figures presenting the isolation and phylogenetic trees (including the extended phylogeny for additional isolates) do not quantify how laminarin alters community composition during enrichment. Since the authors did not quantify pre- and post-enrichment community composition to demonstrate that laminarin selectively increases PVC abundance relative to other taxa (community-level sequencing before and after laminarin addition), the observed isolate recovery (shown in the isolation and phylogeny figures) cannot distinguish substrate selectivity from subsequent purification bias. Demonstrating fold enrichment of PVC (e.g., via 16S rRNA profiling or metagenomics) could have been insightful for substantiating that laminarin preferentially selects PVC in mixed communities. Hence, the isolation outcomes show feasibility but not quantifiable selectivity.

Response: We thank you for this incisive comment, which correctly distinguishes between demonstrating the feasibility of isolation and providing quantitative evidence of substrate selectivity. We agree that in the initial description of our work, the claim regarding laminarin's selective role relied primarily on isolation outcomes, which could theoretically be influenced by purification bias. We are pleased to address this concern directly with new data incorporated into the revised manuscript.

As you suggested, we had indeed performed community-level 16S rRNA amplicon sequencing to compare the microbial communities between basal medium control and basal medium supplemented with laminarin, thereby quantifying the specific shift in community composition induced by laminarin enrichment. This methodological gap, which you identified, is directly addressed by the new data presented in Figure 7a and 7b. The details are as follows: (1) **Quantitative enrichment:** Figure 7a demonstrates that laminarin amendment leads to a significant and quantifiable increase in the relative abundance of the PVC superphylum (specifically *Lentisphaerota*, *Planctomycetota*, and *Verrucomicrobiota*) across all three deep-sea environments tested (cold seeps, hydrothermal vents, and seamounts). Concurrently, we observed a substantial decline

in *Pseudomonadota* and *Actinomycetota*, indicating a true niche displacement driven by laminarin, rather than random isolation outcomes. This community-level profiling fulfills your request for a direct comparison between cultures with and without laminarin, confirming that laminarin drives the selective enrichment of PVC taxa from mixed communities. (2) **Substrate specificity vs. purification bias:** By demonstrating that the relative abundance of PVC bacteria increases specifically in laminarin-amended cultures compared to the unamended controls, and that this increase occurs before any plating or isolation steps, we can confidently rule out "purification bias" as the primary driver of our results. The enrichment occurs at the community level in the liquid culture, directly in response to the laminarin carbon source, and is absent in basal medium controls without laminarin. (3) **Broad applicability:** Furthermore, Figure 7b quantifies the number of PVC bacteria enriched across different habitats, showing that laminarin is consistently effective, particularly in cold seep samples. This quantitative support strengthens our hypothesis that laminarin-based strategies are broadly applicable for accessing these recalcitrant taxa. We believe that the inclusion of these amplicon sequencing data directly answers your query. The data transform our conclusion from simply showing "feasibility" to demonstrating quantifiable selectivity. The isolation of novel strains WC47 and WC29 (Figure 7c) thus serves as the successful endpoint of a well-defined, selective enrichment process.

According to your suggestion, we have added this description as "**Laminarin facilitates the isolation of deep-sea PVC superphylum bacteria.** The ability of laminarin to selectively enrich *Planctomycetota* strain WC338 and *Lentisphaerota* strain WC36 from deep-sea cold seep sediments suggests that laminarin-based cultivation strategies might be broadly applicable to the isolation of other recalcitrant bacterial taxa across diverse deep-sea environments. To test this hypothesis, we enriched sediment samples from cold seeps, hydrothermal vents, and seamounts for one month in basal medium supplemented with laminarin. Amplicon sequencing revealed that laminarin amendment led to a significant enrichment of the PVC

superphylum, specifically *Lentisphaerota*, *Planctomycetota*, and *Verrucomicrobiota*. Concurrently, we observed a substantial decline in the relative abundance of *Pseudomonadota* and *Actinomycetota*, suggesting a niche displacement under laminarin-replete conditions (Fig. 7a). Furthermore, *Fusobacteriota*—a lineage recently recognized for its role in the degradation of complex organic matter[55]—were significantly enriched in cold seep and seamount samples, indicating that they may contribute either directly to the primary degradation of laminarin or indirectly through the utilization of metabolic intermediates. To date, only two *Planctomycetota* strains, (*Gimesia benthica* E7 and *Poriferisphaera heterotrophicis* ZRK32)[31,32] and one *Lentisphaerota* strain (*Lentisphaera profundi* SAORIC-696)[56] have been successfully isolated and characterized from deep-sea habitats, highlighting the challenges associated with accessing and studying these deep-sea PVC superphylum bacteria. Given the limited number of cultured PVC bacteria from deep-sea environments, we focused on analyzing the number of PVC bacteria that were enriched. We found that many PVC bacteria were enriched from sediments using laminarin, with the most from cold seep samples, followed by hydrothermal vent and seamount (Fig. 7b), indicating that laminarin is a very effective material for enriching and isolating deep-sea PVC bacteria. Laminarin-supplemented enrichment cultures enabled the isolation of additional PVC strains from other deep-sea environments: *Planctomycetota* strain WC47 from hydrothermal vent sediment and *Lentisphaerota* strain WC29 from seamount sediment (Fig. 7c), complementing the strains WC338 and WC36 previously obtained from cold seeps. The successful isolation of diverse PVC superphylum members via laminarin enrichment supports previous metagenomic and metatranscriptomic evidence suggesting their metabolic capability to degrade laminarin." in the revised manuscript (Lines 389-420).

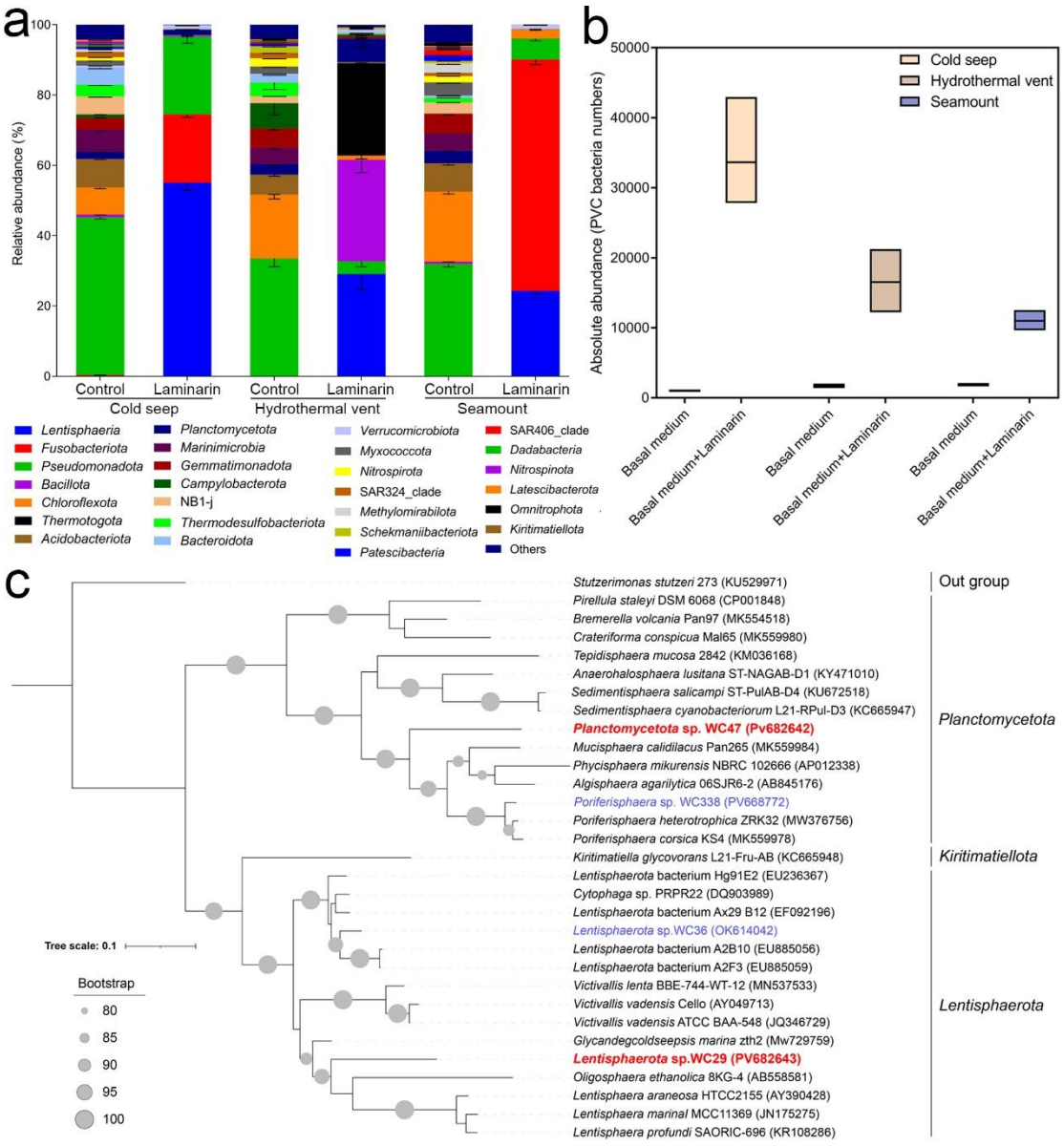


Fig. 7 | Laminarin facilitates the isolation and cultivation of PVC superphylum bacteria from deep-sea sediments. a, Relative abundance of bacterial phyla in deep-sea sediment communities after 30 days of incubation in basal medium (Control) or basal medium supplemented with 3.0 g/L laminarin (Laminarin). Data represent mean±SD (n=3 biologically independent replicates) for cold seep, hydrothermal vent, and seamount samples. **b,** Absolute abundance of PVC superphylum bacteria enriched from these sediments with laminarin as the primary carbon source. **c,** Maximum-likelihood phylogenetic tree of *Planctomycetota* strain WC47 and *Lentisphaerota* strain WC29. Scale bar, 0.1 substitutions per nucleotide position.

Comment 5: In this context, the prevalence estimates for catabolic genes are not adjusted for MAG completeness/contamination, nor validated by read-based profiling, which risks biased presence/absence calls that underpin the majority inference. In particular, prevalence is computed at the bin level without modeling detection

probability as a function of completeness (false negatives) or contamination/chimerism (false positives) (Figure 2), even though many bins are of medium quality where such effects are common (Figure 1b). Without a read-based cross-check and quality-stratified analyses using established protocols, the fraction exceeding 50% could be misestimated, affecting the core threshold logic supporting the claim. I suggest that the authors either perform a quality-stratified prevalence analysis using the reported completeness/contamination metrics (≥90% vs. ≥50% thresholds) and recompute the Figure 2 prevalence for each gene class, or acknowledge the computational limitation of the current parameterization used for the MAGs analysis.

Response: We thank you for raising this important methodological point regarding MAG-based prevalence estimation. We acknowledge the inherent limitations of MAG-based prevalence estimation, which can be influenced by bin completeness and contamination, potentially leading to false negatives from incomplete genomes or false positives from contamination. We acknowledge that our prevalence estimates—derived from MAGs with completeness ≥50% and contamination <10%—are subject to inherent limitations, as bin quality can influence gene detection. While this threshold is commonly used in deep-sea metagenomic studies (Zhang et al, 2023; Dong et al, 2019), we recognize that more refined approaches (e.g., detection probability modeling) could improve accuracy. Therefore, we present our findings as conservative estimates. Accordingly, the reported prevalences should be interpreted as indicative of genomic potential within the sampled communities, rather than precise quantitative measures. Future work integrating complementary approaches such as read-based profiling will be valuable to validate and extend these observations.

According to your suggestion, we have added this detailed description as “While our MAG-centric analysis provides a robust framework for assessing genomic potential, we acknowledge that the prevalence estimates presented here are inherently influenced by bin completeness and contamination—factors that may lead to under- or overestimation of gene frequencies, particularly among medium-quality bins.

Future work integrating complementary read-based profiling and quantitative activity measurements will be necessary to fully resolve the distribution and expression of these catabolic pathways *in situ*." in the revised manuscript (Lines 227-233).

References related to this response:

Zhang, C. et al. The majority of microorganisms in gas hydrate-bearing subseafloor sediments ferment macromolecules. Microbiome 11, 37, (2023).

Dong, X. *et al.* Metabolic potential of uncultured bacteria and archaea associated with petroleum seepage in deep-sea sediments. *Nat. Commun.* **10**, 1816, (2019).

Comment 6: The metatranscriptomic heatmap (Figure 3) showed high log_2-transformed TPM values for multiple CAZyme families across many genomes, supported by site-level details in Supplementary Data 8-13. This provides strong evidence that carbohydrate-related genes are transcriptionally active in deep-sea sediments and aligns with the claim's focus on active pathways. However, the figures and text do not connect these expression profiles with direct measurements of extracellular enzyme activity or substrate turnover. The authors did not directly measure extracellular polysaccharide hydrolysis rates or secreted enzyme activities to confirm that the observed CAZyme transcripts indicate active degradation. Without activity measurements, the conclusion that pathways are actively breaking down environmental polysaccharides remains inferential rather than proven, potentially overstating the link between mRNA levels and functional turnover. Additionally, the metatranscriptomic expression analysis relies solely on TPM-based heatmaps without cross-sample normalization or replicate-level statistical inference, leaving the 'high expression' designation statistically unsupported. The lack of confidence intervals or tests against reference gene sets weakens the inference that these pathways are broadly active. Hence, I suggest the authors justify the current findings by grounding the inference in observed patterns and isolate data.

