**NEMO TOOLS**

Next-generation monitoring & mapping tools to assess marine ecosystems & biodiversity

Milestone M2.4

**Biodiversity estimates, gene pathways and functional characteristics of whole communities**

**Greece 2.0**
NATIONAL RECOVERY AND RESILIENCE PLAN

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# Executive Summary

Milestone 2.4 (Biodiversity estimates, gene pathways and functional characteristics of whole communities) is connected to the entire WP2, following relevant ecological analyses of molecular datasets. The sampling, methodological, bioinformatic, and analyses approaches and the OTUs atlases are depicted and described in previous WP2 related Deliverables (D2.1 for the sampling techniques, D2.2 for the bioinformatics and ecological analyses, D2.3 for the integrated OTUs atlases) and Milestones (M2.1: sampling, M2.2: method testing, M2.3: raw read datasets). This milestone summarizes the communities' biodiversity endpoints which include selected alpha-diversity indicators and shows functional diversity estimators as the predominant gene pathways in the examined Gulfs. Detailed taxonomic and functional biodiversity characteristics of the examined communities will be presented in Deliverable D2.4.

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## CONTRIBUTORS

**TABLE 1 NAMES AND ROLES OF CONTRIBUTORS TO THIS DELIVERABLE.**

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# Biodiversity estimates

Alpha-diversity patterns varied widely across samples and regions for both bacterial and eukaryotic domains. Bacterial richness reached >1900 OTUs in a sample, with offshore waters showing the highest richness and Shannon diversity. Across gulfs, Thermaikos and Saronikos exhibited higher bacterial diversity compared to Kavala, while offshore samples showed rich and even communities. Depth had a modest effect on bacteria, with slightly higher diversity in deeper waters in many stations but without a consistent trend. Sampling method influenced results: Gauzes yielded the most variable bacterial richness, Sterivex filters showed stable intermediate values, and Nucleopore filtering often detected high richness with slightly lower evenness (Figure 1).
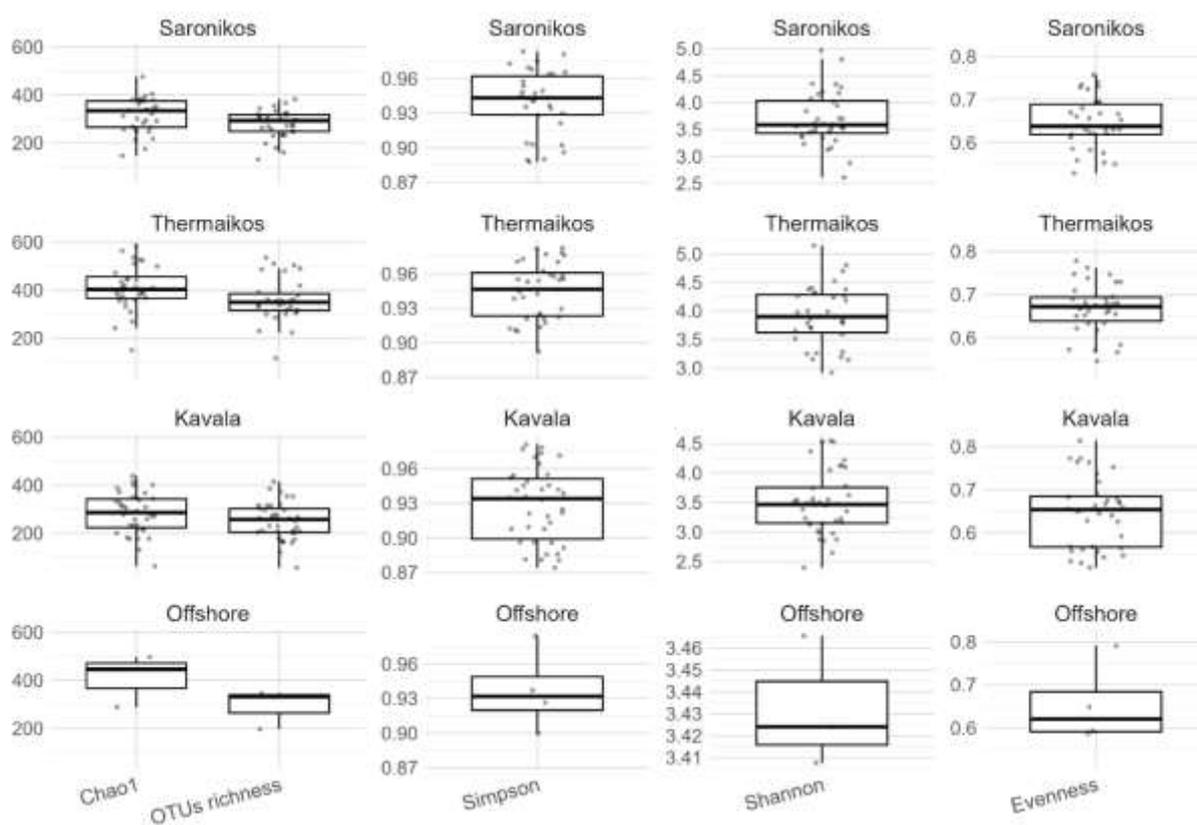


