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Anthropogenic structuring of microbial communities in deep coastal sediment

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by

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ABSTRACT

Anthropogenic structuring of microbial communities in deep coastal sediment

by

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Marine sediment microbial communities are significantly influenced by anthropogenic activities, which alter their composition and function. They also play an invaluable role in determining the fate of contaminants in the sediment environment. The San Pedro (SP) Basin in the Southern California Bight is a prime case study to explore this phenomenon, having been subjected to extensive contamination from sewage input to the bordering Palos Verdes (PV) Shelf and offshore dumping. Historical records have confirmed the occurrence of offshore dumping of industrial wastes, and we observe some of these waste products, such as petroleum (tar) and dichlorodiphenyltrichloroethane and its derivatives (DDX), in high abundance in the SP Basin.

This study analyzes sediment cores from the SP Basin to assess the structuring of the microbial community and its relationship to contaminants. Using 16S rRNA gene sequencing, this study reveals variations in microbial community composition corresponding to a range of environmental gradients such as sediment depth, water depth, sedimentation rates, and inputs from the PV shelf, with emphasis on variations that correspond to anthropogenic impacts. To my

knowledge, this work is the first to characterize microbial community structure of deep coastal basin sediments at high spatial resolution on a regional scale. Taxa such as Proteobacteria and Myxococcota decrease in abundance with sediment depth, while Chloroflexi, Firmicutes, and Archaea tend to increase. A decline in Shannon diversity indices with increasing sediment depth and distance from the shelf suggests that factors such as sediment age and organic matter (OM) characteristics play crucial roles. Non-metric multidimensional scaling (NMDS) analysis highlights correlations between sediment age, water depth, excess carbon load, and microbial community structure. Some taxa like Anaerolineae and Desulfobacterota show increased abundance in regions with high levels of contaminants as indicated by excess carbon or DDX, which may reflect their established roles in processes such as reductive dehalogenation and anaerobic hydrocarbon degradation.

This research underscores the profound impact of anthropogenic inputs on sediment microbial communities and their essential roles in biogeochemical processes. Future studies will employ metagenomic analyses alongside additional geochemical analyses to further explore the functional potential of these communities. Understanding the dynamics of microbial communities in contact with toxic contaminants is crucial for developing effective strategies to mitigate pollution and preserve marine ecosystems.

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1. Introduction

Marine sediment microbial communities are extremely complex systems, shaped by and acting to shape their environment. They are engineers of sediment ecosystem functionality and stability. Sediment microbial communities respond to environmental conditions such as pH, redox conditions, inputs of organic matter (OM) or contaminants, and nutrient availability. In turn, each of these factors is influenced and modified by the members of the microbial community. Sediment microbes play a significant role in global biogeochemical cycling, determining the fate of deposited OM and contaminants, and the replenishment of nutrients in the water column (Wakeham & Canuel, 2006). Their composition can be indicative of ecological health and resilience in response to environmental stressors (Rodríguez et al., 2021; Zhang et al., 2020).

The complexity of deposited OM produces variable sedimentary environments with unique microbial communities. Coastal sediments have the added complexity of terrestrial and anthropogenic inputs (Hedges & Keil, 1995). The San Pedro (SP) Basin in the Southern California Bight is a compelling case study for deciphering the complex relationship between microbial communities and anthropogenic inputs over time. Hypoxic conditions in the basin structure terminal metabolism, controlling community composition and limiting OM breakdown (Jessen et al., 2017). Sediment microbial communities play a central role in metabolizing and detoxifying pollutants in coastal sediments (Schimel & Schaeffer, 2012). Their composition reflects ecosystem health and resilience in response to environmental

stressors. Understanding microbial community responses and potential pollution mitigation is pivotal for ecosystem preservation and bioremediation.

1.1. History of Pollution in the Southern California Bight

The coastline of the Southern California Bight has been the subject of intense anthropogenic inputs related to rapid population growth and industrialization, with sewage outflows serving as a major source of anthropogenic input to the marine environment (Bond et al., 1973; Chartrand et al., 1985; Interstate Electronics Corporation Oceanics Division, 1973; Montrose Chemical Corporation of California, 1998). Before technological developments in wastewater treatment and the imposition of strict regulatory standards, the Palos Verdes (PV) Shelf bordering the SP Basin became home to extremely high concentrations of pollutants including technical-grade dichlorodiphenyltrichloroethane (DDT). Neutralized acid waste

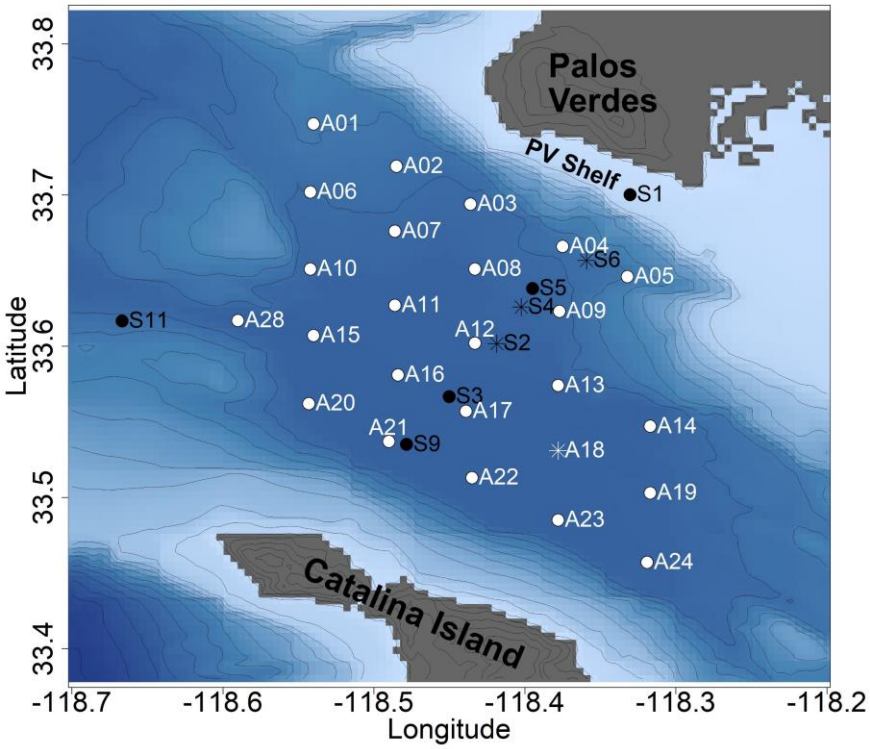

Figure 1. San Pedro Basin bathymetry. Points indicate sampling stations that have 16S sequencing data. The SPB transect (S-Stations) is shown in black. Stars indicate stations with full resolution sequencing data. Cores for microbial community analysis were collected at 30 additional stations, not shown.

containing DDT was disposed of there for decades through the Los Angeles County Sanitary District's sewage system by the Montrose Chemical Corporation of California, emanating at Whites Point Outfall on the PV Shelf (Montrose Chemical Corporation of California, 1998; U.S. Army Corps of Engineers, Seattle District, 2019). As a result, the contaminated shelf was designated a Superfund site by the Environmental Protection Agency under the Comprehensive Environmental Response, Compensation, and Liability Act (Montrose Chemical Corporation, 2017). Anthropogenic inputs to the PV Shelf, including resuspension and redistribution of previously contaminated sediment, continue to impact the PV Shelf and SP Basin today (Schmidt et al., 2024).

In addition to sewage waste disposal, there was a systematic effort to dispose of industrial waste in the deep ocean (Bakalian, 1984; Interstate Electronics Corporation Oceanics Division, 1973). From 1947-1972, the California Salvage Company dumped industrial waste into the deep SP Basin waters including concentrated sulfuric acid waste containing residues from the industrial synthesis of technical grade DDT. Additional waste products dumped in the SP Basin by Cal Salvage and other companies include chemical, radioactive, refinery, and oil drilling wastes. Although Cal Salvage had designated dumpsites in the SP Basin, there is evidence of repeated short dumping resulting in an expanded area of contamination observed in SP Basin sediments (Chartrand et al., 1985; Kivenson et al., 2019; Venkatesan et al., 1996). Our ongoing sampling efforts through extensive sediment coring have been conducted to characterize the extent of the contamination. Schmidt et al. (2024) describes findings from a transect across the SP Basin (Figure

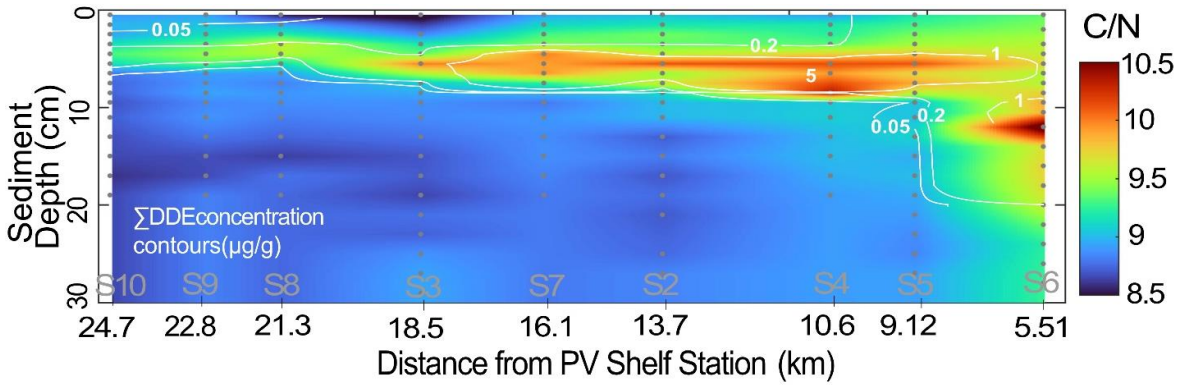

Figure 2. Heat map spanning the SP Basin transect of the organic C/N ratio overlaid by ΣDDE (Dichlorodiphenyldichloroethylene, an aerobic dehydrohalogenation product of DDT) (Schmidt et al. 2024).

1, S-stations), with analysis of a large sediment archive representing a high-resolution grid of SP Basin underway (Figure 1, A-stations).

Chemical analyses performed on the transect of sediment cores from the SP Basin revealed a notable horizon with an elevated organic carbon-to-nitrogen ratio (C/N), extending from the continental slope towards Catalina (Figure 2). This deposition of this horizon, according to C-14, Pb-210, and Cs-137 profiles, corresponds to the mid-20th century, a time of rapid population growth in the Los Angeles area and the release of a wide array of anthropogenic carbon compounds into the environment. Notably, it coincides with peaks in concentrations of persistent organic pollutants (POPs) such as Polychlorinated Biphenyls (PCBs), DDT and associated compounds (DDT+), and petroleum products and is corroborated by chemical dating methods and historical records of industrial dumping. This peak suggests that, in certain areas, up to 20% of the sediment's organic carbon loading may have anthropogenic origins (Schmidt et al., 2024). As we continue to uncover the extent of contamination and its ecological implications, this research serves as a

crucial foundation for developing strategies to protect and restore these vital marine habitats.