Response: We thank you for this thoughtful critique regarding the metatranscriptomic evidence for CAZyme activity. We agree that TPM-based heatmaps alone, without cross-sample normalization or replicate-level statistical inference, provide limited support for claims of "high expression". We also acknowledge that mRNA abundance

does not directly equate to extracellular enzyme activity or substrate turnover rates. To address these concerns, we have substantially revised our analysis and manuscript as follows: (1) **Addressing statistical weakness:** We have incorporated a targeted differential expression analysis, normalizing GH and GT transcript levels against 10 conserved marker genes. This provides fold-change data (Fig. 3, Supplementary Data 14-15), which offers a statistically grounded measure of induction, moving beyond simple TPM normalization. These additions provide the statistical robustness that the reviewer rightly noted was lacking in the original submission. (2) **Grounding inferences:** The revised text now explicitly states that the observed significant induction (based on fold-change analysis) provides some *in situ* transcriptional evidence supporting the functional relevance of these genes, especially within the PVC lineages in cold seep samples (Supplementary Fig. 4). (3) **Explicit acknowledgment of limitations and justification of conclusions:** We have added a description explicitly acknowledging that we did not directly measure extracellular enzyme activities or polysaccharide hydrolysis rates, and that mRNA abundance alone does not prove functional turnover. However, we note that our conclusions regarding active polysaccharide degradation are supported by convergent lines of evidence: (i) widespread genomic potential for CAZyme production across multiple lineages; (ii) *in situ* transcriptional activity of these genes, now supported by replicate-level statistical inference; (iii) subsequent cultivation and physiological validation of laminarin-degrading PVC isolates. This multi-evidence approach, while not a substitute for direct activity measurements, provides a robust foundation for the inferred ecological roles. We thank you for helping us strengthen our metatranscriptomic analysis and appropriately contextualize our conclusions. We believe these updates successfully ground our findings in quantifiable data and appropriately frame the conclusions regarding active polysaccharide degradation.

According to your suggestion, we have modified this description as “To assess the functional relevance of these genes in deep-sea *in situ* environments, initial metatranscriptomic analysis showed widespread transcription of diverse CAZyme

families—predominantly GHs and GTs—across most MAGs via the normalized transcripts per million (TPM), underscoring active polysaccharide degradation (Supplementary Fig. 3, Supplementary Data 8-13). Crucially, to move beyond simple transcript abundance and address statistical support, we performed a targeted differential expression analysis by normalizing GH and GT transcript levels against 10 conserved marker genes[47] (fold change analysis, Fig. 3 and Supplementary Data 14-15). These upregulated GH and GT families were primarily associated with dominant lineages, including *Thermodesulfobacteriota*, *Bacteroidota*, *Alphaproteobacteria*, *Planctomycetota*, and *Omnitrophota*, with predominant enrichment in Site F cold seep sediments. Crucially, the expression profiles of PVC bacteria revealed a clear niche adaptation: GH-encoding genes in three *Planctomycetota* and one *Omnitrophota* MAGs were predominantly upregulated in cold seep sediments, particularly Site F, suggesting a potential role for these lineages in polysaccharide degradation within cold seep habitats (Supplementary Fig. 4). Together, these results demonstrate that polysaccharide degradation in deep-sea sediments is mediated by distinct microbial lineages exhibiting habitat-specific patterns of CAZyme expression. Notably, *Planctomycetota* and *Omnitrophota* belong to the PVC superphylum, a group previously reported for its potential to degrade polysaccharides in other environments[29,48]. While we did not directly measure extracellular enzyme activities or polysaccharide hydrolysis rates—and thus mRNA abundance alone does not directly quantify functional turnover—the convergence of genomic potential and *in situ* transcriptional activity, supported by replicate-level statistical inference, provides evidence that these lineages may contribute to polysaccharide degradation in deep-sea environments. These findings reveal that this understudied PVC superphylum bacteria may contribute to polysaccharide degradation in deep-sea environments alongside other lineages, and provide an empirical framework for the targeted cultivation of previously recalcitrant PVC bacteria." in the revised manuscript (Lines 249-277).

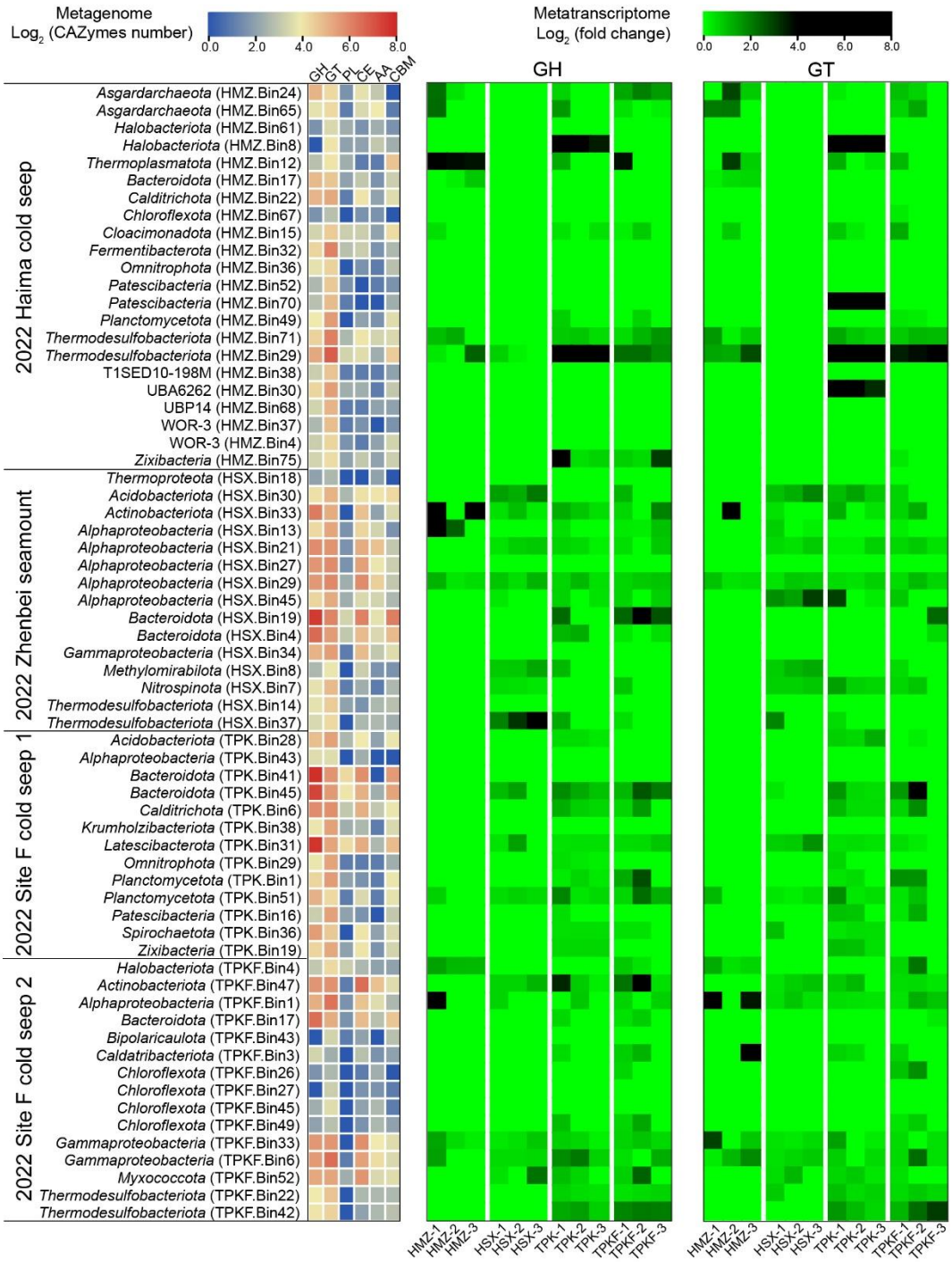
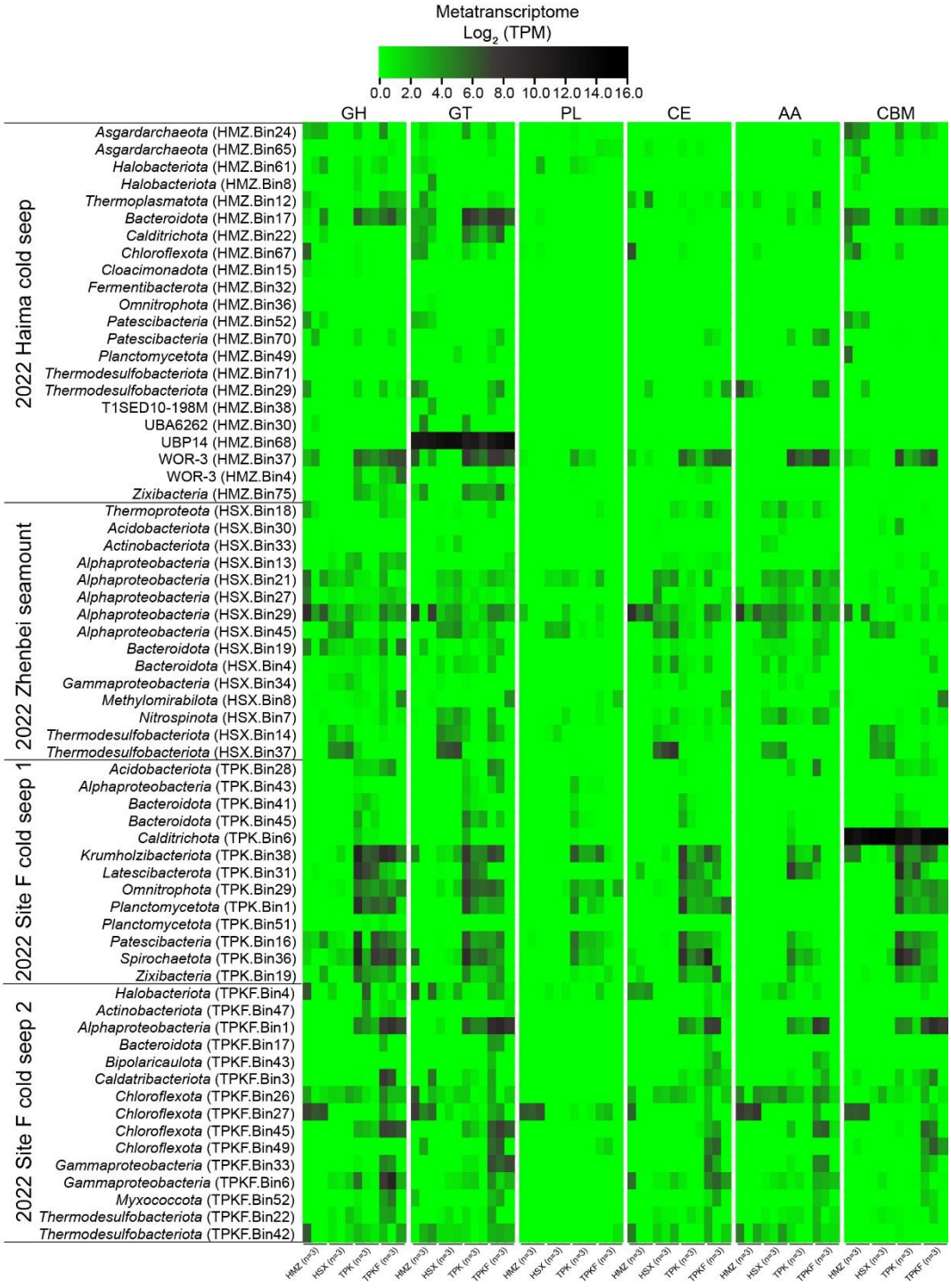
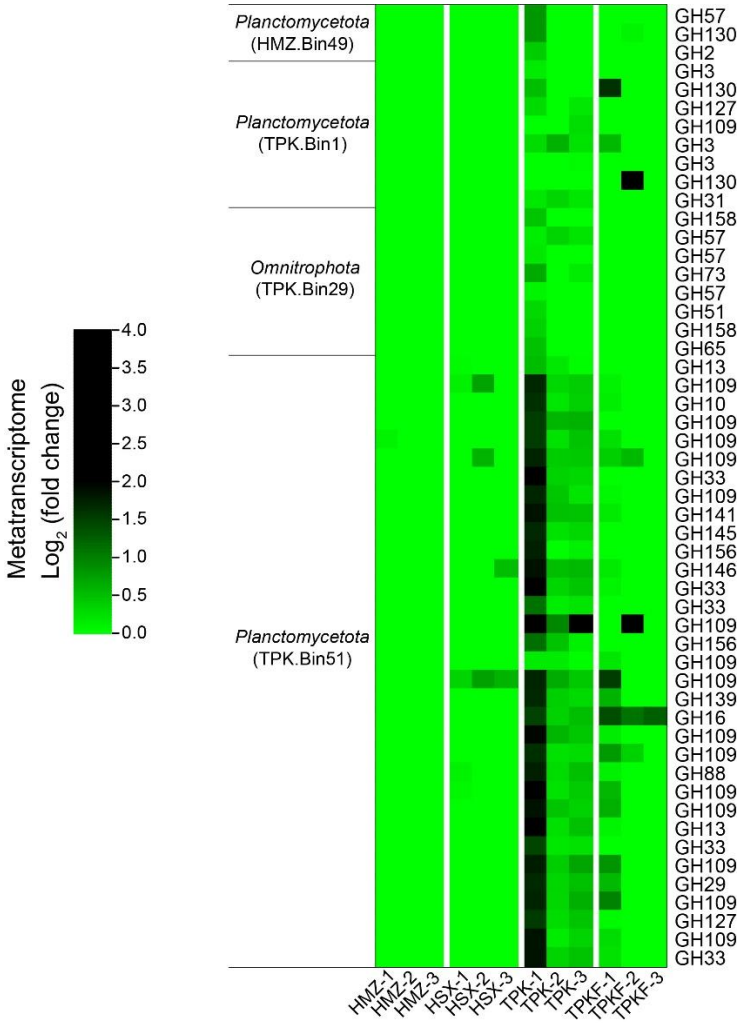


Figure 3 | Relative abundance of potential carbohydrate-active enzyme genes across deep-sea microbial communities. The left panel shows the abundance of key carbohydrate-active enzyme (CAZyme) families (GH, glycoside hydrolase; GT, glycosyltransferase; PL, polysaccharide lyase; CE, carbohydrate esterase; AA, auxiliary activity; CBM, carbohydrate-binding module) within deep-sea MAGs. MAGs are organized into distinct phylogenetic clusters from four deep-sea sites: 2022 Haima cold seep (HMZ), 2022 Zhenbei seamount (HSX), 2022 Site F cold seep 1 (TPK), and 2022 Site F cold seep 2 (TPKF). The right panel displays the relative abundance of genes encoding GH and GT within these MAGs from metatranscriptomic data (three replicates per site). The relative abundance of each gene was normalized against the average abundance of the 10 selected marker genes and quantified as

log_2-transformed fold change (log_2 fold change) based on metatranscriptomic data (three replicates per site).



