

Hadal trench sediments as key ecological reservoir for prokaryotic evolution

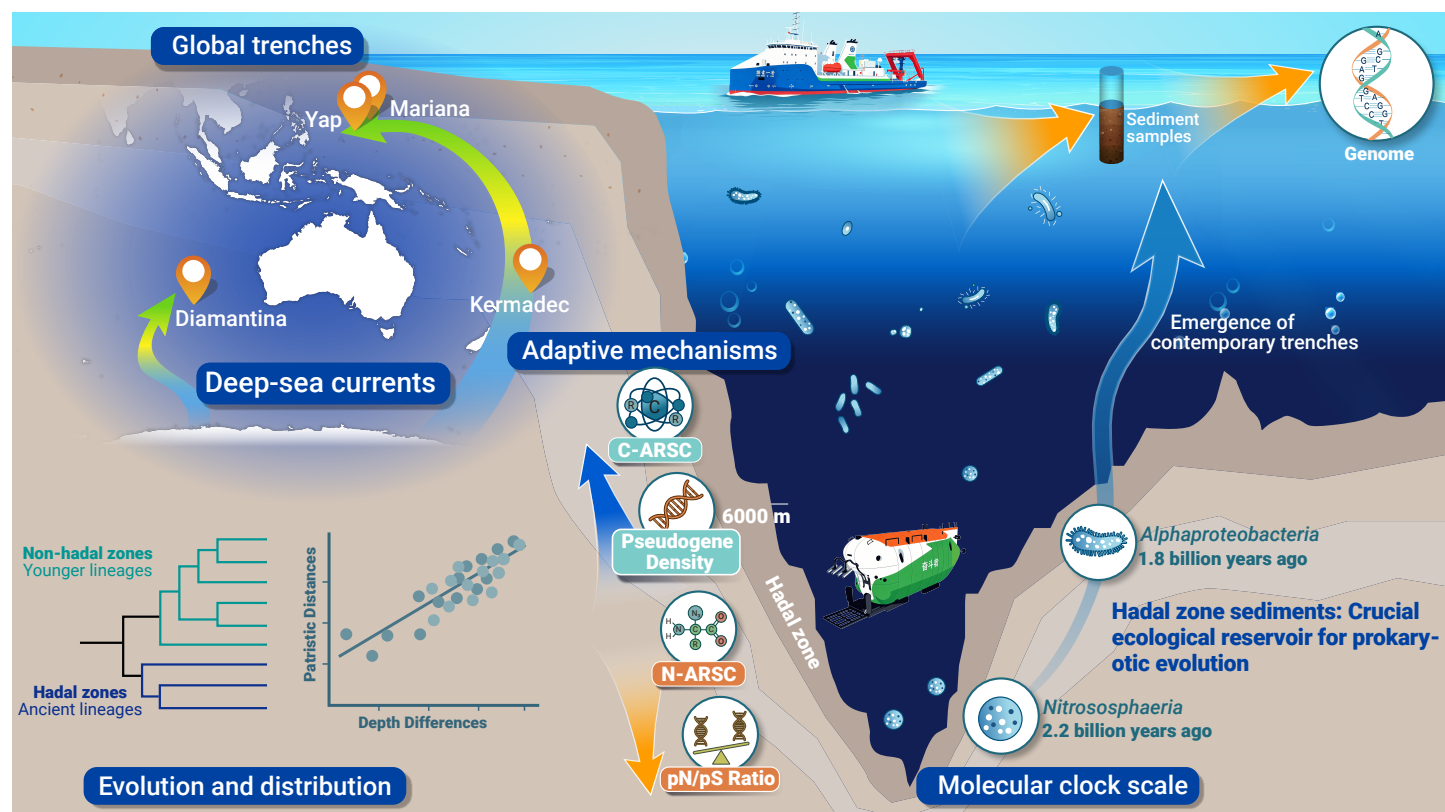
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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Hadal zones serve as an ancient reservoir, harboring more ancient microbial lineages than non-hadal zones.
- Evolutionary distances correlate with depth differences, evidenced by vertical shift in genomic features.
- Hadal microbes likely exchange across trenches via deep-sea currents to maintain genetic connectivity.
- Divergence of typical ancient lineages predates the formation of contemporary oceanic trenches.

Hadal trench sediments as key ecological reservoir for prokaryotic evolution

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The evolutionary ecology of prokaryotes within oceanic trenches remains largely unexplored. Here, we analyzed 19,194 medium-quality prokaryotic metagenomic assembled genomes (MAGs) from sediments of the Diamantina, Kermadec, Yap and Mariana trenches. Hadal zones hosted relatively ancient phylogenetic lineages across most prokaryotic phyla compared to non-hadal zones. A linear correlation between depth differences and the pairwise patristic distances was observed inter/intra trenches, indicating a depth-dependent evolutionary boundary. This pattern was further reflected by a clear vertical shift of genomic features, including carbon/nitrogen atoms per amino-acid-residue side chain (C/N-ARSC), pseudogene density and non-synonymous to synonymous mutation ratio (pN/pS). Close phylogenomic relationships for MAGs among different trenches suggested a rapidly and continuously exchange, which was very likely facilitated by deep-sea currents. Molecular dating of hadal *Nitrososphaeria* and *Alphaproteobacteria* revealed a divergence of respective 2.2 and 1.8 billion years ago, earlier than the emergence of contemporary trenches, emphasized the hadal zone sediments as a crucial ecological reservoir for prokaryotic evolution.

INTRODUCTION

Oceanic trenches are among the deepest and most extreme environments on Earth, characterized by extreme conditions such as immense hydrostatic pressure, low temperatures, and complete darkness.¹ Based on depth, the vertical profile of a trench can be divided into two main zones: the abyssal zone (up to 6,000 m), which features steep-sided slopes, and the hadal zone (beyond 6,000 m), accounting for the deepest 45% of the oceanic depth range.² Within these trench zones, various physicochemical features (such as salinity, temperature, pressure, and nutrient concentration) can vary significantly.³ Physicochemical characteristics can also differ greatly among different trenches due to influences from surrounding environments.⁴ Despite their physical isolation, trenches still host a remarkable diversity of microorganisms adapted to these extreme conditions.⁵ Therefore, understanding the evolutionary ecology of trench microbes is essential for elucidating their vertical and spatial evolutionary strategies, as well as the evolutionary differences of microorganisms between trench and other ecosystems.

For most of Earth's history, life was predominantly microbial, with archaeal and bacterial cells playing crucial roles in mediating biogeochemical cycles through their unique metabolisms and ecologies.^{6,7} The genomes of contemporary microorganisms reflect over 3 billion years of evolution, shaped by accumulated adaptations to planetary changes. Consequently, these genomes preserve vital information about Earth's history, referred to the genomic record.⁸ As one of the most unique ecosystems, oceanic trenches are relatively isolated from various geological processes and climatic fluctuations on the Earth's surface.⁹ Recent studies have revealed high taxonomic novelty,¹⁰ as well as significant differences in microbial community structure and metabolic functions in oceanic trenches, highlighting dramatic variations across different depths and between distinct trench systems.^{11,12} Although remarkably high prokaryotic novelty potentially driven by homogeneous selection and dispersal limitation has been revealed from the Mariana Trench,¹⁰ it remains unclear whether those novel taxa represented ancestral lineages preserved consistently in this extreme environment, or emerged as entirely novel evolutionary clades triggered in the adaptive process. Further-

more, the evolutionary ecology of microbes within and between trenches remains largely unexplored. Considering that hadal trenches were geographically isolated and extreme biosphere, we therefore hypothesized that the hadal trench sediments harbor large amount of ancient lineages, which might be ancestor of the extant taxa.