1.2. Fate of DDT in the San Pedro Basin and other environments

DDT exists in numerous forms in the SP Basin. The term DDX will be used from here on to refer to DDT and its two most common daughter products dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD). DDE is the predominant form of DDX on the PV shelf and, due to the redistribution of shelf sediments, it is the primary form of DDX being deposited continuously to SP Basin surface sediments (Schmidt et al., 2024). DDE can form from abiotic or biotic dehydrohalogenation reactions in oxic conditions (Pfaender & Alexander, 1972). It can be broken down aerobically, but in anoxic conditions, it is commonly considered a dead-end metabolite, resistant to further degradation (Hay, 1997; Nadeau et al., 1994). DDD is the product of anoxic reductive dehalogenation, thought to be primarily mediated by facultative anaerobic microorganisms (Aislabie et al., 1997). Clay-associated iron oxides may also play a role in the production of DDD where Fe(II), often produced by sediment microorganisms, reduces DDT to DDD (Fialips et al., 2010; Glass, 1972). In anoxic environments, DDD can be broken down further into less toxic and persistent compounds such as DDMU, DDMS, and DDNU, which are detected in SP Basin sediments (Stack et al., 2024).

DDX compounds in sediments likely lead to shifts in microbial community composition, as DDT and its metabolites are toxic to cells (Megharaj et al., 1999). DDE and DDD are considered to be more harmful than their parent compound, yet

most known DDT breakdown pathways, particularly anaerobic pathways, begin with either of these two compounds (Boush et al., 1975; Ko & Lockwood, 1968; Megharaj et al., 1999). Transformation of DDT to DDD is advantageous over DDE due to its further biodegradability, driving research on enhancing reductive dehalogenation processes in anoxic soils and sediments (Li et al., 2010; Velasco et al., 2020). One method that allows for the enrichment of native microbes is the addition of electron donors. Amendments of simple (e.g. Glucose, lactic acid) and complex carbon substrates (e.g. Alfalfa, rice straw) to DDT-contaminated anoxic soils have been shown to enhance reductive dehalogenation and the production of DDD by increasing reduction potential (Burge, 1971; Castro & Yoshida, 1971; Guenzi & Beard, 1967; Velasco et al., 2020).

1.3. Roles of Microbes in Coastal Marine Sediments

Marine sediment environments cover the majority of Earth's solid surface and host an estimated 10^{29} microbial cells (Kallmeyer et al., 2012). This staggering number is roughly equivalent to the number of microbial cells found in seawater and to those found in soil. These sedimentary environments play a pivotal role in global biogeochemical cycling and carbon sequestration. They act as significant carbon sinks, particularly in coastal regions characterized by high sedimentation rates, high OM inputs, and low oxygen content. In these conditions, microbial metabolism rapidly depletes the oxygen supply, and microbes must use alternative terminal electron acceptors (TEAs) for respiration. This is more pronounced in some seasonally hypoxic coastal basins, such as the SP Basin and the Santa Barbara (SB) Basin along the California Coast, where even the topmost layer of sediment

may be anoxic at certain times of the year. Anthropogenic activities have introduced a new dimension to these ecosystems. The influx of human-derived pollutants into coastal sediments leaves many unanswered questions about their fate and their ecological risk in deep coastal sediments. Contaminants in the environment may be degraded by biotic or abiotic processes, sequestered through burial over time, or enter marine food chains where they can bioaccumulate as they move up through trophic levels. Microbes may modulate the fate of contaminants by transforming them into other, often less toxic, forms or they may influence their bioavailability (Bayona & Albaigés, 2006; Ren et al., 2018). This underscores the urgent need to deepen our understanding of these complex environments, particularly in the face of increasing environmental pressures.

Sediment microbes may possess the potential to transform or detoxify contaminants, highlighting their potential for mitigating anthropogenic impacts. Reductive dehalogenation is a versatile and ecologically significant process that has garnered interest for its potential use in removing halogenated contaminants, such as polychlorinated biphenyls (PCBs), trichloroethylene (TCE), and chlorinated pesticides like DDT. Some microbes exhibit the capacity for dehalorespiration, using halogenated organics as TEAs in highly reduced environments. Non-respiratory dehalogenation is a co-metabolic process for some methanogenic, acetogenic, sulfate-reducing, or iron-reducing microbes, with non-specific enzymes acting to dehalogenate compounds while providing no energy to the microbe (Holliger & Schumacher, 1994). Members of the phyla Firmicutes, Proteobacteria, Desulfobacterota (previously Deltaproteobacteria), and Chloroflexi are known to

perform facultative dehalogenation, while obligate dehalogenators exist only within Dehalococcoidia belonging to Phylum Chloroflexi, except for the Firmicute *Dehalobacter restrictus* (Fincker & Spormann, 2017).

Sediment environments exhibit a distinct stratification of electron acceptor availability. TEAs tend to be sequentially consumed in the order of highest to lowest reduction potential. In sediment environments where oxygen and nitrate are depleted, halogenated organics may be thermodynamically favorable electron acceptors with redox potentials as much as three times that of sulfate (Dolfing & Harrison, 1992; Gohil & Ogram, 2020). It has been shown that respiratory reductive dehalogenation is inhibited by the presence of alternative electron acceptors such as sulfate and nitrate. Since the first step in the degradation of halogenated compounds is typically the dehalogenation step, the overall biodegradability of organohalide compounds may be affected by the presence of alternative electron acceptors (Häggblom & Milligan, 2000). Competition for H_2 between sulfate reducers and dehalorespirers has been proposed as a mechanism for the inhibition of reductive dehalogenation by sulfate (Madsen & Aamand, 1991). Given the broad substrate specificity seen in many reductive dehalogenase enzymes, they may function on DDT and the presence of DDD in the SP Basin suggests that reductive dehalogenation is occurring. This work aims to identify if potential reductive dehalogenators have any relationship to the distribution of DDX and other contaminants.

The introduction of toxic pollutants through anthropogenic activities also raises significant concerns about the consequences for biogeochemical cycling and

overall marine health. The amount and type of OM, including contaminants, present in sediment is one of the most important drivers of community structure and function. The OM makeup of marine sediment is complex, with different pools being transformed at different rates (Westrich & Berner, 1984). OM reaching the seafloor is often energetically depleted, corresponding with higher C/N values, because it is degraded throughout the water column as it sinks (Hedges & Keil, 1995). Upon reaching the seafloor, organic nitrogen undergoes remineralization to ammonia, a vital nutrient. In sediments, nitrate acts as a crucial terminal electron acceptor in the anaerobic oxidation of OM, facilitating denitrification, which converts nitrate back to N_2, thereby regulating the bioavailability of nitrogen and impacting global organic nitrogen budgets. Sulfate-reducing bacteria play a central role in the anaerobic remineralization of OM. The sulfate-methane transition zone (SMTZ), where sulfate and methane intersect, acts as a significant sink for methane, preventing its escape into the overlying water column, and thereby influencing the global sulfur and carbon cycles (Orsi, 2018). Despite the critical functions of sediment microbial communities, they are under constant severe energy constraints due to sparse OM input, the limited diffusion of TEAs from the water column, and competition among anaerobic microbes.

The prolonged coexistence of a diverse microbial assemblage with a high concentration of anthropogenic contaminants for decades makes this setting ideal for investigating the evolutionary and metabolic potential as well as spatial and biogeochemical structuring of sediment microbial communities. This work aims to characterize the microbial community of the heavily contaminated SP Basin through

exploratory 16S rRNA analysis, with a focus on how community structure is impacted by anthropogenic contamination measured through chemical analysis, CHN analysis, and chemical dating at the same locations (Schmidt et al., 2024). The distribution of dominant taxa in the basin will be reported, with emphasis on groups that correlate with the distribution of contaminants. This analysis will inform future metagenomic analysis of SP Basin sediment and contribute to the understanding of the broader ecological role of marine sediment microbial communities in the coastal ecosystem and the fate of pollutants in marine environments.

2. Methods

2.1. Sample Collection

Samples were collected from SP Basin on four *R/V Yellowfin* expeditions on 07 November 2022, 15 December 2022, 6-8 March 2023, and 24-28 July 2023. Sediment cores with a diameter of 10 cm were collected with a multicorer. Cores were processed for chemical analysis as reported by Schmidt et al. (2024). Cores for microbial community analysis were sectioned in 2 cm intervals to 20 cm depth. Intervals were homogenized and stored in triplicate 2 mL cryovials. The sectioning materials were rinsed with seawater and cleaned with 70% ethanol between each interval. All samples were temporarily stored at 4 °C and stored long-term at -80 °C.

2.2. DNA Extraction and Sequencing

Samples were sent to the Microbiome Core at UC San Diego for DNA extraction, PCR, and sequencing. DNA extractions were automated on KingFisher

Flex robots (Thermo Fisher Scientific, USA) and performed according to published protocols with the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit (Thermo Fisher Scientific, USA) (Marotz et al., 2021). Each extraction plate and all downstream processing steps included blank controls and mock communities (Zymo Research Corporation, USA). PicoGreen fluorescence assay (Thermo Fisher Scientific, USA) was used for DNA quantification. 16S rRNA gene amplification was performed according to the Earth Microbiome Project protocol (Thompson et al., 2017). The V4 region of the 16S rRNA gene was amplified with unique forward primer barcodes (515fB-806r) using miniaturized volumes with single reactions per sample (Marotz et al., 2019; Minich et al., 2018; Walters et al., 2016). The library was prepared with equal volumes of each amplicon and sequenced on the Illumina MiSeq sequencing platform with paired-end 250 bp cycles at the UC San Diego Institute for Genomic Medicine.

For each station, duplicate samples from the 0-2 cm, 6-8 cm, and 18-20 cm intervals were selected to represent the surface, peak contaminant concentrations, and deep intervals. At some stations, additional samples were sent to capture the interval with the most contamination due to differences in sedimentation rate. For the near-shelf stations (Figure 1: A01-A05), samples from 10-12 cm intervals were selected. At deep-basin stations (A14, A19-A24, A28) samples for the 4-6 cm intervals were selected. For stations S6, S2, S4, and A18 duplicate samples for all depth intervals were sent to represent full-resolution cores at near-shelf, peak DDX, peak C/N, and the deep-basin stations, respectively.

2.3. Data Processing

16S rRNA sequencing data was downloaded and processed on the Bridges2 cluster at the Pittsburgh Supercomputing Center. The quality-filtering, denoising, and subsequent taxonomic identification and assignment of Amplicon Sequence Variants (ASVs) using the publicly available SILVA database (v138.1) were executed using the package Dada2 (v1.18.0) in R (v.4.3.2).

A baseline C/N ratio for each core was determined by selecting the C/N value of the depth interval just below the peak in carbon load, interpreted to represent pre-anthropogenic C/N. Excess C/N was calculated by subtracting the baseline C/N from the C/N ratio in each sample. The percentage of excess carbon was calculated assuming a maximum C/N value of 14.95, which was measured at the PV Shelf station. Excess carbon values for the shelf and near-shelf stations were not calculated because the cores did not reach deep enough to capture pre-anthropogenic or baseline C/N. Because sampling for chemical analysis was done using different intervals (1 cm from 0-10 cm, and 2 cm from 10-20 cm), interval matching was performed by averaging the values of chemical analyses for intervals corresponding to each 16S sequencing interval.

2.4. Statistical analyses

All statistical analyses were performed in R (v.4.3.2). The package Phyloseq (v1.40.0) was used to calculate the relative abundance at different taxonomic levels and for other statistical analyses (McMurdie & Holmes, 2013). The number of reads was normalized to the median sequencing depth and Amplicon Sequence Variants (ASVs) with <5 reads across the dataset were excluded from further analysis.