Supplementary Figure 3 | Metatranscriptomic profiling of relative carbohydrate-active enzyme gene abundances across deep-sea microbial communities. Transcript abundances are shown in transcripts per million (TPM). To ensure comparability between samples, TPM values were normalized and quantified as log_2-transformed TPM (log_2 TPM) based on metatranscriptomic data (three replicates per site).



Supplementary Figure 4 | Metatranscriptomic profiling of relative glycoside hydrolase gene abundances across deep-sea microbial communities. The relative abundance of each gene was normalized against the average abundance of the 10 selected marker genes and quantified as log_2-transformed fold change (log_2 fold change) based on metatranscriptomic data (three replicates per site).

Reference related to this response:

Teng, Z. J. et al. Biogeographic traits of dimethyl sulfide and dimethylsulfoniopropionate cycling in polar oceans. Microbiome 9, 207, (2021).

Comment 7: Nonetheless, the manuscript is well-written and thoroughly references the relevant literature. I encourage the authors to address the issues mentioned earlier and clarify or adjust the main claims highlighted in the study. Additionally, I have a few minor comments and some further suggestions to improve the manuscript. Below are my additional comments and suggestions for each section.

Response: We sincerely thank you for these positive assessments of our manuscript and for the thorough evaluation of the relevant literature. We are grateful for these

constructive suggestions, which have significantly helped us sharpen the clarity and precision of our core findings. We have carefully addressed all subsequent comments and incorporated the requested adjustments throughout the manuscript. We believe these revisions have greatly strengthened the narrative and technical rigor of our study.

Comment 8: Abstract: Line 28: I suggest the authors refer to "the deep-sea microbiome" rather than "deep-sea microbes," as the assessment focuses on community-level functional potential rather than individual taxa.

Response: We appreciate your thoughtful suggestion regarding the terminology in the Abstract. We agree that our study's focus on community-level functional potential is more accurately captured by referring to the "deep-sea microbiome." According to your suggestion, we have modified this description as "However, the functional capacity of the deep-sea microbiome to metabolize complex organic matter across diverse regions remains poorly understood." in the revised manuscript (Lines 29-31).

Comment 9: Line 39: The phrase "genomic and transcriptomic potential for COM metabolism" already conveys functional capacity. The latter reference to "robust degradation capacity" is somewhat redundant and could be streamlined for clarity and conciseness.

Response: We thank you for this constructive suggestion regarding the phrasing of our functional findings. We agree that the distinction between "genomic and transcriptomic potential" and "robust degradation capacity" could be more effectively integrated to avoid redundancy. We have revised this section to create a clearer narrative flow: (1) We retained the reference to "potential" when discussing the broader community-wide findings. (2) We streamlined the description of our specific isolates (WC338 and WC36) to emphasize how our growth and transcriptome data provide experimental validation of these genomic predictions. The revised text is more concise, clearly separating the community-level potential from the strain-level experimental characterization of their catabolic machinery.

According to your suggestion, we have modified this description as "Our results reveal spatio-temporal community heterogeneity driven by geochemical gradients, alongside widespread potential for COM metabolism. Notably, the PVC (*Planctomycetota-Verrucomicrobiota-Chlamydiota*) superphylum exhibits extensive polysaccharide degradation capabilities, exemplified by the isolation of *Planctomycetota* strain WC338 and *Lentisphaerota* strain WC36 via laminarin enrichment. Growth experiments and transcriptomics confirm their obligate laminarin dependence and characterize the underlying catabolic machinery—specifically, the deployment of distinct glycoside hydrolase (GH) families, which are broadly distributed and prevalent across the PVC superphylum." in the revised manuscript (Lines 34-43).

Comment 10: Line 39-40: GH16 and GH2 are not "distinct enzymes" but glycoside hydrolase families; better to phrase accordingly.

Response: We thank you for this critical clarification regarding nomenclature. We agree that GH16 and GH2 are defined as glycoside hydrolase families rather than individual enzymes. We have modified this description in the manuscript accordingly to ensure terminological precision and clarity.

Comment 11: Introduction: The introduction section was well-written. However, I still recommend improving/revising specific presentations, as they could create a more direct narrative and make it easier to read. Lines 93-111: I recommend revising this section to improve narrative flow and reduce density. Several sentences are long and contain multiple ideas, which may obscure the central message. Streamlining the presentation, for example, by more clearly separating ecological distribution, deep sea relevance, and culturing limitations, would help create a more direct and readable narrative. Additionally, some details (e.g., listing multiple environments or phyla) could be condensed without loss of meaning.

Response: We appreciate the reviewer's insightful feedback regarding the narrative flow and clarity of the Introduction. We agree that the previous presentation was overly dense and that streamlining the transition from ecological distribution to the

culturing bottleneck significantly enhances the central message. In accordance with your recommendation, we have comprehensively revised this section to achieve a more direct narrative. Specifically, we have implemented the following improvements: Streamlined the taxonomic and ecological listing: We condensed the enumeration of phyla and environments to avoid unnecessary density while maintaining the context of the PVC superphylum's broad distribution. Improved narrative structure: We restructured the paragraph to logically delineate three key components: the unique biological traits of the PVC superphylum, their hypothesized ecological significance in deep-sea environments, and the critical limitation imposed by the current scarcity of cultured isolates. Enhanced readability: By breaking down complex, multi-idea sentences, we have ensured that the transition toward the study's objective—isolating laminarin-degrading strains—is clear and compelling.

According to your suggestion, we have modified this description as "The PVC superphylum—comprising *Planctomycetota*, *Verrucomicrobiota*, *Chlamydiota*, and related phyla—represents a bacterial lineage with unique cellular traits reminiscent of eukaryotes[19-21]. Among these, *Planctomycetota* are the most extensively studied and are ubiquitously distributed across diverse environments[22-26]. They are also hypothesized to dominate deep-sea sediment communities[27], reaching relative abundances of up to 28% in regions such as the Gulf of Mexico[18]. Metagenomic and distributional evidence has suggested their apparent involvement in nitrogen cycling and the breakdown of complex organic matter delivered as marine snow[18,28,29]. However, to date, only two *Planctomycetota* strains have been successfully isolated from deep-sea environments[30-32], hindering comprehensive assessment of their physiology and metabolic capabilities[33]. Despite their apparent ecological importance, our understanding of *Planctomycetota* function in the deep sea remains limited. Obtaining deep-sea isolates from the PVC superphylum is therefore essential for elucidating their role in degrading complex polysaccharides such as laminarin." in the revised manuscript (Lines 93-106).

Comment 12: Methods: Line 497-506: This section provides the necessary sampling information, but the presentation is dense and could be streamlined for clarity. The repeated listing of sites and years makes the paragraph difficult to follow. I recommend reorganizing the description to clearly separate the 2018 and 2022 expeditions, avoiding redundancy in site names, and presenting coordinates and depths in a more concise, tabular, or grouped format. Additionally, there appears to be a typographical error in "a2022 Site F cold seep 2." Clarifying the sampling strategy (e.g., rationale for site selection or differences between seep, vent, and seamount sites) would further strengthen this section.

Response: We thank you for these helpful suggestions to improve the clarity of the sampling description. We agree that the original presentation was overly dense and difficult to follow. In response, we have substantially streamlined this section by moving the detailed sampling information (site names, coordinates, depths, and sampling years) to Supplementary Data 1, as already indicated in the revised manuscript.

Regarding the rationale for site selection: These sampling sites were selected to represent three distinct deep-sea habitat types—cold seeps, hydrothermal vents, and seamounts—each characterized by different geochemical regimes and organic matter inputs. Cold seeps (Site F and Haima) are typically influenced by hydrocarbon-rich fluids, hydrothermal vents (Sites H1 and H2) by high-temperature, sulfur-rich fluids, and seamounts (Zhenbei) by enhanced currents and organic matter deposition. This habitat diversity allows for a comparative assessment of microbial community composition and organic matter degradation potential across contrasting deep-sea ecosystems. The inclusion of Site F in both 2018 and 2022 also enables a temporal comparison within the same cold seep system.

According to your suggestion, we have added this detailed description as "Sediment samples were collected from seven deep-sea sites representing three distinct habitat types—cold seeps (Site F and Haima), hydrothermal vents (Sites H1 and H2), and a seamount (Zhenbei)—during two expeditions in 2018 and 2022 by *RV*

KEXUE. These habitats were selected to capture variation in geochemical regimes and organic matter inputs: cold seeps are influenced by hydrocarbon-rich fluids, hydrothermal vents by high-temperature sulfur-rich fluids, and seamounts by enhanced currents and organic matter deposition. Specifically, deep-sea sediment samples were collected using the remotely operated vehicle (ROV) *Faxian*. Surface sediments were retrieved with push corers deployed by the ROV, with careful attention to preserving the integrity of the uppermost sediment layer. Only the top 1 cm of each sediment core was retained for downstream analyses. To minimize disturbance and prevent contamination from deeper strata, the push corers were inserted to a controlled depth and monitored to avoid core overfilling. This approach ensured the recovery of pristine surface sediment material suitable for subsequent geochemical and microbiological investigations. These sediment samples were immediately stored at -80 °C following rapid collection. Detailed information for each sample, including geographic coordinates, depth, and habitat type, is provided in Supplementary Data 1." in the revised manuscript (Lines 431-447).

Comment 13: Lines 507-524: The description of amplicon and metagenomic sequencing is currently too brief and lacks essential information about the downstream data-processing steps. Please provide additional methodological details, even if referencing previous studies. For example, clarify how raw reads were quality-filtered, how contigs were assembled (individually per sample or co-assembled by site/year), how bins were generated and refined, and which tools/parameters were used for taxonomic and functional annotation. While citing prior work is acceptable, the manuscript should still include enough information for readers to understand the analytical workflow and ensure reproducibility.

Response: We thank you for this constructive suggestion. We agree that the original description was insufficient for ensuring reproducibility. In response, we have substantially expanded the Methods section to provide comprehensive details of all downstream data processing steps. According to your suggestion, we have added this detailed description of amplicon and metagenomic sequencing as "**Amplicon**

sequencing To understand the microbial community structure of these seven sediment samples, 16S rRNA gene sequencing analysis was performed as previously described[59]. Briefly, the total DNA was extracted from these four sediment samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, USA) according to manufacturer's instructions. The quality and concentration of DNA were determined by 1.0% agarose gel electrophoresis and a NanoDrop2000 spectrophotometer (Thermo Scientific, USA) and kept at -80 ℃ prior to further use. The V3-V4 region of the 16S rRNA gene was amplified from total DNA using the forward primer 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and the reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The library was prepared using the TruSeqTM DNA Sample Prep Kit (Illumina, USA) and sequenced on the Illumina Nextseq2000 platform (Illumina, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). After demultiplexing, the resulting sequences were quality filtered with fastp v0.19.6[60] and merged with FLASH v1.2.11[61]. Then the high-quality sequences were de-noised using DADA2 plugin[62] in the Qiime2 v2024.2 pipeline[63] with recommended parameters, which obtains single nucleotide resolution based on error profiles within samples. DADA2 denoised sequences are usually called amplicon sequence variants (ASVs). Taxonomic assignment of ASVs was performed using the Naive bayes consensus taxonomy classifier implemented in Qiime2 and the SILVA database v138.1.

Metagenomic sequencing, assembly, and binning

Total microbial DNA was extracted from deep-sea sediment samples using the E.Z.N.A.® Stool DNA Kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's instructions. DNA integrity was assessed by 1% (w/v) agarose gel electrophoresis, and concentration was determined using a Qubit® 3.0 fluorometer (Invitrogen, Carlsbad, CA, USA). For each sample, 1.0 μg of genomic DNA was sheared using a Covaris S220 focused-ultrasonicator (Woburn, MA, USA) to generate fragment sizes of approximately 450 bp. Sequencing libraries were constructed using the NEB Next® Ultra™ DNA Library Prep Kit for Illumina (New England BioLabs,

Ipswich, MA, USA) according to the manufacturer's protocol. Paired-end sequencing (2 × 150 bp) was performed on the Illumina HiSeq X platform (Illumina, San Diego, CA, USA). To ensure consistency in data processing, all raw sequencing reads were analyzed using a standardized bioinformatics pipeline established by Shanghai Biozeron Biological Technology Co., Ltd. (Shanghai, China). Raw sequence reads underwent quality trimming was performed by SOAPnuke v1.5.6 (parameters: -l 20 -q 0.2 -n 0.05 -Q 2 -d -c 0 -5 0 -7 1)[64] and the clean data were assembled using MEGAHIT v1.2.9 (parameters:--min-count 2 --k-min 33 --k-max 83 --k-step 10)[65]. Genome binning was performed using three complementary algorithms. Coverage information for each sample was generated by mapping clean reads to the assembled contigs using Bowtie2 v2.3.5 with default settings. The resulting coverage profiles were used as input for binning with MaxBin2 v2.2.7 (-markerset 40)[66], MetaBAT2 v2.12.1 (-m 1500 and --unbinned parameters)[67] and Concoct v0.4.0 (default parameters)[68]. The initial bin sets were refined and consolidated using MetaWRAP v1.3.2 (default parameters)[69] with default settings to generate the final metagenome-assembled genomes (MAGs). The completeness and contamination of each MAG were assessed using CheckM v1.0.18[70] with the lineage-specific workflow. Only bins with estimated completeness >50% and contamination <10% were retained for downstream analysis." in the revised manuscript (Lines 448-494).

Comment 14: Lines 563: Did the authors explore model tree optimization methods? What model was used for the tree?