To investigate microbial evolution in oceanic trenches, we collected 262 sediment samples for metagenomic sequencing from the Diamantina, Kermadec, Yap and Mariana trenches, spanning a depth range of 2,000 m to 11,000 m. Multiple trenches were selected to provide broad geographic representation across both hemispheres and to capture key environmental gradients within the hadal zone (e.g., depth extremes from ~8,000 m to ~11,000 m).^{9,13,14} They differ in regional surface productivity, sediment input, and geological location, allowing us to distinguish between evolutionarily conserved adaptations to high pressure and those shaped by local trench-specific conditions.^{5,15} Based on metagenomic datasets derived from sediments of those trenches, we reconstructed 19,194 medium-quality prokaryotic metagenomic assembled genomes (MAGs) to elucidate the phylogenomic relationships, genomic features, divergence times, and evolutionary strategies of trench adapted prokaryotic genomes.

MATERIALS AND METHODS

Sample collection

Sediment samples used in this study were collected using a push core from the Diamantina (Max depth of 8,047 m) and Kermadec (Max depth of 10,047 m) trenches during cruise TS29 from Nov. 2022 to Mar. 2023 on R/V "Tan Suo Yi Hao" (Figure 1). Push cores with a maximum sediment depth of 40 cm below the seafloor (cmbsf) were retrieved using the manned submersible the HOV "Fen Dou Zhe". All collected sediments were sliced into layers with each of 2 ~ 3 cm, and were immediately frozen at -80°C on board and then transferred to the laboratory in dry ice for subsequent DNA extraction. The *in situ* hydrographical parameters (i.e., depth and location) were recorded by HOV during sampling. The metagenomic datasets from this study were compiled with surface sediment samples collected from the Yap (Max depth of 7,600 m) and Mariana trenches (Max depth of 10,946 m) (Table S1).

Metagenomic sequencing, assembly and binning

A total of 262 genomic DNAs were extracted from sediment samples using the DNeasy PowerSoil Pro kit (QIAGEN, USA) according to the manufacturer's instructions. DNA quality was measured using the Qubit dsDNA Assay kit in combination with a Qubit 2.0 Fluorometer (Life Technologies, USA) and verified by 1% agarose gel electrophoresis. Sequencing libraries were generated using the NEBNext Ultra DNA Library Prep kit for Illumina (NEB, USA) and sequenced using a NovaSeq 6000 platform (Illumina, USA). Clean data (150-bp paired-end reads) were obtained by removing the adapters and barcodes, as well as reads containing poly-N or that were of low-quality from the raw data.

The clean reads of each sample were assembled using MEGAHIT v1.2.9 with parameters '--k-min 21 --k-max 141 --k-step 10'.¹⁶ Genomic binning was implemented using MetaWRAP v1.3 binning module based on maxbin2, metatb2, and concoct methods, with 1.5 kb as the contig length cut-offs.¹⁷ The metagenome-assembled genomes (MAGs) were refined using the 'bin_refinement' module of MetaWRAP v1.3,¹⁷ and their quality and taxo-

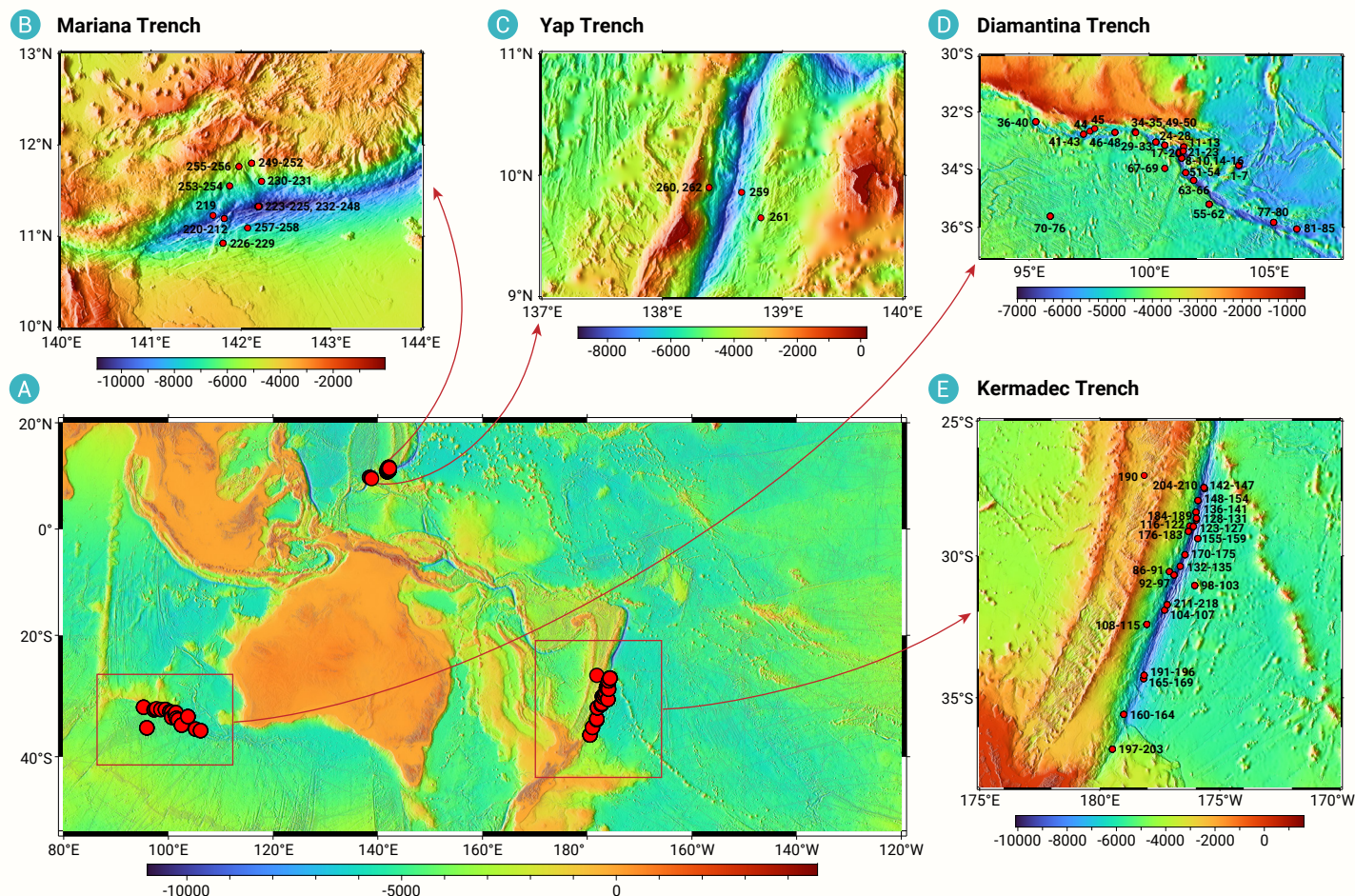


Figure 1. Geographical distribution of the sampling sites (A) Maps illustrating the locations where surface sediment samples were collected and analyzed in this study (B: Mariana trench; C: Yap trench; D: Diamantina trench; E: Kermadec trench). Each sample was annotated with a number for clarity, and the corresponding sample names and information were summarized in Table S1.

nomic information were obtained using CheckM v1.1.2 and Genome Taxonomy Database Toolkit (GTDB-TK) v1.6.0, respectively.^{18,19} The MAGs with completeness $\geq 50\%$ and contamination $\leq 10\%$ were used for further analysis.