Shannon diversity measures were calculated at the ASV level after pre-processing steps using the *plot_richness()* function. NMDS analysis was performed using the *plot_ordination()* function with Bray-Curtis dissimilarity, focusing only on the A-stations to capture spatial variation and limit bias from different sequencing runs. Each NMDS analysis was conducted for individual depth intervals to capture the more subtle gradients in the data. Some outliers were removed when duplicates from the same sample had dissimilar NMDS placements.

Distributions of taxa were made by taking the average abundance of each group across duplicate samples. Distribution maps of taxa and environmental parameters were created in ArcGIS Pro using the Natural Neighbor and Contour tools in the 3D Analyst Tools package.

3. Results and Discussion

3.1. Characteristics of San Pedro Basin Sediment Microbial Community

After quality control, trimming, and denoising, 156,332 ASVs remained and were identified as 86 unique phyla. In the full dataset, 90% of the abundance can be attributed to the top 20 phyla (Table 1). The relative abundance of each of these 20 phyla across the SP Basin transect at the surface, 6-8 cm, and 18-20 cm intervals are shown in Figure 3. Some taxa, such as Proteobacteria, Myxococcota, Bacteroidota, and Sva0485, decrease in relative abundance with sediment depth at all stations. Others such as Chloroflexi, Firmicutes, Asgardarchaeota, and Crenarchaeota, increase in relative abundance with depth at all stations. These

changes with sediment depth are more pronounced in samples farther from the shelf. For example, at station S6 near the shelf, the abundance of Asgardarchaeota increases from 0.9% at the surface to 1.3% at 6-8 cm and to 2.4% at 18-20 cm. In contrast, at station S11 far from the shelf, it increases from 0.7% to 2% to 5.9% in the respective intervals. NB1-j is most abundant in surface samples farthest from the shelf and shows no noticeable change in relative abundance with depth near the shelf but decreases drastically in relative abundance with depth at stations farther from the shelf. The spatial (Figure A1) and depth distributions (Figure A2) of these groups can be found in the Appendix.

Phylum	Average Abundance (%)
Desulfobacterota	18.47
Proteobacteria	13.06
Planctomycetota	9.67
Chloroflexi	8.75
Nanoarchaeota	6.21
Bacteroidota	5.77
Acidobacteriota	4.99
Verrucomicrobiota	3.53
Sva0485	2.40
NB1-j	2.10
Firmicutes	2.04
Myxococcota	2.01
Asgardarchaeota	1.96
Actinobacteriota	1.74
Crenarchaeota	1.55
Thermoplasmatota	1.49
Latescibacterota	1.22
Nitrospirota	1.16
Calditrichota	1.03
Patescibacteria	1.01

Table 1. Average abundance of 20 most abundant phyla across all SP Basin Samples.

Differences in microbial community diversity varied based on sediment depth interval and location in the basin. In this dataset water depth is a proxy for distance from the PV Shelf, reflecting slower sedimentation rates. Surface interval samples have similar diversity values across the basin, declining slightly with water depth, but samples in the 6-8 cm and 18-20 cm intervals have a sharper decline in diversity with increasing water depth (Figure 4a). Sediments from the 0-2 cm interval are young at all stations ranging from about 0-4 years old near the PV shelf to 0-16 years old farther from the shelf, whereas the 18-20 cm interval ranges from

approximately 70 to 300 years old, correlating with a decline in Shannon diversity indices (Figure 4b). The decline in community diversity with distance from the shelf could be the result of several factors including the age of sediment, the type and

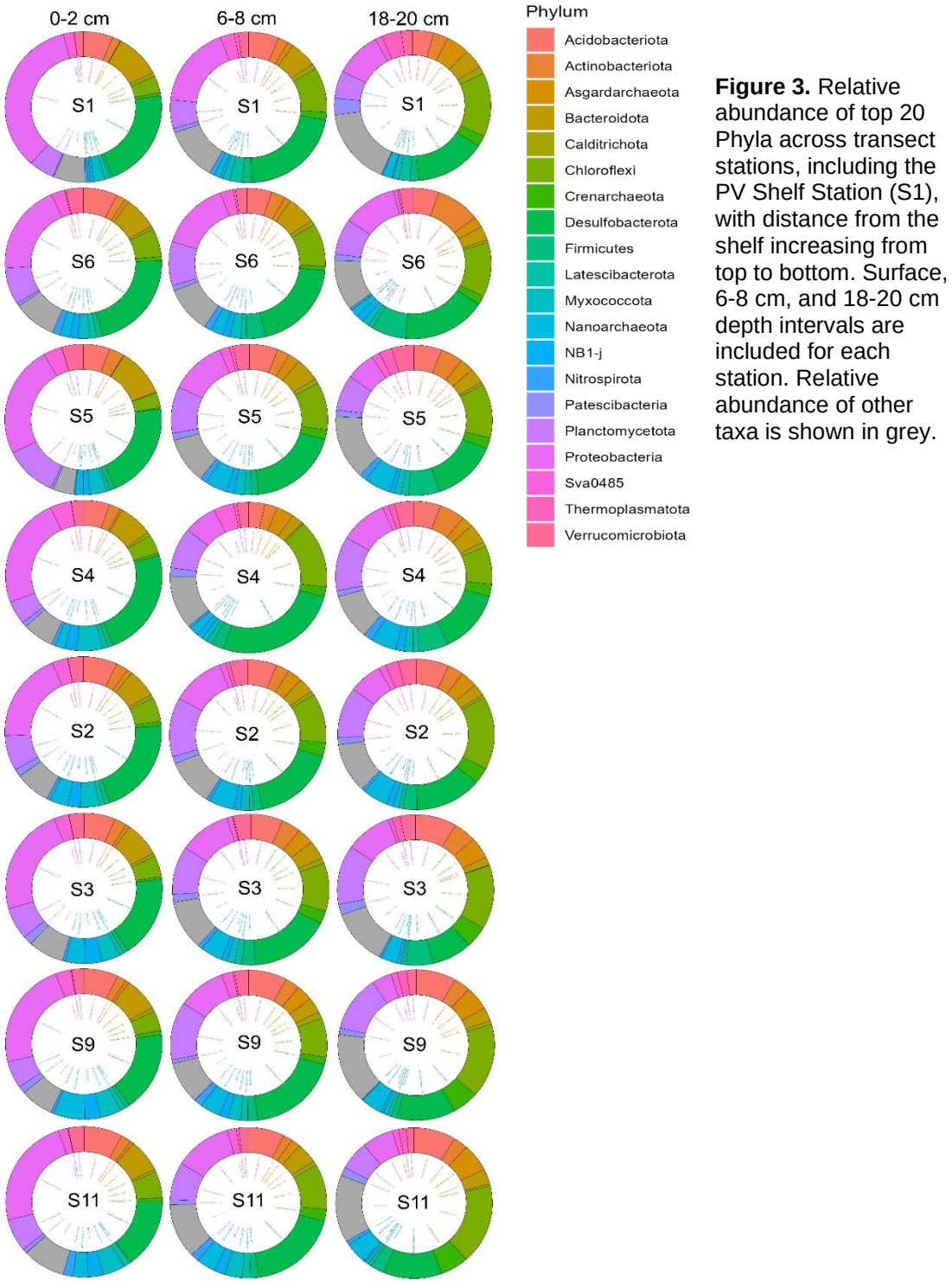

Figure 3. Relative abundance of top 20 Phyla across transect stations, including the PV Shelf Station (S1), with distance from the shelf increasing from top to bottom. Surface, 6-8 cm, and 18-20 cm depth intervals are included for each station. Relative abundance of other taxa is shown in grey.

amount of OM at the time of deposition, or the makeup of sedimentary materials. The same trend of declining diversity with depth can be seen at most stations across the SP Basin and is more pronounced at deeper stations, suggesting an association with sediment age.

The length of time since sediment deposition determines the age of buried OM, which is closely related to its reactivity. The multi-G model can be used to describe the phenomenon where the most labile OM is consumed first, followed by the next pool of more recalcitrant OM at a slower rate (Westrich & Berner, 1984). Older material is significantly less reactive due to its molecular complexity or its physical interactions with minerals, and more specialized communities are required to access or degrade it (Burdige, 2007). These more complex substrates typically cannot be entirely remineralized by one group, but through syntrophic relationships among multiple taxa with each performing a subset of the breakdown pathway for a given compound (Schink & Stams,

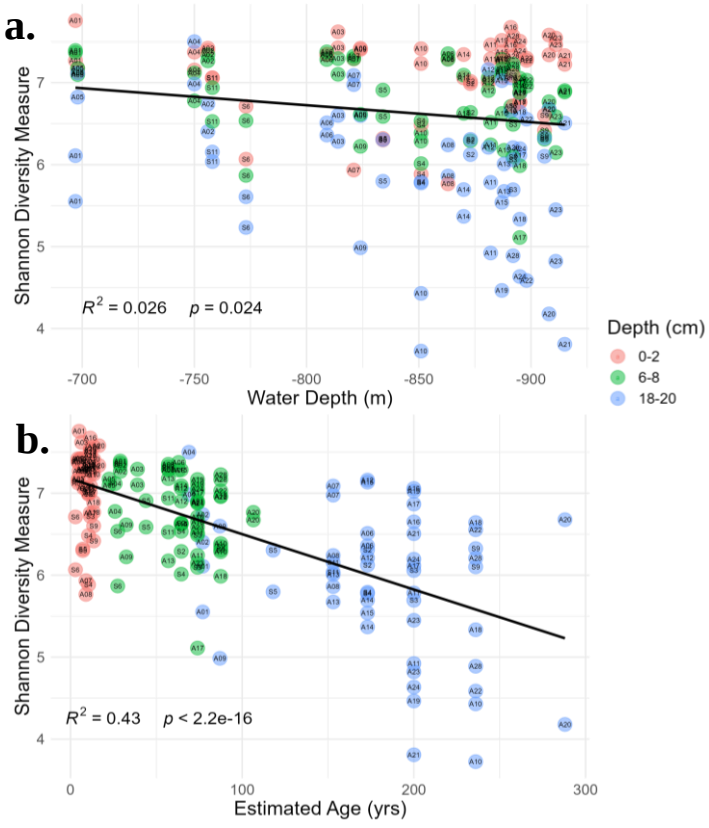

Figure 4. Shannon Diversity Measures. Points are labeled with their station and colored by depth interval. Correlation of diversity indices with **a.** water depth and **b.** estimated age is shown.

2013; Suominen et al., 2021). This, in addition to the increasing limitation of terminal electron acceptors with sediment depth, can lead diversity indices to be stable or even increase as more niches become available for the consumption of organic matter through diverse metabolic pathways. Another explanation for declining diversity with depth is the level of disturbance, which can also be related to water depth (lower O_2 in the deeper water column can mean less bioturbation) and sediment depth (Böer et al., 2009). Low-level disturbance tends to promote diversity in sediments, but little to no disturbance can lead to energy limitation resulting in a decline in diversity (Urakawa et al., 2000). Differences in sediment microbial community diversity in the SP Basin are likely attributable to multiple factors related to sedimentation rate, amount and age of OM, as well as level of disturbance.