Response: We thank you for this insightful question. Yes, we employed model selection to determine the optimal substitution model for phylogenetic reconstruction. Specifically, we used the W-IQ-TREE web server (http://iqtree.cibiv.univie.ac.at) to perform automatic model selection based on the aligned sequences. The K3P+I+G4 model was identified as the best-fitting model according to the Bayesian Information Criterion (BIC). This model was then used to construct the phylogenetic tree presented in the manuscript. We have now clarified this information as "Phylogenetic trees were constructed using the maximum likelihood method via the W-IQ-TREE

web server (http://iqtree.cibiv.univie.ac.at)[80] with automatic model selection. The K3P+I+G4 model was selected as the best fit based on the Bayesian Information Criterion (BIC).” in the revised manuscript (Lines 580-583) to ensure transparency and reproducibility.

Comment 15: Data availability: I suggest that the authors also deposit the codes, scripts, and workflows used in the analyses (e.g., for amplicon processing, metagenomic assembly/binning, and transcriptomic analyses) in a public repository such as GitHub or Figshare. Providing these resources would greatly enhance transparency and reproducibility.

Response: We thank you for this valuable suggestion. In response, we have deposited all codes, scripts, and workflows used for data analysis—including amplicon processing, metagenomic assembly and binning, and transcriptomic analyses—in a public GitHub repository. These materials are now freely available at: https://github.com/zd200572/16s_bacteria_RNAseq_metgenome_metagenomic_pipelines. We have also added this this related description as “All codes, scripts, and workflows used for data processing and analysis (including amplicon processing, metagenomic assembly and binning, and transcriptomic analyses) are publicly available at GitHub: https://github.com/zd200572/16s_bacteria_RNAseq_metgenome_metagenomic_pipelines.” in the Data availability section (Lines 678-682) of the revised manuscript to enhance transparency and reproducibility. We believe this addition will facilitate broader use and validation of our analytical workflows by the community.

Reviewer #2 (Remarks to the Author):

Comment 1: The manuscript submitted by Zheng et al. proposes an integrated multi-omics study to demonstrate the role of members of the PVC superphylum (Planctomycetota-Verrucomicrobiota-Chlamydiota) into the degradation of complex organic matter in the deep-sea sediments. Their study focusses on the catabolism of Laminarin, a conspicuous glycan produced by macro and micro-algae that

significantly contribute to the pool of particulate organic carbon in the oceans. The methods used by the authors are particularly well suited, as they approach this issue with multiple methods (metagenomics, metatranscriptomics, cultivation and strain isolation, genomics, transcriptomics). Together, their results converge towards a central role of PVC members in the mineralization of complex organic molecules in the marine environment.

Response: We thank you for this positive comment of our work. We are pleased that you recognize the suitability of our multi-omics approach—integrating metagenomics, metatranscriptomics, cultivation, strain isolation, comparative genomics and transcriptomics—and agrees that our findings collectively support an important role for PVC members in the degradation of complex organic matter in deep-sea sediments. We appreciate your assessment and have carefully addressed the specific comments below.

Comment 2: The methods and results interpretations are sound and their demonstration is compelling. Below, I list some remarques that may be addressed by the authors to improve the manuscript and facilitate the comprehension by a general readership and data retrieval and sharing for reproducibility.

Response: We sincerely appreciate your positive feedback and constructive comments. These suggestions have been invaluable in improving the quality of our manuscript. We have thoroughly revised the manuscript based on the feedback provided, and all changes in the revised version are highlighted in blue for easy identification (shown as below).

Comment 3: Title: I find the title much too broad and not corresponding to the actual results of the work. I suggest a title more focused on the PVC and laminarin. Organic matter complexity and reactivity is a very broad topic in marine microbiology, and the present study only partially addresses this issue. It would better fit the general conclusion “Collectively, these findings delineate specialized adaptations within the PVC superphylum for polysaccharide degradation, significantly expanding our understanding of deep-sea microbial roles in global carbon cycling” in the abstract

Response: We thank you for this constructive suggestion. We agree that the original title was overly broad and have revised it to more accurately reflect the specific focus of our study. The new title, "Multi-omics and cultivation reveal laminarin-degrading PVC bacteria in the deep sea", directly highlights the key findings: the identification of laminarin-degrading capabilities within the PVC superphylum, supported by both multi-omics evidence and cultivation-based validation. We believe this revised title is now appropriately focused and aligns well with the scope of our work.

Comment 4: Figure 1: Reading "geographic distribution" in the caption, I was looking for MAG distribution in the figure. I would suggest "sample location and phylogenetic analysis of MAGs".

Response: We thank you for this helpful suggestion to improve the clarity of the figure legend. According to your suggestion, we have modified this figure legend as "Sample location and phylogenetic analysis of deep-sea metagenome-assembled genomes (MAGs)" in the revised manuscript (Line 939).

Comment 5: L. 172: Authors mention 316 MAGs reconstruction from 7 metagenomes. However, they then mention the same number of MAGs after clustering (ANI95%). There must be an error here: numbers of MAG pre clustering should be higher than post clustering.

Response: We thank you for this careful observation. You are absolutely correct: the number of MAGs prior to clustering should indeed be higher than the number after clustering at 95% ANI. We apologize for this oversight and have revised the text accordingly. Following the reviewer 1's suggestion, we have re-analyzed the raw metagenomic sequence data from the seven deep-sea sediments, ensuring strict consistency across the assembly, binning, and quality filtering steps using identical software parameters. This rigorous re-analysis yielded a revised and internally consistent set of genomes. We have modified this related description as "Metagenomic sequencing of the seven deep-sea sediments yielded 238 high- to medium-quality (completeness ≥50%, contamination ≤10%) microbial metagenome-assembled genomes (MAGs)[39] after de novo assembly and binning.

These 193 bacterial and 45 archaeal MAGs spanned 37 known and eight unclassified phyla (Fig. 1b and Supplementary Data 2).” in the revised manuscript (Line154-158).

Comment 6: L. 189: The sentence: “which exclusively the high abundance in hydrothermal vent sediments” doesn’t make sense.

Response: We thank you for pointing out the grammatical error in the original sentence. As suggested, we have revised the sentence to correct the grammatical structure. We have modified this related description as “This specialization aligns with previous reports of *Nanobdellota* as a prominent lineage within the DPANN superphylum, which often exhibits high relative abundances exclusively in hydrothermal vent ecosystems” in the revised manuscript (Lines 175-178).

Comment 7: L. 279: “of this understudied the PVC superphylum”. Remove the?

Response: We thank you for spotting this grammatical error. As suggested, we have removed the article “the” in the revised manuscript, and the phrase now correctly reads “of this understudied PVC superphylum”.

Comment 8: L. 291: It would help if you would just mention that you sequenced the genome of these isolates early in this paragraph.

Response: We thank you for this excellent suggestion aimed at improving the logical flow of the Results section. We agree that explicitly stating the availability of genomic data earlier enhances the context for subsequent phylogenetic and polysaccharide degradation gene analysis. We have incorporated your guidance by adding a sentence immediately preceding the taxonomic description of strains WC338 and WC36 as “To further investigate their taxonomic characteristics, we sequenced and analyzed their complete genomes.” in the revised manuscript (Lines 287-288). This upfront clarification establishes that both phenotypic and taxonomic data are grounded in high-resolution genomic information from the outset, ensuring methodological transparency and improving the narrative coherence when transitioning to the BLASTn and phylogenetic analyses.

Comment 9: L. 337: “Strain WC36 formed a distinct, deeply branching lineage that occupied a position equidistant from both the order Lentisphaerales and the order Victivallales Given the taxonomic threshold for order delineation (typically around 83.55%)46, the significant phylogenetic distance and distinct branching pattern observed for strain WC36 suggest that it may represent a novel order within the class Lentisphaeria.”. I somehow disagree with this since the WC36 branch has bootstrap values under 80%. It means that there is considerable uncertainity regarding its phylogenetic placement in the current tree. This could easily be fixed with a phylogenomic approach, using tools from GTDB-tk for instance. It surprising that authors did not use these approach given the availability of whole genome sequences.

Response: We sincerely thank you for pointing out the critical weakness in the phylogenetic assessment of strain WC36. We fully acknowledge that the low bootstrap support (<80%) observed on the 16S rRNA-based tree for the placement of strain WC36 rendered our initial inference of a novel order highly speculative. In direct response to this insightful recommendation, we have performed a comprehensive phylogenomic reanalysis using the Genome Taxonomy Database Toolkit (GTDBTk), leveraging the availability of high-quality whole-genome sequences. The resulting GTDBTk-derived phylogeny provides a much more robust placement for strain WC36. This analysis confirms its position within the order *Victivallales*. Furthermore, the 16S rRNA gene identity to its closest known relatives, *Victivallis lenta* (85.95%) and *Victivallis vadensis* (85.14%), falls definitively below the commonly accepted 16S rRNA gene sequence identity threshold (86.5%) required for family demarcation. Consequently, by combining the robust support of the whole-genome phylogeny with the established 16S divergence metric, we now confidently revise our conclusion: Strain WC36 represents a potential novel family within the order *Victivallales*, rather than a novel order. This revised taxonomic assignment is now firmly supported by superior phylogenomic evidence and has been updated throughout the manuscript’s classification discussion.

According to your suggestions, we have modified this related description as “A maximum-likelihood phylogenetic tree based on genomes revealed that strain WC338 clusters robustly within the genus *Poriferisphaera* (*Planctomycetota*), forming a distinct, monophyletic clade separate from its closest described relatives, such as *Poriferisphaera corsica* KS4 (98.06%, 16S rRNA gene identity) and *Poriferisphaera heterotrophicis* ZRK32 (97.19%) (Fig. 4). This sequence divergence falls below established species demarcation thresholds (98.65%)[49], thus formally designating strain WC338 as a novel species within *Poriferisphaera*. In parallel, strain WC36 was taxonomically placed within the order *Victivallales* (*Lentisphaerota*). Strain WC36 forms a deeply branching lineage, with sequence identity to its nearest neighbors, such as *Victivallis lenta* BBE-744-WT-12 (85.95%) and *Victivallis vadensis* Cello (85.14%), falling below the recognized threshold for family delineation (86.5%)[50]. This significant phylogenetic distance suggests that strain WC36 may represent a novel family within the order *Victivallales*, substantially expanding the known taxonomic breadth of this understudied order. The co-occurrence of these functionally enriched isolates points to a potential active role of PVC superphylum bacteria in deep-sea polysaccharide cycling.” in the revised manuscript (Lines 288-303).

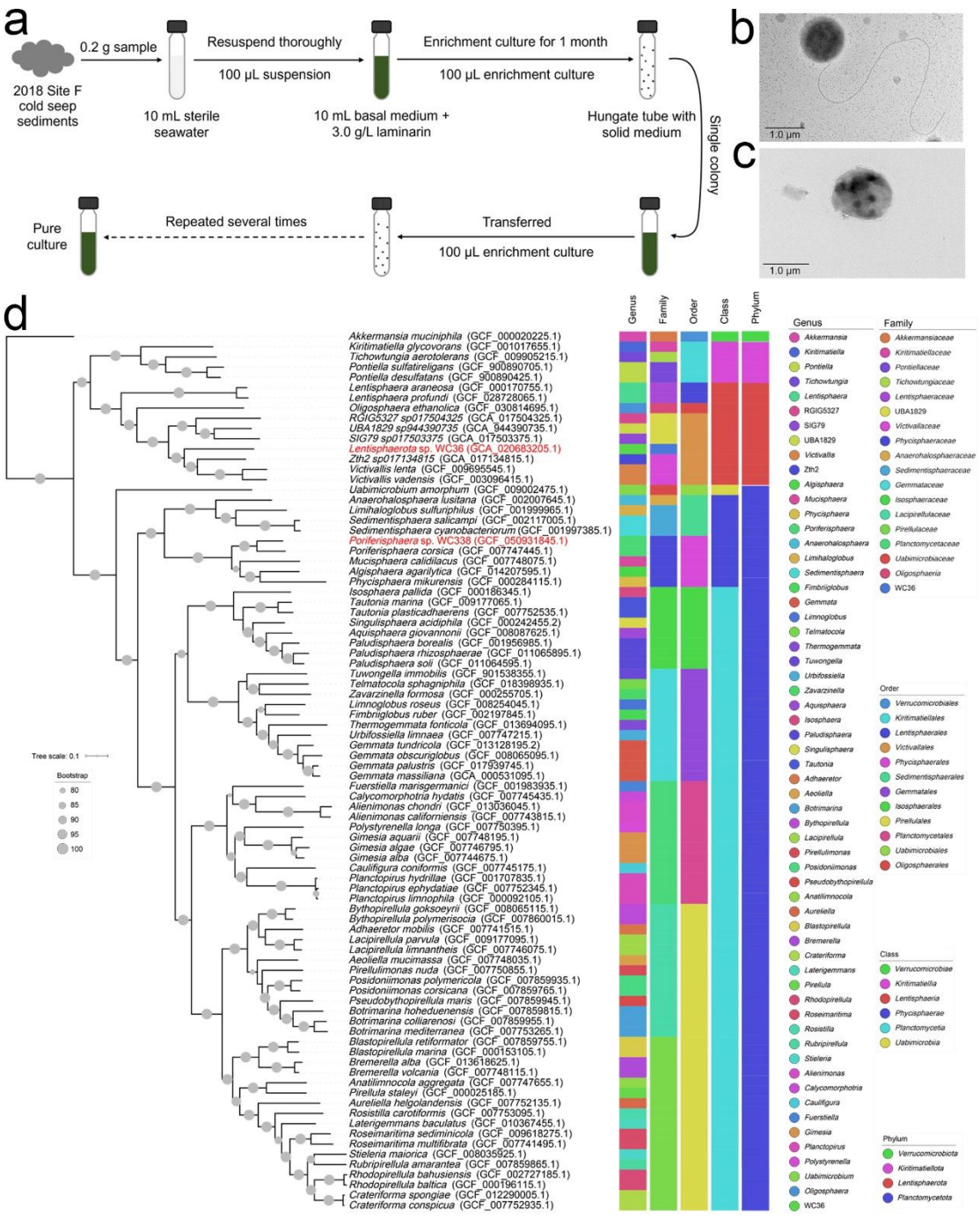


Figure 4 | Isolation, morphology, and phylogenetic analysis of cultured deep-sea PVC superphylum bacteria. a Isolation strategy for PVC superphylum bacteria. **b, c** Transmission electron microscopy (TEM) micrographs of *Poriferisphaera* sp. WC338 (b) and *Lentisphaerota* sp. WC36 (c). **d** Maximum likelihood phylogenetic tree of deep-sea *Poriferisphaera* sp. WC338 and *Lentisphaerota* sp. WC36 was reconstructed using the Genome Taxonomy Database Toolkit (https://github.com/Ecogenomics/GTDBTk). The tree is inferred and reconstructed under the maximum likelihood criterion and nodes with greater than 80% bootstrap support are labeled with a grey circle (expressed as percentages of 1,000 replications). Bar, 0.1 substitutions per nucleotide position.