Phylogenomic tree construction

The maximum-likelihood (ML) phylogenomic tree, based on the multiple sequence alignment of 120 bacterial and 122 archaeal marker genes were constructed using the GTDB-Tk v1.6.0.¹⁹ To further verify the topology among MAGs, the phylogenomic trees of MAGs were also constructed using 59 universal single-copy markers (Table S2).²⁰ Open reading frames (ORFs) for each MAG were predicted using Prodigal v2.6.3 with the '-p meta' parameter.²¹ These ORFs were subsequently aligned with the references of 59 markers using PSI-BLAST, applying threshold criteria of query coverage greater than 80% and percentage identity exceeding 60%.²² The identified sequences were aligned and trimmed using MAFFT v7.222 and trimAl v1.4.rev15 with the auto options, respectively.^{23,24} A ML phylogenomic tree was constructed using the IQ-TREE v2.1.2²⁵ under MFP+MERGE model, and a total of 1,000 ultrafast bootstrap replicates were sampled to evaluate the robustness of the phylogeny.

Genomic content and features analysis

The assembled genome size, gene number, coding density, and GC content were calculated using CheckM v1.1.2.¹⁸ Pseudogenes were predicted from the genomes using the Pseudofinder v1.1.0.²⁶ Nonsynonymous to synonymous mutation ratio (pN/pS) was calculated using the profile module of the inStrain program v1.9.0 based on all predicted genes.²⁷ The number of carbon atoms per amino-acid-residue side chain (C-ARSC) and the number of nitrogen atoms per amino-acid-residue side chain (N-ARSC) were retrieved using custom scripts.

Construction of phylogenomic tree for *Nitrososphaeria* and *Alphaproteobacteria*

For phylogenomic construction of *Nitrososphaeria*, a total of 111 representative genomes, including 16 *Crenarchaeota*, 6 *Aigarchaeota*, 25 *Euryarchaeota*, and 64 *Thaumarchaeota* genomes (Table S3), were downloaded from the NCBI genome, IMG/M and PATRIC databases. These, along with the 18 *Nitrososphaeria* genomes derived from the cold-seep sediments, were used to construct the phylogenetic tree based on 62 markers selected (Table S4) present in at least 100 of the 129 genomes used in this study.²⁸ On the other hand, a total of 80 genomes (Table S5) were selected for phylogenomic reconstruction of *Alphaproteobacteria* according to prior studies.^{29,30} About 22 conserved genes (Table S6) encoded by both the mitochondrial and alphaproteobacterial genomes (nuclear-encoded) were used to determine their phylogenetic relationship. The ORFs of genomes were predicted using Prodigal v2.6.3 and against the conserved marker genes using method mentioned above.²¹ The processed markers for respective *Nitrososphaeria* and *Alphaproteobacteria* were aligned using MAFFT v7.222²³ and trimmed using trimAl v1.4.rev15²⁴ with the auto option, and then concatenated as input sequences for constructing a ML phylogenetic tree using IQ-TREE v2.1.2²⁵ with 1,000 ultrafast bootstrap replicates.

Molecular dating of *Alphaproteobacteria* and *Nitrososphaeria*

A Bayesian estimate of the *Alphaproteobacteria* and *Nitrososphaeria* (also referred as *Thaumarchaeota*) divergence time on the constructed ML concatenated tree was conducted using MCMCTree v4.10.7.³¹ Six calibration fossil records (Table S7) were used to calibrate the divergence time of *Nitrososphaeria* in the tree as described previously.^{28,32,33} Molecular dating of *Alphaproteobacteria* was conducted following previous procedures and protocols.^{29,34} Further details were provided in the supplementary methods.

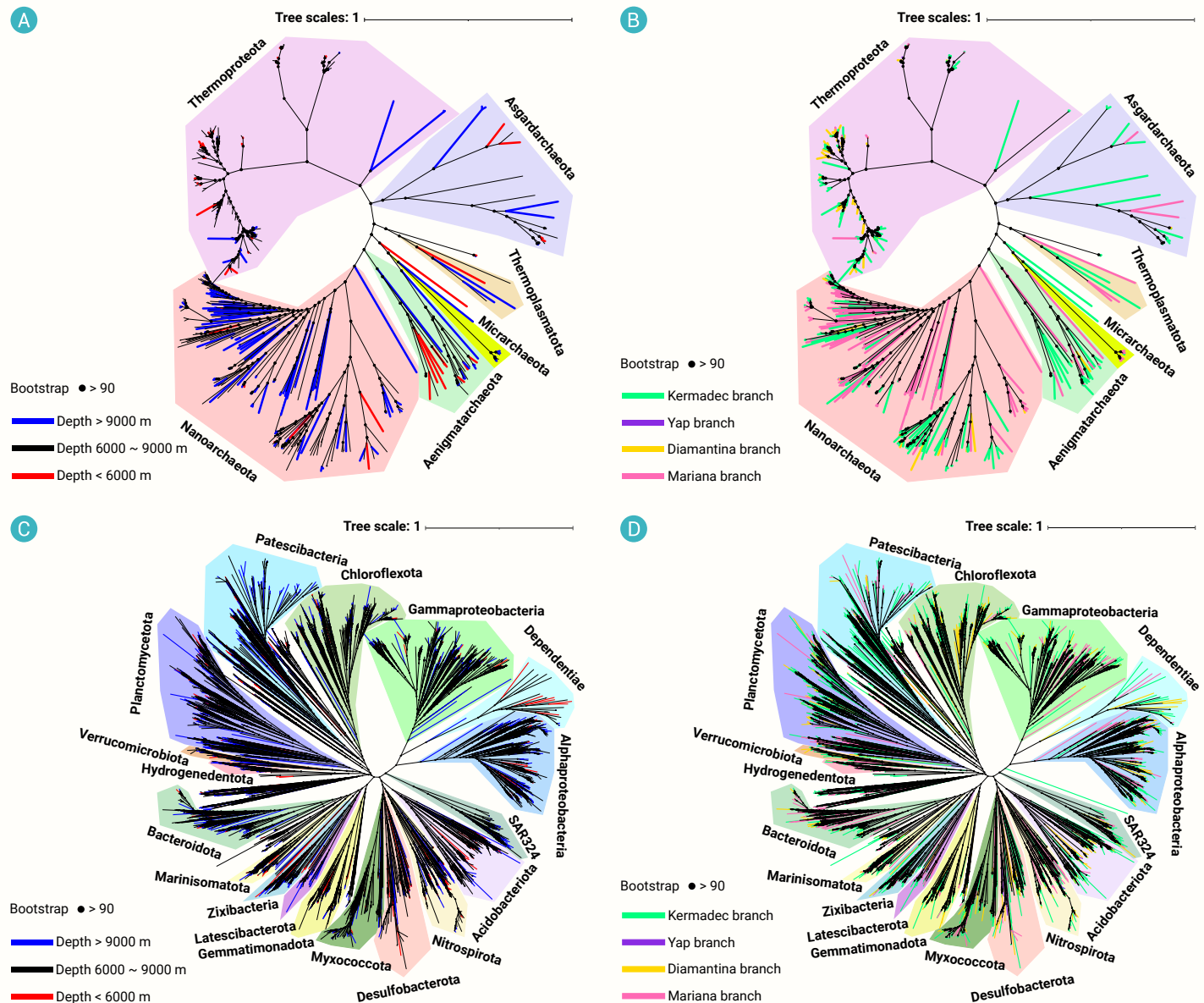


Figure 2. Ancestral branches of archaeal and bacterial phyla predominantly associated with the hadal zone The phylogenomic trees were constructed using 120 and 122 widely accepted conservative genes for archaeal (A and B) and bacterial MAGs (C and D). The branches were color-coded based on the depth ranges from which the MAGs were isolated: Blue for depths greater than 9,000 m, black for depths between 6,000 and 9,000 m, and red for depths less than 6,000 m (A and C). Additionally, branches were annotated with the specific trenches of isolation: Green from the Kermadec Trench, purple from the Yap Trench, yellow from the Diamantina Trench, and pink from the Mariana Trench. Within each phylogenomic tree, clusters affiliated with the same phylum were highlighted in distinct colors.