By correlating non-metric multidimensional scaling (NMDS) analysis on microbial community abundance data with environmental parameters, the effect of sediment age on microbial communities can be seen at all depth intervals (Figure 5). NMDS analyses were conducted on each depth interval independently to capture more subtle gradients in the microbial community abundance data, as the predominant microbial community driver in this dataset is sediment depth. The NMDS1 axis, which captures the largest gradient in microbial community variation, is positively correlated with estimated age in all intervals. This relationship appears to be strongest at the surface interval (Figure 5a), followed by the 6-8 cm (Figure 5b) and 18-20 cm intervals (Figure 5c). This suggests that the most substantial changes in microbial community structure due to age happen in younger sediment, possibly due to more rapid changes in OM accessibility and recalcitrance.

As discussed above, water depth serves as a suitable proxy for distance from the shelf and can be correlated with sediment age, C/N ratio, and DDE concentrations depending on the character of the input during the deposition of the corresponding depth interval (Figure 5). In the surface interval, water depth is correlated with estimated age (R^2 = -0.59), C/N (R^2 = 0.50), and DDE (R^2 = 0.37). Water depth in the 6-8 cm interval is correlated with estimated age (R^2 = -0.71). In

Figure 5. NMDS of microbial communities across all A-stations at individual depths. The correlation matrices show correlations of environmental parameters (DDX, C/N, excess carbon, water depth, and estimated age) with themselves and NMDS axes for the depth intervals **a.** 0-2 cm, **b.** 6-8 cm, and **c.** 18-20 cm.

the 18-20 cm interval, water depth is correlated with C/N (R^2 = 0.81), estimated age (R^2 = -0.71), and DDE (R^2 = 0.53). The correlation of water depth with C/N and DDE in the 18-20 cm interval is due to the inclusion of near-shelf stations with rates of sedimentation so high that anthropogenic influences can be seen much deeper than in mid-to-deep-basin stations. Excess carbon would also probably be correlated with water depth in the 18-20 cm interval if values of excess carbon for the near-shelf stations could be calculated. The NMDS1 axis in each depth interval is most highly correlated with water depth, likely reflecting the combined impact of these correlated parameters on microbial community structure.

Water depth may also affect terminal electron acceptor (TEA) availability, as oxygen concentration tends to be stratified in the SP Basin and is often hypoxic in the deepest parts. Water column oxygen was not measured at the time of collection, but oxygen time series measurements made by the San Pedro Ocean Time Series (SPOT) do not typically show significantly different oxygen concentrations across this depth range (~700 m to ~900 m). However, given the dynamic nature of the basin, the occurrence of bioturbation may be a better indicator of oxygen availability to sediment biota. Savrda et al. (1984) found that bioturbation was much less prevalent in deep-basin areas than in mid-basin areas, which may indicate that differences in water column oxygen across SP basin stations are impactful.

In the surface interval, NMDS1 is correlated with water depth, estimated age, and DDE (Figure 5a), which together suggest that shelf input is a major driver of variation in microbial communities of surface sediment. This may suggest anthropogenic inputs as a microbial community driver, as indicated by the presence

of DDE reflecting redistribution of contaminated shelf sediment. However, modern inputs of contaminants from the shelf are much reduced compared with times before the implementation of secondary sewage treatment and more strict legislation preventing ocean disposal. In an undisturbed system, it makes sense for shelf input to act as a driver due to variations in the amount and quality of organic matter deposited, as well as the type of sediment which can impact the bioavailability of deposited organic matter and other parameters. NMDS2 in the surface and 18-20 cm intervals are uncorrelated with known environmental parameters.

In the 6-8 cm interval, NMDS1 and NMDS2 are correlated with water depth and estimated age, but NMDS1 is not well correlated with other parameters. Instead, NMDS2 is correlated with C/N and excess carbon. The largest driver of microbial community here is likely sediment age and the amount and quality of organic matter input during the deposition of this layer. In much of the SP Basin, this depth interval was deposited during peak anthropogenic inputs from sewage and offshore dumping activities. NMDS2 may indicate anthropogenic inputs as a detectable driver of microbial community structure in SP Basin sediments.

3.2. Relationship of Taxa with Contamination in the San Pedro Basin

DDX quantification and CHN analysis were performed for each of the 33 stations chosen for microbial community analysis. The percentage of total organic carbon in excess was calculated based on the baseline Carbon-to-Nitrogen ratio (C/N) in each core. Excess carbon is presumed to originate from anthropogenic sources, supported by chemical dating that aligns with periods before secondary sewage treatment and during offshore dumping efforts. At some of these stations and additional locations, tar was observed in the sediment – at 17 or more stations – stretching mainly along a linear trend (Figure 6). The absence of nearby oil seepage and the localization of tar between depths of 5 cm to 14 cm suggest it resulted from historical offshore dumping. The highest excess carbon values in the 6-8 cm interval may be associated with such tar. The most extreme case was at station S4, where nearly 40% of the total organic carbon in the most tar-contaminated depth interval was in excess, likely due to the large quantity of tar found there.

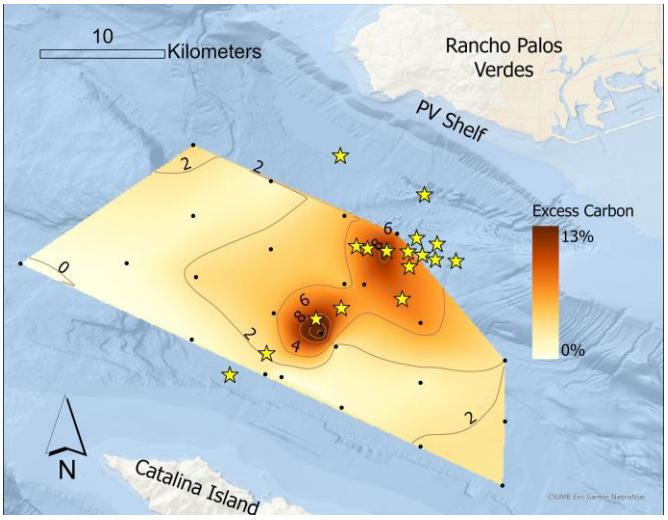

Figure 6. Heatmap of excess carbon in the 6-8 cm interval across SP Basin generated using data from the stations with microbial community analyses. Stars indicate locations where tar was observed.

3.2.1. Chloroflexi

The Phylum Chloroflexi is of particular interest in the field of marine contamination and biodegradation. Chloroflexi are a very diverse group, existing in

a range of environments including freshwater and marine systems, soil and sediment, and even gut microbiomes. They are predominant members of marine sediment microbial communities globally. Metagenomic analyses have indicated the widespread occurrence of acetate oxidation with carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS), and carbon fixation through the Wood-Ljugdahl Pathway (WLP) within Chloroflexi genomes recovered from marine sediments (Fincker et al., 2020). Much work has been done to understand their role in the degradation of PCBs and other chlorinated contaminants due to their ability to perform reductive dehalogenation as a respiratory process which may provide a selective advantage in TEA-limited environments. The Class Dehalococcoidia is particularly well studied because they are the only known group with obligate dehalorespirers, though only in terrestrial environments (Matturro et al., 2017; Nuzzo et al., 2017; Sewell et al., 2017; Wang et al., 2014). Fincker et al. (2020) also demonstrated through metagenomic analysis that dehalorespiration may be more broadly distributed within Chloroflexi than previously thought, with the main difference between Dehalococcoidia and non-Dehalococcoidia reductive dehalogenases being their electron transport partner. In dehalorespiring Dehalococcoidia genomes, an uptake hydrogenase (Hup) and Dehalococcoidia-type formate dehydrogenase-like enzyme complex with the reductive dehalogenase enzyme (RdhA) (Kublik et al., 2016). Formate dehydrogenase-like genes with unknown functions are widespread in subseafloor Chloroflexi genomes. Their hypothesized role is to feed electrons to RdhA from an electron-donating partner. In the absence of Hup, this partner is unknown (Sewell et al., 2017). A diverse array of

these formate dehydrogenase-like genes in subseafloor Chloroflexi genomes may suggest that dehalorespiration is possible with a broader range of substrates (Fincker et al., 2020).

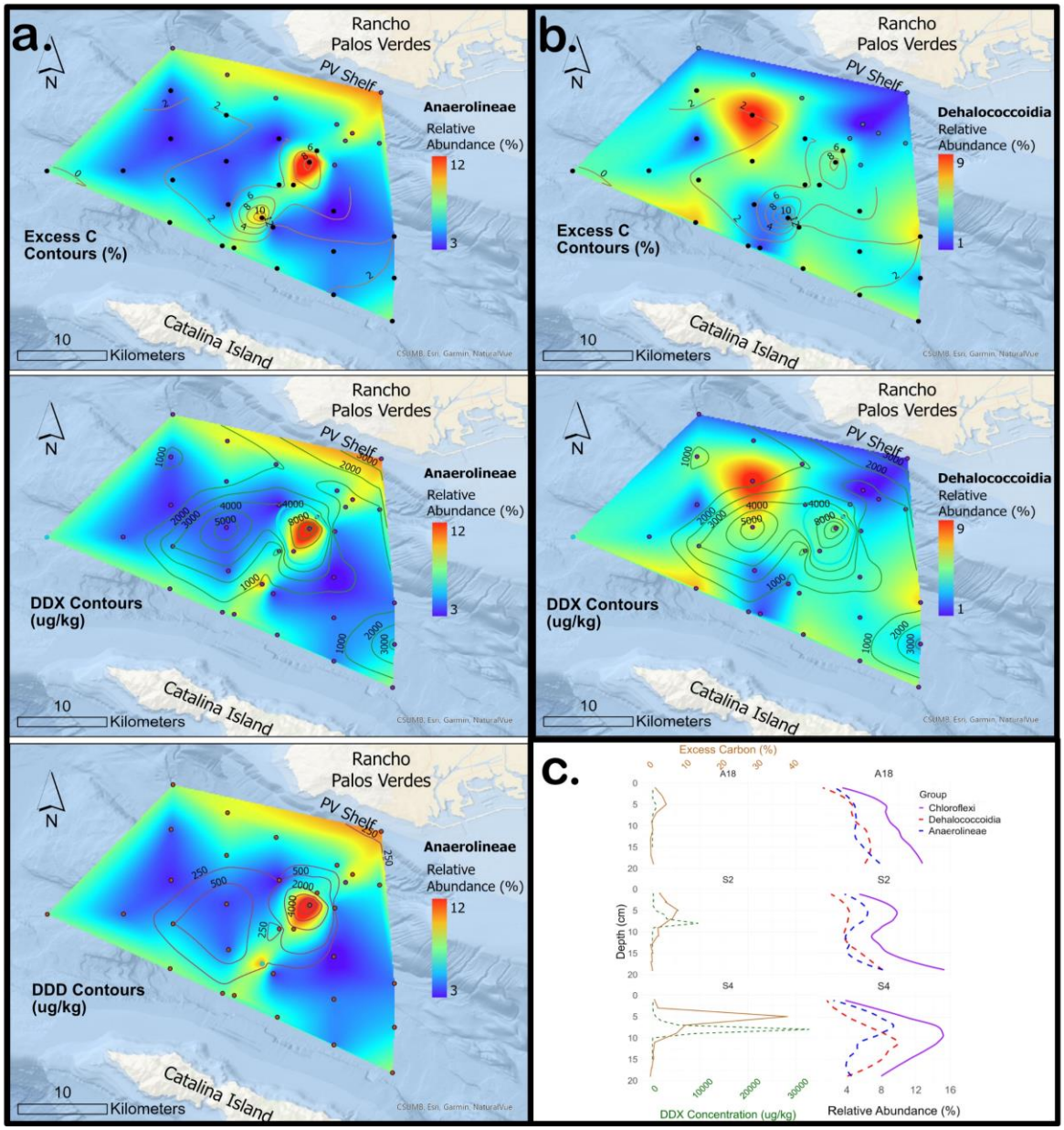

Figure 7. Spatial and depth distributions of Chloroflexi and environmental parameters. **a.** Heatmap of Anaerolineae distribution in the 6-8 cm interval with overlays of excess carbon (%), DDX concentration (ug/kg), and DDD concentration (ug/kg). **b.** Heatmap of Dehalococcoidia distribution in the 6-8 cm interval with overlays of excess carbon (%) and DDX concentration (ug/kg). **c.** Depth distributions of DDX concentration (ug/kg) and excess carbon (%) (left), and Chloroflexi abundance including the classes Dehalococcoidia and Anaerolineae (right).