Comment 10: L. 439: “Our analysis encompassed 171 pure cultures of bacteria” lead the reader to think that thay are part of this study. Maybe use “171 available genomes in the X database” instead?

Response: We thank you for this helpful clarification. We have revised the sentence to accurately reflect the source of the data. The original wording has been replaced with “Our analysis encompassed 171 available genomes in the NCBI database of the PVC superphylum bacteria” in the revised manuscript (Lines 370-371). This modification eliminates potential ambiguity and correctly indicates that these genomes were obtained from a public repository rather than newly sequenced in this study.

Comment 11: L 492: The manuscript really misses a sampling material and method section: How were the sediment recovered? Which sediment horizons were sampled?

Response: We thank you for this important observation. In response, we have now added a detailed description of the sediment sampling procedure in the Materials and Methods section as “Sediment samples were collected from seven deep-sea sites representing three distinct habitat types—cold seeps (Site F and Haima), hydrothermal vents (Sites H1 and H2), and a seamount (Zhenbei)—during two expeditions in 2018 and 2022 by *RV KEXUE*. These habitats were selected to capture variation in geochemical regimes and organic matter inputs: cold seeps are influenced by hydrocarbon-rich fluids, hydrothermal vents by high-temperature sulfur-rich fluids, and seamounts by enhanced currents and organic matter deposition. Specifically, deep-sea sediment samples were collected using the remotely operated vehicle (ROV) *Faxian*. Surface sediments were retrieved with push corers deployed by the ROV, with careful attention to preserving the integrity of the uppermost sediment layer. Only the top 1 cm of each sediment core was retained for downstream analyses. To minimize disturbance and prevent contamination from deeper strata, the push corers were inserted to a controlled depth and monitored to avoid core overfilling. This approach ensured the recovery of pristine surface sediment material suitable for

subsequent geochemical and microbiological investigations." in the revised manuscript (Lines 431-444).

Comment 12: L: 512: OTU instead of OUT

Response: We thank you for pointing out this typographical error. More importantly, in response to this comment and the valuable suggestions from Reviewer 1, we have re-evaluated our amplicon data analysis strategy. Accordingly, we have replaced the traditional OTU (operational taxonomic unit) clustering approach with a more precise ASV (amplicon sequence variant) inference method. The entire analysis pipeline has been updated using the DADA2 algorithm to infer exact ASVs, which offers single-nucleotide resolution and improves the reproducibility and comparability of our results. All relevant figures, tables, and statistical analyses have been updated accordingly, and the revised Methods section now reflects this change. We believe this methodological improvement substantially strengthens the resolution and robustness of our microbial community analysis.

Comment 13: L. 518: authors refer to a bioarxiv for their method description, meaning that the said methods have not been reviewed yet. Although I generally agree with methods described in this pre-print, I think it would be more appropriate to describe the methods in full here, unless the archive is now published.

Response: We thank you for drawing attention to the citation of a pre-print (bioRxiv) referenced preprint has now been formally peer-reviewed and formally published in an established journal (Wang et al., 2025). Correspondingly, we have updated the revised manuscript to cite this official, published version, thereby ensuring all methodological descriptions are referenced to peer-reviewed literature. Furthermore, we have supplemented the relevant section with concise details of the procedure itself to enhance immediate comprehension, as suggested.

Reference related to this response:

Wang, C., Zheng, R. & Sun, C. Deep-sea viral diversity and their role in host metabolism of complex organic matter. *Nat Commun* **16**, 10134, (2025).

Comment 14: In general, I find difficult to follow the methods section due to many references to other articles. A minimum of information would facilitate the reading.

Response: We appreciate your feedback regarding the readability of the Methods section due to extensive referencing. To enhance clarity and provide necessary standalone detail, we have significantly expanded the Materials and Methods section in the revised manuscript.

Comment 15: Also, authors mention analysis on strain genomes, but do not mention the genome sequencing in the results nor in the material and methods. A section "genome sequencing is needed here"

Response: We sincerely appreciate your positive suggestion. We have now added a detailed description of the genome sequencing procedures in the Materials and Methods section as "**Genome sequencing, assembly, and annotation.** Deep-sea strains WC338 and WC36 were cultivated anaerobically at 28 °C for 5 d in basal medium supplemented with 3.0 g/L laminarin. Cells were harvested by centrifugation, and genomic DNA was extracted using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. DNA purity, integrity, and concentration were assessed using three complementary approaches: (1) agarose gel electrophoresis (1% w/v) to visualize degradation or contamination; (2) spectrophotometric analysis (NanoDrop, Thermo Fisher Scientific, Waltham, MA, USA) to evaluate $OD_{260/280}$ ratios; and (3) fluorometric quantification using the Qubit® dsDNA Assay Kit with a Qubit® 3.0 fluorometer (Invitrogen, Carlsbad, USA). Whole-genome sequencing was performed at Novogene Bioinformatics Technology Co., Ltd. (Beijing, China) using a hybrid strategy combining short- and long-read technologies. Short-insert paired-end libraries (350 bp insert size) were constructed using the NEB Next® Ultra™ DNA Library Prep Kit for Illumina (New England BioLabs, Ipswich, MA, USA) and sequenced on the Illumina NovaSeq 6000 platform (PE150). For long-read sequencing, native genomic DNA was processed without amplification and sequenced on the Oxford Nanopore PromethION platform. Base calling of raw nanopore signals (fast5) was performed

using Guppy v3.1.5, and resulting fastq files were demultiplexed by barcode. Read quality metrics were evaluated using NanoPlot v1.29.1. High-quality short and long reads were co-assembled using Unicycler v0.4.8 with a hybrid assembly approach. For each strain, the assembly converged to a single circular contig, which was manually validated and circularized by trimming terminal overlaps. Gene prediction and functional annotation were carried out by aligning predicted protein sequences against multiple public databases using BLAST (E-value $\leq 1\times10^{-5}$, minimum alignment coverage $\geq 40\%$). The following databases were used: Pfam (release 33.1), GO (release 2022-01)[82], KEGG (release 104.1)[83], COG (release 2021-11)[84], NCBI NR (release 2022-03), TCDB (release 2021-01), and Swiss-Prot (release 2021-05)[85].” in the revised manuscript (Lines 604-631).

Comment 16: L. 562: When aligning 16S, using a 2d aware rRNA aligner such as SINA is generally a better option.

Response: We thank you for this valuable suggestion. In the revised manuscript, we have reconstructed the 16S rRNA phylogenetic tree using a secondary-structure-aware aligner (SINA), which offers a more accurate alignment by incorporating rRNA structural information. The updated analysis is presented as “Full-length 16S rRNA gene sequences from strains WC338, WC36, WC47, WC29 (obtained from their genomes), and related taxa (from NCBI) were aligned using the secondary-structure-aware aligner (SINA, v1.2.12)” in the revised manuscript (Lines 577-580), and we believe this improvement strengthens the phylogenetic framework of our study.

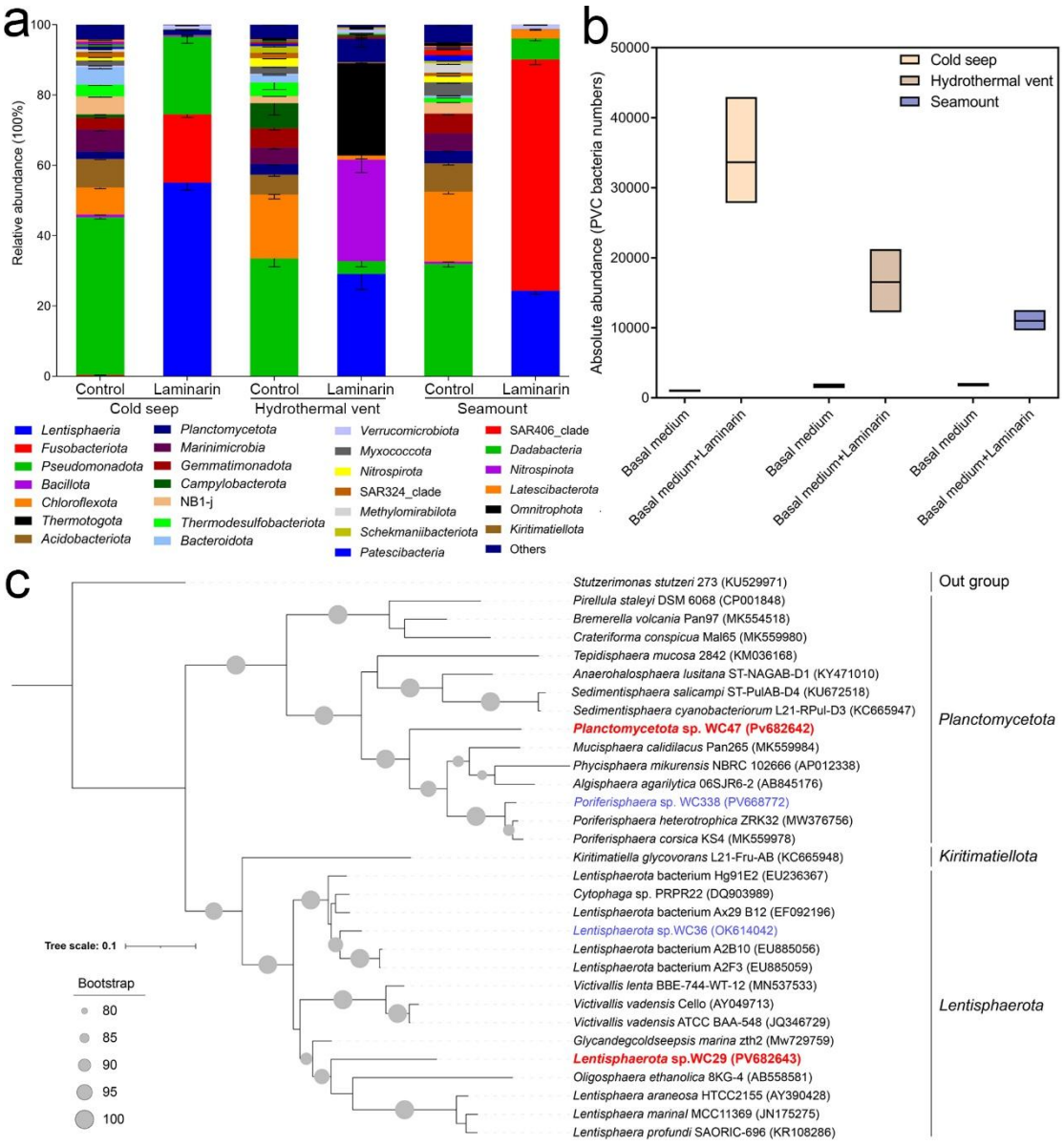


Fig. 7 | Laminarin facilitates the isolation and cultivation of PVC superphylum bacteria from deep-sea sediments. a, Relative abundance of bacterial phyla in deep-sea sediment communities after 30 days of incubation in basal medium (Control) or basal medium supplemented with 3.0 g/L laminarin (Laminarin). Data represent mean±SD (*n*=3 biologically independent replicates) for cold seep, hydrothermal vent, and seamount samples. **b,** Absolute abundance of PVC superphylum bacteria enriched from these sediments with laminarin as the primary carbon source. **c,** Maximum-likelihood phylogenetic tree of *Planctomycetota* strain WC47 and *Lentisphaerota* strain WC29. Scale bar, 0.1 substitutions per nucleotide position.

Reference related to this response:

Pruesse, E., Peplies, J. & Glöckner, F. O. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* **28**, 1823-1829, (2012).

Comment 17 : L. 589: This section seems to lack accession numbers for metagenome reads and metatranscriptome reads. These must be available to the scientific community for publication.

Response: We thank you for highlighting the necessity of data accessibility. We confirm that all sequencing reads have been made publicly available in the NCBI Short Read Archive (SRA). We have added this related description as "The raw amplicon sequencing reads for seven deep-sea sediment samples have been deposited to NCBI Short Read Archive (accession numbers PRJNA1434112 and PRJNA1281722). The raw metagenomic sequencing reads for these seven deep-sea sediment samples are available under NCBI SRA accession numbers PRJNA1417947 and PRJNA1230403. The raw metatranscriptomic sequencing reads for 2022 Site F cold seep 1, 2022 Site F cold seep 2, 2022 Haima cold seep, and 2022 Zhenbei seamount samples are available under NCBI SRA accession number PRJNA1230164. Complete genome sequences for strains WC338 (CP194516) and WC36 (CP085689), and their respective 16S rRNA gene sequences (WC338: PV668772; WC36: OK614042; WC47: PV682642; WC29: PV682643), are deposited in GenBank. Raw transcriptomic reads for strains WC338 and WC36 are available under NCBI SRA accession numbers PRJNA1337914 and PRJNA1337918. The raw amplicon sequencing reads of the laminarin enrichment experiment are available under NCBI SRA accession number PRJNA1434106. All codes, scripts, and workflows used for data processing and analysis (including amplicon processing, metagenomic assembly and binning, and transcriptomic analyses) are publicly available at GitHub: https://github.com/zd200572/16s_bacteria_RNAseq_metgenome_metagenomic_pipelines." in the revised manuscript (Lines 665-682).

Responses to the Reviewers

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

Comment 1: I am happy to say that the authors have carefully and comprehensively addressed my major concerns. In particular, I appreciate that they harmonized analyses across datasets and removed overstated ecological claims. Also, the added community-level enrichment data substantially improved the statistical grounding and transparency of their interpretations. The revised manuscript now presents a well-supported and appropriately cautious assessment of PVC bacteria as contributors, rather than dominant drivers, to laminarin degradation in deep-sea sediments.