16S rRNA gene amplification and analysis

The microbial DNA from another batch of trench sediments (from the same cruise; Table S8) were used for 16S rRNA gene amplification with a primer pair set of Uni341F (5'-CCTACGGGGRGCGAG-3') and Uni805R (5'-GACTACNVGGGTATCTAATCC-3'), and sequenced on the HiSeq 2500 System (Illumina, San Diego, CA, USA).³⁵ After prefiltration, the taxonomy of remaining medium-quality sequences was assigned with the Silva database v123 using the QIIME2 (2023.5) affiliated feature-classifier command.³⁶ Amplicon Sequence Variants (ASVs) with a 99% sequence similarity cutoff was used for phylogenetic analysis with QIIME2 (2023.5). The obtained microbial composition was input for machine learning analysis to reveal the enrichment of microbial species in non-hadal and hadal zones (see supplementary file for details).

Statistical analysis and deep-circulation calculation

The phylogenetic and phylogenomic trees were submitted to iTOL for further modification. Pairwise genomes with an average nucleotide identity (ANI) greater than 90% were identified using fastANI v1.34 and Mash v2.3.^{37,38}

Patristic distances for these genomes were extracted from the phylogenomic trees using TreeSwift v1.1.44 and visualized using ggplot2 with linear fitting performed using the vegan package in R.³⁹ The vertically averaged horizontal velocity of the deep circulation below 3,500 m was calculated, and the time required was further estimated based on the velocity together with the distance between locations.⁴⁰

RESULTS AND DISCUSSION

Phylogenomic relationship of all MAGs

Through the bioinformatic workflow (Figure S1), the MAGs reconstructed from all trench sediments were used to construct phylogenomic trees based on 120 and 122 widely accepted conservative genes for archaeal and bacterial MAGs, respectively (Figure 2). The archaeal MAGs from hadal sediments primarily represent ancestral branches of archaeal MAGs from non-hadal sediments across several phyla, including *Thermoproteota*, *Asgardarchaeota*, *Thermoplasmata*, *Micrarchaeota*, *Aenigmataarchaeota* and *Nanoarchaeota* (Figure 2A). These ancestral branches of archaeal phyla were predominantly associated with the hadal zone of Kermadec and Mariana trenches

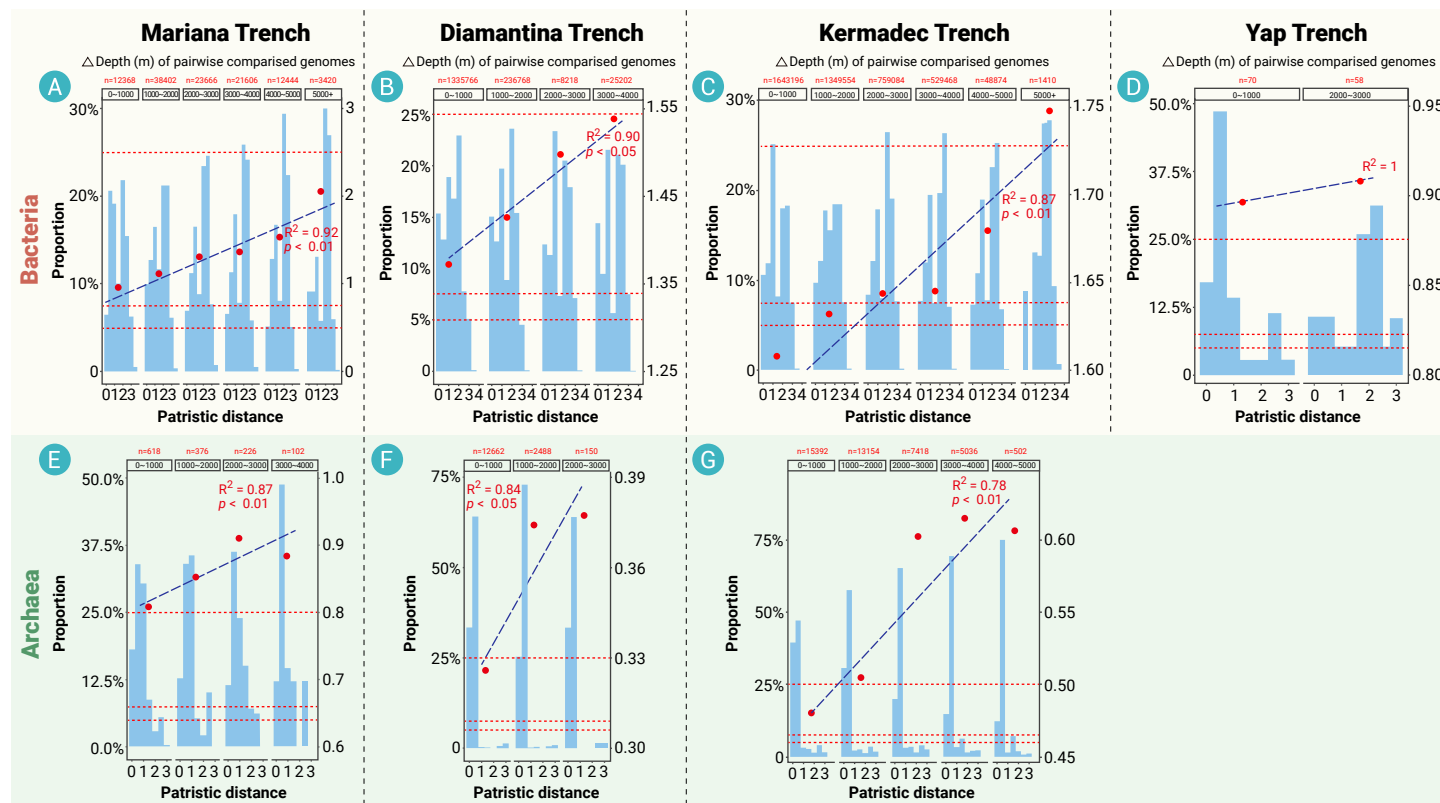


Figure 3. Depth difference exhibited linear relationships with patristic distance within each trench for bacteria (upper panel) and archaea (lower panel). The proportion of patristic distances between pairwise MAGs with ANI > 90% was displayed as grey bars, categorized into different ranges (0–1, 1–2, 2–3, 3–4) based on specified depth differences between the compared MAGs: 0–1,000 m, 1,000–2,000 m, 2,000–3,000 m, 3,000–4,000 m, and > 5,000 m ($\Delta\text{depth} = |\text{depth of MAG1} - \text{depth of MAG2}|$). Proportion values were represented on the left y-axis. Red dots indicated the average patristic distance between pairwise MAGs within each depth difference range, with these values corresponding to the right y-axis. Note: the red dots and the dotted line represent the average patristic distance and its linear fit, respectively.