In the SP Basin, classes Dehalococcoidia and Anaerolineae are by far the most abundant members of Chloroflexi. Of the 8001 Chloroflexi ASVs, 4701 are identified as Dehalococcoidia, and 2941 are identified as Anaerolineae. Most of these ASVs exist in very low abundance. A single ASV makes up 13.6% of Anaerolineae abundance, followed by the next most abundant ASVs at 8.6%, 6.7%, and 2.7%, with over 50% of Anaerolineae abundance being made up of 17 Anaerolineaceae ASVs. The top Dehalococcoidia ASVs make up 5.2%, 3.6%, 3.3%, 2.0%, and 1.7% of Dehalococcoidia abundance, with 90 ASVs making up 50% of their total abundance. Both classes are about equally represented in the whole dataset, but their spatial distributions differ. In the 6-8 cm interval, Dehalococcoidia distribution is uneven throughout the basin, declining slightly in abundance near the shelf. It appears to have no spatial association with excess carbon or DDX concentration (Figure 7b). Anaerolineae are abundant on the borders of the basin, including the shelf, near-shelf stations, and near Catalina. There are notable peaks in Anaerolineae abundance in mid-basin stations with corresponding peaks in excess carbon (Figure 7a). These stations are located along the transect where tar was observed in this depth interval (Figure 6).

Anaerolineae is known for its capacity to degrade complex organic compounds. Fincker et al. (2020) performed a comparative analysis on Chloroflexi metagenome-assembled genomes (MAGs) from several subseafloor environments, including the Santa Monica Mounds in the Southern California Bight. Both Anaerolineae and Dehalococcoidia live anaerobic lifestyles, although some Anaerolineae MAGs have the capacity for aerobic respiration. Fincker et al. (2020)

did not detect the potential for nitrate or sulfate reduction in any of the studied MAGs. However, some Chloroflexi have shown the potential for nitrite and, less commonly, nitrate reduction (Hug et al., 2013). Dehalococcoidia is known to perform dehalorespiration which is more energetically favorable than acquiring energy through fermentation or the Wood-Ljungdahl pathway (WLP). Anaerolineae has not been reported to dehalorespire, but several Anaerolineae MAGs did have rdhA. Anaerolineae lacked Hup, the hydrogenase enzyme typically associated with rdhA in Dehalococcoidia, but it did frequently contain formate dehydrogenase-like genes which may complex with rdhA to conserve energy during reductive dehalogenation (Fincker et al., 2020). Without the complex, rdhA can still carry out reductive dehalogenation but without energy conservation. This can be a co-metabolic process, a means of accessing the hydrocarbon backbone to serve as a substrate, or it can be useful as a form of redox balance or a mechanism for fermentation.

Anaerolineae is prevalent in marine sediments and studies have suggested that they specialize as initial fermenters in carbohydrate-rich sediments (Suominen et al., 2021). Their potential for utilizing a wide range of carbon substrates may explain their increased abundance in areas with high excess carbon. Interestingly, at the peak in excess carbon and Anaerolineae abundance, there is a corresponding peak in the concentration of DDD, the reductive dehalogenation product of DDT. Members of Anaerolineae commonly have reductive dehalogenation potential, so the high concentration of DDD may result from the activity of Anaerolineae. DDD may be a co-metabolic product or a product of fermentation, using DDT to replenish reducing equivalents. If Anaerolineae is

capable of dehalorespiration as suggested by Fincker et al. 2020, the presence of DDT may lend a selective advantage because it would constitute a significant increase in the amount of energy that can be conserved from a given substrate.

3.2.2. Desulfobacterota

Desulfobacterota are the most dominant group in the SP Basin sediments on average. Desulfobacterota is commonly found to be a dominant member of coastal marine sediment communities. They are the most prominent members of the sulfate-reducing microorganisms (SRM) in these regions. SRM account for as much as half of the remineralization of organic matter in sediments (Jørgensen, 1982). Desulfobacterota are detected in marine sediments globally and have been seen to dominate in surface sediments underlying hypoxic waters (Mahmoudi et al., 2015). Three Desulfobacterota classes dominate in the SP Basin sediments. Desulfobacteria is the most common (2897 ASVs. 50% of abundance is attributed to 18 ASVs), followed by Desulfobulbia (843 ASVs. 50% of abundance is attributed to 8 ASVs), and Syntrophobacteria (50 ASVs. 44% and 40% of abundance is attributed to the top 2 ASVs). Most MAGs identified within these groups are obligate anaerobes and have complete pathways for sulfate and sulfite reduction, acetogenesis, and carbon fixation through CODH-ACS and WLP. Some

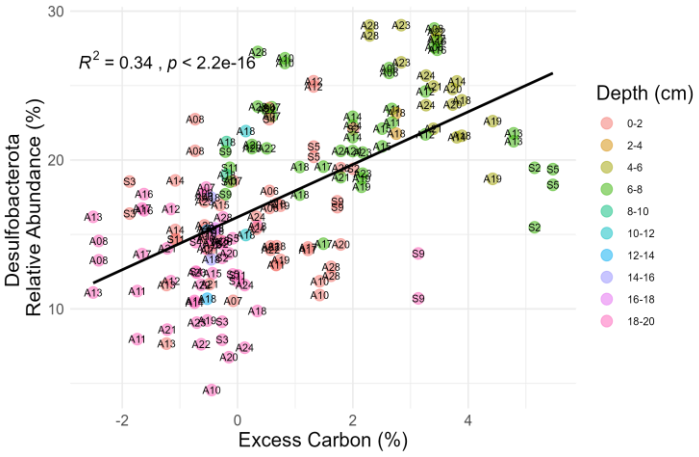

Figure 8. Correlation of Desulfobacterota Relative Abundance with Excess Carbon values in all SP Basin samples.

members of these groups also encode anaerobic hydrocarbon degradation genes (Langwig et al., 2022). Additionally, some members of Desulfobacterota, including *Desulfomonile Tiedjei* in the Class Desulfobacteria, have been observed to dehalorespire on TCE and 3-chlorobenzoate (Cole et al., 1995). However, the Genus SEEP-SRB1 accounts for the majority of Desulfobacterota abundance in the dataset (~47%). SEEP-SRB1 is a diverse clade associated with the anaerobic degradation of hydrocarbons in methane and non-methane hydrocarbon seeps. rdhA has not been identified in this group (Petro et al., 2019).

The abundance of Desulfobacterota is positively correlated with excess carbon using all SP Basin samples (Figure 8). The separation of the 4-6 cm and 6-8 cm samples from the other depth intervals is likely due to the increased organic carbon load in those samples, though it can't be ruled out that this trend could be attributed to an adaptation to living in this depth interval. This trend is not visually

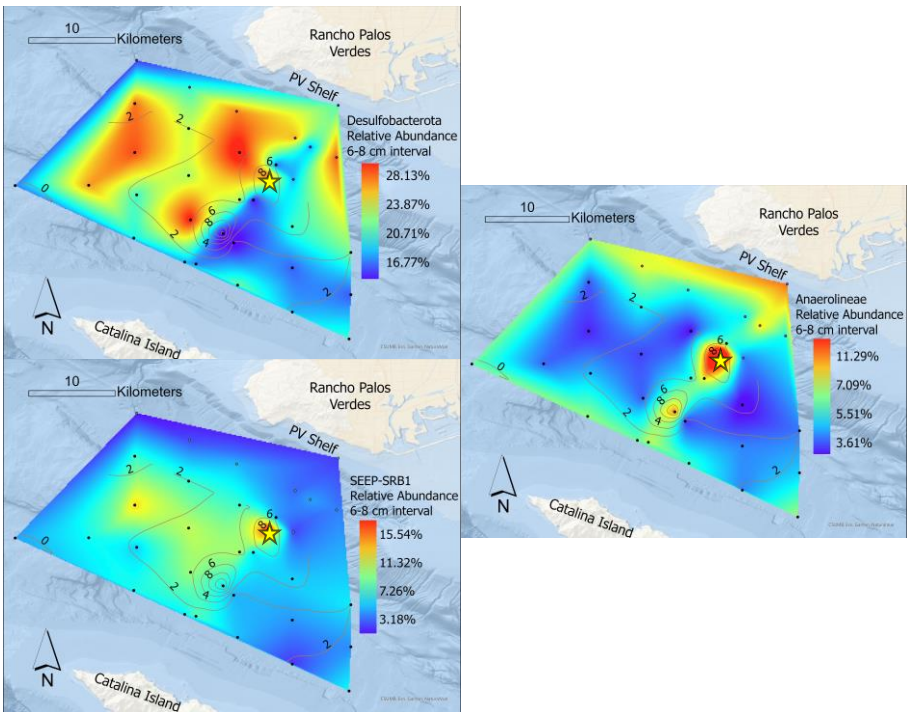

Figure 9. Distribution of Desulfobacterota, SEEP-SRB1, and Anaerolineae in the 6-8 cm interval of the SP Basin with contours of percent excess carbon. The star is located at station S4 where the highest volume of tar was observed.

apparent on a regional spatial scale (Figure 9). Given the complexities of spatial variation and depth distributions, it is more likely that Desulfobacterota is enriched in regions with high excess carbon due to their broad carbon metabolism and role in anaerobic hydrocarbon degradation, especially considering the widespread observations of tar. The apparent decline in their relative abundance in some areas with high levels of excess carbon may be related to the enrichment of Anaerolineae in response to tar if the two groups compete for substrates. Anaerolineae genomes are frequently observed to contain complete degradation pathways for complex carbon substrates, which would allow them to grow independently of syntrophy (Fincker et al., 2020). Extremely concentrated tar was observed at station S4, highlighted with a star in Figure 9, where both taxa coexist in high relative abundance and SEEP-SRB1 makes up 60% of Desulfobacterota abundance.

4. Conclusions

This work investigated the structure of microbial communities in the deep coastal sediment of the polluted San Pedro Basin and reported on the spatial and depth distribution of some of the most prominent taxa and their relationship with contaminants. Diversity indices differed across the SP Basin relating in all studied depth intervals primarily to the input of material from the PV Shelf, which consists of varying types and amounts of sedimentary materials, terrestrial and organic matter, and anthropogenic compounds across space and time. NMDS analyses indicated that the primary drivers of variation in microbial community structure were water depth and sediment age. Water depth is correlated with indicators of shelf input, and its impact on microbial community composition may reflect the combined

impact of factors influenced by shelf input, including sedimentation rate, estimated age, and organic matter inputs. This result was anticipated because shelf input, regardless of anthropogenic contributions, is likely to be a major driver of the microbial community. The sediments with the highest levels of contamination were in the 4-6 cm and 6-8 cm intervals. NMDS axes for the microbial community in the 6-8 cm interval in samples across the SP Basin were correlated with the level of excess organic carbon in the sediment, indicating that anthropogenic inputs are likely drivers of microbial community structure at a regional scale.