**Figure 1.** Boxplots of the distribution of the alpha-diversity indices of bacterial communities in the examined Locations. The horizontal black bars depict the median values, and the dots depict individual samples. Outliers were filtered per-index using 1.5xIQR thresholds.

For eukaryotes, alpha-diversity was generally lower and more uneven than that of bacteria. Richness reached to nearly 500 OTUs in the most diverse samples. Offshore waters and few Kavala sites showed the highest eukaryotic richness and Shannon indices, reaching values >4. Saronikos consistently showed the lowest eukaryotic diversity, often driven by dominance of few taxa. Bloom events strongly

reduced evenness in surface waters, leading to some samples being dominated by one OTU contributing >80% of the total number of reads. Deep samples were occasionally more diverse and balanced when surface layers were bloom-dominated. Methodological effects were pronounced: Sterivex filters performed most consistently, Nucleopore filters ranked second and excelled in offshore samples, while Gauzes often under-captured eukaryotic taxa (Figure 2).
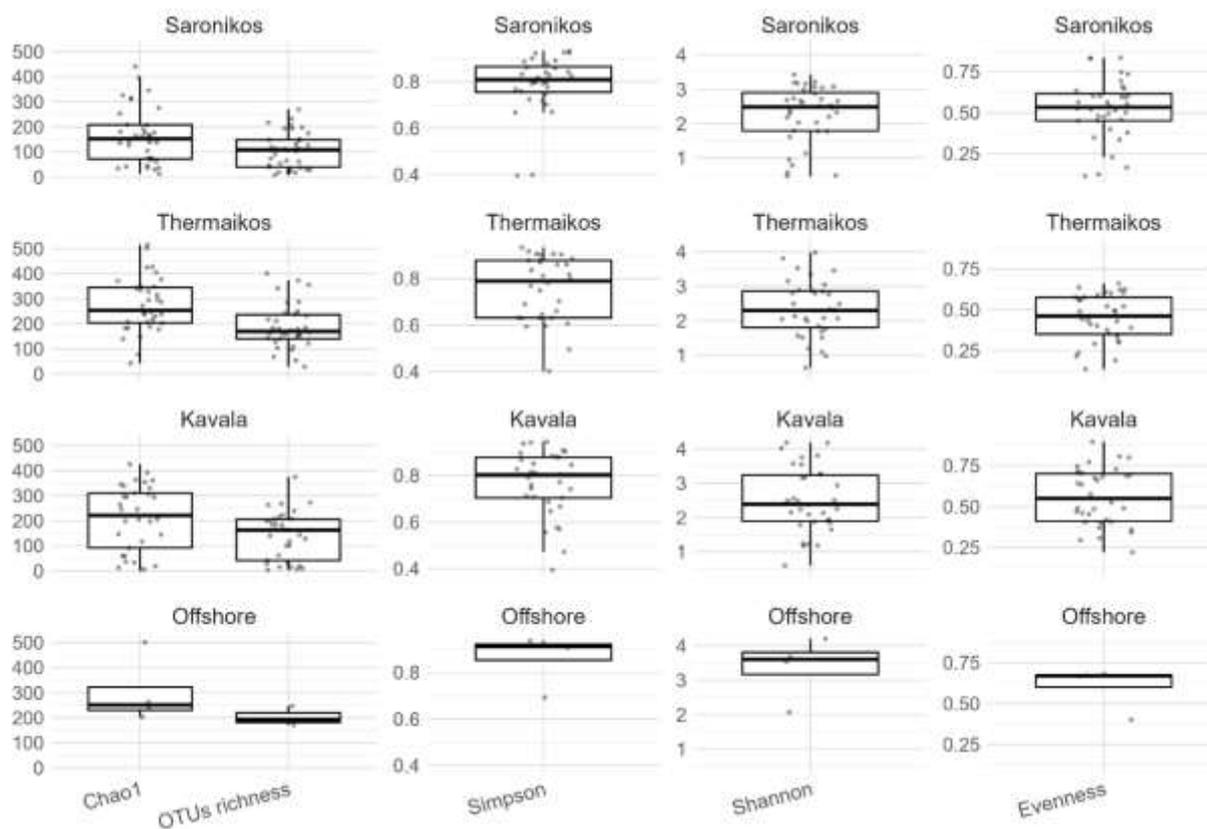


**Figure 2.** Boxplots of the distribution of the alpha-diversity indices of eukaryotic communities in the examined Locations. The horizontal black bars depict the median values, and the dots depict individual samples. Outliers were filtered per-index using 1.5xIQR thresholds.