(Figure 2B). Similarly, the phylogenomic trees of bacterial MAGs revealed a comparable pattern, with hadal MAGs forming ancestral branches in many phyla, including *Planctomycetota*, *Patescibacteria*, *Chloroflexota*, *Gammaproteobacteria*, *Dependentiae*, *Alphaproteobacteria*, *SAR324*, *Acidobacteriota*, *Nitrospirota*, *Desulfobacterota*, *Myxococcota*, *Gemmatimonadota*, *Latescibacterota*, *Zixibacteria*, *Marinisomatota*, *Bacteroidota*, *Hydrogenedentota*, and *Verrucomicrobiota* (Figure 2C). These ancestral branches of bacterial phyla were also predominantly associated with the hadal zone of Kermadec and Mariana trenches (Figure 2D). Consistent phylogenetic patterns were demonstrated from the phylogenomic trees of all MAGs based on 59 single-copy marker genes (Figure S2). These findings seem to support the deep to shallow emergence hypothesis for the current distribution of marine microbes. Hadal taxa evolved *in situ* with representatives subsequently colonizing shallower zones, similar to that of ammonia-oxidizing archaea.²⁸ Depth represents one of the most biologically consequential dimensions in marine ecosystems, where a vertical succession of microbial communities existed at the abyssal-hadal boundary (ca. 6,000 m),⁴¹ and hadal zone harbored distinct microbial assemblages and unique diversity.⁴² Notably, the phylogenomic relationship of MAGs from different trench sediments could be markedly close, possibly resulted from a long-time adaptation to the relatively stable and isolated hydrographic conditions of hadal zone. These microbes in hadal trenches were very likely originated from a common ancient ancestor and have remained in an evolutionary stasis within the isolated hadal environment. Thus, hadal zone was suggested to be acted as a reservoir and/or refuge of ancient microbial lineages, protecting them from the dynamic evolutionary pressures experienced in shallower oceans, potentially providing insights into the origin and early evolution of life on Earth.³ This was in agreement with the recent findings that homogeneous selection was a dominant ecological driver to favor specific adapted taxa across geographically isolated trenches.¹⁰ Moreover, it should be admitted that MAG-based approaches may introduce artifacts such as strain mixtures or mis-binning,^{18,43} therefore future validation through cultivation and single-cell genomics is essential to confirm the adaptive evolution of hadal lineages.

Evolutionary matrix of the MAGs within and between trenches

Based on the phylogenomic trees of MAGs (using 120 archaeal and 122 bacterial conservative genes), the relationship between evolutionary distance and depth differences for pairwise MAGs with ANI > 90% was analyzed within each trench (Figure 3). The results showed that, within each trench, there was a linear relationship between the average evolutionary distance and depth differences for pairwise MAGs with ANI > 90%, with a linear fit (R^2) greater than 0.8 for both bacteria and archaea. This highlighted that depth difference was critical yet neglected evolutionary force, but this pattern might be constrained to deep sea, considering the highly varied hydrographical conditions in the upper ocean, especially the euphotic zone. However, for comparison between trenches, bacterial MAGs with ANI > 90% showed a linear relationship between evolutionary distance and depth differences only in the comparison groups between the Kermadec and Diamantina trenches, as well as the Yap and Mariana trenches. In contrast, pairwise archaeal MAGs with ANI > 90% exhibited linear relationships across all comparison groups (Figure 4). The relatively homogeneous distribution of bacteria might be attributed to the horizontal gene transfer, which occurred more frequently in bacteria than archaea, thereby leading to higher chances of genetic recombination and rapid adaptation.⁴⁴

Genomic characteristics of MAGs

To further investigate the genomic characteristics of MAGs from different trenches, several genomic features, including C-ARSC, N-ARSC, pseudogene density, pN/pS ratio, as well as GC content were analyzed (Figure 5). In many bacterial phyla (*SAR324*, *Acidobacteriota*, *Nitrospirota*, *Myxococcota*, *Zixibacteria*, *Marinisomatota*, and *Bacteroidota*) and archaeal phyla (*Nanoarchaeota*, *Thermoproteota*, and *Asgardarchaeota*), pseudogene density increased with depth in the Kermadec and Mariana trenches, whereas N-ARSC showed a decreasing trend. The high pseudogene density suggested that these microbes had undergone genome streamlining, eliminating non-essential genes to conserve energy and resources for trench adaptation.⁴⁵ Pseudogenization primarily targets costly or redundant systems, such as signal trans-