The distributions of several groups corresponded to the distribution of contaminants. Members of the class Anaerolineae in the phylum Chloroflexi are significantly enriched in regions with high levels of excess carbon. Anaerolineae are known to be dominant primary fermenters of diverse carbohydrates in marine sediments, and metagenomic analyses have highlighted their broad carbon metabolism. Their abundance is also correlated with high concentrations of DDD. While several processes can result in the reductive dehalogenation of DDT, the amount of DDD in this sediment is anomalously high, making up nearly half of the total DDX content of the sediment. This may indicate that Anaerolineae are involved in the reductive dehalogenation of DDT. Future work will leverage metagenomic sequencing to investigate the presence of rdhA in recovered Anaerolineae genomes and whether they are enriched in the most contaminated areas. Other genes that may confer dehalorespiration as a function of rdhA will also be investigated, such as the Hup enzyme and formate dehydrogenase-like enzymes which form a complex

with RdhA to leverage the reduction potential of organohalide compounds to produce a proton gradient for ATP generation.

Observations of tar are likely responsible for the strongest peaks in excess carbon content in the SP Basin sediments and appear to be a strong driver of microbial community structure in the affected samples. The presence of tar may be associated with increased microbial interaction with other contaminants through the enrichment of impactful taxa as shown by enhanced DDD production in tar-contaminated samples. Desulfobacterota relative abundance is positively correlated with excess carbon. The genus SEEP-SRB1 makes up a significant portion of Desulfobacterota abundance in the SP Basin, and its association with anaerobic hydrocarbon degradation potentially explains its enrichment at tar-contaminated stations. Metagenomic analyses will shed light on the presence of anaerobic hydrocarbon degradation genes and whether they are enriched on a per-genome basis in the most contaminated samples.

Microbial community structure and function vary across the SP Basin due to many overlapping environmental gradients. It is of particular importance to elucidate their response to anthropogenic inputs. Deep coastal sediments play a crucial role in carbon sequestration and biogeochemical cycling. Functional analyses are necessary to elucidate changes in the potential for biogeochemical cycles in sediment communities. This work may also benefit from additional measurements. Metagenomics and metatranscriptomics will help bridge the gap between abundance data, genomic potential, and actual activity. Measuring sulfate reduction rates and conducting porewater analyses to quantify sulfate, sulfide, and Fe(II) can

help determine the reduction potential of sediments and inform prevailing metabolic processes. While biogenic Fe(II) can reduce DDT to DDD, Fe(II) sorbed to clay particles is more reactive than dissolved Fe(II) (Li et al., 2010). Grain size analysis will reveal clay content, which affects the sorption of Fe(II) as well as substrates and electron acceptors, including Fe(III), which is used in microbial degradation of contaminants in subsurface environments (Shelobolina et al., 2004).

This work is the first to characterize sediment microbial community structure of a deep coastal basin environment at high spatial resolution on a regional scale. Accompanying geochemical analyses provide a useful backdrop for understanding the drivers of community structure, but little can be inferred about microbial community function from deep sequencing of the 16S rRNA gene alone. The work shown here provides guidance and informs hypotheses for a forthcoming directed metagenomic analysis of SP Basin and broader SCB sediment microbial communities.

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Appendix

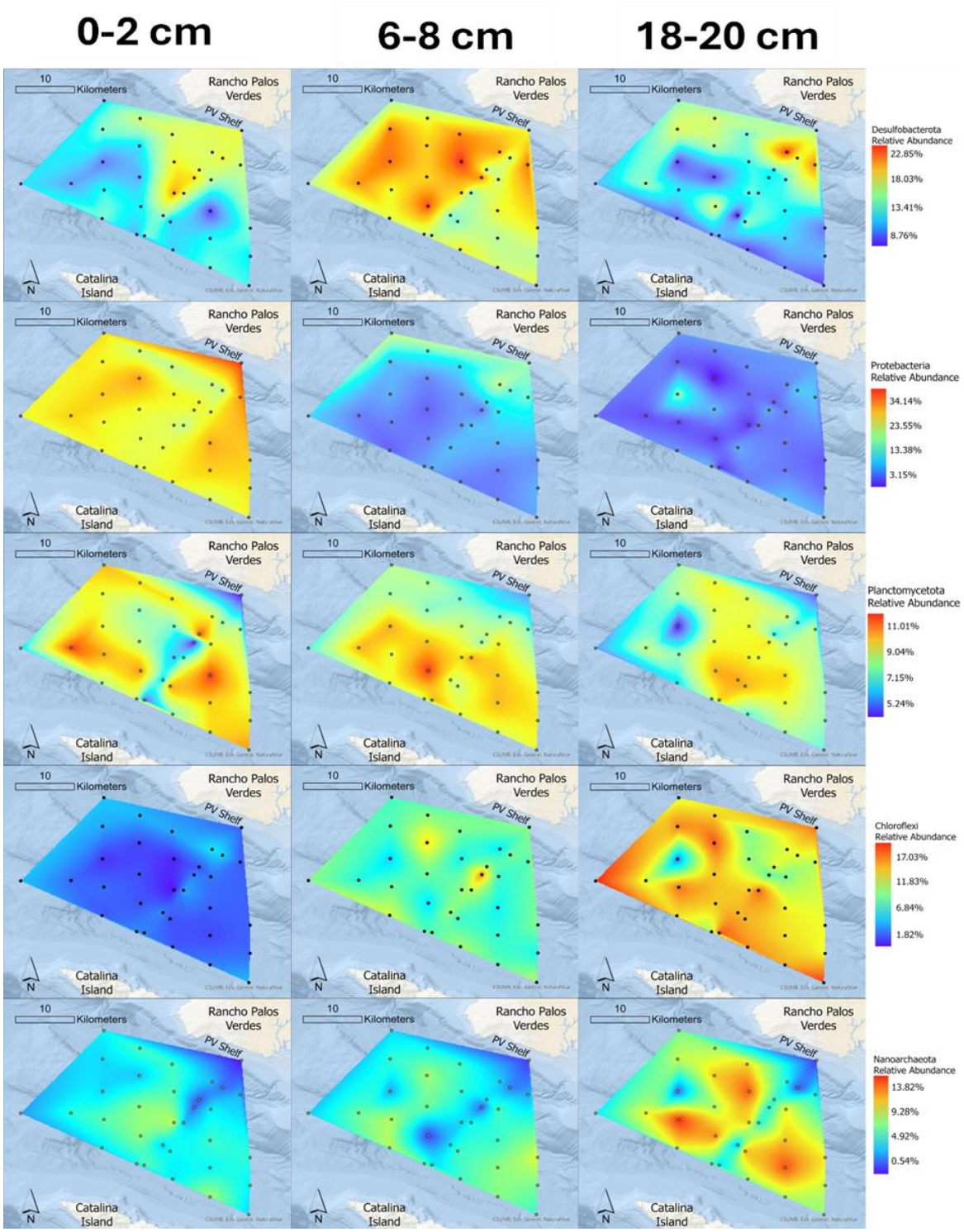
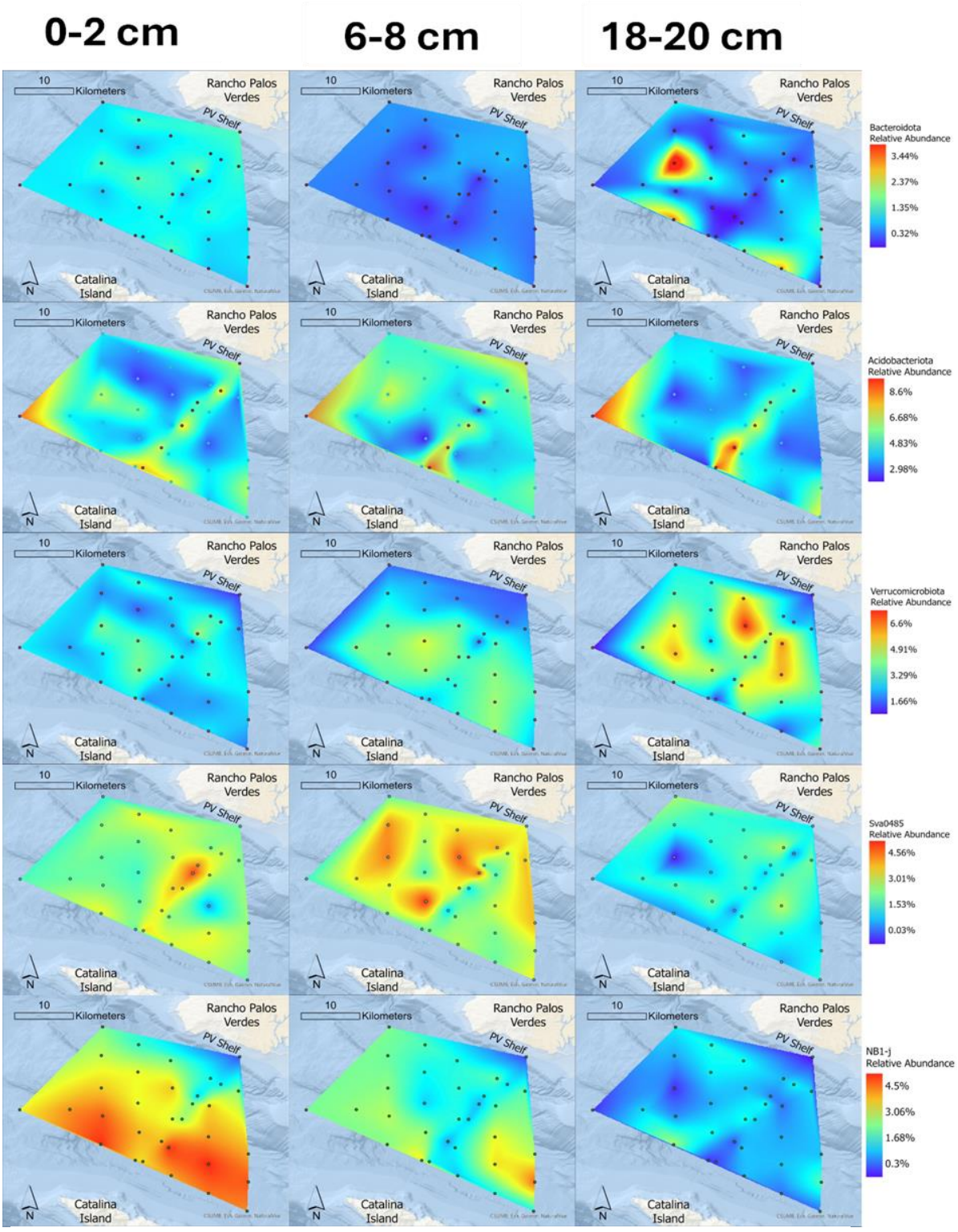


Figure A1. Spatial distributions of the top 20 Phyla in the 0-2 cm, 6-8 cm, and 18-20 cm intervals (left to right).