Overall, bacterial communities were richer and more even than eukaryotic ones across all sites. Both domains showed strong spatial structuring, with oligotrophic offshore environments supporting the highest measured a-diversity.

# Gene pathways and functional characteristics of whole communities

The three examined gulfs (Kavala, Saronikos and Thermaikos) exhibited broadly similar functional profiles, with most Clusters of Orthologous Genes (COGs) assigned to core metabolic pathways. Various metabolic pathways represented the most abundant category in all regions with >40 % contribution to the total number of COGs, with Saronikos showing the highest counts. The second most represented category is microbial metabolism in diverse environments, again slightly higher contribution of COGs to the total number being retrieved in Saronikos and Thermaikos. Biosynthesis of secondary metabolites also ranked high, with Kavala showing somewhat elevated representation. Carbon metabolism and 2-oxocarboxylic acid metabolism (01210) display comparable values across the gulfs, reflecting conserved carbon utilization potential. Pathways related to fatty acid metabolism and aromatic compound degradation show lower but consistent representation across all three locations. Biosynthetic pathways such as amino acids, cofactors, nucleotides, and nucleotide sugars exhibit moderate abundances. Nitrogen and sulphur cycling pathways represent the lowest detected categories, with very small relative gene contributions in all gulfs (Figure 3).
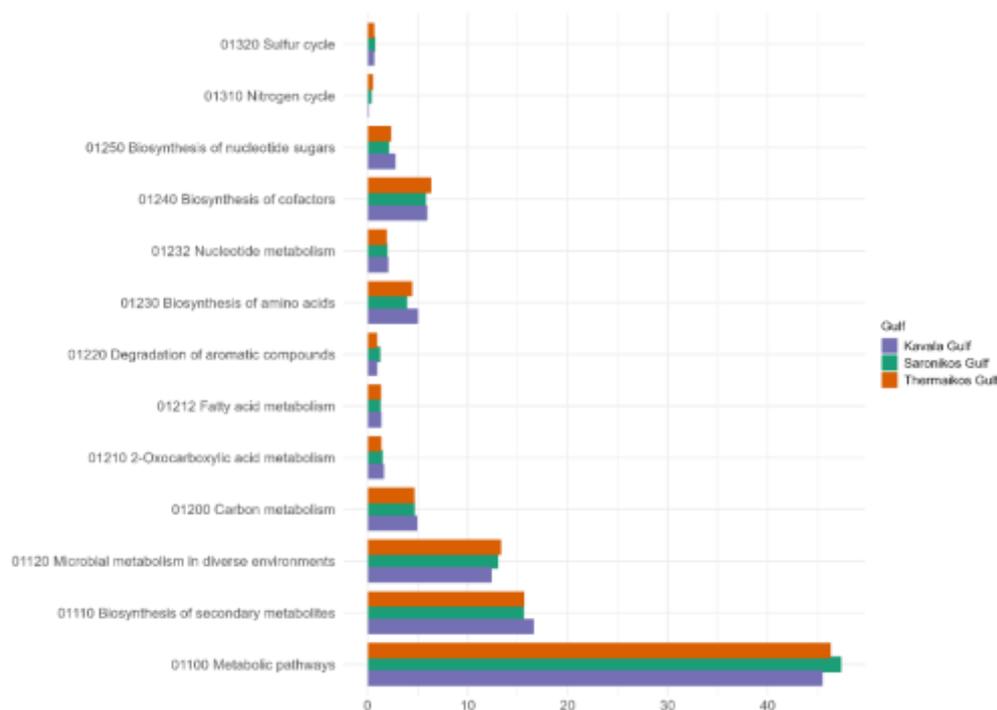


**Figure 3.** The relative contribution of Clusters of Orthologous Genes (COGs) mapped to global and overview KEGG pathways for Saronikos, Thermaikos, and Kavala Gulfs.

Differences among gulfs are quantitative rather than qualitative, indicating environmental gradients rather than distinct community functions.

The dominance of pathways related to carbon turnover and biosynthesis suggests active microbial processing of organic matter across all regions. Collectively, the data indicate stable metabolic capability across the gulfs with minor localized enhancements tied to specific biogeochemical processes.