Bacteria

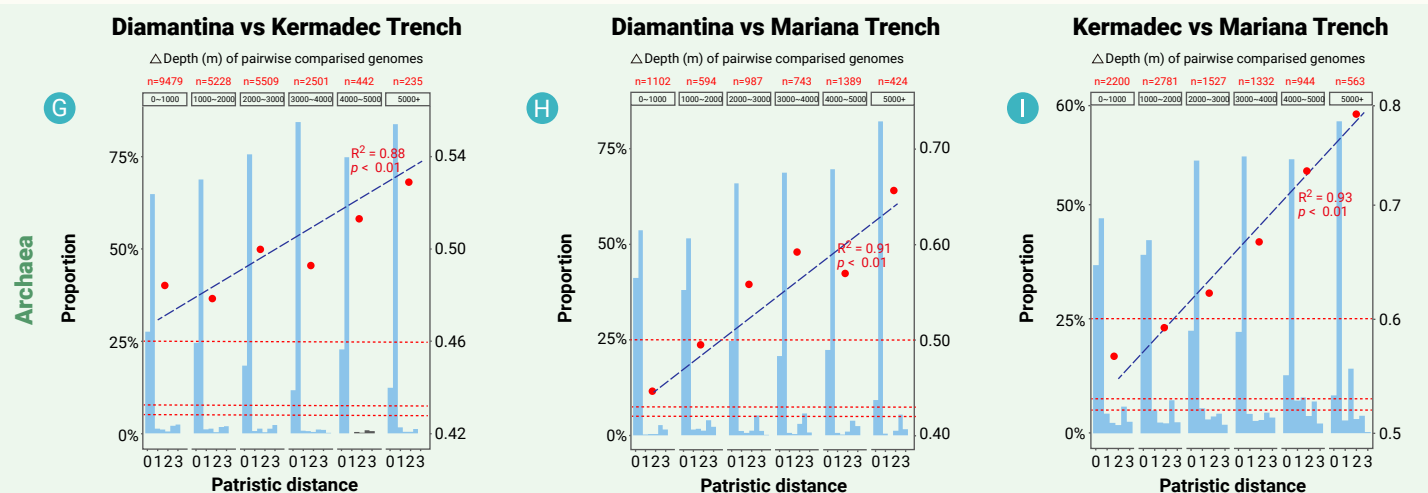
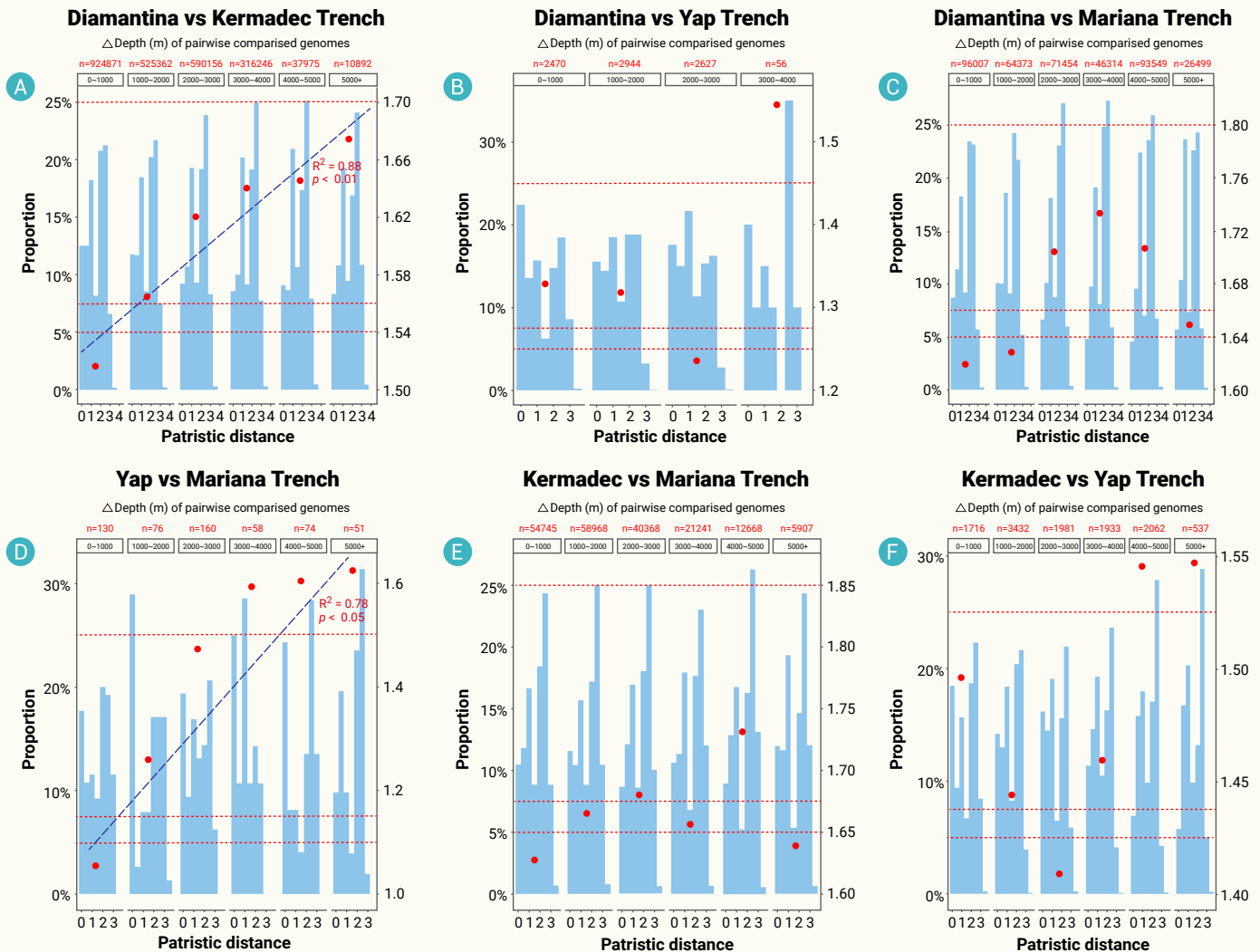


Figure 4. Depth difference exhibited linear relationships with patristic distance between trenches for bacteria (upper panel) and archaea (lower panel) The proportion of patristic distances between pairwise MAGs with ANI > 90% was displayed as grey bars. Proportion values were represented on the left y-axis. Red dots indicated the average patristic distance between pairwise MAGs within each depth difference range, with these values corresponding to the right y-axis. Note: the red dots and the dotted line represent the average patristic distance and its linear fit, respectively.

duction and flagellar motility, underscoring a shift from generalist to specialist lifestyle in the hadal environment.⁴⁶ The decrease of N-ARSC in the hadal zone suggested a low nitrogen availability was critical to the genome evolution, because preference for low G+C codon usage in amino acid biosynthesis pathways was an adaptive strategy to reduce the nitrogen cost of protein synthesis, which was particularly advantageous in nitrogen-limited environ-

ments.⁴⁷ The dramatic variation of C-ARSC of MAGs between bacterial and archaeal MAGs and across different trenches reflected a greater degree of genomic plasticity and adaptation to specific conditions. In contrast, the average pN/pS ratio decreased with increasing depth in some archaeal phyla and nearly all bacterial phyla, as well as for archaeal *amoA* and bacterial *nrxA* genes (Figure S3), possibly resulted from the fact that an adaptive optimum

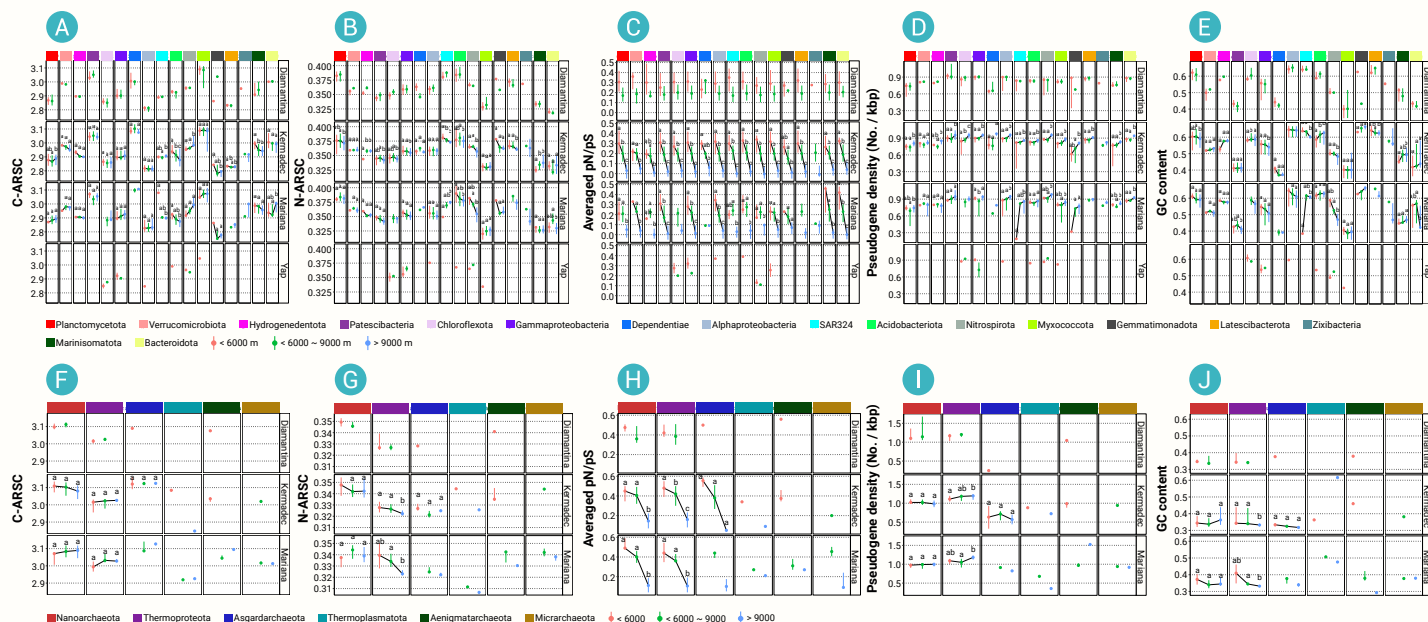


Figure 5. Genomic characteristics of MAGs showing adaptive evolution to depth/trench-associated nutrient availability and physicochemical variations The number of carbon atoms per amino-acid-residue side chain (C-ARSC) (A), number of nitrogen atoms per amino-acid-residue side chain (N-ARSC) (B), average pN/pS ratio (C), pseudogene density (D), and GC content (E) of bacterial MAGs and of archaeal MAGs (F-J) were categorized by depth range, phyla, and trenches. Wilcoxon test, a and b represent significant differences ($p < 0.05$).