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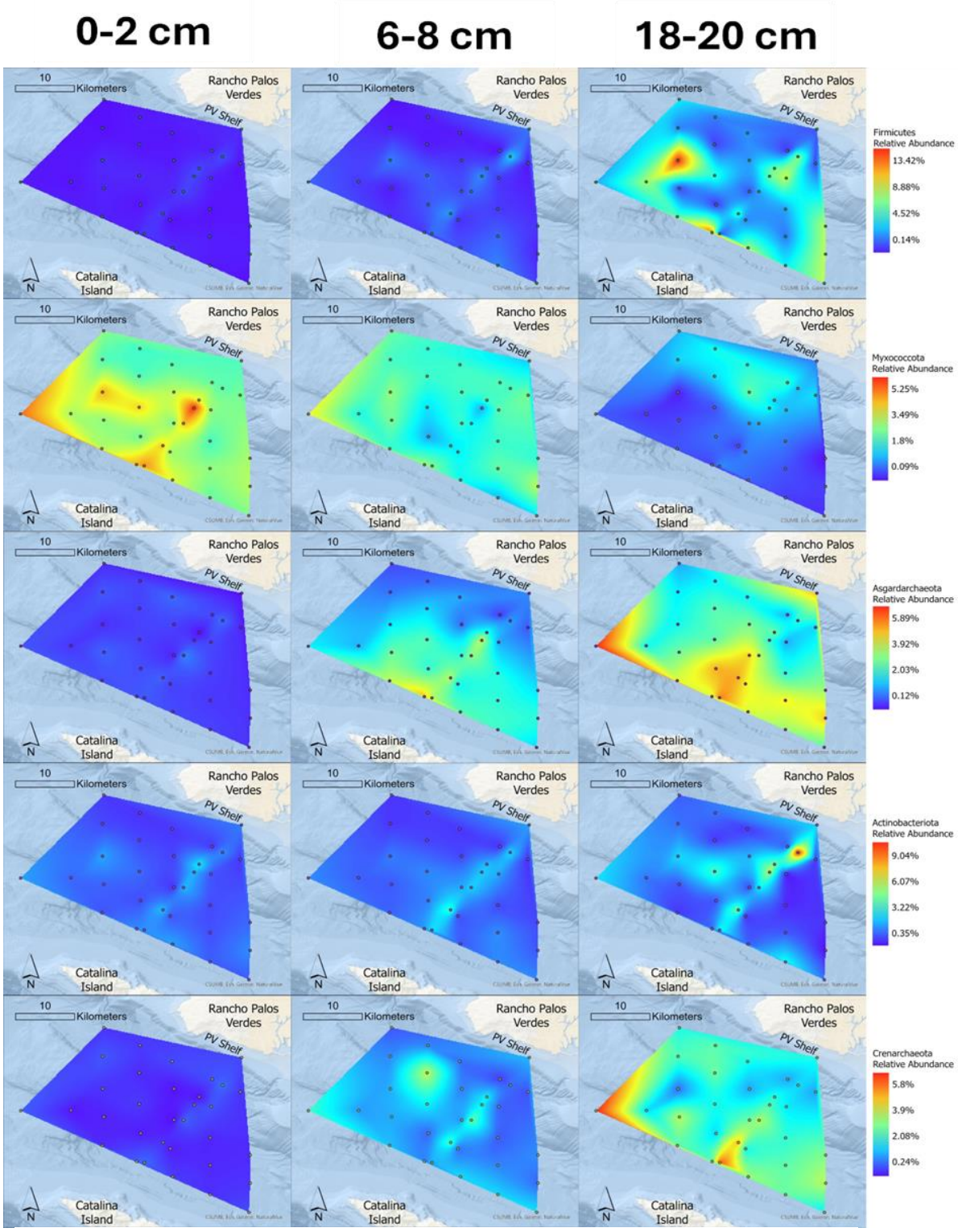

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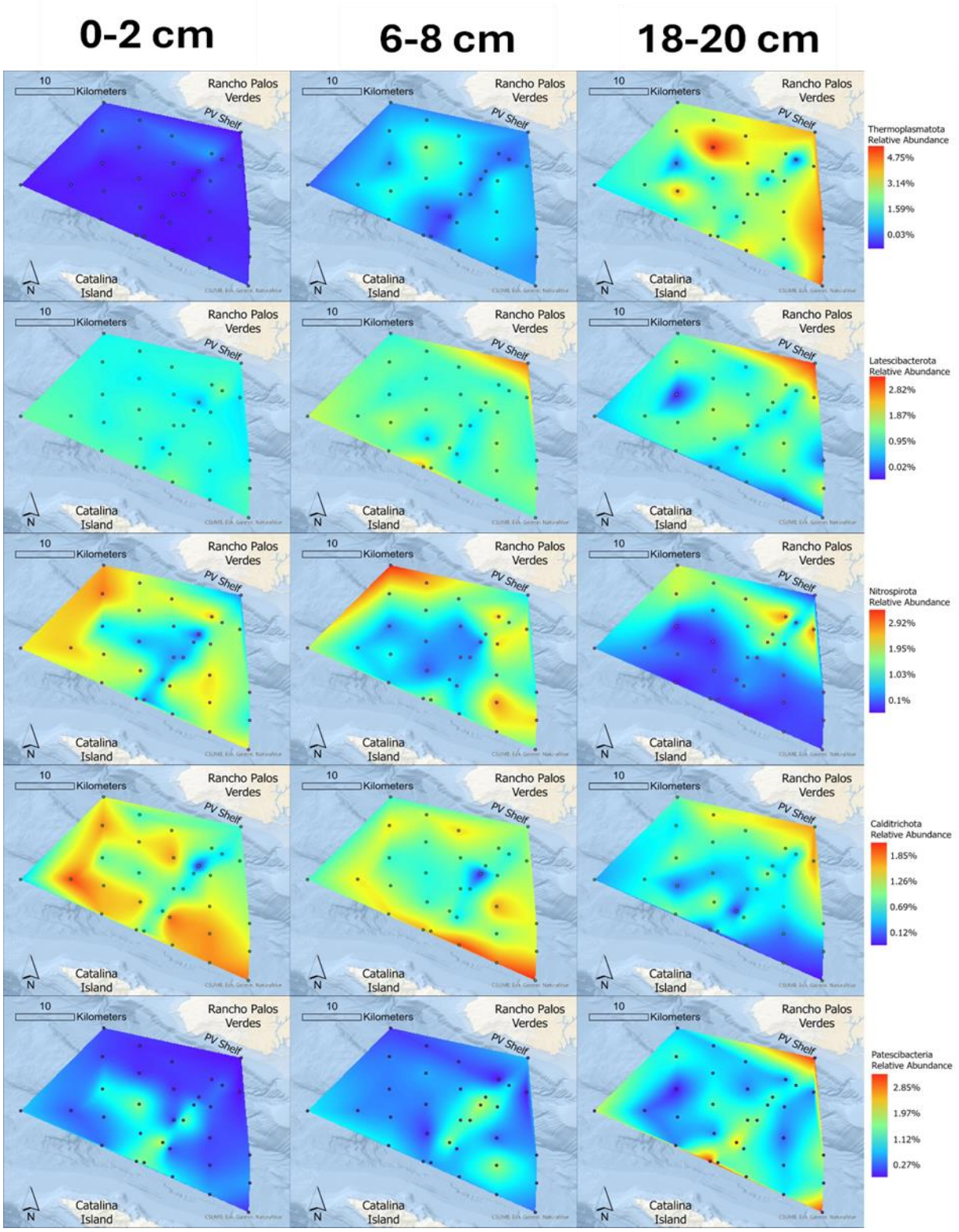

Figure A1 (continued)**.**

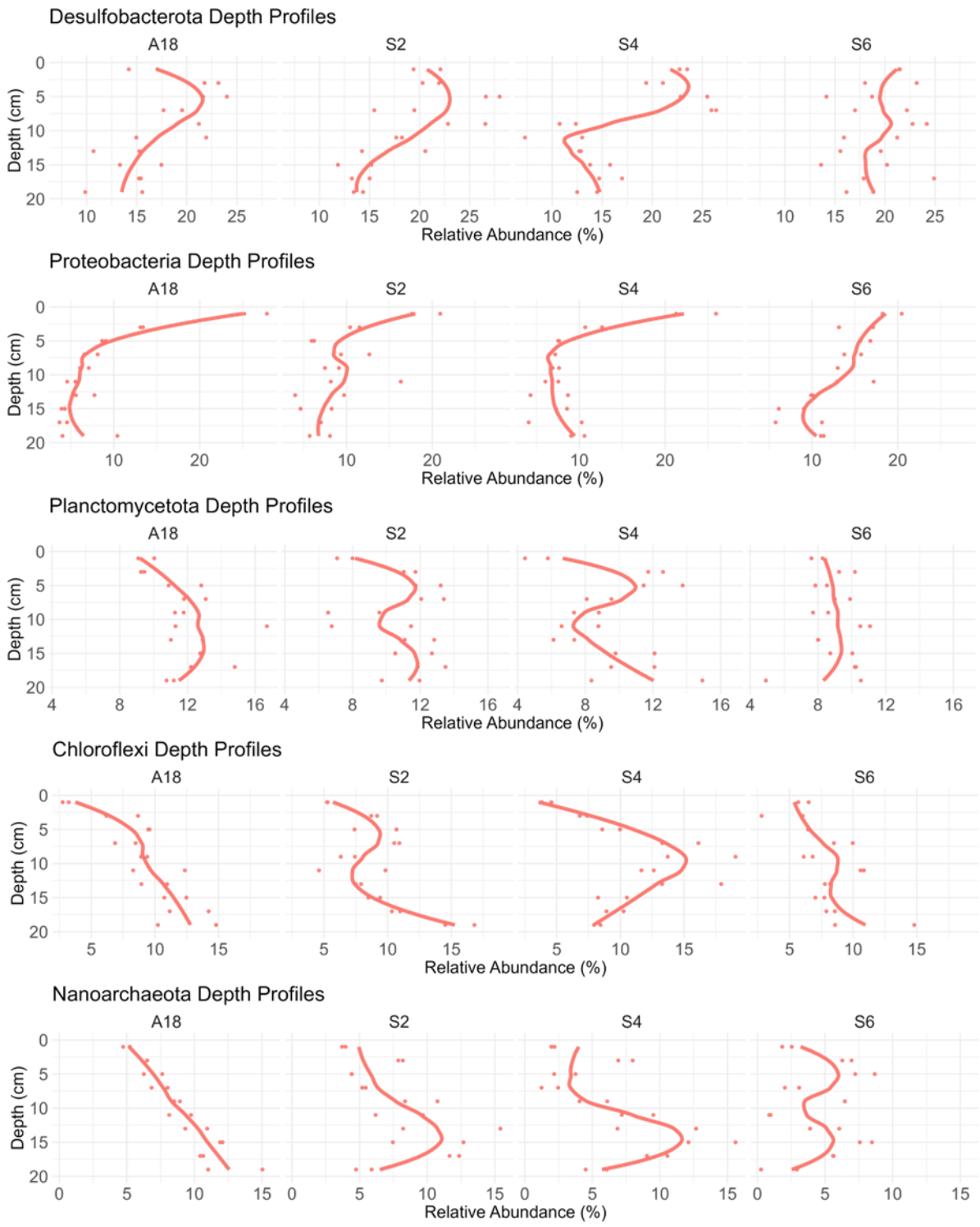

Figure A2. Depth distributions of top 20 phyla at stations A18, S2, S4, and S6 which represent stations with low anthropogenic impact, high excess carbon, high tar content, and near-shelf with high sedimentation rates, respectively.