Response: We thank you for the positive and constructive feedback. We are pleased that the harmonization of cross-dataset analyses, removal of overstated claims, and inclusion of community-level enrichment data have satisfactorily addressed the major concerns. We also note with appreciation the reviewer's acknowledgment that our revised conclusion—describing PVC bacteria as contributors rather than dominant drivers—is now well supported and appropriately cautious.

Comment 2: Still, I have minor issues, primarily related to clarity. For example, I suggest further tempering of statements like "highlight the crucial role of the PVC superphylum bacteria in the marine carbon cycle", to "potentially important role" or "previously underappreciated role", to keep the wordings aligned with the stated limitations. Please check the manuscript for similar statements.

Response: We thank you for this insightful and constructive comment. We have carefully considered your suggestion regarding the clarity of our statements. As you recommended, we have tempered overclaiming language throughout the manuscript. Specifically, we have replaced phrases as following: "highlight the crucial role of the PVC superphylum bacteria" has been changed to "highlights the previously underappreciated role of PVC superphylum bacteria" (Lines 364-365); "crucial

drivers" to "important drivers" (Line 108); and "significantly upregulated" to "upregulated" (Line 331), along with other similar adjustments. We also systematically reviewed the entire manuscript and revised similar statements where necessary to ensure that all conclusions are appropriately supported by the experimental results and that no claims are overstated.

Comment 3: Also, the figures read as compacted. I suggest for some in-text information to be reduced further, and specifically for Figure 7, adding significance markers (e.g., $p < 0.05$) would be helpful for easier interpretation.

Response: We thank you for this constructive suggestion. We have carefully revisited all figures to address the issue of overcrowding and to improve interpretability. As recommended, we have added significance markers (e.g., $*P < 0.05$, $**P < 0.01$) to Figure 7 (shown below), which now allows for easier visual interpretation of statistical comparisons.

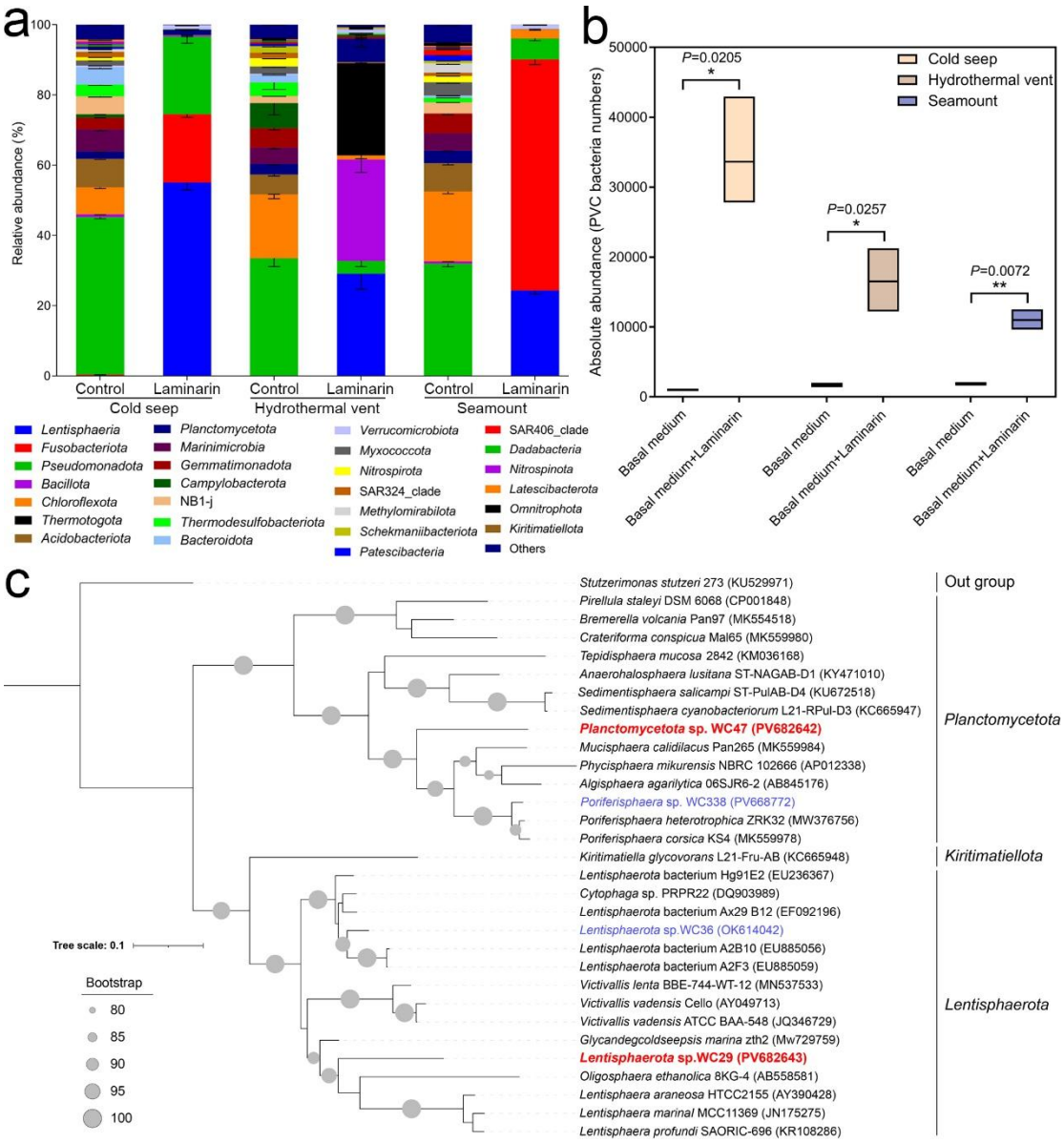


Fig. 7 | Laminarin facilitates the isolation and cultivation of PVC superphylum bacteria from deep-sea sediments. a Relative abundance of bacterial phyla in deep-sea sediment communities after 30 days of incubation in basal medium (Control) or basal medium supplemented with 3.0 g/L laminarin (Laminarin). Data represent mean±SD (n=3, three biologically independent replicates) for cold seep, hydrothermal vent, and seamount samples. **b** Absolute abundance of PVC superphylum bacteria enriched from these sediments with laminarin as the primary carbon source. Data are presented as mean values ± SD ($n = 3$, three replicates). *$P < 0.05$, **$P < 0.01$, with one-way ANOVA and Dunnett's multiple-comparisons test. **c** Maximum-likelihood phylogenetic tree of *Planctomycetota* strain WC47 and *Lentisphaerota* strain WC29. Scale bar, 0.1 substitutions per nucleotide position.

More broadly, we have reduced in-text information within figures where possible to avoid compactness. Specifically: **(1) For Figure 2** (shown below), we have

replaced the gene and pathway annotations at the bottom of the figure with letter-based labels and moved the detailed sample information to the figure legend. **(2) For Figure 3** (shown below), we have replaced the different sites at the bottom of the figure with Roman numerals and moved the detailed information to the figure legend.

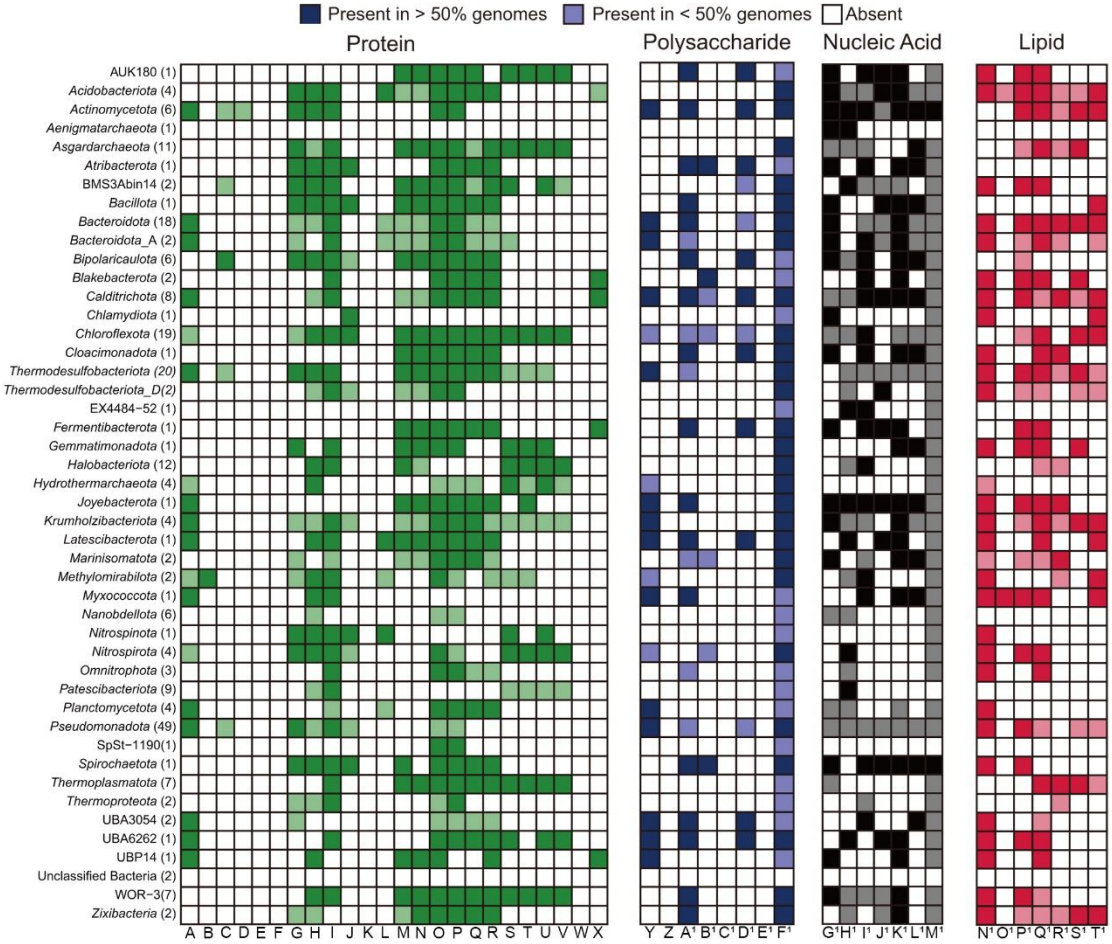


Fig. 2 | Distribution of key catabolic genes across deep-sea MAGs reveals diverse degradation capabilities of complex organic matter. Key genes responsible for the catabolism of proteins, polysaccharides, nucleic acids, and lipids were detected in MAGs. Color intensity indicates gene prevalence within each cluster: dark shading denotes presence in >50% of MAGs, and light shading indicates presence in <50% of MAGs. The total number of MAGs per cluster is shown in parentheses. A: extracellular peptidases; B: glutamate/aspartate transport system; C: general L-amino acid transport system; D: glutamate transport system; E: cystine transport system; F: arginine/ornithine transport system; G: putative polar amino acid transport system; H: branched-chain amino acid transport system; I: peptides/nickel transport system; J: oligopeptide transport system; K: arginine/lysine/histidine/glutamine transport system; L: gdh; M: iorA; N: iorB; O: korA; P: korB; Q: korC; R: korD; S: porA; T: porB; U: porC; V: porD; W: vorA; X: vorB; Y: extracellular CAZYmes; Z: maltose/maltodextrin transport system; A^1: putative multiple sugar transport system; B^1: ribose transport system; C^1: putative chitobiose transport system; D^1: glucose/mannose transport system; E^1: PTS system, D-glucosaminate-specific II component; F^1: glycolysis; G^1: cytosine/cytidine deaminase; H^1: extracellular nuclease; I^1:

guanine/adenine/adenosin deaminase; J[1]: nucleobase transport; K[1]: nucleoside phosphorylase; L[1]: nucleoside transport; M[1]: pentose phosphate pathway; N[1]: phospholipid transport system; O[1]: fatty acid transport; P[1]: fadD; Q[1]: acd and bcd; R[1]: crt and paaF; S[1]: fadN and paaH; T[1]: fadA and atoB. Pathway identification was performed with the software METABOLIC. All annotations are provided in Supplementary Data 3.