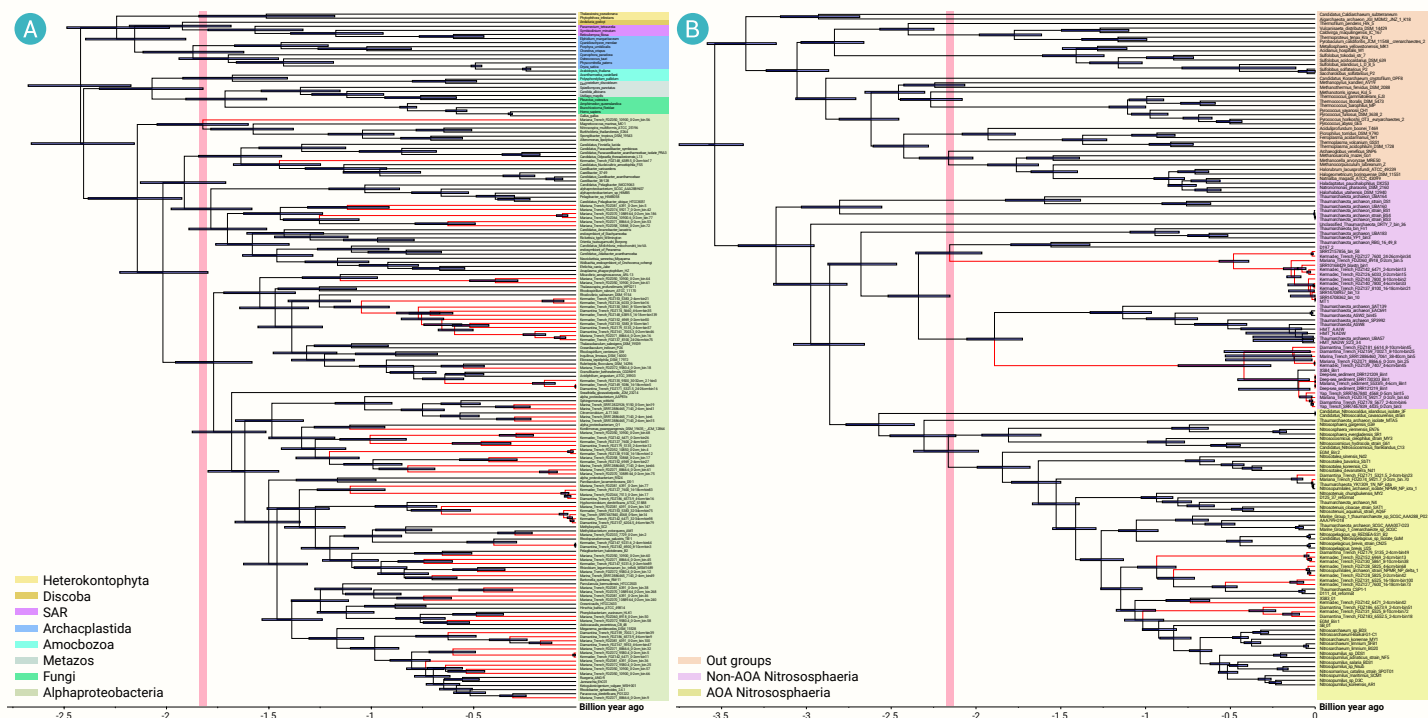


Figure 6. Divergence time of two representative supergroups of bacterial *Alphaproteobacteria* (A) and archaeal *Nitrososphaeria* (B) through adaptive evolution based on molecular dating The branches of MAGs from this study were highlighted in red.

was reached in the hadal zone, which acted as stronger purifying selection pressure for purging non-synonymous mutations and to maintain the protein structure and functional stability for microbial survival.^{48,49} These genomic features indicated that microbes in trench sediments had employed various adaptive traits and strategies to cope with depth/trench-associated nutrient availability and physicochemical variations.⁵⁰

Molecular dating of the representative bacterial and archaeal lineages

Based on previously reported methods, MAGs affiliated with bacterial representative lineages of *Alphaproteobacteria* and archaeal representative

lineages of *Thaumarchaeota*, were used to calculate their divergence times, along with other reference genomes, since these two predominant lineages have well-established temporal calibration points.^{29,33}

Notably, the phylogenomic relationships of MAGs belonging to *Alphaproteobacteria* and *Thaumarchaeota* from different trenches could be dramatically close. The phylogenomic trees constructed for *Alphaproteobacteria* and *Thaumarchaeota* showed that the MAGs from the hadal zone were ancestral branches of the MAGs from non-hadal zones. The divergence time of the hadal zone-adapted strains of *Alphaproteobacteria* was approximately 1.8 billion years ago, comparable to the evolution dating with eukaryotic fossils,²⁹