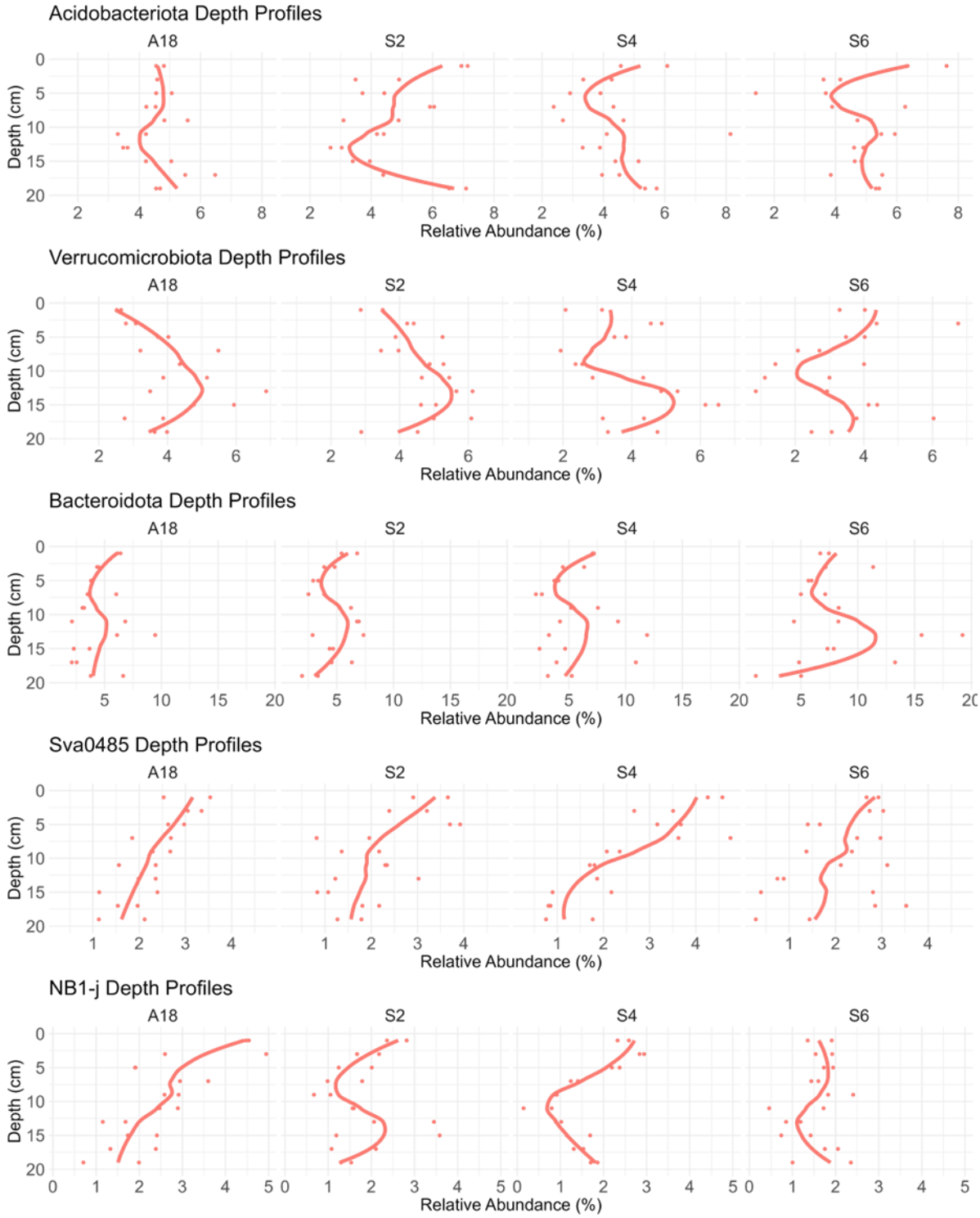

Figure A2 (continued)**.**

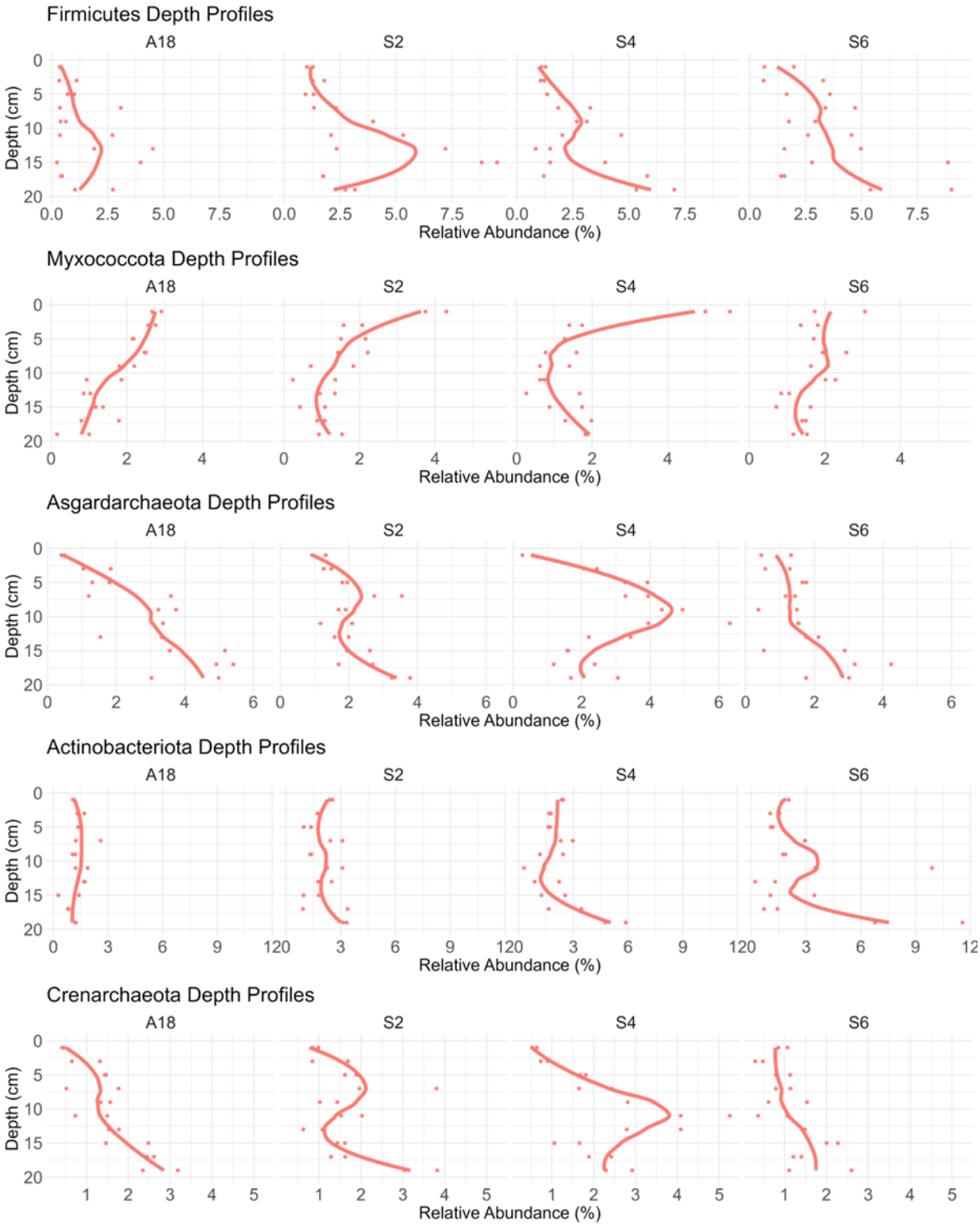

Figure A2 (continued)**.**

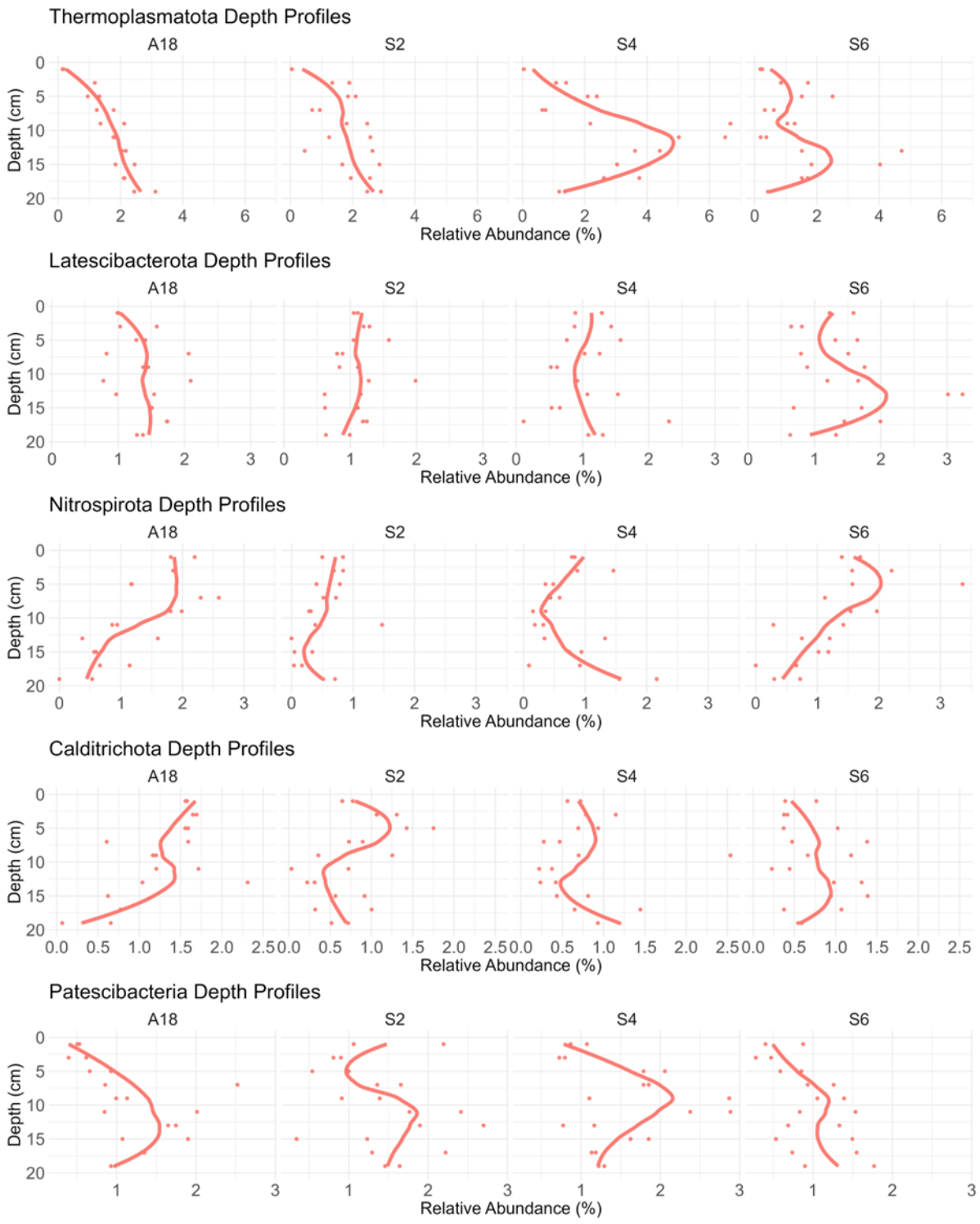

Figure A2 (continued)**.**