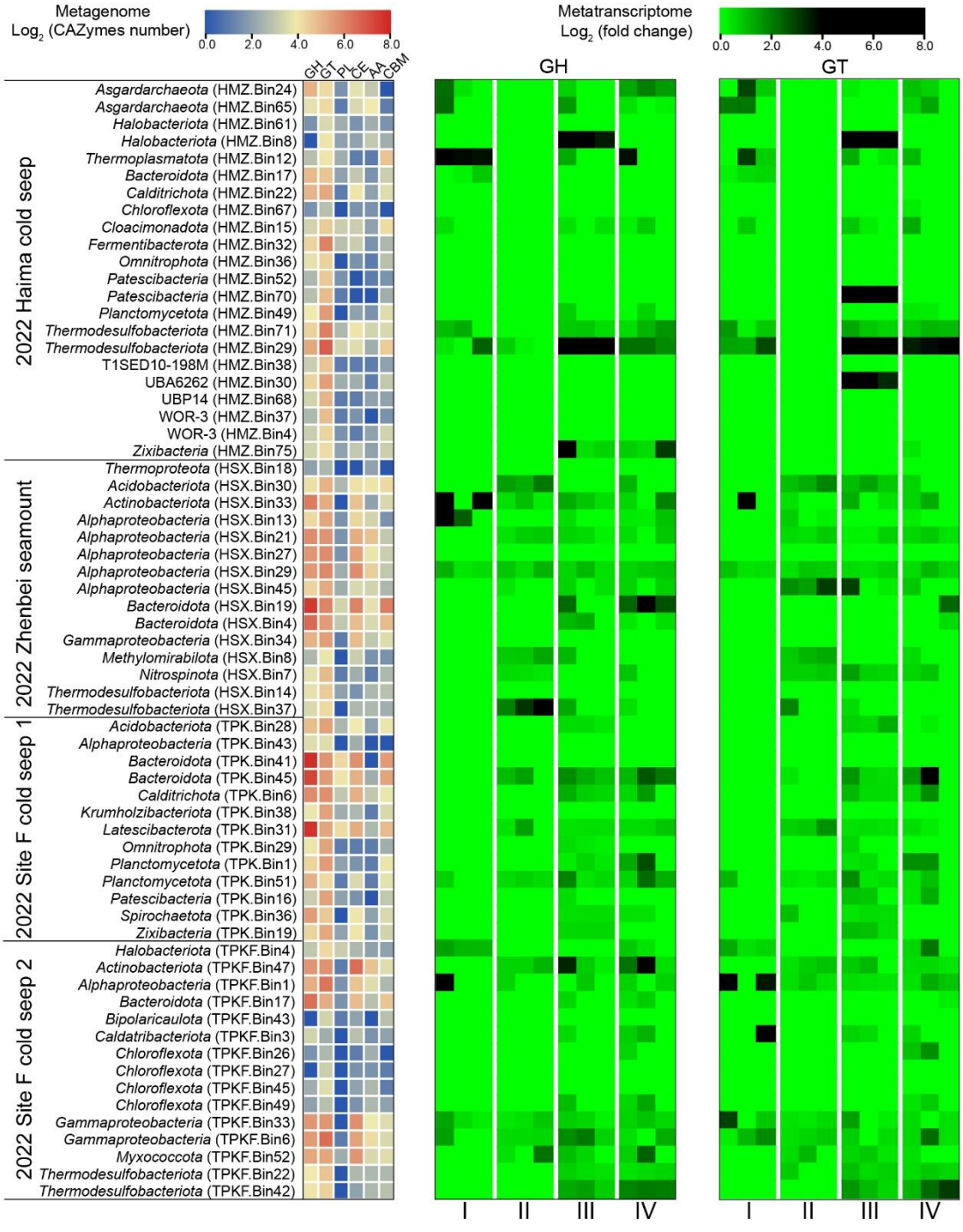


Fig. 3 | Relative abundance of potential carbohydrate-active enzyme genes across deep-sea microbial communities. The left panel shows the abundance of key carbohydrate-active enzyme (CAZyme) families (GH, glycoside hydrolase; GT, glycosyltransferase; PL, polysaccharide lyase; CE, carbohydrate esterase; AA, auxiliary activity; CBM, carbohydrate-binding module) within deep-sea MAGs. MAGs are organized into distinct phylogenetic clusters from four deep-sea sites:

2022 Haima cold seep (HMZ), 2022 Zhenbei seamount (HSX), 2022 Site F cold seep 1 (TPK), and 2022 Site F cold seep 2 (TPKF). The right panel displays the relative abundance of genes encoding GH and GT within these MAGs from metatranscriptomic data (three replicates per site). The relative abundance of each gene was normalized against the average abundance of the 10 selected marker genes and quantified as $\log_2$-transformed fold change ($\log_2$ fold change) based on metatranscriptomic data (three replicates per site). Sites are denoted as follows: I, HMZ; II, HSX; III, TPK; IV, TPKF.

All revised figures have been updated and resubmitted. We believe these changes have made the figures more concise and easier to interpret, in line with the reviewer's helpful comments.

Comment 4: Hence, I only recommend minor editorial revisions to improve readability and figure clarity, but no additional analyses are required, and I happily recommend the study for acceptance after these minor corrections.

Response: We thank you for your positive assessment and for recommending the study for acceptance after minor revisions. In response to the suggestion for minor editorial revisions, we have carefully reviewed the entire manuscript to improve readability and figure clarity. All changes have been marked in the revised manuscript. We appreciate the reviewer's constructive input throughout the review process.

Reviewer #2 (Remarks to the Author):

Comment 1: I congratulate the authors for extensively addressing the multiple points raised by the two reviewers. While reading the new version, I however found a few sections for which a few changes would be interesting to better align the claims and the messages of the manuscript with the experimental work carried by the authors.

Response: Thanks for your suggestion to better align the claims and messages with the experimental work. Accordingly, we have revised the relevant sections to ensure that all statements are appropriately supported by the data and that no claims are overstated. All changes are marked in the revised manuscript.

Comment 2: L56-70: In this part of the introduction, authors justify their work with the argument that deep subseafloor life is based in part on the degradation of recalcitrant organic matter. But later, the authors mention benthic communities and

COM, and then move on to Laminarin as a part of COM. This is a little confusing and suggest that Laminarin is an important contribution to this recalcitrant organic matter and is buried in the deep subseafloor. I didn't find any evidence in the cited literature that Laminarin is recalcitrant, nor that it is present in the subseafloor. In addition, samples of this study come from benthic surface. I think this part of the intro may be slightly reorganized or maybe removed.

Response: We thank you for this careful and constructive observation. We agree that the original introduction (lines 56-70) contained confusing logic and unsupported claims suggesting that laminarin is recalcitrant or present in the deep subseafloor. To address this concern, we have removed the entire paragraph as recommended. In addition, we have revised the manuscript to ensure that no statements imply laminarin is recalcitrant or buried in the subseafloor, consistent with the available literature and the benthic surface origin of our samples. According to your suggestions, we have modified this description as "The deep seafloor contains large amounts of organic matter—such as proteins, polysaccharides, nucleic acids, lipids, and humic acid fractions—that sustain diverse heterotrophic microbial communities[1-3]. Among the myriad components of polysaccharides, laminarin stands out as one of the most abundant polysaccharides globally, comprising approximately 26% of marine particulate organic carbon (POC)[4,5]." in the revised manuscript (Lines 56-60). We believe these revisions have eliminated the logical confusion and better aligned the introduction with the scope of our study.

Comment 3: L. 115-118: similar remark as for L.56-70.

Response: We thank you for this constructive comment. According to your suggestions, we have removed this sentence in the revised manuscript.

Comment 4: L. 168-181: This paragraph describes differences in Archaeal distribution between cold seeps and hydrothermal vents. I wouldn't describe these as "striking", but rather expected instead. These are two very different ecosystems (energetic metabolism, chemical environment, temperature…). I don't see how this

section supports the main message of the article and would encourage the authors to remove or rewrite it.

Response: We thank you for this constructive suggestion. We agree that the described archaeal distribution patterns are expected given the distinct environmental conditions of cold seeps and hydrothermal vents, and that the term "striking" was inappropriate. Furthermore, we acknowledge that this section does not directly support the main message of our study, which focuses on bacterial degradation of laminarin and the PVC superphylum. Therefore, we have removed the entire paragraph (Lines 168-181) to improve the focus and coherence of the manuscript. We have also added a brief transition sentence as "These MAGs spanned a wide range of taxonomic lineages and enabled a comprehensive assessment of the genomic potential for COM degradation across different deep-sea habitats." at the end of the preceding paragraph to directly introduce our subsequent analyses of COM degradation potential (Lines 153-155). We thank you for helping us sharpen the manuscript's narrative.

Comment 5: L. 212: eDNA generally refers to environmental DNA, not necessarily extracellular. Maybe consider removing?

Response: We thank you for this careful observation. We have removed "eDNA" and now write "extracellular DNA" in the revised manuscript.

Comment 6: L. 182-233: this section describes the catabolic potential of different members of the community for which MAGs were reconstructed. My opinion is that it is hard to establish general rules such as "This genomic deficit suggests a limited capacity for the direct extracellular hydrolysis and utilization of complex polysaccharides by deep-sea archaea." with a limited number of MAGs (238 Bac and 45 Arch). For comparison, other studies of the deep sea environment have collected one order of magnitude more MAGS to discuss metabolic potential, see for instance (Lu et al. 2024; St. John et Reysenbach 2026; Schauberger et al. 2024).

Response: We thank you for this important methodological comment. We agree that the number of archaeal MAGs (n=45) in our dataset is limited compared to larger-scale studies (Lu et al. 2024; St. John et Reysenbach 2026; Schauberger et al.

2024). We therefore acknowledge that broad generalizations about “deep-sea archaea” as a whole are not warranted based solely on our data.

To address this concern, we have revised the text to restrict all conclusions regarding archaeal catabolic potential to the specific MAGs recovered in this study. For example, the sentence “This genomic deficit suggests a limited capacity for the direct extracellular hydrolysis and utilization of complex polysaccharides by deep-sea archaea” has been changed to “This genomic deficit may suggest a limited capacity for the direct extracellular hydrolysis and utilization of complex polysaccharides among the archaeal MAGs recovered in this study”. Similar revisions have been made throughout the section to avoid overgeneralization. All changes are highlighted in the revised manuscript.

Moreover, we would like to clarify that the core focus of our study is not large-scale MAG-based metabolic inference, but rather the experimental validation of laminarin degradation by cultivated PVC superphylum bacteria from the deep sea. The MAG analysis serves as an initial genomic survey to contextualize our cultivation and functional experiments, rather than as the primary evidence for our main conclusions. Our key findings—demonstrating that novel isolates of *Planctomycetota* and *Lentisphaerota* possess a previously unrecognized capacity for laminarin degradation—are supported by growth experiments and transcriptomics, not solely by MAG data.

We have also retained the existing limitations paragraph at the end of this section, which acknowledges the influence of bin completeness and contamination on prevalence estimates, further contextualizing our findings. We thank the reviewer for helping us improve the precision of our claims.

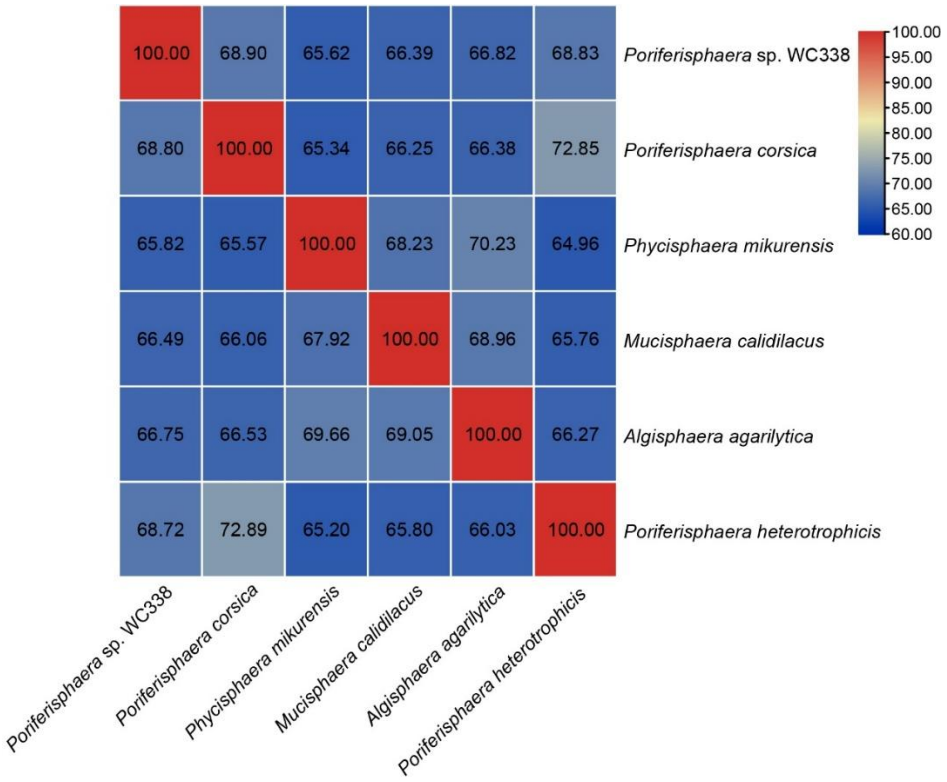
Comment 7: L. 235: the sentence suggest that all polysaccharides derive from cell wall and are recalcitrant. I would remove the coma after “polysaccharides,” to prevent this misinterpretation.

Response: We thank you for this careful observation. We agree that the original phrasing could be misinterpreted as suggesting that all polysaccharides derive from

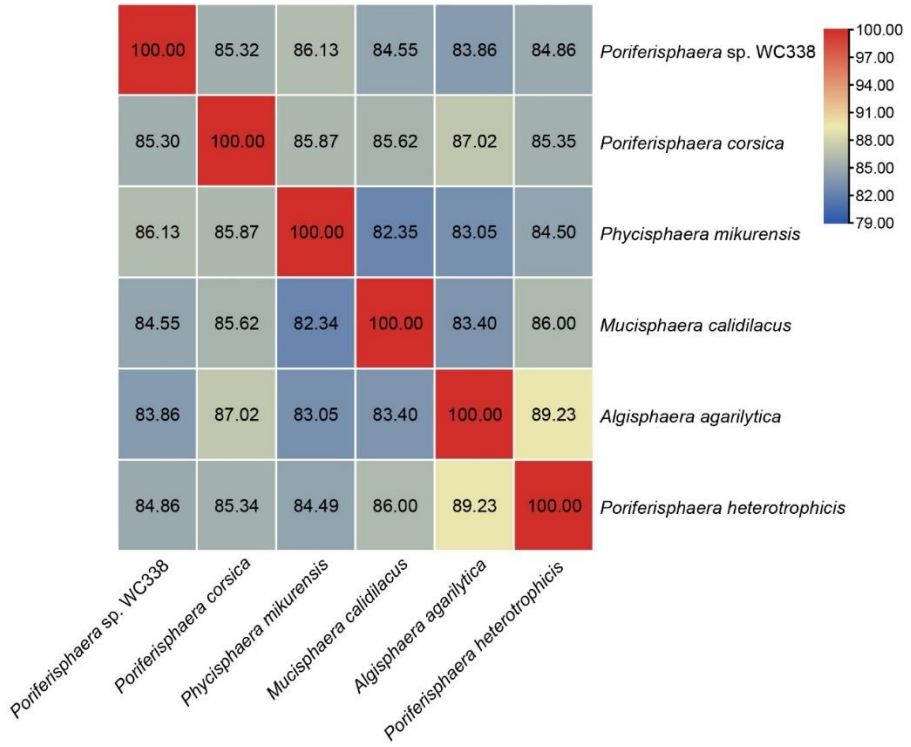
cell walls and are recalcitrant. According to your suggestions, we have removed the comma after “polysaccharides” as recommended.

Comment 8: L. 291-303: Here, authors use 16S rRNA genes (v3-v4) sequences to discuss is their isolates are new species. Why not using ANI % as this is a superior metric to delineate new species?