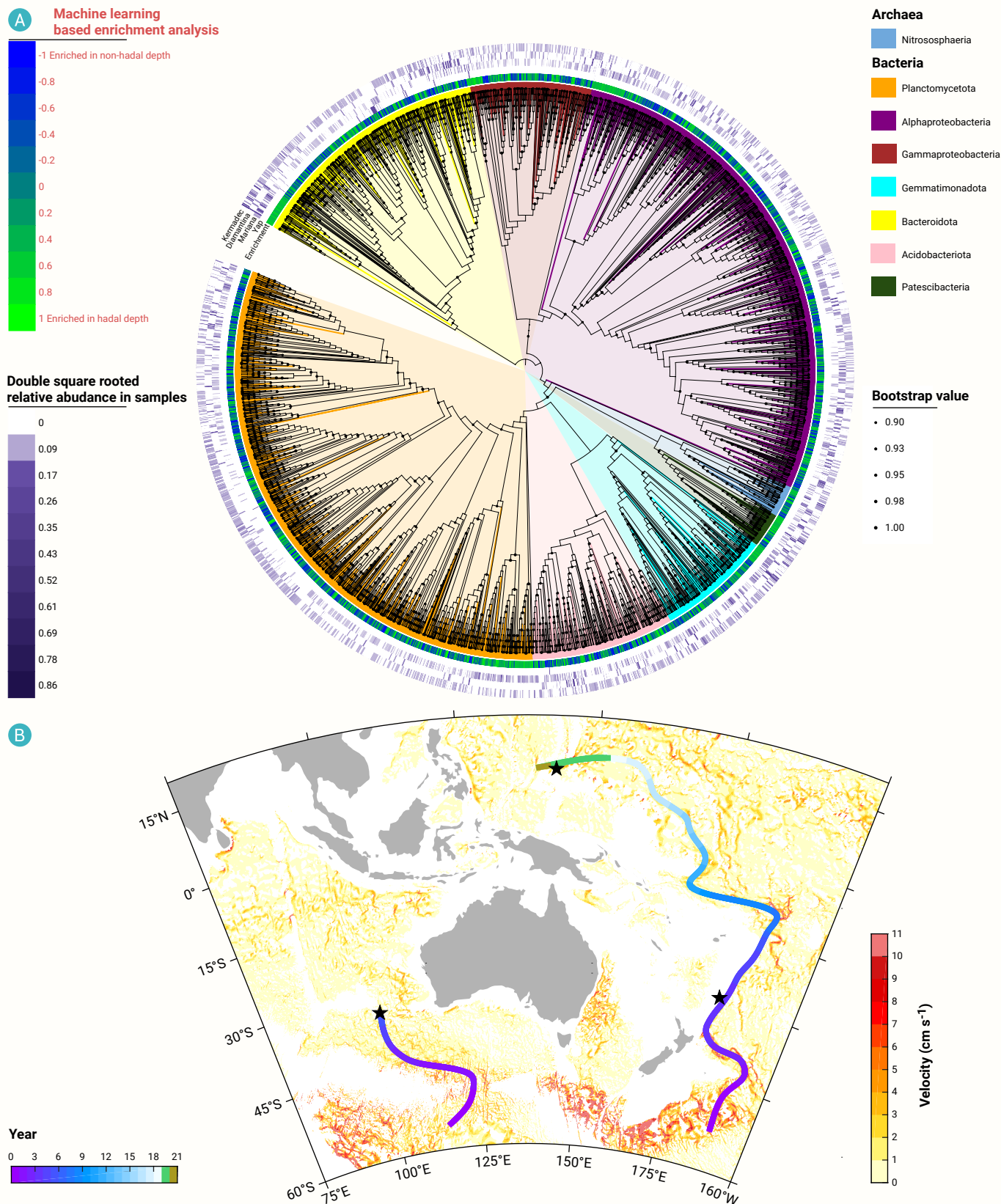


Figure 7. Oceanic currents as key driving force for the long-distance transportation of ancient microbial lineages among different hadal zones (A) Phylogenetic tree of 16S rRNA amplicon sequence variants (ASVs) across different depth ranges and trenches. The transition from blue to green reflected the machine learning-based enrichment levels of each 16S rRNA ASVs, where green indicated enrichment in hadal depths (> 6,000 m) and blue signified enrichment in non-hadal depths. The transition from white to purple illustrated the variation in relative abundance of 16S rRNA ASVs across each trench. The branches of the phylogenetic tree were color-coded to represent different phyla. (B) Direction and speed of currents below 3,500 m linking four individual trenches. The model simulation was based on satellite monitoring data, with dark purple indicating the starting points of major currents.

while that of *Thaumarchaeota* was around 2.2 billion years ago (Figure 6). Both microbial groups were divergent predated the geological timescale of the trenches, similarly for snailfish in the Mariana Trench.⁵¹ Moreover, the calculations were repeated multiple times, and the divergence times of the branches in both *Alphaproteobacteria* and *Thaumarchaeota* showed significant overlap, indicating that the molecular dating results for both lineages were accurate and repeatable (Figure S4). It was very likely that a common ancestor from trench sediments gave rise to unique genetic lineages through a complex evolutionary and adaptive history. However, the underlying evolutionary processes still need further investigation.

Potential linkages among trenches

To further validate the phylogenomic results, we applied 16S rRNA genes with machine learning approach to validate the evolutionary patterns of bacterial and archaeal MAGs. The results showed that the ancestral branches of *Nitrososphaeria*, *Planctomycetota*, *Alphaproteobacteria*, *Gammaproteobacteria*, *Gemmatimonadota*, *Bacteroidota*, *Acidobacteriota* and *Patescibacteria* were predominantly enriched in the hadal depth sediments of the Kermadec and Mariana trenches (Figure 7A). This shared enrichment pattern of evolutionarily deep-branching taxa across distant trenches provides strong, independent biological evidence supporting the occurrence of dispersal and subsequent colonization.

Oceanic current has been recognized as a key driving force for the long-distance transportation of viruses, microeukaryotes and planktons,^{52,53,54} likely promoting genetic connectivity among different deep-sea ecosystems. To explore possible mechanisms underlying the close phylogenomic relationships between MAGs from different trench sediments, satellite monitoring data were used to predict the oceanic currents below 3,500 m. Based on the prediction model, we further predicted the direction and speed of these currents to estimate the time required for transporting particles from one trench to another (Figure 7B). Deep layer currents originating from the Kermadec Trench would reach the Mariana Trench approximately 20 years later. Additionally, the strong linkage between the Kermadec and Diamantina trenches was likely via the Antarctic Circumpolar Current, which explained the strong linkage observed among bacteria within trenches of the same hemisphere as well.⁵⁵ These deep-layer flows likely facilitate genetic and ecological connectivity among geographically isolated hadal ecosystems. The concordance between observed cross-trench distribution of ancestral lineages (Figure 7A) and predicted deep-sea current circulation (Figure 7B) offers a coherent and comprehensive explanation for the close phylogenomic relationships across trenches, emphasizing the role of ocean circulation in shaping microbial evolution in the hadal zone.

CONCLUSION

The analysis of 19,194 MAGs from multiple trenches revealed that hadal sediments harbor relatively ancient phylogenomic lineages of prokaryotes. The genomic features and phylogenomic distances of prokaryotic MAGs were strongly associated with depth, suggesting depth-dependent evolutionary strategies. The divergence times of two representative supergroups of bacteria and archaea predate the emergence of contemporary oceanic trenches, indicating a long evolutionary history of prokaryotes in hadal environments. The close phylogenomic relationships between hadal MAGs from different trenches could be attributed to the continuous and rapid material exchanges between trenches facilitated by deep-sea currents. These findings supported that hadal trench sediments as key ecological reservoir for prokaryotic evolution, and offer valuable insights into the evolutionary and adaptation of deep-sea microbes to the hadal biosphere.

WEB RESOURCES

The number of carbon atoms per amino-acid-residue side chain (C-ARSC) and the number of nitrogen atoms per amino-acid-residue side chain (N-ARSC) were retrieved using custom scripts (<https://github.com/yilic/aoa-in-methane-seep-sediment>).

For phylogenomic construction of *Nitrososphaeria*, a total of 111 representative genomes, including 16 *Crenarchaeota*, 6 *Aigarchaeota*, 25 *Euryarchaeota*, and 64 *Thaumarchaeota* genomes (Table S3), were downloaded from the NCBI genome (<https://www.ncbi.nlm.nih.gov/genome/>),

IMG/M (<https://img.jgi.doe.gov/cgi-bin/m/main.cgi>) and PATRIC (<https://www.patricbrc.org/>) databases.

Both microbial groups were divergent predated the geological timescale of the trenches (<https://www.britannica.com/science/deep-sea-trench>).

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AUTHOR CONTRIBUTIONS

Y.L. and H.J. designed the research. Y.L., Y.X., H.L., P.W., and W.Z. performed data analyses. X.P. and X.X. provided data. Y.L. wrote the first draft with input from all the other authors. All authors contributed to the manuscript and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

ETHICAL STATEMENT AND PATIENT CONSENT

Not applicable.

DATA AND CODE AVAILABILITY

The raw metagenomic sequencing reads have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1111327, PRJNA1187693 and PRJNA1111327. For samples from Mariana Trench, metagenomic data sets were downloaded from NODE (<https://www.biosino.org/node/project/detail/OEP004067>). MAGs can be accessed from the GitHub website for repeating the analysis (linkage: <https://github.com/ylicf/microbial-Genomes-and-metagenomic-assembly-from-Kermadec-and-Diamantina-trench-sediments/tree/main/bins>).

SUPPLEMENTAL INFORMATION

It can be found online at <https://doi.org/10.59717/j.xinn-life.2026.100209>

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