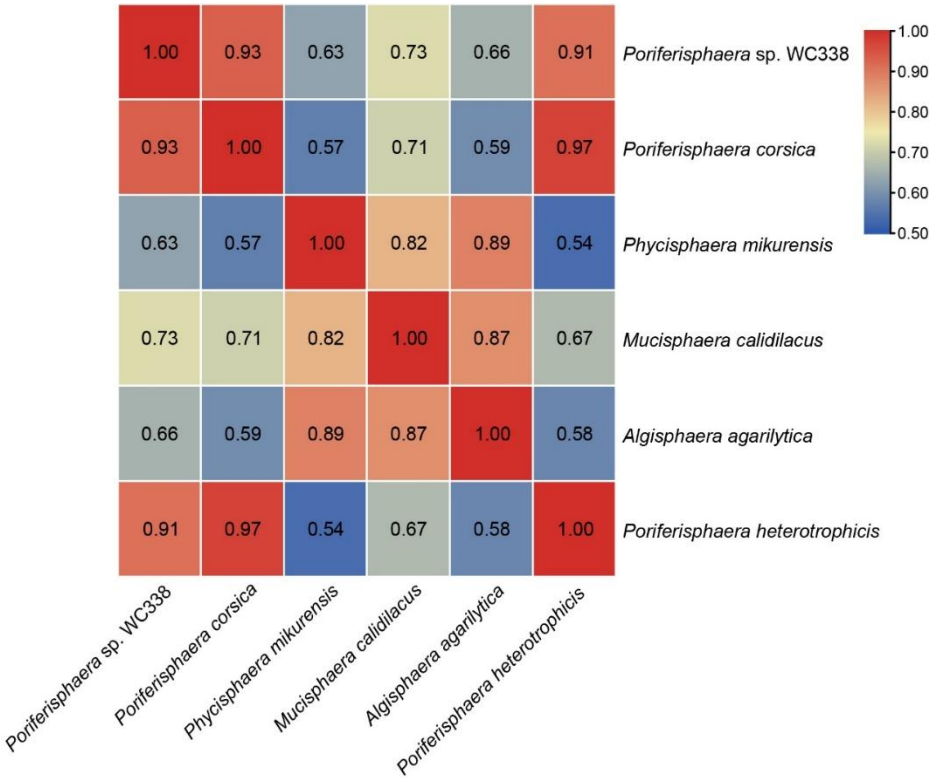
Response: Thanks for your insightful comments. As the primary focus of this study is not on species classification, we initially relied on 16S rRNA gene sequence similarity and phylogenetic tree analysis to propose the potential novelty of these strains. According to your suggestion, we have expanded the taxonomic characterization by incorporating genome-based analyses, including ANIm, ANIb, and Tetra. We believe these additional analyses provide a more robust and precise classification framework. We have added the related description as “To further corroborate their taxonomic status, we calculated average nucleotide identities (ANIb, based on BLAST+; ANIm, based on MUMmer) and tetranucleotide signatures (Tetra) for both strains. The results showed that the genomes of strains WC338 (ANIb, 68.8; ANIm, 86.13; Tetra, 0.93; Supplementary Figs. 5-7) and WC36 (ANIb, 65.01; ANIm, 89.1; Tetra, 0.69; Supplementary Figs. 8-10) fell well below the established cut-off values (ANIb: 95%, ANIm: 95%, Tetra: 0.99) for species classification, strongly suggesting that these strains represent novel taxa as currently defined.” in the revised manuscript (Lines 275-282). The additional figures have been included in the revised manuscript as follows:



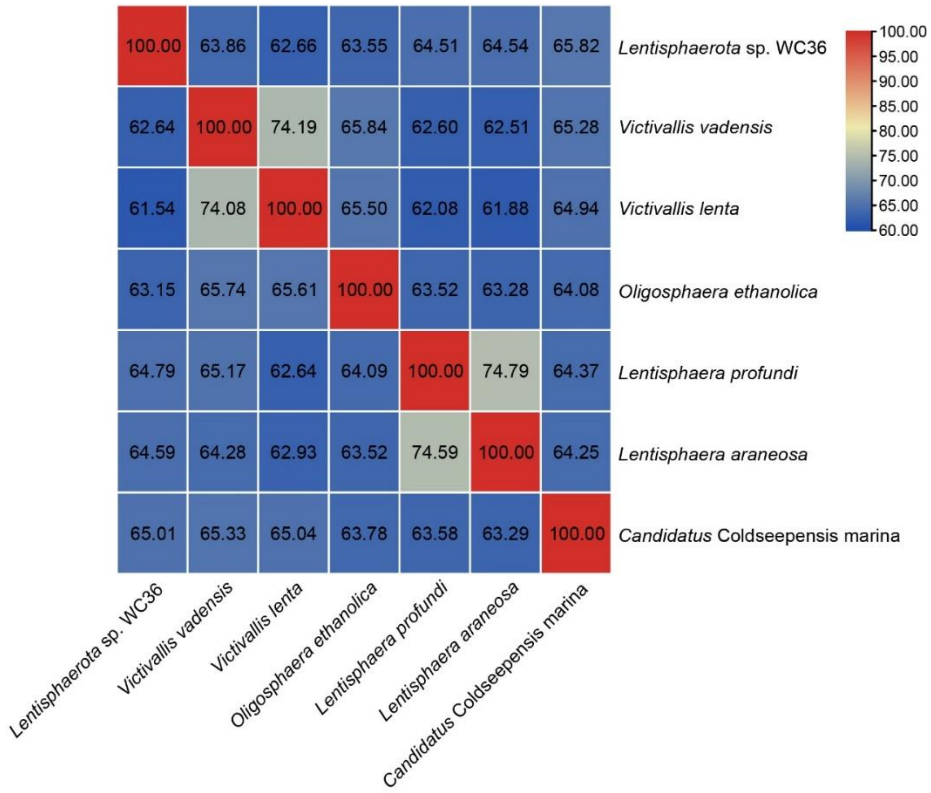
Supplementary Fig. 5 | Average nucleotide identity based on BLAST+ (ANIb) of *Poriferisphaera* sp. WC338 compared with those of other closely related species.



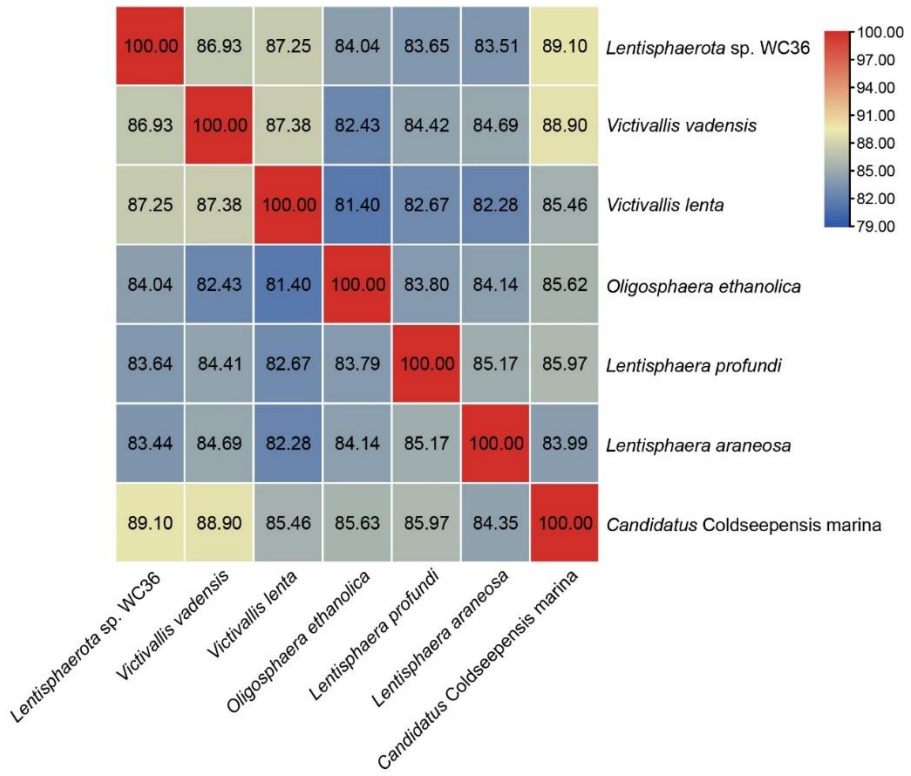
Supplementary Fig. 6 | Average nucleotide identity based on MUMmer (ANIm) of *Poriferisphaera* sp. WC338 compared with those of other closely related species.



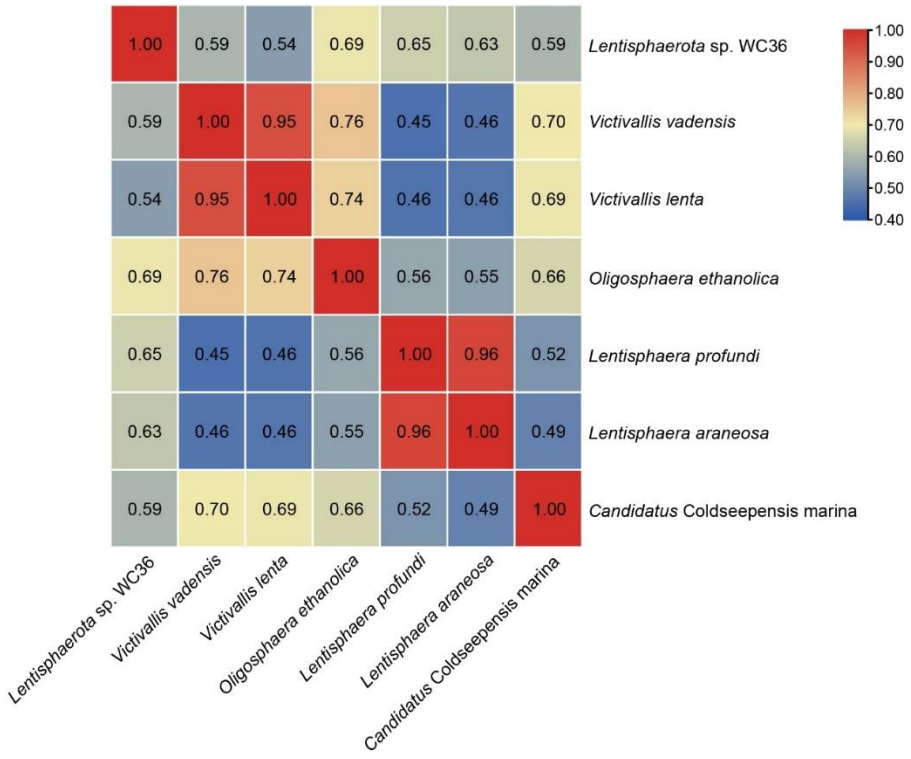
Supplementary Fig. 7 | Tetranucleotide signatures (Tetra) of *Poriferisphaera* sp. WC338 compared with those of other closely related species.



Supplementary Fig. 8 | Average nucleotide identity based on BLAST+ (ANIb) of *Lentisphaerota* sp. WC36 compared with those of other closely related species.



Supplementary Fig. 9 | Average nucleotide identity based on MUMmer (ANIm) of *Lentisphaerota* sp. WC36 compared with those of other closely related species.



Supplementary Fig. 10 | Tetranucleotide signatures (Tetra) of *Lentisphaerota* sp. WC36 compared with those of other closely related species.

Comment 9: L. 385: Here, authors argue that "this broad distribution of laminarin-degrading genes highlights the crucial role of the PVC superphylum bacteria in the marine carbon cycle and underscores their potential importance in the breakdown of algal biomass, a major component of oceanic organic matter". However, this conclusion is based on the distribution on laminarin degradation genes only in the PVC superphylum. If these genes are present in other phyla, the PVC role may not be so central. Authors should probably show that GH16 and GH2 are not significantly present in genomes from other phyla to support this claim.

Response: We thank you for this insightful comment. We agree that the original phrasing "crucial role" was not sufficiently supported by our data, given that laminarin-degrading genes (e.g., GH16 and GH2) are also wide present in other bacterial phyla (such as *Bacteroidota*) (Viborg et al, 2019). To address this concern, we have substantially revised the manuscript to temper our claims accordingly. According to your and Reviewer 1's suggestions, we have changed this description from "highlights the crucial role" to "highlights the previously underappreciated role" (Lines 364-365). We have modified this description as "This broad distribution of laminarin-degrading genes highlights the previously underappreciated role of PVC superphylum bacteria in the marine carbon cycle and underscores their potential importance in the breakdown of algal biomass, a major component of oceanic organic matter[6]. Moreover, laminarin degradation in deep-sea sediments is likely mediated by a diverse consortium, with PVC bacteria contributing alongside other specialized lineages such as *Bacteroidota*[48]. The relative contribution of PVC bacteria compared with these other lineages remains to be quantified." in the revised manuscript (Lines 363-370).

Moreover, we have changed "crucial drivers" to "important drivers" (Line 108); and "significantly upregulated" to "upregulated" (Line 331), along with other similar adjustments. We have also systematically reviewed the entire manuscript and revised similar overstatements where necessary, ensuring that all conclusions are

appropriately supported by the experimental results and that no claims are overstated. All changes are highlighted in the revised manuscript.

We thank you for helping us improve the accuracy and clarity of our claims.

Reference related to this response:

Viborg AH, Terrapon N, Lombard V, et al. A subfamily roadmap of the evolutionarily diverse glycoside hydrolase family 16 (GH16). *J Biol Chem*. 2019;294(44):15973-15986.

Point-by-point response to Reviewers

REVIEWER COMMENTS

Reviewer #2 (Remarks to the Author):

Comment: I once more congratulate the authors for carefully addressing reviwer's comments. After reading this last version, I believe this manuscript is ready for publication. I only found a small typo L170 where a sentence needs revision:“in MAGs from gas hydrate-bearing subseafloor and the Helgoland mud are”

Response: We thank you for the positive and constructive feedback. We have modified this description as “contrasting sharply with previous reports of widespread absence of such pathways in MAGs from gas hydrate-bearing subseafloor and the Helgoland mud[33,34].” in the revised manuscript (Line 170).